# STUDIES ON INFLUENCING FACTORS OF PHYTOPLANKTON FUNCTIONAL GROUPS COMPOSITION AND ECOLOGICAL STATUS OF THE DONGTA SPAWNING GROUNDS IN THE PEARL RIVER, CHINA

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Abstract. To explore the spatial and temporal distribution characteristics of phytoplankton functional groups (PFGs) of the Dongta spawning grounds in the Pearl River, China, quarterly surveys of this area were carried out during March, 2016 to December, 2018, and the ecological status was analyzed and evaluated according to PFGs assemblage index (Q index). 26 PFGs were identified, and the dominant PFGs were C, D, J, K, L<sub>0</sub>, MP, N, P, S1, S2, T, W1, W2, X2, Y in this area. Phytoplankton Q indexes varied from 1.83 to 3.73, with the mean value 2.91, which showed that the ecological status of the Dongta spawning grounds was "medium" and "good", but parts occasionally were "tolerable". Temporally, the general ecological status was good in March and June, but it was medium in September and December. Basing on our study, the results showed that: (1) factors including water velocity (V), DO, Chla, phosphate, oxidation reduction potential, total phosphorus, electrical conductivity, total dissolved solid and salinity were significantly correlated with the dominant PFGs; (2) the phytoplankton Q index was significantly correlated with the V. It was suggested that after the construction and operation of Datengxia Water Dam and its upper reaches cascade dams, attention should be paid to regulate the water flow and velocity in the lower reaches of the river by means of ecological operation, so as to ensure the functions of the spawning grounds and protect the fishery conservation zones in the mainstream of the Pearl River. **Keywords:** Dongta spawning grounds, phytoplankton functional groups, assemblage index, water quality, relationship analysis

### Introduction

Phytoplankton is the main primary producer in water areas, which releases oxygen through photosynthesis and forms the basis of material circulation and energy transfer in aquatic ecosystems (Reynolds, 1984). In addition, phytoplankton is an important indicator for water environments because of its small size, rapid growth, short life

cycle, highly unified living space and water environment, and rapid response to environmental changes (Huisman et al., 2004; Istvanovics et al., 2010). Its community composition and population changes can directly and quickly reflect the living environment (Reynolds, 2006; Istvanovics et al., 2010; Wang et al., 2017). Traditional phytoplankton ecology is studied mainly by identifying algae species based on original Linnaean classification system, followed by the analysis of the variation of phytoplankton species composition, density, biomass and dominant species, to reflect and evaluate the environmental quality of specific waters (Reynolds, 2006). However, the process was time-consuming and laborious. Besides, dominant species, diversity index and other indicators could only reflect one or several aspects of the environmental status, the results were often having large difference, the interpretation of the results and conclusions was usually arbitrary and subjective, and could not reflect the habitat conditions (Reynolds et al., 2002). In order to accurately reflect the real functions of phytoplankton in the aquatic ecosystem, Reynolds et al. (2002) and Padisák et al. (2006) referenced the research methods on land plants, after gradually expanding and improving these approaches, and finally formed a relatively complete theory of phytoplankton functional groups (PFGs, coda) methods. These methods combined phytoplankton habitat characteristics with community ecological process, simplified the complexity of traditional classification system, and provided a powerful tool for explaining the selection mechanism of phytoplankton community and predicting community succession results more rationally (Padisák et al., 2009). In the world, PFGs classification methods had become one of the main techniques to study the ecological structure and function of rivers, reservoirs and lakes (Crossetti et al., 2008; Xiao et al., 2011; Abonyi et al., 2012; Devercelli et al., 2013; Cupertino et al., 2019; Wang et al., 2020b). At the same time, a new ecological status estimation method for waters basing on PFGs assemblage index (Q index), was proposed to assess ecological status of different types of water (Padisák et al., 2006). Q index combines the weight of functional groups relative to the total biomass, with a factor number for each assemblage related to the type of water body. The Q index method had been successfully applied in ecological status assessment for extensive regional waters (Wang et al., 2011; Zhu et al., 2013; Santana et al., 2017).

Dongta spawning grounds is located in the upstream of the Xunjiang River, from the junction of Qianjiang and Yujiang Rivers to Dongta Village, Xun Wang Town, Guiping County, with a length of about 7 km, which lies in the area of E 110.0956°, N 23.4054° and E 110.0967°, N 23.4003° to E 110.1387°, N 23.4560° and E 110.1443°, N 23.4550°. It is known as the second largest spawning grounds of the four Chinese farmed carps, i.e. black carp, grass carp, silver carp and bighead carp, and the most abundant fish biodiversity in the main stream of the Pearl River, China (Shuai et al., 2016). The Dongta spawning grounds had important economic value and aquatic biological resources protection value for aquatic living resources, especially the Chinese carp fish (Li et al., 2009, 2021). At present, the Datengxia Water Control Project, upstream of Dongta spawning grounds, is under construction. It is only about 15 kilometers away from the downstream boundary of the Dongta spawning grounds. Previous studies showed that dam construction would have many important impacts on water velocity, sediment and biological resources in the upstream and downstream of the river, and affected the distribution of phytoplankton (Zeng et al., 2006; Zhou et al., 2011; Zuo et al., 2019; Maavara et al., 2020). Therefore, the influence of Datengxia hydroelectric power plant during the construction and water storage period on the changes of water environment and biological resources in Dongta spawning grounds should be attracted early attention. In the past, the division of fish spawning grounds was mainly based on the status of fishery resources, and the location and boundary area of spawning grounds were determined through investigation or detection of fish resources in various sections of rivers (Tan et al., 2011a, b; Li et al., 2021). It was difficult to scientifically explain the formation and change mechanism of fish spawning grounds, and to formulate corresponding protection and restoration measures.

Good water environment in the Dongta spawning grounds is the basically conditions to ensure fish spawning and breeding, which is also beneficial to realize its protection function (Fellman et al., 2015; Deng et al., 2019; Li et al., 2021). Since the PFGs could explain and predict the water condition, farther more, it had far-reaching impact on fishery resources distribution, the present study selected the habitat of the Dongta spawning grounds in the Pearl River, through monitoring and analysis its environmental factors and PFGs, as well as the relationship between the environment variables and PFGs, to analyze the distribution characteristics of functional groups and its main influencing factors, and to understand the ecological status of the Dongta spawning grounds. Comprehensive conclusions would be discussed and proposed that how to ensure the functions of the spawning grounds and protect the fishery conservation zones in mainstream of the Pearl River after the construction and operation of Datengxia Water Dam and its upper reaches cascade dams.

### Materials and methods

### Study site and sampling

Surface water samples were collected in March, June, September and December of the year 2016, 2017 and 2018 in the Dongta spawning grounds of the Pearl River, China (*Fig. 1*), and the investigation was launched on the last ten days of the sampling month. The sampling stations of the Dongta spawning grounds were Bailan (BL, downstream and near the Datengxia Dam, E 110.0352°, N 23.4675°), Guiping (GP, under the Qianjiang bridge of the Guiping County, E 110.0807°, N 23.4103°), Yujiang (YJ, near the river mouth of the Yujiang connection to the mainstream of the Pearl River, E 110.1021°, N 23.3961°), Dongta (DT, Dongta Village, Xun Wang Town, Guiping County, E 110.1773°, N 23.4690°).

