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Article

Empirical Formula for Determining Freshwater Zooplankton Biomass from Pigs Manures of the Piggeries in Forest Guinea

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Abstract : To identify an ideal dose of pig manure from piggeries in the Forest Guinea for the production of zooplankton in rural fish farms for larval breeding, three doses including a control of 0 (T₀), 300 (T₁) and 600 (T₂) g/m³ were tested for forty-five days. The experimental device of three buckets with a capacity sixty liters per treatment was each filled with 40 liters of demineralized water and immediately fertilized with the different doses. Three days after fertilization, phytoplankton was seeded followed by zooplankton seventy-two hours later. The initial seeding density was 35±7.62 ind/L or (23±1.13 ind/L of copepod nauplii or 1.84±0.13 µg/L (*Thermocyclops sp.*), 4±0.1 ind/L or 1.88±0.1 µg/L copepod adults (*Thermocyclops sp.*), 1±0.007 ind/L or 2.7±0.7 µg/L cladocerans (*Moina sp. and Daphnia sp.*) and 3±1.48 ind/L or 0.21 ± 1.48 of rotifers (*Brachionus sp and Asplanchna sp.*). The results show that doses of 300 (T₁) and 600 (T₂) g/m³ of pig slurry gave good primary production, the highest of which was obtained with T₂ (4.63±2.48 mg/L) with a strong correlation with zooplankton biomass (r² between 0.96 and 0.98). Biomasses recorded in T₂ were 594.35±24.93 µg/L rotifers, 589.73±18.98 µg/L copepods and 449.95±18.15 µg/L cladocerans. This dose could be considered ideal for rural fish farmers in forested Guinea.

Keywords: Forest Guinea; pig manure; phytoplankton; zooplankton; biomass

1. Introduction

Food and agriculture are essential to achieve all the Sustainable Development Goals (SDGs) directly related to fisheries and aquaculture (SDG 14) [1]. However, many sub-Saharan African countries struggle develop and transform fish farming into viable economic activity. Indeed, despite the many potentials they have, they only contribute less than 0.5% the 1.5% the tonnage of African aquaculture production [2]. The majority African countries, the scarcity, unavailability food and the cost this food really slow down aquaculture general and fish farming particular [3,4]. In addition, the success larval rearing has been limited by the early use artificial meals [5]. One major constraints recognized and which hinders the development this activity in Guinea is the lack on the

local market high-performance feeds at different growth stages prices accessible to fish farmers [6,7]. This is a crucial factor in slowing down the development Guinean fish farming despite investments in recent decades [8]. In addition, the import surcharge on products intended for animal feed restricts imports fish feed, as well those the flours needed for the local production feed. Faced with this situation, it is necessary to set up feed production systems adapted the socio-economic situation employers, species fish be fed and especially according availability locally organic fertilizers. In Forest Guinea, the wettest region Guinea, the greatest risk is that a drop in the level water tables climate change, with a significant number fish farming activities according a preliminary study carried out by APDRA under the direction a member the Intergovernmental Panel on Climate Change [9]. It is therefore urgent to design and implement fish production systems that have a positive impact on water sources. Indeed, according [10] and [11], dry food is absorbed by the water column, prevents light penetration, creates deposits and an anxious environment, and would pollute the production environments unlike the use zooplankton. The use live food (zooplankton) produced from organic fertilizers of animal origin for the larval rearing freshwater fish is an essential alternative. According to [5], live food stimulates food intake and would facilitate the establishment enzyme complexes in fish at the larval stage. According to [12], a good diet for larval rearing aquaculture species is essential, especially for growth, survival and disease resistance larvae. Zooplankton is used as feed for fish larvae in aquaculture due its high nutrient content, easy digestibility and richness in polyunsaturated fatty acids (PUFAs) and unsaturated fatty acids (UFAs) [13]. Previous studies on the use of organic fertilizers such as poultry droppings, cow dung [14] (Agadjihouèdè et al., 2011) and rabbit droppings [15] for the production aquatic zooplankton have been conducted. However, the composition organic fertilizers animal origin in macro and microelements varies from one environment another and is dependent on the diets which livestock are subjected and influences algal and zooplankton productivity. This is what justifies the present study whose main objective is the determination of the optimal dose of organic fertilizer (pig manure) obtained in the piggeries the forest Guinea. Specifically, it is a question (i) evaluating the influence organic fertilizer on the abundance the three major zooplankton groups, (ii) evaluating the biomass of zooplankton from the different doses organic fertilizers (iii) determining the optimal dose of organic fertilizer (pigs manure) for a production freshwater zooplankton.

