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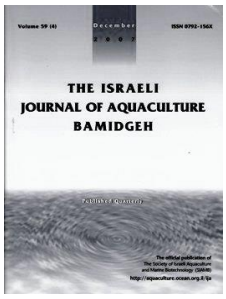

Combined Effects of Dietary Phosphorus Level and Polyculture on Fish Production, Water Quality, and Plankton Composition, in Intensive Culture of Crucian Carp

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Keywords: *Carassius auratus*; diet phosphorus; polyculture; fish production; water quality

Abstract

The combined effects of dietary phosphorus (P) levels and co-culture of silver and bighead carp on fish production, water quality, and the planktonic community in intensive culture of crucian carp (*Carassius carassius)* were studied in this enclosure experiment. There were four treatments with three replicates each: crucian carp (50 fish) fed with low (8.7 g/ kg) or high phosphorous (13.5 g/ kg) diets (L1 and H1), and polyculture (50 crucian carp + 10 silver carp + 10 bighead carp) fed with low or high-phosphorus diets (L3 and H3). Results suggested that polyculture with filter feeders suppresses crucian carp growth, especially when supplied with low phosphorus diet. Polyculture significantly enhanced the total fish production when crucian carp were fed a diet with high P levels. Dietary P level and polyculture with silver and bighead carp did not affect dissolved oxygen (DO), pH, water transparency, NH_4^+-N, total nitrogen, or any form of phosphorus ($p < 0.05$), while the presence of filter feeders significantly suppressed plankton. The present experiment suggested that silver carp is more suitable for polyculture with crucian carp, and a higher P level is needed to support plankton and reduce competition with crucian carp.

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Introduction

Phosphorus (P) is one of the most significant elements in aquaculture, not only for fish growth but also to maintain stability in aquaculture systems. Failure to meet P requirements in fish results in poor growth and low feed efficiency. It also suppresses the mineral content of fish (Roy, 2003). Supplying adequate P in feed is important to satisfy fish requirement for maximum growth (Bureau and Cho, 1999). A relatively high P concentration in the culture system would also support the growth of plankton communities to maintain good water quality as well as provide a quality food source for other consumers (Sun et al., 2017, Ji et al, 2017)). However, too much P may stimulate a cyanobacterial bloom, which may produce toxic compounds and degrade the culture ecosystem by increasing ammonia or nitrite, and decreasing dissolved oxygen after bloom crashes (Liu, et al., 2015; Sun et al., 2011; Paerl and Tucker, 1995). Due to the low retention of dietary P (Rahman et al., 2008), most consumed P is excreted by fish and stays in the culture system (Lazzari et al, 2008; Sun et al, 2017), which contributes to the production of excess algal blooms.

Polyculture with planktivore fish is considered an efficient method of P utilization and algal bloom control by the consumption of phytoplankton (Fernanda and Proença, 2017). Filter feeding silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) are widely used for the biomanipulation of planktonic communities, especially cyanobacterial bloom in eutrophic waters (Xie and Liu, 2001; Zhao et al., 2017). For this reason, polyculture of silver carp and bighead carp in commercial ponds together with other cultivated fish is very common in China and elsewhere (Neori et al. 2017). Crucian carp (*Carassius auratus*) is widely farmed in China because of its flavour (Yin et al, 2017). It is always cultured as the main species in association with silver carp and bighead carp (Yin et al; 2017). However, few studies have explored how filter-feeding fish affect the culture system and growth performance of the culture fish, especially when the fish are given different dietary P levels. The purpose of this study was to evaluate the effect of the co-culture of silver carp and bighead carp with crucian carp on aquaculture water quality, and plankton biomass and composition, and growth performance of crucian carp under different dietary P levels.