### Determination of water physical and chemical factors

Water velocity was measured using water current meter (Global water, USA). Water transparency (SD) was measured using a Secchi disk according to a standard method (Zhang and Huang, 1991). Water temperature (WT, °C), pH, dissolved oxygen (DO, mg/L), oxidation–reduction potential (ORP, mV), conductivity (Cond,  $\mu$ S/cm) and total dissolved solids (TDS, g/L) were measured using a smart portable multi-parameter water quality analyzer (YSI, USA). Approximately 500 ml water was filtered using a Whatman GF/C filter membrane and was then used to measure the chlorophyll-a content (Chla,  $\mu$ g/L) according to a previously described method (The State Environmental Protection Administration, 2002). The concentrations of ammonium (NH<sub>4</sub>-N), nitrate (NO<sub>3</sub>-N), NO<sub>2</sub>-N, total nitrogen (TN), total phosphorus (TP) and

Silicate (SiO<sub>3</sub>-Si) were determined using a flow injection water quality analyzer (Skalar, Netherlands) by standard procedures. The concentration of un-ionized ammonia (NH<sub>3</sub>, mg/L) was calculated using NH<sub>4</sub>-N, pH and WT according to previously studying method (Liu et al., 2021).



*Figure 1.* The sampling stations of the Dongta spawning grounds in the Pearl River, China. In the figure, BL, GP, YJ, DT, SZ standing for Bailan, Guiping, Yujiang, Dongta and Shizui, respectively

### Phytoplankton sample collection and analysis

For each phytoplankton sample, 1 L of water was collected 0.5 m below the surface using a 5-L HQM-1 sampler, put into a polyethylene bottle, and fixed immediately with neutral Lugol's solution (5%). The phytoplankton samples were taken to laboratory, and concentrated to 30 mL using the settling technique. Phytoplankton was quantified using a light microscope (Olympus CX21, Japan) at 400× magnification. The units (cells, colonies and filaments) were enumerated in random fields, and at least 100 fields or 200 individuals of the most frequent species were counted (Zhang and Huang, 1991), and phytoplankton species were identified according to Hu and Wei (2006). Phytoplankton biomass was calculated from the biovolume of each species, assuming unit specific gravity, by geometrical approximation according to Hillebrand et al. (1999).

Phytoplankton taxa were classified into characteristic PFGs according to Reynolds et al. (2002) and Padisák et al. (2009). PFGs contributing more than 10% to the total biomass of each sample were categorized as dominant PFGs; meanwhile, some algal species usually contributed more than 10% to the density of the sample, and the PFGs the algae belonging to were also considered as dominant functional groups.

#### Evaluation of the water ecological status

The functional group approach had been applied in estuary, lake and reservoir ecosystems (Crossetti and de M. Bicudo, 2008; Wang et al., 2011; Santana et al., 2017; Cao et al., 2018), and can potentially be developed to assess the water quality more

consistently. According to phytoplankton functional classification and typology of lakes, Padisák et al. (2006) developed an assemblage index, Q index, to assess ecological status of different lake types established by the Water Framework Directive (WFD). Q index combines the weight of functional groups relative to the total biomass, with a factor number for each assemblage related to the type of water body. The calculation of Q is the following:

$$Q = \sum_{i=1}^{n} (p_i F_i)$$
(Eq.1)

Where  $p_i = n_i/N$ ,  $n_i$  is the biomass of the *i*th group, while *N* is the total biomass;  $F_i$  represents the assignment value of the *i*th PFG in the water body, and *i* represents the water quality index to range between 0 (the worst) and 5 (the best). Ecological status based on *Q* index can be classified into 5 grades: 0–1: bad; 1–2: tolerable; 2–3: medium; 3–4: good; and 4–5: excellent. The *Q* index method had a sufficiently solid theoretical basis, and it had been successfully applied in ecological status assessment for several reservoirs recently (Crossetti and de M. Bicudo, 2008; Santana et al., 2017).

#### Data treatment

Statistical analysis was carried out using the SPSS 18.0 package (one-way ANOVA). Variables of phytoplankton and chemical data were visualized using the EXCEL 2010, Origin (8.0) and R (R Studio) program with vegan package. To analyze the influence of these environmental factors on PFGs, Redundancy Analysis (RDA) was performed to investigate the relationship between the main environmental parameters and the dominant coda, using the R package Vegan. During the analysis, the PFGs biomass data were Hellinger transformed, as well as the environmental parameters were standardized, to reduce the effects of extreme values. A forward selection of environmental factors was applied to avoid using collinear environmental factors in the same constrained ordination model (Wang et al., 2020b). Only those parameters contributing significantly (P < 0.05) to PFGs dynamics were considered as the main influencing variables.

#### Results

### Physical and chemical variables

The results of water environmental variables in the Dongta spawning grounds were shown in *Table 1*. The mean water velocity in the survey area was 0.9 m/s, with the lowest velocity in December (mean value 0.5 m/s) and the highest velocity in June (mean value 1.4 m/s). Water pH values were most in a range of 7.43 ~ 8.64, and the pH value in June was slightly lower than other period. The change of water temperature varied obviously seasonally. The water salinity varied in the range of 0.08‰ to 0.17‰. The mean value of water TDS content in three years was roughly the same, but it was obviously higher in wet season than in other periods. ORP of the study area had a widely variation range, with the minimum value of 15.50 mV and maximum value of 730.50 mV. The water Cond showed an obvious changes with hydrological period. Water DO content varied in the range of 5.33~9.43 mg/L, meeting the oxygen conditions needed for fish living. Affected by rainfall in the sampling periods, the water transparency varied in a large range, with the mean values were about 92 cm, 30 cm,

74 cm and 98 cm in the four quarters, respectively. TN content was higher than 1.0 mg/L in most periods with its maximum value 2.74 mg/L, and the quarterly average value was 2.11 mg/L, 1.89 mg/L, 1.94 mg/L and 2.02 mg/L, respectively, which had an obviously increase in wet season. The variation trend of NO<sub>3</sub>-N, NH<sub>4</sub>-N and SIN (soluble inorganic nitrogen, NO<sub>3</sub>-N + NO<sub>2</sub>-N + NH<sub>4</sub>-N + NH<sub>3</sub>) was similar to that of TN. Silicate concentration showed an obvious change in the hydrological period, and the concentration in wet season (June and September) was significantly higher than that in level season and dry season (March and December). The contents of NO<sub>2</sub>-N and NH<sub>3</sub> were at a low level, but peaked in some periods. The ratio of nitrogen to phosphorus was significantly different, with the minimum value of 4.31 and maximum value of 131.54.

## Phytoplankton community composition

Identification of phytoplankton samples collected from the Dongta spawning grounds showed that a total of 176 phytoplankton species, including a few varieties and forma, were distributed among the following 7 major taxonomic categories: Bacillariophyceae (69 taxa), Chrysophyceae (59 taxa), Euglenophyceae (23 taxa), Cyanophyceae (14 taxa), Dinophyceae (4 taxa), Cryptophyceae (4 taxa) and Xanthophyceae (3 taxa). The Percentages of the 7 categories to the total species were 39.20%, 33.52%, 13.07%, 7.95%, 2.27%, 2.27% and 1.70%, orderly.