2. Material and methods

2.1. Experimental design

The experiment was conducted September 2023 at a site (7°43'57.6"N ; 08°50'4.3"E altitude 7 m above sea level) located at the University of N'Zérékoré. The experimental design is made of 9 plastic buckets of 60 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 three (03) treatments including one control such as: T₀, T₁, T₂, corresponding respectively to 0, 300, 600 of dry pig manure (PM) in one (01) m³ of water replicated thrice. Buckets were filled with 40 liters of demineralized water and immediately fertilized [16]. Pig droppings were obtained from an integrated fish production farm in the urban commune of N'Zérékoré. Manures contained 16.13%, 0.36% and 0.72% of N, P and K respectively.

2.2. 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 Water Quality Meter (Version AZ86031) à ± 0.01 pH and ± 0.1°C by mg/L). At each sampling site, 250 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 [17], [18] and [19] while dosage of nutritive salts was realized according to [20] following below formula, with a spectrophotometer of molecular absorption (HACH DR/ 2800).

$$Chl - a \left(\frac{\mu g}{L} \right) = \frac{(A_{0665} - A_{0750}) - (A_{a665} - A_{a750}) \times 11.49 \times v \times 2.43}{V \times L}$$

with, A_{0665} : Absorption at 665 nm before acidification; A_{0750} : Absorption at 750 nm before acidification; A_{a665} : Absorption at 665 nm after acidification; A_{a750} : Absorption at 750 nm after acidification; 2.43: factor to associate the reduction in absorbance to the initial concentration of chlorophyll; 11.49: absorption coefficient of chlorophyll-a, V: volume of the sample filtered (l), L: length of the cuvette (cm) and v: volume of the extract in ml

2.3. Phytoplankton and Zooplankton 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, medium were seeded with phytoplankton according to [16] by transferring ten (10) liters of polyculture (*Heterotis niloticus*, *Heterobranchus isopterus* and *Oreochromis niloticus*) pond water from the Federation of Fish Farmers of Forest Guinea (FFFFG) previously filtered with plankton net of 30 μ m mesh to eliminate zooplankton.

Zooplankton was seeded three (03) days after microalgae seeding, otherwise six days after fertilizing. Indeed, according to [21], this period is sufficient for microalgae development. To harvest zooplankton, three hundred (100) liters of the previous pond were filtered with a net of 30 μ m, and then concentrated in 250 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. In the absence of suitable equipment in the laboratory (sensitive scales) for weighing zooplankton species during the work, data from the literature were used to estimate zooplankton biomass: for each species, the numbers of individuals were transformed into biomass using the average individual dried masses calculated [22,23] and used by [24], which are 0.07 μ g/ind for rotifers, 0.08 μ g/ind for nauplii-stage copepods and 0.47 μ g/ind for adult copepods; and 2.7 μ g/ind (dry weight) for adult cladoceran [25]. The initial seeding density was 35 ± 7.62 ind/L either (23 ± 1.13 ind/L nauplii of copepods either 1.84 ± 0.13 μ g/L of (*Thermocyclops sp.*), 4 ± 0.1 ind/L either 1.88 ± 0.1 μ g/L adults of copepods (*Thermocyclops sp.*), 1 ± 0.007 ind/L either 2.7 ± 0.7 μ g/L of cladocerans (*Moina sp.* and *Daphnia sp.*) and 3 ± 1.48 ind/L either 0.21 ± 1.48 of rotifers (*Brachionus sp* and *Asplanchna sp.*). These three great groups of zooplankton are generally phytophage.

