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Comparison of Phytoplankton Growth and Species Composition in Pangasiid Catfish Monoculture and Pangasiid Catfish/Silver Carp Polyculture Ponds

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Excessive growth of phytoplankton is a common and severe problem in intensively farmed pangasiid catfish (*Pangasius hypophthalmus*) culture ponds. It can lead to cyanobacterial blooms, reduced fish growth, bad-tasting fish flesh, and lower market demand. To investigate how to manage undesirable phytoplankton growth, we evaluated three stocking strategies in nine rural fishponds (0.020-0.022 ha) owned by various farmers: a pangasiid catfish monoculture (treatment 1, T_1), and pangasiid catfish-silver carp (*Hypophthalmichthys molitrix*) polycultures at two stocking ratios of 1:1 (treatment 2, T_2) and 2:1 (treatment 3, T_3). The total density of all ponds was approximately 30,000 fishes/ha. Monoculture (T_1) resulted in significantly higher ($p < 0.05$) nutrient levels (nitrate and phosphate) in ponds than did polyculture (T_2 and T_3). Nutrient loads increased with culture time, resulting in increased growth of phytoplankton, including Cyanophyceae (9 genera), Chlorophyceae (15 genera), Bacillariophyceae (8 genera), and Euglenophyceae (3 genera). The introduction of silver carp as a co-species helped to regulate phytoplankton growth and to improve the water quality of pangasiid catfish culture ponds.

Key words: Aquatic environment, Pangasiid catfish, Phytoplankton growth, Polyculture, Silver carp

Introduction

Phytoplankton plays an important role in aquaculture, but excessive growth is generally undesirable and troublesome for fish production. The most serious problems caused by excessive phytoplankton growth in fishponds are deficiencies in dissolved oxygen, sudden and massive phytoplankton die-offs, and bad-tasting fish flesh (Boyd, 1990). Phytoplankton populations periodically collapse, and the subsequent decomposition of dead phytoplankton causes oxygen deficiencies in aquaculture ponds (Barica, 1975; Seymour, 1980). Some blue-green algae synthesize compounds that have an earthy-musty flavor and odor, such as geosmin and 2-methylisoborneol, which are released into the water and absorbed by fish, giving their flesh an odd flavor (Lovell and Sackey, 1973). Perschbacher (1995) reported that large cyanobacteria, especially those of the genera *Anabaena, Aphanizomenon, Microcystis*, and *Oscillatoria*, also produce surface scum that often leads to algae die-offs and further deterioration of water quality. Thus, unmanaged phytoplankton growth poses major constraints on the profitability of aquaculture. The composition of phytoplankton in a water body is determined by a combination of climatic, geographical, physical, chemical, and biological factors. A change in one or more of these factors may result in a major change in phytoplankton composition. For example, changes in nutrient loading have led to significant shifts in phytoplankton

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*Corresponding author: bioengchoi@korea.com composition in many water bodies (Cronberg, 1982; Daley and Pick, 1990). Indeed, changes in phytoplankton composition in large freshwater lakes are good indicators of trophic status and environmental quality (Reynolds, 1996). Pangasiid catfish (*Pangasius hypophthalmus*), a popular aquaculture species, was introduced to Bangladesh from Thailand in the 1990s. Its production expanded dramatically throughout the country because of high consumer demand and a good market price. Today, the fish is widely farmed in intensive monocultures. However, this practice often results in high nutrient loads via the fish's metabolic wastes and the microbial decomposition of unused feed, leading to frequent and dense phytoplankton blooms. Eventually, catastrophic phytoplankton die-offs impair water quality, leading to lower quality catfish and less profit. The introduction of the planktivorous silver carp (*Hypophthalmichthys molitrix*) as a co-species may help to regulate this process and to improve the aquaculture of pangasiid catfish. We compared phytoplankton composition and growth in pangasiid catfish monocultures and pangasiid catfish-silver carp polycultures in nine ponds to assess whether polyculture may help to manage undesirable phytoplankton growth in pangasiid catfish ponds.