The quarterly phytoplankton community composition during March 2016 to December 2018 were showed in Figure 2A. In March, a total of 117 phytoplankton species, including a few varieties and forma, were distributed among the following 7 major taxonomic categories: Bacillariophyceae (46 taxa), Chlorophyceae (39 taxa), Euglenophyceae (15 taxa), Cyanophyceae (7 taxa), Dinophyceae (3 taxa), Cryptophyceae (4 taxa) and Xanthophyceae (3 taxa). In June, a total of 130 phytoplankton species, were distributed among the 7 major taxonomic categories: Bacillariophyceae (53 taxa), Chlorophyceae (39 taxa), Euglenophyceae (18 taxa), Cyanophyceae (12 taxa), Dinophyceae (2 taxa), Cryptophyceae (4 taxa) and Xanthophyceae (2 taxa). In September, a total of 118 phytoplankton species, were distributed among 7 major taxonomic categories: Bacillariophyceae (42 taxa), Chlorophyceae (43 taxa), Euglenophyceae (16 taxa), Cyanophyceae (8 taxa), Dinophyceae (2 taxa), Cryptophyceae (4 taxa) and Xanthophyceae (2 taxa). In December, a total of 98 phytoplankton species, were distributed among 7 major taxonomic categories: Bacillariophyceae (32 taxa), Chlorophyceae (38 taxa). Euglenophyceae (13 taxa), Cyanophyceae (7 taxa), Dinophyceae (2 taxa). Cryptophyceae (4 taxa) and Xanthophyceae (2 taxa). And the phytoplankton species composition in each sampling station were showed in Figure 2B-F.

# Phytoplankton abundance

The phytoplankton cell density in the sampling area ranged from  $8.40 \times 10^4$  cell/L to  $4.08 \times 10^6$  cell/L. The average density was  $5.01 \times 10^5$  cell/L. The maximum density appeared at the GP station in December 2018, and the minimum value appeared at the SZ site in June 2018 (*Fig. 3A*). The variation range of phytoplankton biomass was  $0.14 \sim 2.55$  mg/L, with an average of 0.94 mg/L. The maximum biomass appeared at the BL station in September 2016, and the minimum biomass appeared at the DT station in December 2017 (*Fig. 3B*).

Environmental	Mean and variation ranges											
factors*	March	June	September	December								
V (m/s)	$0.86 \pm 0.10$ (0.30~1.50) <sup>a**</sup>	1.42 ± 0.11 (0.70~2.20) <sup>b</sup>	$0.81 \pm 0.07$ (0.50~1.30) <sup>a</sup>	$0.53 \pm 0.06$ (0.30~0.90) °								
рН	$8.00 \pm 0.07$ (7.67~8.64)	$7.88 \pm 0.08$ (7.45~8.48)	$8.02 \pm 0.04$ (7.60~8.23)	$8.03 \pm 0.10$ (7.43~8.64)								
WT (°C)	$\begin{array}{c} 18.48 \pm 0.25 \\ (17.48 {\sim} 20.55)^{a} \end{array}$	$\begin{array}{c} 27.14 \pm 0.38 \\ (23.98{\sim}29.43)^{\text{b}} \end{array}$	$28.29 \pm 0.23 \\ (26.95 \sim 29.68)^{c}$	$20.84 \pm 0.43$ (19.09~24.61) <sup>d</sup>								
Sal (‰)	$0.13 \pm 0.01$ (0.08~0.15) <sup>ab</sup>	$0.12 \pm 0.01$ (0.08~0.15) <sup>b</sup>	$\begin{array}{c} 0.14 \pm 0.003 \\ (0.11 \text{~~} 0.16)^{\text{ac}} \end{array}$	$0.15 \pm 0.004$ (0.11~0.17) <sup>c</sup>								
TDS (g/L)	$0.18 \pm 0.01$ (0.13~0.21) <sup>a</sup>	$0.18 \pm 0.01$ (0.15~0.22) <sup>a</sup>	$\begin{array}{c} 0.19 \pm 0.005 \\ (0.14 {\sim} 0.22)^{ab} \end{array}$	$\begin{array}{c} 0.20 \pm 0.005 \\ (0.15  0.23)^{\text{bc}} \end{array}$								
ORP (mV)	82.31 ± 13.29 (30.40~151.90) <sup>a</sup>	93.19 ± 13.05 (15.50~167.70) <sup>a</sup>	$146.85 \pm 32.86$ (37.70~348.70) <sup>ab</sup>	$\begin{array}{c} 198.93 \pm 52.95 \\ (25.30{\text{-}}730.50)^{\text{b}} \end{array}$								
DO (mg/L)	$7.92 \pm 0.20$ (6.64~8.93) <sup>a</sup>	$7.34 \pm 0.24$ (5.33~8.31) <sup>ab</sup>	$7.07 \pm 0.20$ (5.75~8.31) <sup>b</sup>	$7.81 \pm 0.24 \\ (6.47 \sim 9.43)^{a}$								
Cond (µS/cm)	265.60 ± 9.95 (177.00~312.90) <sup>a</sup>	$\begin{array}{c} 272.04 \pm 10.09 \\ (176.00 {\sim} 337.89)^{ab} \end{array}$	$314.35 \pm 7.33$ (238.00~356.19) <sup>c</sup>	$\begin{array}{c} 291.76 \pm 6.46 \\ (236.00  325.49)^{\text{bc}} \end{array}$								
SD (cm)	$92.00 \pm 5.87$ (70.00~150.00) <sup>a</sup>	$29.67 \pm 3.53$ (10.00~50.00) <sup>b</sup>	$74.00 \pm 12.71$ (30.00~190.00) <sup>a</sup>	$97.67 \pm 6.53$ (60.00~140.00) <sup>ac</sup>								
TP (mg/L)	$0.08 \pm 0.02$ (0.02~0.25) <sup>a</sup>	$0.10 \pm 0.01$ (0.03~0.20) <sup>a</sup>	$0.18 \pm 0.02$ (0.02~0.31) <sup>b</sup>	$0.25 \pm 0.01$ (0.14~0.32) <sup>c</sup>								
PO <sub>4</sub> -P (mg/L)	$0.05 \pm 0.01$ (0~0.17) <sup>a</sup>	$0.06 \pm 0.02$ (0.01~0.18) <sup>a</sup>	$0.12 \pm 0.02$ (0.01~0.26) <sup>b</sup>	$0.12 \pm 0.02$ (0.01~0.28) <sup>b</sup>								
TN (mg/L)	$2.11 \pm 0.06 \\ (1.75 \sim 2.51)$	$1.89 \pm 0.08$ (1.01~2.40)	$1.94 \pm 0.12 \\ (0.64 \sim 2.65)$	$2.02 \pm 0.08 \\ (1.55 \sim 2.74)$								
NO <sub>3</sub> -N (mg/L)	$1.71 \pm 0.07$ (1.25~2.08) <sup>a</sup>	$1.25 \pm 0.10$ (0.15~1.79) <sup>b</sup>	$\begin{array}{c} 1.35 \pm 0.13 \\ (0.29 \ \sim 2.06)^{\rm bc} \end{array}$	$\frac{1.60 \pm 0.09}{(1.04 \sim 2.06)^{\rm ac}}$								
NO <sub>2</sub> -N (mg/L)	$0.04 \pm 0.02$ (0.00~0.21)	$\begin{array}{c} 0.04 \pm 0.02 \\ (0.00 \ \sim \ 0.32) \end{array}$	$0.03 \pm 0.01$ (0.00~0.09)	$0.06 \pm 0.01$ (0.00~0.18)								
NH <sub>4</sub> -N (mg/L)	$\begin{array}{c} 0.21 \pm 0.05 \\ (0.01 {\sim} 0.50)^{ab} \end{array}$	$\begin{array}{c} 0.33 \pm 0.04 \\ (0.04 \text{~~} 0.55)^{\text{b}} \end{array}$	$0.31 \pm 0.05$ (0.11~0.69) <sup>b</sup>	$\begin{array}{c} 0.14 \pm 0.03 \\ (0.00 \text{~~} 0.33)^{a} \end{array}$								
NH <sub>3</sub> (mg/L)	$0.01 \pm 0.003$ (0.00~0.03) <sup>a</sup>	$\begin{array}{c} 0.03 \pm 0.01 \\ (0.00 \text{~} 0.07)^{\text{b}} \end{array}$	$\begin{array}{c} 0.02 \pm 0.003 \\ (0.00 \text{~~} 0.05)^{\text{b}} \end{array}$	$\begin{array}{c} 0.005 \pm 0.001 \\ (0.00{\sim}0.02)^{\rm a} \end{array}$								
Si (mg/L)	$6.89 \pm 0.80$ (2.42~10.16) <sup>ab</sup>	$7.01 \pm 0.98$ (2.78~12.27) <sup>ab</sup>	$9.41 \pm 1.15$ (3.54~16.07) <sup>a</sup>	$\begin{array}{c} 6.11 \pm 0.79 \\ (1.70  11.76)^{\text{b}} \end{array}$								
SIN (mg/L)	$1.97 \pm 0.06$ (1.35~2.40) <sup>a</sup>	$1.65 \pm 0.12$ (0.63~2.26) <sup>b</sup>	$1.71 \pm 0.13$ (0.43~2.35) <sup>ab</sup>	$\frac{1.80 \pm 0.08}{(1.17 \sim 2.18)^{ab}}$								
N/P	$40.72 \pm 8.69$ (9.44~131.54) <sup>a</sup>	$26.10 \pm 3.99 (9.16 \sim 56.54)^{ab}$	$19.42 \pm 6.66$ (4.31~105.63) <sup>b</sup>	$8.78 \pm 0.78$ $(5.06 \sim 14.43)^{b}$								
Chla (ug/L)	$3.59 \pm 0.24$ (1.89~4.88) <sup>a</sup>	$2.34 \pm 0.21$ (1.05~3.81) <sup>b</sup>	$2.81 \pm 0.26$ (1.33~5.00) <sup>b</sup>	$1.54 \pm 0.10$ (0.70~2.28) <sup>c</sup>								

