Microorganisms used as indicators of hygiene and sanitary quality in *Perna perna* mussels from mariculture in three traditional communities in Niterói, RJ, Brazil

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Abstract

Bivalve molluscs are invertebrate organisms of economic relevance in several regions of the world. Due to their commercial importance, this study aimed to monitor the hygiene and sanitary quality of *Perna Perna* mussels (Linnaeus, 1758), from commercial farming and extractivism in Jurujuba, Boa Viagem and Centro, Niterói, Rio de Janeiro, Brazil. Collections were carried out once a month, from January to December 2022, in 1,080 animals, for bacteriological evaluation by the MPN of coliforms at 35°C, 45°C and *Escherichia coli*, according to the "Fluorocult LMX" Broth method. The results obtained in fresh mussel samples of coliforms at 45°C (*E. coli*) ranged from < 3.0 to 4.0 x 10^2 MPN/100 g, and animals from Jurujuba had the highest geometric mean of contamination and a higher 90th percentile level. A percentage of 22.2% of samples with satisfactory quality for consumption was obtained, according to the National Safe Bivalve Molluscs Program (MoluBiS). It is concluded that it is necessary to implement the aforementioned Program in the state of Rio de Janeiro to monitor sanitary conditions, so that the minimum requirements to guarantee the safety and quality of bivalve molluscs for human consumption are provided.

Keywords: coliforms; contamination; bacteriological; bivalve mollusk.

Practical Application: Bivalve molluscs are used to assess the contamination of marine environments, to measure parameters linked to microbiological contaminants, as they are filtering organisms, with this condition there is a need to implement the National Safe Bivalve Molluscs Program, which aims to establish the minimum requirements necessary to ensure the safety and quality of bivalve molluscs for human consumption.

1 INTRODUCTION

Bivalve molluscs of the *Mytilidae* family are widely used in assessing contamination of marine environments, particularly to measure parameters linked to microbiological contaminants, as they are filter-feeding sessile organisms, participate in the nutrient cycling, give rise to food webs, and occur in the same location throughout the year (Beyer et al., 2017; Gosling, 2015; Mersch et al., 1996; Świacka et al., 2019).

Guanabara Bay receives the majority of untreated effluents, with raw domestic sewage totaling 17 m³/s. There are over 14,000 industries around the Bay, whose effluents may contain heavy metals such as lead, chromium, zinc, and mercury (Semads, 2001).

The production of fresh mussels in the Guanabara Bay region accounts for 50 to 70% of the mussels collected in Jurujuba and other regions. Extractivism consists of collecting mussels on rocky shores (Lage & Jablonski, 2008).

The importance of the bacteriological analysis recommended for monitoring the estimate of the average density of *Escherichia coli* in the edible part of bivalve molluscs is due to the fact

that this microorganism is used as an indirect indicator of contamination by other enterobacteria, following the One Health approach and pollution by anthropic action in the environment where the animals are produced. In turn, the absence of good handling practices in obtaining the food matrix, conservation methods, as well as the quality of the source water may favor contamination and microbial growth (Anon, 2017; Feldhusen, 2000; Walker et al., 2018).

One way of conditionally using bivalve molluscs with a certain degree of contamination is to subject them to complementary treatment, allowing for the elimination of pathogenic microorganisms (Anon, 2017).

Therefore, Ordinance SDA/MAPA No. 884, of September 6th, 2023, which set out the National Safe Bivalve Molluscs Program (MoluBiS), aims to establish the minimum requirements necessary to guarantee the safety and quality of bivalve molluscs for human consumption (Brasil, 2023).

The current program recommends defining areas for the extraction and production of bivalve molluscs as approved

extraction (Class A control areas: all-time series results less than or equal to 700 MPN of *E. coli* and 80% of results less than or equal to 230 MPN of *E. coli*), conditionally approved extraction (bivalve molluscs from Class B control areas: all-time series results that are less than or equal to 46,000 MPN of *E. coli* and 90% of samples that have a result less than or equal to 4,600 MPN of *E. coli*; or Class C: all-time series results that are less than or equal at 46,000 MPN of *E. coli*). Control areas that do not meet pre-defined classification criteria must be designated as suspended (prohibited extraction).

