Effect of *Microcystis aeruginosa* blooming on the production of Nile tilapia (*Oreochromis niloticus*) in fish ponds

Mostafa A. I. Elgammal[1] Ashraf A. Ramadan[2]

1- Fish limnology Department 2- Fish Genetics Department

Central Laboratory for Aquaculture Research, Abbassa, Abou- Hammad, Sharkia, Egypt.

Corresponding researcher e-mail address: drelgammal@yahoo.com

ABSTRACT

Reasons for low fish farm production at Eltel El-kabeer area, Ismailia Governorate were unknown. At the concerned area, fish survival rates have been dropped to reach less than 10% for several consecutive years. Some of the conducted studies, concerning the high mortality rates in the concerned area, claimed that that phenomenon was initially referred to cyanobacterium blooming (*Microcystis aeruginosa*) thought to be ingested by fish during feeding, or assimilated through gills during breathing.

Twelve earthen ponds (each of 2000 m^2 area, 100-120 cm water depth, fertilized by 125 kg chicken manure / pond / week for a period of 16 weeks, and stocked with 5000 Nile tilapia fry with a weight ranged 5-7 gm) were used in the study and were divided equally into two studied groups. The first pond group was full of *Microcystis aeruginosa* bloom, while the second pond group was free of *Microcystis aeruginosa* bloom. Fish were stocked in the two previously mentioned studied pond groups, fed on feeding rate at 3 % of live body weight. Commercial floating fish diet with 25% protein content was used in fish feeding. Physicochemical parameters, plankton population, fish survival, fish production, and toxicity test for different fish size (5, 20, 50, 150 grams). In addition, *Artemia* also were studied.

There was a significant difference between the two studied pond groups concerning all physical and chemical water characteristics investigated. Those investigated water characteristics were: dissolved oxygen, visibility, salinity, total suspended solid (TSS), alkalinity, total phosphorus and Ammonium (NH_4). On the other hand, there was no significant difference between the two studied pond groups concerning each of temperature, pH, electric conductivity (EC), Hardness, nitrate (NO_3) ammonium (NH_3) and chlorophyll "a". In general, all investigated values were within the suitable range for tilapia growth.

The abundance of phytoplankton in number was highly significant in the first research pond group concerning each of Cyanophyta, Euglenophyta, and *Microcystis aeruginosa* in blooming, and was not significant in Baciloarophyta and Chlorophyta. In addition, Zooplankton was significant in numbers concerning *Cladocera*, *Rotifers* and Copepoda and was not significant in total zooplankton

number. Fish and *Artemia* exposed to pond with *Microcystis* blooming died within 24 hours.

The research drew the attention towards conducting more future research studies in this concern as a way to control the toxic algae of *Microcystis aeruginosa* in fish ponds.

Key words: *Microcystis aeruginosa*, fish production, water quality, phytoplankton, zooplankton.

INTRODUCTION

Bloom of Cyanobacterium *Microcystis aeruginosa* is a ubiquitous phenomenon in eutrophic lakes, fish ponds, reservoirs and polluted water in many countries of the world. Many strains of *Microcystis* are known to produce Cyanobacterial hepatotoxins called microcystin. The toxin, a soluble peptide, is lethal to many kinds of aquatic organisms and damages zooplankton, fish, and also liver of higher animals (Gan *et al*., 2010; Penaloza *et al*., 1990; Sivonen, 1990; Watanabe *et al*., 1989).

There has been an apparent increase in the occurrence of harmful algal blooms (Hallegraef, 1993; Newman & Barrett, 1993, Geng & Xie 2008; Soares *et al*., 2010). In general, there are three classes of algal blooms which are ranked according to their level of toxicity: (1) those which are fundamentally harmless until their breakdown by certain bacteria deprives fish and invertebrates of oxygen; (2) those which produce toxins which are accumulated through the food chain resulting in various types of shellfish poisoning in humans; (3) those which produce toxins which are non-toxic to humans but are toxic to fish and invertebrates.. Although presence of the previously mentioned algal blooms may be noticed throughout the year in water bodies, they proliferate abundantly from summer to autumn (Carmichael, 1995).

Among some important bloom forming Cyanobacteria came those of *Anacystis*,*Anabaena*, *phanizomenon*, *Rivularia* and *Microcystis*, and others. Among the *Microcystis*, the cyanobacaterium is considered the most widespread and frequently encountered organism. It is well known with its ability to destroy the pristine quality of water and cause many problems such as occurrence of nasty odor, fish kill, deterioration of recreational worth and clogging of filter in water supply system. In addition, it also releases toxins such as microcystin (Carmichael and An, 1999) which is toxic to each of fish, aquatic invertebrates, domestic animals and human beings. The development of Cyanobacterium bloom may also be partly explained due to unpalatability since *Microcystis* are hardly grazed upon by zooplanktons. The ability of *Microcystis* species to photosynthesize at rates higher than green alga may also facilitate the dominance of *Microcystis* in water bodies (Osami *et al*., 1994). Field and laboratory studies indicated that grazing by some zooplankton can be disrupted by toxic Cyanobacteria (Lampert, 1987; De Bernardi; and Giussani, 1990; Sellner *et al*., 1993; Boon *et al*., 1994; Christoffersen,

1996; Rohrlack *et al*., 1999; Paerl *et al*., 2001; Ghadouani *et al*., 2003).

In many systems which experience dense and/or toxic blooms, mesozooplankton (>200 mm) such as Cladocerans and Copepods can be impacted, experiencing reduced feeding, reduced food assimilation or even mortality (Paerl *et al.*, 2001 and Ghadouani *et al*., 2003). Concerning degree of zooplankton graze, Cyanobacteria can be influenced by many factors including toxin concentrations, strains of Cyanobacteria species, species of zooplankton, and various environmental conditions (Paerl, 1988; Sellner *et al*., 1993; Boon *et al.*, 1994; Christoffersen, 1996).

Recent studies of field isolated Daphnia have shown that individuals isolated from eutrophic environments, which are presumed to have been chronically exposed to toxic Cyanobacteria, are better in its ablity to graze and grow in the presence of cultures of toxic *Microcystis* sp. than individuals that have not been exposed to blooms (Hairston *et al*., 2001; Sarnelle & Wilson, 2005).

