MICROBIOLOGICAL CONDITION OF THE CATFISH *Sciades herzbergii* FROM BACANGA LAGOON, NORTHEASTERN BRAZIL

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ABSTRACT

Fishing in Bacanga Lagoon supplements the income or even guarantees the survival of several families that live in this river basin on Maranhão Island (São Luís), which is densely populated and shows signs of water pollution. The present study aimed to assess the quality of recently captured catfish *Sciades herzbergii*, through microbiological analyses of muscles. In general, the samples of Bacanga Lagoon showed higher coliform values than those observed in fish collected in the control area (Pau Deitado, Paço do Lumiar, state of Maranhão). Samples collected in January showed a high content of total coliforms and coliforms at 45ºC, which suggests a relationship between microbial contamination and the rainy season. *Escherichia coli* was the most abundant bacteria species in the samples from Bacanga Lagoon. The results showed poor sanitary conditions of the Bacanga Lagoon and demonstrated fecal contamination of the fish captured due to the high amount of untreated effluents discharged in the lagoon.

Key words: coliforms; pemecou sea catfish; sewage.

QUALIDADE MICROBIOLÓGICA DO BAGRE *Sciades herzbergii* CAPTURADO NA LAGUNA DO BACANGA, NORDESTE DO BRASIL

RESUMO

A pesca na laguna do Bacanga é uma atividade que garante o sustento e complementação de renda para diversas famílias que habitam nessa bacia hidrográfica da Ilha do Maranhão (São Luís) densamente ocupada e com sinais de poluição de suas águas. O trabalho objetivou avaliar a qualidade do bagre *Sciades herzbergii* através de análises microbiológicas do músculo de peixes recém-capturados. No geral, as amostras do Bacanga apresentaram valores de coliformes significativamente maiores que os observados nos peixes coletados na área controle (Pau Deitado, Paço do Lumiar, MA). Amostras coletadas nos meses de janeiro apresentaram elevada quantidade de coliformes totais e coliformes *a 45ºC* sugerindo relação da contaminação microbiana com o período de chuvas, sendo que *Escherichia coli* foi a espécie mais abundante nas amostras do Bacanga. Os resultados indicaram as péssimas condições sanitárias da laguna do Bacanga e demostraram a contaminação de origem fecal nos peixes capturados em função do elevado volume de efluentes lançados sem tratamento na laguna.

Palavras-chave: coliformes; bagre guribu; efluentes domésticos.

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INTRODUCTION

Fish consumption is growing worldwide because fish is a nutrient-rich food, with high protein and essential fatty acids and low cholesterol (VILA NOVA *et al*., 2005). Fish are also considered a functional food able to decrease the risk of heart diseases (RAMOS FILHO *et al*., 2008; DOI *et al*., 2015). The consumption of fish may also be influenced by either socioeconomic factors or regional characteristics (TRONDSEN *et al*., 2003). The world per capita consumption increased from 9.9 kg in 1960 to 18.4 kg in 2009 (FAO, 2012), and in Brazil, recent data point out to an average consumption of 14.5 kg per inhabitant/year in 2013 (BRASIL, 2014).

The fish quality (healthiness) considers its natural microbiota. The more contaminated is the water where the fish is captured; the richer in microorganisms is the microbiota. Hence, the fish quality reflects the microbiological quality of the environment (AL-HARBI and UDDIN, 2005; GUZMÁN *et al*., 2004; JAY, 2005; NASCIMENTO *et al*., 2001). There is great concern about food quality and sanitary conditions during food production, as there is an increasing number of foodborne diseases and immunodeficient or immunocompromised people that are susceptible to those diseases (OLIVEIRA *et al*., 2008; SOUZA *et al*., 2006).

According to the World Health Organization, foodborne diseases can be defined as infectious or toxic diseases caused by agents introduced into the organisms through the ingestion of contaminated food or water (WHO, 2010). In general, foodborne diseases are identified through gastrointestinal symptoms, especially diarrhea, vomiting, and abdominal pain. Besides bacteria, other microorganisms, such as protozoans and viruses can cause diseases through fish consumption. However, bacteria have higher relevance and occurrence than other organisms, and they are the only group whose content in food is limited by Brazilian law (BRASIL, 2001).

