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Effluents from Fish Farming Ponds: A View from the Perspective of Its Main Components

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Abstract: Among the animal protein production activities, world aquaculture has the highest growth rate, and is mainly practiced in ground-excavated ponds. However, with great productivity comes the concern about the increasing generation of effluents, mainly at the moment of fish removal, when high loads of organic matter and nutrients are released into the environment. Thus, this study evaluated the quality of effluents through the principal component analysis (PCA) in samples from nurseries of different sizes in four sampling scenarios. Analysis was performed during the process of fish removal in Nile Tilapia intensive fish farming sites at various properties in the Western region of Paraná State in Brazil. Twenty physical and chemical parameters were analyzed in each effluent sample using standard methods of effluent analysis. The results indicated that the concentrations of Suspended Solids (SS), Total Solids (TS), Chemical Oxygen Demand (COD), and Total Phosphorus (TP) increased significantly at the end of the fish removal process, which caused a progressive deterioration in the effluent released into the environment. Hence, regulating water management during cultivation, as well as mitigating the effects of effluent generated in fish removal, is indispensable to maintain the legality, profitability, and sustainability of this sector.

Keywords: aquaculture; environmental sustainability; Nile Tilapia; PCA

1. Introduction

Fish is an excellent source of protein, vitamins, and minerals of high quality; its consumption brings countless benefits to human health [1], with a recommended amount of 12 kg per capita per year [2]. The world's aquaculture production of fish is around 100 million tons/year. Brazil rank 14th in fish production, with an output of approximately 563 thousand tons/year, 84% of which are from inland waters. Moreover, projections for production are promising, and indicate an increase of 104.4% in output and 32.3% of consumption per capita for 2015–2025 [3].

This growth is mainly due to large investments in the sector from private companies and cooperatives in Brazil, focusing on round fish in the Northern region, and on tilapia (*Oreochromis niloticus*) in the Central–Southern region.

In general, fish farming is an agricultural activity with economic importance. Production occurs intensively with the daily use of rations and high cultivation densities. Consequently, this promotes an increase in nitrogen and phosphorus in the water as the result of fish excretion and feed leftovers. Thus, the release of nutrient-rich effluents that occur during cultivation (due to water renewal rates), at the moment of fish removal, and at the end of cultivation, produce impacts on the environment.

According to Cyrino, et al. [4], these impacts are minimal compared to those resulting from domestic and industrial effluents.

However, there is concern about the pollutant capacity of this activity, because nutrients released through effluents can contribute, along with other punctual or diffuse forms of pollution, to the eutrophication of receiving water bodies. Punctual sources of pollution are caused by drains, industrial discharges, or inefficient sewage treatment, while diffuse sources refer to surface runoff brought by rains [5,6]. Omofunmi, et al. [7] studied the impacts of the discharge of effluents generated from catfish farming (*Clarias gariepinus*) in a river in Southwestern Nigeria by evaluating the water quality from a physical, chemical, biological, and aesthetic point of view. The authors concluded that the interference of the studied effluent on river quality was related to the draining methods used, drained water volume at harvest, and concentration of organic matter and nutrients.

The fish farming activities in the Western region of Paraná, Brazil, use constant water exchange from 1% of the water volume at the beginning of the culture period to about 10% at the end. The total water volume is drained in ponds before the harvest, and effluents accumulated during the cultivation are released in the receiving body in large volumes and in a short period of time. Several studies have evaluated water quality and effluent treatment during culturing periods in fish farming activities [8–12]; however, the characterization of effluents at the time of fish removal should be performed according to the culture system used.

According to information collected in 24 fish slaughterers in Western Paraná, 180 tons/day of tilapia are harvested in ground-excavated ponds with an average yield of 45 ton/ha/cycle. The cultivation cycle in these sites lasts on average 200 days, using balanced rations, water renewal, and mechanical aeration for the supplementation of dissolved oxygen. Considering this productivity, approximately 1000 ha of water surface is needed to meet the demands of fish commercial processing facilities installed in the region.

The activity of fish farming as an economical source of income has stood out in Paraná since 1990 when many ground-excavated ponds were built without concerns for technical aspects, size, and format. From a technical point of view, the great challenge is the sustainable use of resources, ensuring environmentally appropriate conditions for the animals with minimal consumption of resources [13].

Commonly, fish farming is performed in rectangular or round ponds. In rectangular ponds, the flow of water depends on the characteristics of the water inlet and tank geometry; in these ponds, the formation of dead zones without circulation is common, and interferes with fish distribution and consequently their behavior and performance [14]. On an experimental scale, it is known that factors including that interfere with the quality of water include shape and flow [13], the width-to-length ratio [15], water inlet positions, and quantities of water in and out of the tank [16]. However, on a real production scale, little is known about the influence of the morphological characteristics of ponds on the water quality.

Thus, this study characterized the effluents during the fish removal process in ground-excavated ponds of different sizes used for fish farming. The principal component analysis (PCA) was performed on the studied parameters to summarize the effluent quality and infer, in an integrated way, the influences of the different moments associated with the process of fish removal in farms of small, medium, and large sizes.

2. Materials and Methods

2.1. Study Area

Samples were collected in the Western region of the State of Paraná in Brazil, which is a fish production pole in ground-excavated ponds for intensive cultivation of Nile Tilapia. The sampling period was from June to October of 2016, in nine areas belonging to an integrated production system located in the municipalities of Tupãssi, Nova Aurora, Cafelândia, and Ubiratã (Figure 1), where twelve ponds were evaluated.

As it is an integrated production system, food management and water renewal rates are standardized, regardless of the size of the ponds. The water supply came from the subsurface flow with incipient values in the compounds evaluated in the effluent produced by the fish farming.