In general, those studies indicated that *Daphnia* are able to adapt to the presence of toxic Cyanobacteria, others have found these grazers were not able to graze on Cyanobacteria, even when frequently exposed (Walls *et al.*, 1997). It was suggested that microzooplankton (20–200 mm) may graze on toxic Cyanobacteria (Paerl *et al.*, 2001 and Leonard & Paerl, 2005), and microzooplankton grazing rates on toxic Cyanobacteria have yet to be quantified. Sublethal effects resulting from microcystin exposure include liver damage in carp growing in New Zealand lakes (Carbis, *et al*., 1997) and decreased opercula movement in tilapia fed toxic Microcystis (Keshavanath, *et al*., 1994).

The main objectives of the research were identified as the following:

1- To determine causes of fish mortality increase in some fish farms that may lead to low fish production.

2- To define effect of *Microcystis aeruginosa* on the production of Nile tilapia *(Oreochromis niloticus)* in fish ponds.

MATERIALS AND METHODS

The research was conducted in Eltal El-Kebeer area, Ismailia Governorate / Egypt. Twelve earthen ponds, divided into equal pond groups (6 ponds each) each of 2000 m^2 total area, of were used in carrying out the research. Ponds were drained, cleaned and supplied with fresh water from Ismailia canal branched from Nile River. water depth in those research ponds ranged (100-120 cm), with a stable average water depth of approximately one meter. In those research pond groups, supply and drainage pipes were equipped with nylon screen to prevent fish escape and / or entry. The first pond group (filled with *Microcystis aeruginosa* bloomings), the second groups (free from *Microcystis aeruginos*a). The study started from 15 April till 17 October 2010.

All ponds, in the two studied / research groups, were fertilized each

by 125 kg chicken manure/pond/week and after 16 weeks, the commercial floating diet contains 25% protein was used to feed the fish in two groups 3% live body weight. The used stocking rate for each studied pond was 5000 Nile tilapia (*Oreochromis niloticus*) fry, mono sex, with an average weight ranged 5-7 grams. The stocked fish

and monthly at rigidly determined pond sites. Mean values of the different parameters, under examination, were expressed as changes during the conducted research period (table 1). A column sampler constructed from a PVC pipe (5-cm diameter, 1.5-m long) was used to collect water samples for phytoplankton, total suspended solids

Table 1: Methods of analysis of Chicken manure used to fertilize the experimental earthen ponds and chemical, physical and plankton properties of water fish ponds

Parameters	Time	Method	Source
Chicken manure analyses			
Dry matter	Every batch of manure	Oven dried (80^{o}C overnight)	APHA (1985)
Total Kjeldahl Nitrogen	Every batch of manure	Modified Micro-Kjeldahl method	Yoshida *et al*., (1976)
Total phosphorus	Every batch of manure	Acid digestion	Yoshida *et al*., (1976)
Potassium	Every batch of manure	spectrophotometers	(AOAC 1990)
Total ash	Every batch of manure	Ignition at 600^{o}C for 2 hours	APHA (1985)
Organic carbon	Every batch of manure	%Carbon =(100-ash%)/1.8	Golueke (1977)
water Pond analysis			
Total suspended solids (TSS)	Every week	Oven drying (105oC for 4 h)	APHA (1985
Total alkalinity	Every 2weeks	Titration with o.1 N sulfuric acid	APHA (1985
Total hardness	Every 2weeks	Titration with EDTA	APHA (1985)
Total phosphorus	Every week	Acid digestion	Yoshida *et al*., (1976)
Nitrate mg/l NO_3	Every week	Cadmium reduction method	Boyd (1992)
Total ammonia mg/l (NH_4,NH_3)	Every week	Indophenol or Phenat method	Boyd (1992)
Temperature & DO measurement	Daily 5 day/week	DO meter	YSI (model 58)
pH	Every week	pH meter	Corning (model 345)
Salinity&(EC Water Conductivity)	Every week	Conductivity meter	YSI (model 33)
Chlorophyll a	Every week	Acetone extraction method	APHA (1985
Phytoplankton counts	Every 2 weeks	Counting by using a microscope	APHA (1985)
Zooplankton counts	Every 2 weeks	Counting by using a microscope	APHA (1985)

were harvested and weighed after 24 weeks. The obtained measurements came in accordance with the following:

a- Fish survival rate = (initial numbers of fish stocked - numbers of dead fish / initial numbers of fish stock) 100

b- Fish production (kg) = harvested fish weight (kg)

In addition, physicochemical and biological properties of water ponds were studied throughout the predetermined investigation periods, samples were taken daily, weekly

(TSS). A stick sampler, with an attached 300 ml biological oxygen demand bottle, was used in collecting water samples from a depth of 10 cm for all other parameters. Mean individual final weight, daily weight gain, yield and survival data were analyzed using analysis of variance, ANOVA (Zar, 1984). Significant differences were at an alpha level of 0.05.

Fish exposed to water pond with *Microcystis aeruginosa* bloom

Laboratory reared fish. (5, 20,50,150 gm) were exposed to water from the blooming ponds by gavages

and intra-peritoneal (IP) injection (Carbis, *et al*., 1996) with only an exception in using fish size with 5 grams each and they were exposed to bloom water pond only. Pond water blooming was concentrated 15-fold by centrifugation at 100 g for 10 min. The pellet was resuspended, triturated by mortar and pestle, and 0.5 ml of this suspension was administered intraperitoneally (n. 5) and orally (n. 5). Fish were held in 25 liter aquaria containing de-chlorination tap water under static conditions and observed for 48 hours. In each case, appropriate de-chlorination water controls were utilized. Fish died following these procedures were subjected to similar diagnostic testing as described above.

***Artemia* bioassay**

Brine shrimp medium (BSM) stock solution,

BSM stock solution composed of sodium chloride (NaCl) 300g, Calcium chloride dehydrate (CaCl $2H_2O$) 3g, Magnesium chloride hexhydrate (MgCl2.6 H_2O) 15g, Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) 5g, Potasium chloride (KCl) 8g, Glycine 60g, Disodium glycerophosphate 30g. All the stock solution chemicals were dissolved in 1.25 liters of distilled water dissolved each chemical separately and kept in brown glass bottle in refrigerator (-4^OC)

Brine shrimp eggs were supplied by Ocean Star International Inc. (Snowville, USA). Larvae were used within just a day after hatching. Following extraction of lyophilized cell masses (50 mg) of *Microcystis aeruginosa* with methanol, the extract was evaporated to dryness in a vacuum. The dried extract was dissolved in 250 ml filtered brine shrimp medium (BSM) stock solution, reaching a concentration as 200 mg/ml in terms of lyophilized cell masses, then further diluted with BSM to give five concentrations of 100, 50, 20, 10, and 2 mg/ml. Assays were performed on Petri dish (5cm diameter) with 10~20 brine shrimp larvae in 5 ml of BSM per dish. The brine shrimp larvae in each dish were tested using 5 ml per concentration level of extract. They were observed for 24 h to calculate mortality (Meyer *et al*., 1982).

