

Effect of pig dung fertilizer on zooplankton production

Hervé H. AKODOGBO^{1*}, Clément A. BONOU² & Emile D. FIOGBE¹

¹Research Laboratory on Wetlands (LRZH), Department of Zoology, Faculty of Sciences and Technics, University of Abomey-Calavi (UAC), B.P. 526 Cotonou, Benin.

²Research Laboratory in Applied Biology (LARBA), Polytechnic School of Abomey-Calavi, University of Abomey-Calavi (UAC), B.P. 526 Cotonou, Benin.

*Corresponding Author; E-mail: hakodogbo@yahoo.fr

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ABSTRACT:

Objectives: To test the effect of pig dung fertilizer on zooplankton production in research station on wetlands at the University of Abomey-Calavi in Benin.

Methodology and Results: The fertilization was carried out using pig dung inside treatment T₁ buckets, whereas the control medium (T₀) was not fertilized. The medium was seeded with phytoplankton. Each bucket was seeded with zooplankton with initial density of 52 individual/l. The zooplankton density evolution was followed through sampling every seven days from the seeding. The trophic and physico-chemical parameters were recorded. The pig dung utilization improved the chemical properties of the medium water. That fertilization had a significant effect ($p < 0.05$) on plankton production. Thus, the fertilized media offered the best phytoplankton biomass and the best zooplankton maximum density of 1071 individual/l.

Conclusions and application of findings: The zooplankton production is realizable with pig dung. The dynamic of zooplankton population, points out copepods dominance which are rotifers and cladocerans predators.

Key-words: pig dung, fertilization, zooplankton production.

INTRODUCTION

Aquaculture development, especially fish culture, is currently an important economical activity for African countries. So, for a successful outcome, larvae rearing requires some live food (zooplankton), mostly for the species with small eggs whose larvae carry a small size vesicle of which the vitellus is rapidly resorbed. This is particular about catfishes, *Clarias gariepinus* and *Heterobranchus longifilis*. Legendre & Teugels (1991) and Legendre (1992) have shown larvae of the *H. longifilis*, descended from very small eggs, weighing not more than 2 mg at the vitellin resorption end, either 48 hours after hatching and present at food diet only composed of zooplankton till 5-6 days. It's this necessary to supply to these fish larvae some live prey (zooplankton) in order to allow

them to pass this zooplanktonophage stage at their best. This importance of live prey in the larvaculture has increased the laboratory production interest (closed/controlled system) or in the opened systems (uncontrolled). But the production in laboratory requires high financial means, unlike the opened system which is a cheaper alternative (Tavares *et al.*, 2009). Live prey utilization in rearing fields was an already widespread practice in many Asian countries (fukusho, *et al.*, 1976; kurcha *et al.*, 1977), European (Geiger, 1983; Barnabé, 1991; Awais & Kestemont, 1992; Fiogbé *et al.*, 2003) and American (Whitehouse & Lewis, 1973; Lewis, 1979; Herbert, 1995). Some rare studies have been carried out in the West African region (Legendre *et al.*, 1987; Saint

Jean *et al.*, 1994; Wade *et al.*, 1999; Dhawan & Kaur, 2002; Francis *et al.*, 2003; Orji & Chibugwu, 2010; Ekelemu & Nwabueze, 2011). Most of these different studies frequently realized in the laboratory with monospecific rearing, constraining and requiring some specialists, have displayed their limits (Saint-Jean *et al.*, 1994). These zooplankton production operations have been done inside fertilized media (organic or inorganic fertilizers) or rich in seaweeds. Among the organic fertilizers used were animal manure of which poultry dropping and cow dung which have been often used, and sheep and horse

manure which were less utilized and at last pig dung which was rarely used. In Benin, only one zooplankton production study (Agadjihouédé *et al.*, 2010a, 2010b, 2011) had been conducted with the poultry dropping. Such a situation was primarily related to a lack of mastering simple and cheap techniques of zooplankton production. The studies could be diversified and developed in this field. This justifies the present study which aims to test the effect of fertilization with the pig dung, a pollutant for the environment, on outdoors zooplankton plurispecific production.

