SANDY LORENA PULECIO SANTOS

PATHOLOGICAL ASSESSMENT OF FISHES AND WATER QUALITY OF BILLINGS DAM

São Paulo

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Dissertation submitted to the Postgraduate Program in Experimental and Comparative Pathology of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Master's degree in Sciences

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CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO ANATOMOPATOLOGICA E DETERMINAÇÃO DO ESTADO DE SAÚDE DOS PEIXES DO RESERVATORIO DA BILLINGS ", protocolada sob o CEUA nº 3318010216, sob a responsabilidade de **Lilian Rose Marques de Sá** *e equipe; Sandy Lorena Pulecio Santos; Ana Carolina Camachos López; Gilson Alves Quinaglia; Ivy Tasso Gomes; Vera Lisa Generosa da Silva Paiva* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 15/03/2017.

We certify that the proposal "PATHOLOGICAL EVALUATION AND HEALTH STATUS OF FISH OF BILLINGS DAM", utilizing 427 Fishes (males and females), protocol number CEUA 3318010216, under the responsibility of **Lilian Rose Marques de Sá** *and team; Sandy Lorena Pulecio Santos; Ana Carolina Camachos López; Gilson Alves Quinaglia; Ivy Tasso Gomes; Vera Lisa Generosa da Silva Paiva* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 03/15/2017.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 03/2016 a 02/2018 Área: Patologia - Toxicologia Veterinária

Origem:	Não aplicável biotério						
Espécie:	Peixes	sexo:	Machos e Fêmeas	idade:	0001 a 1000 semanas	N:	78
Linhagem:	traíras			Peso:	100 a 3000 g		
Origem:	Não aplicável biotério						
Espécie:	Peixes	sexo:	Machos e Fêmeas	idade:	001 a 100 semanas	N:	246
Linhagem:	acará			Peso:	001 a 3000 g		
Origem:	Não aplicável biotério						
Espécie:	Peixes	sexo:	Machos e Fêmeas	idade:	001 a 100 semanas	N:	103
Linhagem:	tilápias			Peso:	001 a 3000 g		

Resumo: A água é um recurso não renovável de vital importância para o abastecimento humano e o equilíbrio dos principais ecossistemas do planeta. De modo que o impacto da qualidade das águas no meio ambiente é indiscutível, e cada vez é mais reconhecida à relevância de monitorar a qualidade das fontes hídricas. O reservatório da Billings é reconhecido pela sua importância para o abastecimento de água para a Região Metropolitana de São Paulo (RMSP) e também pelas atividades de pesca para o consumo humano. Os peixes são parte importante dos ecossistemas hídricos e da biota dos reservatórios de água como Billings, na atualidade o uso dos peixes como bioindicadores é um dos métodos mais relevantes para avaliar a qualidade da água e seus possíveis efeitos sobre a população humana. O tamanho dos peixes e a análise dos seus órgãos permitem fazer inferência sobre a saúde do ambiente por meio de estudos de macroscopia e histopatología, pois poluentes geralmente causam danos e respostas que vão desde nível celular e bioquímico ao nível do comportamento, o crescimento e reprodução. O objetivo principal do projeto é a avaliação do estado de saúde por meio da análise anatomopatológica de três espécies de peixes que são amplamente utilizadas no consumo humano e que habitam o reservatório da Billings: tilápias, acará (Geophagus brasiliensis) e traíra (Hoplias malabaricus), e baseado nas características macro e microscópicas determinar a espécie que melhor poderia seria empregada na avaliação da qualidade da água do reservatório.

Local do experimento: Laboratório de Gastroenterologia e Patologia Ambiental, Departamento de Patologia, FMVZ-USP

São Paulo, 30 de dezembro de 2017

Profa. Dra. Anneliese de Souza Traldi Presidente da Comissão de Ética no Uso de Animais Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo	Roseli da Costa Gomes Secretária Executiva Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

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Date: _____/_____/_____

Committee Members

Prof. ____

Institution:_________________________ Decision: ________________________

Prof. ____

Institution: _________________________ Decision: ________________________

Prof. ____

Institution: _________________________ Decision: ________________________

A mis papás, Clara y César. Muchas gracias por su amor infinito, apoyo e incentivo siempre.

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A Molly, por ella!

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RESUMO

PULECIO-SANTOS, S. **Avaliação anatomopatológica de peixes e qualidade da água do Reservatório Billings**. [**Pathological assessment of fishes and water quality of Billings Dam**]. 2018. 81p. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2018.

Os reservatórios de água, como a represa Billings, são monitorados com o propósito de proteger e conservar os recursos hídricos baseando-se na análise periódica de água e do sedimento, porém não são utilizados de rotina peixes como bioindicadores. O objetivo do estudo foi caracterizar a qualidade de água da represa Billings, considerando a análise integrada dos parâmetros de água e sedimentos somados à avaliação anatomopatológica de tilápias (n=103), acarás (*Geophagus brasiliensis,* n=246), e traíras (*Hoplias malabaricus,* n=78). Amostras de água, sedimento e peixes foram coletadas em seis pontos da represa durante o verão e a primavera de 2014 e 2015. Métodos analíticos padronizados foram utilizados para avaliação da água e do sedimento, e a avaliação anatomopatológica dos peixes, incluiu o índice Fulton, lesões macroscópicas e histopatológicas de brânquias, fígado, rins e baço. As análises de água e sedimentos caracterizaram a água como ambiente eutrofizado e poluído por metais. Os parâmetros da água mostraram melhora no ano de 2015, porém os resultados físicoquímicos, biológicos, de toxicidade crônica e de metais estiveram acima da média histórica e dos limites permitidos pela legislação. A avaliação anatomopatológica revelou que as três espécies de peixes responderam às condições ambientais de formas diferentes, apresentando índices mais altos de lesão em 2015. A brânquia foi o principal órgão afetado em todas as espécies de peixes. O principal padrão de resposta tecidual foi o regressivo e reversível. Os índices das trairas foram os maiores e esta espécie foi considerada a melhor espécie para o biomonitoramento da represa Billings. A análise integrada de parâmetros de água, sedimento e dos peixes permitiu a avaliação da qualidade da água da represa Billings, e pode representar uma das formas de avaliar as condições ambientais bem como a qualidade dos peixes consumidos na região.

Palavras-chave: abastecimento hídrico, biomonitoramento ambiental, peixes brasileiros, patologia de peixes, toxicologia ambiental.

ABSTRACT

PULECIO-SANTOS, S. **Pathological assessment of fishes and water quality of Billings Dam**. [**Avaliação anatomopatológica de peixes e qualidade da água do Reservatório Billings**]. 2018. 81 p. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2018.

The environmental agencies has been monitoring water reservoirs such as the Billings dam with the purpose of protection and conservation of water resources based on periodical water and sediment analyses, but it does not use fish as bioindicators routinely. The study aimed to characterize water quality of the Billings dam based on integrated analyses of water and sediment parameters in addition to pathological assessment of tilapias (n=103), acaras (*Geophagus brasiliensis*, n=246), and trairas (*Hoplias malabaricus*, n=78). Samplings of water, sediment and fish were executed in six sites of the dam in summer and spring of 2014 and 2015. Analytical methods for water and sediment parameters were carried out according to standard methods, while the pathological evaluation of fish involved the Fulton index, gross lesions evaluations, the macroscopic index and histopathological diagnosis of gills, liver, kidney, and spleen lesions, and their histopathological indices. Water and sediment analyses characterized the water as a eutrophic environment and polluted by metals. Water parameters showed improvement in 2015, but physicochemical, biological, chronic toxicity results, and metals criteria were in part above historic average and legal limits required. The pathological evaluation revealed that the three fish species responded to environmental conditions in different ways and presented worse indices in 2015. Gills were the main organ affected in all species studied and the principal tissue response was regressive and reversible pattern. The indices of trairas were the highest and this species was considered the best for biomonitoring the Billings dam. Comprehensive approach of water, sediment and fishes parameters allowed the evaluation of water quality of the Billings dam and it may represent one of the ways to evaluate its environmental conditions as well as the quality of the fish consumed in the region.

Key words: water supply, environmental biomonitoring, Brazilian fish, fish pathology, environmental toxicology.

SUMMARY

INTRODUCTION

Water is a valuable natural resource in the central focus of global health, environment, and sustainable development. Climate change and its effects on water supply and quality are worldwide problems, the consequences of which have affected even Brazil, a country widely known for its extensive areas of rainforests. The severe and long drought in northeastern and southeastern Brazil form 2012 to 2015 caused a profound economic, social and environmental impact which led to water rationing for months, especially in São Paulo, the biggest city of Brazil (COELHO; CARDOSO; FIRPO, 2015; ESCOBAR, 2015; GETIRANA, 2016).

Currently, the implementation of measures with the purpose of the protection and conservation of water resources has increased and is becoming more relevant. Population growth and urbanization add additional pressure on water sources and raise the demand for available water, affecting water quality and different aquatic species, which can also be used for human consumption (MALMQVIST; RUNDLE, 2002; VAN DER OOST; BEYER; VERMEULEN, 2003; FONSECA et al., 2016).

Anthropogenic factors modify the physical, chemical, and biological processes associated with water sources. The use of fish as bioindicators for the management and treatment of water sources is relevant and has been used in different countries. They are used because of their biological sensitivity and direct exposure to multiple pollutants present in water bodies (JENKINS, 2004; REDDY; RAWAT, 2013). As widely known, one of the most common vehicles by which pollutants transfer to humans besides water is food. Defining the degree of contamination of water sources and how they can affect a living organism is not a simple matter, and physicochemical analyses of water do not provide enough information for the management and control of water quality (DE LA TORRE; FERRARI; SALIBIÁN, 2005), reason why fish were used in the present research.

Fish are sentinel organisms, with biological responses that can vary and be quantified according to exposure to pollutants (GAGNON; HODSON, 2012). Among the tools to measure these responses is the toxicological pathology, which using techniques such as histopathology allows to determine morphological effects and alterations produced by harmful substances (ADAMS; CRABBS, 2013)

Metals are compounds widely used by human civilization; they are the only toxic pollutants that occur naturally. Despite the restrictions of their use in various contexts, human exposure to them is inevitable, however, due to human action, the toxic effects of these might be potentiated (GOSSLAU, 2016). In addition, the biological cycles of plants and animals result in the incorporation of these substances in the human diet, water, and atmosphere, increasing the distribution of these compounds in the environment and intensifying the possible adverse effects and the concern over these ecotoxicological effects (WHO, 2006; GOSSLAU, 2016).

The consumption of fish per capita in Brazil is 11.17 kg/year, which is considered low, whereas the world consumption per capita is near 20.6 kg/year (FAO, 2014; MURUSSI et al., 2016). Tilapia is one of the most employed species in aquaculture because of its quick growth rate, the characteristics of its meat, its resistance to extreme conditions and the ease of its reproduction (MARTIN; VALENTINE; VALENTINE, 2010; MAITHYA et al., 2012).

Currently, three species of tilapia are found in the Billings dam: *Oreochromis niloticus, Sarotherodon* sp, and *Tilapia rendalli.* The three of them were introduced in Billings for fishing as well as for ornamental purposes. These are the most abundant species in commercial fishing in the Billings dam and represent 80% of fish extracted from the dam (PETRERE; WALTER; MINTE-VERA, 2006; SECRETARIA DE MEIO AMBIENTE DE SÃO PAULO, 2010; AVARI, 2013).

Geophagus brasiliensis, known as cara or acara, is an omnivorous species with diurnal habits (ABELHA; GOULART, 2004), widely distributed in southern Brazil, the northern region of Argentina, and Paraguay (WIMBERGER, 1991). It is a native Brazilian species, a natural inhabitant of lentic environments, such as lakes and reservoirs, and highly adaptable to warm and cold regions (VOIGT et al., 2015). It usually remains in the same region as the tilapia, and is a very resistant species (ABELHA; GOULART, 2004; DI GIULIO; HINTON, 2008).

The *Hoplias malabaricus* popularly known as trahira or traira is a species which occurs in all the basins of South America including the Billings dam, and can be found in almost all the continental water bodies of Brazil; it inhabits diverse spaces such as small or large rivers, as well as spaces with stagnant water and little movement or even waterfalls (MALABARBA, 2009; OYAKAWA; MATTOX, 2009). This species is carnivorous and its food habit is based mainly on the consumption of other fish; it has been described as an ambush predator of nocturnal habits (CELI et al., 2001; OYAKAWA; MATTOX, 2009). It is a sedentary species, its life cycle takes place in relatively small geographic areas so it can spend long periods without feeding and then consume food in a voracious manner (SANT’ANNA; GOITEIN, 2009).

The species analyzed in this work have been previously used in several similar studies and in experimental reviews demonstrating their usefulness as indicators of environmental conditions in different contexts including the Billings dam (VAN DYK; PIETERSE; VAN VUREN, 2007; GARCIA-SANTOS et al., 2007; MELA et al., 2007; AZAZA; DHRAÏEF; KRAÏEM, 2008; MOHAMED, 2008; SANT'ANNA; GOITEIN, 2009; OLIVEIRA et al., 2011; NASCIMENTO et al., 2012; AHMED et al., 2013; REZENDE et al., 2013; LIEBEL; TOMOTAKE; OLIVEIRA-RIBEIRO, 2013; CASTRO et al., 2014; JAYASEELAN et al., 2014; MORAIS et al., 2016).

The 2014 and 2015 were atypical years with precipitation levels up to 22% lower than the average of the previous 19 years (CETESB, 2014, 2015; COELHO; CARDOSO; FIRPO, 2015). Hydrological conditions affect discharge, transformation, and diffusion of pollutants in the aquatic environment such as Billings dam (SMITH et al., 2011; EL-KHOURY et al., 2015; AZIMI; ROCHER, 2016; WEI et al., 2016).

In order to understand the status of the Billings reservoir in the context of a prolonged drought, integrative analyses of physicochemical, microbiological, toxicological and pathological parameters are important. Furthermore, the study of health status of fish from the Billings dam is relevant due to its possible impact on human health and as bioindicators of environmental quality to be used for future public actions (SOARES; MOZETO, 2006; CUNHA et al., 2011; OLIVEIRA, 2012; AVARI, 2013; CARDOSO-SILVA et al., 2014)

The study aimed to characterize the water quality of the Billings dam based on integrated analyses of water and sediment parameters in addition to pathological assessment of three species of indigenous fish.

2. MATERIALS AND METHODS

The protocol of the study was approved by the Commission on Ethics in Animal Use of the School of Veterinary Medicine and Animal Science of the University of São Paulo, according to the guidelines of the Brazilian College on Animal Experimentation with protocol number 3318010216. The study was linked and part of the FEHIDRO AT 603 project, and has authorization for activities with scientific purpose from the Brazilian Ministry of the Environment through the Authorization and Information on Biodiversity System - SISBIO, under registration number 25609-4.

2.1. STUDY AREA

Billings Reservoir (23°47' S, 46°40' W) is located on the southeast region of São Paulo (Fig. 1), it is one of the largest and most important water sources in the Metropolitan Region of São Paulo. The dam has a storage volume of 1.2 billion m^3 and an inundation area of 127 km^2 with a water system capacity of 4.8 m^3/s. Billings has a drainage basin area of 560 km^2; climate is tropical and subtropical in the region, with an average temperature of 19°C and an average precipitation of 1300 – 3500 mm/year. The water is used as drinking supply of about 1.2 million people, energy generation, fishing, navigation, recreation and flood control of São Paulo city (CETESB, 2010).

The soil occupancy and main land use surround the Billings dam are: 14.6% urban area with rapidly growing population, 5.6% small farms, condominium and scattered houses, and approximately 50% of the land is covered by primary Atlantic Forest, and secondary forest of commercial pines and eucalyptus, pasture land, agriculture and wetlands. Billings' population concentrates 97% in urban areas, 20% of them live in precarious housing conditions. The main water flow passes through the longest central axis of the dam and exits at the Summit Control (S2- BILLL02900)(CETESB, 2015).

Billings' water is polluted in several ways and at different geographical points, pollution is mostly due to urban explosion, disrespect for regulations, illegal occupation of protected areas leading to the discharge of domestic, industrial and agricultural wastewater into the dam, inadequate waste disposal, and increased loss of Atlantic Forest (CETESB, 2010).

The water at the Billings reservoir is considered class 2, which means that these waters can be used for human consumption or supply after being treated; to protect the aquatic communities; they can be used for recreational purposes, for irrigation of vegetables, fruit plants, parks and gardens, and for aquaculture and fishing activities (CONAMA, 2005).

The water and fish samplings were carried out once in summer and once in spring of 2014 and 2015 (Table 1). Figure 1 illustrates the general conformation of the basin, the nearby towns and the sites used in this study for water and fish sampling. The points S1 to S4 are separated from the points S5 and S6 by a physical barrier, which does not allow the passage of fish from one side to another. However, it is not a watertight barrier and there may be a partial passage of water. In addition, due to the geographic conformation of the reservoir and its ramifications, it has been suggested that each extension presents particular characteristics and dynamics.

