Journal of Cleaner Production 375 (2022) 134106

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Evaluation of the control effect of bighead carp and silver carp on cyanobacterial blooms based on the analysis of differences in algal digestion processes

Zhicong Wang [a,1], Qidong Wang [b,1], Jinglong Wang [a,c], Hui Wei [a,c], Jing Qian [b,c], Yinzhe Zhang [b,c], Kai Feng [b,c], Qinyi Chen [a,c], Jing Yuan [b], Jiashou Liu [b], Dunhai Li [a,*]

[a] Key Laboratory of Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, PR China
[b] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, PR China
[c] University of Chinese Academy of Sciences, Beijing, 100049, PR China

ARTICLE INFO

Handling Editor: Zhen Leng

Keywords:
Filter-feeding fish
Cyanobacterial blooms
Fish intestine
Chlorophyll fluorescence
Phytoplankton community

ABSTRACT

The phytoplankton community in Qiandao Lake has typical seasonal succession characteristics, and Cyanophyta, especially the genus *Microcystis*, are dominant in summer. To evaluate the control effects of filter-feeding fish (silver carp and bighead carp) on cyanobacterial blooms, the changes in algal species composition along the fish intestine were analyzed by microscopic examination, chlorophyll fluorescence, high-throughput DNA sequencing and pigment ratios. The microscopic examination results showed that both silver carp and bighead carp have a certain control effect on bloom-forming cyanobacteria in spring. The digestion of silver carp in summer could still effectively remove cyanobacteria and reduce the dominance of *Microcystis* in the phytoplankton community, while the digestion of bighead carp seemed to have little effect on cyanobacteria. The results of the chlorophyll fluorescence method were similar to those of the microscopic analysis, which indicated that silver carp could reduce the cyanobacterial biomass in spring, while bighead carp could significantly promote the dominance of cyanobacteria ($p < 0.001$). High-throughput DNA sequencing showed that there were significant differences in filter-feeding plankton between silver carp and bighead carp. The midgut may be the main gathering site of intestinal microorganisms, and cyanobacterial digestion mainly occurs in the intestinal segments between the foregut and midgut. The analysis of pigment ratios indicated that selective digestion of phytoplankton by silver carp significantly reduced the dominance of Cyanophyta in its hindgut ($p < 0.01$), while bighead carp had the opposite effect ($p < 0.05$), especially in seasons other than spring. The results of the above four methods are basically the same, that is, the digestion effect of silver carp on cyanobacteria is better than that of bighead carp. Therefore, the stocking proportion of silver carp should be appropriately increased in some meso-eutrophic waterbodies.

1. Introduction

Eutrophication has become a major environmental problem in many lakes and reservoirs in the world and has attracted great attention from many scholars. Cyanobacterial blooms are common in eutrophic water bodies, and most of them are notorious for their toxins and odors. At present, many methods for cyanobacterial bloom control have been reported, such as removal by flocculant (Yuan et al., 2016; Li and Pan, 2013), inactivation by oxidant (Wang et al., 2012; Li et al., 2021), and growth inhibition by phoslock (Wang et al., 2017). Although these methods can quickly and effectively remove cyanobacterial blooms, they may lead to the extracellular release of cyanotoxins, thus posing a serious threat to water ecological security and human health (Mucci et al., 2020). Most importantly, none of these methods can reduce the nutrient load of water bodies, and the cyanobacterial organic matter (COM) deposited in sediments may further aggravate lake

* Corresponding author.
E-mail addresses: wangzc@ihb.ac.cn (Z. Wang), wangqd@ihb.ac.cn (Q. Wang), 1559777925@qq.com (J. Wang), weihui0820@163.com (H. Wei), jingq1992@163.com (J. Qian), zhangyinzhe@ihb.ac.cn (Y. Zhang), 375726569@qq.com (K. Feng), 1456654239@qq.com (Q. Chen), yuanj@ihb.ac.cn (J. Yuan), jsliu@ihb.ac.cn (J. Liu), lidh@ihb.ac.cn (D. Li).
[1] Zhicong Wang and Qidong Wang contributed equally to this work.

https://doi.org/10.1016/j.jclepro.2022.134106
Received 24 April 2022; Received in revised form 29 August 2022; Accepted 11 September 2022
Available online 17 September 2022

eutrophication by promoting the formation of Fe–P (Wang et al., 2019). Currently, using an aquatic food web to remove cyanobacterial blooms is a widely accepted method because of its environmentally friendly features (Yu et al., 2013). In particular, the stocking and harvest of filter-feeding fish can not only significantly decrease the biomass and dominance of cyanobacteria, but also convert this COM into fish products with great economic value (Ke et al., 2007; Fujibayashi et al., 2018).

Before 1985, Lake Donghu in Wuhan city occurred experienced serious cyanobacterial blooms. However, these blooms suddenly disappeared from Lake Donghu after 1985 and did not reappear for at least 14 years (Liu and Xie, 1999). This strange phenomenon has attracted the research of many Chinese scholars, and Liu and Xie (1999) proposed that it might be attributed to the feeding actions of filter-feeding fish such as silver carp and bighead carp on bloom-forming cyanobacteria and further demonstrated this hypothesis through an enclosure experiment with silver carp and bighead carp. Limnologists traditionally considered that filter-feeding fishes affected water quality through a unidirectional trophic cascade from nutrients → phytoplankton → zooplankton → fish (Straskraba, 1965). However, Xie and Liu (2001) found that phytoplankton could be directly consumed by filter-feeding fish, and this new nontraditional biomanipulation has been tested and used to remove cyanobacterial blooms in many Chinese lakes, including Lake Dianchi in Yunnan Province, Lake Chaohu in Anhui Province, and Lake Taihu in Jiangsu Province (Guo et al., 2015; Yi et al., 2016). Some of the cyanobacteria are killed, either by being disrupted by the pharyngeal teeth or by the fish's gut enzymes or by bacteria in the foregut (Kolar et al., 2007). Silver carp and bighead carp are common and important species in Chinese aquaculture. Since bighead and silver carp use their highly specialized gill rakers and epibranchial organs to feed by filtering small particles from the water column, cyanobacteria might be the most important food source during the outbreak of cyanobacterial blooms. Additionally, we have observed that the intestines of silver carp and bighead carp in Lake Taihu are full of cyanobacteria (Wang et al., 2015).

However, there are also many failed applications of nontraditional biomanipulation; for example, the biomass of phytoplankton did not decrease as expected after silver carp and bighead carp were raised in the Villerest Reservoir in France (Domaizon and Devaux, 1999) and the Saidenbach Reservoir in Germany (Radke and Kahl, 2002). A previous study found that the photosynthetic activity of undigested *Microcystis* recovered rapidly after being excreted from the digestive system of carp and re-entered the water column, and its growth rate increased significantly (Wang et al., 2015). Furthermore, a simulation experiment performed in enclosures showed that silver carp and bighead carp could significantly reduce the biomass of large colonial *Microcystis*, but dramatically promoted the dominance of small-sized green algae and the total biomass of phytoplankton (Shen et al., 2020). The possible reason for the acceleration of algal growth caused by stocked filter-feeding fish may be the enhancement of nutrient cycling related to the excretion and sediment resuspension by fish (Vanni, 2002; Zhang et al., 2016). However, a recent study showed that silver carp can indirectly promote the growth of nanophytoplankton through zooplankton predation rather than nutrient cycling and suggested that silver carp alone is not feasible for removing algae and creating clean water (Shen et al., 2021). Based on the detrimental effects of silver carp and bighead carp, some scholars have proposed that integrated biomanipulation, including traditional and nontraditional biomanipulation, can more effectively control cyanobacterial blooms than any single biomanipulation, which indicates that integrated biomanipulation should be used instead of single biomanipulation in the ecological restoration of tropical and subtropical lakes (Peng et al., 2021). Furthermore, combined biomanipulation projects, including submerged plants, fish, macrobenthos, and zooplankton, have a significant positive effect on the removal of internal nutrients and chl-*a* (Chen et al., 2020).

In summary, there are still many disputes and uncertainties about whether nontraditional biomanipulation can effectively control cyanobacteria. It has been reported that the photosynthetic activity of *Microcystis* can recover rapidly after passing through the digestive tract of silver carp, and its population shows compensatory growth in the presence of fish predation (Wang et al., 2015). The success or failure of nontraditional biomanipulation applications may also depend on the latitude of lakes (tropical or subtropical) (Wang et al., 2021) and the degree of lake eutrophication (Mao et al., 2020). These controversial studies do not affect a basic consensus on nontraditional biomanipulation; that is, filter-feeding fish can indeed decrease the biomass and proportion of large colonial cyanobacteria to a certain extent, even if they cannot significantly reduce the total biomass of phytoplankton in eutrophic lakes, especially during periods without cyanobacterial blooms (Guo et al., 2015). However, the selective inhibition of bloom-forming cyanobacteria by filter-feeding fish and its specific mechanism have not yet been reported. We cannot definitively answer the following questions: (1) what is the difference between silver carp and bighead carp in terms of cyanobacterial control, and (2) which part of the fish gut performs digestive function? Qiandao Lake is a typical lake where algal blooms have been successfully controlled by the stocking and harvest of silver carp and bighead carp (Liu et al., 2007), so it was selected to carry out the present research to reveal the control mechanism of cyanobacterial blooms.

