Relating the bivalve shellfish harvesting area classification criteria in the United States and European Union programmes

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ABSTRACT

Estimation of the level of risk of faecal contamination of shellfish harvesting areas is undertaken by monitoring faecal indicator bacteria in seawater samples under the United States programme and shellfish flesh samples under the European Union (EU) programme. Determining the relationship between the two approaches is important for assessing the relative level of public health protection and regulating international trade. The relationship was investigated using both statistical modelling and simple compliance assessment on large international data sets of paired seawater and shellfish samples. The two approaches yielded the same conclusions: EU class A is more stringent than the US Approved category for all species; the US Restrictive standard is more restrictive than EU class B for some bivalve species. Therefore, the classifications under the two programmes are not exactly equivalent.

Key words | *Escherichia coli*, European Union, faecal coliforms, free-trade, shellfish, United States

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INTRODUCTION

Bivalve molluscan shellfish obtain nutrients from seawater by filtering. As well as their food sources, they can filter and concentrate contaminants. These include pathogenic microorganisms that can cause, amongst other illnesses, gastroenteritis in consumers (Wittman & Flick 1995; Lees 2000). Most of these pathogens originate from faecal contamination of the water.

Many countries have controls on commercial shellfish production to reduce the risk to consumers from pathogens and other contaminants. With respect to pathogens from faecal sources, the risk of contamination is usually assessed by the use of faecal indicator organisms. These are usually either faecal coliforms or *Escherichia coli*. A harvesting area is classified according to a series of results from the area. This classification determines whether harvesting is permitted and, if so, whether shellfish from the area have to undergo some form of treatment prior to sale (Lee & Murray 2010).

There are two main harvesting area classification systems. One, based on total or faecal coliforms in the water, is used in the United States (FDA 2013). The other, based on *E. coli* in shellfish flesh, is used in the European Union (EU) (Official Journal of the European Communities 2004). The classification criteria are shown in Tables 1 and 2. Countries exporting to the EU or USA must use a system that complies with the requirements of the destination market. Some countries that export to both regions run both systems in parallel; others have developed a system that is essentially an amalgamation of the two.

It is therefore important for both international trade and public health protection to determine the relationship between classifications under the two systems by comparing concentrations of faecal indicator bacteria in the shellfish flesh and the surrounding seawater. This has been investigated previously (European Commission 1996; Lees & Nicholson 1997). However, the present work extends the principles of the previous work to a much larger data set.

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Table 1 | National Shellfish Sanitation Program (NSSP) criteria for classifying shellfish harvesting areas

	Total coliforms per 100 mL water		Faecal coliforms per 100 mL water		
Classification	**Geometric mean[a]**	**90% compliance[b]**	**Geometric mean**	**90% compliance[b]**	**Treatment required**
Approved areas[c]	≤70	≤230	≤14	≤43	None
Restricted areas[d]	≤700	≤2,300	≤88	≤260	Purification or relaying in an approved area
Prohibited areas	No sanitary survey or conditions for approved/restricted areas not met[e]				Harvesting not permitted

[a]Or median.
[b]Values for five-tube decimal dilution test; different 90% compliance are given for the three-tube MPN (most probable number) and mTEC membrane filtration tests.
[c]Determination of approved area status must be based on a minimum of 15 samples from each monitoring station.
[d]Conditionally restricted areas may be declared where these are subject to predictable contamination events: such areas are closed for harvesting during contamination events and for a period afterwards to permit natural cleansing.
[e]Considerations other than the concentration of contaminants may be used to declare an area prohibited.

