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Assessment of the impact of agricultural by-products on the dynamics of phytoplankton functional groups on a rice-fish farm in Bonoufla, Côte d'Ivoire

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Abstract

The objective of this study was to determine the influence of agricultural by-products on the dynamics of phytoplankton functional groups' abundance on a rice-fish farm. To this end, experiments were conducted in rice-fish ponds by introducing three categories of agricultural by-products: maize bran (RSM), rice bran (RSR), mixed agricultural by-products (RPC) and a control with no exogenous feed (RC). Data were collected monthly between May 2019 and November 2020 during the Pre-Grow-out and Grow-out phases of *Oreochromis niloticus.* Phytoplankton were sampled by water filtration using a 20 µm mesh plankton net. Algal cells were identified and counted. The Reynolds Functional Groups (RFG) classification was used to form the Phytoplankton assemblages with the dominant microalgae. Physicochemical parameters likely to influence algal proliferation were measured using a HQ40D multi-parameter and nutrient salts were determined using spectrometric methods. It was found that the phytoplankton flora of the rice-fish ponds was composed of 235 algal taxa, with a higher diversity of Chlorophyta and Euglenophyta. The specific richness of phytoplankton was virtually identical from one feeding treatment to the next. The phytoplankton in these ponds were grouped into 08 functional groups. The group J, represented by species of the genus *Coelastrum, Lacunastrum, Desmodesmus* and *Scenedesmus*, and group W2, represented by species of the genus *Trachelomonas* and *Euglena*, were the most abundant in all rice-ponds and in all months. The relative density of these functional groups increased monthly and significantly during each rearing phase with the RPC treatment compared with the other treatments. The proliferation of taxa in these functional groups was positively correlated with the concentration of nutrient salts, pH and dissolved oxygen content of the water in the rice-fish ponds. These results show that the J and W2 phytoplankton assemblages are effective indicators for monitoring organic pollution of rice-fish pond water.

Key words: Phytoplankton functional groups, Rice-fish farming, Agricultural by-products, Nutrient salts, Côte d'Ivoire.

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INTRODUCTION

Rice-fish ponds are shallow, artificially stagnant, flooded rice fields where fish and rice plants are the important components that we want to grow optimally at the same time (Mortillaro *et al.,* 2022). These ponds have several trophic levels with a diversity of organisms (Mortillaro and Dabbadie, 2018). Among these organisms, microalgae form the basis of pelagic food chains (Azam and Malfatti, 2007). In fish farming, planktonic microalgae are a key component in the functioning of rearing structures and form an important part of the natural feed of certain farmed fish such as tilapia (Ouattara *et al.,* 2009; Kra *et al*., 2023). These planktonic microalgae, known as phytoplankton, are photosynthetic organisms invisible to the naked eye that live freely and passively suspended in the water column (Stickney *et al*., 2000; Groga, 2012).

As in fish farming, the simultaneous production of fish and rice in rice-growing basins makes use of exogenous feeds such as industrial feeds, fish meal and by-products of plant and animal origin (Zié *et al.,* 2022; Kamagaté *et al.,* 2020; Niamien *et al.,* 2017; Bamba, 2007). According to surveys by (Kamagaté *et al.,* 2020; Kimou *et al.,* 2016; Yao *et al.,* 2017), local feeds based on agricultural and agrifood by-products are the most widely used in rice and fish production in Côte d'Ivoire. The use of exogenous feeds is inevitably accompanied by an increase in nutrient loads and leads to an increase in the trophic status of fishponds (Kopp *et al.,* 2016*;* Dochin *et al.*, 2020). The main symptoms of this state throughout the world are the massive development of phytoplankton. However, the high concentration of phytoplankton is accompanied by a reduction in the dissolved oxygen content of the water in the farming structures and can lead to serious economic losses for the fish farmer (Briand *et al*., 2003; Rahman *et al*., 2012). Consequently, knowledge of phytoplankton dynamics in fish farming environments is essential for effective management. In Côte d'Ivoire, the numerous studies carried out on microalgae in fish and rice-farming ponds have focused on analysing the diversity and dynamics of phytoplankton taxonomic assemblages (Da, 1992; Dabbadie, 1996; Kouassi, 2004; Bamba, 2007; Avit *et al*., 2012; Groga *et al*., 2019; Kouadio *et al*., 2020; Soro 2020; Kra *et al*., 2021). All these studies do not take into account the functional group of phytoplankton in farmed ponds. However, the functional group approach established by Reynolds *et al*. (2002) is more effective for characterising the environmental parameters of phytoplankton in water bodies (Baillot, 2013). So, this approach has been used in the country's large tropical lakes such as Taabo Lake and Dohou Lake to diagnose the ecological quality of the water (Kouamé *et al.,* 2021; Camara *et al*., 2022). It therefore appears to be better suited to understanding the dynamics of phytoplankton community patterns in small fish and rice-farming ponds. This approach provides a good description of the algal assemblage in terms of habitat characteristics, environmental tolerance and trophic status of lakes and ponds compared with other systems (Reynolds, 2006; Padisák *et al.,* 2009). The concept adopted today is designed to group together species with similar morphological and physiological characteristics, as well as those with similar ecological requirements (Reynolds, 2006; Padisák *et al.,* 2009; Salmosa *et al.,* 2015).

The aim of this study was to analyse the influence of agricultural by-products used in fish feed on the dynamics and determinism of phytoplankton functional groups' abundance in rice-fish ponds on a farm in west-central Côte d'Ivoire.

MATERIALS AND METHODS

Geographical location and description of the Kouadiokro-Bonoufla rice-fish farm

The study was carried out at the Kouadiokro-Bonoufla rice-fish farm. The farm is located in the Department of Vavoua at latitude 7°11'40" N and longitude 6°31'38" W (Figure 1). The climate in this Department is tropical and humid, with a dry season from October to March and a rainy season from April to September, with a rainfall peak in June and another in September (Ligban *et al*., 2009). The Kouadiokro-Bonoufla rice-fish farm consists of nine ponds. Pond E1 has a surface area of 675 sq.m and the other eight ponds have surface areas of between 200 and 360 sq.m (Kouadio *et al.,* 2023). A dam upstream of the farm supplies water to all the ponds. This dam is itself supplied with water by a small river running through a cocoa plantation. The Kouadiokro-Bonoufla rice-fish farm is subject to strong anthropogenic pressures (Kouadio *et al.,* 2022).