Some microorganisms that contaminate fish are pathogenic for humans. Others, though, do not cause disease, but are indicators of poor hygienic conditions and their presence in a sample suggests that pathogens may also be present. Among the microorganisms that indicate hygienic quality are total coliforms and coliforms at 45ºC (thermo-tolerant). Bacteria of the genera *Escherichia, Enterobacter, Citrobacter,* and *Klebsiella* are members of the coliform group (FRANCO, 1996).

The aquatic environment influences the natural microbiota of fish. The presence of microorganisms, such as *Salmonella* and *Staphylococcus*, indicate the occurrence of cross-contamination between the fecal material of animals or humans and the fish along the productive chain (GERMANO *et al*., 1993; BALLESTEROS *et al*., 2016). In this case, the bacteria that are more frequently found in contaminated fish and deserve greater concern are *Salmonella spp. Shigella spp. Escherichia coli, Yersinia enterocolitica, Vibrio parahaemolyticus, Staphylococcus aureus*, *Bacillus cereus, Clostridium perfringens*, and *Clostridium botulinum* (VIEIRA, 2004). Ninety percent of the coliforms at 45 ºC are *Escherichia coli* (LANDGRAF, 1996). *Escherichia coli* is considered the main indicator of fecal contamination of food (ALMEIDA FILHO *et al*., 2006; SILVA *et al*., 2001; VIEIRA, 2004). It is usually related to the contamination of the site where the fish was collected, transport and processing conditions, as well as other processes and material that might have been in contact with fresh fish (AGNESE *et al*., 2001).

Bacanga River Basin (São Luís, state of Maranhão) is one of the most densely populated areas of Maranhão Island. The area harbors intensive traditional fishing, especially where the barrage that connects the lagoon (formed by damming) to São Marcos Bay.

When the floodgates are open, the water is renovated and the number of fish increases. According to data from an interview carried out by SOARES (2013) with local fishermen, most of them (60%) capture over 5 kg of fish a day, but some (5%) obtain more than 20 kg/day. However, the amount of fish decreases considerably when the floodgates remain closed for long periods. Low water renovation worsened the conditions of chemical variables, which makes the environment unfavorable for fishing some species. Among the most fished species in Bacanga, the silverfish (*Diapterus* sp.), white mullet (*Mugil curema*), Atlantic anchoveta (*Cetengraulis edentulus*), and catfish (*Sciades herzbergii*) stand out (SOARES, 2013).

Although Bacanga River Basin still provides fish to the surrounding population, it has suffered from the impacts of disorganized urbanization, in addition to the discharge of untreated effluents into its waters. This process not only creates several socio-environmental problems, such as dissemination of waterborne diseases, but also harms the aquatic communities through pollution, eutrophication and, ecological unbalance (PIMENTA *et al*., 2002). To improve fishing management in this river basin and guide decisions about traditional fishing, we

need to assess the quality of the fish captured by the population in the surroundings of Bacanga Lagoon using a microbiological analysis. This way, it would be possible to take measures to avoid compromising the health of people who consume those fish.

METHODS

We obtained catfish *S. herzbergii* directly from local fishermen in Bacanga Lagoon, São Luís, and in the Pau Deitado village, Paço do Lumiar (considered a control site), both located in Maranhão Island (state of Maranhão, northeastern Brazil) (Figure 1).

We carried out sampling in January and August 2014 and January 2015 in both sites. We captured ten specimens of the catfish *S. herzbergii* with gillnets, in a total of 60 individuals. Right after collection, we placed the fish in isothermal boxes filled with ice (filtered water), applying the same procedure used by local fishermen.

Next, we took the fish to the Laboratory of Ecotoxicology (DEOLI/UFMA), where they were washed in distilled water and had their total length (30.76 ± 3.96 cm) and total weight (265.03 ± 134.44 g) measured. Next, the fish's muscle tissue was removed to check for total coliforms and 45 ºC tolerant coliforms.