Figure 1. Map of Billings reservoir and sites of sampling of water, sediment and fishes; S1 BILL02100, S2 BILL 02900, S3 BILL02500, S4 BITQ00100, S5 RGDE02900, S6 RGDE02200

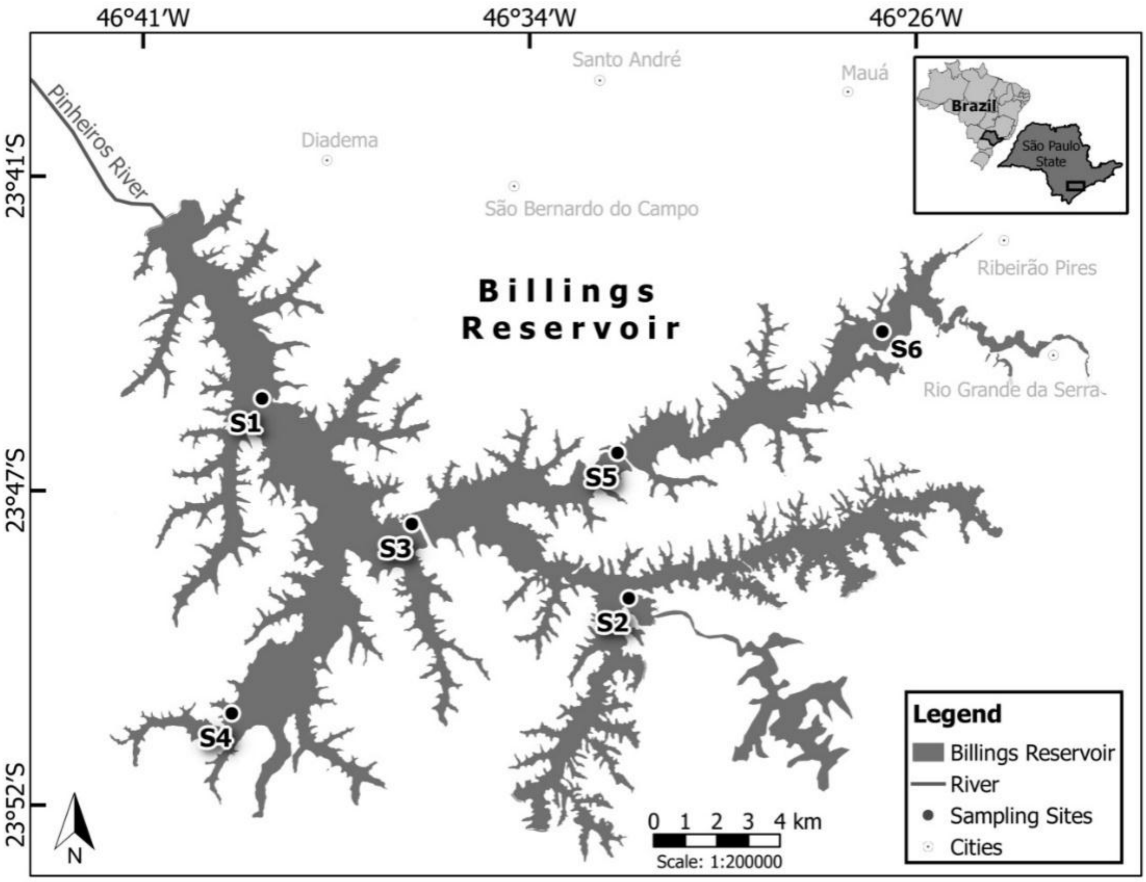


Table 1. List of water and fish sampling sites with coordinates

Sampling site	Coordinates
S1 BILL02100	23° 45’ 16’’ S; 46° 38’ 04’’ W
S2 BILL02900	23° 49’ 16’’ S; 46° 31’ 30’’ W
S3 BILL02500	23° 47’ 27’’ S; 46° 35’ 54’’ W
S4 BITQ00100	23° 50’ 41’’ S; 46° 39’ 20’’ W
S5 RGDE02900	23° 46’ 16’’ S; 46° 32’ 03’’ W
S6 RGDE02200	23° 44’ 23’’ S; 46° 26’ 44’’ W

2.2. WATER AND SEDIMENT SAMPLES

Water and sediment samples were collected according to the "National Guide of Collection and Samples Preservation: Water, Sediment, Water Communities and Wastewater", as recommended by the National Water Agency of Brazil and the Environmental Agency of the State of São Paulo (CETESB, 2011). The samples were collected for the first sample period in March and April, and for the second period in November and December.

The parameters used for the analysis were: conductivity, water temperature, air temperature, turbidity, pH, biochemical oxygen demand (BOD), dissolved oxygen (DO), organic nitrogen, nitrate, nitrite, ammoniacal nitrogen, total solid, total dissolved solid, total phosphorus, total chloride, *Escherichia coli* counts, chlorophyll-a, number of cyanobacterial cells, both water and sediment were analyzed for metals and metalloids aluminum, arsenic, cadmium, chromium, copper, nickel, lead, and zinc. The data reported by the CETESB of chronic toxicity was also included.

The methods of analysis were performed according to the American Public Health Association (APHA); American Water Works Association (AWWA); Water Environment Federation (WEF), Standard Methods for the examination of Water and Wastewater (APHA; AWWA; WEF, 2011). All laboratory analyses of water and sediment were conducted by the CETESB and the results are free to public access. The limits accepted were established by the CONAMA law 357/05 (CONAMA, 2005), which determines minimum water pattern guidelines based on water use and procedures for the assessment of dredged material. The data were compared with international acceptable limits of the World Health Organization (WHO, 2011a) and the United States Environmental Protection Agency (EPA, 2001).

For the water analysis, indices such as the Water Quality Index (IQA), Eutrophication Index (IET), and a water quality index for the protection of aquatic life (IVA) used by the CETESB for water classification were included, as well as the results of chronic toxicity evaluation (CETESB, 2015).

The water data reports included correspond to the dates of the collections of the fish and water sampling.

2.3. FISH SAMPLE COLLECTION

The capture of fish was performed by local fishermen and technicians of the CETESB Sampling Division using trammel nets with different meshes, which were placed in the sampling points in the late afternoon of the day prior to sampling. A trammel net consists of two/three layers of netting with slack small mesh inner netting between two layers of large mesh netting. Fish were captured in the same sampling sites of the monitoring network by CETESB according to the Guide for Collection and Sample Preservation (CETESB, 2011). A total of 427 individuals were collected including tilapias, acaras, and trairas.

2.4. FISH NECROPSY AND BIOMETRIC INDICES

The individuals and tissues of the three species were evaluated and processed simultaneously. The fish were weighted (grams), its standard and total length (cm) were measured, and the Fulton index (weight/standard length^3 x 100) was calculated for each individual. The gross exam was conducted based on (YAMONG, 2003).

The macroscopic evaluation index of health (HAI) was calculated, based, and adapted from Adams et al. (1993) considering gross findings of gills, liver, kidney, spleen, and skin. Every individual was examined macroscopically, taking into consideration external injuries, body condition, fat deposits, and changes in the celomic cavity and organs.

2.5. TISSUE PROCESSING AND HISTOPATHOLOGICAL EVALUATION

The fragments from gills, liver, kidney and spleen were collected and fixed in a neutral 10% formalin solution. The fixed tissues were dehydrated with ethanol in crescent concentrations, infiltrated with xylene and, embedded in paraffin, cut to a width of 5µm and dyed with hematoxylin and eosin (HE). When necessary, specific colorations such as Perls blue stain, Masson’s Trichomic, Ziehl-Nielsen, and Period Acid Schiff were used. Microscopic examination was performed under light microscopy, Eclipse NiU, Nikon with digital camera DSU3 in 40x, 100x, 200x, 400x and 1000x. Two observers unaware of the identification of the animal and the collection site conducted the analyses.

The analysis of organs and the calculation and analysis of pathological changes indices were adapted from Bernet et al. (1999). Accordingly, the histopathological changes were classified into five reaction patterns: circulatory, degenerative or regressive, inflammatory, proliferative, neoplastic, and for this study, a sixth pattern related to a parasitic infestation was included. Every alteration had an importance factor ranging from 0 to 3, associated with pathologic relevance. The extension of the lesion was ranging also from 0 to 3. Injury indices were calculated, in order to enable the standardization of histopathology findings, and the comparison between individuals. An index was calculated for each organ (I-Org) and a total index (I-Tot) was obtained from the sum of the I-Org of gills and liver..

2.6. STATISTICAL ANALYSIS

Statistical analysis was performed using RStudio programs. For the variables and indices obtained from fish evaluation, Wilcoxon, Kruskall-Wallis, linear and multiple regression tests were performed. Correlation analyses were performed between the health indices calculated for the fish and the water quality indices. Data with $p<0.05$ were considered statistically significant. Histopathological parameters were descriptively presented in tables with absolute (n) and relative (%) frequency.

3. RESULTS

The results were divided in four parts, one for water and sediment, and one for each of the three fish species, in order to facilitate the understanding of the data.

3.1. PARAMETERS OF WATER QUALITY AND SEDIMENT

3.1.1. PHYSICOCHEMICAL AND MICROBIOLOGICAL VARIABLES

No statistical difference was found among the water variables of the four sampling periods during the two years of the study. The average values and standard deviation of the values obtained for all water sampling sites during 2014 and 2015 are found in tables 2 and 3.

The BOD, total phosphate, chlorophyll-a, and number of cyanobacteria were the main variables outside of the parameters. The BOD remained above allowed values during the four collection periods in S1 with an outcome of 9, 8, 6 and 6 respectively; S4 in summer of 2014 and spring of 2015 resulting in 15 and 21 respectively; and S6 in spring of 2014 and summer of 2015 with values of 7 and 6 respectively. The total phosphate values remained above the allowed value in all the sampling sites during the four collection periods. Chlorophyll-*a* values were above the limit value in S1 for all collections except summer 2015; In S4 during all collections; and in S6 during spring 2014 and summer 2015. The number of cyanobacteria was above the allowed values during the two years in sites S1 and S2, during 2015 in S3, and during 2014 and spring of 2015 in S4. These parameters (BOD, phosphate, chlorophyll – *a*, cyanobacteria*)* are the main indicators when determining contamination by sewage in water.

Table 2. Average and standard deviation of physicochemical and biological water parameters in sampling site in 2014

Parameters	S1	S2	S3	S4	S5	S6	CONAMA 357/ 2005
Condutivity µS/cm	215 ± 25.46	154.50 ± 3.54	181.50 ± 10.61	185.50 ± 24.75	113.50 ± 16.26	123.00 ± 22.63	
Water temperature °C	24.44 ± 0.07	24.21 ± 0.13	24.32 ± 0.22	24.50 ± 0.45	24.50 ± 0.14	25.10 ± 0.71	
Air temperature °C	25.95 ± 2.90	22.75 ± 3.18	24.10 ± 3.39	25.25 ± 2.47	24.60 ± 3.68	24.25 ± 3.18	
Turbidity UNT	27.20 ± 21.07	13.29 ± 6.38	18.69 ± 16.14	30.85 ± 27.08	3.51 ± 0.93	10.29 ± 8.37	<100
pH	7.68 ± 0.09	8.42 ± 1.05	7.74 ± 0.28	8.37 ± 0.92	7.09 ± 0.30	8.06 ± 1.58	6 to 9
BOD (5.20) mg/L	8.50 ± 0.71	4.00**	4.50 ± 0.71	9.50 ± 7.78	<3*	7.00**	<5
Dissolved O2 mg/L	6.19 ± 1.62	7.99 ± 2.26	5.95 ± 0.95	8.15 ± 0.54	6.06 ± 2.18	8.41 ± 2.66	>5
Ammoniacal Nitrogen mg/L	1.09 ± 1.12	0.17 ± 0.04	0.46 ± 037	0.23 ± 0.16	0.45 ± 0.13	0.81 ± 0.33	<0.5
Total organic N mg/L	2.93 ± 1.70	1.01 ± 0.37	1.40 ± 0.61	1.2**	0.85 ± 0.08	1.30 ± 0.57	
Nitrate N mg/L	0.81***	<0.2*	0.23***	0.19**	0.38**	<0.2*	<10
Nitrite N mg/L	<0.1*	<0.1*	<0.1*	<0.1*	<0.1*	0.15**	<1
Total solids mg/L	175 ± 41.01	119.00 ± 1.41	132.00 ± 5.66	144.00 ± 0.00	<100*	138.00**	
TDS mg/L	163.00 ± 43.84	115.00 ± 4.24	123.00 ± 1.41	144.00**	<100*	100.00**	<500
Total phosphate mg/L	0.27 ± 0.08	0.05 ± 0.01	0.11 ± 0.02	0.12 ± 0.03	0.07 ± 0.04	0.09 ± 0.04	<0.03
Total chloride mg/L	18.70 ± 2.55	14.00 ± 0.57	16.30 ± 1.41	16.70 ± 2.69	12.80 ± 2.12	14.35 ± 3.04	<250
Escherichia coli UFC/100mL	140.50 ± 129.40	2.50 ± 2.12	13.50 ± 13.44	27.00 ± 36.77	26.50 ± 19.09	95.00 ± 114.55	<600
Chlorophyll-a µg/L	235.89 ± 106.79	50.05 ± 20.89	41.89 ± 40.56	161.72 ± 180.82	14.87 ± 0.24	49.11 ± 50.01	<30
Cyanobacteriae (Number of cells)	905437.5 ± 1005657.87	406400.00 ± 162698.20	18375.00****	106560.00 ± 141209.22	34590.00 ± 26247.80	ND	<50000

BOD: Biological Oxygen Demand. TDS: Total Dissolved Solids. * Below detection limit in summer and spring. ** Below limit detection in summer period. *** Below limit detection in spring period. **** No data for summer period. ND: No data. Limit detection of BOD: < 3 mg/L, Ammoniacal nitrogen: <0,1 mg/L, Total organic nitrogen: <0,5 mg/L, Nitrate: <0,2 mg/L, Nitrite: <0,1 mg/L, Total solids: <100 mg/L, TDS: <100 mg/L. CONAMA 357/2005: Current Law of the State of São Paulo (CONAMA, 2005).

Table 3. Average and standard deviation of physicochemical and biological water parameters in sampling sites in 2015

Parameters	S1	S2	S3	S4	S5	S6	CONAMA 357/ 2005
Condutivity μS/cm	213.50 ± 14.85	161.00 ± 4.24	202.50 ± 7.78	185.50 ± 0.71	103.55 ± 3.466	99.5 ± 0.71	
Water temperature °C	24.82 ± 1.29	24.99 ± 1.00	25.09 ± 2.28	23.29 ± 0.32	23.86 ± 0.06	26.25 ± 0.21	
Air temperature °C	25.05 ± 0.21	24.74 ± 0.35	25.10 ± 0.99	23.55 ± 1.48	24.80 ± 0.42	27.25 ± 1.20	
Turbidity UNT	6.98 ± 3.13	6.10 ± 1.72	5.99 ± 2.84	5.59 ± 2.35	2.87 ± 2.09	5.73 ± 1.51	<100
pH	7.38 ± 0.14	8.15 ± 0.05	7.53 ± 0.21	7.66 ± 0.04	7.51 ± 0.45	7.75 ± 0.62	6 to 9
BOD (5.20) mg/L	6.00 ± 0.00	4.00***	5.00***	21.00**	<3*	6.00***	<5
Dissolved O2 mg/L	ND	8.22 ± 1.87	6.55 ± 0.75	7.41 ± 0.13	6.38 ± 2.10	8.58 ± 1.11	>5
Ammoniacal Nitrogen mg/L	5.68 ± 2.25	0.21**	0.28 ± 0.20	<0.1*	0.42 ± 0.44	0.32 ± 0.07	<0.5
Total organic N mg/L	0.95 ± 1.03	1.06 ± 0.65	1.23***	1.39 ± 0.57	1.32***	0.79 ± 0.18	
Nitrate N mg/L	2.69**	<0.2*	0.55 ± 0.07	0.32***	0.31**	0.3**	<10
Nitrite N mg/L	<0.1*	<0.1*	<0.1*	<0.1*	<0.1*	0.13**	<1
Total solids mg/L	141.00 ± 1.41	114***	140.00 ± 2.83	134.00 ± 28.28	128.00***	100***	
TDS mg/L	126.00 ± 14.14	<100*	139.00 ± 4.24	124.00 ± 28.28	114.00***	100.00***	<500
Total phosphate mg/L	0.27 ± 0.05	0.04 ± 0.01	0.12 ± 0.02	0.08 ± 0.04	0.03 ± 0.00	0.06 ± 0.03	<0.03
Total chloride mg/L	18.25 ± 0.78	15.10 ± 0.28	17.90 ± 0.71	15.70 ± 0.85	10.35 ± 0.21	10.15 ± 0.07	<250
Escherichia coli UFC/100mL	642.00 ± 732.56	2.00 ± 1.41	10.50 ± 12.02	15.50 ± 0.85	15.50 ± 20.50	319.50 ± 368.40	<600
Chlorophyll-a μg/L	76.68 ± 57.88	29.05 ± 2.02	37.92 ± 12.05	15.50 ± 14.85	11.85 ± 0.38	31.81 ± 14.74	<30
Cyanobacteriae (Number of cells)	296775.00 ± 286540.88	182050.00 ± 85842.76	150195.00 ± 103916.41	177.98 ± 194.36	24735.00 ± 13201.68	52492.50 ± 16274.06	<50000

BOD: Biological Oxygen Demand. TDS: Total Dissolved Solids. * Below detection limit in summer and spring. ** Below limit detection in summer period. *** Below limit detection in spring period. **** No data for summer period. ND: No data. Limit detection of BOD: < 3 mg/L, Ammoniacal nitrogen: <0,1 mg/L, Total organic nitrogen: <0,5 mg/L, Nitrate: <0,2 mg/L, Nitrite: <0,1 mg/L, Total solids: <100 mg/L, TDS: <100 mg/L. CONAMA 357/2005: Current Law of the State of São Paulo (CONAMA, 2005).

The effects of chronic toxicity reported did not show statistical difference by collection period. S2 was positive for chronic toxicity in the four periods, S3 during 2014, S6 showed no effects of chronic toxicity in any of the samples (Table 4).

Table 4. Chronic toxicity reported for each sampling site during the four periods of collection

Sampling Period	Sampling Site	Chronic Toxicity	Sampling Period	Sampling Site	Chronic Toxicity
Summer 2014	S1	-	Summer 2015	S1	+
	S2	+		S2	+
	S3	+		S3	+
	S4	+		S4	-
	S5	+		S5	-
	S6	-		S6	-
Spring 2014	S1	-	Spring 2015	S1	-
	S2	+		S2	+
	S3	-		S3	-
	S4	-		S4	-
	S5	-		S5	+
	S6	-		S6	-

Font: (CETESB, 2014; CETESB, 2015). +: positive; -:negative.

In superficial waters, levels of aluminum (<0.1 mg/L), arsenic (<0.01 mg/L), cadmium (<0.0007 mg/L), chromium (<0.02 mg/L), nickel (<0.009 mg/L) and zinc (<0.02 mg/L) remained below detection limit. Dissolved copper remained below detection limits (<0.009) except in 2015 in points S5 and S6, which values were 0.01 mg/L in summer, 0.05 mg/L in spring, 0.03 mg/L in summer, and 0.02 mg/L in spring respectively.

In sediment, in the concentrations of metals and metalloids analyzed, no significant statistical difference was found between sampling sites and collection periods. The levels of aluminum, arsenic, cadmium, chromium, copper, nickel, lead and zinc were above the limits allowed as it appears in table 5. All metals analyzed reached PEL concentrations, most frequently arsenic, chromium, nickel.

There was no significant association among physicochemical, biological, and metal levels in water and sediment.

