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A French study on the efficiency of sanitary shucking of scallops contaminated by the lipophilic toxins

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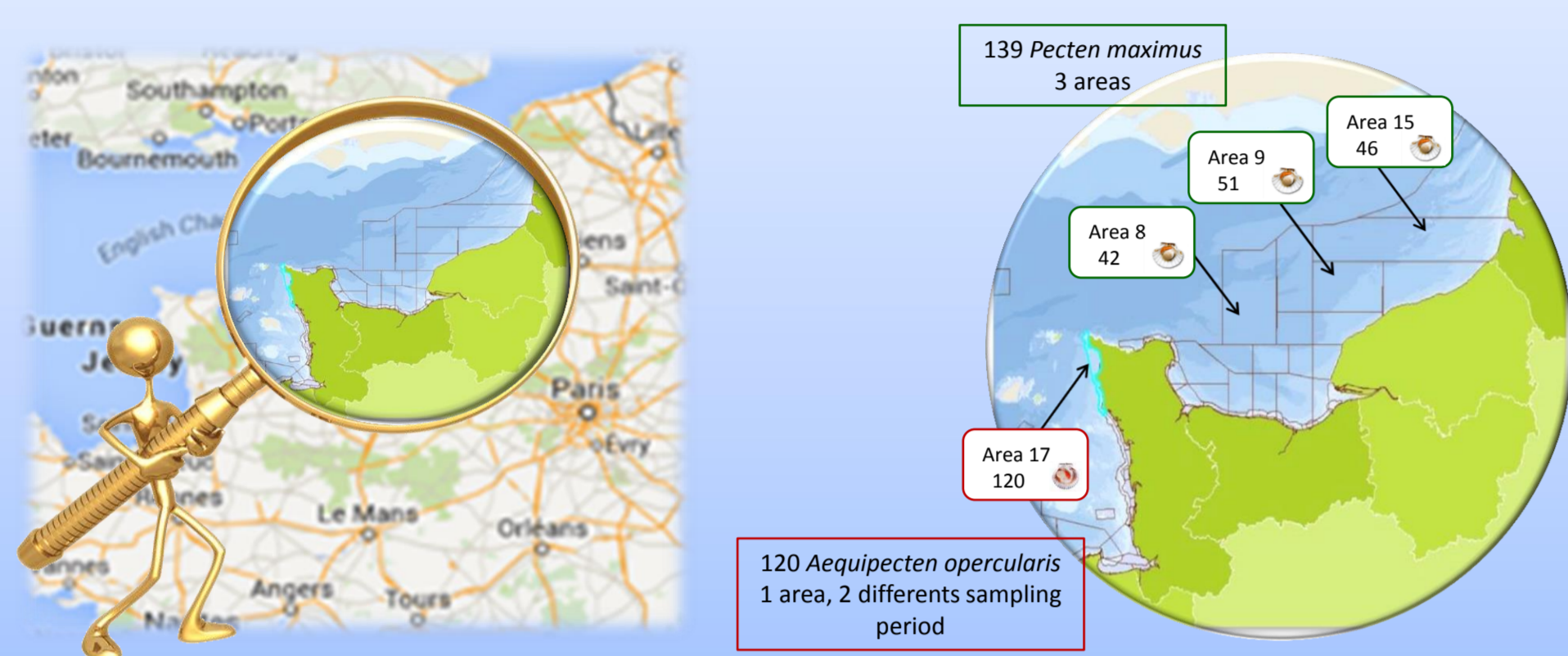
Context

Following episodes of contamination of *Pecten maximus* and *Aequipecten opercularis* by okadaic acid lipophilic toxins (autumn 2014 in eastern Cotentin, Normandy, and summer 2016 in North West Cotentin respectively), the French National Reference Laboratory for Marine Biotoxins was commissioned by the General Directorate of Food of the Ministry of Agriculture, Agri-Food and Forestry to carry out a study on the distribution of these toxins in the shellfish.

