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# Billings reservoir water used for human consumption presents microbiological contaminants and induces both behavior impairments and astrogliosis in zebrafish

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ABSTRACT

The Billings reservoir is the largest water-storage facility in the São Paulo Metropolitan Region, with only a small part of the reservoir used for water supply. Recently, the São Paulo Metropolitan Region has experienced the greatest water collapse ever recorded. Thus, the intensification of use of the Billings reservoir should be considered. The objective of this study was to evaluate the quality of the water from different areas of the Billings reservoir related to human consumption (water supply and fishing): Rio Pequeno, Rio Grande, and Bororé rivers. We performed microbiological and physical studies on one water sample collected at each of these sites. Adult zebrafish were exposed to such water samples and their behaviors were evaluated. Finally, we studied central glial fibrillary acidic protein (GFAP) expression, which is related to neuroinflammatory processes. Water samples from Rio Pequeno, Rio Grande, and Bororé presented microbiological contamination for *Escherichia coli* and heterotrophic bacteria. Water from the Rio Pequeno river induced both motor/exploratory impairments and anxiogenic-like behavior in zebrafish. Water from the Bororé river induced behaviors in zebrafish related to respiratory impairments (hypoxia) as well as higher alarm reaction. Zebrafish exposed to water from the Bororé also presented astrogliosis, which seems to have happened in detrimental of the high heterotrophic bacterial contamination. Rio Grande and Bororé water increased the lethality rates. Considering the present results of microbiological contaminants and behavior impairments, lethality, as well as astrogliosis in zebrafish, the water from Rio Pequeno, Rio Grande, and Bororé rivers should be considered unacceptable for human use in their untreated state. The Basic Sanitation Company of the State of Sao Paulo should consider adopting rigorous processes of microbiological water treatment. Authorization for fishing at Bororé river should be reconsidered.

## 1. Introduction

The Billings reservoir is the largest water-storage facility in the São Paulo Metropolitan Region (Wengrat and Bicudo, 2011). It has an area of 127 $km^2$, a total volume of 1228.7 × $10^6$ $m^3$ with a water surface of 10,814.20 ha, and a maximum depth of 18 m (Petrere et al., 2006; Rezende et al., 2014). The complex has a dendritic pattern and an elongated and narrow central body with eight arms: Rio Grande, Rio Pequeno, Capivari, Pedra Branca, Taquacetuba, Bororé, Cocaia, and Alvarenga rivers (Rodrigues et al., 2010; Wengrat and Bicudo, 2011). The Billings reservoir was conceived as a water storage for hydroelectric power plant (Petrere et al., 2006). However, currently it has multiple uses, including public water supply, energy generation, amateur and commercial fishing, and recreation (Gemelgo et al., 2009; Wengrat and Bicudo, 2011).

Population and industrial growth that occurred in a disorganized and indiscriminate way, polluted the Billings reservoir, including domestic and industrial sewage, such as heavy metals from automotive and metallurgical industries, as well as agricultural residues (Gemelgo et al., 2009; Hortellani et al., 2013). The diversion of the Tietê and Pinheiros rivers to the reservoir to increase the hydroelectric capacity also have significantly contributed to increase the pollution of its waters (Wengrat and Bicudo, 2011). Therefore, only the Taquecetuba and Rio Grande arms are used by the Basic Sanitation Company of the State of Sao Paulo for drinking water supply (SABESP, Sao Paulo, Brazil). Currently, the reservoir is responsible for supplying 1.6 million people with drinking water, however, it is estimated that 4.5 million people could be supplied by its waters (SABESP, 2015b; Wengrat and Bicudo, 2011).

Recently, the São Paulo Metropolitan Region has experienced the greatest water collapse ever recorded (Dobrovolski and Rattis, 2015;

