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Integrated assessment of biomarker responses and microbiological analysis of oysters from São Luís Island, Brazil

E.B. Ribeiro [a,*], L.S. Bastos [b], L.S. Galeno [c], R.S. Mendes [c], F. Garino Jr. [d], R.N.F. Carvalho-Neta [e], F.N. Costa [f]

[a] Universidade Estadual do Maranhão (UEMA), Centro de Ciências Agrárias-CCA, Programa de Pós-Graduação em Ciência Animal, Cidade Universitária Paulo VI, Caixa Postal 09, São Luís, MA, Brazil
[b] Universidade Federal do Maranhão (UFMA), Programa de Pós-Graduação em Saúde e Ambiente, Avenida dos Portugueses, 1966, Bacanga, São Luís, MA 65080-805, Brazil
[c] Universidade Estadual do Maranhão (UEMA), Centro de Ciências Agrárias-CCA, Curso de Medicina Veterinária, Cidade Universitária Paulo VI, Caixa Postal 09, São Luís, MA, Brazil
[d] Universidade Estadual do Maranhão (UEMA), Centro de Ciências Agrárias-CCA, Departamento de Patologia, Cidade Universitária Paulo VI, Caixa Postal 09, São Luís, MA, Brazil
[e] Universidade Estadual do Maranhão (UEMA), Centro de Ciências Exatas e Naturais - CECEN, Departamento de Química e Biologia, Programa de Pós-Graduação em Recursos Aquáticos e Pesca, Cidade Universitária Paulo VI, Caixa Postal 09, São Luís, MA, Brazil
[f] Universidade Estadual do Maranhão (UEMA), Centro de Ciências Agrárias-CCA, Departamento de Patologia, Cidade Universitária Paulo VI, Caixa Postal 09, São Luís, MA, Brazil

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ABSTRACT

This study was conducted to evaluate the use of biochemical biomarkers and microbiological analysis to identify levels of oyster contamination at different ports in São Luís Island (Maranhão), Brazil. Oysters were analyzed for total coliforms, thermotolerant coliforms, *Escherichia coli* and *Aeromonas* spp. In addition, tissue was removed from the digestive gland to determine the glutathione-*S*-transferase (GST) and catalase (CAT) activity. The highest percentage of microbiological contamination of oyster samples occurred during the rainy season. The activity of GST and catalase in oysters was also higher in the rainy season, coinciding with the greatest abundance of total and thermotolerant coliforms. Among the prospective biomarkers, GST showed the best results for identification of areas with higher levels of contamination.

1. Introduction

Bivalve molluscs have been suggested as possible biomonitors for the international program of monitoring pollutants in marine environments since the 1970s (Goldberg, 1975). Many features make bivalve molluscs interesting animals for assessment of the environmental concentrations of contaminants, including their occurrence in estuaries and coastal areas, their sessile nature, long lifetime, widespread distribution, common occurrence in high density and ability to accumulate contaminants in their tissues (Cunningham and Tripp, 1975).

The *Crassostrea* genus of oysters comprises an important food source and major source of income for fishing communities in Brazil, especially in Maranhão (Monteles et al., 2010). In the capital of Maranhão, oysters are often consumed raw, which represents a risk to human health.

One of the effects of contaminants on aquatic animals is the occurrence of oxidative stress (Farombi et al., 2007). Free radicals produced by the presence of toxic substances in the body react with lipids, proteins or nucleic acids and may result in severe biochemical or genetic injury (Jing et al., 2006). Biochemical changes commonly used to assess the impact of contaminants on the aquatic environment of the Maranhão coast include GST and catalase (Carvalho-Neta and Abreu-Silva, 2010). Catalase has been used as a biochemical biomarker because it is an important enzyme in the antioxidant hydrogen peroxide decomposition produced in greater amounts during the biotransformation process (Ventura et al., 2002). Glutathione-*S*-transferase is a biochemical biomarker involved in cell detoxification of electrophilic compounds that is important for protecting against degenerative diseases caused by exposure to contaminated environments (Babbitt, 2000).

