

Compliance of brown mussel (*Perna perna*) production areas in the South of Brazil with the bacteriological criteria of the shellfish hygiene systems in the European Union and United States of America: assessing the impacts on consumer safety

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ABSTRACT

Levels of faecal indicator organisms (FIOs) monitored in surface water and brown mussels collected at 28 production areas in Brazil from August 2012 to October 2013 were used to assess compliance with the bacteriological standards of the shellfish hygiene classification systems used in the European Union (EU) and USA. This classification determines the level of post-harvesting purification needed to reduce the risk of illness in consumers. The results indicate that 36% of production areas would be class A under the EU system and 75% would be 'Approved' under the US system. Mathematical models showed that a 90th percentile of FIO levels in water of 43 MPN (most probable number) 100 mL⁻¹ (standard for 'Approved' areas under the US system) would correspond to an 80th percentile of FIO levels in mussels of 572 MPN 100 g⁻¹. This concentration is more than double that in the class A standard in the EU system. These results have important implications for public health since no post-harvesting purification is required for any of these classification categories. Areas compliant with the US 'Restricted' and EU class B standards, however, provide similar levels of consumer safety.

Key words | faecal indicator organisms, hygiene, legislation, *Perna perna*, trade

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INTRODUCTION

Filter-feeding bivalve molluscan shellfish accumulate micro-organisms, including human pathogenic bacteria and viruses, when grown in sewage-polluted waters and can present a significant health risk when consumed raw or lightly cooked. To protect human health, shellfish safety authorities commonly monitor faecal indicator organisms (FIOs) in shellfish production areas (SPAs) in order to classify them. This classification determines whether the area can be used for production and, if so, the level of post-harvest treatment (depuration, relaying, heat treatment) that needs to be applied to harvested shellfish prior to sale for human consumption (WHO & FAO 2012).

In general, there are two approaches to monitoring the microbiological quality of SPAs depending on the type of sample. The approach used in the European Union (EU) and countries with trade agreements with the EU is to monitor *Escherichia coli* in shellfish flesh while the approach used in the USA and countries with a memorandum of understanding with the USA is to monitor total coliforms or faecal coliforms in surface waters (Table 1). The EU Scientific Veterinary Committee Working Group on Faecal Coliforms in Shellfish studied the equivalence between these two systems (EU Working Group 1996, 2010) and concluded that class A in the EU system is more

Table 1 | Microbiological standards for the classification of SPAs in the EU and USA

| Type of sample | Standard | Classification | Post-harvest treatment required |
|------------------|---|-------------------------|---|
| USA ^a | | | |
| Water | Geometric mean ≤ 14 MPN 100 mL ⁻¹ and 90th percentile ≤ 43 MPN 100 mL ⁻¹ | Approved | Harvesting permitted and no post-harvesting treatment required |
| Water | Geometric mean ≤ 88 MPN 100 mL ⁻¹ and 90th percentile ≤ 260 MPN 100 mL ⁻¹ | Restricted | Purification or relaying in an approved area required |
| Water | Conditions for approved/restricted areas not met | Prohibited | Harvesting not permitted |
| EU ^b | | | |
| Shellfish | 80th percentile ≤ 230 MPN 100 g ⁻¹ and maximum ≤ 700 MPN 100 g ⁻¹ | Class A | Harvesting permitted and no post-harvesting treatment required |
| Shellfish | 90th percentile $\leq 4,600$ MPN 100 g ⁻¹ and maximum $\leq 46,000$ MPN 100 g ⁻¹ | Class B | Harvesting permitted and purification, relaying or cooking by an approved method required |
| Shellfish | Maximum $\leq 46,000$ MPN 100 g ⁻¹ | Class C | Harvesting permitted and relaying or cooking by an approved method required |
| Shellfish | $> 46,000$ MPN 100 g ⁻¹ | Prohibited ^c | Harvesting not permitted |

^aUS Food and Drug Administration. National Shellfish Sanitation Program (NSSP): Guide for the Control of Molluscan Shellfish 2015 Revision. Geometric mean or median can be considered.

