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Laboratory studies for preventing the growth of *Microcystis aeroginosa* using *Chlorella* and *Scenedesmus* in the Nile tilapia culture

Dawah, A[1]., Meslhy, S. and [2]El‡ Naggar, G. [2]

[1]Central Lab. For Aquaculture Research Abbassa, Agricultural Research Center Egypt
[2] WorldFish Center Abbassa, Sharkia, Egypt

Abstract

Cyanobacteria (blue-green algae) are a diverse group of photosynthetic, prokaryotic organisms found in fresh water and marine environments. Their cell structure resembles that of gram negative bacteria, but as a rule they live photoautotrophically.

The time to control a toxic algae bloom is before the bloom develops. This study is a trial for the prevention of *Microcystis sp.* growth using *Chlorella* and *Scenedesmus* mass culture via indoor experiment. The experiment was conducted in 12 glass aquaria; of one hundred liters capacity each. All aquaria were filled with canal water, having a known species composition count of phytoplankton, chlorophyll "a , "b , "c and c-phycocyanin content. These aquaria were seeded with green algae (*Chlorella elliposoides* & *Scenedesmus bijuga*) at initial density; 20 x 10^3 cells ml^{-1} and left for 10 days to propagate the algae. 10 Nile tilapia (*Oreochromis niloticus*) with initial weight of 30±3 gm was stocked in each aquarium. The experimental water was fertilized by chicken manure as 3 mg l^{-1} to allow the propagation of the green algae (*C. elliposoides* and *S. bijuga* sp.). Aeration was supplemented, provided by a regenerative blower and stones submerged at the bottom of each aquarium. First 3 aquaria groups in each replicate were inoculated by *Microcystis aeroginosa* collected from pond supper from the problem as 10, 30 and 50 x 10^3 cells ml^{-1} as T_1, T_2 and T_3, respectively. 4th aquaria served as a control without inoculation (T_0). The experiment was maintained for another 10 days after inoculation.

The numbers of *Microcystis* (blue green algae) decreased rapidly after 5 days of incubation and continued to decline till disappear after 10 days of incubation in the aquaria inoculated with 50 x 10^3 cells ml^{-1} of *Microcystis* cells (T_3) as a high dose. On the other hand, the growth of green algae (*Chlorella* and *Scenedesmus* sp.) increased gradually in numbers from first day of incubation with *Microcystis* cells to the end of the experiment.

The c-phycocyanin was not detected in the control and T_1 after 5 days of incubation and in all treatments after 10 days of incubation.

Although, all treatments and control seeded with the same numbers of *Chlorella* and *Scenedesmus* sp. but there was significantly difference between the control and the other treatments in the number of green algae and in the chlorophyll b content. The number of green algae and chlorophyll b content increased gradually where the control < T_1 < T_2 < T_3 after 5 days of incubation to the end of experiment. The green algae abundance was strong positively correlated with the count of diatoms (r = 0.75) and strong negatively correlated with dinoflagellates (r = -0.75).

Introduction

Fertilizing a pond with organic or inorganic fertilizer is often done to initiate an algal bloom, which is another term for an increased population of algae. The natural and artificial fertilizers provide principal chemical nutrients necessary for algal growth and reproduction. The principal nutrients are nitrogen (N), phosphorus (P), and potassium (K). The microscopic plants that make up the phytoplankton population take up these chemical nutrients and at the proper temperature undergo rapid population growth (Conte, 1990).

Algal blooms are essential to successful fish production because of the dissolved oxygen they produce through photosynthesis during the daylight hours and their uptake (assimilation) of nitrogenous waste products. In a sense, the fish help keep the bloom alive by fertilizing it, and the bloom helps keep the fish alive by producing oxygen and by breaking down and absorbing fish wastes, rendering them harmless to the fish population (Brunson, et al., 2003).

A number of problems are associated with dense algal blooms. In waters that have a low or moderate buffering capacity (alkalinity), dense blooms create wide fluctuations in pH during the day. Occasionally, phytoplankton populations cause pH to reach afternoon values of 10 or above which depress fish growth and health. Algal die-offs can result in high ammonia concentrations that can affect fish appetites and growth rates for extended periods. This can result in reduction of the growing season for fish producers each time an algal bloom dies back in a pond (Brunson, et al., (2003).

