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Zooplankton Diversity and Abundance in Extensive Fish ponds during the Rearing of tilapia *Oreochromis niloticus* juveniles Fed with Rice Bran (West Africa, Côte d'Ivoire)

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Abstract
The objective of this study is to determine the effect of pond fertilization (using chicken droppings and rice bran treated or untreated) on the water quality as well as its effect on the composition, diversity and abundance of zooplankton organisms during an experimental culture of *Oreochromis niloticus*. A total of 30 zooplankton taxa were identified, belonging to four groups, 15 families and 17 genera plus some unspecified families and genera. Zooplankton abundance varied according to treatments, with highest values observed in ponds fertilized with chickens droppings and fermented rice bran (42-52 ind.l^{-1}) and *Mesocyclops* sp., *Thermocyclops neglectus, Moina micrura* and *Diaphanosoma excisum* as main species.

Keywords: rice bran, chickens droppings, fertilization, zooplankton production

Introduction
Zooplankton provides a crucial source of food to larger, more familiar aquatic organisms such as fish and crustaceans. Besides, most zooplanktonic organisms have an herbivorous-detritivorous diet and exert a strong grazing impact on the phytoplankton biomass [1]. It is the initial prey item for almost all fish larvae which fish begin preying on small organisms such as rotifers and the early developmental forms of planktonic crustaceans (Nauplii and copepodite) [2] and the range of available food particles increases with the growth of larvae (Cladocera, adult forms of Copepoda, Gastropod larvae, and others). In general, zooplankton is the main food base and the most valuable source of natural protein for developing fish, dry food (inert) have shown their limit [3]. Additionally, plankton communities play a major role in the biogeochemical cycles of many important processes such as the carbon cycle, nitrification, denitrification, remineralization, and methanogenesis. Beside, zooplankton constitutes a sensitive tool for monitoring environmental changes aquatic ecosystem. Indeed, the zooplankton populations react to trophic [4, 5], and can be used as biological indicators for water quality and eutrophication [6, 7, 8]. In freshwater ecosystem, Rotifers *Brachionus angularis, B. falcatus, B. calyciflorus, Hexarthra intermedia, Keratella cochlearis,* and *Polyarthra* sp., were all considered like typical of meso-eutrophic environments [8] while copepod *Thermocyclops decipiens* is associated to eutrophic waters [8].
The fish has an interesting biological value and constitute an excellent source of essential amino acid. In Africa, fish is sometimes the most financially affordable protein source in poor households in urban and suburban areas, and it is considered as « a rich food for the poor people » and contributes around 50% of total animal protein in the diets of many Africans [9]. To optimize its production through aquaculture, *Artemia* sps was the organisms which mostly used as a live prey feed in world [10, 11]. Its utilization in Africa, mostly in rural tropical area is difficult because of constraining hatching conditions, its high cost and unavailability on the local market [12]. So, the utilization of other local live prey with a high potential of production is an alternative. One of the solutions, inexpensive, is to stimulate zooplankton production in fish farming by the use of organic fertilizers. In fact, provision of these live foods to fish larvae is appreciated as an important aspect because they supply nutrients to the larvae and as well as exogenous enzymes important for the digestion of other feeds, and enhance the development of

larvae's pancreas [13]. Organic fertilizers are typically derived from natural substances such as waste product from living organisms (animal and vegetal) such as cow dung, pig dung, chicken droppings, rabbit dropping, rice bran, maïze bran, azolla, etc.. [14].

Like any other productive activity, fish farming causes environmental impacts, among them the increase in nutrient concentrations originating from the waste that is directly released in the water, changes in the trophic web and the balance of aquatic communities, and eutrophication [15, 16]. On the other hand, organic fertilizer has its excesses in that it contains a high concentration of ammonia which could be toxic to aquatic life if too much is added to a pond. Like zooplankton responds quickly to environmental changes because of their short life cycle, their species composition is more likely to indicate the quality of the water which they are found. It is broadly admitted that of Brachionidae family and *Brachionus* species are majorly and regularly met in eutrophics tropical waters [17] while *Trichotria tetratis* is considerate as the pollution indicator as they were found in the lake which was rich in phosphorus and other heavy metal ion [18]. So, knowledge concerning the effect of organic manure utilization on zooplankton production (diversity and abundance) variation can be used for the management of the natural productivity of ponds containing fish. Therefore this study was conducted to determine the effect of pond fertilization (using chicken droppings and rice bran) on the water quality as well as its effect on the composition, diversity and numerical abundance of zooplankton organisms during an experimental culture *Oreochromis niloticus*.