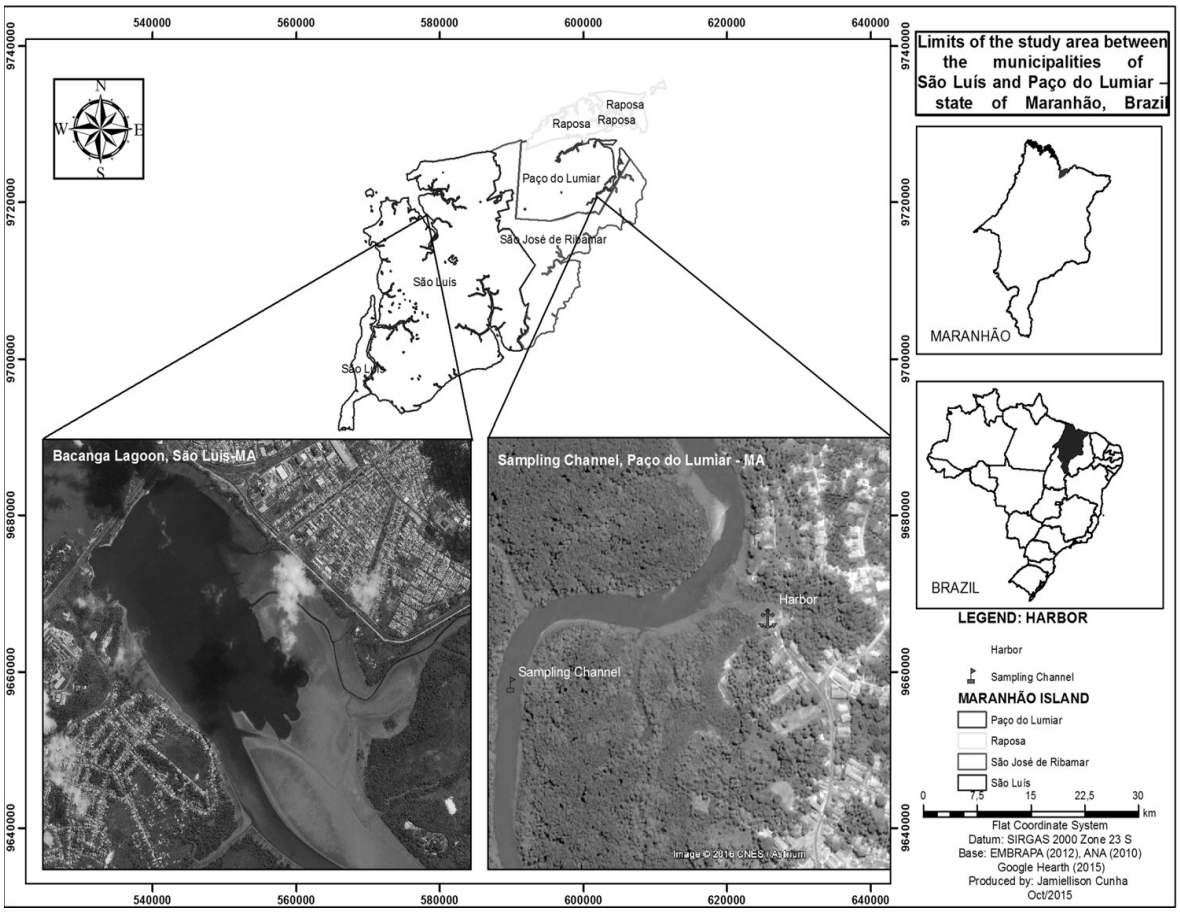

Figure 1 . Location of the study sites: Bacanga Lagoon in the municipality of São Luís (Maranhão/Brazil) and Pau Deitado in the municipality of Paço do Lumiar (Maranhão/Brazil). Source: EMBRAPA (2012), ANA (2010). Produced by Jamielison N. Cunha (2014).

Quantification of total coliforms and coliforms at 45ºC and identification of *Escherichia coli* and other bacteria of the family Enterobacteriaceae.

