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Effect of Different Doses of Rabbit Manure on the Abundance, Growth Rate and Production of Zooplankton in Plurispecific System for Fish Larvae Feeding

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Abstract In order to determine the optimal dose of rabbit manure for plurispecific production of fresh water zooplankton in controlled area, the effects of six different doses of dry rabbit manure (T_1 = 300 g/m^3, T_2 = 600 g/m^3, T_3 = 900 g/m^3, T_4 = 1200 g/m^3, T_5 = 1500 g/m^3, T_0 = 0g/m^3) on physico-chemical and biological parameters of areas were monitored for 27 days in cultures realized in plastic buckets of 80 L capacity each. Manures are from rabbits fed with diet previously revealed favorable to their growth, and is made of 2% of dried cassava, 30% of maize bran, 10% of palmist cake, 10% of soya cake, 5% of cotton cake, 2% of shell, 10% of malt, 5% of beer yeast, 10% of *Panicum maximum* and 1% of salt. Six days after fertilization, buckets were seeded with zooplankton by 26 individuals per liter (ind/L). Results obtained show that the supply of rabbit manure has improved chemical properties and microalgae production (phytoplankton) with treatments T_1, T_2, T_3, T_4 and T_5. Optimal conditions of zooplankton production through phytoplankton are obtained with doses in T_2 and T_4 which present the best specific growth rate. The daily production through the highest phytoplankton biomass (chlorophyll-a) has been obtained with dose in T_5. Thus, doses 600 g/m^3 and 1200 g/m^3 of rabbit manure may be considered optimal for a plurispecific production of fresh water zooplankton. It was noted that Rotifers were predominant in abundance in almost the different culture areas.

Keywords Rabbit manure; Optimal dose; Zooplankton; Plurispecific production

1 Background

The natural feed appreciated by fish larvae is essentially made of zooplankton and artificial dry feed are not always appropriated to this premature stage (Arimoro, 2005). Consequently, zooplankton such as cladocerans, copepods and rotifers are used for larval rearing by many researchers in controlled area (Awaïss et al., 1992). Artemia (live food) is the most used for certain marine species whose hatching, development and growth were realized in pond (Agadjihouèdé et al., 2011). Nevertheless, it remains less effective in fresh water fish larvae. Artemia as larvae food is inaccessible to rural fish farmers (Fiogbe et al., 2003). Indeed, its use doesn't always give good zootechnical performances to fresh water fishes. Zooplankton production in controlled area is carried out in many studies, by the way of phytoplankton production using different types of fertilizer (chemical, organic genuine vegetal or animal) (Adande et al., 2015). Chemical fertilizers (minerals) are the former used for phytoplankton production for the purpose of zooplankton production (Boyd et al., 2008) while organic fertilizers genuine vegetal or animal are the second used (Dalme et al., 2011). Between these two categories of fertilizer, the first type is dangerous to the environment and also expensive while the second type is less expensive and does not present any risk for the environment (Agadjihouèdé et al., 2011; Adande et al., 2017). These findings justify nowadays the easy use of organic fertilizers genuine animal such as cow and poultry manure (Agadjihouèdé et al., 2010), and pig dung (Ekelemu et al., 2011), in zooplankton production. The quantities of these organic fertilizers used influence too much the productivity of phytoplankton and indirectly of zooplankton and; according to the literature; there is an optimal dose for which a maximum production of zooplankton could be obtained. Indeed, different doses of manure have been tested in experiments on zooplankton production by many authors. Saint-Jean et al. (1994), for the production of Moina micrura used 0.05 g of poultry manure per liter of water; Agadjihouèdé et al. (2010) used in aquarium designed for zooplankton production 0.6 g of poultry manure per liter of water; by the same way, Akodogbo et al. (2014) used 0.3 g of pig dung per liter of water in plastic buckets during a plurispecific process of zooplankton production. Indeed, high production and survival rate of fish larvae are realizable from a low cost production of nutritive algae and zooplankton (Sipaúba-Tavares et al., 2007). That is the reason why, the aim of this study is to carry out the plurispecific production of fresh water zooplankton for larvae of *Clarias gariepinus* and *Heterobranchus longifilis*. The study looks to evaluate the progression of zooplankton density as a function of different doses of rabbit manure.

