Revista O Mundo da Saúde

Detecting bacteria of the coliform group in the soft tissue of Mytella guyanensis extracted in Cananéia/SP, Brazil

Fatima Alessandra Deanna Buono Campos*
Larissa Yoshida Roselli**
Edison Barbieri*

Abstract

This study aimed to evaluate the microbiological quality of water and tissue samples of *Mytella guyanensis* (Mollusk: Bivalve), a species of relevant commercial interest and is a direct part of the income and food of traditional communities in coastal regions. This study was carried out in two communities in the Cananeia Estuary known as Retiro and Itanhoapina. During the period of 2017, where the species is collected for human consumption, water and bivalve samples were collected in the localities in order to determine the concentrations of total and thermotolerant coliforms. The samples of water and *M. guyanensis* were analyzed to determine the Most Probable Number (MPN) of Coliforms, based on the Multiple Tube Technique. The results of the analyses showed low concentrations of coliforms in the water samples, with a similar result for the tissue samples of the individuals collected. The water analysis showed a geometric mean of 34.81 total coliforms and 20.70 MPN 100 mL^{-1} of thermotolerant coliforms. The averages of coliforms in the soft tissues of *M. guyanensis* were 190 MPN g^{-1} for total coliforms and 174 MPN g^{-1} for thermotolerant coliforms. The concentrations of coliforms in the tissue of the collected specimens showed a positive correlation with temperature and a negative correlation with salinity. There were significant differences between seasonality in relation to the concentration of total and thermotolerant coliforms in the tissue. The summer rainy season showed the highest seasonal average of coliforms in *M. guyanensis*. All values obtained for MPN of coliforms were below the limit established by the legislation.

Keywords: Thermotolerant coliforms, Estuary, Microbiology Contamination, *Mytella guyanensis*.

INTRODUCTION

The municipality of Cananéia is part of the Complex Estuarino-Lagunar de Iguape-Cananeia-Ilha Comprida-Paranaguá, a region rich in mollusks; mainly the bivalve mollusk, *Mytella guyanensis* (Lamarck, 1819). It is also known as, golden beak (bico de ouro) and whose natural banks are located beginning from the northern portion of Cananéia Island, extending in a southern direction, following the coastline of Trapandé Bay and Ararapira Canal, and entering the contiguous region of Paranaguá, in the State of Paraná[1,2,3]. *Mytella guyanensis* are filtering organisms, or rather, pollutant bioaccumulators, therefore having the capacity to absorb toxins, chemical and biological pollutants, including heavy metals and microorganisms present in the water. They can filter 19 to 50 liters of water per hour, with little or no selective capacity[4,5,6].

DOI: 10.15343/0104-7809.202044084091

*Instituto de Pesca- APTA-SAA -Governo do Estado de São Paulo. Santos/ SP, Brasil.
**Universidade Estadual Paulista - UNESP – Programa Biodiversidade Aquática, Campus do Litoral Paulista. São Vicente – SP, Brasil
E-mail: edisonbarbieri@yahoo.com.br

Bivalve mollusks have a relevant commercial interest and are part of the diet of many communities, being removed from natural banks by shellfish gatherers and sold in the region close to where they occur. The bivalve, *M. guyanensis*, is a direct part of the income and diet of shellfish fishermen from coastal regions. The species is commercially exploited, being removed from natural banks in a disorderly manner and without any health control by local authorities.

This species has been well studied with respect to aspects of its population dynamics, habitat characterization[7], reproduction[8], and physiological aspects related to different environmental variables, mainly temperature[9] and salinity[10,11]. However, with regard to its sanitary aspect, there are still few studies on the concentration of coliforms in its tissues, especially in the Cananeia estuary. This species of mollusk is a filtering organism which, when in areas that receive polluted effluents from untreated domestic sewage, can accumulate contaminating microorganisms in its interior, in quantities much higher than the indices found in water where they live[12]. For this reason, there is an urgent need to monitor the concentrations of Coliforms in this mollusk, in order to avoid public health problems.

