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Kouadio Atto Delphin
Laboratory of Environmental Science and Technology, UFR Environment, University Jean Lorougnon Guédé, Côte d'Ivoire

Groga Noël
Laboratory of Agricultural Improvement and Valorization, UFR Agroforestry, University Jean Lorougnon Guédé, Côte d'Ivoire

Konan Kouakou Seraphin
Laboratory of Environmental Science and Technology, UFR Environment, University Jean Lorougnon Guédé, Côte d'Ivoire

Ndjouondo Gildas Parfait
Department of Biology, Higher Teacher Training College, University of Bamenda, Cameroun

SALLA Moreto
Laboratory of Environmental Science and Technology, UFR Environment, University Jean Lorougnon Guédé, Côte d'Ivoire

Corresponding Author:
ouadio Atto Delphin
Laboratory of Environmental Science and Technology, UFR Environment, University Jean Lorougnon Guédé, Côte d'Ivoire

Impact of agricultural by-products inputs to the juveniles of *Oreochromis niloticus* (Linnaeus, 1758) on phytoplankton diversity in rice-fish ponds (Central West, Côte D'ivoire)

Kouadio Atto Delphin, Groga Noël, Konan Kouakou Seraphin, Ndjouondo Gildas Parfait and Salla Moreto

Abstract
The influence of agricultural by-products inputs to juveniles of *Oreochromis niloticus* on phytoplankton composition and density in rice-fish ponds was assessed from water samples collected between April 10 to July 18, 2019. The experiment was carried out in four types of rice-fish ponds, namely, ponds without exogenous food input (RC), ponds with maize bran input (RSM), ponds with rice bran input (RSR) and ponds with various agricultural by-products combined input (RPC). Phytoplankton sampling was carried out by filtering 45 litres of water using a 20 µm plankton net with a 20 µm void and environmental parameters that could influence phytoplankton blooms were determined *in situ*. A total of 194 taxa divided into five phytoplankton phyla and 51 genera were inventoried. Species richness was higher in the RSM and RPC ponds than in the others with 101 and 92 taxa, respectively. Mean phytoplankton density was significantly higher in rice-fish ponds RPC (60.6 10^6 Individuals/L) and RSM (55.1 10^6 Individuals/L) with a strong dominance of Chlorophyta and Euglenophyta. These high densities were positively correlated with high nutrient concentrations.

Keywords: Agricultural by-products, phytoplankton, rice-fish farming, Côte d'Ivoire

1. Introduction
In Côte d'Ivoire, the aquaculture production estimated at about 1105 tons in 2000 [1], is far from satisfying the national demand for fishery resources. In order to make up for this deficit, the country is trying as best it can to succeed in tilapia culture by developing its many bodies of water and combining fish farming with rice cultivation [2]. The integration of pond fish farming into rice production systems, which is emerging worldwide as one of the alternative solutions to reduce food insecurity in rural areas [4, 5], is very recent in Côte d'Ivoire [3]. However, the cost of fish feed associated with the complexity of rice-fish ponds remains a major constraint to the emergence of this practice in Côte d'Ivoire. To cope with this constraint, most pond fish producers, especially those in the Central-Western region of Côte d'Ivoire, use various types of feed formulated from agro-industrial by-products in an uncontrolled manner [6, 7]. However, in aquaculture, the supply of feed to fish is the main source of proliferation and change in the diversity of algae, particularly phytoplankton [8]. Phytoplankton is the starting point of the food chain and thus a determining element in the functioning of the farming structures [8, 9]. It is the natural food for zooplankton and certain species of farmed fish [10, 11]. However, an excessive and uncontrolled proliferation of phytoplankton is worrying [12, 13] and leads to nocturnal deoxygenation of ponds with biological consequences [14]. As a result, several algal studies have been carried out in Côte d'Ivoire in fish ponds [15, 16, 17, 18, 19]. But to date, work on phytoplankton in rice-fish farming environments is rare and limited to their occurrence [20, 21].
The objective of this study is therefore to estimate the effect of exogenous inputs based on cereal by-products on phytoplankton diversity in rice-fish farming environments.

2. Materials and methods

2.1 Study area and experimental design

Located in the department of Vavoua (Central-Western Ivory Coast) between 7°10 and 7°12 North latitude and between 6°30 and 6°40 West longitude, the Kouadiokro-Bonoufla fish farming station enjoys a Guinean-type climate. Rainfall in this zone is between 1200 and 1600 mm per year, with a maximum from June to July and a minimum from December to March [22]. The annual temperature in the department of Vavoua varies between 19 and 33°C [23].

This fish farm consists of nine ponds located downstream of a dam (Figure 1). The rice-fish ponds (E2 to E9) used for this study range in size from 200 to 360 m^2. The experimental set-up consisted of two completely randomized blocks with two replicates. Rice seedlings were transplanted at 25 cm spacing and the stocking density of *Oreochromis niloticus* fry was 20 fish /m^2. The food provided to the fish in these replicates was corn bran, rice bran and a combination of several agricultural by-products. The latter combined food consisted of rice bran, corn bran, palm oil, cooking salts, shell ash, cottonseed cake and soybean meal.

