

Water quality of small fish ponds associated with swine culture

Luciana Aparecida Paula Martins^{1*}, Rodrigo Ney Millan², Eduardo da Silva Martins² & Aníbal da Fonseca Santiago¹

¹ Universidade Federal de Ouro Preto, campus Morro do Cruzeiro S/N, 35400-000, Ouro Preto MG Brazil
 ² Universidade do Estado de Minas Gerais, unidade Frutal, Av. Prof. Mario Palmerio, 1001, Bairro Universitário, 38200-000, Frutal MG Brazil.

Abstract. The water quality of fish ponds with tilapia (*Oreochromis niloticus*) culture, associated with swine culture activities in a farm in Frutal, Brazil, was evaluated. Water samples were retrieved monthly, during 9 months, in three fish ponds with inflows and outflows (6 sites) to quantify limnological and microbiological variables, such as dissolved oxygen, pH, electrical conductivity, total dissolved solids, temperature, ammonia, nitrate, nitrite, total phosphorus, orthophosphates and thermotolerant coliforms. Results from different sample sites were statistically analyzed, according to data distribution for each variable. Three variables (dissolved oxygen, total phosphorus and thermotolerant coliforms) failed to comply with standards recommended by the Brazilian legislation. Since adjacent swine farms near the two ponds impacted water quality with regard to the above parameters, a treatment system in Pond 2 or a reduction of swine activity in the ponds is recommended to maintain adequate water quality for fish farming.

Keywords: tilapia culture; limnology; thermotolerant coliforms

Resumo. Qualidade da água de viveiros de piscicultura de pequeno porte associados à suinocultura. O presente trabalho avaliou a qualidade da água de viveiros de piscicultura com cultivo de tilápias (*Oreochromis niloticus*), associada à suinocultura, em uma propriedade rural no município de Frutal, Brasil. Foram feitas coletas mensais durante 9 meses, na entrada e saída de 3 viveiros (6 pontos de coleta), para quantificação de variáveis limnológicas e microbiológicas, como: oxigênio dissolvido, pH, condutividade elétrica, sólidos totais dissolvidos, temperatura, amônia, nitrato, nitrito, fósforo total, ortofosfato e coliformes termotolerantes. Os resultados dos diferentes pontos de coleta foram submetidos a análises estatísticas, conforme o modo de distribuição dos dados de cada variável analisada. Três variáveis (oxigênio dissolvido, fósforo total e coliformes termtolerantes) estavam em desacordo com os padrões vigentes da legislação. Uma vez que a suinocultura adjacente a dois dos viveiros impactou a qualidade da água em relação a estes parâmetros, um sistema de tratamento na saída do viveiro 2 e/ou a diminuição da quantidade de suínos junto aos viveiros é recomendado, visando manter uma água de qualidade adequada para o cultivo dos peixes.

Palavras-chave: tilapicultura; limnologia; coliformes termotolerantes

Introduction

The development of integrated fish farming in Brazil has been triggered by two factors, namely, the great availability of organic matter on small farms and the fragile economic conditions of the farmer who often depends exclusively on only this specific type of activity (Palhares & Coldebella, 2012).

Feeding costs may range between 40% and 70% of total fish production costs, depending on certain variables, such as type of cultivation system, production scale, productivity and price of inputs.

^{*}Corresponding author: <u>duelu2004@gmail.com</u>

Consequently, fish farming, associated with that of other animals, or rather, using organic matter to feed the fish, may reduce feeding costs (Andrade *et al.*, 2015).

In the wake of the development of aquaculture in Brazil, limnological studies have been undertaken to monitor and control water quality. In fact, the area of study is of great importance to forward effective measures for a more sustainable production. The effect of water quality on fish health and physiological conditions varies according to species, size and age. Temperature, pH, dissolved oxygen, suspended solids, nitrogen, phosphorus and ammonia are among the most important parameters for water monitoring (Urbinati & Carneiro, 2004).

