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Potential toxic effect of ammonia in reservoirs with tilapiculture in cages

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Abstract
The use of reservoirs for aquaculture is yet a common practice in many countries and due to its potentially polluting impact is considered the main activity that restricts the multiple uses of water reservoirs. Ammonia is the main nitrogen product of protein catabolism and can cause chronic and acute toxic effects even at relatively low concentrations. Therefore, this compound should be closely monitored in fish farming. In the present study, the water quality of six tilapia production systems located in three reservoirs of São Paulo state (Brazil) was monitored during 2014-2016. The proximity between farms negatively affected farming, with ammonia concentrations above the safe limit. The pH had significantly affected the fractionation of the total ammoniacal nitrogen. Our results indicated that natural conditions (i.e. drought) and anthropogenic activities in the watershed of the reservoirs affected the water quality more significantly than the aquiculture practices in the net cages.

Keywords: environment; management; multiple uses; water quality

1. Introduction

Large reservoirs originally built to provide water or energy supply are currently multipurpose and serve for numerous activities that stimulate the regional development, such as recreation and aquaculture [1]. Aquaculture is the fastest growing food production system worldwide and the use of reservoirs for this practice is still common in many countries, such as Brazil. The Nile tilapia (*Oreochromis niloticus*) is widely used in aquaculture due to its fast-grow and tolerance to a range of environmental conditions [2]. Brazil is the world's fourth-largest tilapia producers, with 357,639 tons produced in 2017 [3]. The State of São Paulo is the third largest fish producer in Brazil, due to the large volume of dams with the capacity and potential for net cage aquaculture. Reservoirs used for aquaculture in São Paulo have already a high degree of eutrophication[4] mainly due to several decades of untreated domestic sewage and industrial effluents discharge. Therefore, their multiple uses are often compromised and dependent on complex interactions and adequate management that avoid the disrupting of the ecological balance of the water bodies [5, 6]. On the other hand, aquaculture and particularly food management practices have a great polluting potential that might negative impact on the water quality of the reservoirs. Overfeeding or imbalanced diets reduce the fish nutrient absorption, increasing the inputs of particulate organic matter and nutrients in the systems. This has direct effects on water quality by increasing the phytoplankton, reducing water transparency and the concentrations of dissolved oxygen to critical levels at dawn, which consequently compromise fish health [7]. Ammonia is the main product of fish excretion and represents approximately 90% of the protein catabolism obtained through food. Therefore, it is directly related to the fish feed rate and the protein content in the diet. Approximately 40-60% of the nitrogen that the fish obtain through the food is excreted as ammonia in 24 hours [8].

Ammonia has a high acute and chronic toxicity at both cellular and tissue levels, affecting excretory and osmoregulatory processes, respiration and growth, as well as increasing the susceptibility to diseases [9] (Arana 2004). The sequence of symptoms of acute ammonia intoxication are hyperactivity, seizures, lethargy, balance loss and death [10]. Ammonia is in equilibrium with the ammonium ion in an aqueous media. This equilibrium can be affected by pH, temperature, ionic strength and pressure, being the first two the most important factors in natural waters 11-14.

Unlike ammonia, the ammonium ion ($NH4^+$) has a low toxicity with no risks for fish farming, however, is more readily assimilable by phytoplankton, which increases the occurrence of blooms. Therefore, the maintenance of a good water quality is essential for fish health and welfare as well as for the conservation of the aquatic environment. In the present study, we monitored the water quality of six aquaculture systems for the production of Nile tilapia located in three water reservoirs of São Paulo state, Brazil, during the years 2014-2016, to assess the effect of net cage aquaculture on the water quality of the reservoirs. Our aims were to 1) evaluate if the concentrations of the physical and chemical factors analyzed, especially of ammonia, were adequate for Nile tilapia farming, 2) identify which factor, pH or temperature, have exerted a more significant effect on the ammonia concentration, and 3) determine whether spatial variations (upstream and downstream from the farms) were more significant than the temporal ones (related to internal processes of the reservoir and to the hydrographic basin).

