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GROWTH RATE OF AFRICAN CATFISH (*Clarias gariepinus*) AND PLANKTON DIVERSITY IN PONDS UNDER ORGANIC AND INORGANIC FERTILIZATION

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ABSTRACT

Aquaculture offers the opportunity for safeguarding local and global food security in the face of declining capture fisheries. However, the form of aquaculture that is commonly practised in Kenya is characterized by the use of agrochemicals such as fertilizers that negatively impact biodiversity especially when effluents from fish ponds drain into water bodies. This study aimed to determine differences in growth rate of *Clarias gariepinus,* an important aquaculture fish in Kenya, to assess plankton diversity, and to identify phytoplankton species associated with pollution under organic and inorganic fertilization regimens using chicken manure, Diammonium phosphate and urea, respectively. Average growth rate calculated per day was higher in the organically-fertilized ponds at 0.06 cm/day, followed by inorganically-fertilized ponds at 0.05cm/day and then, the control at 0.04 cm/day. Average weight gain was higher in organically-fertilized ponds at 0.08 g/day followed by ponds fertilized with inorganic fertilizer at 0.07 g/day and the control, at 0.06g/day. There were significant differences in growth rate across fertilization regimens (length: $F_{2, 264} = 24.06$, p = 0.0399; weight: $F_{2, 264} = 20.89$, p = 0. 0457). Specifically, although differences in growth rate of fish in organically and inorganically fertilized ponds were not significant, fish in fertilized ponds were on average, longer and weighed more than those in the control pond. Jaccard's similarity index for phytoplankton was highest (0.38) between organically-fertilized ponds and control but lowest (0.25) between inorganically-fertilized ponds and control. Use of chicken manure produced the highest diversity of zooplankton (Shannon-Weiner's H in organically-fertilized pond = 1.886; inorganic = 1.044, and control = 0.935). The use of DAP and urea produced the highest proportion of phytoplankton species associated with pollution. These results do not support the commonly reported notion that ponds fertilized using inorganic fertilizers are more productive. Findings suggest that the use of inorganic fertilizers may threaten biodiversity in aquatic ecosystems through the production of toxic algae.

Key words: Aquaculture, fertilization, *Clarias gariepinus*, growth rate, plankton diversity

INTRODUCTION

Global aquaculture production has been growing for the last several decades with a production level estimated at 80 million tonnes in 2016 while capture world fisheries has remained static [1] or declining in some African countries such as Kenya [2]. In Kenya, total fish production from aquaculture as of 2010 was 12,000 MT/year, representing 7% of the total fish production and this statistic is from non-integrated farms where ponds are fertilized by inorganic fertilizers [2,3]. According to the United Nations Food and Agriculture Organization (FAO), sustainable agricultural practices offer opportunities to address projected shortfalls in food production in the face of climate change [1]. Global aquaculture will, thus, need to continue growing in order to ensure sufficient supply of fish and other aquatic foods to meet the needs of the increasing human population [1].

Although aquaculture has the potential to address shortfalls in capture fisheries, the most commonly practiced form of aquaculture, non-integrated aquaculture, uses agrochemicals such as fertilizers that have been shown to pollute the environment in ways that adversely affect biodiversity [1]. The commonly used inorganic fertilizers in aquaculture in Kenya include diammonium phosphate (DAP) and Urea [4]. These fertilizers are readily available and are used to improve phosphorus and nitrogen levels in the water in order to stimulate primary productivity. In contrast, integrated aquaculture either cuts down the amounts of agrochemicals or altogether, eliminates the use of such chemicals. In addition, integrated aquaculture involves reutilization of resources in addition to having a low space requirement. The most common form of integrated aquaculture system practiced is livestock-fish farming where animals like chicken, pig and duck have been used to produce manure that is used to fertilize ponds with the aim of improving both primary productivity and zooplankton proliferation [5]. Taken together, these core practices of integrated aquaculture augment agricultural productivity in ways that lower the magnitude of threat to biodiversity compared to non-integrated aquaculture [6,7].

Although a large number of farmers use agrochemicals such as inorganic fertilizers apparently because such chemicals are assumed to improve productivity [2,5], differences in productivity as a function of fertilization types remain largely unknown. However, many farmers still use these inorganic fertilizers that are known to be associated with pollution, yet the use of such chemicals may imperil not only fish but also other species including plankton, the key primary producers in aquatic ecosystems [8,9]. For instance, inorganic fertilizers directly affect fish by increasing nutrient level in water, resulting in eutrophication. Eutrophication, in turn, is associated with fish kills, which are partly attributed to proliferation of toxic algae species [8].