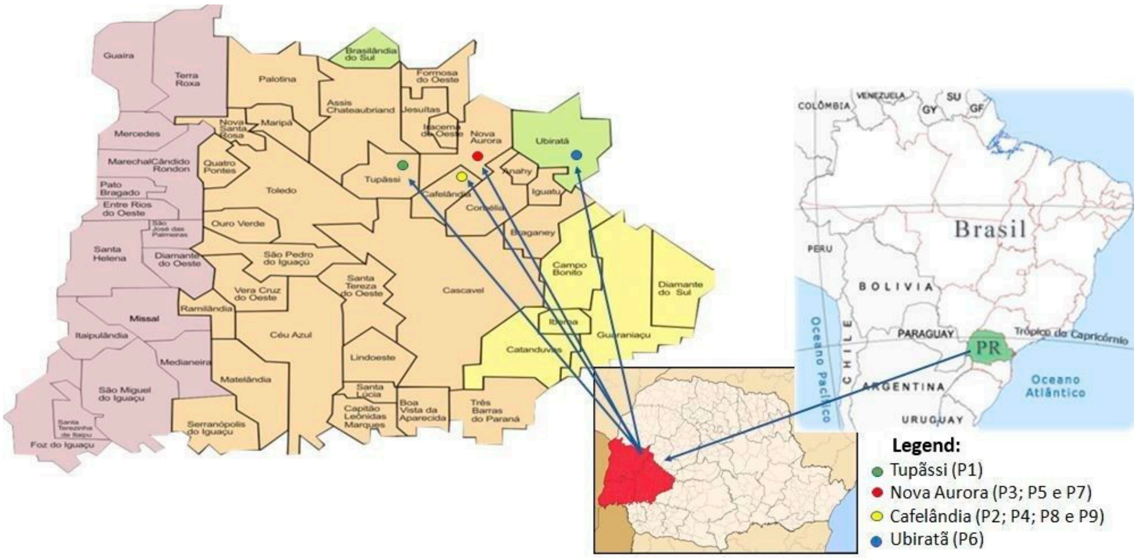

Figure 1. Map of Brazil showing the location of municipalities in Western Paraná where effluents from fish farming were sampled.

2.2. Sampling and Analysis of Effluent Samples

The ponds were defined and divided according to size classes: class I: ponds with an area of up to 3000 m^2 (Small); class II: ponds with areas from 3001 to 7000 m^2 (Medium); and class III: ponds with an area of 7000 m^2 (Large). Ponds were considered sampling units, and each was assessed through 4 replicates in order to evaluate the impact of size on the quality of effluents generated. In all size classes, cultivation was conducted at 5 fish/m^2 density, with a final average weight of 0.9 kg, totaling 45 ton/ha/cycle in 200 days of cultivation.

Effluent samples were collected at different times during the fish removal process, defined as “scenarios” within the same pond. Different collection times were, therefore, considered as replicate samples within the same site. These scenarios were characterized as C1: Full; C2: Medium; C3: Net; and C4: Final (Figure 2). The term “Full” represents the initial time in the fish removal process, before any intervention or collection of water was conducted, and before the beginning of water drainage; the term “Medium” represents the time when approximately 60% of the pond water was drained, however, without any intervention on the environment; the term “Net” represents the time after the first net trawling for fish harvesting was executed. In this time the revolving of sediment possibly occurs, resulting in suspension of organic matter and alteration in the water’s physical and chemical composition; and the term “Final” represents the final drainage time, when there is approximately only 5% of effluent in the pond.

The following physical and chemical analyses were performed: Alkalinity ($CaCO_3$) mg/L, CDO mg/L, BDO mg/L, Total Hardness (TH) mg/L $CaCO_3$, Total Phosphorus (TP) mg/L, Nitrite (NO_2^-) mg/L, Nitrate (NO_3^-) mg/L, Ammoniacal Nitrogen (NH_3) mg/L, Kjeldahl Nitrogen (TN) mg/L, Soluble Orthophosphate (PO_4) mg/L, Sedimentable Solids (SES) mL/L, Suspended Solids (SS) mg/L, and Total Solids (TS) mg/L. The analyses were performed according to the Standard Methods for the examination of water and wastewater [17].

Simultaneously to water sample collection, the following parameters were measured in situ: Dissolved Oxygen (DO) mg/L, Dissolved Oxygen in percentage of saturation (DO%), pH, Electrical Conductivity (EC) µS/cm, Total Dissolved Solids (TDS) mg/L, and Temperature (°C); these measurements were taken with the following portable devices: YSI Pro20, YSI F-1010PH, and YSI F-1030A, respectively.

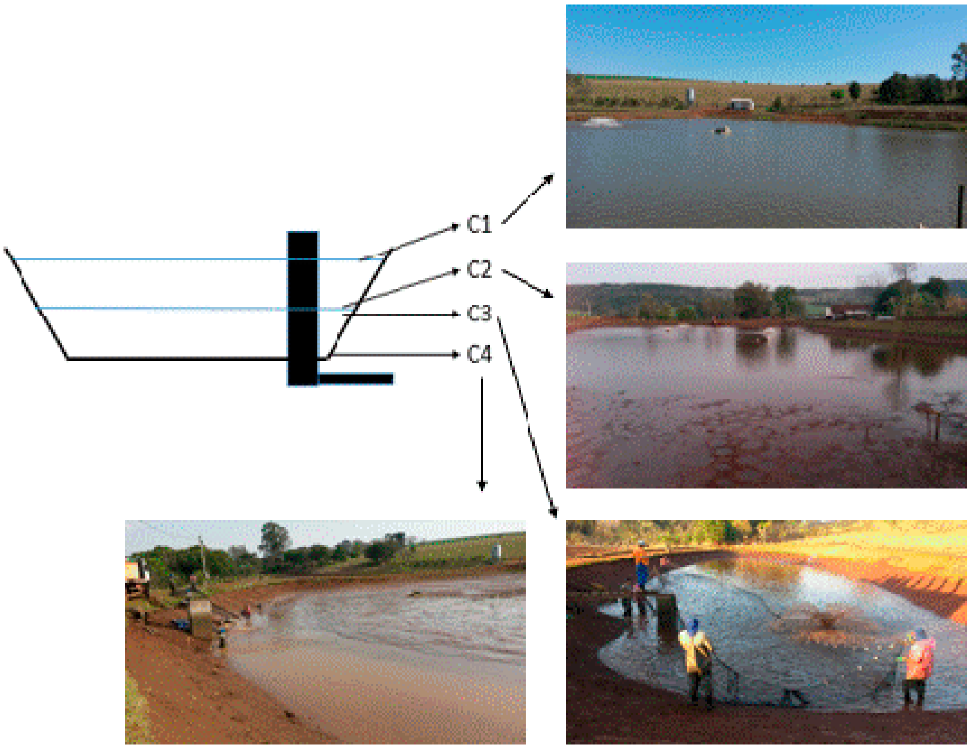

Figure 2. An illustrative diagram of the different time-point samplings of effluent. Where: C1: Full; C2: Medium; C3: Net; and C4: Final.

