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Multi linear Regression among the Zooplankton Community Counts, Water Quality Parameters and Nutrients Input under Biofloc System Conditions

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ABSTRACT

A 70-day experiment was carried out to estimate mathematical equations that describe the effect of water quality and nutrient input on different zooplankton community count under biofloc conditions. The experiment was designed to deliver three diets with different dietary C/N ratios (8.8, 11.5 & 12) by manipulating the dietary protein (CP) and/or dietary lipid (L). The three diets were; control diet (C; 30% CP, 8% L), $P_{23}L_8$ diet (23% CP, % 8 L) and $P_{23}L_{10}$ diet (23% CP, 10% L). Control was the only treatment supplemented with an external carbon source (starch) to reach a C:N ratio of 10:1. The control treatment with high dietary protein level showed the highest count for different zooplankton groups, especially for total Protozoa. Protozoa taxa showed a significant regression model ($R^2_{adjusted}$= 0.814, P<0.001) relation with both TSS and nitrogen input. While, the total rotifers showed no correlation with any of the independent variables except for a polynomial relation with *Alona intermedia*,(P= 0.01). The only cladoceran species recorded in the water samples was *Alona intermedia* and it showed a significant regression model ($R^2_{adjusted}$ = 0.363, P<0.001) with Phosphorus input and alkalinity. Phosphorus input and alkalinity seemed to explain 56% of the copepods count (P=0.003). Ostracoda species showed a significant positive relation with both energy input and Protozoa species of *Centropyxis aculeate* ($R^2_{adjusted}$ =0.302 P= 0.003). It could be concluded that, alkalinity, TSS and mineral input are the major variables that affected the zooplankton community density, especially the large sized groups.

INTRODUCTION

Application of more intensified, responsible and sustainable aquaculture has become a need for almost all countries around the world, especially in aired regions. The required balance among water resources, fish density, environmental impact and feed expenses resulted in the adoption of fish culture systems away from traditional or sophisticated aquaculture system. Biofloc system is an unsophisticated fish culture system; as for instance the reticulating aquaculture system (RAS), and unlike traditional aquaculture systems in which more fish densities are cultured with zero water exchange.

The biofloc system largely depends on activating the growth of heterotrophic bacteria to manipulate nitrogen toxic effluent in fish tanks. A C: N ratio of 10:1 -20:1 is required for the activation of heterotrophic bacteria in biofloc tanks **(Avnimelech, 2011)**. With less extent, other organisms in the system may manipulate the nitrogen effluents as nitrifying bacteria and alge **(Dauda, 2020)**.With more developing of the system, a complex community of different organisms as bacteria, phyto and zooplankton community are aggregated in the form of what is known as floc particles. They are a result of dietary recycled nutrient and are considered as a secondary feed source for fish.

Flocs are a protein - lipid natural rich open access source for cultured fish, and therefore help in limiting the feed expenses **(Hargreaves *et al*., 2013; Emerenciano *et al*., 2017)**. The protein content of the dried flocs ranges between 17.2 – 42.5%, while 1.26 -7.5% was determined to be the range of lipid content **(Emerenciano *et al*. 2012; Ekasari *et al*. 2014; Mabroke *et al*., 2019)**. Zooplankton community is one of the main groups that affect the nutritional quality of the flocs. Rotifers contain about 33.6 -36.4% protein **(Øie *et al*., 1997)**, while *Artemia* sp. contains about 51.24% **(Hamsah *et al*., 2017)**. Remarkably, 44-52% is generally the range recorded of protein content of copepods **(Lavens & Sorgeloos, 1996)**. The more presence of species with rich nutritional composition such as rotifers, *Artemia* and copepods, the more nourishing are the flocs for cultured animals.

The structure of the zooplankton community seems to be affected by water quality parameters, feed frequency, complexity of carbon source and the nutrient- input of biofloc system **(El-Shafiey *et al*., 2018; Mabroke *et al*., 2021)**. No information is available on the correlation between zooplankton community and the different biofloc system conditions. Many efforts have been paid to optimize the biofloc condition for optimal fish growth. Hence, more efforts are required to maximize the developing of zooplankton community under biofloc system to enhance fish growth.

This study aimed to detect mathematical equations that describe the effect of biofloc conditions regarding water quality and nutrient input in fish tanks on different species of zooplankton, resulting in fish growth under biofloc system.