A variety of schemes have been proposed for managing phytoplankton blooms, but most approaches have either been unreliable or impractical. Copper sulfate and certain chelated or complexed copper products are currently the only algicides labeled for use in aquaculture ponds. Copper algicides are not ideal for off-flavor management because they are not selectively toxic to odor-producing blue green algae. Copper also interacts strongly with other water quality variables, and one important consequence of those interactions is that copper products become more toxic to fish and algae as water hardness and alkalinity decrease (Tucker and van der Ploeg, 1999).

Copper is also toxic to invertebrates, such as snails, and also most of the zooplankton in a pond (daphnia, rotifers, etc.), so if you are relying on the zooplankton as a food source (i.e. fry ponds), you may not want to use copper

Potassium permanganate, $KMnO_4$, is a chemical oxidizing agent that will react with any organic matter in a pond including algae, bacteria, fish, particulate and dissolved organic, and organic bottom sediments. Potassium permanganate is an expensive treatment. Therefore, it is important to properly estimate water volume to achieve both a cost-effective and biologically effective treatment. Underestimating water volume will result in an insufficient concentration of chemical, and retreatment would be necessary. Overestimating water volume can result in a greater-than-desired concentration of chemical, and may injure or even kill fish.

Copper or permanganate treatments can cause oxygen concentrations to drop, which may result in fish kills. Pond algae are a major source of oxygen production and by removing it this source of oxygen is also removed. In addition, oxygen will be consumed as the algae decompose. If you are treating to kill algae, either treat in a series of smaller doses over time or have emergency aeration available.

The time to control a toxic algae bloom is before the bloom develops. Preventing fertilizers, animal wastes and other sources of nutrients from reaching the water is the best prevention. Reducing nutrient and pollution runoff from the land has generally been accepted as vitally important in greatly reducing, though not eliminating, the frequency, toxicity and longevity, of harmful algal blooms. High phosphorus is often a precursor to an algal bloom. Nutrient-rich bodies of water such as estuaries, eutrophic lakes, agricultural ponds or catch basins may support a rapid growth of algae. Under ideal conditions a clear body of water can become very turbid with an algal bloom within just a few days (Webster, 1996).

Virtually all freshwater blooms are caused by cyanobacteria and some of them are toxic. Freshwater algae rarely cause more than oxygen depletion problems due to high density blooms. The severity of the blooms varies from year to year depending on the climate; blooms tend to be worst in particularly dry summers or during droughts and least severe in wetter summers.

In the previous studies we could control the algal blooms using tannic acids or using *Chlorella* and *Scenedesmus* sp. (green algae) treatments but these treatments might cause oxygen depletion due to algae die off by tannic acid or increase the algae density by using *Chlorella* and *Scenedesmus* sp. So might be better to prevent the algal blooms than treatments.

This study is a trial for the prevention of *Microcystis sp.* growth using *Chlorella* and *Scenedesmus* mass culture via indoor experiment.

Materials and methods

Isolates of *Chlorella elliposoides* & *Scenedesmus bijuga* were obtained from Nile water samples according to Pascher (1915). The microalgae were subculture in a solid Bold's basal medium (BBM) (Bischoff & Bold, 1963). Stock cultures of *C. elliposoides* & *S. bijuga* were prepared at WorldFish Center in two litters capacity flasks in the laboratory for 5-6 days, then inoculated in carboy cultures at a density of 1 x 10^5 cells ml^{-1}. The carboy cultures were used as inoculate for two different phases of production in indoor and outdoor glass aquaria. The transfer of the algal cells to fish aquaria was achieved at a density of 5 x 10^6 cells ml^{-1}.

Indoor experiment was carried out in natural light using 12 glass aquaria as four groups (each aquarium has 100 liters capacity) at WorldFish Center. 3 treatments and control groups were carried out in triplicates. 10 Nile tilapia (*Oreochromis niloticus*) with initial weight of 30±3 gm was stocked in each aquarium. The experimental water was fertilized by chicken manure as 3 mg l^{-1} to allow the propagation of the green algae (*Chlorella elliposoides* and *Scenedesmus bijuga* sp.). Aeration was supplemented, provided by a regenerative blower and stones submerged at the bottom of each aquarium.

All aquaria filled with canal water, having a known species composition count of phytoplankton, chlorophyll “a , “b , “c and c-phycocyanin content. These aquaria were seeded with green algae (*C. elliposoides* & *S. bijuga*) at initial density; 20 x 10^3 cells ml^{-1} and left for 10 days to propagate the algae where the average mean of green algae reached 1.2 x 10^6 cells ml^{-1} in all aquaria. First 3 aquaria groups in each replicate were inoculated by *Microcystis aeroginosa* collected from pond supper from the problem as 10, 30 and 50 x 10^3 cells ml^{-1} as T_1, T_2 and T_3, respectively. 4th aquaria served as a control without inoculation (T_0). The experiment was maintained for another 10 days after inoculation. Sampling for chemical, physical and biological analysis in all treatments and control were carried out at starting time, 5, 10 days intervals.