Table 1. Means and ranges of environmental variables in the Dongta spawning grounds

\*V, water velocity; WT, water temperature; Sal, salinity; TDS, total dissolvable solid; ORP, oxidationreduction potential; DO, dissolved oxygen; Cond, conductivity; SD, water transparency; TP, total phosphorus; PO<sub>4</sub>-P, phosphate; TN, total nitrogen; NO<sub>3</sub>-N, nitrogen nitrate; NO<sub>2</sub>-N, nitrite nitrogen; NH<sub>3</sub>, un-ionized ammonia; Si, Silicate, SiO<sub>3</sub>-Si; NH<sub>4</sub>-N, ammonium nitrogen, SIN, soluble inorganic nitrogen, SIN; N/P, ratio of nitrogen to phosphorus; Chla, chlorophyll-a \*\*Different letters indicated significantly different, P < 0.05



Figure 2. Phytoplankton species composition in the Dongta spawning grounds. (A) The quarterly phytoplankton species composition during March 2016 to December 2018. (B)
Phytoplankton species composition of Bailan sampling station during March 2016 to December 2018. (C) Phytoplankton species composition of Guiping sampling station during March 2016 to December 2018. (D) Phytoplankton species composition of Yujiang sampling station during March 2016 to December 2018. (E) Phytoplankton species composition of Dongta sampling station during March 2016 to December 2018. (E) Phytoplankton species composition of Dongta sampling station during station during March 2016 to December 2018. (F) Phytoplankton species composition of Shizui sampling station during March 2016 to December 2018. In the figure, M, J, S, D standing for March, June, September and December, respectively

### Phytoplankton functional groups

According to the PFGs methods, the planktonic algae in the Dongta spawning grounds could be divided into 26 functional groups, including A, B, C, D, F, G, H1, J, K, L1, LM, L0, M, MP, N, P, S1, S2, T, W1, W2, Ws, X1, X2,  $X_{Ph}$  and Y, and the dominant phytoplankton functional groups were 15 coda, including C, D, J, K, L0, MP,

N, P, S1, S2, T, W1, W2, X2 and Y. The frequency analysis showed that 13 functional groups, including W1, MP, P, Y, K, X2, J, C, D, F, W2, T and G, were common functional groups in this area, with their frequencies of occurrence ranging from 50 to 100%; The occurrence frequency of functional groups L<sub>0</sub>, B, S1 and X<sub>Ph</sub> ranged from 25 to 50%, belonging to the sub-common functional groups in this area; The frequency of functional groups L<sub>1</sub>, H, M, LM, Ws, N and X1 was between 5 and 25%, belonging to the uncommon functional groups; The frequency of functional groups S2 and A was less than 5%, which belonged to the rare or occasional functional groups in this area (*Fig. 4A*). The biomass of each functional group was shown in *Figure 4B*. It can be seen that W1, P, Y, D, MP, W2, C, J, K and F coda had large average biomass values, which were the most important PFGs in the Dongta spawning ground.



Figure 3. Phytoplankton density and biomass in each sample stations of the Dongta spawning grounds. In the figure, BL, GP, YJ, DT, SZ standing for Bailan, Guiping, Yujiang, Dongta and Shizui, respectively

The distribution of dominant PFGs in the Dongta spawning grounds was shown in *Table 2*. Based on the three years' investigation, the temporal characteristics of dominant PFGs had some difference. In March, there were 11 dominant functional groups including C, D, K, MP, N, P, S1, W1, W2, X2 and Y; the dominant functional groups in In June were D, MP, P, T, W1, W2 and Y; the dominant functional groups in September were D, L<sub>0</sub>, MP, P, S2, W1, W2 and Y; and D, J, K, L<sub>0</sub>, MP, P, S1, W1, W2,

X2, Y coda were dominated in December. Spatially, six dominant PFGs were dominated BL station, which were D, L<sub>0</sub>, MP, P, W1, Y; nine functional groups including D, K, MP, P, S1, W1, W2, X2 and Y, were dominated in GP station; ten dominant functional groups including D, K, MP, N, P, T, W1, W2, X2 and Y, were dominantly found in YJ station; eleven dominant functional groups (C, D, J, K, L0, MP, P, W1, W2, X2, Y) were dominantly found in DT station. Similarly, eight dominant functional groups, including D, MP, P, S1, S2, W1, W2 and Y were found at SZ station. The quarterly biomass of dominant phytoplankton functional groups in each sampling station of the Dongta spawning grounds were showed in *Figure 5*.



Figure 4. Occurrence frequency analysis of phytoplankton functional groups and their biomass in the Dongta Spawning Grounds. (A) Occurrence frequency of phytoplankton functional groups; (B) Biomass of phytoplankton functional groups

### Phytoplankton functional groups assemblage index and water ecological status

Through habitat analysis, representative species, taxonomic status and F factor assignment of each PFG identified in the Dongta spawning grounds were listed in *Table 3*. The phytoplankton Q index was calculated according to Padisák et al. (2006) and Zhu et al. (2013).