MATERIALS AND METHODS

Study site

The present study was carried out at the Fish Nutrition Lab (FNL), Department of Animal Production, Faculty of Agriculture, Cairo University, Egypt

Fish and experimental conditions

Nile tilapia was obtained from a commercial farm located in Kafr El-Sheikh Governorate, Egypt. A total number of 126 the mono-sex Nile tilapia (*Oreochromis niloticus*), with an average weight of 145.87g ± 1.44, were randomly selected and stocked at nine fiberglass tanks with a water capacity of $1m^2$. Each tank was filled up to reach 500L (100 L matured biofloc and 400 L of well water) and were installed under

polycarbonate greenhouse. Each tank was stocked at a density of 14 fish corresponding to the density of 4 kg m^{-3}. The experiment extended for 70 days.

Experimental design and feeding

The experiment was designed in triplicate to deliver three diets with three different dietary C/N ratios (8.8, 11.5 and 12) to the tilapia tanks. The diets with different C: N ratios were performed by manipulating the dietary protein (CP) and/or dietary lipid (L). The three diets were the control diet (C; 30%CP, 8% lipid), $P_{23}L_8$ diet (23% CP, % 8 lipid) and $P_{23}L_{10}$ diet (23% CP, 10% lipid). The control was the only treatment supplemented with an external carbon source (starch) to reach a C: N ratio of 10:1. For 6 days per week, fish in different treatments were fed by hand twice daily (10 a.m. and 5 p.m.) till apparent satiation.

Table 1. Proximate chemical composition of the experimental diets (as fed)

Proximate composition	Control	$CP_{23}L_8$	$CP_{23}L_{10}$
	C/N ratio		
	8.8	11.5	12
Moisture %	8.23	8.62	8.33
Crude protein %	30.80	23.00	22.60
lipid %	8.68	8.25	10.57
Ash %	5.10	5.40	4.70
Phosphorus %[a]	0.39	0.45	0.41
Total carbohydrate [b]	47.24	54.75	53.80
Gross energy (kcal/kg) [c]	4497	4324	4482
P/E (mg/kj)	16..40	12.70	12.10

[a] **calculated**

[b] **Total carbohydrate content was determined by the difference: total carbohydrate = 100 − (% crude protein + % crude fat + % total ash +% Moisture).**

[c] **Dietary gross energy was calculated using the conversion factors of 5.65, 9.45 and 4.1 kcal /g for protein, lipids and carbohydrates, respectively. Hepher *et al*. (1983)**

Fish gain was recorded biweekly. Feed intake and the dietary nutrient inputs of the biofloc system were calculated biweekly to detect the relation between the occurring development of zooplankton species in different experimental tanks, the nutrient input and water quality.

Water quality

Water temperature and dissolved oxygen were recorded using SensoDirectOxi 200 device, while the pH was measured using Milwaukee-PH600 Digital pH meter tester pocket Pen every three days. The pH was maintained between 7.5 and 8.5 using $NaHCO_3$. The Total ammonia nitrogen (TAN) values was detected using water analysis photometer (MultiDirectLovibond) every two days, while nitrite (NO2-N) was detected using water analysis photometer (MultiDirectLovibond) on weekly basis. The biofloc volume was determined using Imhoff cone; as floc volume in the bottom of the cone was measured after 15 minutes of sedimentation twice a week **(Avnimelech, 2009)**. Alkalinity was monitored twice a week by titration with sulfuric acid **(Boyd & Tucker, 1992)** till

the pH reaches a point of 4.5. Total suspended solids (TSS) were monitored twice a week by water analysis photometer (MultiDirectLovibond).

Zooplankton

Water samples were collected five times during the experimental period on a biweekly basis to detect the zooplankton count of different species. A total of 30 observations (5 biweekly observation× 2 replicates (as odd replicate was omitted) × 3 treatments) were determined for each zooplankton species and were used in the statistical analysis. A total count for different zooplankton taxa was used in the current statistical approach to find a mathematical relation between tanks' condition and the general development of the taxa. In the same context, the total count of each taxon was used to avoid the appearance of one species in a single replicate that does not show up in the other. This finding was detected in some species especially in the Copepod taxa. Zooplankton samples were collected by filtering 5 liters of surface water using plankton net with size of 55 µm mesh, diameter of 25cm and length of 80 cm. Each collected sample was transferred to a labeled clean bottle and immediately fixed with 5% formaldehyde. In the laboratory, three subsamples (one ml for each) of the homogenized plankton samples were transferred into a counting cell and zooplankton species were identified under a binocular research microscope with magnification varying from 100X to 400X. The zooplankton species were identified following the descriptions of **Edmondson (1963)**, **Wallace and Snell (1991)** and **Foissner and Berger (1996)**. Zooplankton population density was then calculated as the number of individuals per liters following the equation in **APHA (1995)**