2. Materials and Methods

2.1 Study area

This study was performed in three reservoirs with commercial systems for Nile tilapia farming (net cages) located in the cities of Ipaussu (Chavantes reservoir, Paranapanema River Basin), Zacarias (Nova Avanhandava reservoir, São Jerônimo and Santa Bárbara River Basins), Bela Vista and Santa Fe do Sul (Ilha Solteira reservoir, São José dos Dourados and Can Can River Basins), state of São Paulo).

Samplings were carried out at five dates (October 2014; March and October 2015; March and October 2016) in the fish farms (F) and their respective downstream (D) and upstream (U) locations. The mean residence time were: approximately 300 days in Chavantes [15], 46.7 days in Ilha Solteira [16] and of 50 days and 120 days in Nova Avanhandava during the rainy and dry seasons, respectively [17].

2.2 Water physical, chemical, and biological variables

The pH, water temperature, and dissolved oxygen were measured *in situ* with a portable YSI multiprobe. Water samples for the analysis of total ammoniacal nitrogen (TAN), ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-) and chlorophyll a were collected at 1m of depth, using a Van Dorn bottle. Samples for the analysis of the nitrogen forms were immediately filtered in a vacuum pump using a GF/ F filter (0.47 µm pore size) and frozen-stored until processed. In the laboratory, the ammoniacal nitrogen and and the nitrite and nitrate were quantified [18, 19].

2.3 Dissolved oxygen saturation

The oxygen concentration was expressed in terms of saturation percentage considering the effects of temperature and atmospheric pressure. The estimation of the atmospheric pressure in each of the reservoirs was according to the following equation [20]:

$$P = 101.3x\left(\frac{288-0.0065xA}{288}\right)^{5.257} \quad (1)$$

Where P is the pressure (In ATM) and A is the altitude (in m). The effect of temperature on the oxygen saturation was estimated according to equation 2 [21]:

$$DO_0 = e^{\left[-139.34411+\left(\frac{1.575701x10^5}{T}\right)-\left(\frac{6.642805x10^7}{T^2}\right)+\left(\frac{1.243800x10^{10}}{T^3}\right)-\left(\frac{8.621979x10^{11}}{T^4}\right)\right]} \quad (2)$$

The effect of atmospheric pressure on the oxygen saturation was estimated [21, 22] where F_p is the correction factor for atmospheric pressure; P is the atmospheric pressure (in atm); P_w is the saturation vapor pressure (in atm); θ_0 is the second virial coefficient for oxygen; T is the temperature (in K) and t is the temperature (in °C):

$$F_p = \frac{(P-P_w)(1-\theta_0 P)}{(1-P_w)(1-\theta_0)} \quad (3)$$

$$P_w = e^{\left[11.8571-\left(\frac{3840.7}{T}\right)-\left(\frac{216961}{T^2}\right)\right]} \quad (4)$$

$$\theta_0 = 0.000975 - 1.426x10^{-5}t + 6.436x10^{-8}t^2 \quad (5)$$

The percentage of saturation was calculated according to equation 6, where % DO is the percentage of dissolved oxygen saturation and DO_{med} is the oxygen concentration of the sample (in mg L^{-1}).

$$\%DO = \left(\frac{DO_{med}}{DO_0 x F_p}\right)x100 \quad (6)$$

2.4 Chemical equilibrium of ammoniacal nitrogen

In an aqueous medium, the ammonia is in equilibrium, according to the equation below:

$$NH_4^+ + H_2O \leftrightarrow NH_3 + H_3O^+ \quad (7)$$

The equilibrium constant (k_a) is given by:

$$k_a = \frac{[NH_3]+[H_3O^+]}{[NH_4^+]} \quad (8)$$

The equilibrium constant is a function of temperature, pressure and ionic strength of a solution. The correction of the equilibrium constant as a function of temperature can be performed by derivations of the equations of Arrhenius[21], van't Hoff [23] or empirical equations[11] which are commonly used in the mathematical modeling of water quality [21, 24, 25].

$$pk_a = 0.09018 + \frac{2729.92}{T_2} \quad (9)$$

The percentage of free ammonia (% NH3) that compose the TAN was calculated [11]:

$$\%NH_3 = \frac{1}{1+10^{(pk_a-pH)}} \quad (10)$$

2.5 Statistical analyses

Data were tested for normality with the Shapiro-Wilk test ($\alpha > 0.01$). The upstream and downstream locations were compared with the non-parametric Wilcoxon signed-ranked test, whereas differences between fish farms were evaluated with the non-parametric Kruskal-Wallis test [26]. A cluster analysis using Ward's method and Euclidean distance was employed to evaluate whether the physical and chemical factors analyzed were more significantly influenced by spatial or temporal conditions. Group consistency was verified using the clustering algorithm of K-means.