The following formula was used to compute for the required volume of stock green algae to be added into the aquaria.

$$\text{Volume to be added} = \frac{(\text{desired density} - \text{existing density}) \times \text{volume of water in aquarium}}{\text{Density of stock culture}}$$

The laboratory investigations were done (by the end of 5th and 10th days of treatment) at WorldFish Center where chlorophyll, C-phycocyanin and phytoplankton were estimated. Also, the physicochemical characteristics of water were analyzed.

Chlorophyll a, b, and c contents were determined in 100 ml water photometrically by using spectrophotometer. Calculation of the chlorophyll a, b, and c was carried out using the equation adopted by APHA (1985).

Spectrophotometrically, the C-phycocyanin (CPC) concentration was calculated using Beer's law and an extinction coefficient of 7.9 L g^{-1} cm^{-1} (Svedberg & Katsurai, 1929):

$CPCgL^{-1} = A_{625}/7.9\ Lper\ gcm$ x 1 cm.

Quantitative estimation of phytoplankton was carried out by the technique adopted by APHA (1985) using the sedimentation method. The results were then expressed as counts per ml. The phytoplankton cells were identified to four division as green algae (chlorophyceae), blue-green algae (cyanophyceae), diatom (bacillariophyceae), and (dinoflagellates), for identification of the algal taxa, [Fritsch (1979) and Komarek and Fott (1983)].

Water temperature (^oC) and dissolved oxygen (DO, mg/l) were measured using an oxygen electrode, water samples were collected to measure both the hydrogen ions (pH) by using the ACCUMET pH meter (model 25) and total ammonia (mg/l) by using HACH Comparison (1982). Total alkalinity (as $CaCO_3$ mg/l), total hardness (mg/l) and nitrate (NO_3) were determined according to Boyd and Tucker (1992).

One-way ANOVA was used to evaluate the significant difference of the different treatments and duration. A probability at level of 0.05 or less was considered significant. Means and standard errors were also estimated. All statistics were run on the computer, using the SAS program (SAS, 2003).

Results

Results demonstrated in Table (1) and Fig (1) revealed that the inhibitory effect of *Chlorella* and *Scenedesmus* sp. on the growth of *M. aeroginosa*. The numbers of *Microcystis* (blue green algae) decreased rapidly after 5 days of incubation and continued to decline till disappear after 10 days of incubation in the aquaria inoculated with 50 x 10^3 cells ml^{-1} of *Microcystis* cells (T_3) as a high dose. On the other hand, the growth of green algae (*Chlorella* and *Scenedesmus* sp.) increased gradually in numbers from first day of incubation with *Microcystis* cells to the end of the experiment.

Similar results were observed in the treatments using *Chlorella* and *Scenedesmus* sp. with *Microcystis* incubation at 30 x 10^3 cells ml^{-1} (T_2). The growth of *Microcystis* was inhibited within 5 days of incubation and not detected after 10 days of incubation. The *Microcystis* was not detected in the group incubated with 10 x 10^3 cells ml^{-1} of *Microcystis* (T_1) at 5 days of incubation. No *Microcystis* was detected in the control group (T_0) using *Chlorella* and *Scenedesmus* sp. only.

The c-phycocyanin was not detected in the control and T_1 after 5 days of incubation and in all treatments after 10 days of incubation. There was significantly difference between T_2 and T_3 after 5 days of incubation but there was insignificantly different ($p < 0.05$) Table (2) and Fig (2). Although, all treatments and control seeded with the same numbers of *Chlorella* and *Scenedesmus* sp. but there was significantly difference between the control and the other treatments in the number of green algae and in the chlorophyll b content. The number of green algae and chlorophyll b content increased gradually where the control < T_1

$< T_2 < T_3$ after 5 days of incubation to the end of experiment. This is means that the *Microcystis* cells may be exhibit the growth of *Chlorella* and *Scenedesmus* sp. (green algae) which they could produce the antibacterial substances that inhibit the growth of *Microcystis*.

A multiples correlation analysis including 9 biological variables was carried out for the experiment (Table 3). The green algae abundance was strong positively correlated with the count of diatoms ($r = 0.75$) and strong negatively correlated with dinoflagellates ($r = -0.75$). Also, the chlorophyll b content showed strong positive correlation with the count of diatoms ($r = 0.85$) and pigment fraction namely chlorophyll "c ($r = 0.78$) during the experimental periods.