Table 2 | EU criteria for the classification of shellfish harvesting areas

Class[a]	**Microbiological standard[b]**	**Post-harvest treatment required**
A	Live bivalve molluscs from these areas must not exceed 230 MPN *E. coli* per 100 g of flesh and intra-valvular liquid[c]	None
B	Live bivalve molluscs from these areas must not exceed the limits of a five-tube, three dilution MPN test of 4,600 *E. coli* per 100 g of flesh and intravalvular liquid in more than 10% of samples[d]	Purification, relaying or cooking by an approved method
C	Live bivalve molluscs from these areas must not exceed the limits of a five-tube, three dilution MPN test of 46,000 *E. coli* per 100 g of flesh and intravalvular liquid[e]	Relaying or cooking by an approved method

[a]The competent authority has the power to prohibit any production and harvesting of bivalve molluscs in areas considered unsuitable for health reasons.
[b]The reference method is given as ISO 16649–3.
[c]By cross-reference from Regulation (EC) No 854/2004, via Regulation (EC) No 853/2004, to Regulation (EC) No 2073/2005.
[d]From Regulation (EC) No 1666/2006.
[e]From Regulation (EC) No 854/2004.

MATERIALS AND METHODS

The data used in the analyses were derived from official monitoring programmes and had been provided by the authorities responsible for the programmes. The studies had been undertaken over an extended period of time. Samples had been taken according to the protocols under the relevant programme and submitted to the official laboratories responsible for testing the samples for that programme. All of the programmes have requirements for the use of standard methods and the application of laboratory quality assurance (Official Journal of the European Communities 2004; EU Working Group on the Microbiological Monitoring of Bivalve Mollusc Harvesting Areas 2010; FDA 2013). The performance characteristics of individual methods were not supplied with each data set: lower limits of detection (LoD) were inferred from the data.

All data were presented as paired results of either faecal coliforms or *E. coli* in water and shellfish matrices, each pair being from samples taken at approximately the same time and location. Previous examination of a UK data set at Cefas had shown that the median faecal coliform to *E. coli* ratio was 1.00 for bivalve shellfish ($n = 13{,}058$); independently, the Environment Agency in England and Wales has determined that faecal coliform and *E. coli* concentrations in seawaters are broadly equivalent (Environment Agency 2003). In the present work, the values for both indicators were assumed to be equivalent.

Sampling within each programme had not been undertaken according to a statistical design for environmental

covariates but was assumed to have been undertaken at random across a range of conditions. The date ranges of sampling varied between the different sets and also within some of the sets with respect to the areas and bivalve species. Not all bivalve species were represented in each data set.

EU data set

The original EU data set was available as a series of comma-separated values (.csv) text files and covered five EU countries (France, Ireland, Italy, The Netherlands, UK). The data originated from monitoring undertaken in support of the implementation of the Shellfish Hygiene Directive in 1993 (Official Journal of the European Communities 1991). Specific sampling dates were only available for the UK data set: the results were from a survey undertaken between 1991 and 1994. The EU data set contained paired faecal indicator results for flesh and seawater for three 'species': European flat oyster (*Ostrea edulis*), Pacific oyster (*Crassostrea gigas*) and mussels (*Mytilus edulis* and *Mytilus galloprovincialis*). The data set contained results for both faecal coliforms and *E. coli* but usually only for one faecal indicator per sample observation.

The data were screened to remove results that were not identified to sampling site and to remove sites for which there were fewer than 10 pairs of results. This left 69 sites across three countries: France (21), The Netherlands (2) and UK (46). Where sites had data for more than one species, the few measurements for the minority species at a site were discarded.

US data set

The US data were supplied to the authors in 2010 in an Excel spreadsheet with paired samples identified to sampling site. They originated from several studies undertaken between 1963 and 1996. Rows that did not contain paired values were omitted and then sites for which there were fewer than 10 paired results were excluded. The data set contained only faecal coliform results.

Combined data set

The EU and US data sets were concatenated and a separate data set from New Zealand (mussels: species not given) was also included. No sampling dates were given for the New Zealand results but they had been provided for the original EU study (European Commission 1996). The final combined set contained data for 1,564 paired observations at 58 separate stations in five countries – France, The Netherlands, New Zealand, UK and USA – with at least 10 paired results per site. A summary of the number of paired results by country and species is shown in Table 3.

Data were requested from environmental sampling, not from laboratory studies. It is assumed that all data complied with this requirement. Inclusion of data from laboratory studies (e.g. microcosm experiments) or purification systems would potentially bias the outcomes.