Estimated at 21%, aquaculture production level of African catfish, *Clarias gariepinus,* in Kenya is second to that of *Oreochromis niloticus* which is at 71% [2,4]. The species, *C. gariepinus,* is a generalist omnivore that is known to feed on natural foods in both its natural and captive environments [10]. African catfish are of great importance as they grow quickly, attain a large size with more flesh and few spines and are also able to withstand a wide range of environmental conditions, thus increasing this taxon's potential to contribute to food security [5]. Given its potential, the species is an excellent candidate for evaluating any differences in productivity as a function of fertilization regimen. The objectives of this study were, first, to determine differences in growth rate between *C. gariepinus* raised in ponds fertilized using chicken droppings and those raised in ponds fertilized by DAP and urea, and second, to determine differences in plankton diversity and occurrence of phytoplankton genera associated with pollution between ponds fertilized by chicken droppings and in ponds fertilized by inorganic fertilizer.

MATERIALS AND METHODS

Study site

The research study was carried out at Me-Farm, situated along Kisumu-Nairobi Road approximately 10 km from Kisumu Town at -0.1500° latitude and 34.8333° longitude. The area is characterized by clay-loam alluvial soils with soil pH ranging from 4.5 to 10.4. The water used in the ponds was from underground and had a pH range of 6.7 to 8 during the duration of the study period. Data were collected for four months from June to September, 2015.

Experimental design

One month old fingerlings, weighing approximately 1g of *C. gariepinus* with an average total length of 6 cm, were bought from Mabro Fish Farm in Usenge Town and transported in oxygen filled polythene bags to the experimental station at Me-Farm, a distance of approximately 100 km. Fingerlings were allowed to acclimatize to water temperature for 30 minutes before being released into the ponds. Fingerlings were raised in five experimental earthen ponds measuring 2x2x1m^2 identified as A, B, C, D, and E. Ponds A and B were fertilized using chicken droppings at the rate of 200g/week/pond whereas ponds C and D were fertilized using inorganic fertilizers, DAP at the rate of 8g/week/pond and urea at the rate of 12g/week/pond following Ngugi *et al.* [4]. Fertilization using inorganic fertilizers was informed by common practice among aquaculturists in Kenya. Pond E was not fertilized and served as the control.

Each pond carried 28 fishes according to stocking recommendation by Ngugi *et al*. [4]. Fish were given supplementary commercially formulated fish feed purchased in Kisumu Town containing 35% protein, 20% carbohydrate, 10% lipid and 5% crude fiber. Feeding rate was calculated depending on the average weight of the fish and water temperature at the rate of 0.074% grams of feed/gram of body weight [11]. Fish in all the ponds received the same type of feed.

Determining growth rate of *Clarias gariepinus*

Length and weight measurements were carried out fortnightly in each pond. Fish were caught randomly from the ponds using a seine net with a mesh size of 0.4 mm. Sampling effort consisted of 3-4 sweeps of the seine net per pond while the same sampling effort was employed so as not to create differences in stress across ponds. Between 5 and 10 fish were measured from each pond on each sampling day. The collected fish were placed in a container with pond water and after measurement placed

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in a second container having the same pond water. Fish were measured on site and returned to their respective ponds.

The total length of fish, from the mouth tip to the end of the tail fin, was measured using a 30 cm ruler. The following formula was used to determine specific growth rate in length:

$$\text{Specific growth rate in length} = \frac{\text{Current mean length (cm)} - \text{Previous mean length (cm)}}{\text{Time (days)}}$$

The weight of fish was measured to the nearest 0.1 g using a portable digital battery-operated weighing balance (model CL 201J, OHAUS Corporation, New Jersey, USA). The following formula was used to determine specific growth rate by weight:

$$\text{Specific growth rate in weight} = \frac{\text{Current mean weight} - \text{Previous mean weight(g)}}{\text{Time(days)}}$$

Determination of plankton diversity

Water was sampled fortnightly from all the ponds and analysed for phytoplankton and zooplankton diversity at the Kenya Marine and Fisheries Research Institute in Kisumu for three months from July to September, 2015. For phytoplankton analysis, a 20 ml vial was used for water collection and the organisms were preserved in 5% formalin, after which 0.5 ml of sampled water was analysed using the Utermohl technique [12] and examined under a compound microscope (Leica Microsystems Gmbh, Wetzier, Germany) with magnification level between ×100 to ×150. Phytoplankton species were identified up to the family and genus level using an identification key [13].

