

*Full Length Research Paper*

## **Microbiota of two species of commercially important fish in the Amazon region (Belém-Pará-Brazil): Butterfly peacock bass (*Cichla ocellaris*) and piramutaba (*Brachyplatystoma vailanti*)**

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The Amazon offers great potential for fishery activities but the fish fauna's specific microbiota is not yet known. This paper identified the bacterial flora composition and the influence of this process on the microbiological spoilage in economically important fish species in the Amazon region: butterfly peacock bass (*Cichla ocellaris*) and piramutaba (*Brachyplatystoma vailanti*). To this end, microbiological characterization was performed: counts of total mesophilic aerobic bacteria, psychrotrophic bacteria and coliforms at 35 and 45°C. Bacteria were also isolated through seeding in agar surface using violet red bile glucose (VRBG) for enterobacteria strains and Baird-Parker Agar with egg-yolk tellurite for *Staphylococcus* species, both with incubation at 36°C for 48 h. The bacteria isolated were identified using the API 20E kit (Enterobacteria), and Gram-positive with API Staph (Staphylococci). Finally, the limit temperature for strain growth was tested using spectrophotometry readings at 554 nm at 10 and 15°C at three different times: 0, 3 and 6 h. The mesophilic aerobic bacteria counts for fresh fish samples ranged from 6.03 - 8.23 log CFU/g for piramutaba and 4.52 - 7.24 log CFU/g for butterfly peacock bass. The count ranges of psychrotrophic aerobic bacteria found were 6.14 - 8.56 log CFU/g and 4.52 - 7.24 log CFU/g for piramutaba and butterfly peacock bass, respectively. They also had an average score above 10<sup>3</sup> MPN/g for total coliforms. Sixteen different strains were isolated. The most predominant were *Staphylococcus hominis*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia* and *Enterobacter intermedius*. When subjected to temperatures of 10 and 15°C, the strains did not achieve growth for 6 h at a 95% significance level.

**Key words:** Fish, *Cichla ocellaris*, *Brachyplatystoma vailanti*, microflora.

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### **INTRODUCTION**

The coast of the state of Pará (Brazil) offers great potential for fishery activities due to the numerous rivers and estuaries that empty into the Atlantic Ocean, forming

a complex aquatic environment with high biological productivity. The substantial biomass of fish species in this region is exploited by both artisanal and industrial

fleets (Isaac et al., 2009). Butterfly peacock bass (*Cichla ocellaris*) and piramutaba (*Brachyplatystoma vailantii*) feature among these species and are two of the favorite targets of fishing in the region given their considerable importance from both an economic and nutritional perspective. However, as marine fish, freshwater fish are extremely perishable food commodities. Enzymatic and chemical reactions are usually responsible for the initial loss of freshness whereas microbial activity is responsible for the overt spoilage and thereby establishes product shelf life (Gram, 1995; Gram and Huss, 1996). In some cases, chemical changes such as auto-oxidation or enzymatic hydrolysis of the lipid fraction may result in off-flavors, while, in other cases, tissue enzyme activity can lead to unacceptable softening of the fish. The spoilage of fresh fish by microbial activity is usually due to its microbiota located mainly in the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. It can also be the consequence of fish cross-contamination associated with inappropriate handling and storage (Cruz-Romero et al., 2008). The poikilotherm nature of fish allows bacteria to grow in a broad temperature range. Thus, the microbiota of temperate-water fish is dominated by psychrotrophic, aerobic or facultative anaerobic Gram-negative, rod-shaped bacteria, and in particular, by *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Shewanella putrefaciens*, *Flavobacterium*, *Cytophaga*, *Vibrio*, *Photobacterium* and *Aeromonas* (Lalitha and Surendran, 2006; Pantazi et al., 2008). The microbiota in tropical freshwater fish is dominated by Gram positive bacteria such as *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Enterobacter*, *Flavobacterium*, *Flexibacter*, *Pseudomonas*, *Psychrobacter*, *Citrobacter*, *Micrococcus*, *Staphylococcus*, *Streptococcus* and *Moraxella* spp. (Gram and Huss, 1996; Apun et al., 1999; Austin, 2002; ICMSF, 2005). The specific microbiota of butterfly peacock bass and piramutaba is not yet known. During the processing of the two species, the microorganisms present in the gut and on the skin can spread to the processing equipment, the workers and sterile flesh fillets. The objective of this paper was to identify the microbiota and study the growth temperature of isolated microorganisms to improve the refrigeration temperature conditions in two fish species from the Amazon region: butterfly peacock bass (*C. ocellaris*) and piramutaba (*B. vailantii*).