Materials and Methods

Experimental sites and culture conditions

Nine ponds in the village of Digharkanda in the Sadar Upazila of the Mymensingh District, Bangladesh, were monitored for 98 days between May and August 2002. The ponds were 5-10 years old and similar in size (0.020-0.022 ha). Before the start of each experiment, previously stocked fishes were removed from the ponds by netting, and the ponds were treated with lime (CaO) at the rate of 247 kg/ha 10 days prior to stocking with fish fingerlings. We performed three treatments, each replicated three times, following a randomized complete block design (RCBD). Each block contained three nearby ponds. Pangasiid catfish and silver carp were stocked at the ratio of 1:0, 1:1, and 2:1 in treatments 1 (T_1), 2 (T_2), and 3 (T_3), respectively. The total density of each pond was approximately 30,000 fishes/ha. Same-sized pangasiid catfish (21.01-21.67 g) and same-sized silver carp (4.90-4.93 g) fingerlings were bought from a local vendor and used for stocking. A commercial pellet feed containing 28% crude protein and 6% lipid was fed to pangasiid catfish at the rate of 8% body weight per day during the first month, 6% during the second month, and 4% thereafter. The feed was given twice daily, one-half in the morning (09:00 to 09:30) and the other half in the evening (17:00 to 17:30). The amount of feed given was adjusted biweekly according to the weight gain of the fish.

Phytoplankton and water quality determination

Physicochemical water quality parameters such as surface water temperature, transparency, dissolved oxygen, pH, and total alkalinity were measured between 9:00 and 11:00 each sampling day. Phytoplankton, chlorophyll-*a*, nitrate nitrogen (NO_3-N), and phosphate phosphorus (PO_4-P) were measured biweekly. To study phytoplankton, a known volume of water from each pond was collected and concentrated to approximately 200 mL using a plankton net (mesh size 15 μm). The samples were stored in plastic bottles and preserved in 5% buffered formalin for laboratory analysis. For qualitative and quantitative analysis of phytoplankton, 1 mL of sample (drawn using a dropper) was added to a Sedgwick-Rafter counting cell. The phytoplankton in 20 randomly selected squares were identified and counted under a microscope. The mean number of phytoplankton in each treatment was recorded and expressed numerically per liter of water (cells/L). The phytoplankton per liter of original water was estimated following the methods of Rahman (1992). The qualitative analysis of phytoplankton was made following the methods of Prescott (1964) and Bellinger (1992). To measure chlorophyll-*a*, NO_3-N, and PO_4-P, water samples were collected from each pond using a 1-m plastic tube (4 cm diameter), transferred to separate 250 mL bottles, and labeled. The samples were then filtered in the laboratory through GF/C filter paper (Whatman). Chlorophyll-*a* levels were determined using a spectrophotometer after acetone extraction (Greenberg et al., 1992). NO_3-N and PO_4-P were measured using a data logging spectrophotometer (Odyssey-2500, HACH, USA), using NitraVer 5 and PhosVer 3 powder pillows, respectively. Surface water temperature, transparency, dissolved oxygen, and pH were measured weekly using a thermometer, Secchi disk, portable waterproof dissolved oxygen meter (HI 9142, Hanna Instruments, Portugal), and portable pH meter (HI 8424, Hanna Instruments, Portugal), respectively.

All data on phytoplankton growth and water quality were analyzed using one-way analysis of variance (ANOVA) followed by the least significant difference test (LSD) for paired comparisons (Gomez and Gomez, 1984). Differences were considered significant at $p < 0.05$.

Results

Water quality

The surface water temperatures of all ponds were similar and varied between 28.0 and 31.8°C throughout the study period (Table 1). Water pH fluctuated narrowly and ranged from 6.49 to 7.43 (Table 1). Alkalinity did not change significantly during the experimental period. The mean dissolved oxygen concentration differed significantly ($p < 0.05$) among the treatments and was lowest in T_1 (pangasiid catfish monoculture) and highest in T_2 (pangasiid catfish-silver carp polyculture at a 1:1 ratio; Table 1). In general, dissolved oxygen tended to decrease over time for all treatments (Fig. 1). The concentrations of NO_3-N and PO_4-P varied significantly among the treatments ($p < 0.05$); both were highest in T_1 and lowest in T_2, their concentrations increased over time (Fig. 1), and the rate of increase was highest in T_1.