Materials and Methods

Experimental design

The experiment was conducted from July 17 -Sep. 20, in 12 enclosures (each 4 m long ×4 m wide ×1.3 m deep) located in a pond in an aquaculture farm (Zhuhai 519100, China). The polyethylene enclosures, supported by steel pipe frames, were impervious to water. They were open to the atmosphere and to the sediment but were isolated from the surrounding pond water. After a two-week acclimation period, 600 crucian carp of similar size (71.00 grm±0.58) were evenly distributed into the 12 enclosures, while silver carp (50.0 grm±0.4) and bighead carp (25.5 grm±0.8) were randomly distributed into 6 enclosures, each of which was stocked with 10 silver carp and bighead carp. Two experimental diets fed to crucian carp containing low (LP, 8.7 g/ kg) and high levels (HP, 13.5 g/ kg) of total P (see Table 1) were assigned to triplicate polyculture enclosures and mono crucian carp enclosures. The monoculture treatments included crucian carp fed with low and high P levels (L1 and H1), and polyculture of the three fish fed with low and high P (L3 and H3) levels (Table 2). Fish were fed at a feeding rate of approximately 1.5-2% body weight of the crucian carp, four times per day (07:00 am, 11:00 am, 14:00 pm and 17:00 pm) to apparent satiety. There was no water exchange during the experimental period.

Table 1 The formulation of the experimental diets

Ingredients (g/ kg)	*Diets*	
	LP	*HP*
White fish meal	100	100
Soybean meal	400	400
Rapeseed meal	130	130
Peanut meal	52	52
Flour	275	255
Soybean oil	20	20
Soy lecithin	10	10
Monocalcium phosphate[1]	0	20
Salt	20	20
Mineral premix[2]	5	5
Vitamin premix[3]	5	5
Choline chloride	3	3
Chemical composition		
Crude protein	365.3	368.7
Crude lipid	61.7	60.2
Total phosphorus	8.7	13.5

The feed was produced by the Fengda Feed factory, 24 Dongti Road, Doumen District, Zhuhai 519100, P.R. China.
Where LP represent diets with low P content and HP represent diets with high P content.
[1] Commercial feed additives.
[2] Mineral premix (mg/ kg diet): $FeSO_4.7H_2O$: 348.5 mg; $CuSO_4.5H_2O$: 6 mg; $ZnSO_4.7H_2O$: 108.2 mg; $MnSO_4.H_2O$: 20.5 mg; KI: 14.5 mg; $NaSeO_3$: 12.5 mg; $CaCO_3$: 4490 mg.
[3] Vitamin premix (mg/ kg diet): retinal palmitate, 60 mg; cholecalciferol, 10 mg; DL-a-tocopherol acetate, 100 mg; menadione, 40 mg; thiamine-HCl, 25 mg; riboflavin, 25 mg; D-calcium pantothenate, 80 mg; pyridoxine-HCl, 20 mg; meso-inositol, 1000 mg; D-biotin, 40 mg; folic acid, 7.5 mg; para-aminobenzoic acid, 25 mg; niacin, 100 mg; cyanocobalamin, 0.05 mg; corn starch, 3467 mg.

Table 2 The formulation of the experimental diet

Items	*Treatments*			
	L1	*H1*	*L3*	*H3*
Crucian carp (inds /enclosure)	50	50	50	50
Bighead carp (inds/enclosure)	0	0	10	10
Silver carp (inds /enclosure)	0	0	10	10
Total fish (inds /enclosure)	50	50	70	70
Diets	LP	HP	LP	HP

Where LP means diets with low P content and HP means diets with high p contents
Where L1, H1,L3, H3 represents different treatments.

Fish sampling and analysis

Before harvesting, the crucian carp were fasted for approximately 24 hours. Then all the fish in each enclosure were counted and weighed. Survival and growth performance parameters including weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR), were calculated according to the methods described by Sun et al. (2017). Net production was calculated according to the following equation:

Net fish production=（final crucian carp weight + final bighead carp weight + final silver carp weight） -（initial crucian carp weight + initial bighead carp weight + initial silver carp weight）

Water sampling, physicochemical and plankton analysis

Prior to the start of the experiment, samples were taken from the enclosures on July 17 to assess the pre-treatment conditions. Samples were taken at 20-day intervals in the morning (8:00-10:00 am) during the experimental period. DO (dissolved oxygen, YSI 550A), pH (Jenway model 3020 meter) and SD (transparency, Sechii depth) were determined in situ at 50 cm under the surface. Water samples (1 L) were taken by Niskin samplers filtered through GF/F Whatman filters (pore size of 0.45 μm) for the analysis of total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), dissolved nitrogen (TDN), soluble reactive phosphorus (SRP), and total dissolved phosphorus (TDP). Total nitrogen (TN) and total phosphorus (TP) were determined with unfiltered water. The methods for analysing TAN, NO_2^--N, TN, TP were described by Sun et al. (2017). The methods for analysing NO_3-N and TDN were similar to those used for