2.3. Data Analysis

Initially, the physical and chemical data were submitted to descriptive exploratory analysis through means, variabilities, frequency dispersions, and bivariate correlations. From this analysis, data were transformed into square root to better approximate the normal distribution, reducing the influence of few observations with exceedingly high values. Because of the high degree of observed bivariate linear correlations, the evaluation of these characteristics was conducted in an integrated way through the technique of extraction of components of greater variability known as Principal Component Analysis (PCA).

PCA was developed by Pearson in 1901 to summarize groups of linearly correlated variables; it expresses a large part of the total variability contained in the data using a few components generated from linear combinations of input variables [18]. These components, called principal components (PC), are synthetic variables that represent the covariation gradient for the set of variables that are correlated with them. Therefore, these components were generated and correlated to the variables that generated them (Pearson's correlation) in order to identify the group of variables that they represent. These components were subsequently evaluated in relation to pond sizes and sampling scenarios through the repeated measures analysis of variance—RM-ANOVA. The RM-ANOVA is an adequate technique to evaluate information obtained in the form of repeated observations about the same sampling unit [19], as was the case for measurements performed in the different scenarios. However, RM-ANOVA requires the assumption of sphericity, i.e., that the correlation structure between the several repeated measures is homogeneous, for the tests to be valid. Thus, the Mauchly's test of sphericity was used, and in the case of non-homogeneity, we performed and interpreted the Greenhouse–Geisser (GG) and Huynh–Feldt (HF) corrective procedures. The Tukey's test for the comparison of means was performed in the case of significant effects, considering a 5% level of significance ($p < 0.05$). The analyses were performed using the Statistic software version 7.1 [20].

3. Results and Discussion

The process of fish removal in ground-excavated ponds is carried out through the total drainage of water when the effluent is released into the environment. In this study, several parameters of effluent quality showed alterations during the fish removal process (Table 1). During the cultivation period, the water was constantly renewed at rates ranging from 1 to 10% per day, with lower rates at the beginning of the culture, and higher at the end. Fish were usually fed up to three times a day with balanced feeds containing 32% of crude protein. Feed leftovers and fish feces accumulated at the bottom of the ponds throughout the 200 days of culture. The feces and feed leftovers reflected the concentrations of TP, TN, and COD observed in C1 and their increase in the C2, C3, and C4 scenarios regardless of the ponds' sizes (Figure 3A,E,G).

The COD/BOD ratio is related to the degree of effluent degradability. When this ratio is <3.5, the biodegradable fraction is high, when it is >3.5, the inert fraction is predominant [21]. Therefore, the greater the biodegradable fraction, the easier the biological decomposition. However, when the inert fraction is elevated, the physical and chemical treatment of the effluent becomes necessary to reduce the BOD and COD in the discharged effluent. Moreover, the biological degradation of organic matter by decomposing bacteria depends on several factors including temperature, dissolved oxygen available at the bottom of the tank, pH, and alkalinity, which is necessary for bacterial survival. Thus, it can be observed that in the C1 scenario, independent of pond sizes, the COD/BOD ratios were <3.0, indicating that the present organic matter is biodegradable, and can be easily degraded by decomposing bacteria [21]. However, as the process of fish removal goes on from the C1 to C4 scenarios, the COD/BOD ratio increased, indicating that the organic matter fraction becomes less biodegradable at the end of the process, making it's biological decomposition increasingly difficult.

The results also indicated that small ponds have higher COD/BOD ratios than those of medium and large sizes (Table 1). The BOD results from the C1 scenario (Table 1) are considered to be related to the water at the end of cultivation, and were given as 6.98, 27.03, and 23.9 mg/L for small, medium, and large size ponds, respectively, with a density of 5 fish/m^2 and final weight of 900 g for the same species. Boyd [22], Boyd and Gautier [23], Boyd [24] reported that the restrictions imposed by directives that regulate water quality for BOD may be 30 mg/L or less depending on the inspecting institution. These values can be restricted to maximum discharging limits imposed on the receiving body. Frimpong, et al. [25] found BOD results of up to 12.61 mg/L using floating rations with the density of 2 fish/m^2 and final weight of 300 g in Nile Tilapia cultures. Therefore, the observed BOD values in C1 are acceptable for this standard culture.

The draining procedure is the same in all pond sizes. However, this process lasts 24 h in small ponds, and up to 36 h in large ponds. Fish are concentrated in a smaller volume of water during the process, which increases density and promotes water agitation that leads to the suspension of solids present in the bottom. This causes an increase in the concentration of COD, SES, SS, TS, TP, NH_3, and TN, and a decrease in DO concentration (Figure 3). This alteration possibly results from a reduction in water volume and increased fish movement in the C1 and C2 scenarios. In the C3 scenario, the alterations are aggravated by the disturbance caused by the net trawling for harvesting fish. In the C4 scenario, the water volume is small, and the amount of organic matter present in the sediment causes great alterations in the observed parameters.

The changes in the concentrations of $CaCO_3$, TH, NO_3^-, NO_2^-, and PO_4 were lower in relation to the others parameters evaluated. Alkalinity and total hardness are related to the presence of calcium carbonate in the water; the decreased water volume during the process of fish removal and alterations in the environment did not interfere with these concentrations. Due to the high ability of alkalinity to maintain a stable pH, pH also did not change during the process of fish harvesting.

The alkalinity results were lower than 30 mg/L in all sampling scenarios; the recommendation for fish cultivation to avoid pH variations is 25–100 mg/L [26]. Alkalinities between 40–80 mg/L are beneficial in nitrification processes [27].

The presence of NO_3^- and NO_2^- in the water is related to the nitrification and denitrification processes; however, the duration of the process of fish harvesting is not long enough to alter these parameters. In addition, the movement in the bottom and decreased DO hinder the nitrification and denitrification processes. The PO_4 concentrations ranged from 0.01 to 0.07 mg/L among the scenarios, with the highest concentration in C4, in medium size ponds. The concentration of this parameter is related to the concentration of total phosphorus (TP), which is the portion that can be absorbed by biological metabolism without the need for conversion, where TP is a trophic indicator. When TP > 0.05 mg/L, the environment is considered eutrophic [21].