3. Results

Our results showed that 41.2% of the temperature data were lower than 27ºC, which could affect tilapia growth causing damages to its welfare and consequently economic losses. The pH was neutral to alkaline and the oxygen saturation ranged from 62-172%. All nitrogen forms showed significant differences between upstream and downstream locations of the fish farms, except for TAN in farm 4 and for nitrite in farms 5 and 6 (Fig. 1).

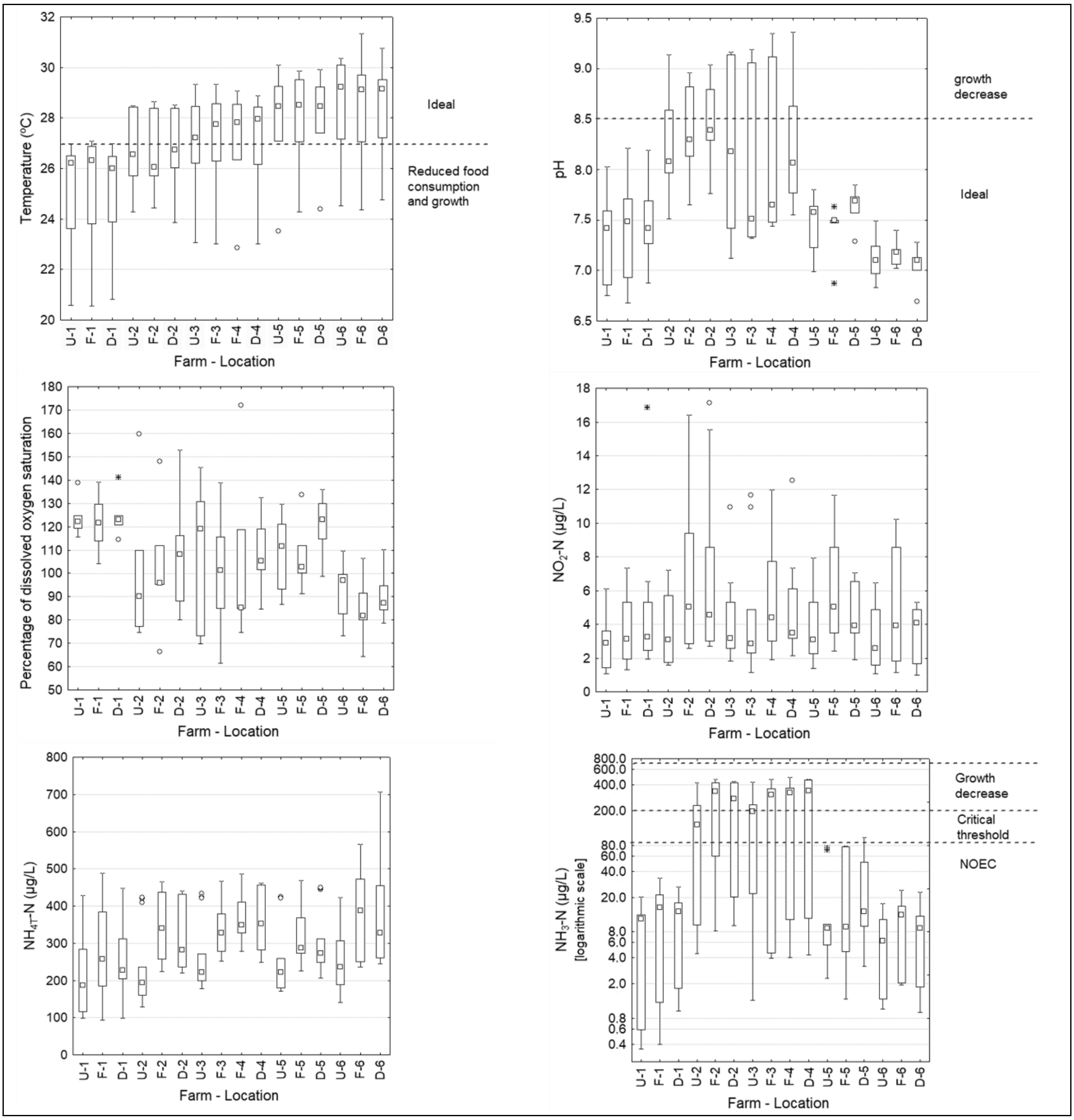

Fig 1: Boxplots of the temperature, pH, percentage of dissolved oxygen saturation, and nitrite, ammonium and ammonia concentrations at the reservoirs. References: boxes: interquartile distance; squares: median; whiskers: minimum and maximum values; circles: outliers; asterisks: discrepant values; D: downstream, F: fish farm, U: upstream; 1-6: tilapia farms

As shown in Fig. 1, all farms presented low nitrite concentrations, with a maximum of 1.6 x 10^{-2} mg L^{-1} and a median of 3.9 x 10^{-3} mg L^{-1}, indicating appropriate conditions for tilapia farming. Of the total ammonia data registered (N=255), 26% were above the no observed effect concentration (NOEC) and 91% of them were critical for farming, as in the case of farms 2, 3 and 4. The upstream median concentration of ammonia, which was already above the NOEC and even above the critical limit, strongly influenced the concentration of this nitrogen form in the farms (Fig. 1). The distribution model of the TAN fractions showed that pH and temperature had a more significant effect on the increase of the ammonia fraction. Figure 2 showed that pH and temperature had an exponential and linear effect, respectively, on the distribution of NH3 and $NH4^+$ fractions.

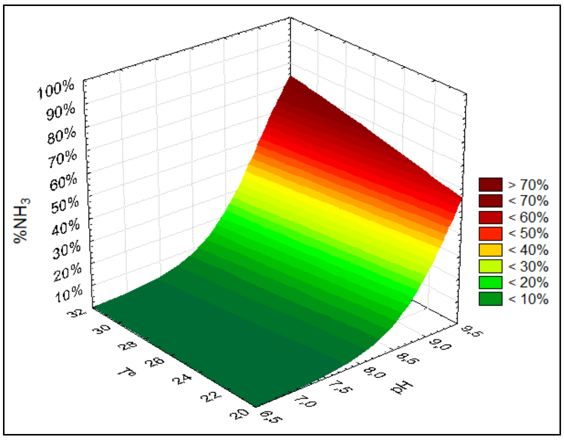