Materials and Methods

This study was conducted from May to September 2015 in the center-ouest of Côte d'Ivoire (Daloa) in 12 ponds (average 613 m²) during *Oreochromis niloticus* culture experience. During these experiences, six ponds have been fertilized with chickens droppings (CD): 15 kg of dry matter (DM) per 100 m^2 of pond in the beginning of the experience (two weeks before the stocking with fish) and a maintenance dose of 1.25 kg of DM of chickens droppings by are (100 m^2) per week all along the experimentation [19]. In continuation, fish have been fed daily at 9h 00 min and at 15h 00 min, with a food ration of 2 kg/are/day, from the third week of experience until the end. The six other ponds have not been fertilized to chickens droppings. Thereafter, the experience consisted in having in every share (fertilized and no fertilized with the chickens droppings) a pond without rice bran (WtRB), a pond with rice bran unprocessed (RB), a pond with boiled rice bran (BRB), a pond with toasted rice bran (TRB), a pond With fermented Rice Bran to palm wine (FRBpw), and a pond with fermented Rice Bran with water (FRBw). Pond without rice bran constitutes witnesses (control) pond. The six other ponds were, different treatments have been applied: fertilizing or no with rice bran treated. During this study, a DANTE multi parameter type was used for measuring of physico-chemical parameters like temperature, pH, dissolved oxygen concentration and conductivity. Water transparency was also measured *in situ* using a graduated Secchi disc. Nutrient salt (PO_4^{3-}, NH_4^+, NO_2^-, NO_3^-) were determined by molecular absorption spectrophotometric with a DR 2800 spectrophotometer. The zooplankton organisms were collected monthly with a cylindro-conical net (64 µm in mesh opening size). Samples were immediately preserved in a buffered solution (pond water and formaldehyde at a final concentration of 5%). Zooplankton was identified according to following authors [20-26]. ANOVA were used to test the effects of treatments on the zooplankton density, species richness and ecological diversity index. Data have been transformed in log10 (x+1) prior to analysis to increase normality. For this analysis, Statistica 7.1 computer was used.

Results and Discussion

Physico-chemical parameters

Ponds water temperature varied between 27.70 and 30.22°C while pH varied between 5.40 and 6.24 according to treatment (Table 1). So, pH in all experimental ponds was relatively acid (< 7). Ponds waters were well oxygenating, with values varied from 6.88 to 8.11 $mg.l^{-1}$ concerning the dissolved oxygen. Conductivity varied from 51 to 117 $µS.cm^{-1}$, with highest values obtained in obtained in ponds fertilized with chicken droppings associated to fermented rice bran to palm wine. Transparency in all ponds was low (< 100 cm). Chlorophyll *a* concentration varied from 19 to 66.27 µg/l, with highest values obtained in pond treated with fermented Rice Bran to palm wine (58.67 µg/l) and by fermented Rice Bran with water (66.27 µg/l). Concerning the essential nutrient parameters (Ammonium; nitrite, nitrate), highest ammonium concentration were obtained in ponds treated with toasted rice bran, boiled rice bran, fermented rice bran with water (11.41-13.70 µg/l) versus 3.75 to 8.62 µg/l in the other treatments. Highest nitrate concentration were observed in ponds fertilized with Rice Bran associated to Chickens Droppings, ponds no treated with Rice Bran, fermented rice bran with water and ponds fermented rice bran to palm wine (11.21 to 15.45 µg/l) versus 4.73 to 9.33 µg/l.

Zooplankton Community composition

A total of 30 zooplankton taxa were identified in the 12 experimental ponds divided in four groups: 4 Copepoda (13.33% of total diversity), 5 Cladocera (16.67%), 17 Rotifera (56.67%) and 4 others zooplankton forms (13.33%) (Table II). Copepods were represented by one familly (cyclopidae) and two genera (*Mesocyclops* and *Thermocyclops*) plus unspecified copepod nauplii. Cladoceran taxa (5) belonging to three families (Chydoridae, Daphniidae and Sididae) and five genera (*Alona*, *Chydorus*, *Ceriodaphnia*, *Diaphanosoma* and *Moina*). Rotifera dominate qualitatively zooplankton community with 16 taxa (57% of total richness) belonging to 8 families and 10 genera. Brachionidae family presented the highest richness (3 genera and 8 species, followed by Filinidae, with 3 species (*Filinia longirostris*, *F. opoliensis* and *F. terminalis*). The others families were all monospecifics. The group "others zooplankton forms" was mainly represented by aquatic insect larvae and one Ostracoda taxon.