Wastes derived from animal production (such as pigs, poultry and cattle) may be used as fertilizers in fish ponds. Animal manure is directly discharged into the fish pond, where the nutrients sustain the growth of plankton and other microorganisms consumed by fish. Consequently, additional feeding is reduced (Dang & Alsgaard, 2012). However, this practice has high environmental risks if performed incorrectly, or rather, if it is done without taking into account the capacity of soils and nurseries (Xu & Boyd, 2016). In addition, nutrients from swine breeding, for example, may enrich ponds with nutrients which, when discharged into the receiving water body, cause serious problems, such as eutrophication, and introduce enteric pathogens (Lopera Barrero et al., 2006; Mlejnková & Sovová, 2012). Effluents from fish farming generally have low concentrations of pollutants when compared to those produced by domestic ones (20 to 25 times more diluted). However, the flow is greater and continuous, providing a source of waste in the environment (Naylor et al., 2003).

Since water quality affects fish productivity, it should be monitored to ensure the quality of the effluent generated in the ponds. Phosphorus and nitrogen, for example, are aquaculture residues that may cause considerable problems in water bodies (Green *et al.*, 2002). Phosphorus is the most limiting nutrient for the primary production of algae. Nitrogen is one of the most important elements in the metabolism of aquatic ecosystems. It affects the formation of proteins since it is a limiting factor for the primary production of ecosystems. Further, under certain conditions (for example, in a nonionized form, NH₃), it becomes toxic to aquatic organisms (Pereira & Mercante, 2005). The excessive disposal of nitrogen and phosphorus in

water causes eutrophication and algal blooms (Cyrino *et al.*, 2010).

The presence of thermotolerant coliforms in the residues is another issue when pig wastes are disposed of to increase primary productivity in fish farming. This fact indicates contamination of water by pathogenic bacteria of enteric origin, such as *Campylobacter*, *Helicobacter* and *Legionella*. Contamination of water by bacteria of intestinal origin may also indicate the presence of other groups of pathogenic microorganisms, such as viruses, protozoa and helminths (Al Harbi, 2003).

Current study evaluates the water quality of fish farms associated with swine farming in a rural property in the municipality of Frutal MG Brazil, by analyzing physical, chemical and microbiological variables. The technical feasibility of this association is also assessed to verify whether pig excrement impairs the water quality of ponds and produces harmful effluent.

Materials and Methods

Period and place of collection: Current research was undertaken on a farm about 20 km from the town of Frutal, Brazil, at UTM 693759 E and 7790173 S. There are three fish ponds (V1, V2 and V3), two of them (V1 and V2) adjacent to the swine breeding section. Inflows and outflows of each fish pond were sampled, totaling six collection sites (P1 - P6) (Figure 1).

The swine-breeding sector comprised 30 pigs stocked in a 95 m² area, at a density of 0.31 swine m⁻ ². Pond 1 features an area of 345.5 m² and it is supplied by water from the stream Veadinho that passes prior to the swine sector. Pond 2 has the largest area (510.2 m²), with supply similar to that of Pond 1, close to the swine-breeding sector and with the same proportion of animals. Pond 3 has an area of 160.6 m² and is supplied by water from Pond 1. Swine density related to the fish ponds' total area was about 0.03 swine m⁻². Swine excrement enters the ponds by driving, without any pre-treatment. Fish ponds have an average depth of 1.5 m. The Nile tilapia (*Oreochromis niloticus*), the fish species cultivated, has an average storage density of two fish m⁻² per fish pond. Feeding takes place once a day, in the morning, with an approximate amount of 1,200 g for each fish pond. All fish ponds are associated with swine culture. Pond 2 receives waste from two swine-breeding sectors. Water samples collected monthly, from June 2015 to February 2016, from the subsurface of each site, totaling 54

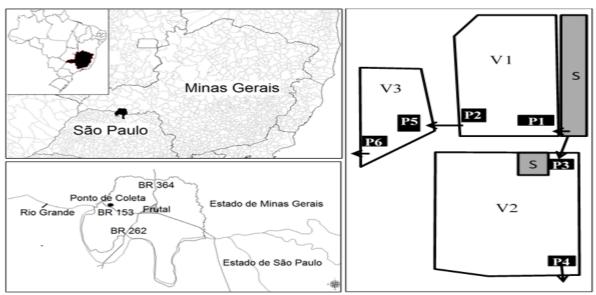


Figure 1. Map showing integrated fish farm with swine culture, where: V1-V3 = fishponds; S = swine culture; P1-P6 = sample sites.

samples. All samplings from the first to the last site were retrieved between 08:30 and 10:00 am.