2 Materials and Methods

2.1 Experimental site

The experiment was conducted on the Diversification of Fish Farming Station of the Laboratory of Research on Wetlands that is located in the Department of Zoology of the Faculty of Science and Techniques, University of Abomey-Calavi (Benin). It is located between 6° 24' 53.3" N; 2° 20' 18.5" E and 6° 24' 53.4" N; 18.5" E and 9 m.a.s.l.

2.2 Experimental design

The experimental design is made of 18 plastic buckets of 80 L capacity each, exposed at free air on the previously described site. For this purpose, seventy-two hours before experiment starting up, buckets were cleaned and disinfected. It is made of six (06) treatments including one control such as: T_0, T_1, T_2, T_3, T_4, T_5 corresponding respectively to 0, 300, 600, 900, 1200, 1500 g of dry rabbit manure (RM) in one (01) m^3 of water replicated thrice. Buckets were filled with 40 liters of demineralized water and immediately fertilized. Manures are from rabbits fed with a low price diet but provided good performances on rabbits rearing (Adande et al., 2017; in press) and is made of 2% of dried cassava, 30% of maize bran, 10% of palmist cake, 10% of soya cake, 5% of cotton cake, 2% of shell, 10% of malt, 5% of beer yeast, 10% of *Panicum maximum* and 1% of salt. Manures contained 15.02%, 1.26% and 0.84% of N, P and K respectively.

2.3 Phytoplankton seeding process

After fertilizing, production areas were left for three days in order to enable nutrients releasing by washing so that they will be available to phytoplankton (microalgae). After this period, areas were seeded with phytoplankton according to Agadjihouèdé et al. (2010) and Akodogbo et al. (2015) by transferring ten (10) liters of polyculture (*Clarias* and *Tilapia*) pond water previously filtered with plankton net of 25 µm mesh to eliminate zooplankton.

2.4 Zooplankton seeding process

Zooplankton was seeded three (03) days after microalgae seeding, otherwise six days after fertilizing. Indeed, according to Guiral et al. (1994), this period is sufficient for microalgae development. To harvest zooplankton, three hundreds (300) liters of the previous pond were filtered with a net of 25 µm, and then concentrated in 500 mL of water. Each bucket was seeded with 25 mL of this filtrate. A sub-sampling of 50 mL was taken and formolled in order to perform individuals counting and zooplankton diversity study. The initial seeding density was 26 ± 0.23 ind/L otherwise (5 ± 0.13 ind/L nauplii of copepods (*Thermocyclops sp.*), 4 ± 0.23 ind/L adults of copepods (*Thermocyclops sp.*), 2 ± 0.14 ind/L of cladocerans (*Moina sp.* and *Daphnia sp.*) and 15 ± 0.41 ind/L of rotifers (*Brachionus sp.* and *Asplanchna sp.*). These three great groups of zooplankton are generally phytophage. According to Sprules (1980) and Pourriot et al. (1982), a great number of copepods become carnivorous at a given stage of their development. After its seeding on day 0 (D_0), zooplankton growth in different treatments was monitored every three days, at 8 a.m., for 24 days. During this period, a sampling was carried out every three days in order to follow biomass progression. Each sampling of zooplankton was preceded by homogenization of the culture, and then a taking of five (05) liters of water that are filtered with plankton net (25 µm mesh). The harvest was preserved according to Ara (2001) with 4-5% of formol in bottles of 100 mL until its analysis.

2.5 Image capture and zooplankton counting

Zooplankton counting was carried out according to Liady et al. (2015), by image treatment using Image Pro-Plus® software (Figure 1). Pictures were first captured from a microscope Optim Jeulin (X10) equipped by a photograph device (Figure 2). The technique of image treatment offering possibility to enlarge images during counting process enabled to distinguish easily rotifers from 120 µm. Different individuals of zooplankton were counted following species representativeness (at least 100 individuals were counted) in 1-2 mL of sample that were successively observed in a plastic saltcellar at densities that do not provoke overlapping among individuals.