2.4. Statistical analysis

From data collected, density (D) was obtained by using these equations: $D = \frac{n \times V_2}{V_1 \times V_3}$ where n: number of individuals counted, V_1 : volume (l) of aliquot, V_2 : volume (l) of sample concentrated, V_3 : volume (l) of water filtered. 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.

Linear regression relationship equation was applied to determine the relationships between chlorophyll-a versus total biomass zooplankton of this study as follow ; $Y = aX + b$ with slop intercep whereas, Y represents the biomass zooplankton and X represent the chlorophyll-a, whereas, a : was a constant value and b : was the regression coefficient [26]. Excel software was used for data organization and requests enabled data extraction for statistical analyses and graphic making up by using Paleotological Statistic (PAST) version 4.02 accessible on website <http://folk.uio.no/ohammer/past/>.

3. Results

3.1. Abiotic parameters

The analysis of variance of the results for the effects of the different doses (treatments), considering the concentrations, shows a large significant difference ($F_{(10, 30)} = 8.26$; $p=0.00$) between the different treatments and physicochemical parameters. With the exception of temperature, which did not vary between treatments ($F_{(2, 6)} = 2.96$; $P>0.05$), the mean values of the other parameters varied from one treatment to another from 6.12 to 76.72 for conductivity, from 0.17 to 0.40 for pH, from 4.86 to 50.29 for TDS and from 0 to 0.04 for salinity (Table 1). As shown in Table 1, mean values for parameters such as electrical conductivity, salinity, TDS and dissolved oxygen were higher in the fertilized media than in the T₀ control treatment, with a high significant difference ($p < 0.00$). With the exception of pH, the highest mean values of the various parameters mentioned above were recorded in the T₂ medium followed by T₁ with a significant difference ($p < 0.05$).

Table 1. Physico-chemical parameters of the different treatments.

Parameters	Cond ($\mu\text{s}/\text{cm}$)	pH	TDS	T°C	DO (mg/L)	Sal (mg/L)
T ₀	225±6.12c	7.33±0.37c	162.4±4.86c	28.34±0.38a	4.7±0.17c	0.21±0.00a
T ₁	574.13±76.72b	6.73±0.40abc	277.6±50.29b	27.85±0.36a	6.03±0.05ab	0.33±0.01ab
T ₂	616.26±24.91a	6.4±0.17ab	397.73±39.03a	29.36±0.08a	6.03±0.05ab	0.36±0.04ab

Cond: conductivity, DO: dissolved oxygen, Sal: salinity, Transp: transparence, TDS: total dissolved solids. T₀ (the control), T₁, T₂ corresponding respectively to 0, 300, 600 g of dry pig manure (PM). Mean values affected by different letters on the same colonne are significantly different at 5% threshold.

Mean nutrient concentrations varied significantly between treatments ($F_{(6, 18)} = 320.27$; $p = 0.00$). The highest concentrations were obtained in treatment T₂ with an average of 2.19 ± 0.12 , 0.38 ± 0.03 , 0.26 ± 0.02 and 2.55 ± 0.1 respectively of N-NH₃, N-NO₂, N-NO₃ and P-PO₄ (Figure 1), followed by T₁ and T₀.

