



Physical and chemical water parameters and *Streptococcus* spp. occurrence in intensive tilapia farming in the State of Espírito Santo, Brazil

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ABSTRACT. This study evaluated the influence of intensive farming of tilapia on physical and chemical parameters and on the occurrence of *Streptococcus* spp. in the water of the lake and of cages. Throughout a year, monthly samplings were taken in the rainy and dry seasons for a year, at two sampling sites, lake and net cages. For the determination of water quality, physical and chemical water parameters were evaluated and compared to the standards established by Conama Resolution no. 357/2005. The presence of *Streptococcus* spp. in the water was determined by plating on blood Agar and biochemical screening. Mean values of water parameters were tested using the Kruskal-Wallis test comparing sampling sites and seasons. Ammoniacal nitrogen (ammoniacal-N), total phosphorus (total-P) levels and occurrence of *Streptococcus* spp. have increased in the water of the net cages. The mean values of several parameters have decreased during the rainy period, except for pH, temperature and ammoniacal-N. Total-P and dissolved oxygen levels, during dry and rainy periods, respectively, exceeded the standard established for freshwater class 2, recommended for aquaculture, which can be harmful to the fish. Therefore, constant monitoring of the physical, chemical and microbiological water parameters is recommended since the Juara lake is also used for recreational purposes.

Keywords: lake, aquaculture standards, net cage.

Parâmetros físico-químicos da água e ocorrência de *Streptococcus* spp. em criação intensiva de tilápia no Estado do Espírito Santo, Brasil

RESUMO. Este estudo avaliou a influência de uma criação intensiva de tilápias na alteração dos parâmetros físico-químicos e ocorrência de *Streptococcus* spp. na água. Durante um ano em dois períodos, seco e chuvoso, as amostras de água foram colhidas mensalmente em dois locais, lagoa e tanques-rede. Parâmetros físico-químicos para determinar a qualidade da água foram avaliados por indicadores estabelecidos na Resolução Conama n. 357/2005. A presença de *Streptococcus* spp. na água foi verificada por plaqueamento em ágar sangue e triagem bioquímica. Os dados foram analisados pelo teste de Kruskal-Wallis comparando-se as médias dos locais e dos períodos de amostragem. Verificou-se aumento da concentração de nitrogênio amoniacal (N-amoniacal), fósforo total (P-total) e *Streptococcus* spp. na água dos tanques-rede. No período chuvoso houve redução de vários parâmetros, exceto pH, temperatura e N-amoniacal. A concentração de P-total no período seco e do oxigênio dissolvido no período chuvoso excedeu o padrão estabelecido para água doce classe 2, recomendado para aquicultura, fato que pode prejudicar a criação de peixes. Diante do exposto, recomenda-se o monitoramento dos parâmetros físico-químicos e microbiológico da água, visto que a lagoa Juara é utilizada também para fins recreativos.

Palavras-chave: lagoa, padrões para aquicultura, tanques-rede.

Introduction

World tilapia production was 2,382,998 tons in 2008, and Brazil was ranked 6th with production of 96,000 tons (FAO, 2010). This high productivity is mainly due to the expansion of intensive farming characterized by monoculture, artificial feeding and high stocking density (SHOEMAKER et al., 2000).

According to studies investigating nutrient levels and changes of the aquatic environment due to

intensive fish farming, nitrogen and phosphorous are considered as the main pollutants (BUENO et al., 2008; GUO et al., 2009). The high levels of nitrogen and phosphorous in the water trigger the eutrophication process, thus making the water quality inadequate for fish farming (GUO et al., 2009).

The total ammoniacal nitrogen found in the environment in non ionized form is toxic to fish because it is easily spread through the gills causing

behavioral, physiological and histological changes (EVANS et al., 2006). Low levels of dissolved oxygen in the water, as well as temperature are factors that predispose fish to diseases caused by pathogens present in the water. Stress in fish leads to a low response of its immunological system (MATA et al., 2004).

Among fish diseases, streptococcal infections have increased worldwide during the last decade as a consequence of the intensification of aquaculture, being responsible for significant economic losses. Fish streptococci is a generic term used to describe similar diseases, where different genera of gram-positive cocci are involved, including streptococcus, lactococcus and vagococcus (MATA et al., 2004).