Fig 2: Surface graph of the percentage of free ammonia, considering the minimum and maximum values of temperature and pH registered in the present study

For example, considering a median TAN of 280.65 $\mu g\ L^{-1}$ and a temperature of 27ºC, when varying the pH between a minimum of 6.7 and a maximum of 9.4, the concentration of ammonia increased exponentially from 0.9 to 174.6 $\mu g\ L^{-1}$ at a rate of 1.94, which represent 0.3% to 67% of the TAN fraction, respectively. In the case of temperature, considering the median TAN and a pH of 7.5, when varying from a minimum of 20.6°C to a maximum of 31.4°C, the ammonia concentration increased linearly from 3.6 to 7.7 $\mu g\ L^{-1}$ at a rate of 0.37.

All farms had differences with statistical significance ($p < 0.05$) between upstream and downstream concentrations in some of the physical and chemical factors analysed, according to the Wilcoxon´s test. Although the pH between the upstream and downstream locations were similar or showed small differences, the Wilcoxon´s test indicated significant differences between farms 1, 2 and 5 ($p < 0.01$), and between farms 4 and 6 ($p < 0.05$). The concentrations of nitrogen forms were higher downstream than upstream, indicating the influence of farming on the water quality of the reservoirs. Kruskal-Wallis test showed differences in the water quality between farms ($p < 0.01$; Fig. 1). Farm 1 presented a pH close to neutral, higher DO and lower ammonia concentrations, while similar water conditions were observed between farms 2, 3 e 4. Cluster analysis showed three main groups based on the physical and chemical conditions of the sampling dates (Fig. 3). Group 1 corresponded to Oct 2015; Group 2 to Mar and Oct 2016, while Group 3 to Oct 2014 and Mar 2015. These groups were consistent with the K-means algorithm.