## MATERIALS AND METHODS

### Fish samples

Four successive commercial-sized fish samples (n=4) (butterfly

peacock bass and piramutaba) were collected between March and April 2013 at Ver-o-Peso Market (Belém, Pará, Brazil). At the time of collection, the samples were placed in sterile bags kept under refrigeration (around 10°C) and transported to the Laboratory of Food Microbiology (Federal University of Pará - UFPA) for further analysis.

### Microbiological characterization

Twenty five grams of each sample (ventral part of the filet) were aseptically collected and added with 225 mL of 0.1% sterile peptone water (SPW), thus obtaining 1:10 dilution, which were homogenized in a stomacher (STOMACHER 400 CIRCULATOR SEWARD) at 2,300 rpm for 30 s. Next, counts of total mesophilic aerobic bacteria, psychrotrophic bacteria and coliform at 35 and 45°C were performed according to Brazil (2003). The total mesophilic aerobic and psychrotrophic bacteria counts were carried out in pour plate using plate count agar followed by incubation at 35°C/48 h for mesophilic and 7°C/10 day for psychrotrophic bacteria. Coliforms at 35 and 45°C were counted through the most probable number (MPN), with three sets of three tubes. Lauryl sulfate tryptose broth (LST) was used as a presumptive medium and incubated at 35°C for 24-48 h. After reading, the positive tubes were transferred to brilliant green bile broth 2% (GB) and EC broth. The former was incubated at 35°C/24-48 h for confirmation of total coliforms and EC broth tubes were incubated in a water bath at 45.5°C/24 h for confirmation of thermotolerant coliforms.

### Bacterial isolation

The homogenized matter used for microbiological characterization was subsequently used for bacteria isolation. The isolation to obtain pure cultures was carried out through seeding in agar surface using violet red bile glucose (VRBG) for enterobacteria strains and Baird-Parker with egg-yolk tellurite for *Staphylococcus* species, both with incubation at 36°C/48 h. Next, one plate was selected for each medium and 5-10 colonies per plate were randomly chosen. The selected colonies were striated in VRBG or Baird-Parker agar plates to obtain a pure culture. After incubation at 36°C/48 h, a colony was transferred from each plate to brain heart infusion (BHI) with 10% glycerol and stored in a freezer to be used in further tests.

### Bacterial strain identification

The bacteria isolated were previously identified with Gram stain tests. Next, Gram negative strains were identified using the API 20E kit (Enterobacteria), and Gram positive strains with API Staph (Staphylococci). The procedure was in accordance with the manufacturer's recommended procedures (Biomérieux, France) (Harrigan, 1998).

### Limit temperature for growth (adapted from Bordignon-Junior et al., 2012)

Strains were reactivated in BHI broth for 24 h at 36°C. After that, the isolates identified were transferred to a new BHI broth (1:15 mL)

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**Table 1.** Mean values of microbiological characterization in fresh fish.

