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Microbiological featuring of two neotropical fish species from the quilombola area of maranhão state, Brazil

Caracterização microbiológica de duas espécies de peixes neotropicais oriundas de área quilombola maranhense, Brasil

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ABSTRACT: The aim of study is to feature the microbiological quality of two neotropical fish species from the quilombola area of Maranhão State, Brazil. In order to do so, 21 samples of *Hoplerythrinus unitaeniatus* and 21 samples of *Cichlasoma bimaculatum* were captured in flooded environment. Collected fish were euthanized in laboratory environment; muscle fragments were removed for microbiological analyses focused on enumerating molds and yeasts, viable strict and facultative mesophilic microorganisms and coagulase-positive staphylococci; on counting total and thermotolerant coliforms; and on investigating *Escherichia coli* and *Salmonella* sp. Microbiological results were compared to the Brazilian legislation, which establishes the list of microbiological standards for food products. Among the assessed fish, 9.52% were classified as non-acceptable for human consumption, based on the *Salmonella* parameter. Enumerated coagulase-positive staphylococci ranged from < 10 to 3.9 x 10^4 CFU/g; 9.52% of assessed fish were classified as having intermediate standard for human consumption, whereas 4.76% were classified as non-acceptable for such a purpose. *E. coli* counting ranged from 3.6 to > 1,100MPN/g; 4.76% of assessed fish were classified as having intermediate standard for human consumption, whereas 4.76% were classified as non-acceptable for such a purpose. Total and thermotolerant coliforms' counting and the enumeration of viable strict and facultative aerobic microorganisms, as well as of molds and yeasts, have evidenced high microbial population rates; this finding suggests poor hygienic conditions at capture site, contaminated raw material and risk of incidence of enteropathogens. This finding has evidenced imbalance in the investigated environment, as well as compromised aquatic biodiversity.

KEYWORDS: Traditional communities; fish; microbiology; indicator microorganisms.

RESUMO: Objetivou-se caracterizar a qualidade microbiológica de duas espécies de peixes neotropicais oriundas de área quilombola maranhense, Brasil. Para isso, foram capturadas 21 amostras de *Hoplerythrinus unitaeniatus* e 21 amostras de *Cichlasoma bimaculatum* de ambiente alagável. No laboratório, os peixes foram eutanasiados, procedida a retirada dos fragmentos musculares e realizada as análises microbiológicas: enumeração de bolores e leveduras, micro-organismos mesófilos aeróbios estritos e facultativos viáveis e de estafilococos coagulase positiva, quantificação de coliformes totais e termotolerantes, pesquisa de *Escherichia coli* e *Salmonella* sp. Os resultados microbiológicos obtidos foram comparados com a legislação brasileira que estabelece a lista de padrões microbiológicos para alimentos. Dos peixes avaliados, 9,52% foram considerados inaceitáveis para consumo humano para o parâmetro *Salmonella*. A enumeração de estafilococos coagulase positiva variou de <10 a 3,9 x 10^4 UFC/g, sendo 9,52% dos peixes considerados com padrão intermediário e 4,76% inaceitáveis para consumo. A quantificação de *E. coli* variou de 3,6 a >1.100 NMP/g, sendo 4,76% considerados com padrão intermediário e 4,76% inaceitáveis para consumo. A quantificação de coliformes totais e termotolerantes e a enumeração de micro-organismos aeróbios estritos e facultativos viáveis e bolores e leveduras revelou elevadas populações microbianas o que sugere más condições higiênicas do local de captura, matéria-prima contaminada e risco da presença de enteropatógenos, demonstrando desequilíbrio no ambiente estudado com comprometimento da biodiversidade aquática.

PALAVRAS-CHAVE: comunidades tradicionais; pescado; microbiologia; micro-organismos indicadores.

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INTRODUCTION

Fishing and aquaculture in Maranhão State are important activities providing animal protein of high biological value for human consumption (MONTELES; FUNO; CASTRO, 2010). Western Maranhão State' Lowland stands out among areas with the highest fish production in the State; this region comprises a hydrographic system integrated by a set of rivers, lakes and floodplains, which gives it the status of the largest lacustrine basin in Northeastern Brazil (PEREIRA; RODRIGUES; VIEGAS, 2016).

