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Comparison of the European Union (EU) and the United States National Shellfish Sanitation Programme (NSSP) microbiological standards in relation to EU Regulation 2015/2285.

Nick Taylor, Rachel Hartnell, Ron Lee & David Lees

European Union Reference Laboratory (EURL) for monitoring bacteriological and viral contamination of bivalve shellfish, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, Weymouth, Dorset, DT4 8UB, United Kingdom.

Scope

This report describes analyses undertaken to establish the effect of the introduction of Regulation (EU) 2015/2285 amending Regulation (EC) No 854/2004 on the official control of microbiological monitoring and classification of bivalve mollusc harvesting areas in the EU with respect to comparisons between EU 'Class A' and US 'Approved' classifications. This analysis was requested by the US FDA and EU Commission to inform the discussion on equivalence of standards in the context of trade negotiations. This report builds on previous work (Annex 1) undertaken at the EURL examining the equivalence of US and EU legislation for sanitary production of live bivalve molluscs for human consumption, and also a preliminary evaluation of any changes consequential on the introduction of (EU) 2015/2285.

Associated Documents

EURL (2016) Comparison of Current and Proposed European Union Classification Criteria for Category A Shellfish Harvesting Waters (Annex I)

EURL (2010) Addendum to the report on the equivalence of US and EU legislation for sanitary production of live bivalve molluscs for human consumption. https://eurlcefas.org/media/8456/addendum_to_the_report_on_the_equivalence_for_the_sanitary_production_of_live_bivalve_molluscs_for_human_consumption_2010.pdf

EU Scientific and Veterinary Committee Working Group on Faecal Coliforms in Shellfish (1996) Report on the equivalence of US and EU legislation for sanitary production of live bivalve molluscs for human consumption https://eurlcefas.org/media/8596/reportontheeuanduslegislation.pdf

Summary findings

- Analyses of the agreed US-EU dataset reveals that although more sites comply with the new class A criteria this is not a statistically significant change (see also EURL 2016).

- Previous analysis (EURL 2010) concluded that EU class A is a more stringent standard than US approved. Further analyses of the agreed US-EU dataset indicates that the revised EU Class A criteria remains more stringent than the US approved standard.

- Confirming this finding, analysis of the pooled US-EU dataset shows that a greater number of sites comply with the US approved standards than either the current or revised EU category

A standard – i.e. either EU standard is more stringent (see also EURL 2016a).

- Although EU standards are more stringent overall there is little overlap between the sites compliant with EU and US standards i.e. it is not possible to predict whether a site will pass or fail one scheme based on the other.
- Preliminary evaluation suggests the disparity between water and shellfish data can be explained by variability in mollusc filtration rates and the different dynamics of bacterial indicators within water and mollusc samples. It was attempted to illustrate this using mathematical modelling. This demonstrated that under certain conditions, sites with low levels of faecal indicators in the water (<=14 per L) can potentially result in levels in shellfish that exceed 700 MPN/100g F.I.L, and that equally, sites exceeding the US Approved standard could pass the EU Class A standard if shellfish filtration rates are low.

Background

In the US, sanitation controls for molluscan shellfish are set out in the NSSP (FDA 2013). In the EU controls governing placing live bivalve shellfish on the market are prescribed in EU Regulations (European Communities 2004, 2005 and 2015). These control systems utilise different approaches to managing shellfish safety, however both use levels of faecal indicator organisms as a proxy for the risk of exposure to faecally borne pathogens. To achieve 'Approved' status the geometric mean faecal coliform count per water sample from an area must not exceed 14 MPN/100 ml, and the ninetieth percentile must not exceed 43 MPN/100 ml (FDA 2013). Under existing EU Regulations, bivalve shellfish samples from a 'Class A' area must contain no more than 230 *E. coli* MPN/100g of shellfish F.I.L and intervalvular liquid (F.I.L) (European Communities 2004, 2005). Live bivalve molluscs harvested from growing areas classified as 'Approved' in the US, and production areas classified as 'Class A' in the EU can be placed directly on the market for live consumption. Previously, US 'Approved' and EU 'Class A' systems, whilst acknowledged as philosophically different in approach have been considered broadly equivalent with respect to the level of public health protection that each confers (EURL 2010).

From 1st January 2017 EU legislation will be revised to align with Codex Alimentarius criterion for live bivalve molluscs placed on the market (three-class plan where n=5, c=1, m=230 and M = 700 *E. coli* MPN/100g F.I.L. (CAC 2014)). The Codex Alimentarius criterion will also be reflected in the rules on the classification of Class A production areas. Therefore, in the EU after 1st January 2017 production areas where 80% samples ≤230 *E. coli* MPN/100g F.I.L. with the remaining 20% of samples ≤700 *E. coli* MPN/100g F.I.L. will be classified as 'Class A' evaluated according to a defined review period[1].

The work presented in this report builds on initial analysis presented by the EURL in 2016 (Annex 1)

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[1] The Competent Authority will define a review period. To take account of variability in concentration of faecal indicators and environmental factors impacting microbiological quality of fisheries recommendations from the EURL are that the review period for an established classification should be no less than 3 years with a minimum of 24 results. Reviews can be undertaken annually or on a rolling basis.

and broadly replicates the approach taken in the analyses undertaken on equivalency of EU and US previously (EURL 1996, 2010). This analysis was agreed at a teleconference between US and EU officials on 20th April 2016. The relationship between the US geometric mean faecal coliform limit of 14 MPN/100ml of water and mean levels observed in shellfish are examined, prior to comparing how the 90th percentile value of faecal coliforms in the water of 43 MPN/100ml (which largely drives the US standards) compares to existing and revised EU upper thresholds of 230 and 700 *E. coli* MPN/100g F.I.L. respectively. We also examine overall levels of association between pass/fail rates at the site level with the application of a mathematical modelling approach to help further understand the relationship between the EU and US standards.

All analyses were undertaken on an agreed paired bivalve shellfish and water faecal coliform dataset circulated to the FDA and EURL colleagues following the teleconference with the technical working group on equivalency on the 20th April 2016.