TN, while TDP was analysed with the same method used for TP. Particulate nitrogen (PN) and phosphorus (PP) were calculated according to the following equations:

PN=TN-TDN and PP= TP-TDP

For phytoplankton analysis, water samples were taken at the same time as the water samples for chemistry analysis. The 300ml samples were preserved with 1% acidified Lugol's solution and concentrated to 30 ml after 48 hours of sedimentation. Algae >2 μm were counted with a Sedgwick Rafter chamber under a microscope at magnification of ×200-×400. The size of the phytoplankton was also measured, and phytoplankton biomass were calculated from geometric volumes of each algal taxon assuming a specific gravity of 1 mg/mm^3.

Zooplankton samples were collected by filtering 10 L water through a 60-μm mesh size plankton net and then transferred to a 50ml plastic bottle and preserved with 1% formalin solution. Cladocera, copepoda, and rotifers were identified and counted with a 1ml micrometric plate.

Statistical methods

All results are shown as the mean ± SE. Data from each treatment were subjected to one-way ANOVA using SPSS 22.0 statistical software. When overall differences were significant (P < 0.05), Duncan's multiple-range test was used to compare the means between individual treatments.

Results

Growth performance

Growth performance of crucian carp, silver carp, and bighead carp is shown in Table 3. No fish died during the experimental period. Crucian carp in the monoculture enclosures fed with a high level of dietary phosphorus (H1) showed the highest final body weight, WGR, and SGR, and the lowest FCR ($p<0.05$), followed by H3. The growth of crucian carp was seriously suppressed when fed diets with low levels of P and cultured together with the silver and bighead carp ($p<0.05$). Compared with bighead carp, silver carp grew better and had a much higher WGR and SGR. Silver carp in enclosures fed a higher level of dietary P had a much higher final weight, WGR and SGR than those fed a lower level of dietary P. Treatment H3 showed the highest total fish production and lowest FCR, followed by treatment H1, and the total production and FCR of treatments L1 and L3 did not show significant differences.

Table 3. Growth performance of crucian carp, silver carp and bighead carp

Growth index	*Treatments*			
	L1	*H1*	*L3*	*H3*
Crucian carp				
Initial weight (g)	70.7±0.3	71.0±1.2	71.3±0.3	71.3±0.3
Final weight (g)	134.4±1.8^b	139.3±0.8^a	128.9±0.96^c	134.5±1.5^b
WGR (%)	90.2±3.1^a	96.4±3.5^a	80.7±1.5^b	88.5±2.0^{ab}
SGR (%/day)	1.0±0.0^a	1.1±0.03^a	1.0±0.01^b	1.0±0.02^{ab}
FCR (g)	1.7±0.05^b	1.6±0.03^b	1.9±0.03^a	1.8±0.04^b
Bighead carp				
Initial weight (g)			25.5±0.8	25.5±0.8
Final weight (g)			30.3±0.3	32.9±2.4
WGR (%)			19.0±1	29.2±9.3
SGR (%/day)			0.28±0.0	0.40±0.1
Silver carp				
Initial weight (g)			50.0±0.4	50.0±0.4
Final weight (g)			81.4±3.3^b	111.7±1.8^a
WGR (%)			62.7±6.5^b	123.4±3.6^a
SGR (% /day)			0.8±0.1^b	1.3±0.0^a
Total fish				
Net production (g/enclosure)	3184±98^c	3417±72^b	3241±17^{bc}	3848±55^a
FCR	1.7±0.1^a	1.6±0.0^b	1.7±0.0^{ab}	1.4±0.0^c

Values are the mean±MSE (n=3) of three replicates. Values within the same row with different superscript small letters are significantly different ($P<0.05$). Where L1, H1, L3, H3 represent different treatments.

Water quality

During the culture period, the water temperature ranged from 28.5-32.4°C in all enclosures. DO concentration, pH, and SD varied from 6.89-8.84 mg/L, 7.4-8.4 and

10cm-64cm, respectively. There were no significant differences among different treatments (p>0.05).