Zooplankton were collected by using 30 litres of water collected from each pond, filtered and concentrated to a 20 mL volume using a 60 µm zooplankton net. The concentrated samples of zooplankton were preserved in 5% formalin to which two drops of Lugol solution was added. A concentration of 1 mL of the preserved zooplankton samples were introduced into a Sedwick Rafter counting chamber (Olympus BH2, OHAUS Corporation, New Jersey, USA), for examination under a light microscope (Leica Microsystems, Wetzier, Germany). Identification of zooplankton was done up to genus level [14].

Data analysis

Growth rates of *C. gariepinus* for each fertilization regimen were calculated and presented as mean increase in length and weight per day. Differences in length and weight across fertilization regimens were analysed using repeated measures ~~of~~ analysis of variance. Diversity of zooplankton under the two fertilization regimes as well as that in the control pond was analysed using the Shannon-Weiner Diversity (H) Index. The formula for H:

$$\mathbf{H = -\sum [(pi) * ln(pi)]}$$

where, pi = number of individuals of species i/total number of samples; H typically ranges from 1 to 4 such that the higher the index, the higher the species diversity. The

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Shannon Weiner diversity was then converted (using exp(H)) to effective species number for ease of interpretation as recommended by Jost [15]; it denotes the number of equally abundant species necessary to produce the observed value of diversity. Phytoplankton diversity was analysed using Jaccard's index, which is computed using the formula: $J = s_c/s_a + s_b + s_c$ where, s_a and s_b are the numbers of species unique to ponds a and b, respectively, and s_c is the number of species common to the two ponds. Descriptive statistics were used to summarize data on the occurrence of phytoplankton species associated with polluted waters. For all statistical tests, statistical significance was evaluated at $p \leq 0.05$. Statistical tests were performed using R Version 2.14.1.

RESULTS AND DISCUSSION

Growth of *Clarias gariepinus*

Fish in the organically and inorganically-fertilized ponds had comparable total lengths but were on average, longer than those in the control pond. The longest fish was from the organically-fertilized pond with a total length of 14.2 cm, followed by inorganically-fertilized pond at 14.1 cm, and 12.3 cm from the control pond after 4 months (Table 1). Mean total length was also higher in the organically-fertilized pond (9.39 cm, range 5.5-14.2), inorganically-fertilized (9.16 cm, range 5.7-14.1), and control (8.30 cm, range 6.0-12.3) (Figure 1). Average growth rate (length) per day was highest in the organically-fertilized pond, followed by the inorganically-fertilized pond and lastly the control. The growth rates were in the order of 0.06 cm/day in the organically-fertilized pond, 0.05 cm/day in the inorganically-fertilized pond and 0.04 cm/day in the control pond. Mean total length was significantly different across ponds ($F_{2, 264} = 24.06$, $p = 0.040$); the results showed that there was a significant difference in mean total length between organically-fertilized pond and control and between inorganically-fertilized pond and control but not between organically-fertilized and inorganically-fertilized ponds. Data on fish growth rate are summarized in Table 1.

