Shellfish monitoring for lipophilic phycotoxins in France, recommendation for an updated sampling strategy

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Abstract

In France, the sampling strategy for the official monitoring of lipophilic phycotoxins in bivalve shellfish relies on the definition of risk areas and high risk periods, during which a systematic weekly analysis of toxins in shellfish is performed. Since 2010, high risk periods are defined as follows: the occurrence of one result above the European regulatory limit (160 µg equivalent okadaic acid/kg shellfish) over the last 3 years leads to that month being considered a high risk period. This definition was established according to a statistical analysis of the official monitoring results for the period 2003-2008, based on the mouse bioassay (MBA) as the official analytical method. As of the 1st January 2010, the MBA has been replaced by LC-MS/MS. In 2014, a new statistical analysis was performed, based this time on results for the period 2010-2013 for which quantitative LC-MS/MS data are available. We tested the robustness of the definition set in 2010 and identified a new methodology to improve our sampling strategy for lipophilic toxins in bivalve shellfish, based on Bayesian inference.

Keywords: monitoring; shellfish; lipophilic phycotoxins; Bayesian inference

Introduction

The sampling strategy for the official environmental monitoring of phycotoxins in bivalve shellfish in France differs according to the family of toxins and to the type of zone (coastal or offshore). For PSP and ASP toxins, the strategy relies on the monitoring of phytoplankton in seawater. The detection of toxic species above an alert threshold acts as a trigger for the analysis of toxins in shellfish. For lipophilic toxins, the phytoplankton is not a reliable indicator. A systematic weekly analysis of toxins in shellfish is performed in risk areas during high risk periods, firstly proposed in 1999 by Ifremer (the French Research Institute for Exploitation of the Sea). Outside these high risk periods, the strategy relies on phytoplankton analysis, which is the method used for ASP and PSP. The methodology to identify high risk periods for lipophilic toxins has been reviewed in 2010. The occurrence of one result above the European regulatory limit (160 µg equivalent okadaic acid/kg shellfish) over the last 3 years in the area leads to that month being considered as a high risk period in this area. An area with at least one month as risk period is considered as a risk area. This definition has been recommended by ANSES (the French Agency for Food, Environmental and Occupational Health & Safety) based on a statistical analysis of the official monitoring results for the period 2003-2008. At that time, the mouse bioassay (MBA) was the official analytical method but as of the 1st January 2010, it has been replaced by LC-MS/MS. In 2014, ANSES carried out a new statistical analysis, based this time on results for the period 2010-2013 for which quantitative LC-MS/MS data are available (and not only qualitative positive/negative results from the MBA). The objective was to evaluate the performance of the current definition of high risk periods and to look for a new definition that could improve the efficiency of the sampling strategy.

Material and Methods

Based on the data for the 3-year period 2010-2012, we compared the predicted high risk periods to actual periods in 2013 (reference year) with results above the EU regulatory limit and evaluated the sensitivity and the specificity:
- for the current system: based on qualitative data (result above the EU regulatory limit during the last 3 years? YES/NO)
- and for an alternative system: based on quantitative data, evaluation of the probability of

A. Lincoln MacKenzie [Ed]. *Marine and Freshwater Harmful Algae 2014*. Proceedings of the 16th International Conference on Harmful. Algae. Cawthron Institute, Nelson, New Zealand and the International Society for the Study of Harmful Algae (ISSHA)

the area/period to have 1 result > EU regulatory limit. The data analysis involved:

- Lognormal fitting of the data taking into account censoring (by cumulative distribution function)
- Testing 2 models: 1) maximum of likelihood (fitdistrplus package, R.3.03) 2) Bayesian inference (package rjags): higher number of situations can be fitted with this model compared to the maximum of likelihood (e.g. only 1 data available for the area/period), uncertainty is function of the number of analysis done, but interpretation of uncertainty is not always easy.
- The risk manager setting the acceptable level above which the area/period is considered as being at high risk (p-value).