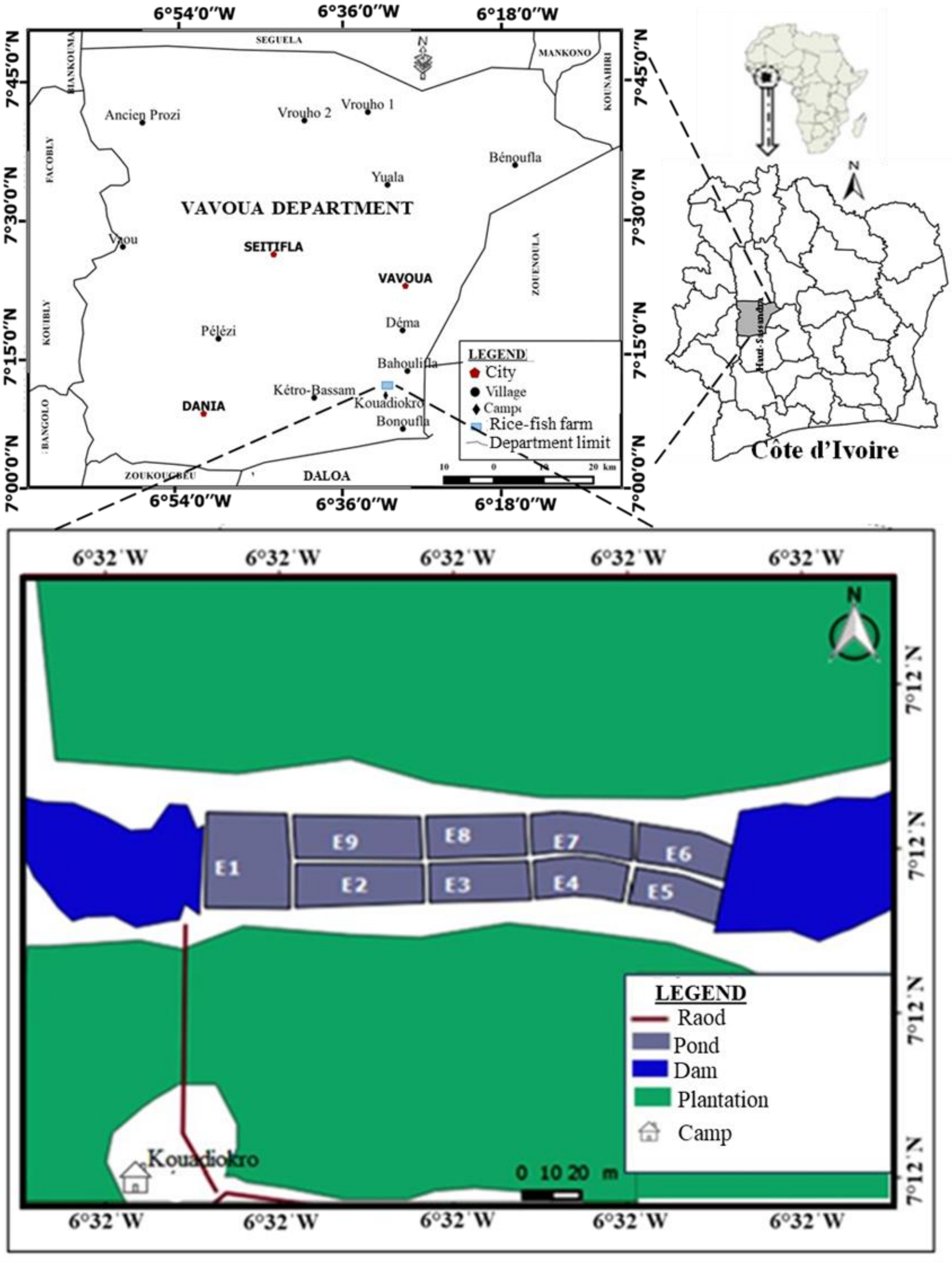

Figure 1: Geographical location of the Kouadiokro-Bonoufla rice-fish farm (Kouadio *et al.,* 2022)

Experimental set-up

The experimental set-up used in this study was the same as that of Kouadio *et al.* (2022). Eight ponds were created by digging trenches and refuge areas for the combined production of rice and fish. The ponds were left to dry for one month. Then, *Oryza sativa* rice seedlings of the Wita-9 variety with a 4-month crop cycle were transplanted into these ponds to carry out the fish Pre-Grow-out phase. After this rearing phase, *Oryza glaberrima* rice seedlings with a 6-month crop cycle were also transplanted into the same ponds for the fish Grow-out phase. These two varieties were chosen because their cropping cycle covers the duration of Pre-Grow-out and Grow-out of *O. niloticus* in extensive rearing, respectively. At the start of each rearing phase, the two-week-old seedlings were transplanted two by two into pots with a spacing of 20 cm between pots in the same row and 25 cm between the rows, using the technique described in (Kouakou *et al.*, 2016). The seedlings were transplanted on the same day and the ponds were re-watered two weeks after transplanting (Figure 2). The depth of water in each pond was gradually increased as the size of the rice increased, until water depths ranging from 0.5 to 1 m were reached.

Figure 2: View of a pond transplanted, re-watered and stocked for Pre-Grow-out

The ponds were stocked 30 days after rice transplanting. Fish stocking densities were 20 fry/sq.m (6 ± 1.02 g) for the pre-grow-out phase and 1.5 male juveniles/sq.m (38 ± 0.09 g) for the grow-out phase. These stocked ponds were used to create a randomised block design with two replicates and four feeding treatments for each phase of fish rearing (Figure 3). They were distributed as follows:

- two rice-fish ponds with no feed (RC);
- two rice-fish ponds with maize bran (RSM);
- two rice-fish ponds with rice bran (RSR);
- two rice-fish ponds with a mixture of by-products (RPC). This mixture consists of rice bran, palm oil, cooking salts, shellfish ash, maize bran and cotton and soya cake.

The fish in the RSM, RSR and RPC ponds were fed 250 g of the corresponding feed in the mornings (9 a.m) and evenings (3 p.m).

Bromatological analyses of the various by-products fed to the fish were carried out at the Central Analysis Laboratory of NANGUI ABROGOUA University using the Kjeldahl, gravimetric and calorimetry methods. For these analyses, two assays were carried out for each foodstuff and the average values for each bromatological compound were recorded in Table I.