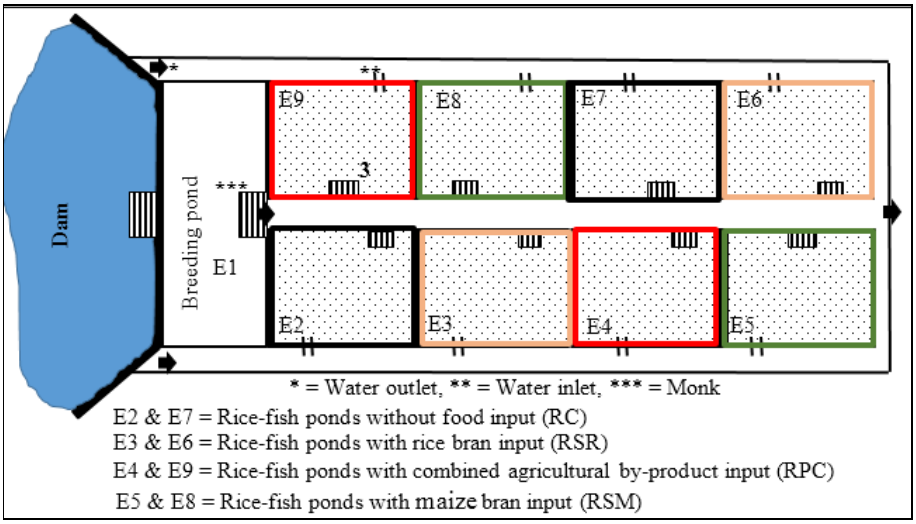


Fig 1: Diagram of the experimental device used for this study at the Kouadiokro-Bonoufla fish farm (Vavoua, Central-West, Côte d'Ivoire)

2.2 Data collection

Data for this study were collected in rainy seasons between April 10 and July 18, 2019 during the juvenile phase of fish (*O. niloticus*). The water samples were taken in three campaigns (start-pre-fattening, mid-fattening and finepre-fattening) between 7 h 30 min and 9 h00 min along the diagonal of each rice-fish pond. Thus, during each campaign, 45 L water samples were taken and filtered using a 20 µm plankton net. After filtration, the water sub-samples were collected in the 50 mL pill boxes and immediately fixed with formaldehyde at a 5% concentration. During these campaigns, the transparency was measured at midday by vertical immersion of a Secchi disc, the values of pH, dissolved oxygen, temperature, dissolved solid content and conductivity of the water in the rice-fish ponds were measured *in situ* with a multiparameter HQ40d. One liter (1 L) of water from these ponds was also collected and sent to the Laboratory for the determination of nutrient salts (NO_3-, NH_4^+ and PO_4^{3-}).

2.3 Phytoplankton stand analysis

The algal cells were observed with the OPTIKA photomicroscope according to the technique of Atanle *et al.* [24] and photographed. The identification of the taxa photographed was carried out by combining several works [25, 26, 27, 28, 29, 30, 31, 32, 33]. Cell enumeration was carried out using an inverted microscope of the OPTIKA type by the Utermöhl method [34] and standard NF EN 15204 [35].

To evaluate the effect of agricultural by-products on phytoplankton population, algal density and indices such as species richness [36], frequency of occurrence [37], Shannon's diversity [38], Pielou's equitability [39] and Jaccard's similarity [40] indices were used.

2.4 Statistical analysis of data

The values of most of the parameters measured in this study were averaged for each type of pond. To evaluate the significant effect of the different inputs, some data were subjected to analyses of variance (ANOVA1 factor at 5% threshold) and others to Kruskal-Walli or Tukey tests using the "ade4" package in R 2.8 software [41]. A canonical analysis of ReDondances (RDA) was performed using the "vegan" package of R 2.8 [41] to highlight the correlation between dominant taxa and environmental variables.

3. Results

3.1 Physico-chemical parameters of pond water

With the exception of dissolved oxygen content and pH, the values of the different parameters recorded were higher in the rice-fish ponds where combined agricultural byproducts (RPC) were added than in the other ponds (Table 1). Temperature values measured in the different ponds were significantly identical (ANOVA, Tukey's test, $p > 0.05$) and ranged from 26.32 ± 1.45 (RSM) to 26.81 ± 1.18 °C (RPC), while values for dissolved oxygen, pH, dissolved solids content, conductivity, turbidity and transparency were significantly different between ponds (ANOVA, Tukey's test, $p < 0.05$). The concentrations of the nutrient compounds (NO_3^-, NH_4^+ and PO_4^{3-}) differ significantly from pond to pond (Kruskal-Wallis test, $p < 0.05$). The NO_3^- and NH_4^+ levels were highest in rice-fish ponds with combined by-product input (RPC) and rice bran inputs (RSR), while the lowest concentrations were obtained in rice-fish ponds without food input (RC). At the PO_4^{3-} level, the highest concentration (0.52 ± 0.28 mg/L) was also noted in rice-fish ponds with combined by-product input (RPC), while the lowest level (0.26 ± 0.15 mg/L) was recorded in rice-fish ponds (RC).

Table 1: Average values of physical water parameters of rice-fish ponds

	Rice-fish Ponds				
	RC	**RSR**	**RPC**	**RSM**	**p-valu**
Temp (°C)	26.41 ± 1.09^{a}	26.73 ± 1.34^{a}	26.81 ± 1.18^{a}	26.32 ± 1.45^{a}	0.44
DO (mg/L)	3.79 ± 0.55^{c}	3.40 ± 0.50^{b}	2.06 ± 0.34^{a}	2.28 ± 0.46^{a}	0.00
pH	6.25 ± 0.35^{c}	5.53 ± 0.48^{b}	5.10 ± 0.54^{a}	5.28 ± 0.43^{ab}	0.00
Cond (µS/cm)	149.54 ± 29.94^{a}	198.98 ± 29.94^{ab}	216.04 ± 70.88^{bc}	177.91 ± 41.72^{c}	0.00
Turb (NTU)	157.51 ± 20.8^{b}	226.16 ± 22.44^{a}	242.92 ± 64.31^{a}	193.06 ± 37.34^{c}	0.00
TDS (mg/L)	92.70 ± 15.63^{b}	133.76 ± 27.74^{a}	162.44 ± 25.17^{c}	133.83 ± 24.93^{a}	0.00
Transp (cm)	32.01 ± 5.22^{b}	36.03 ± 10.47^{b}	23.43 ± 4.63^{a}	26.96 ± 3.46^{a}	0.00
NO_3^- (mg/L)	0.24 ± 0.25^{b}	0.74 ± 0.17^{a}	1.01 ± 0.34^{c}	0.58 ± 0.19^{a}	0.00
NH_4^+(mg/L)	0.32 ± 0.18^{a}	0.51 ± 0.18^{bc}	0.58 ± 0.28^{c}	0.39 ± 0.19^{ab}	0.00
PO_4^{3-}(mg/L)	0.26 ± 0.15^{a}	0.33 ± 0.23^{a}	0.52 ± 0.28^{b}	0.37 ± 0.22^{ab}	0.00