MATERIAL AND METHODS

Experimentation plan: Six (06) plastic buckets with a capacity of 80 litres (l) were used for zooplankton plurispecific production. Three (03) of these buckets contents were fertilized with pig dung (feed with *Azolla* mixed to the rice and palm oil) at ratio of 15g of manure dry weight (Agadjihouédé *et al.*, 2010a) in 40 litres of drilling water whereas the three other containing also 40 litres of drilling water were not fertilized (control). Seeding in phytoplankton (10 litres of pond water green enough filtered on a silk of 50 µm) all the buckets three (03) days after the fertilization. After that, the harvested zooplankton were concentrated in a pond using a plankton net of 50 µm three days later (D₀). 5 ml of this concentrate was fixed with formaldehyde at 5% for enumeration on the microscope. Each bucket was seeded with zooplankton, at 15 ml of this concentrate (D₀) and finally calculated from the under-sample formalised, of respective densities of each zooplankton group from which a culturing rate of 28 ind/l of copepods was obtained (nauplii+ copepodites + adults), 13 ind/l of rotifers and 11 ind/l of cladocerans in each bucket.

Zooplankton harvest : The zooplankton were sampled every seven (07) days after the culturing for 21 days (D₂₁). 10 l of water from each bucket was taken, after homogenization of the culture medium, and filtration on a silk of 50 µm for zooplankton harvest. After fixing the filtrate with formaldehyde at 5%, the filtrate under-samples were observed under light microscope

(Pierron, S/N S294452 / X4). The zooplankton organisms were enumerated to evaluate the densities of the different zooplankton groups. Zooplankton biomass was calculated, multiplying each zooplankton group density by their average dry weight. The dry weight of rotifers, of copepodites and copepods adults; copepods nauplii and of the cladocerans were 0.18; 0.08; and 1.32 g respectively (Legendre, 1987; Gras & Saint-Jean, 1981b).

Physico-chemical and trophic parameters follow up: 500 ml of water was taken from each bucket in plastic bottles (0.5 l of capacity) for different chemical analyses (ammonium, nitrates, nitrites, and phosphates, respectively by Nessler-330 methods, of reduction with Cadmium-335, of diazotation-371 and of Phorver 3-490 with HACH Spectrophotometer). 500 ml of water from each bucket in plastic bottles was also taken, and placed inside aluminium paper to prevent sample photosensitivity, for the chlorophyll *a* measurement (trophic parameter) by Pechar method, 1987. The measurement was made in situ of the physical parameters such as the pH, the conductivity and water temperature with the dissolved oxygen.

Statistical analyzes: The statistical analysis of obtained results was performed with statistic logiciel SAS version 9.2 by analysis of variance method with one classification criteria (ANOVA I) (Scherrer, 1984; Dagnelie, 1984). The LSD (Least Significant Difference) of Fisher (Saville, 1990) was used to compare the different means.

RESULTS

Variation of physico-chemical, trophic and zooplankton parameters: The tables 1 and 2 summarize

the different physico-chemical, trophic and zooplankton parameters in the rearing medium.

Table 1: physico-chemical, trophic and zooplankton parameters in the control medium (T₀).

Parameters	D ₀	D ₇	D ₁₄	D ₂₁	Means Average	Standard Deviation	Variations Coefficients (%)
pH	6.7	6.02	5.89	5.86	6.12	0.39	6.44
Temperature (°C)	28.63	28.78	28.7	28.05	28.54	0.33	1.17
Conductivity (µS/cm)	517.5	537.5	568.67	608	557.92	39.47	7.07
Dissolved oxygen (mg/l)	6.07	5.74	5.73	5.99	5.88	0.17	2.95
NH ₄ ⁺ (mg/l)	0.06	0.07	0.05	0.13	0.08	0.04	44.91
NO ₂ ⁻ (mg/l)	0.009	0.02	0.009	0.008	0.012	0.006	48.25
NO ₃ ⁻ (mg/l)	5.72	11.29	6.75	7.28	7.76	2.44	31.48
PO ₄ ³⁻ (mg/l)	0.5	0.96	0.93	0.16	0.64	0.38	59.83
Chlorophyll a (µg/l)	138.87	206	131.15	101.2	144.32	44.22	30.64
Rotifers (ind/l)	13	21	25	14	18	6	31.70
Copepods (ind/l)	28	56	71	110	66	34	51.76
Cladocerans (ind/l)	11	40	31	21	26	13	49.57
zooplankton total density (ind/l)	52	117	127	145	110	40	36.36
zooplankton total Biomass (µg/l)	21.05	73.57	63.513	47.17	51.326	22.93	44.676