Determination of limnological variables: Temperature (Temp), pH, electrical conductivity (Cond), dissolved oxygen (DO) and total soluble solids (TSS) were determined with a HANNA HI 9828 multiparameter probe. Further, 500 mLpolyethylene bottles were used to collect water at the subsurface of each site to quantify nutrients. Samples were transported to the laboratory in iceboxes and frozen for further analysis of nutrients. Total phosphorus (TP), orthophosphate (OP), nitrate (NO₃), nitrite (NO₂) and total ammonia nitrogen (TAN) were determined according to Golterman & Koroleff (1975)and Hansen (1999).Thermotolerant coliforms (TC) were quantified by multiple-tube technique in A1 medium (APHA, 2005). Samples were collected at the subsurface of each site with sterile 1000 mL vials and transported to the laboratory in iceboxes. Crude water was diluted to 10⁻⁴ in medium A1. Samples were incubated at 35°C for three hours and then at 44.5°C in a water bath, for another 21 hours. Number of coliforms was given as MPN 100 mL⁻¹.

Statistical analysis: Mean and standard deviation of physical, chemical and microbiological variables of water were calculated and plotted in graphs, and analysis of variance (ANOVA) was performed. When significant differences were verified, they were compared by Tukey's test at 5% significance. Principal components analysis (PCA) determined temporal and spatial changes in the physical and chemical conditions of the water,

including Temperature, pH, Dissolved Oxygen (DO), Conductivity (Cond), Total Soluble Solids (TSS), Total phosphorus (TP), orthophosphate (OP), nitrate (NO $_3$), nitrite (NO $_2$) and total ammonia nitrogen (TAN). All statistical analyses were performed with Statistica 10.0 (Statsoft, 2011).

Results

Limnological variables: Temperature, pH and electrical conductivity of pond water were at the proper rates and within fish-breeding standards recommended bv Brazilian legislation. parameters failed to present any significant difference (p>0.05) between sites. The water's mean temperature ranged between 23.5 and 24.4°C. The pH was acid during the research period, with mean rate varying between 6.6 and 6.8. Electrical conductivity remained between 60.0 and 61.7 µS cm⁻¹ 1, with no significant difference (p>0.05) between sites. Dissolved oxygen (DO) ranged between 2.8 to 3.9 mg L⁻¹ (Figure 2).

Total soluble solids did not present any significant difference (p>0.05) between sites, ranging between 60.7 and 62.0 mg $\rm L^{-1}$ (Figure 2), complying with CONAMA Resolution 357/2005, which establishes a maximum of 500 mg $\rm L^{-1}$ (Brasil, 2005).

Total phosphorus was the only nutrient with significant difference between sampling sites (p <0.05), with rates ranging between 74.0 μ g L⁻¹ (P6) and 112.0 μ g L⁻¹ (P2). Orthophosphate was found in low amounts (below 20.0 μ g L-1) at all sites, with no significant difference (p> 0.05) between sites (Fig. 3)

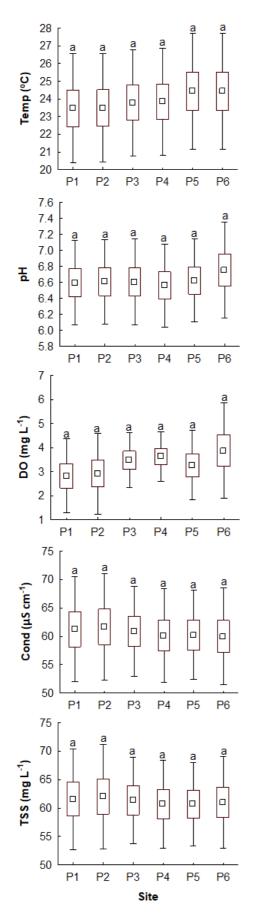


Figure 2 (opposite column). Boxplot graph showing average rates of temperature (Temp), pH, dissolved oxygen (DO), electrical conductivity (Cond) and total soluble solids (TSS) at sites, where: square = mean; rectangle = standard mean error; vertical bars = standard deviation. Equal letters on vertical bars indicate similarity between sites after multiple comparisons of means.

