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Stoichiometry of Carbon, Nitrogen, and Phosphorus (C-N-P) of Phytoplankton and Fish in Fish Ponds During the Grow-Out Phase

Kokora Yah Reine Marina^{1*}, Seu-Anoï Netto Mireille¹, Yeo Kandana Marthe²

¹Laboratoire d'Environnement et de Biologie Aquatique (LEBA), Université Nangui ABROGOUA, Abidjan, Côte d'Ivoire.

²Centre de Recherche en Ecologie (CRE), Université Nangui ABROGOUA, Abidjan, Côte d'Ivoire.

Abstract

The present study aims to determine the stoichiometry of phytoplankton and fish using industrial and local feed. Fish samples and phytoplankton *samples* were taken from six grow-out ponds. Carbon, nitrogen, and phosphorus concentrations and their ratios were determined and analyzed to characterize the stoichiometry of phytoplankton and fish. The carbon/phosphorus, carbon/nitrogen, and phosphorus/nitrogen stoichiometric ratios of phytoplankton ranged from 89.24 to 214.36, respectively. 5.05 and 8.87; 21.97 and 31.63, those of fish range between 38.43 and 45.56; 3.60 and 4.05, 9.71, and 18.66. The correlation shows that the weight of fish receiving commercial feed is significantly correlated with phosphorus and nitrogen in both locally fed and unfed fish. The carbon/phosphorus, carbon/nitrogen, and phosphorus/nitrogen stoichiometric ratios of phytoplankton in ponds receiving local feed and control ponds are higher compared to those in ponds receiving commercial feed. The same trend was observed in the fish. These analyses showed that fish receiving commercial feed have higher phosphorus levels compared to locally fed and unfed fish, which are richer in nitrogen. These results indicate that the fish have the same nutrient and carbon compositions as those found in their natural environments.

Keywords: Carbon; Nutrients, Phytoplankton; Fish; Stoichiometry

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*Corresponding author : Kokora_Yah Reine Marina ; Email: kokorareine12@gmail.com, Laboratoire d'Environnement et de Biologie Aquatique (LEBA), Université Nangui ABROGOUA, Abidjan, Côte d'Ivoire

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INTRODUCTION

Fish provides an important source of dietary protein and partially meets the growing global food demand. However, the gradual decline in fish stocks is linked to the overexploitation of aquatic natural resources (Anougbo et al., 2019). Aquaculture was identified as the best alternative to meet this growing demand (FAO, 2016). However, this sector has not yet reached a viable economic scale in Africa (Lazard et al., 1991). In Côte d'Ivoire,

despite the existence of favorable factors such as the availability of good-quality water bodies and experienced extension services, the development of fish farming remains limited. One of the factors behind this low level of development is the cost of quality feed (Mirah & Jica 2019). This situation has forced many fish farmers to resort to feed based on lower-cost agri-food by-products (Yao et al., 2017). Distributed in powder form, this feed generates feed losses and enriches the environment with nutrients, which in turn stimulate plankton production, particularly

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phytoplankton (Anougbo et al. 2019). Indeed, phytoplankton plays a role pre - responding At breast of the aquatic eco - systems since he product of oxygen grace has there photo synthesis needed has there survival de so r g a n i s m e s heterotrophe of a part, he constituted on the other hand a source of food For fish (Pinel - Alloul al., 1998; Angelier, 2000; Schlumberger & Bouretz, 2002). Regarding nutrients, nitrogen (N) and phosphorus (P) control phytoplankton biomass in ponds Burgess (2018), and carbon (C) is an important source in photosynthesis (Groga, 2012). Consequently, these can lead to changes in phytoplankton composition and affect fish structure. Much work has been carried out in fish ponds in Côte d'Ivoire. Among these studies, the most recent is that of YAO et al. (2017). While studies have been conducted by Brou et al. (2018), Zié (2025), and Diabagaté (2025), no research has yet been carried out on the stoichiometric distribution of carbon, nitrogen, and phosphorus in phytoplankton and fish in fishponds in Côte d'Ivoire. To achieve good fish production at a lower cost, the objective of this study is (1) to evaluate the stoichiometry of nutrients in phytoplankton

and fish in fishponds receiving exogenous feed (local and industrial) and (2) to explore the influence of nutrients on fish growth.