Bivalve molluscs from approved areas may be intended for consumption without the need for depuration in a depuration plant, upon prior inspection. However, those from Class B areas (conditionally approved extraction) that are not transferred to relaying areas must be subjected to heat treatment or depuration in a depuration plant (Brasil, 2023).

Depuration is a technique used to reduce microbial contamination of filter-feeding molluscs to levels acceptable by MoluBiS for human consumption by keeping the animals in tanks with clean water (Epagri, 2013).

It is a controlled process where bivalve molluscs are placed in seawater treated with agents such as chlorine, ozone or ultraviolet light for a few hours to reduce the microorganisms present in their tissues through natural filtration (Guimarães Filho et al., 2022b; Rong et al., 2014).

Therefore, the study aimed to carry out bacteriological analyses in accordance with MoluBiS, which aims to ensure the safety and quality of bivalve molluscs for human consumption (Brasil, 2023).

2 MATERIAL AND METHODS

2.1 Study area

Collections were performed once a month, from January to December 2022, encompassing 1,080 animals from the communities of Jurujuba, Boa Viagem and Centro, Niterói, where there is commercial farming and extraction of mussels, particularly *Perna* (*Linnaeus,* 1758).

The molluscs were collected randomly (30 animals at each collection) from Jurujuba (22°55'53" S/43°09'38" W), Boa Viagem (22°54'36" S/43°07'45" W), and Center (22°52'16" S/43°09'30" W; 22°52'08" S/43° 11'02" W; 22°53'27" S/43°11'40" W), as depicted in Figure 1, to carry out bacteriological analyses.

2.2 Field sample

Bacteriological analyses are in accordance with the standards of MoluBiS, with the Most Probable Number (MPN) of coliforms at 45°C (*Escherichia coli*). The samples analyzed were carried out in triplicate, from January to December 2022, except for Boa Viagem and Centro, which were in the closed season for mussels from September 1st to December 31st, 2022.

The mussel samples were transported in refrigerated containers (with filtered ice in polyethylene packaging) in isothermal
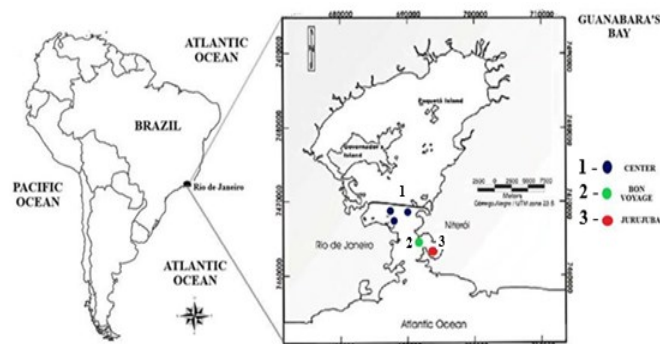
Figure 1. Map showing the South Atlantic and Guanabara Bay with the markers where *P. Perna* was collected in the three communities of Niterói, Rio de Janeiro, Brazil: (1) Centro, (2) Boa Viagem, and (3) Jurujuba.

boxes to the Laboratory of Microbiological Control of Products of Animal Origin (*Laboratório de Controle Microbiológico de Produtos de Origem Animal* – CMPOA), of the Department of Food Technology of Faculdade de Veterinária da Universidade Federal Fluminense (Niterói, RJ). The molluscs were cleaned with the aid of a brush and under running water to remove sediments and encrusted organisms, and then dried in the open air in a previously disinfected plastic tray.

Then, for bacteriological analysis, six mussels were opened with the aid of a sterilized instrument next to a Bunsen burner to remove the shells and collect them from the analytical unit to obtain the quantity required for analysis of 25 grams (ISO, 2003; Salfinger & Tortorello, 2015).

Analyses on mussel samples were carried out in triplicate and diluted in 0.1% peptone saline solution in a 1:1 ratio. Serial decimal dilutions were carried out up to 10^{-8}, in which MPN of *Escherichia coli* was determined, obtained according to the rapid detection method described by Merck (2000), modified by Franco and Mantilla (2004) with "Fluorocult" Broth LMX.