Several populations of pathogenic bacteria can be found in water[13,14] and sometimes are concentrated in filtering bivalve mollusks in places contaminated with domestic sewage[12,13]. Thus, high concentrations of pathogenic bacteria in bivalves can lead to serious public health problems[15]. Innumerable pathologies can occur through the ingestion of mollusks from contaminated waters, such as gastroenteritis[15], usually caused by infection with *Vibrio cholerae*, *Shiggella sp.*, *Salmonella typhi* and *Escherichia coli*[11,16]. In the case of *Mytella guyanensis*, most of the times consumed in natura, it can cause diseases if they are contaminated by bacteria and/or by the presence of toxins[17].

The microbiological standards of food quality, including those of marine origin, such as fish, mollusks and crustaceans are regulated by the Resolution of the National Health Surveillance Agency, ANVISA Resolution RDC Nº. 12/01, based on the concentrations of Coliforms at 45ºC, coagulase postive *Staphylococcus coagulases* and *Salmonella sp.*[18]. The quality of water destined for the cultivation of aquatic organisms, including brackish and saltwater ones, is evaluated through the Resolution of the National Environment Council, CONAMA Resolution Nº 357/05, which considers the thermotolerant coliforms and *Escherichia coli* as parameters.

In the United States of America, Asia, Europe and Australia there have been several records of diseases associated with the consumption of bivalve mollusks due to microorganisms or biotoxins[19]. In Brazil, there is still no casuistic that shows a correlation that statistically points out the problem in this regard[20]. For these reasons, there is an urgent need to monitor regions and bivalve organisms for bacteria. Therefore, the objective of this study was to microbiologically evaluate the hygienic-sanitary quality of *Mytella guyanensis* sold by two communities in the municipality of Cananéia.

MATERIALS AND METHODS

Study area and collection method:

Samples of *Mytella guyanensis* and estuarine waters were collected in two locations called Retiro/Itanhoapina (25°06`48``S - 48°02´03``W), located in the municipality of Cananéia (SP), in the 2017 period, when the extraction of *Mytella guyanensis* is developed in muddy shoals. The collected locations were geo-referenced with the aid of a GPS (Global System Position) device, chosen for characterizing the sampling station and due to the seafood collection area (figure 1). The analysis of the environmental variables: temperature (°C), pH, salinity and dissolved oxygen (mg/L), were obtained in the collection area itself with the aid of the multi-parameter measuring equipment, YSI-63.