Legend: Temp : temperature; DO : dissolved oxygen, Ph : hydrogen potential, TDS : dissolved solids content, Cond : conductivity, Turb : turbidity, Transp : transparency. The values of the lines with different alphabetical exponents (a, b and c) are significantly different (ANOVA, $p < 0,05$).

3.2 Effect of agricultural by-products on phytoplankton composition

A total of 194 taxa divided into five phyla and 51 genera were collected (Table 2). These phyla are Cyanophyta with 14 taxa (7.21%) and 10 genera, Euglenophyta with 83 taxa (44.33%) and six genera, Chlorophyta with 68 taxa (35.05%) and 18 genera, Bacillariophyta with 23 taxa (11.86%) and 15 genera and Dinophyta with 3 taxa (1.55%) and 2 genera. The species richness of the rice-fish ponds RSM and RPC is higher with 101 and 92 taxa respectively than that of the rice-fish ponds RSR (83 taxa) and RC (86 taxa). Euglenophyta taxa are more represented in all ponds and with a high proportion in the rice-fish ponds RSR (56.63%) and RPC (56.52%) (Figure 2). Dinophyta are less present with proportions of 2.32% (RC), 2.97% (RSM), 1.08% (RPC) and 1.40% (RSR). In these ponds, 56 constant taxa were recorded against 15 accessory taxa and 123 accidental taxa. The high phytoplankton diversity was noted in the rice-fish ponds RC (H' = 3.44 ± 0.62 bit/cells) compared to the other rice-fish ponds (Table 3). The equitability index was also higher in the rice-fish pond RC (Table 3). However, there was no significant difference (Kruskal-Wallis test, $p > 0.05$) between the Shannon diversity and Piélou equitability values obtained in the different ponds. Jaccard's similarity index values are low and vary between 0.28 and 0.40 between all rice-fish ponds (Table 4). These ponds compared are therefore different on the basis of occurrence of phytoplankton species. However, the lower values of the similarity index between ponds RC and RPC and RSM ponds (0.28) show a high heterogeneity between these ponds.

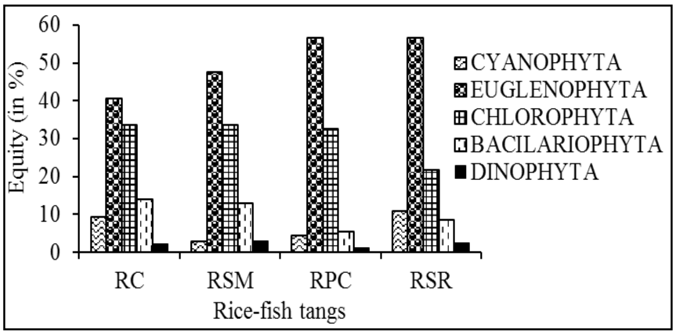


Fig 2 : Proportional representation of the different algae phyla according to rice-fish ponds. Legend: RC = Rice-fish pond without food input, RSR = Rice-fish pond with rice bran input, RPC= Rice-fish pond with combined by-products input, RSM = Rice-fish pond with maize bran input.

Table 2: Taxonomic list of phytoplankton inventoried at the Kouadiokro-Bonoufla fish farming station (Vavoua, Central-West - Côte d'Ivoire). Acro = acronym, F = frequency of occurrence, *** = constant taxon; ** = accessory taxon; * = accidental taxon, + = presence of the taxon.

Taxa	Rice-fish ponds					
	Acro	**F**	**RC**	**RSM**	**RPC**	**RSR**
Cyanophyta (7.21%)						
Chroococcus limneticus Lemmermann	Chli	*				+
Chroococcus minutus (Kützing) Nägeli	Chmi	*	+			
Aphanocapsa roseana De Bary	Apro	***	+			+
Aphanocapsa sp.	Apsp	***			+	+
Merismopedia punctata Meyen	Mepu	***	+			+
Merismopedia sp.	Mesp.	*	+			+
Microcystis aeruginosa (Kützing) Kützin	Miae	***	+	+	+	+
Microcystis sp.	Misp.	***	+			+
Nostoc entophytum Born. et Flah.	Noen	*	+			
Oscillatoria sp.	Ossp.	***	+			+
Phormidium sp.	Phsp.	*			+	
Planktothrix sp.	Plsp.	*		+		
Pseudanabaena sp.	Pssp.	***		+	+	
Spirulina princeps W. & G.S. West	Sppr	*				+
Euglenophyta (44.33%)						
Euglena dicentra Skuja.	Eudi	**			+	+
Euglena ehrenbergii G.A. Krebs	Eueh	*	+	+	+	+
Euglena ehrenbergii var. *baculifera* (Thompson) Gojdics	Eueb	*	+			+
Euglena hemichromata Skuja	Euhe	**			+	
Euglena gracilis (Klebs)	Eugr	**	+		+	