Materials and Methods

Description of the study site

The work took place at the Béliér Ecological Farm (FEB) located between 6°50'46"North and 5°8'15"West 3 km from Kacoubroukro and 14 km from Yamoussoukro (06° 49'47 ")N and 05°16'15"W. The farm is supplied with water by gravity from a dam (6°50'57.6"N 5 ° 09'25.5"W) which covers the water needs of this 10-hectare farm consisting of 61 ponds with a surface area of 800 m² (20 × 40 m) and an average depth of 0.8 m, of which 40 are functional. The site selection was guided by accessibility, the availability of ponds, and the position of the dam that supplies the fish ponds. Ponds E1, E2, E3, E4, E5, and E6, which were used for grow-out, have the respective codes AG1, AG2, AP1, AP2, AN1, and AN2.

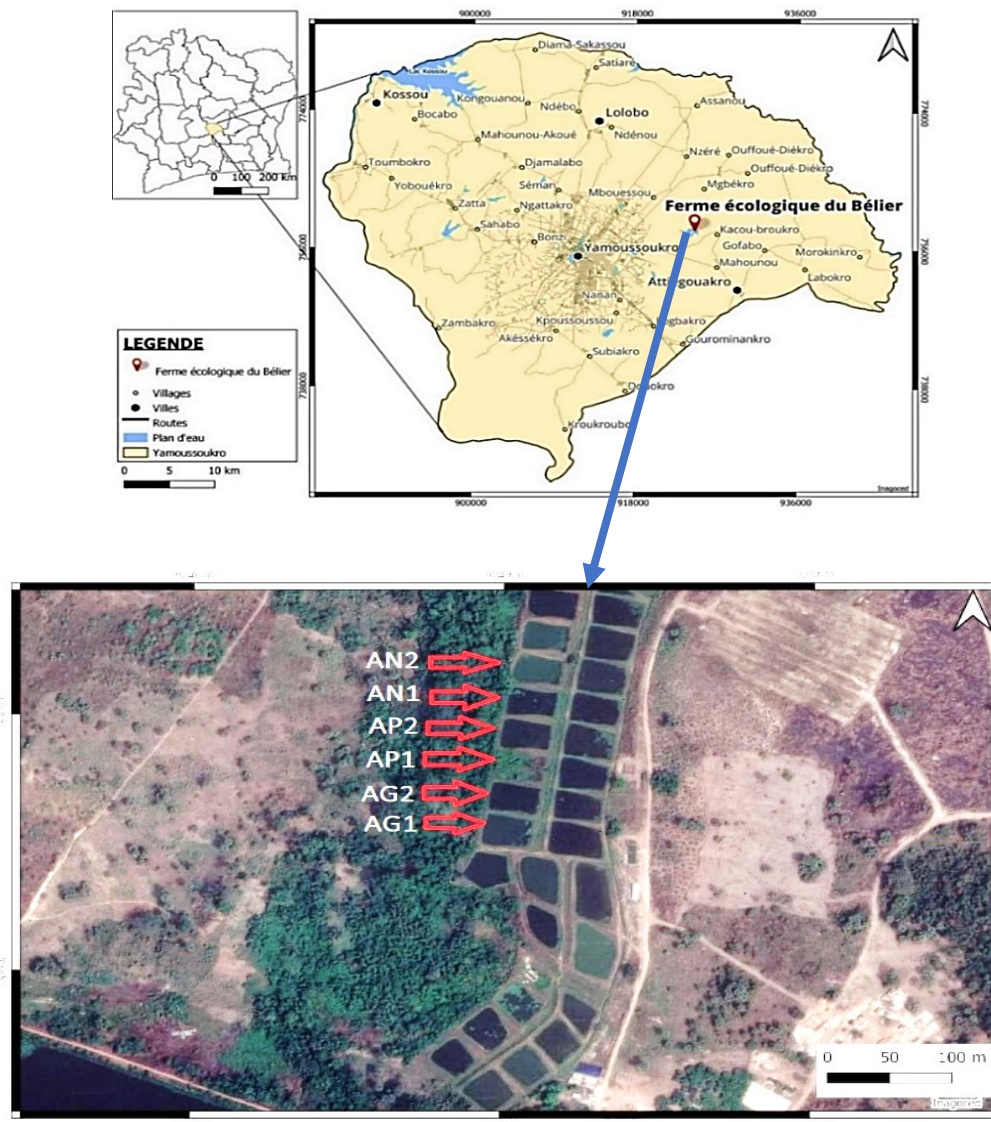


Figure 1: Location of the Béliér ecological farm

Experimental foods

Two exogenous feeds (local and commercial) were used throughout the fattening phase. The local diet, produced by local suppliers, was formulated with raw materials consisting of low-grade rice flour, wheat bran, soybean meal, vitamins, table salt, and a vitamin-mineral complex. The commercial diet contained soybean flour, wheat, rice bran, fishmeal, poultry meat meal, salmon oil, and a premix of vitamins and minerals from imported feeds.

Sampling and follow - up breeding

This study was conducted in six ponds, and sampling was carried out once a month for three months. Fish with an average weight of 25 g were stocked at a density of 1.25 individuals / m², i.e., 1000 fish per pond. During feeding time, the fish were fed in ratios of 5 % and 4 % of their weight were applied. The juveniles were fed every 4 hours between 8am and 4pm, i.e. 3 meals per day. Using two 20 mm and 60 mm mesh sizes, twenty (20) fish were sampled every month and at chance in each ponds whose weights were determined individually. Fish in ponds AG1 and AG2 were fed 1924.15 g of commercial feed (extruded pellets), those in ponds AP1 and AP2 received 1464.6465 g of locally sourced feed, and fish in control ponds AN1 and AN2 were not fed any exogenous feed to allow for estimation of the natural contribution of the rearing environment. Each experiment was replicated.

C, N, P concentration of phytoplankton and fish