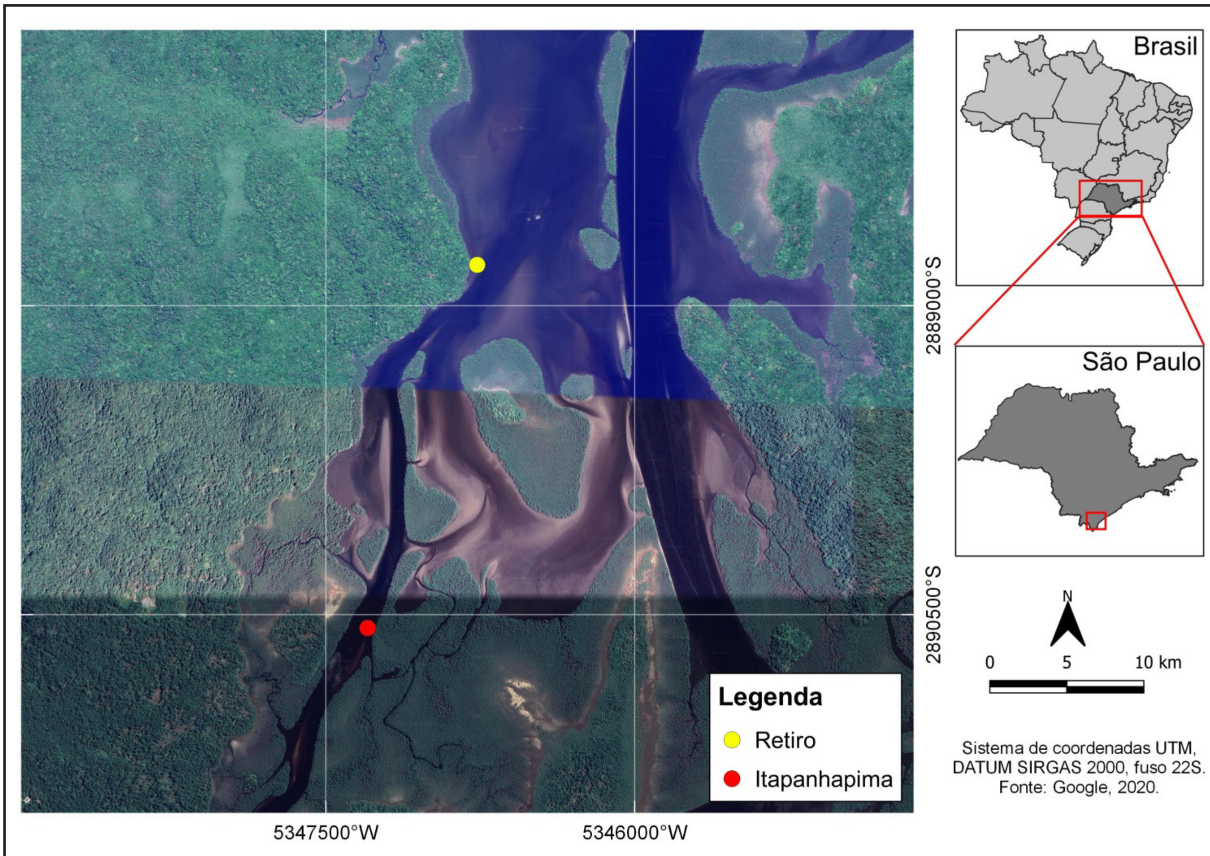

Figure 1 – Sites where water samples were collected for microbiological analysis in Cananéia, SP.

A total of 30 samples were collected weekly with the aid of a Van Dorf claw, at a depth of 1 meter for a period of one year to study the relationship of salinity and the concentration of coliforms in the soft tissues of *M. guyanensis* in both locations mentioned. After collection, the samples were placed in autoclaved borosilicate neutral glass vials, stored in a refrigerated isothermal box for transportation and analyzed within a maximum period of 6 hours from the first field sampling. To study the concentration of coliforms in the tissue of that mollusk, in relation to seasonality, 16 specimens were collected per season of the year, totaling 64 individuals analyzed.

Microbiological analysis

The analysis of water and soft tissue were performed in the laboratory of the Instituto de Pesca-Cananéia. The water samples and *Mytella guyanensis* were submitted to two stages for coliform detection. They were submitted to analyses for the determination of the MPN of Total and Thermotolerant Coliforms, following the methodology described by the Standard Methods For The Examination Of Water And Wasterwater[21].

The water samples were analyzed without dilution and were diluted in buffered water with a 1:9 ratio, obtaining serial decimal dilutions from 10^{-1} to 10^{-3} (performed in duplicates). The soft tissue of *Mytella guyanensis* was collected in aseptic conditions and, in a sterile container, measured 20 g, homogenized in 180 mL of buffered water and removed the necessary aliquots for each analysis, starting the dilution at 10^{-1} in series of 3 tubes.

The presumptive test provided a preliminary estimate of the concentration of the bacterial group based on enrichment in a minimally restrictive medium. The samples were diluted according to APHA (2005), with 5 replicates for each dilution in addition to the control tubes, in order to identify possible false-positive results. Then, 10 mL of the samples were inoculated in Lactated Broth Sodium Sulphate in two concentrations, 1 mL and 0.1 mL in a simple concentration and incubated at 35ºC for 24 - 48 h. The tubes that showed gas production in the Durham tube and acidification were considered positive and the cultures were used to perform the confirmatory test[12].

The tubes considered positive in the previous test were inoculated in tubes of selective and inhibitory media, to determine the total coliforms and to differentiate the thermotolerant coliforms. For total coliforms, the samples were inoculated in Broth Green Bile Brilliant (2% lactose), incubated at 35ºC for 48 h. For thermotolerant coliforms, the samples were inoculated in Broth EC (specific medium to *Escherichia coli*) used as an indicator of pathogenic enteric bacteria for 24 h at 44.5°C. due to the presence of gas in the Durham tube indicating a positive test[12,22].