Sampling period

Data were collected monthly between 7:30 a.m and 12:30 p.m during the Pre-Grow-out (juvenile) and grow-out (adult) phases of *O. niloticus*. Three sampling campaigns were carried out between May and July 2019 during the Pre-Grow-out phase. The six other sampling campaigns were carried out between June and November 2020 during the *O. niloticus* grow-out phase. These periods were defined taking into account the rice-growing season in the study area (April to December), the crop cycle of the transplanted rice varieties and the duration of the final rearing phases of *O. niloticus*.

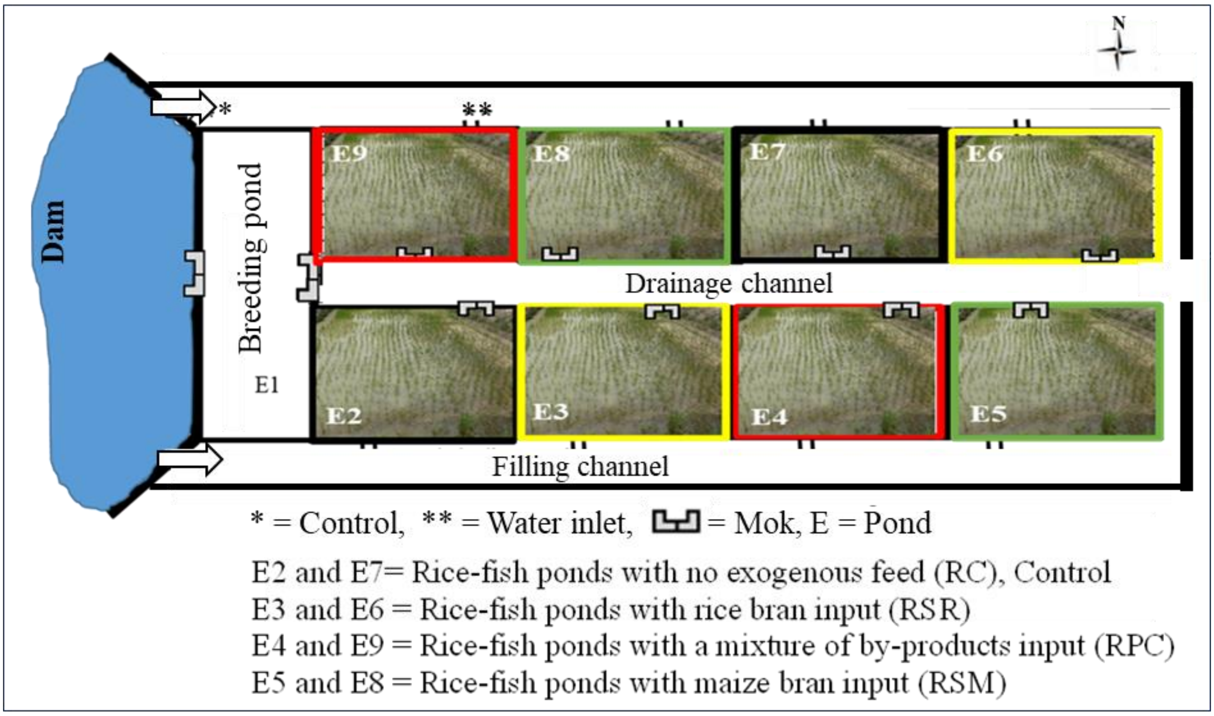

Figure 3: Diagram of the experimental set-up (Kouadio *et al*., 2022)

Table I: Average values of bromatological compounds of agricultural by-products used during the pre-grow and grow-out phase of fish.

Biochemical composition (% of dry matter)	Agricultural by-products		
	Rice bran	Corn bran	Mixture of by-products
Dry matter (%)	91.23	86.37	86.96
Crude protein (%)	13.31	14.21	23.1
Fat (%)	5.63	9.78	9.74
Ash (%)	5.19	7.02	8.05
Total carbohydrates (%)	64.44	55.36	46.97
Non-nitrogenous extractives (%)	25.60	31.82	36.61
Fibre (%)	38.43	23.54	12.36
Gross energy (kj/g)	16.55	12.71	17.55
Digestive energy (kj/g)	11.30	11.08	13.15

Water physicochemical parameters measuring

Parameters such as pH, dissolved oxygen (DO), temperature, total dissolved solids, turbidity and electrical conductivity were measured *in situ* in each rice-fish pond along its diagonals. The measurements were taken with the probes of a multi-parameter HQ40d, which had been calibrated beforehand. When the measurements were taken, an appropriate probe for each of these parameters was calibrated with the device switched on and immersed in the water. Once the value of the selected parameter had stabilised, it was recorded in a notebook. During each campaign, the transparency of the water was measured at midday. The measure was by gently dipping the Secchi disc into the pond water and noting down the depth at which it disappears.

Water samples of 500 ml from each pond were taken in plastic bottles and wrapped in aluminum foil. These samples were kept in a cooler with an internal temperature of 4°C. The nitrogen and phosphorus compounds in these water samples were measured in the Laboratory of the Oceanological Researches Centre. Nutrient salts were determined 48 h after taking the water samples. Spectrometric analysis of nitrogen and phosphorus compounds was carried out according to the protocols described by Kouamé (2021) in compliance with the standards of AFNOR (1994).

Figure 4: Filtration of water samples using the plankton net

Phytoplankton sampling

Phytoplankton from the rice-fish ponds were collected using two sampling methods. The first method, designed for qualitative analyses, involved taking 45 L of water with a bucket in each rice-fish pond, following the diagonals. These samples were filtered using a 20 µm mesh plankton net (Figure 4). After filtration, sub-samples of the water obtained were inverted into 50 ml pillboxes labelled according to the code for farming structures, then fixed immediately with 5% formalin. The second method was devoted to quantitative analyses of phytoplankton. This involved taking a litre of water from each farm using a sterilised plastic jar. These quantities of water were left to settle and the pellets were poured into pillboxes and treated with formalin as before.