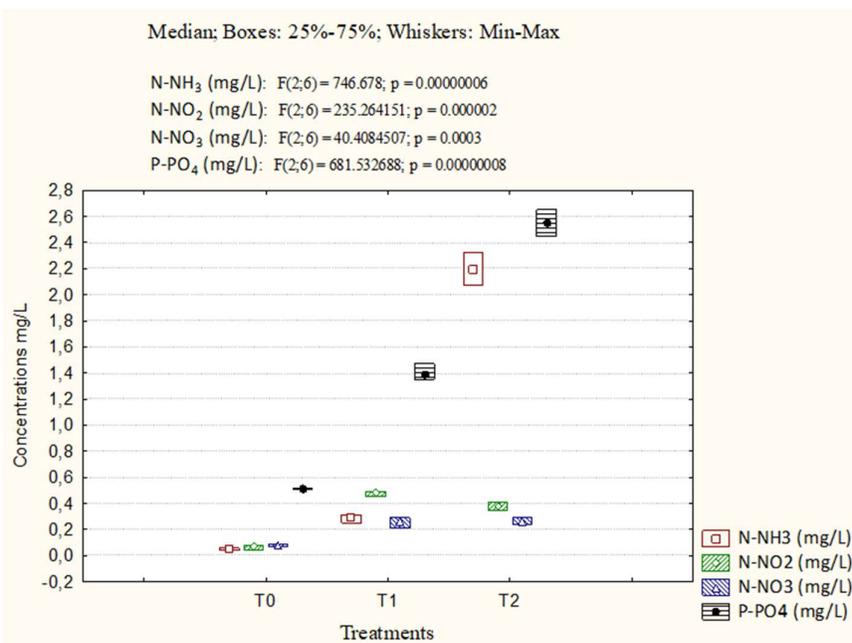


Figure 1. Mean concentrations of nutrients in the different production media. T₀ (the control), T₁, T₂ corresponding respectively to 0, 300, 600 g of dry pig manure (PM).

3.2. Biotic parameter

Figure 2 shows the different chlorophyll-a means for the different treatments, with significant differences ($F_{(2, 6)} = 85.60$; $p = 0.00$). Treatment T₂ recorded the highest Chl-a concentration at 4.63 ± 2.48 mg/L. In the same way, a high positive correlation ($R = 0.982$, $R^2 = 0.96$, $F = 198.9702$, $p < 0.00$) was observed between chlorophyll-a concentration and pig manure dose.

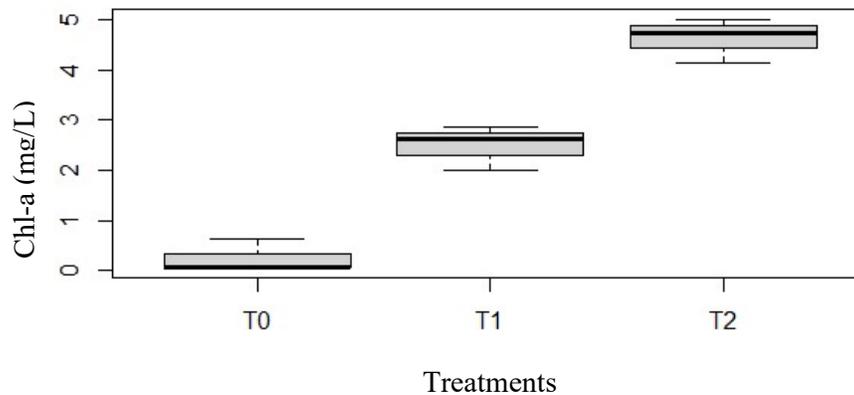


Figure 2. Mean concentrations of chlorophyll-a in the different production media. T₀ (the control), T₁, T₂ corresponding respectively to 0, 300,600 g of dry pig manure (PM).

3.3. Multispecies zooplankton biomass

The biomasses of rotifers, copepods, copepod nauplii and cladocerans in the different treatments evolved in three main phases: a latent phase after seeding, from day 0 to day 7, followed by an exponential phase from day 8 to day 27, when they reached their maximum biomasses before decreasing until day 45 (Figure 4), regardless of the dose pig manures used. Maximum biomass was 49.15 ± 31.05 $\mu\text{g/L}$, with a significant difference ($F_{(28, 84)} = 51.41$; $p = 0.00$). However, the evolution of copepod nauplii biomass did not follow the same trend (Figure 3D). Indeed, copepod nauplii reached their maximum biomass on day 24 after seeding in treatment T₁ (74.50 ± 2.04 $\mu\text{g/L}$) followed by treatment T₀ (39.98 ± 1.17 $\mu\text{g/L}$) and on day 33 for treatment T₂ (47.44 ± 0.88 $\mu\text{g/L}$) with a difference ($F_{(28, 84)} = 157.97$; $p = 0.00$) (Figure 3D). Biomass trends for rotifers, adult copepods and cladocerans are higher in treatment T₂ (Figure 3 A, B, C) followed by T₁, except in treatment T₀ (Figure 3D).