Maximum concentration for total ammonia nitrogen reached 134 μ g L⁻¹, well below the limit established by CONAMA Resolution 357/2005 (Brasil, 2005), namely, 3,700.0 μ g L-1 for water with pH lower than 7.5. Highest mean nitrite concentration was 49.0 μ g L⁻¹ (Figure 3), or rather, about 20 times lower than the rate recommended by legislation, namely, 1,000 μ g L⁻¹ (Brasil, 2005).

Thermotolerant coliforms: All sites revealed concentration of thermotolerant coliforms which were higher than the maximum limit established by the legislation for Class II waters intended for aquaculture (Brasil, 2005), namely, 1,000 MPN 100 mL⁻¹ of water. P3 site presented a mean coliform concentration of 2.6384x10⁴ MPN 100 mL⁻¹, with significant difference (p <0.05), when compared to sites P2, P5 and P6, respectively with averages 0.4425x10⁴, 0.2413x10⁴ and 0.2117x10⁴ MPN 100 mL⁻¹ (Figure 4).

Principal components analysis: Principal Component 1 (PC1) accounted for 55% of the original data variability and Principal Component 2 (PC2) accounted for 22%. On the negative side of PC1, the variables Cond, TSS, NO₂, TP and OP were associated with P1 and P2. Further, P5 and P6 were positioned on the positive side of PC1, where variables total ammonia nitrogen, pH, temperature and dissolved oxygen were associated (Figure 5). Variables thermotolerant coliforms and nitrate were retained on the negative side of PC2, being associated with P3 and P4 (fish pond 2) (Figure 5).

Discussion

Rebouças & Lima (2014) reported that the best temperature range for fish growth lies between 25 and 32°C. In the case of tilapia, the optimum temperature range for growth lies between 25°C and 30°C (Hepher *et al.*, 1983; Sipaúba-Tavares & Braga, 2008). Jian *et al.* (2003) emphasize that water temperature is an important variable because it is associated with oxygen consumption, growth and survival of organisms. The temperature registered by Baccarin *et al.* (2000) in a study on fish ponds in Jaboticabal, Brazil, ranged between 18 and 24°C, or rather, within the limits of tolerance. Maximal temperature is very similar to data in current study.

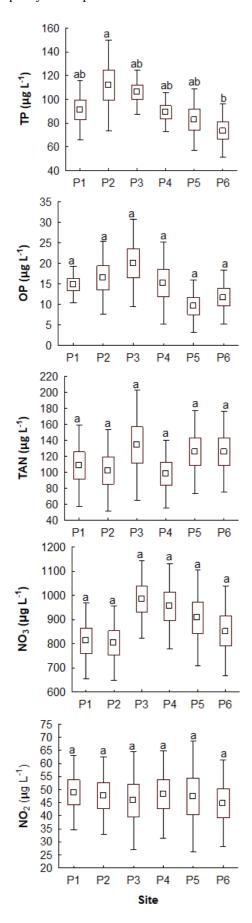


Figure 3 (opposite column). Boxplot graph showing average rates of total phosphorus (TP), orthophosphate (OP), total ammonia nitrogen (TAN), nitrate (NO $_3$) and nitrite (NO $_2$) at sites (P1-P6), where: square = mean; rectangle = standard mean error; vertical bars = standard deviation. Equal letters on vertical bars indicate similarity between sites after multiple comparisons of means.

Low rates of DO registered in current study may be due to the use of swine residues in ponds (organic matter). The decomposition of organic matter depletes DO due to microbial respiration and nitrification (Jamu & Piedrahita, 2002). Fish are expected to develop more slowly, but no deaths occurred during the sampling period. Baccarin et al. (2000) registered results well below 4.0 mg L⁻¹, probably due to the excess feed released in the tanks. Silva & Ferrari (2012) also reported rates below 4.0 mg L⁻¹, in winter and morning collections, in which oxygen availability was lower. According to Masser et al. (1993), concentrations below 4.0 mg L⁻¹ may cause stress in fish, reduce food consumption and increase pre-disposition for disease, causing growth decrease and liabilities to producer. Sipaúba-Tavares (1995) reports that pH between 6.5 and 9.5 is the ideal range for fish ponds. The studied area in question lies within this recommended range.

According to Mainardes-Pinto & Mercante (2003), conductivity should vary between 23.0 and 71.0 μ S cm⁻¹, as reported in current assay. Similar rates were reported by Paggi (2006), with sites sampled below 66.0 mg L⁻¹, at an average. According to the author, high rates were due to the sediment removal from fish and to reduction in the water volume.