2. The association between mean faecal coliform levels in water and shellfish

Although the EU does not base its classification system on a mean value of faecal coliforms in F.I.L. and the US standard is largely driven by the 90th percentile limit for water samples, it is still useful to understand the level of association and relationship between mean faecal coliform counts in water and shellfish since this can give an appreciation of the comparability of these measures. Using the dataset analysed in previous reports (summarised in Annex 2), we can explore the relationship between the numbers of faecal coliforms detected in the water column and the mean number detected in shellfish (per gram of F.I.L) harvested from the same areas. Graphical exploration of these data (after $\log_{10}$ transformation) presented in Figure 1 suggest a positive correlation between the two, with an upward trend being evident despite the relatively high level of variability between the two measures.

Applying a linear model (Box 1) to these data shows that both the amount of variability in the faecal coliforms present in shellfish explained by the number in the water column, and the observed upward trend are statistically significant. The R^2 value suggests that the numbers of faecal coliforms present in the water explain 40% of the variability in faecal coliforms present in shellfish. Including shellfish species in the model (Box 2) increases the R^2 value to 0.52, showing that species explains another 12% of the variability observed in the dataset.

The linear model (Box 2) shows that although there were significant differences in the mean value of faecal coliforms expected in some of the different species of shellfish compared to Pacific oysters (used as the reference species in this study), with the exception of mussels, the rate at which faecal coliforms increased in the shellfish relative to the water was the same (i.e. the slope of the line for all species except mussels was not significantly different). The statistically significant interaction term between faecal coliform counts in mussels and the water show the rate of increase in faecal coliforms ($\log_{10}$) in the shellfish compared to the water was higher than observed in oysters, suggesting a faster rate of bioaccumulation.

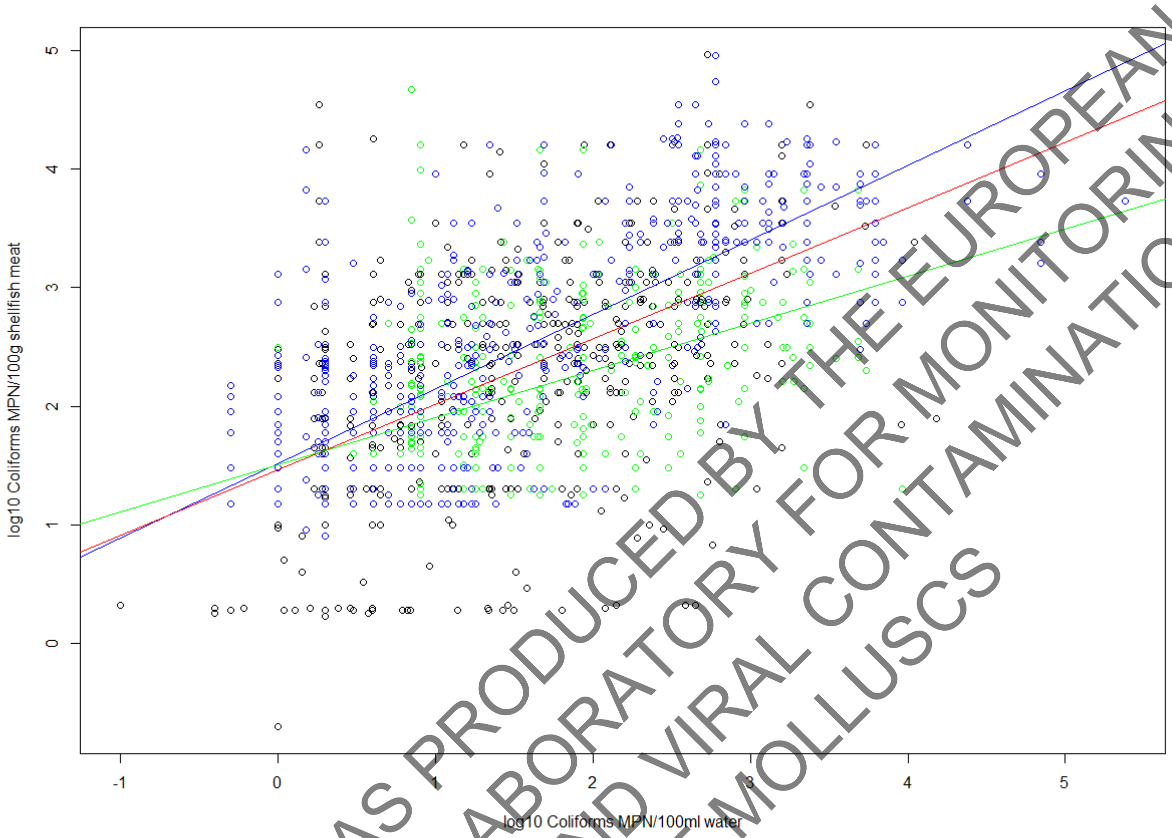

Figure 1. The relationship between faecal coliform (log$_{10}$) levels in water and shellfish F.I.L. Red = mean trend, Blue = Mussels, Green = Pacific Oysters, Black = other species. Lines = model predictions (box 1), points = observed data.

Box 1. Linear model of the relationship between faecal coliforms (log$_{10}$) present in shellfish and water.

```
glm_cor2=(lm(data$logfc_sf~data$logfc_water))

                   Df Sum Sq Mean Sq F value    Pr(>F)
data$logfc_water    1 548.65  548.65  1063.9 < 2.2e-16 ***
Residuals        1577 813.23    0.52

Call: lm(formula = data$logfc_sf ~ data$logfc_water)

Residuals:
    Min      1Q  Median      3Q     Max
-2.6065 -0.4541 -0.0363  0.4510  2.9366

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)       1.46375    0.03161   46.30   <2e-16 ***
data$logfc_water  0.55283    0.01695   32.62   <2e-16 ***

Residual standard error: 0.7181 on 1577 degrees of freedom
Multiple R-squared:  0.4029,  Adjusted R-squared:  0.4025
F-statistic:  1064 on 1 and 1577 DF,  p-value: < 2.2e-16
```