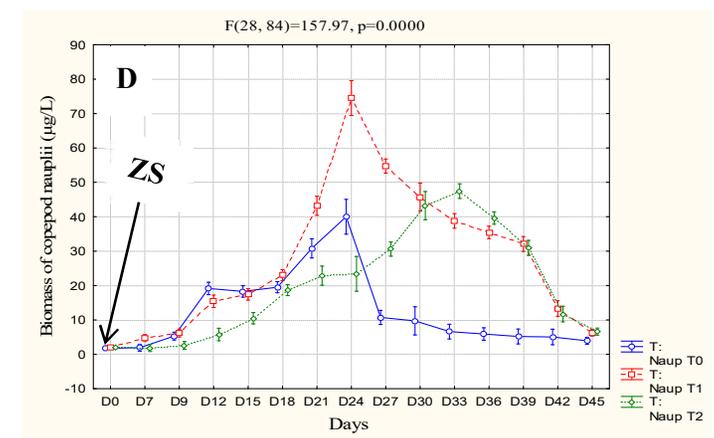
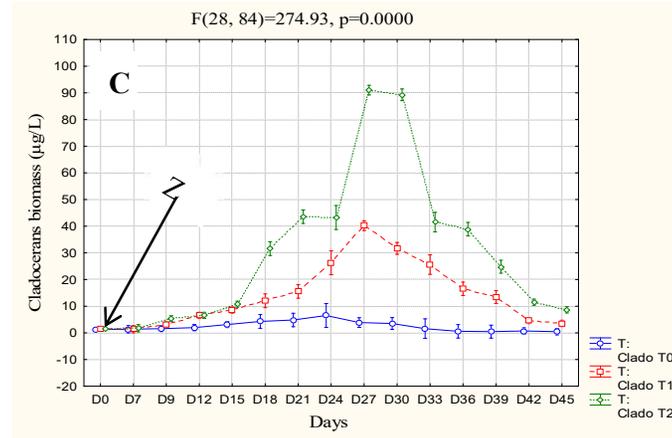
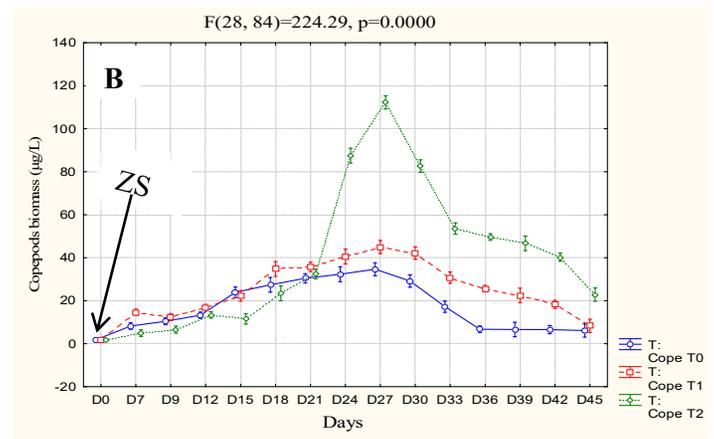
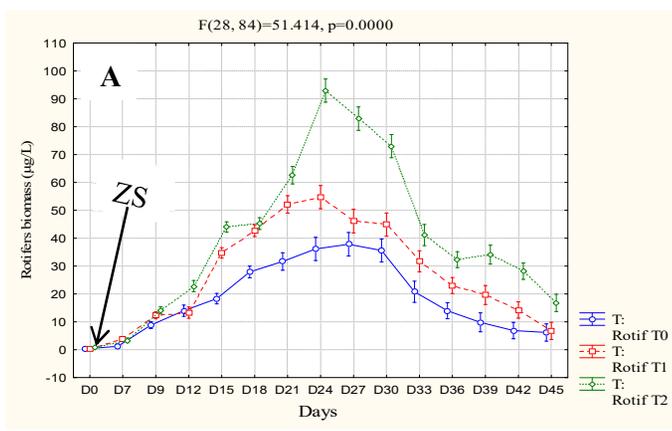