No. of zooplankton species = (c x v′) / (v″ x v‴)

Where: - c= number of organisms counted;

v′= volume of concentrated sample in ml;

v″ = volume counted in ml

v‴= volume of the grab sample in liters

Statistical analysis

Backwards stepwise linear-regression was used to analyze the effect of independent variables; water quality parameters (alkalinity and TSS) and nutrient input in fish tanks (nitrogen, ash, energy, and phosphorus; P) on dependent variables (Protozoa, Rotifera, Cladocera (*Alona intermedia)*, Ostracada, and Copepoda taxa count). To meet the assumption of the data residuals homoscedasticity, the dependent variables data were transformed. Log_{10} were calculated for total Protozoa count and *Centropyxis aculeate* (n=29) as one case were excluded because of the case-wise diagnosing test (standard residuals > 3 stander deviation). The square root was calculated for *Arcella vulgaris*, *Alona intermedia* and Ostracada (n=30). For copepod data, the average of each two replicates was used (n=15). PAleontological STatistics (PAST; Version 3.22) statistical program was used to check the assumption of linearity among the dependent and independent variables. While, Jeffreys's Amazing Statistics Program (JASP; Version

14.0) was used for performing the linear-regression analysis. Figures were performed using both PAST and Excel programs. The scatter plot of residuals was used versus the predicted values produced by JASP program to chek homoscadicity. When the existence of heteroscadisity was suspected, the test of Breusch–Pagan was performed according to Bobbitt (2020) using EXCEL. The result of the Breusch–Pagan test was adopted to confirm the homoscadicity assumption.

RESULTS

The periodical average count of different zooplankton species of different treatments is presented in Table (2) and Figs. (1 & 2). Rotifers were the dominant species after two weeks of feeding tilapia with different experimental diets C, $CP_{23}L_8$, $CP_{23}L_{10}$ being 62, 77, 58 %, respectively. Starting from the 4th week till the end of the 10th week, the Protozoa were the dominant group among other zooplankton taxa. Cladosra, Copepod and Ostracoda showed less count and percentage among other zooplankton taxa. Throughout the experimental period, the total counts of Rotifera, Cladocera, Copepoda and Osrtracoda showed a decreased pattern, which was in parallel with the increased count of the Protozoa sp. (Fig.1).

Table 2 . Average periodical count of different zooplankton taxa and species of different experimental treatments.