Treatment of faecal coliform and *E. coli* results

Previous analysis of a large number of paired faecal coliform and *E. coli* results from shellfish samples showed a median ratio of 1 for the two faecal indicators. The data for both indicators were therefore treated as equivalent in the present analyses and where a mixed data set has been used the outputs are identified as *E. coli* in the results section.

Table 3 | Distribution of number of paired results by species and country

Country	Species					
	C. gigas	*C. virginica*	*O. edulis*	*M. arenaria*	*M. mercenaria*	Mussels
France	348					24
The Netherlands						306
New Zealand						40
UK	101		149			303
USA		129		106	58	

Treatment of censored values

Some of the data sets reported censored values as the respective lower or upper limits of detection (i.e. not adjusted). In the US data set, at least a proportion of these had been adjusted by increasing or decreasing the relevant limit of detection by one significant number. In none of the data sets were censored values explicitly identified as such. It was therefore decided to use the data sets as supplied.

Assumptions about statistical distributions

The National Shellfish Sanitation Program (NSSP) standard requires an estimate of the 90th percentile of results from a site. This can be derived empirically and non-parametrically from the set of samples taken over time and assumed to be serially independent and reflecting the range of variation. However, theory and repeated experience of such sampling indicate that sets of sample values follow a log-normal distribution, and percentiles can be calculated from the standard parameters of the underlying normal distribution. Comparing values from the two methods on the current data indicated very close agreement over the range of values (unpublished result). No bias is introduced by using one or the other estimate.

Logistic regression

These analyses were undertaken on the EU data set; 90%-ile *E. coli* (or faecal coliform) values were empirically estimated for the water results at each site. Percentage compliance of the shellfish results at each site was determined against 230 *E. coli*/100 g (for class A) and 4,600 *E. coli*/100 g (for class B). Weighted logistic regression (fitted as a generalized linear model using Stata version 11 (Statacorp 2009)) was undertaken of percentage compliance (separately for classes A and B) at each site against the 90%-ile *E. coli* value in seawater at that site. Separate models were determined for each of the three 'species' and for all species combined.

Compliance comparison

Compliance assessment was undertaken on the combined data set using the following criteria.

(a) Water
  (i) Approved: geometric mean $\leq$14 faecal coliforms (FC) or *E. coli*/100 mL and 90%-ile $\leq$43 FC or *E. coli*/100 mL.
  (ii) Restricted: geometric mean $\leq$88 FC or *E. coli*/100 mL and 90%-ile $\leq$260 FC or *E. coli*/100 mL.
  (iii) Not approved or restricted: geometric mean >88 FC or *E. coli*/100 mL and/or 90%-ile >260 FC or *E. coli*/100 mL.

(b) Shellfish
  (i) Class A strict: all results $\leq$230 FC or *E. coli*/100 g.
  (ii) Class A pragmatic: 95% results $\leq$230 *E. coli*/100 g.
  (iii) Class B: 90% results $\leq$4,600 *E. coli*/100 g and all results $\leq$46,000 *E. coli*/100 g.
  (iv) Class C: do not comply with A or B but all results $\leq$46,000 *E. coli*/100 g.
  (v) Not A, B or C: one or more results >46,000 *E. coli*/100 g.

Geometric means were determined as $10^{(\text{mean log10 values})}$; 90%-iles were determined by the method given in the NSSP (FDA 2013): $10^{(\text{mean log10} + 1.28*\text{SD log10})}$.

RESULTS

Assessment of the combined data set

A number of different bacteriological methods had been used to enumerate the faecal indicators across the various countries: these were dictated by national or programme requirements. Some of the US results for faecal coliforms in *Mercenaria mercenaria* had been obtained using an investigative method which did not conform to the method specified in the NSSP. Each data set contained a concentration of results at one or more low values. One interpretation is that these values correspond to the lower limits of detection for the test as used for that data set. Inspection of the combined data set led to the conclusion that the lower limit of detection (in particular) for both the water and shellfish testing varied between subsets of the data (by countries or US states, by year and even by date/sample). These factors complicated analysis of the data and logistic regression analysis was

confined to the EU data set. The combined data set was only used for the practical compliance assessment element of the study.