Table 2: physico-chemical, trophic and zooplankton parameters in fertilized medium (T₁).

Parameters	D ₀	D ₇	D ₁₄	D ₂₁	Means Average	Standard Deviation	Variations Coefficients (%)
pH	6.69	5.95	6.66	6.84	6.53	0.4	6.11
Temperature (°C)	28.68	28.72	28.23	27.82	28.36	0.42	1.5
Conductivity (µS/cm)	557.67	552.3	577.83	611.3	574.79	26.72	4.65
Dissolved oxygen (mg/l)	5.72	5.38	5.61	5.79	5.62	0.18	3.19
NH ₄ ⁺ (mg/l)	0.23	0.37	0.26	0.28	0.28	0.06	21.20
NO ₂ ⁻ (mg/l)	0.04	0.05	0.03	0.02	0.03	0.01	42.21
NO ₃ ⁻ (mg/l)	6.72	16.45	7.65	6.67	9.38	4.74	50.55
PO ₄ ³⁻ (mg/l)	5.17	6.02	5.86	5.33	5.59	0.41	7.26
Chlorophyll a (µg/l)	143.07	391.6	214.39	135.2	221.07	119.1	53.88
Rotifers (ind/l)	13	248	171	72	126	104	83
Copepods (ind/l)	28	204	262	237	183	106	57.93
Cladocerans (ind/l)	11	619	33	16	170	299	176
zooplankton total density (ind/l)	52	1071	466	325	479	431	89.98
zooplankton total Biomass (µg/l)	21.05	911.8	106.47	62.05	275.33	425.7	154.62

Physico-chemical parameters: The variance analysis with on only criteria (ANOVA I) applied to the different parameters values (table 1 and 2) revealed significant differences of ammonium, nitrites, nitrates and phosphates rates between the fertilized and unfertilized

medium ($p < 0.05$). But the difference was not significant for the temperature, the conductivity, the pH and dissolved oxygen between the fertilized and the unfertilized medium ($p > 0.05$).

Chlorophyll a : The figure 1 shows the evolution of chlorophyll a concentration in function of time in the

fertilized medium and the control.

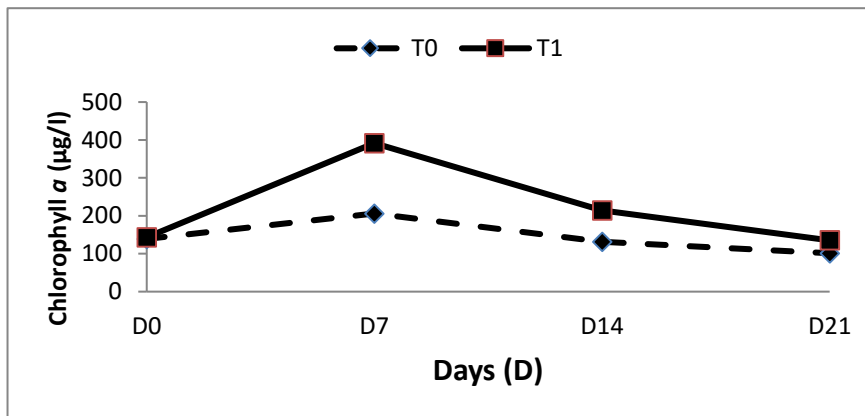


Figure 1: evolution of chlorophyll a concentration in function of time in the fertilized medium and the control

The variations of chlorophyll a concentrations presented the same appearance in the two types of medium. But the chlorophyll a concentrations were higher in the fertilized medium than in the control. The seaweed peak was obtained at D₇ before decreasing at the end of the experiment (figure 1).