	Control					$CP_{23}L_8$					$CP_{23}L_{10}$				
	2wks	**4wks**	**6wks**	**8wks**	**10wks**	**2wks**	**4wks**	**6wks**	**8wks**	**10wks**	**2wks**	**4wks**	**6wks**	**8wks**	**10wks**
Protozoa															
Centropyxis aculeate	32460	193800	376200	433800	314520	2700	9600	35400	37200	17640	12780	38400	61800	23400	19800
Arcella vulgaris	16260	6000	4200	240	0	3000	10200	11400	1140	1260	3300	3600	3000	2100	1800
Vorticella campanula	600	0	0	0	60	0	0	0	0	0	0	0	0	0	0
Total	49320	199800	380400	434040	314580	5700	19800	46800	38340	18900	16080	42000	64800	25500	21600
Total%	**35**	**97**	**96**	**93**	**69**	**8**	**49**	**60**	**96**	**84**	**33**	**85**	**86**	**77**	**48**
Rotifera															
Lecan bulla	82200	1860	9000	24000	94800	54600	18000	28800	360	1920	26940	6600	7620	6000	15600
Lecanclosterocerca philodena sp.	2400	60	0	60	26400	0	300	0	0	60	180	0	0	0	120
	2460	0	3600	3600	21600	120	0	300	420	900	180	60	360	1200	7380
Colurellaobtusa	240	0	600	0	0	1200	660	240	0	0	600	120	300	0	120
Brachionus angularis	0	0	0	0	0	180	0	0	0	0	120	0	0	0	0
Total	87300	1920	13200	27660	142800	56100	18960	29340	780	2880	28020	6780	8280	7200	23220
Total%	**62**	**1**	**3**	**6**	**31**	**77**	**47**	**38**	**2**	**13**	**58**	**14**	**11**	**22**	**51**
Cladocera															
Alona intermedia	4200	3000	120	720	420	9720	420	840	660	660	2640	240	1860	0	60
Total%	**3**	**1**	**0**	**0**	**0**	**13**	**1**	**1**	**2**	**3**	**5**	**0**	**2**	**0**	**0**
Copepoda															
Nauplius larvae	180	0	0	0	0	0	120	0	60	0	120	60	0	0	0
Cyclopoid copepodite	0	180	0	0	0	300	180	60	0	0	0	0	120	0	0
Acanthocyclopstrajani	0	60	0	0	60	0	60	0	0	0	180	120	0	120	0
Total	180	240	0	0	60	300	360	60	60	0	300	180	120	120	0
Total%	**0.13**	**0.12**	**0.00**	**0.00**	**0.01**	**0.41**	**0.90**	**0.08**	**0.15**	**0.00**	**0.62**	**0.36**	**0.16**	**0.36**	**0.00**
Ostracoda															
Ostracoda sp.	300	1800	1200	2580	360	600	660	1200	120	0	1500	240	720	300	360
Total%	0.21	0.87	0.30	0.55	0.08	0.83	1.64	1.53	0.30	0.00	3.09	0.49	0.95	0.91	0.80
Total zooplankton	141300	206760	394920	465000	458220	72420	40200	78240	39960	22440	48540	49440	75780	33120	45240

Generally, the control treatment with high dietary protein level and external carbon supplementation showed the highest count for different zooplankton groups, especially for the total Protozoa (Table 2, Fig. 2). Except for Rotifer taxa, significant multilinear regression models were recognized among zooplankton group counts, water quality

condition and nutrient input in the tanks of the tilapia. The regression results and model equations are represented in Tables (3 & 4). Protozoa showed a positive regression relation with both TSS and nitrogen input ($R^2_{adjusted}$= 0.814, P<0.001).

Table 3. Results of backward stepwise linear regression analyses as dependent variables are the total count of Protozoa, Rotifera, Cladocera (*Alona intermedia*), Copepoda and Ostracoda

Zooplankton taxa		n	Beta	R^2	$R^2_{adj.}$	p
Total Protozoa (taxa)	Model					<0.001
	Nitrogen input	29	0.625	0.828	0.814	< 0.001
	TSS		0.742			< 0.001
Protozoa	Model					< 0.001
Centropyxis aculeate	Nitrogen input	29	0.503	0.736	0.716	< 0.001
	TSS		0.759			< 0.001
Protozoa	Model					0.002
Arcella vulgaris	Ash input	30	0.290	0.361	0.314	0.121
	TSS		-0.394			0.039
Cladocera	Model					<0.001
(Alona intermedia)	Phosphorus input	30	0.265	0.407	0.363	0.116
	Alalinity		0.479			0.007
Copepoda	Model					0.003
	Phosphorus input	15	0.499	0.623	0.560	0.028
	Alkalinity		0.423			0.056
Ostracoda	Model					0.003
	Energy input	30	0.403	0.350	0.302	0.016
	Centropyxis aculeate		0.369			0.027

n: number of observations; Beta, standardized regression coefficient; R^2,coefficient of discrimination (R squared); R^2_{adj} , adjusted R squared; P, probability.

The highest development was recorded when nitrogen input and TSS values ranged between 25-35 g and 400-600 mgL^{-1}, respectively (Fig.3). With respect to species, protozoan species of *Centropyxis aculeate* and *Arcella vulgaris* showed significant linear models. Both models were significantly associated with the values of TSS (Tables 3 & 4) but in a different way. *Centropyxis aculeate* showed a positive relation with the TSS values, while *Arcella vulgaris* showed the opposite.