The TAN concentration ranged from 0.45-1.15 mg/L during the experimental period, and there were no significant differences (P>0.05) among different treatments (Figure 1). The NO_2^--N concentration in different treatments varied between 0.01-0.21 mg/L, respectively. At the last two sampling dates, the NO_2^--N concentration in H1 was significantly lower than that in L3 and H3 (P <0.05). The NO_3^--N and TDN contents showed an increasing trend during the experimental period. They were much higher than those of TAN and NO_2^--N. During the last three sampling periods, L3 and H3 had significantly higher contents of NO_3^--N and TDN than H1 and L1 (p <0.05). However, H3 and L3 had significantly lower contents of PN during most of the experimental period. The TN concentration ranged from 5.36-12.65 mg/L during the culture period, and there were no significant differences under different treatments.

The SRP concentration ranged from 0.0-0.18 mg/L, showing a decreasing trend first and maintaining stability later during the culture period (Figure 2). At the end of the experiment, the SRP in H3 was significantly higher than that in the other treatments. The TDP content showed a similar trend as SRP. PP and TP showed an increasing trend during the experimental period. The contents of TDP, PP, and TP did not show significant differences under different treatments (P >0.05).

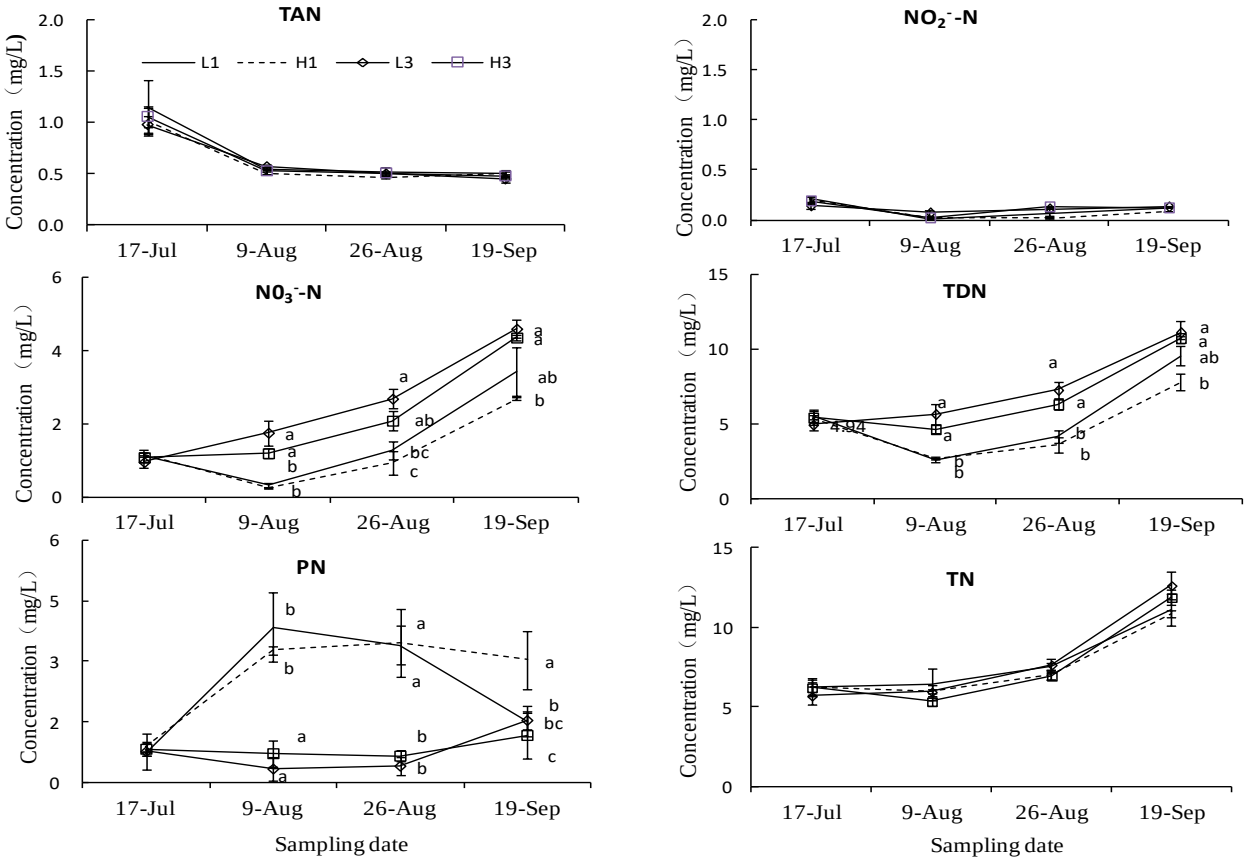

Figure 1. Dynamics of total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), total dissolved nitrogen (TDN), particulate nitrogen (PN) and total nitrogen (TN) and in four treatments during the experimental period. The values for each date are the mean concentration from three replicates. The vertical bars refer to the standard errors associated with the means. Labels with different lowercase letters indicate significant differences among the treatments in the same sampling dates (P<0.05). The absence of labels indicates no significant differences.

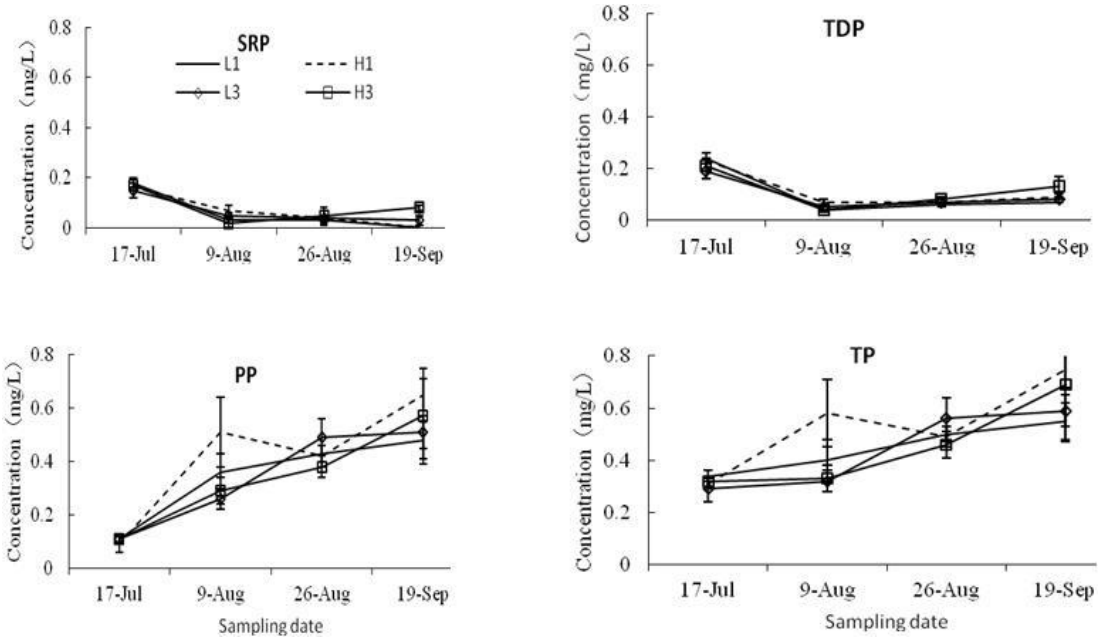

Figure 2. Dynamics of soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP), particulate phosphorus (PP) and total phosphorus (TP) in four treatments during the experimental period. The values for each date are the mean concentration from three replicates. The vertical bars refer to the standard errors associated with the means.