Figure 3. Evolution of the density different zooplankton groups on treatments. (ZS : zooplankton seeding), Rotif : rotifers, Cope : Copepod, Clado : Cladocera, Naup : Copepod nauplii, T0 (the control), T₁, T₂ corresponding respectively to 0, 300,600 g of dry pig manure (PM).

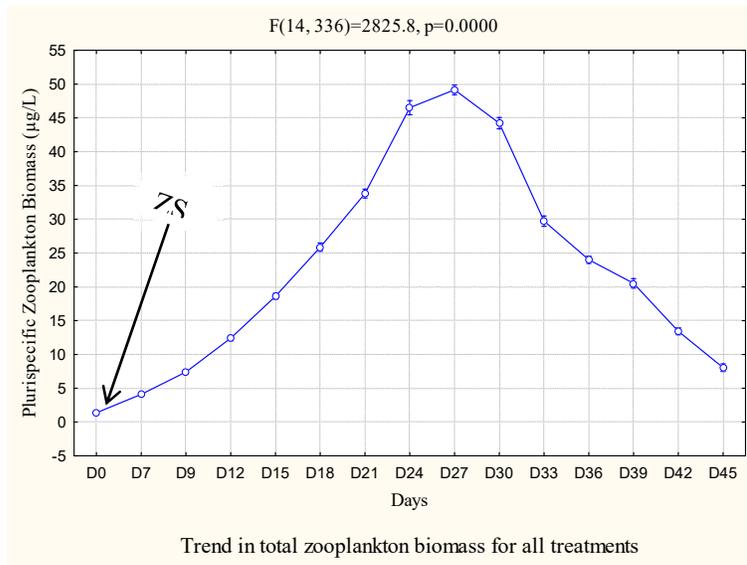


Figure 4. : Evolution of total zooplankton biomass basis pig manure as a function of days. (ZS : zooplankton seeding) .

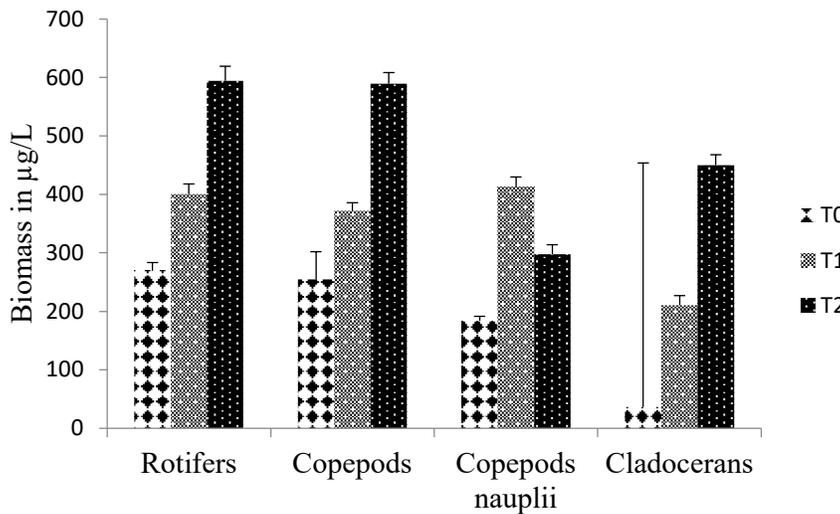


Figure 5. : Estimation total biomass produced by treatment to zooplankton groups.