Box 2. Linear model results exploring the relationship between faecal coliforms (log$_{10}$) present in shellfish and the water column adjusting for species.

```
glm_cor2=(lm(data$logfc_sf~data$logfc_water*data$species))
                                 Df Sum Sq Mean Sq  F value    Pr(>F)    
data$logfc_water                  1 548.65  548.65 1344.264 < 2.2e-16 ***
data$species                      5 143.51   28.70   70.322 < 2.2e-16 ***
data$logfc_water:data$species     5  30.16    6.03   14.777 3.461e-14 ***
Residuals                      1567 639.56    0.41                       

Call: lm(formula = data$logfc_sf ~ data$logfc_water * data$species)
Residuals:
     Min       1Q   Median       3Q      Max 
-1.90503 -0.42905 -0.05126  0.39901  2.82793 

Coefficients:
                                           Estimate Std. Error t value Pr(>|t|)    
(Intercept)                                 1.50362    0.06410  23.458  < 2e-16 ***
data$logfc_water                            0.39746    0.03575  11.118  < 2e-16 ***
data$speciesC. virginica                   -0.02585    0.11358  -0.228    0.820    
data$speciesM. arenaria                     0.54335    0.12752   4.261 2.16e-05 ***
data$speciesM. mercenaria                  -1.07645    0.13926  -7.730 1.91e-14 ***
data$speciesMussels                         0.01662    0.07491   0.222    0.824    
data$speciesO. edulis                       0.67953    0.14640   4.642 3.75e-06 ***
data$logfc_water:data$speciesC. virginica   0.10195    0.06706   1.520    0.129    
data$logfc_water:data$speciesM. arenaria   -0.10246    0.06771  -1.513    0.130    
data$logfc_water:data$speciesM. mercenaria -0.05705    0.09610  -0.594    0.553    
data$logfc_water:data$speciesMussels        0.23053    0.04074   5.659 1.81e-08 ***
data$logfc_water:data$speciesO. edulis     -0.13224    0.08053  -1.642    0.101    

Residual standard error: 0.6389 on 1567 degrees of freedom
Multiple R-squared:  0.5304,	Adjusted R-squared:  0.5271 
F-statistic: 160.9 on 11 and 1567 DF,  p-value: < 2.2e-16
```

Using the linear model (Box 1), it was possible to make predictions regarding the mean number of faecal coliforms that would be expected in shellfish based on levels in the water and *vice versa*. Using the US geometric mean threshold for approved waters of <=14 faecal coliforms MPN/100ml of water, the model predicts a mean of 125 faecal coliforms per 100 grams of shellfish F.I.L (across shellfish species, the most conservative value is 91 MPN/100g for oysters). As the distribution of faecal coliform counts in shellfish is known to be log normally distributed, this value can be compared to the mean of faecal coliforms expected to be in a population where the upper faecal coliform limit is either 230 or 700 MPN/100g F.I.L, i.e. the existing and revised EU standards. This is achieved using the following equation:

$$\mathbf{GM=10\hat{}^{\log10(TH)/2}}$$

Where: GM=geometric mean and TH=upper threshold value (this equation gives the same as the square root of the threshold value).

Evaluating this equation using the existing and revised EU upper threshold values of 230 or 700 give mean values of 15.17 and 26.46 respectively, substantially lower than the mean of 125 that is associated with the US mean water count of 14. Substituting these mean values for shellfish faecal coliform counts back into the linear model (Box 1) it was possible to estimate associated expected mean values for waterborne faecal coliform counts of just 0.31 or 0.84 MPN/100ml respectively.

Though the increased EU upper limit of 700 faecal coliforms per gram of shellfish F.I.L does lead to an increase in the expected mean count compared to the previous limit of 230, the mean values associated with this limit remains well below the mean value (125 MPN/100g F.I.L) associated with the US mean limit of 14 waterborne faecal coliforms (MPN/100ml). The new criterion therefore appears to bring the EU methods more in line with the US criterion, whilst still remaining more stringent.

3. The association between upper permitted faecal coliform levels in water and shellfish

To evaluate the relationship between the US 90th percentile limit of 43 MPN/100ml for water samples and the probability of a shellfish being below the EU thresholds of 230 or 700 MPN/100g F.I.L, logistic regression models (assuming a logit link function and quasibionomial error distribution due to data being over-dispersed) were used (Box 3 and 4). For each station the proportion of shellfish testing below the EU thresholds were used as the dependent variable, and the natural log of the 90th percentile for waterborne faecal coliform counts was calculated and used as the explanatory variable. The resulting models were then also used to predict the proportion of shellfish expected to be below the EU threshold based on the US upper 90th percentile value of 43 MPN/100ml.

The models presented in Boxes 3 and 4 show that a significant amount of the variability in the proportion of shellfish testing below the EU thresholds was explained by the 90th percentile value of the faecal coliform counts in the water. For both thresholds there was a significant downward trend, meaning that as the 90th percentile value increased the probability of a shellfish passing either of the EU thresholds decreased. Based on a 90th percentile value of 43 MPN/100 ml for faecal coliforms in the water the models predict that 80.27% and 92.47% of shellfish would be expected to pass the 230 and 700 MPN/100g F.I.L thresholds respectively (Figure 2). Though this increase in pass rate at the level of the shellfish is statistically significant (X-squared = 98.2123, df = 1, p-value < 2.2e-16); under both the old and new EU thresholds, a site providing these results would not pass the EU criteria for category A classification as the threshold constitutes an absolute limit. This suggests that a substantially lower 90th percentile value (than 43) for faecal coliforms in the water would be required in order for a site to meet the EU requirement for no shellfish to test above either the 230 or 700 MPN/100g F.I.L faecal coliform limits