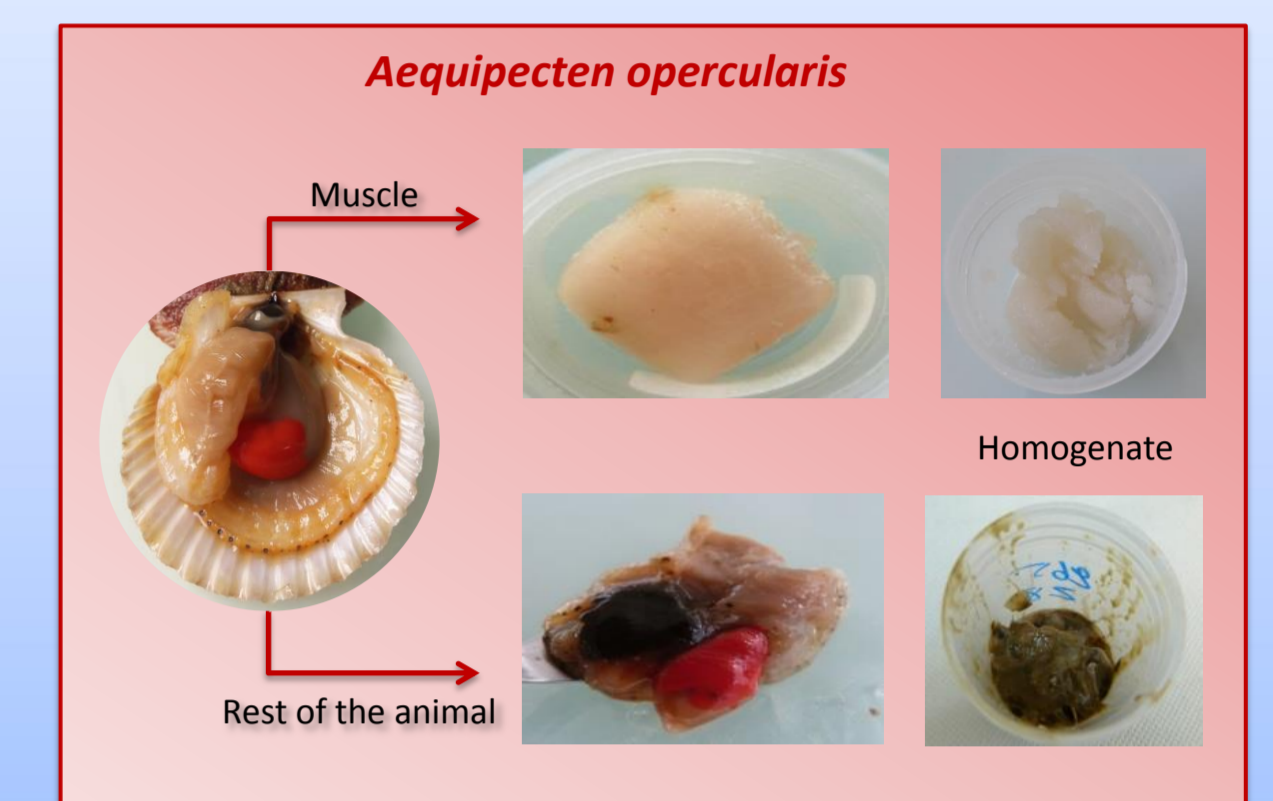
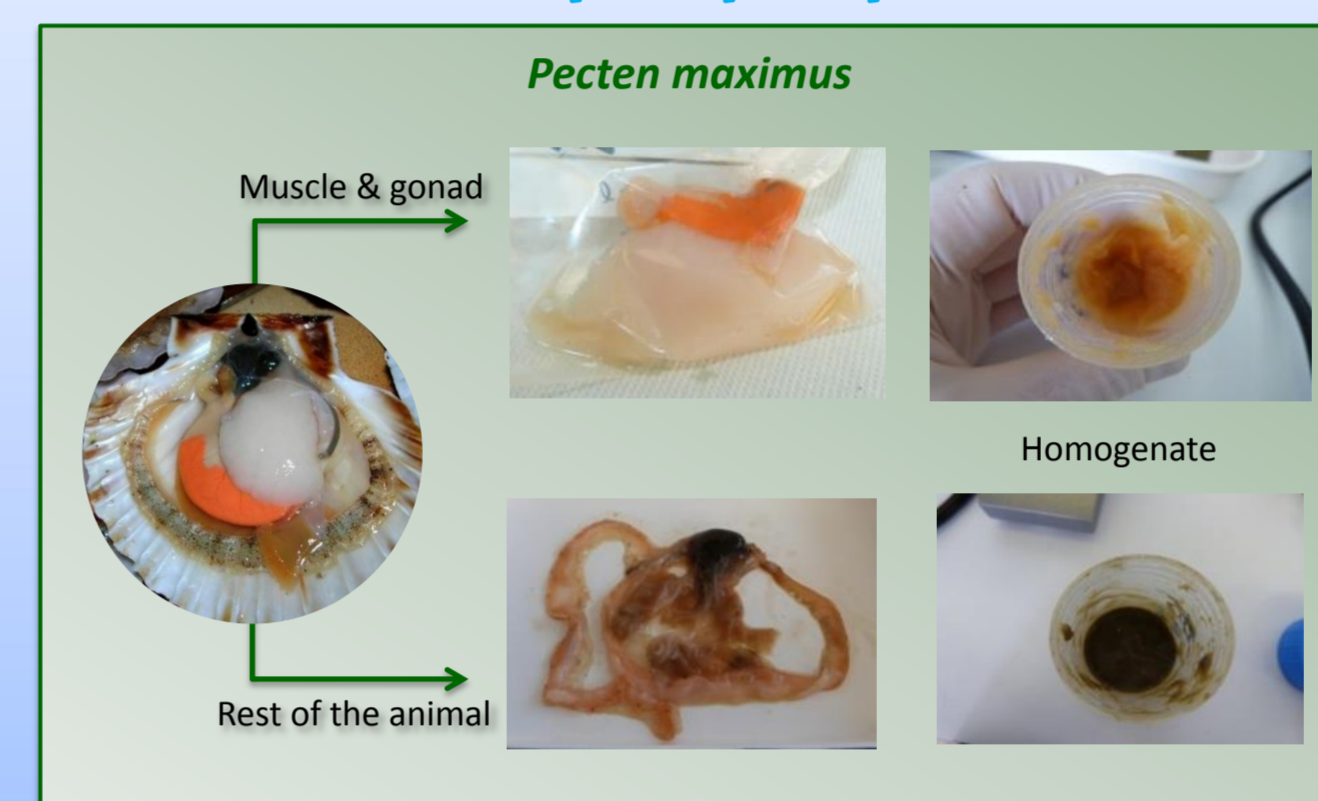
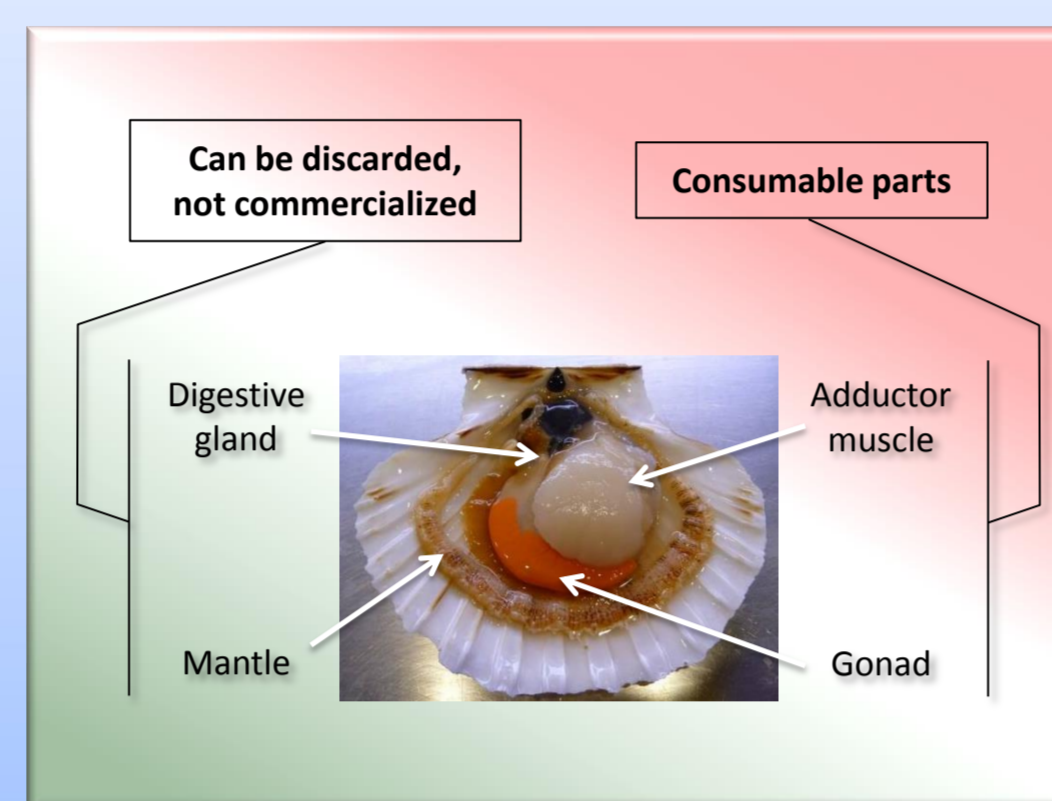
One hundred and thirty-nine individuals of *Pecten maximus*, harvested in 3 contaminated areas of the Baie-de-Seine, and one hundred and twenty individuals of *Aequipecten opercularis*, from two different lots, were analyzed for regulated lipophilic toxins (Regulation (EC) No. 853/2004) by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Analyses involved two different parts of each individual, namely "roe-on" (adductor muscle and gonad)/"roe-off" (adductor muscle only) and "rest of the animal".