	С	D	J	K	L <sub>0</sub>	MP	Ν	Р	<b>S1</b>	<b>S2</b>	Т	W1	W2	X2	Y
March	+ *	+		+		+	+	+	+			+	+	+	+
June		+				+		+			+	+	+		+
September		+			+	+		+		+		+	+		+
December		+	+	+	+	+		+	+			+	+	+	+
BL		+			+	+		+	+			+			+
GP		+		+		+		+				+	+	+	+
YJ		+		+		+	+	+			+	+	+	+	+
DT	+	+	+	+	+	+		+				+	+	+	+
SZ		+				+		+	+	+		+	+		+

**Table 2.** Spatial and temporal distribution of dominant phytoplankton functional groups inthe Dongta spawning grounds

\*"+" representing the appearance of this phytoplankton functional group



Figure 5. The quarterly biomass of dominant phytoplankton functional groups in the Dongta spawning grounds. In the figure, BL, GP, YJ, DT, SZ standing for Bailan, Guiping, Yujiang, Dongta and Shizui, respectively

The Q index of PFGs at each sampling station in Dongta spawning grounds ranged from 1.83 to 3.73, with an average of 2.91 and the median of 2.86 (*Fig. 6*). In March, the Q index varied from 2.77 to 3.46, with mean value of 3.05. The lowest Q index was observed in March 2017, and the highest was observed in March 2018. In June, Q index varied from 2.55 to 3.73, with a median value of 2.87 and mean value of 3.05. YJ station had the lowest value in June 2017, and SZ site had the highest value in June 2018. In September, Q index varied from 1.83 to 3.43, with a median value of 2.84 and mean value of 2.82. The SZ station in September 2017 was the lowest, and the BL station was the highest in September 2016. In December, Q index varied from 2.31 to 3.12, with a median value of 2.73 and average value of 2.72. YJ station had the lowest value in December 2016, and SZ site had the highest value in December 2018. To the sample stations, the Q index variation range of BL station was 2.53~3.64, with mean value of 2.99. The Q index of Guiping samples ranged from 2.45 to 3.49 with mean value of 2.91. The Q index of YJ ranged from 2.31 to 3.23, with an average of 2.83. The Q index of DT area ranged from 2.65 to 3.67, with an average of 2.92. The Q index of SZ site ranged from 1.83 to 3.73, with an average value of 2.90. It can be seen that the Q index difference between March and December was small (less than 1), but the difference between September was larger (over 1.5), and June' Q index difference was in the middle range.



*Figure 6.* Phytoplankton functional groups assemblage index (Q index) at each sampling station in the Dongta spawning ground. In the figure, BL, GP, YJ, DT, SZ standing for Bailan, Guiping, Yujiang, Dongta and Shizui, respectively (\*P < 0.05)

According to the phytoplankton Q index, it can be seen that the overall ecological status of the waters in the Dongta spawning grounds was in the range of "tolerable" to "good". Most of investigation periods, the ecological status of the Dongta spawning grounds was "medium" or "good", but it was "tolerable" occasionally with some areas Qindex less than 2. The ecological status in March and June was in the range of "medium" to "good", and the ecological status in these two periods was similar without significant difference (P > 0.05). The average Q indexes were 3.05 in these two sampling periods, which presented ecological status was "good" as a whole. In September, with an average Q index of 2.82, but there was a great difference in ecological status. A few sampling sites were "tolerant", while most of the sites were at the "medium" to "good", which was significantly different from March and June (P < 0.05). In December, the mean value of Q index was 2.72, and the water body was at the level of "medium" to "good", but the significance was lower than that of March, June and September (P < 0.05). The average annual Q index of the five sampling sites were 2.99, 2.91, 2.83, 2.92 and 2.90, respectively. There was no significant difference among the five stations (P > 0.05), and their quarterly average values Q index closing to 3.0, and their ecological status were in the critical state of "medium" to "good" (Table 4).

Functional group	The main phytoplankton representative species	Taxonomic group	F factor
Y	Cryptomonas sp.	Cryptophyceae	3
MP	Nitzschia sp.	Bacillariophyceae	4
MP	Cymatoplcura sp.	Bacillariophyceae	4
MP	Stauroneis sp.	Bacillariophyceae	4
MP	<i>Cymbella</i> sp.	Bacillariophyceae	4
MP	Gomphonema sp.	Bacillariophyceae	4
MP	Navicula sp.	Bacillariophyceae	4
$L_0$	Peridiniosis niei	Dinophyceae	2
$L_0$	Peridinium sp.	Dinophyceae	2
L <sub>0</sub>	Ceratium hirundinella	Dinophyceae	2
В	Cyclotella sp.	Bacillariophyceae	4
В	Aulacoseira sp.	Bacillariophyceae	4
С	Cyclotella meneghiniana	Bacillariophyceae	3
С	Asterionella formosa	Bacillariophyceae	3
D	Synedra sp.	Bacillariophyceae	3
X2	Chroomonas sp.	Cryptophyceae	3
X2	Chlamydomonas sp.	Chlorophyceae	3
J	Pediastrum sp.	Chlorophyceae	2
J	Scenedesmus sp.	Chlorophyceae	2
Р	Fragilaria sp.	Bacillariophyceae	4
Р	Aulacoseira granulata	Bacillariophyceae	4
K	Aphanocapsa sp	Cyanophyceae	2
G	Eudorina sp.	Chlorophyceae	2
G	Pandorina sp.	Chlorophyceae	2
F	Oocystis spp.	Chlorophyceae	5
F	Kirchneriella sp.	Chlorophyceae	5
H1	Anabaena flos-aquae	Cyanophyceae	0
М	Microcystis sp.	Cyanophyceae	0
Ν	Cosmarium sp.	Zygnemaphyceae	3
S1	Pseudanabaena sp.	Cyanophyceae	0
W1	<i>Euglena</i> sp.	Euglenophyceae	2
А	Rhizosolenia sp	Bacillariophyceae	5
L <sub>1</sub>	Geitlerinema sp	Cyanophyceae	3
$L_{M}$	Ceratium sp	Dinophyceae	2
S2	Spirulina sp	Cyanophyceae	0
Т	<i>Tabellaria</i> sp	Bacillariophyceae	2
W2	Trachelomonas	Euglenophyceae	4
Ws	Strombomonas sp	Euglenophyceae	3
X1	<i>Chlorella</i> sp	Zygnemaphyceae	2
$X_{Ph}$	Phacotus sp	Zygnemaphyceae	4

*Table 3.* The phytoplankton functional groups, representative species, taxonomic group and their F factor of the Dongta spawning grounds

	March			June	Sep	tember	Dee	cember	Annual			
	Q**	E-S***	Q	E-S	Q	E-S	Q	E-S	Q	E-S		
$BL^*$	3.03	Good	3.08	Good	2.99	2.99 Medium		Medium	2.99	Medium		
GP	3.10	Good	3.06	Good	2.80	Medium	2.65	Medium	2.91	Medium		
YJ	3.07	Good	2.85	Medium	2.82	Medium	2.58	Medium	2.83	Medium		
DT	3.02	Good	3.11	Good	2.83	Medium	2.72	Medium	2.92	Medium		
SZ	3.02	Good	3.17	Good	2.64	Medium	2.77	Medium	2.90	Medium		

*Table 4.* Quarterly average values of phytoplankton functional groups assemblage index and the ecological status at each sampling station of the Dongta spawning grounds

\*BL, GP, YJ, DT, SZ standing for Bailan, Guiping, Yujiang, Dongta and Shizui, respectively \*\*Q, phytoplankton functional groups assemblage index (Q index); \*\*\* E-S, ecological status

#### Correlation analysis of environmental factors

Results of correlation analysis of environmental factors showed that TP and PO<sub>4</sub>-P were significantly correlated with Sal, TDS, ORP, Cond, DO, V, pH, SD environmental factors (P < 0.05). In addition, WT, SD, TP and NH<sub>3</sub> were significantly correlated with water velocity (*Table 5*).