Furthermore, the highest average zooplankton biomass in dry matter was obtained with treatment T₂ (600g/m³) (rotifers 594.35±24.93, copepods 589.73±18.98 and cladocerans 449.95±18.15 µg/L) (Figure 5).

Table 4. Linear regression association between total biomass zooplankton and of chlorophyll-a (µg/L).

Characters		Regression coefficients					t-test at 5% significant (p<0.05)		Correlation type
X	Y	a	b	r	r ²	N	p-value	CT	
Chl-a (µg/L)	Biomass Rotifers	74.05	239.39	0.99	0.98	1999	0.07	***	

Biomass Copepods	76.18	217.98	0.98	0.96	0.34	***
Biomass copepods nauplii	26.50	232.93	0.50	0.25	0.67	**
Biomass Cladocerans	94.33	0.35	0.99	0.98	0.32	***

CT = Correlation type; *** shows the strong correlation ($r > 0.70$); ** shows moderate correlation ($r = 0.51-0.69$); * represent weak correlation ($r < 0.50$); shows insignificant correlation when $p > 0.05$; a : intercept of regression line, b : slope of regression line, r: correlation coefficient.

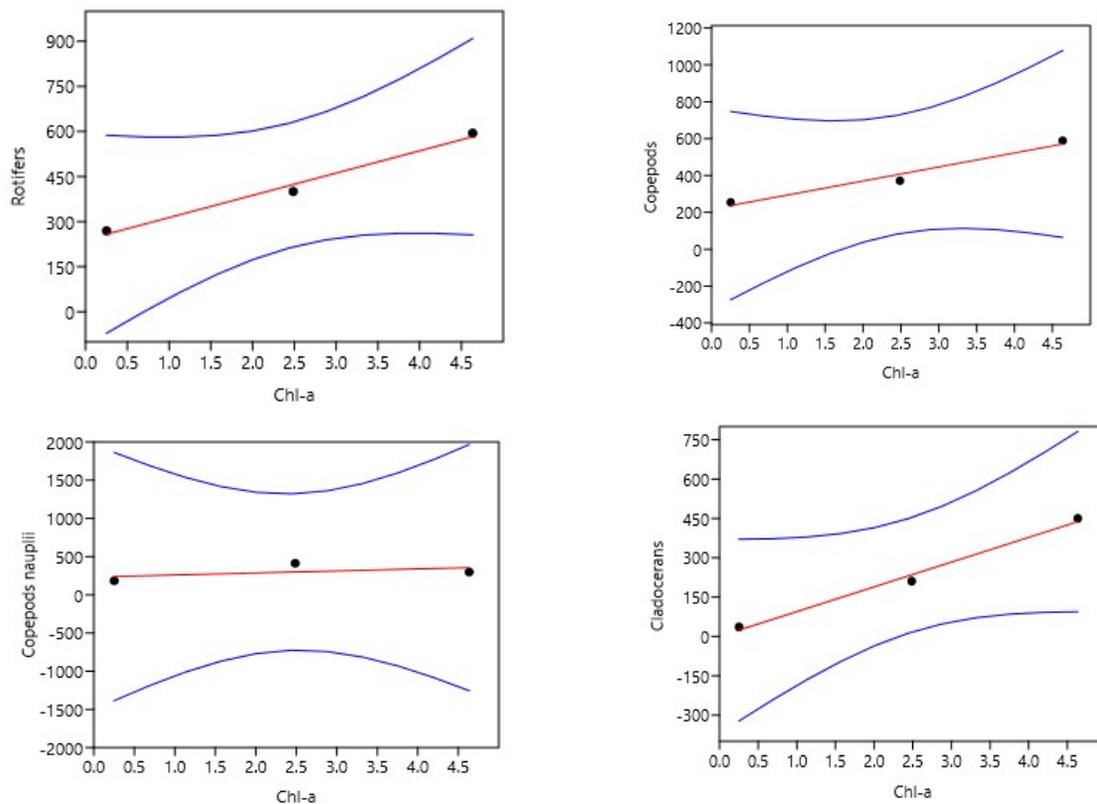


Figure 6. Linear regression association between total biomass rotifers, copepods, copepods nauplii, cladocerans and concentration of chlorophyll-a.

