

## Comparison of two Techniques on the Optimization of Zooplankton Production from Pig Dung: Renewed and Non-Renewed Medium

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### Abstract

Present study aimed at the comparison of two production techniques of the zooplankton mass production (renewed and non-renewed medium) so as to determine the most efficient. In fact, the experimentation was realized in triplicate in plastic buckets, grouped together in three treatments ( $T_1$ ,  $T_2$  and  $T_3$ ) which were fertilized and a control ( $T_0$ ) during 27 days. The buckets were seeded in zooplankton with an initial density of 37 individual.l<sup>-1</sup>( $D_0$ ). From  $D_{12}$ , those of  $T_2$  and  $T_3$  were periodically fertilized and have respectively sustained a partial and periodical renewal and fishing (50%) in the production medium. The results have shown that the optimization in non-renewed medium has given the best zooplankton production ( $p < 0.05$ ). Thus, the mean zooplankton density was higher in treatment  $T_3$  ( $896 \pm 977 \text{ ind.l}^{-1}$ ) as compared to  $T_2$  ( $631 \pm 440 \text{ ind.l}^{-1}$ ) where the medium was renewed. The best technique is the one made in the non-renewed medium as it also favored a continuous zooplankton production for a long period whereas the production in renewed medium is discontinuous and for a short duration. The adoption of this technique permitted to high densities of small sized live prey (rotifers) for several weeks, for the aquaculture hatcheries.

**Keywords:** Comparison, Non-Renewed medium, Optimization, Pig dung, Renewed medium, Zooplankton.

### 1. Introduction

Fish farming natural resources are increasingly rare and threatened due to the overexploitation of water ponds linked to the people demographic boom. To feed the human population, it is therefore imperious to promote aquaculture, including fish farming whose development goes essentially through the success of larval rearing which requires the availability of zooplankton [1,2,3]. Yet, the most used zooplankton for the feeding of the fish larvae was *Artemia* [4,5]. But, the utilization, mostly in developing countries is difficult because of these cysts hatching conditions, its high cost and its availability on the local market [6]. It is then important to make intensive zooplankton production at low cost for the fish farming development.

Besides, intensive culture systems from the periodical diminishing of the zooplankton density permitted the local zooplankton mass production. It is concerned with the culture in renewed medium which consisted in the elimination of a part of the production medium and its replacement by new water [7] and the culture in non-renewed medium which consisted in fishing a part of the zooplankton population [8,9,10]. These productions, often monospecific, are constraining [11,12]. It is

therefore indispensable to determine a method less constraining and efficient for the zooplankton production. This is what shows the importance of present study which main objective is the comparison of two techniques (renewed medium and non-renewed medium) for the optimization of local plurispecific zooplankton production with pig dung so as to determine the most efficient. It would permit to the rural pisciculturists to ensure a simple and intensive production of the local live food (zooplankton) during a long period and would consequently favor the reduction of fish larvae production costs.

### 2. Materials and Methods

#### 2.1 Experimental design

The experiment was constituted of twelve (12) plastic buckets with 80 liters of capacity, disposed in free air, at wetlands research station, University of Abomey-Calavi, Benin, in which were respectively poured 40 liters of drilling water and 10 liters of pond water. These buckets were grouped into three triplicates of three treatments ( $T_1$ ,  $T_2$  and  $T_3$ ) and a control ( $T_0$ ). The buckets of treatment  $T_1$  were fertilized once whereas those of treatments  $T_2$

and T<sub>3</sub>, in addition to the initial dose have been periodically fertilized. In addition, the production medium of treatment T<sub>2</sub> was partially and periodically renewed, whereas those of T<sub>3</sub> have sustained a partial fishing of organisms.

Before putting the water in the buckets, these latter were have been washed with bleaching water and dried for 24 hours. The following day, they have received 40 liters of drilling water. Immediately after this, buckets of treatments T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were fertilized by pig dung with the optimal dose of 600 g.m<sup>-3</sup> [13]. Three (03) days after the fertilization, all the buckets were seeded in phytoplankton with 10 liters of pond water green enough filtered with a silk of 50 µm. Three days later (D<sub>0</sub>), sufficient period to allow the growth of phytoplankton [14], of zooplankton, harvested in a pond, with 50 µm plankton net, was seeded in each bucket with an initial density of 37 ind.l<sup>-1</sup> (7 ind.l<sup>-1</sup> of rotifers; 28 ind.l<sup>-1</sup> of copepods and 2 ind.l<sup>-1</sup> of cladocerans). From D<sub>12</sub>, the contents of the T<sub>2</sub> buckets were renewed at 50% with pond water filtered with a silk of 50 µm [12] whereas those of T<sub>3</sub> have sustained a partial fishing (50%) of their population every three days [15]. The fertilization has been renewed with the third (1/3) of the initial dose every three days in the treatments T<sub>2</sub> and T<sub>3</sub> buckets [12,15].