For the phytoplankton samples, 5 to 10 L of water were filtered through an 83 µm mesh (particulate matter assumed to be dominated by phytoplankton) and collected. In each pond, 5 healthy fish were randomly selected and sacrificed after 24 hours of starvation. The fish were immediately placed on ice and transported to the laboratory where they were weighed, gutted, and dried to a constant weight. Using an ultracentrifuge grinder (Resch ZM 100), the whole fish were ground into a powder to obtain a homogeneous mixture. Organic carbon (C) and nitrogen (N) from the phytoplankton and fish were measured using an analyzer, and phosphorus (P) samples were analyzed after HCl digestion using the molybdenum blue method with an auto-analyzer (Higgins et al., 2006). Phytoplankton stoichiometry is linked to nutrient concentrations in the environment (Prater et al., 2017). Element concentrations and ratios in fish were expressed as molar ratios. The stoichiometric ratios obtained were compared to those of the authors cited in Table 1.

Table 1: Stoichiometry of autotrophs in freshwater ecosystems

Stoichiometric ratios	C:N:P	C:P	C:N	N:P
Phytoplankton (freshwater)	166:20:1	307	10.2 ± 3.0	30.2 ± 16.0
Fish (freshwater)	95:16:1	95.9	7.7	16.1

Statistical analysis of data

For the analysis of the results obtained, the assumption of normality of the data distribution was used to compare the differences in stoichiometric ratios (C:P, C:N, N:P). Therefore, the Shapiro- Wilk test was used to test the normality of the data distribution. Parameters with a normal distribution were subjected to analysis of variance (one-way ANOVA). Conversely, parameters with an abnormal distribution were subjected to the non-parametric Kruskal -Walli's test followed by the Mann-Whitney U test for multiple comparisons. These different tests were used at a significance level of 95% ($p < 0.05$) and were performed using R 3.4.3 with the RStudio interface. This descriptive factorial statistical method allows for the graphical representation of the maximum amount of information contained in a large dataset (Philippeau, 1992). The principal components follow the descending order of the amount of information they explain and are obtained by diagonalizing a matrix which, depending on the nature of the initial variables, is either the correlation matrix or the covariance matrix (Legendre & Legendre, 1979). In this study, the correlation matrix was used with focused principal component analysis (PCA).