Table 5. Average and standard deviation of concentration of metals and metalloids in sediment for site of sampling in 2014 and 2015

Metals / metalloids (mg/Kg)	S1*	S2	S3	S4	S5*	S6*****	Criteria for the Assessment of Sediment Quality [1]		EPA[2]
							TEL	PEL	
Aluminum	63900.00 ± 13378.46	ND	ND	ND	70519 ± 5004.90	ND	ND	ND	ND
Arsenic	19.45 ± 0.92	17.85 ± 10.52	28.25 ± 12.50	13.30 ± 5.63	20.35 ± 0.35	2.65	5.9	17	9.8
Cadmium	3.25 ± 0.17	<0.50**	1.87 ± 1.59	<0.50***	0.6	<0.50	0.6	3.5	0.99
Chromium	176.00 ± 9.90	73.65 ± 41.49	160.50 ± 73.41	77.22 ± 29.16	54.95 ± 19.02	12.1	37.3	90	43.4
Copper	215.50 ± 0.71	32.15 ± 17.69	91.50 ± 80.63	33.30 ± 12.57	5373.50 ± 637.10	7.01	35.7	197	31.6
Nickel	79.35 ± 1.77	38.65 ± 21.95	55.60 ± 40.79	48.25 ± 15.14	30.95 ± 0.92	6.33	18	35.9	22.7
Lead	99.70 ± 7.50	29.5 ± 3.11	66.70 ± 23.96	39.40 ± 10.00	50.60 ± 2.69	47.3	35	91.3	35.8
Zinc	308.00 ± 261.63	93.55 ± 40.38	272.50 ± 206.30	88.40 ± 19.38	110.5 ± 4.95	79.7	123	315	121

ND: no data. ND: No data. * Sampled in spring of 2014 and summer of 2015. ** Below the limit detection except in summer of 2015 with 0.52 mg/Kg. *** Below the limit detection in all sampled periods. **** Below the limit of detection in summer of 2015. ***** Sampled only in summer of 2014. [1]CANADA, 2007; [2]EPA, 2001.

3.1.2. Water Quality Indices

Table 6 shows the results of water indices IQA, IET and IVA, and their classification by sampling sites. When comparing each one of these indices there was no statistical difference between the year and period of sampling.

Table 6. Water quality indices and the distribution for sampling period and sampling point

Sampling Period	Sample Site	IQA	Category	IET	Category	IVA	Category
Summer 2014	S1	65	Good	73	Hyper eutrophic	8.6	Very bad
	S2	85	Optimal	60	Eutrophic	5.4	Bad
	S3	75	Good	63	Eutrophic	5.4	Bad
	S4	58	Good	63	Eutrophic	6.6	Bad
	S5	74	Good	54	Mesotrophic	5.4	Bad
	S6	82	Optimal	60	Eutrophic	3.2	Good
Spring 2014	S1	60	Good	71	Hyper eutrophic	6.2	Bad
	S2	77	Good	62	Eutrophic	4.5	Fair
	S3	79	Good	61	Eutrophic	4.2	Fair
	S4	86	Optimal	62	Eutrophic	4.2	Fair
	S5	81	Optimal	61	Eutrophic	4.2	Fair
	S6	60	Good	66	Super eutrophic	6.4	Bad
Summer 2015	S1	55	Good	61	Eutrophic	7.6	Very bad
	S2	87	Optimal	59	Eutrophic	4.4	Fair
	S3	82	Optimal	61	Eutrophic	5.4	Bad
	S4	84	Optimal	62	Eutrophic	4.2	Fair
	S5	76	Good	57	Mesotrophic	4.4	Fair
	S6	69	Good	63	Eutrophic	4.2	Fair
Spring 2015	S1	70	Good	69	Hyper eutrophic	7.4	Very bad
	S2	85	Optimal	59	Eutrophic	4.4	Fair
	S3	76	Good	65	Super eutrophic	5.2	Bad
	S4	65	Good	69	Hyper eutrophic	5.2	Bad
	S5	91	Optimal	57	Mesotrophic	5.6	Bad
	S6	84	Optimal	84	Hyper eutrophic	4.2	Fair

The IQA reported remained within the good (58.33%) and optimal (41.67%) categories in the four sampling periods, with an average of 76±10.18 during the year 2014, and 79±10.56 during the year 2015.

The IET had an average of 62±5.06 in 2014 and 61.5±7.52 in 2015, being categorized as hypereutrophic (20.83%), super eutrophic (8.33%), eutrophic (58.33%), and mesotrophic

(12.50%). When comparing the IET of points S5 and S6, located in the arm of Rio Grande to the other points, a significant difference was found (p = 0.003615), showing different water quality for the arm of Rio Grande area.

The IVA during the two years was categorized as good (4.16%), fair (41.67%), bad (41.67%), and very bad (12.50%). The average value was 5.4±1.45 in 2014 and 4.8±1.20 in 2015.

3.2. TILAPIAS

One hundred and three tilapias were studied, 47 males, 52 females and two individuals of undetermined sex. Table 7 shows the fish obtained in each collection period in respect to the year and season, as well as the number of male and female individuals for each one.

Table 7. Distribution and sex of the tilapias captured for the four collection periods in summer and spring

Sampling Period		Male	Female	Undetermined	Total
2014	Summer	10	14	0	24
	Spring	10	12	0	22
2015	Summer	7	12	2	21
	Spring	20	16	0	36
Total		47	54	2	103

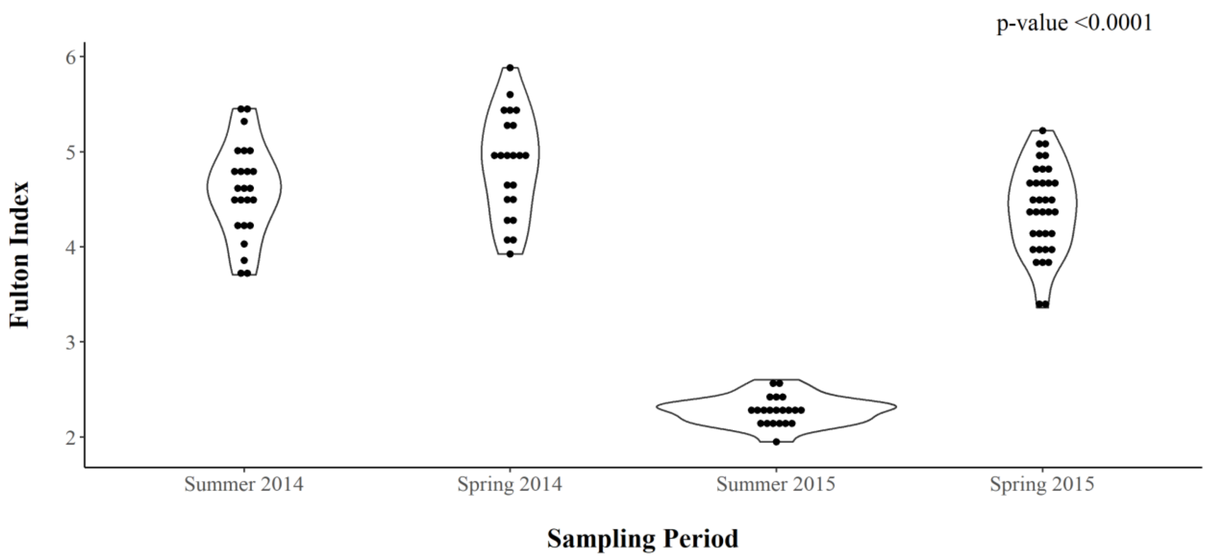
3.2.1. BIOMETRY AND FULTON INDEX

The average weight (grams) for the individuals of the year 2014 was 252.25 ± 111.21, and 232 ± 120.1 for 2015. The average standard length (cm) was 17.7 ± 2.56 for the year 2014, and 19 ± 3.55 for the year 2015. There was no difference between 2014 and 2015 taking into account the average weight and standard length of tilapias.

The Fulton Index for individuals collected in 2014 had an average value of 4.71 ± 0.53, and of 4 ± 1.09 in 2015. There is a statistically significant difference between the years 2014 and 2015 (p=0.0002827) for Fulton index, which tended to be lower during 2015.

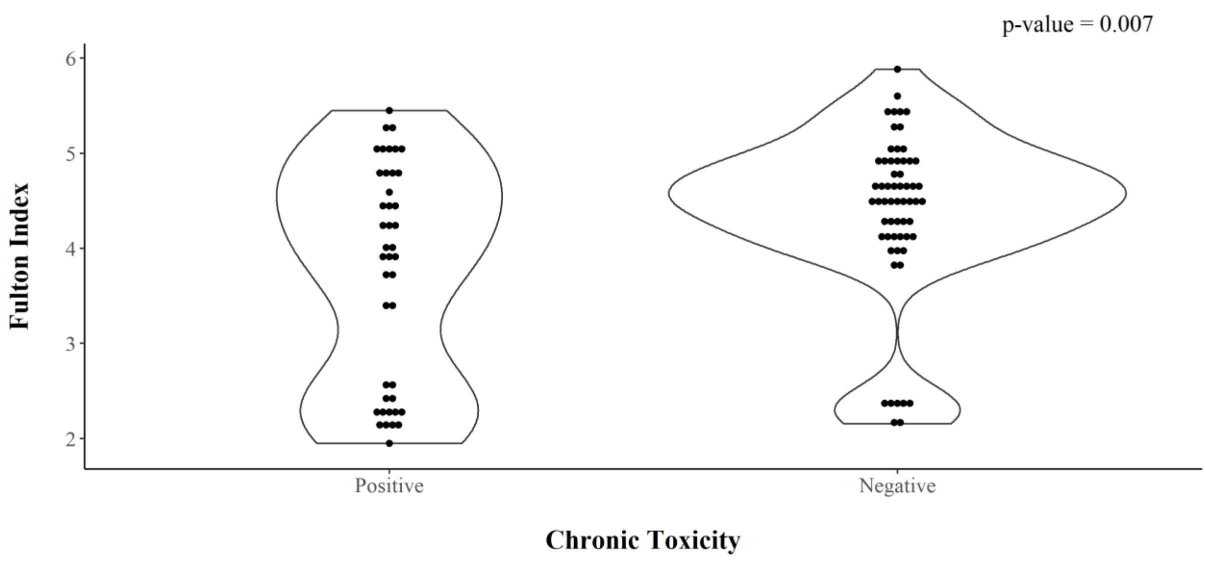
The Fulton index did not present a significant statistical relation with neither physicochemical and biological variables, nor with the IQA, IET and IVA water quality indices. The four collection periods compared to each other resulted in a significant statistical difference (p=0.000000005171), being the summer collection period of 2015 (Graphic 1) in which the Fulton index was by far the lowest (worse index) compared to the other three collections.

Graphic 1. Distribution of the Fulton index for individuals during the four sampling periods



When comparing the Fulton index with the chronic toxicity reported (CETESB, 2014, 2015), there was evidenced significance (p=0.00731) between the effects of chronic toxicity and a lower Fulton index (Graphic 2).

Graphic 2. Distribution of the Fulton index for individuals related to the effect of chronic toxicity in water



3.2.2. Necropsy Findings

Skin lesions were found in 6.80% (7/103) of the individuals. In terms of the amount of fat in the celomic cavity, absence was observed in 12.62% (13/103); a low quantity in 51.46% (53/103); moderate in 27.18% (28/103), and abundant in 1.94% (2/103) of the tilapias. Parasites were observed in the celomic cavity in 3.88% (4/103). Mucus in gills was found in moderate to abundant amounts in 10.68% (11/103) of the individuals. In liver, whitish foci were observed in 16.50% (17/103), rounded edges in 6.80% (7/103) and infiltration of fat in the liver was found in 0.97% (1/103). In the spleen multiple whitish foci were observed in 3.88% (4/103). The macroscopic findings are shown in table 8 and figure 2.

Table 8. Relative and absolute frequency of gross findings in body inspection of tilapias, acaras and trairas

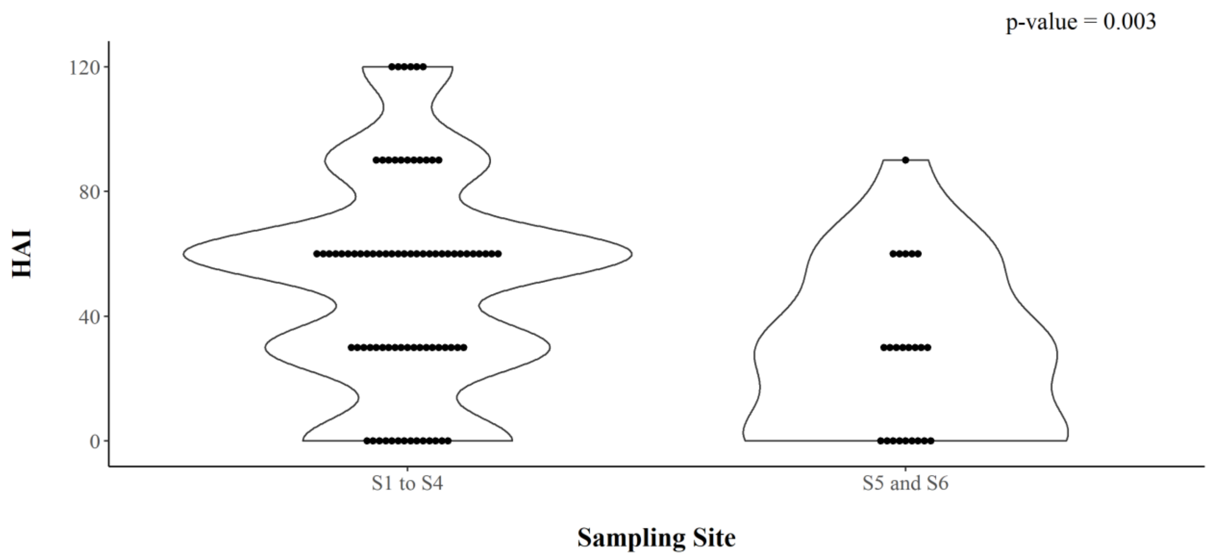
Gross findings	**TILAPIAS n (%) n=103**	**ACARAS n (%) n=246**	**TRAIRAS n (%) n= 78**
Mucus on gills	11 (10.68)	17 (6.91)	50 (69.44)
Low fat in celomic cavity	53 (51.46)	52 (21.14)	40 (55.56)
Parasites in the celomic cavity	4 (3.88)	7 (2.85)	38 (52.78)
Moderate fat in celomic cavity	28 (27.18)	106 (43.09)	26 (36.11)
White nodules in liver	17 (16.50)	13 (5.28)	8 (11.11)
Skin Injuries	7 (6.80)	7 (2.85)	4 (5.56)
Abundant fat in celomic cavity	2 (1.94)	79 (32.11)	3 (4.17)
Absence of fat in celomic cavity	13 (12.62)	5 (2.03)	3 (4.17)
Infiltration of fat in the liver	1 (0.97)	55 (22.36)	0 (0)

3.2.3. Health Assessment Index (HAI)

The HAI average was on 60 ± 31.41 for the year 2014, and 30 ± 35.69 on 2015. There was no statistical difference between HAI and sampling site, collecting period or year.

The evaluation of HAI and water-related indices did not show a significant statistical association. Tilapias from points S5 and S6 (Rio Grande) presented a moderately significant difference ($p = 0.003$) with respect to the fish collected in the other points (Graphic 3**)**. Points located in arm of Rio Grande showed lower HAI.

Graphic 3. HAI of tilapias captured in sampling sites S1 to S4 compared with the index of individuals captured in sites S5 and S6 (Rio Grande)



3.2.4. HISTOPATHOLOGICAL FINDINGS

The histopathological lesions and their frequency of occurrence amongst this population in gills, liver, kidney and spleen are presented in tables 9, 10, 11 and 12, and in the figures 5, 6, 7 and 8, respectively.

The most frequent microscopic findings were: in the gills: epithelial hypertrophy 81.37% (88/102), rupture of pillar cells 75.49% (77/102), lymphocytic infiltrate 56.86% (58/102), epithelial hyperplasia 51.96% (53/102), and epithelial lifting in 50% (51/102); in the liver: steatosis 58% (58/100), cellular edema 55% (55/100), lymphocytic infiltrate 29% (29/100), congestion 26% (26/100), and granulomas 17% (17/100); in kidney: congestion in 54.55% (42/77), lymphocytic infiltrate 54.55% (42/77), hemorrhage 33.77% (26/77), granulomas 32.47% (25/77), hyaline droplets in tubular epithelium 25.97% (20/77); and in spleen: lymphoid depletion 87.76% (86/98), congestion 73.47% (72/98), and granulomas 26.53% (26/98).

Table 9. Relative and absolute frequency of gill histopathological lesions in tilapias, acaras and trairas

Lesions in Gills	**TILAPIAS n (%) n=102**	**ACARAS n (%) n=204**	**TRAIRAS n (%) n= 53**
Rupture of pillar cells	77 (75.49)	110 (53.92)	53 (100)
Epithelial hypertrophy	83 (81.37)	109 (53.43)	46 (86.79)
Congestion	32 (31.37)	79 (38.73)	30 (56.60)
Epithelial hyperplasia	53 (51.96)	54 (26.47)	23 (43.40)
Epithelial lifting	51 (50.00)	81 (39.71)	16 (30.19)
Aneurysms	8 (7.84)	37 (18.14)	16 (30.19)
Lymphocytic Infiltrate	58 (56.86)	46 (22.55)	14 (26.42)
Fusion of secondary lamellae	22 (21.57)	24 (11.76)	6 (11.32)
Parasites	9 (8.82)	49 (24.02)	4 (7.55)
Granulomas	14 (13.73)	26 (12.75)	1 (1.89)
Metaplasia	8 (7.84)	22 (10.78)	1 (1.89)
Hemorrhage	17 (16.67)	5 (2.45)	1 (1.89)
Atrophy	9 (8.82)	2 (0.98)	0 (0.00)
Autolisis	0 (0%)	1/210 (0.47)	19/78 (24.35)
Not represented	1/103 (0.97)	5/210 (2.38)	6/78 (7.69)

Table 10. Relative and absolute frequency of liver histopathological lesions in tilapias, acaras and trairas

Lesions in Liver	**TILAPIAS n (%) n=100**	**ACARAS n (%) n=208**	**TRAIRAS n (%) n= 58**
Cellular swelling	55 (55)	72 (34.62)	39 (67.24)
Steatosis	58 (58)	167 (80.29)	33 (56.90)
Bile duct fibrosis	16 (16)	60 (28.85)	30 (51.72)
Congestion	26 (26)	123 (59.13)	19 (32.76)
Lymphocytic Infiltrate	29 (29)	52 (25.00)	12 (20.69)
Granulomas	17 (17)	12 (5.77)	8 (13.79)
Hemorrhage	6 (6)	3 (1.44)	3 (5.17)
Intercellular fat infiltration	0 (0.00)	104 (50.00)	1 (1.72)
Necrosis	0 (0.00)	0 (0.00)	1 (1.72)
Parasites	0 (0.00)	10 (4.81)	1 (1.72)
Autolisis	1/103 (0.97)	2/210 (0.95)	19/78 (24.35)
Not represented	1/103 (0.97)	0/210 (0)	1/78 (1.28)

Table 11. Relative and absolute frequency of kidney histopathological lesions in tilapias, acaras and trairas