2.3 Statistical analysis

Statistical analyses of the data were performed with the use of Jamovi software (Version 2.3). The results of the analyses of coliforms at 45°C and *Escherichia coli* were subjected to descriptive statistics. Additionally, the Kruskal-Wallis non-parametric test (ANOVA) was employed to compare contamination levels across different regions over the course of a year. In case of a 5% significance level, multiple comparison tests were applied (Campos, 1983), with $\alpha = 0.05$.

For statistical calculation purposes, whenever the results obtained in the table were < 3 MPN/g, they were replaced by the immediately lower number, *i.e.*, 2 MPN/g.

3 RESULTS AND DISCUSSION

The results obtained in the bacteriological analyses of mussel meat (*Perna perna*) from the three communities (Jurujuba, Centro, and Boa Viagem) complied with the parameters of Ordinance SDA/MAPA No. 884, of September 6th, 2023, which establishes MoluBiS.

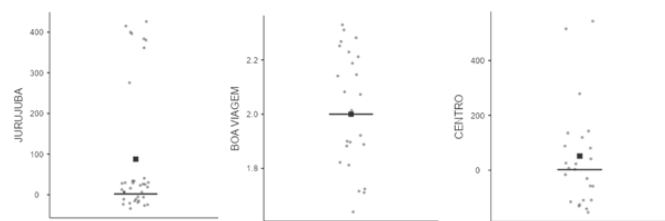MPN of coliforms at 45°C (*E. coli*) in mussel samples ranged from < 3.0 to 4.0 x 10^2 MPN/100 g, with samples from the Jurujuba region obtaining the highest geometric mean of contamination (Table 1), with a percentage of 77.8% in Jurujuba, 87.5% in Centro, and 100% in Boa Viagem, as an approved product (Table 2). The indication of conditionally approved product accounted for 22.2% in Jurujuba and 12.5% in Centro.

The highest counts were observed in Jurujuba in the months of April, June, July, October, and November, likely due to its geographical characteristics hindering efficient water circulation (Figure 2) thereby posing challenges in the event of discharges of domestic sewage and other waste; in Centro, in May, July, and August; and in Boa Viagem, the results were < 3 MPN/g, meeting the criteria for an approved product (all results in the time series less than or equal to 700 MPN of *E. coli* and 80% of the results less than or equal to 230 MPN of *E. coli*) in all analyses performed.

Therefore, this region had 100% of the results as approved product in all analyses carried out, owing to its proximity to the entry of marine currents into Guanabara Bay (Figure 2) This is represented by the velocities of sea currents obtained in hydrodynamic modeling (Environmental Hydrodynamic Base System – SisBaHiA 9b), in the course of the waters at times of high and low tides in Guanabara Bay (Rosman, 2000; Santos, 2018).

According to a study that monitored the quality of *Perna perna* mussels farmed along the coast of São Paulo (Galvão, 2004), coliform counts at 45°C ranged from 3.2 to 3.3 x 10^4 MPN/100 g, values higher than those found in this study (< 3 to 2.4 x 10^4 MPN/100 g).

E. coli counts obtained in oysters from six different regions of the Ribeirão da Ilha district in the South Bay of Ilha de Santa Catarina (Ramos et al., 2010) varied between < 3.0 and 2.6 x 10^3 MPN /100 g. Similarly, in another study conducted in the same farming area in Ribeirão da Ilha (Pereira et al., 2006), contamination levels were observed in *Crassostrea gigas* oysters. These values found in the two studies are higher than those obtained in the present study (< 3.0 to 4.0 x 10^2 MPN/100 g).

In the communities of Jurujuba, Boa Viagem, and Centro, there were differences in the frequencies (Figure 3) of the status of Approved, Conditionally Approved, and Prohibited, statistics

Figure 2. Illustration of the distribution of the velocities of sea currents in Guanabara Bay in the hydrodynamic modeling domain (Environmental Hydrodynamic Base System – SisBaHiA 9b). Mussel collection location from the three communities of Niterói, Rio de Janeiro, Brazil (1) Centro, (2) Boa Viagem, and (3) Jurujuba.

Table 1. Counts of Coliforms at 35°C and 45°C (*E. coli*) in *Perna perna* mussels during collections in the communities of Jurujuba, Boa Viagem, and Centro, Niterói, where there is commercial farming and extractivism.