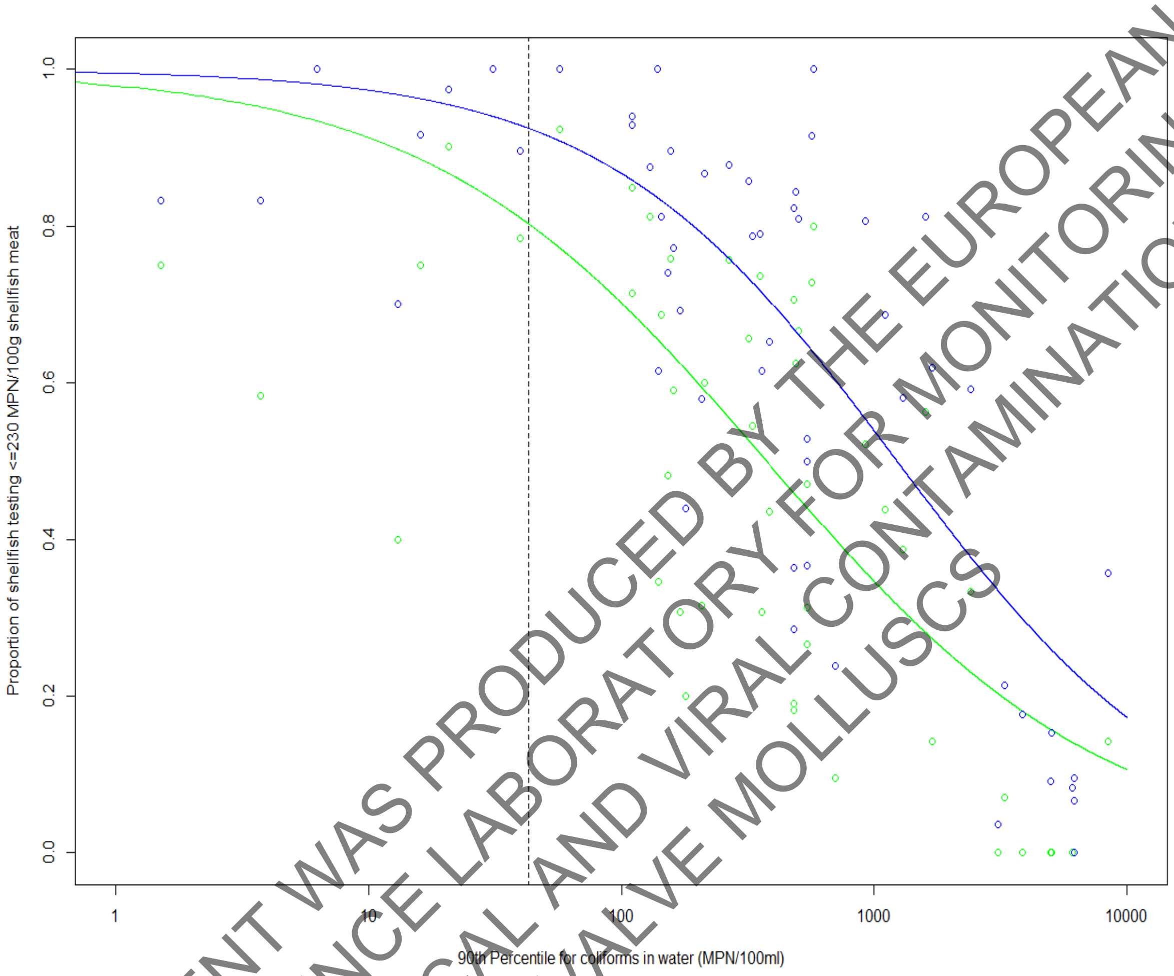

Figure 2. The relationship between the proportion of shellfish from sampling stations testing below the old (230 MPN/100g = green) and new (700 MPN/100g = blue) EU thresholds for faecal coliform counts in shellfish F.I.L, and, the 90th percentile value from faecal coliform counts from matched water samples. Lines denote logistic regression model predictions (See Box 3 for green and Box 4 for blue), points relate to observed data values. Black dashed line denotes the current US limit for a 90th percentile water value of 43 MPN/100ml.

Using the models in Box 3 and 4 it is possible to predict these thresholds (for the purpose of this analysis we assume no more than 5% exceed the threshold). This analysis suggests that to achieve a 95% pass rate under the old EU scheme the 90th percentile for faecal coliforms in the water should not exceed 3.99 MPN/100ml water. To achieve a 95% pass rate under the new EU scheme the 90th percentile in the water should not exceed 23.96 MPN/100ml water, obviously a substantial increase but still well below the current US limit of 43 MPN/100ml.

Under the new EU classification standards, in addition to the 700 MPN/100g faecal coliform limit in shellfish F.I.L, a second criteria must also be met which specifies that 80% of samples in the shellfish population must not exceed 230 MPN/100g. This condition corresponds very well to the figures presented in the above analysis, which show that 80.27% of shellfish samples associated with the US

90th water percentile limit of 43 MPN/100ml would be expected to test below the 230 MPN/100g threshold.

Box 3. Logistic regression model showing the relationship between the proportion of shellfish at a station tested as having <=230 faecal coliforms MPN/100g of shellfish F.I.L and the log 90th percentile value from water samples testing for faecal coliforms (MPN/100ml).

```
Call: glm(formula = y_N230 ~ log(A90th), family = quasibinomial)

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  3.84134    0.53500   7.180 1.74e-09 ***
log(A90th)  -0.64815    0.09086  -7.133 2.09e-09 ***

(Dispersion  parameter  for  quasibinomial  family  taken  to  be
5.272608)
    Null deviance: 662.76  on 57  degrees of freedom
Residual deviance: 282.80  on 56  degrees of freedom
Number of Fisher Scoring iterations: 5

Analysis of Deviance Table
Response: Proportion of shellfish testing below 700 EU threshold

           Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                          57     662.76
log(A90th)  1   379.97        56     282.80 < 2.2e-16 ***
```

Box 4. Logistic regression model showing the relationship between the proportion of shellfish at a station with <=700 faecal coliforms per gram of shellfish F.I.L and the log 90th percentile value from water samples testing for faecal coliforms (per litre)

```
Call: glm(formula = y_N700 ~ log(A90th), family = quasibinomial)

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   5.3183     0.6797   7.824 1.51e-10 ***
log(A90th)   -0.7473     0.1066  -7.011 3.32e-09 ***

(Dispersion  parameter  for  quasibinomial  family  taken  to  be
5.560164)
    Null deviance: 674.48  on 57  degrees of freedom
Residual deviance: 267.32  on 56  degrees of freedom
Number of Fisher Scoring iterations: 5

Analysis of Deviance Table
Response: Proportion of shellfish testing below 700 EU threshold

           Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                          57     674.48
log(A90th)  1   407.16        56     267.32 < 2.2e-16 ***
```

4. The association between EU and US area classification methods

A previous report (Annex 1) documents the influence of changes to the EU microbiological standards on the number of stations meeting the requirements for 'Class A' status. That report suggested that though there was a small increase in the number of stations in the available dataset that met the class A standard under the EU new system, this increase was not statistically significant and still resulted in fewer stations meeting this standard than under the US classification system. The report also suggested little association between the stations meeting the Class A/Approved status under the two schemes. Here, further analysis was conducted using logistic regression models to evaluate the level of association between the probability of a station meeting the EU standards for Class A status based on whether a station had been categorised as approved or not under the US classification system. The models compare the probability of passing either the old (Box 5) or new (Box 6) EU standards given a site has passed the US criteria for approved classification.