Lesions in Kidney	**TILAPIAS n (%)** n=77	**ACARAS n (%)** n=86	**TRAIRAS n (%)** n=38
Hyaline droplets in tubular epithelium	20 (25.97)	22 (25.58)	19 (50.00)
Congestion	42 (54.55)	41 (47.67)	13 (34.21)
Lymphocytic Infiltrate	42 (54.55)	39 (45.35)	5 (13.16)
Granulomas	25 (32.47)	2 (2.33)	5 (13.16)
Hemorrhage	26 (33.77)	11 (12.79)	3 (7.89)
Parasites	0 (0.00)	0 (0.00)	2 (5.26)
Interstitial Edema	13 (16.88)	10 (11.63)	1 (2.63)
Tubular degeneration	5 (6.49)	29 (33.72)	4 (5.19)
Autolisis	2/103 (19.41)	3/210 (1.43)	11/78 (14.10)
Not represented	23/103 (2.33)	121/210 (49.19)	28/78 (35.80)

Table 12. Relative and absolute frequency of spleen histopathological lesions in tilapias, acaras and trairas

Lesions in Spleen	**TILAPIAS n (%) n=98**	**ACARAS n (%) n=203**	**TRAIRAS n (%) n=58**
Lymphoid Depletion	86 (87.76)	152 (74.88)	48 (82.76)
Congestion	72 (73.47)	105 (51.72)	5 (8.62)
Granulomas	26 (26.53)	1 (0.49)	1 (1.72)
Autolisis	0 (0)	1/210 (0.48)	9/78 (11.54)
Not represented	5/103 (4.85)	6/210(2.86)	10/78 (12.82)

3.2.5. Histopathological Indices

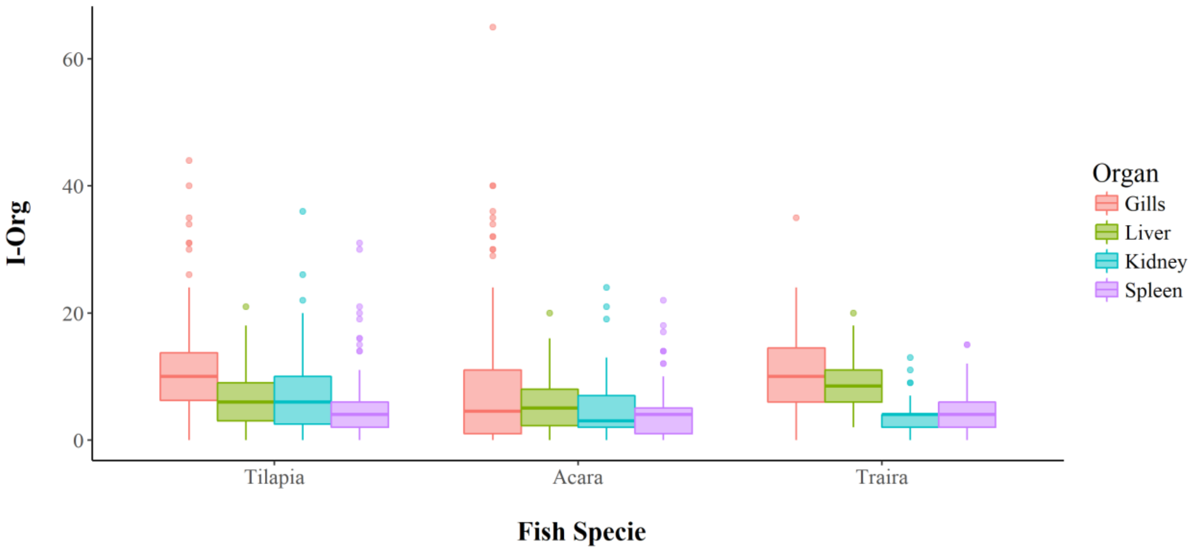
The highest index of histopathological injury for organ (I-Org) considering the year was the gill, with an I-Org of 10 ± 4.34 in 2014, and 10 ± 10.31 in 2015. The highest reaction pattern corresponds to progressive lesions: epithelial hypertrophy and hyperplasia of secondary lamellae. The I-Org of gill showed neither statistical correlation with the water quality indices, nor statistical difference between the two years of collection.

The kidney comes in second place with an average I-Org of 6 ± 4.62 in 2014, and 7.5 ± 7.30 in 2015. The reaction pattern with the highest index was the inflammatory one, being the presence of interstitial lymphocytic inflammatory infiltrate the most frequent finding.

The liver is positioned third with I-Org values of 3.5 ± 2.59 in 2014, and 8 ± 4.47 in 2015. The reaction pattern with the highest index was that of regressive changes, which included esteatose degeneration, one of the most frequent lesions. The I-Org of the liver had no association with water quality indices, and presented a statistical difference between 2014 and 2015, being higher in the latter (p = 9.775 e-05).

The spleen presented the lowest I-Org amongst the evaluated organs with 4 ± 2.54 in 2014, and 4 ± 6.94 in 2015. The reaction pattern with the highest index was that of regressive lesions, which also includes lymphoid depletion, the most common lesion in this population (Graphic 4).

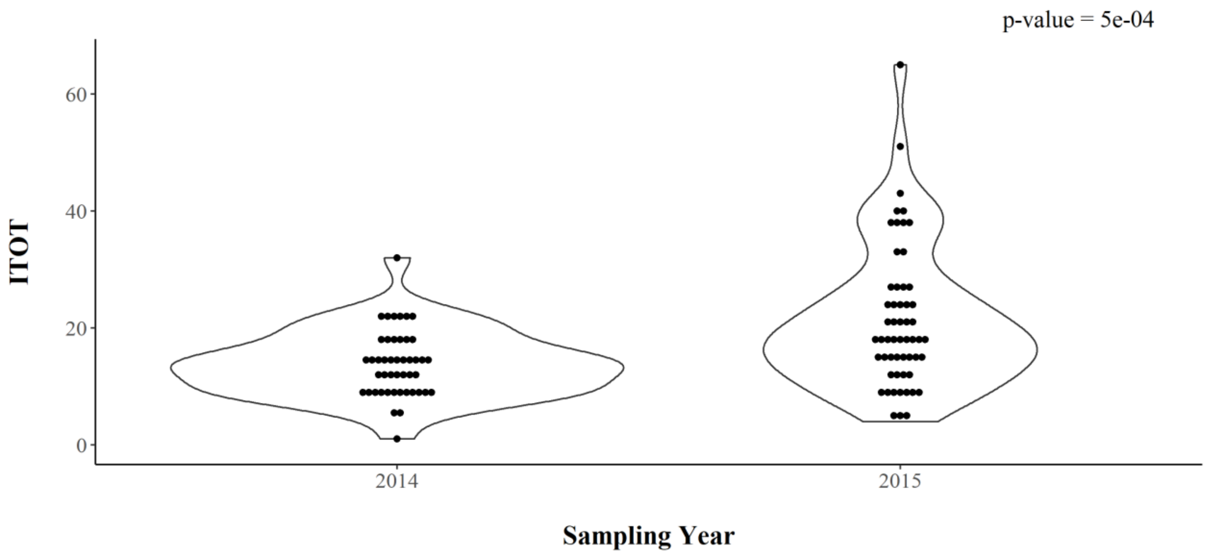
Graphic 4. Distribution of I-Org for tilapias, acaras and trairas



There was no statistical relation between the ITOT and the water quality indices. The ITOT calculated for the year 2014 averaged 14 ± 5.69, and 18.50 ± 12.18 in the individuals captured in 2015.

The ITOT in the two years showed a significant statistical difference (Graphic 5), and the ITOT had a tendency to rise in 2015 (p= 0.000563) meaning that the fish presented more histopathological lesions.

Graphic 5. Distribution of ITOT of tilapias in years 2014 and 2015



When comparing the ITOT of individuals from sites S5 and S6, located in the arm of Rio Grande, with the other sampling sites S1, S2, S3 and S4, there was not a statistical difference.

3.3. ACARAS

A total of 246 fish were caught, from which 84 were females, and 162 males. In summer of 2014 124 individuals were collected, and 39 in the spring of the same year. In the summer of 2015 45 individuals were collected, and 38 in spring. All the collected individuals were used for macroscopic and biometric analysis. For histopathological analysis 210 of these individuals were processed, distributed as shown in the table 13.

Table 13. Distribution and sex of the acaras captured for the four collection periods in summer and spring

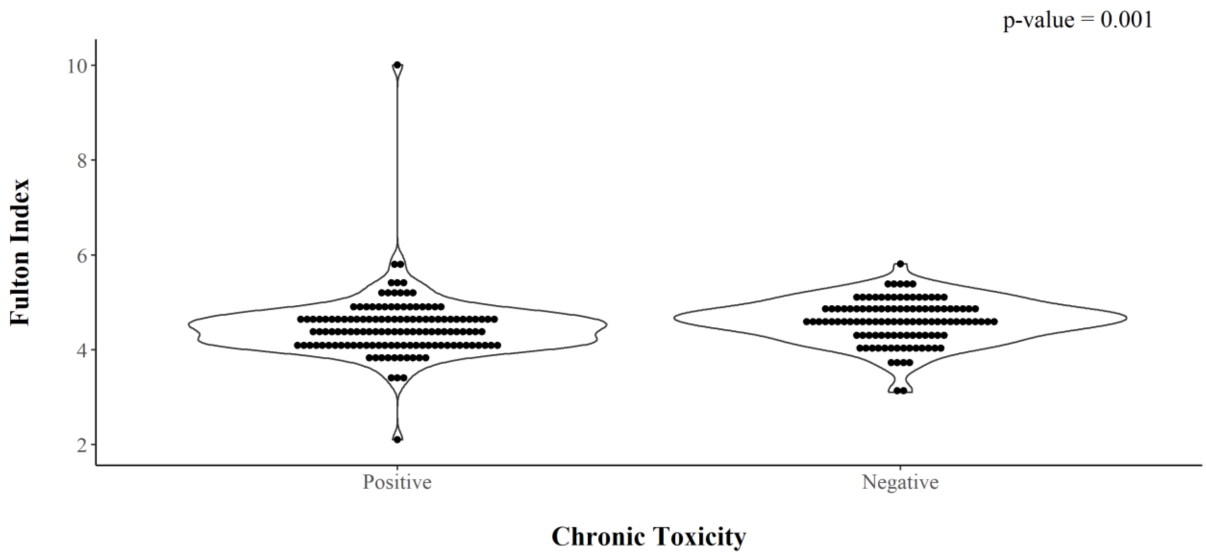
Sample period	Male	Female	Total
Summer 2014	53	35	88
Spring 2014	24	15	39
Summer 2015	21	24	45
Spring 2015	30	8	38
Total	128	82	210

3.3.1. BIOMETRY AND FULTON INDEX

The average weight (grams) was 144 ± 48.85 in 2014, and 145 ± 43.8 in 2015. The standard length was on average 14.5 ± 1.53 in 2014, and 14.8 ± 1.36 in 2015. No statistically significant difference between weight and standard length was found when comparing males and females, or different collection times.

In 2014, the Fulton index averaged 4.61 ± 0.68; and in 2015, 4.44 ± 0.5. A trend of lower body condition factor was observed during 2015, which was also observed in the tilapia. There was a small statistical difference ($p = 0.033$) between the two years studied. When comparing the factor of corporal condition with the chronic toxicity reported, a discrete statistical significance was observed ($p=0.001$), therefore, when no effects of chronic toxicity were observed in the water, a tendency of the fish to present a higher body condition factor was found, as shown in the graphic 6. The data was similar for tilapias.

Graphic 6. Distribution of the Fulton index of acaras related to the effect of chronic toxicity in water



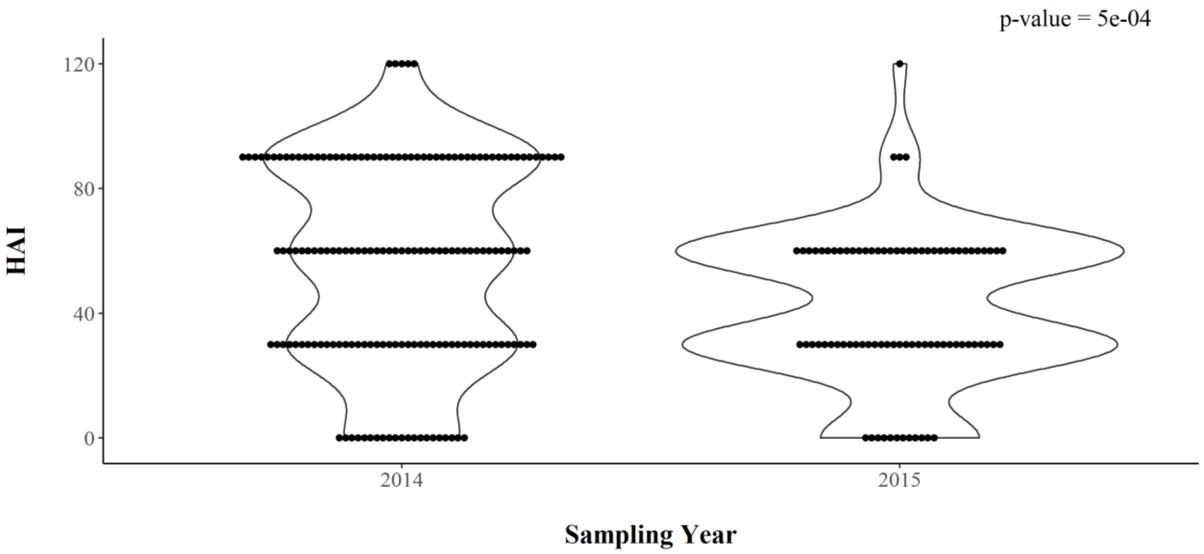
3.3.2. NECROPSY FINDINGS

Skin lesions were found in 2.85% (7/246) individuals, parasites in celomic cavity in 2.85% (7/246), absence of fat deposits in celomic cavity in 2.03% (5/246), low fat levels in 21.14% (52/246), moderate fat deposits in 43.09% (106/246) and abundant fat in 32.11% (79/246). Liver fat infiltration was observed in 22.36% (55/246), hepatomegaly in 5.69% (14/246), whitish nodules in liver 5.28% (13/246). In gills, the presence of mucus was found in 6.91% (17/246). Table 8 and figure 3 the macroscopic findings observed during the necropsy.

3.3.3. Health Assessment Index (HAI)

The HAI average was on 60 ± 32.48 for the year 2014 and on 30 ± 24.66 for 2015. No correlation was found between HAI and water-related indices. There was no statistical difference between HAI and sampling site. A statistically significant difference was found between the two years of the study as observed in graphic 7 ($p = 0.0004701$) with lower indices for the second year, which was not observed in the tilapia.

Graphic 7. HAI of acaras captured in 2014 compared with the tilapias captured in 2015



3.3.4. Histopathological Findings

The main histopathological lesions observed during the year 2014 and 2015 are listed in the tables 9, 10, 11 and 12, and in the figures 5, 6, 7 and 8 for gills, liver, kidney and spleen, respectively.

The most frequent microscopic findings in the gills were: pillar cell rupture 53.92% (110/204), epithelial hypertrophy 53.43% (109/204), interstitial edema 39.71% (81/204), congestion 38. 73% (79/204) and epithelial hyperplasia 26.47% (54/204); In liver: steatosis 80.29% (167/208), congestion 59.13% (123/208), intercellular fat infiltration 50% (104/208), cellular swelling 35.10% (73/208) and bile duct fibrosis 28.85% (60/208); In kidneys the most common findings were: congestion in 47.67% (41/86), interstitial lymphocytic infiltrate 45.35% (39/86), hemorrhage 12.79% (11/86), interstitial edema 11.63% (10/86) and granulomas in 2.33% (2/86); In spleen: lymphoid depletion in 74.88% (152/203), congestion in 51.72% (72/203) and granulomas in 0.49% (1/203).

3.3.5. Histopathological Indices

The organ with the highest I-Org was the gill; in 2014 the mean lesion index was 3 ± 3.89, and averaged 12 ± 10.95 in 2015. The reaction pattern with the highest index was that of regressive and non-reversible lesions, which includes rupture of pillar cells, the most common lesion for this population. The I-Org of gills did not present a correlation with the water quality variables, and presented a statistical difference between the years of the 2014 and 2015 collections, being the highest I-Org values those of the latter year (p = <2.2e -16).

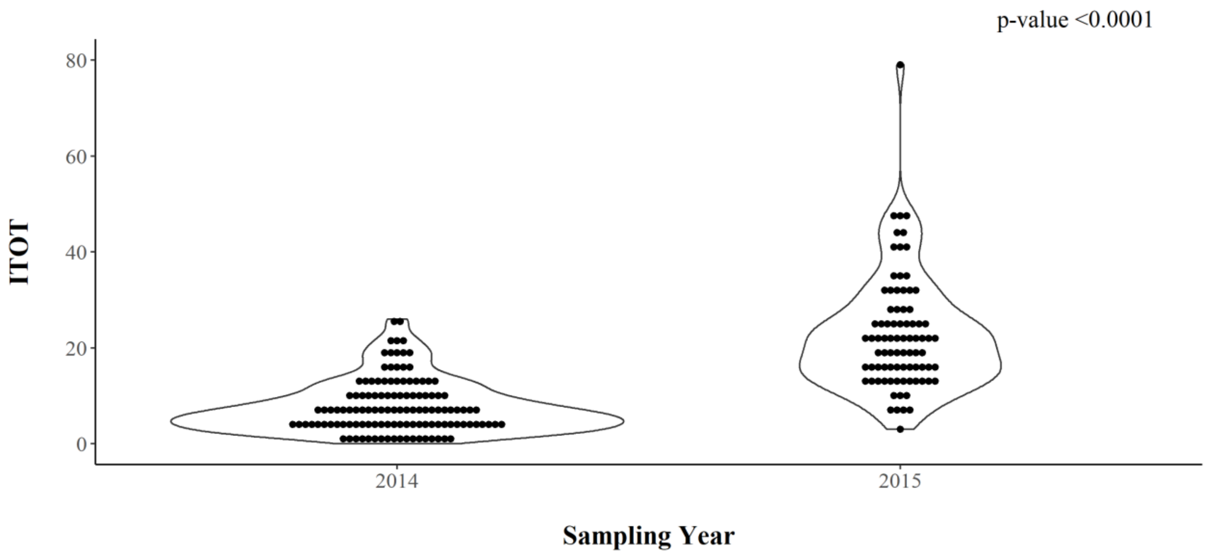
In the second place, the liver presented an I-Org of 4 ± 3.41 for the year 2014, and of 8.5 ± 3.84 for 2015. The highest reaction pattern for the liver was the regressive and reversible lesion category that includes hepatocyte steatosis. The I-Org of the liver did not present a statistical correlation with the water quality variables, and it was statistically higher in the year 2015 (p = 5.183e - 13).

The kidney presented an I-Org of 4 ± 4.72 in the first year, and 7 ± 5.45 during the year 2015. The highest reaction pattern for the kidney was regressive and reversible pattern such as tubular degeneration and congestion was the most frequent.

The spleen presented the lowest I-Org with an average value of 4 ± 3.83 in 2014 and 2 ± 2.39 in 2015 (Graphic 4). The highest reaction pattern in the spleen was that of regressive lesions, which includes lymphoid depletion, the most frequent alteration found in this organ.