REGION	Coliforms at 35°C		Coliforms at 45°C (*E. coli*)	
	Geometric Mean (MPN/100g) *	90th Percentile (MPN/100g) *	Geometric Mean (MPN/100g) *	90th Percentile (MPN/100g) *
Jurujuba	34.5	900	6.4	400
Boa Viagem	7.1	771	< 3	< 3
Centro	72.4	3.220	3.9	281

*MPN/g = Most probable number in 100 grams; The means did not show statistically significant differences at a 5% level ($p > 0.05$); as for the results of coliforms at 35°C, there are no legislation standards, but in samples of mussels *in natura* some results were significant, as stated in the literature.

Table 2. Frequencies (%) of Jurujuba, Boa Viagem, Centro and the parameters used as approved, conditionally approved, and prohibited for coliforms at 45°C (*Escherichia coli*) as a sanitary hygiene indicator, according to the National Safe Bivalve Molluscs Program (MoluBiS).

JURUJUBA (MPN/100 g)	Sample No.	% of Total	Status according to Interministerial Normative Ordinance MPA/MAPA No. 07, of May 8, 2012
< 3	28	77.8	Approved
300	1	2.8	Conditionally Approved
400	7	19.4	Conditionally Approved
BOA VIAGEM (MPN/100 g)	**Sample No.**	**% of Total**	
< 3	24	100.0	Approved
CENTRO (MPN/100 g)	**Sample No.**	**% of Total**	
< 3	21	87.5	Approved
400	3	12.5	Conditionally Approved

The status of Approved refers to Class A control areas: all-time series results less than or equal to 700 MPN of *E. coli* and 80% of results less than or equal to 230 MPN of *E. coli*; the status of Conditionally approved refers to Class B control areas: all-time series results are less than or equal to 46,000 MPN of *E. coli* and 90% of samples have a result of less than or equal to 4,600 MPN of *E. coli* or C : all-time series results are less than or equal to 46,000 *E. coli* MPN.

Figure 3. Graphics from the regions of Jurujuba, Boa Viagem, and Centro demonstrating the frequency at which the Approved status refers to Class A control areas: all-time series results less than or equal to 700 MPN of *E. coli* and 80% of results less than or equal to 230 MPN of *E. coli*; and Conditionally Approved refers to Class B control areas: all-time series results are less than or equal to 46,000 MPN of *E. coli* and 90% of samples had a result of less than or equal to 4,600 MPN of *E. coli* or C : all-time series results are less than or equal to 46,000 MPN of *E. coli*.

of contamination averages, as can be seen in Table 2. Like the Jurujuba region, a higher geometric mean and a higher 90th percentile were observed.

Farias et al. (2010) and Ramos et al. (2010) found higher MPN values in oyster samples, especially during the rainy season (summer), unlike this study, which identified significant contamination during autumn, winter, and spring, likely due to accumulated rainfall in the week prior to collection.

Guimarães Filho et al. (2022a) noted in their bacteriological analyses for bivalve mollusc meat for *E. coli* densities, elevated levels (> 1.1×10^{18} MPN/g) in the winter period, in Armação de Búzios (coastal region of the state of Rio de Janeiro), surpassing the limits established in accordance with MoluBiS.

4 CONCLUSION

The bacteriological analyzes of mussel meat (*Perna perna*) from the three communities (Jurujuba, Centro, and Boa Viagem) showed a higher percentage of mussels in the approved status (concentration lower than 230 MPN/100 g of edible part), according to the standards of MoluBiS for coliforms at 45°C (*Escherichia coli*) during analyses carried out in 2022. The consistent results of < 3 MPN/g under approved status for the Boa Viagem region in all analyzes carried out suggests that this location is close to the entry of marine currents into Guanabara Bay.

Although no specific standards for coliforms at 35°C in samples of fresh mussels have been established, some results were significant, as shown in the literature.

Therefore, it is necessary to implement the MoluBiS Program in the state of Rio de Janeiro to effectively monitor hygiene-sanitary conditions, ensuring the minimum requirements necessary to guarantee the safety and quality of bivalve molluscs for human consumption.

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