We weighed muscle samples (10-25 g), aseptically transferred them to sterile polyethylene bags, and transported them to the Laboratory of Food and Water Microbiology of the Department of Chemical Technology (UFMA) to determine the most probable number ($MPNg^{-1}$) of total coliforms (TC) and

coliforms at 45 ºC (C45). We used the technique of multiple tubes, following BRASIL (2003), in which we used 225 mL of saline solution at 0.85% of NaCl as a diluent solution (dilution 10^{-1}), and successive dilutions 10^{-2}, 10^{-3}, and 10^{-4}.

Next, to identify species of the family Enterobacteriaceae, we inoculated the samples that showed a positive result for C45. We used the biochemical tests: Indol, Methyl Red – Voges-Proskauer (VM-VP), Simmons Citrate, Semi-agar SIM Medium, Urea Broth, KCN Broth, Malonate Broth, amino acids (lysine and ornithine), and sugars (arabinose, lactose, maltose, raffinose, and sucrose), following VANDERZANT and SPLITTSTOESSER (2001). We log-transformed (log10) the values of MPN g^{-1} of TC and C45 to fit a normal distribution. We compared logarithmic averages with a *t* test at a significance level of 5% in the software Past 3.0.

Accumulated rainfall data referring to the study period were obtained from the meteorological station Vale (MET ONE Sensor 370), located at the terminal of Ponta da Madeira, São Luís, assigned by the Directorate of Integrated Management and Infrastructure, Northern Sustainability Management of Vale SA.

RESULTS

The samples collected in Bacanga Lagoon in January 2014 showed high prevalence of coliforms, whose values were above the threshold suggested by AGNESE *et al*. (2001) for total coliforms (gray line in Figure 2). Those authors recommended the adoption of actions for better control in the cases of values between 50 and 100 MPN g^{-1} (warning value). Concerning the observed values of C45, 30% of the fish were above the threshold determined by BRASIL (2001), which is 10^2 MPN g^{-1} (black line in Figure 2).

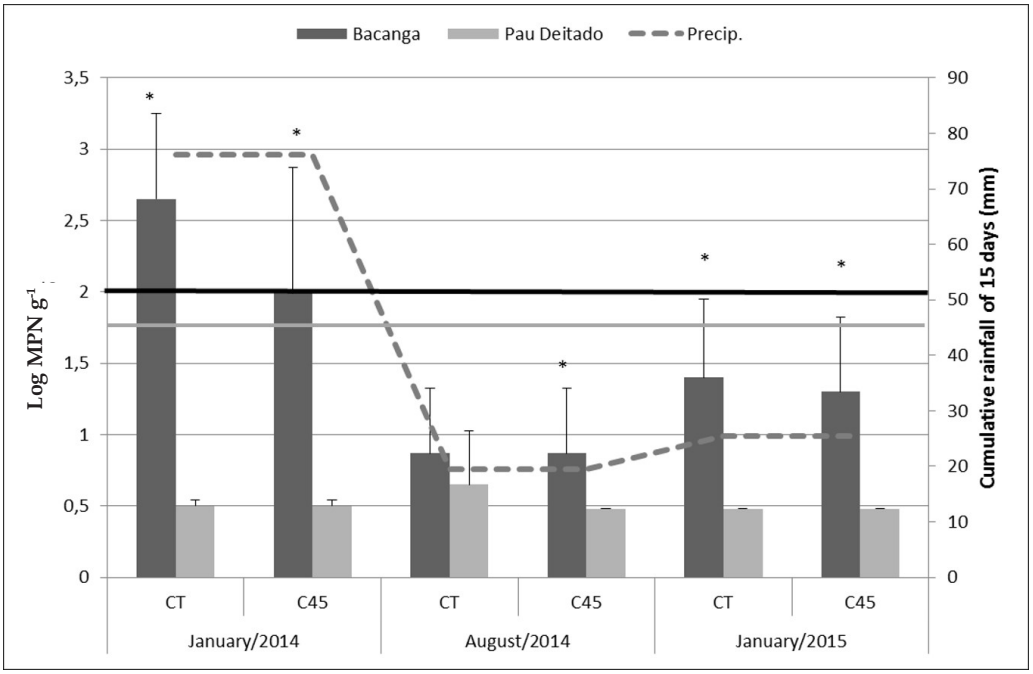

Figure 2. Logarithmic average (+ standard deviation) of the most probable number (MPN) of total coliforms (TC) and coliforms at 45 ºC (C45) in muscles of S. *herzbergii* captured in Bacanga Lagoon and Pau Deitado, northeastern Brazil, in January and August 2014 and January 2015. * indicates different values between fishing sites (test *t*, $p<0.05$). The black line indicates the maximum tolerable threshold for C45 (BRASIL, 2001) and the gray line indicates a "warning value" for TC (AGNESE *et al*., 2001).