The influence of some physical and chemical water parameters on the stress, disease resistance and mortality rate of fish economically important for aquaculture had been previously reported. Non-ionized ammonia levels between 0.32-0.37 mg L⁻¹ for 24 hours did not increase the susceptibility of Nile tilapia to the species *S. agalactiae* (EVANS et al., 2006). Sublethal levels of dissolved oxygen, lower than 2 mg L⁻¹ compared to a normal level of 6 mg L⁻¹, increased the susceptibility of African catfish and Nile tilapia to the infection caused by *Edwardsiella*

ictaluri (WELKER et al., 2007) and *S. agalactiae* (EVANS et al., 2006).

The pathogenicity of these bacteria genera to fish and the close association with the physical and chemical parameters of water quality led to the investigation of the possible impact of fish farming in cages in the Juara lake on the environment, and its influence on the presence of *Streptococcus* spp. in the system.

Material and methods

Study site

The study was conducted in a lake of approximately 2.8 km² area and 6 km length, located (20°06'17"S, 40°14'15"W) 5 km far from the Atlantic Ocean, in Espírito Santo State, southeastern Brazil (Figure 1). This intensive farming of Nile tilapia (*Oreochromis niloticus*) has 154 net-cages with volume of 4 m³ each, distributed into five rows and average depth of 3 m, without predominant flow (Figure 1A). The study was conducted in 6 net-cages, which were populated with 125 juveniles per m³ with initial average weight 29.22 ± 6.9 g.