2. Materials and methods

2.1. Study area and sampling sites

Qiandao Lake, the Xin'anjiang Reservoir, is located in Chun'an County, Hangzhou City, Zhejiang Province, China (N29°11'~30°02', E118°34'~119°15'). The average water depth of the reservoir is 30.44 m. At the normal water level, the water surface covers an area of approximately 580 km^2, with a storage capacity of 17.8 billion m^3. At the highest water level, it has 1078 land bridge islands with an area of more than 0.25 km^2. Qiandao Lake has important functions such as tourism, drinking water sources, ecological regulation and fisheries. The water quality and eutrophication trend of Qiandao Lake have attracted much attention because Xin'an River, the inflow river upstream of the reservoir, is rich in nitrogen and phosphorus nutrients. A total of 13 sampling sites are set in the reservoir area, covering the upstream Xin'an River (S1–S3), the reservoir (S4–S12) and the downstream of the reservoir (S13) (Fig. 1). Lake surveys of basic environmental factors, phytoplankton, and algal composition in fish guts at the above sampling sites were conducted in summer (August 6th, 2018), autumn (November 21st, 2018), winter (January 8th, 2019) and spring (May 10th, 2019).

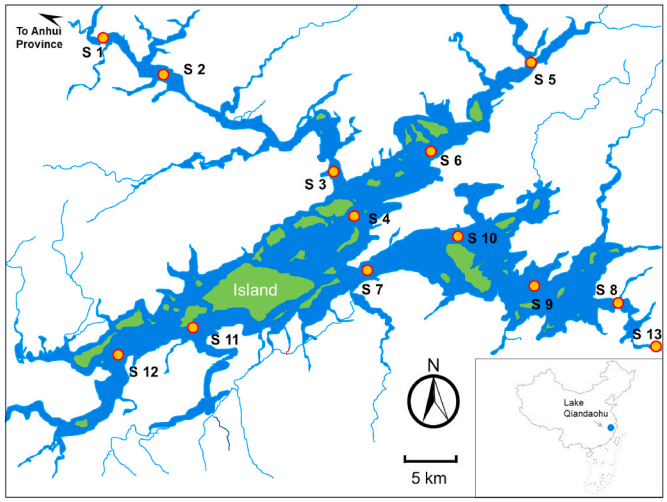

Fig. 1. Locations of the sampling sites in Qiandao Lake.

2.2. Measurement of physical and chemical factors

Dissolved oxygen (DO), water temperature (WT), conductivity (Cond.) and pH values were measured on site using a multiparameter water quality analyzer (YSI 550, USA). The collected water samples for detecting nutrient concentrations were immediately stored at 4 °C and measured within 24 h. Total nitrogen (TN) was determined by the alkaline potassium persulfate ($K_2S_2O_8$+NaOH) digestion method and calculated according to the spectral absorption at 220 nm and 275 nm (GB11893-89) (Greenberg et al., 2012). Total phosphorus (TP) was determined by the molybdenum-antimony anti-spectrophotometric method (Beattie et al., 1978). Nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), ammonia nitrogen (NH_4^+-N), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) were measured with water samples filtered with a GF/C glass fiber membrane (Whatman). The measurement of NO_3^--N was similar to that of TN except that $K_2S_2O_8$+NaOH digestion was not performed. NO_2^--N was detected with N-(1-naphthyl)-ethylenediamine and calculated with absorbance at 540 nm (Xu et al., 2005). NH_4^+-N was measured according to the standard method (APHA, 1995). Measurements of TDP and SRP were similar to those of TP. The filtered samples were directly used to measure SRP without $K_2S_2O_8$ digestion, while TDP required potassium persulfate digestion. The physicochemical factors of water quality in Qiandao Lake in different seasons are shown in Table 1.

2.3. Phytoplankton community and fish biomass

At each site, 1 L water samples were collected from the surface, 0.5 m depth and 1.0 m depth. After mixing them, 2.5 L was placed into a PVC sample bottle, and then 2% Lugol's solution was added immediately to fix the sample. After standing for 48 h, the supernatant was removed by siphoning, and the pellet was concentrated to 30 mL for the determination of phytoplankton community structure. A 0.1 mL concentrated sample was placed into a phytoplankton frame counter, and the algae were identified and counted in columns 2, 5, and 8 of the counter (10 rows × 10 columns) under a light microscope. At least 500 algal cells were counted from each sample, and at least 200 cells were counted from non-dominant species. Finally, the biomass of various algae was calculated based on the counted data and concentrated volume. A trammel net (outer mesh size is 35 cm, and inner mesh size is 8.8 cm) was used to collect fish samples of silver carp and bighead carp for analysis of the food composition in fish guts, and fish were evaluated with a BioSonics DT-X EXTREME split beam echosounder with a frequency of 120 kHz. The densities and biomass of fish in the water body of Qiandao Lake in different seasons are shown in Table Supplement 1.

2.4. Sampling and processing of fish intestinal contents

Several silver carp and bighead carp in each season of spring, summer, autumn and winter were selected, the basic fish information, such as sampling number, body length, and body weight, are shown in Table 2, and their intact intestines were carefully collected. The intestines were cut into three segments, namely, the foregut, midgut and hindgut. The foregut refers to the intestine segment from the esophagus to the first fold, the hindgut refers to the intestine segment from the last fold to the anus, and the midgut is the intestine segment between the foregut and the hindgut. The contents of each intestine segment were removed and placed into 10 mL centrifuge tubes (solid matter ≥6 mL) and immediately stored at 4 °C, and the chlorophyll fluorescence was measured with Phyto-PAM (Walz, Germany) within 24 h. In addition, 3 silver carp and 3 bighead carp were randomly selected. Their intestines were cut into 20 sections from front to back and immediately refrigerated at 4 °C, and then the chlorophyll fluorescence and pigment composition of phytoplankton in each section of intestinal contents were measured within 24 h. After sampling the intestinal contents of the 20 sections, the intestinal contents of every 2 adjacent sections were combined immediately to obtain 10 samples. These 10 samples were stored in liquid nitrogen until high-throughput DNA sequencing was performed.

Table 2
The length and weight of fish collected in Qiandao Lake in different seasons.

	Silver carp		Bighead carp	
	body length (cm)	body weight (kg)	body length (cm)	body weight (kg)
Spring	68.53 ± 4.74 (n = 17)	3.69 ± 0.76 (n = 17)	92.13 ± 16.68 (n = 15)	11.23 ± 5.73 (n = 15)
Summer	60.95 ± 6.91 (n = 50)	2.39 ± 0.93 (n = 50)	75.56 ± 12.03 (n = 50)	5.32 ± 3.35 (n = 50)
Autumn	61.53 ± 3.44 (n = 50)	2.40 ± 0.29 (n = 50)	94.99 ± 8.73 (n = 42)	11.45 ± 3.98 (n = 42)
Winter	71.57 ± 3.36 (n = 50)	4.07 ± 0.61 (n = 50)	86.68 ± 12.90 (n = 50)	8.28 ± 3.84 (n = 50)

2.5. Chlorophyll fluorescence measurement

Approximately 0.5 mL of the intestinal contents sample was resuspended with 5 mL purified water, filtered with a 10-mesh screen after discarding the surface flotsam in the sample, and then treated with 30 W ultrasonic for 60 s. A 2 mL sample was pipetted into the sample reaction chamber of Phyto-PAM (Walz, Germany), and the maximum fluorescence (F_m) was measured with 3000 μmol photons m^{-2} s^{-1} pulse light after dark adaptation for 30 min. Before measurement, the Phyto-PAM was calibrated with *Microcystis* (FACHB7806), *Scenedesmus* (FACHB469) and *Asterionella Formosa*, which represent the phyla Cyanophyta, Chlorophyta and Bacillariophyta, respectively. According to the operation instructions of Phyto-PAM, the F_m values of Cyanophyta, Chlorophyta and Bacillariophyta were measured simultaneously when saturated pulse light was triggered and were labelled F_{m-Cyan}, F_{m-Chlo} and F_{m-Baci}, respectively. The dominance index of the above three phyla was calculated according to formulas (1) - (3) and recorded as DI_{Cyan}, DI_{Chlo} and DI_{Baci}.

$$DI_{Cyan} = F_{m-Cyan} / (F_{m-Cyan} + F_{m-Chlo} + F_{m-Baci}) \quad (1)$$

$$DI_{Chol} = F_{m-Chlo} / (F_{m-Cyan} + F_{m-Chlo} + F_{m-Baci}) \quad (2)$$

Table 1
The physicochemical factors of water quality in Qiandao Lake in different seasons.

	TN (mg/L)	TP (mg/L)	NH_4^+-N (mg/L)	COD_{Mn} (mg/L)	Chl-*a* (μg/L)	Conductivity (μS/cm)	pH	Algal biomass (× 10^6 cell/L)	Temp °C
Spring	0.866 ± 0.319	0.024 ± 0.014	0.05 ± 0.029	1.96 ± 0.59	6.72 ± 8.62	108.3 ± 11.7	8.36 ± 0.34	1.79 ± 1.04	18.3 ± 3.3
Summer	1.019 ± 0.228	0.031 ± 0.019	0.18 ± 0.052	2.55 ± 0.81	6.77 ± 5.24	137.8 ± 15.4	9.59 ± 0.25	31.93 ± 54.27	30.6 ± 4.7
Autumn	0.926 ± 0.151	0.024 ± 0.022	0.063 ± 0.026	1.58 ± 0.23	2.58 ± 0.7	112.1 ± 17.6	10.17 ± 0.12	1.75 ± 0.85	19 ± 0.1
Winter	0.866 ± 0.197	0.024 ± 0.008	0.05 ± 0.019	1.96 ± 0.18	6.72 ± 2	108.3 ± 14.4	8.36 ± 0.1	3.06 ± 2.12	18.3 ± 0.3

$$DI_{Baci} = F_{m-Baci} / (F_{m-Cyan} + F_{m-Chlo} + F_{m-Baci}) \quad (3)$$

2.6. Extraction and measurement of pigments

Six milliliters of 90% acetone solution was added to 10 mL centrifuge tubes containing 0.5 mL of intestinal contents, and immediately, these tubes were shaken well and placed in a 4 °C refrigerator for extraction for 24 h. Then, the samples were centrifuged, and the supernatant was taken to measure the absorbance values OD_{750}, OD_{663}, OD_{645} and OD_{630} by spectrophotometry at 750 nm, 663 nm, 645 nm and 630 nm, respectively. The concentrations of various chlorophylls were calculated according to the following formulas (4) - (6):

$$C_a = 11.64 \times OD_{663} - 2.16 \times OD_{645} + 0.10 \times OD_{630} \times V_1/(V_2 \times L) \quad (4)$$

$$C_b = 20.97 \times OD_{645} - 3.94 \times OD_{663} - 3.66 \times OD_{630} \times V_1/(V_2 \times L) \quad (5)$$

$$C_c = 54.22 \times OD_{663} - 14.8 \times OD_{645} + 5.53 \times OD_{630} \times V_1/(V_2 \times L) \quad (6)$$

where, C_a, concentration of chlorophyll *a* (chl-*a*, mg/L);C_b, concentration of chlorophyll *b* (chl-*b*, mg/L); C_c, concentration of chlorophyll *c* (chl-*c*, mg/L);V_1, the volume of extract (mL);V_2, the volume of filtered water, it is arbitrary value here; L, the optical path length of the colorimetric dish (mm); D, the corrected absorbance of the extract.