Zooplankton species richness and diversity index varied according to treatments. It varied between 7 and 23, with highest values respectively in pond fertilized with chickens droppings associated to fermented rice bran to palm wine (18 taxa), with chickens droppings associated to fermented rice bran with water (20 taxa) and with fermented rice bran to palm wine without chickens droppings (23 taxa). Shannon diversity index varies from 1.36 to 2.11 $bit.ind^{-1}$ (Mean: 1.72 $bit.ind^{-1}$). It should be noted that highest Shannon diversity index values was obtained in ponds without fertilization with chickens droppings and fertilized with fermented rice bran to palm wine (2.11 $bit.ind^{-1}$). Equitability index varies from 0.54 to 0.77 (Mean: 0.66).

Zooplankton community structure, abundance variation with treatment and season

Zooplankton abundance varied significantly ($p < 0.01$) according to treatment (5 to 52 ind.l^{-1}) (Figure 1A), with lowest abundance in ponds fertilized or no with chickens droppings and treated with toasted rice bran (TRB) (5-7 ind.l^{-1}) and highest values observed in ponds fertilized with chickens droppings and treated on the one hand by fermented rice bran to palm wine (FRBpw/CD) (42 ind.l^{-1}) and on the other hand by fermented rice bran with water (FRBw/CD) (52 ind.l^{-1}).

Table 1: Physico-chemical parameters measured experimental ponds during *Oreochromis niloticus* culture

	TRB/CD	TRB	RB/CD	RB	BRB/CD	BRB	WtRB/CD	WtRB	FRBw/CD	FRBw	FRBpw/CD	FRBpw
Temperature (°C)	29.22	29.48	29.60	29.18	28.95	29,27	29.28	27.70	27.65	29.18	30.22	29.62
pH	5.68	5.67	6.06	6.24	6.02	5,73	6.08	5.94	6.56	5.40	6.07	5.79
Dissolved Oxygen (mg/l)	7.43	7.47	7.21	7.72	7.25	7,44	7.28	8.11	8.03	6.88	7.28	7.00
Conductivity (µS/cm)	59.05	52.93	74.20	80.65	93.45	65,28	76.85	207.45	217.08	61.55	76.62	50.97
Transparency (cm)	0.57	0.68	0,41	0.31	0.40	0,57	0.35	0.49	0.34	0.61	0.54	0.55
Rate of dissolved solid (ppm)	36.20	26.05	28.87	33.64	38.72	27,78	28.52	24.98	39.77	216.25	32.33	50.18
Chlorophyll a (µg/l)	19.90	25.57	40.48	32.73	25.88	19,07	41.30	18.65	66.27	24.94	58.67	24.25
Ammoniun (NH_4^+) (µg/l)	5.552	11.409	6.667	5.701	5.898	13,415	1.391	5.152	5.028	8.618	3.735	13.705
Nitritre (NO_2^-) (ppm)	0.019	0.010	0.014	0.021	0.021	0,007	0.019	0.013	0.016	0.020	0.016	0.021
Nitrate (NO_3^-) (ppm)	4.911	7.041	11.835	7.900	9.331	6,937	4.729	15.452	8.857	11.213	9.033	14.284

(CD: Chickens Droppings; TRB: Toasted Rice Bran; RB: Rice Bran; BRB: Boiled Rice Bran; WtRB: Without Rice Bran; FRBw: Fermented Rice Bran with water; FRBpw: Fermented Rice Bran to palm wine; /: Associate to…)

Table 2: Zooplankton taxa collected in the different ponds with specific treatments; + = presence