Phytoplankton identification

The phytoplankton cells were observed under a light microscope. Drops of water from each collected sub-sample were taken using a micropipette and mounted between the slide and the coverslip. For each water subsample, at least five slides were prepared to ensure reproducibility (Atanlé *et al.,* 2012). The slides were observed under the microscope and the taxa encountered were measured by micrometre and photographed. The slides were first observed using the microscope's 20x objective for an overall view, then using the 40x or 60x objectives to observe the details of the organisms (cell size, length of filaments). The taxa observed were identified using the morphological keys described by Komárek and Anagnostidis (1999, 2005); Kim *et al.* (2000); Komárek *et al*. (2005); Osório *et al.* (2018); Zongo *et al*. (2008); Wołowski *et al*. (2013); John *et al.* (2021); Komárek *et al*. (2014); Salla (2015) and Tucci *et al*. (2019). The names of the taxa were updated according to the classification proposed in Algaebase (Guiry and Guiry, 2024). Taxa identified were grouped by phylum using to the systematics of John *et al.* (2021).

Phytoplankton enumeration

Phytoplankton cells were counted in accordance with the Utermöhl method (Utermöhl, 1958) modified (standard NF EN 15204) by Laplace-Treyture *et al.* (2007). Algal individuals were counted in 5.5 ml water subsamples using an inverted microscope and a sedimentation tube. Cell counts were carried out on 45 randomly selected eye fields without repetition in order to comply with the 5% counting fidelity (AFNOR EN 15204/T 90-179 standard) by counting at least 400 algal objects (individuals). Each cell colony, 100 µm filament and isolated cell was counted as an individual. The density of a taxon i (Di) was calculated according to the same standard using this formula:

$$D_i = \frac{X_i}{C}\left[\frac{Ad}{aV}\right]$$

where : $a = \pi R^2_{40x}$

With : Di = density of a taxon i, expressed in Individuals/l;

Xi = number of individuals counted for a taxon i;

a = area of a field observed under a microscope;

C = number of fields visited with the 40x lens;

R40x = radius of the field at the 40x objective (0.25 mm);

A = surface area of the sedimentation dish where the cells accumulate;

d = dilution factor;

V = volume of sedimented sample (5.5 ml).

Phytoplankton functional grouping

The functional assemblages considered in this work are the functional groups developed by Reynolds *et al.* (2002) and the update proposed by Padisák *et al.* (2009). The functional groups are inspired by phytosociology (Baillot, 2013). They are referred to as codons, and each codon consists of a letter or a letter with a letter or number (codon A, codon LM, codon S1, codon J, etc.). Each codon groups together a set of phytoplankton species usually found together in a given habitat and sharing the same ecological requirements (Reynolds *et al.,* 2002). In other words, they differentiate phytoplankton on the basis of adaptations and specialised requirements, such as a strong affinity for nutrient salts (Prévost, 2014). For this study, phytoplankton taxa with densities greater than or equal to 10% of the total algal density of each pond were considered to be the dominant taxa. These dominant taxa were used to form the functional groups. The relative abundance (RA) of taxa in each functional group (FG) of a rice-fish pond was determined from the following

$$RA = \frac{A_{FG}}{A_{TFG}} x 100$$

relationship

Where: RA = Relative abundance of individuals in FG i;

A_{FG} = Abundance or number of individuals of an FG i ;

A_{TFG} = Abundance or total number of individuals of all the FGs in a pond.

Statistical analysis

The physicochemical parameters measured during each rearing phase were first subjected to the Shapiro-Wilk and Levene tests to check the normality and homogeneity of the variances of each variable, respectively. Next, the non-parametric Kruskal-Wallis test was applied to the variables to assess the significance of the variation in the parameters. Where this was significant, the Mann-Whitney rank comparison test was then applied to determine the specific differences between the variables taken by pairs. Differences were considered significant at the threshold of $\alpha = 0.05$ ($p < 0.05$) for all tests performed. All these univariate tests were performed on the Rstudio interface of the R 3.6.3 software using the "ade4" package (Core, 2021).The influence of physicochemical parameters on the dynamics of phytoplankton functional groups constituted by the dominant taxa in the ponds was analysed using CANOCO 4.5 software (Ter Braak and Šmilauer, 2002). To this end, data matrices (functional groups/ponds and physicochemical parameters/ponds) were created on both sides on the basis of the abundances of the various dominant taxa. The matrices designed with the data from each rearing phase were imported into the software and Monte Carlo permutation tests with a threshold of 0.05 were carried out a priori to check that the abundance of functional groups and physicochemical parameters were not linearly related. Canonical Redundancy Analyses (RDA) were then applied to explain the determinism of the proliferation of phytoplankton functional groups in the rearing structures.

RESULTS

Variations in physicochemical parameters between the two rearing phases

Table II shows the variation in the mean values of the physicochemical parameters of the water in the rice-fish ponds during the rearing phases of *O. niloticus*. This table shows that the means of temperature, conductivity, turbidity, pH, total dissolved solids and nitrite ion concentration of the rearing structures did not vary significantly from one phase to another in the same rice-fish pond (Mann-Whitney test, $p > 0.05$). However, the averages of transparency, dissolved oxygen, nitrate ions, ammonium, orthophosphate, total phosphorus and total nitrogen in the water varied significantly from one phase to another in the same rice-fish pond.

Taxonomic diversity of phytoplankton in rice-fish ponds

Microscopic analysis of water samples collected from rice-fish ponds during the pre-grow-out and grow-out phases of *Oreochromis niloticus* identified a total of 235 phytoplankton taxa (Table III). These taxa are grouped into 7 taxonomic groups (Bacillariophyta, Chlorophyta, Chrysophyta, Cyanobacteria, Dinoflagellata, Euglenophyta and Xanthophyta). The Chlorophyta and Euglenophyta are the most diverse taxonomic groups in the water of all the rice-fish ponds, with species richness ranging from 87 to 93 and 44 to 58 taxa respectively. Xanthophyta and Dinoflagellata are the least diverse taxonomic groups in the water of the rice-fish ponds. The species richness of phytoplankton in the control rice-fish ponds (RC) was 196 taxa, whereas it ranged from 190 (RSM) to 203 (RSR) in the rice-fish ponds that had received the agricultural byproducts.