The estimates for the slope (Estimate: 0.9226 and 2.136 respectively) of the relationship for the old and new EU classification systems suggest a positive association between the two standards, however neither of these trends are statistically significant (p= 0.526 and 0.293 respectively). In summary this analysis confirms the findings of the previous report (Annex 1), suggesting that passing the US standard for approved classification is not a good predictor of whether a site will pass either the old or new EU standards and *vice versa*.

Box 5. Logistic regression model of the relationship between probability of a site passing the old EU microbiological standard for class A shellfish harvesting waters and US category A standards.

```
glm_USA=(glm(new_A_2~as.factor(USA_cat_2), quasibinomial,
weights=no_samples))

Call:glm(formula = new_A_2 ~ as.factor(USA_cat_2), family =
quasibinomial, weights = no_samples)

Coefficients:
                      Estimate Std. Error t value Pr(>|t|)
(Intercept)            -3.5674     0.9373  -3.806 0.000352 ***
US approved = T         0.9226     1.4445   0.639 0.525598

(Dispersion parameter for quasibinomial family taken to be
28.19645)
    Null deviance: 497.12  on 57  degrees of freedom
Residual deviance: 486.34  on 56  degrees of freedom
Number of Fisher Scoring iterations: 6

Analysis of Deviance Table
Response: Probability of a site passing EU standard for class A

                     Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                                57     497.12
as.factor(USA_cat_2)  1   10.775        56     486.34   0.5365
```

Box 6. Logistic regression model of the relationship between probability of a site passing the new EU microbiological standard for Class A shellfish harvesting waters and US Approved standards

```
Call:glm(formula = old_A_2 ~ as.factor(USA_cat_2), family =
quasibinomial, weights = no_samples)

Coefficients:
                      Estimate Std. Error t value Pr(>|t|)
(Intercept)             -4.781      1.686  -2.835  0.00636 **
US approved = T          2.136      2.013   1.061  0.29313

(Dispersion parameter for quasibinomial family taken to be
28.19752)

    Null deviance: 335.86  on 57  degrees of freedom
Residual deviance: 299.67  on 56  degrees of freedom
Number of Fisher Scoring iterations: 4

Analysis of Deviance Table
Response: Probability of a site passing EU standard for class A

                     Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                                    57     335.86
as.factor(USA_cat_2)  1    36.19        56     299.67   0.2573
```

5. Investigating the disparity between EU and US shellfish classification systems

The levels of faecal coliform contamination in a shellfish over time can be described mathematically as a function of the number of faecal coliforms in the water column, the rate at which a shellfish takes faecal coliforms out of the water, and the rate at which these faecal coliforms are passed back out of the shellfish (or decay within the shellfish). This can be described by the following differential equation:

$$dN/dt=\lambda E-\mu N$$

Where:

- N = the total number of faecal coliforms in an individual shellfish (final results must be adjusted according to shellfish weight (g) and the number of grams tested)
- E = the average number of faecal coliforms in the environment (per litre of water)
- λ = the rate at which faecal coliforms are removed from the environment - i.e. the number of litres of water filtered per hour)
- μ = the rate at which faecal coliforms number are reduced in shellfish - i.e. the clearance rate – 1/time (hours)

By rearranging this equation, we can say that whilst λE > µN we would expect the number of faecal coliforms in the shellfish to increase. This also implies that as numbers of faecal coliforms in the shellfish increases there will become a point where λE = µN, at which point the population of faecal coliforms in the shellfish will stop increasing, and, if we assume E to be constant, will reach equilibrium. Under these conditions the number of faecal coliforms present in an entire shellfish can be predicted by:

$$N = \lambda E / \mu$$

This equation was used to run simulations to illustrate the relationship between faecal coliforms in the water and faecal coliforms in the shellfish under different scenarios and across a range of different filtration and clearance rates (Figure 3). The plots show that the bacterial loading of shellfish at equilibria will increase as both the filtration rate and time taken for shellfish to clear bacteria increase. Additionally, the higher the level of bacteria present in the water, the faster this increase will occur (compare the top and bottom plots in Figure 3). This suggests that that in both US approved and non-approved waters, the likelihood of passing the EU standard for class A waters are dependent on the local filtration and clearance rates of the shellfish, and are therefore not directly associated. Where both rates are high, shellfish are likely to fail the EU class A standards regardless of the level of bacteria in the water (whether above or below the US approved limits), and conversely, will pass the standards if both rates are low. These relationships between bacterial levels in the water and shellfish filtration and clearance rates may explain the lack of association observed between US approved, and EU class A waters identified within the test dataset.