A description of the data used in the analysis is as follows:

- Years: 2010, 2011, 2012, 2013
- LC-MS/MS analysis
- Concentration in shellfish meat, sum of AO+DTXs+PTXs
- based on EFSA toxic equivalence factors
- Number of monitored marine areas: 77
- Number of data (measured concentration): 5 434
- Minimum: 3 µg eq OA/kg shellfish meat
- Maximum: 37 296 µg eq OA/kg shellfish meat
- Number of censored data (< limit of detection): 2 962.

Results and Discussion

Figure 1 illustrates the diversity in the data available according to the shellfish area. For some areas there is a lot of data all year around whereas for other there is data for only 2 or 3 months.

Table 1 shows the high risk periods according to the current system in some shellfish areas for 2013. In high risk periods, there is a weekly sampling of shellfish in the marine area for lipophilic toxins testing by LC-MS/MS. Outside these periods (not high risk periods), there is a water sampling every 2 weeks for plankton monitoring. If the number of cells of *Dinophysis* is above an alert level, there is a shellfish sampling in week n+1 for toxin testing.

In addition to this strategy, for 10 areas (part of the vigilance system), there is a systematic monthly sampling of shellfish all year.

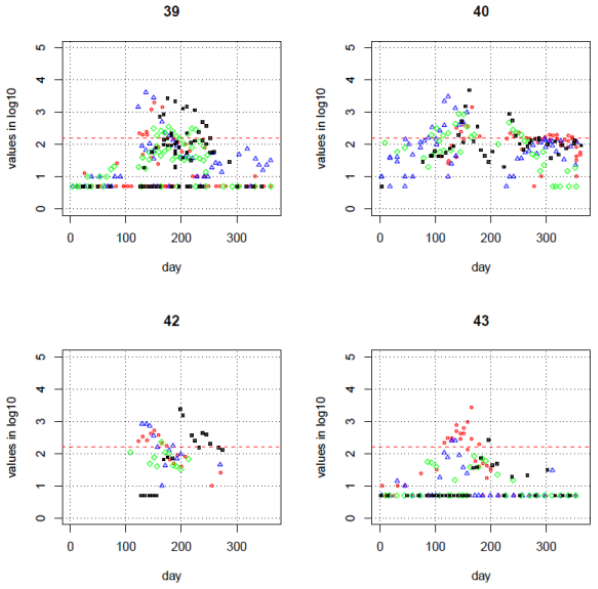

Fig. 1: An illustrative example of toxicity data (y axis: µg OA/kg) for the period 2010-2013 in some areas (39, 40, 42, 43) by day of year.
Legend. 2010: red, 2011: blue, 2012: green, 2013:black, horizontal red dot line: EU regulatory limit.

Table 1: Current system with high risk periods for 2013 by area and month (for areas #3 to 57 as an example). Legend. ND: no data in 2010-2012, by default defined as not a high risk period, 0: defined not a risk period with data, 1: defined as high risk period.

month area	1	2	3	4	5	6	7	8	9	10	11	12
3	0	0	0	0	0	ND	ND	ND	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
9	ND	ND	ND	ND	ND	0	ND	0	0	0	0	0
10	0	0	0	0	1	1	0	1	1	1	0	0
12	0	0	ND	ND	0	ND	ND	ND	0	0	0	0
13	0	0	0	ND	ND	ND	ND	ND	ND	0	0	0
14	ND	ND	0	ND	0	0	ND	0	0	0	0	0
15	0	ND	0	ND	0	0	0	0	0	0	0	0
16	0	0	0	0	ND	ND	ND	ND	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	ND	ND	ND	ND	ND	0	ND	0
24	0	0	0	0	ND	ND	ND	ND	0	0	0	0
26	ND	ND	ND	ND	0	ND	ND	ND	ND	ND	ND	ND
32	ND	ND	ND	0	1	0	0	0	1	1	0	ND
33	0	0	0	0	ND	0	ND	ND	0	0	0	0
34	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
37	ND	ND	0	1	1	ND	ND	0	0	ND	0	0
38	0	0	ND	1	1	1	0	1	1	1	1	0
39	0	0	0	0	1	1	1	1	0	0	0	0
40	0	0	0	1	1	1	0	1	1	1	1	1
42	ND	ND	ND	0	1	1	1	ND	0	ND	ND	ND
43	0	0	0	1	1	1	0	0	0	0	0	0
44	ND	ND	ND	0	1	1	1	0	ND	ND	ND	ND
45	ND	ND	ND	0	1	1	0	ND	ND	ND	ND	ND
46	ND	ND	ND	0	1	1	0	ND	ND	ND	ND	ND
47	0	0	0	1	1	1	1	1	0	0	0	0
48	ND	ND	ND	0	1	1	0	ND	ND	ND	ND	ND
49	0	0	0	1	1	1	1	0	0	0	0	0
50	ND	ND	ND	ND	0	ND	ND	ND	ND	ND	ND	ND
51	ND	ND	ND	ND	1	1	1	ND	ND	ND	ND	ND
52	ND	ND	ND	ND	1	1	ND	0	0	ND	ND	ND
53	ND	ND	ND	0	1	1	0	0	0	0	ND	ND
54	0	0	0	0	0	0	0	0	0	0	0	0
55	ND	ND	ND	ND	0	0	0	ND	ND	ND	0	ND
56	ND	ND	ND	ND	0	ND	ND	ND	ND	0	ND	ND
57	ND	ND	ND	ND	0	0	ND	ND	ND	0	ND	ND