2.7. Extraction and analysis of DNA

The DNA of intestinal contents was extracted by an OMEGA kit (E.Z.N.A™ Mag-Bind Soil DNA Kit) and performed according to kit instructions. Agarose gel was used to detect the integrity of DNA, and the genomic DNA was quantified by Qubit 2.0 DNA detection kit to determine the amount of DNA added to the PCR. High-throughput sequencing of DNA was performed, and the specific primers of prokaryotes were 341F (5′- CCTACGGGNGGCWGCAG-3′) (Klindworth et al., 2013) and 805Rmod (5′-GACTACNVGGGTWTCTAATCC-3′) (Apprill et al., 2015). The specific primers of eukaryotes were V4F (5′-GCGGTAATTCCAGCTCCAATA-3′) and V4R (5′-GATCCCCHWACTTTCGTTCTTGA-3′) with barcodes (Song et al., 2016). Computer sequencing: After DNA purification and recovery, the Qubit 3.0 DNA detection kit quantified the recovered DNA to facilitate sequencing after mixing in the same amount of 1:1. Ten nanograms of DNA was sampled, and the final sequencing concentration was 20 pmol. USEARCH was used to remove the sequence of the nonamplified region in the preprocessed sequence; then, the sequencing error was corrected, and UCHIME was employed to identify chimeras. Sequences without chimeras were subjected to BLASTN analysis with the representative sequence in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and alignment values below the threshold were removed due to nontarget region sequences. All detected sequences were clustered according to the sequence distance, and then these sequences were divided into different operational taxonomic units (OTUs) based on the sequence similarity. Statistical analysis of OTUs at a 97% similar level was carried out, and the representative sequences in OTU clustering were obtained. The sequence with the highest abundance was defaulted as the representative sequence of OTUs for various analyses. At the same time, when there were more than 5 samples, the OTU corresponding to only one read was removed.

2.8. Statistical analysis

All data were analyzed by using Statistical Product and Service Solutions (version 11.5, SPSS Inc., Chicago, IL, USA). Significant differences between samples were statistically compared using one-way analysis of variance (ANOVA), and Duncan's multiple comparisons for measuring means were performed. The correlation was analyzed with the Pearson test (two-tailed) at $p = 0.05$. Any differences between the mean values at $p < 0.05$ were considered statistically significant.

3. Results and discussions

3.1. Phytoplankton community and its relationship with fish

In spring, cyanobacteria and green algae accounted for 23.7% and 32.2% of the total population densities in Qiandao Lake, respectively (Fig. 2A), and the dominant genera were *Microcystis*, *Cryptococcus* and *Cyclotella* (Fig. S1). In summer, the relative abundance of cyanobacteria increased to 75.7% (Fig. 2B), and *Microcystis* was the only dominant genus (Fig. S1). In autumn, diatoms gradually dominated, accounting for 45.2% of the total abundance (Fig. 2C), and the dominant species were *Cyclotella* sp. and *Microcystis* sp. (Fig. S1). The dominance of *Cyclotella* continuously increased in winter (Fig. S1), and its proportion reached 82.7% (Fig. 2D). The phytoplankton community structure in Qiandao Lake showed a typical seasonal succession pattern, which is similar to that in most mesotrophic lakes, with bloom-forming cyanobacteria dominating in summer (Wang et al., 2016, 2019). The cyanobacterial biomass in Xin'an River reached 2.05×10^8 cells/L in summer, of which *Microcystis* accounted for 85.8%. However, in the middle and lower reaches of the reservoir, the average proportion of *Microcystis* biomass was only 10.4%. These results suggested that the biomass and population dominance of *Microcystis* in Qiandao Lake decreased gradually from upstream to downstream. It has been reported that fish filter-feeding is one of the important reasons for the disappearance of cyanobacterial blooms (Liu and Xie, 1999). Therefore, it is particularly necessary to study the relationship between cyanobacterial blooms and fish communities.

Cyanobacterial dominance (DI_{Cyan}) was positively correlated with soluble nitrogen (NH_4^+-N, NO_3^--N, NO_2^--N), conductivity (Cond.), total dissolved solids (TDS), dissolved oxygen (DO) and fish biomass (Fig. 3A). This correlation is mainly reflected in the upstream statistical results (Sample 1 and sample 2). Upstream, both cyanobacterial biomass and DI_{Cyan} were relatively high (Fig. 3B), which suggested that the impact of environmental and biological factors on the formation of DI_{Cyan} gradually weakened with the decrease in cyanobacterial biomass. Therefore, we can speculate that the formation of DI_{Cyan} in eutrophic lakes is not only directly regulated by environmental factors, but also closely related to fish biomass. Linear regression analysis showed that the higher the fish biomass, the greater the DI_{Cyan} (ANOVA - test, $P < 0.01$). Obviously, this finding contradicts the previous application of filter-feeding fishes to control cyanobacterial blooms (Guo et al., 2015; Yi et al., 2016), but it may be due to a general rule that the direction of foraging migration of fish is where algae are abundant. The answer to the question of whether fish feeding has an impact on cyanobacterial blooms and what kind of impact it has should depend on the further analysis of fish intestinal contents.

3.2. Microscopic analysis of cyanobacteria digestion

Gill rakers are the main organ for filter feeding fish to acquire food. When silver carp and bighead carp ingest food, the two rows of gill rakers on each gill arc continuously open and close with the activity of the mouth. When water with food flows through the gill rakers, water and smaller particles are discharged from the gap of gill rakers, while algae and zooplankton are filtered and trapped. These trapped foods, which include phytoplankton and zooplankton, then enter the fish gut under the constant impact of water flow and palatine fold fluctuation (Fig. S2). In spring and winter, although some cyanobacteria and green algae were consumed by silver carp and bighead carp, diatoms were the main food of these fish in terms of population density and species number (Fig. 4). The algal composition in the intestinal contents showed that *Cyclotella stelligera* and *Melosira granulata* were the dominant species, which was consistent with the community structure of phytoplankton in the water column in winter and spring (Fig. S1). Fig. 4 shows that the proportions of various cyanobacteria in spring sharply

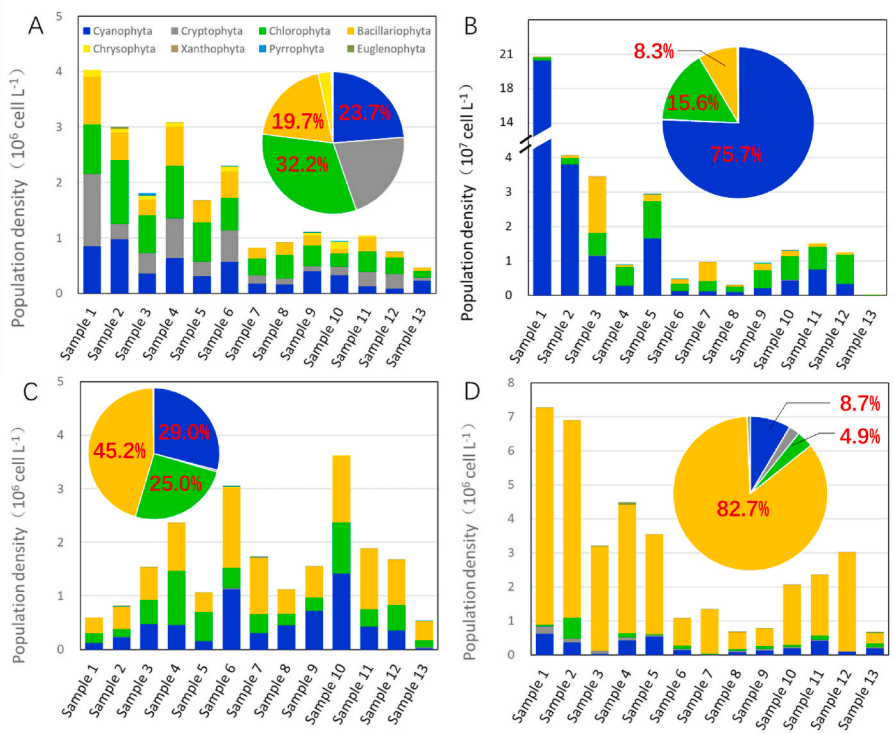

Fig. 2. Phytoplankton community structure in Qiandao Lake in four seasons. (A) in spring, (B) in summer, (C) in autumn and (D) in winter.