Chlorophyll-*a*

The chlorophyll-*a* content differed significantly between T_1 and the other treatments; it was highest in T_1 (549.20 ± 120.02 µg/L) and lowest in T_2 (267.44 ± 72.62 µg/L; $p < 0.05$). There was no significant differrence in chlorophyll-*a* content between T_2 and T_3 ($p > 0.05$; Table 1).

Phytoplankton growth and density

Phytoplankton growth differed significantly among the treatments and was highest in T_1 ($p < 0.05$; Fig. 1). The growth rate increased with increasing NO_3-N and PO_4-P concentrations (Fig. 1). Density also varied significantly and was highest in T_1 ($289.02 \pm 64.77\times 10^3$ cells/L) and lowest in T_2 ($141.85 \pm 36.70\times10^3$ cells/L; $p < 0.05$). Cell density did not differ significantly between T_2 and T_3 ($p > 0.05$; Table 2).

Phytoplankton composition

Phytoplankton was identified to the genus level. There were 35 genera in four classes: Cyanophyceae (9 genera), Chlorophyceae (15 genera), Bacillariophyceae (8 genera), and Euglenophyceae (3 genera; Table 2).

Cyanophyceae

Cyanophyceae (blue-green algae) were the second most abundant and most dense phytoplankton. The cell density differed significantly among treatments and was highest in T_1 ($194.34 \pm 49.20\times10^3$ cells/L; 67.24% of total phytoplankton) and lowest in T_2 ($50.45 \pm 10.44\times10^3$ cells/L; 35.56% of total phytoplankton; $p < 0.05$). The cell density increased in all treatments up to week 12 and then tended to decrease. The highest density was in T_1, followed by T_3 and then T_2 (Fig. 2). The genera identified were *Microcystis, Chrococcus, Aphanocapsa, Gomphosphaeria, Aphanizomenon, Sphaerocystis, Anabaena, Oscillatoria*, and *Merismopedia.* Of these, *Chrococcus, Anabaena*, and *Aphanizomenon* were the most abundant.

Chlorophyceae

Chlorophyceae (green algae) were the most abundant and second most dense phytoplankton. The cell density did not differ significantly among treatments, but was highest in T_2 ($54.86 \pm 10.58\times10^3$ cells/L; 38.68% of total phytoplankton) and lowest in T_3 ($50.67 \pm 15.17\times10^3$ cells/L; 32.35% of total phytoplankton; $p > 0.05$). The cell density was highest in T_1 during week 4 and in T_2 during week 12 (Fig. 2). The genera identified were *Pediastrum, Oocystis, Chlorella, Scenedesmus, Botryococcus, Tetraedron, Zygnema, Desmidium, Closterium, Coelastrum, Characium, Staurastrum, Treubaria, Ankistrodesmus*, and *Ulothrix*. Of these, the most abundant genera were *Chlorella, Scenedesmus*, and *Pediastrum*.

Bacillariophyceae

Bacillariophyceae were the third most abundant and dense phytoplankton. The cell density did not differ significantly among treatments, but was highest in T_1 ($30.62 \pm 3.09\times10^3$ cells/L; 10.59% of total phytoplankton) and lowest in T_3 ($25.45 \pm 5.04\times10^3$ cells/L; L; 16.25% of total phytoplankton; $p > 0.05$). The cell

Table 1. Mean values ± SE (n=42) of water quality parameters in ponds of different treatments. Figures in the same row having the same superscripts are not significantly ($p > 0.05$) different

Parameters	Treatment 1	Treatment 2	Treatment 3
Temperature (°C)	30.38 ± 0.23	30.23 ± 0.10	30.20 ± 0.11
Transparency (cm)	$18.99^b \pm 1.09$	$28.90^a \pm 2.28$	$25.44^a \pm 2.41$
pH	6.85 ± 0.05	7.03 ± 0.05	6.92 ± 0.05
Dissolved oxygen (mg/L)	$3.71^b \pm 0.08$	$4.70^a \pm 0.17$	$4.21^a \pm 0.09$
Total alkalinity (mg/L)	99.21 ± 5.98	100.67 ± 4.17	99.54 ± 4.83
NO_3-N (mg/L)	$2.18^a \pm 0.06$	$1.68^b \pm 0.06$	$1.94^b \pm 0.15$
PO_4-P (mg/L)	$1.98^b \pm 0.07$	$1.37^a \pm 0.01$	$1.83^b \pm 0.04$
Chlorophyll *a* (µg/L)	$549.20^a \pm 120.02$	$267.44^b \pm 72.62$	$297.25^b \pm 66.02$