Results

Stoichiometric analysis of carbon, nutrients (C, N, P) and their ratios in phytoplankton and fish in the growth phase

Tables 2 and 3 present the stoichiometric ratios of carbons, nutrients present in phytoplankton and fish. At the grow-out stage, the average C/P ratios of phytoplankton in ponds receiving commercial feed (89.24; 100.94) and locally produced feed (158.47; 159.18) are low compared to those receiving no feed (214.36; 202.47) (Table 2). Regarding the average C/N ratios, all ponds show fairly similar values (AP1: 5.09; AP2: 5.05; AG1: 5.49; AG2: 4.57; AN1: 8.87; AN2: 7.77). The average N/P ratios of ponds receiving powdered feed (AP1 and AP2) (31.63; 31.18) are higher than those receiving pelleted feed (21.97; 21.97) and those that did not receive feed (24.06; 27.35). The average C/P ratios of fish fed with pelleted feed (AG1: 45.99; AG2: 47.68) are low compared to those fed with powdered feed (AP1: 63.30; AP2: 61.83) and those not fed (AN1: 74.68; AN2: 73.57) (Table 3). The average C/N ratios (AP1: 3.61, 30; AP2: 3.58; AG1: 4.01; AG2: 4.09; AN1: 3.61; AN2: 3.56) of all fish are low. Regarding the average N/P ratios (18.67 ;18.66), the fish receiving powdered feed (AP1 and AP2) have higher ratios than those receiving pelleted feed (9.80; 9.60) and those not receiving exogenous feed (12.67; 11.74). Kruskal -Wallis test on the mean C/P ratios (p); C/N ($p = 0.0428$) and N/P ($p < 0.005$) of phytoplankton (Figure 2) and fish (Figure 3) indicated that the concentrations of these ratios were not significantly different within the ponds ($p < 0.005$).

Table 2: Stoichiometric ratios of nutrients (C, N, P) in phytoplankton during the growth phase

Stations	Statistics	Chemical content			Stoichiometric ratios		
		C (mol)	P (mol)	N (mol)	C:P	C:N	N:P
AP1	MIN	49.78	0.36	12.12	58.64	2.67	22
	MAX	154.12	0.85	19.33	268.57	8.25	49.12
	MOY	87.25	0.61	17.82	159.18	5.09	31.63
	ECAR	36.52	0.21	2.81	77.18	2.43	10.3
AP2	MIN	50.24	0.37	12.54	59.81	2.64	22.63
	MAX	153.07	0.84	19.39	259.37	8.22	47.4
	MOY	87.05	0.61	17.83	158.47	5.05	31.18
	ECAR	35.95	0.20	2.61	78.92	2.32	9.01
AG1	MIN	57.27	0.53	16.64	55.94	1.93	9.2
	MAX	200.98	2.48	29.75	161.93	9.24	37.32
	MOY	124.91	1.36	24.11	100.94	5.49	21.97
	ECAR	61.62	0.69	5.02	44.14	2.9	10.93
AG2	MIN	57.27	0.53	16.64	55.94	1.92	9.2
	MAX	199.93	2.48	29.88	161.93	9.15	37.54
	MOY	105.42	1.31	25.02	89.24	4.57	23.42
	ECAR	53.89	0.69	5.46	42.28	2.78	10.75
AN1	MIN	34.36	0.18	6.85	45.72	2.49	10.91
	MAX	143.7	0.75	13.82	550.57	15.13	40.5
	MOY	75.58	0.45	9.11	214.36	8.87	24.06
	ECAR	42.35	0.19	2.53	181.62	4.99	12.51
AN2	MIN	33.84	0.18	6.78	45.03	2.49	10.91
	MAX	143.7	0.75	13.82	550.57	15.13	40.5
	MOY	67.6	0.43	9.88	202.47	7.77	27.35
	ECAR	44.37	0.19	3.19	186.55	5.55	13.57

Table 3: Stoichiometry of nutrients (C, N, P) and their ratios in fish during the grow-out phase