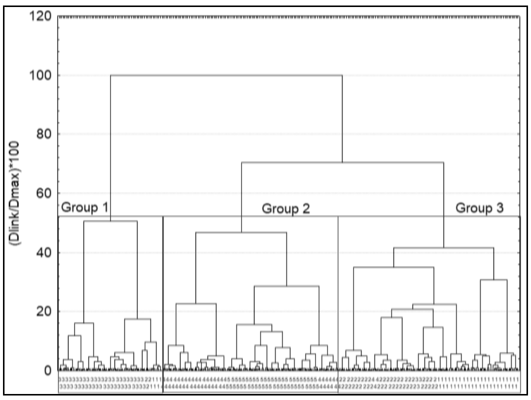

Fig 3: Cluster analysis of the physical and chemical variables at the downstream, fish farm and upstream locations (N=255), using Ward´s method and Euclidean distance. 1-5: sampling dates

4. Discussion

Tilapias are tropical fish that find thermal comfort between 27°C and 32°C. Temperatures outside this range lead to a reduced appetite and growth rate. And below 20°C, the appetite of tilapias is extremely low and the risks of diseases are increased, while below 14°C, mortality may occur [27]. Therefore, under the temperature conditions found in the studied farms, the growth of tilapias could be affected, causing damages to their welfare and consequently economic losses. The water pH and the oxygen concentrations registered were considered within the adequate range for tilapia welfare. Tilapia farming, pH should be maintained between 6 and 8.5 [27]. Tilapias are able to tolerate low oxygen conditions for a few hours. Nevertheless, when the DO saturation reaches 45-50%, they reduce their activity and oxygen consumption whereas at 20-10%, they reduce growth and are susceptible to disease or even death [27]. Some nitrogen forms are of great relevance in fish farming because of their toxic potential. In the studied reservoirs, we registered low nitrite concentrations, which are safe for tilapias. Tilapias are one of the cultivated fish the most sensitive to nitrite. Nitrite sublethal concentrations between 0.3 and 0.5 $mg\ L^{-1}$ are able to reduce the growth and resistance to diseases [28]. The ammonia lethal concentration ($LC_{50\text{-}96h}$) for tilapias was 2.65 ± 0.09 $mg\ L^{-1}$ [29]. These authors also showed that 0.73 and 1.46 $mg\ L^{-1}$ of ammonia caused a decrease in fish growth of 50% and 100%, respectively. Similar results were found in juveniles of the blue tilapia (*Oreochomis chrysops*), with an $LC_{50\text{-}96h}$ for the ammonia of 2.88 $mg\ L^{-1}$ [30]. Ammonia concentrations of 0.2 $mg\ L^{-1}$ are critical, above which the performance and health of tilapias begin to be impaired [28].

