Comparison of microbiological contamination level between different species of shellfish

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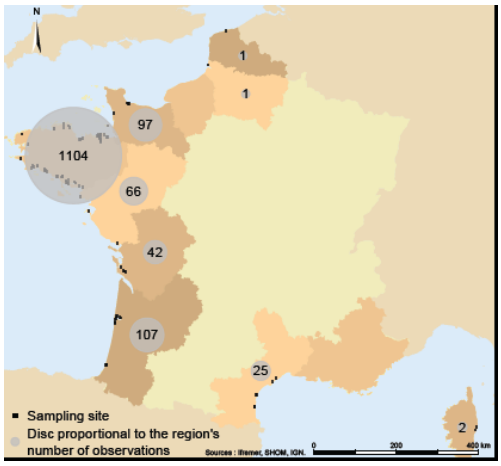
Introduction

Each area from which shellfish are collected to be commercialised has to be classified and monitored on microbiological parameter in order to protect consumer health, according to requirements of regulation (EC) n°854/2004. The microbiological monitoring is carried out by Ifremer since 1989 throughout the REMI, French microbiological monitoring network for shellfish growing areas. REMI allows notably to evaluate and monitor faecal contamination levels (*Escherichia coli* /100 g Flesh and Intravalvular Liquid –FIL of shellfish production areas.

In France, 323 areas are delimited. Each one of these areas can be classified either for one, two or three groups of shellfish (group 1 : gastropod, echinoderm and tunicate ; group 2 : filter feeding burrowing bivalves (cockle, clams…) ; group 3 : filter feeding non burrowing bivalve : oyster and mussel). Finally, 458 areas are classified for a group of shellfish. Some on these areas are monitored for two groups, **corresponding to at least two different species** in a concomitant way (same sampling point, same time of sampling).

The present study was conducted in order to optimize the sampling strategy, allowing to keep a high level of consumer's health protection while minimizing the costs of surveillance. Its aim is to identify if one or more species can be used as indicator species for microbiological contamination of other species present.



Map 1 : Location of monitoring points sampled for two species of molluscs and number of observation per point.

Material and methods

This study is based on the analysis of REMI data collected from 01/01/1989 to 31/12/2010. For all couples of taxa, common sites and dates were identified. Among 100 107 records present in the national database Quadrige², a total of 1 525 couples were extracted, representing all the couples of shellfish species that were concomitantly present at the sametime. Sampling points included 84 locations (Map 1), most of the data are from Britanny.

In order to insure the statistical significance of results, we arbitrarily decided to work with couple of species with more than 30 results. Seven couples of taxa have been kept concerning five species : two filter burrowing, *Cerastoderma edule* and *Tapes spp*, two non filter burrowing, *Mytilus spp* and *Crassostrea gigas* and one gastropod (Tab. 1).

Table 1 : Number of results for couples of taxa with more than 30 results at common sites and dates

	Tapes spp	*Mytilus spp*	*Crassostrea gigas*	*Patella vulgata*
Cerastoderma edule	70	345	316	26
Tapes spp		55	528	No data
Mytilus spp			105	80
Crassostrea gigas				No data

Depending on the results on *E. coli* two types of statistic approach have been done

Results ≥ limit of quantification (LOQ) →

❶Results are transformed in **base-10 logarithms**.

❷**Scatter plots** with an **orthogonal regression** are done in order to study the unique reciprocal relationship between concentrations measured for two different taxa.

❸ Differences of log-concentration are tested to be null using the non-parametric **Wilcoxon rank test** (α= 0,05). The null hypothesis of this test is the nullity of the median of the differences.

❹ The **Bonferroni correction** is used to address the problem of multiple comparisons.

❺ The robust and non-parametric **Hodges-Lehmann** estimate the median of the log-differences is given.