Statistical Methods

Pearson's Linear correlation was used to verify the existence of a correlation between coliforms in water and oyster tissue with salinity and temperature. For seasonality, the statistical analyses used were the Kruskal-Wallis analysis, performed to test normality, and Levene's test to verify homoscedasticity. After this procedure, ANOVA was used to analyze the variance, analyzing whether there was a difference between the coliform MPN and seasonality.

RESULTADOS

The results of the estuary water analysis showed a geometric mean of 64.81 total coliforms and 50.70 MPN.100mL^{-1} of thermotolerant coliforms. The averages of coliforms in the soft tissues of *M. guyanensis* were 90 MPN.g^{-1} for total coliforms and 74 MPN.g^{-1} for thermotolerant coliforms.

There was a negative correlation between the increase in salinity and the values of Thermotolerant Coliforms (Person`s r= -0.91, $p<0.001$) present in the soft tissue of *M. guyanensis* (figure 2). The same negative correlation trend was observed between the values of Total Coliforms (Person`s r= -0.85, $p<0.001$) present in the soft tissue of the bivalves in relation to salinity (figure 3).

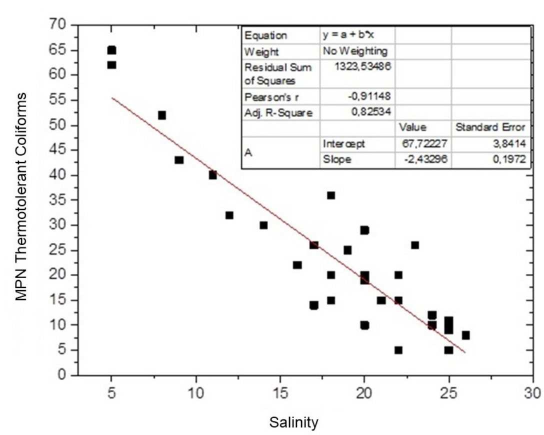

Figure 2 – Correlation between the MNP of thermotolerant coliforms present in the soft tissue of *Mytella guyanensis* and the variation in salinity (n = 30, r = -0.91).

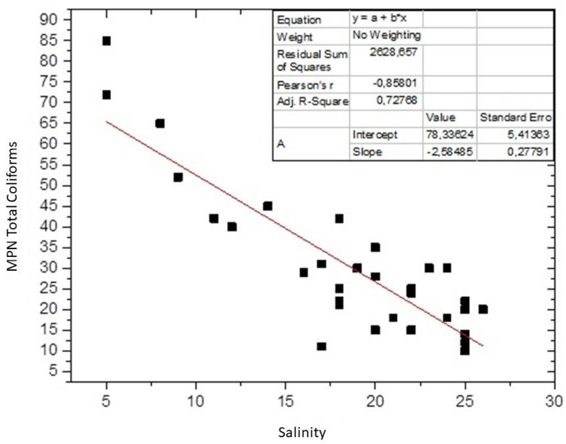

Figure 3 – Correlation between the MNP of total coliforms present in the soft tissue of *Mytella guyanensis* and the variation in salinity (n = 30, r = -0.85).

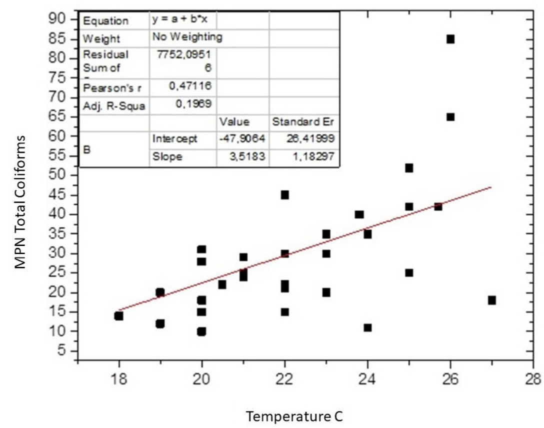

Figure 5 – Correlation between the MPN of thermotolerant coliforms present in the soft tissue of *Mytella guyanensis* and the temperature variation (n=30, r=0.47).

The analysis of the relationship of Thermotolerant Coliforms and temperature, showed that the MPN of Thermotolerant Coliforms present in the soft tissue of *Mytella guyanensis* presented a positive correlation with temperature (Person`s r= 0.48, p<0.001), as well as the results obtained for the MPN of Total Coliforms present in the soft tissue of *Mytella guyanensis* (Pearson's r= 0.47, p<0.001) (figures 4 and 5).