## 2.2 Zooplankton production follow-up

Zooplankton was sampled from the D<sub>7</sub>, every three days [15], until the 27<sup>th</sup> production day. In each bucket, 5 liters after homogenization of culture medium, water sample was taken and then filtered through a silk of 50 µm for the zooplankton harvest; this filtrate was fixed with 5% formaldehyde. Some under-samples of this harvest were taken with an Eppendorf pipette (capacity: 1000ml) and observed under a light microscope (PIERON S/N S 294452 X 4). The present zooplankton organisms were enumerated to evaluate the densities (D) of the different zooplankton groups. The daily production (P), the intrinsic increase rate (Kr) and the doubling time (Td) of the zooplankton population were calculated from the following formula:

$$\begin{aligned} -D &= (N/V_1) \times (V_2/V_3) ; \\ -P &= (N_1 - N_0)/t ; \\ -Kr &= (\ln N_1 - \ln N_0)/t; \\ -Td &= \ln_2/Kr; [16]. \end{aligned}$$

Whereas N = number of individuals counted in an under-sample; V<sub>1</sub> = observed volume (under-sample); V<sub>2</sub> = concentration volume; V<sub>3</sub> = filtered water volume; N<sub>1</sub> = final number per liter; N<sub>0</sub> = initial number per liter and t = production time.

## 2.3. Measurement of physico-chemical and trophic parameters

The physical and chemical parameter of the culture medium (temperature, pH, conductivity and dissolved

oxygen) were *in situ* measured, respectively with a multiparameter conductimeter W340i and an oxymeter ANNA (HI 9143 Microprocessor Auto Cal Dissolved Oxygen Meter). Diverse chemical analyses of the water in each bucket were then carried out with 500 ml of water sample were collected in plastic bottle. Then, the ammonium, the nitrates, the nitrites and the phosphates were respectively measured by the Nessler-380 methods, to Cadmium-335 reduction, to Diazotation-371 and to Phosver-490 with the spectrophotometer HACH.

Similarly, 500 ml of water sample has been drawn from each bucket into other plastic bottles (0.5 l of capacity), has allowed appreciating the phytoplankton quantity through the measure of chlorophyll *a* (trophic parameter). Each bottle was packed inside aluminium paper to prevent sample photosensitivity. The chlorophyll *a* measurement has been achieved by spectrophotometer according to Pechar method [17].

## 2.4. Statistical analysis

The statistical analysis of the obtained results was performed with statistic logiciel SAS version 9.2 by analysis of variance method with one criteria (ANOVA I) [18]. The LSD (Least Significant Difference) of Fisher [19] was used to compare the different average.

## 3. Results

### 3.1 Variation of physico-chemical and trophic parameters

Table 1 summarizes the physico-chemical and trophic mean values of different treatments. According to Table 1, the mean value of water in buckets was around 31.25 ± 0.68°C. The pH mean values were around 6.11 ± 0.46 and slightly fluctuated; those of dissolved oxygen were 5.63 ± 0.36 mg.l<sup>-1</sup>. The conductivity and average concentrations of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup> varied from one treatment to another. They were higher in the buckets of treatment T<sub>3</sub> (non-renewed medium). As for the NO<sub>2</sub><sup>-</sup> the obtained concentrations were low in all medium. The variance analysis with one classification criteria (ANOVA I) applied to the values of different parameters revealed significant differences of conductivity, ammonium, dissolved oxygen and phosphates rates between the treatments T<sub>2</sub> and T<sub>3</sub> (p < 0.05). But the difference was not significant for the temperature, pH, nitrates and nitrites between these treatments (p > 0.05).