Results < limit of quantification (LOQ) → Contingency table

Results

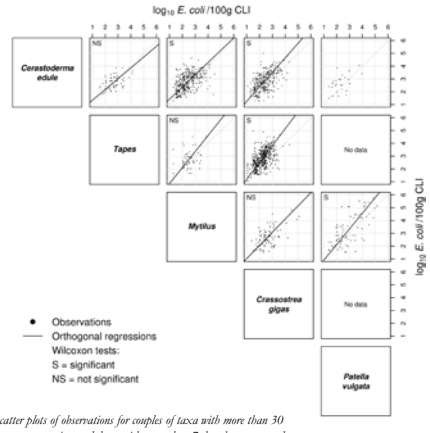


Figure 1: Scatter plots of observations for couples of taxa with more than 30 observations at common sites and dates with more than 7 days between two dates.

Comparisons in log10 E. coli/100g FIL	Median of differences [95% confidence interval]	p-values	Adjusted p-values
Cerastoderma edule - Tapes	0.094 [-0.063 ; 0.254]	0.2373	> 1
Cerastoderma edule - Mytilus	0.388 [0.313 ; 0.463]	< 1e-04	< 1e-04
Cerastoderma edule - Crassostrea gigas	0.48 [0.403 ; 0.555]	< 1e-04	< 1e-04
Tapes - Mytilus	0.244 [0.017 ; 0.459]	0.0241	0.1688
Tapes - Crassostrea gigas	0.614 [0.566 ; 0.663]	< 1e-04	< 1e-04
Mytilus - Crassostrea gigas	0.102 [-0.05 ; 0.259]	0.1677	> 1
Mytilus - Patella vulgata	0.383 [0.169 ; 0.581]	0,0007	0.0052

Table 2: Wilcoxon tests (α= 0,05) and median estimates for comparisons in log_{10} E. coli/100g CLI between couples of taxa. Adjusted p values are computed according to Bonferronni.

		Cerastoderma edule ≥ LOQ	Cerastoderma edule < LOQ
Mytilus	≥ LOQ	354	20
	< LOQ	139	86

		Cerastoderma edule ≥ LOQ	Cerastoderma edule < LOQ
Crassostrea gigas	≥ LOQ	326	29
	< LOQ	188	145

		Crassostrea gigas ≥ LOQ	Crassostrea gigas < LOQ
Tapes	≥ LOQ	540	593
	< LOQ	72	348

Table 3: Contingency tables

Discussion & conclusion

As a result of the statistical treatment (Fig.1, Tab. 2), three pairs of species show no significant differences in their level of microbiological contamination. This applies to the following pairs:

- *Cerastoderma edule/ Tapes spp, Tapes spp/ Mytilus spp, Mytilus spp / Crassostrea gigas.*

For four pairs of shellfish species, significant differences in levels of contamination are highlighted:

- ***Cerastoderma edule / Mytilus sp., Cerastoderma edule / Crassostrea gigas, Tapes spp/ Crassostrea gigas, Mytilus spp /Patella vulgata***

Based on the following property of the logarithm : log (A)- log (B) = log (A/B), the ratio of concentration between species can be estimate. **Thus, the contamination level of:**

- ***Cerastoderma* is about 2.5 times higher than *Mytilus spp.***
- ***Cerastoderma edule* is about 3 times higher than *Crassostrea gigas.***
- ***Tapes spp* is about 4 times higher than *Crassostrea gigas.***
- ***Mytilus spp* is about 2.5 times higher than *Patella vulgata.***

For non-quantifiable data, ie data below the LOQ of the method used, the data pairs of species showing significant differences were examined. Previous results are confirmed by contengency tables (tab 3) which indicates for example that for 593 results "< LOQ" on Crassostrea gigas, results on Tapes were quantified. For 348 results, both were "< LOQ" and 72 indicates a results "<LOQ" for Tapes while results had been quantified for *Crassostrea gigas.*

From this study based on 1525 couples of data collected by the REMI since 1989, a significant difference of the microbiological contamination level between taxa has been demonstrated. This difference does not allow for modelling of microbiological contamination, but identifies species that can be considered as sentinel for other species.

These results confirm the existence of groups of shellfish. *Cerastoderma edule* is a sentinel species for group 2 (burrowing bivalves), and either *Mytilus spp* or *Crassostrea gigas* can be used to represent group 3 (non burrowing bivalves).

Cerastoderma spp can be used as indicator for all commercial species present in the area (*Mytilus spp, Crassostrea gigas, Tapes spp*).