Samples	Mesophilic aerobic bacteria(log CFU/g)	Psychrotrophic bacteria (log CFU/g)	Total coliforms (MPN/g)	Thermotolerant Coliforms(MPN /g)
Piramutaba 01	6.23±0.03 <sup>bcd</sup>	6.14±0.13 <sup>c</sup>	1,100 <sup>a</sup>	15 <sup>c</sup>
Piramutaba 02	8.23±0.01 <sup>a</sup>	8.56±0.02 <sup>c</sup>	1,100 <sup>a</sup>	1,100 <sup>a</sup>
Piramutaba 03	6.03±0.01 <sup>cd</sup>	6.46±0.00 <sup>c</sup>	1,100 <sup>a</sup>	1,100 <sup>a</sup>
Piramutaba 04	6.61±0.65 <sup>bc</sup>	7.97±0.03 <sup>a</sup>	1,100 <sup>a</sup>	240 <sup>b</sup>
B. peacock bass 01	4.52±0.74 <sup>e</sup>	6.40±0.06 <sup>d</sup>	1,100 <sup>a</sup>	1,100 <sup>a</sup>
B. peacock bass 02	5.35±0.07 <sup>de</sup>	6.66±0.06 <sup>a</sup>	1,100 <sup>a</sup>	1,100 <sup>a</sup>
B. peacock bass 03	6.16±0.01 <sup>cd</sup>	6.52±0.008 <sup>c</sup>	1,100 <sup>a</sup>	240 <sup>b</sup>
B. peacock bass 04	7.24±0.03 <sup>ab</sup>	8.33±0.04 <sup>b</sup>	1,100 <sup>a</sup>	1,100 <sup>a</sup>

\*Different letters in each column means difference at 95% level of significance.

and maintained at different temperatures: 10 and 15°C during three different times: 0, 3 and 6 h. Spectrophotometric readings were performed (Spectrophotometer Model Nova 2000 UV) at 554 nm.

#### Statistical analysis

Tukey's test was applied to evaluate the difference of means among microorganism groups (mesophilic and psychrotrophic bacteria, total and thermotolerant coliforms) found in different fishes. The optical density data were subjected to ANOVA considering different groups of isolated microorganisms. The software Statistica 8.0 was employed considering a 95% level of significance.

## RESULTS AND DISCUSSION

### Microbiological characterization

The mesophilic aerobic bacteria counts for fresh fish samples ranged from 6.03 - 8.23 log CFU/g for piramutaba and 4.52 - 7.24 log CFU/g for butterfly peacock bass (Table 1). Brazil (2001) does not establish micro-biological standards for mesophilic bacteria count in fresh fish. However, the International Commission on Microbiological Specifications for Foods (ICMSF, 1986) recommends the limits for mesophilic aerobic should not exceed values of  $10^7$  CFU/g or  $\text{cm}^2$  in chilled fish samples for human consumption.

Aerobic mesophilic bacteria, when present in large numbers, indicate unsanitary conditions. The high count of this microorganism in food may result from unsatisfactory storage conditions, with potential danger to health (Morton, 2001; Coelho et al., 2010). The fish samples analyzed had a mean score of  $10^6$  and  $10^5$  for piramutaba and butterfly peacock bass, respectively. A similar result was observed by Fernandez and Barbosa (2010), who reported counts of  $10^4$  -  $10^5$  CFU/g for mesophilic bacteria in sardines. Li et al. (2013) found values around  $10^3$  CFU/g in large yellow croaker

(*Pseudosciaena crocea*) from China. Vishwanath et al. (1998) observed a total bacteria plate count range of  $10^6$  -  $10^7$  CFU/g for *Muscodor albus* (Manipur, India). Oku and Amakoromo (2013) found bacteria count values of  $10^8$  -  $10^{10}$  CFU/g for fresh fish (Yenagoa metropolis, Nigeria). Thong Thi et al. (2013) found total mesophilic counts on raw pangasius fish around 5.1 log CFU/g. Previous studies by Shewan (1977), Guizani et al. (2005) and Ercolini et al. (2009) have indicated that mesophilic microorganisms are dominant in tropical fish species. The count of psychrotrophic aerobic bacteria found ranged from 6.14 to 8.56 log CFU/g for piramutaba and from 4.52 to 7.24 log CFU/g for butterfly peacock bass (Table 1).