Logistic regression

Figures 1 and 2 show the weighted logistic regression curves for the three species individually and the combined data, for class A and class B compliance against Approved and Restricted standards, respectively. The lines linking the combined-species curve to axis values show the predicted compliance in bivalve molluscs at the US 90%-ile limits for the equivalent category (i.e. class A versus US Approved and class B against US Restricted). Reference lines are also drawn to show nominal 95% compliance with 230 *E. coli*/100 g (class A: Figure 1) and 90% compliance with 4,600 *E. coli*/100 g (class B: Figure 2) in bivalve molluscs. The percentage compliance values are presented in Tables 4 and 5.

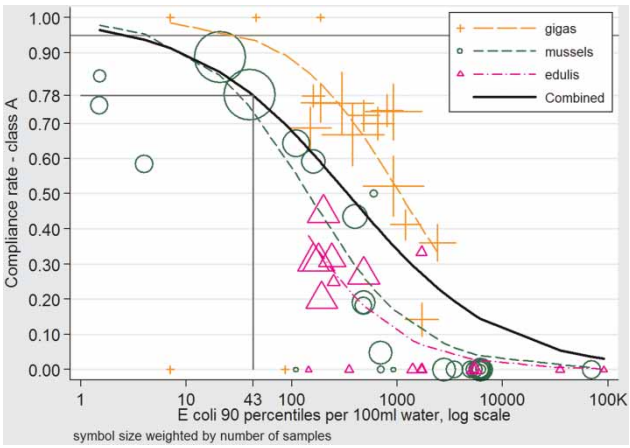

Figure 1 | Logistic regression of class A compliance versus 90%-ile *E. coli* in seawater.

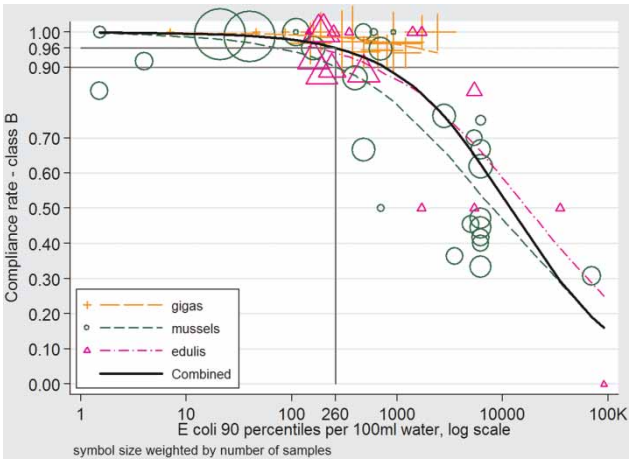

Figure 2 | Logistic regression of class B compliance versus 90%-ile *E. coli* in seawater.

Predicted compliance with 230 *E. coli*/100 g for areas meeting the US Approved standard was therefore lower than the absolute limit implied in the EU food hygiene legislation for the combined data set and the three individual species (Official Journal of the European Communities 2004).

Predicted compliance with 4,600 *E. coli*/100 g for areas meeting the US Restricted standard was generally higher than the 90% required in EU food hygiene legislation and ranged from equivalence for mussels to markedly higher compliance for *C. gigas*.

Comparison of actual compliance: combined EU and US data set

All species

Table 6 shows the number of sites that would have met the individual US and EU classifications on the basis of assessment of water and bivalve results, respectively. The areas

Table 4 | Predicted compliance with 230 *E. coli*/100 g flesh (class A) at a 90%-ile value of 43 *E. coli*/100 mL seawater (the US Approved area standard)

Species	Percentage compliance with 230 *E. coli*/100 g
Mussels	73
C. gigas	94
O. edulis	63
Combined	78

Table 5 | Predicted compliance with 4,600 *E. coli*/100 g flesh (class B) at a 90%-ile value of 260 *E. coli*/100 mL seawater (the US Restricted area standard)

Species	Percentage compliance with 4,600 *E. coli*/100 g
Mussels	90
C. gigas	99
O. edulis	94
Combined	96

Table 6 | Observed compliance with EU and US classification criteria: all species

	EU status				
US status	A	B	C	Prohibited[a]	Total
Approved	2	5	1	0	8
Restricted	0	12	2	0	14
Prohibited[b]	0	16	17	3	36
Total	2	33	20	3	58

[a]Does not comply with the requirements for A, B or C (see footnotes to Table 2).
[b]Does not comply with the requirements for Approved or Restricted.

conforming to EU class A complied with US Approved status while the opposite was not true. One area that would have been classified as C under the EU approach complied with US Approved. However, almost half of areas conforming to class B under the EU criteria did not meet the requirements for either Approved or Restricted under the US approach and harvesting would not have been allowed.