Ponds	Statistics	Chemical content			Stoichiometric ratios		
		P	N	C	C/P	C/N	N/A
AG1	MIN	0.51	8.29	27.91	34.21	3.11	10.68
	MAX	1.83	27.57	86.23	64.8	4.03	16.3
	MOY	1.14	14.33	50.92	45.63	3.61	12.67
	ECAR	0.54	7.8	25.68	10.48	0.41	2.53
AG2	MIN	0.6	8.77	26.49	32.09	3.02	10.6
	MAX	1.84	25.87	82.55	65.11	4.03	16.23
	MOY	1.14	14.32	51.07	45.49	3.58	12.66
	ECAR	0.51	6.87	23.52	10.94	0.43	2.39
AP1	MIN	0.5	3.21	12.7	24.78	3.43	6.26
	MAX	0.84	10.97	37.64	51.62	5.17	14.95
	MOY	0.63	6.17	24.44	38.32	4.09	9.6
	ECAR	0.14	3	9.64	11	0.59	3.43
AP2	MIN	0.5	3.42	13.35	24.69	3.4	6.33
	MAX	0.85	11.26	38.31	52.18	5.04	15.34
	MOY	0.63	6.33	24.72	38.54	4.01	9.81
	ECAR	0.14	3	9.7	11.12	0.57	3.43
AN1	MIN	0.41	6.16	21.06	34.21	3.11	10.68
	MAX	0.78	8.99	34.47	64.8	4.03	16.3
	MOY	0.59	7.35	26.43	45.63	3.61	12.67
	ECAR	0.12	1.14	4.63	10.48	0.41	2.53
AN2	MIN	0.42	4.88	18.75	35.77	3.11	10.6
	MAX	0.78	8.64	34.58	46.73	4.07	14.91
	MOY	0.6	6.99	24.8	41.5	3.56	11.74
	ECAR	0.12	1.42	5.47	4.39	0.39	1.61

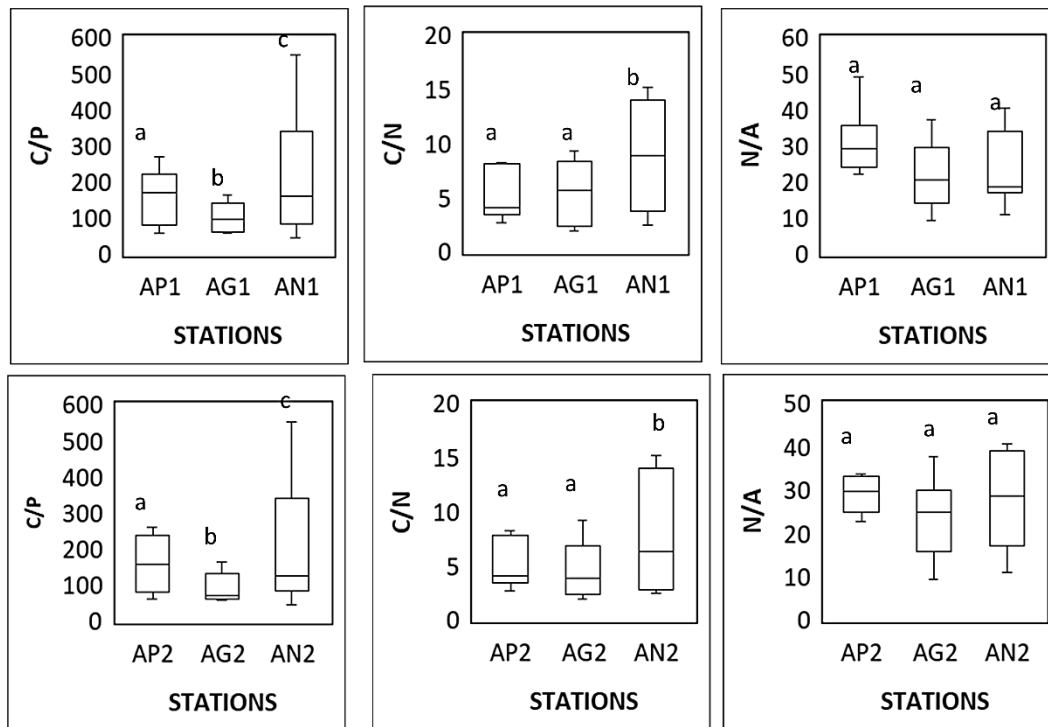


Figure 2: C/P, C/N, N/P ratio of phytoplankton during growth (C/P: carbon/phosphorus; C/N: carbon/nitrogen; N/P: nitrogen/phosphorus)