Table 4. Regression equation of zooplankton groups, water quality parameters and nutrient input in tilapia tanks

Log_{10} *protozoa count = 2.209 + 0.059 Nitrogen input +0.003 TSS*

log_{10} *Centropyxis aculeate count= 1.892 +0.057 Nitrogen input + 0.004 TSS*

$\sqrt[3]{Arcella\ vulgaris\ count}$ *=32.220 + 1.880 Ash input -0.107 TSS*

$\sqrt[3]{Alona\ intermedia\ count}$ *= – 41.158 +16.240 Phosphorus input +0.170 Alkalinity*

Copepod count = –331.771+134.787 Phosphorus input+0.661 Alkalinity

$\sqrt[3]{Ostracoda\ SP.\ count}$ *=–24.681+0.015 energy input+ 4.475e-5 Centropyxis aculeate count*

The only cladoceran species recorded in the water samples was *Alona intermedia* and it showed a significant regression model ($R^2_{adjusted}$ = 0.363, P<0.001) with phosphorus input and alkalinity. On the other hand, Copepoda species showed the most

tenuous pattern among the zooplankton group in the three experimental treatments. Compared to the late weeks of this study, the early ones witnessed a higher recorded density (Fig. 1), followed by a severe reduction preceding the end of the experiment. A significant model (P=0.003) was estimated for Copepod taxa included alkalinity and phosphorus input in the tilapia tanks.