^bThe European Parliament and of the Council: EC Regulations 853/2004 and 1666/2006 and Commission Regulation (EU) 2015/2285.

^cThis class is not specifically given in EU regulations.

stringent than the 'Approved' category in the US system and that standards for class B in the EU and 'Restricted' in the US systems are broadly equivalent. In contrast, Burkhardt *et al.* (2009) concluded that classes A and B used in the EU system do not conform to the 'Approved' and 'Restricted' categories used in the USA. Lee & Reese (2014) conducted a similar analysis and confirmed the conclusions of the EU working group. These studies were based on monitoring data relating to European flat oyster (*Ostrea edulis*), Pacific oyster (*Crassostrea gigas*), hard clam (*Mercenaria mercenaria*), soft clam (*Mya arenaria*) and mussels of the genus *Mytilus* (*M. edulis* and *M. galloprovincialis*). Species-specific differences in compliance levels were noted in all the above-mentioned studies and these differences could impact on the levels of public health protection afforded by the two systems. Thus, compliance assessments for species not considered in previous studies are important to underpin trade deals with countries that wish to export different shellfish species to the EU or USA and do not meet the specific requirements of the importing country or region (e.g. when the type of sample required by the monitoring programmes differ), since exporting countries must satisfy the relevant legislative requirements of the importing state.

The brown mussel *Perna perna* is widely distributed along the coasts of the America, Africa and Asia (Fernandes *et al.* 2008). Brazil is the world's most important producer of this species (~20,000 tonnes/year) (see www.fao.org – Global Aquaculture Production, accessed March 2017). In 2013, an international workshop held in Brazil discussed the EU microbiological requirements following expressions of interest from the local industry in exporting shellfish to EU markets (Souza *et al.* 2014). At the workshop, it was considered that there is insufficient information to understand if hygiene controls based on monitoring of FIOs in *P. perna* provide equivalent levels of consumer safety to international controls based on other species. The aim of this study was therefore to assess compliance of brown mussel production areas with the bacteriological standards for classification of SPAs used in the EU and USA. This study provides new data relating to a species that has not been studied and for a geographical area that has not been considered in previous studies.

METHODS

This study used a database of levels of FIOs monitored in shellfish and surface waters at 28 sampling points located

in SPAs in the North and South Bays, Santa Catarina, Brazil. The study area was described by Garbossa *et al.* (2014). To our knowledge, this is the largest database of FIO levels in 'paired' mussel and seawater samples ($n = 321$) available in Brazil. The shellfish and water samples were collected monthly from August 2012 to October 2013. Levels of faecal coliforms were quantified in water samples using the most probable number (MPN) method given in ISO 9308-2:1990. Levels of *E. coli* were quantified in shellfish using the MPN method given in ISO 16649-3:2005. We assumed that levels of faecal coliforms and *E. coli* are equivalent in each matrix (bivalves or water) and the term FIO is used in this study to represent both. The microbiological results were used to classify the SPAs according to standards set out in the EU and US legislation and associated guidance (Table 1).

Levels of *E. coli* in shellfish flesh at the lower limit of quantification of the MPN method (<20 MPN 100 g^{-1}) were adjusted to 19 MPN 100 g^{-1} and the 90th percentile for the water results obtained at each sampling point were determined using the equation $10^{(\text{mean log}_{10} + 1.28 \cdot \text{SD log}_{10})}$ (www.fda.gov). Generalised linear models with quasibinomial distribution (QGLMs) were developed considering the 90th percentile of FIO levels in water at the sampling points as the explanatory variable and the percentages of FIO results in shellfish flesh ≤ 230 MPN 100 g^{-1} (80th percentile standard for EU Class A), 700 MPN 100 g^{-1} (maximum standard for EU class A) and 4,600 MPN 100 g^{-1} (90th percentile standard for EU class B) at each site as the response variables. Additionally, simple generalised linear models (SGLMs) were developed considering the 90th percentile of FIO levels in water as the explanatory variable and the 80th percentile, 90th percentile and maximum FIO levels in mussels as the response variables. The microbiological levels were \log_{10} -transformed prior to statistical analysis to improve parametricity.