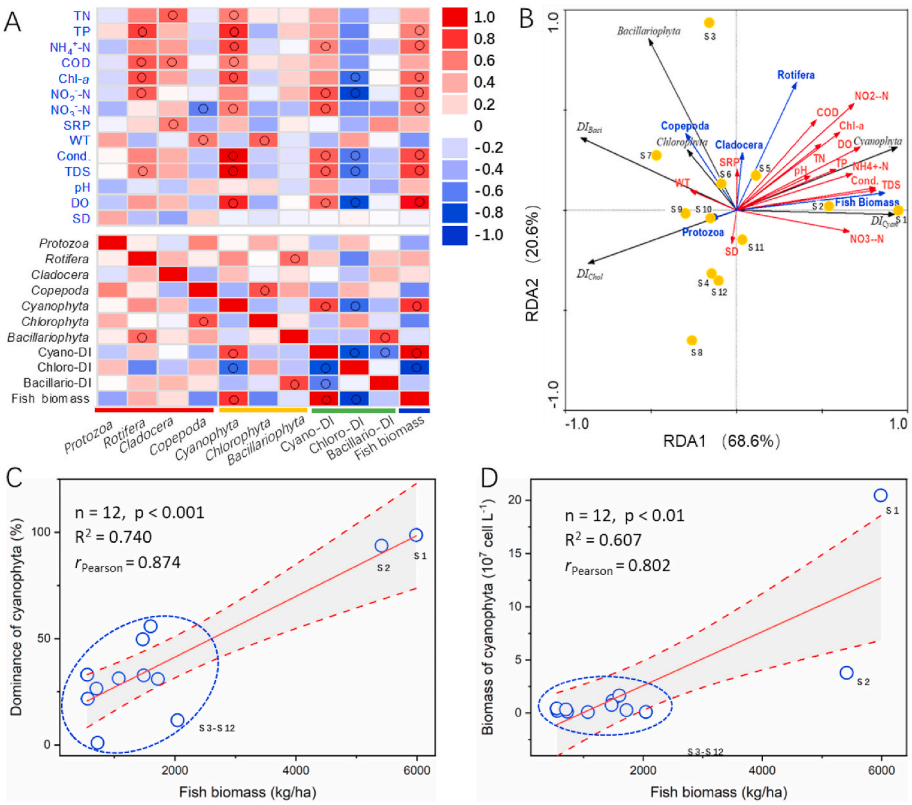

Fig. 3. Statistical analysis between environmental factors and biological factors in Qiandao Lake in summer. (A) Pearson correlation analysis for physicochemical factors and biological factors, black circle means there is a significant correlation between various factors, that is, $p < 0.05$; (B) ordination biplot of the redundancy analysis (RDA) for algal dominance and environmental variables, red arrows indicate the physicochemical factors of water, black arrows indicate the algae and their dominances, and yellow solid dots indicate sample sites; (C) linear regression analysis for fish biomass and cyanobacterial dominance; (D) linear regression analysis for fish biomass and cyanobacterial biomass.

decreased in the midgut and/or hindgut in comparison with those in the foregut. For example, in spring, the proportions of *Microcystis flos-aquae*, *Anabaena flos-aquae* and *Aphanizomenon* sp. decreased from 5 to 10%, 5–10%, and 0.5–5% in the foregut of silver carp to 0.5–5%, <0.5%, and <0.5% in the hindgut, respectively. Also, the proportions of *Microcystis flos-aquae*, *Anabaena flos-aquae* and *Aphanizomenon* sp. decreased from 10 to 30%, 5–10%, and 0.5–5% in the foregut of bighead carp to 5–10%, 0.5–5%, and <0.5% in the hindgut, respectively. The number of diatom

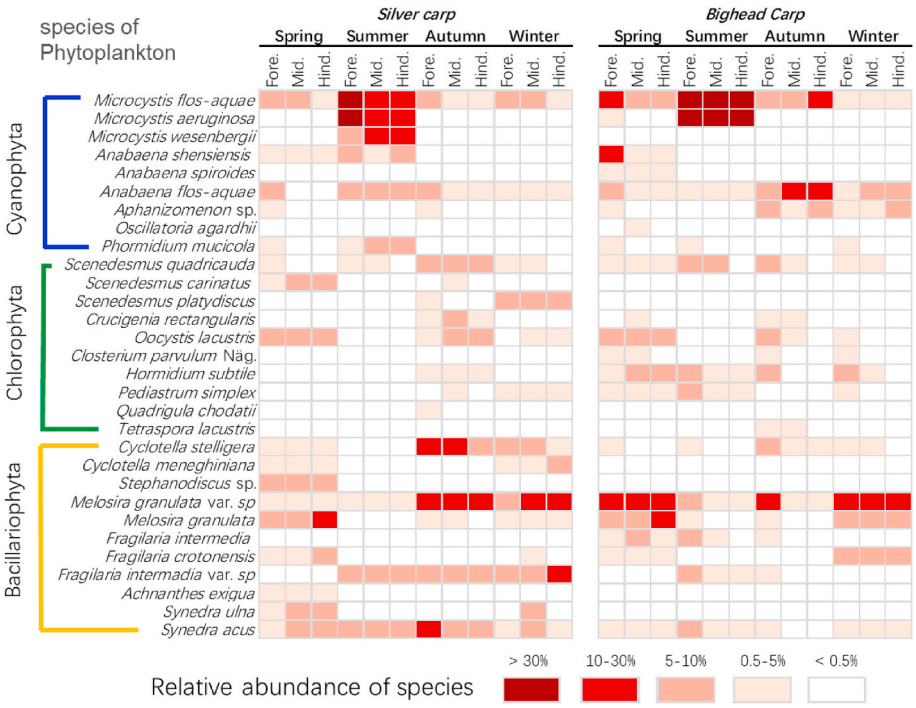

Fig. 4. Abundance of dominant phytoplankton species in different intestinal segments (foregut, midgut and hindgut) of silver carp or bighead carp caught in all seasons (spring, summer, autumn and winter) in Qiandao Lake. Fore., foregut; Mid., midgut; Hind., hindgut.

species consumed by silver carp, especially some smaller species, such as *Cyclotella stelligera*, *Cyclotella meneghiniana* and *Stephanodiscus* sp., is much higher than that of bighead carp, which may be because silver carp can filter much smaller particles from the water than bighead carp (Cohen and Hernandez, 2018). In autumn and winter, there was no significant difference in the composition of dominant algal species in the intestinal contents of the foregut, midgut and hindgut of the two fish, but the abundance of some diatoms, such as *Melosira granulate* and *Fragilaria crotonensis*, in the hindgut of silver carp showed an increasing trend, which may be due to the weak digestion of diatoms or strong digestion of cyanobacteria in the intestines of silver carp. In spring, the abundance of *Microcystis* in the gut of both fish decreased in the hindgut compared with the foregut, indicating that both filter-feeding fish had a certain control effect on the development of *Microcystis* blooms.

In the summer, when cyanobacteria are dominant, the feeding and digestion of algae by filter-feeding fish is particularly important, which is the key to the success of nontraditional biomanipulation (Xie and Liu, 2001). *Microcystis* is the dominant species in the foregut of both fish in summer (Fig. 4), which suggests that both fish can consume a large amount of *Microcystis*. However, the decrease in *Microcystis* abundance only occurred in the hindgut of silver carp, not bighead carp. All these results imply that only silver carp can efficiently digest *Microcystis* during summer *Microcystis* blooms, even though both fish feed on most of the colonial *Microcystis*, which is consistent with our previous research (Wang et al., 2015). Compared with bighead carp, silver carp fed less eukaryotic algae and had a stronger digestion ability for *Microcystis*. Therefore, it is inferred that silver carp has a selective digestion effect on *Microcystis* rather than eukaryotic algae. In autumn, with the decline in cyanobacterial biomass in the water column, the abundance of *Microcystis* in fish intestines also decreased. However, both the biomass and the dominance of *Microcystis* in the gut were still strongly reduced by the digestion of silver carp, while they were greatly promoted by the digestion of bighead carp in autumn.

3.3. Analysis of cyanobacterial digestion with chlorophyll fluorescence

Chlorophyll fluorescence measurement is an important technology for noninvasive detection of the processes and efficiency of plant photosynthesis (Schreiber et al., 1995). As a typical chlorophyll fluorescence analyzer, Phyto-PAM can be used to determine the relative proportions of Cyanophyta, Chlorophyta and Bacillariophyta in mixed algal samples (Beecraft et al., 2021). Since there are some differences in cell wall composition and algal colony morphology, the three types of algae should have different degrees of digestion when they pass through the fish gut, which will be expressed as changes in relative chlorophyll fluorescence intensity. In spring, the proportions of Cyanophyta and Bacillariophyta significantly decreased ($p < 0.01$ and $p < 0.05$, respectively) (Fig. 5A, I), while Chlorophyta significantly increased ($p < 0.01$) (Fig. 5E) along the intestine of silver carp, which indicates that digestion of silver carp significantly inhibited Cyanophyta dominance and promoted the population growth of Chlorophyta, and these inhibition and promotion effects were enhanced with increasing fish weight (Fig. S3). However, the digestion of bighead carp in spring seems to have no effect on the biomass of various algae in the gut, which is proven by the fact that the proportions of the above three algal phyla are nearly unchanged from the foregut to the hindgut (Fig. 5M, Q, U). In summer, autumn and winter, the initial fluorescence intensities of these three seasons were nearly the same, and there was almost no change along the intestine (Fig. 5B–D, F–H, J-L), which suggests that silver carp has no obvious feeding and digestion selectivity for various algae, including cyanobacteria. This conclusion seems to be inconsistent with the results in Fig. 3, which may be due to the high chlorophyll fluorescence intensity associated with Cyanophyta when *Microcystis* was dominant in summer, resulting in relatively low differential resolution between samples. Based on these results and analysis, we can speculate that the application of chlorophyll fluorescence in determining algal composition is greatly limited by the algal community structure, especially when a single species is dominant. Similar to silver carp, the feeding and digestion of bighead carp in summer also had little effect on cyanobacterial abundance (Fig. 5N). However, in autumn and winter, the digestion of bighead carp can significantly inhibit the increase in Chlorophyta and Bacillariophyta ($p < 0.001$) (Fig. 5S, T, W, X) and significantly promote the increase in Cyanophyta ($p < 0.001$) (Fig. 5O, P), and this promotion of cyanobacteria was enhanced by increasing fish