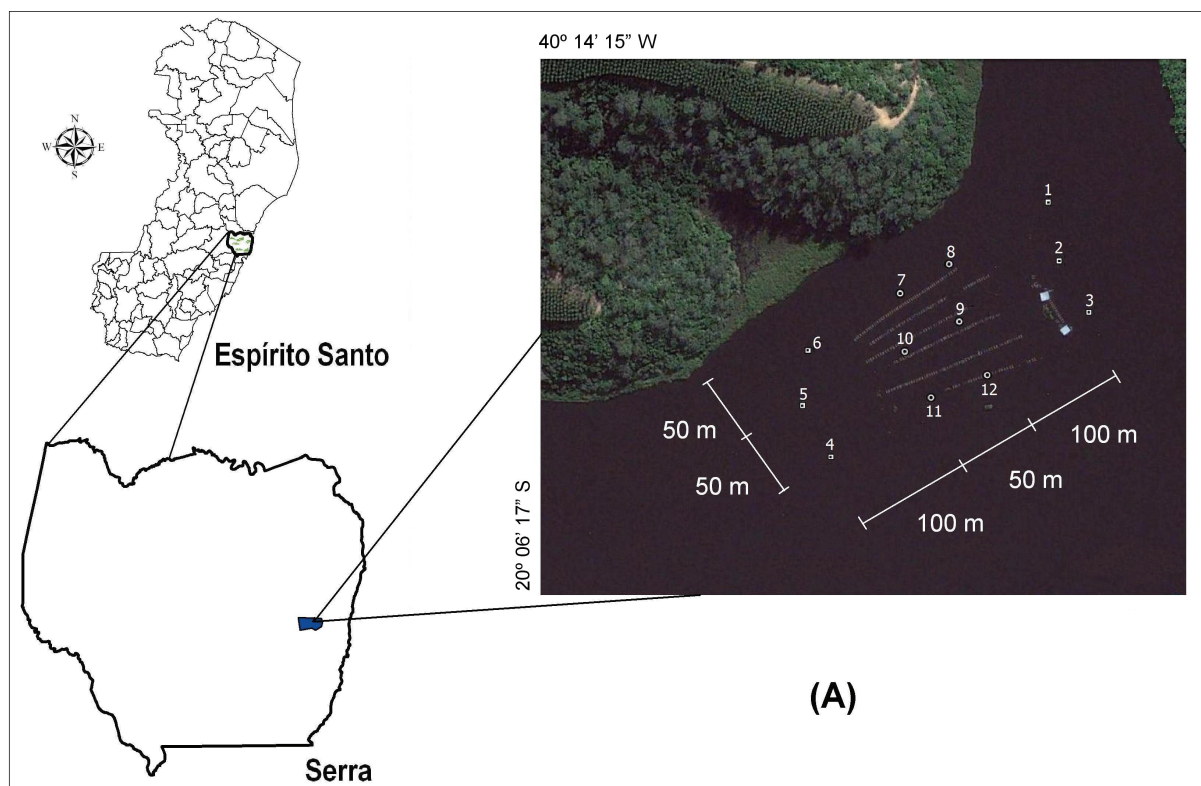


Figure 1. Lake Juara, State of Espírito Santo, Brazil, where the study was conducted. (A) Composition of satellite images obtained from Google Earth and layout of the sampling sites: 1, 2, 3, 4, 5, 6 = Lake and 7, 8, 9, 10, 11, 12 = Cages.

The feed contained minimum level of crude protein, between 28 and 22%, and minimum level of total phosphorus, between 0.8 and 0.6%. The feed provides from 2 to 4% of the biomass according to the water temperature and consumption, and was supplied once or twice a day divided accordingly.

Proceedings

Sampling and analysis were both carried out monthly throughout the year, divided into two periods, dry (April to September, 2008) and rainy (October, 2008 to March, 2009) seasons. During each visit, one water sample was collected from each one of the 6 cages, and a sample of lake water at 6 pre-determined points, 50 m away from each other at two sampling sites 100 m apart from one another, in the surroundings of the cages (Figure 1A). Therefore, for each site, a total of 36 samples were collected in each period. Water samples were collected at 20 cm below the surface. A 500 mL sample was collected in sterile glass bottles for microbiological analysis, and another 500 mL sample was collected in polyethylene bottles for physical and chemical analyses. Samples were properly labeled and placed in coolers filled with ice to keep the temperature below 10°C until using.

In order to determine the presence of *Streptococcus* spp. in the water, the sample was homogenized manually by inverting the flask 25 times. An aliquot was transferred to a Petri dish previously prepared with Tryptic Soy Agar (TSA) enriched with 5% defibrinated horse blood to obtain plates with isolated colonies. The plates were incubated in an atmosphere modified with 5% of CO₂ at 37°C (HOLT et al., 1994) for 48 to 96 hours.

From each plate, gray punctiform, white grayish and bright colonies with halo of beta-hemolysis or nonhemolytic ones were sub-cultured in TSA plus 5% defibrinated horse blood and incubated at 37°C for 48 hours to obtain pure culture. The isolates were Gram stained. Colonies that showed isolated Gram-positive cocci, in pairs or chains, were transferred to inclined tubes with TSA and incubated at 37°C during 24 hours. After that, the catalase proof was performed, if negative, the sample was then tested for growth in sodium chloride at 6.5%, when no growth was detected they were considered *Streptococcus* spp.

All tests were validated by testing a reference sample of *S. agalactiae* (ATCC 13813) supplied by Fiocruz, Rio de Janeiro State.

Simultaneously to samplings, between 9 and 10 in the morning, some water parameters were determined *in situ* such as, temperature, dissolved oxygen levels using an oxymeter AT-150 and water transparency using a 30 cm-diameter Secchi disk. The pH was determined in the laboratory. Alkalinity, nitrate and nitrite, ammoniacal nitrogen and total phosphorus were also determined according to the method proposed by APHA (1995).

The data obtained for each parameter were evaluated according to indicators determined by Brazilian legislation, Conama Resolution no. 357 (BRASIL, 2005), for freshwater systems of class 2, aquaculture and fishing activities.

Rainfall data were provided by the National Institute of Meteorology. The mean air temperature varied from 22.8 to 25.5°C. The average rainfall during the study periods were 37.7 and 240.8 mm, for the dry (April to September, 2008) and rainy (October, 2008 to March, 2009) seasons, respectively.

Statistical analysis

The Kruskal-Wallis analysis was performed to check for possible significant differences of the physical, chemical and the microbiological parameters, which were analyzed according to sampling sites and study period. Statistical probability equal or inferior to 5% was considered significant. The software SAEG (Federal University of Viçosa, Brazil) was used to analyze the results.