The Brazilian legislation establishes no maximum limit for psychrotrophic microorganisms in fish for human consumption. But considering the ICMSF (1986), piramutaba and butterfly peacock bass species showed values above the maximum limit ( $10^7$  CFU/g).

Britto et al. (2007) evaluated the bacteriological deterioration of whole jaraqui (*Semaprochilodus* spp.) captured in the Amazon region and found a count of psychrotrophic aerobic bacteria ranging from 2 - 3 log CFU/g. Similar results were found by Hanninen et al. (1997), who found freshwater fish samples contaminated by *Aeromonas* spp. Sallam et al. (2007) found psychrotrophic bacteria count in raw Pacific saury around 3.95 log CFU/g, while Thong Thi et al. (2013) found total psychrotrophic count to be around 4.3 log CFU/g in raw pangasius fish (*Pangasius hypophthalmus*).

The growth of psychrotrophics also indicates poor sanitary conditions. Psychrotrophic bacteria grow in foods refrigerated between 0 - 7°C, with optimum growth temperature around 20°C. Some psychrotrophics may be pathogens, such as *Aeromonas hydrophila*, some strains of *Bacillus cereus*, *Clostridium botulinum* type E, B and F, *Listeria monocytogenes*, *Vibrio cholerae*, *Yersinia enterocolitica*, some strains of enteropathogenic *Escherichia coli*, and other pathogens such as *Salmonella* spp. and *C. perfringens* type C. Some strains of *Bacillus*

**Table 2.** Identification of *Staphylococcus* strains isolated from piramutaba.

Strains isolated	Number of strains isolated	Strains isolated (%)	ID (%)
<i>Staphylococcus lentus</i>	01	4.76	99.5
<i>Staphylococcus epidermis</i>	01	4.76	97.9
<i>Staphylococcus aureus</i>	04	19.04	88.4-99.6
<i>Staphylococcus hominis</i>	05	23.80	81.3-94.9

**Table 3.** Identification of *Staphylococcus* strains isolated from butterfly peacock bass.

Strains isolated	Number of strains isolated	Strains isolated (%)	ID (%)
<i>Staphylococcus cohnii</i> ssp. <i>cohnii</i>	01	4.76	98.3
<i>Staphylococcus saprophyticus</i>	01	4.76	80.2
<i>Staphylococcus lentus</i>	01	4.76	99.9
<i>Staphylococcus xylosus</i>	01	4.76	97.9
<i>Staphylococcus hominis</i>	06	28.57	81.3-92.3

*cereus* and *Staphylococcus aureus*, which have lower development temperatures between 7 – 15°C can grow if temperature abuse occurs during storage (Cousin et al., 2001).

In refrigerated fish, the psychrophilic and psychrotrophic bacteria play direct roles in fish deterioration because they multiply well in these conditions (Franco et al., 1996). The butterfly peacock bass and piramutaba collected had an average score above 10<sup>3</sup> MPN/g for total coliforms (Table 1). Although this does not indicate the presence of pathogens, total coliforms are important indicators of potential product deterioration and its mean shelf life (Agnese et al., 2001).

The total and thermotolerant coliforms are indicators of hygienic quality, not representing direct contact of the product with human or animal feces, but reporting on the degree of microbial pollution to which the food has been exposed. This score thus indirectly reflects the quality of production practices.

Brazil (1997, 2001) set the value of 10<sup>2</sup> MPN/g as the maximum acceptable standard for thermotolerant coliforms in fish and fishery products. However, it is observed that the average of the samples collected from piramutaba and butterfly peacock bass are above the limits established by the Brazilian legislation, indicating the possibility of water contamination as well as the existence of some source of organic matter containing animal or even human feces, which compromises the quality of the fish.