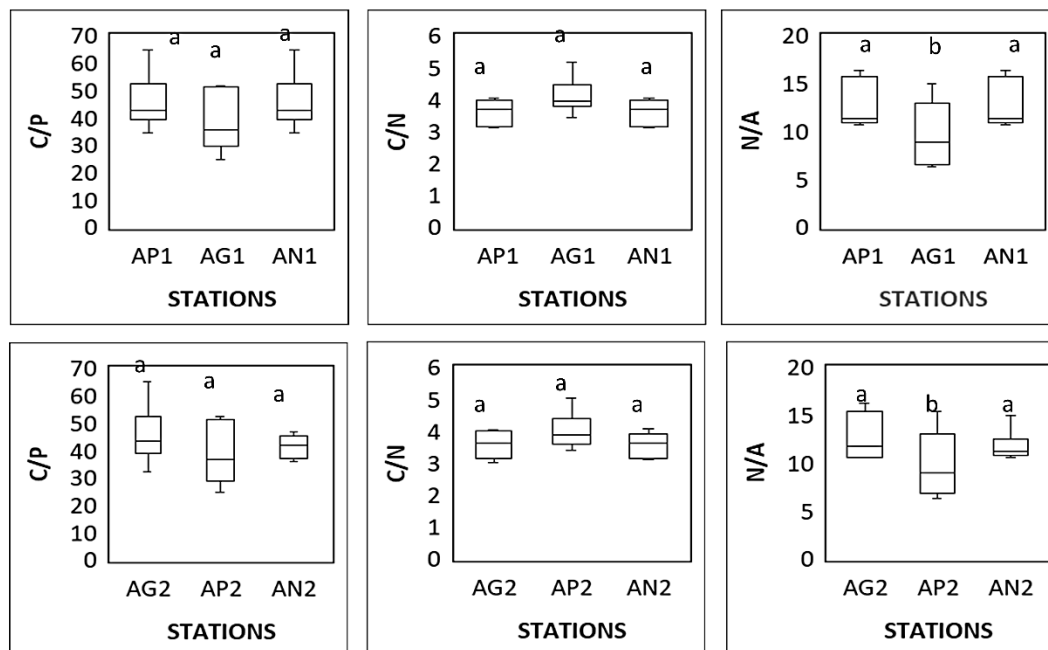


Figure 3: C/P, C/N, N/P ratio of fish during growth (C/P: carbon/phosphorus; C/N: carbon/nitrogen; N/P: nitrogen/phosphorus)

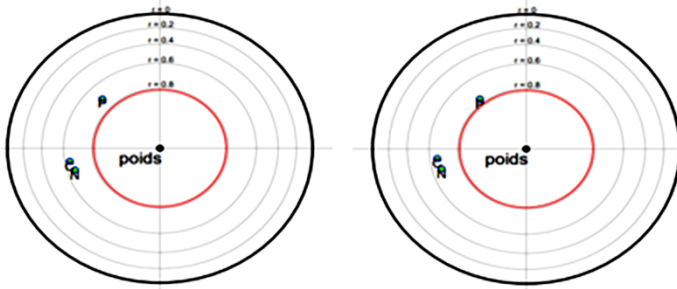


Figure 4: Focused principal component analysis of nitrogen (N), phosphorus (P), carbon (C) and weight of fish fed with pelleted feed during grow-out

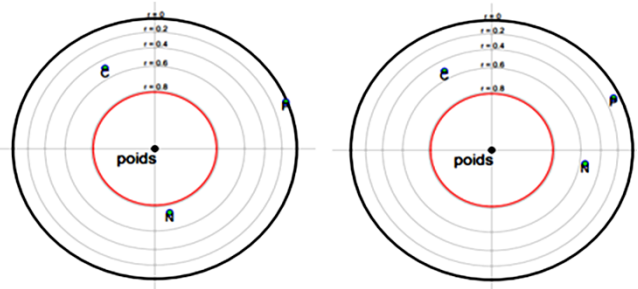


Figure 5: Focused principal component analysis of nitrogen (N), phosphorus (P), carbon (C) and weight of fish fed with powdered feed at grow-out.