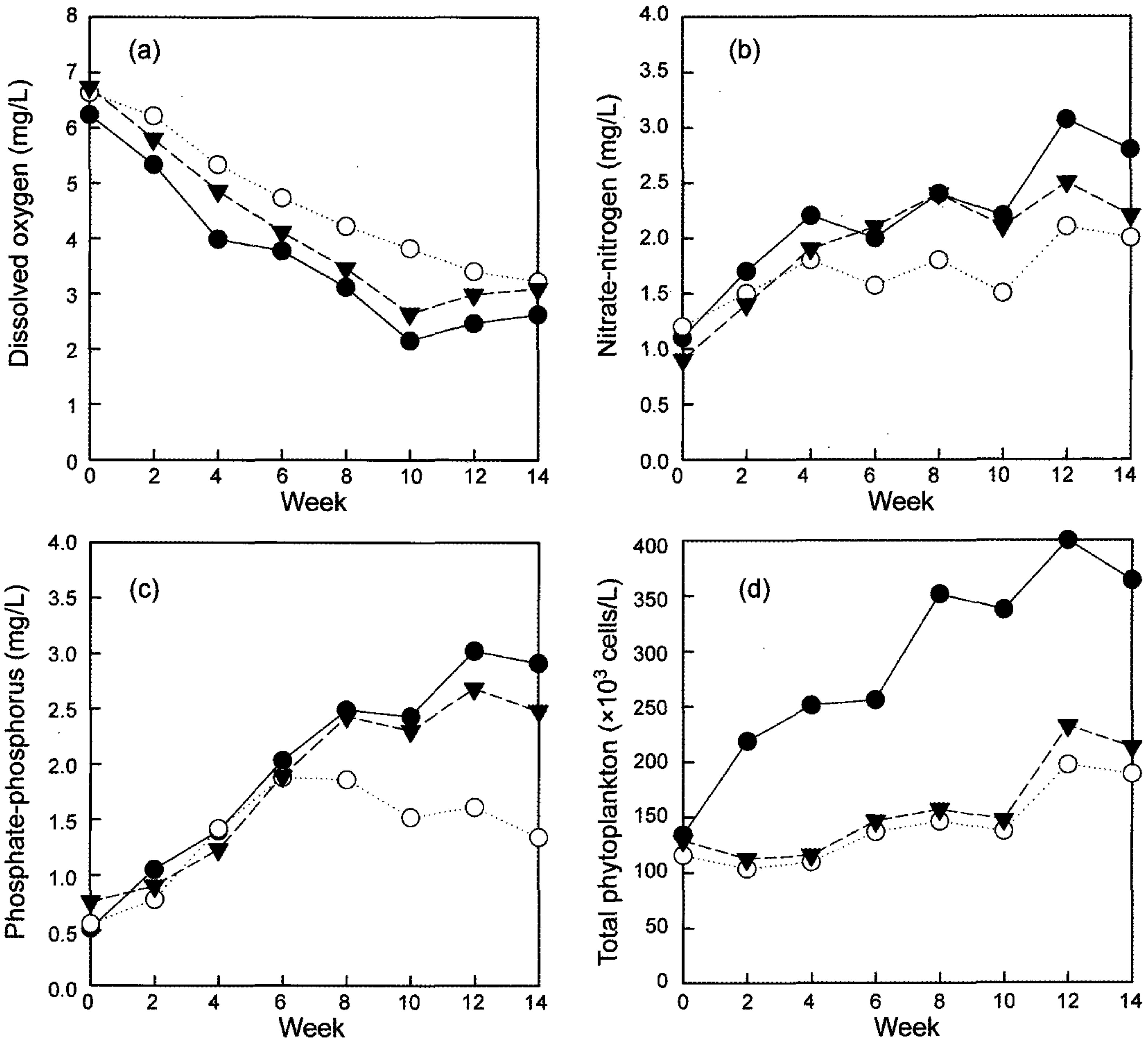

Fig. 1. Biweekly variation of dissolved oxygen (a), nitrate-nitrogen (b), phosphate-phosphorus (C) and total phytoplankton (d) in three different treatments, T1 (●), T2 (○) and T3 (▼) during the study period.