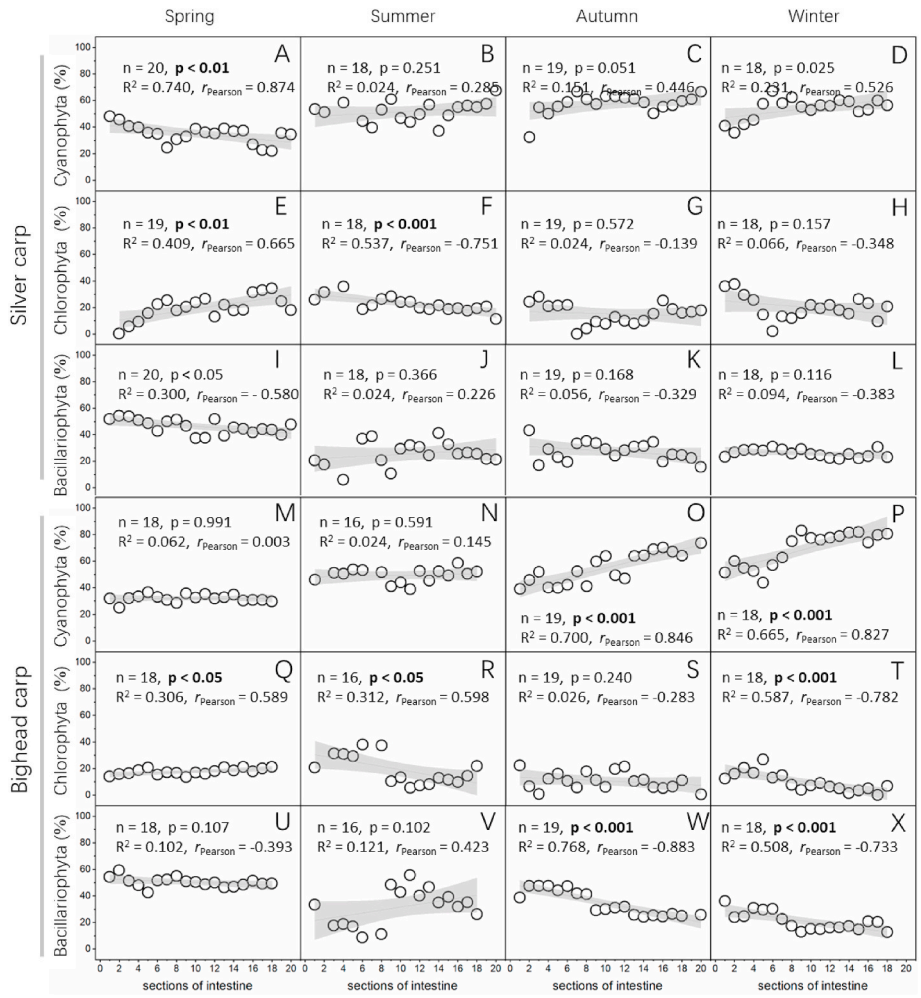

Fig. 5. Change trends of the chlorophyll fluorescence intensity of phytoplankton along the intestine of silver carp or bighead carp caught in four seasons in Qiandao Lake.

weight (Fig. S4), which indicates that bighead carp is not suitable for controlling cyanobacterial blooms, especially in autumn and winter.

Plants can carry out photosynthesis mainly because there are light harvesting pigments in their photosynthetic system, which can efficiently capture solar energy and be used for carbon fixation (Johnson, 2016). The use of pigment-based CHEMTAX for quick surveys of phytoplankton communities was recommended as a useful supplement or alternative tool to microscopy for freshwater ecosystem management (Yu et al., 2021). According to the differences in fluorescence emitted by the excited states of these light harvesting pigments, the phylum and relative biomass of algae can be determined by a fluorescence detector (Beecraft et al., 2021). Since algae generally emit strong chlorophyll fluorescence, some errors may occur in the process of converting fluorescence intensity into algal biomass. For example, the dark environment in the gut may lead to low-light syndrome and the degradation of D1 protein (Keren et al., 1997), eventually leading to an underestimation of algal biomass.

3.4. Analysis of cyanobacterial digestion by DNA high-throughput sequencing

In recent years, DNA high-throughput sequencing has been widely used in the identification of phytoplankton community structure (Qiao et al., 2020). Based on the OTU analysis at the genus level of prokaryotes, bacteria and cyanobacteria are the main components (>80%) of prokaryotes in fish intestinal contents. Along the foregut to midgut of silver carp, the proportion of bacterial OTUs increased rapidly from 24% to 66%, while that of cyanobacteria decreased sharply from 65% to 19% (Fig. 6A); the proportion of bacterial OTUs in the hindgut decreased rapidly from 66% to 17%, while that of cyanobacterial OTUs increased significantly from 19% to 78% (Fig. 6A). These results are consistent with the findings that the midgut may be the main gathering site of intestinal microbes (Li et al., 2018), and cyanobacterial digestion mainly occurs in the intestinal segments between the foregut and midgut. A gradual decline in DNA concentration from fore-to hind-gut in silver carp might in part be due to gradual degradation of DNA that is released to the gut after the pharyngeal teeth mechanically disrupt cell membranes. However, cyanobacteria can also be digested by bile or its combination with intestinal contents (Šetlíková et al., 2020). The OTU results of eukaryotes showed that the α-diversity of the algal community in the intestine of silver carp was significantly higher than that of bighead carp ($p < 0.001$, Fig. S5). The main components of plankton consumed by silver carp were unicellular eukaryotic algae, while those of bighead carp were zooplankton (Fig. 6B), which is consistent with a previous report (Kolar et al., 2007; Su et al., 2020). Generally, a small mesh reduces the flow rate, so narrow gill rakers for silver carp mean that less water is filtered and that evasive organisms can more effectively evade the pumping of water, and larger zooplankton are unlikely to be eaten by silver carp. Cluster analysis of OTUs at the genus level of prokaryotes in fish guts showed that silver carp and bighead carp could not cluster into two significantly different categories, and even OTUs of prokaryotes in the foregut could not be clearly distinguished from those

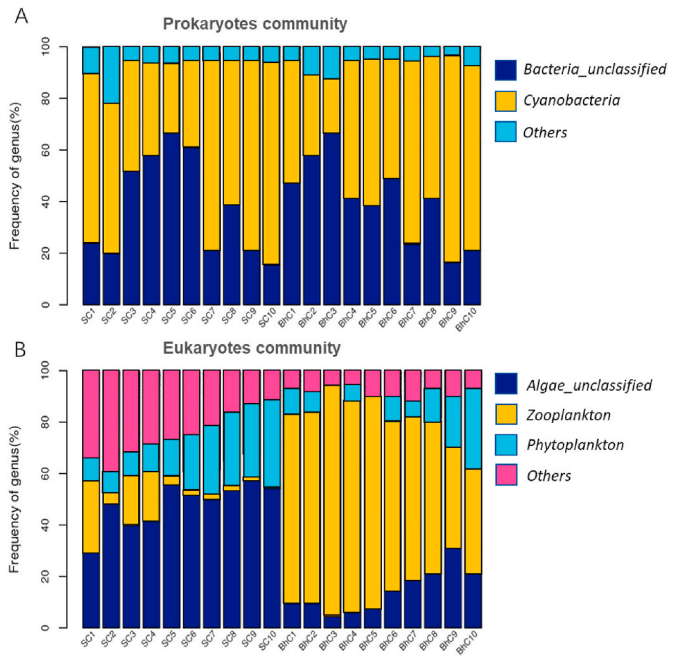

Fig. 6. The community structure of (A) prokaryotes and (B) eukaryotes obtained by the method of high-throughput DNA sequencing in different intestinal segments of silver carp and/or bighead carp. SC, silver carp; BhC, bighead carp.

in the hindgut (Fig. 7A, C). This result may be attributed to the indigestible properties of cyanobacterial cell walls (Hoiczyk and Hansel, 2000). However, the OTUs of eukaryotes can be clustered into two significant categories, belonging to silver carp and bighead carp (Fig. 7B and D and Fig. S6A), which is likely due to the afore-stated greater range of particle sizes that big carp can effectively filter. With the rise of water temperature in spring and summer, the physiological metabolism and intestinal microbial community of fishes will also change, which will lead to seasonal differences affecting the algal ingestion and digestion processes.