Table II : Variations in mean water physicochemical parameters between the two rearing phases of *Oreochromis niloticus*

Physicochemical parameters	Phase breeding	Rice-fish ponds				p-value inter-phase
		RC	RPC	RSM	RSR	
Temperature (°C)	Pre-Grow-out	26.41 ± 1.09[a]	26.81 ± 1.18[a]	26.32 ± 1.45[a]	26.73 ± 1.34[a]	> 0.05
	Grow-out	26.95 ± 1.36[a]	26.78 ± 1.69[a]	27.16 ± 1.73[a]	27.13 ± 1.49[a]	
Transparency (cm)	Pre-Grow-out	32.01 ± 5.22[a]	23.43 ± 4.63[b]	26.96 ± 3.46[b]	36.03 ± 10.47[a]	< 0.05
	Grow-out	35.67 ± 4.67[b]	21.56 ± 4.06[a]	24.92 ± 3.58[a]	27.67 ± 4.66[b]	
Dissolved oxygen (mg/l)	Pre-Grow-out	3.79 ± 0.55[a]	2.06 ± 0.34[a]	2.28 ± 0.46[a]	3.40 ± 0.50[a]	< 0.05
	Grow-out	4.26 ± 0.91[b]	2.83 ± 0.78[b]	3.02 ± 0.88[b]	3.43 ± 1.08[a]	
pH	Pre- Grow-out	6.25 ± 0.35[a]	4.95 ± 0.54[a]	5.28 ± 0.43[a]	5.53 ± 0.48[a]	> 0.05
	Grow-out	6.45 ± 0.48[a]	5.12 ± 0.75[a]	5.57 ± 0.73[a]	5.87 ± 0.62[a]	
Conductivity (µS/cm)	Pre-Grow-out	149.54 ± 29.94[a]	216.04 ± 70,88[a]	177.91 ± 41.72[a]	198.98 ± 29.94[a]	> 0.05
	Grow-out	144.80 ± 31.53[a]	224.33 ± 36,78[a]	188.60 ± 34.50[a]	205.99 ± 33.53[a]	
Turbidity (UTN)	Pre-Grow-out	157.51 ± 20.80[b]	242.92 ± 64.31[a]	193.06 ± 37.34[a]	226.16 ± 22.44[a]	> 0.05
	Grow-out	151.47 ± 28.80[b]	232.49 ± 46.61[a]	200.10 ± 37.07[a]	220.60 ± 33.42[a]	
Total dissolved solids (mg/l)	Pre-Grow-out	92.70 ± 15.63[a]	162.44 ± 25.17[a]	133.83 ± 24.93[a]	133.76 ± 27.74[a]	> 0.05
	Grow-out	110.50 ± 18.69[a]	163.94 ± 27.12[a]	149.09 ± 31.21[a]	142.13 ± 24.82[a]	
Nitrites (mg/l)	Pre- Grow-out	0.02 ± 0.00[a]	0.09 ± 0.04[a]	0.05 ± 0.02[a]	0.03 ± 0.00[a]	> 0.05
	Grow-out	0.03 ± 0.01[a]	0.12 ± 0.07[a]	0.08 ± 0.03[a]	0.04 ± 0.01[a]	
Nitrates (mg/l)	Pre-Grow-out	0.24 ± 0.25[a]	1.01 ± 0.34[a]	0.58 ± 0.19[a]	0.74 ± 0.17[a]	< 0.05
	Grow-out	0.44 ± 0.18[b]	1.76 ± 0.66[b]	1.39 ± 0.58[b]	1.01 ± 1.31[b]	
Ammonium (mg/l)	Pre-Grow-out	0.32 ± 0.18[b]	0.58 ± 0.28[a]	0.39 ± 0.19[a]	0.51 ± 0.18[b]	< 0.05
	Grow-out	0.22 ± 0.08[a]	1.08 ± 0.45[b]	0.64 ± 0.57[b]	0.44 ± 0.38[a]	
Orthophosphates (mg/l)	Pre-Grow-out	0.26 ± 0.15[a]	0.52 ± 0.28[a]	0.37 ± 0.22[a]	0.33 ± 0.23[a]	< 0.05
	Grow-out	0.44 ± 0.19[b]	2.91 ± 0.85[b]	0.97 ± 0.20[b]	1.14 ± 0.35[b]	
Total phosphorus (mg/l)	Pre-Grow-out	0.39 ± 0.16[a]	1,10 ± 0,33[a]	0,63 ± 0,19[a]	0.56 ± 0.15[a]	< 0.05
	Grow-out	0.55 ± 0.16[b]	1,54 ± 0,45[a]	1,17 ± 0,27[b]	1.68 ± 0.14[b]	
Total nitrogen (mg/l)	Pre-Grow-out	10.96 ± 1.89[a]	14.53 ± 3.15[a]	11.80 ± 2.15[a]	13.10 ± 2.44[a]	< 0.05
	Grow-out	13.20 ± 1.16[b]	16.70 ± 2.59[b]	16.03 ± 2.61[b]	16.25 ± 2.01[b]	

RC = no exogenous feed intake, RSR = rice bran intake, RPC = combined by-product intake, RSM = maize bran intake). The different superscripts on the values of a parameter in the same column in the table indicate a statistically significant difference (Mann-Whitney test) between the two phases (±: standard deviation)

Table III: Specific richness of phytoplankton taxonomic groups in rice-fish ponds

Taxonomic groups	Rice-fish ponds			
	RC	RPC	RSM	RSR
Bacillariophyta	20	23	26	26
Chlorophyta	87	87	90	93
Chrysophyta	04	01	01	02
Cyanobacteria	30	22	13	18
Dinoflagellata	09	05	08	08
Euglenophyta	44	58	49	54
Xanthophyta	02	02	03	02
Specific richness	196	198	190	203
Total species richness	235			

Monthly evolution of the abundance of phytoplankton functional groups

The monthly evolution of the relative abundance of the eight functional groups of phytoplankton populations in the rice-fish ponds is presented in Figure 5. During pre-grow-out and grow-out phase *of O. niloticus*, groups J, W1 and W2 were dominant in all rice-fish culture ponds and in all sampling months with proportions greater than 9.5%. Groups X1, H2, MP, LM and LO had significantly low proportions in the ponds in each sampling month. In the RC ponds, the relative abundance of group J increased from 16.79% in the first month (m-1) to 17.9% in the third month (m-3) of Pre-Grow-out and decreased from 21.8% (m-1) to 16.6% in the sixth month (m-6). Those of group W2 changed during Pre-Grow-out and grow-out phase, from 23.81% (m- 1) to 28.05% (m-3) and from 30.70% (m-1) to 32.5% (m-6) respectively. In group W1, it decreased from 28.01% (m-1) to 21% (m-3) and from 23.4% (m-1) to 18.17% (m-6) in pre-grow-out and grow-out, respectively. In ponds receiving agricultural by-products (RPC, RSM and RSR), the relative abundance of groups J, W1 and W2 increased significantly during each rearing phase of *O. niloticus*.