The high concentration of total phosphorus at all sites are above CONAMA Resolution 357/2005 standard, namely, 30.0 µg L⁻¹ (Brasil, 2005), caused by constant contribution of organic matter in the system, generated by fish metabolism and by the effect of swine feeding and manure disposal. The lowest rate in P6 may have been caused by the dilution of total phosphorus in the water due to the dilution effect of passage from one site to the other. Since maximum rate of total phosphorus is 30.0 µg L⁻¹ (Brasil, 2005), all collection sites missed permitted standards. Mercante et al. (2007) also presented high phosphorus rates, higher than those allowed by legislation for waters used in fish farming. The above was caused by high levels of organic and inorganic matter in the ponds. The authors also reported that the enrichment of nutrients, mainly nitrogen and phosphorus, is usual and is due to feeding and fertilizers used in the fish

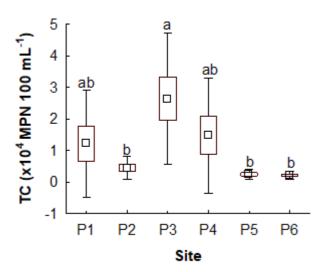


Figure 4. Boxplot graph showing average rates of thermotolerant coliforms (TC) at sites (P1-P6), where: square = mean; rectangle = standard mean error; vertical bars = standard deviation. Equal letters on vertical bars indicate similarity between sites after multiple comparisons of means.

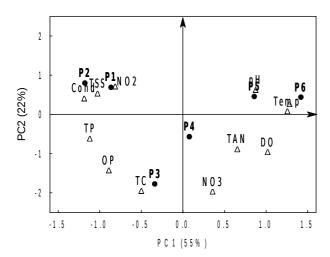


Figure 5. Interpolation of eigenvalues from the matrix of water variables. First two axes from the principal component analysis (PCA). Close circles = sites (P1-P6), open triangles = water variables. Cond = conductivity, DO = dissolved oxygen, NO2 = nitrite, NO3 = nitrate, OP = orthophosphate, TAN= total ammonia nitrogen, TSS = total suspended solids, Temp = temperature, TP = total phosphorus; TC = thermotolerant coliforms.

farming activity. Excessive fertilization causes financial and environmental problems, such as eutrophication and effluent discharge, with excessive nutrient loading in water bodies.

Paggi (2006) reported that orthophosphate rates averaged 68.9 $\mu g \ L^{\text{-1}}$, associating high rates with the degradation of organic matter.

Higher concentration of total ammonia nitrogen (maximum 4,300.0 $\mu g \ L^{-1}$) was registered by Pilarski et al. (2004). The highest average nitrate rate found in current study was 983 µg L⁻¹ (Figure 3), about 10 times below that allowed by CONAMA Resolution 357/2005, namely, 10,000 μg L⁻¹ (Brasil, 2005). Similar results, with nitrate 5000.0 μg L⁻¹, were registered by Pilarski et al. (2004), or rather, low rates due to the predominantly organic system. In addition, total ammonia nitrogen and nitrate are lost through the de-nitrification process. Low DO concentrations and swine manure in the ponds in current study enhanced de-nitrification. According to Pereira & Mercante (2005), de-nitrification occurs mainly at low oxygen conditions. In aquatic ecosystems, the main site of its occurrence is the sediment, with low oxygenation conditions and with large amounts of organic substrate.

Paggi (2006) also reported low nitrite rates. The author concluded that the highest rates were due to the presence of cattle (feces and urine) in the creek adjacent to the tanks. On the other hand, lower rates were due to increase in rainfall, which dilutes the compounds.

Nitrite (NO₂-) and ammonia may be toxic to aquatic organisms. Their sources are the oxidation of ammonia in oxidizing environments and the reduction of nitrate (NO₃⁻) in reducing environments, as the first most common source in aquaculture systems. The nitrogen removal relies on a two-step process of nitrification and denitrification. In the nitrification process, nitrifying bacteria oxidizes ammonia to nitrate using available dissolved oxygen, while denitrification uses denitrifying bacteria to reduce the nitrate to nitrogen gas (Grismer & Collison, 2017). Thus, in the case of nitrogen forms, high nitrate rates and low nitrite and ammonia rates were observed, suggesting that the continuous water flow system may have favored nitrification. In fact, high nitrate levels and low levels of dissolved oxygen were reported, indicating that oxygen increased due to continuous flow of water consumed by the nitrification process.