The similarity of prokaryote composition in each intestinal segment of fish was analyzed by OTUs (Fig. 8A–D). There were significant differences in prokaryote composition between the foregut (segments 1–6) and hindgut (segments −10) of both silver carp (Fig. 8B) and bighead carp (Fig. 8C). Combining these results with the species information in Fig. 6A, we can conclude that the foregut and hindgut are the main intestinal segments where abundant gut microbes congregate and digest cyanobacteria, which was also suggested by a previous study (Li et al., 2018). Fig. 8D shows that the prokaryote community along each section of silver carp intestine is increasingly similar to that of bighead carp hindgut, which suggests that the filter-feeding and digestion of cyanobacteria by silver carp is similar to that of bighead carp. This result is not surprising because the two fish have some overlap in feeding habits (Gu et al., 1996). According to the similarity analysis of OTU abundance, the foregut (segments 1–3) and the hindgut (segments 5–10) of silver carp are clustered into two significantly different regions (Fig. S6A, S6D), which indicates that silver carp has a strong digestion effect on plankton, as shown in previous research (Wang et al., 2015). Whether in the intestines of silver carp or bighead carp, the eukaryotic plankton community structures in the foregut and hindgut are clustered into two

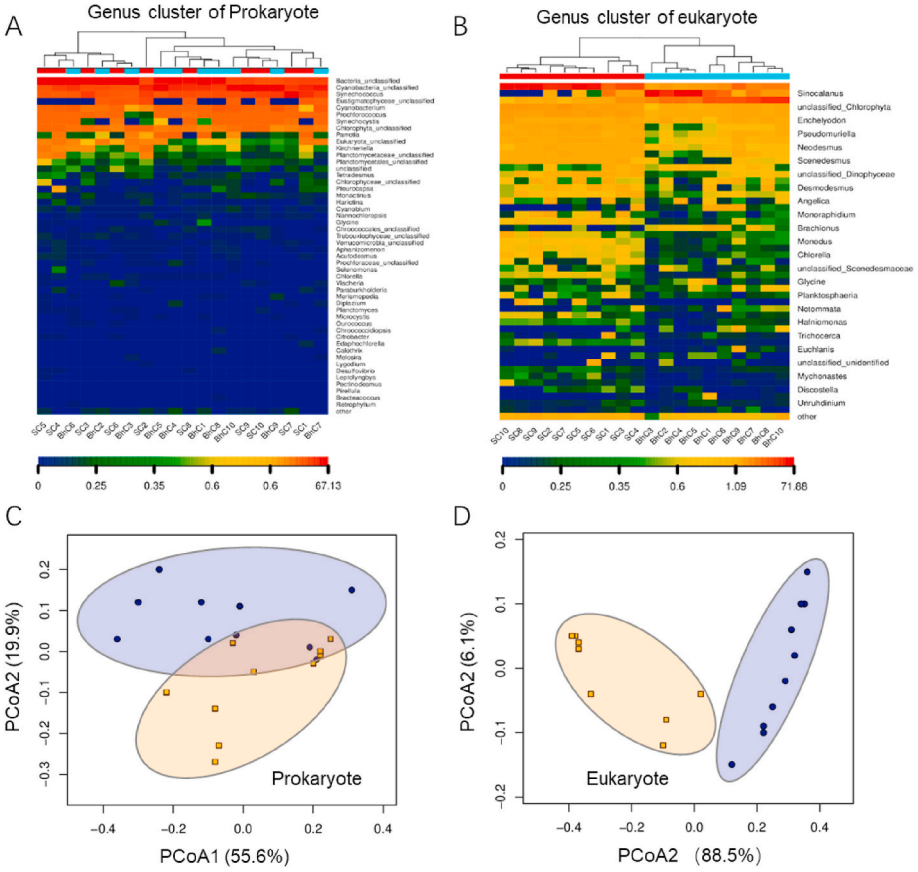

Fig. 7. Cluster analysis and PCA of the community structure at the genus level of prokaryotes and eukaryotes in different intestinal segments of silver carp and bighead carp. (A) Cluster analysis of prokaryote community structure; (B) Cluster analysis of eukaryote community structure; (C) PCA of prokaryote community structure; (D) PCA of eukaryote community structure. SC, silver carp; BhC, bighead carp.

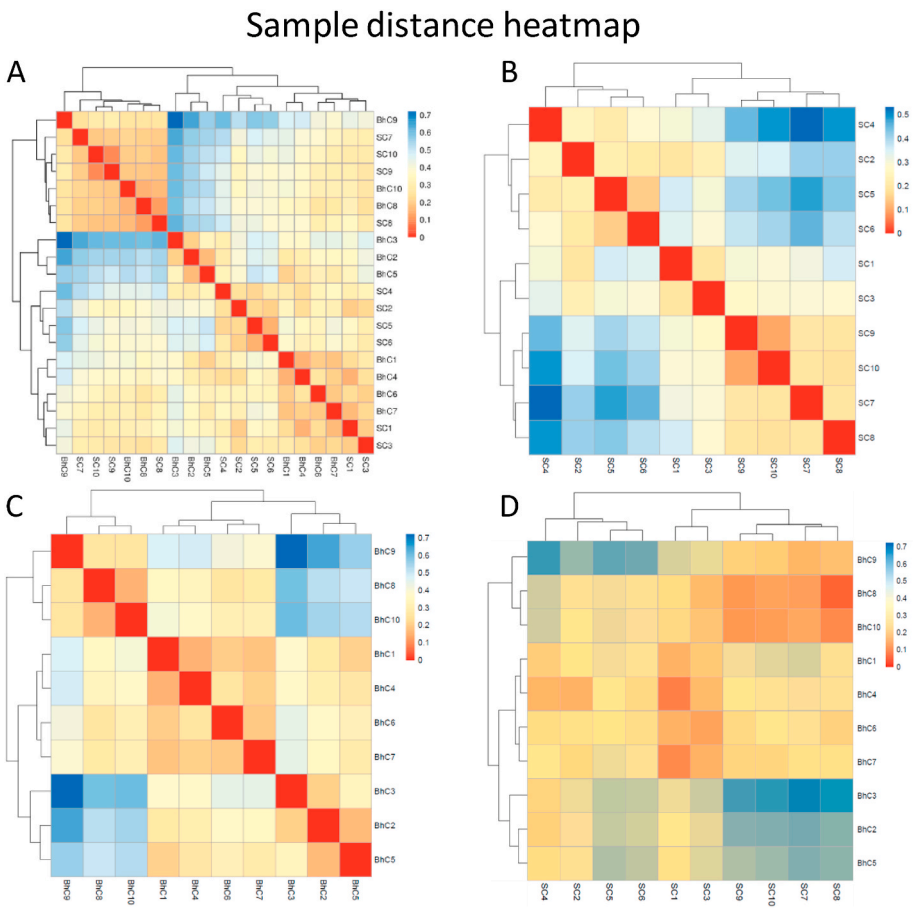

Fig. 8. Heatmap analysis of the community structure similarity of prokaryotes in various intestinal segments of silver carp and/or bighead carp. (A) Community structure similarity of prokaryotes in various intestinal segments of silver carp and/or bighead carp; (B) Community structure similarity of prokaryotes in various intestinal segments of silver carp; (C) Community structure similarity of prokaryotes in various intestinal segments of bighead carp; (D) Community structure similarity of prokaryotes in various intestinal segments of silver carp and bighead carp. SC, silver carp; BhC, bighead carp.

distinct groups (Fig. S6B, S6C), which shows that the digestion of eukaryotic plankton is also mainly performed in foreguts or midguts.

3.5. Analysis of cyanobacterial digestion with pigment ratios

There are great differences in the composition of photosynthetic pigments among Cyanophyta, Chlorophyta and Bacillariophyta (Hu et al., 2010). These three phyla all contain chl-*a*, only Chlorophyta contains chl-*b*, and only Bacillariophyta contains chl-*c* (Dring, 1982; Alam et al., 2017). Since the chl-*b* content in Chlorophyta is different from the content of chl-*c* in Bacillariophyta, we used the relative changes in these pigments to indicate which phyla (Cyanophyta, Chlorophyta or Bacillariophyta) of algae are easier to digest. Therefore, the chl-*b*/chl-*a* ratio and chl-*c*/chl-*a* ratio can be used to represent the relative abundance of Chlorophyta and Bacillariophyta, respectively, in phytoplankton communities. Since the dominant algal species consumed by silver carp and bighead carp are mainly composed of Cyanophyta, Chlorophyta and Bacillariophyta (Fig. 9), the change in the relative abundance of Cyanophyta can be predicted according to the changing trend of Chlorophyta and Bacillariophyta along the intestine.

In spring, chl-*b*/chl-*a* and chl-*c*/chl-*a* increased significantly along the intestines of silver carp and bighead carp, indicating that digestion can lead to a significant increase in proportions of green algae ($p < 0.001$) and diatoms ($p < 0.001$) (Fig. 9A–C) in the intestinal contents, which should be attributed to the fact that fish digestion of cyanobacteria is stronger than that of green algae and diatoms. In summer, autumn and winter, the change trends of the chl-*b*/chl-*a* and chl-*c*/chl-*a* ratios in the intestinal contents of silver carp were similar to those in spring (Fig. 9D, G, J, F, I, L). Compared with the foregut dominated by *Microcystis* in summer, these ratios in the hindgut increased by 167.5% ($p < 0.01$) and 55.3% ($p < 0.001$), respectively (Fig. 9F). Based on these results, we can infer that silver carp has selective digestion of cyanobacteria in all seasons, especially in summer.

In spring and winter, when the temperature is relatively low, the chl-*b*/chl-*a* and chl-*c*/chl-*a* ratios increase rapidly along the foregut and then gradually slow down in the midgut and hindgut, indicating that the selective digestion of silver carp mainly occurs in the foregut in low-temperature seasons. However, in high-temperature seasons, the digestion of phytoplankton by silver carp occurs throughout the intestine, as evidenced by the similar linear increase in chl-*b*/chl-*a* and chl-*c*/chl-*a* ratios in summer and autumn. Why the digestion of silver carp changes with season or temperature is an interesting topic, possibly due to differences in microbial community composition at different temperatures (Zhang et al., 2021) and enzyme activity is also highly temperature dependent, which should be further elucidated in the future. Surprisingly, in summer and autumn, the digestion of algae by bighead carp is completely opposite to that of silver carp; that is, both the chl-*b*/chl-*a* and chl-*c*/chl-*a* ratios are significantly reduced, which suggests that the digestion of bighead carp can decrease the proportion of eukaryotes and promote the dominance of Cyanophyta. This promoting effect of digestion on the dominance of Cyanophyta (mainly *Microcystis*) contradicts previous studies (Guo et al., 2015) but is consistent with the results in Figs. 4 and 5. Although the digestion of bighead carp seems to have little effect on, or even promote, cyanobacterial blooms, the retention of algae in the fish intestine and the compressed algal excreta may inhibit the normal proliferation of cyanobacteria to some extent.