Zooplankton: The zooplankton groups identified in this study were: the rotifers the copepods and the cladocerans (tables 1 and 2). The figure 2 showed the zooplankton total biomass evolution in the rearing medium (control and fertilized) in function of time.

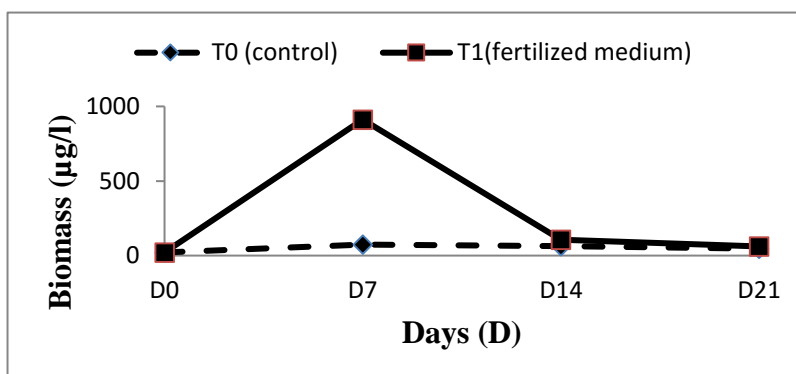


Figure 2: zooplankton total biomass evolution in the rearing medium (control and fertilized) in function of time.

The total zooplankton biomass was more slight in the control medium during all experimentation period compared to the fertilized medium (figure 2). In the fertilized medium, between D₀ and D₇, the average biomass of zooplankton recorded increases from 21.05 µg/l to 911.8 µg/l, approximating 43 times the seeding biomass inside one week. It was the same thing concerning the zooplankton average densities of these fertilized medium (from 52 ind/l to 1071 ind/l) approximating 21 times of the seeding rate in one week

(table 2). After D₇ the zooplankton biomasses and densities progressively decreased till the end of the experiment. A comparison of the averages through Fisher's test applied to the zooplankton biomass and densities (tables 1 and 2) at the threshold of 5% detected a significant difference between the two types of culture medium ($p < 0.05$). The figure 3 shows density evolution of different zooplankton groups in the control medium (non-fertilized) in function of time.

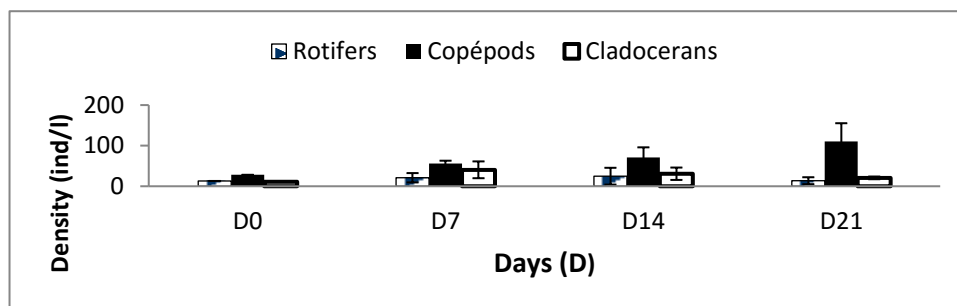


Figure 3: evolution of different zooplankton groups' density in the control medium (non-fertilized) in function of time.

Inside the non fertilized medium (figure 3), the following was noticed:

- The copepods were a majority during all the experiment followed by the cladocerans, only at the seeding where the rotifers dominated the cladocerans;
- The copepods density increases regularly from D₀ to D₂₁; the one of cladocerans increases from D₀ to D₇,

then decreases progressively till the end of experiment. As for the rotifers, they have reached their peak at D₁₄.

The figure 4 shows the evolution of different zooplankton groups' density inside the fertilized medium in function of time.