In January 2015, the microbial contamination was lower considering that only three fish reached the warning value. The presence of bacteria in fish from Bacanga Lagoon was significantly higher ($p < 0.05$) than in fish from Pau Deitado, except for TC in August ($p = 0.26$). Most fish collected in Pau Deitado showed values below 23 MPN g^{-1} in the three sampling events, which indicates low fecal bacteria contamination.

We analyzed fish samples from Bacanga Lagoon that were positive for C45 for the biochemical

identification of bacteria strains. In January 2014, we identified the highest microbial diversity, with five species, whereas in August 2014, we identified only *E. coli* and *E. aerogenes*, and in January 2015, only *E. coli*, with the highest prevalence recorded (83%) among the three sampling events (Table 1).

Table 1 . Percentage of the identified species of the family Enterobacteriaceae isolated from the muscle tissue of *S. herzbergii* captured in Bacanga Lagoon (São Luís, state of Maranhão, northeastern Brazil).

Species	(January/2014)	(August/2014)	(January/2015)
Escherichia coli	41.6%	33.3%	83.3%
Escherichia fergusonii	20.8%	ND	ND
Enterobacter aerogenes	12.5%	8.3%	ND
Klebsiella omithinolytica	16.6%	ND	ND
Klebsiella pneumoniae	4.1%	ND	ND
Klebsiella oxytoca	4.1%	ND	ND
Salmonella sp.	ND	ND	ND

ND: Not Detected.

DISCUSSION

The microbiota associated with living fish reflects the microbiota of the habitat at the moment of capture or during farming. However, this population changes due to the capacity of different microorganisms (mainly bacteria) to multiply themselves in body structures, such as skin (mucus), gills, and digestive duct (ICMSF, 2005). LANZARIM (2011) affirms that besides their natural biota, fish can also be contaminated by pathogenic bacteria coming from improper processing and storage. Environmental conditions, such as temperature variation and salinity, and individual health condition can facilitate or hinder the multiplication of these bacteria. The microbiota with high degenerative potential is concentrated on the fish surface. After the fish dies, its natural defenses cease to exist, and the superficial microbiota starts to invade inner tissues, which accelerates deterioration. Not only environmental factors but also the proximity to pollution sources can influence the microbiota associated with fish. Fish captured close to human settlements tend to have higher bacteria content and higher microbiota diversity than fish from remote areas. In addition, most bacteria that contaminate fish have enteric origin and are present in polluted environments (SILVEIRA, 2005; MIGNANI *et al*., 2013).

There is a positive correlation between the high level of coliforms in the environment and rainfall, as the higher volume of surface runoff can increase the input of fecal organic matter into the water and the resuspension of sediments loaded with coliforms (ALM *et al*., 2003; AN *et al*., 2002; EVANSON and AMBROSE, 2006). The high concentration of TC and C45 observed in the samples of fish muscle tissue from Bacanga Lagoon in January 2014 are probably related to the high rainfall at that time. The microbiota of a living fish is directly related to the microbiota of the water where it lives, which may vary depending on chemical, physical, and biological properties of that water (MOLLERKE *et al*., 2002; ICMSF, 2005). The lower continental water input in August (dry season) leads to a shorter fresh water residence time and percentage in Bacanga Lagoon, which increases the average salinity of the water. HE *et al*. (2007) observed that an increase in salinity contributes to a decrease in fecal contamination indicators, as higher concentration of salts inhibits microorganism growth or even damages them. C45 bacteria do not multiply themselves or remain viable in the aquatic medium for a long time when kept at ambient temperature. Therefore, their presence indicates a recent contamination (CARDOSO *et al*., 2001). The low rainfall in January 2015 resulted in a lower amount of coliforms in fish samples than in the previous year, which corroborates the hypothesis that rainfall constitutes an important vehicle of bacterial contamination in Bacanga Basin.