4. Conclusion

Based on the above results, we believe that silver carp is more suitable for controlling cyanobacterial blooms than bighead carp, especially in summer and autumn when cyanobacterial blooms are most

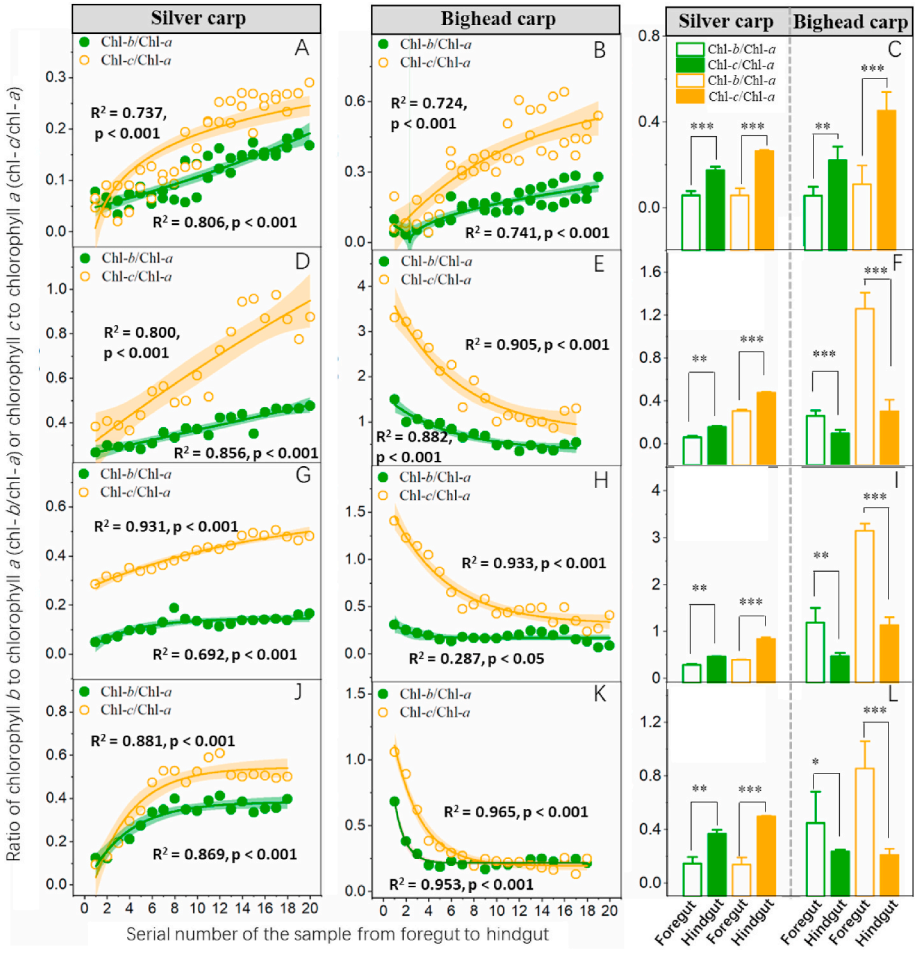

Fig. 9. Change trends of pigment proportion in the intestinal segments of silver carp and bighead carp caught in four seasons in Qiandao Lake. A-C are the change trends of pigment proportion in the intestinal segments of silver carp and bighead carp caught in spring; D-F are the change trends of pigment proportion in the intestinal segments of silver carp and bighead carp caught in summer; G-I are the change trends of pigment proportion in the intestinal segments of silver carp and bighead carp caught in autumn; J-L are the change trends of pigment proportion in the intestinal segments of silver carp and bighead carp caught in winter. A. D, G and J are the changing trends of pigment proportion in the intestine of silver carp; B. E, H and K are the changing trends of pigment proportion in the intestine of bighead carp; C. F, I and L are the comparisons of the proportion of pigments between foregut and hindgut of silver carp or bighead carp. "*" indicates that there is a significant difference between the treatments at the level of $p < 0.05$; "**" indicates that there is a significant difference between the treatments at the level of $p < 0.01$; "***" indicates that there is a significant difference between the treatments at the level of $p < 0.001$.

prominent. The particle size of plankton consumed by silver carp is usually smaller than that consumed by bighead carp due to narrow gill rakers for silver carp; therefore, larger zooplankton in comparison with phytoplankton are unlikely to be eaten by silver carp. Zooplankton play a significant role in controlling algal biomass, which is the key content of traditional biomanipulation. Many studies have reported that traditional biomanipulation can efficiently remove algal biomass by enhancing the feeding effect of zooplankton on phytoplankton, but it is not always applicable to all lakes, especially some eutrophic lakes. Similarly, the nontraditional biomanipulation of direct algae removal by filter-feeding fish is also not successful in all lakes, which may be attributed to the inadvisable stocking density and stocking proportion. Therefore, the relatively high ratio of silver carp to bighead carp can reduce the inhibitory effect of filter-feeding fish on large zooplankton populations, and has both the advantages of nontraditional biomanipulation and traditional biomanipulation. Meanwhile, some bighead carp should be captured before summer to reduce their promoting effect on the formation of cyanobacterial blooms.

CRediT authorship contribution statement

Zhicong Wang: Writing – original draft, Conceptualization, Methodology. **Qidong Wang:** Software, Conceptualization. **Jinglong Wang:** Validation, Investigation. **Hui Wei:** Visualization, Investigation. **Jing Qian:** Formal analysis. **Yinzhe Zhang:** Investigation. **Kai Feng:** Software, Validation. **Qinyi Chen:** Writing – original draft. **Jing Yuan:** Visualization. **Jiashou Liu:** Writing – review & editing. **Dunhai Li:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This study was jointly supported by the National Key Research and Development Program of China (2019YFD0900603) and the National Natural Science Foundation of China (41977285).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2022.134106.

Table S1
Densities and biomass of fish in the water body in Qiandao Lake in different seasons

Sample sites	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	S 10	S 11	S 12
Fish biomass (ind./1000 m^3)	34.61	21.66	16.76	16.53	62.64	20.46	16	13.76	12.43	24.37	29.62	60.13
Fish biomass ($\times 10^3$ kg/ha)	5.98	5.41	1.48	1.71	1.59	0.72	2.04	1.07	0.55	0.55	1.46	0.70
Fish biomass ($\times 10^3$ind./ha)	7.44	6.91	6.52	6.31	18.2	9.01	8.06	7.76	6.86	10.81	10.48	18.99

References

Alam, T., Najam, L., Harrasi, A.A., 2017. Extraction of natural pigments from marine algae. J. Agric. Mar. Sci. 23, 81–91.

APHA, 1995. Standard Methods for the Examination of Water and Wastewater, twentieth ed. American Public Health Association, Washington, DC.

Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat. Microb. Ecol. 75, 129–137.

Beattie, D.M., Golterman, H.L., Vijverberg, J., 1978. An introduction to limnology of Friesian lakes. Hydrobiologia 58, 49–64.

Beecraft, L., Watson, S.B., Smith, R.E.H., 2021. Quantifying the uncertainties in multi-wavelength PAM fluorometry due to innate and irradiance-induced variability of fluorescence spectra. Aquat. Ecol. 55 (1), 169–186.

Chen, Z.Q., Zhao, D., Li, M.L., Tu, W.G., Luo, X.M., Liu, X., 2020. A field study on the effects of combined biomanipulation on the water quality of a eutrophic lake. Environ. Pollut. 265, 115091. Part A).

Cohen, K.E., Hernandez, L.P., 2018. Making a master filterer: ontogeny of specialized filtering plates in silver carp (*Hypophthalmichthys molitrix*). J. Morphol. 279 (9), 925–935.

Domaizon, I., Devaux, J., 1999. Experimental study of the impacts of silver carp on plankton communities of eutrophic Villerest reservoir (France). Aquat. Ecol. 33, 193–204.

Dring, M.J., 1982. The Biology of Marine Plants. Edward Arnold, pp. 1–8.

Fujibayashi, M., Okano, K., Takada, Y., Mizutani, H., Uchida, N., Nishimura, O., Miyata, N., 2018. Transfer of cyanobacterial carbon to a higher trophic level fish community in a eutrophic lake food web: fatty acid and stable isotope analyses. Oecologia 188, 901–912.

Greenberg, A.E., Clesceri, L.S., Eaton, A.D., 2012. Standard Methods for the Examination of Water and Wastewater, twenty-second ed. American Public Health Association, Washington, DC, USA.

Gu, B.H., Schell, D.M., Huang, X.H., Yie, F.L., 1996. Stable isotope evidence for dietary overlap between two planktivorous fishes in aquaculture ponds. Can. J. Fish. Aquat. Sci. 53 (12), 2814–2818.

Guo, L.G., Wang, Q., Xie, P., Tao, M., Zhang, J., Niu, Y., Ma, Z.M., 2015. A non-classical biomanipulation experiment in Gonghu Bay of Lake Taihu: control of *Microcystis* blooms using silver and bighead carp. Aquaculture 46, 2211–2224.

Hoiczyk, E., Hansel, A., 2000. Cyanobacterial cell walls: news from an unusual prokaryotic envelope. J. Bacteriol. 182 (5), 1191–1199.