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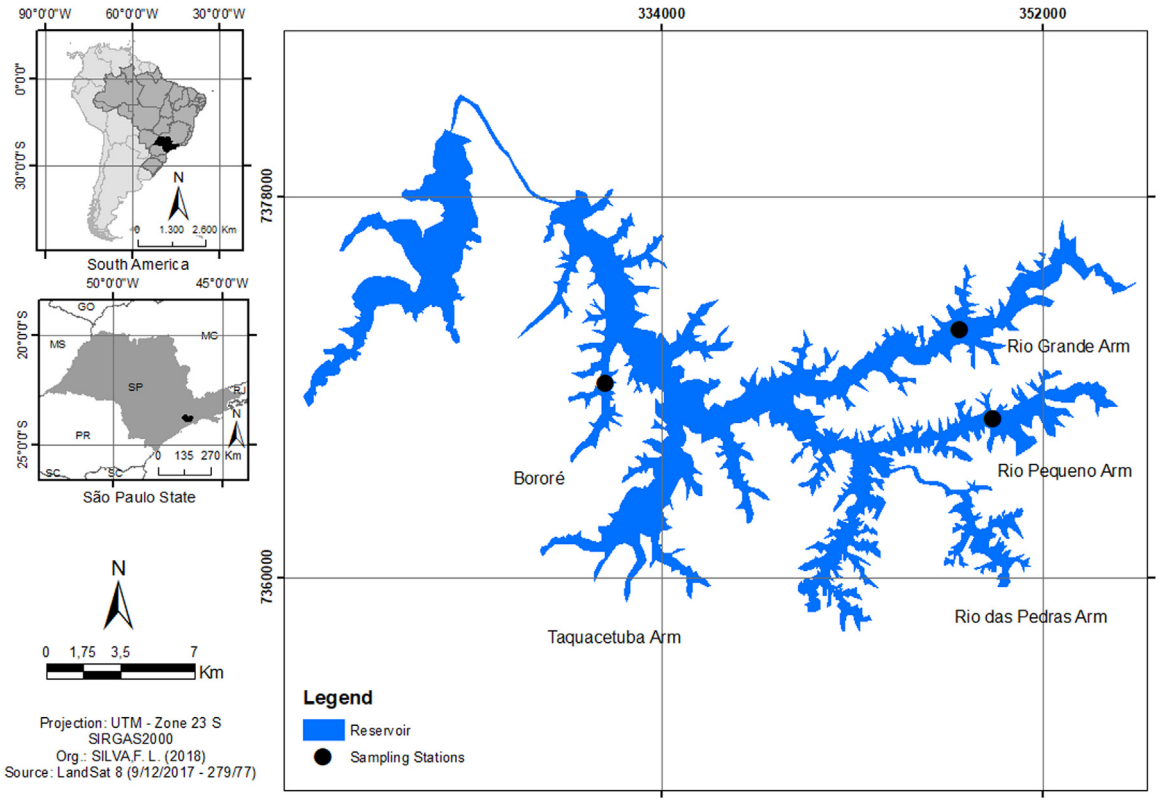


**Fig. 1.** Billings reservoir and the sampling sites. Billings reservoir with its dendritic pattern and an elongated and narrow central body with several arms including the three sampling sites: Rio Pequeno, Rio Grande, and Bororé .

Sousa and Silva, 2014). In July 2014, the Cantareira System, which serves 8.8 million people, was exhausted. This collapse was influenced by low levels of rainfall (Climatempo, 2015; Dobrovolski and Rattis, 2015), the increase in water consumption due to a huge population growth (from 4.8 million in 1960–11.8 million in 2013 in the Sao Paulo city alone), and numerous other consequences of such scenario like the waterproofing of soil (Sousa and Silva, 2014). Moreover, 31.8% of the treated water in Sao Paulo is lost on the way between the distributor and the households (SABESP, 2016). These have all contributed to the periods of serious water shortage that the São Paulo Metropolitan Region has been through.

Even if currently there is more rainfall and the population adheres to a drastic reduction in consumption, a more efficient management plan is badly needed. Today's full reservoirs will not be able to meet the demand in a few years (Sousa and Silva, 2014). Therefore, the water supply collapse is a serious problem in the São Paulo Metropolitan Region making changes in the management plan urgently required. The intensification of use of the Billings reservoir should be considered (Brasil, 2015). The Billings reservoir has a potential 10 times higher as a water reservoir when compared to the Cantareira system, and is specified by non-governmental organizations as an alternative to the supply of the São Paulo Metropolitan Region (SABESP, 2015a).

Zebrafish (*Danio rerio*) has been used as an experimental model for developmental toxicology (Bailey et al., 2013; Hill et al., 2005; Teraoka et al., 2003) including neurotoxicity testing and brain disorders understanding (de Esch et al., 2012; Kalueff et al., 2014). Moreover, behavioral study of zebrafish has been used as toxicity biomarker (Eissa et al., 2010) as well as for translational neuroscience, e.g., to understand neural pathways, physiological biomarkers, and genetic underpinnings of normal and pathological brain function (Kalueff et al., 2013, 2014).

The objective of this study was to evaluate the quality of the water from different areas of the Billings reservoir related to human consumption: Rio Pequeno, Rio Grande and Bororé rivers. Rio Pequeno has been considered a possible point of water collection and supply (Brasil, 2015). Rio Grande is routinely used to supply part of the São Paulo Metropolitan Region. Bororé river is a fishing area. We performed microbiological and physical studies on water samples. Biomarkers, such as total Coliforms, *Escherichia coli*, heterotrophic plate count bacteria, and microcystin are related to human/animal diseases and environmental contamination (Ramos et al., 2014). Moreover, adult zebrafish were exposed to those water samples and their behavior related to motor, exploratory, intoxication, and respiratory system parameters were evaluated, as well as anxiety (Kalueff et al., 2013). The survival rates of zebrafish were also analyzed. Finally, we studied central glial fibrillary acidic protein (GFAP) expression (an astrocyte biomarker), which is related to neuroinflammatory processes.

## 2. Materials and methods

### 2.1. Ethics statement

The present study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals (GCULA) of the National Institutes of Health (NCR, 2011). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Paulista University, Brazil (Permit Number: 374/15). All efforts were made to minimize suffering, reduce the number of animals used, and utilize alternatives to in vivo techniques when available. The method used for euthanasia of zebrafish at the end of experiments was decapitation, accordingly to the Federal Council of Veterinary Medicine and the GCULA (NCR, 2011). The experiments were also performed in accordance with good laboratory practice protocols and quality assurance methods.