Our findings indicate that an accumulation of nutrients in the water results when the effluent from the fish removal process is released into the recipient water body without being treated. This accumulation triggers the eutrophication process and causes ecological damage, e.g., mortality of fish and aquatic plants. In order to minimize environmental impacts, the characteristics of the effluent and the purification capacity of the receiving body should be known.

The CONAMA 357/2005 [28] Brazilian environmental legislation classifies rivers that receive effluents from fish farming in the region under study as class 2, establishing that these waters can be used for human consumption, recreation, irrigation, aquaculture, and fishing activities. In addition, these areas can be considered as protected areas for aquatic communities. Therefore, because of this classification, it is important that the effluents released in this environment meet the recommendations required in the legislation in order to not interfere with the river's potential for use.

Within the context of pollution of natural aquatic environments, the eutrophication caused by nitrogen and phosphorus supplies generates concern in inspection agencies. The results presented (Table 1) show that the effluents generated during the process of fish removal have phosphorus concentrations well above the recommended level, reaching 12 times (0.59 mg/L) the maximum allowed limit (0.05 mg/L of TP) in scenario C1, and increasing to 170 times (8.56 mg/L) in scenario C4. However, it should be taken into account that C4 represents the final moment in the process of fish removal when several parameters were altered, and the volume of effluents was 5% of the total water volume in the pond. Total nitrogen (TN) presented concentrations of 2.08 mg/L in C1 in small-sized ponds, and up to 24.68 mg/L in C4 in medium-sized ponds.

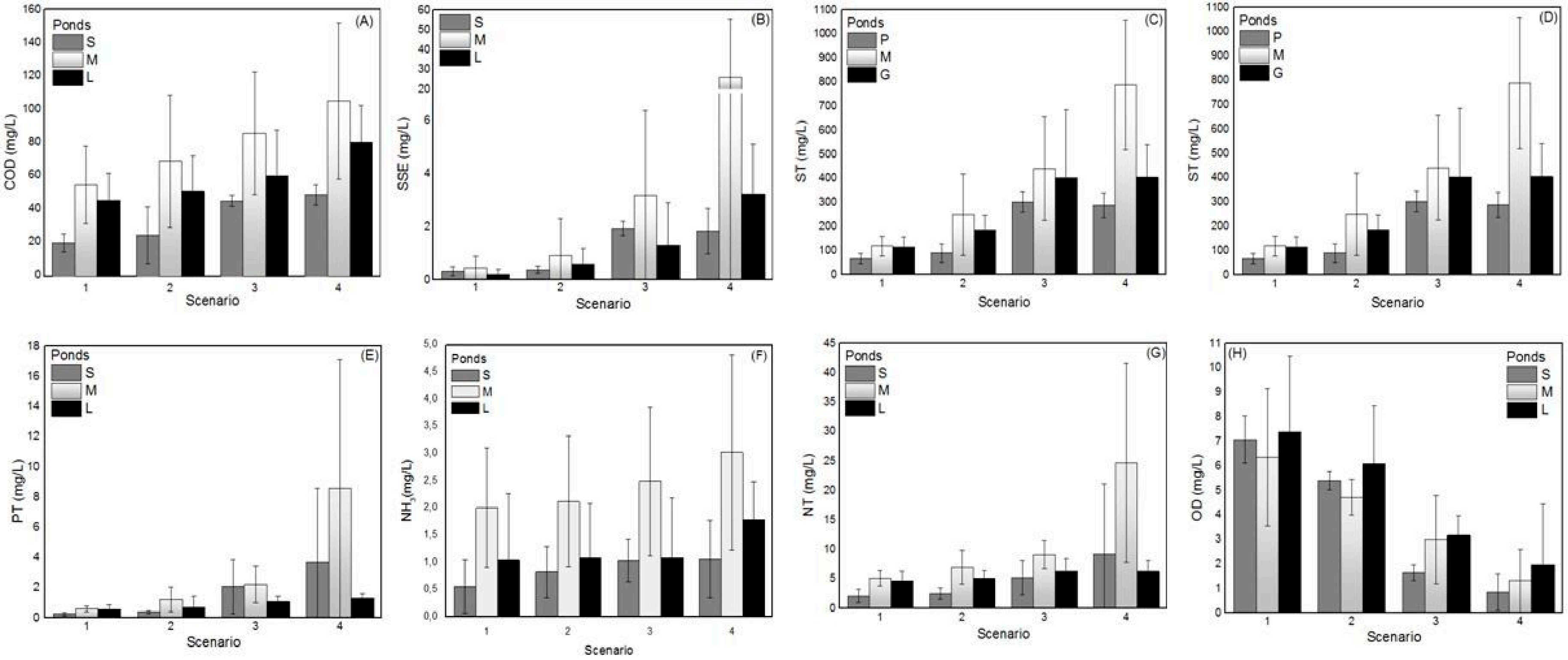

Figure 3. S: Small; M: Medium; and L: Large: (**A**) chemical oxygen demand; (**B**) sedimentable solids; (**C**) total solids; (**D**) suspended solids; (**E**) total phosphorus; (**F**) ammoniacal nitrogen (**G**) total nitrogen; and (**H**) dissolved oxygen.

Table 1. Parameters of effluent quality in different pond sizes and sampling scenarios during the fish removal process in Nile Tilapia ponds *.