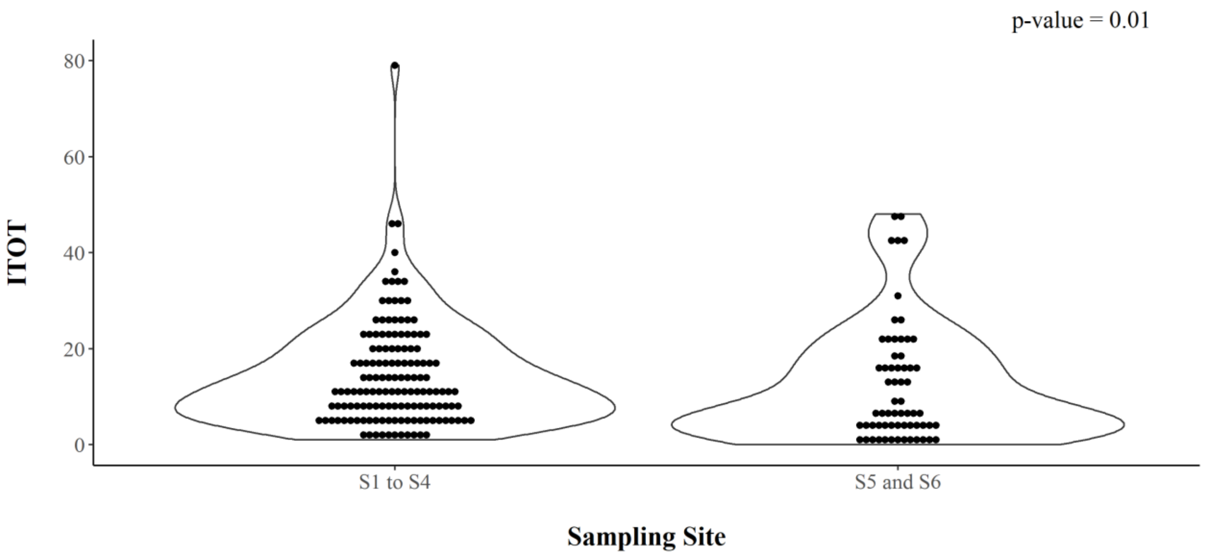
The ITOT did not present a correlation with the water quality indices, the average value for the year 2014 was of 7 ± 5.71, and 21 ± 12 for the individuals captured in 2015. The ITOT presented significant statistical difference between 2014 and 2015 (p = <2.2e-16) being higher in the year 2015 as shown in graphic 8. This was also observed in the tilapia.

Graphic 8. Distribution of ITOT of acaras in years 2014 and 2015



Furthermore, in the S5 and S6 sites (Rio Grande) slightly lower ITOT values were calculated with respect to values corresponding to the other sample locations S1-S4 ($p = 0.0146$), result which was also observed in the tilapia (Graphic 9).

Graphic 9. ITOT of acaras captured in sampling sites S1 to S4 compared with the index of individuals captured in sites S5 and S6 (Rio Grande)



3.4. TRAIRAS

A total of 78 individuals were captured, 32 of which were females, 45 were males and it was not possible to determine the sex of one of them. The distribution of the population is shown in table 14.

Table 14. Distribution and sex of the trairas captured for the four collection periods in summer and spring

Sample period	Male	Female	Undetermined	Total
Summer 2014	24	5	1	30
Spring 2014	15	13	0	28
Summer 2015	6	1	0	7
Spring 2015	7	6	0	13
Total	52	25	1	78

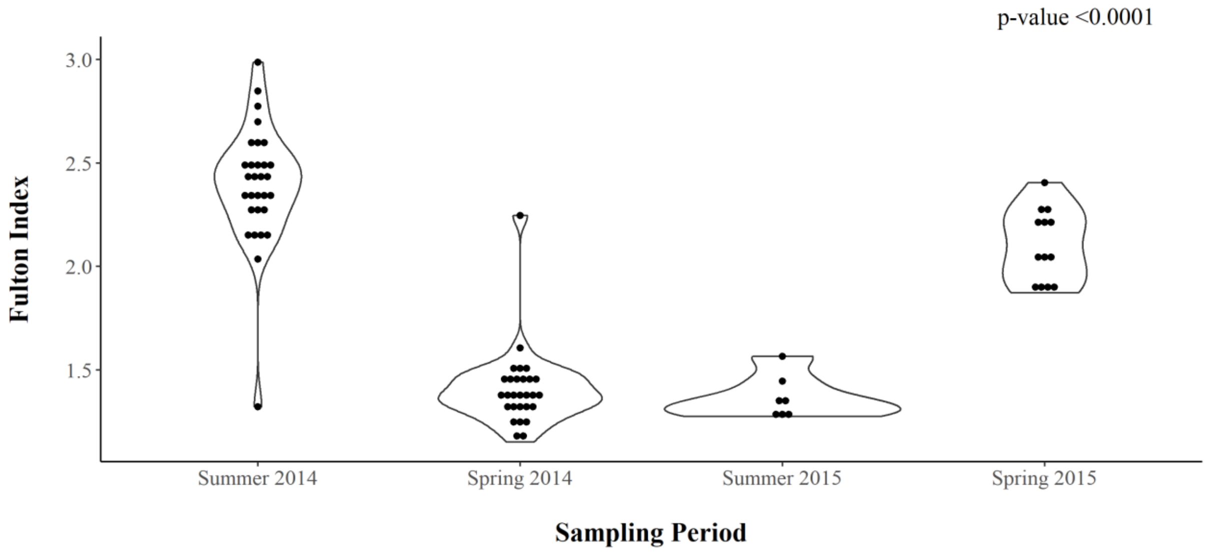
3.4.1. BIOMETRY AND FULTON INDEX

The average weight (grams) for individuals during the collection period of summer of 2014 was 490 ± 137.89 with an average standard length (centimeters) of 27.75 ± 2.10; in the spring of the same year the average weight was 529 ± 208.32 g., and the length was 32.95 ± 3.7 cm. In the summer of 2015, the average weight was 642.5 ± 182.51 g., and the length was 36.5 ± 3 cm. In the spring of the same year the weight was 506 ± 185.68 g., and the average length was 30 ± 3.3 cm.

The Fulton index of the individuals collected in the summer of 2014 averaged 2.42 ± 0.3, and in the spring of the same year, it was 1.3 ± 0.19. In the year 2015 in the summer the average was of 1.35 ± 0.1, and in the spring it was 2.1 ± 0.17.

The Fulton index did not present a statistical correlation with the indices related to water quality. Statistical differences were found between Fulton index of the spring collection periods of 2014 and that of the spring of 2015 ($p = 0.0022$), and between the values of summer and spring of 2015 ($p = 0.0173$) as shown in the graphic 10. This result was different in the other species, which showed a difference between years 2014 and 2015, with a lower index in 2015.

Graphic 10. Distribution of Fulton index of trairas captured in the four sampling periods in 2014 and 2015



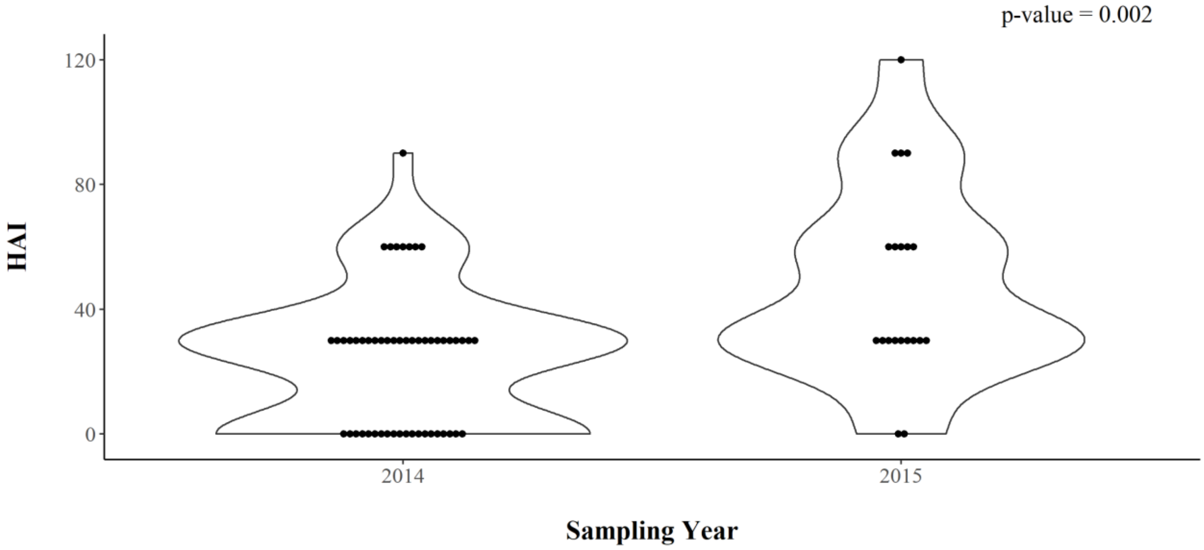
3.4.2. Necropsy Findings

Skin lesions were found in 5.56% (4/72) of the individuals. Parasites in celomic cavity in 52.78% (38/72), absence of fat deposits in celomic cavity in 4.17% (3/72), low fat in 55.56% (40/72), moderate fat deposits in 36.11% (26/72) and abundant fat in 4.17% (3/72). In liver, whitish nodules were observed in 11.11% (8/72). On gills, mucus was observed in 69.44% (50/72) (Table 8). Macroscopic lesions were in figure 4.

3.4.3. Health Assessment Index (HAI)

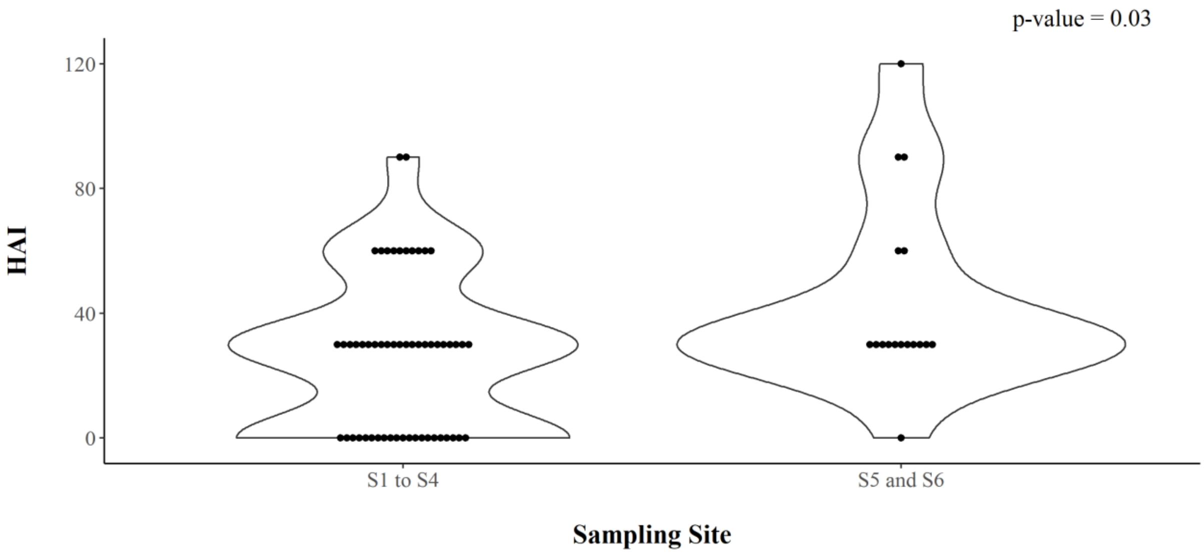
No statistically significant correlation was observed between HAI and indices related to water quality. The average HAI was 30 ± 22.49, and 30 ± 31.39 for 2014 and 2015, respectively. A statistically significant difference was observed between the HAI calculated for 2014 and 2015 (p = 0.0018) with the indices in the individuals collected during 2015 showing a notable tendency to be higher (Graphic 11), this tendency being exclusively observed in this species, in this study.

Graphic 11. HAI of trairas captured in 2014 compared with trairas captured in 2015



When comparing the HAI values of the sampling sites S1 to S4 to those obtained in points S5 and S6 (Rio Grande), there was a significant statistical difference (p = 0.028) with a higher HAI tendency in the Rio Grande sites as shown in graphic 12, while tilapias and acaras presented a lower HAI in 2015.

Graphic 12. HAI of trairas captured in sampling sites S1 to S4 compared with the index of individuals captured in sites S5 and S6 (Rio Grande)



3.4.4. Histopathological Findings

The histopathological lesions observed are presented in the tables 9, 10, 11 and 12, and in the figures 5, 6, 7 and 8 for gills, liver, kidney and spleen, respectively. The most frequent microscopic findings in the gills were: rupture of pillar cells 100% (53/53), epithelial hypertrophy 86.79% (46/53), congestion 56.60% (30/53), epithelial hyperplasia 43.40% (23/53), and interstitial edema 30.19% (16/53). In liver, cellular swelling was observed in 67.24% (39/58), steatosis in 56.90% (33/58), bile duct fibrosis in 51.72% (30/58), congestion in 32.76% (19/58), and lymphocytic infiltrate in 20.69% (12/58). In kidney, the most common finding was the presence of hyaline droplets in tubular epithelium in 50% (19/38), congestion in 34.21% (13/38), interstitial lymphocytic infiltrate in 13.16% (5/38), granulomas in 13.16% (5/38), and hemorrhage in 7.89% (3/38). In the spleen, the most frequent finding was lymphoid depletion, 82.76% (48/58), followed by congestion in 8.62% (5/58), and the presence of granulomas in 1.72% (1/58).

3.4.5. Histopathological Indices

The organ with the highest index of histopathological lesion (I-org) was the gill, with an average value for 2014 of 8 ± 4,14, and of 17 ± 8.49 for 2015. The highest reaction pattern in the gills was regressive lesions pattern and non-reversible, which includes rupture of pillar cells, the most commonly observed lesion. The I-Org of the gills did not present a statistical relationship with any of the water quality indices; although there was no statistical difference with respect to the year of collection, it had a tendency to be higher in the summer of 2015 ($p = 0.0893$), and in spring of the same year ($p = 0.0682$).

The second organ with the highest I-Org was the liver that for 2014 presented a mean value of 8 ± 3.61, and of 12.5 ± 3,97 for 2015. The highest reaction pattern in liver was the regressive pattern and the most frequent lesion being cellular swelling. The I-Org of the liver had no statistical correlation with the water quality indices, and a difference between the collection periods was observed, being higher during the two summer periods of 2015 (2.434 and -05), and spring of 2015 ($p = 0.000254$).

In the third place, the spleen with an average I-org for the year 2014 of 4 ± 2.87, and of 4 ± 4.05 for the year 2015. The highest reaction pattern in the spleen was, as in the two previously evaluated species studied, the pattern of regressive lesions, being the depletion of lymphoid cells the most frequent lesion, as well as in the other two other species.

Finally, the kidney with I-org average for 2014 of 3 ± 1.73 and of 5 ± 3.83 for 2015. The highest reaction pattern in the kidney was that of regressive lesions, which includes the presence of hyaline deposits in the tubular epithelium, the most common lesion in these individuals. (Graphic 4).

The average ITOT value calculated for the year 2014 was of 16 ± 7.26, and 28 ± 8.94 in the individuals captured in 2015. The ITOT did not present a statistical correlation with any of the water quality indices. It presented statistical difference between the years 2014 and 2015 (p = 3.283 e -5), being higher during 2015 (Graphic 13), like in the other two species studied, the ITOT presented a higher value in this species compared with the tilapias and acaras. There was no statistical difference between the ITOT of points S1 - S4 and points S5 and S6 (Rio Grande).