Results and discussion

Physical and chemical parameters

The nomenclature adopted in this study was as follows: dissolved oxygen (DO), non-ionized form of ammonia (N-NH₃); ammonium ion, the ionized form (N-NH₄⁺), ammoniacal nitrogen (ammoniacal-N) refers to both nitrite (N-NO₂⁻) and nitrate (N-NO₃⁻), and total phosphorus (total-P).

Table 1 shows significant differences ($p \leq 0.05$) between the periods, for the physical and chemical parameters evaluated, with the exception of pH. Between sampling sites, significant differences ($p \leq 0.05$) were also observed for DO levels in the rainy season, ammoniacal-N in the dry season, and total-P in both periods. Higher levels were observed in the net cage water, except for DO.

Water temperature ranged from 24.8 to 27.9°C, in the dry and rainy seasons, respectively. These seasonal changes correspond to the warmest and coldest months of each period.

Table 1. Mean values of physical and chemical parameters and occurrence of *Streptococcus* spp. in the water of the lake and of cage, in the rainy and dry seasons.

Parameters	Period**	Sites*	
		Lake	Cages
Temperature (°C)	dry	24.8 ^A	25.1 ^A
	rainy	27.6 ^B	27.9 ^B
DO (mg L ⁻¹)	dry	7.0 ^A	6.5 ^A
	rainy	4.6 ^{Ba}	3.8 ^{Bb}
pH	dry	7.1	7.1
	rainy	7.0	7.1
Alkalinity (mg L ⁻¹)	dry	27.8 ^A	28.4 ^A
	rainy	24.9 ^B	24.3 ^B
Transparency (cm)	dry	61.5 ^A	53.9 ^A
	rainy	51.6 ^B	48.7 ^B
N-NO ₂ ⁻ (mg L ⁻¹)	dry	0.007 ^A	0.0083 ^A
	rainy	0.0029 ^B	0.0033 ^B
N-NO ₃ ⁻ (mg L ⁻¹)	dry	0.1248 ^A	0.1577 ^A
	rainy	0.0537 ^B	0.0529 ^B
Ammoniacal-N (mg L ⁻¹)	dry	0.2022 ^{Aa}	0.2316 ^{Ab}
	rainy	0.3024 ^B	0.3231 ^B
Total-P (mg L ⁻¹)	dry	0.0678 ^{Aa}	0.0881 ^{Ab}
	rainy	0.0223 ^{Ba}	0.0287 ^{Bb}
Isolation (%) <i>Streptococcus</i> spp.	dry	42 ^A	47 ^A
	rainy	15 ^{Ba}	28 ^{Bb}

*Means followed by different lowercase letters in the rows are statistically different ($p \leq 0.05$); **Means followed by different uppercase letters in the columns, for the same parameter, are statistically different ($p \leq 0.05$).

DO levels varied between 7.0 and 4.6 mg L⁻¹ in the lake, and between 6.5 and 3.8 mg L⁻¹ in the net cages, in the dry and rainy seasons, respectively. These values are within the limits required by law for freshwater bodies class II (not lower than 5 mg L⁻¹ O₂) (BRASIL, 2005), except in the rainy season when the levels ranged from 4.6 to 3.8 mg L⁻¹, therefore lower than the limit of 6.0 mg L⁻¹ (WELKER et al., 2007), considered ideal for intensive farming of Nile tilapia. The lowest DO concentration ($p \leq 0.05$) was observed in the net cages during the rainy season. In this period, rainfall was 240.8 mm and the vegetation was partially submersed. According to Correll (1998) this leads to an increase of organic matter in the system and its decomposition by aerobic organisms, causing a decrease of DO in the water, and consequent fish loss. The DO values refer to average readings performed between 9 and 10 in the morning. Moreover Mercante et al. (2005) stated that these values are probably lower during the night, due to phytoplankton respiration.