Bivalve molluscs used in food can be subjected to microbiological analyses using standardized methodologies for the identification of a variety of pathogens. However, in Brazil, there is no specific legislation focused on the microbiological evaluation of bivalve molluscs that are consumed raw. Thus, the care of these foods should be reinforced and consumers should be informed about the hygienic and sanitary quality and the danger of consuming fresh food without purifying treatment. Accordingly, research is needed to compare biochemical biomarkers associated with microbiological analysis to identify the effects of contaminants on the health of organisms and the environment. The main goal of this study was to use biochemical biomarkers and microbiological analysis to identify levels of oyster contamination at different ports in São Luís Island (Maranhão), Brazil.

* Corresponding author.
E-mail addresses: elianeribeiro.biologa@gmail.com (E.B. Ribeiro), lucianabastos79@gmail.com (L.S. Bastos), lygiagaleno@outlook.com (L.S. Galeno), leafar-2310@hotmail.com (R.S. Mendes), garinofjr@hotmail.com (F. Garino), raimundafortes@yahoo.com.br (R.N.F. Carvalho-Neta), franeidec@yahoo.com.br (F.N. Costa).

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2. Materials and methods

2.1. Study area and sampling procedures

The survey was conducted from December 2013 to January 2015. Samples were collected during the rainy season (February to June) and the dry season (July to October) according to INMET (2016). The study area corresponded to the main ports of fish landings of São Luís Island, MA, which consists of the municipalities of São Luís, Paço do Lumiar, São José de Ribamar and Raposa (Fig. 1).

In São Luís Island were identified of the traditional four points of oyster extraction by fishing communities. The ports are: Braga Port (S02°25′215″, W044°05′660″) and Raposa Port (S02°24′985″, W044°06′243″) in the municipality of Raposa, and, Cumbique Port (S02°27′531″, W044°07′507″) and Pau Deitado Port (S02°31′818″, W044°05′080″) in the city of Paço do Lumiar.

Sixteen samples of oysters that were each composed of 12 organisms were collected from each location. Samples of oysters were transported under refrigeration to the Microbiology Laboratory of Food and Water of the State University of Maranhão (UEMA).

Salinity and water temperature data were measured concurrently with oysters. Additionally, rainfall data (monthly mean for the period 1961–1990) for São Luís Island at the period of sample were obtained from INMET (2016).

2.2. Microbiological analysis

In the laboratory, oysters were opened with sterilized surgical scissors and the visceral mass and intervalvar liquid was removed. Samples were then analyzed for total coliforms, thermotolerant coliforms, *Escherichia coli* and *Aeromonas* spp. in accordance with the methods described by the American Public Health Association (APHA, 2001).

2.3. Analysis of biomarkers

One gram of digestive gland tissue was removed from each oyster, placed in an Eppendorf microtube and deposited in a dry shipper containing −185 °C liquid nitrogen for later analysis. For analysis, samples were homogenized in phosphate buffer and then centrifuged, after which the supernatant was used to determine the enzymatic activity of GST and catalase (CAT).

For analysis of GST activity, the homogenized sample was centrifuged again for 70 min. The enzyme activity of the supernatant was measured using a spectrophotometer at a wavelength of 340 nm and 25 °C for 2 min according to Keen et al. (1976) and the modification described by Camargo and Martinez (2006). Briefly, 20 μl of the sample was added to a glass cuvette containing 10 μl of reduced glutathione (GSH) and 10 μl of 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate in 960 μl of potassium phosphate buffer (0.1 M) at pH 7.0. The activity of CAT was measured at 240 nm and 25 °C for 1 min based on the decomposition rate of hydrogen peroxide (H_2O_2) according to Beutler (1975) and the modification described by Ventura et al. (2002). For analysis, 10 μl of sample was added to a quartz cuvette containing 990 μl of reaction medium.