All chemical parameters of cultures remained all time with minor changes, but there were significantly differences in oxygen and nitrate content between T_3 and other treatments ($p < 0.05$). No mortality appeared during the experiment. The total harvest of Nile tilapia was insignificantly different between control and all other treatments at the end of experiment (Table 4).

Discussion

Cyanobacteria (blue-green algae) are a diverse group of photosynthetic, prokaryotic organisms found in fresh water and marine environments (Schoof and Packer, 1987). Their cell structure resembles that of Gram negative bacteria, but as a rule they live photoautotrophically.

This information indicates that it might be possible to use *Chlorella* and *Scenedesmus* sp. in Nile tilapia ponds not only for control of *Microcystis* but also to control of bacterial disease and maintain ecological balance in the pond (Tendencia et al., 2005)

Chlorella and *Scenedesmus* are unicellular phytoplankton genus belonging to the phylum Chlorophyta. There are many species of *Chlorella* and *Scenedesmus* in both fresh water and sea water. Certain freshwater *Chlorella* and *Scenedesmus* are cultured as health foods for humans and animals because of the proteins, vitamins and other substances they contain. They have also been used in medicinal products and as an antibiotic (Suwapepan 1984; Hill and Nakagawa 1981). The results of this study revealed clearly that *Chlorella* and *Scenedesmus* could inhibit growth of the *Microcystis*. Pratt et al., (1944) suggested that *Chlorella* produced an antibacterial substance called chlorellin, also, Jones 1988 suggested that *Chlorella* produces more than one antibiotic substance and that one of these may be chlorophyllid, a precursor of chlorophyll. In aquaculture they are used as food for aquatic animal larvae as well as a biological control agent. Therefore, the efficacy of growth inhibition may be due to released antibacterial substances. This is consistent with Tendencia and dela Pena (2003) who reported that luminous bacteria were not detected in flasks with *Chlorella* sp. after 2 days. Also, Tendencia et al., (2005) showed that the luminous bacterial counts in tanks with *Chlorella* sp. were lowest only from day 1 to day 5.

These micro-algae are found in pond water and could enhance upon exposure to sunlight. It is possible that these micro-algae could have antibacterial activity against some Gram negative bacteria (Lio-Po et al., 2002).

Survival and growth of many fish larvae such as sand goby and hybrid catfish was found to increase when *Chlorella* was added to nursing ponds (Viputanumart et al., 1986. Watcharagonyotin et al., 1992). In shrimp ponds, rearing water containing *Chlorella* is

considered ideal for disease prevention and it could inhibit growth of pathogenic bacteria isolated from diseased shrimp (*Vibrio harveyi, V. parahaemolyticus* and *V. penaeidida*) Direkbusarakom et al., (1997) . Among the problems associated with the use of algal cells as fish feed is that the low digestibility of the algal cells makes the algal biomass unsuitable for rearing fishes. The rigid cell walls of the green algae make them even more difficult for the fishes to digest (Soeder, 1976). Segner et al. (1987) even reported that the larvae of milk fish (*Chanos chanos*) were found to suffer 100% mortality when reared on *Chlorella* sp. Moreover mixed diets containing several species of microalgae have been reported to give better results for some organisms (Hu 1990).

It could be concluded that, addition of chlorelaa and scendesmus sp. At the beginning of the production season (or before the preferable time of cyanobacteria bloom may inhibit the growth and multiplication of *Microcytsits* in the production pond. In order to maintain a stable bloom of *Chlorella* in Nile tilapia ponds, further studies are required.

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Table (1): Phytoplankton divisions (cell x 10^3 ml^{-1}) for effective dose of *Chlorella* and *Scenedesmus* sp. (20 x 10^3 ml^{-1}) after inocubated by *Microcystis aerogonosa* on day 5 and 10 during experiment periods