The aim was to determine intra-batch inter-individuals variability on the one hand and the distribution of toxins between the two parts of the scallops on the other. Results were evaluated statistically in order to assess the efficiency of sanitary shucking of scallops contaminated with okadaic acid and explore the possibility of placing on the market the 'muscle and gonad' only or the muscle only from *Pecten* and *Aequipecten* initially contaminated by DSP toxins.

Harvest areas



Sample preparation

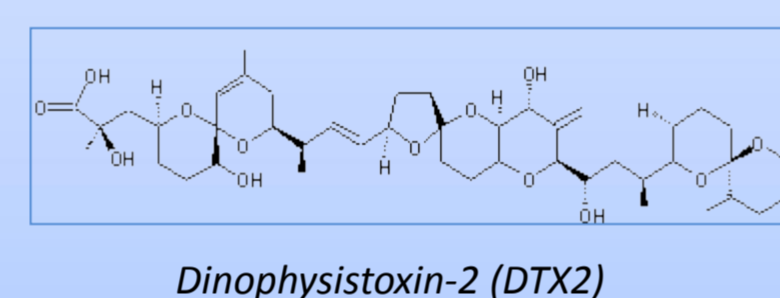
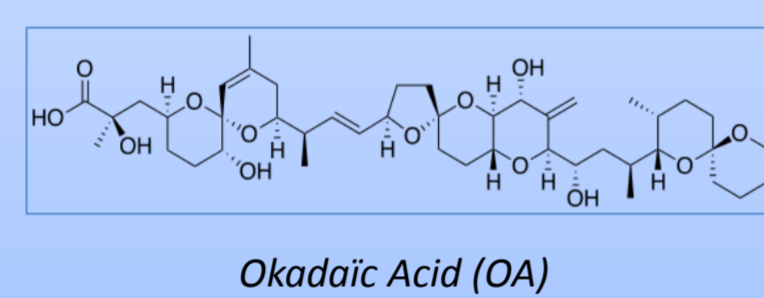


Method : EURL-SOP 'Lipophilic toxins by LC-MS/MS' - Intra-Laboratory Validation



LC Instrument: Agilent 1200 series
MS Instrument: AB Sciex API 4000

- Column: RP C8
- LC gradient elution, acidic conditions
- Ionisation: Electrospray (negative)



Toxin	Analytical limits		Performance characteristics				Expanded measurement uncertainty, U (k=2, %)	
	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%)	Repeatability CV (%)		Intermediate precision CV (%)		
Free OA	3,9	10	90	7,9 (10-20 µg/kg)	3,8 (80-240 µg/kg)	17,8		38
Total OA	3,9	10	101	10,6 (10-240 µg/kg)		24,7 (10-20 µg/kg)	15,2 (80-240 µg/kg)	53 (10-20 µg/kg) 31 (80-240 µg/kg)
Free DTX2	2,4	10	102	10,0 (10-80 µg/kg)	6,0 (160-240 µg/kg)	13,1 (10-80 µg/kg)	7,9 (160-240 µg/kg)	28 (10-20 µg/kg) 21 (80-240 µg/kg)
Total DTX2	2,4	10	109	9,5 (10-240 µg/kg)		10,6 (10-240 µg/kg)		21 (10-240 µg/kg)