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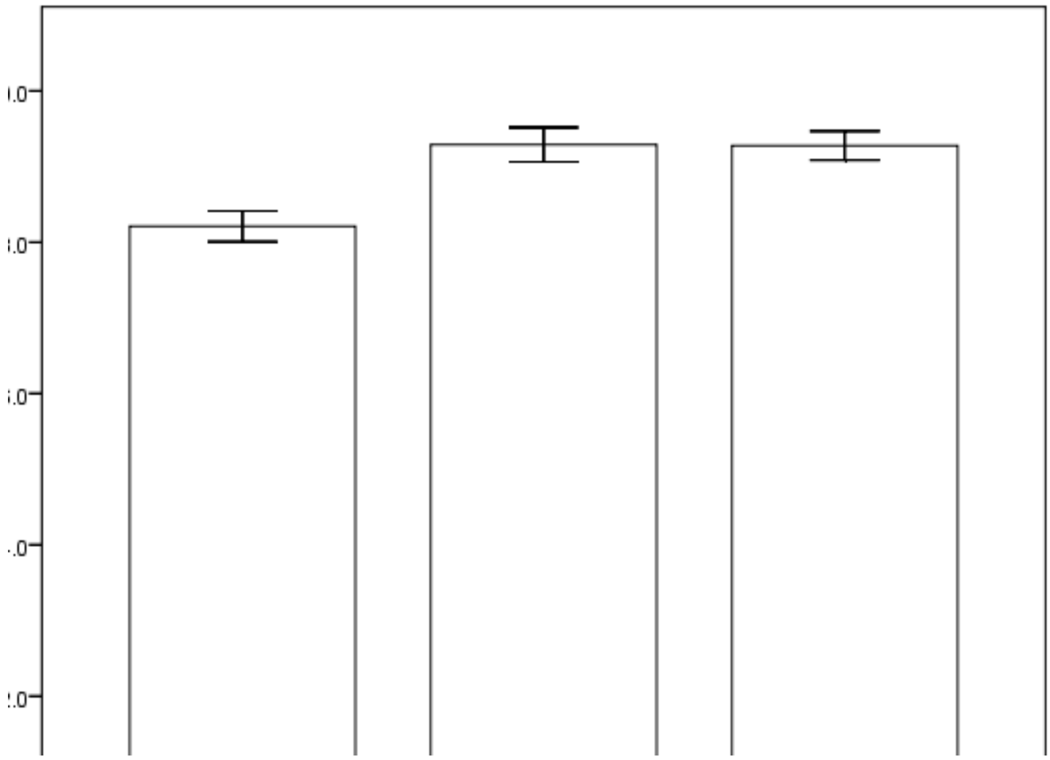

Figure 1: Mean length of *C. gariepinus* in ponds under different fertilization conditions for four months. Error bar represents standard error of mean

Mean weight of fish was highest in organically-fertilized pond (mean = 5.75 g, range 2.1-12.2 g), followed by inorganically-fertilized pond (mean = 5.25 g, range 2.1-12.1g) and lastly control (mean = 4.33 g, range 2.1-9.5 g) (Figure 2) over a period of four months. Average weight gain in grams per day was highest in the organically-fertilized pond. This was followed by the inorganically-fertilized pond and lastly the control. The growth rates were in the order of 0.08g/day in organically-fertilized pond, at0.07g/day in inorganically-fertilized pond and 0.06g/day in control pond (Table 2). The present results showed that mean weight was significantly different across fertilization regimens ($F_{2, 264}$ =20.89, p = 0.0457).

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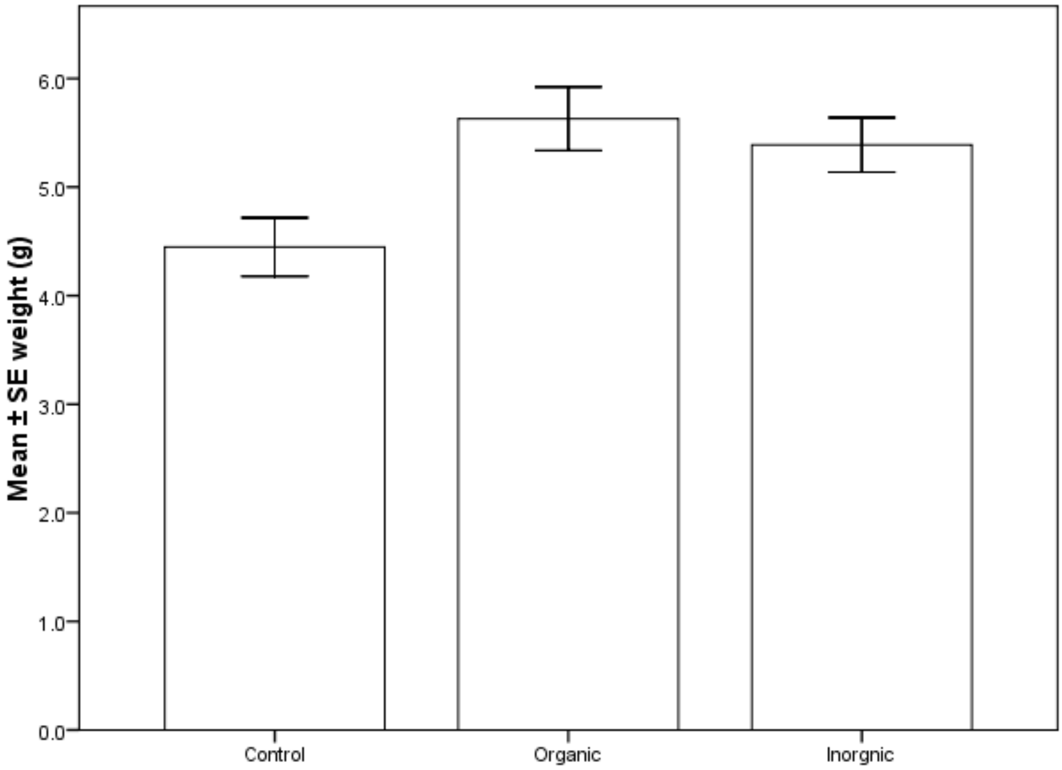