3. Results

3.1. Microbiological aspects of oysters

The temperature data observed were as follows: 29.6 °C ± 1.2 (Pau Deitado Port); 29.4 °C ± 0.7 (Cumbique Port); 29.4 °C ± 1.5 (Braga Port) and 29.5 °C ± 0.4 (Raposa Port). Overall, the salinity values were as follows: 27.4 ± 2.9 g/kg (Pau Deitado Port); 32.7 ± 1.9 g/kg (Cumbique Port); 33.8 ± 0.7 g/kg (Braga Port) and 34.8 ± 1.2 g/kg (Raposa Port).

The highest percentage of contaminated oyster samples occurred in the rainy season (typical value = 470 mm). During this period, total

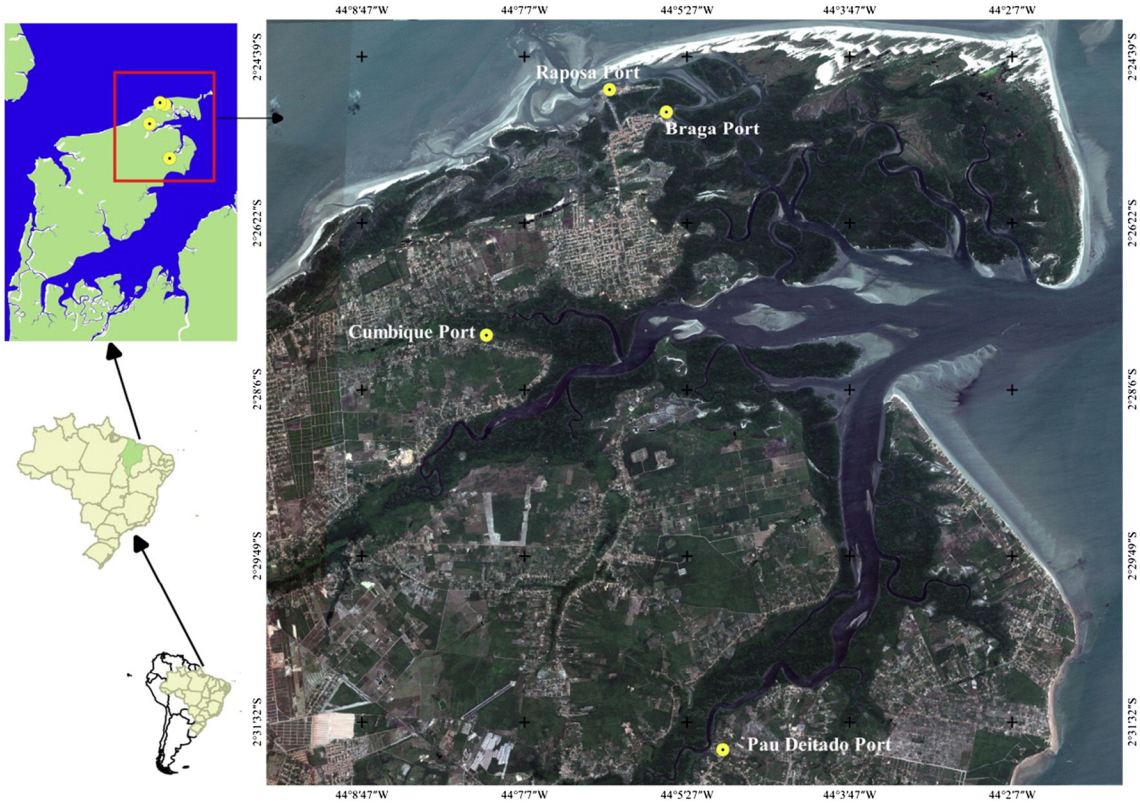

Fig. 1. Location of sampling points in São Luís Island, MA, Brazil.

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