Western Maranhão State's Lowland hosts several traditional quilombola communities for whom artisanal fishing is an important practice, based on traditional empirical knowledge, which provides relevant protein source throughout the year (BERNARDES; BOTELHO; MOTTA NETO, 2011). *Cichlasoma bimaculatum* (black acara) and *Hoplerythrinus unitaeniatus* (jeju) are important fishing-based food resources for quilombola communities living in Maranhão State (VIANA et al., 2014). Both species are often found in shallow-water environments and close to submerged or marginal vegetation (LEAL et al., 2010; FROESE; PAULY, 2016).

However, artisanal fishing in Maranhão State has been strongly affected by social and ecological changes, which result in loss of fish species on a yearly basis. In addition to several environmental impacts – such as deforestation and pollution due to domestic sewage discharge, among others – experienced by the aforementioned state, it is worth emphasizing domestic and livestock animals' breeding under precarious sanitary conditions, such as pigs, buffaloes and cattle, which can host bacterial etiological agents (DE JESUS et al., 2022).

Despite fish's unquestionable benefits to human health, and the fact that they integrate the food culture of different peoples (SARTORI; AMANCIO, 2012), fish are quite susceptible to the action of viruses, fungi, parasites and bacteria capable of putting both animal and human health at risk, since many of these agents are potentially zoonotic (OLIVEIRA, 2005).

According to the historical series conducted by the Health Surveillance Secretariat (SVS - Secretaria de Vigilância em Saúde) of the Brazilian Ministry of Health (MS - Ministério da Saúde), 469,482 individuals were reported to have been exposed to water- and foodborne diseases (WFBD) in Brazil, from 2007 to 2016; 118,104 of them got sick and 109 have died (BRASIL, 2018). However, one must take into consideration that national data are underestimated, since not all exposed individuals are located for epidemiological studies. In addition, there is clear underreporting of WFBD events in the country (FAULA; SOARES; DIAS, 2015). This underreporting issue becomes even more worrying in specific social groups and subgroups, who live in environments subjected to inappropriate sanitary conditions, who are occasionally concentrated in ethnically diverse populations and who experience lack of public services, such as quilombola communities.

The aforementioned historical series has shown that 90.5% of etiological agents involved in WFBD outbreaks were of the bacterial type; 70.3% of them accounted for unidentified bacterial species, 7.5% were *Salmonella* spp., 7.2% were *Escherichia coli* and 5.8% were *Staphylococcus aureus*. With respect to contaminated food types, fish, seafood and processed food accounted for 0.8% of reported outbreaks, although 66.8% of cases were classified as unidentified-origin food, whereas 9% were mixed-nature food (BRASIL, 2018). In light of the foregoing, the aim of the current study was to feature the microbiological quality of two neotropical fish species from the quilombola area of Maranhão State, Brazil.

MATERIAL AND METHODS

Research Ethics

The current research was approved by the Animal Ethics and Experimentation Committee (CEEA - Comitê de Ética e Experimentação Animal) of State University of Maranhão (UEMA - Universidade Estadual do Maranhão), under protocol n. 08/2021. It was carried out in compliance with Resolutions n. 879/2008 and 1000/2012 by the Federal Council of Veterinary Medicine (CFMV - Conselho Federal de Medicina Veterinária) and with Federal Law n. 11794/2008, which provide on Ethical procedures in Animal Experimentation.

Study Site

The study site comprised a lake region located in the rural area of Anajatuba County, Maranhão State. The aforementioned county belongs to Maranhão State's Lowland region and it is located between the following geographic coordinates: Latitude 03°15'50" S and Longitude 44°37'12" W.

Humid tropical climate, with dry winter season, prevails in this region: overall, the dry season in it lasts from six to seven months; among them, three to four months are extremely dry, since they record less than 8% rainfall on a daily basis. On the other hand, the rainy season lasts from five to six months; at least two months can be considered very rainy, since they record more than 30% of the region's total annual rainfall rate (CUNHA; SILVA, 2002).

Fish Sample Collection

Local fishermen collected the assessed fish in marginal lakes (flooded environment) of Ponta Bonita community, in September 2021 (dry season) and February 2022 (rainy season). Forty-two fish specimens were captured - 21 individuals belonged to species *H. unitaeniatus* (jeju) and 21, to species *C. bimaculatum* (black acara) – based on using actively operated (drag/siege) 4-mesh (20 mm) gillnet and 4-mesh (20 mm) casting net, based on Silva et al. (2022).