### Relationship between phytoplankton functional groups and environmental factors

Redundancy analysis (RDA) were done between environmental factors and the dominant PFGs to find the main influence factors, and the results were shown in *Figure 7*. In the biplot diagrams for redundancy analysis, the interpretation degree of the measured environmental indicators for the first two axes of PFGs reached 50.72%. The results of showed that: factors including DO, Chla, V, PO<sub>4</sub>-P, ORP, TP, Cond, TDS and Sal were significantly correlated with the dominant PFGs (Monte Carlo test, P < 0.05; *Fig. 7A*); V was reversely related with most of the dominant PFGs, meanwhile, it had a reverse relationship with most environmental factors except Chla and DO in the biplot diagrams; V had a significant positive correlation with codon MP (P < 0.05), and was significant negatively correlated with codon J (P < 0.01, *Fig. 7B*).

### Relationship between phytoplankton Q index and water velocity

Correlation analysis results showed that phytoplankton Q index was positively correlated with water velocity, with a correlation coefficient of 0.263 (P < 0.01). Further, linear fitting between Q index and V was done which showed a good correlation (*Fig.* 8).

### Discussion

### Distribution characteristics of PFGs in the Dongta spawning grounds

PFGs corresponded to the characteristics of water habitats. The dynamic sequence of phytoplankton was mainly the result of the interaction of environmental factors such as thermal stability, hydrodynamic characteristics, nutrient status, physiological adaptation characteristics, light conditions, zooplankton and fish grazing pressure, and hydrological dynamics (Reynolds, 2006; Abonyi et al., 2012). These environmental factors induced the phytoplankton appear or disappear in a particular habitat selection

mechanism, and also impacted on PFGs. Generally, the more PFGs in the water, the higher diversity of habitats in this water environment (Padisák et al., 2006; Wang et al., 2011a; Santana et al., 2017; Zuo et al., 2019;). With the change of water habitat conditions, the composition of PFGs had obvious characteristics of temporal and spatial succession (Zhu et al., 2013; Wang et al., 2020b). In the present study, after three years of sampling investigation and microscopic analysis in different quarters, the planktonic algae of the Dongta spawning grounds were classified to 26 PFGs, including A, B, C, D, F, G, H1, J, K, L1, LM, L<sub>0</sub>, M, MP, N, P, S1, S2, T, W1, W2, W<sub>S</sub>, X1, X2, X<sub>Ph</sub>, Y, and the dominant PFGs were C, D, J, K, L<sub>0</sub>, MP, N, P, S1, S2, T, W1, W2, X2, Y. Compared with other global water bodies (Zhu et al., 2013; Santana et al., 2017; Zuo et al., 2019), the waters of the Dongta spawning grounds had more numerous of PFGs and dominant functional groups, which showed that the water quality in this area was relatively good. In addition, the current of this spawning grounds was fast flowing with many reefs in river channel, which formed a continuous turbulent environment, and become a good spawning ground for cyprinid fish, principally the four Chinese farmed carps, i.e. black carp, grass carp, silver carp and bighead carp, green grass silver carp and bighead carp (Tan et al., 2011a).



Figure 7. Relationship between dominant phytoplankton functional groups and environmental factors at each sampling point of Dongta spawning Ground. (A) Biplot diagrams for redundancy analysis between environmental factors and the dominant PFGs; (B) fitting relationship between water velocity and dominant phytoplankton functional group J and MP. WT, water temperature; DO, dissolved oxygen; Cond, conductivity; TDS, total dissolvable solid; SD, water transparency; ORP, oxidation-reduction potential; TP, total phosphorus; PO<sub>4</sub>-P, phosphate; TN, total nitrogen; Si, Silicate, SiO<sub>3</sub>-Si; Chla, chlorophyll-a. NH<sub>4</sub>-N, ammonium nitrogen, NO<sub>3</sub>-N, nitrogen nitrate; NO<sub>2</sub>-N, nitrite nitrogen; V, water velocity; Sal, salinity; NH<sub>3</sub>, un-ionized ammonia; SIN, soluble inorganic nitrogen, SIN; N/P, ratio of nitrogen to phosphorus. \* P < 0.05; \*\*, P < 0.01; \*\*\* P < 0.001</li>

Factors***	WT	Sal	TDS	ORP	DO	Cond	SD	PO <sub>4</sub> -P	ТР	TN	NO <sub>3</sub> -N	NO <sub>2</sub> -N	NH <sub>4</sub> -N	NH <sub>3</sub>	Si	SIN	N/P	Chla	V
pН	-0.103	0.299*	0.394**	-0.166	0.409**	0.120	0.049	-0.337**	-0.179	0.175	0.202	-0.360**	-0.025	0.352**	0.240	0.158	0.238	-0.221	-0.157
WT		-0.022	0.062	0.095	-0.437**	0.360**	-0.464**	0.242	0.079	-0.234	-0.402**	-0.108	0.394**	0.495**	$0.256^{*}$	-0.241	-0.186	-0.073	0.371**
Sal			0.681**	0.174	0.086	0.775**	0.208	0.346**	0.475**	$0.304^{*}$	0.306*	-0.009	-0.044	0.009	$0.300^{*}$	$0.296^{*}$	-0.316*	-0.116	-0.208
TDS				0.202	0.181	0.618**	0.066	0.273*	0.407**	0.217	0.182	0.048	-0.030	0.151	0.150	0.188	-0.224	-0.224	-0.202
ORP					-0.415**	0.179	-0.065	0.677**	0.551**	-0.246	-0.251	-0.098	-0.100	-0.124	-0.134	-0.321*	-0.228	-0.113	-0.145
DO						-0.058	0.184	-0.598**	-0.398**	0.087	0.181	-0.068	0.024	0.151	-0.078	0.192	$0.292^{*}$	-0.322*	-0.171
Cond							0.069	0.350**	0.396**	0.175	0.023	0.015	0.309*	0.236	0.334**	0.168	-0.318*	-0.089	-0.044
SD								-0.003	0.158	-0.044	0.198	0.138	-0.376**	-0.378**	-0.418**	0.048	0.011	0.140	-0.499**
PO <sub>4</sub> -P									0.833**	-0.052	-0.049	0.182	-0.054	-0.134	0.038	-0.052	-0.537**	-0.006	-0.097
ТР										0.105	0.136	0.291*	-0.226	-0.248	0.072	0.077	-0.704**	-0.210	-0.286*
TN											$0.782^{**}$	0.202	0.047	0.033	0.347**	0.857**	0.054	0.015	-0.100
NO <sub>3</sub> -N												-0.004	-0.281*	-0.198	0.140	0.901**	-0.004	0.107	-0.155
NO <sub>2</sub> -N													-0.198	-0.244	-0.057	0.050	-0.240	-0.016	-0.087
NH4-N														0.755**	0.418**	0.140	0.166	-0.245	0.205
NH <sub>3</sub>															0.365**	0.124	0.195	-0.267*	0.310*
Si																$0.330^{*}$	-0.131	-0.085	0.214
SIN																	0.040	-0.008	-0.072
N/P																		0.093	0.023
Chla																			0.173

Table 5. Correlation between environmental factors of the Dongta Spawning founds

\*Significantly correlated at 0.05 level. \*\*Significantly correlated at 0.01 level

\*\*\*V, water velocity; WT, water temperature; Sal, salinity; TDS, total dissolvable solid; ORP, oxidation-reduction potential; DO, dissolved oxygen; Cond, conductivity; SD, water transparency; TP, total phosphorus; PO<sub>4</sub>-P, phosphate; TN, total nitrogen; NO<sub>3</sub>-N, nitrogen nitrate; NO<sub>2</sub>-N, nitrite nitrogen; NH<sub>3</sub>, un-ionized ammonia; Si, Silicate, SiO<sub>3</sub>-Si; NH<sub>4</sub>-N, ammonium nitrogen, SIN, soluble inorganic nitrogen, SIN; N/P, ratio of nitrogen to phosphorus; Chla, chlorophyll-a