### Bacterial strain identification

Among the 36 isolates, it was observed that 58.33% were Gram positive and the other 41.67% were Gram

negative. From the results of the Gram stain test, the kits were selected to identify the strains.

The data show that Gram negative bacteria belonged to seven different species described in Tables 2 and 3. The most predominant were *Staphylococcus hominis* for butterfly peacock bass (28.57%) and piramutaba (23.81%) and *Staphylococcus aureus* (19.05%), found only in piramutaba.

Staphylococci are not part of the normal fish microbiota (Huss, 1988; Van den Broek et al., 1984). This indicates that if *S. aureus* is found in fish, it most likely originates from human sources (Bulushi et al., 2010). Moreover, the presence of staphylococci in fish is an indication of (a) postharvest contamination due to poor personnel hygiene, or (b) disease in fish (Austin and Austin, 2007; Huss, 1988). In Japan, fish-borne microbes, including *S. aureus*, are a major cause of food poisoning (Cato, 1998) both because of the very high consumption of fish and because of the common practice of eating raw fish (Huss, 1988). Other authors reported food poisoning by *S. aureus* due to fish consumption (Cato, 1998; CDC, 2011; Huss, 1988; Iwamoto et al., 2010; Sokari, 1991; Ayulo et al., 1994; Rodma et al., 1991).

*S. hominis* is rarely implicated in food poisoning, because it does not multiply quickly in this environment. However, it may contaminate food since humans are carriers of microorganisms and some of these may be related to certain human infections (Pereira and Pereira, 2005; Cunha et al., 2006). The presence of microorganisms in fish products may also indicate the occurrence of food contamination due to poor hygiene in handling and lack of preservation techniques, since *S. hominis* is not part of microbiota of these aquatic organisms.

The Gram negative bacteria found belong to nine

**Table 4.** Identification of *Enterobacteriaceae* strains isolated from piramutaba.

Strains isolated	Number of strains isolated	Strains isolated (%)	ID (%)
<i>Stenotrophomonas maltophilia</i>	01	6.67	65.6
<i>Enterobacter intermedius</i>	02	13.34	72.4-92.5
<i>Klebsiella pneumoniae ssp. pneumoniae</i>	01	6.67	94.4
<i>Serratia marcescens</i>	01	6.67	71.9
<i>Buttiauxella agrestis</i>	01	6.67	93.6
<i>Pantoea spp. 1</i>	01	6.67	87.8

**Table 5.** Identification of *Enterobacteriaceae* strains isolated from butterfly peacock bass.

Strains isolated	Number of strains isolated	Strains isolated (%)	ID (%)
<i>Stenotrophomonas maltophilia</i>	03	20.00	80.4-92.4
<i>Enterobacter intermedius</i>	01	6.67	93.3
<i>Enterobacter amnigenus 2</i>	01	6.67	82.2
<i>Klebsiella pneumoniae ssp. pneumoniae</i>	01	6.67	90.0
<i>Serratia liquefaciens</i>	01	6.67	82.6
<i>Serratia ficaria</i>	01	6.67	98.4

% ID indicates the profile similarity of isolates with the standards established by the kits, according to the manufacturer.

different species of enterobacteria as shown in Tables 4 and 5. The most prevalent were *Stenotrophomonas maltophilia* (20.0%) and *Enterobacter intermedius* (13.34%) in butterfly peacock bass and piramutaba, respectively.

Both *S. maltophilia* and *E. intermedius* have not been found to cause food poisoning. However, the presence of these bacteria is worrying since they may be associated with fish deterioration. Oku and Amakoromo (2013) obtained twelve bacterial isolates from raw tropical freshwater fish samples. The bacteria belonged to five genera identified as: *B. subtilis*, *Corynebacterium*, *Lactobacillus*, *Pseudomonas* and *S. aureus*.