Item (cell x 10^3 ml^{-1})	Initial*	Days	T_0 control	T_1 10 x 10^3 cell ml^{-1}	T_2 30 x 10^3 cell ml^{-1}	T_3 50 x 10^3 cell ml^{-1}
Blue green algae	0±0	5	$0±0^{B}$	$0±0^{B}$	$0.05±0.01^{Ba}$	$1.2±0.1^{Aa}$
		10	0±0	0±0	$0±0^{b}$	$0±0^{b}$
Green algae	1205±50	5	$1350±60^{Bb}$	$1530±50^{ABb}$	$1580±30^{Ab}$	$1620±40^{Aa}$
		10	$1545±65^{Ba}$	$1670±30^{ABa}$	$1790±70^{Aa}$	$1795±85^{Aa}$
Diatoms	7.75±0.55	5	$11.8±0.3^{Ca}$	$14±0.2^{ABb}$	$12.95±0.15^{Bb}$	$13.55±0.25^{Ab}$
		10	$13.3±0.5^{Ba}$	$16.1±0.7^{Aa}$	$16±0.2^{Aa}$	$15±0.2^{ABa}$
Dinoflagellates	0.04±0.01	5	$0.09±0.004^{Aa}$	$0.007±0.0005^{Bab}$	$0.006±0.001^{Bab}$	$0.007±0.001^{Bab}$
		10	$0.025±0.003^{Ab}$	$0.001±0^{Bb}$	$0.002±0.001^{Bc}$	$0.003±0.0005^{Bb}$
Total standing crops	1212.79±5.56	5	$1361.9±59.7^{Bb}$	$1544.01±50.2^{Ab}$	$1593.01±30.14^{Ab}$	$1634.76±39.85^{Aa}$
		10	$1558.33±5^{Ba}$	$1686.1±30.7^{ABa}$	$1806±10.2^{Aa}$	$1810±84.8^{Aa}$

Normal count withen the canal water as blue green algae = (0.022±0.005), green algae = (0.2±0.05), diatoms = (0.06±0.003), dinoflagellates = (0.007±0.001) and total standing crops = (0.289±0.05)

*** At day 11 injection of 10, 30 and 50 x 10^3 ml^{-1} from *Microcystis aeroginosa* as dose rate**

% Inh = percentage inhibition

A, B, C, D. Values-having different script at the same row are significantly (P<0.05) different

a, b, c, d. Values-having different script at the same column are significantly (P<0.05) different

Table (2): Some pigments contents (µg ml^{-1}) for effective dose of *Chlorella* and *Scenedesmus* sp. (20 x 10^3 ml^{-1}) after inocubated by *Microcystis aerogonosa on* day 5 and 10 during experiment periods

Item (µg ml-1)	Initial*	Days	T_0 control	T_1 10 x 10^3 cell ml^{-1}	T_2 30 x 10^3 cell ml^{-1}	T_3 50 x 10^3 cell ml^{-1}
Chlorophyll "a"	4.15±0.2	5	6.75±0.12^{Db}	7.27±0.07^{Cb}	7.95±0.065^{Bb}	8.85±0.085^{Ab}
		10	8.32±0.41^{Ba}	8.99±0.065^{Ba}	10.3±0.25^{Aa}	10.8±0.11^{Aa}
Chlorophyll "b"	1.58±0.05	5	2.16±0.06^{Ca}	2.38±0.05^{BCb}	2.72±0.09^{Bc}	3.53±0.2^{Ab}
		10	3.16±0.08^{Cb}	3.58±0.43^{CBa}	4.4±0.06^{Ba}	5.26±0.25^{Aa}
Chlorophyll "c"	0.078±0.002	5	0.079±0.002^{Cab}	0.094±0.004^{BCb}	0.165±0.015^{Ba}	0.25±0.04^{Aa}
		10	0.112±0.02^{Ca}	0.16±0.01^{BCa}	0.2±0.016^{Ba}	0.26±0.02^{Aa}
C-phycocyanin	0±0	5	0±0^{C}	0±0^{C}	0.007±0.002^{B}	0.023±0.002^{A}
		10	0±0	0±0	0±0	0±0

Normal amount withen the canal water as chlorophll "a" = (0.033±0.002). chlorophyll "b" = (0.023±0.005), chlorophyll "c" = (0.01±0.0) and c-phycocyanin = (0.007±0.001)
*** At day 11 injection of 10, 30 and 50 x 10^3 ml^{-1} from *Microcystis aeroginosa* as dose rate**
% Inh = percentage inhibition
A, B, C, D. Values-having different script at the same row are significantly (P<0.05) different
a, b, c, d. Values-having different script at the same column are significantly (P<0.05) different

Table (3): Correlation coeficients of biological parameters for effective dose of Chlorella and Scenedesmus sp. (20 x 103 ml-1) after infection by Microcystis aerogonosa at all periods Listed are only the coefficient of the significant correlations (p< 0.05)