*Figure 8. Relationship between phytoplankton functional groups assemblage index (Q index) and water velocity in the Dongta spawning grounds. V, water velocity* 

In the Pearl River Basin, May to September was the main rainy season, with rainfall accounting for 70% of the annual rainfall (Li et al., 2021). The main dry season was from December to March of the following year. During dry period, rainfall was scarce and the river bed was exposed in part of the river. In this study, there were 11 dominant PFGs in the Dongta spawning grounds' waters in March and December, and 7 and 8 dominant PFGs in June and September, respectively. It shows that the number of dominant PFGs in the dry season was obviously more than that in the wet season. The reason may mainly due to the increasing rainfall, turbid water, and increased river runoff in the rainy season, which was not conducive to the growth and reproduction of most algae, resulting in fewer functional groups during this period. Similarly, a study by Salmaso and Zignin (2010) found a negative correlation between phytoplankton biomass and runoff. Six dominant PFGs existed throughout the year in the Dongta spawning grounds, which were D, MP, P, W1, W2 and Y coda. Among them, codon D is tolerant to scouring action, and the representative algae include Stylus acuminata, Nitzschia, etc. Codon P is tolerant to moderately low light, and its main habitat is continuous or semi-continuous mixed water layer. The representative algae mainly include Fragillaria, etc. Codon MP is tolerant to mixed agitation, the main habitat is frequent agitation, turbid water; Codon Y is also tolerant to low light; and coda W1 and W2 represent the habitat conditions of "water bodies that obtain organic matter from farmland or sewage" and "medium or eutrophic water bodies", respectively. From the perspective of the composition of functional groups, these PFGs that exist throughout the year constitute the basic PFGs of the waters of Dongta spawning grounds, and also represented the habitat conditions and pollution factors (Revnolds, 2002; Padisák et al., 2009). Geographically, the Dongta spawning grounds undertake runoff from a large area of the upper reaches of the Pearl River, including the main rivers such as Nanpan River, Beipan River, Hongshui River and Liujiang River. Because the river bank from Wuxuan to Bailan is the high mountain and gorge area, the river width downstream of Bailan increases greatly, and a large amount of rainwater runoff begin to burst, forming a faster water velocity and violent scouring force, which led to higher water turbidity and weaker underwater light. Therefore, D, MP, P, Y groups suitable for this habitat (Padisák et al., 2009), became the dominant PFGs. As the geographical conditions from Bailan to Shizui is flat, which is adjacent to the Guiping County urban area and several towns. The concentrated residential, developed field farming, established multiple factories and enterprises, produce domestic sewage, agricultural fertilizers, and factory sewage, which would also be carried into the river by runoff, causing the concentration of nutrients and organic matter in the water body to increase (Abonyi et al., 2012; Guo et al., 2016). Thus, the dominance coda (W1 and W2) adapted to this environment increasing gradually.

Temporally, there was no significant difference between PFGs in March and December, with the difference rate of 18.18%. Similarly, there was no significant difference between PFGs in wet season, with the difference rate of 25%, indicating that the ecological environment in this region was relatively stable in dry season or wet season. Codon C was the unique functional group of March's PFGs, the algae in this group mainly include Asterionella formosa and Cyclotella meneghiniana, etc. The habitat condition indicated by these coda was medium or small nutrient-rich waters beginning to stratify (Padisák et al., 2009). It was corresponded to the river's state. Since March was the deep dry periods of the Pearl River basin, as the decreasing of rainfall and runoff, and the downstream impoundment of Changzhou Hydraulic Complex (Tan et al., 2011a, b), the Dongta spawning grounds water velocity reduced, most river area in static or half static state, water may form a weak layer in this period. In June, codon T was dominant. The environmental characteristics indicated by T group were continuous mixing layer and light as the limiting factor (Padisák et al., 2009). It was consistent with the characteristics of water body in this period, either. June was the most important rainy season in the Pearl River Basin, and the inflow of heavy rainfall and surface runoff introduced the water velocity and sediment content in this period reach the peak of the whole year (Wang et al., 2017, 2020a), thus forming the complete mixing of water body and weak underwater light. In September, codon S2 was the unique functional group, and one of the important habitat characteristics indicated by S2 was "water body warmth" (Padisák et al., 2009). The actual monitoring also showed that the water body temperature was the highest in September. It was consistent with the regional environment and climate characteristics of this period. In September, the rainfall in the Pearl River Basin gradually decreased, and the sediment content decreased, but this period was still a high temperature period in southern China. Besides, the sunny and warm weather was dominant in this period, so the water body had good light transmission, sufficient light and warm water, leading the adaptive PFGs proliferating.

Comparing with the 5 sampling positions, the number of dominant PFGs in BL station were the least. According to the above information, the BL sample site was located at the junction of canyon and open river section, where the water velocity was higher than other areas all the year round, and the PFGs suitable for this environment were limited. The DT station had the most PFGs, which may be caused by: the sampling site was located in the core area of the Dongta spawning grounds, which area had the widest river width, with the rock forest of gully region (with high water velocity) and the broad waters of aquatic plants growing in the region (with low water velocity). The multifarious habitat provided the important place of producing sticky egg and fish

breeding, and the varied topography also provided different algae with their appropriate habitat conditions (Abonvi et al., 2012; Nagy-László et al., 2020). For example, apart from the coda MP, P and W1, algae suitable for slow water velocity, such as *Chlorella* meniensis (codon C), Chlorella convolvula and Chlorella sclerotii (codon G), were also noticeably appeared in this area. Therefore, the number of PFGs was higher than other regions. This study also showed that the YJ station contained some PFGs, such as coda N and T, the main representative species being *Euastrum* spp and *Tribonema*. spp, which were significantly different from other sites. Unlike the other stations, YJ sampling site located in the river mouth of Yujiang River tributaries into the Pearl River. The Yujiang River flowing through the large or medium cities, such as Nanning city and Guigang city of Guangxi Zhuang Autonomous Region, China. Densely populated residents, many ports and factories along the river banks, which possesses many nutrients sources into water, but there was no water exchange with the Pearl River main stream. Therefore, the YJ area had an independent water environment. Cosmarium belonging to codon N and Tribonema belonging to codon T were large algae, which were more suitable for living in static water environment (Padisák et al., 2009). Since the runoff of Yujiang River was lower than the main stream of Pearl River, and the estuary area was relatively wide, so the water velocity was low, which provides conditions for the growth and reproduction of these groups.