The specific microbiota of fresh butterfly peacock bass and piramutaba is not yet known. However, it is known that the flora in tropical fish often carries a slightly higher load of Gram-positive and enteric bacteria than fish from temperate water, but it is otherwise similar to that flora dominated by psychrotrophic Gram negative, rod-shaped bacteria belonging to the genera *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Shewanella*, *Flavobacterium*, *Vibrionaceae*, *Aeroomonadaceae* and, to a lesser degree, *Bacillus*, *Micrococcus*, *Clostridium*, *Lactobacillus* and *Corynebacterium* (Liston, 1980; Apun et al., 1999; Austin, 2002; ICMSF, 2005). Enterobacteriaceae genera have been frequently isolated from the digestive tracts and flesh of freshwater fish (Austin, 2002; Yagoub, 2009; Gonzalez-Rodriguez et al., 2002; Paludan-Müller et al., 1998). Apun et al. (1999) show some Enterobacteriaceae strains such as *K. pneumoniae*, *E. aerogenes*, and *E. coli* have been isolated from the intestines of tropical

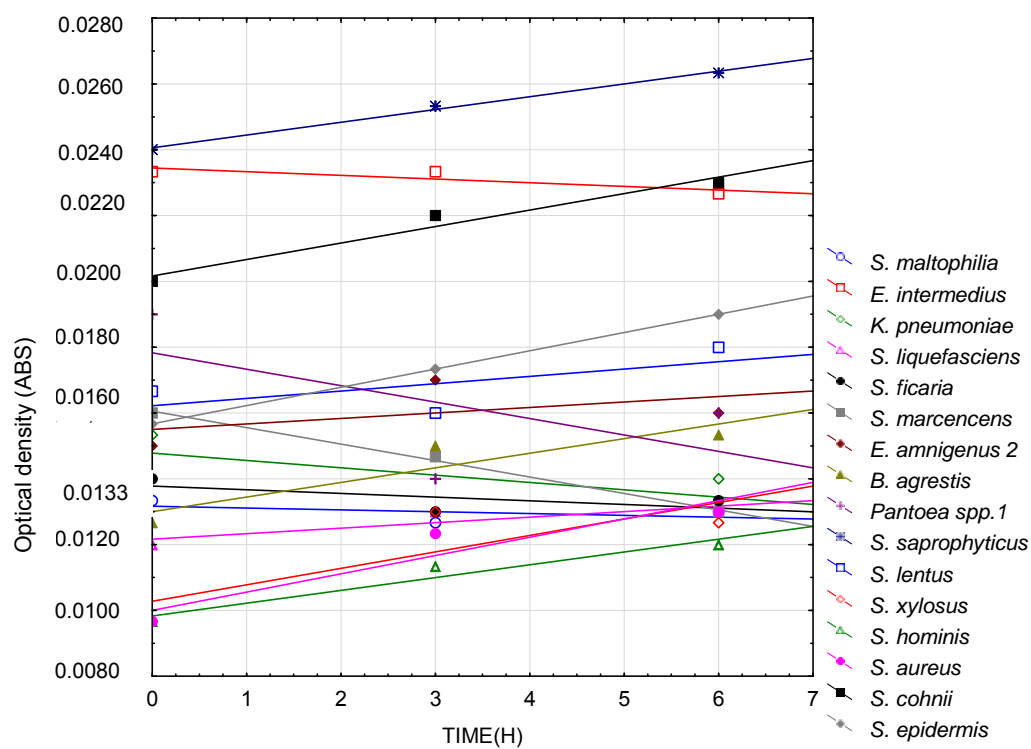
freshwater fish. *Serratia* spp. have also been found in *Pangasius* filets (Thong et al., 2013). At ambient temperature (25°C), the microbiota is dominated by mesophilic *Vibrionaceae* (Gorczyca and Pek, 1985; Gram et al., 1990) and, particularly if the fish are caught in polluted waters, mesophilic Enterobacteriaceae become dominant (Gram, 1992).