In Brazil, the Decree nº 12,486, from 20th October 1978 of the state of São Paulo established standards for the presence of bacteria of the coliform group with fecal origin, with a maximum value allowed of 10^2 g^{-1} for total coliforms in fish (BRASIL, 1978). However,

AGNESE *et al.* (2001) stated that values of total coliforms above 50 MPN g^{-1} in fish flesh are enough to carry out a stricter hygiene control. The Brazilian National Health Surveillance Agency - Anvisa (BRASIL, 2001) established maximum values for C45 in processed fish and fish-derived products, but did not consider in natura fish. However, in the case of products not included in the resolution RCD nº 12, Technical Regulation on Microbiological Standards for Food - Anvisa, the type of product and its processing are used fit it into the patterns established for a similar product. Hence, we considered the patterns for "chilled or frozen fish-derived products" established by the resolution, whose maximum threshold for C45 is 10^2 MPN g^{-1}. Other authors, such as GATTI JÚNIOR (2011), LIUSON (2003), MARTINS (2006), PACHECO *et al*. (2004), and SANTOS (2006) adopted the same pattern.

Several studies have shown contamination by coliforms in fish from different environments. FERREIRA *et al*. (2014) analyzed the microbiology of the Spanish mackerel *Scomberomerus brasiliensis* (marine) (n=60) landed in the municipality of Raposa (state of Maranhão). They recorded in 13.3% of the samples C45 values that varied from 3 to 95 MPN $g^{-1,}$ with the presence of *E. coli* in 1.67% of the samples. SANTOS (2006) recorded values of 4.3 x 10^1 MPNg^{-1} of C45 in mullet (*Mugil* sp.) and horse mackerel (*Caranx* sp.) from municipal markets of São Paulo. RALL *et al*. (2008) analyzed fresh fish traded in the city of Botucatu (n=33), state of São Paulo, and observed variations from <3 to 93 MPN g^{-1} in C45 in seven samples (21.2%). RESENDE-LAGO *et al*. (2013) analyzed fresh fish from supermarkets in Ribeirão Preto, also in the state of São Paulo, and found values from <10^2 to 10^4 MPN g^{-1} for TC, and values below 10^2 MPN g^{-1} for C45.

AGNESE *et al.* (2001) obtained higher values when assessed sanitary conditions of 26 fresh fish samples traded in Seropédica (state of Rio de Janeiro) and found CT values varying from 4 to 2.4 x 10^3 MPN g^{-1} and C45 values varying from <3 to 2.3 x 10 MPN g^{-1}. Those authors also identified *E. coli* in 34.6% of the samples. DAMS *et al*. (1996) recorded C45 values ranging from <3 a 2.4 x 10^5 in samples of striped weakfish (*Cysnoscion striatus*). MARTINS *et al*. (2002) found C45 values between <10 and 1.2 x 10^4 MPN g^{-1} in fish traded in fish-and-pay enterprises in Toledo (state of Paraná).

The previously cited authors obtained their samples during fish production, distribution, and trade. They considered that flaws in processing, poor hygiene conditions, and inadequate sanitation practices were the probable causes of high coliform concentration in fish. PACHECO *et al*. (2004) claim the need for proper hygienic-sanitary conditions from fishing to the final distribution to the consumer to ensure a high-quality fish. According to FERREIRA *et al*. (2014), there can be a cross-contamination by *E. coli* through the contact with ice for preservation, water reuse to wash fish and use of vessels at poor hygienic conditions. VIEIRA (2004) and SATO *et al*. (2005) reported in their studies that the success of fish production depends on the trio: time, temperature, and hygiene, and complying with just one item of the trio is not enough to obtain a high-quality fish. In the present study, coliform concentration reached values up to 2.4 x 10^3 MPN g^{-1} (January 2014). This fact is worrisome as the fish were captured directly from their habitat, which indicates a high input of domestic sewage into the lagoon and environmental conditions favorable to the permanence of those bacteria in the environment.