Euglena limnophila Lemm.	Euli	*			+	
Euglena obtusa Schimitz	Euob	*				+
Euglena polymorpha Dangeard	Eupo	**		+	+	+
Euglena proxima Dang. (Lemm.)	Eupr	***		+	+	+
Euglena rostrifera L.P. Johnson	Euro	*		+		
Euglena sanguinea Ehrenberg	Eusg	*			+	+
Euglena satelles Braslavskaja Spektorov	Eusa	***	+	+	+	+
Euglena sp.1	Esp.1	*	+	+		
Euglena sp.2	Esp.2	*				+
Euglena sp.3	Esp.3	**	+	+		+
Euglena sp.4	Esp.4	*		+		
Euglena sp.5	Esp.5	*				+
Euglena sp.6	Esp.6	*			+	
Euglena splendens Dangeard	Eusp	*		+	+	
Euglena tuberculata Svirenko	Eutu	*			+	+
*Euglena viridis (*Müller) Ehrenberg	Euvi	*	+			+
Lepocinclis acus (Müller) Marin & Melkonian	Leac	***	+	+	+	+
Lepocinclis fusiformis (Carter) Lemm.	Lefu	***	+	+	+	+
Lepocinclis ovum (Ehrenberg) Lemm.	Leov	***	+	+	+	+
Lepocinclis oxyuris fo. charkowiensis Hüber-Pestalozzi	Leox	*		+	+	+
Lepocinclis oxyuris Marin et Melk. var. *oxyuris*	Leoo	*			+	
Lepocinclis playfairiana Deflandre	Lepl	***			+	+
Lepocinclis salina Fritsch	Lesa	***	+	+	+	+
Lepocinclis sp.1	Lsp.1	*		+	+	
Lepocinclis sp.2	Lsp.2	*		+		
Lepocinclis sp.3	Lsp.3	*	+			+
Lepocinclis sp.4	Lsp.4	*		+	+	
Lepocinclis spirogyra (Ehrenb.) Marin & Melk. var *spirogyra*	Lspi	*			+	
Lepocinclis spirogyroides Marin et Melk.	Lesp	*			+	+

Table 2: continued

Taxa	Rice-fish ponds					
	Acro	**F**	**RC**	**RSM**	**RPC**	**RSR**
Euglenophyta						
Lepocinclis texta (Dujardin) Lemm.	Lete	***	+	+	+	+
Lepocinclis turbiniformis Defl.	Letu	***		+	+	+
Menoidium sp.	Mesp.	*	+			
Phacus angulatus Pochmann	Phan	*		+		
Phacus brevicaudatus (Klebs) Lemm.	Phbr	*			+	
Phacus caudatus Hübner	Phca	***		+	+	+
Phacus helicoides Pochm.	Phhe	***			+	+
Phacus inconspicuus Defl.	Phin	**		+	+	
Phacus lefevrei Bourrelly	Phle	***		+	+	
*Phacus longicauda (*Ehren.*)* Duja.	Phlo	***	+	+	+	+
Phacus longicauda (Ehren.) Duja. var. *longicauda*	Phll	***	+	+	+	+
Phacus longicauda var. *attenuatus (*Pochmann*)* Huber-Pest.	Phla	*			+	
Phacus longicauda var. *insecta* Koczwara.	Phli	***	+	+	+	+
Phacus longicauda var. *rotunda* (Pochm.) Huber-Pest.	Phlr	***	+	+	+	+
Phacus longicauda var. *tortus* (Pochm.)	Phlt	*		+		
Phacus onyx Pochm.	Phon	*			+	+
Phacus orbicularis Hübner	Phor	***	+	+	+	+
Phacus platalea Drezepolski	Ppla	***	+	+	+	
Phacus pleuronectes (Müll.) Duja.	Phpl	***	+		+	+
Phacus pleuronectes (O.F.Müll.) var. *triquetra* Klebs	Phpt	*		+		
Phacus ranula Pochm.	*Phra*	*		+		
Phacus sesquitortus Pochm.	Phse	*			+	
Phacus sp.1	Phsp.1	*				+
Phacus sp.2	Phsp.2	*		+		
Phacus swirenkoi Skvortzov	Phsw	***			+	+
Phacus tortus (Lemm.) Skvortzov	Phto	***		+	+	+
Phacus triqueter (Ehren.) Dujardin	Phtr	*	+		+	
Phacus undulatus (Skvortzov) Pochm.	Phun	***	+		+	+
Strombomonas fluviatilis (Lemm.) Defl.	Strf	***	+	+	+	+
Strombomonas schauinslandii (Lemm.) Defl.	Strs	*		+		
Strombomonas sp.	Strp.	*		+		
Strombomonas sp.1	Stsp.1	*		+		
Strombomonas verrucosa (Daday) Defl.	Stve	*	+			
Trachelomonas abrupta Svirenko	Trab	**	+		+	

Trachelomonas bacillifera Playf.	Trba	*				+
Trachelomonas curta Da Cunha	Trcu	***		+	+	+
Trachelomonas duplex (Defl.) Couté & Tell	Trdu	*				+
Trachelomonas hispida (Perty) Stein emend. Defl.	Trhi	***	+	+		
Trachelomonas hispida var. *coronata* Lemm.	Trhc	*				+
Trachelomonas lefevrei Defl.	Trle	***	+	+	+	+
Trachelomonas mirabilis Defl.	Trmi	*				+
Trachelomonas pisciformis var. *bicoronata* Couté & Iltis	Trpi	*				+
Trachelomonas raciborskii Woloszynska	Trra	*		+		
Trachelomonas scabra Playf.	Trsc	*	+	+		
Trachelomonas sp.1	Trsp.1	***	+	+	+	
Trachelomonas sp.2	Trsp.2	***	+	+	+	
Trachelomonas sp.3	Trsp.3	*			+	+
Trachelomonas sp.4	Trsp.4	*	+			
Trachelomonas volvocina Ehrenb.	Trvo	***	+	+	+	+
Trachelomonas volvocina var. *derephora* Conrad &Van Meel	Tvod	*		+		
Trachelomonas volvocinopsis Svirenko	Tvos	***	+	+		+