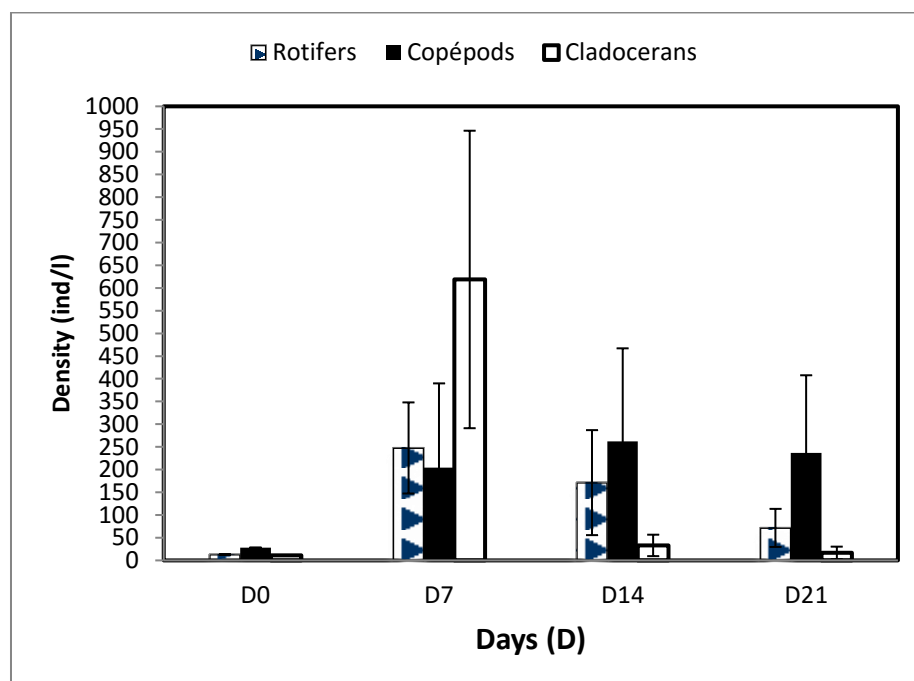


Figure 4: evolution of different zooplankton groups' density inside the fertilized medium in function of time.

In the fertilized medium (figure 4), that the following was noticed:

- The rotifers and the cladocerans reached their peaks after one week of the culture. The rotifers rate progressively decreased till the end of the experiment whereas that of the cladocerans fell very quickly;
- The copepods densities weakly vary from D₇ to D₂₁. But these organisms have reached their peak at D₁₄.

Considering the figures 3 and 4, it was recorded that at each sampling the different zooplankton groups from fertilized medium displayed densities higher than the ones of the non-fertilized medium.

Relation between zooplankton biomass, the chlorophyll a and dissolved salts : The figure 5 presents the compared evolution of zooplankton biomass, of the chlorophyll a and the dissolved salts rates during the experiment.