The toxicity threshold concentration, expressed as dry weight of *Microcystis aeruginosa* mass per milliliter of BSM, was defined as the lowest concentration that kills all tested brine shrimp within 24 hours. Each test was run in triplicate, and BSM was used as the control.

RESULTS AND DISCUSSION

Effective water management in fishponds is one of the most important factors contributing to the success of fish culture, reducing the occurrence of fish disease, enhancing better fish growth, and increasing survival

The obtained analyses of organic fertilizers (chicken litter before and through the application), total average values of dry matter; total nitrogen; phosphorus and potassium, were 89.52; 1.64; 1.38 and 0.76 % respectively.

Physicochemical investigation for water fish ponds showed no significant differences in water temperature between the first group

ponds and the second group ponds (table 2). The result provided an indicator implied that the blooming of *Microcystis aeruginosa* had no effect on water temperature in the ponds and that the effect of air temperature is critical factor in that aspect (Boyd, 1990).

The Secchi disk visibility in water for the first group ponds was decreased significantly (18.08 ± 1.4 cm) when compared to unaffected ponds (25.13 ± 1.5 cm). While the dissolved oxygen concentration in the first group ponds (9.6 ± 1.43 mg/l) was increased significantly than the second group ponds (7.8 ± 1.4 mg/l). This may be explained as a result of high abundance of phytoplankton and blooming of *M. aeruginosa* in the first group ponds, the high concentration of dissolved oxygen in parallel with the increased photosynthetic activity of phytoplankton population. In this respect, Talling (1986) reported that oxygen supersaturation, due to photosynthetic activity, is often recorded with abundant phytoplankton. In general, Secchi disk values and dissolved oxygen concentrations, recorded in first and second group ponds, were always higher than the critical minimum concentration for fish growth (Balarin & Haller, 1982; Dianna *et al.*, 1991).

Results presented (table 2 & figure 1) Showed also no significant changes in water pH in first group ponds and second group ponds. This may be explained as a result of the limited amount of organic fertilizer which was not enough to affect the pH of the large water volumes directly, although the total numbers of phytoplankton was with no significant differences between the two groups. The pH was higher in first group ponds (fig.1) on June than the second group ponds, where the increase of blooming of *M. aeruginosa* on June led to an increase in the photosynthesis that involved the uptake of the carbon dioxide from the water by algae and increased the pH values (Boyd 1990; Abdalla 1997).

As shown in Table (2) and figure (1), Water alkalinity was significantly decreased in the first group (258.33 ± 16 mg/l) ponds when compared to the second group ponds (284.82 ±7 mg/l); but total hardness was not significant between the first group ponds (253± 11 mg/l) and the second group ponds (231 ± 17 mg/l). The obtained values for both total hardness and alkalinity were always suitable, or ideal, for microbial phytoplankton and fish growth (Krenkel & Novotny, 1980; Piper *et al.*, 1982).

The mean levels of ammonium-NH_4 and total phosphorus was decreased significantly in the first group than second group ponds as shown in (Table 2) which was in parallel with an increase in phytoplankton and blooming of *M. aeruginosa* because of phytoplankton uptake ammonium-NH_4 and phosphorus *for their growth* (Boyd, 1992). The measured nitrate NO_3 and NH_3 in water fish ponds (table 2 and figure 1) indicated that there was no significant difference between the two groups. The salinity (mg/l) and total suspended solid (mg/l) were decreased significantly in the first

group than the second group ponds. On the other hand, there was no significance difference between the two studied pond groups concerning electric conductivity (EC). .

Based upon the obtained results, range of all parameters was always good or ideal for water productivity and fish production (Boyd 1992; Khalil, 1990; Abdalla, 1997).

It was found (table 2 and figure 1) that there were no significant differences, concerning chlorophyll "a" concentration in water expressed in µg/l, between the two studied pond groups in all months of investigation (June and September were an exception). It was found that the first group ponds recorded higher values than the second group ponds (total averages were 88.48 & 56.97µg/l. in first and second group ponds respectively). In addition, values of chlorophyll "a" were (141.9 & 46.6µg/l) on June and (200.95 & 156.10 µg/l) on September paralleled with abundance of phytoplankton and increase of the blooming of *M. aeruginosa* in those mentioned months. The obtained results came to confirm those of Boyd, (1990); Zhang, and Geng, (2011).

The different structures of plankton community might be due to different degrees of tolerance by different organisms to the available chemical and environment conditions, in addition to their differences in nutritional requirement and fish (planktivorous) utilization of some preferred species (Northcote, 1988)

In regard with different algal species in the two research groups of fish ponds belonged to Cyanophycophyta, Chlorophyta, Bacilarophyta and Euglenophyta, it was found that total count of phytoplankton in the first group ponds was more significant difference at $P<0.05$ than the total numbers of phytoplankton in the second groups at most months of the research. The cyanophycophyta groups had more significant difference in first groups than the second groups ($P<0.05$). The percentage of *M. aeruginosa* in total phytoplankton in the first groups was higher than that of the second group. The higher percentage was recorded 83.6% & 65.7% in June and September respectively, but the lowest percentage was in October with 18.7 % for the first group ponds. The species was rarely found in second group ponds. The bloom appeared obviously on (8-15 June) and (18-22 September). Despite their presence may be noticed throughout the year in water bodies, they proliferate abundantly from summer to autumn. This also may occur as a reason for the toxicity of *M. aeruginosa* which causes an increase in the mortality of *O. niloticus* in the first groups and a decrease in the phytoplankton consumed by fish. This phenomenon caused due to the increased numbers of phytoplankton that appear as the phytoplankton blooming (Carmichael, 1995; Zhang, & Geng, 2011).