Table 2. Density of different phytoplankton groups (mean ± S.E; $\times 10^3$ cells/L) in different treatments. Each value is the mean of three ponds collected on eight sampling dates (n=24). Figures in the same row having the same superscripts are not significantly ($p > 0.05$) different

Phytoplankton group	Treatment 1	Treatment 2	Treatment 3
Cyanophyceae	$194.34^a \pm 49.20$	$50.45^b \pm 10.44$	$74.23^b \pm 13.40$
Chlorophyceae	52.62 ± 9.94	54.86 ± 10.58	50.67 ± 15.17
Bacillariophyceae	30.62 9 ± 3.0	29.59 ± 8.46	25.45 ± 5.04
Euglenophyceae	$11.44^a \pm 2.33$	$6.95^b \pm 0.75$	$6.27^b \pm 1.70$
Total phytoplankton (cells/L)	$289.02^a \pm 64.77$	$141.85^b \pm 36.70$	$156.62^b \pm 30.14$

density was highest in T_1 during week 2 and in T_2 during week 12 (Fig. 2). The genera identified were *Cyclotella, Fragilaria, Navicula, Nitzschia, Tabellaria, Gyrosigma, Melosira*, and *Synedra*, of which *Nitzschia, Navicula*, and *Cyclotella* were the most abundant.

Euglenophyceae

Euglenophyceae were the least abundant and dense phytoplankton. The cell density differed significantly among treatments and was highest in T_1 ($11.44 \pm 2.33 \times 10^3$ cells/L) and lowest in T_2 ($6.95 \pm 0.75 \times 10^3$ cells/L; $p < 0.05$). Overall, Euglenophyceae were scarce in all treatments. The genera identified were *Euglena, Phacus*, and *Trachelomonas*.

Relative growth of the phytoplankton groups

There were noticeable relationships in growth among the phytoplankton groups (Fig. 3). Cyanophyceae inhibited the growth of the other groups, which decreased in abundance as the Cyanophyceae population increased. There was no such relationship