Alkalinity was significantly higher ($p \leq 0.05$) in the dry season (Table 1). According to Mercante et al. (2005) the rainfall dilutes calcium concentrations; therefore, the low precipitation (37.7 mm) in the dry season might have increased the concentrations of ions carbonate and bicarbonate in the water. However, these concentrations were within the range considered of good buffering capacity between 20 to 300 mg L⁻¹ CaCO₃ (ALBANEZ; MATOS, 2007). Furthermore, Boyd and Tucker (1998) reported that higher levels of ions carbonate and bicarbonate in the water increase the

alkalinity, making more difficult to change the water pH. Water pH remained between 7.0 and 7.1, within the range established for freshwater bodies class II (6.0 to 9.0) (BRASIL, 2005) and aquaculture. On the other hand, Pereira and Mercante (2005) observed that pH higher than 8 is ideal for the occurrence of fish infection because it potentializes ammonia toxicity.

Transparency values measured by Secchi depth ranged from 61.5 to 48.7 cm in the dry and rainy season, respectively (Table 1). According to Schmittou (1993), water transparency should not be below 40 cm in the net cages used for fish farming. The lowest value in the rainy season can be explained by the increased amount of organic matter resulting from the decomposition of the submerged vegetation during this period.

The nitrate, final product of the nitrogen cycle, is considered harmless to fish in lagoons and natural systems. However, in closed systems, with very little water renewal or without it, the accumulation can become harmful if higher than 250 mg L⁻¹ (FRANCIS-FLOYD et al., 2005). In this study, N-NO₃⁻ concentration was significantly higher ($p \leq 0.05$) in the dry season, 0.1577 mg L⁻¹ compared to 0.0529 mg L⁻¹ in the rainy season (Table 1), but remained below the level considered toxic for fish and the limit established for freshwater bodies class II, of 10.0 mg L⁻¹ (BRASIL, 2005).

High concentrations of nitrate may be due to illegal release of domestic sewage into the water (ALVES et al., 2008). Other important source of nitrate are the fertilizers, which if improperly used can reach the watercourses, especially during rainy months (ESTEVEZ, 1998). The presence of riparian vegetation in this lake has probably minimized the leaching of this nutrient into the water and therefore, the lower concentration in the rainy period can be due to its dilution by high rainfall, 240.8 mm.

Nitrite is toxic to fish even at levels as low as 0.1 mg L⁻¹ (FRANCIS-FLOYD et al., 2005). Average concentration of N-NO₂⁻ varied from 0.0083 to 0.0029 mg L⁻¹, during dry and rainy periods, respectively, and remained lower than the limit of 1.0 mg L⁻¹, established by Conama Resolution no. 357/2005 for freshwater bodies class II (BRASIL, 2005).

Higher concentrations of ammoniacal-N and total-P in the net cages may be due to nitrogen and phosphorus present in the diet not consumed that remained in the water, as well as fish excrement, algal growth and other organisms that may cause clogging of the meshes of the net cages, increasing the concentrations of these nutrients (GUO et al., 2009).

Ammoniacal-N concentration ranged from 0.2022 mg L⁻¹ in the lake to 0.2316 mg L⁻¹ in the net cages, during the dry season, and from 0.3024 to 0.3231 mg L⁻¹, respectively, in the rainy season. Concentrations that remained below the limit of 3.7 mg L⁻¹ at pH lower than 7.5, established by Conama Resolution no. 357/2005 for freshwater bodies class II (BRASIL, 2005). Abdelaziz and Mamal (2010), studying tilapias, observed that when ammonia water concentration was higher than 2 mg L⁻¹ there was massive mortality, while concentration higher than 1 mg L⁻¹ causes losses, especially juveniles, when there is prolonged exposure (several weeks).

Total-P concentrations were 0.068 mg L⁻¹ in the lake, and 0.088 mg L⁻¹ in the net cages, above the limit of 0.03 mg L⁻¹ established for freshwater bodies class II (BRASIL, 2005). In the rainy season, the mean values were lower and closer to the minimum allowed by the legislation. These lower concentrations may be related to the dilution caused by rainfall. The increased total-P concentration during the dry season may be due to low average rainfall, 37.7 mm, compared to 240.0 mm in the rainy season. This fact contributed to the excessive growth of macrophytes, mainly “taboa” (*Typha domingensis*), on the banks of the lake. As the water level rose during the rainy season, the decaying of submersed organic matter together with higher mean temperatures, 27.9°C, may have caused the depletion of DO in the water, diminished the nitrifying process and, consequently increased ammoniacal-N level in the system (CORRELL, 1998).

