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Identification of *Pseudo-nitzschia australis* and *P. multiseries* in the Bay of Seine. Was there a relation to presence of domoic acid in king scallops in autumn 2004?

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In late 2004, the French phytoplankton monitoring network (REPHY of Ifremer) detected domoic acid (DA) above the EU-regulatory limit of 20 µg DA/g tissue in king scallops (*Pecten maximus*) from the Bay of Seine in the Channel (Fig. 1). Shellfish harvesting sites were closed for several months due to slow depuration of the scallops.

Accumulation of DA in French shellfish was previously correlated (as in June 1999) with blooms of the pennate diatom *Pseudo-nitzschia*, dominated most often by species of the « *pseudodelicatissima/cuspidata* complex » [1] or exceptionally by species of the « *seriata* complex ». Scanning electron microscopy (SEM) analyses were performed and *P. pseudodelicatissima* and *P. multiseries* were identified respectively [2]. More recently, the toxic species *P. calliantha* (which belongs to the « *pseudodelicatissima/cuspidata* complex ») was shown to be also present in French coastal waters (Billard, unpublished data).

At the end of October 2004, just before the opening of the harvesting period, and when DA was detected in king scallops from the Bay of Seine, field samples were examined to look for the diatoms suspected to be responsible for this contamination. A first investigation by light microscopy (LM) of bottom seawater did not detect the DA-producing benthic diatoms *Amphora coffaeformis* or *Nitzschia navis-varingica*. On the other hand, cells and empty frustules of at least four species of *Pseudo-nitzschia* were present in low numbers. The most abundant featured narrow cells with sigmoid ends (Fig. 2) as in *P. multistriata* or wide symmetrical valves as in *P. fraudulenta*, and the least abundant showed wide asymmetrical (type A) or narrowly fusiform valves with coarse fibulae and interstriae (type B). The usually toxic « *pseudodelicatissima/cuspidata* complex » was absent.

During November 2004 a specific study was undertaken in several Bay of Seine sites and samples examined included : contents of scallop gastrointestinal tracts, sediment, bottom seawater and surface plankton (20 µm mesh size nets). Thorough LM analyses showed that only rare frustule fragments were present in scallops whereas rare sigmoid cells and a few empty frustules of the four species of *Pseudo-nitzschia* were recorded in the sediment samples ; in bottom waters and surface nets, neither of the two morphotypes A and B was recorded. Three Bay sites sampled on November 29-30th for isolation of living cells and EM identification showed scarcity of *Pseudo-nitzschia*: two types only were recognized by LM, thin sigmoid cells and larger cells with symmetrical valves. The latter species, more abundant at the most coastal of the three sites, was isolated into unialgal culture and unambiguously identified by SEM as *P. fraudulenta* (Fig. 3).

Since *P. fraudulenta* is generally considered non-toxic and other putative toxic *Pseudo-nitzschia* species were scarce in autumn of 2004, and in order to understand what had been responsible for the DA contamination, phytoplankton samples collected routinely in the Bay of Seine by the REPHY network during the previous summer were examined. A moderate *Pseudo-nitzschia* bloom was detected from mid-July to September, with a peak on September 14th, 2004 at 56,000 cells l^{-1}.

Light microscopy observations showed that the *Pseudo-nitzschia* summer assemblages included only three morphotypes : *P*. cf. *fraudulenta*, but also types A and B. Either type A or B was dominant during the period of mid-July to September as shown by estimation of cell densities of individual types. Type A was not only characterized by asymmetrical valves but also by 7.5-8 µm wide cells, with important overlapping ends (25-28% of cell length) as in *P. australis* or *P. seriata* ; type B with narrowly fusiform (4.5-6 µm wide) and coarsely silicifed valves showed overlap of cell ends about 27-30% of length, as in *P. pungens* or *P. multiseries*.

Species identification by transmission electron microscopy (TEM) was carried out on acid-cleaned frustules following the method of Lundholm & Moestrup [3]. Examination of a subsample of the September 14th 2004 surface water collection allowed to formally identify three species of *Pseudo-nitzschia* : *P. pungens*, *P. multiseries* and *P. australis* (Fig. 4). The first two, which are roughly similar in shape, are easily distinguished by their ornamentation, *P. pungens* with 2 rows of poroids between thick interstriae and *P. multiseries* with striae showing 3-4 rows of tightly packed poroids. *P. australis*, with large asymmetrical valves, features thin interstriae (18 interstriae in 10 µm and approximately the same number of fibulae) and 2 rows of poroids per valve stria (4-5 pores in 1µm). Following EM analyses, type A cells may therefore be assimilated to *P. australis*, while type B cells were either *P. pungens* or *P. multiseries*.