Then, fish were packaged and transported alive in styrofoam box filled with water from the capture environment to UEMA's Aquatic Resources Reproduction Laboratory (LARAQUA - Laboratório de Reprodução de Recursos Aquáticos), where they were placed in a tank filled with water, under constant oxygenation, for 12 hours, until further biological material analysis and processing in the Food and Water Microbiology Laboratory of the Agricultural Sciences Center – CCA/UEMA.

Biological Material Processing: euthanasia and muscle fragment collection

Fish were euthanized by perforating the upper part of their heads with pointed instrument (scalpel blade). Subsequently, all 42 specimens were aseptically dissected in order to have their longitudinal skeletal striated muscles (dorsal and ventral muscles) removed to enable collecting muscle fragments.

Laboratory Procedures

Initially, 25 ± 0.2 g of each sample were weighed and added to 225 mL of 0.1% buffered peptone water for fish's microbiological analysis; it corresponded to the first dilution (10^{-1}). Subsequently, two other successive dilutions (10^{-2} and 10^{-3}) were prepared in test tubes filled with 9 ml of 0.1% buffered peptone water.

Microbiological featuring was performed by enumerating molds and yeasts, as well as viable strict and facultative mesophilic aerobic microorganisms and coagulase-positive staphylococci, by counting total and thermotolerant coliforms, and by investigating *Escherichia coli* and *Salmonella* sp. All analyses were conducted based on procedures and recommendations by the American Public Health Association (DOWNES; ITO, 2001).

Enumerating molds and yeasts

The herein adopted technique comprised deep plating of 1 mL of serial dilutions (10^{-1}, 10^{-2} and 10^{-3}), based on using Potato Dextrose Agar (PDA) acidified with 10% tartaric acid solution. After the procedure was over, plates were incubated in oven at 25°C ± 2°C, for five (05) days. Results were expressed as colony forming units (CFU) per gram.

Enumerating viable strict and facultative mesophilic aerobic microorganisms

The herein adopted technique comprised deep plating of 1 mL of serial dilutions (10^{-1}, 10^{-2} and 10^{-3}), based on using Plate Count Agar (PCA). It was followed by plates' incubation (in inverted position) in bacteriological oven at 35°C ± 1°C, for 48±2h. Only plates presenting from 30 to 300 colonies were taken into consideration for counting; their arithmetic mean was multiplied by the respective dilution factor; results were expressed as Colony Forming Units/g of sample (CFU/g).

Enumerating coagulase-positive staphylococci

Coagulase-positive staphylococci analysis was performed by inoculating 0.1 ml of each dilution (10^{-1}, 10^{-2} and 10^{-3}) in Baird-Parker Agar (BP), based on surface scattering technique. Then, plates were incubated (in inverted position) at 37°C, for 24-48h. Plates holding 30 to 300 colonies were selected and five typical (black or gray, convex, with opaque/clear zone around them) and atypical staphylococci colonies were selected for Gram, catalase and coagulase tests.

Selected colonies were incubated in test tubes filled with 2 mL of brain-heart infusion (BHI) broth, incubated at 37°C, for 24 hours, and subjected to coagulase test. Their plasma clotting ability was confirmed based on coagulase enzyme activity.

Counting total and thermotolerant coliforms

Multiple tubes' technique was used to count total and thermotolerant coliforms. In order to do so, 1-mL aliquots of each dilution were inoculated in a series of three inverted Duhran tubes filled with 9 mL of Lauryl Sulfate Tryptose (LST) broth. The aforementioned tubes were incubated at 35°C, for 24-48 hours. A view of each positive tube (turbidity and visible gas formation in the Durham tube) was transferred to tubes filled with Brilliant Green (BG) and *Escherichia coli* (EC) broths, which were incubated at 35°C and 45°C, respectively, both for 24-48 hours. The number of positive tubes was recorded and MPN was determined based on a table suitable for the adopted dilutions; results were expressed as MPN of total and thermotolerant coliform/g.