Parameter	Small				Medium				Large				VPM [1]
	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	
$CaCO_3$ (mg/L)	11.9 ± 4.87	12.08 ± 4.04	13.13 ± 3.47	15.63 ± 9.03	26.00 ± 9.70	20.50 ± 6.14	21.00 ± 4.24	26.25 ± 6.70	17.20 ± 10.37	17.68 ± 9.23	17.6 ± 8.64	18.75 ± 6.50	-
TH (mg/L)	9.60 ± 1.99	10.35 ± 1.81	16.75 ± 4.99	19.75 ± 7.27	21.80 ± 9.15	20.00 ± 7.83	19.30 ± 7.23	23.00 ± 7.07	18.75 ± 7.63	19.25 ± 7.50	18.75 ± 7.41	18.25 ± 10.40	-
BOD (mg/L)	6.98 ± 2.95	7.48 ± 3.83	11.93 ± 1.72	12.00 ± 1.41	27.03 ± 17.48	33.55 ± 26.04	49.2 ± 33.26	38.75 ± 15.39	23.9 ± 13.15	29.75 ± 14.08	28.0 ± 12.91	27.75 ± 12.28	-
COD (mg/L)	19.25 ± 5.50	23.75 ± 17.17	44.50 ± 3.32	48.25 ± 6.02	54.25 ± 23.34	68.50 ± 39.87	85.25 ± 36.86	104.7 ± 47.0	44.75 ± 16.46	50.25 ± 21.53	59.5 ± 27.69	80.00 ± 21.92	-
COD/BOD	2.76	3.18	3.73	4.02	2.01	2.04	1.73	2.70	1.87	1.69	2.13	2.88	-
TP (mg/L)	0.26 ± 0.11	0.39 ± 0.11	2.06 ± 1.78	3.67 ± 4.92	0.59 ± 0.20	1.22 ± 0.82	2.20 ± 1.21	8.56 ± 8.54	0.56 ± 0.29	0.68 ± 0.37	1.07 ± 0.74	1.30 ± 0.31	0.5
PO_4 (mg/L)	0.027 ± 0.03	0.02 ± 0.01	0.01 ± 0.00	0.03 ± 0.03	0.03 ± 0.04	0.02 ± 0.02	0.01 ± 0.01	0.07 ± 0.10	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	-
NO_3^- (mg/L)	0.49 ± 0.16	0.40 ± 0.08	0.42 ± 0.15	0.36 ± 0.21	0.54 ± 0.21	0.99 ± 0.78	0.79 ± 0.61	0.47 ± 0.41	1.22 ± 1.06	1.18 ± 0.82	1.01 ± 0.79	0.47 ± 0.53	10
NO_2^- (mg/L)	0.03 ± 0.02	0.21 ± 0.19	0.03 ± 0.02	0.04 ± 0.03	0.46 ± 0.34	0.45 ± 0.41	0.43 ± 0.37	0.19 ± 0.14	0.24 ± 0.23	0.25 ± 0.15	0.21 ± 0.24	0.20 ± 0.26	1
NH_3 (mg/L)	0.55 ± 0.49	0.82 ± 0.47	1.03 ± 0.36	1.06 ± 0.71	2.00 ± 1.10	2.12 ± 1.20	2.49 ± 1.37	3.23 ± 1.79	1.04 ± 1.22	1.08 ± 1.00	1.09 ± 1.10	1.78 ± 0.70	20
TN (mg/L)	2.08 ± 1.14	2.48 ± 0.99	5.18 ± 2.90	9.25 ± 11.87	5.10 ± 1.28	6.98 ± 2.83	9.08 ± 2.40	24.68 ± 16.91	4.68 ± 1.61	5.05 ± 1.31	6.30 ± 2.16	6.25 ± 1.85	-
SES (mL/L)	0.33 ± 0.17	0.38 ± 0.13	1.93 ± 0.26	1.83 ± 0.85	0.45 ± 0.44	0.90 ± 1.40	3.18 ± 3.19	26.05 ± 29.43	0.20 ± 0.20	0.58 ± 0.59	1.90 ± 1.61	3.23 ± 1.87	1
SS (mg/L)	27.00 ± 1.51	48.75 ± 20.29	257.0 ± 50.28	238.3 ± 66.0	66.75 ± 34.70	201.5 ± 162.9	382.0 ± 202.0	690.1 ± 247.5	67.75 ± 24.32	119.25 ± 52.6	337.7 ± 282.2	337.5 ± 141.5	-
TS (mg/L)	66.75 ± 21.08	88.75 ± 38.74	302.0 ± 41.74	288.0 ± 51.63	119 ± 40.96	249 ± 170.24	440 ± 216.03	788.0 ± 270.1	114.25 ± 42.2	186.5 ± 60.18	403 ± 282.82	404.5 ± 134.7	-
TDS (mgL)	22.5 ± 5.00	46.25 ± 21.75	76.25 ± 16.01	57.5 ± 5.00	40.00 ± 16.83	40.0 ± 14.72	58.75 ± 21.75	66.25 ± 8.54	40.00 ± 10.80	51.25 ± 25.94	61.25 ± 23.58	45.00 ± 9.13	500
DO (mg/L)	7.07 ± 0.96	5.40 ± 0.98	1.64 ± 0.32	0.86 ± 0.74	6.36 ± 2.80	4.72 ± 0.72	2.99 ± 1.81	1.31 ± 1.27	7.38 ± 3.09	6.08 ± 2.40	3.17 ± 0.79	1.97 ± 2.48	≥ 5
DO%	92.37 ± 5.63	60.53 ± 7.78	18.00 ± 3.70	10.5 ± 10.47	76.60 ± 29.57	54.55 ± 9.29	33.76 ± 20.27	14.18 ± 14.19	90.38 ± 27.37	70.48 ± 29.69	37.38 ± 10.22	22.23 ± 27.57	-
EC (µ.S/cm)	50.0 ± 10.0	93.75 ± 48.02	152.5 ± 27.54	118.75 ± 8.54	82.50 ± 32.27	82.50 ± 27.84	117.5 ± 42.52	133.8 ± 14.4	8.25 ± 25.29	105 ± 50.50	123.75 ± 49.6	91.25 ± 20.16	-
pH	6.96 ± 0.40	6.81 ± 0.55	6.35 ± 0.12	6.29 ± 0.12	6.89 ± 0.28	6.74 ± 0.11	6.63 ± 0.23	6.59 ± 0.20	6.79 ± 0.21	6.50 ± 0.37	6.43 ± 0.37	6.34 ± 0.21	5–9
T °C ef [2]	25.50 ± 2.89	15.50 ± 1.45	18.00 ± 5.35	22.88 ± 8.19	23.75 ± 4.57	15.50 ± 1.73	14.50 ± 1.73	18.25 ± 4.57	24.25 ± 2.22	18.00 ± 2.58	16.75 ± 0.96	26.75 ± 6.65	-
T °C ar [3]	21.70 ± 1.96	19.25 ± 0.55	18.55 ± 2.74	21.33 ± 5.81	21.83 ± 3.25	20.33 ± 1.25	19.90 ± 1.07	19.18 ± 1.14	21.60 ± 1.74	21.78 ± 1.70	21.68 ± 1.80	24.50 ± 5.13	-

* Results expressed as means and standard deviations. [1] Maximum allowed value e. - Not specified by the CONAMA n° 357/05 Resolution [28], complemented by the CONAMA n° 430/2011 Resolution [29] for the discharge of effluents in Class 2 rivers. [2] T °C ef: effluent temperature; [3] T °C air: air temperature.