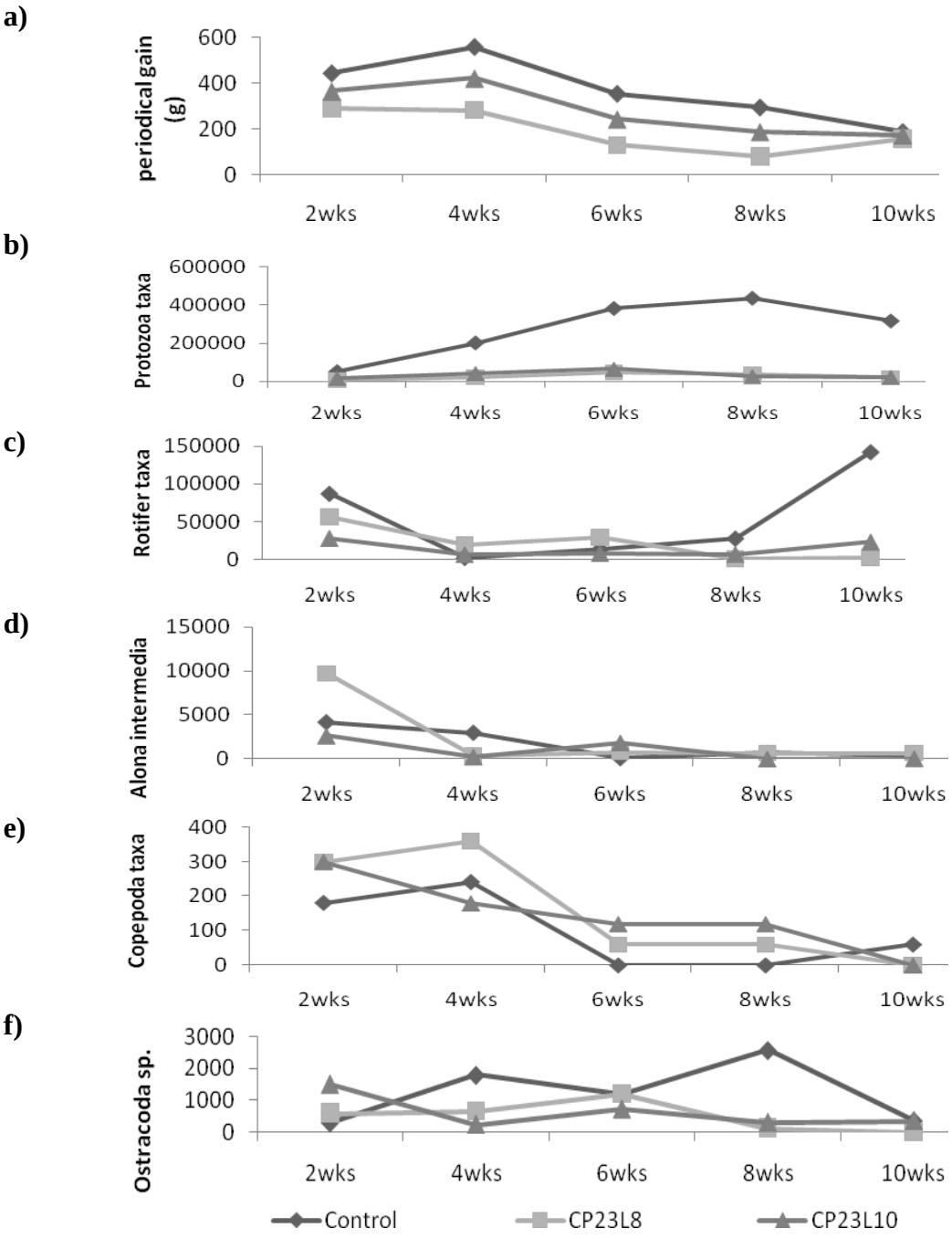

Fig . 1 . The periodical effect of different experimental treatments C, $P_{23}L_8$ and $P_{23}L_{10}$ on tilapia gain (a), Protozoa(b), Rotifera (c), Cladocera (*Alona intermedia*)(d), Copepoda(e) and Ostracoda taxa (f).

Despite that the effect of alkalinity on total Copepod count was not significant as phosphorus input; the model seems to explain 56% of the Copepod count by including both phosphorus input and alkalinity. Furthermore, the Ostracoda group showed a significant linear model (P= 0.003) with both energy input and Protozoa species of *Centropyxis aculeate*; both variables explain 30 % of the Ostracoda total count.

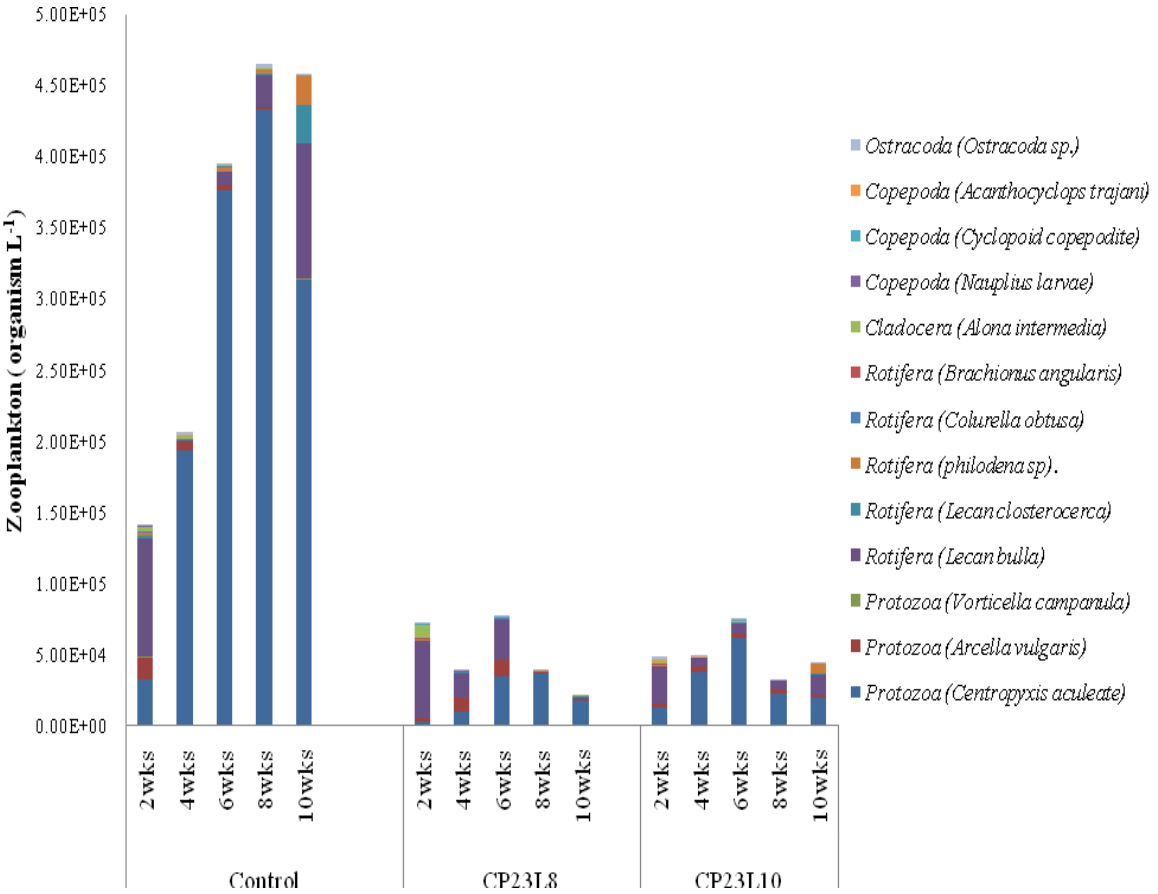

Fig.2. Periodical average count of different zooplankton species in different experimental tank