The Euglinophycophyta was recorded the higher numbers in the first group ponds in parallel with the high numbers of *M aeruginosa*. The blooming of Euglinophycophyta increased significantly in the first groups than second groups ($p<0.006$)

through all period of research investigation (Table 3). On the other hand, there was no significant difference between the first groups and the second groups concerning numbers of Chlorophycophyta and Bacilarophyta (table 3). The difference in structures of phytoplankton communities within fish ponds in the two studied groups could be explained on basis of different degrees of tolerance for different categories of phytoplankton organisms to the present nutritional requirements (Hanson *et al*., 1987; Northcote, 1988)

Table 5 showed that the zooplankton obtained from water fish ponds in the two studied groups. The identified zooplankton species were those of rotifers, cladocera and copepoda. The obtained findings showed no significant difference between the two research pond groups regarding total count of zooplankton. The research results (table 4) indicated that there was a less significant difference in the first group ponds than the second group ponds in content of rotifer which may be explained as a positive correlation between the decreased phytoplankton numbers and the increase in *M aeruginosa* numbers. The accrued finding confirmed that of Wylie and Currie (1991) and Qin and Culver (1992). On the other hand, the Cladocera and Copepoda numbers were significantly increased in the first group than second group which may be explained as Cladocera and Copepoda have the ability adapt to the presence of toxic Cyanobacteria (Walls *et al.,* 1997; Trabeau *et al*., 2004). It was suggested that they may graze on toxic Cyanobacteria (Paerl *et al.*, 2001; Leonard & Paerl, 2005; Zhang & Geng, 2011),

It was indicated (table 6) that survival rate of *O. niloticus* was decreased significantly (5.5%) in the first group ponds in comparison with the second group ponds (80.36%), and the lower fish production (31.08 kg) in the first group ponds versus the second group ponds (560.75 kg). The lower survival rates and production paralleled with the blooming of *M aeriginosa* in the first group ponds.

Although fish injected by *M aeriginosa* died within 48 hours, the gavages fish didn't die through using the same concentration of *M aeriginosa*. Dead fish was negative for bacterial pathogens.

The percentage of LC_{50} death of *Artemia* at 24 hours exposed to the toxicity threshold concentration, expressed as dry weight of *M aeriginosa* as tabulated in table (7) was death in all concentration except that of 2 mg/ml. This may be explained as an effect of toxicity *M aeriginosa* for both of fish and *Artemia*. The results came in harmony with Keshavanath, *et al*., (1994); Lahti *et al.*(1995); Vezie *et al.,* (1996) that stated toxicity of Cyanobacteria bloom to brine shrimp and fish

The study indicated that all the implemented physicochemical parameters were suitable (ideal) for water productivity and fish production, but the blooming of *M aeriginosa* in the first ponds group was thought to be the only reason for the increase in mortality, the low survival rates and low production.

The result supported that of Carbis *et al*. (1996).

REFERENCES

Abdalla, A. A. F. (1997): Effects of increased nitrogen fertilization on water quality and yield of Nile tilapia, *Oreochromis niloticus*, and Common Carp, *Cyprinus carpio*, in earthen ponds in Egypt. Journal of Applied Aquaculture, 7(4):1 -14.

APHA (1985): Standard Methods for Examination of water and waste water, 16th ed. American Public Health Association, Washington, D.C. 1268 pp.

AOAC (1990): Official Methods of Analysis of Association of Official Analytical Chemists, 15th edn. AOAC, Arlington, VA, 1298 pp.

Boon, P. I.; Bunn, S.E.; Green, J. D.; Shiel, R. J. (1994): Consumption of cyanobacteria by fresh-water zooplankton—implications for the success of top-down control of cyanobacterial blooms in Australia. Aust. J. Mar. Fresh Res. 45:875–887.

Boyd, C. E. (1990): Water Quality in Ponds for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, Birmingham Publishing.

Boyd, C. E. (1992): Shrimp pond bottom soil and sediment management. Pages 166-181 in J. Wyban, editor. Proceedings of the Special Session on Shrimp Farming. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Carbis, C.R.; Mitchell, G.F.; Anderson, J.W. and Mc Cauley, I. (1996): The effects of microcystins on the serum biochemistry of carp, *Cyprinus carpio* L., when the toxins are administered by gavages, immersion and intra-peritoneal routes. Journal of Fish Diseases 19:151-159.

Carbis, C.R.; Rawlin, G.T.; Grant, P.; Mitchell, G.F.; Anderson, J.W. and McCauley, I. (1997): A study of feral carp, *Cyprinus carpio* L., exposed to Microcystis aeruginosa at Lake Mokoan, Australia, and possible implications for ® sh health. Journal of Fish Diseases 20, 81-91.

Carmichael, W.W. (1995): The toxins of cyanobacteria. Sci. Am. Jan. 270, 78–86.

Carmichael, W.W. and An, J. S. (1999): Using an enzyme linked immunosorbent assay (ELISA) and a protein phosphatase inhibition assay (PPIA) for the detection of microcystins and nodularins. Nat. Toxins 7:377–385.

Christoffersen, K. (1996): Effect of microcystin on growth of single species and on mixed natural populations of heterotrophic nanoflagellates. Nat. Toxins 4:215–220.

De Bernardi, R. and Giussani, G. (1990): Are blue-green algae a suitable food for zooplankton?

An overview. Hydrobiologia 200/201, 29–41.

Dianna, J.S., Lin, C.K., and Schneeberger, P.J., (1991): Relationships among nutrient inputs, Water nutrient concentrations primary production, and yield of *Oreochromis niloticus* in ponds, Aquaculture, 92,323,1991.

Gan N, Mi L, Sun X, Dai G, Chung, F.L, Song, L (2010) Sulforaphane protects Microcystin-LR-induced toxicity through activation of the Nrf2-mediated defensive response. Toxicol Appl Pharmacol 247:129–137

Geng, H. and Xie, P. (2008): Experimental studies on the effects of toxic *Microcystis aeruginosa* PCC7820 on the survival and reproduction of two freshwater rotifers *Brachionus calyciflorus* and *Brachionus rubens*. Ecotoxicology 17:709–715

Ghadouani, A.; Pinel-Alloul, B. and Prepas, E. E., (2003): Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. Freshwater Biol. 48:363–381.

Golueke, C. G. (1977): Biological reclamation of solid wastes. Rodale Press, Pennsylvania, USA.

Hairston, Jr. M. G., Holtmeier, C. L.; Lampert, W.; Weider, L. J.; Post, D. M.; Fisher, J. M.; Caceres, C. E.; Fox, J. A. and Gaedke, U. (2001): Natural selection for grazer resistance to toxic cyanobacteria: evolution of phenotypic plasticity. Evolution, 55: 2203–2214.