# The relationships between phytoplankton functional groups and environmental factors in the Dongta spawning grounds

The RDA analysis results showed that environmental factors such as DO, Chla, V, PO<sub>4</sub>-P, ORP, TP, Cond, TDS and Sal were significantly correlated with the dominant PFGs. Both Rodrigues et al. (2018) and Qu et al. (2019) pointed out that phytoplankton communities were more impacted by the hydrological index than other factors. In our RDA ordination diagram, V was reversely correlated with many environmental factors other than Chla and DO, as well as the dominant PFGs other than MP, D and P. Large number of hydroecological studies had shown that the water velocity was an important impact factor to change the biological and non-biological factors of the water environment (Zeng et al., 2006; Devercelli, 2013). The speed of water flow affects the input and output of nutrients in rivers, such as sediment content, transparency, suspended particulate matter and dissolved oxygen content. In our study, V was significantly positively correlated with water temperature, and significantly negatively correlated with SD and TP, while water temperature, transparency and TP played an important role in the growth of phytoplankton (Reynolds, 2006; Santana et al., 2017). In addition, previous studies showed that phosphorus was the limiting nutrient relative to nitrogen in the middle and upper reaches of the Pearl River (Liu et al., 2019). In other regions of China, such as the Three Gorges Basin of the Yangtze River, the source and flow areas of tributaries were rich in phosphate minerals and phosphorus processing enterprises, resulting in phosphorus heavy load in rivers and becoming major pollution factors. After the impoundment of the Three Gorges Dam, with the slowing down of water velocity (Zeng et al., 2006; Zhu et al., 2013), a large amount of nutrients accumulates in the reservoir bays, and finally algal blooms appeared in several tributaries (Zhou et al., 2011). However, the Pearl River basin was different from the Yangtze River basin in that there were few phosphate ore and phosphorus products processing enterprises in its basin. Due to the low input of nutrients, the accumulation of nutrients was relatively slow after the construction of the Dam. Therefore, the

changes of algal structure in the water, especially the appearance of algal blooms, may not be as obvious as after the construction of the Three Gorges Dam. But water dams changed the river hydrological dynamics, and the aquatic organisms' structure transformation would be inevitable (Tan et al., 2011; Jung et al., 2014; Steinschneider et al., 2014; El-Karim, 2015; Li et al., 2021). Such as after the completion of the Danjiangkou Reservoir since 1958, its water environment had changed in these 60 years: (1) A tendency to gradually increase the reservoir eutrophication degree (Zhu et al., 2008); (2) The whole reservoir phytoplankton density increased 16 times; (3) Phytoplankton composition changed from Bacillariophyta to Bacillariophyta-Cyanophyta-Chlorophyta type, and then was gradually replaced by Bacillariophyta-Pyrrophyta-Cryptophyta-Cyanophyta (Shen et al., 2011). Similarly, in other rivers, a series of environmental problems had also been reported due to the changes in hydrology and water regime of the upstream and downstream rivers after damming (Zhou et al., 2011; Leslie et al., 2014; Steinschneider et al., 2014; Jung et al., 2014; Deng et al., 2019). Basing on the present study, analysis and lots of relevant literature, we concluded that water velocity was a very important factor influencing phytoplankton functional groups composition and river ecological status. However, the dominant influence of flow velocity should be fatherly studied in the future.

# Water ecological status of Dongta spawning grounds based on phytoplankton functional groups assemblage index

PFGs assemblage index (Q indexes) reflected the ecological status of water from the perspective of composition and the habitat conditions of phytoplankton species. In this study, the Q indexes in Dongta spawning grounds were obtained by investigating the BL, GP, YJ, DT and SZ sampling stations to evaluate the water ecological status. On the whole, the ecological status of the Dongta spawning grounds was fair to middling among the reported study areas, showing a "medium" to "good" ecological status and occasionally "tolerant" status in some areas (Padisák et al., 2006). There was no significant difference in Q index among the five sampling stations, meaning exciting smaller habitats in the main stream of the Pearl River.

The sampling sites of BL, GP, DT and SZ were located in the main stream of the Pearl River. The spatial distance between them was small, and the climate, human geography and other factors were very close. Moreover, due to the high water velocity in this region, the whole region had good connectivity between upper and lower levels, and the ecological status changes had no much difference. Previous studies indicated that the greater the spatial distance, the greater the difference in regional ecological environment (Thomaz et al., 2007; Graco-Roza et al., 2020; Wang et al., 2020b). For the YJ station, due to the low water velocity, the proportion of green algae in the algal community structure increased and the Q index was slightly lower than other sites, but the geographical proximity did not make it significantly different from other sample sites. The results of this study showed that the ecological conditions of the SZ station had changed in larger ranges, and "tolerable" condition appeared in some periods. The actual investigation found that there were some sand digging and transport ships in this area, and a large number of fishery boats rested and the fishermen lived near the bank. As a result, some mechanical interference, domestic sewage and garbage would be generated. Once it encountered a period of high temperature and low rainoff, the output could not be timely, and the environment would become worse. Temporally, there were significant differences in Q index in different periods. March and June were grouped

together, and their Q indexes were significantly higher than other periods; while September and December's Q indexes were grouped respectively. The analysis indicated that the main reason was that the water temperature in March was low, biological activities were not active, because February was generally during the Chinese Lunar New Year holiday, human production activities had been drastically reduced, and pollutants discharged accordingly had been reduced (Guo et al., 2016). March was the deep dry period, though water velocity dropped drastically in this period, but after a long period of normal and dry periods, most of the nutrients and pollutants in the water were used or degraded, so the water ecology showed the "good" condition. June was the main rainy period, when the inflow of rainwater runoff allowed the river to be replenished with fresh water and facilitated the output of nutrients. So the ecological state of this period was also in "good" condition. The same results mentioned by Okogwu and Ugwumba (2012) basing on their studies in the Cross River showed that a sudden increase in runoff resulted in the dilution effect on nutrient contents of the river. and water quality improved. In our study, September and December were in the transitional period, and they were also the most prosperous period of people's production and living in this area. As water supply gradually decreased and the influx of pollutants related to people's production increasing, the ecological status would be reduced (Abonyi et al., 2012), which displayed as "medium" conditions in these two periods. As other studies and proposals (Leslie et al., 2014; Steinschneider et al., 2014; Chen and Li, 2015), it was recommended that after the completion and operation of the Datengxia Dam and the upstream cascade dams, attention should be paid to the regulation of the water volume and water flow velocity of the downstream reaches in the way of ecological operation, to ensure the function of the spawning grounds and fishery conservation zones in the Pearl River.

# Conclusion

There were 26 PFGs identified in the Dongta spawning grounds, and the dominant PFGs were C, D, J, K, L<sub>0</sub>, MP, N, P, S1, S2, T, W1, W2, X2, Y. Phytoplankton Q indexes were varied from 1.83 to 3.73, with the mean value 2.91, the corresponding result showed that most of the time, the ecological status of the Dongta spawning grounds was "medium" and "good", but parts occasionally was "tolerable". Spatially, the mean values of Q index in Bailan, Guiping, Yujiang, Dongta and Shizui sampling stations were 2.99, 2.91, 2.83, 2.92 and 2.90, respectively, which were in the critical state of "medium" to "good", but without statistically significant difference among the 5 sampling sites. Temporally, the Q index average values in March and June were the same for 3.05 showing the "good" ecological status, and they were significantly higher than September and December. Factors including DO, Chla, V, PO<sub>4</sub>-P, ORP, TP, Cond, TDS and Sal were significantly correlated with the dominant PFGs; especially, V was reversely related with most of the dominant PFGs and most environmental factors except Chla and DO; besides, V had a significant positive correlation with codon MP, and was significant negatively correlated with codon J. Basing on the present study and analysis, we conclude that water velocity was an very important factor influencing phytoplankton functional groups composition and river ecological status, but the dominant influence of flow velocity should be fatherly studied in the future. Nevertheless, it is also suggested that after the construction and operation of Datengxia Water Dam and its upper reaches cascade dams, attention should be paid to regulating

the water flow and velocity in the lower reaches of the river by means of ecological operation, so as to ensure the functions of the spawning grounds and protect the fishery conservation zones in mainstream of the Pearl River. This study provided important guidance for drawing up protection measures for the ecological status of the Dongta spawning grounds of its downstream after the completion of Datengxia Water Control Project.

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