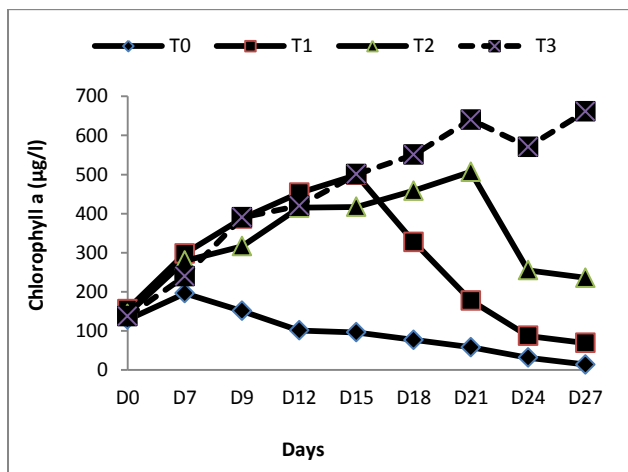
The mean values of chlorophyll *a* concentration (Table 1), were higher in fertilized buckets (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>), whereas they were too low in unfertilized medium (T<sub>0</sub>). In fact, those values were more important in the treatment T<sub>3</sub> buckets. The variance analysis with one classification criteria (ANOVA I) applied to the different chlorophyll *a* concentration values revealed significant differences between the different treatments (p < 0.05). The evolution of chlorophyll *a* concentration during the

**Table 1** Physico-chemical characteristics and chlorophyll *a* concentration of different treatments

	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Temperature (°C)	31.10 ± 0.66 <sup>a</sup>	31.21 ± 0.59 <sup>a</sup>	31.41 ± 0.77 <sup>a</sup>	31.28 ± 0.70 <sup>a</sup>
pH	6.18 ± 0.41 <sup>a</sup>	6.16 ± 0.33 <sup>a</sup>	5.95 ± 0.66 <sup>a</sup>	6.16 ± 0.43 <sup>a</sup>
Dissolved oxygen (mg.l <sup>-1</sup> )	5.36 ± 0.20 <sup>a</sup>	5.57 ± 0.31 <sup>b</sup>	5.88 ± 0.54 <sup>c</sup>	5.72 ± 0.41 <sup>d</sup>
Conductivity (µS.cm <sup>-1</sup> )	71.90 ± 4.01 <sup>a</sup>	127 ± 7.03 <sup>b</sup>	135.81 ± 24.29 <sup>c</sup>	151.99 ± 35.92 <sup>d</sup>
NH <sub>4</sub> <sup>+</sup> (mg.l <sup>-1</sup> )	0.13 ± 0.05 <sup>a</sup>	0.44 ± 0.27 <sup>b</sup>	0.67 ± 0.88 <sup>c</sup>	1.03 ± 0.50 <sup>c</sup>
NO <sub>2</sub> <sup>-</sup> (mg.l <sup>-1</sup> )	0.007 ± 0.003 <sup>a</sup>	0.010 ± 0.005 <sup>a</sup>	0.011 ± 0.006 <sup>a</sup>	0.011 ± 0.003 <sup>a</sup>
NO <sub>3</sub> <sup>-</sup> (mg.l <sup>-1</sup> )	4.99 ± 1.38 <sup>a</sup>	6.46 ± 4.19 <sup>b</sup>	10.73 ± 3.11 <sup>c</sup>	11.06 ± 4.90 <sup>d</sup>
PO <sub>4</sub> <sup>3-</sup> (mg.l <sup>-1</sup> )	1.23 ± 0.60 <sup>a</sup>	7.94 ± 1.67 <sup>b</sup>	8.12 ± 4.39 <sup>b</sup>	12.56 ± 7.90 <sup>c</sup>
Chlorophyll <i>a</i> (µg.l <sup>-1</sup> )	95.02 ± 57.83 <sup>a</sup>	272.95 ± 157.73 <sup>b</sup>	336.51 ± 119.52 <sup>c</sup>	456.78 ± 178.07 <sup>d</sup>

The values affected with the same letter in exponent on the same line were not significant different ( $p > 0.05$ ).

experimentation (Figure 1) has shown that the culture medium of treatment T<sub>1</sub> and T<sub>2</sub> have reached their peak on 15<sup>th</sup> and 21<sup>st</sup> production day and then fall progressively till the end of the experimentation. On the other hand the chlorophyll *a* rate in treatment medium T<sub>2</sub> has increased during all the experimentation.