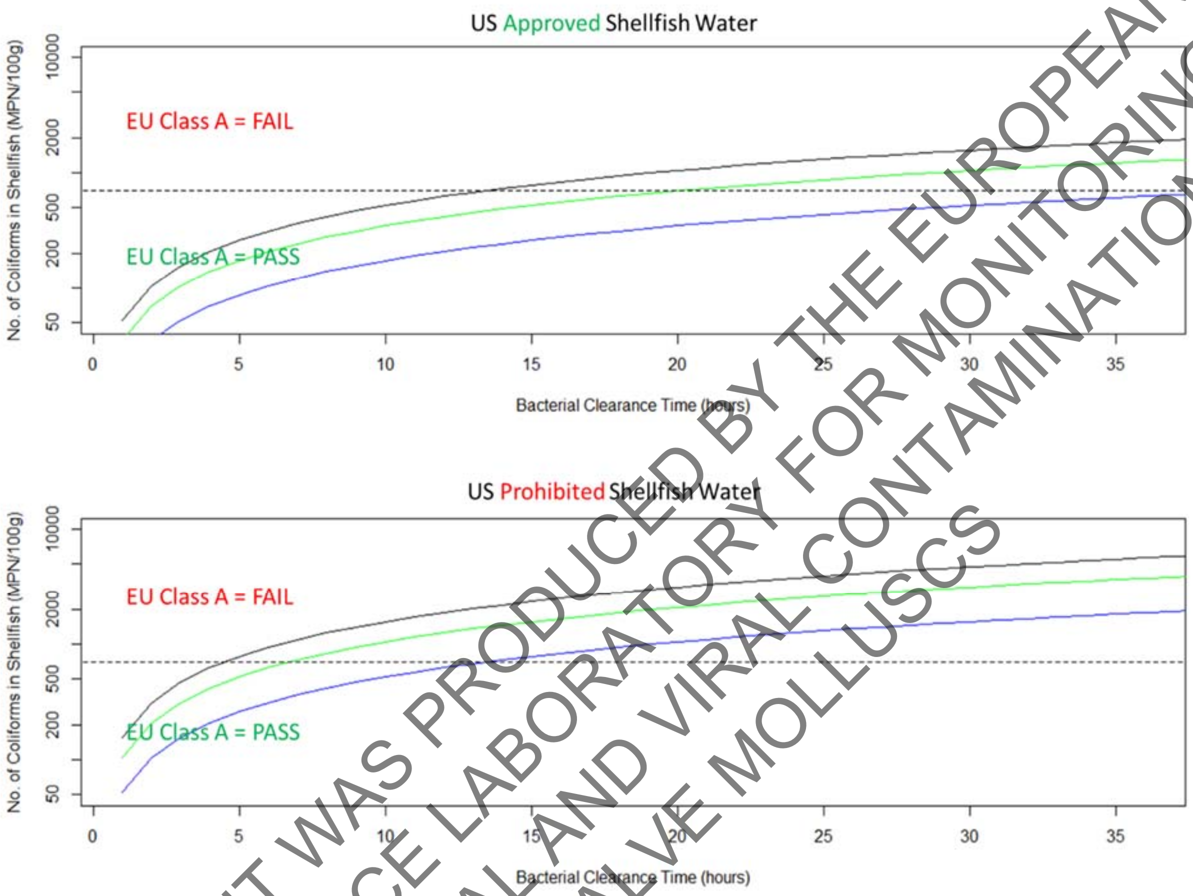

Figure 3. Illustration of the relationship between the bacterial loading of shellfish against bacterial clearance times based on slow (blue line), medium (green line) and fast (black line) filtration rates in US approved (top plot) and prohibited (bottom plot) shellfish growing waters. Black dashed line shows the new EU threshold for category A shellfish growing waters.

Conclusions

This report suggests that at level of the shellfish and the site, the new EU standards for class A waters remain more stringent than the standards required for US approved shellfish growing waters. Comparison of the mean faecal coliform burden in water and shellfish demonstrates a significant associate between the two, and shows that the current US mean limit is likely to cause the mean burden in shellfish to exceed the mean value in shellfish associated with the new EU class A 700 faecal coliform (MPN/100g) maximum limit.

The analysis also showed that the proportion of shellfish likely to pass the new EU upper threshold of 700 faecal coliforms (MPN/100g) based on the US 90th percentile limit of 43 faecal coliforms (MPN/100ml water) was only 92%, which is not sufficient for a site to pass the new EU class A standard (as 700 MPN/100ml represents an absolute threshold requiring a 100% pass rate). This suggests the US 90th percentile limit would have to be reduced substantially in order to meet the EU standard and explains why substantially more sites met the US approved criteria in the agreed dataset than meet the EU class A criteria under either the existing or revised standards.

The lack of association between sites passing the US approved category criteria, and, the EU class A standards, can be likely explained by variability in shellfish filtration and clearance rates at sites. This study shows that where these rates are high a site is likely to fail the EU standard regardless of whether a site has met the US approved criteria. Conversely where these rates are low, a site may pass the EU standard unless faecal coliforms counts in the water are far in excess of the US approved status upper limits. It is likely that there is a relatively small window of overlap where conditions are conducive to sites meeting both the US approved and EU class A standards, potentially explaining the disparity between the two systems observed in the trial dataset.

In summary, the modified EU standard remains more stringent than the US approved shellfish water standards, and does not lead to a significant change in the number of sites achieving class A status.

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European Union Reference Laboratory
for monitoring bacteriological and viral
contamination of bivalve molluscs

Annex 1:

Comparison of Current and Proposed European Union Classification Criteria for Category A Shellfish Harvesting Waters

Nick Taylor, Ron Lee, Rachel Hartnell & David Lees

European Reference Laboratory (EURL) for monitoring microbiological contamination of bivalve shellfish, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, Weymouth, Dorset, DT5 2ES.

In the US sanitation controls for molluscan shellfish are set out in the National Shellfish Sanitation Program (NSSP) (FDA 2013). In the EU controls governing placing live bivalve shellfish on the market are prescribed in EU Regulations (European Communities 2004, 2005 and 2015). These control systems utilise different approaches to managing shellfish safety however both use levels of faecal indicator organisms as a proxy for the risk of exposure to faecally borne pathogens that may be present in production areas. Growing areas classified as 'approved' according to the US approach and production areas classified as 'category A' according to the EU system can be placed live directly on the market.

Production areas in the US are monitored and classified based on the levels of faecal coliforms detected in the seawater. To achieve approved status, the geometric mean faecal coliform count per water sample from an area must not exceed 14 cfu/100 ml, and the ninetieth percentile must not exceed 43 cfu/100 ml (FDA 2013). In the EU, category A waters are classified based on the level of the faecal coliform bacteria, *Escherichia coli*, present in the bivalve shellfish F.I.L. Under existing EU Regulations bivalve shellfish samples from a category A area must contain no more than 230 faecal coliforms per 100g of shellfish F.I.L (European Communities 2004, 2005).