Groups	Familly	Taxons	Treatments											
			TRB/CD	TRB	RB/CD	RB	BRB/CD	BRB	WtRB/CD	WtRB	FRBw/CD	FRBw	FRBwp/CD	FRBwp
Copepods	Cyclopidae	*Mesocyclops* dussarti.	+	+	+	+	+	+	+	+	+	+	+	+
	"	*Thermocyclops neglectus*	+	+	+	+	+	+	+	+	+	+	+	+
	Unspecified	Unspecified harpacthicoid									+			
	Unspecified	Nauplii	+	+	+	+	+	+	+	+	+	+	+	+
Rotifers	Asplanchnidae	*Asplanchna* sp.	+										+	+
	Brachionidae	*Anuraeopsis fisa*												+
	"	*Brachionus angularis*					+	+	+	+	+		+	+
	"	*B. calyciflorus*					+	+	+		+	+		+
	"	*B. caudatus*	+			+			+	+	+		+	+
	"	*B. falcatus*	+	+	+	+	+	+	+	+	+	+	+	+
	"	*B. plicatilis*				+				+		+		
	"	*Brachionus* sp.						+						
	"	*Platyonus patulus*						+						
	Epiphanidae	*Epiphanes* sp.			+		+				+			
	Filiniidae	*Filinia opoliensis*			+		+		+	+	+	+	+	+
	"	*F. longiseta*					+		+		+		+	+
	"	*F. terminalis*									+		+	+
	Gastropidae	*Gastropus* sp.						+						
	Lecanidae	*Lecane* spp.	+		+		+		+			+	+	+
	Testudinelidae	*Testudinella* sp.												+
	Trichocercidae	*Trichocerca* spp.	+		+		+				+	+	+	+
Cladocerans	Chydoridae	*Alona* sp.	+								+			+
	"	*Chydorus* sp.							+					+
	Daphnidae	*Ceriodaphnia cornuta*				+	+			+	+		+	+
	"	*Moina micrura*	+	+	+	+	+	+	+	+	+	+	+	+
	Sididae	*Diaphanosoma excisum*	+	+	+	+	+	+	+	+	+	+	+	+
Others	Unspecified	Ostracodes	+			+	+		+	+			+	+
	Chironomidae	Chironomidae Larvae				+	+	+	+	+	+	+	+	+
	Chaoboridae	Chaoborus Larvae						+			+			+
	Unspecified	Others aquatic insects larvae		+	+	+	+	+	+	+	+	+	+	+
Total taxa	15	30	12	7	11	13	17	14	16	14	20	13	18	23

Furthermore, our results show that abundance in control pond (no fertilized with chickens droppings and non-treated to rice bran: WtRB) (22 ind.l^{-1}) was higher than abundances estimated in the ponds fertilized or no with the chicken droppings and respectively treated with toasted rice bran (TRB) (5-7 ind.l^{-1}), rice bran no treated (RB) (11-16 ind.l^{-1}), boiled rice bran (BRB) (16-21 ind.l^{-1}) (Figure 1A). On average, zooplankton abundance was dominated by Copepoda (mean: 56% of zooplankton total abundance, 12.57 ind.l^{-1}), followed by Cladocera (mean: 30%, 6.75 ind.l^{-1}) and (mean: 27 %, < 1 ind.l^{-1} in the rainy season) and by Rotifera (mean: 12%, < 3 ind.l^{-1}) (Figure 1A). This general tendency, is observed in all experimental ponds excepted in ponds fertilized only with fermented rice bran with water, where copepods (42%) were followed by rotifer (36%). Copepoda were represented by copepodites and adult stage of *Mesocyclops dusarti* (39% of total copepod abundance) and *Thermocyclops neglectus* (21%) and their nauplii stages (probably) (39%) (Figure 1B). Copepods highest abundances were obtained in ponds fertilized with chickens droppings and treated, on the one hand to fermented rice bran with water and on the one hand by fermented rice bran to palm wine (21 ind.l^{-1}) while lowest copepods abundance were observed in ponds fertilized and no fertilized with chickens droppings, and treated to the toasted rice bran (< 6 ind.l^{-1}) (Figure 1B). Cladocera community is composed of 5 species, but *Moina micrura* and *Diaphanosoma excisum* accounted for 96.53% of total abundance, with 69.62% for the first specie (Figure 1C). Rotifers group presents the highest diversity, but it was dominated by five taxa (*Brachionus plicatilis*: 33.55%, *B. falcatus*: 25.54%, *B. calyciflorus*: 8.26%, *Filinia opoliensis*: 6.97% and *B. angularis*: 5.96%) (Figure 1D). *B. plicatilis* presents the highest density in pond fertilized with fermented rice bran with water (10.54 ind.l^{-1}) while *B. falcatus* showed highest abundances in ponds fertilized with fermented rice bran to palm wine associated to chickens droppings (2.15 ind.l^{-1}), with chickens droppings (1.21 ind.l^{-1}) and with boiled rice bran associated to chickens droppings (1.76 ind.l^{-1}). *B. calyciflurus* was obtained with highest abundance in pond treated with chickens droppings (1.95 ind.l^{-1}) while *Filinia opoliensis* presented the highest abundance in pond fertilized with fermented rice bran to palm wine associated to chickens droppings (1.14 ind.l^{-1}) (Figure 1D).