Individual chains of type A cells were isolated from a Lugol-fixed water sample collected in mid-August 2004 and processed for molecular analysis. The ITS1, 5.8S and ITS2 region and part of the 28S of the nuclear ribosomal DNA were sequenced following PCR amplifications. The resulting sequences were compared with known sequences of *Pseudo-nitzschia* species from other geographic regions available in databases (Fig. 5) and shown to be identical (100% identity) with two sequences of Scottish strains of *P. australis* [4] and with other strains of *P. australis* from U.S.A. [5, 6], New Zealand [U92260, Haywood, A.J., *et al.*, direct submission] and Denmark [AF417651, Lundholm, N. *et al.*, direct submission]. Results of the molecular analyses therefore confirm once more that type A cells were of *P. australis* and, furthermore that this species was present in mid-August in the Bay of Seine.

Pseudo-nitzschia multiseries had never been recorded before in the Bay of Seine, while this is the first report for the occurrence of *P. australis* in France. Both these species, known to be highly toxic, co-occurred during the summer bloom of 2004 in the Bay. They are suspected to be the main source of DA in the king scallops, while *P. fraudulenta* and the *P. multistriata*-like cells could be potential secondary sources of DA.

Acknowlegments

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References

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Figures

Fig. 1. Map with localization of the Bay of Seine.

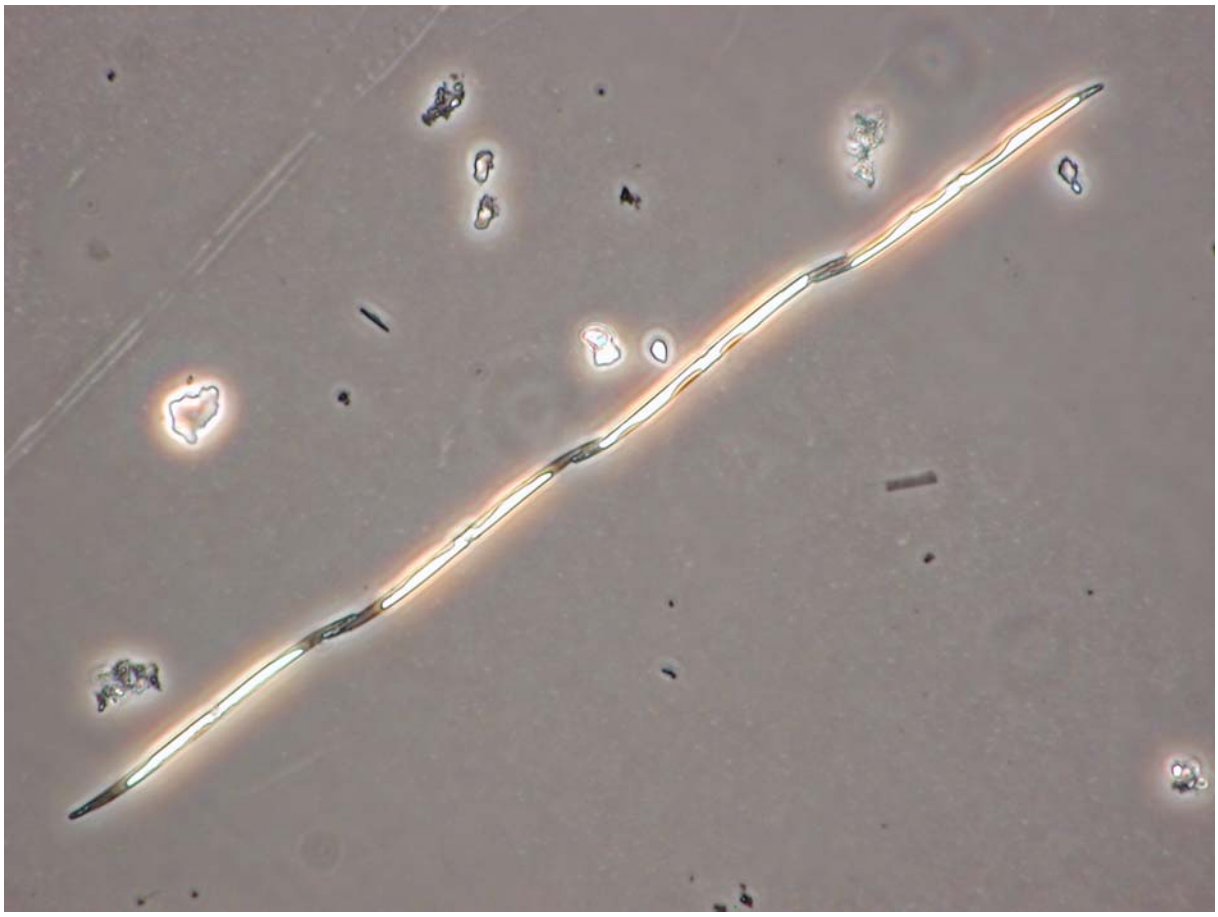

Fig. 2 . Light micrograph of a chain of Pseudo-nitzschia cf. multistriata.