Though the methods for classifying bivalve shellfish waters in the US and EU are different, previous studies conducted by the European Reference Laboratory for Monitoring Bacteriological and Viral Contamination of Bivalve Shellfish (EURL) have shown that approved and category A waters are broadly equivalent, though the criteria for EU category A waters appears somewhat more stringent than those for US approved classification. Recently the EU has approved a change in the criteria for the classification of category A shellfish waters, essentially to align category A status with the international Codex Alimentarius criterion for *E. coli* for live or raw bivalve shellfish placed in the market (CAC 2014). Under the revised criteria, a production area can be classified as category A if at least 80% of shellfish sampled from an area do not exceed 230 *E. coli* per 100g F.I.L, and 100% do not exceed 700 faecal coliforms per 100g shellfish F.I.L over a defined review period (European Communities 2015). The implementation date for this legislation is 1st January 2017.

This study aimed to compare the results obtained using the current and future EU criteria for classifying category A shellfish waters with that of US approved areas. This study reanalysed the paired seawater-shellfish EU/US dataset used in the original EURL shellfish classification equivalency study. The revised EU criteria for category A waters were examined to determine whether this would lead to a significant change in the proportion of waters classified as category A, and this was compared to the number of areas determined as approved using the US classification criteria. The dataset was comprised of 58 sampling stations, at which between 10 and 153 shellfish and water samples were obtained (median=19, mean=27.7). The faecal coliforms bacteria per 100ml of water and per 100g of shellfish F.I.L were used to determine the classification of the station under the US, and the current and future EU classification systems.

Table 1 shows the results from stations classified as approved or category A under the US, current and future EU criteria. The results (Figure 1) showed that 8 of the 58 (13.8% - 95% CI: 6.9 to 25.2 %) stations met the criteria for approved classification under the US scheme. Only 3 of the 58 (5.2% - 95% CI: 1.2% to 14.7%) stations met the criteria for category A status under the existent EU classification system. This rose to 5 of the 58 (8.6% - 95% CI: 3.3 to 19.1%) stations under the revised EU classification criteria. Though there was a small increase in the number of sites classified as category A under the future EU criteria, Fisher's exact test (which given the low number of stations classified as category A was deemed more appropriate than a Chi-squared test or logistic regression) showed this difference not to be statistically significant (p=0.7167) suggesting an area is equally likely to be classified as category A under both systems.

Table 1. *Key results relating to faecal coliform(FC) levels at shellfish waters meeting the criteria for US approved classification or EU category A status under the current and future legislation..*

Station	Proportion of shellfish with FC<=230	Proportion of shellfish with FC<=700	Gemometric mean of waterbourne FC's	90th Percentile of waterbourne FC's	Current EU category	Proposed EU category	US classification
RI- B	1	1	4.37	31	A	A	APPROVED
RI- C	1	1	1.8	6.22	A	A	APPROVED
MA21	1	1	11.55	138.9	A	A	OTHER
MA11	0.8	1	240.58	577	OTHER	A	OTHER
RI- A	0.92	1	11.16	57.12	OTHER	A	OTHER
12W1	0.4	0.7	5.71	13	OTHER	OTHER	APPROVED
ME- C7	0.75	0.92	4.02	15.98	OTHER	OTHER	APPROVED
s1	0.78	0.9	6.3	39.8	OTHER	OTHER	APPROVED
s2	0.9	0.97	4	20.8	OTHER	OTHER	APPROVED
st 1	0.75	0.83	1.63	1.5	OTHER	OTHER	APPROVED
st 2	0.58	0.83	2.62	3.75	OTHER	OTHER	APPROVED

Applying the Fishers exact test to compare the likelihood of being classified as approved under the US system or category A under either the current or future EU criteria, showed that there was no significant difference in the proportion of sites classified under any of these schemes (US vs Current EU: p=0.2035, US vs future EU: p=0.5577). Though the proportions of sites classified as approved or category A (i.e. stringency) were not significantly different, only 2 of the 8 (25% - 95% CI: 6.3 to 59.9%) of sites classified as approved under the US system were common to either of the EU class A criteria. Both the current and future EU classification systems identified the same 3 stations as reaching the category A standard, however, the revised EU scheme identified a further 2 sites as meeting the category A standard. Fishers exact test showed no significant (p=1) difference in the equivalence (i.e.

commonality between stations) between the EU classification schemes compared to the US system, though this analysis is based on a very small sample size [based on 2 of 3 category A stations (66.7% - 95% CI: 20.2 to 94.4%) also being classed as US approved under the current criteria, and 2 of 5 category A stations (40%- 95% CI: 11.6 to 77.1%) also being classed as US approved under the proposed criteria].