Figure 2: Mean weight of *C. gariepinus* raised under different fertilization conditions over four months. Error bar represents standard error of mean

The higher growth rate of *C. gariepinus* recorded in fertilized ponds may be attributed to increased growth of both phytoplankton and zooplankton. Orji and Udonwu [14], for example, showed that both organic and inorganic fertilizers improve plankton abundance. Improved plankton abundance implies increased food availability or fish and, thus, resources to facilitate growth. Similarly, although there were no significant differences in growth rate between fish raised in ponds fertilized by DAP and Urea and those in ponds fertilized by chicken droppings, the marginally higher growth rate in ponds fertilized using chicken droppings compared to those raised in ponds fertilized with inorganic fertilizers may be accounted for by the fact that organic fertilizers result in proliferation of zooplankton that are preferred by *C. gariepinus* [17,18]. However, it cannot be concluded that the differences in growth rates presented in this study persisted throughout the life of the fish because differences in growth rate were assessed for four months.

Plankton diversity

Phytoplankton diversity

Ponds fertilized by inorganic fertilizer had 29 genera and had the highest number of phytoplankton species in the study followed, by the ponds fertilized by organic fertilizer that had 10 genera, while the unfertilized pond (control) had 8 genera. Jaccard's similarity coefficient was highest between organic and control (0.38), followed by organic and inorganic (0.33) and lowest between inorganic and control (0.25).

There was no significant difference, under a null expectation of equal occurrence, in frequency of phytoplankton genera between organically-fertilized pond and the control pond ($X^2 = 0.22$, $p>0.05$). In contrast, significant differences in frequency of phytoplankton genera were observed between inorganically-fertilized and control ($X^2 = 11.918$, $p<0.05$) and inorganically-fertilized and organic ($X^2 = 9.26$, $p<0.05$) ponds.

A list of phytoplankton genera associated with pollution that were identified in the current study are shown in Table 3. Worth noting is the fact that all the phytoplankton associated with pollution that were identified in the current study were found in the ponds fertilized using inorganic fertilizer.

Zooplankton diversity
Three groups of zooplankton genera were observed. These were Copepods, Cladocerans, and Rotifers and their abundance across all ponds were in the order of Copepods>Rotifers>Cladocerans. There was no significant difference in Rotifers ($X^2 = 4.63$; $P>0.05$) across all the ponds, but differences were observed for Copepods ($X^2 = 32.08$; $P<0.05$) and Cladocerans $X^2 = 10.3$; $P<0.05$) in all the ponds. Zooplankton species diversity, based on Shannon-Wiener diversity index, and effective number for zooplankton species identified in the study are shown in Table 4 and the index shows higher species diversity in ponds fertilized with chicken droppings than in those fertilized by DAP and urea and in the unfertilized pond.

The comparatively higher abundance of phytoplankton diversity in fertilized ponds observed in the current study is consistent with findings from Oparaku [18], who reported higher phytoplankton abundance in pond fertilized by inorganic fertilizer, NPK, compared to ponds fertilized by either biogas sludge, cow dung, or poultry manure. However, although ponds fertilized by inorganic fertilizers registered high phytoplankton species diversity, these ponds contained 100% of species that are associated with pollution in aquatic environments. A greater cause for concern are the results showing that even the control pond had 36% of such phytoplankton species, suggesting that either the soil, water or chicken droppings used contained some level of nutrients that were conducive for growth of toxic species of phytoplankton.

The high species diversity of zooplankton under chicken manure fertilization suggests that organic fertilizers promote the growth of zooplankton. Comparably, Mosha and Kang'ombe [19] found that zooplankton diversity was higher under fertilization with chicken manure than under inorganic fertilization. It can, thus, be argued that the choice of organic manure to use in aquaculture should consider forage requirements of each fish species. To the extent that organic manure enhances growth and proliferation of zooplankton, it should be the fertilizer of choice for *C. gariepinus*.