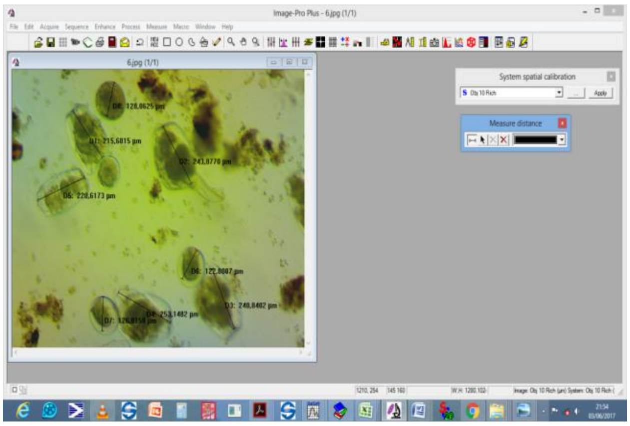

Figure 1 Zooplankton counting process

Figure 2 Technique of image capture

2.6 Physico-chemical and trophic parameters

During experiment, temperature, pH, conductivity and dissolved oxygen have been measured in situ every sampling day at noon with a multi-parameter borer CALYPSO (Version Soft/2015, SN-ODEOA 2138) à ± 0.1°C and by mg/L). At each sampling site, 500 mL of the culture was filtered per bucket on the one hand for dosage of chlorophyll-a and for dosage of nutritive salts (ammonium, nitrate, nitrite and orthophosphate) on the other hand. Dosage of chlorophyll-a was carried out according to SCOR-UNESCO (1966) while dosage of nutritive salts was realized according to Rodier et al. (2016), with a spectrophotometer of molecular absorption (HACH DR/2800).

2.7 Comparison of different treatments performances

Performances of the six treatments were compared following two methods:

The first consisted to the comparison of different densities obtained in treatments; the second consisted to compare specific growth rates of organisms during their exponential growth stage according to Liady et al. (2015). In this second method, growth curves of zooplankton in non- renewed area presenting identical stages to those observed in microorganism's cultures in non-renewed area, identical mathematical laws were considered. Thus, in each treatment, the exponential growth stage of density was considered and described by the formula (Waldbauer, 1968). From this formula, specific growth rate (µ) was determined by monitoring the progression of the logarithm of the relative density as a function of the time; it corresponds to the slope of the straight line obtained.

2.8 Statistical analysis

From data collected, density (D) and daily production (P) were obtained respectively by using these equations:

$$D = \frac{n \times V_2}{V_1 \times V_3} \text{ and } P = \frac{D_t - D_0}{t}$$

Where n = number of individuals counted; V_1 = volume of aliquot; V_2 = volume of sample concentrated; V_3 = volume of water filtered; D_0 = initial population density; D_t = population density at time t (in hour)

Collected data were analyzed using STATISTICA software (Statsoft inc., Tulsa, OK, USA). All significant levels were fixed at $P<0.05$. The influence of treatments was studied using a one-way ANOVA; in case of need, significance of differences among means was tested using the test LSD of Fisher.