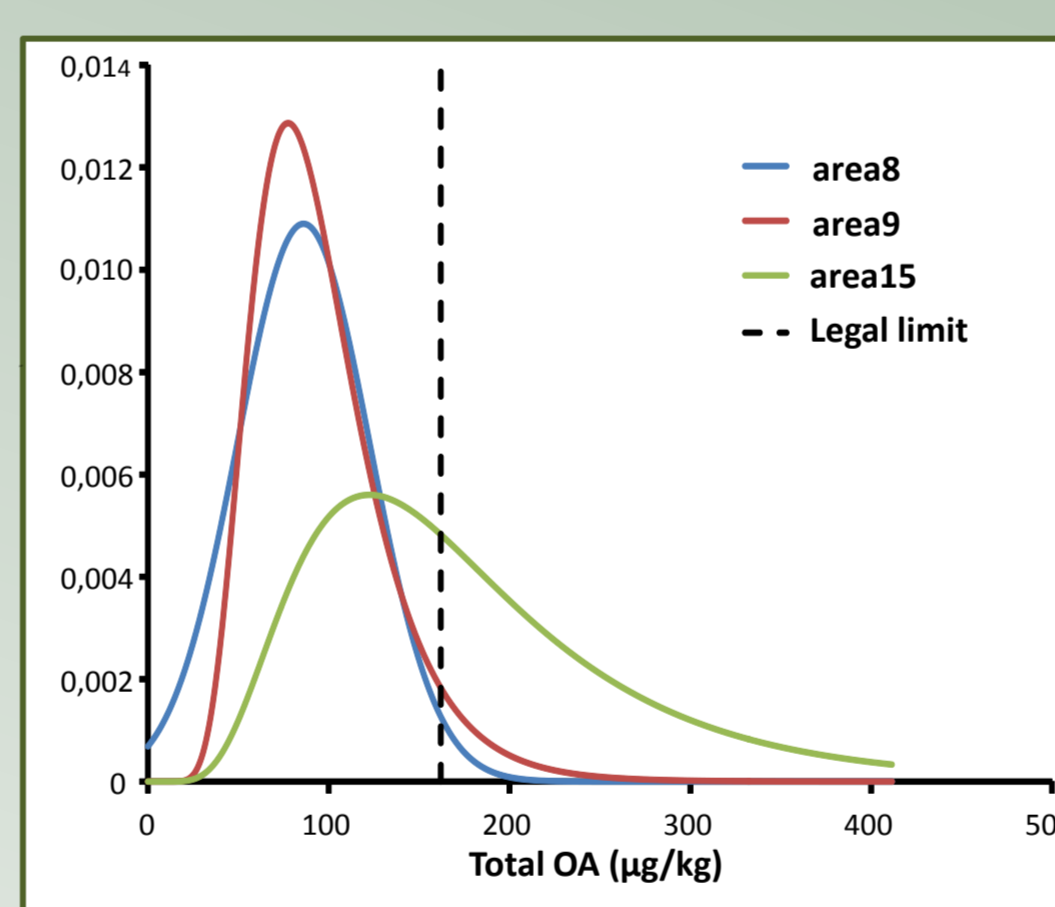
Range of validity: 10 to 240 µg/kg

Distribution of the lipophilic toxins

Average content of the Okadaic Acid group toxins (AO) in *Pecten* (µg/kg) [Min - Max]

	Digestive gland & mantle	Adductor muscle & gonad	Whole body (by calculation)
Harvest area 8	170 [29 - 335]	≤LD	86 [15 - 165] CV : 41 %
Harvest area 9	202 [101 - 439]	≤LD	97 [48 - 206] CV : 36 %
Harvest area 15	372 [74 - 894]	≤LD	180 [37 - 404] CV : 51 %

Distribution of total OA in the 3 harvest areas



Total OA content in 'muscle & gonad' and probability of exceeding limit values

Harvest area	Area 15	Area 9
Median	1.14 µg/kg IC 95% [0.6 - 1.77]	0.89 µg/kg IC 95% [0.86 - 1.06]
Probability > 10 µg/kg in muscle & gonad	0.02 IC 95% [0.004 - 0.05]	0 IC 95% [0 - 8.10 ⁻¹⁶]
Probability > 160 µg/kg in muscle and gonad	2.1 ⁻⁶ IC 95% [1.10 ⁻⁹ - 8.10 ⁻⁵]	0 IC 95% [cannot be estimated] *