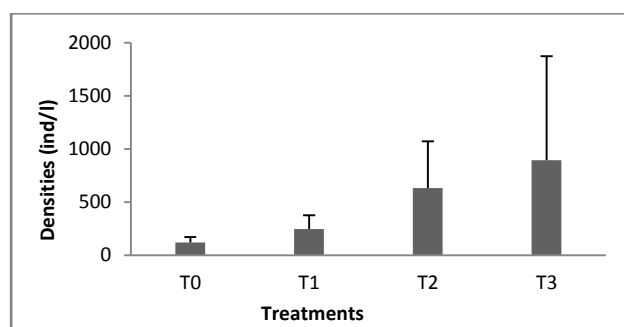


**Fig.1** Evolution of chlorophyll *a* concentration of different treatments in function of time

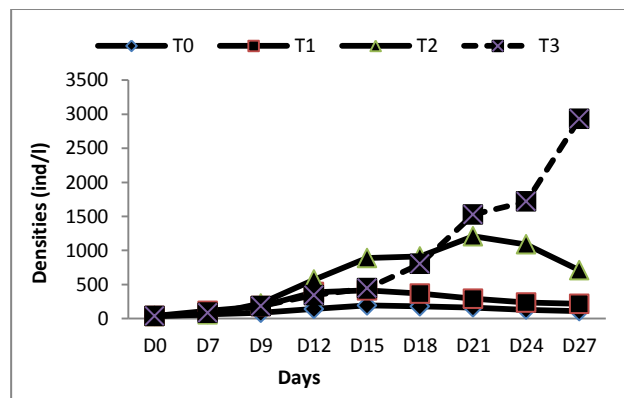
**3.2 Variation of zooplankton densities**

The analysis of Figure 2 showed that the total zooplankton average densities during the experimentation, were higher for the treatment T<sub>3</sub> medium (896 ± 977 ind.l<sup>-1</sup>), it was followed by the ones of T<sub>2</sub> (631 ± 440 ind.l<sup>-1</sup>). The variance of analysis with one classification criteria (ANOVA I) revealed a significant difference between the total zooplankton average densities in the different treatments ( $p < 0.05$ ).

Results presented in Figure 3 revealed the evolution of the total zooplankton densities with the time.



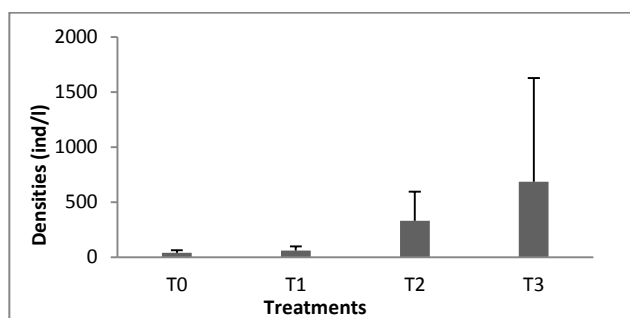
**Fig.2** Zooplankton total average densities by treatment



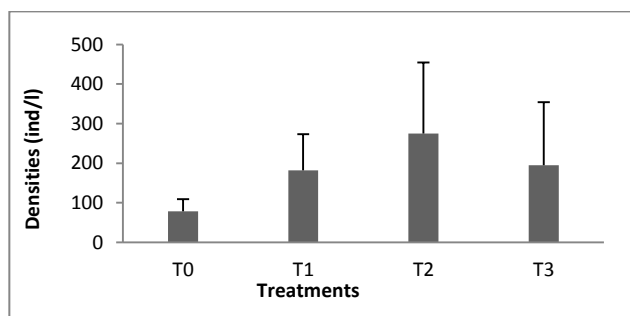
**Fig.3** Evolution of total zooplankton average densities of different treatments in function of time

From D<sub>15</sub>, the zooplankton density was ameliorated in treatments T<sub>2</sub> and T<sub>3</sub>; but it slightly increased in T<sub>2</sub> with a peak (1207 ind.l<sup>-1</sup>) at D<sub>21</sub> and then decreased slightly till the end of the experimentation at D<sub>27</sub> (708 ind.l<sup>-1</sup>). This density sustained an exponential increase in T<sub>3</sub> from D<sub>15</sub> (444 ind.l<sup>-1</sup> to D<sub>27</sub> (2929 ind.l<sup>-1</sup>). On the other hand, the one of T<sub>1</sub> decreased progressively from D<sub>15</sub> where they reached their peak (413 ind.l<sup>-1</sup>) till the end of the experimentation at D<sub>27</sub> (215 ind.l<sup>-1</sup>). From D<sub>0</sub> to D<sub>27</sub>, the