Composition of phytoplankton functional groups

Of all the phytoplankton taxa collected, 16 taxa were dominant (taxon density ≥ 10% of total algal density) in the rice-fish ponds during the pre-grow-out and grow-out phases of *O. niloticus*. Referring to the phytoplankton functional groups proposed by Reynolds *et al.* (2002) and Padisák *et al.* (2009), these dominant taxa were grouped into 08 functional groups (Table IV). The groups include X1, J, H2, MP, LM, LO, W1 and W2. Of these functional groups, 05 were identified in the Pre-Grow-out phase and 08 in the grow-out phase. Functional groups J, LM, LO, W1 and W2 were observed during these two rearing phases. Groups H2, MP and X1 were identified only during the grow-out phase. Taxa representative of groups J and W2 were the most numerous. Group J was represented by 06 dominant taxa (contribution ≥ 10% of total density) of Chlorophyta of the genus *Coelastrum, Lacunastrum, Desmodesmus* and *Scenedesmus.* Group W2 was represented by 03 dominant Euglenophyta taxa of the genus *Trachelomonas* and *Euglena.* Functional groups X1, H2, MP, L_M and L_O were characterised respectively by *Akistrodesmus bibraianus* (Chlorophyta). *Anabaena affinis, Oscillatoria subbrevis, Microcystis aeruginosa* (all Cyanobacteria) and *Peridinium bipes* (Dinoflagellata). Euglenophyta of the genus *Lepocinclis* were the most representative of group W1. The various phytoplankton taxa representative of the functional groups is autotrophic or mixotrophic and generally sensitive to low light conditions.

Relationship between physicochemical parameters and the dynamics of phytoplankton functional groups

Canonical Redundancy Analyses (RDA) (Figure 6A and 6B) show that the abundance dynamics of each phytoplankton functional group during the two rearing phases of *O. niloticus* depends on the different gradients of environmental factors in the rice-fish ponds. During the pre-grow-out phase, RDA axes 1 and 2 accounted for 91% of the total variance (Figure 6A). pH and dissolved oxygen were negatively correlated with the first axis, which accumulated 75.4% of the total inertia.

Table IV: Occurrence and Functional characteristics of dominant phytoplankton taxa in the water column of rice-fish ponds

Taxons	Functional groups	Rice-fish ponds								Functional characteristics according to Reynolds *et al.* (2002)			
		RC		RPC		RSM		RSR		Habitats	Tolerances	Sensitivities	Energy Source
		Pre	Gro	Pre	Gro	Pre	Gro	Pre	Gro				
CHLOROPHYTA													
Ankistrodesmus bibraianus	X1		+		+		+			Shallow mixed layers in enriched conditions	Stratification	Nutrient deficiencies filter feeding organisms	Autotrophic
Coelastrum cambricum	J	+	+	+	+	+	+	+	+				
Lacunastrum gracillimum	J	+	+	+	+	+	+	+	+				
Scenedesmus naegeli	J		+		+		+		+	Shallow, enriched lakes ponds and rivers	-	Settling into low light	Autotrophic
Desmodesmus opoliensis	J	+	+	+	+	+	+	+	+				
Desmodesmus communis	J		+		+		+		+				
Desmodesmus armatus var. *longispina*	J	+			+	+		+					
CYANOBACTERIA													
Anabaena affinis	H2		+		+		+		+	Dinitrogen-fixing Nostocaleans of larger mesotrophic lakes	Low nitrogen	Mixing, poor light	Autotrophic
Oscillatoria subbrevis	MP		+		+		+				-	-	Autotrophic
Microcystis aeruginosa	L_M	+	+	+	+	+	+	+	+	Summer epilimnia in eutrophic lakes	Very low Carbon	Mixing, poor stratification light	Autotrophic
DINOFLAGELLATA													
Peridinium bipes	L_O	+	+	+	+	+		+	+	Summer epilimnia in mesotrophic lakes	Segregated nutrients	Prolonged or deep mixing	Mixotrophic
EUGLENOPHYTA													
Trachelomonas volvocina	W2	+	+	+	+	+	+		+				
Euglena ehrenbergii var. *africana*	W2	+	+	+	+	+	+		+	Shallow mesotrophic lakes	?	?	Mixotrophic
Euglena sp.	W2		+		+		+		+				
Lepocinclis ovum	W1	+	+	+	+	+	+	+	+	Small organic ponds	High BOD	Grazing	Mixotrophic
Lepocinclis fusiformis	W1	+	+	+	+	+	+	+	+				

Pre = Pre-Grow-out phase; Gro = Grow-out phase; BOD = Biochemical oxygen demand.