Hu, X.P., Su, R.G., Zou, W.M., Ren, S.J., Wang, H.T., Chai, X.P., Wang, Y.M., 2010. Research on the discrimination methods of algae based on the fluorescence excitation spectra. Acta Oceanol. Sin. 29 (4), 116–128.

Johnson, M.P., 2016. Photosynthesis. Essays Biochem. 60 (3), 255–273.

Ke, Z.X., Xie, P., Guo, L.G., Liu, Y.Q., Yang, H., 2007. In situ study on the control of toxic *Microcystis* blooms using phytoplanktivorous fish in the subtropical Lake Taihu of China: a large fish pen experiment. Aquaculture 265 (1–4), 127–138.

Keren, N., Berg, A., van Kan, P.J.M., Levanon, H., Ohad, I., 1997. Mechanism of photosystem II photoinactivation and D1 protein degradation at low light: the role of back electron flow. Proc. Natl. Acad. Sci. USA 94, 1579–1584.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1. https://doi.org/10.1093/nar/gks808.

Kolar, C.S., Chapman, D.C., Courtenay, W.R., Housel, C.M., Williams, J.D., Jennings, D. P., 2007. Bigheaded Carps: a Biological Synopsis and Environmental Risk Assessment, vol. 33. American Fisheries Society Special Publication, p. 208.

Li, L., Pan, G.A., 2013. Universal method for flocculating harmful algal blooms in marine and fresh waters using modified sand. Environ. Sci. Technol. 47, 4555–4562.

Li, X.H., Yu, Y.H., Li, C., Yan, Q.Y., 2018. Comparative study on the gut microbiotas of four economically important Asian carp species. Sci. China Life Sci. 61 (6), 696–705.

Li, X., Zeng, J., Yu, X., 2021. Different response pattern of cyanobacteria at development and maintenance stage to potassium permanganate oxidation. J. Hazard Mater. 419, 26492. -26492.

Liu, J.K., Xie, P., 1999. Unraveling the enigma of the disappearance of water bloom from the East lake (Lake Donghu) of Wuhan. Resour. Environ. Yangtze Basin 8 (3), 312–319.

Liu, Q.G., Chen, Y., Li, J.L., Chen, L.Q., 2007. The food web structure and ecosystem properties of a filter-feeding carps dominated deep reservoir ecosystem. Ecol. Model. 203 (3–4), 279–289.

Mao, Z.G., Gu, X.H., Cao, Y., Zhang, M., Zeng, Q.F., Chen, H.H., Shen, R.J., Jeppesen, E., 2020. The role of top-down and bottom-up control for phytoplankton in a subtropical shallow eutrophic lake: evidence based on long-term monitoring and modeling. Ecosystems 23, 1449–1463.

Mucci, M., Iame, A., Elisabeth, J., Lurling, M., 2020. Chitosan as a coagulant to remove cyanobacteria can cause microcystin release. Toxins 12, 0711. https://doi.org/10.3390/toxins12110711.

Peng, G.G., Zhou, X.J., Xie, B., Huang, C., Uddin, M.M., Chen, X.X., Huang, L.F., 2021. Ecosystem stability and water quality improvement in a eutrophic shallow lake via long-term integrated biomanipulation in Southeast China. Ecol. Eng. 159, 106119 https://doi.org/10.1016/j.ecoleng.2020.106119.

Qiao, L., Chang, Z.Q., Li, J., Chen, Z., 2020. Phytoplankton community succession in relation to water quality changes in the indoor industrial aquaculture system for *Litopenaeus vannamei*. Aquaculture 527, 735441.

Radke, R.J., Kahl, U., 2002. Effects of a filter-feeding fish [silver carp, *Hypophthalmichthys molitrix* (Val.)] on phyto- and zooplankton in a mesotrophic reservoir: results from an enclosure experiment. Freshw. Biol. 47, 2337–2344.

Schreiber, U., Bilger, W., Neubauer, C., 1995. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze, E.D., Caldwell, M.M. (Eds.), Ecophysiology of Photosynthesis, vol. 100. Springer Study Edition, Berlin, Heidelberg, pp. 49–70. https://doi.org/10.1007/978-3-642-79354-7_3. Springer.

Šetlíková, I., Maciarzová, S., Bláha, M., Policar, T., 2020. Silver carp (*Hypophthalmichthys molitrix*) can non-mechanically digest cyanobacteria. Fish Physiol. Biochem. 46, 771–776.

Shen, R.J., Gu, X.H., Chen, H.H., Mao, Z.G., Zeng, Q.F., Jeppesen, E., 2021. Silver carp (*Hypophthalmichthys molitrix*) stocking promotes phytoplankton growth by suppression of zooplankton rather than through nutrient recycling: an outdoor mesocosm study. Freshw. Biol. 66, 1074–1088.

Shen, R., Gu, X., Chen, H., Mao, Z., Zeng, Q., Jeppesen, E., 2020. Combining bivalve (*Corbicula fluminea*) and filter-feeding fish (*Aristichthys nobilis*) enhances the bioremediation effect of algae: an outdoor mesocosm study. Sci. Total Environ. 727, 138692.

Song, L., Wu, J., Liu, W.D., Song, Y.G., Wang, N.B., 2016. Diversity of marine nanophytoplankton and picophytoplankton in Changxing island offshore waters of Bohai Sea. Res. Environ. Sci. 29, 1635–1642.

Straskraba, M., 1965. The effect of fish on the number of invertebrates in ponds and streams. Mitt. Int. Ver. Limnol. 13, 106–127.

Su, C.Q., Hu, W.J., Hu, Z.J., Zhang, Z., Wedchaparn, O., Zhao, L.J., Liu, Q.G., 2020. Comparison of high-throughput sequencing analysis of gut contents between silver carp *Hypophthalmichthys molitrix* and bighead carp *Hypophthalmichthys nobilisin* mesotrophic and eutrophic lakes. Mar. Freshw. Res. 71 (7), 761–770.

Vanni, M.J., 2002. Nutrient cycling by animals in freshwater ecosystems. Annu. Rev. Ecol. Systemat. 33 (1), 341–370.

Wang, C.H., He, R., Wu, Y., Lurling, M., Cai, H.Y., Jiang, H.L., Liu, X., 2017. Bioavailable phosphorus (P) reduction is less than mobile P immobilization in lake sediment for eutrophication control by inactivating agents. Water Res. 109, 196–206.

Wang, L., Chen, J., Su, H.J., Ma, X.F., Wu, Z.X., Shen, H., Yu, J., Liu, J.R., Wu, Y., Ding, G.Y., Xie, P., 2021. Is zooplankton body size an indicator of water quality in (sub)tropical reservoirs in China? Ecosystems. https://doi.org/10.1007/s10021-021-00656-2.

Wang, Z.C., Huang, S., Li, D.H., 2019. Decomposition of cyanobacterial bloom contributes to the formation and distribution of iron-bound phosphorus (Fe-P): insight for cycling mechanism of internal phosphorus loading. Sci. Total Environ. 652, 696–708.

Wang, Z.C., Li, D.H., Cao, X.Y., Song, C.L., Zhou, Y.Y., 2016. Photosynthetic characteristics and inferred changes in thylakoid membrane fluidity determine bloom succession between Anabaena and Microcystis in eutrophic lakes. J. Appl. Phycol. 28, 2353–2365.

Wang, Z.C., Li, Z.J., Zhou, Y.Y., Li, D.H., 2015. Photosynthetic activity of *Microcystis* in fish guts and its implication for feasibility of bloom control by filter-feeding fishes. Fresenius Environ. Bull. 24 (7), 2384–2393.

Wang, Z.C., Li, D.H., Qin, H.J., Li, Y.X., 2012. An integrated method for removal of harmful cyanobacterial blooms in eutrophic lakes. Environ. Pollut. 160, 34–41.

Xie, P., Liu, J.K., 2001. Practical success of biomanipulation using filter-feeding Fish to control cyanobacteria blooms: a synthesis of decades of research and application in a subtropical hypereutrophic lake. Sci. World J. 1, 337–356.

Xu, J., Xie, P., Zhang, M., Yang, H., 2005. Variation in stable isotope signatures of seston and a zooplanktivorous fish in a eutrophic Chinese lake. Hydrobiologia 541 (1), 215–220.

Yi, C.L., Guo, L.G., Ni, L.Y., Luo, C.Q., 2016. Silver carp exhibited an enhanced ability of biomanipulation to control cyanobacteria bloom compared to bighead carp in hypereutrophic Lake Taihu mesocosms. Ecol. Eng. 89, 7–13.

Yu, J.L., Li, Y.M., Liu, X.L., Li, K.Y., Chen, F.Z., Gulati, R.D., Liu, Z.W., 2013. The fate of cyanobacterial detritus in the food web of Lake Taihu: a mesocosm study using C-13 and N-15 labeling. Hydrobiologia 710 (1), 39–46.

Yu, X.Q., Yang, J.R., Chen, J.X., Isabwe, A., Yang, J., 2021. On the use of chemotaxonomy, a phytoplankton identification and quantification method based on pigment for quick surveys of subtropical reservoirs. Environ. Sci. Pollut. Res. 28, 3544–3555.
Yuan, Y.T., Zhang, H.G., Pan, G., 2016. Flocculation of cyanobacterial cells using coal fly ash modified chitosan. Water Res. 97, 11–18.
Zhang, S.Y., Zhong, Q.B., Jiang, Y.H., Li, M., Xia, S.B., 2021. Temperature-induced difference in microbial characterizations accounts for the fluctuation of sequencing batch biofilm reactor performance. Biodegradation 32, 595–610.
Zhang, X., Liu, Z., Jeppesen, E., Taylor, W., Rudstam, L., 2016. Effects of benthic-feeding common carp and filter-feeding silver carp on benthic-pelagic coupling: implications for shallow lake management. Ecol. Eng. 88, 256–264.