Investigating Escherichia coli

E. coli was investigated based on its isolation and confirmation through conventional methodology, as described below:

1. First stage: enriching the samples

Bacterial inocula from each positive EC tube were inoculated in MacConkey Agar (MCK). The culture was incubated at 37°C, for 24 hours. All colonies showing different morphotypes, and five colonies (at most) with the same morphotype, were transferred from the representative plates. Each colony was inoculated in Trypticase Soy Agar (TSA), as well as in brain-heart broth (BHI), and incubated at 37°C, for 24 hours.

2. Second stage: identifying the isolated colonies

The identification of species *E. coli* in each pure culture was confirmed through the following biochemicalphysiological tests: (i) glucose-based gas production; (ii) lactose fermentation; (iii) lysine decarboxylation; (iv) tryptophan-based indole production; (v) non-degradation of urea; and, (vi) lack of hydrogen sulfide production from sulfur amino acids, based on using Rugai's medium modified by Pessoa and Silva (1972). In addition, Methyl Red (MR) and Voges Proskauer (VP) biochemical tests were carried out.

Investigating Salmonella sp.

Samples held in 225 ml of 0.1% buffered peptone water were incubated at 37°C, for 24 hours, to investigate the incidence of *Salmonella* sp. These samples were transferred to two different selective enrichment broths (Rappaport-Vassiliadis and Tetrathionate-Novobiocin) and incubated at 37°C and 42°C, for 24 hours, respectively. Subsequently, each sample was seeded in Petri dishes covered with Hektoen Enteric Agar (HEA) and xylose lysine deoxycholate (XLD), and incubated at 37°C, for 24 hours. Typical colonies formed on the plates were confirmed based on biochemical and serological tests. Initially, colonies were subjected to the following tests: lysine decarboxylation, lactose and/or sucrose fermentation and H_2S production, in Lysine Iron (LIA) and Triple Sugar Iron (TSI) Agars. Cultures typical of genus *Salmonella* in these media were subjected to agglutination test based on using polyvalent anti-Salmonella serum.

Data analysis

Laboratory results were processed and interpreted, stored in database, sorted and presented in tables to enable better visualizing the set of variables. Descriptive statistical analysis was also performed to find absolute and relative frequencies. Fisher's test was carried out in InStat free software to check whether there were significant differences (at 5% significance level) in microbiological contamination between assessed periods and sampled species.

RESULTS AND DISCUSSION

Hygienic-sanitary indicator microorganisms addressed in Brazilian legislation

Normative Instruction (NI) n. 60 by the National Health Surveillance Agency (ANVISA), from December 23rd, 2019, provides on the microbiological quality standard for food products and determines that for raw fishery (fish, crustaceans and mollusks) and giblets (roe, gizzard, swim bladder) - be them seasoned or not, fresh, chilled or frozen - to be acceptable for consumption, they must meet the microbiological criterion, according to which, 25 grams of the analyzed sample cannot present *Salmonella* sp. (BRASIL, 2019). Based on this criterion, 90.74% (n= 38/42) of the analyzed fish were acceptable for human consumption, whereas 9.52% (n= 04/42) were non-acceptable for such a purpose (Table 1).

Current findings are worrisome, since *Salmonella* incidence in food products is a significant epidemiological factor for WFBD outbreaks. According to the European Food Safety Authority (EFSA, 2010), a large number of salmonellosis cases can affect public health in developed and developing countries where this disease is featured as emerging issue. According to Huber et al. (2004), species belonging to family Enterobacteriaceae, such as *Salmonella*, stand out among bacterial agents distributed in aquatic ecosystems. Based on on-site visits to the place where the assessed fish were captured, as well as on monitoring the procedure adopted to remove specimens from marginal lakes, the contamination of four samples with *Salmonella* is attributed to the environment, since different wild, domestic and livestock species lived in the assessed area. Therefore, these animals' waste contributes to pollute the lake environment and they can be the source of the observed contamination.