3 Results

3.1 Water physico-chemical parameters

Different physico-chemical parameters of treatments varied strongly (Table 1 and Figure 3) during experiment period except temperature and pH which remained relatively constant. By the same way, conductivity is high in fertilized media with a significant difference among treatments ($F_{(5, 12)} = 33.92$; $p<0.00$). Dissolved oxygen (DO) is most important in treatments T_2 and T_5 followed by T_4 and T_3 compared to T_0 with a significant difference ($F_{(5, 12)}$ = 69.42; p<0.00) (Table 1). This oxygen concentration shows the photosynthetic activity of algae and consequently their presence in culture media. Moreover, salinity and TDS followed the same trend with a significant difference between T_0 and the other treatments respectively ($F_{(5, 12)}$ =11.60; p<0.00 and $F_{(5, 12)}$ = 7.79; p<0.00). Besides, the high value of transparency of Secchi's disk in treatment T_0 contrary to others explained clearly its low proportion in suspended matter and algae. Concentrations of N-NH3 obtained were important in all treatments except T_0 and varied between 0.06 ± 0.12 mg/L (T_0) and 2.82 ± 0.75 mg/L (T_5) (Figure 3). Concerning mean values of nitrite and nitrate, they varied respectively between 0.07 ± 0.11 mg/L and 0.08 ± 0.1 mg/L for T_0; 0.75 ± 0.35 mg/L and 0.81 ± 0.14 mg/L for T_5 (Figure 3). By the same way, orthophosphate concentration is high in all treatments except in T_0 and followed the same trend with a mean value in range of 0.5 ± 0.13 mg/L (T_0) and 3.15 ± 0.16 mg/L (T_5) (Figure 3). These different concentrations of in the different treatments were highly significant ($F_{(15, 36)}$ = 842.99; p<0.00). It results from this analysis that the organic fertilizer (rabbit manure) used had strongly affected the water quality of different culture areas in plurispecific production of fresh water zooplankton. Thus, it could be used as a fertilizer for primary production.

Table 1 Physico-chemical parameters of different treatments

Parameters	T_0	T_1	T_2	T_3	T_4	T_5
T°C	33.74±1.70a	34.02±1.81a	34.30±1.70a	34.34±1.87a	34.48±1.86a	34.51±1.78a
Cond (µs/cm)	37.66±23.17e	597.82±34.10b	604.50±23.17b	607.19±22.12b	625.58±36.08ab	664.45±50.63a
pH	6.87±0.94b	7.29±0.79a	7.33±0.94a	7.12±0.92a	7.09±1.05a	7.16±1.05a
DO (mg/L)	3.81±0.72f	7.48±1.95e	9.22±0.72bc	8.45±2.40bc	8.82±2.95ab	9.42±3.33a
Sal (mg/L)	0.08±0.01e	0.31±0.02b	0.32±0.01b	0.32±0.01b	0.33±0.01ab	0.36±0.03a
TDS	64.60±8.77e	296.92±17.07b	302.56±8.77b	303.43±7.47b	318.76±16.81ab	338.61±27.11a
Transp (cm)	27.91±0.37a	22.50±1.13b	16.85±1.51c	17.63±1.41d	14.43±1.38e	12.98±1.50f

Note: Cond = conductivity, DO = dissolved oxygen, Sal = salinity, Transp = transparency, TDS = total dissolved solids. Mean values affected by the same letter on the same line are not significantly different at the threshold of 5% according to LSD of Fisher