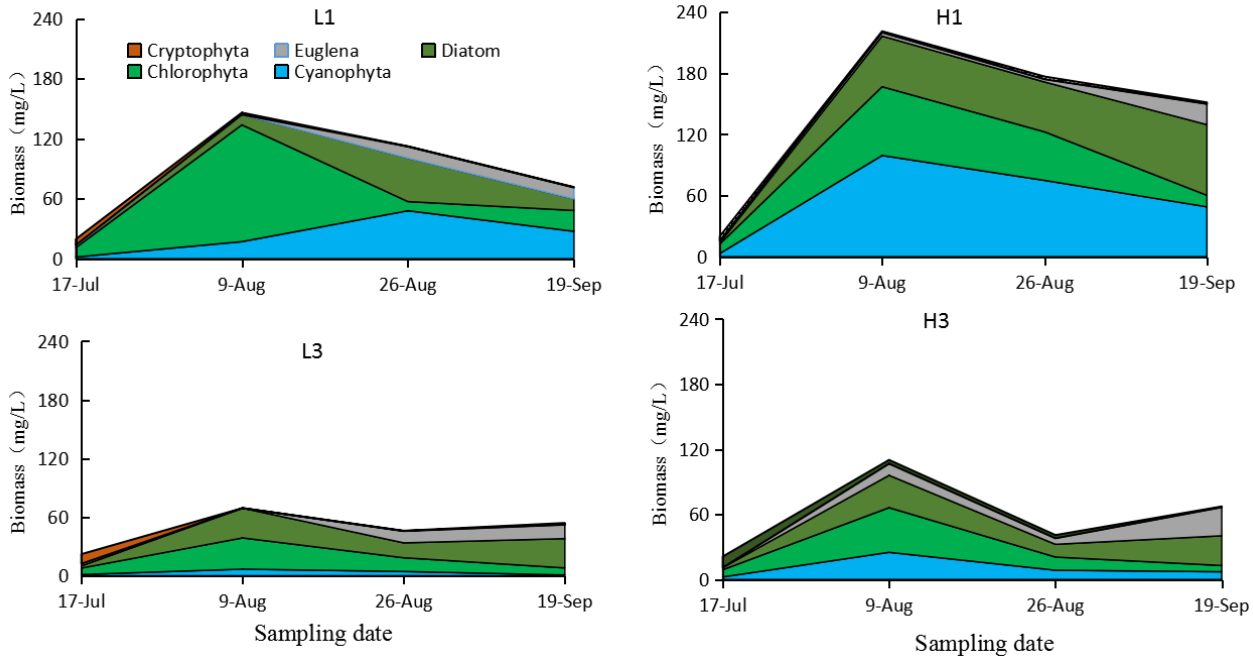

Figure 3. Dynamics of phytoplankton biomass in four treatments during the experimental period. The values for each data are the mean biomass from three replicates.

Figure 3 shows the dynamics of phytoplankton biomass composition in different treatments during the experiment. The variation of the phytoplankton community and biomass showed a similar pattern between L1 and H1 and between L3 and H3, respectively. Initially, phytoplankton in all the enclosures was composed mainly of Chlorophyta (*Oocystis* and *Chlorella*) and Cryptophyta (*Cryptomona*), with an average total phytoplankton biomass of approximately 20 mg/L. The phytoplankton biomass increased rapidly as the feeding experiment began. The average total biomass of phytoplankton during the feeding period was highest in treatment H1 ($p<0.05$), followed by L1, but there was no significant difference ($p>0.05$) between L1 and H3 or between L3 and H3 (Figure 4). The average Cyanophyta (mainly composed of *Microcystis* and *Osillatoria*) and Chlorophyta (mainly composed of *Closterium* and *Chlorella*) biomass of L1 and H1 was significantly higher than that of treatments H3 and L3 ($p<0.05$). H1 also showed a significantly higher average diatoms biomass (mainly composed of *Melosira* and *Synedra*) than all the other treatments. However, the biomass of other phytoplankton (mainly composed of *Euglena*) in H3 was significantly higher than other treatments ($p<0.05$).