RESULTS AND DISCUSSION

Under the US system, most production areas (75%) would be classified as 'Approved' and much smaller percentages of areas would be classified as 'Restricted' (19%) or 'Prohibited' (7%). Under the EU system, 10 production areas (36%)

would be class A, 14 (50%) would be class B and four (14%) would be class C. None of the production areas would be classified as 'Prohibited' under the EU system (Table 2). These results indicate that a larger proportion of live shellfish would be allowed to be placed on the market without any post-harvest purification treatment if the production areas were classified using the US criteria than those if the production areas were classified using the EU criteria.

Concerning the microbiological criteria for classification, for categories not requiring post-harvest purification (i.e. class A in the EU and 'Approved' in the USA), the QGLMs show that the 90th percentile of FIOs in water of 43 MPN 100 mL^{-1} (standard for US 'Approved' areas) corresponds to 66% of FIO results in shellfish ≤ 230 MPN 100 g^{-1} and to 80% of FIO levels in shellfish ≤ 700 MPN 100 g^{-1} . In comparison, to comply with the class A standard of the EU system, the percentages of FIO results in shellfish should be 80% and 100%, respectively. The SGLMs show that a 90th percentile of FIO levels in water of 43 MPN 100 mL^{-1} (standard for 'Approved' areas under the US system) would correspond to an 80th percentile of FIO levels in mussels of 572 MPN 100 g^{-1} . This concentration is more than double the class A standard in the EU system (Figure 2). Considering that no post harvesting treatment (i.e. depuration or heat treatment) is required for any of these categories, these results have important implications for public health since theoretically higher loads of pathogens are expected to be found in mussels classified following the US system and may also impact on trade if these shellfish are exported to a country that uses one of these classification systems.

Table 2 | Number of mussel production areas in Santa Catarina (Brazil) compliant with the classification criteria used in the EU and USA

| Classification under the US system | Classification under the EU system | | | | Total number of production areas |
|------------------------------------|------------------------------------|----|---|------------|----------------------------------|
| | A | B | C | Prohibited | |
| Approved | 8 | 11 | 2 | 0 | 21 |
| Restricted | 2 | 2 | 1 | 0 | 5 |
| Prohibited | 0 | 1 | 1 | 0 | 2 |
| Total number of production areas | 10 | 14 | 4 | 0 | |

Concerning the microbiological criteria for classification categories that require post-harvest purification treatments, the QGLMs predict that areas complying with the 90th percentile standard of 'Restricted' classification under the US system ($260 \text{ MPN } 100 \text{ mL}^{-1}$) would have 89% of the FIO results in shellfish $\leq 4,600 \text{ MPN } 100 \text{ g}^{-1}$ (Figure 1). This value is very close to the 90% percentile standard of class B in the EU system. Furthermore, the SGLMs predict that, when the 90th percentile of FIO levels in water is equal to the standard for 'Restricted' areas in the US system ($260 \text{ MPN } 100 \text{ mL}^{-1}$), the 90th percentile in mussels is estimated to be $3,309 \text{ MPN } 100 \text{ g}^{-1}$, which is less than the standard for class B in the EU system ($4,600 \text{ MPN } 100 \text{ g}^{-1}$) (Figure 2). These results indicate that the criteria for class B in the EU system are slightly less stringent than the criteria for 'Restricted' areas in the US