Table 2: Comparison of observed data and predicted high risk periods in 2013 (for areas #3 to 57 as an example).

month area	1	2	3	4	5	6	7	8	9	10	11	12
3	0	0	0	0	0	NA	NA	0	0	2	0	0
6	0	0	0	0	0	0	0	0	NA	0	0	0
9	0	0	0	0	NA	NA	NA	0	0	2	0	0
10	0	0	0	0	1	1	0	3	3	3	0	0
12	NA	NA	NA	NA	NA	NA	NA	0	NA	NA	0	0
13	0	0	NA	NA	NA	NA	NA	0	NA	NA	0	0
14	0	NA	NA	NA	NA	NA	NA	0	NA	NA	1	1
15	0	0	NA	NA	NA	0	0	0	0	0	0	NA
16	0	0	0	0	NA	NA	NA	NA	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	
21	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	0
24	0	0	0	0	NA	NA	NA	NA	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
32	NA	NA	NA	0	1	0	0	0	1	1	0	NA
33	0	NA	NA	NA	NA	0	0	0	0	0	0	0
34	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA
37	NA	NA	0	1	-1	NA	0	0	NA	NA	NA	NA
38	0	0	0	1	1	3	0	3	3	1	1	0
39	0	0	0	NA	1	3	3	3	2	0	0	0
40	0	NA	0	1	3	3	0	3	3	1	1	1
42	NA	NA	NA	NA	1	1	3	2	2	0	NA	NA
43	0	0	0	1	1	1	2	0	0	0	0	0
44	NA	NA	NA	NA	1	1	3	2	0	NA	NA	NA
45	NA	NA	NA	NA	1	1	2	NA	NA	NA	NA	NA
46	NA	NA	NA	NA	1	1	2	0	0	0	NA	NA
47	0	0	0	1	1	3	3	3	2	6	6	1
48	NA	NA	NA	NA	1	1	2	NA	0	0	NA	NA
49	0	0	0	1	1	3	3	0	0	2	0	NA
50	NA	NA	NA	NA	NA	NA	0	NA	NA	0	NA	NA
51	NA	NA	NA	NA	1	1	3	NA	NA	0	NA	NA
52	NA	0	NA	NA	-1	-1	NA	NA	NA	NA	NA	NA
53	NA	NA	NA	NA	1	1	NA	NA	NA	NA	NA	NA
54	0	0	0	0	0	2	2	0	0	0	0	0
55	NA	NA	NA	NA	NA	NA	NA	0	NA	0	NA	NA
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0

Legend:
Green 3: true positive predicted (TPP): result > EU regulatory limit in 2013 and predicted as high risk period
white 0 : true negative predicted (TNP): no result > EU regulatory limit in 2013 and predicted as not high risk period
red 2: false negative predicted: result > EU regulatory limit in 2013 but NOT predicted as high risk period
blue 1: false positive predicted: no result > EU regulatory limit in 2013 but predicted as high risk period.
NA: no data.