Despite being known as fecal coliforms, not all coliforms at C45 have an exclusively fecal origin. Strains of the genera *Klebsiella* and *Enterobacter* (non-pathogenic species) can come from the soil and plants. On the other hand, *E. coli* is probably the only bacteria with an exclusively fecal origin and has been used as microbial indicator in estuarine and marine environments (SILVA, 2014).

In the present study, *E. coli* was the most frequent species in samples, and considered one of the most relevant agents of foodborne diseases for public health, considering that they can be related to gastroenteritis in humans.

According to PERETTI and ARAÚJO (2010), the food safety management in Brazil is carried out by the State through the monitoring of the quality of products and services, technical regulations, and control actions. However, those authors highlight that this management is inefficient due to the limitations of the regulatory agencies and the lack of care during the production of safe food by the productive sector. Fish safety is related to the presence of risks (physical, chemical, and biological) at acceptable levels, i.e., without the potential to cause adverse health effects. Fish industry has directed its quality management systems to reach these objectives through the adoption of sanitary actions, such as Good Production Practices (of Management, in the case of aquaculture) and Hazard Analysis and

Critical Control Point (HACCP). However, these practices are still far from what occurs in traditional fishing (subsistence). The sampling site, for example, has received untreated effluent discharge with a high amount of contaminants including pathogenic agents, such as *E. coli*. This way, the lack of management to implement basic sanitation services efficiently has affected the quality of the fish captured at this site. The deficient education of the population that discharges their residues directly into the Bacanca Lagoon also affects fish quality.

Fortunately, most pathogenic bacteria are destroyed during cooking or thermal treatment, because do not resist extreme temperatures. Most cases of food intoxication are associated with the consumption of raw fish or fish submitted to an inefficient thermal treatment. Some authors cite practices that help increase fish conservation; such as PIMENTEL and PANETTA (2003) who focus on the importance of the use of ice in fish conservation, as in the absence of ice, fish deterioration is accelerated. The Ordinance # 185, established on 13th May 1997, regarding the Technical Regulation on the Identity and Quality of Fresh Fish (whole and eviscerated), determines that "fish packaging must use an amount of finely crushed ice enough to ensure a temperature close to the fusion point in the inner part of the muscle" (BRASIL, 1997).

The ice must be used immediately after fish capture to reduce the speed of bacteria proliferation. Practices, such as beheading, which eliminates gills that are among the main focus of deterioration (ORDÓÑEZ, 2005), and evisceration (GERMANO and GERMANO, 2001) help preserve fish for a longer time.

In regions where there is a greater habit of consuming *in natura* fish, there is a positive relationship with the cases of food outbreaks in comparison with other localities (HAMADA-SATO *et al.*, 2005). It is worth highlighting the increase in the habit of consuming raw fish among Brazilians in exotic dishes, such as sushi, sashimi, ceviche, marinate, etc. (CÁRDIA and BRESCIANI, 2012).

CONCLUSION

The high concentration of coliforms at 45 ^{o}C in fish captured in Bacanga Lagoon suggests unsatisfactory sanitary conditions. The bacteria *E. coli* identified in pemecou sea catfish puts at risk the consumers of those fish, as the presence of such bacteria in food is related to the occurrence of diseases transmitted through food and water. Unfortunately, this scenario has been observed in several poor regions where large human populations, precarious sanitary conditions, and recurrent contamination of the water and food prevail. The consumption of fish contaminated with enterobacteria represents a potential health risk to the consumer, mainly when consumption occurs without proper thermal treatment.

Technology transfer projects aiming at improving sanitary conditions of the artisanal fishing must be implemented at the Bacanga River Basin. The diffusion of knowledge in the community about Good Management Practices (GMP) and the Hazard Analysis and Critical Control Points (HACCP) for fisheries will certainly impact this activity positively. Before these actions are applied, it is important to warn the population about the risks of consuming fish from polluted environments. Besides, there is an urge to implement sanitation policies in the river basin area to avoid risks to the population that consumes and trade the fish captured in the waters of the ecosystems contaminated with domestic effluents.

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