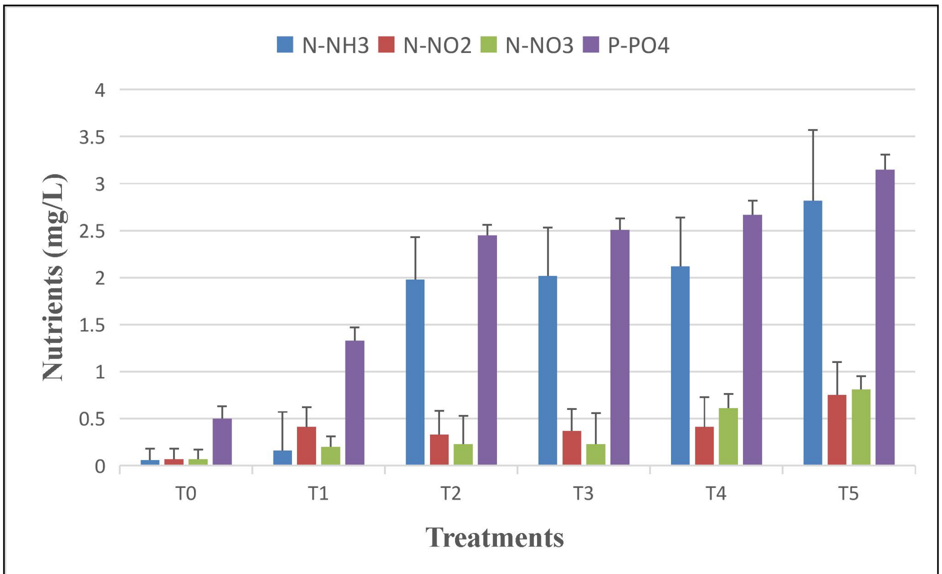

Figure 3 Nutrients proportion in different culture middle

3.2 Trophic parameter

Figure 4 expresses different concentrations of chlorophyll-a obtained during experiment in each treatment. There was a high significant difference among treatments ($F_{(40, 96)}$ = 1003.5; p<0.00). Also, we observed a strong linear correlation between the increase of chlorophyll-a rate and different doses of rabbit manure (R = 0.88; R^2 = 0.78;

R^2 Adjusted = 0.76; $F_{(1, 16)}$ = 55.608; p<0.00) tested. These different phytoplankton biomasses could serve for fresh water zooplankton development and indirectly for fish production.

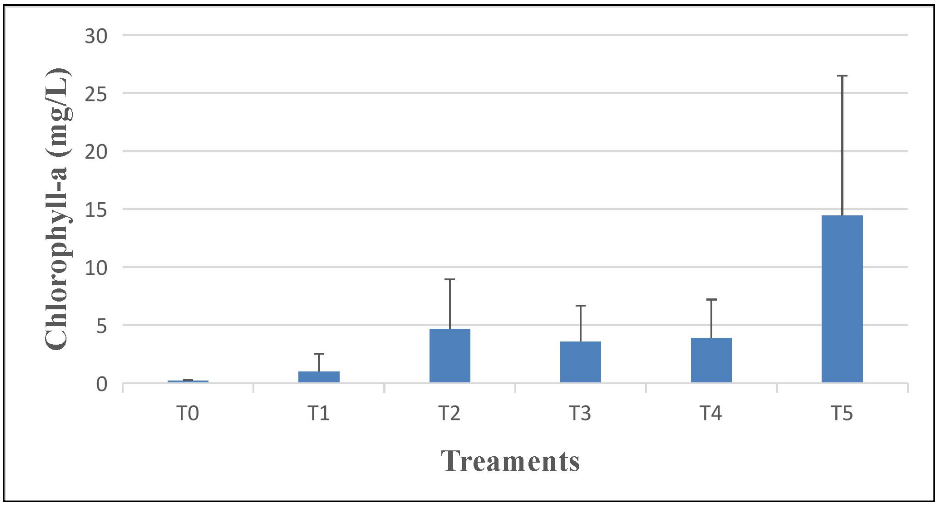

Figure 4 Chlorophyll-a rate of different treatments

3.3 Density progression and phytoplankton production

Treatments T_2 and T_4 showed the best densities of zooplankton. Indeed, the density passed from 15 ind/L to 12,207 ind/L for rotifers (*Brachionus sp.* and *Asplanchna sp.*) and 3467 ind/L for eggie rotifers. There was a significant difference among treatments ($F_{(7, 84)}$ = 41,685; p<0.00). Similarly, the density increased from 2 ind/L to 840 ind/L of cladocerans (*Moina sp.* and *Daphnia sp.*) with a significant difference ($F_{(7, 84)}$ = 738.18; p<0.00), but there was no difference between T_0 and T_1 (p>0.00). Nauplii of copepods (*Thermocyclops sp.*) increased from 5 ind/L to 140 ind/L with a significant difference ($F_{(7, 84)}$ = 329.10; p<0.00) between T_2 and T_3 on the one hand, and T_1 and T_0 and the other treatments on the other hand. However, there was no significant difference among T_2, T_4 and T_5 (p>0.00). In addition, density of adult copepods (*Thermocyclops sp.*) increased from 4 ind/L to 260 ind/L with a significant difference ($F_{(5, 12)}$ = 47.15; p<0.00) and followed the same progression trend as nauplii. Besides, treatment T_0 (non-fertilized) presented a low density regarding different groups of zooplankton, what could be explained by the impoverishment of the area in nutritive elements. Rotifers, adult copepods and cladocerans reached their optimum at the 9th day while nauplii of copepods and eggie rotifers reached their optimum at the 6th day (Figure 5). The decrease of the density began on the 12th day in all treatments and the 9th day for nauplii and eggie rotifers (Figure 5B; Figure 5D). Figure 5F presents the total density of zooplankton in different treatments.