Table 2: continued

Taxa	Rice-fish ponds					
	Acro	**F**	**RC**	**RSM**	**RPC**	**RSR**
Chlorophyta (35.05%)						
Actinastrum gracillimum G.M. Sm.	*Acgr*	*		+		
Actinastrum sp.	Acsp.	***	+	+	+	
Actinastrum sp.1	Acsp.1	*			+	
Actinotaenium dilplosporum Lundell	Acdi	*	+			
Ankistrodesmus sp.	Ansp.	***	+		+	
Closterium cynthia De Notaris	Clcy	**	+			+
Closterium gracile Brébisson ex Ralfs	Clgr	*				+
Closterium jenneri Ralfs	Clje	*	+			
Closterium lineatum Ehrenb. ex. Ralfs	Clli	***	+	+	+	+
Closterium littorale F. Gay	Clit	*		+		
Closterium moniliferum Ralfs	Clmo	*		+		
Closterium parvulum NÄgeli	Clpa	*	+			
Closterium praelongum Bréb.	Clpr	*		+		
Closterium sp.	Clsp.	*		+		
Closterium sp.1	Clsp.1	*	+	+		
Closterium sp.2	Clsp.2	*		+		+
Closterium sp.3	Clsp.3	*	+			
Closterium sp.4	Clsp.4	*			+	
Closterium strigosum Bréb.	Clst	*		+		
Coelastrum cambricum W. Archer	Ceca	***	+	+	+	+
Coelastrum sp.	Cesp.	*			+	
Coelastrum recticulatum (Dang.)	Cere	*	+			
Cosmarium binum Nordstedt	Cosb	***	+	+	+	+
Cosmarium galeritum Nords. var. *subtumidum* Borge	Cosg	*	+			
Cosmarium subauriculatum West & G.S. West	Cosu	**	+	+		
Cosmarium sp.	Coss.	*		+		
Dictyosphaerium sp.	Disp.	**	+		+	
Euastrum elegans (Turpin.) Ralfs	Euel	*			+	
Euastrum pseudopectinatum Schmidt	Eups	*	+			
Kirchneriella lunaris (Kirchner) Moebius var. *irregularis* G.M	Kilu.	*	+			
Oedogonium sp.	Oesp.	*	+			
Pandorina sp.	Pasp.	*				+
Pediastrum biradiatum Meyen	Pebi	*		+		
Pediastrum boryanum (Turpin) Meneghini	Pebo	*		+		
Pediastrum duplex Meyen var. *duplex* Meyen	Pedd	***	+	+	+	+
Pediastrum duplex Raciborski	Pedu	***		+	+	+
Pediastrum duplex var. *gracilimum* W.West & G.S.West	Pedg	***	+	+	+	+
Pediastrum duplex var. *rugulosum* Racib.	Pedr	*			+	
Pediastrum duplex var. *subgranulatum* Racib.	Peds	*		+		
Pseudostaurastrum sp.	Pseu.	*			+	
Scenedesmus carinatus (Lemm. E.) Chodat R.	Scca	*			+	
Scenedesmus acuminatus (Lagerheim) Chodat	Scac	*		+		
Scenedesmus acutiformis Schröder	Scact	*			+	
Scenedesmus bernardii G.M.Smith	Scbe	***		+	+	+
Scenedesmus communis Hegewald	Scco	*			+	
Scenedesmus disciformis (Chodat) Fott & Komárek	Scdi	*	+			

Scenedesmus ecornis (Ehrenb.)	Scec	*			+	
Scenedesmus naegeli Brébisson	Scna	***	+		+	+
Scenedesmus opoliensis P.G. Richter	Scop	***	+	+	+	+
Scenedesmus opoliensis var. *mononensis* Chodat	Scom	***		+	+	+

Table 2: continued

Taxa	Rice-fish ponds					
	Acro	**F**	**RC**	**RSM**	**RPC**	**RSR**
Chlorophyta						
Scenedesmus protuberans Fritsch & Rich.	Scpr	**	+		+	+
Scenedesmus quadricauda Chodat	Scqc	*			+	
Scenedesmus quadricauda var. *longispina* (Chodat) G.M.S	Scql	***	+	+		+
Scenedesmus javanensis Chodat	Scja	***		+		+
Scenedesmus quadricauda var. *quadrispina* (Turpin) Bréb.	Scqs	*	+			
Scenedesmus sp.	Scsp.	*	+			
Scenedesmus tropicus Crow	Sctr	*			+	
Spirogyra sp.	Spsp.	*		+		
Staurastrum brachioprominens var. *archerianum* Bohlin	Stab	*	+			
Staurastrum volans West & G.S. Wes	Svol	*		+		
Staurastrum setigerum Cleve	Sset	**		+		
Staurodesmus brevispina (Ralfs) Croasdale	Sbre	**	+		+	
Staurodesmus sp.	Stasp	*			+	
Tetraedron incus (Teiling) G.M. Smith	Tein	*		+		
Tetraedron trilobulatum (Reinsch) Hansgirg	Tetr	***		+	+	+
Tetraedron sp.	Tesp.	*		+		
Tetraedron arthrodesmiforme West fo. *typicum* Wolosz.	Tear	*		+		
Tetraedron trigonum (Nägeli) Hansg.	Tetr	**		+	+	
Bacillariophyta (11.86%)						
Amphora sp.	Amsp.	*	+			
Cyclotella litoralis Lange & Syvertsen	Cyli	*		+		
Cymbella dadwinensis Foged	Cyda	**			+	+
Epithemia sp.	Epsp.	*				+
Frustulia sp.	Frsp.	*		+		
Gomphonema gracile Ehrenb.	Gogl	*	+			
Gonatozygon pilosum Wolle	Gopi	*	+			
Hantzschia sigma Hustedt	*Hasi*	*		+		
Orthostichae sp.	Orsp.	*		+		
Pinnularia acrosphaeria W.Sm.	Piac	***	+	+		+
Pinnularia gibba Ehrenb.	Pigi	*				+
Pinnularia legumen Ehrenb.	Pile	*	+			
Pinnularia interrupta W. Sm.	Piin	**	+	+		
Pinnularia major (Kützing) Rabenhorst	Pima	**	+		+	
Pinnularia sp.	Pisp.	***	+	+	+	+
Pinnularia sp.1	Pisp.1	*		+	+	
Pinnularia sp.2	Pisp.2	*	+	+		+
Pinnularia viridis (Nitzsch) Ehrenb.	Pivi	*	+			
Rhoicosphenia sp.	Rhsp.	*		+		
*Stephanodiscus astraea (*Ehrenberg) Grunow	Stas	*	+	+		
Surirella tenera Gregory	Sute	***	+	+	+	
Thalassiosira sp.	Thsp.	*		+		
Ulnaria ulna (Nitzsch) Comp.	Ulul	*				+
Dinophyta (1.55%)						
Peridinium cinctum (O. Müller) Ehrenb.	Pecin	***	+	+	+	+
Peridinium sp.	Pesp.	*	+	+		
Protoperidinium sp.	Prsp.	*		+		+
Specific richness		194	86	101	92	83