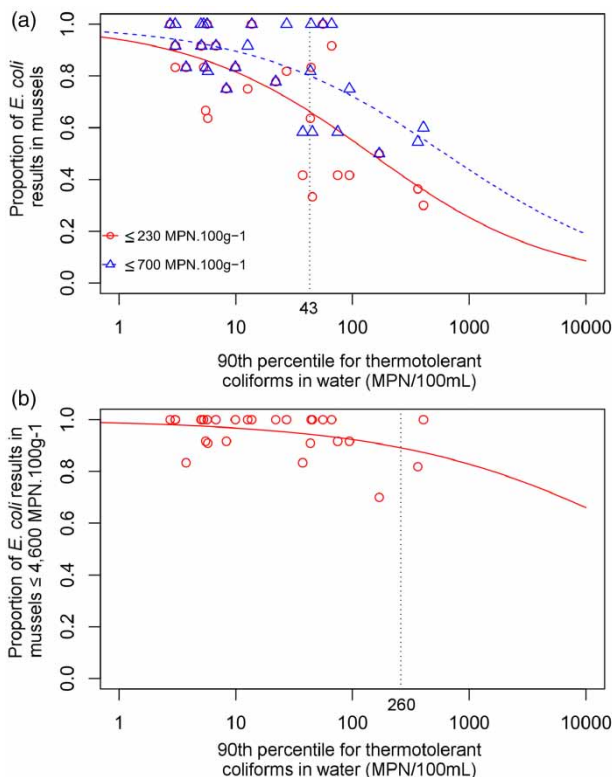


Figure 1 | Relationships between the 90th percentile of FIOs in seawater and the percentages of FIO results compliant with the class A (A) and B (B) thresholds of the EU classification system. The dashed lines indicate the 90th percentile standards for 'Approved' (A) and 'Restricted' (B) classifications under the US system. Quasibinomial generalised linear models: A ($\leq 230 \text{ MPN } 100 \text{ g}^{-1}$): $y = \exp(2.768 - 0.557 \cdot \log_{10}x) / 1 + \exp(2.768 - 0.557 \cdot \log_{10}x)$. A ($\leq 700 \text{ MPN } 100 \text{ g}^{-1}$): $y = \exp(3.355 - 0.5218 \cdot \log_{10}x) / 1 + \exp(3.355 - 0.5218 \cdot \log_{10}x)$. B ($\leq 4,600 \text{ MPN } 100 \text{ g}^{-1}$): $y = \exp(4.3056 - 0.3957 \cdot \log_{10}x) / 1 + \exp(4.3056 - 0.3957 \cdot \log_{10}x)$.

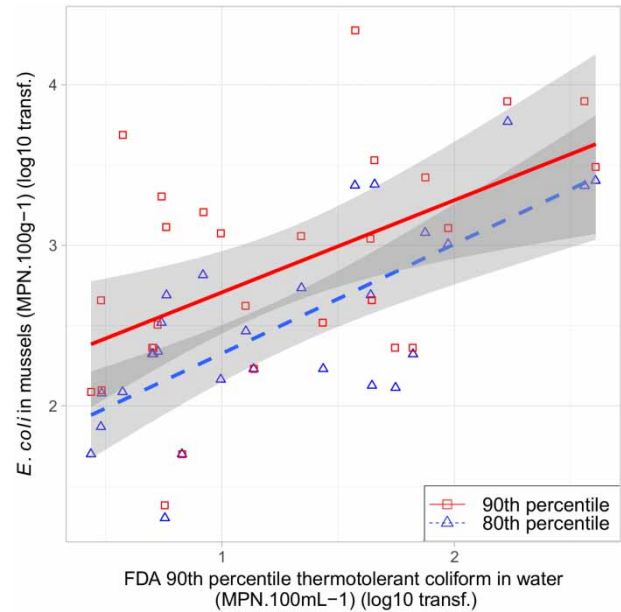


Figure 2 | Relationship between the 90th percentile of FIO levels in water and the 80th percentile (A) and 90th percentile (B) of FIO levels in mussels. Linear regression models: A: $r^2 = 0.53$; $r = 0.72$, $p < 0.0001$; $y = 1.644 + 0.6817 \cdot x$. B: $r^2 = 0.27$; $r = 0.52$, $p = 0.0044$; $y = 2.1323 + 0.5744 \cdot x$.

system. It is important to note that the apparent equivalent levels of consumer safety provided by EU class B/US restricted areas applies where the required post-harvest treatment is enforced, since classification of production areas, in itself, does not necessarily provide consumer protection.

CONCLUSIONS

This study shows that brown mussels harvested from production areas in Santa Catarina that would be classified as 'Approved' using the US system criteria may have worse microbiological quality than those from areas that would be classified as A using the EU system criteria. Brown mussels harvested from areas that would be class B in the EU system provide equivalent levels of consumer safety than those from areas that would be classified as 'Restricted' under the US system.

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