3.4 Effect of treatment on the production and specific growth rates of zooplankton

Daily production of different group of zooplankton per treatment is reported in Table 2. Thus, the daily production was higher in all fertilized treatments than in the control (T_0) (p<0.05) (Table 2). The highest production was obtained in treatment T_2 followed by T_4 (Table 2). This high production of different groups of zooplankton in T_2 revealed that this area offered the most suitable conditions to the development of these organisms. Specific growth rates of different groups of zooplankton determined on the exponential growth stage are mentioned in Table 2. Their comparison showed a high significant difference among treatments regarding rotifers ($F_{(5, 12)}$ = 6,568.6; p=0.00), cladocerans ($F_{(5, 12)}$ = 25,705; p=0.00) and copepods ($F_{(5, 12)}$ = 3,586.5; p=0.00). The best specific growth rates (μ) were obtained in treatment T_2 followed by T_4 (Table 2). From the multiple analysis of treatments effect (test LSD of Fischer), it results that treatment T_2 followed by T_4 presented the best specific growth rates.

The supply of rabbit manure as organic fertilizer would be the source of this performance of zooplankton.

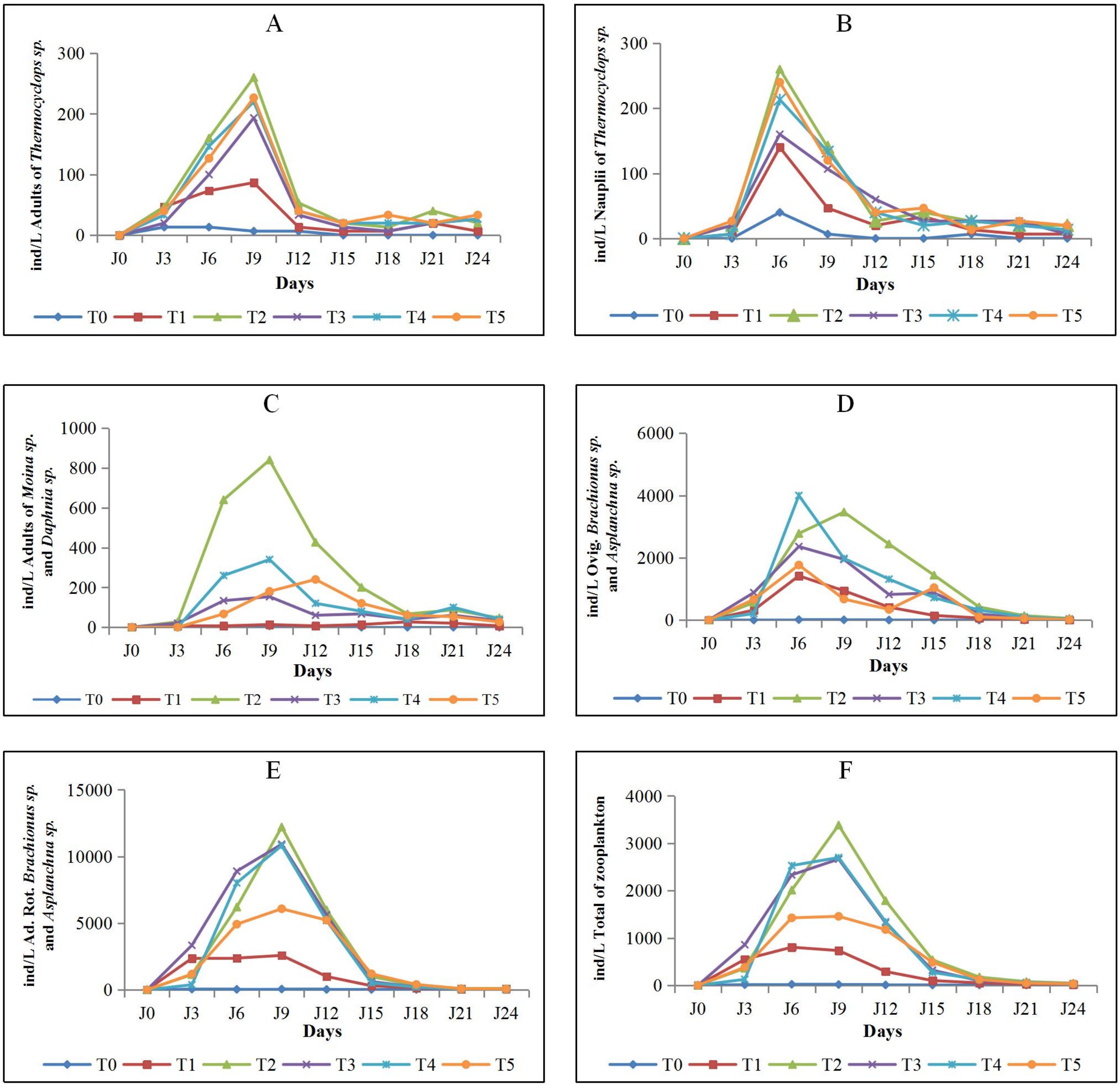

Figure 5 Variation of zooplankton densities in different treatments

Table 2 Production and specific growth rates of different groups of zooplankton

Traitements	Rotifer (*Brachionus sp* and *Asplanchna sp.*)			Cladoceran (*Moina sp.* and *Daphnia sp.*)			Copepods (*Thermocyclops sp.*)		
	P (ind/j)	μ (j^{-1})	R^2	P (ind/j)	μ (j^{-1})	R^2	P (ind/j)	μ (j^{-1})	R^2
T_0	2±0.48	0.10f	0.78	7±1.54	0.00ef	-0.10	7±11.54	0.00g	0.00
T_1	108±0.80	0.34e	0.90	13±1.54	0.00e	0.00	87±30.55	0.34f	0.99
T_2	509±1.27	1.48a	0.94	840±20	1.53a	0.82	260±20	1.28a	0.91
T_3	455±0.83	0.94c	0.87	153±30.55	1.09c	0.88	193±30.55	1.19b	0.92
T_4	451±1.27	1.30b	0.81	340±20	1.47b	0.97	220±20	0.89c	0.94
T_5	253±0.83	0.81d	0.84	180±20	0.54d	0.97	227±29.03	0.89ce	0.98

Note: μ = specific growth rate, R^2 = coefficient of correlation, P = daily production. Means followed by the same letter are not significantly different (P>0.05) according to the LSD test of Fisher