The concentration of 0.14 $mg\ L^{-1}$ of ammonia are already able to adversely affect the growth and estimated a NOEC of 0.068 $mg\ L^{-1}$ [31]. These authors recommended that concentrations for fish farming should be below 0.1 $mg\ L^{-1}$. Was verified that at different times of exposure [32] the LC50 and NOEC of ammonia for the Nile tilapia the concentrations were $LC_{50\text{-}48h}$ of 1.46 $mg\ L^{-1}$, $LC_{50\text{-}72h}$ of 1.33 $mg\ L^{-1}$ and $LC_{50\text{-}96h}$ of 0.98 $mg\ L^{-1}$ whereas the NOEC for 24 and 48 hours was 0.131 $mg\ L^{-1}$, for 72 hours of 0.120 $mg\ L^{-1}$ and for 96 hours of 0.088 $mg\ L^{-1}$ [32]. These results should be interpreted with caution because the toxicity of a compound expressed as CENO, LC_{50} or EC_{50} is based on laboratory tests in which organisms are in rest and subjected to fixed concentrations[14]. When fishes are subjected to exhaustive exercises, stress and after feeding, the ammonia excretion increased and are more sensitive to external ammonia concentrations. In the present study, ammonia concentrations were mainly affected by pH and temperature. Other factors could also affect the $NH3/NH4^{+}$ equilibrium but to a lesser extent, considering that the ionic force is commonly related to salinity and pressure [12, 13]. An increase in pressure up to 100 atm (equivalent to approximately 1000 m of depth) raises the pk_a to 0.014, which has a minimal effect on the %NH3 [13, 31]. These authors also compared the effect of temperature and ionic strength and found that by varying the temperature between 10°C and 30°C in fresh and saline water, the ionic strength had a 50 times lower effect than the temperature. The pH, besides having a significant effect on the fractionation of the ammoniacal nitrogen, also affects the excretory activity of fish. Ammonia (NH3), which is the main nitrogenous waste excreted by fish, is more permeable in biological membranes due to its lipophilic characteristics than the ammonium ion ($NH4^{+}$). The NH3 that is excreted in a neutral to acidic medium reacts rapidly with the hydronium ion ($H3O^+$) to form water and $NH4^+$, which hindered the return of nitrogen to the body, acting as a mechanism of detoxification. Thus, fish would have a greater difficulty in excreting NH3 under alkaline conditions [8], as in the case of farms 2, 3 and 4 located in the Nova Avanhandava reservoir. These results evidenced that the monitoring and control of pH in the reservoirs are of key relevance for fish farming. Cluster analysis showed that differences in the physical and chemical conditions between sampling dates (i.e., temporal differences) were more relevant than spatial differences (e.g. between upstream and downstream locations or between farms). These results might be attributed to water deficit, since the rainfall registered during Oct and Nov 2014 (first sampling) was 1.3 mm and 3.8 mm respectively in the Ilha Solteira reservoir region. During the same period, also low rainfall was registered in the regions of Chavantes and Nova Avanhandava. Normal rainfall conditions for Oct in the Tietê region based on data from 1961 to 1990 [33] showed values of 117.6 mm. The low rainfall may have caused a decrease in the carrying capacity of the studied reservoirs due to an increase in the residence time. A low carrying capacity favour the exportation of organic matter and nutrients [17]. Therefore, the drought that drastically affected the State of São Paulo promoted very atypical conditions that might have contributed to the temporal differences observed in the physical and chemical conditions. Consequently, it is difficult to make conclusive inferences for the study period about the possible impacts of aquaculture on a temporal scale, although drought and anthropogenic activities that occurred in the watershed seemed to have affected the water quality more significantly than the fish management practices in the reservoirs. In addition, the proximity between farms 2, 3 and 4, which are located in the Nova Avanhandava reservoir, created conditions that impaired farming, with low oxygen levels and high values of pH and ammonia. Net cages are structures that allow the continuous water flowing and are intended to confine farmed fish in aquatic environments, providing protection against predators and competitors and high-quality water and food [34]. It is important to consider that due to uneaten feed and faecal material produced by the fish, the load of the particulate organic matter is increased [35], with the consequent increase in nutrients and reduction of dissolved oxygen concentrations in the reservoirs [36-38]. Therefore, an adequate spacing between the cages is highly recommended for allowing environmental autodepuration, via biogeochemical (assimilation and adsorption) and physical processes (dilution).

5. Conclusions

The natural (strong drought) and anthropogenic effects occurred in the water basin affected water quality significantly more than the activities of tilapia production in the studied reservoirs. Quantified ammonia concentrations were considered potentially harmful to the fish production, however, such results were strongly influenced by upstream water, where ammonia concentrations were already inadequate for Nile tilapia cultivation. The distribution model of the NAT fractions shows that pH has a more significant effect on the increase of the ammonia fraction, among the minimum and maximum values of pH and temperature, so it is important for the culture to know and control the mechanisms that increase the pH of the water of the reservoirs. Efficient mechanism of water resources management should be implemented to maintain the ecological balance of reservoirs and guarantee their multiple uses.

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