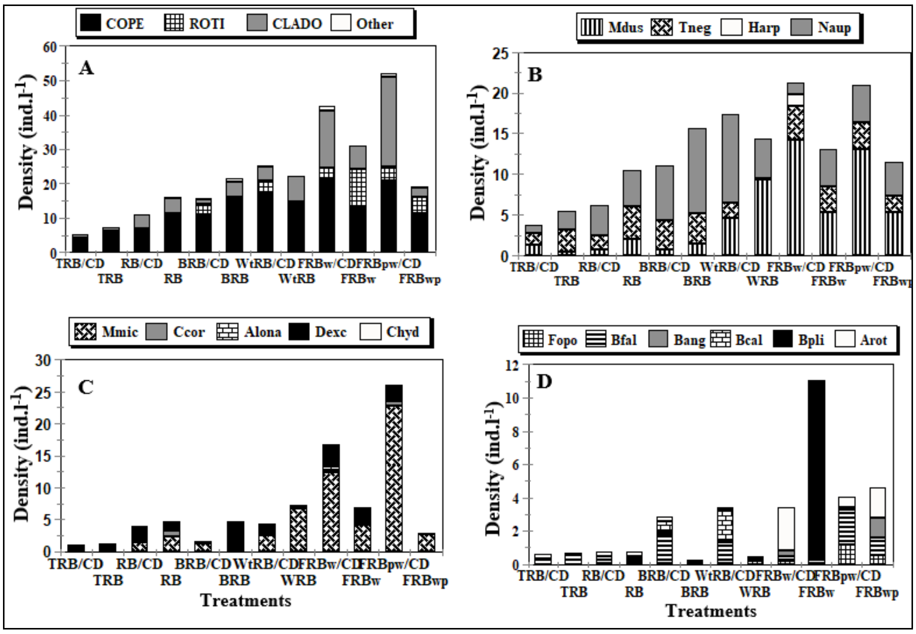

Fig 1

Figure 1. Relatives abundance of main zooplankton groups (A), Copepods taxa (B), Cladocerans taxa (C), and Rotifers taxa (D) by treatment during *Oreochromis niloticus* culture (CD: Chickens Droppings; TRB: Toasted Rice Bran; RB: Rice Bran; BRB: Boiled Rice Bran; WRB: Without Rice Bran; FRBw: Fermented Rice Bran with water; FRBwp: Fermented Rice Bran to palm wine; /: Associate to…, Fopo: *Filinia opoliensis*, Bfal: *Brachionus falcatus*, Bang: *Brachionus angularis*, Bcal: *Brachionus calicyflorus*, Bpli: *Brachionus plicatilis*, Mdus: *Mesocyclops dussarti*, Tneg: *Thermocyclops neglesctus*, Harp: Unspecified harpacthicoid, Naup: copepods nauplii, Mmic: *Moina micrura*, Ccor: *Ceriodaphnia cornuta*, Alona: *Alona* sp., Dexc: *Diaphanosoma excisum*, Chydo: Chydorus sp.).