Graphic 13. Distribution of ITOT of trairas in years 2014 and 2015

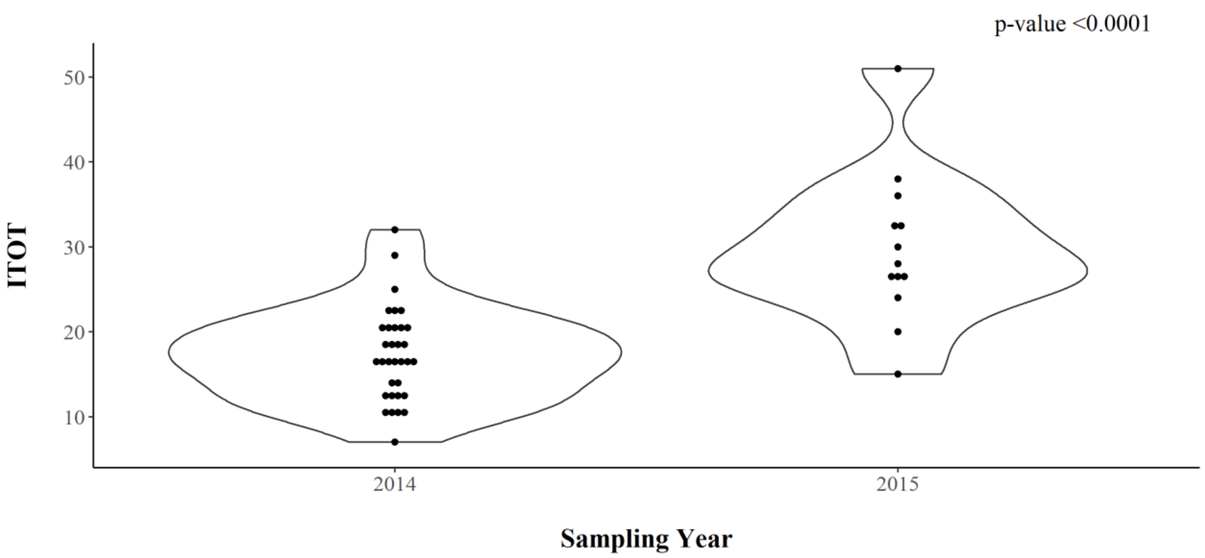
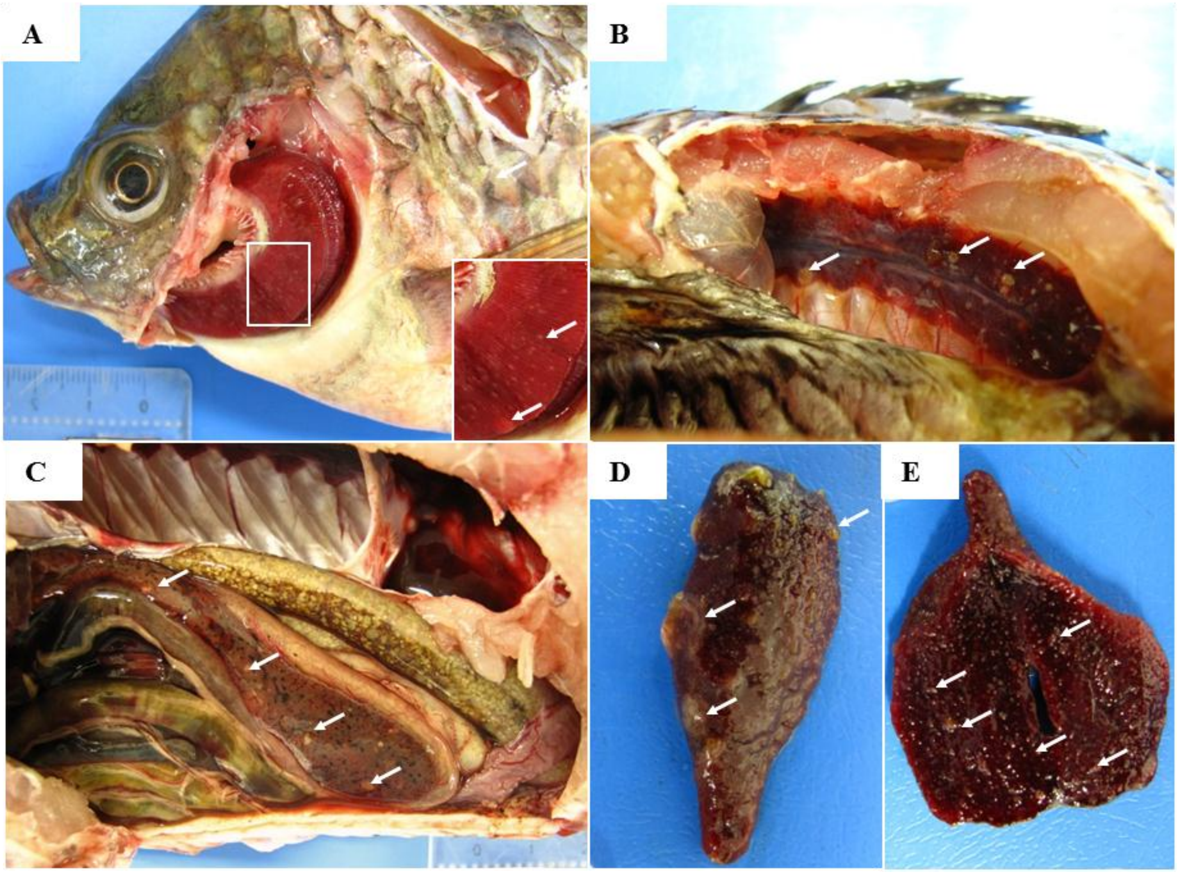
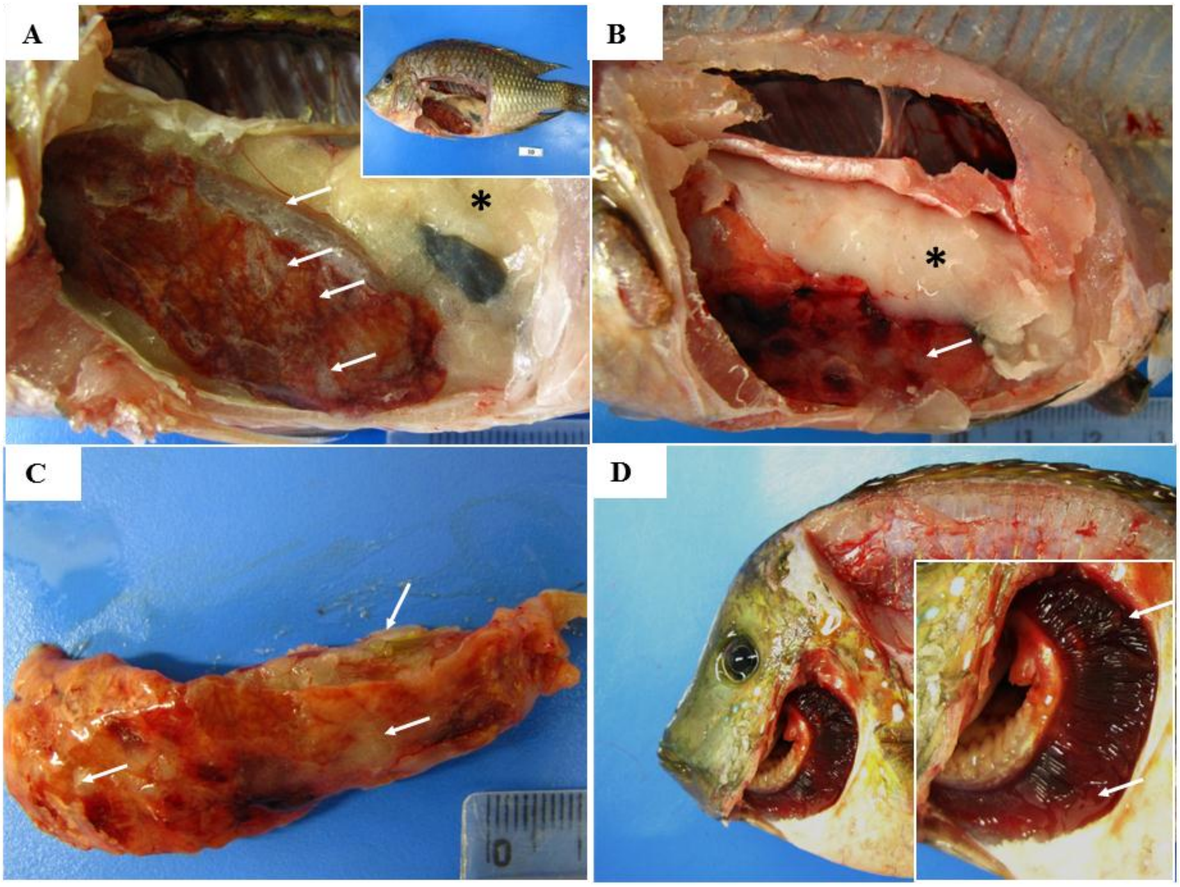


Figure 2. Gross lesions in tilapias



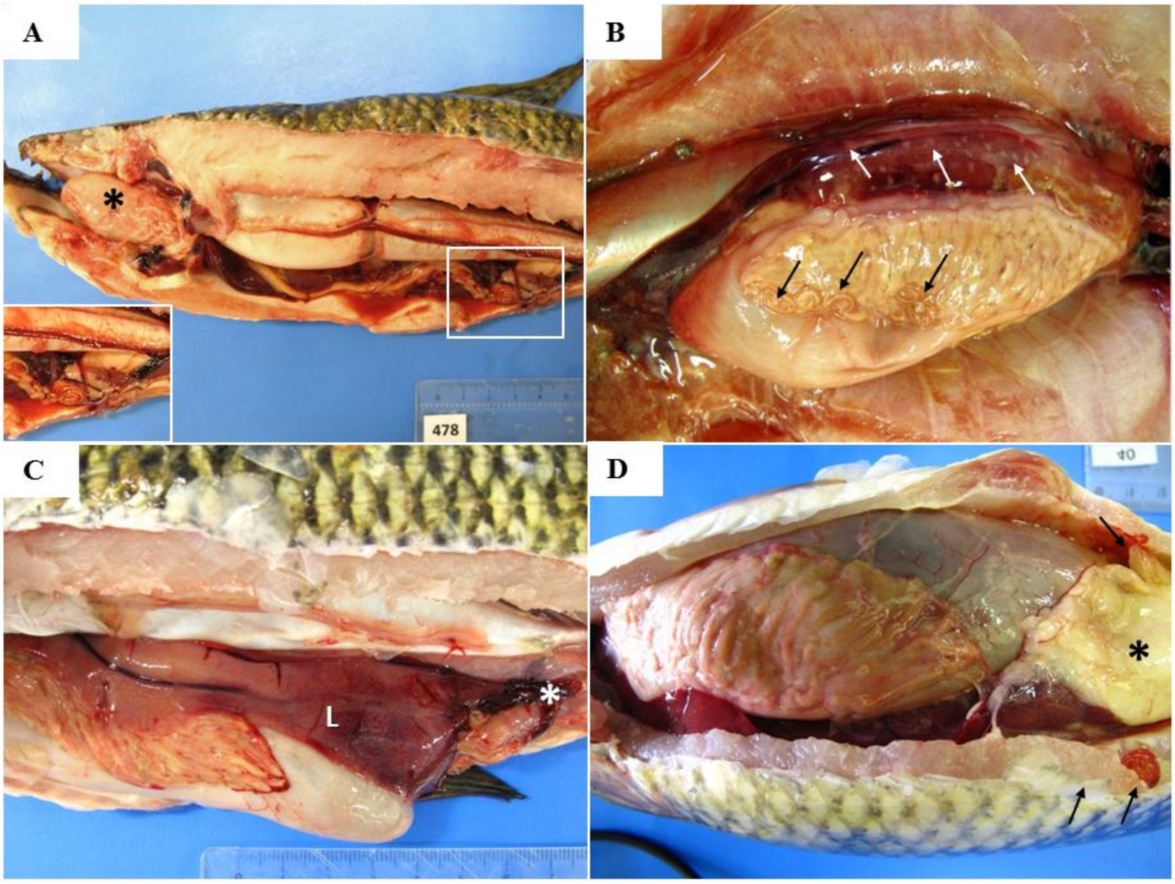
A. Gills *in situ*, left side view. Multiple whitish foci (arrows) in the irregular surface and covered by mucus. Multiple granulomas in gills. **B.** Caudal kidney *in situ*. Multiple whitish foci on its surface (arrows). Multiple granulomas in kidney. **C.** Celomic cavity with main organs *in situ*. Liver, multiple whitish foci disseminated (arrows). Multiple granulomas in liver. **D.** Spleen. Splenomegaly and multiple whitish foci (arrows) and irregular surface. Multiple granulomas in spleen. **E.** Spleen, longitudinal section. Multiple whitish foci disseminated (arrows). Multiple granulomas in spleen.

Figure 3. Gross lesions findings in acaras



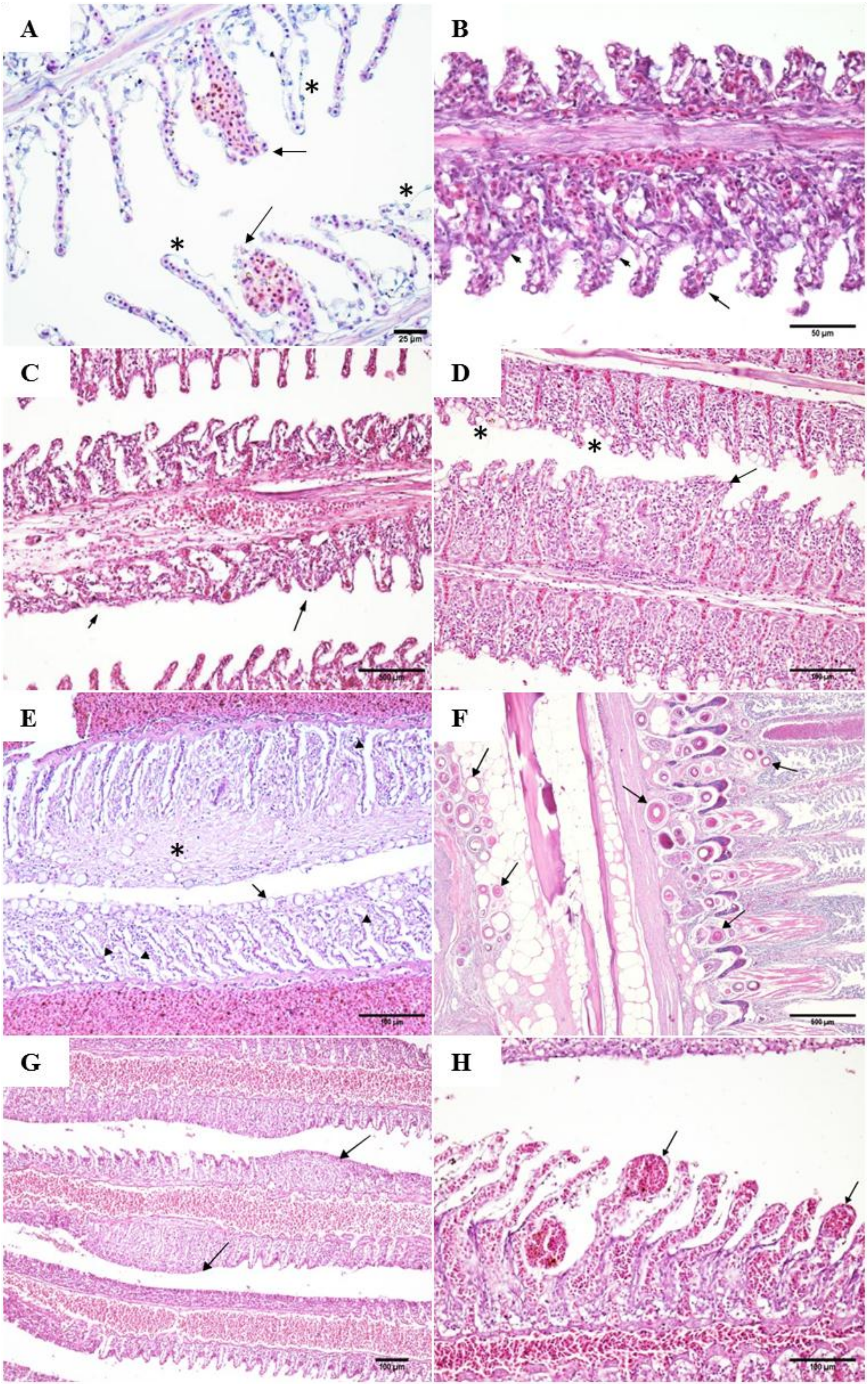
A. Celomic cavity, liver. Abundant deposit of adipose tissue in celomic cavity (*). Hepatomegaly, liver showing multiple areas with infiltrated fat (arrows). Insert on top right, side view of acara at gross. **B.** Celomic cavity, liver. Liver with diffuse fat infiltration (arrows) and hemorrhage foci, abundant deposition of visceral adipose tissue (*). **C**. Liver. Abundant adipose tissue infiltrating the hepatic parenchyma (arrows). **D.** Gills in situ. Foci of irregular surface covered by mucus (arrows).

Figure 4. Gross lesions in trairas



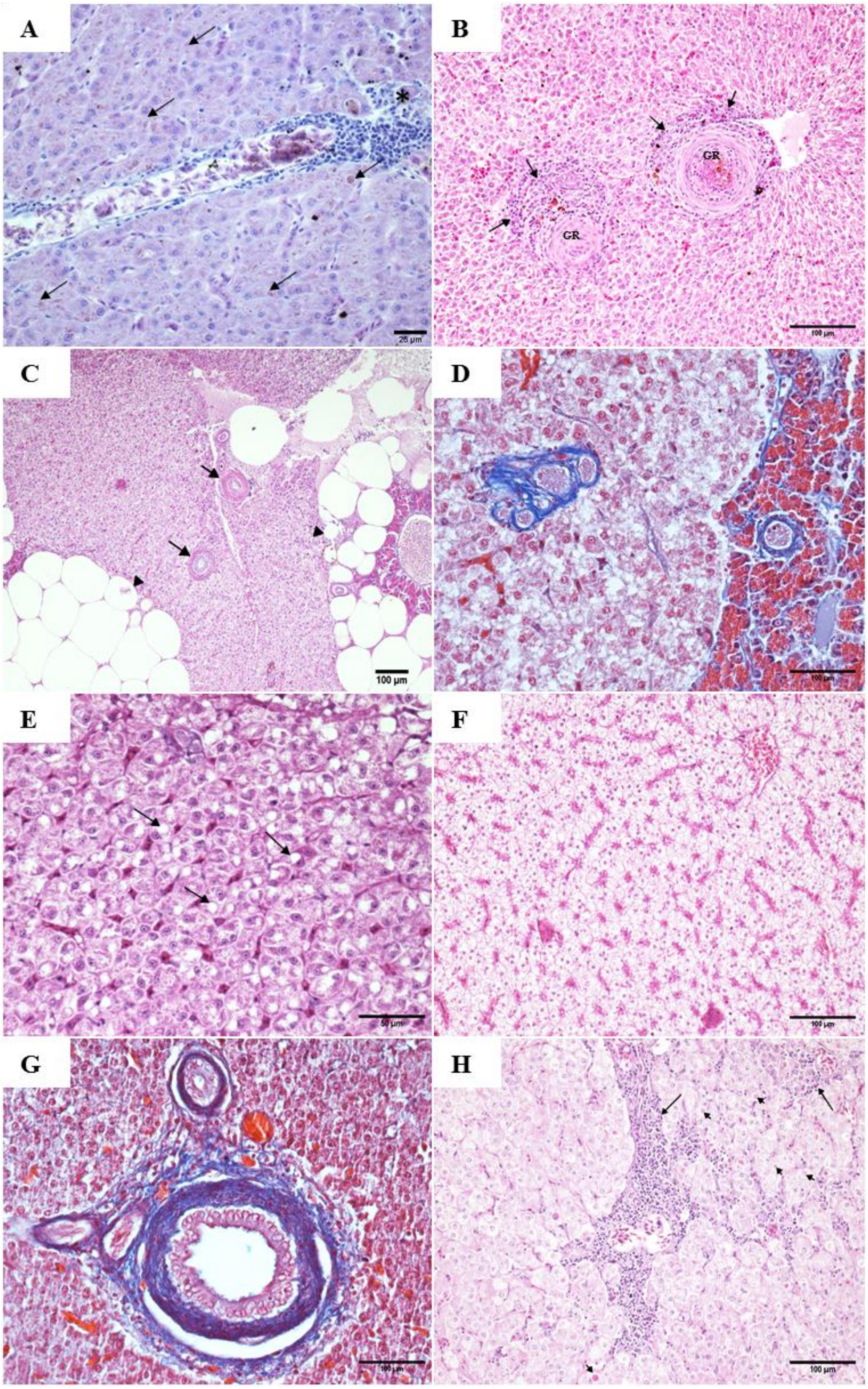
A. Side view of cross section of body, traira. Cranial displacement of stomach into oral cavity (*). Observe scarce deposits of fat in the cavity. White box, insert figure, presence of parasites in the celomic cavity. **B.** Celomic cavity. Presence of parasites on gastric serosa (black arrows), multiple whitish spots on the hepatic surface (white arrows). **C.** Celomic cavity, Liver (L). Hepatic degeneration. Hemorrhage focus in the caudal portion of the liver (*). **D.** Celomic cavity. Deposit of fat in cavity (*). Presence of parasites in a coelomic cavity and a parasite in muscle (arrows).