		BG.	G.	D.	Dino.	Tot	CHLA	CHLB	CHLC	CPC
Blue green algae	BG.	1.00								
Green algae	G.	0.03	1							
Diatoms	D.	-0.16	0.75	1						
Dinoflagellates	Dino.	-0.15	-0.75	-0.69	1					
Total standing crops	Tot	0.03	0.99	0.75	-0.75	1				
Chlorophyll "a"	CHLA	-0.41	0.67	0.88	-0.63	0.67	1			
Chlorophyll "b"	CHLB	-0.4	0.58	0.85	-0.55	0.58	0.96	1		
Chlorophyll "c"	CHLC	-0.4	0.39	0.67	-0.59	0.39	0.79	0.78	1	
C-phycocyanin	CPC	0.95	-0.01	-0.22	-0.18	-0.01	-0.45	-0.46	-0.4	1

Table (4): Some chemical parameters for effective dose of *Chlorella* and *Scenedesmus* sp. (20 x 10^3 ml^{-1}) after infection by *Microcystis aerogonosa* on day 5 and 10 during experiment periods

Item	Initial	Days	control	10 x 10^3 cell ml^{-1}	30 x 10^3 cell ml^{-1}	50 x 10^3 cell ml^{-1}
		5	8.15 ± 0.15^{A}	8.15 ± 0.05^{A}	8.1 ± 0.2^{A}	7.55 ± 0.05^{B}
Dissolved oxygen (mg/l)	7.2±0.2	10	8.35 ± 0.05^{A}	8.15 ± 0.05^{A}	8.23 ± 0.08^{A}	7.75 ± 0.15^{B}
		5	21.1 ± 0.5^{A}	21.25 ± 0.7^{A}	21.7 ± 0.1^{A}	21.9 ± 0.1^{A}
Temperature (ºC)	21.3±0.2	10	21.75 ± 0.25^{A}	21.2 ± 0.6^{A}	21.2 ± 0.4^{A}	$22.25\pm.015^{A}$
		5	8.95 ± 0.05^{A}	8.73 ± 0.03^{A}	8.85 ± 0.25^{A}	8.8 ± 0.2^{A}
pH	8.0±0.05	10	8.95 ± 0.25^{A}	8.45 ± 0.15^{A}	9.05 ± 0.45^{A}	9.1 ± 0.1^{A}
		5	0.18 ± 0.02^{A}	0.18 ± 0.04^{A}	0.2 ± 0.04^{A}	0.215 ± 0.005^{A}
Ammonia (mg/l)	0.07±0.01	10	0.305 ± 0.005^{A}	0.31 ± 0.01^{A}	0.25 ± 0.01^{B}	0.26 ± 0.0^{B}
		5	235 ± 15^{A}	230 ± 10^{A}	232 ± 2^{A}	232.5 ± 7.5^{A}
T. alkalinity (mg/l)	220±2	10	217 ± 1^{C}	256 ± 4^{A}	252 ± 2^{A}	237 ± 3^{B}
		5	231 ± 1^{A}	223 ± 1^{AB}	207 ± 1^{B}	228 ± 12^{AB}
T. hardness (mg/l)	230±6	10	207 ± 1^{A}	215 ± 5^{A}	217 ± 1^{A}	211 ± 1^{A}
		5	0.05 ± 0.01^{B}	0.09 ± 0.01^{B}	0.09 ± 0.01^{B}	0.25 ± 0.05^{A}
NO3 (mg/l)	0.100±0.02	10	0.1 ± 0.02^{A}	0.11 ± 0.01^{A}	0.06 ± 0.02^{A}	0.15 ± 0.05^{A}
		5	0.239 ± 0.18^{A}	0.01 ± 0.002^{A}	0.015 ± 0.003^{A}	0.028 ± 0.016^{A}
Available phosphorus	1.40±0.03	10	0.017 ± 0.001^{A}	0.006 ± 0.002^{A}	0.01 ± 0.005^{A}	0.01 ± 0.005^{A}
Initial body weightg (g/10 fish)		10	292 ± 5^{A}	299.5 ± 6.5^{A}	328.5 ± 31.5^{A}	327 ± 17^{A}
Final body weight (g/10 fish)		10	313 ± 7^{A}	325 ± 5^{A}	351 ± 31^{A}	348.5 ± 17^{A}
Total harvest (g/aquarium)		10	21 ± 2^{A}	25.5 ± 1.5^{A}	22.5 ± 0.5^{A}	21.5 ± 0.5^{A}

A, B, C, D. Values-having different script at the same row are significantly (P<0.05) different