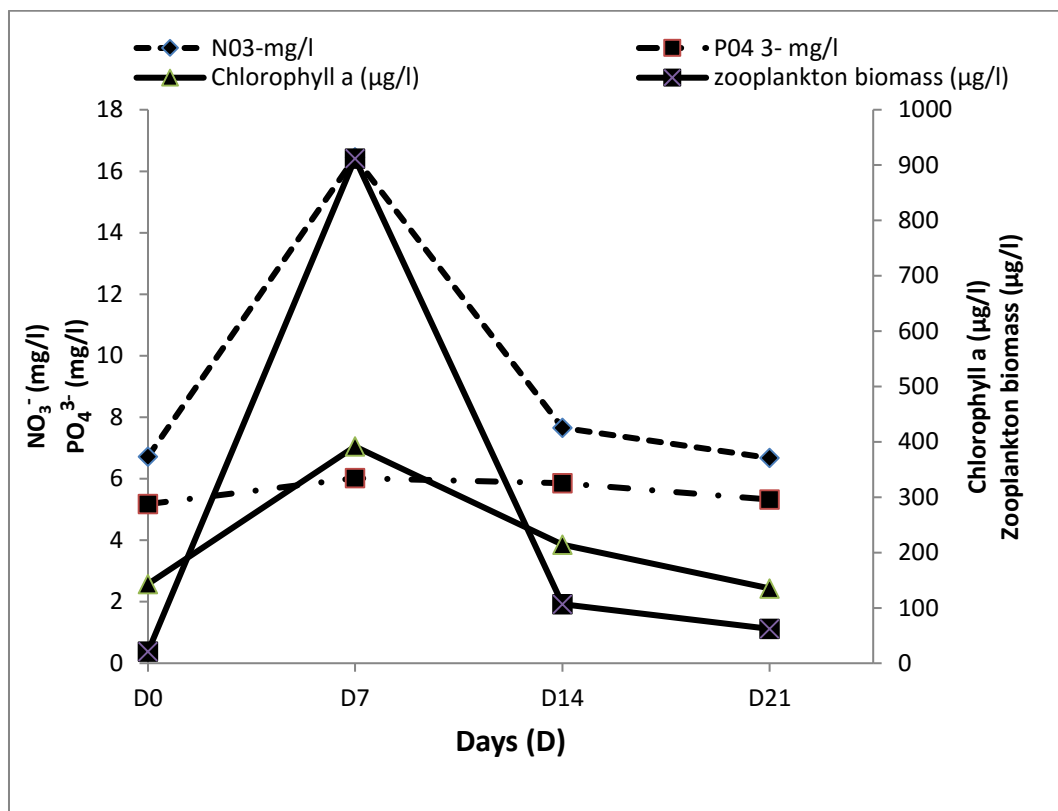


Figure 5: evolution of zooplankton biomass, of the chlorophyll a and the dissolved salts rates during the experiment.

Rates of NO₃⁻, PO₄³⁻, the chlorophyll a and the biomass evolution of the fertilized medium zooplankton, get the same look during the experiment (figure 5). These rates values have reached their peak at D₇ and they have progressively decreased till the end of the experiment. The correlation coefficients between NO₃⁻ and chlorophyll a (0.98), between PO₄³⁻ and the chlorophyll a (0.87) and between chlorophyll a and zooplankton biomass (0.97)

are positive and very strong. There is a positive linear correlation between these parameters. The nutritive salts and the chlorophyll a are thus linked. It's the same situation between this latter and the zooplankton biomass. So chlorophyll a concentration evolution of the medium depends on that of the dissolved salts; in the same case, the zooplankton biomass rate is function to that of the chlorophyll a.

DISCUSSION

Pig dung offers satisfying conditions for plurispecific zooplankton rearing. In fact, the average pH inside the fertilized buckets (6.53 ± 0.4) allows the good zooplankton development because its value stands around the optimum which is 6.5 (Carballo *et al.*, 2008). But this value is slightly inferior than the one obtained by Agadjihouédé *et al.* (2010a) with the poultry dropping (7.7 ± 0.2). This difference could be explained by the utilized fertilizer nature. In the same way, this pig dung allows a supply in nutrients for the rearing medium. So, the ammonium concentrations, the phosphates, the nitrates and nitrites ions are higher in fertilized buckets and are significantly different from the ones of the control buckets (non fertilized). These results are comparable to those

obtained by Dhwan *et al.* (2002) with pig dung and with those obtained by Shep (1994) and Agadjihouédé *et al.* (2010a) with poultry dropping. This enrichment of fertilized medium in nutritive salts might be due to mineral salts liberation by the organic matters (pig dung) after their mineralization. But the dissolved salts rates fall in these medium could be linked to the manure exhaustion in nutritive substances which have stayed inside water for twenty-one (21) days. This confirms Berard (1993) works that showed the soluble matters of organic fertilizers completely mineralized in the water within twenty (20) days. Phosphates average rates of fertilized buckets (between 5.17 mg/l and 6.02 mg/l) are comparable to those obtained by Sevrin-Reyssic (1994) in the basins of

seaweed cultures and of *Daphnia* livestock with pig manure (between 3.7 mg/l and 9.9 mg/l). The works of Agadjihouédé et al. (2010a) realized in the aquariums of zooplankton production with poultry dropping have provided average values ranged between 3.64 and 9.53 mg/l which were also conformed to our results.