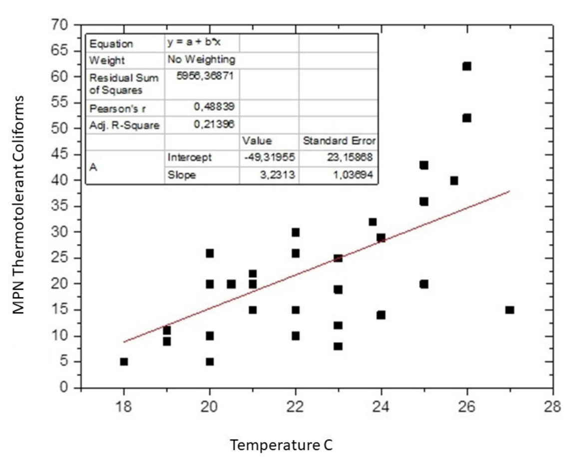

Figure 4 – Correlation between the MPN of thermotolerant coliforms present in the soft tissue of *Mytella guyanensis* and the temperature variation (n = 30, r = 0.48).

Regarding seasonality, the analyzed soft tissues of *Mytella guyanensis* showed higher levels of Thermotolerant Coliforms in the summer (174 MPN/100mL), compared to the other seasons. The summer differed statistically from the other seasons studied for both thermotolerant coliforms and total coliforms (figures 6 and 7). As for the Total Coliforms, the soft tissues of *Mytella guyanensis* also showed high levels in the summer (190 MPN/100mL), and when compared with the other seasons, there were statistical differences between them (figure 7).

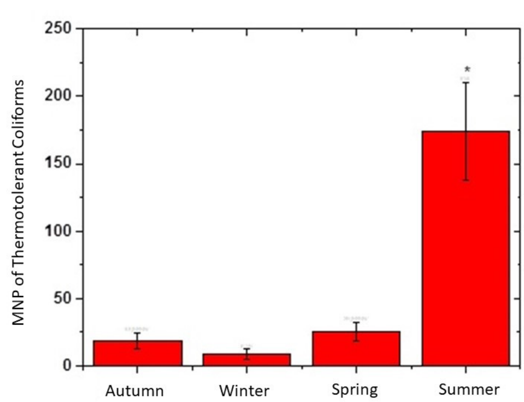

Figure 6 – Mean variation of the MPN of thermotolerant coliforms present in the soft tissue of *Mytella guyanensis* in relation to seasonality. The bars are the respective standard deviations (n=16).

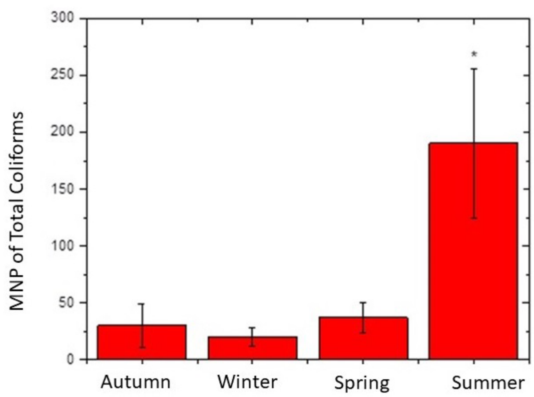

Figure 7 –Variation of the mean MPN of total coliforms present in the soft tissue of *Mytella guyanensis* in relation to seasonality. The bars are the respective standard deviations (n=16).

DISCUSSÃO

The concentrations of coliforms found in *M. guyanensis* were higher than those found in water, unlike the results of studies by Farias et al.[24] and Kolm & Absher[25], which indicated greater contamination in water than in mollusks. Results observed in the study by Moreira et al.[26] carried out in Paraty (RJ), showed that the concentration of fecal bacteria was higher in mollusks than in the water. Similar findings were observed by Kolm & Absher[25], in a study carried out in the Paranaguá estuarine complex (PR), where they observed that in the summer the concentration of coliforms in oyster tissue was higher than that in the water. These corroborate the results found in the present study.