DISCUSSION

The periodical tilapia gain showed a general decreased pattern with the decreased count of large sized zooplankton (Rotifera, Cladocera, Copepoda and Ostracada). The periodical gain reduction was in parallel with the increased count of small sized species (Protozoa). A severe decrease in those taxa was more recognized in tanks that delivered low protein diets and no carbon source supplementation (Fig. 1). Protozoa and Rotifera represented the main groups regarding count under biofloc condition in the present experiment. This finding coincides with that of **Suloma *et al.* (2021)** who revealed that

Rotifer and Protozoa were the most abundant species in the biofloc of eel culture and deduced that their count increased with increasing the dietary protein level.

In the current study, the total Rotifer count showed no correlation with any of the independent variables except for a polynomial relation recorded with *Alona intermedia* (p=0.01). Reversely, **Zidan *et al.* (2017)** reported a significant correlation between Rotifer taxa and the dietary contents of selenium and phosphorus. With more accumulation of the organic matter and the gradual increase of TSS values, an increase in protozoa total count was noticed. *Centropyxis aculeate* was the predominant species regarding Protozoa taxa that showed a significant linear relation with the increase of TSS. **Singh (2009)** reported that, Protozoan count increased in the presence of organic matter. Ciliated Protozoa could become the dominant group because of its ability to consume free bacteria **(Gerardi*et al*., 1995; Madoni, 2017)** under biofloc condition. The latter finding support the validity of the multilinear regression models estimated in the present study for total Protozoa and *Centropyxis aculeate* count. Nitrogen input and TSS values significantly explained 81% and 72% of total Protozoa and *Centropyxis aculeate* count, respectively. The model could also explain the high Protozoan count in the control treatment where diet with higher protein content (30% CP) was delivered in the presence of carbon supplementation.

Alona is a Cadoceran species that rapidly responses to changes in environmental conditions (**Cortez-Silva *et al*., 2021**). This adaptive capacity has important implications for egg production and life cycle, especially in transitory environments (**Cortez-Silva *et al*., 2021**). It was reported that *A. intermedia* is more found in rich electrolyte water (**NBIC, 2021**), which can explain the significant effect of both phosphorus input and alkalinity on such species. Remarkably, the more developing the biofloc system becomes, the more the decrease in alkalinity and pH values is recorded. This occurs because of the consumed carbon by the bacteria. Consequently, a decrease of *A. intermedia* was observed with the development of the biofloc system, especially in treatments with no carbon supplementation. Moreover, it was recorded that the highest value of *A. intermedia* was detected at alkalinity level of 250 mg/L CaCO3 and phosphorus input of 3-3.5 (g) (Fig. 4). The Copepod taxa were noticed in the early phase of this experiment, but with a more developing stage of the system and it showed a severe decrease (Fig. 1). The model included phosphorus input and alkalinity that described 56% of the Copepod count. With the decrease of alkalinity, the lower count of Copepod in the tilapia tanks was recognized. The highest count was recognized when P input was between 3- 3.5g in the experimental tanks (Fig. 5). It is worthy to mention that, limited phosphorus source reduced the secondary production of copepod (*Acartia tonsa*), as no compensatory growth was recognized **(Malzahn & Boersma, 2012)**. Naupllii is the first larval stage of Copepod that was early detected during this experiment and disappeared at late stages .This could be attributed to the limitation of P input in the early experimental stage. Copepod nauplii have high phosphorus demands due to rapid growth **(Faithfull &**

Goetze, 2019). A negative correlation was detected between some copepod genera such as *Cyclops* sp. (r=-0.54), *Mesocyclops* sp. (r=-0.19), *Diaptomus* sp. (r=-0.32) and Co3 alkalinity. While, a positive correlation with HCO_3 – alkalinity was noticed recording values of r =0.47, 0.51, 0.58 , respectively **(Islam & Bhuiyan, 2007)**. The previous authors recorded an adverse observation for *Merocyclops* sp.; showing a positive correlation with CO_3-alkalinity (r=0.35) and negative correlation with HCO_3 alkalinity (r=-0.76). Energy intake and *Centropyxis aculeate* count explained 30 % of the Ostracoda sp. Count (Table 3 & Fig. 6). **Liperovskaya (1948)** suggested that the protozoan (*Vorticella* sp.*)* is one of the organisms that were found in the guts of the ostracod (*Eucypris crassa*).