Table 3 : Average values of Shannon's index and Piélou's equitability in the different rice-fish ponds.

	Rice-fish ponds				
	RC	**RSM**	**RPC**	**RSR**	***p-valu***
Shannon's index (bit/cells)	3.44 ± 0.62[a]	3.47 ± 0.66[a]	3.23 ± 1.08[a]	3.38 ± 0.71[a]	0.98
Piélou's equitability index	0.88 ± 0.05[a]	0.78 ± 0.07[a]	0.77 ± 0.02[a]	0.80 ± 0.04[a]	0.53

Legend: RC = rice-fish pond without food input, RSR = rice fish pond with rice bran input, RPC = rice fish pond with combined by-product input, RSM = rice fish pond with maize bran input.The same alphabetic exponent (a) indicate a no significant difference between the values at 5% significance level based Kruskal-Wallis test, $p > 0.05$.

Table 4 : Similarity between the different rice-fish pond.

	Jaccard's similarity index			
RC	--			
RSR	0.33	--		
RPC	0.28	0.4	--	
RSM	0.28	0.3	0.32	--
	RC	RSR	RPC	RSM

Legend: RC = rice-fish pond without food input, RSR = rice-fish pond with rice bran input, RPC: = rice-fish pond with combined by-product input, RSM = rice-fish pond with maize bran input.

3.3 Effect of agricultural by-products on phytoplankton density

Overall phytoplankton density ranged from 21.88×10^6 to 68.82×10^6 Individuals/L (Figure 3). The minimum densities were obtained in rice-fish ponds without exogenous food (RC) with an average of 30.5×10^6 Individuals/L while they were higher in rice-fish ponds that received combined by-products (RPC) and those that received maize bran (RSM) with an average of 60.6×10^6 and 55.1×10^6 Individuals/L, respectively. The ANOVA test indicates a significant difference ($p < 0.05$) between the phytoplankton densities recorded in the different rice-fish ponds. The relative density of the different phytoplankton phyla collected shows a small proportion of the Dinophyta density in all rice-fish ponds (Figure 4). However, the algal density recorded in the rice-fish ponds RC is dominated by Chlorophyta (35.04%) and Bacilariophyta (25.96%) while the algal density obtained in the other rice-fish ponds is dominated by Chlorophyta and Euglenophyta with a strong preponderance in the rice-fish pond RC.

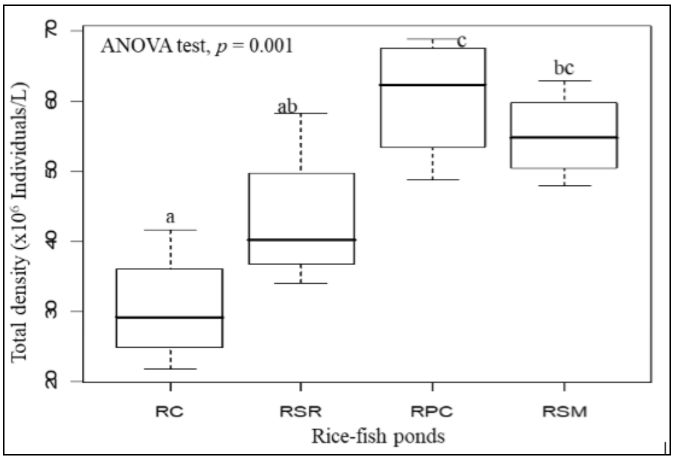


Fig 3: Total phytoplankton density in the different ponds. Legend: RC = rice-fish pond without food input, RSR = rice-fish pond with rice brann input, RPC = rice-fish pond with combined by-products input, RSM = rice-fish pond with maize bran input. The different letters (a, b and c) on the moustache boxes indicate a significant difference at 5% significance level based on one way ANOVA followed by Tukey's test.