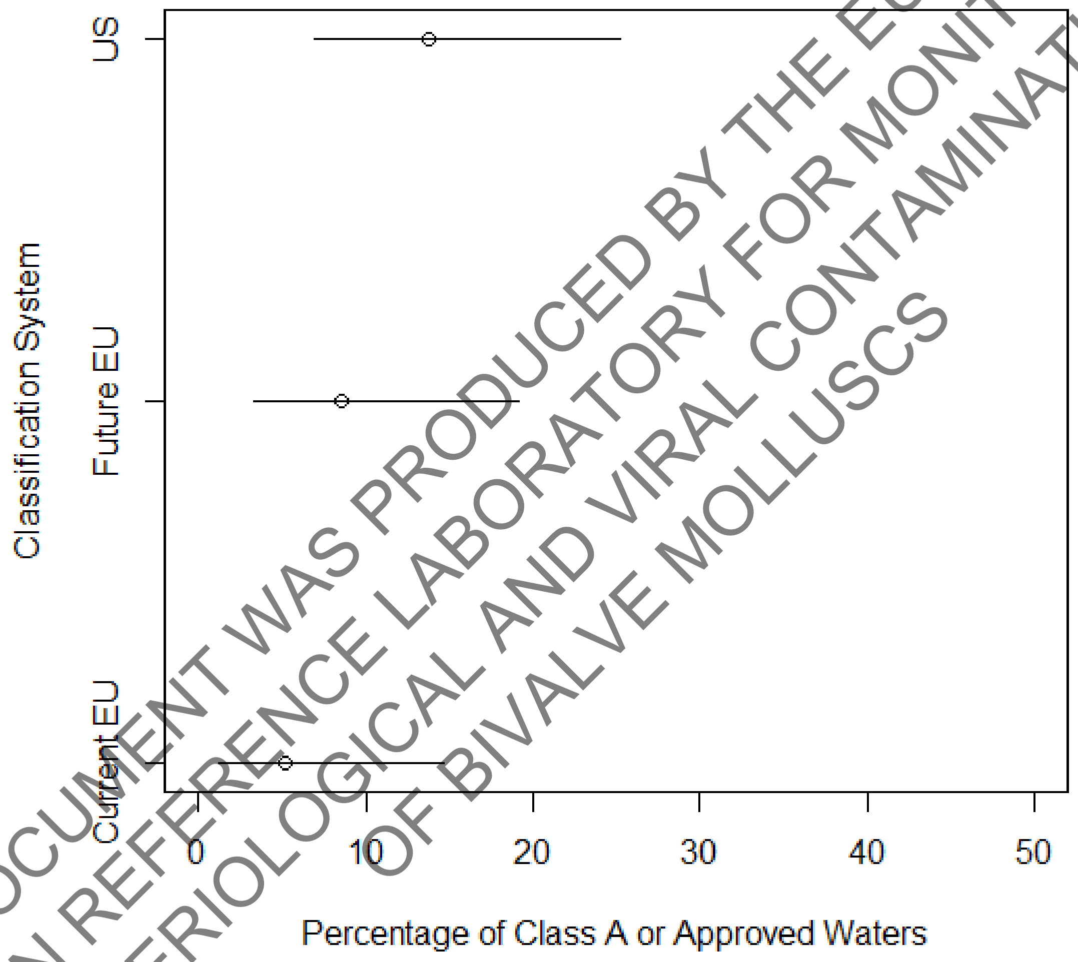

Figure 1. Proportion of shellfish stations classified as approved under the US classification systems or class A under the current and revised EU classification systems. Points show the observed percentage of sites surveyed meeting class A/approved status, lines show the 95% confidence intervals associated with these values.

Of the 8 stations classified as approved under the US scheme, 6 (75%- 95% CI: 40.1 to 93.7%) did not meet the criteria for category A under either the current or revised EU schemes. Of these 6 sites, 5 met neither of the criteria adopted under the new EU classification scheme. The remaining site met the requirement to have 80% of samples with no more than 230 faecal coliforms per 100g shellfish F.I.L, but 3% of samples exceeded the 700 faecal coliform maximum limit. Only 1 of the 3 (33%- 95% CI: 56.3 to 79.8%) category A stations classified under the current EU scheme would not have been approved under the US scheme, this station would have met the US criteria based on the geometric mean, but would not have met the 90th percentile criteria. Under the revised EU classification system, 3 of the 5 (60%- 95% CI: 22.9 to 88.4%) category A sites would not have met the US approved classification. Two of these would have met the US criteria relating to the geometric mean from water samples not exceeding 230 faecal coliforms per 100ml of water, but would have not met the 90th percentile upper threshold limit. The remaining station did not meet either of the US approved classification criteria.

In summary, the revised EU criteria to determine category A waters (European Communities 2015) did not lead to a significant increase in the proportion of stations classified under this category compared to the current legislation. Under both EU schemes, fewer sites were classified as category A than as approved under the US system. The difference in the proportion of sites classified as approved or category A was not however statistically significant, suggesting that the three schemes are equivalent and will result in a similar number of areas reaching the highest classification status. Statistical analysis suggests that the revised EU criteria do not cause a significant change in the degree of equivalence with US approved areas.

References

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Annex 2: Number of shellfish samples (by species) collected per data station

	C. gigas	C. virginica	M. arenaria	M. mercenaria	Mussels	O. edulis
12W1	0	0	0	0	10	0
71012	17	0	0	0	0	0
71013	32	0	0	0	0	0
71020	29	0	0	0	0	0
B012A	0	0	0	0	23	0
B012B	0	0	0	0	11	0
B012D	0	0	0	0	21	0
B012E	19	0	0	0	0	0
B012F	20	0	0	0	0	1
B012G	0	0	0	0	22	0
B013A	0	0	0	0	0	26
B013B	0	0	0	0	0	25
B013D	0	0	0	0	0	26
B013E	0	0	0	0	0	19
B013F	0	0	0	0	0	27
B013G	0	0	0	0	0	26
B031A	21	0	0	0	0	0
B031B	25	0	0	0	0	2
B032G	0	0	0	0	21	6
B032L	0	0	0	0	10	3
B032M	0	0	0	0	11	3
B032O	0	0	0	0	21	0
B032P	0	0	0	0	28	0
B035A	16	0	0	0	0	0
B044A	0	0	0	0	18	0
B044B	0	0	0	0	10	0
B044C	0	0	0	0	15	0
B044D	0	0	0	0	18	0
B044E	0	0	0	0	12	0
B044F	0	0	0	0	11	0
B044H	0	0	0	0	17	0
B044I	0	0	0	0	21	0
B044M	0	0	0	0	13	0
cs	0	0	0	0	30	0
LA	0	33	0	0	0	0
MA11	0	0	0	10	0	0
MA21	0	0	0	10	0	0
ME- C1	0	0	14	0	0	0
ME- C2	0	0	16	0	0	0
ME- C3	0	0	16	0	0	0
ME- C4	0	0	17	0	0	0
ME- C5	0	0	16	0	0	0
ME- C6	0	0	15	0	0	0
ME- C7	0	0	12	0	0	0
MS- 302	0	31	0	0	0	0
MS- 303	0	33	0	0	0	0
MS- 304	0	32	0	0	0	0
O61026	70	0	0	0	0	0
O61027	67	0	0	0	0	0
O61034	74	0	0	0	0	0
O61035	59	0	0	0	0	0
RI- A	0	0	0	13	0	0
RI- B	0	0	0	12	0	0
RI- C	0	0	0	13	0	0
s1	0	0	0	0	153	0
s2	0	0	0	0	153	0
st 1	0	0	0	0	12	0
st 2	0	0	0	0	12	0