Salmonella has been the target of studies conducted with fish in different countries, due to its relevance for public health. Hughes; Gillespie; O'Brien (2007) conducted a survey in England, between 1992 and 2003, to investigate intestinal infection outbreaks associated with food. Results have shown that this bacterial agent accounted for 53% of infection cases and that approximately 6% of the total number of cases was associated with fish and fish-product intake. Given the frequent series of salmonellosis cases associated with fish intake, EFSA (2010) reported that this fishery type is a relevant vehicle for *Salmonella* contamination, as well as emphasized its prevalence values in the following countries: Belgium (14.3%); Spain (1.6%), Italy (1.2%), Greece (0.9%) and Germany (0.5%).

Study conducted by Bouchrif et al. (2009) in Morocco, Africa, has shown that fish recorded the second largest number of positive samples for *Salmonella* among several analyzed food products. Based on the study carried out by Nadimpalli et al. (2019) in Cambodia, Asia, ten (10/60) Salmonella samples were isolated from raw fish meat sold in two public markets.

This bacterium accounts for 800 thousand to 4 million cases of infectious diseases in the United States of America (USA), on a yearly basis (GAZAL et al., 2018). Scallan et al. (2011) collected data on foodborne diseases recorded between 2000 and 2008; non-typhoid *Salmonella* spp. accounted for approximately 35% of hospitalizations and for 28% of death cases.

Table 1. Investigating the incidence of *Salmonella* sp. in 21 *Cichlasoma bimaculatum* and 21 *Hoplerythrinus unitaeniatus* individuals from wetland environment in Ponta Bonita Quilombola Community, Anajatuba County, Maranhão State.

Microbiological Standard	Fish Species				Total
	Cichlasoma bimaculatum		*Hoplerythrinus unitaeniatus*		
	RS	DS	RS	DS	
Acceptable (Lack of *Salmonella*/25g)	17	02	02	17	38
Non-acceptable (Incidence of *Salmonella*/25g)	01	01	01	01	04
Total	18	03	03	18	42

Wherein: n = number of samples; RS= rainy season; DS= dry season; Microbiological Standard = Normative Instruction n. 60/2019 by National Health Surveillance Agency.

According to Fernandes et al. (2018), most fish intake-associated salmonellosis cases observed in humans are caused by serovar *Salmonella enterica* subsp. *Enterica,* serovar Typhimurium *Salmonella enterica* subsp. *enterica*, and serovar Enteritidis. *Salmonella* prevalence in freshwater fish ranges from 3.4% to 64%, depending on the quality of water and on good production practices. Therefore, the prevalence value identified in the present study (9.52%) is in compliance with reports in the aforementioned studies.

According to the microbiological criterion for coagulase-positive staphylococci enumeration, the Brazilian legislation sets values ranging from 10^2 to 10^3 CFU/g (BRASIL, 2019). Based on this criterion, the enumeration of this sanitary indicator in the current study ranged from < 10 to 3.9 x 10^4 CFU/g in the assessed fish; 85.71% (n= 38/42) of fish were acceptable for human consumption, 9.52% (n= 04/42) presented intermediate standard, and 4.76% (n= 02/42) were considered non-acceptable for human consumption (Table 2).

According to Novotny et al. (2004), there is high coagulase-positive staphylococci incidence in fish sold in Brazil. Costa et al. (2018) conducted a study focused on investigating the resistance profile of *Staphylococcus aureus* isolated from *Cynoscion acoupa* (blacktail basher) sold at a public fair in Macapá City – Amapá State; they identified this group of microorganisms in 75% (n=15/20) of the analyzed samples. Silva Junior et al. (2015) have analyzed *Cynoscion* sp. ("*pescada branca*") sold at Perpétuo Socorro fair, Macapá City, Amapá State; they found coagulase-positive staphylococci in 50% of the analyzed samples.

Coagulase-positive staphylococci incidence in 14.28% of the analyzed fish samples (n= 06/42 - total number of samples with intermediate and non-acceptable microbiological standard) may be associated with poor hygienic practices (equipment and utensils), with incorrect handling by local fishermen during fish capture and removal from fishing gear, as well as with local environmental conditions. Sales; Silva (2012) have pointed out that coagulase-positive staphylococci are often found in humans' skin and mucous membranes, whereas Plata; Rosate; Wegrzyn (2009) reported that from 20% to 30% of the world population overall carries this pathogen.