Figure 5. Photomicrography of lesion in gill sections of tilapias, trairas and acaras



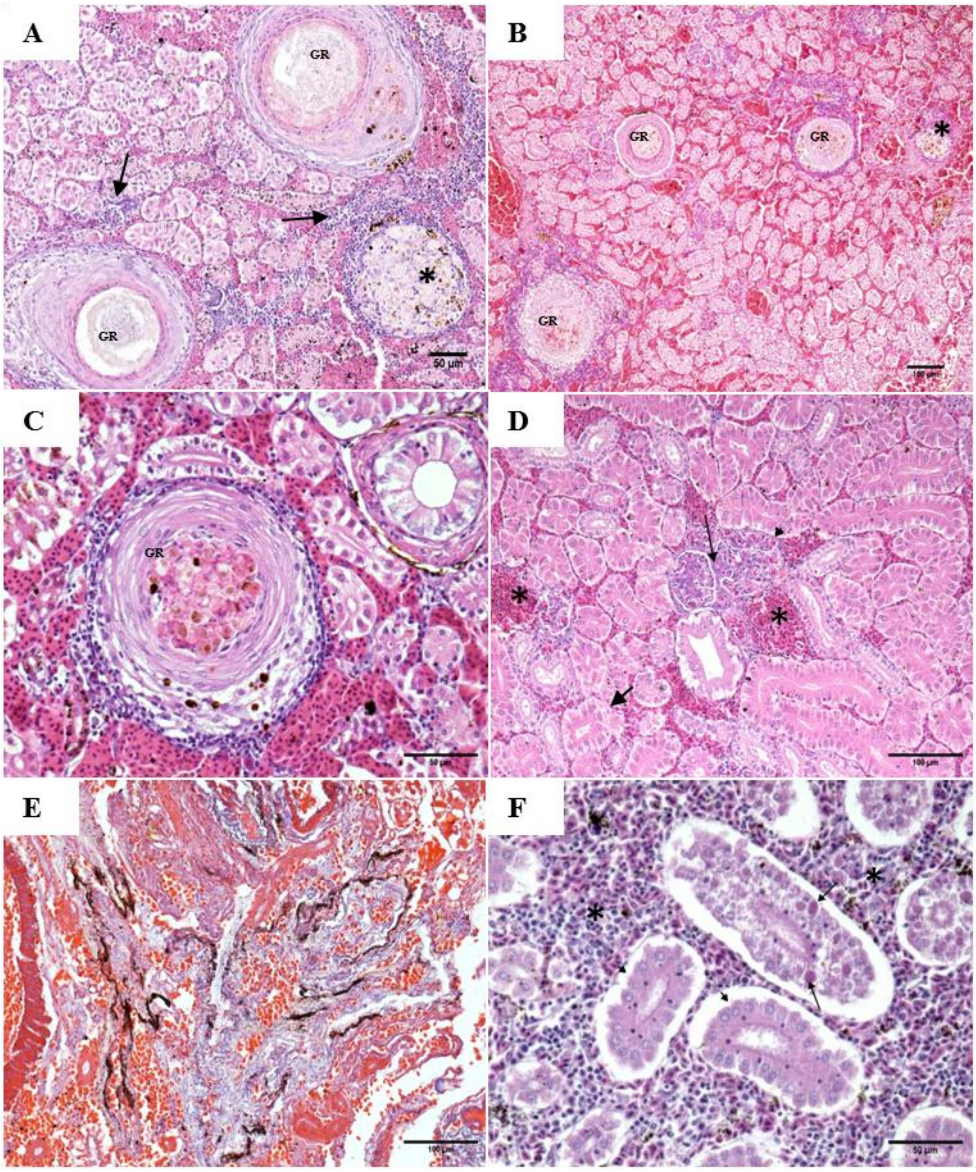
Photomicrography of gill sections. A. Tilapia. Aneurysms-Telangiectasia in secondary lamellae (arrows), lifting of secondary lamellae epithelium (*) 40X H&E. B. Tilapia. Rupture of pillar cells (arrows), secondary lamella atrophy, lamellar epithelial hypertrophy (arrowhead) 40X H&E. C. Tilapia. Fusion of secondary lamellae (short arrow), secondary lamella atrophy (arrow) 20X H&E. D. Tilapia. Hyperplasia of mucous cells (*), fusion of secondary lamellae loss of normal structure (arrow) 20X H&E. E. Acara. Fusion of secondary lamellae and rupture of pillars cells of secondary lamellae (arrowheads), necrosis (*), hyperplasia of mucous cells (arrow), 20X H&E. F. Acara. Multiple granulomas in secondary lamellae (arrows) 4X H&E. G. Traira. Fusion and atrophy of secondary lamellae (arrows) 10X H&E. H. Traira. Aneurysms in secondary lamellae (arrows) 20X H&E.

Figure 6. Photomicrography of lesions in liver sections of tilapias, trairas and acaras



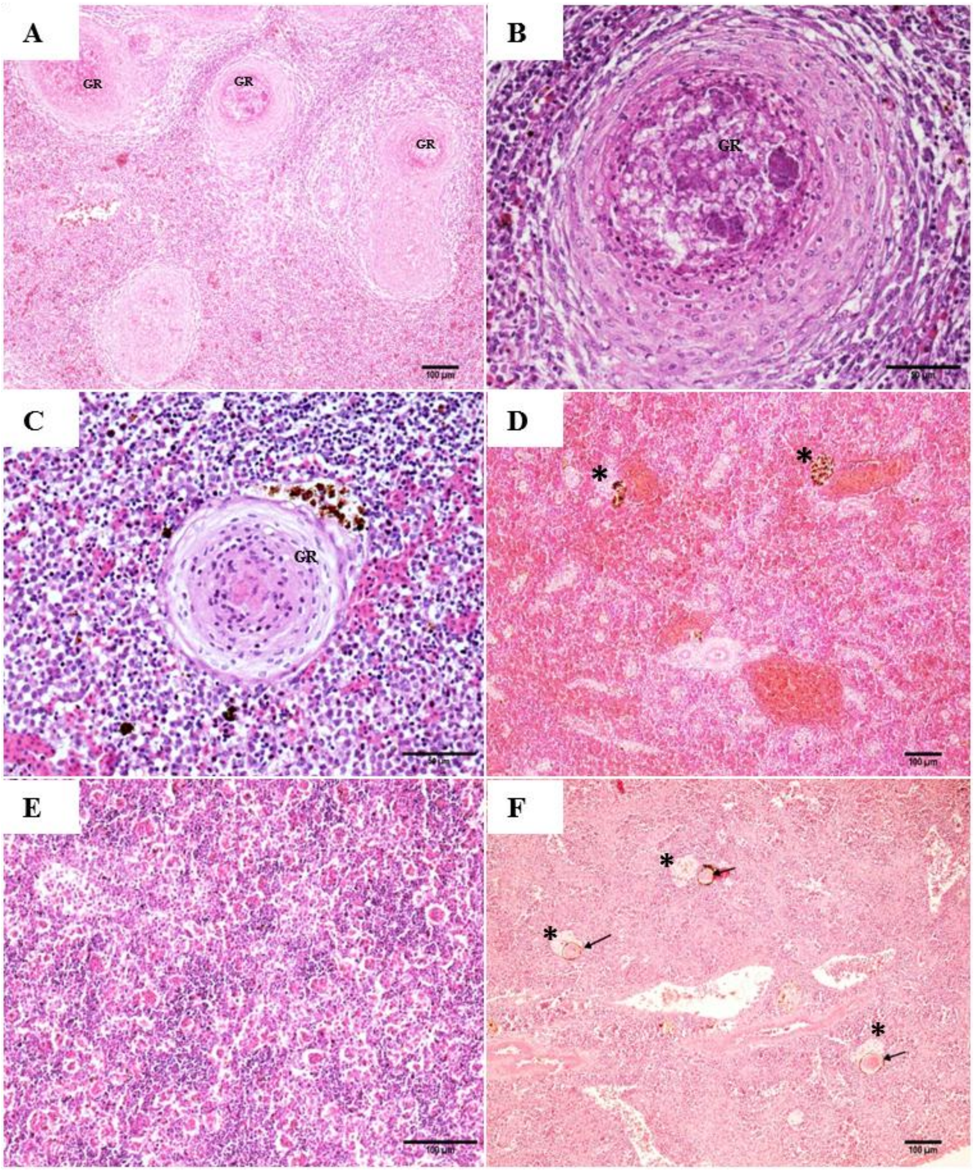
Photomicrography of liver sections. A. Tilapia. Moderate intracellular deposits of lipofuscin and hemosiderin (arrows), discrete perivascular lymphocytic infiltrate (*), 40X H & E. B. Tilapia. Granulomas (GR), lymphocytic infiltrate surrounding granulomas (arrows), 20X H & E. C. Acara. Multifocal fat infiltration in liver (arrowheads), fibrosis around bile ducts (arrows), 20X H & E. D. Acara. Periductal fibrosis (blue color), 40X Masson's trichromic. E. Acara. Discrete microgoticular steatosis (arrows), 40X H & E. F. Traira. Diffuse cellular swelling 20X H & E. G. Traira. Periductal Fibrosis, 40X Masson's Trichromic. H. Traira. Perivascular lymphocytic infiltrate (arrows), intracellular deposits of lipofuscin and hemosiderin (short arrow), 20X H & E.

Figure 7. Photomicrography of representative lesions in kidney sections of tilapias, trairas and acaras



Photomicrography of kidney sections. A. Tilapia. Multiple granulomas (GR), center of melanomacrophages (*), lymphocytic infiltrate (arrows) 20X H & E. B. Tilapia. Granulomas (GR), diffuse congestion, 10X H & E. C. Tilapia. Granuloma (GR), 40X H & E. D. Acara. Tubular degeneration (short arrow), increased glomerular cellularity (arrowhead), glomerular synechia (arrow), diffuse congestion (*), 20X H & E. E. Acara. Interstitial fibrosis, 20X Masson's Trichromic. F. Traira. Hyaline droplets in cells of tubular epithelium (arrows), discrete tubular degeneration (short arrows), lymphocytic infiltrate (*) 40X H & E.

Figure 8. Photomicrography of representative findings in spleen sections of tilapias, trairas and acaras



Photomicrography of spleen sections. A. Tilapia. Granulomas (GR), 10X H & E. B. Tilapia. Granuloma (GR), 40X H & E. C. Tilapia. Granuloma (GR) with melanomacrofagos in the periphery (*), 40X H & E. D. Acara. Lymphoid depletion, melanomacrophage centers (*) 10X H & E. E. Acara. Lymphoid depletion, melanomacrophage centers (*) 20X H & E. F. Traira. Lymphoid depletion, granulomas (arrows) surrounded by melanomacrophage centers (*), 10X H & E.

4. DISCUSSION

Studies that monitor water reservoirs and perform water and sediment analyses periodically, are part of public politics adopted by environmental agencies like the CETESB, they are fundamental due to the presence of xenobiotic agents in water and sediment, and can be decisive in a chronic poisoning process. At the same time, although the use of fish as bioindicators of environmental quality has been recognized as an important and useful tool, environmental agencies do not use them routinely. In this context, the research was conducted aiming an integrated analysis of water quality of the Billings dam using water and sediment parameters and performing a pathology analysis of three species of fish as biomarkers.

The geographical location of the reservoir and its surroundings is a crucial factor that determines the physicochemical characteristics of its surface waters and sediment (CETESB, 2010; CARDOSO-SILVA et al., 2014). The water of the Billings reservoir did not present a statistical difference in relation to the physicochemical parameters in the years 2014 and 2015 in the points analyzed, despite the prolonged drought that the region experienced (ESCOBAR, 2015). Regarding previous years, Cunha et al., (2011) evaluated the physicochemical parameters of the reservoir water during 34 years (1977 to 2010) and showed how throughout this period the water was also characterized as eutrophic, due to anthropic activities, population growth and irregular use of the land.

The water crisis suffered by the State of São Paulo during the years 2014 and 2015 generated a high impact on different socio-economic areas, notably impacting the Metropolitan Region of São Paulo (CETESB, 2014, 2015; COELHO; CARDOSO; FIRPO, 2015; ESCOBAR, 2015). Furthermore, water quality was more impacted, based on physicochemical, microbiological and toxicity parameters during 2014, showing more alterations compared to 2015 which also presented a lower quality compared to the average since year 2010 (CETESB, 2014, 2015).

In addition, Pires et al., (2015) recently analyzed the limnological parameters that characterized the reservoir as a eutrophic environment, a conclusion which previous studies had already reached (CARDOSO-SILVA et al., 2014; CETESB, 2014, 2015). Meanwhile, although the eutrophic state is characteristic of the reservoir, the concentrations of total phosphate and BOD, in the period covered by this study, were higher than the historic average (CETESB, 2015) which was also reflected in an increased chlorophyll-*a* concentration.

In this context, the evaluation of the cyanobacteria cell count also showed a significant increase in the study period, showing that the water quality of the reservoir worsened and there was an increased risk of occurrence of cyanobacteria blooms and fish mortality, as shown in the reports of the CETESB of 2014 and 2015 (CETESB, 2014, 2015). The eutrophication process can occur naturally or artificially, being the last one the case of the studied area, its anthropogenic origin lies mainly in the outflow of domestic, agricultural, livestock, or industrial waste(SMITH, 2003; MACEDO; LÚCIA; SIPAÚBA-TAVARES, 2010; TUNDISI; MATSUMURA-TUNDISI, 2010). During 2014, 23 cases of fish mortality were reported and in 2015, there were 5 reports (CETESB, 2015). The increase in the incidence of fish mortality in the reservoir during 2014 could be associated with the effects of eutrophication, which leads to the proliferation of algae species and have toxic effects on fish (KARIM et al., 2003; KARAOUZAS et al., 2017).

The compartmentalization of the reservoir and the partial physical barrier between the Rio Grande arm (S5 and S6) and the other sites of sampling (S1 to S4) were highlighted in this study and showed that in the Rio Grande region the concentrations of phosphorus, the chlorophyll-*a* concentration, and the cyanobacteria counts were lower in comparison with the other sites. That had already been indicated by other authors (CARDOSO-SILVA et al., 2014; PIRES et al., 2015), although S5 and S6 classified as mesotrophic to hypereutrophic in the study period.

The IQA values in the present study did not show either a statistical difference between the sampling sites nor with respect to the time of sampling. However, the IQA was higher in 2015 compared to 2014, which were lower when compared with the historic averages (CETESB, 2014). This data may be due to the low precipitations in those two years resulting in a lower amount of water pumped from the Pinheiros River to the Billings reservoir. On the other hand, the pumped water was higher in 2015 reflected in the IQA average, due to a greater concentration of pollutants in the points closest to the Pinheiros entrance, mainly in point S1 (CETESB, 2015).

The IVA was worse in 2014, and lower in 2015 compared with the historic average. The IVA indicates the quality of water to protect aquatic fauna and flora in general, considering the presence and concentration of toxic substances and their degree of toxicity, pH and dissolved oxygen, as well as the IET (CETESB, 2016). The IVA for this period remained in the categories of fair to very bad in most samples (4,16% was classified as good), without showing statistical differences among location and sampling time. In this way, the IVA showed that the water quality was worse but showed discrete improvement in 2015.

The effect of chronic toxicity detected in several samples considered for this study can be attributed to the presence of sewerage and other xenobiotics, such as metals and metalloids. Copper, for instance, is associated with the application of algaecides in point S5, while for points S1, S2, and S3 the toxic effect was associated with the presence of cyanobacteria and their toxic effects (CETESB, 2014, 2015). Copper-based algaecides were used close to points S1, S3, and S5, where on several occasions the concentration exceeded the limits of the PEL and can cause fish toxicity. The toxicity in fish related to copper concentration has already been presented by other authors in different conditions of analysis (ARELLANO; STORCH; SARASQUETE, 1999; RODRÍGUEZ DE LA RUA et al., 2005; EPA, 2010).

The environmental agency has recognized the increased and irregular use of land around the reservoir, which has a reduced number of aqueducts and sewerage infrastructure, and the agency of water supply and sanitization has implemented actions to improve these conditions for more than two decades (CETESB, 2010). It should be emphasized among recent actions an increase of 2% in the proportion of population with sewage treatment compared to 2014, reaching a 63% coverage of the sewage treated in the state of São Paulo, and the implementation of more monitoring points for sediment and water within the different water reservoirs of the state (CETESB, 2015). The sewage in the water reflected directly in the water quality analysis, so phosphorus, BOD, and dissolved oxygen were above the limits allowed for waters classified as class 2, in accordance with the current regulations CONAMA, 357 of 2005.

The metals and metalloids such as arsenic, cadmium, chromium, copper, nickel, lead, and zinc were found above the allowed concentrations in the majority of the samples, and in several cases exceeded the limits of PEL, accepted by the EPA (2001) and the Canadian Council of Ministers of the Environment (2007). The concentrations of heavy metals in environment as Billings dam can associated mainly with industrial effluents, soil erosion, and mineral dissolution (JAIN; ALI, 2000; MANDAL; SUZUKI, 2002; RATNAIKE, 2003; EPA, 2010; WHO, 2011b, 2017a, 2017b).

Water and sediment analyses showed that water quality is compromised and threatened mainly by anthropogenic actions over time, and got worse during the drought.

Water-related indices (IQA, IET, IVA) evaluated for long periods help the monitoring and improvement of water quality, which in turn allows taking informed actions and preparing plans in order to improve the quality of water for public use. Specially, IVA and chronic toxicity are useful tools to know the possible effect of water pollutants on biological organisms, and their effects by exposure over time.

In this sense, fish are widely used tools for environmental studies in natural and experimental conditions, since they have shown a technical response to changes in their environment and in relation to water quality (AZAZA; DHRAÏEF; KRAÏEM, 2008; MARCHAND et al., 2009; SANT'ANNA; GOITEIN, 2009; GAGNON; HODSON, 2012; GOMES et al., 2012; AHMED et al., 2013; CASTRO et al., 2014; MORAIS et al., 2016). The three species of fish analyzed had already been used before with the purpose of aquatic environmental biomonitoring in Brazil and other countries (GARCIA-SANTOS et al., 2007; MELA et al., 2007; LINDE et al., 2008; LINDE-ARIAS et al., 2008a, 2008b; MOHAMED, 2008; OLIVEIRA et al., 2011; LIEBEL; TOMOTAKE; OLIVEIRA-RIBEIRO, 2013; REZENDE et al., 2013).

Tilapias, acaras, and trairas have been previously used together as bioindicators in the Billings reservoir for measuring metals in tissue(OLIVEIRA et al., 2011); there have also been studies of an isolated species such as the one of tilapia for histopathological characterization in a pollutant environment (REZENDE et al., 2013; JAYASEELAN et al., 2014), and the one of acara for determination of metals in tissues (FURLAN, 2011). According to the reviewed literature, there are no studies in the Billings Reservoir that have carried out pathological analyses of three fish species together along with water and sediment analyses for a characterization of the Billings water quality.