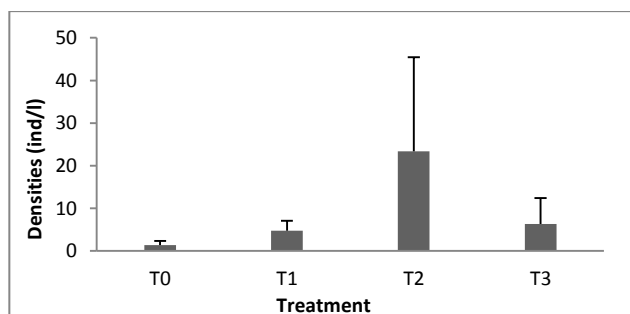
daily production, intrinsic increase rate and doubling time of zooplankton population for the treatment T<sub>2</sub> were respectively 24.85 ind.l<sup>-1</sup>.d<sup>-1</sup>; 0.11 in 24 hours and 6.34 days whereas those of treatment T<sub>3</sub> were respectively 107.13 ind.l<sup>-1</sup>.d<sup>-1</sup> ; 0.16 in 24 hours and 4.28 days. The daily production of treatment T<sub>3</sub> during that period was 8 times the one of treatment T<sub>2</sub>. From D<sub>15</sub> to D<sub>27</sub> the daily production, intrinsic increase rate and doubling time of zooplankton population of treatment T<sub>3</sub> were respectively 207.12 ind.l<sup>-1</sup>.d<sup>-1</sup>; 0.16 in 24 hours and 4.41 days; whereas, zooplankton production of the treatment T<sub>2</sub> has fallen during that period.



(a) Rotifers



(b) Copepods

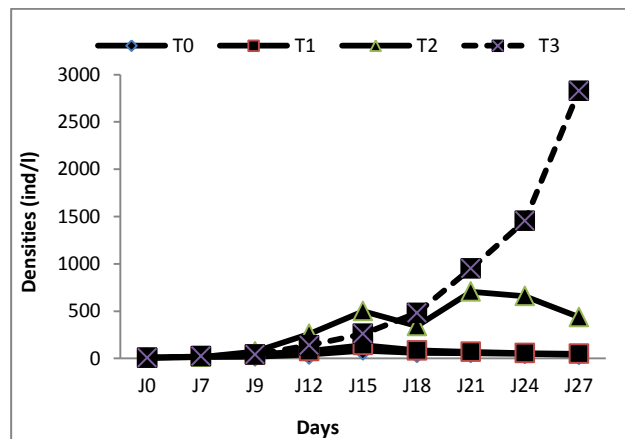


(c) Cladocerans

**Fig.4** Different zooplankton groups' density by treatments

Figure 4 showed that the average densities of different zooplankton densities (rotifers, copepods and cladocerans) by treatment. Thus, the rotifers were more important in treatment T<sub>3</sub> (687 ± 941 ind.l<sup>-1</sup>) which was

followed by the T<sub>2</sub> (333 ± 265 ind.l<sup>-1</sup>). As for the copepods and the cladocerans, they were higher in treatment T<sub>2</sub>. In fact, the evolution of the rotifers density of different treatments in function of time (Figure 5) showed that the rotifers have an exponential growth from D<sub>15</sub> (259 ind.l<sup>-1</sup>) at D<sub>27</sub> (2829 ind.l<sup>-1</sup>) in T<sub>3</sub> whereas they sustained a short amelioration during that period in T<sub>2</sub>.



**Fig.5** Evolution of total rotifers average densities of different treatments in function of time

**4. Discussion**

The average temperature of buckets was around 31.25 ± 0.68°C during the study time, this temperature was conform to that which permitted the production of high density of rotifers (28 and 32°C) obtained during the fresh water rotifers culture, *Brachionus calyciflorus* [20]. Likewise, Ludwig [21] has shown that the rotifers could tolerate a temperature which is around 31°C. The pH mean value was 6.11 ± 0.46 and slightly fluctuated. It has permitted a good zooplankton development in production medium, as this value was around the optimum of 6.5 [22]. It was also comprised between the one obtained by Kabir et al., [11] for a good growth of rotifers (6-8°C). The conductivity and the mean concentrations of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup> and PO<sub>4</sub><sup>-</sup> were the highest in treatment T<sub>3</sub>; as the latter were not renewed; there has been an accumulation of fertilizers which liberated mineral salts. The physico-chemical water quality changed in function of the quantity of fertilizers introduced in the medium [23]. These results were due by the positive effect of pig dung on the nutritious quality of water [24].