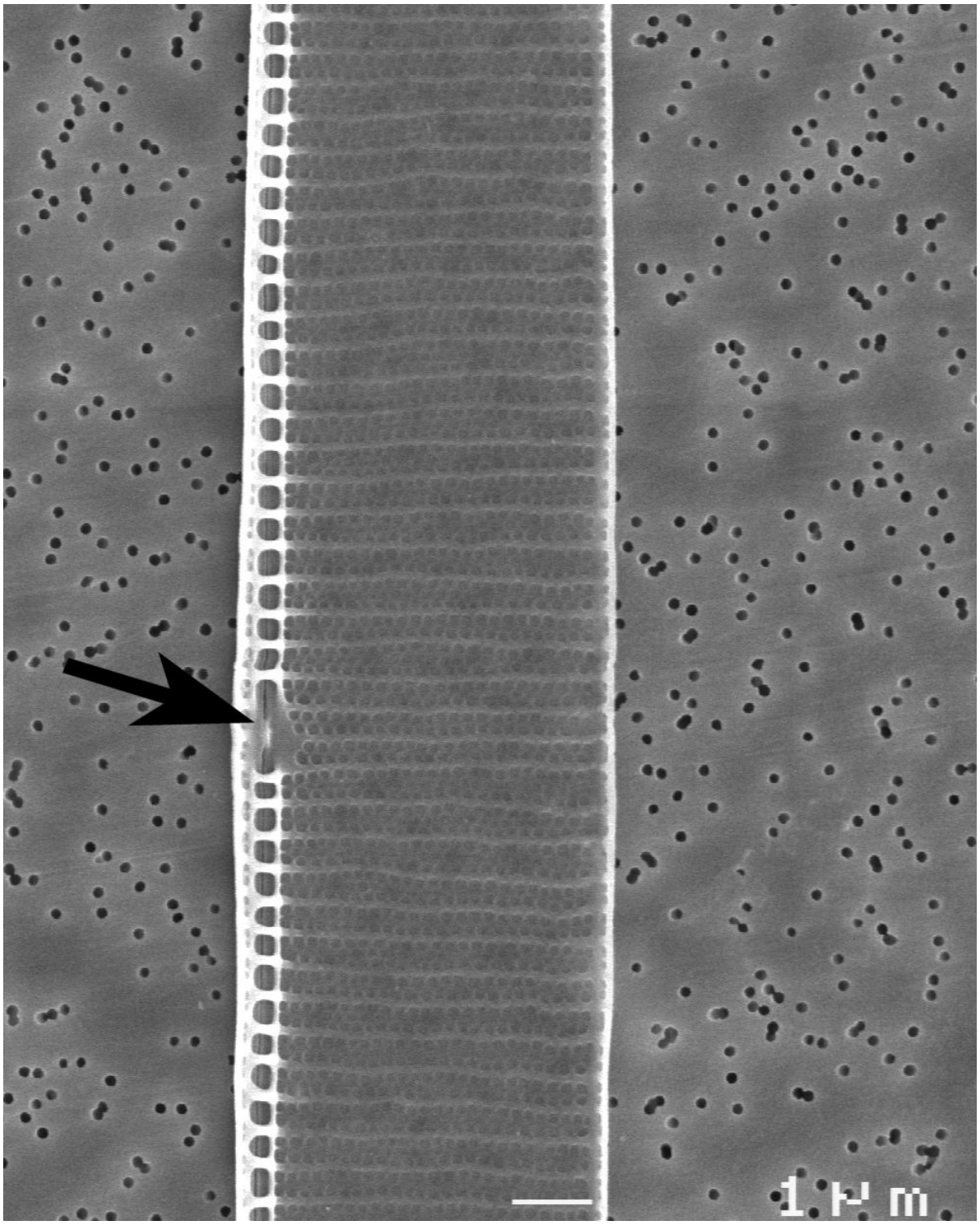

Fig. 3. Pseudo-nitzschia fraudulenta, SEM micrograph of valve showing central interspace, fibulae, interstriae and the typical poroid arrangement.