#### Limit temperature for growth

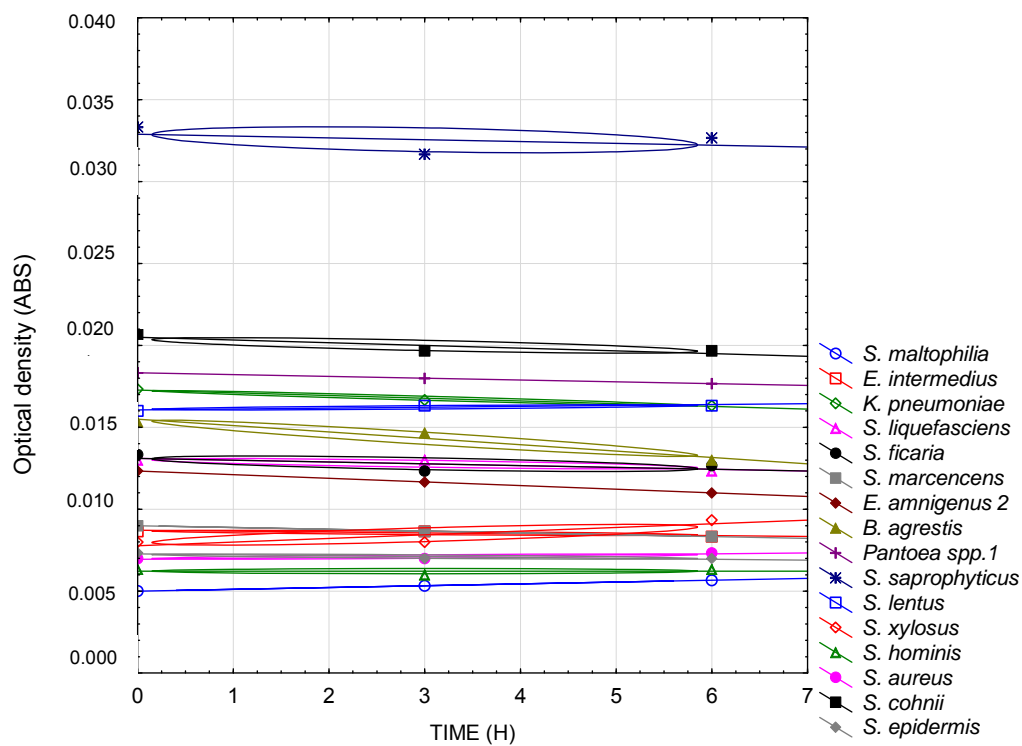
The 16 strains (Figures 1 and 2; Tables 6 and 7) from butterfly peacock bass and piramutaba, when subjected to temperatures of 10 and 15°C, did not achieve growth for 6 h at a 95% significance level.

Denton and Kerr (1998) stated that *S. maltophilia* growth does not occur at temperatures lower than 5°C. However, Margesin and Schinner (1991) reported isolation of an *S. maltophilia* strain from an alpine environment capable of growth at 10°C. According to Schmitt et al. (1990), *S. aureus* is capable of growing in a wide range of temperatures, from 7 - 48.5°C with an optimum from 30 to 37°C. Valero et al. (2009) reported *S. aureus* growth can be inhibited at refrigeration temperatures (around 8°C). Schmitt, Schuler-Schmid and Schmidt-Lorenz (1990) stated the lowest temperature limit for growth was about 7°C for seven days. Raw fish should be kept at 10°C throughout processing to inhibit the growth and toxin production of pathogenic bacteria (FDA, 2011).