Nitrogen is an indispensable element for the growth of algae, and consequently, its high concentrations contribute to the process of eutrophication. Furthermore, the transformation processes from ammonia to nitrite and nitrate consume oxygen, which leads to an oxygen reduction in the environment. The legislation imposes restrictions on the release of N in the form of ammonia, nitrite, and nitrate. It is notable that intensive farming systems can cause impacts on the environment mainly due to residues related to food leftovers and feces produced by fishes. These residues are sources of N and P, which are the principal nutrients responsible for eutrophication, with emphasis on P as the limiting nutrient for primary freshwater production [4]. Balanced feeds with adequate concentrations of available phosphorus decrease the excretion and release of phosphorus in the environment, improving water quality [30].

The search for knowledge on the biology of cultivated species and feed sources that can be efficiently converted into animal protein has been the focus of research on fish nutrition. Phosphorus is indispensable for bone formation and metabolic activities. However, rations must meet fish requirements in adequate concentrations [31]. In evaluations to determine the centesimal composition of artificial feed for tilapia juveniles (<10 g) and post-juveniles (>10 g) (*Oreochromis niloticus*), Boscolo et al. [32,33] concluded that 0.74% of total phosphorus is required for juveniles and 0.70% for post-juveniles.

The intensively practiced agriculture in the studied region also contributes to the supply of nutrients in aquatic environments. Hu, et al. [32] indicated that the nutrient supply in aquatic environments comes from several sources such as domestic sewage, industries, animal feces, aquaculture activity, leaching, and surface runoff. Rivers are sources of water for fish farming, and may already have high concentrations of P and N due to other forms of contamination. Nevertheless, intensive fish farming can produce effluents that are rich in nitrogen and phosphorus, producing eutrophic effluents. Effluents from fish farming have a high oxygen biochemical demand in addition to high concentrations of suspended solids, nitrogen, and phosphorus, which make it very similar to domestic effluents [33].

The use of management practices to improve effluent quality involve actions to reduce effluent volume, efficient feed management, erosion control to reduce suspended solids, and adequate use of fertilizers. These actions are ways to control problems such as algal bloom, pH variation, and biochemical oxygen demand. The disordered growth of aquatic macrophytes and cyanobacteria can occur in aquatic ecosystems with high concentrations of nitrogen and phosphorus. In the studied region, phosphorus available in the environment does not only come from the activity of fish farming, but mainly from leaching of areas of intense agricultural activity that are characteristic of the region [6]. Palácio, et al. [6] observed that the phosphorus concentration rose 150 times higher than the approved level during the course of a river in this region. The authors attributed this value to the use of fertilizers in soybean and corn crops in areas without fish farming activities. Thus, the waters that reach the fish farms are possibly already rich in nutrients. The ammoniacal nitrogen (NH_3) in the C4 scenario had concentration levels of up to 3.23 mg/L, however, which was within the allowed range for the effluent. The main nitrogen compound excreted by fish is ammonia, as it is part of the protein metabolism process, aiding in the microbial decomposition process of leftovers, feces, and organic fertilizers [33]. The excess of organic matter in the ponds' sediment, and the lack of conditions for decomposition, cause an imbalance in the nitrification and denitrification processes, and can contribute to the accumulation of toxic ammonia and nitrite during cultivation [34,35].

The total dissolved solids (Table 1) presented values below the maximum values permitted by the environmental legislation (500 mg/L) in all sampling scenarios and pond sizes, showing a tendency to increase with the progress of harvesting fish. Electrical conductivity is directly related to the concentrations of total dissolved solids. The increased EC with the progress of the fish removal process indicates a great availability of nutrients in the effluents, which is evidenced by the observed N and P levels. According to Ribeiro, et al. [36], the electrical conductivity identifies polluting sources and increases linearly with the concentration of salts in the aquatic environment. The electrical conductivity values are recommended to remain between 20 μS/cm and 150 μS/cm for fish farming [37].

The analysis of each parameter (Table 1) allowed for the identification of alterations during the fish removal process in the different pond sizes. However, it was not possible to evaluate only through univariate analysis or through the comparison of means because these variables were correlated with each other. This was due to the data variability obtained from effluents in the different pond sizes.

Therefore, the principal component analysis allowed us to identify and correlate significant parameters, allowing us to evaluate how the fish farming and fish removal processes can be managed in order to improve the quality of effluents released in the receiving body.

Confirming the observations already described in the univariate approach of the fish farming effluent, the principal components analysis identified that the main gradient of variation contained in the data (PC1) accounted for approximately 40% of the total variability, and was positively associated with chemical oxygen demand (COD), sedimentable solids (SES), suspended solids (SS), total solids (TS), total phosphorus (TP), total nitrogen (TN), and ammoniacal nitrogen (NH_3), and negatively related to dissolved oxygen (DO) and its percentage of saturation (DO%) (Table 2).

In the evaluation of the sources of variation in this gradient through RM-ANOVA evidenced that both pond sizes and sampled scenarios influenced the set of variables associated with PC1 in a significant way, with no interaction between pond size and effluent quality in fish removal scenarios (Table 3).

The Tukey's test for a posteriori comparison of means identified that the medium-size ponds presented the worst effluent quality (Figures 4a and 5). Regarding the scenarios, the quality of effluents showed a slight tendency (not significant) of worsening between the full and medium scenarios. However, effluents showed significantly lower qualities after the first net trawling and at the end of the fish removal process (Figure 4b). The second variation component (PC2) accounted for 16% of the variability and was positively associated with biological oxygen demand, nitrite, and nitrate, and negatively associated with the water electrical conductivity (EC) (Table 2). This component had a significant influence only from the measurement scenario (Table 3), separating again the full (C1) and middle (C2) scenarios from those after the first net trawling (C3) and final net trawling (C4) (Figure 4b). Components PC1 and PC2 represented 56% of the data variability. The other generated principal components presented a low explanation of variability (<10% each), without strong associations with the variables or significant relationships with pond sizes or scenarios.

The identification of the medium size ponds with the worst indexes of effluent quality may be related to design aspects of ponds or rates of water renewal that are uncontrolled variables while storage densities and food management were standardized. However, this indication is important for defining pond size in future projects, and can be improved in further studies.