Table 2 shows the comparison of observed data and predicted high risk periods in 2013. Table 3 shows the proposed new systerm, based on Bayesian inference and providing the probability (p-value) of results above the EU regulatory limit by marine area and month for 2013, based on all available quantitative data in 2010, 2011 and 2012. The prior is a probabilistic distribution reflecting the level of knowledge we have before taking into account the data. In this study we chose a non-informative prior probabilistic distribution for estimating the risk for 2013 before adding the data (2010-2012), setting that we assume to know quite nothing about the area and the period without the knowledge of the data. Then, the inference is only linked by the data and the prior is not supposed to influence the results. After making inference with the data set, we estimate the risk for each area and period.The risk manager sets the acceptable level (p-value, e.g. 10%, 5%, 1%) and identifies high risk periods.

Table 3: probability (p-value) of results above the EU regulatory limit by marine area and month for 2013, based on Bayesian inference (new system). ND, NA: no data.

month area	1	2	3	4	5	6	7	8	9	10	11	12
3	NA	NA	0,00	NA	NA	ND	ND	ND	0,00	0,00	0,00	0,01
6	0,00	NA	0,00	NA	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
9	ND	ND	ND	ND	ND	NA	ND	0,00	0,01	0,00	0,03	0,01
10	0,00	0,00	0,00	0,00	0,07	0,10	0,01	0,17	0,15	0,12	0,08	0,04
12	NA	NA	ND	ND	0,00	ND	ND	ND	0,00	0,00	0,01	0,01
13	0,05	0,00	0,00	ND	ND	ND	ND	ND	ND	0,00	0,01	NA
14	ND	ND	0,00	ND	0,00	0,00	ND	NA	0,00	0,00	0,00	0,00
15	0,00	ND	0,00	ND	NA	NA	0,00	0,00	0,00	NA	NA	NA
16	0,00	0,00	NA	NA	ND	ND	ND	ND	0,00	NA	0,00	NA
18	0,00	NA	0,00	NA	0,00	NA	NA	NA	NA	0,00	NA	NA
21	NA	NA	NA	NA	ND	ND	ND	ND	ND	0,00	ND	0,00
24	0,00	NA	NA	0,00	ND	ND	ND	ND	NA	0,00	0,00	NA
26	ND	ND	ND	ND	0,00	ND	ND	ND	ND	ND	ND	ND
32	ND	ND	ND	0,00	0,11	0,00	NA	0,01	0,11	0,10	0,02	ND
33	NA	0,00	0,00	0,00	ND	0,00	ND	ND	0,00	0,00	NA	0,00
34	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
37	ND	ND	0,00	0,12	0,29	ND	ND	0,00	0,04	ND	0,02	0,00
38	NA	0,00	ND	0,22	0,31	0,23	0,03	0,10	0,17	0,09	0,12	0,00
39	0,00	0,00	0,01	0,00	0,23	0,15	0,15	0,08	0,02	0,01	0,02	0,01
40	0,05	0,10	0,10	0,43	0,43	0,69	0,12	0,20	0,19	0,12	0,21	0,12
42	ND	ND	ND	0,13	0,65	0,23	0,02	ND	0,03	ND	ND	ND
43	0,00	0,00	0,02	0,09	0,30	0,24	0,04	0,00	NA	0,00	0,01	0,00
44	ND	ND	ND	0,02	0,22	0,31	0,07	0,01	ND	ND	ND	ND
45	ND	ND	ND	NA	0,10	0,08	0,04	ND	ND	ND	ND	ND
46	ND	ND	ND	0,03	0,25	0,12	0,12	ND	ND	ND	ND	ND
47	0,02	0,01	0,00	0,09	0,33	0,36	0,21	0,09	0,03	0,00	0,00	0,01
48	ND	ND	ND	0,02	0,21	0,17	0,05	ND	ND	ND	ND	ND
49	0,00	NA	0,00	0,10	0,29	0,29	0,19	0,03	0,02	0,02	0,00	0,00
50	ND	ND	ND	ND	0,07	ND	ND	ND	ND	ND	ND	ND
51	ND	ND	ND	ND	0,22	0,20	0,08	ND	ND	ND	ND	ND
52	ND	ND	ND	ND	0,21	0,20	ND	0,08	0,03	ND	ND	ND
53	ND	ND	ND	NA	0,14	0,11	0,10	NA	NA	NA	ND	ND
54	NA	0,00	0,00	0,00	0,05	0,12	0,09	0,01	0,05	0,02	0,03	0,01
55	ND	ND	ND	ND	0,02	0,01	0,00	ND	ND	ND	0,00	ND
56	ND	ND	ND	ND	0,04	ND	ND	ND	ND	0,01	ND	ND
57	ND	ND	ND	ND	0,04	0,02	ND	ND	ND	0,02	ND	ND