Hallegraef, G.M., (1993): A review of harmful algal blooms and their apparent global increase. Phycologia 32: 79–99.

Hanson, L.A.; Johansson, L. and Persson, L. (1987): Effect s of fish Grazing on nutrient release and succession of primary producers. Limnol. Oceanogr., 32: 723-729.

Keshavanath, P., Beveridge, M. C. M., Baird, D. J., Lawton, L. A., Nimmo, A.. and Codd, G. A. (1994): The functional grazing response of a phytoplanktivorous fish *Oreochromis niloticus* to mixtures of toxic and non-toxic strains of the cyanobacterium Microcystis aeruginosa. Journal of Fish Biology, 45: 123-129.

Khalil, M. T. (1990): The physical and chemical environmental of lake Manzala, Egypt. Hydrobiologia 196: 193-199.

Krenkel, P.A. and Novotny, V. (1980): Water Quality Management. Academic Press, New York.

Lampert, W. (1987): Laboratory studies on zooplankton-cyanobacteria interactions derived from enclosure studies. N. Z. J. Mar Freshwater Res. 21: 483–490.

Lahti, K., J. Ahtiainen, J. Rapala, K. Sivonen and S. I. Niemela (1995): Assessment of rapid bioassay for detecting

cyanobacterial toxicity. Lett. Appl. Microbiol. 21: 109-114.

Leonard, J. A. and Paerl, H. W. (2005): Zooplankton community structure, micro-zooplankton grazing impact, and session energy content in the St. Johns river system. Florida as influenced by the toxic Cyanobacterium *Cylindrospermopsis raciborskii*. Hydrobiologia, 537: 89–97.

Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E. and McLaughlin, J. L. (1982): Brine shrimp: a convenient general bioassay for active plant constituents. J. Med. Plant Res. 45: 31-34.

Newman, J. R. and Barrett, P. R. F. (1993): Control of *Microcystis aeruginosa* by decomposing barley straw. J. Aquatic Plant Man. 31:203- 206.

Northcote, T.G. (1988): Fish in the structure and function of freshwater ecosystems; a "top-down" view. Can. J. Aquatic. Sci., 45: 361-379.

Osami, Y.; Ohkubo, N.; Tomoku, N. and Okada, M., (1994): Effects of irradiance and temperature on photosynthetic activity of cyanobacterium *Microcystis* sp. Environ. Technol. 15: 389–394.

Paerl, H.W. (1988): Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnol. Oceanogr. 33:823–847.

Paerl, H.W.; Fulton, R. S., Moisander, P. H.; and Dyble, J. (2001): Harmful freshwater algal blooms with an emphasis on cyanobacteria. Sci. World. 1:76–113.

Penaloza, R.; Rojas, M., Vila, I. and Zambrano, F. (1990): Toxicity of a soluble peptide from Microcystis sp. to zooplankton and fish. Freshwater Biol 24:233–240.

Piper, R.G.; McElwain, I.B.; Orme,L.E.; McCraren, J. P.; Fowler, L.G. and Leonard, J. R. (1982): Fish hatchery management. US Department Interior, fish and Wildlife Service, Washington, D.C.517 pp.

Qin, J. and Culver, D. A. (1992): The survival and growth of larval walleye, Stizostedion vitreum, and trophic dynamics in fertilized ponds. Aquaculture, 108: 257-276.

Rohrlack, T.; Dittmann, E.; Henning, M., Borner, T. and Kohl, J. G. (1999): Role of microcystins in poisoning and food ingestion inhibition of Daphnia galeata caused by the cyanobacterium Microcystis aeruginosa. Appl. Environ. Microbiol. 65:737–739.

Sarnelle, O. and Wilson, A.E. (2005): Local adaptation of Daphnia pulicaria to toxic cyanobacteria. Limnol. Oceanogr. 50:1565–1570.

Sellner, K.G.; Brownlee, D.C.; Buundy, M. H.; Brownlkee, S.G.; Braun, K.R. (1993): Zooplankton grazing in a

Potomac River cyanobacteria bloom. Estuaries 16, 859–872.

Sivonen, K. (1990): Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. Appl. Environ. Microbiol. 56:2658–2666.

Soares M. C. S.; Lurling, M.; Huszar, V. L. M. (2010): Responses of the rotifer Brachionus calyciflorus to two tropical toxic cyanobacteria (*Cylindrospermopsis raciborskii* and *Microcystis aeruginosa*) in pure and mixed diets with green algae. J Plankton Res. 32: 999–1008.

Talling, J. F. (1986): The seasonality of phytoplankton in African lakes. Hydrobiologia, 138: 139–160.

Thostrup, L. and Christoffersen, K. (1999): Accumulation of microcystin in Daphnia magna feeding on toxic Microcystis. Archiv Fur Hydrobiologie, 145:447–467.

Trabeau, M.; Bruhn-Keup, R.; Mcdermott C.; Keomany, M.; Millsaps, A.; Emery, A. and De Stasio, JR. B. (2004): Midsummer decline of a Daphnia population attributed in part to cyanobacterial capsule production. J. Plankton Res. 26:949–961.

Vezie, C., F.; Benouffla, K.; Sivonen, G. B. and Laplanche, A. (1996): Detection of toxicity of cyanobacterial strains using Artemia salina and Microtox assays compared with mouse bioassay results. Phycologia. 35(Suppl.6):198-202.

Walls, M., LaurenMaatta, C., Ketola, M., Ohraho, P., Reinikainen, M. and Repka, S. (1997): Phenotypic plasticity of Daphnia life history traits: the roles of predation, food level and toxic cyanobacteria. Freshwater Biol. 38:353–364.

Watanabe, M. F., Harada K. I., Matsuura K., Watanabe M. and Suzuki, M. (1989): Heptapeptide toxin production during the culture of two *microcystis* species (Cyanobacteria). J. Appl. Phycol. 1:161–5.

Wylie, J. L. and Currie, D. J. (1991): The relative importance of bacteria and algae as food source for crustacean zooplankton. Limnol. Oceanogr, 36:708–728.

Yoshida, S.; Forno, D. A.; Cock, J. H. and Gomez, K. A. (1976): Laboratory manual for physiological studies of rice, 3rd edition. The International Rice Research Institute, Los Baiios, Laguna, Philippines.