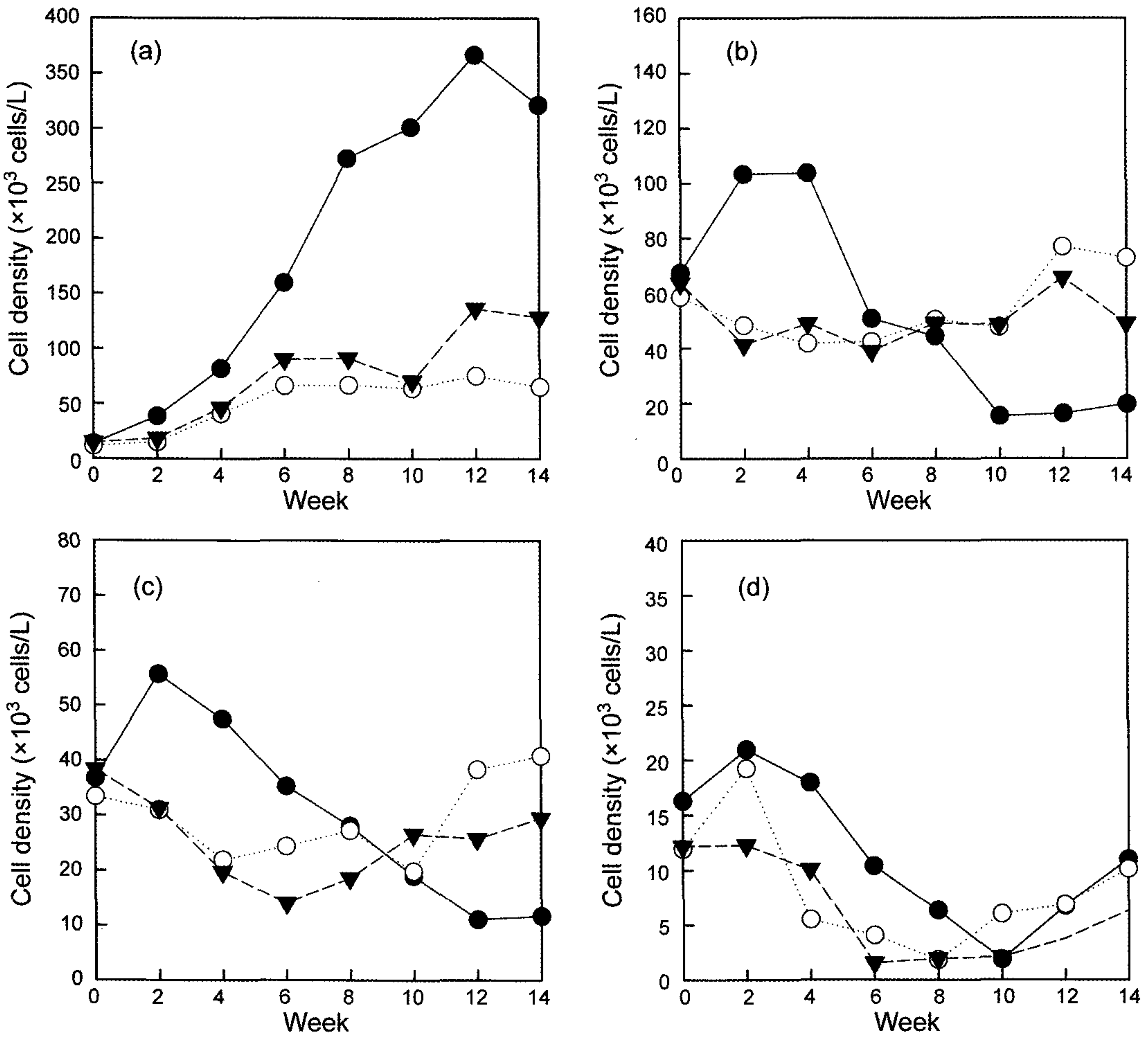

Fig. 2. Biweekly variation of Cyanophyceae (a), Chlorophyceae (b), Bacillariophyceae (c) and Euglenophyceae (d) cell density in different treatments, T1 (●), T2 (○) and T3 (▼) during the study period.

among the Chlorophyceae, Bacillariophyceae, and Euglenophyceae. When the Cyanophyceae were subdominant or rare, the populations of these other three groups increased simultaneously.

Discussion

Pangasiid catfish monoculture ponds had higher levels of phytoplankton than did pangasiid catfish–silver carp polyculture ponds. Phytoplankton growth in natural habitats is regulated by various environmental factors such as temperature, pH, and nutrient concentrations (Uye and Takamatsu, 1990). Each environmental factor has an optimum level for promoting phytoplankton growth; those of temperature, pH, and NO_3-N and PO_4-P concentrations in aquaculture ponds range from 20.0 to 37.5°C, 7.0 to 8.5, 0.06 to 0.1 mg/L, and 0.2 to 0.4 mg/L, respectively (Mollah and Haque, 1978, 1995). Phytoplankton requires nutrients, sufficient light, and favorable temperatures to grow (Fogg, 1975; Bold and Wynne, 1978). The environmental parameters were optimal for phytoplankton growth in all treatments. Nonetheless, phytoplankton growth and composition varied among the treatments, possibly because of variation in the environmental parameters. The concentrations of both NO_3-N and PO_4-P were significantly higher in T_1 than in T_2 and T_3 ($p < 0.05$). These higher nutrient loads could have stimulated more phytoplankton growth in T_1 than in T_2 and T_3. More pangasiid catfish fingerlings were stocked in T_1 than T_2 and T_3 (50% higher than in T_2 and 33% higher than in T_3 because they were replaced by silver carp in these treatments). Thus, the amount of feed required for pangasiid catfish in T_1 was much higher than in T_2 and T_3. Therefore, unused feed and metabolic wastes in T_1 were higher, which could have led to excess NO_3-N and PO_4-P via microbial decomposition. This finding is in agreement with that of Boyd (1982), who reported that uneaten fish food supplied more nutrients