Discussion

A total of 30 zooplankton taxa were collected in the different experimental ponds. Taxa diversity is comparable to findings of Aka *et al.* [27] in collected 49 small reservoirs of northern Côte d'Ivoire (30 taxa) and the diversity obtained by Yte *et al.* [28] in Gagnoa low ground (centre-west region of the Côte d'Ivoire) (31 taxa). This diversity is also comparable to those gotten by Elégbé *et al.* [29] (30-31 taxa) during the Co-Culture experiences of *Clarias gariepinus* and *Oreochromis niloticus* in traditional aquaculture system "*Whedos*", with fertilization with an imported food « Skretting » (45% of protein), a local aliment (made in Benin with 27% of protein) and a mixed aliment (50% imported and 50% local). Lowest diversity registered in the present study and in others ponds, wetlands and reservoirs studied may be linked to diver's factors as hydro systems area, aquatic ecosystem type, and the catchment area of the aquatic ecosystem. The result of the present study showed that, in the experimental ponds, the highest diversity was observed among Rotifera, with 16 taxa (57% of total richness) belonging to 8 families and 10 genera. Brachionidae family presented the highest richness (3 genera: *Anuraeopsis*, *Brachionus* and *Platyonus*; and 8 species: *Anuraeopsis fisa*, *Brachionus angularis*, *B. calyciflorus*, *B. caudatus*, *B. falcatus*, *B. plicatilis*, *Brachionus sp.*, *Platyonus patulus*), followed by Filinidae, with 3 taxa: *Filinia longirostris*, *F. opoliensis* and *F. terminalis*. Similar results, with rotifer as most diversified zooplankton group, Brachionidae family and *Brachionus* genus presented, respectively, high family and genus diversity were reported in several freshwater ecosystem of Côte d'Ivoire as the 49 reservoirs in northern of Côte d'Ivoire [27], the low ground of Gagnoa [28], Similar results were also reported in other tropical freshwater ecosystems as the lake Azili (Benin) [31], fish ponds ecosystem (Benin) [29]. According to Badsi *et al.* [32], a strong representation of rotifers in aquatic ecosystem can be considered as an indicator of high biological trophic level. Besides, Rotifers are able to ingest small particles such as bacteria and organic detritus often abundant in eutrophic environments [33]. In addition, the predominance of rotifer species was attributed to the fact that they are opportunistic, small size, with short life cycles and high tolerance to a variety of environmental factors [32].

Our results show that lowest zooplankton abundance was obtained in ponds fertilized or no with chickens droppings (5-7 ind.l^{-1}) and highest values observed in ponds fertilized with chickens droppings and treated on the one hand by fermented rice bran to palm wine (42 ind.l^{-1}) and on the other hand by fermented rice bran with water (52 ind.l^{-1}). Besides, during these experiences highest zooplankton diversity were observed in ponds fertilized with chickens droppings and treated on the one hand by fermented rice bran to palm wine (20 taxa) and on the other hand by fermented rice bran with water (20 taxa). This result suggests a fertilizing effect of treatment by chickens droppings combined to fermented rice bran on zooplankton production. This result may be linked to the fact that fermented rice bran was probably easily mineralized and transformed in nutritious salts (nitrate, phosphate and potassium) that stimulate the primary production, valued in this survey through the *chlorophyll a* which presented the highest values in these ponds (58.67-66.23 µg/l), which in turn enhance the accelerated growth of zooplankton (42-52 ind.l^{-1}) as noted by Seyer [34]. Fertilizing pond with fermented rice bran favored mainly the growth of copepods *Mesocyclops dussarti*. (22-34% total zooplankton abundance) and *Thermocyclops neglectus* (6-10%) on the one hand, and cladocera *Moina micrura* (29-44%) and *Diaphanosoma excisum* (5-8%) on the other hand. Utilization of organic fertilizers which increase zooplankton abundance is also reported in several studies [40-42]. This study has revealed also that zooplankton abundance in control pond (no fertilized with chicken droppings and non-treated to rice bran) (22 ind.l^{-1}) was higher than abundances estimated in the ponds fertilized or no with the chicken droppings and respectively treated with toasted rice bran (5-7 ind.l^{-1}), rice bran no treated (11-16 ind.l^{-1}), boiled rice bran (16-21 ind.l^{-1}). Generally zooplankton abundance was higher in ponds fertilized than in control ponds [34-36]. Our results may be linked to the fact that, rice bran has a ratio C/N (with C for Carbone and N for azote) > 25 (Mean: 35.24) and would be therefore less mineralized due to its rigidity and the size of these particles [37]. So, in the ponds fertilized respectively with toasted rice bran, rice bran no treated, boiled rice bran; it could produce a low mineralization, with as consequence a low production of phytoplanktonic biomass which in turn don't enhance the zooplankton production. In contrast, ponds fertilized with chickens droppings and fermented rice bran, fermentation could promote the mineralization of the rice bran and stimulate zooplankton production.

Conclusion

This study allowed to inventory the zooplankton population and to estimate it abundance in ponds fertilized with different organic manures en tropical zone. It reveals that fertilization with fermented rice bran promotes significantly zooplankton production (qualitatively and quantitatively) in ponds.

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