Interspecies comparison of E. coli accumulation in bivalve shellfish using data obtained from official control monitoring under EU Regulation 854/2004

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Introduction

Monitoring of *E.coli* in live bivalve shellfish to establish faecal contamination status is required under EU Regulation 854/2004 in order to set an appropriate level of harvesting area classification. In England and Wales, it has been found that species growing at the same site may show differences in the levels to which they accumulate contamination to the extent that they may fall into different classification categories.

The aim of this study was to determine how consistent these inter-species *E. coli* concentration differences might be across a range of sites. Where differences are found to be consistent and/or predictable then it might be possible to rationalise monitoring programmes e.g. monitor one species to represent two or more. This would have the benefit of conserving monitoring resources and might lessen delays in classification by allowing some species to be classified without specific monitoring.

Possible causes of differences in *E.coli* content between species growing at the same site are essentially:

- Difference in species response to uptake of *E.coli* in terms of overall filtering capacity
- Time effects in terms of differences between species in speed of uptake and removal of contaminants in response to temporal variation in water quality.
- Differences in mode of growth (e.g. trestle grown vs. sea/river bed grown) may have the effect of exposing shellfish to different pollution impacts by holding the shellfish at different tidal heights, particularly if they are grown in the intertidal zone (see Figure 1)

This study examined the historic data accumulated at sites with two or more species and statistically assessed the significance of any differences.

Figure 1 Growing methods: Is there a difference in contamination between bed grown and trestle grown shellfish?
(Species shown: Oyster racks/trestles (upper), Mytilus *spp. on sea bed (lower)*

Figure 2 Location of monitoring points selected in England

Materials and methods

Sites for analysis were selected according to the following criteria:

- Site contained two or more separately monitored species.
- The relevant Representative Monitoring Points (RMP's) shared at least ten same-day sampling results (for statistical assessment purposes).

To assist with a simple first stage visual assessment of the data, logged (base 10) shellfish flesh *E. coli* results were graphically represented for each species at each site using box plots (see example graph in Figure 4). After this initial assessment paired t-tests were performed to specifically assess the difference between paired observations (a p-value of 0.05 was used to determine statistical significance).

A simple comparison was made between geometric means for each species and the results of this comparison plotted as a bar graph to show the number of instances where the geometric mean for one species was greater than that for another e.g. *Mytilus* spp. > *C. gigas* (see Figure 5). The number of occasions where the differences between paired data was statistically significant according to a 2 sided paired t test was shown on the same bar graph.

Geometric mean ratios were tabulated for all species pair comparisons (see Table 1, paired t test significant outcomes are highlighted in red).

Results

It was not possible to make comparisons between the *E. coli* geometric mean data for all species due to the lack of sufficient instances where certain species combinations existed e.g. a comparison between *Tapes philippinarum* and *Mercenaria mercenaria* could not be made at all and there were only two instances where data for *C. edule* and *T. philippinarum* could be compared. For the latter, on both occasions *C. edule* > *T. philippinarum* although the differences were not found to be statistically significant.

Some species comparisons showed equivocal results with, for example, *O. edulis* and *C. gigas* showing 1 instance of *C. gigas* > *O. edulis* and 4 instances of *O. edulis* > *C. gigas* although none of these differences were found to be statistically significant according to the paired t test outcomes. Similarly a comparison between *O. edulis* and *Mytilus* spp revealed 2 occasions where *O. edulis* > *Mytilus* spp. , none of which were statistically significant, and 3 occasions where *Mytilus* spp. > *O. edulis*, 2 of these being statistically significant.

The most consistent difference in terms of *E. coli* accumulation was found to be between *Mytilus* spp and *C. gigas*. In all 17 instances where these two species were growing together, *Mytilus* spp. accumulation was greater than *C. gigas* with 9 of these comparisons being significantly different according to a paired t-test (see Table 1).

Discussion and conclusion

From the data presented in Table 1 and Figure 5 a tentative ordering in terms of *E. coli* accumulation could be proposed as follows:

(*C. edule* & *T. philippinarum* & *Mytilus spp.*) > (*O. edulis* & *C. gigas*)

O. edulis > *M. mercenaria*

Further work (either in analysing existing datasets from elsewhere or from specific experimental work) would be necessary in particular to confirm the relationship between *C. edule, T. philippinarum* and *Mytilus spp.* The other species comparisons would also benefit from further evaluation to clarify the extent (if any) of any differences. Furthermore, it would be useful to characterise where inconsistencies in the orderings above may exist i.e. to determine whether differences in growth mode or other factors can account for any reversal of these general relationships.

In terms of geometric means, the ratio of differences found between *C. gigas* and *Mytilus* spp. ranged between 0.29 to 1 and 0.6 to 1 for sites where the paired data were shown to be significantly different by the paired t test (as highlighted in red in Table 1). In other words, average *E. coli* accumulation in *Mytilus* spp. was between 1.7 and 3.4 times greater than in *C. gigas.*

From the data presented it is proposed that *C. gigas* could be represented by monitoring of *Mytilus* spp. alone. From the shellfish industry perspective and considering the greater marketability of class A shellfish, this would only be appropriate if class A compliance was considered unlikely at the outset and classification at class B would be sufficient for local shellfish industry needs. If class A compliance is considered possible then specific monitoring of *C. gigas* would be necessary.

*Figure 3 Shellfish species, a)*Cerastoderma edule, *b)*Mercenaria mercenaria, *c)*Tapes philippinarum, *d)*Crassostrea gigas, *e)*Ostrea edulis *and f)*Mytilus *spp.*

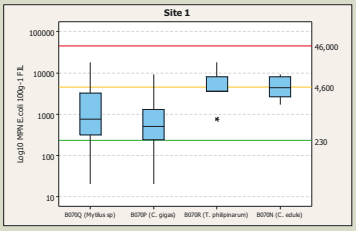

Figure 4 Example species E. coli *data comparison box plot at a single site*

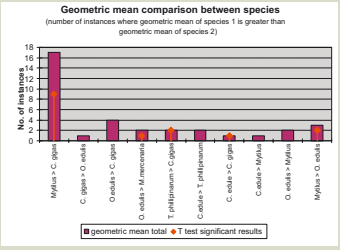

Figure 5 Geometric mean comparison between species along with paired t-test significance results (2 sided)

Site	*O. edulis*	*C. gigas*	*Mytilus spp.*	*C. edule*	*T. philippinarum*	*M. mercenaria*	Difference factor
1	476					190	2.51
2		271	393				0.69
3		202	288				0.70
4		196	334				0.59
	293	196					1.49
	293		334				0.88
5		261	726				0.36
6		937	2043				0.46
7			51	73			0.70
8		587	801				0.73
9	323		317				1.02
10	432		680				0.64
11		64			178		0.36
12	93	71					1.31
13		141	289				0.49
14				396	311		1.27
		130			311		0.42
		130		396			0.33
15	113					58	1.95
16				4317	4215		1.02
		526	940				0.56
17		34	92				0.37
18	239		382				0.63
	239	230					1.04
		230	382				0.60
19	36		32				1.13
20		259	809				0.32
21		1083	3738				0.29
22	111	152					0.73
23		49	66				0.74
24		49	80				0.61
25		269	867				0.31
26		1640	3467				0.47
27	65	46					1.41
28		692	813				0.85

Table 1 Geometric mean comparison between species pairs including factor of difference between the two (t-test significant outcomes p<0.05 highlighted in red)