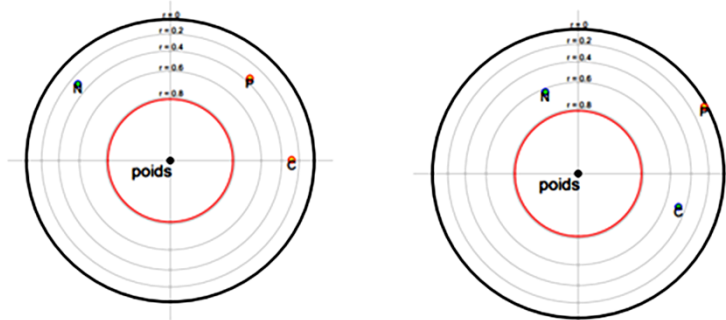


Figure 6: Focused principal component analysis of nitrogen (N), phosphorus (P), carbon (C) and weight of unfed fish during grow-out

At magnification, the weight final means (Pmf) fish fed with powdered feed in the first trial (AP1) vary between 44.86 ± 5.25 g and 158 ± 12.48 g, and those in the second trial (AP2) fluctuated between 45.7 ± 6.4 g and 160.4 ± 11.53 g. As for the final average weights for unfed fish (AN1 and AN2), the average weights range from 32.82 ± 2.36 g to 135 ± 16.4 g and from 36.58 ± 2.56 g to 146 ± 17.57 g, respectively. However, the average weights of fish fed with pelleted food (AG1: 95.82 ± 8.85 g; 388.8 ± 19.84 g) and (AG2: 97.05 ± 7.3 g; 397.6 ± 18.58 g) are higher

than those of fish fed with powdered food and unfed fish. Focused principal component analysis (FPCA) of fish weight and chemical elements showed the influence of nutrients (N, P) on fish weight. Weight was positively and significantly correlated with phosphorus in ponds receiving pelleted feed ($r = 0.8$). The weight of fish fed powdered feed was positively and significantly correlated with nitrogen ($r = 0.8$). For unfed fish, weight was positively and significantly correlated with all elements.

Discussion

The average C/P (95.09–158.81) and C/N (5.03–5.07) ratios of phytoplankton in ponds that received feed were lower than those that did not (C/P: 208.42; C/N: 8.86). This could be explained by the fact that nutrients from the feed reduced the C/P and C/N ratios. Indeed, Welti et al. (2017) show that nutrient inputs into streams increase carbon loss. The mean N/P ratios of phytoplankton in ponds receiving pelleted feed (22.70) are low compared to those receiving powdered feed (31.41) and those not fed (25.71). This could be explained by the fact that pelleted feeds are richer in phosphorus compared to powdered feeds, which are rich in nitrogen. Kaushik (2005) argues that processed food contains a significant amount of phosphorus due to fishmeal, which is rich in phosphorus. According to Zié (2022) feed from local suppliers contains a significant amount of nitrogen. These average C/P, C/N, and N/P ratios show that ponds receiving exogenous feed accumulate more nutrients. Regarding fish, the average C/P (AG: 38.43; AN: 43.57; AP: 45.56) are all less than the McIntyre & Flecker value (C/P 95.9), McIntyre &

Flecker (2010), similar to those of Guo et al. (2018) who obtained a C/P ratio of 40.91 in *Oreochromis niloticus*. Through this report, Guo et al. (2018) showed that the growth of *Oreochromis niloticus* is linked to a significant amount of phosphorus. This suggests that the fish obtained have a significant amount of phosphorus. The average C/N ratios (AP: 3.60; AG: 4.05; AN: 4) of all fish are low compared to that (5.9) of McIntyre & Flecker (2010). This shows that these fish also contain a significant amount of nitrogen. Because Kaushik (2005) it is stated that fishmeal is the main source of nitrogen, indicating a significant amount of nitrogen in the fish. However, the average N/P ratios (AG: 9.71; AN: 12.21; AP: 18.66) of the fish in the ponds fed with pelleted feed are lower than those in the other two ponds. These results could be explained by the fact that the fish fed with pelleted feed contain a significant amount of phosphorus, while those fed with powdered feed are rich in nitrogen. The average C/P, C/N, and N/P ratios demonstrate that the fish fed with commercial feed are richer in phosphorus, while those fed with powdered feed and those that did not receive any external feed are richer in nitrogen. Indeed, the fish have adapted to the food

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available in their environment because the fish have the same nutrient composition as those found in their environment. Several studies demonstrate that the body stoichiometry of fish can vary depending on ecological and environmental conditions (Benstead et al., 2014; Sullam et al., 2015).

Conclusion

These analyses showed that phytoplankton and fish in ponds receiving commercial feed have a high phosphorus content compared to fish fed with local feed and those not fed at all, which are richer in nitrogen. These results indicate that the fish have the same nutrient and carbon compositions as those found in their natural environments.

Conflict of interest

The authors declare that there is no conflict of interest.

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