***Streptococcus* spp. occurrence**

The results obtained for *Streptococcus* spp. isolates are shown in Table 1. The highest occurrence of *Streptococcus* spp. was observed during dry season, with no significant difference between sampling sites ($p \geq 0.05$), 47% in the net cages and 42% in the lake. However, during rainy season, the occurrence of this bacteria was significantly higher ($p \leq 0.05$) in the net cages (28%) compared to (15%) in the lake.

The great number of samples positive for *Streptococcus* spp. in the dry period, coincided with higher levels of total-P and lower temperatures (Table 1), and was not significantly different ($p \geq 0.05$) between sampling sites. In the rainy season, the temperature increased, but without significant difference ($p \geq 0.05$) between the lake and net cages, and the occurrence of *Streptococcus* spp. and levels of total-P have decreased. Nevertheless, the values were significantly higher ($p \leq 0.05$) in the net cages. Similar results for total-P were reported by Guo et al. (2009). Thus, in the cages, the damming as a

function of the high number of fish and of clogging of the meshes due to deposition of organic waste, such as feed leftovers, excreta, growth of algae and other microorganisms may have favored the increase of *Streptococcus* spp. Perera et al. (1997) suggested the existence of a permanent source of streptococci in water. Therefore, under favorable conditions such as large amount of organic matter, these bacteria multiply in the water. The biological decomposition of organic matter consumes oxygen from the water (CHENG et al., 2004). Anoxic waters are the main cause of losses of aquatic animal, while favoring the increase of anaerobic organisms (CORRELL, 1998).

Salvador et al. (2003) have related higher temperatures with higher incidence of streptococcal infections in fish. These authors also reported higher number of Nile tilapia with clinical signs and isolates of *Streptococcus* spp. during the transition period between winter and spring, which decreased in the subsequent months despite the water temperature in the network where the net cages were installed remained high, maximum of 26.5°C in January. In the present study, the highest occurrence of this bacteria was verified during the dry season in the water of both lake and net cages, when the temperatures were lower (Table 1), this fact may have been favored by the highest concentration of total P in the water. Phosphorus is a mineral element essential to all life forms. Extracellular enzymes hydrolyze organic forms of phosphates; the orthophosphate is the only form of phosphorus that autotrophic organisms assimilate and the result of an excessive production is the eutrophication process. This high productivity also leads to an increase of bacterial populations (CORRELL, 1998).

During the dry season, the DO values were higher and coincided with a greater isolation of *Streptococcus* spp. in the waters, while during the rainy season the remarkable depletion of DO level coincided with a lower isolation rate ($p \leq 0.05$) (Table 1). This genus is classified as facultative anaerobe bacteria (HOLT et al., 1994); therefore, its multiplication is independent of the amount of oxygen in the medium. Although, in fish, the higher frequency of isolation of this genus is reported during periods of low dissolved oxygen (BOWSER et al., 1998; SALVADOR et al., 2003). According to Evans et al. (2006) the increased susceptibility to infection caused by streptococci is related to stress and immunosuppression situations when the fish are exposed to sublethal rates of dissolved oxygen. The depletion of DO in the water, during the rainy season, may have limited the number of nitrifying bacteria in relation to heterotrophic bacteria on the

water surface (HARGREAVES, 1998), but the dilution of the water during the rainy period, when rainfall was 240.8 mm, may have contributed to reduce the population of the latter, especially *Streptococcus* spp.

Conclusion

All parameters evaluated, except pH, changed with respect to the season. The concentration of DO, ammonia-N nitrogen, total-P and the occurrence of *Streptococcus* spp. in the water were affected by the management adopted. The parameters total P and dissolved oxygen levels measured in the water, during dry and rainy season, respectively, have not achieved the required standard. In periods of intense rainfall, fish mortality may occur. Also, it is suggested further investigation to determine whether the intensive farming continues to increase the nutrient levels and *Streptococcus* spp. in the system, once the Juara lake is also used for recreational purposes.

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