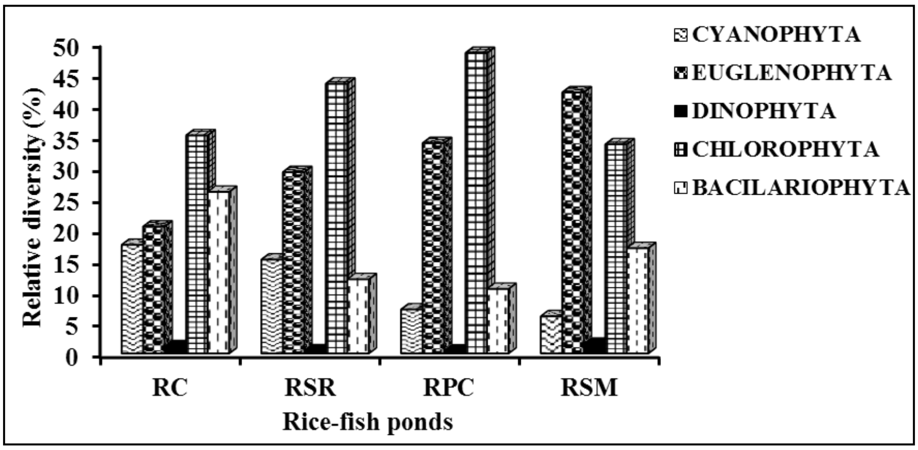
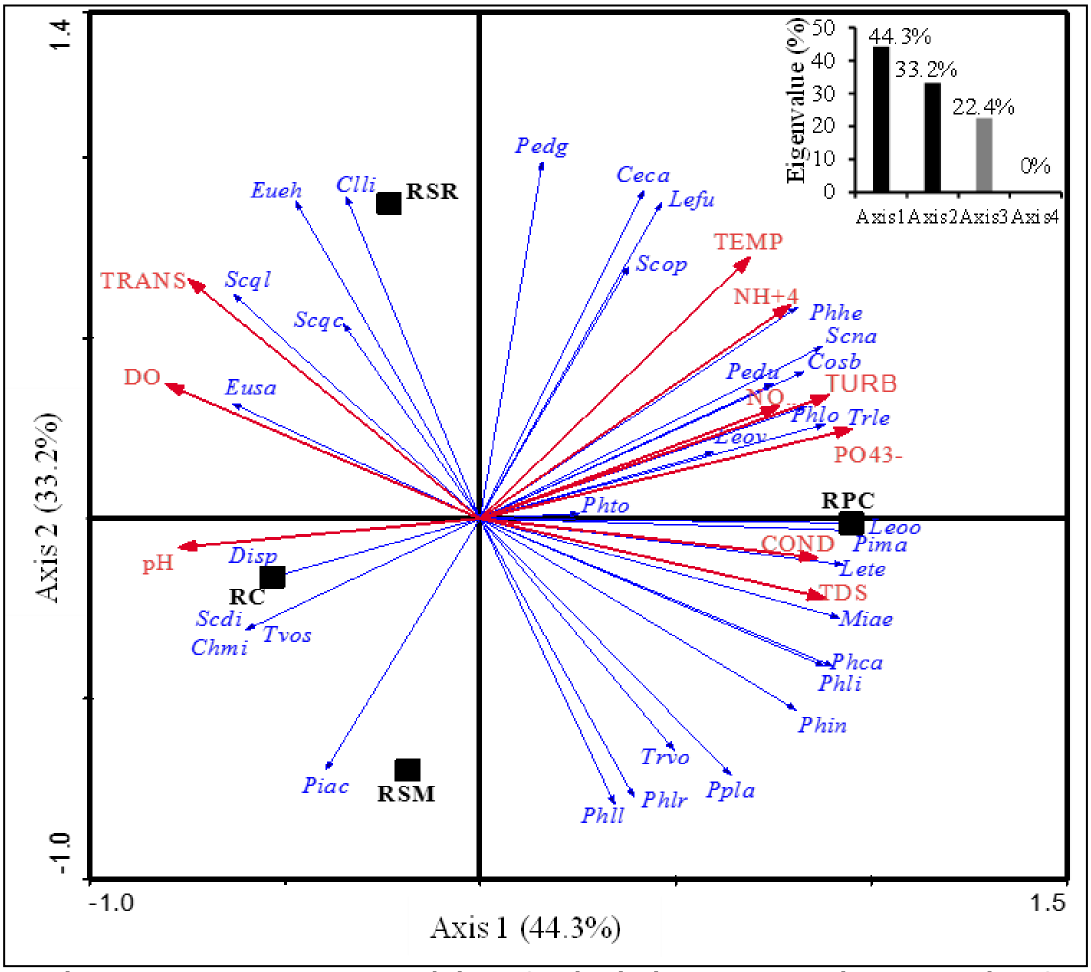


Fig 4: Proportion of phytoplankton phyla in rice-fish ponds. Legend : RC = rice-fish pond without food input, RSR = rice-fish pond with rice bran input, RPC = rice-fish pond with combined by-products input, RSM = rice-fish pond with maize bran input.

3.4 Determining the distribution of phytoplankton in individual ponds

Based on the density of the 197 taxa harvested, 33 species with densities greater than or equal to 2.50% of the absolute density were selected and cross-referenced with environmental variables. Axis 1 and 2 of the canonical Redundancy Analysis (RDA) absorbed 77.5% of the variance (Figure 5). The first axis, which accumulates 44.30% of the total variance, is positively correlated with dissolved solids (TDS), conductivity (COND), turbidity (TURB) and nutrient compounds (PO_4^{3-}, NO_3^- and NH_4^+). This axis opposes on the one hand the high level of nutrient compounds to high pH values and on the other hand the high values of TDS and COND to those of dissolved oxygen (DO) and transparency (TRANS). The proliferation of *Microcystis aeruginosa* (Miae), *Pinnularia major* (Pima), Euglenophyta such as *Phacus longicauda* var. *insecta (*Phli), *P. caudatus (*Phca), P. *longicauda (*Phlo), *P. helicoides (*Phhe), *Lepocinclis texta (*Lete), *L. oxyuris* var. *oxyuris (*Leoo) and *Trachelomonas lefevrei (*Trle) and Chlorophyta such as *Pediastrum duplex (*Pedu), *Scenedesmus naegeli (*Scna) and *Cosmarium binum (*Cosb) is strongly influenced in the rice-fice pond RPC by the high concentrations of nutrient compounds and the values of turbidity, conductivity and TDS.