All four samples showing intermediate microbiological standard, as well as the two samples showing non-acceptable standard, have evidenced a worrisome situation, since coagulase-positive staphylococci produce thermostable enterotoxins in food products. Food products with intermediate microbiological profile require more rigorous processing - in terms of cold storage methods and cooking techniques - to avoid microbial multiplication and survival (CHAVES et al., 2015). Hygiene - both for fishermen/handlers and for utensils and equipment used to catch fish (fishing gear) - is another aspect that must be taken into account to avoid recontamination with these microorganisms.

Staphylococci contamination in fish is often attributed to factors external to animals (environment, water, personnel involved in the captures, equipment and utensils in direct contact with specimens). However, Ali (2014) has reported incidence of this microorganism in *Cyprinus carpio* (common carp) and *Silurus glanis* (wels catfish) skin, liver, intestines and muscles. Coagulase-positive staphylococci were isolated from skeletal striated muscles of specimens investigated in the current study.

With respect to microbiological criterion "counting *Escherichia coli*", Brazilian legislation establishes values ranging from 50 to 5x10^2 MPN of *Escherichia coli*/g for products that are not consumed raw (BRASIL, 2019), as the ones assessed in the current study. Values recorded for this health indicator ranged from 3.6 to > 1,100 MPN/g in the analyzed fish; 90.48% (n= 38/42) of fish were acceptable for human consumption, 4.76% (n= 02/42) presented intermediate standard and 4.76% (n= 02/42) were considered non-acceptable for human consumption (Table 3).

Table 2. Coagulase-Positive Staphylococci enumeration in 21 *Cichlasoma bimaculatum* and 21 *Hoplerythrinus unitaeniatus* individuals from wetland environment in Ponta Bonita Quilombola Community, Anajatuba County, Maranhão State.

Microbiological Standard	Fish Species				Total
	Cichlasoma bimaculatum		*Hoplerythrinus unitaeniatus*		
	RS	DS	RS	DS	
Acceptable (≤10^2 UFC /g)	16	02	01	17	36
Intermediary (10^2 UFC /g to 10^3 UFC /g)	01	01	01	01	04
Non-acceptable (>10^3 UFC /g)	01	00	01	00	02
Total	18	03	03	18	42

Wherein: n = number of samples; RS= rainy season; DS= dry season; CFU= Colony Forming Unit; Microbiological Standard = Normative Instruction n. 60/2019 by National Health Surveillance Agency.

Table 3. Counting *Escherichia coli* in 21 *Cichlasoma bimaculatum* and 21 *Hoplerythrinus unitaeniatus* individuals from flooded environment in Ponta Bonita Quilombola Community, Anajatuba County, Maranhão State.

Microbiological Standard	Fish Species				Total
	Cichlasoma bimaculatum		*Hoplerythrinus unitaeniatus*		
	RS	DS	RS	DS	
Acceptable (< 50 MPN/g)	16	03	02	17	38
Intermediary (< 50 to 5x10^2 MPN/g)	00	00	01	01	02
Non-acceptable (5x10^2 MPN/g)	02	00	00	00	02
Total	18	03	03	18	42

Wherein: n = number of samples; RS= rainy season; DS= dry season; MNP= Most Probable Number; Microbiological Standard = Normative Instruction n. 60/2019 by National Health Surveillance Agency.

E. coli found in 9.52% of analyzed fish samples (total number of samples with intermediate and non-acceptable microbiological standard) may be associated with conditions of the aquatic habitat where the analyzed specimens were captured. According to Barbosa et al (2010), the incidence of this microorganism, even at low concentrations, indicates risk of pathogen transmission to humans.

Laboratory analysis of *E. coli* helps finding potential risk of food poisoning through water and food provided for consumption purposes. Thus, the meaning attributed to the incidence of *E. coli* in 9.52% of samples analyzed in the current study must be addressed based on two basic premises, according to Franco; Landgraf (2002): (i) it indicates microbial contamination of fecal origin and, therefore, unsatisfactory sanitary conditions at capture place; and, (ii) these bacteria can integrate pathogenic/diarrheagenic to humans and animals.