4 Discussion

The mean temperature in buckets surrounded 34.51 ± 1.78°C during the full period of experiment with a pH value in the range of 6.87 ± 0.94 and 7.33 ± 0.94. The limit of tolerance of fresh water zooplankton (rotifer for instance: Brachionus calyciflorus) is in the range of 15 and 31°C. The optimal value of pH is situated between 6 to 8 (Ludwig, 1993). Temperatures recorded during experiment were close to those found by Park et al. (2001) and Kabir et al. (2010) that fluctuate between 28 and 32°C during fish larvae feeding with great densities of rotifers. The conductivity was function of the area richness in minerals (NH_4^+, PO_4^-, NO_3^-, NO_2^-). It was higher than those obtained by Akodogbo et al. (2015) with pig dung and close to what was reported by Bokossa et al. (2014) with pig manure. This difference may be explained by the greatest richness of rabbit manure (compared to pig dung) in mineral salts necessary to primary production that is the base of organisms' development. These results are comparable to those obtained by Agadjihouèdé et al. (2011) with poultry manure. Indeed, the use of rabbit manure had significantly increased nutrients concentration in the water and accordingly enabled a good primary production for zooplankton development. These results are comparable to those obtained by Dalme et al. (2011) during zooplankton production process from the use of animal wastes. The effects of rabbit manure are revealed by the concentration of chlorophyll-a in treatments T_1, T_2, T_3, T_4 and T_5. Concentration of chlorophyll-a obtained in these different treatments were higher than those reported by Canovas et al. (1991) that is 2 mg/L as minimal value for a good phytoplankton production. Thus, treatments T_1, T_2, T_3, T_4 and T_5 are favorable to zooplankton development. The most important zooplankton concentration was obtained in treatment RM_{1500} and could be explained by its strong load in organic matter due to the strongest concentration of orthophosphate and ammonium. Indeed, phosphorus in the shape of orthophosphate (assimilable) is the prime element required for vegetal (algae) development (Reynold, 1980; Schlumberger et al., 2002). In addition, the low transparency value obtained in treatments T_2, T_3, T_4 and T_5 indicate the strong load in algae in treatments these areas particularly in T_3 and T_5. This great load could be the source of low density recorded in these treatments. However, oxygen levels recorded in different doses were slightly higher than those recorded by Agadjihouèdé et al. (2011), in the range 5.5±0.9 and 6.10±1.21. Such difference could be due to different in time of parameters measurement. Highest chlorophyll-a rates were obtained in doses of T_5 though, the density of zooplankton was low. Reversely, the treatment T_2 provides excellent nutritive conditions to zooplankton.

4.1 Production and specific growth rates of zooplankton

Preferable densities of zooplankton were given by doses in T_2 and T_4 (600 g/m^3 or 0.6 g/L and 1200 g/m^3 or 1.2 g/L). Several studies were based on the relationship between algae biomasses and zooplankton production (Sendacz et al., 2006; Ekelumu et al., 2011). This relationship could be characterized by optimal concentrations lower than which zooplankton growth is exponential and upper than which this growth is inhibited (Ovie et al., 2002; Liady et al., 2015). This matter could explain low productions of zooplankton observed in T_5 and T_3 compared to those observed in T_2 and T_4. The inhibition of zooplankton growth by high biomasses of algae might be due to their respiration (especially at night) that provokes zooplankton asphyxia. Indeed, high densities of algae tend to impact negatively the availability of oxygen for zooplankton that is competed by bacteria that also need it to deteriorate (mineralize) rabbit manure in order to make nutritive elements available to algae. In addition, the development of an important algae biomass is not sufficient for good fish farming; the nature of algae species and their height is to be considered (Lazzaro et al., 1995; Barbe et al., 2010). In the same way, treatments T_2 and T_4 provided the best specific growth rates with a best daily production among the different zooplankton species in culture areas with an abundance of rotifers. Indeed, this abundance of rotifers is a trump for larval rearing by considering their small height in relation to the diameter of larvae muzzle. Specific growth rate recorded were upper than those obtained by Agadjihouèdé et al. (2014) that are 0.74, 0.85 and 0.21 respectively for *Brachiomus calyciflorus*, *Moina micrura* and *Thermocyclosps sp.* This difference could be explained by the nature of fertilizer that is function of the quality of the animal diet. It results from this analysis that treatment T_2 (600 g/m^3) would be the favorable dose for an optimal plurispecific production of fresh water zooplankton.

5 Conclusion

This experiment showed that the doses of 600 g/m^3, 1200 g/m^3 and 1500 g/m^3 in rabbit manure provided very good zooplankton productions. Nevertheless, the doses of 1200 g/m^3 and 1500 g/m^3 could provoke eutrophication of the culture area. For an optimal production of zooplankton, the dose of 600 g/m^3 in rabbit manure offering the best conditions for zooplankton production could constitute the optimal dose of dry rabbit manure to be proposed in controlled area. Being aware that of the major challenges in fish farming is the feeding at different growth stages, it could be suggested the possibility to adopt different doses of rabbit manure as a function of the individual height of zooplankton to be produced in majority. Unfortunately, with almost the different doses applied, it was noted that rotifers were predominant in abundance.

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