Zar, J. H. (1984): Bio-statistical analysis, 2nd edition. Prentice-Hall International, Inc. Englewood Cliffs, New Jersey, USA.

Zhang, X. and Geng, H. (2011): Effect of *Microcystis aeruginosa* on the rotifer *Brachionus calyciflorus* at different temperatures Bull Environ. Contam. Toxicol, Nov 6. [Epub ahead of print].

Table (2): The total average values of different water quality parameters in water fish ponds in first group ponds (affected) and second group ponds (un-affected) by *Microcystis aeraginosa*

parameters	first group ponds	second group ponds
Temperature (°C)	27.77A ± 2.12	28.93A ± 2.01
pH Value	8.74 A + 0.29	8.54 A ± 0.32
Secchi disk (SD cm)	18.08 B ± 1.4	25.13 A ± 1.5
Dissolved Oxygen (mg/l)	9.6 A ± 1.43	7.8 B ± 1.4
Electric conductivity	969.04 A ± 0.178	1065.47A ± 0.271
Salinity(mg/l)	0.047B ± 0.01	0.31A ± 0.08
Chlorophyll "a("Ug/l)	88.48.28A ± 120	56.97.92A ± 123.14
Total suspended solid (mg/l)	0.958A ± 0.673	0.753B ± 0.637
Ammonia (NH_4 mg/l)	0.416 B ± 0.2	0.717 A ± 0.2
Nitra rate (NO_3 mg/l)	1.8A ± 0.447	1.71A ± 0.0428
Total Phosphorus (mg/l)	0.711B ± 1.63	1.049 A ± 1.1842
Alkalinity (mg/l)	258.33B ± 16	284.82A ± 7
Hardness (mg/l)	253A ± 11	231A±17
Total Phytoplankton (No/l)	2396480.0 A ± 9574	479195.8 B ± 7863
Total zooplankton (No/l)	2803625A ± 10551	2564880A ± 9401

- Data are represented as means ± standard error.
- Values with the same letters show non-significant differences between the two groups and values with the different letters show significant differences at ($p < 0.05$)

Table 3: Variation of the total numbers of Phytoplankton groups (Number of organisms /L) and *Microcystis aeraginosa* numbers number of cells /l) and percentage from total count of phytoplankton in water of first group ponds (affected) and second group ponds (unaffected) by *Microcystis aeraginosa*

	CYANOPHYCOPHYTA													
	without *M.aeraginosa*		***M.aeraginosa***		**BACILAROPHYTA**		**EUGLENOPHYTA**		**CHLOROPHYTA**		**TOTAL PHYTO**		**% *M.aeraginosa***	
	First group ponds	Second group ponds	First group ponds	Second group ponds	First group ponds	Second group ponds	First group ponds	Second group ponds	First group ponds	Second group ponds	First group ponds	Second group ponds	**First group ponds**	**Second group ponds**
May	4400B ± 400	25700A ± 819	79300A ± 4051	9000B ± 61	9900B ± 900	12400A ± 980	105600A ±1000	56360B ± 48941	51000B ± 374	98640A ± 849	250200A ± 2743	202100A ± 112 9	31.7	4.5
June	535290A ± 4400	47520B ± 8198	580000A ± 4051	2560B ± 361	55510B ± 990	70760A ± 906	226060A ±1050	79760B ± 4841	319720B ± 3474	509120A ± 488	6936580A ± 2743	709720B ±112691	83.6	0.4
July	665100A ±162576	52280B ± 18421	69400A ± 1598	5640B ±5.680	11240B ± 972	68420A ± 292	255200A ±1938	48720B ± 242	150320B ± 683	506740A ± 1133	1775860A ± 2771	681800B ± 1089	39.1	0.8
August	405480B ±117276	49400A ± 17623	46600A ± 2679	1000B ±0.520	99780A ± 318	73100B ± 886	196920A ± 4896	110800B ± 3560	489120A ± 1378	511500A ± 975	1657300A ± 1956	745800B ± 2584	28.1	0.1
September	676540A ± 1575	67860A ± 237	1982000 A ± 9451	5900B ± 4605	27260B ± 285	47260A ± 381	153920A ± 553	11720B ± 932	177440B ± 395	210240A ± 8259	3017160A ± 239498	275120B ± 100671	65.7	2.1
October	340470A ± 608	39330A ± 129	138750A ± 3365	2995B ± 30	18580B ± 229	2983A ± 156	129760A ±748	34040A ± 271	114220B ± 2426	154440A ± 1767	741780A ± 2300	260635B ± 1 638	18.7	1.1

- Data are represented as means ± standard error.
- % of *Microcystis aeraginosa* = means numbers of *Microcystis aeraginosa* / means numbers of total phytoplankton x 100
- Values with the same letters show non-significant differences between the two groups and values with the different letters show significant differences at ($p < 0.05$)

Table (4): Variation and distribution of zooplankton (%) in water fishponds in first group ponds (affected) and second group ponds (unaffected) by *Microcystis aeraginosa*

	CLADOSRA%		ROTIFER %		COPEPODA%		TOTALZOO%	
	First group	Second group	First group	Second group	First group	Second group	First group	Second group
May	6.0	28.3	11.3	37.1	82.7	34.6	100.0	100.0
June	59.8	1.2	10.6	98.0	29.6	0.9	100.0	100.0
July	0.3	0.0	6.1	69.7	93.6	30.3	100.0	100.0
August	46.4	7.5	44.5	90.7	9.1	1.8	100.0	100.0
September	1.6	0.0	67.5	50.0	30.9	50.0	100.0	100.0
October	12.5	3.4	8.2	91.2	79.3	5.4	100.0	100.0
Total average%	21.1	6.7	24.7	72.8	54.2	20.5	100.0	100

- Cladosra% = means numbers of Cladosra / means numbers of total zooplankton x 100
- Rotifer % = means numbers of Rotifer / means numbers of total zooplankton x 100
- Copepoda% = means numbers of Copepoda / means numbers of total zooplankton x 100

Table 5: Variation of the total numbers of zooplankton (Number of organisms /l) in water fishponds in first group ponds (affected) and second group ponds (unaffected) by Microcystis aeraginosa