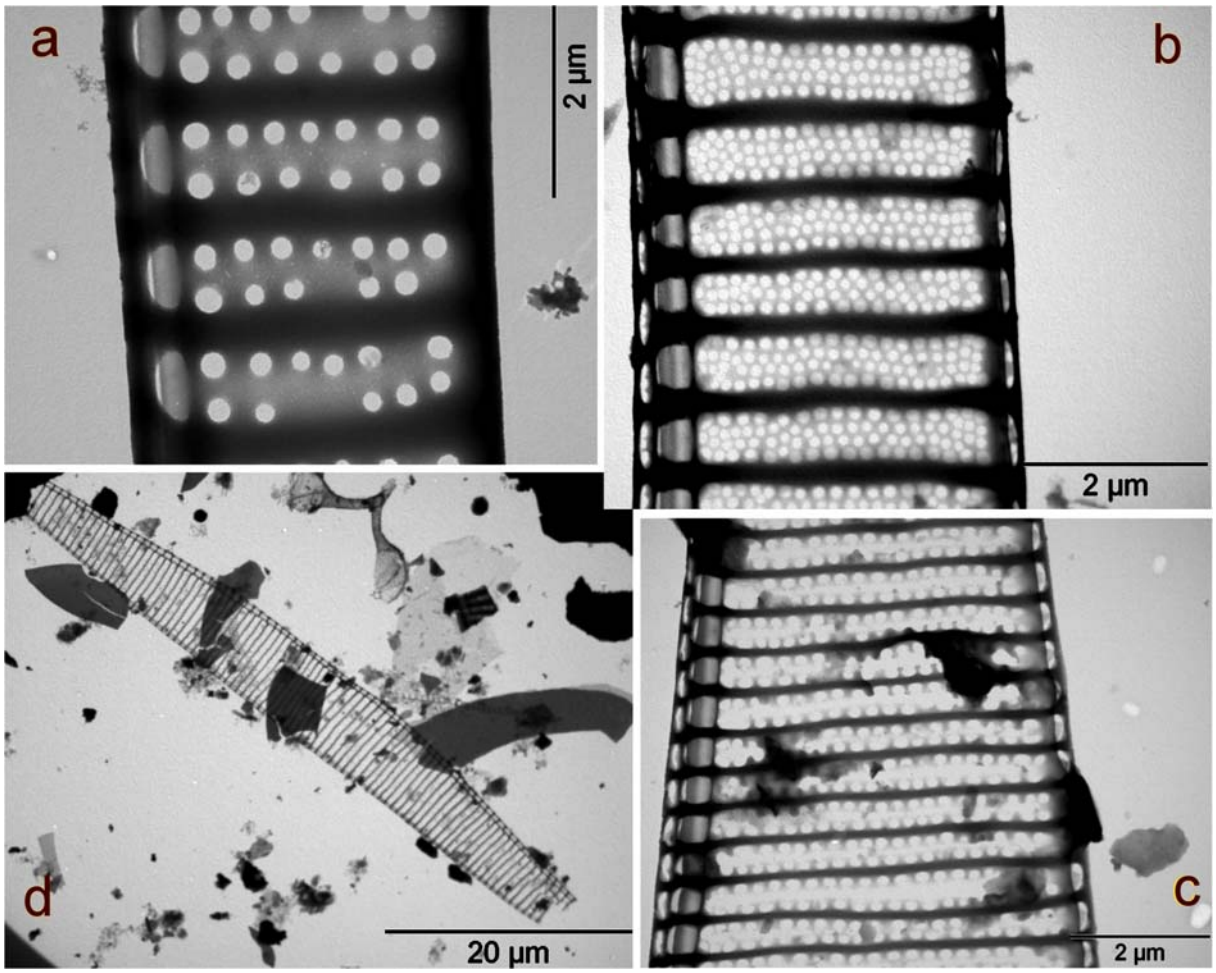

Fig. 4. TEM micrographs of valve views of Pseudo-nitzschia spp. with arrangement of poroids. a) P. pungens. b) P. multiseries. c-d) P. australis, with d) showing asymmetrical outline of the valve.

Fig. 5. Pair-wise distance calculations using MEGA 3.1 (Neighbor joining, Kimura 2-parameters), accession numbers of the aligned sequences, and one of the two similar obtained matrices.

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]
[1]								
[2]	0.000							
[3]	0.000	0.000						
[4]	0.000	0.000	0.000					
[5]	0.000	0.000	0.000	0.000				
[6]	0.000	0.000	0.000	0.000	0.000			
[7]	0.000	0.000	0.000	0.000	0.000	0.000		
[8]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

[1] P. australis Bay of Seine-28S partial, [2] AY452530-P.australis-28Spartial, [3] AY452529-P.australis-28Spartial, [4] AF440768-P.australis-28Spartial, [5] AF417651-P.australis-28Spartial, [6] U92260-P.australis-28Spartial, [7] U41393-P.australis-D1-D3, [8] U40850-P.australis-D1-D3.
[1] P. australis Bay of Seine-ITS1-2, [2] AY452528-P.australis-ITS1-2, [3] AY452527-P.australisITS1-2, [4] AY559850-P.cf.australis-18Spart-28Spart, [5] AY257842-P.australis-18Spart-28Spart