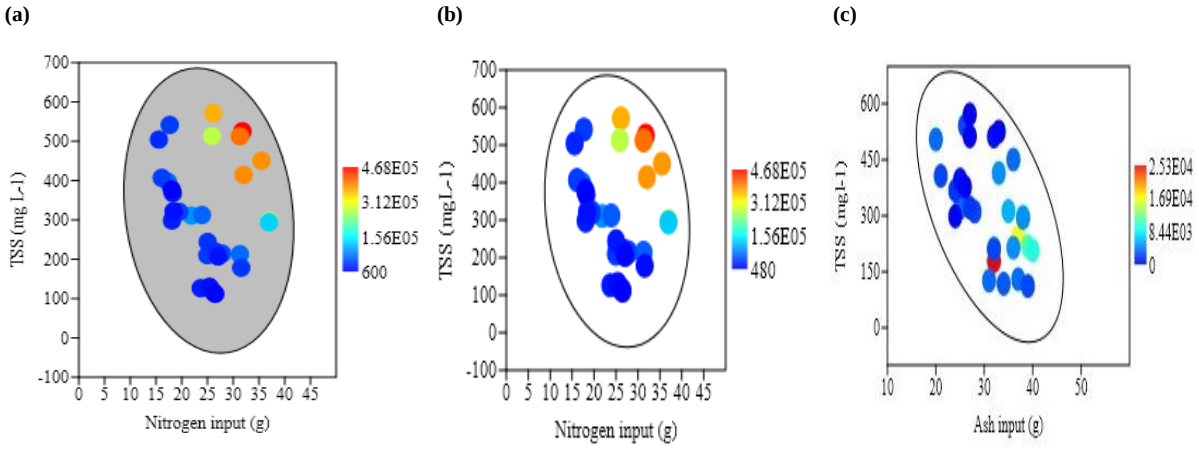

Fig. 3. Response of total Protozoa taxa (a) , *Centropyxis aculeate (b)* to TSS and nitrogen input and *Arcella vulgaris (c)* to TSS and Ash input .

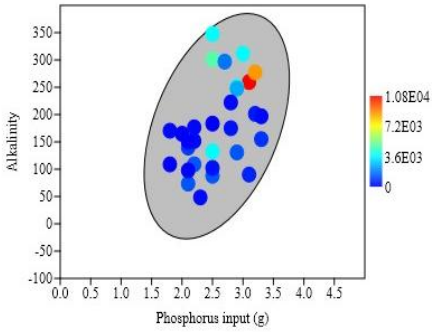

Fig. 4.Response of *A. intermedia* to Alkalinity and phosphorus input

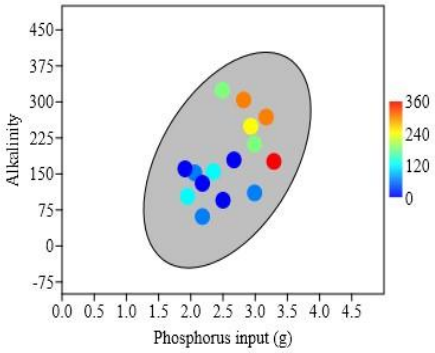

Fig.5. Response of Copepod taxa to Alkalinity and phosphorus input

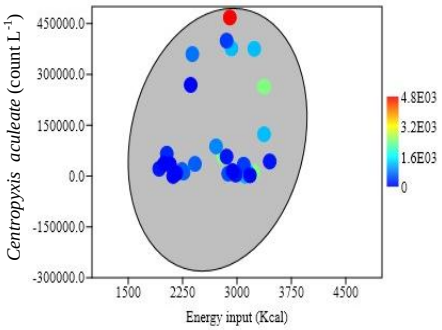

Fig.6. Response of Ostracoda taxa to energy input and *Centropyxis aculeate* count

CONCLUSION

It could be concluded that nutrient input as well as water quality parameters affected composition, dominance and abundance of zooplankton community species. Alkalinity and TSS are the major variables that affected the zooplankton community count, especially the large sized groups. Further studies are needed to study the effect of the levels and fluctuation of TSS and alkalinity on the large sized zooplankton count to attain fish growth performance.

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