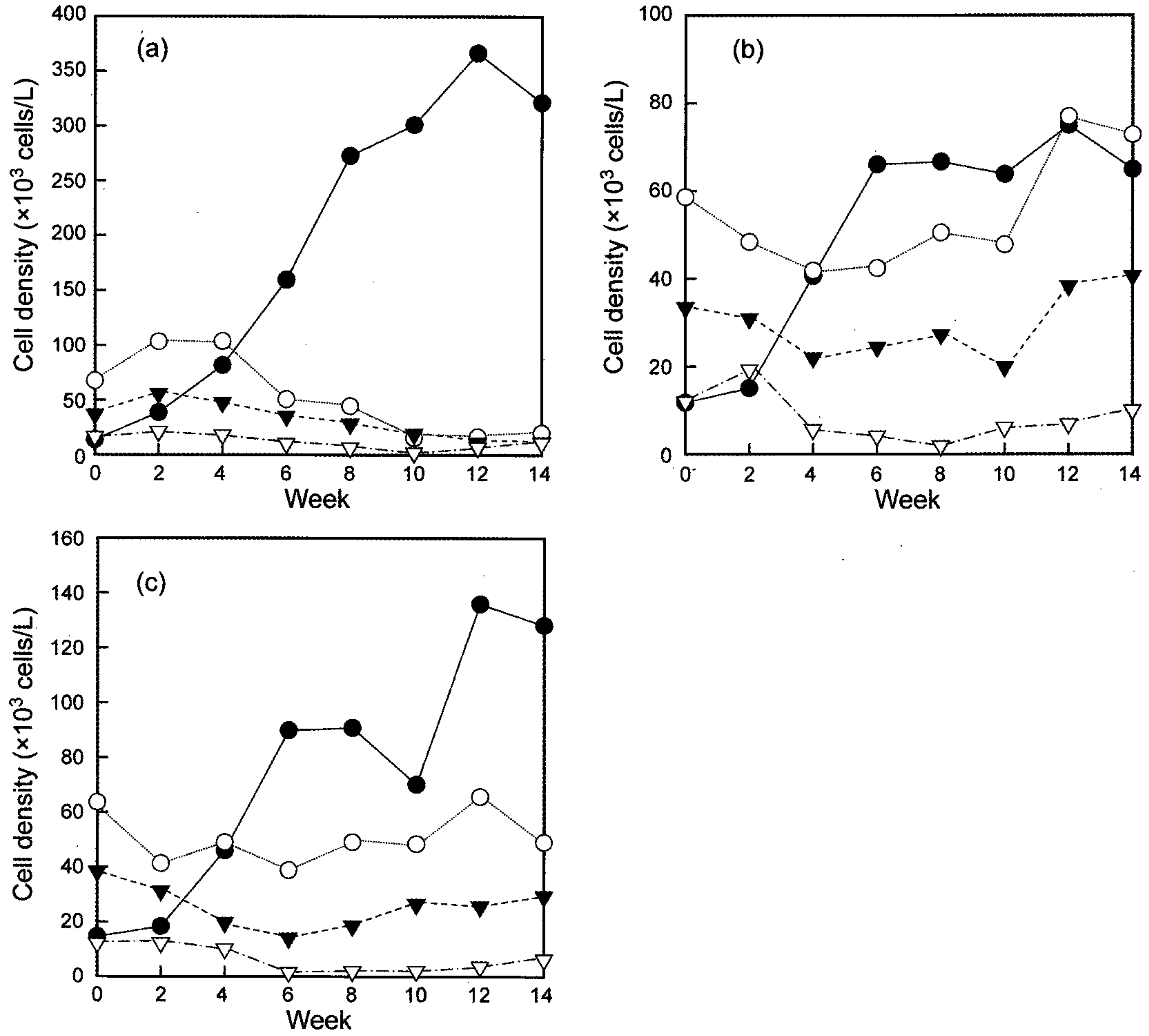

Fig. 3. Growth relation among Cyanophyceae (●), Chlorophyceae (○), Bacillariophyceae (▼) and Euglenophyceae (▽) in three different treatments, T1 (a), T2 (b) and T3 (c) during the study period.