However, it is important to remember that the fish



**Figure 1.** Optical density (absorbance) measured through spectrophotometry (554 nm) at 10°C after 6 h.



**Figure 2.** Optical density (absorbance) measured through spectrophotometry (554 nm) at 15°C after 6 h.

**Table 6.** ANOVA for optical density measured through spectrophotometry (554 nm) at 10°C after 6 h.

<b>Bacterial strains/temperature</b>	<b>F</b>	<b>p</b>
<i>Staphylococcus cohnii</i> ssp. <i>cohnii</i>	1.2353	0.3554
<i>Staphylococcus saprophyticus</i>	0.1878	0.8335
<i>Staphylococcus lentus</i>	0.4179	0.6762
<i>Staphylococcus epidermis</i>	0.9740	0.4302
<i>Staphylococcus xylosus</i>	3.9565	0.0802
<i>Staphylococcus aureus</i>	1.7931	0.2452
<i>Staphylococcus hominis</i>	3.5455	0.0962
<i>Stenotrophomonas maltophilia</i>	0.6000	0.5787
<i>Enterobacter intermedius</i>	0.0125	0.9876
<i>Enterobacter amnigenus</i> 2	0.8182	0.4851
<i>Klebsiella pneumoniae</i> ssp. <i>pneumoniae</i>	0.6727	0.5450
<i>Serratia liquefaciens</i>	1.0000	0.4219
<i>Serratia ficaria</i>	0.1207	0.8884
<i>Serratia marcensces</i>	2.4400	0.1677
<i>Buttiauxella agrestis</i>	2.4400	0.1677
<i>Pantoea</i> sp.	4.0714	0.0763

**Table 7.** ANOVA for optical density measured through spectrophotometry (554 nm) at 15°C after 6 h.

<b>Bacterial strains/temperature</b>	<b>F</b>	<b>P</b>
<i>Staphylococcus cohnii</i> ssp. <i>cohnii</i>	0.0234	0.9769
<i>Staphylococcus saprophyticus</i>	0.4220	0.6737
<i>Staphylococcus lentus</i>	0.0910	0.9143
<i>Staphylococcus epidermis</i>	0.0357	0.9651
<i>Staphylococcus xylosus</i>	0.6400	0.5598
<i>Staphylococcus aureus</i>	0.0232	0.9771
<i>Staphylococcus hominis</i>	0.0588	0.9434
<i>Stenotrophomonas maltophilia</i>	0.6000	0.5787
<i>Enterobacter intermedius</i>	0.3330	0.7290
<i>Enterobacter amnigenus</i> 2	0.3429	0.7228
<i>Klebsiella pneumoniae</i> ssp. <i>pneumoniae</i>	0.3330	0.7290
<i>Serratia liquefaciens</i>	0.0597	0.9426
<i>Serratia ficaria</i>	0.1111	0.8966
<i>Serratia marcensces</i>	0.6000	0.5787
<i>Buttiauxella agrestis</i>	0.1069	0.9003
<i>Pantoea</i> sp.	0.2730	0.7702

studied are sourced from tropical regions, that is, from waters with elevated temperatures. It is suggested that the strains found have adapted to moderate temperatures. Thus, refrigeration conditions are adverse for the multiplication of such microorganisms. Hence, butterfly peacock bass and piramutaba from the Amazon region could maintain their quality considering the microbiological

aspects under refrigerated conditions (time and temperature) considering the bacteria isolated in this study. However, due to the high psychrotrophic count (4.52 – 8.56 log CFU/g), greater than the 7 log CFU/g established by the ICMSF (1986), other conservation measures (besides refrigeration) are required to prevent the multiplication of these microorganisms and fish deterioration.

## Conclusion

The microbiological evaluation suggests Amazonian fish species marketed at the port of Ver-o-Peso Market have high counts of total mesophilic and psychrotrophic bacteria. Furthermore, it was observed that some isolated mesophilic microorganisms did not grow under refrigeration temperatures of over 6 h. However, due to the high concentrations of psychrotrophic bacteria, these fish require other conservation methods to ensure the microbiological quality.

## Conflict of interests

The authors declare there is no conflict of interests.

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