Farias et al.[24] found that the MPN values in the water samples and in the organisms were higher mainly in the rainy season (summer); results similar to the present study. The concentration of microorganisms in *M. guyanensis* indicates the level of contamination at the time of collection, but this can vary from one animal to another and also depends on environmental and meteorological conditions, as well as whether the organism was filtering or at rest at the time of collection[27].

It was found that all concentrations of thermotolerant coliforms detected during this study were within the limits established by the European Union Quality Assurance Program - EUSQAP[28], Codex Alimentarius[29], the International Commission for Microbiological Specifications for Food[30] and finally the National Control Program Hygienic-Sanitary of Bivalve Mollusks (PNCMB). Although it was registered that in some periods of the year the levels of these bacteria were higher, the values obtained never exceeded the limits established by the PNCMB.

The range of salinity variation in estuaries is always very high, and it is possible to find bacteria in this ecosystem that have adaptations to tolerate high salinities, as well as those that live in waters with a low salt content[31]. In the present study, it was found that there was a negative correlation between the salinity and the MPN of total and thermotolerant coliforms, similar to that observed by Ramos et al.[32], in the estuarine region of Lagoa dos Patos, where the bacterial concentration was lower in waters where high salinity occurred. In contrast, Silva et al.[33], studying fecal bacteria in the estuary of Rio Cocó, CE, found that there was no correlation between salinity and coliform concentrations. Moreover, a study by Kolm and Andretta[31] demonstrated that in estuaries where salinity variations are very large, there is a tendency for coliforms to decrease as salinity increases, since these organisms are not very resistant to high concentrations of salt.

Other factors may contribute to fluctuations in the concentrations of coliforms in the water, including an increase in temperature, since simultaneously there is an increase in the metabolism of these bacteria, accelerating their reproduction and increasing the bacterial concentration in the waters in which they are found. Estuaries can present very high temperature variations[34], conditioning higher concentrations of coliforms where the thermal values are higher, which may explain the positive correlation observed in this work between coliforms and water temperature and which agrees with the results obtained by Doi et al.[35] and Ristori et al.[36].

As for seasonality, it was found that there was a trend towards a statistically higher concentration of coliforms in the summer – rainy season, compared to other seasons. Other studies have reached similar results when evaluating the concentration of thermotolerant coliforms in oysters[24,35,37]. Studies by Salles et al.[15], Batista and Harari[34], Doi et al.[35] indicated that estuarine waters may have high levels of thermotolerant coliforms in summer, as recorded in the present study, which can be explained by the positive correlation between thermotolerant coliform concentration and temperature.

CONCLUSION

The MPN variations of total and thermotolerant coliforms showed a positive correlation with temperature and a negative correlation with salinity, the seasonal variations observed were associated with different temperatures, and in summer there was a higher concentration of coliforms in oyster tissues. Despite this increase, all concentrations obtained were below the standard stipulated in current legislation.

In conclusion, microbiological monitoring of bivalve mollusks that are destined for human consumption is important to certify the healthiness of the product and also the environmental health, in order to avoid public health problems.