Previously used biomarkers found in the examined literature include: fish population, weight evaluation, Fulton index, macroscopic fish evaluation (RØNSHOLDT, 1995; NEFF; CARGNELLI, 2004; PANGLE; SUTTON, 2005; MOZSÁR et al., 2015; MORADO; ARAÚJO; GOMES, 2017), macroscopic index of lesions (WATSON; CRAFFORD; AVENANT-OLDEWAGE, 2012; SARA et al., 2014), microscopic evaluation of lesions and histopathological indices(BERNET et al., 1999; NERO et al., 2006a, 2006b; MCHUGH et al., 2011). Biomarkers related to body condition, gross lesions and microscopic evaluation of gills, liver, kidney, and spleen were used under this study.

Analyses of fish body condition based on the Fulton index as a biomarker of environmental contamination have been used to infer the water quality of other reservoirs (PANGLE; SUTTON, 2005; AGBOHESSI et al., 2015; MORADO; ARAÚJO; GOMES, 2017), according to the reviewed literature, there are no reports of previous studies using this index in the Billings dam. In this analysis, it was observed that tilapias, acaras, and trairas show differences in the Fulton index between periods and collection points and that the Fulton index alone did not have an association with water and sediment parameters.

The Fulton index for tilapias and acaras showed the same tendency to be lower in 2015 than in 2014. The factors pointed out to explain the data were in part consequence of alterations in food consumption, being either fasting or a decrease in food consumption for tilapias with the Billings water conditions, since authors reported that weight gain and growth in tilapias were reflected both in captivity, and experimentally (HODGKISS; MAN, 1977; ABDEL-TAWWAB et al., 2006). On the other hand, the lower Fulton index presented by acaras in 2015 could be due to their exposure to environmental stress conditions because of pollution or weather conditions. The data is compatible with the results obtained by (Linde-Arias et al., (2008b) in a River of Paraiba do Sul, Brazil. In contrast, it is possible to consider that the values of the Fulton index for acaras were higher in 2014 due to a higher organic load available for consumption (MORADO; ARAÚJO; GOMES, 2017), differently for tilapias.

In trairas, this difference between the Fulton index of the years 2014 and 2015 did not occur. Nevertheless, it was observed between the summer and spring collections in both years, being higher during the summer periods of 2014, and spring of 2015. These changes in this index may be associated with the reproductive processes of the species, which are markedly seasonal and are higher during warm periods (BALBONI; COLAUTTI; BAIGUN, 2009). Then, the lowest Fulton index during the summer of 2015 could be an abnormal pattern to the expected for this species, possibly in response to the water crisis and to stressful environmental conditions.

Gross evaluation allows inferences about the health status of fish in different environmental conditions (ADAMS; BROWN; GOEDE, 1993; BLANAR et al., 2009). The macroscopic findings in tilapias showed a pattern of low fat deposits in the celomic cavity although this species tends to deposit peri-visceral fat with available nutritional sources (VIOLA et al., 1988). Frequent white foci in the liver were one of the most common findings in this species and it has been associated with the presence of granulomas in this study and by another author (JANTRAKAJORN; WONGTAVATCHAI, 2016).

Acaras presented moderate and abundant deposits of fat in the celomic cavity as the most common finding at gross evaluation, along with the infiltration of fat in the liver; this allows to infer that this species, being in common environmental conditions with tilapias and trairas, has a greater tendency to store fat, a finding not found in the literature consulted and which still lacks a clear explanation. The steatosis and multifocal fat infiltration in the liver were relatively frequent in acaras, and not compatible with histological descriptions of the hepatic normal morphology of this species (SALES et al., 2017).

The presence of parasites in the celomic cavity was the most remarkable gross finding in trairas, covering a 52.78% of the individuals, and showing a different trend compared to the other two species studied. The susceptibility and high frequency of parasitism observed in this species had been previously described (WEIBLEN; BRANDÃO, 1992; MARTINS et al., 2003). The presence of parasites in trairas had been formerly associated to eutrophic and metal-contaminated waters (BLANAR et al., 2009), as well as to stressful conditions which may increase their susceptibility to infection (LAFFERTY, 1997).

Gills partially covered by mucus were another frequent gross lesion in trairas. Mucus in gills can alter gas exchange and cause respiratory stress (NOGA, 2010), and may be interpreted as a non-specific response and/or may be due to infectious agents, changes in pH of water, and exposure to toxic agents (FERGUSON, 2006; ROBERTS; PALMEIRO, 2008; SOTO et al., 2009; NOGA, 2010; ABDEL-MONEIM; AL-KAHTANI; ELMENSHAWY, 2012; FONSECA et al., 2016). The reason for mucus presence in gills is unknown in the studied trairas from the Billings dam, considering that there are many factors related to an increased mucus production on gills.

Interesting data resulted from the determination of HAI of each species of fish and its absence of association with water and sediment parameters. Tilapias didn't show association with location point, sampling period, or year, acaras, on the other hand, presented a lower HAI in 2015 differing from tilapias and trairas, which had a higher HAI in 2015. Furthermore, the HAI of tilapias was different and lower in S5 and S6 compared with S1 to S4, result that was interpreted as a response to the concentration of pollutants due to the drought, especially in S1, due to a higher concentration of pollutants pumped from the sites of sampling closest to the Pinheiros River (CETESB, 2015).

The HAI for acaras was higher in 2014 when water quality was worse compared to the historic average and to the year 2015. Acaras may have presented gross alterations faster than the tilapias and trairas analyzed. The HAI index results showed how the responses of each species vary even experiencing the same environmental conditions and this should be taken into account when it is necessary to choose one species of fish as bioindicator.

The frequency and grade of autolysis observed in trairas were unexpected data, which led to the exclusion of tissues from the histopathological evaluation; this feature can be used as one of the relevant criteria to consider in the choice of species to be used as bioindicators. Factors that may interfere in the degree of autolysis include: the time of death of the individuals after being caught in the net, the size of the individuals, the enzymes present in their gastrointestinal tract, and the temperatures at which they were exposed, which can affect the action of these enzymes on their own tissues (MUKUNDAN; ANTONY; NAIR, 1986).

Microscopic evaluation of gills, liver, kidney and spleen of fish showed similar lesions in the three species, but in different grade and frequency of occurrence consequently resulting in a notable difference in the I-Org determined for each organ and species. Evaluating I-Org for each species allows determining the severity of response of each organ to the same environmental conditions. Gills were the main organ affected in all the studied fish. In tilapia, the order according to the degree of intensity in the pathological alterations was: gills, kidney, liver, and spleen, the former being the most affected and the latter the least affected. In the acaras the order was: gills, liver, kidney, and spleen; and in the trairas it was gills, liver, spleen, and kidney. Thus, when using these three species as bioindicators and performing histopathological analyses, the analysis of gills is of great importance, as reported by the literature (VAN DER OOST; BEYER; VERMEULEN, 2003). The previous results are important because they indicate the organs that could be analyzed considering what species is chosen as bioindicator.

The most related research studies using fish as bioindicators analyzed both gills and liver (ARELLANO; STORCH; SARASQUETE, 1999; ASHRAF, 2005; NERO et al., 2006b; COSTA et al., 2009; SHIOGIRI et al., 2012; RODRIGUES et al., 2017). Gills are relevant due to their wide surface exposed to water, are more sensitive to toxic substances in water, showing high rates of injury during acute toxic exposure; and liver is taken into account due to its relevance in the metabolic function, biotransformation, accumulation, and excretion of toxic agents (CHOVANEC; HOFER; SHIEMER, 2003).

Microscopic analysis of the pattern of lesions in each organ allowed highlighting that regressive and non-reversible lesions were the most common pattern diagnosed besides there were regressive, non-reversible, and progressive lesions in gills, as observed before in other study (REZENDE et al., 2013).

There was a difference between the frequencies of occurrence of the microscopic lesion patterns diagnosed in the gills of each species. Gills of tilapias showed more progressive lesions such as respiratory epithelial hypertrophy of the secondary lamellae, while regressive irreversible lesions like the rupture of pillar cells, were more recurrent in trairas and acaras. Hypertrophy and hyperplasia of the respiratory epithelium are lesions that cause reduction of the gas exchange area and alterations in the electrolyte balance (ROBERTS, 2012) and which can lead to lamellar fusion and mucoid metaplasia. These lesions are responses to chronic exposure to low levels of irritation, and in this context, they can be identified as a response to the presence of contaminants in the water including that of metals (VAN DER OOST; BEYER; VERMEULEN, 2003; POLEKSIC et al., 2010; ABDEL-MONEIM; AL-KAHTANI; ELMENSHAWY, 2012; AHMED et al., 2013; LIEBEL; TOMOTAKE; OLIVEIRA-RIBEIRO, 2013; BARIŠIĆ et al., 2015; GALINDO-RIAÑO et al., 2015; MARCON et al., 2016; MURUSSI et al., 2016).

The rupture of pillar cells resulting in the presentation of aneurysms was the most common finding in acaras and trairas, it had been described before as a consequence to exposure to contaminants in other species of fish (MCHUGH et al., 2011; BARIŠIĆ et al., 2015) and also as caused by pathogens (ROBERTS, 2012). Trairas presented aneurysms in secondary lamellae as their most common lesion. Aneurysms become irreversible and can lead to the rupture of epithelial cells and cause hemorrhages (CAMARGO; MARTINEZ, 2007; PAULINO; SOUZA; FERNANDES, 2012). They can be more common in carnivorous fish, due to their greater tendency to accumulate contaminants (NASCIMENTO et al., 2012).

The regressive lesion was the most common response pattern in the liver for the three studied species. In tilapias (58%) as well as in the acaras (80.29%) the most frequent lesion was steatosis, which can be caused by different agents and is a reversible lesion related to the exposure of fish to different xenobiotics such as pesticides (AGBOHESSI et al., 2015), metals, and metalloids (ARELLANO; STORCH; SARASQUETE, 1999; SHAW; HANDY, 2006; COSTA et al., 2009; GALINDO-RIAÑO et al., 2015) or which can also be an effect of nutritional deficiencies (ROBERTS, 2012). The causes previously pointed out may be considered as possible explanations for steatosis in the fish analyzed in this research.

Tilapias evidenced a presence of granulomas in the liver, lesion which has been described previously as caused by different infectious agents including zoonotic agents such as *Mycobacterium marinum,* and *Franciscella* sp. (WOLF; SMITH, 1999; MAUEL et al., 2005; SOTO et al., 2009; BIRKBECK; FEIST; VERNER-JEFFREYS, 2011; ROBERTS, 2012). However, it was not possible to determine the etiological agent responsible by conducting a conventional histopathological study or with the use of histochemistry (Ziehl Neelsen, Periodic Acid Schiff), complementary techniques for the diagnosis like PCR and microbiological culture would need to be executed to determinate the etiology (MAUEL et al., 2005; FERGUSON, 2006; SOTO et al., 2009).

Steatosis was observed in the liver of tilapia as another of the most common findings (58%), and different experimental studies have stated its presence is due to exposure to metals such as copper, cadmium, zinc, and nickel (PEDLAR et al., 2002; ATHIKESAVAN et al., 2006; VAN DYK; PIETERSE; VAN VUREN, 2007; COSTA et al., 2009; SHIOGIRI et al., 2012), which in this case were found in increased concentrations in the sediment.

Multifocal to coalescent liver fat infiltrations, the most common lesion in acaras, were related to the gross lesion described. Moreover steatosis was a common lesion for acaras and trairas, but cellular swelling of hepatocytes was frequent finding in trairas was likewise frequent finding in the later. Steatosis may be associated with a high consumption of lipids in the diet (ROBERTS, 2012) and with exposure to pollutants (TEH; ADAMS; HINTON, 1997). Fish that have very severe steatosis may also present an altered hepatic function increasing their susceptibility to toxicity (ROBERTS, 2012). Swelling hepatocytes were diagnosed in the three species at different frequencies and have been described as a common and unspecific histological lesion characterized by high levels of glycogen, which can be associated with hepatocellular hemosiderin (SALES et al., 2017). However, this data has also been reported when fish are exposed to different agents (PEDLAR et al., 2002; ATHIKESAVAN et al., 2006; VAN DYK; PIETERSE; VAN VUREN, 2007; COSTA et al., 2009; SHIOGIRI et al., 2012).

Fibrosis around the bile ducts was a frequent histological lesion in trairas unlike in the other species. It can be observed in fish that inhabit contaminated areas, as trairas, and also in cases of infection by protozoa (FERGUSON, 2006), which was not the case in this study.

Periductal fibrosis has been previously described without finding an associated viral, parasitic or bacterial etiology (SCHMIDT-POSTHAUS et al., 2001).

Tilapias, in contrast to acaras and trairas, presented important kidney lesions. The most common response pattern was the inflammatory, characterized by interstitial lymphocytic infiltrate and granulomas that could be related to an infectious agent present in water. Bacteria and fungi in water may vary according to climatic variations and water quality (SCHMIDT-POSTHAUS et al., 2001).

The common response pattern in acaras was tubular epithelium degeneration, a regressive and reversible lesion in kidneys of the majority of fish, while the common lesion was congestion. Tubular degeneration usually corresponds to the development of an inflammatory process of infectious origin or to the effect of toxic organic compounds such as PCBs, insecticides, herbicides or petroleum hydrocarbons (ROBERTS, 2012).

In trairas, the most common response pattern were regressive lesions, being the presence of hyaline droplets in the tubular epithelium, the most frequent finding (50%), it can be seen in larger quantities in fish in contaminated environments (BERNET et al., 2004).

All three species shared the pattern of regressive response in spleen characterized by lymphoid depletion. The lymphoid depletion may be associated with environment stressful conditions, contaminated water, bacterial infection, and eutrophic environments (TEH; ADAMS; HINTON, 1997; ABU-ELALA et al., 2016). The result coincides with the literature and may be expected for the environmental condition of the Billings dam since it has been characterized as polluted and eutrophic.

In the particular case of this study, the lymphoid depletion described in the three species must be considered as a response to multiple factors such as interaction between individuals, exposure to immunosuppressive contaminants, or to several chemical substances simultaneously and chronically (WESTER; VETHAAK; VAN MUISWINKEL, 1994; VAN DER OOST; BEYER; VERMEULEN, 2003).

The presence of splenic granulomas was also observed in tilapia in higher frequency compared with the other species. The etiology of granulomas was not determined by histochemistry, and like in the case of liver lesions, complementary technics would be necessary to diagnose possible zoonotic infectious agents.

According to this study, it would be recommended to give priority to the analysis of different organs in each species, depending on the intensity of the response. Thus, using tilapias as biomarkers it would be recommended to prioritize gills, followed by kidney, liver, and spleen in the last place.

The ITOT (based on lesions in gills and liver) favors the determination of which of the three species is closest to being the most suitable bioindicator for this context. This index presented differences for each species with respect to the period and the sampling site. In all three species the ITOT value was higher in 2015, which means that the fish collected during that year showed more histopathological lesions; However, no statistical correlation was found between ITOT and water and sediment parameters of these two years.

There was a statistical difference regarding ITOT for points S1 to S4, and S5 and S6 only in acaras ($p = 0.0146$), which display a lower degree of injury in the Rio Grande points. It may be associated with the compartmentalization described before (CARDOSO-SILVA et al., 2014), as well as with the lowest proportion of positive effects of chronic toxicity.

Trairas had the highest ITOT value when exposed to the same environmental conditions, followed by tilapias in second place, and by acaras in third. Then, although the three species analyzed are useful as bioindicators for the Billings dam, trairas would be the most indicated species for such purpose. In this context, the data may indicate that the three species showed different responses to the adverse environmental conditions of the Billings dam. The fact that fish had a worse ITOT in 2015, in addition to the discrete improvement of water quality compared with that of 2014, could indicate that fish reflect environmental changes by presenting worse lesions after a long period of drought. That makes this analysis interesting and may help the environmental agency to choose when and which species to use for environmental evaluation over time, and to strengthen the continuous monitoring evaluation.

The choice of a fish species for monitoring aquatic environments such as the Billings dam should consider other criteria besides I- Org, ITOT, lesion pattern, and intensity of injury response like their abundance, load, size, longevity, water column use, feeding habits, and human consumption among others (GERHARDT, 2014). In addition, biomonitoring a reservoir using indigenous fish species represents advantages because they reflect the effects of water and sediment conditions as long as they live there and through the food chain such as carnivorous species (BERNET et al., 2004; MAGALHÃES; FERRÃO, 2008).

In this context, the three species analyzed display advantages and disadvantages that can be pondered. Tilapia is a widely distributed species in the world and has been used as a bioindicator in several (ASHRAF, 2005; GARCIA-SANTOS et al., 2007; VAN DYK; PIETERSE; VAN VUREN, 2007; AZAZA; DHRAÏEF; KRAÏEM, 2008; MOHAMED, 2008; AHMED et al., 2013; LIEBEL; TOMOTAKE; OLIVEIRA-RIBEIRO, 2013; REZENDE et al., 2013; JAYASEELAN et al., 2014). Therefore, tilapias are an exotic, omnivorous, and introduced species in the reservoir, where is highly available, used for fishing, and consumed (PETRERE; WALTER; MINTE-VERA, 2006). On the other hand, acaras are a native species, omnivorous, and one of the most fished species for human consumption in the reservoir (PETRERE; WALTER; MINTE-VERA, 2006); they showed similar pathological lesions but with a lower intensity as reported here and in previously (NASCIMENTO et al., 2012). Additionally, trairas are an indigenous species, carnivorous, equally fished and available in the reservoir, and they have been reported as an effective bioindicator (PETRERE; WALTER; MINTE-VERA, 2006; AHMED et al., 2013; LIEBEL; TOMOTAKE; OLIVEIRA-RIBEIRO, 2013; REZENDE et al., 2013; JAYASEELAN et al., 2014). Taking into account the exposed features and the data analyzed, traira is a good species to use as a bioindicator for the Billings dam.

Studies that integrate analysis of water, microbiological, toxicity, sediment criteria, and health/pathology of fish, should be carried out in a continuous manner that facilitates detecting changes in these organisms over time as well as changes in the environmental conditions, such as prolonged droughts, use of fertilizers, algaecides, presence of sewage, or improvement due to public actions.

Finally, the analysis highlighted features previously reported of the Billings reservoir and provided new data about the tissue response of different fish during and after the drought that were not reported before.

5. CONCLUSION

The comprehensive approach of water, sediment and fishes parameters allowed the evaluation of water quality of the Billings dam, and it might represent one of the ways to evaluate its environmental conditions as well as the quality of fish consumed in the region. The Billings dam was characterized as a eutrophic environment polluted by heavy metals as result of previous and historical factors such as population growth, irregular land use, and multiple industrial effluents and domestic sewage into the reservoir. The pathological indices applied indicated that the main lesions were regressive, unspecific and reversible, the responses of each species are different in the same environmental conditions, and this fact should be taken into account to choose a species of fish as bioindicator. The consequences of the compromised conditions of the Billings dam on fish consumption and the public health risks it represents deserve to be specifically analyzed in future.

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