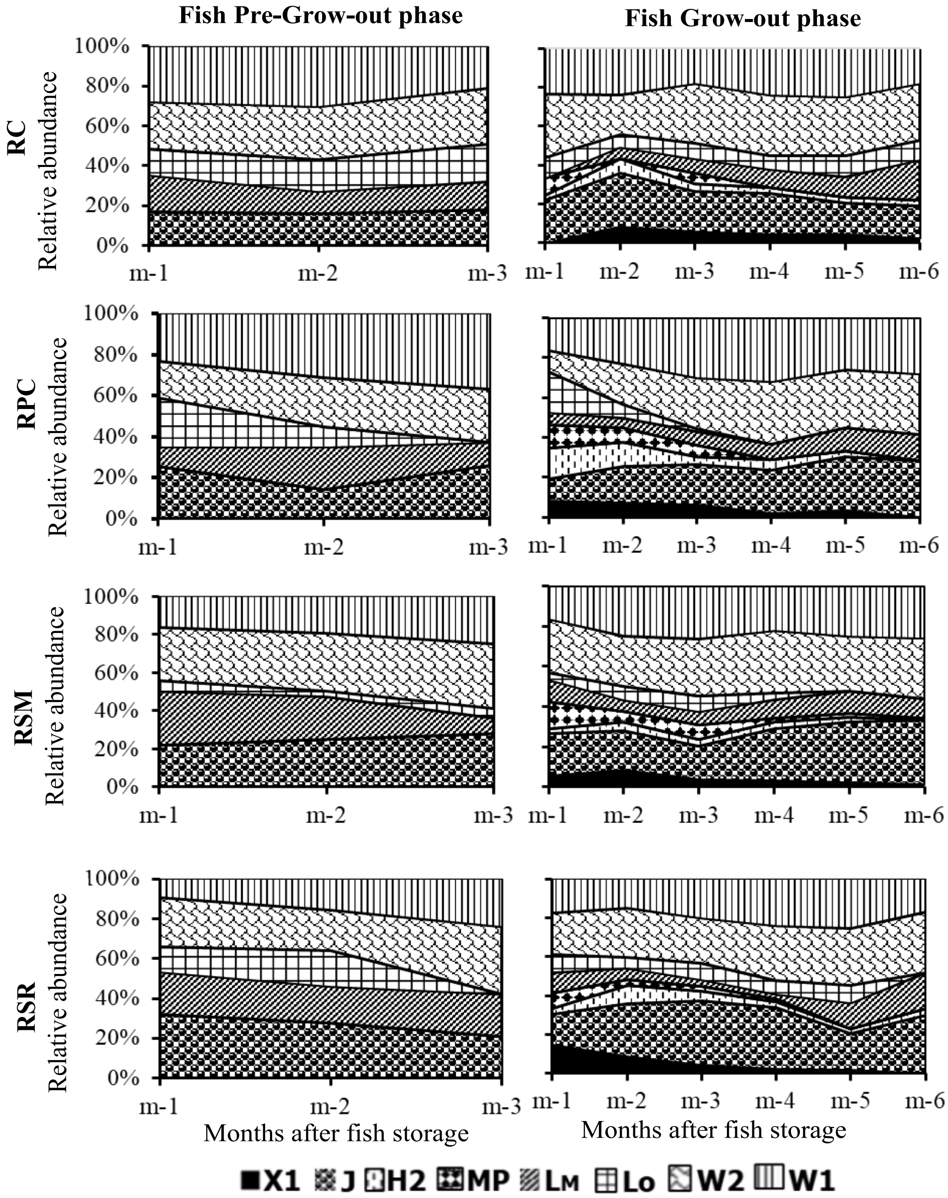

Figure 5 : Monthly dynamics of phytoplankton functional group abundance in rice-fish pond water during *O. niloticus* rearing phases. X1, J, H2, MP, LM, LO, W2, and W1 are Reynolds functional groups.

Conductivity (CE), turbidity (Tub) and concentrations of nutrient salts and total dissolved solids (TDS) were positively correlated with this axis. High values of these parameters are positively associated with high abundances of functional groups J and W2 in the RPC pond. On the second axis, which expresses 15.6% of the total inertia, the functional group LM characterises the RSR pond with the most transparent waters.

For the grow-out phase, the two axes of the canonical projection absorbed 85.3% of the total inertia (Figure 6B). The first axis of this projection accumulated 56.3% of this inertia. Temperature (T°C) is positively correlated with this axis. The high density of the W2 functional group in the RSM pond is strongly linked to the increase in water temperature. The concentrations of total nitrogen (N-t), ammonium ions (NH-4) and orthophosphate (PO-4) are negatively correlated with this axis. The dynamics of phytoplankton belonging to functional groups H2, J, MP and Lo in the RPC pond are positively related to high concentrations of these nitrogen compounds. The second axis, which accounted for 29.1% of total inertia, was positively correlated with conductivity (CE), turbidity (Turb), total phosphorus (P-t) and total dissolved solids (TDS) concentrations. The development of phytoplankton belonging to the functional group LM in the RSR pond is positively attributed to the high values of these chemical parameters. In contrast, pH, dissolved oxygen (DO) and water transparency (Trans) were negatively correlated with this second axis. These three parameters positively favoured the grow-out of phytoplankton taxa representing functional groups X1 and W1 in the control rice-fish ponds (RC).