In addition, pH and dissolved oxygen were the variables that influenced the distribution of *Euglena satelles* (Eusa), *Trachelomonas volvocinopsis (*Trvs), *Dictyosphaerium sp.* (Disp), *Scenedesmus disciformis (*Scdi) and *Chroococcus minutus* (Chmi) in rice-fice pond RC. The second axis which expressed 33.20% of the total variance is positively correlated by *Euglena ehrenbergii* (Eueh), *Closterium lineatum (*Clli) and *Pediastrum duplex* var. *gracilimum* (Pdg). Following this axis these last species characterize the rice-fice pond RSR.



Legend: TEMP = temperature, TURB = turbidity, DO = dissolved oxygen, pH= Hydrogen potential, TDS = dissolved solids content, COND = Conductivity, TRANS = Transparency, NH_4^+ =Ammonium, NO = nitrate, PO_4^{3-} = orthophosphate. RC = rice-fish pond without food input, RSR = rice-fish pond with rice bran input, RPC = rice-fish pond with combined by-products input, RSM = rice-fish pond with maize bran input.

Figure 5 : Ordering in RDA of environmental parameters and taxa on axis 1 and 2.

4. Discussion

Overall, taxon richness is high (194 taxa) in the rice-fish ponds of Bonoufla with higher richness in rice-fish ponds with maize bran input (RSM) and combined by-products input (RPC) compared to rice-fish ponds without food input (CR). This high taxon richness obtained could be the result of environmental conditions conducive to inocula from the feeder dam located upstream of the ponds. On the other hand, the higher specific richness obtained in the rice-fish ponds RSM and RPC could be related to the enrichment of these ponds in nutrient compounds by the loss of nutrients from the exogenous feed supplied to the fish. Indeed, according to Blé *et al.* [8], exogenous nutrients in fish farming would stimulate pelagic trophic pathways by promoting diversity and efficient development of planktonic organisms. The total number of phytoplankton taxa recorded in the present study is very close to those of Kra [19] and Bamba [18] who collected 197 taxa at the CNRA fish farm in Bouaké in 2016 and 190 taxa in at the fish farm in Blondey in 2007, respectively. However, this number of taxa is much higher than those of Avit *et al.* (2012) [20] and Bony *et al.* (2015) [21] who all recorded 19 phytoplankton taxa in the rice-fish ponds of the CNRA in Bouaké. This difference could be attributed on the one hand to the mineralization activities of the ponds and on the other hand to the small amount of filtered water (30 L) by these authors. The total richness is dominated by Euglenophyta (44.33%) with their high diversity in the rice-fish pond RSR (56.63%) and RPC (56.52%). This dominance of Euglenophyta, especially the genera *Euglena* and *Phacus*, is explained by the impact of the distributed food rich in organic matter [42] and therefore by the overall state of water pollution on the rice-fish farm. Indeed, according to Reynolds *et al.* [43], Thomas [44] and Rahman *et al.* [45], Euglenophyta, especially the genera *Euglena*, are known for their predilection for eutrophic environments *sensu lato* rich in putrescible organic substances from their environment. Shannon's index and Piélou's equitability values were high in the different ponds reflecting a high phytoplankton diversity and a high stability of the ponds. According to Frontier [46], in exceptionally diversiffied environments, the Shannon index never exceeds 4.5. The absence of significant difference between the Shannon diversity values and the Piélou equitability values of the different ponds could be justified by the fact that the ponds were filled by stagnant water from the same dam. Indeed, according to Adon *et al.* [47], stagnant water promotes biological processes such as complete cycles of reproduction and development of algae. Jaccard similarity index values between ponds taken 2 to 2 were less than (0.5). This result shows that the ponds compared, in terms of taxon, are very different. According to De Bello *et al.* [48], indices below 0.5 indicate that the habitats of the species are heterogeneous and that different habitat conditions determine a turnover of important species. Concerning phytoplankton density, the highest algal densities and a high preponderance of

Chlorophyta and Euglenophyta were recorded in rice-fish ponds that received exogenous food (RSM, RPC and RSR). These high densities are believed to be due to the effect of fortification of these ponds with nutrient salts through the daily distribution of rice bran and several ingredients of the combined feed that increase the organic matter content in these ponds. Thus, the high density in the RPC ponds coincides with the high NO_3^- (1.01 ± 0.34 mg/l) and PO_4^{3-} (0.52 ± 0.28 mg/l) values. However, the decomposition of this organic matter provides a large amount of nutrients that can be assimilated by the microalgae, especially phosphorus and nitrogen, and promotes their growth [49]. These results agree with the conclusions of Rahman *et al.* [45] and those of Da [15] according to which Euglenophyta and Chlorophyta are very abundant in environments rich in organic and inorganic matter. The distribution of the dominant Euglenophyta and Chlorophyta in the experimental farm is strongly related to turbidity and conductivity, nutrient concentrations and water pH. This relationship is supported by Munawar [50]. According to this author, the concentration of nutrient salts and oxygen in the water affects the growth and photosynthetic activity of phytoplankton. Thus, the high concentrations of nutrient compounds (PO_4^{3-}, NO_3^- and NH_4^+) and the low pH and dissolved oxygen values obtained in the rice-fish ponds RPC justify the high density of Euglenophyta and Chlorophyta in this pond.

5. Conclusion

The data obtained showed that phytoplankton diversity and density were higher in rice-fish ponds where agricultural by-products were distributed to juveniles of *O. niloticus* with a dominance of Euglenophyta and Chlorophyta. Taking into account the high density of these branches for a sustainable and less expensive rice-fish culture requires a control of the frequency of feed intake based on agricultural by-products. Further work with a sampling frequency of several days during the complete rearing cycle of fish *O. niloticus* (from rearing to grow-out) during the climatic seasons could allow more accurate conclusions on the influence of agricultural by-products on change in phytoplankton population and environmental conditions in rice-fish farming environments.

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