to algae in channel catfish ponds because catfish assimilate up to 40% of the nitrogen and 65% of the phosphorus that they consume. Similarly, Cole and Boyd (1986) found increased average concentrations of NO_3-N with increased feeding rate. Boyd (1985) found that increased levels of inorganic nutrients enhanced phytoplankton biomass in catfish ponds. Conversely, there was less excess feed in T_2 and T_3 and thus less NO_3-N and PO_4-P, which may have prevented the excessive growth of phytoplankton. Moreover, the grazing pressure of silver carp maintained phytoplankton growth at lower levels in T_2 and T_3 than in T_1. Smith (1985, 1988) and Radke and Kahl (2002) reported that silver carp were effective in reducing populations of large microalgae. Dunseth and Smitherman (1977) found that fewer algae grew in ponds stocked with silver carp compared to control ponds with catfish alone. The composition of phytoplankton is regulated by several factors, including the availability of nutrients, the growth rate of the algal species, and the specific rate of loss attributed to grazing, sedimentation, and dilution. We identified a total of 35 phytoplankton genera in four classes: Cyanophyceae, Chlorophyceae, Bacillariophyceae, and Euglenophyceae. These findings are similar to those of Ehshan et al. (1997), Rahman et al. (1999), and Hasanat et al. (2000), who reported 33-39 genera in ponds and lakes of Bangladesh. Boyd (1990) reported that phytoplankton in aquaculture ponds includes mainly members of the four taxonomic groups Chlorophyta (green algae), Cyanophyta (blue-green algae), Euglenophyta (euglenaphytes), and Bacillariophyta (bacillariophytes), as we found. Of these groups, Cyanophyceae was the most abundant in pangasiid catfish monoculture (T_1). The higher NO_3-N and PO_4-P concentrations in that treatment may be related to the better growth of this group of phytoplankton. Park et al. (1993) reported that increases in dissolved NO_3-N favor the growth of *Microcystis* at Lake Suwa in Japan. We found that as

Cyanophyceae became dominant, other groups of phytoplankton decreased in abundance. This finding agrees with those of previous studies (Lam and Silvester, 1979; Stockner and Cronberg, 2000) that reported that the high nutrient-scavenging capacity, buoyancy properties, and toxic substances of Cyanophyceae help them to become dominant. We found that Chlorophyceae (green algae) was the second most dominant group, which agrees with previous findings that green and blue-green algae are usually the most abundant phytoplankton in fishponds (Tucker and Lloyd, 1984). There was significant variation in chlorophyll-*a* content among the treatments throughout the study period. It reached a maximum in T_1, in which phytoplankton density was highest, and a minimum in T_2, in which phytoplankton density was lowest. Khatrai (1984) found a positive relationship between phytoplankton growth and chlorophyll-*a* content. The dissolved oxygen was lowest in T_1, in which the phytoplankton density was highest. This was possibly due to higher consumption of dissolved oxygen by more fish, as well as to microbial decomposition of a higher amount of unused feed, metabolic wastes, and dead phytoplankton.

In conclusion, phytoplankton growth and water quality were evaluated for pangasiid catfish monocultures and pangasiid catfish-silver carp polycultures. Monoculture resulted in the highest phytoplankton growth, dominated by Cyanophyceae, increased NO_3-N and PO_4-P concentrations, and decreased dissolved oxygen concentrations compared to polyculture. Pangasiid catfish-silver carp polycultures at a 1:1 ratio and a total density of 30,000 fishes/ha resulted in the lowest phytoplankton growth, lowest NO_3-N and PO_4-P concentrations, and highest dissolved oxygen in the culture ponds. We demonstrated that a pangasiid catfish-silver carp polyculture is better than a pangasiid catfish monoculture in terms of managing phytoplankton and maintaining a good aquatic environment. Future studies using multiple species of fish would help to elucidate further the possible benefits of polyculture in terms of economic gain and environmental maintenance.

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