In rectangular ponds, the water flow depends on the geometry and forms of the water inlet. It is common to find heterogeneous environments in these ponds caused by a lack of circulation that creates dead zones [13]. The sedimentation of fecal solids and feed leftovers occurs where there is no water circulation; these solids increase the BOD and cause variations in the DO gradient [38].

Studies concerning the ideal size and shape of pond tanks for fish farming are scarce. Oca and Masaló [15] evaluated the width-to-length ratio in the formation of dead volumes. Evaluations using particle tracking velocity (PTV) techniques indicate that a single water inlet in a rectangular tank provides more areas with dead volumes [16].

The characteristics mentioned with reference to the design aspects of the ponds concur with the results found on the quality of effluents, as the evaluated ponds are rectangular and have a single water inlet. The formation of dead zones inside ponds interferes with water quality, and consequently has a direct influence on effluent quality. Further studies that demonstrate the impact of design on effluent quality in fish farming should be undertaken to seek the means for increased activity sustainability.

Table 2. Matrix of correlations between variables and the main components generated in the principal components analysis.

	PC1	**PC2**	**$CaCO_3$**	**BOD**	**COD**	**TH**	**NO_3^-**	**NO_2^-**	**SES**	**SS**	**TS**	**TP**	**NH_3**	**TN**	**PO_4**	**pH**	**DO**	**DO%**	**EC**
Autovalue	2.66	1.83																	
Explanation (%)	39.46	15.6																	
$CaCO_3$ (mg/L)	0.35	0.42																	
BOD (mg/L)	0.55	0.69	0.26																
COD (mg/L)	0.79	0.36	0.28	0.72															
TH (mg/L)	0.33	0.48	0.54	0.46	0.19														
NO_3^- (mg/L)	−0.01	0.58	−0.21	0.59	0.23	0.36													
NO_2^- (mg/L)	0.15	0.78	0.2	0.77	0.37	0.48	0.62												
SES (mg/L)	0.68	−0.05	0.08	0.3	0.52	−0.05	−0.17	−0.06											
SS (mg/L)	0.88	−0.04	0.17	0.49	0.75	0.11	−0.02	0.08	0.64										
TS (mg/L)	0.89	−0.03	0.18	0.51	0.76	0.13	0	0.09	0.66	0.99									
TP (mg/L)	0.75	−0.06	0.16	0.27	0.5	0.06	−0.16	−0.06	0.89	0.62	0.64								
NH_3 (mg/L)	0.62	0.35	0.66	0.52	0.67	0.18	−0.17	0.27	0.43	0.53	0.53	0.42							
TN (mg/L)	0.81	0.09	0.31	0.42	0.59	0.16	−0.07	0.06	0.85	0.7	0.71	0.95	0.52						
PO_4 (mg/L)	0.5	0.23	0.2	0.31	0.47	0.08	−0.03	0.11	0.72	0.35	0.37	0.73	0.53	0.67					
pH	−0.44	0.31	0.2	−0.13	−0.17	−0.24	−0.21	0.07	−0.15	−0.29	−0.31	−0.22	0.19	−0.18	−0.06				
DO (mg/L)	−0.59	0.55	−0.03	0	−0.22	−0.15	0.25	0.26	−0.26	−0.45	−0.45	−0.36	−0.11	−0.34	0.03	0.5			
DO%	−0.62	0.54	−0.04	−0.02	−0.25	−0.16	0.26	0.24	−0.27	−0.48	−0.47	−0.37	−0.15	−0.35	0.01	0.51	0.99		
EC (µS/cm)	0.5	−0.57	−0.04	−0.06	0.15	−0.06	−0.2	−0.21	0.24	0.45	0.45	0.26	0.1	0.24	0.05	−0.43	−0.47	−0.49	
TDS (mg/L)	0.51	−0.55	−0.02	−0.05	0.17	−0.06	−0.2	−0.2	0.25	0.45	0.44	0.26	0.12	0.24	0.07	−0.43	−0.45	−0.48	0.99

Table 3. Statistical test (F and ε) and associated probabilities (*p*) obtained in the analyses of variances of repeated measures applied to the main components (PC) generated in the principal components analysis.

	PC1 *						**PC2 ****	
Variation Source	**F**	***p***	**GG (ε)**	***p***	**HF (ε)**	***p***	**F**	***p***
Size	12.85	0.002	-	-	-	-	3.46	0.077
Scenario	35.77	<0.001	0.49	<0.001	0.57	<0.001	14.70	<0.001
Size: Scenario	1.89	0.119	0.49	0.180	0.57	0.169	0.62	0.710

Greenhouse–Geisser (GG) and Huynh–Feldt (HF) corrections are presented for the case of non-sphericity. Mauchly's test for sphericity: * $p = 0.004$; ** $p = 0.39$.

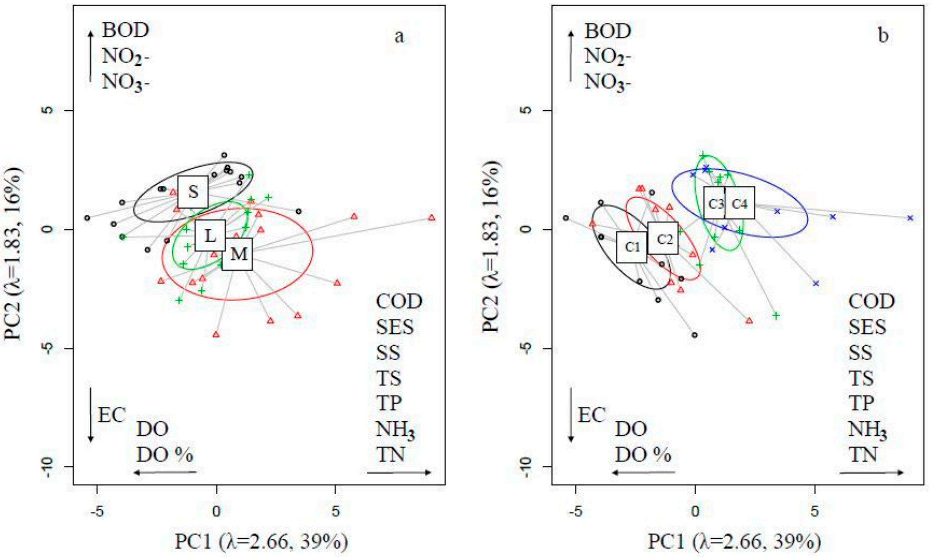

Figure 4. Scores of the main components 1 (PC1) and 2 (PC2) obtained in relation to pond sizes (**a**) and sampling scenarios (**b**). Eigen values (λ) followed by percentages of explanation are presented close to the axes. Arrows indicate the relationship between PCs and variables: BOD: biochemical oxygen demand; COD: chemical oxygen demand; NO_3^-: nitrate; NO_2^-: nitrite; SES: sedimentable solids; SS: suspended solids; TS: total solids; TP: total phosphorus; NH_3: ammoniacal nitrogen; TN: total nitrogen; DO: dissolved oxygen; DO%: percentage of dissolved oxygen saturation; and EC: electrical conductivity.