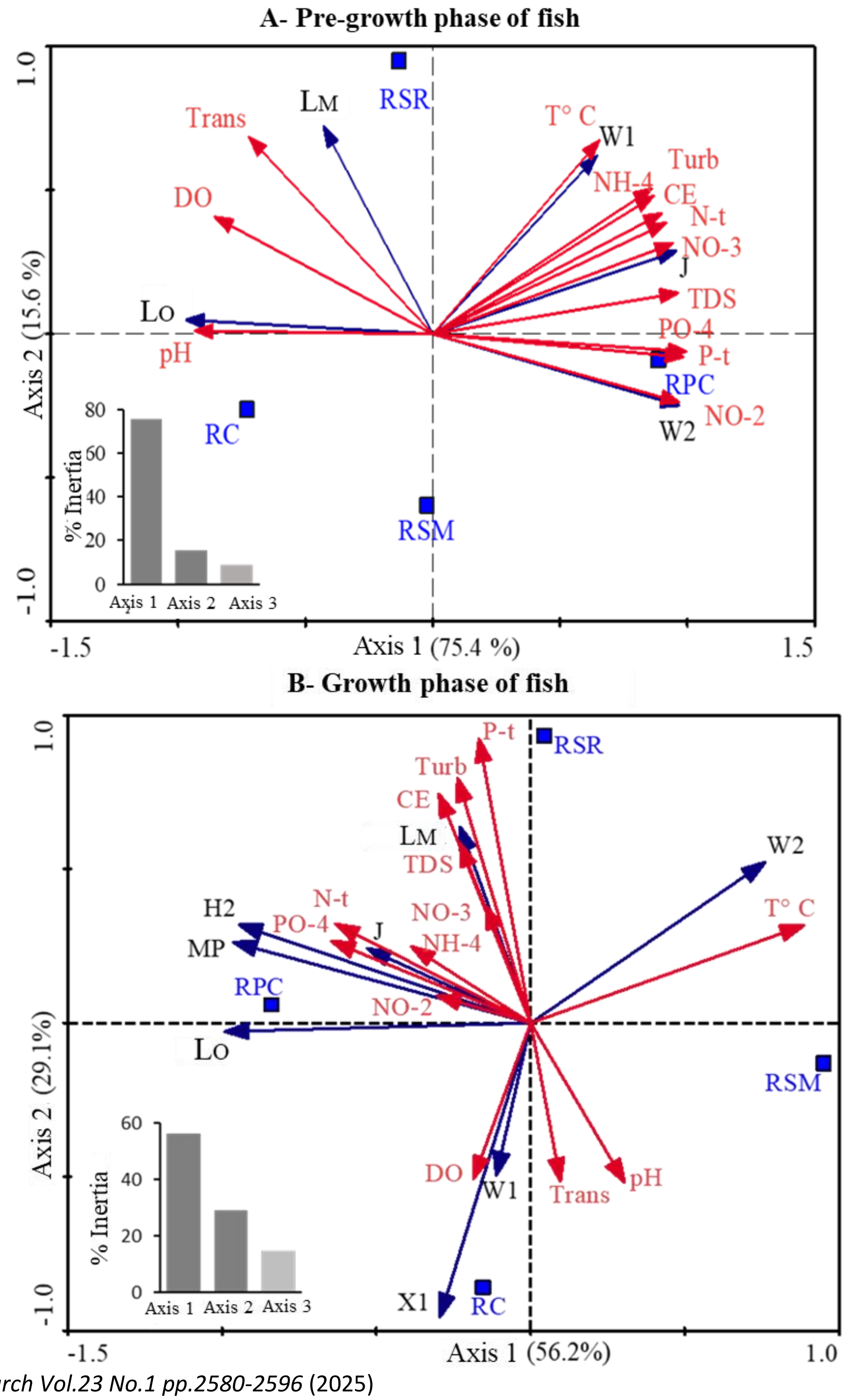

Figure 6: RDA ordination of environmental parameters and the main functional groups of phytoplankton in the water of rice-fish ponds during the Pre-Grow-out (A) and Grow-out (B) phases of *O. niloticus*. Key: T°C= Temperature ; CE = Conductivity ; Turb = Turbidity ; TDS = Total dissolved solids ; N-t = Total nitrogen ; P-t = Total phosphorus; NH-4 = Ammonium ions; NO-2 = Nitrites ions; NO-3 = Nitrates ions; PO-4 = Orthophosphate; pH = Hydrogen potential; DO = Dissolved oxygen; Trans = Water transparency; W2, W1, J, LO, LM, H2, MP and X1 are Reynolds functional groups; RSR, RC, RPC and RSM are rice-fish ponds.

DISCUSSION

A total of 235 phytoplankton taxa were identified in the water of the rearing structures on the Kouadiokro-Bonoufla rice-fish farm, as a whole. This number suggests a high diversity of microalgae in the rice-fish ponds. This high diversity of microalgae could be linked to the availability of nutrients in the rice-fish ponds, which would favour the complete development cycle of the microalgae. Nutrient availability affects the specific composition of microalgae (Caron *et al.,* 2000). According to Blé *et al.* (2007), exogenous feed in fish farming stimulates trophic pathways by promoting the diversity and rapid development of planktonic organisms. The number of taxa recorded in this study is very high compared to that of Avit *et al.* (2012), who collected 19 phytoplankton taxa in rice-fish ponds at the fish farming research station of the National Centre for Agronomic Research (CNRA) in Bouaké. This number of taxa is also higher than that recorded by Kra *et al*. (2021) (197 taxa) in the fishponds of the aforementioned fisheries research station. These identified taxa are grouped into 7 taxonomic groups, of which the Chlorophyta and Euglenophyta are the most diverse in all the rice-fish farming ponds. The high diversity of these two phyla in fish farming environments has also been noted by Kra *et al*. (2021). According to the work of Adon *et al.* (2017) in White Bandama and that of Groga *et al.* (2020) in Lake Kossou, the diversity of Chlorophyta and Euglenophyta is essentially linked to the richness of the water of the study area in nutrients and putrescible organic substances.

Eight phytoplankton functional groups were identified, with a high representation of taxa belonging to groups J and W2. This result would indicate the existence of several species sensitive to the low light levels caused by the shading of rice plants and adapted to the disturbances associated with fish feeding in rice-fish ponds. In fact, each functional group characterises a given habitat type Baillot (2013). The rice-fish ponds used in this study are small (200 to 360 sq.m), lentic and very shallow (0.5 to 1 m) with high nutrient levels. In addition, the presence of group W1, which includes Euglenophyta of the genus *Lepocinclis*, during the two phases of fish rearing is thought to be due to the increase in the concentration of organic pollution parameters such as orthophosphate ions (PO43-) and 5-day biochemical oxygen demand (BOD5). According to Reynolds *et al.* (2002) and Gani *et al.* (2011), the species in this group are tolerant of increases in BOD5. The relative abundance of the different functional groups fluctuated monthly in all the rice-fish ponds during the Pre-Grow-out and Grow-out phases of *O. niloticus*. This fluctuation could be due to rainfall runoff during the study period, which modifies the concentration of nutrients in lentic waters as observed by Cao *et al.* (2018).

Canonical analyses showed that the abundance dynamics of each phytoplankton functional group during each rearing phase of *O. niloticus* depends on different gradients of environmental factors in rice-fish ponds. The strong correlations between the abundance of phytoplankton functional groups and nitrogen compounds, TDS and transparency in the RPC pond show that the dynamics of phytoplankton assemblages in this pond are positively related to high concentrations of organic pollution parameters. In fact, according to work carried out in the same structures by Kouadio *et al.* (2023), the water in rice-fish ponds is polluted and organic pollution is very high in those receiving a mixture of agricultural by-products. Furthermore, the correlations observed in this study are in conformity with those of Sondergaard *et al*. (2003). These authors found that increasing nitrogen and phosphate inputs were accompanied by algal blooms and increased water turbidity, which can reduce transparency. In fact, the availability of nutrients is the main factor controlling the dynamics of microalgae when light and temperature conditions are adequate (Hecky and Kilham,1988).

CONCLUSION

The phytoplankton communities of the rice-fish ponds on the Kouadiokro-Bonoufla farm are grouped into eight main functional groups. Taxa belonging to groups J and W2 are strongly represented. The relative abundance of these main functional groups increases monthly during each rearing phase with the addition of the mixture of agricultural by-products. Successions are therefore very strongly linked to environmental parameters. This study

highlights the usefulness of phytoplankton functional groups in the study of phytoplankton dynamics in rice-fish ponds. It shows that phytoplankton assemblages J and W2 are effective indicators for monitoring organic pollution in rice-fish ponds.
Further studies need to be carried out using the Morpho-Functional traits of phytoplankton in order to predict the effect of exogenous feeds on the form, physiology and adaptability of microalgae species in fish farming environments.

Conflict of interest

The authors declare that they have no conflict of interest.

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