The various linear regressions between chlorophyll-a levels (primary production) and the biomasses of rotifers, copepods and cladocerans show a very strong correlation (r^2 between 0.96 and 0.98) except for nauplii copepods, which show a moderate correlation ($r^2=0.25$) (Table 4 and Figure 6). This analysis shows that the organic fertilizers used in this study (pig manure) stimulate primary production (Chl-a) and, in turn, increase in zooplankton biomass.

4. Discussion

Temperature plays major role in the uptake nutrients by phytoplankton and the metabolism zooplankton. The average temperatures recorded in this study fall within temperature tolerance range for zooplankton, which between 15°C and 30°C [27]. It is slightly lower than that obtained by [14] which is around $30 \pm 2^\circ\text{C}$ in these zooplankton production environments with poultry manures. This could be explained by the difference in climatic zones. Indeed, the Guinean forest region is the

most humid in Guinea with an average temperature oscillate between 24 and 28°C, which would justify the average temperatures obtained during this study. Similarly, the temperatures recorded during this work are close to those obtained by [28] which is between 27 and 28°C and constitutes according to the author temperature range suitable for reproduction *Brachionus Calyciflorus* rotifers. The almost neutral pH our cultures is close to those obtained by [25,29] which would be between 7.7 to 8, and stimulates the maximum growth proteins and algae therefore chlorophylls and certain species rotifers [30]. The salinity rate obtained was lower than that of [31 between 15 and 20 ppt (parts per thousand) which would be optimal for the production calanoid copepods *Acartia tropica*. This difference would be due to the composition the culture media. Indeed, [31] used seawater enriched with organic carbon after filtering. The conductivity characterizes the richness in nutrients such as N-NH₃, N-NO₂, N-NO₃ and P-PO₄ the media fertilized with pig manures and the highest was obtained treatment T₂. Our results are superior to those obtained by [32] with pig manures obtained after feeding pigs with a special diet. It could be said that pig manures used in this study would be richer in nutrients. For significant primary production (chlorophyll-a), a minimum value of 2 mg/L is required, reported by [33]. The mean chl-a values the T₂ followed by T₁ treatments are significantly above this average. The good primary productivity (Chl-a) recorded in treatment T₂ would be due not only to richness the environment but also to the average temperature of the experimental period which constitutes an important determining factor according to [34, 35]. Since there is good correlation between chl-a rate and zooplankton biomass, our results (Table 4 and Figure 6) confirm those [36, 37]. The strong correlations between the dose (organic fertilizer, r² = 0.96) chlorophyll-a on the one hand and those of the biomasses on zooplankton groups and chlorophyll-a the other hand are better than those obtained by [38] (r² = 0.40 with P < 0.01) in the lakes of Jiangsu and Anhui. The most significant chlorophyll-a rate and biomass rotifers, copepods and cladocerans were obtained in T₂ treatment. They are higher than those obtained by [14,39]. Indeed, the dose 600g/m³ (T₂) pig droppings would have created a favorable environment for growth and reproduction the different groups freshwater zooplankton.

5. Conclusion

Dose of 600g/m³ (T₂) pigs manures provides favorable environment for the development phytoplankton via the rate chlorophyll-a and freshwater zooplankton which uses it effectively for its growth and development. This dose could be proposed to rural fishfarmers to replace dry food and artemia which are expensive and almost unavailable the local market. However, studies on the production phytoplankton and zooplankton with doses higher than those used this study should be done in order to better understand the development behavior plankton.

Author Contributions: Conceptualization, manuscript, editing and methodology A.R.; formal analysis, visualization, writing-original and writing-review, G.D.; Investigation, K.A.O.; resources, review and Data Curation, B.P.; supervision, Writing - review and editing, M.J-C.

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Conflicts of Interest: The authors declare no conflict of interest.

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