Sites with the highest amount of coliforms were those in which direct dumping of swine manure occurred, especially P3 with two adjacent swine sectors. Data indicate that this activity contributes towards water contamination by coliforms above the established limits. Sites P2, P5 and P6 had lower coliform rates when compared to those of site P3. This may have occurred because of a greater distance from the swine sector. Mlejnková & Sovová (2011) reported that integrated farming

may be associated with contamination of pond water and sediments with pathogenic microorganisms from the manure.

Since coliforms indicate other potentially pathogenic intestinal microorganisms (bacteria, such as Campylobacter, Helicobacter, protozoa, viruses, etc.), there is a risk that these microorganisms occur at all sites. Although some studies report coliforms in the intestinal tract of fish, these are not considered natural habitants of their intestinal microbiota (Frazier & Westhoff, 1988). However, according to the authors, improper handling of fish may lead to the contamination of their products, such as fillet, and these contaminants may be transferred to humans. Data from the literature differ on the correlation between fish muscle contamination and water quality. Dang & Dalsgaard (2012) reported high levels of Escherichia coli in the fish intestinal contents but very low levels in the fish muscle tissue. Pilarski et al. (2004) reported that there was no increase trend for MPN of thermotolerant coliforms in carp muscle when compared to that registered in nursery water. This fact indicated that fish microbiological status was not the result of the microbiological situation of pond water. According to these authors, data contrasted with those by Antoniolli (1993) who observed that water influenced the carp's microbiological situation. According to Esposto et al. (2007), the invasion of fish musculature by bacteria may occur when they are produced in ponds containing concentrations of thermotolerant coliforms greater than 10⁴ MPN 100 mL⁻¹, and the potential for invasion of the muscle increases with the duration of fish exposure to contaminated water.

Since rates above 10⁴ MPN 100 mL⁻¹ were observed in current study, there may be a risk of invasion of bacteria in the tilapia muscle. Further, involved in the consumption contaminated fish is not necessarily associated with bacteria in the tissue used for human consumption. Human infection may occur when the edible part is contaminated during fish handling, or rather, transporting microorganisms to fish musculature, equipment, other food and factory facilities. In fact, cross contamination occurs when microorganisms in other parts of the fish, usually viscera, skin and gills, are transferred (Esposto et al., 2007). These authors cautioned against manipulation when gut is removed and during the preparation of the product, since it is a potential route of transmission of pathogenic bacteria (Lorenzon et al., 2010).

Thus, thermotolerant coliform rates in current assay, found at P1, P3 and P4, which were higher than 10⁴ MPN 100 mL⁻¹, would favor invasion of fish musculature by bacteria and contamination due to inadequate manipulation.

Principal Component Analysis indicated eutrophication in fish pond 1 (P1 and P2), with high electric conductivity and concentration of variables TSS, NO₂, TP and OP, related to the disposal of pig dung. The highest concentrations of ammonia in Pond 3 were due to higher stocking fish density that consumed the phytoplankton in addition to feed.

It should be noted that the absence of dumping of pig dung in this fish pond causes higher pH and dissolved oxygen levels in the water, since amount of organic matter to undergo decomposition is lower when compared to other fish ponds. Sipaúba-Tavares et al. (2006) verified that ponds with organic matter had lower oxygen contents, functioning as an energy source for The bacterial growth. latter causes aerobic decomposition, an important factor in decreasing oxygen supply in fishponds. Lowest rates of thermotolerant coliforms occurred at P5 and P6, since they did not receive wastes directly from the adjacent pig farms.

Intense nitrification occurred in Pond 2, since low dissolved oxygen, high nitrate concentration and a marked increase of thermotolerant coliforms, due to waste disposal of two swine farms, were registered.

Conclusion

The physical, chemical and microbiological analyses of water in the fish farms showed concentrations of total phosphorus, dissolved oxygen and thermotolerant coliforms in disagreement with rates established by Brazilian legislation. Data impact the productivity and quality of fish and the water body that receives effluent from fish ponds. Results indicate the need to reduce the number of pigs next to the fish ponds and / or the construction of an effluent treatment system.

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