Progressive decrease in the concentration of chlorophyll *a* observed in treatments T<sub>1</sub> from D<sub>15</sub> was due by the exhaustion of the culture medium, during the time, in nutritious salts [25,26]. The fall of chlorophyll *a* concentration observed in the treatment T<sub>2</sub> from D<sub>21</sub> though the periodical fertilization, was due by the elimination of a part of the nutritious salts and the phytoplankton population during the renewal of these medium. On the other hand, in treatment T<sub>3</sub> medium, the

amelioration of chlorophyll *a* rate and therefore of the phytoplankton, was due to the maintaining of their content which was periodically fertilized. That fertilization liberated permanently nutritious salts required to the phytoplankton development in the production medium. The water renewal has then provoked the dilution of the culture medium. The periodical fertilization in non-renewed medium has a positive effect on the quantity of the liberated mineral salts and consequently on the phytoplankton development. This confirmed that the phytoplankton development depend on nutritious salts [27].

The average zooplankton densities, reared in periodical fertilized medium ( $T_2$  and  $T_3$ ) compared with  $T_1$ , were due to the elevated rates in phytoplankton in these medium which received additional doses of fertilizers. This confirmed the fertilizing effect of pig dung on the zooplankton production [24,28]. The fall of zooplankton density in medium  $T_1$  from  $D_{15}$  was due to the decrease in phytoplankton density, because the zooplankton peak coincided with the phytoplankton one. This proved the zooplankton dependence towards the phytoplankton which constitutes their food [29]. This reduction of zooplankton density was also due to the exhaustion of the fertilizers in nutritious substances required for the phytoplankton development. As in 20 days, the organic matter was completely mineralized in the water [30]. This justified the correlation between the nutritious salts, the phytoplankton population and the zooplankton [24,31]. Likewise, the works of Akodogbo et al., [13] showed that the maintain time of the optimal dose of pig dung was 14 days.

The average zooplankton densities of treatment  $T_2$  has not fallen after  $D_{15}$  because of the medium renewal following the fertilization. We also observed a regular increase of these densities after the first three stripping. These results confirmed the ones obtained by Saint-Jean et al., [12] on the *Moina micrura* production, which showed that the renewal of the half of the medium and the organisms harvest is expressed by a sensitive rise of the zooplankton density after the first two stripping. This renewal eliminated a part of pollutants (metabolites) and avoided the congestion and the sudden death of the population due to the lack of food [32,33]. It improved the zooplankton density which was dominated by the rotifers. This technique diluted the medium and allowed a discontinuous production of zooplankton [34].

The improvement of the total zooplankton densities of treatment  $T_3$  ( $896 \pm 977 \text{ ind.l}^{-1}$ ) in relation to  $T_2$  ( $631 \pm 440 \text{ ind.l}^{-1}$ ) after  $D_{15}$  were due to the higher phytoplankton density in the non-renewed medium and periodically fertilized. The food (phytoplankton) is available for the zooplankton, therefore their good growth. The partial fishing followed by the periodical fertilization also avoided the sudden death of the zooplankton population for different reasons: congestion, lack of food, auto-regulation, decreased of fecundity

[10,33]. It improved the total zooplankton density of production medium with the dominance of rotifers. This harvest eliminated, from the zooplankton population, a part of the big sized organisms of which some were in food competition (cladocerans) with the rotifers and others which were the predators (copepods). That allowed the rotifers development which was small sized species [35,36]. In addition, it eliminated from the production medium, the older individuals which have a low fecundity rate, favoring the appearance and the development of amictic females. In fact, the age of rotifers female parents influenced significantly the fecundity which decreased linearly with the age. The amictic females have a very high increase rate as they are parthenogenetic individuals [37]. This confirmed the higher production of rotifers population in treatment  $T_3$ . This technique permitted the zooplankton continuous production in mass.

Zooplankton mass production in renewed medium permitted to get satisfactory population densities which were maintained during few days (larval rearing period). Whereas those of non-renewed medium permitted to get higher zooplankton population densities and favored their continuous production for a long period (several weeks); it was then efficient. So, the optimization of local zooplankton production from pig dung in non-renewed medium was the best technique to adopt for the hatcheries of fish farming. Nevertheless, it could be combined with the medium renewal after a long fishing period so as to eliminate from the medium, a part of metabolites which constitute pollutants.

## Conclusion

Plurispecific production of local zooplankton from pig dung could be optimized in renewed and non-renewed through zooplankton partial harvest. The best technique is the one realized in non-renewed medium as it gave not only the best plankton densities but favored the continuous zooplankton production for a long period; whereas the production realized in renewed medium was discontinuous and of short duration. The adoption of this technique allowed getting higher densities of small sized live prey (rotifers), for several weeks, for the pisciculture hatcheries in order to decrease the larval production cost.

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