	CLADOSRA		ROTIFERA		COPEPODA		TOTALZOO	
	First group	Second group	First group	Second group	First group	Second group	First group	Second group
May	1200.0B ± 103.3	2185.0A ± 1477.2	2276.7B ± 0.00	2863.3A ± 114.2	16666.7A ± 945.3	2676.7B ± 1048.3	20143.3A ± 108.2	7725.0B ± 788.6
June	8408.3A ± 351.5	901.7B ± 584.98	1485.0B ± 120.4	76255.0A ± 292.9	4161.7A ± 335.4	681.7B ± 352.3	14055.0B ± 259.2	77838.3A ± 892.0
July	118.3A ± 11.3	0.0B ± 0.00	2663.3B ± 660.6	3483.3A ± 857.0	40950.0A ± 223.2	1516.7B ± 151.7	43731.7A ± 396.8	5000.0B ± 117.7
August	15110.0A ± 71.01	2333.3B ± 1760.8	14481.7B ± 451.0	28058.3A ± 1151.8	2966.7A ± 1407.2	550.0B ± 250.3	32558.3A ± 900.5	30941.7A ± 594.1
September	520.0A ± 30.6	0.0B ± 0.0	21430.0A ± 624.3	1100.0B ± 0.0	9802.5A ± 387.0	1100.0B ± 0.0	31752.5A ± 787.0	2200.0B ± 0.0
October	3242.2A ± 54.4	1028.9B ± 68.4	2141.7B ± 127.1	27533.9A ± 7996.7	20592.8A ± 181.3	1625.0B ± 97.4	25976.7B ± 151.4	30187.8A ± 932.8

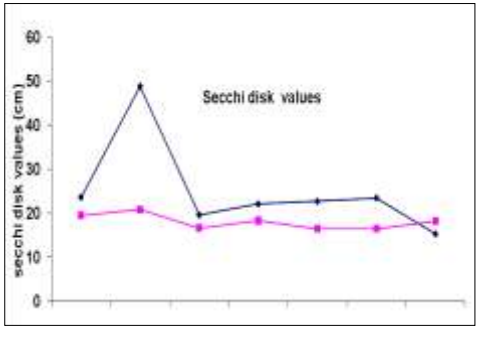
Data are represented as means ± standard error
Values with the same letters show non-significant differences between the two groups and values with the different letters show significant differences at ($p < 0.05$)

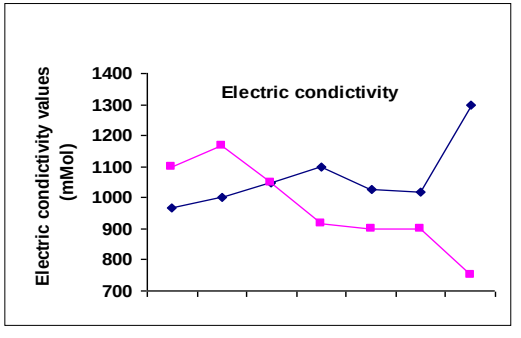
Table (6): Survival rate and fish production in first group ponds (affected) and second group ponds (unaffected) by *Microcystis a eraginosa*

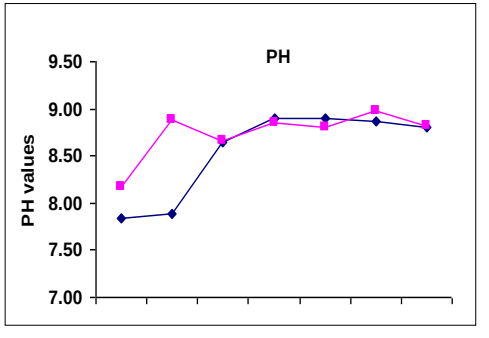
Pond Numbers	First group ponds		Second group ponds	
	Survival %	Production /kg	Survival %	Production /kg
1	12.1	67.76	89.3	571.52
2	6.8	38.08	92.4	591.36
3	1.6	8.96	74.9	479.36
4	3.7	20.72	99.4	636.16
5	3	16.8	85	544
6	6.1	34.16	84.7	542.08

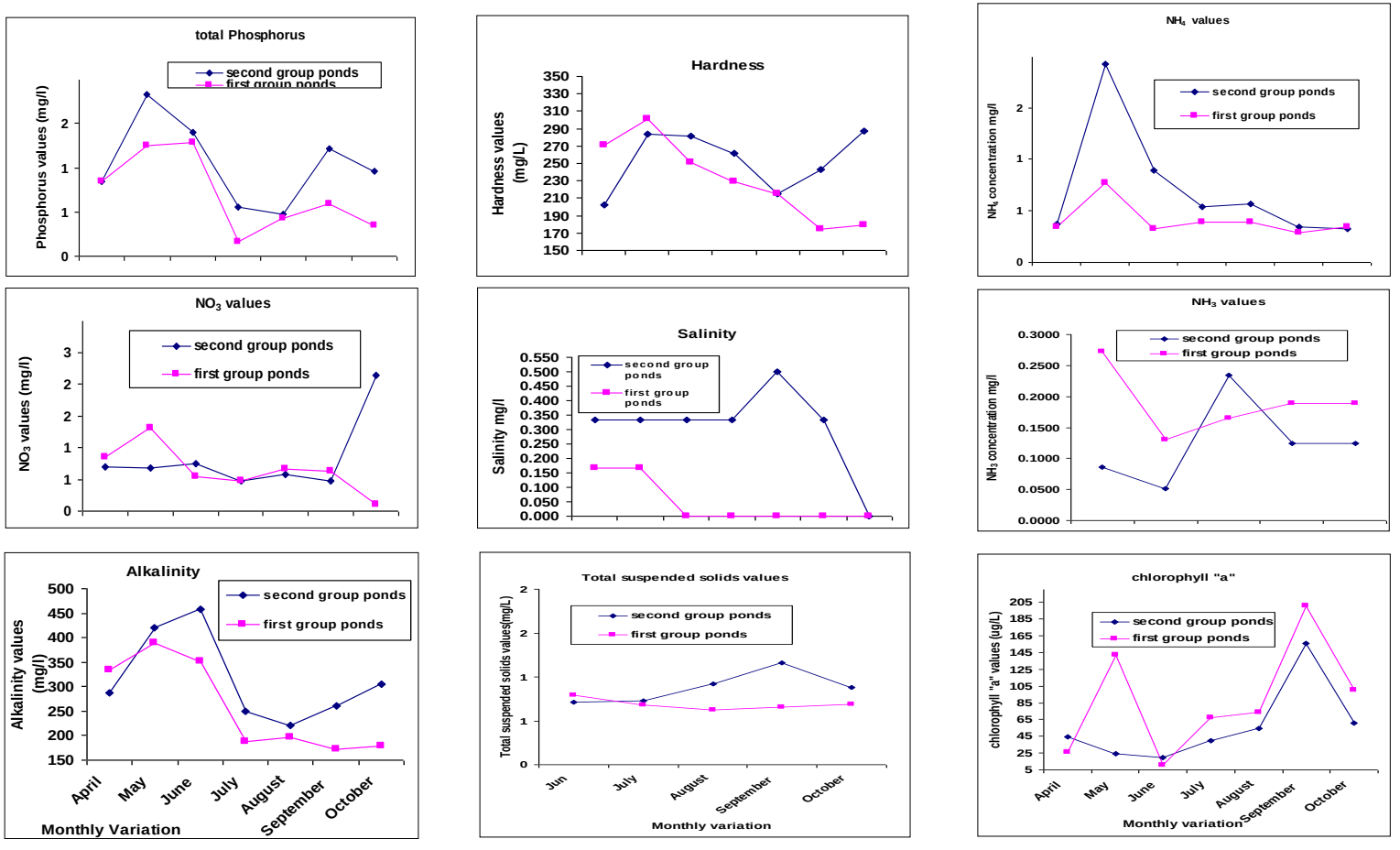
Table (7): Percentage of LC_{50} death of Artemia at 24 hours exposed to the toxicity threshold concentration, expressed as dry weight of *Microcystis aeraginosa*