Pig dung utilization has improved water physico-chemical properties. This confirms Parvez et al. (2006) works and those of Tavares et al. (2009) who have showed organic wastes utilization as fertilizer, improved water physico-chemical properties. This nutrients improvement (phosphates and nitrates) leads to an increase of the medium zooplankton biomass. This pig dung has brought necessary nutrients to an important primary production. In fact, in the aquatic medium phytoplankton organisms multiplication and growth are under nutritious resources control as inorganic nitrogen and the dissolved orthophosphate in the water (Dabbadie, 1996; Schlumberger & Bouretz, 2002). The dissolved nitrogen (N-NH_4) and N-NO_3) and the orthophosphates (PO_4) are utilized for the phytoplankton development (Billard & Marie, 1980; wurtz-Arlet, 1980; Boyd, 1982).

This phytoplankton development is confirmed by the measurement of chlorophyll *a* rate, of which the values are high in the fertilized medium but this rate has progressively decreased since the 7th day (391.6 $\mu\text{g/l}$) till the end of the experiment (135.2 $\mu\text{g/l}$). This drop might be linked to the nutritious elements exhaustion (inorganic N and P-PO_4) released in the water through pig manure and which might then be insufficient to favour a good development of phytoplankton. The same observations have been done by Shep (1994) who noticed during three live preys (*Moina micrura*, *Diaphanosoma excisum* and *Thermocyclops sp*) in ponds that seston reduction (seaweeds, rubbishes or microorganisms) was linked to a lack of N-NH_4 , and by Agadjihouédé et al. (2010a) in the aquariums of zooplankton production with poultry dropping. This preference of seaweeds for nitrogen ammoniacal form was a known phenomenon (Pourriot et al., 1982). Phytoplankton production depends not only of the medium richness in nitrogen and phosphorus, but also of the exerted predation by the zooplankton (Billard

et al., 1980; Wurtz-Arlet, 1980; Boyd, 1982). In fact the zooplankton production obtained in the fertilized buckets is clearly better than the one of the control. This can be early explained by the better primary production mentioned above. The phytoplankton serves as food to zooplankton. Then, for a good zooplankton production in a medium, abundant food in seaweed is needed (Seyer, 2002). In the culture medium, it goes out clearly again the zooplankton is dependent of phytoplankton, which itself depends of nutritious salts available in these medium. A such of relation has been demonstrated and confirmed before through many studies by McQueen et al. (1989), Vanni et al. (1990), Lazzaro & Lacroix (1995) who have found a positive correlation between the enrichment in nutrients and the phytoplankton biomass and then between phytoplankton biomass and the one of zooplankton. The utilization of organic fertilizer has a positive effect on the phytoplankton and zooplankton abundance (Geiger, 1983; Kang'ombe et al., 2006). The zooplankton production in fertilized ponds with organic fertilizer shows that chlorophyll *a* and zooplankton rates are higher in these ponds than in the control (Geiger, 1983). By elsewhere it can be noticed at the 7th day when the zooplankton density is maximal, the cladocerans and the rotifers reach their peaks and dominate the copepods. But during the farming (from D_{14} to D_{21}) the copepods reinforce their presence whereas in the same time the rotifers decrease notably in density; likewise, the cladocerans rate falls in the medium. Such of dynamic can be explained with the predator character of copepods adults on the rotifers and the cladocerans. So the copepods get control on zooplankton populating of the production like what is done in the natural medium. This analysis is conforming to the one done by Bonou (1990) concerning the zooplankton populations in aquaculture ponds in Ivory-Coast and by Agadjihouédé et al. (2010a) on the dynamic of zooplankton production in aquariums. In the same way, Brooks & Dodson (1965) have recorded zooplankton big species dominated the small one in absence of predation by the fishes because they fed themselves efficiently.

CONCLUSION

The zooplankton plurispecific rearing is realizable with pig dung. Water fertilization with these dejections is profitable and improves chemical elements and phytoplankton biomass concentrations of the medium. This fertilization is also responsible of an important

zooplankton production composed of rotifers, copepods and cladocerans. Populations are controlled by the copepods which are the predators of other zooplankton groups.

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