REFERENCES

1. Barbieri E, Bondioli AC, Woiciechovski E, Zapotoski SMK. Microbiology quality of the oysters cultivation water marketed in Cananeia-SP, Brazil. O Mundo da Saúde 2012; 36(4): 541-547.
2. Mignani L, Barbieri, E, Marques HLA, Oliveira AJFC. Coliform density in oyster culture waters and its relationship with environmental factors. Pesquisa Agropecuária Brasileira 2013; 48(8): 833-840.
3. Pinto AB, Pereira CR, Oliveira AJFC. Densidade de *Enterococcus sp* em águas recreacionais e areias de praias do município de São Vicente – SP, Brasil e sua relação com parâmetros abióticos. Mundo da Saúde 2012; 36(4): 587–593
4. Sande D, Melo TA, Oliveira GSA, Barreto L, Talbolt T, Boehs G, Andrioli JB. Prospecção de moluscos bivalves no estudo da poluição dos rios Cachoeira e Santana em Ilhéus, Bahia, Brasil. Brazilian Journal Veterinary Research Animal Science 2010; 47(3): 190-196.
5. Nascimento VFS, Araújo MFF. Ocorrência de bactérias patogênicas oportunistas em um Reservatório do Semiárido do Rio Grande do Norte, Brasil. Revista de Ciências Ambientais. 2013; 7 (1): 91-104.
6. Barros D, Barbieri E. Análise da ocorrência de metais: Ni, Zn, Cu, Pb e Cd em ostras (*Crassostrea brasiliana*) e sedimentos coletados no Estuário de Cananéia, SP (Brasil). O Mundo da Saúde 2012; 36(4): 635-642.
7. Nishida AK, Leonel RMV. Occurrence, population dynamics and habitat characterization of *Mytella guyanensis* (Lamarck, 1819) (Mollusca, Bivalvia) in the Paraíba do Norte river estuary. Boletim do Instituto Oceangráfico 1995; 43(1): 41-49.
8. Christo SW, Ferreira-Jr AL, Absher TM. Aspectos reprodutivos de mexilhões (Bivalvia, Mollusca) no complexo estuarino de Paranaguá, Paraná, Brasil. Boletim do Instituto de Pesca 2016; 42(4):924-936.
9. Onodera FK, Henriques MB, Mortalidade de *Mytella falcata* e *M. guyanensis* submetidos a diferentes temperaturas. Boletim do Instituto de Pesca 2017; 43(1): 106-111
10. Doi AS, Oliveira AJFC, Barbieri E. Determinação de coliformes na água e no tecido mole das ostras extraídas em Cananéia, São Paulo, Brasil. Engenharia Sanitária e Ambiental 2015; 20(1): 111-118
11. Leonel RMV, Silva IN, Salomão LC. Effect of salinity variation on the osmotic concentration and the cation content of the hemolymph of *Mytella guyanensis* and *Mytella charruana* (Mollusca, Bivalvia, Mytilidae). Bolm Fisiol. Anim. 1988; 12(1): 89-97
12. FAO 2010. Depuracion de Bivalvos: aspectos fundamentales y práticos. Documento técnico de pesca 511. http://www.fao.