The averages and standard deviations presented in Table 1 and Figure 3 depict the final PCA results showing the correlation between parameters and the worsening condition in effluent quality during the process of fish removal. This result consequently indicates the need to improve the water quality already observed in the studied scenarios 1. The dissolved oxygen presented a significant decline during the fish removal process due to the decrease in the volume of water in the ponds. Fish concentration subsequently also declined, besides the suspension of solids promoted by the management of nets. The dissolved oxygen considered optimal for tilapia cultivation is between 5 mg/L and 6 mg/L, whereas, an intervention is recommended when it reaches below 3.1 mg/L [39].

The C1 scenario shows adequate DO concentration for the cultivation of tilapia; according to the environmental legislation, which considers the DO level as a limiting factor for the discharge of effluents, scenarios C3 and C4 show inadequate effluent quality.

TP and TN were highly correlated (r = 0.95). These nutrients are the main cause of environmental degradation produced by aquaculture [26,40–42]. The PCA shows that SES is positively correlated with TP (0.89) and TN (0.85), indicating that there should be an intervention on SES as a way to mitigate the environmental impact. SS and TS also have an influence on nutrients, particularly on TN, with correlations of 0.7 and 0.71, respectively. SS and TS are the variables with the highest positive correlation over others presented in PC1.

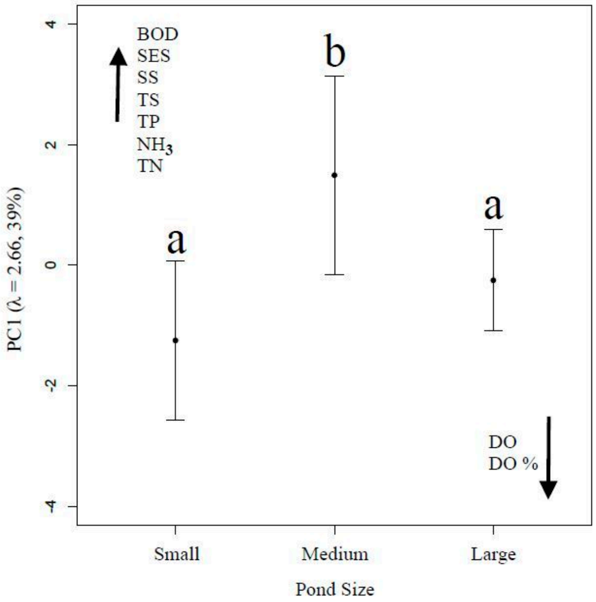

Figure 5. Averages and 95% confidence intervals for the first main component (PC1) relative to pond sizes. Distinct letters on confidence intervals indicate significant differences by the Tukey's test. Arrows indicate the direction of PC relationship with variables: COD: chemical oxygen demand; SES: sedimentable solids; SS: suspended solids; TS: total solids; TP: total phosphorus; NH_3: ammoniacal nitrogen; TN: total nitrogen; DO: dissolved oxygen; and DO%: percentage of dissolved oxygen saturation.

Solids can be removed by sedimentation, sand or mechanical filters, and various physical, chemical, and biological methods that have been or can be used to treat aquaculture effluents. These treatments are also efficient for the removal of phosphorus. However, they require high implementation and maintenance investments, and specific studies in this field are still scarce [43].

The formulation of fish feed is based on proteins of plant origin; the use of enzymes that may favor the uptake and availability of P present in plant proteins, such as phytase, can be used [44]. The reduction of P in feeds can also be promoted with the use of good quality fish meal, or the inclusion of monocalcium phosphate, which presents a high availability of P. Another recommendation for P reduction would be the indication of maximum levels of available phosphorus on ration labels. This initiative could reduce the use of rations with high levels of phosphorus, which would prevent the eutrophication of aquatic environments. In Brazil, the indication of minimum levels of phosphorus is the only requirement.

The concern with environmental sustainability should be taken as a priority in the production of fish in ground-excavated ponds. Fish farming has economic importance in the Western region of Paraná generating jobs and income for farmers. Hence, the characterization of effluents presented in this study may contribute to the implementation of similar fish production systems in other locations seeking alternatives to reduce pollution load. The principal components analysis on the quality of effluents presented here demonstrated that the environmental impacts resulting from the fish farming activity are concentrated at the moment of fish removal; however, care with nutrient supply should also be controlled during the cultivation period.

4. Conclusions

Fish farming in ground-excavated ponds has undergone a series of changes; the main change has been the increase in productivity by means of culture intensification. However, the characterization of effluents generated by the fish farming activity in ground-excavated ponds pointed out to the need for the application of good management practices during cultivation in order to avoid high concentration levels of nutrients released into the environment during the fish removal process. The discharge of effluents with high pollutant capacity threatens the growth of this activity.

The PCA results showed that small and large ponds do not present significant differences in the quality of effluents; however, medium size ponds showed poor effluent quality. These results suggest the advantage of using large ponds whenever possible; these ponds have lower implantation and maintenance costs compared to other sizes, and allow improved area utilization without generating effluents of low quality.

Irrespective of size, management measures associated with sediments from ground-excavated ponds are necessary to mitigate the negative impacts of the released effluent. Through the principal components analysis, the progressive degradation in effluent quality during the fish removal process was identified. It was also found to be associated with elevations in the water suspended solids and total solids concentrations, mainly after the first net trawling to harvest fish. Subsequently, the sediment turnover triggers an increase in nitrogenous and phosphate compounds and COD elevation. The use of decantation ponds is an effective practice to reduce the concentration of these solids. Further studies aimed at the identification of the ideal management to reduce the nutrient load that could cause eutrophication in recipient bodies are still relevant.

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