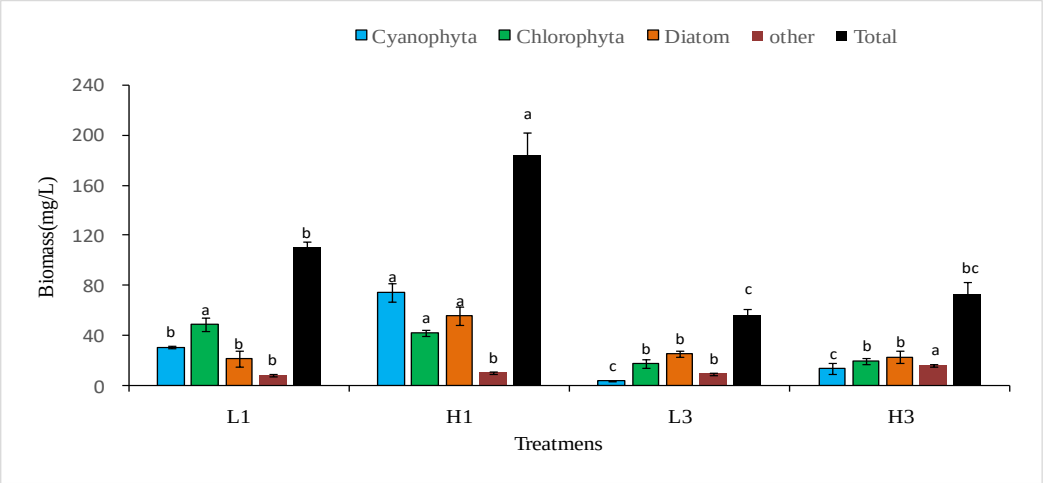

Figure 4. Multicomparison of the mean biomass of different phytoplankton during the feeding period among four treatments. The vertical bars refer to the standard errors associated with the means. Different small letters above the same column indicate significant differences among the treatments ($P<0.05$).

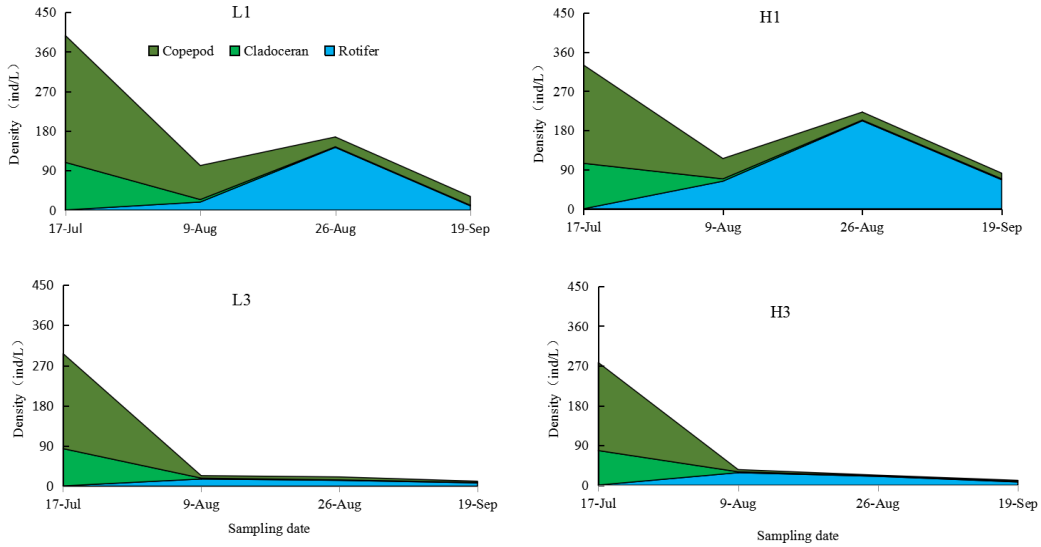

Figure 5. Dynamics of zooplankton composition in four treatments during the experimental period. The values for each date are the mean density from three replicates

Figure 5 shows the dynamics of the density composition of major zooplankton in different treatments during the experiment. Initially, all the enclosures were composed of Copepod and Cladocera. As the feeding experiment proceeded, both Copepod and Cladocera decreased rapidly, and small-sized rotifers became the dominant zooplankton. However, the total zooplankton density was significantly higher in L1 and H1 than L3 and H3 due to a relatively higher density of rotifers ($p<0.05$).

Discussion

Crucian carp was the main fish species cultured in the present experiment. Deficiency of dietary P supressed fish growth. This concept was also demonstrated in this study, since the final weight of H1 and H3 groups was significantly higher than that of L1 and L3, respectively. However, there were significant differences in WGR and SGR between L1 and H1 and between H3 and L3. Compared with monoculture, the growth of crucian carp cultured with the silver and bighead carp was suppressed when fed a low P diet and growth rate was significantly lowered (WGR and SGR); FCR was higher. Although crucian carp mainly consume pelleted diets in pond culture, they might also eat detritus occasionally as additional food (Wang et al. 2015). In the experiments carried out simultaneously in pond enclosures and recirculating tanks with the same diets, crucian carp grew better in the pond enclosures due to the presence of natural food (Chen et al., 2017). In the present research, when fed a diet high in P, natural food was more abundant, and growth of crucian carp was less affected in H3 than L3. Aside from the competition for natural food among the three carp species, silver and bighead carp might share some of the pelleted food. Using C and N stable isotopic analysis it has been suggested that silver and bighead carp can share some of the food with other fish (Xia et al. 2013; Wang et al. 2017).