In conclusion, results of the present study do not support the claim that fertilization of ponds using inorganic fertilizers enhances growth rate of fish more than organic manures, at least for the case of *C. gariepinus*. This study has demonstrated that fertilization of ponds using inorganic fertilizers, in particular DAP and urea, promotes the proliferation of toxic algae suggesting that the use of such fertilizers is a threat to biodiversity in aquatic ecosystems. Policy makers should focus efforts to sensitize farmers who lack support on the notion that fertilization of ponds using inorganic fertilizers enhances growth rate of *C. gariepinus*. However, future studies should focus on whether this is the case with other inorganic fertilizers and with other aquaculture fish species.

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Table 1: **Average length gain per day of *Clarias gariepinus***

	Inorganically-fertilized Pond			Organically-fertilized pond			Control Pond		
Sampling Week	Samples n	Mean length (cm)	Growth Rate (cm/day	Samples n	Mean length (cm)	Growth Rate (cm/day	Samples N	Mean Length (cm)	Growth Rate (cm/day
Week 0	21	7.11	0.00	17	7.09	0.00	10	6.70	0.00
Week 2	20	8.09	0.07	20	7.46	0.03	10	7.00	0.02
Week 4	20	8.71	0.04	20	8.31	0.06	10	7.70	0.05
Week 6	8	10.21	0.11	10	10.40	0.15	5	8.60	0.06
Week 8	8	10.04	-0.01	10	9.68	-0.05	6	8.80	0.01
Week 10	10	10.94	0.06	10	11.59	0.14	5	9.36	0.04
Week 12	10	11.42	0.03	10	12.19	0.04	5	9.94	0.04
Week 14	10	12.14	0.05	10	12.73	0.05	5	10.64	0.05
M/Growth Rate		0.05			0.06			0.04	

Note: n represents number of fish sampled under each fertilization regimen on each sampling date. Variation in n reflects observed differences in the number of fish caught in each pond under similar sampling effort (3-4 sweeps of the seine net per pond)

Table 2: Average weight gain of *Clarias gariepinus* per day

	Inorganically-fertilized Pond			Organically-fertilized pond			Control Pond		
Sampling Week	Samples n	Mean Weight (g)	Growth Rate (g/day)	Samples n	Mean Weight (g)	Growth Rate (g/day)	Samples n	Mean Weight (g)	Growth Rate (g/day)
Week 0	21	2.39	0.00	17	2.76	0.00	10	2.40	0.00
Week 2	20	4.07	0.12	20	3.36	0.04	10	2.54	0.01
Week 4	20	4.86	0.05	20	4.19	0.06	10	4.05	0.11
Week 6	8	6.83	0.14	10	7.20	0.22	5	4.36	0.09
Week 8	8	6.06	-0.06	10	5.20	-0.14	6	4.80	0.03
Week 10	10	7.30	0.09	10	8.64	0.25	5	6.32	0.11
Week 12	10	8.09	0.06	10	9.31	0.05	5	6.85	0.04
Week 14	10	9.07	0.07	10	10.07	0.05	5	7.66	0.06
M/Growth Rate		0.07			0.08			0.06	

Note: n represents number of fish sampled under each fertilization regimen on each sampling date

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Table 3: Phytoplankton genera identified in the ponds

Genus	Organically-fertilized pond	Inorganically-fertilized pond	Control pond
Scenedesmus	Yes	Yes	No
Ankistrodesmus	Yes	Yes	No
Navicula	Yes	Yes	Yes
Microcystis	Yes	Yes	Yes
Nitzschia	Yes	Yes	Yes
Fragilaria	Yes	Yes	No
Synedra	No	Yes	No
Amphora	No	Yes	No
Tabellaria	No	Yes	No
Asterionella	No	Yes	No
Coelostrum	Yes	Yes	Yes

Note. Yes = present and No = Absent

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Table 4: Diversity of zooplankton species under different fertilization regimes

Pond Fertilization	Number of Genera	n	Shannon-Weiner index (H)	Effective Number of Genera
Control	3	22	0.935	3
Inorganic	3	47	1.044	3
Organic	3	76	1.886	7

n- represent the number of zooplankton species in each pond. Effective number is the true diversity of zooplankton in each pond

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