	Percentage of death of Artemia at 24 hour				
Dry wt mg/ml	100 mg/ml	50 mg/ml	20 mg/ml	10 mg/ml	2 mg/ml
Time/ hours	1	4	12	18.5	24
Percentage	100	100	79	38	0









Figures 1: Water quality parameters changed (secchi disk, electric conductivity, pH, total phosphorus, hardness, alkalinity, NO_3, salinity, NH_3, NH_4, , total suspended solids and chlorophyll "a" in water fish ponds in first group ponds (affected) and second group ponds (unaffected) by *Microcystis aeraginosa*

تأثير وفرة طحلب الميكرو سيست أوريجينوزا على الأنتاج السمكي للبلطي في المزارع السمكيه

مصطفى عبد المحسن إبراهيم الجمال[1] أشرف عبد الرحمن رمضان[2]
1- قسم الليمنولوجى 2- قسم وراثة الأسماك
بالمعمل المركزى لبحوث الثروة السمكية

إنخفضت إنتاجية المزارع السمكيه بمنطقةِ التل الكبير ، محافظة ا لإسماعيليه، حيث وصلت نسبة معدل الحياة للأسماك المرباة بأحواض الإستزراع السمكى لتلك المنطقة أقل مِنْ 10% لعدّة سَنَوات متتابعة، وكان أحد الأسباب المحتملة لهذا الإنخفاض يعزى إلى وجود طحلب الميكرو سيست أوريجينوزا السام الذى من الممكن أن يبتلعَ بواسطة الأسماك المستزرعة أثناء التغذية أو عن طريق التنفّس.

تم إجراء هذا البحث خلال الفترة من 15أبريل الى 17 أكتوبر 2010م، على مجموعتين من أحواض الإستزراع السمكى الترابية (كل مجموعة تضم ستة أحواض إستزراع سمكى)، المجموعة الأولى بها طحلب الميكروسيست أوريجينوزا، والمجموعات الثانية خالية منه. هذا وكانت مساحه كُلّ حوض 2000 متر، وعُمق الماءِ 100-120 سنتيمتر، وتم تسميد كل حوض بكمية 125 كيلوجرام سماد زرقِ دواجن / حوض/ إسبوع لمدة 16 إسبوع، و إستزرع كل حوض بعدد 5000 إصباعية بلطي نيلي وحيد الجنس (وزن الأصبعية 5-7 جرام). تمت تغذية أسماك الأحواض السمكية المبحوثة بنسبةِ 3 % مِنْ وزنِ الجسمِ (عليقه طافيه 25 % بروتين). تمت دراسة العوامل الفيزوكيميائيه و البلانكتون والإنتاج السمكي و نسبة النفوق و سمية طحلب الميكروسيست أوريجينوزا على كل من أحجامِ الأسمكِ المختلف ه (5، 20، 50، 150 جرام) والأرتميا.

أوضحت النتائج الفيزوكيميائيه للمياه وجود إختلافاتَ معنوية بين مجموعتى الأحواض الخاصة بالبحث لكل من الأوكسجين الذائب و قرص الشفافيه و الملوحة والأملاح الذائبه و القلويه الكليه والفسفور الكليّ و الأمونيا (NH_4) و لم تسجل كل من درجةِ الحرارة و pH والتوصيل الكهربائي و النترات و الأمّونيا (NH_3) والكلوروفيل "أ" فروق معنويه بين المجموعتين محل الدراسه . بصفة عامة، كانت كل التحليلات والقيم المشار إليها في المدى المناسبِ لنمو البلطي النيلى و البلانكتون.

أوضح البحث وجود فروق معنوية بين مجموعتى أحواض الإستزراع السمكى المبحوثة فيما يتصل بأعداد الهائمات النباتية (الفيتوبلانكتون) لصالح المجموعة الأولى فى كل من الطحالب الخضراء المزرقه، و الأيوجلينا ، و طحلب الميكرو سيست أوريجينوزا . ولم تكن هناك فروق معنويه في أعداد الدياتومات. فيما يتصل بأعداد الزوبلانكتون (الهائمات الحيوانية)، فكانت هناك فروق معنويه في أعداد كل منِ Rotifers و Coepoda لصالح مجموعة أحواض الإستزراع الأولى، فى حين لم تكن هناك فروق معنويه في أعدادِ Cladocera والعدد الكلي للزوبلانكتون (الهائمات الحيوانية) بين المجموعتين. أكدت الدراسه السميه الخاصة بطحلب الميكرو سيست أوريجينوزا أن له تأثيرا مميتا، خلال 24 ساعه، على كل من الأسماك المستزرعة و الأرتيميا.

أوصى هذا البحث، من خلال النتائج المتحصل عليها، ضرورة إجراء مزيد من الدِراساتِ للسَيْطَرَة على الطحالبِ السامّةِ، وبخاصه طحلب الميكرو سيست أوريجينوزا ، في المزارع السمكيه حفاظا على الإنتاج السمكى والإستثمارات الخاصة به.