Based on the aforementioned aspects and on the analysis of indicators and criteria established in NI 60/2019, in comparison to Collegiate Board of Directors' (RDC - Resolução da Diretoria Colegiada) Resolution n. 12/2001 (legislation that previously regulated microbiological standards for food), it is possible noticing that standards set for fresh fishery are stricter and require important changes, such as: (i) histamine test for raw fish, crustaceans, mollusks and giblets (roe, gizzard and swim bladder), be them seasoned or not, fresh, cooled or frozen; and, (ii) analysis of *E. coli* to the detriment of counting thermotolerant coliforms. With respect to this last parameter, fecal contamination in food is indicated through *E. Coli* counting, hence the current requirement in Brazilian legislation.

Therefore, animal excreta contaminating the environment can pollute the water in aquatic ecosystems and reach fish living in them. These fish, in their turn, can emerge as potential routes of pathogenic agents' transmission to humans, since salt or fresh water is the natural environment of a wide variety of bacteria capable of causing infections in humans, although such a potential depends on isolated or synergistic factors like organism's survival, latency and infective dose, and host's susceptibility to harm both animal and human health, due to incidence of undesirable pathogens, as mentioned by Barbosa et al. (2010).

There was no statistically significant difference in microbiological contamination with the three groups of hygienic-sanitary indicator microorganisms addressed in the Brazilian legislation between the two investigated fish species and the assessed seasons. Therefore, it is possible saying that bacterial infections affecting fish are a challenge, regardless of the season, due to the complexity of factors involved in it, i.e., those inherent to the hosts (age, immunological status, intercurrent diseases, among others), to bacterial agents (species and pathogenicity), or to the environment (climate, local hygiene, biosecurity, reservoirs, among others).

Microorganisms indicative of useful shelf life and unsatisfactory hygienic conditions

With respect to results recorded for microorganisms indicating conservation time, 45.23% (n= 19/42) of assessed specimens presented molds and yeasts' values ranging from 1.5 x 10^3 to 4.54 x 10^3 CFU/g, whereas 66.67% (n= 28/42) presented viable strict and facultative aerobic microorganisms in bacterial populations ranging from 816 to 3.9 x 10^4 CFU/g. As for results recorded for microorganisms indicating unsatisfactory hygienic conditions, total coliforms were found in 66.67% (n= 28/42) of analyzed specimens, whereas thermotolerant coliforms were found in 42.86% (n= 18) of them - values ranged from 3.0 to > 1,100 MPN/g.

Although the Brazilian legislation lacks fish-related information regarding the limits of counts tolerated for both organism groups (indicators of storage time and unsatisfactory hygienic conditions), results in the current study suggested poor hygienic conditions of the place, contaminated raw material, as well as risk of incidence of fecal-origin pathogens.

Pathogenic agents transmitted from fish to humans do not often cause damage to fishery and aquaculture production. Therefore, fishermen and/or producers do not feel encouraged to seek adequate sanitary control to ensure the quality of the raw material. However, contaminated fish can work as transmission route for these agents when they are consumed by humans, as well as contaminate other food types and surfaces (BARBOSA et al., 2010) and onset food outbreaks. This issue becomes even more worrisome in specific social groups, who live in environments subjected to inappropriate sanitary conditions, who are occasionally concentrated in ethnically diverse populations and who experience lack of public services.

The incidence of pathogens is one of the main hazards compromising the quality of fishery products. Such an incidence is associated with improper breeding practices, environmental pollution, improper handling (poor water quality, lack of hygienic care with fish, equipment and utensils, and lack of knowledge), as well as with direct input of feces from animals (poultry, swine, cattle, dogs and cats) living close to fishing sites (MORITA et al., 2006).

CONCLUSIONS

Based on the current findings, it was possible concluding that the microbiological featuring of *H. unitaeniatus* and *C. bimaculatum* has evidenced: (i) hygienic-sanitary indicator microorganisms addressed by Brazilian legislation – *Salmonella* sp., *Escherichia coli* and coagulase-positive staphylococci; (ii) microorganisms indicative of useful shelf life - viable strict and facultative aerobic mesophiles, and molds and yeasts; and (iii) microorganisms indicative of unsatisfactory hygienic conditions – total and thermotolerant coliforms. The incidence of these agents has shown imbalance in the investigated environment and impaired aquatic biodiversity. Therefore, it is essential conducting studies of this nature to contribute to the adoption of management measures aimed at avoiding irreparable damage to aquatic ecosystems and the depreciation of quilombola communities' dietary base.

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