Individual species

Table 7 shows the results of the compliance assessment broken down by species. Five of the six site/species combinations that showed Approved but not class A compliance were mussels. Conversely, a greater proportion of the *Crassostrea* sites (both species) showed class B compliance while not conforming to US Approved or Restricted.

Table 7 | Observed compliance with EU and US classification criteria: by individual species

		EU status			
Species	US status	A	B	C	Not A, B or C[a]
C. gigas	Approved				
	Restricted		4		
	Not A or R[b]		8		
C. virginica	Approved				
	Restricted		1		
	Not A or R		3		
O. edulis	Approved				
	Restricted		3	1	
	Not A or R			2	
M. arenaria	Approved		1		
	Restricted		1	1	
	Not A or R		1	2	1
M. mercenaria	Approved	2	1		
	Restricted		2		
	Not A or R				
Mussels	Approved		4	1	
	Restricted		2		
	Not A or R		2	13	2

[a]Does not comply with the requirements for A, B or C.
[b]Does not comply with the requirements for Approved or Restricted.

DISCUSSION

The conclusions of earlier work, using logistic regression analysis based on assessment against the US geometric mean specifications, were that EU class A was broadly equivalent to US Approved and that EU class B was broadly equivalent to US Restricted (European Commission 1996). The results presented here, using the same statistical approach on the same data set, but judging US compliance on the basis of the 90%-ile specifications, indicates that compliance with the US 90%-ile water standard will not necessarily result in bivalves meeting the EU class A standard. With regard to species, the highest predicted compliance with class A was shown by *C. gigas* and the lowest by mussels. In contrast, the US 90%-ile water standard should result in bivalves meeting the EU class B standard: by species, the compliance should be equal to, or exceed, the EU requirement for class B, with mussels just complying (at 90%) and *C. gigas* easily complying (99%).

Determination of actual compliance under each system showed that sites conforming to US Approved status could fall into EU classes A, B or C (with the majority being class B) and most sites (12 out of 14) conforming to US Restricted status conformed to EU class B, with the others conforming to EU class C. These observations would suggest that EU class A is more stringent than US Approved status

and that EU class B is broadly equivalent to US Restricted status. However, the latter observation needs to be clarified by the fact that several sites (mainly *Crassostrea* spp.) complying with class B did not comply with either the Approved or Restricted criteria. This leads to the conclusion that US Restricted status is more stringent than EU class B for some species.

These differences reflect information on the relative concentration of faecal indicators by different bivalve species obtained through other studies based on different approaches (Berry & Younger 2009).

The current work assessed compliance from as few as 10 results per site/species combination. This is less than the requirement under the NSSP (minimum of 15 results) and less than the usual EU approach for maintenance of a full classification. While using fewer results reduces the robustness of results, there is no evidence that it biases either classification, so we think the comparisons are robust.

Further exploration of the relationship between faecal indicator bacteria in shellfish and the surrounding water, and the implications for classifications determined under the US and EU shellfish hygiene programmes, would significantly benefit from data specifically collected for that purpose. This could be approached by means of systematic field studies and/or laboratory studies under controlled conditions using standard laboratory methods for which the performance characteristics have been assessed.

CONCLUSIONS

1. EU class A is more stringent than the US Approved category for all species.
2. The US Restrictive standard is more restrictive than EU class B for some bivalve species.
3. The combined data set used for the present analyses has significant limitations. If there is a need for further comparisons between the two programmes, from the basis of the classification systems, there should be a targeted acquisition of new, appropriate data.

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