org/3/a-i0201s.pdf
13. Ballesteros ER, Andrade VC, Barbieri E, Pinto AB, Oliveira RS, Oliveira AJFC. Qualidade microbiológica de ostras (Crassostrea sp) e de águas coletadas em cultivos e em bancos naturais de Cananéia (SP). Boletim do Instituto de Pesca 2016; 42(1): 134-144.
14. Zampieri DBB, Oliveira RS, Pinto AB, Andrade VC, Barbieri E, Chinellato, RM, Oliveira AJFC. Comparison of bacterial densities and resistance in different beach compartments: should water be our main concern? Mundo da Saúde 2017; 40(A): 461-482.
15. Salles PBD, Macedo YB, Figueiredo EL. Caracterização físico-química e microbiológica da carne do molusco Bivalve Sarnambi (*Phacoides pectinitus*) coletado nas praias em Algodoal e Salinópolis, no Pará. Revista brasileira de Tecnologia Agroindustrial 2017; 11(1): 2245-2261.
16. WHO - World Health Organization. Guidelines for drinking-water quality. Switzerland, 4th ed, 2011, p. 541.
17. Balaban N, Rasooly A. Staphylococcal enterotoxins: a review. International Journal Food Microbiology 2000; 21(1): 1-10.
18. ANVISA - Resolução-RDC Nº 12, de 02 de Janeiro de 2001- http://portal.anvisa.gov.br/documents/33880/2568070/RDC_12_2001.pdf/15ffddf6-3767-4527-bfac-740a0400829b
19. Potasman I, Paz A, Odeh M. Infectious outbreaks associated with bivalve shellfish consumption:a worldwide perspective. Clinical Infection Diseases 2002; 35(5): 921-928
20. Barbieri, E. Sanidade de moluscos bivalves em relação as biotoxinas marinhas. In: Tavares-Dias, M. & Mariano, W.S. (Org.). Aquicultura no Brasil. 1ed.São Carlos: Pedro & João Editora, 2015, v. 1, p. 355-379.
21. APHA. American Public Health Association. 2005. Standard Methods for the Examination of Water and Wastewater. 21ª ed. Washington: American Public Health Association. 1214p.
22. Barbieri E, Collaço FL, Doi AS, Oliveira AJFC, Rezende KFO. Microbiology as an indicator of environmental health of Ilha Comprida lagoons- SP. Mundo da Saúde 2017; 40(4): 507-520.
23. Zar JH. Biostatistical Analysis, 4a ed. Prentice-Hall Inc., New Jersey, 1999, p.231.
24. Farias MF, Rocha-Barrera CA, Carvalho FCT, Silva CM, Reis EMF, Costa RA, Vieira RHS. Condições microbiológicas de Tagelus plebeius (Lightfoot, 1786) (Mollusca: Bivalva: Solecurtidae) e da água no estuário do rio Ceará, em Fortaleza - CE. Boletim do Instituto de Pesca 2010; 36(2): 135-142.
25. Kolm HE, Absher TM. Bacterial density and coliform organisms in waters and oysters of Paranaguá EstuarineComplex, Paraná, Brazil. Boletim do Instituto de Pesca 2008; 34(1): 49 – 59.
26. Moreira AS, Leao MVP, Santos SSF, Jorge AOC, Silva CRG. Qualidade sanitária da água e de bivalves *Iphigenia brasiliensis* (Lamarck, 1818) na praia do Jabaquara, Paraty, RJ. Revista Biociências 2011; 17(1): 66-71
27. Mill A, Schlacher T, Kaouli M. Tidal and longitudinal variation of faecal indicator bacteria in an estuarine creek in south-east Queensland. Marine Pollution Bulletin 2006; 52(8): 881-891.
28. Rodgers CJ. The NSW Shellfish Quality Assurance Program: an operational review. Safe Food Production NSW, Sydney, 2001, p.146.
29. Codex Alimentarius (CODEX). Standard for live and raw bivalve molluscus. Codex Standard 2008, p.1-7.
30. International Commission on Microbiological Specifications for Foods (ICMSF). Microorganismos in food. Sampling for microbiological analysis: Principles and specific applications. 2ed., Toronto, Blackwell Scientific Publications, 1986; v. 278.p. 234
31. Kolm HE, Andretta L. Bacterioplancton in different tides of the Perequê tidal creek, Pontal do Sul, Paraná, Brazil. Brazilian Journal of Microbiology 2003; 34(1): 97-103
32. Ramos RJ, Pereira MA, Miotto LA, Faria LFB, Silveira-Junior N, Vieira CRW. Microrganismos indicadores de qualidade higiênico-sanitária em ostras (*Crassostrea gigas*) e águas salinas de fazendas marinhas localizadas na Baía Sul da Ilha de Santa Catarina, Brasil. Revista do Instituto Adolfo Lutz 2010; 69(1): 29-37.
33. Silva AIM, Vieira RHSF, Menezes FGR, Fonteles-Filho AA, Torres RCO, Sant'Anna E. Bacteria of fecal origin in mangrove oysters (*Crassostrea rhizophorae*) in the Cocó river estuary, Ceará state, Brazil. Brazilian Journal of Microbiology 2003; 34(1): 126-130.
34. Batista S, Harari J. Modelagem da dispersão de coliformes termotolerantes e enterococos em duas enseadas na região costeira de Ubatuba (SP), Brasil. Engenharia Sanitária e Ambiental 2017; 22(2): 403-414
35. Doi AS, Barbieri E, Marques HLA. Densidade colimétrica das áreas de extrativismo de ostras em relação aos fatores ambientais em Cananeia (SP). Engenharia Sanitária e Ambiental 2014; 19(2): 165-171.
36. Ristori CA, Iaria ST, Gelli DS, Rivera ING. Pathogenic bacteria associated with oysters (*Crassostrea brasiliana*) and estuarine water along the south coast of Brazil. International Journal of Environmental Health Research 2007; 17(2): 259–269.
37. PNCNB. 2012. Programa Nacional de Controle Higiênico-Sanitário de Moluscos Bivalves. Instrução Normativa Interministerial MPA/MAPA Nº7 de 08 de maio de 2012. http://www.cidasc.sc.gov.br/inspecao/files/2012/08/20160622154705901.pdf

Received in september 2019.
Accepted in december 2019.