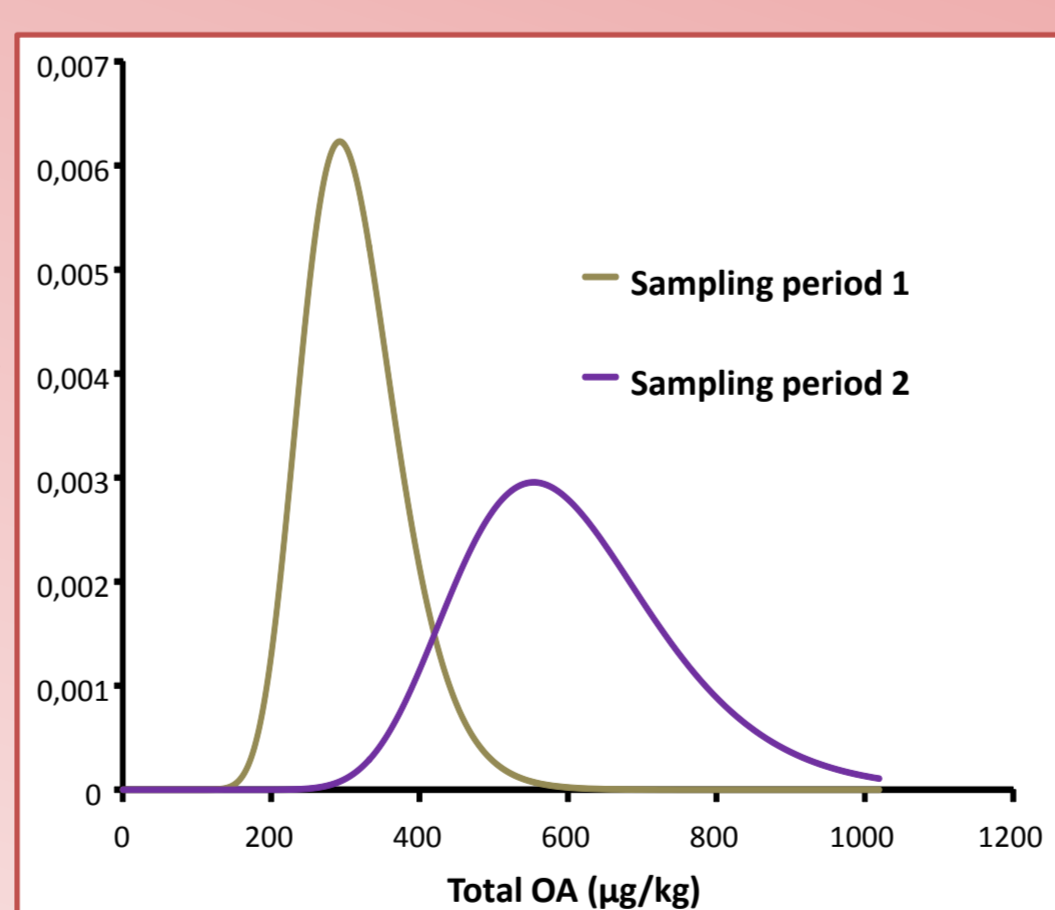
[IC: Confidence Interval]

* Results being extremely low, no quantified assessment is possible

Average content of the Okadaic Acid group toxins (AO+DTX2) in *Aequipecten* (µg/kg) [Min - Max]

	Digestive gland, gonad & mantle	Adductor muscle	Whole body (by calculation)
Sampling period 1	494 [319 - 729]	≤LD	313 [187 - 502] CV : 21 %
Sampling period 2	983 [562 - 1550]	≤LD	597 [357 - 938] CV : 24 %

Distribution of total OA in the 2 sampling periods



Total OA content in 'muscle' and probability of exceeding limit values

Sampling periods	Sampling period 2	Sampling period 1
Median	5.9 µg/kg IC 95% [3.7 - 6.2]	Cannot be estimated *
Probability > 10 µg/kg in muscle	5.10 ⁻¹³ IC 95% [0 - 1.10 ⁻¹⁰]	Cannot be estimated *
Probability > 160 µg/kg in muscle	<10 ⁻¹⁵ IC 95% [cannot be estimated] *	Cannot be estimated *

[IC: Confidence Interval]

* Results being extremely low, no quantified assessment is possible

Conclusions

- In this study, only okadaic acid was measured in contaminated *Pecten maximus*, irrespective of the harvest area.
- In *Aequipecten opercularis*, okadaic acid and DTX2 were involved in the contamination.
- In both cases, muscle or 'muscle & gonad' of contaminated scallops are toxin-free; OA group toxins are concentrated in the rest of the body (mainly in the digestive gland and to a lesser extent in the mantle).
- Modelling implemented shows that for the contamination levels studied probability to exceed a value arbitrary fixed to the LOQ of the method is extremely low and probability to exceed the legal limit of 160 µg/kg is infinitesimally small thereby proving the efficiency of sanitary shucking of scallops contaminated by the lipophilic toxins.