The current system of definition of high risk periods (1 result > EU regulatory limit over the last 3 years) has a sensitivity of 51% and a specificity of 86%.
Criteria definition :
- Sensitivity: probability (predicted+/found +) = TPP/(TPP+False Negative)
- Specificity: probability (predicted-/found-) = TNP/(TNP+False Positive)
+: above the EU regulatory limit of 160 µg eq OA/kg shellfish meat
-: below

In the proposed new definition of high risk periods the sensitivity and the specificity are function of the acceptable level (as shown in Figure 2). Sensitivity can reach 85% (Bayesian inference, p-value of 0.1%).

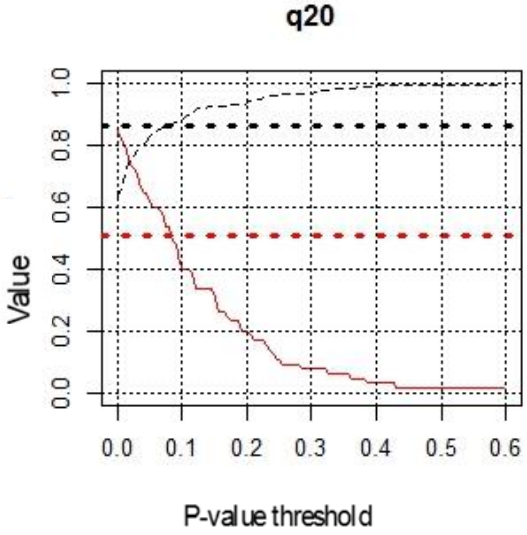

Fig. 2. Sensitivity and specificity of the proposed new system.
Legend:
red line: sensitivity
black line: specificity
dotted red line: sensitivity of the current system
dotted black line: specificity of the current *system*

However, this improvement in terms of sensitivity and specificity implies an increase in the number of shellfish samples necessary (an increased number of high risk periods), as shown in table 4. The prior we used was the same for all months and areas. However it could be feasible to set informative priors, different for some areas or months based on environmental or historical data, and then to better identify at-risk areas.

Conclusion

The strengths of the new system (Bayesian inference is the preferred option) include 1) the use of all the data information (quantitative) and not only a part of it (qualitative), 2) the ability to fit more situations (e.g. when there is only 1 result for a month with a value below, but close to, the EU regulatory limit), 3) the possibility for increased sensitivity, and therefore a better protection of consumer health, 4) the possibility to take into account extra information in the prior, 5) an increased role of the risk manager who can choose the level of protection.

As drawback, this new system implies the need for an increased number of shellfish-meat toxin analysis and consequently, increased costs.
However, this work only relies on one reference year (2013). To confirm our modelling and our recommendations, we plan to conduct a new statistical analysis with the data now availalble for the year 2014. This study is expected to be completed by mid-2015.

Table 4. Sensitivity and specificity of the proposed new system according to the p-value threshold and the numer of high risk periods.

Sensitiviy	P-value threshold	Number of high risk periods	Specificity
0.85	0.10	282	0.63
0.85	0.20	275	0.65
0.83	0.30	271	0.65
0.82	0.70	257	0.67
0.80	1.40	226	0.72
0.78	1.70	217	0.74
0.77	1.80	214	0.74
0.75	2.00	210	0.74
0.74	2.30	199	0.75
0.72	2.90	179	0.78
0.71	3.40	173	0.78
0.68	3.50	170	0.79

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