Silver and bighead carp are filter feeders, consuming both phytoplankton and zooplankton (Dong and li, 1994; Xie and Liu, 2001). Existing research indicates that these two fish have quite similar food niches (Schuyler, 2009; Li et al., 2013). The higher pore size of gill rakers in bighead carp enable it to select larger food particles (Opuszynski, 1981). The less effective ability of bighead carp to collect small algae, as with silver carp, resulted in less competition between them (Yi et al., 2016). In the present study, bighead carp had a much lower growth rate than silver carp, especially when the enclosures were provided with a diet low in P, and food limitations.

The integrated polyculture of silver and bighead carp with other species can potentially remove farming waste in the form of feed residues and fecal matter, and make use of excessive plankton (Xia et al., 2013; Mo et al., 2014). In the present study, the net production of total fish was significantly higher in treatment H3 than H1, while L1 had the lowest net production but did not show a significant difference from L3. The results suggested that P is the key factor that increases system production. When sufficient P was provided, co-culture with silver and bighead carp promoted nutrient utilization and increased total production.

TN increased rapidly after introducing filter-feeding fish (McQueen et al. 1992). However, in the present study, TN was not significantly different among different treatments, but the nitrogen composition was different. Overall, co-cultured treatments had significantly higher TDN, including nitrites and nitrates. However, mono-cultured treatments had significantly higher PN content. In another study, silver carp could convert PN into dissolved forms via excretion because animal excretion contains a considerable amount of urea and results in varying TDN levels (Essington and Carpenter, 2000). In an enclosure experiment carried out in eutrophic Lake Taihu, TDN increased with increased fish biomass (Yi et al. 2016).

A higher dietary P level was presumed to excrete more P into the environment, resulting in higher TP content in the water column, as shown in a pond enclosure experiment carried out by Sun et al. (2017) however in that experiment fish density was much higher (120 fish per enclosure and a higher feeding rate（3%) compared with the present enclosure experiment. In the Sun et al experiment, much more P was added in the diet, which led to greater impact on P content of water column. The effects of filter feeding fish on P concentration in the water were not consistent. In another study it was suggested that introducing filter-feeding fish increased TP content (McQueen et al. (1992)), while in an enclosure experiment carried out in Taihu Lake, TP content

decreased when silver carp density was 35 and 75 g /m^2 (Yi et al., 2016). In the present study, TP concentration did not show significant differences among fish composition and diet P levels. The adsorption of phosphorus into sediments rather than the level of phosphorus in diets determined the phosphorus dynamics in channel catfish (Gross et al. (1998). In the present experiment, P sediment might also act to balance the P in the water.

Although TN and TP were not significantly different among the four treatments, as discussed above, the plankton was significantly affected by the co-culture of fish and dietary P levels. The presence of silver carp and bighead carp significantly decreased the biomass of Cyanophytes, Chlorophytes and diatoms, therefore decreasing total phytoplankton biomass. The density of zooplankton was also suppressed by the filter feeding carp. Dietary P could also affect phytoplankton and zooplankton, but to a lesser extent. Overall, the impact of filter feeding fish on the plankton community was much greater than that of nutrients, as indicated by other authors (Meneze, et al., 2010; Zhao, et al., 2013).

Conclusions

In summary, the combined effects of dietary P levels and polyculture with silver carp and bighead carp on fish production, water quality, and plankton composition in intensive culture of crucian carp suppressed the growth of crucian carp, especially when fed a diet with low P levels. Compared with silver carp, bighead carp were less competitive. Polyculture significantly enhanced total fish production when the crucian carp were provided with diets high in P. Dietary P levels and polyculture with silver carp and bighead carp did not affect physiochemical status, but the presence of filter feeders significantly reduced plankton count. The present experiment suggests that silver carp is more suitable for polyculture than other carp species, and a higher P level is needed to support the plankton and reduce the competition with crucian carp.

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Conflict of Interest and Ethical statement: The authors declare that they have no conflicts of interest in this work and the material has not been published in whole or in part elsewhere.

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