Paralytic Shellfish Poisoning on French Mediterranean coast in the autumn of 1998: *Alexandrium tamarense* as a causative agent

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INTRODUCTION

In France, almost yearly since 1988, intense summer blooms of *Alexandrium minutum* are responsible for PSP toxin accumulation in bivalves along North-Brittany coasts N.E.Atlantic.

For the first time, during the autumn of 1998, a bloom of another species of the genus *Alexandrium* was involved in shellfish toxicity in Thau lagoon Mediterranean coast. Plankton samples indicated that cells of the genus *Alexandrium*, up to 85000 cellsL^{-1}, were almost exclusively present.

In fact, positive toxicity tests were encountered with a toxicity up to 852 µg STXeq. 100g^{-1} of mussel meat, according to AOAC method. Closure of this shellfish breeding area had an economic impact, since it provides around 10% of French shellfish industry (Oysters: 12000T, Mussels: 8000T).

Spatio-temporal distribution of toxicity was monitored for three different shellfish species: mussel, oyster and carpet-shell.

High toxicity levels encountered in mussels were observed with relatively low levels of cells in seawater, contrary to observations with *A. minutum* blooms.

Main objectives of this study were:

To identify the species of the genus *Alexandrium* involved,

To determine toxic profiles for plankton and shellfish samples,

To confirm that identified species was the causative agent for observed shellfish toxicity.

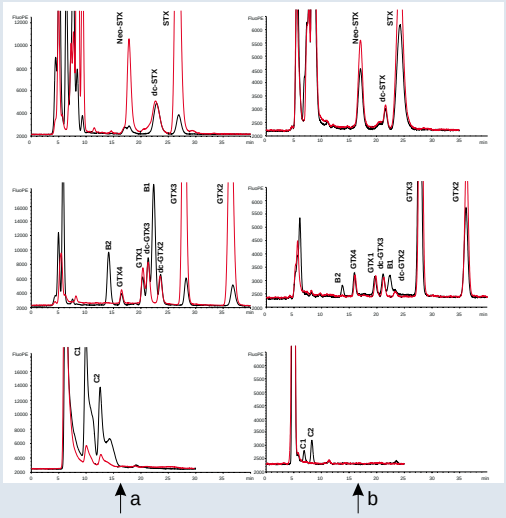


Fig. 7 : LC/FD toxic profiles of a mussel extract (7a) and plankton extract (eq. 1300 cells injected) (7b), collected in Bouzigues site, before (▬) and after (▬) acid hydrolysis. Three different isocratic elutions according to Oshima method.

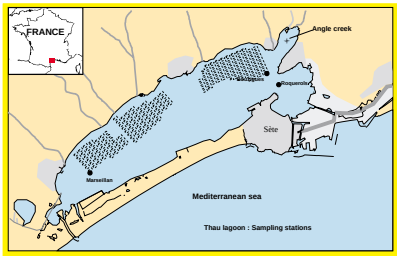


Fig. 1: Thau lagoon map indicating sampling stations.

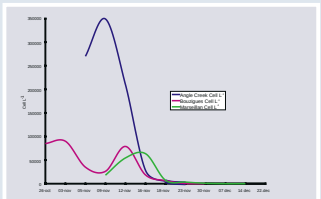


Fig. 2 : *A. tamarense* cells concentration observed in 3 sampling stations.

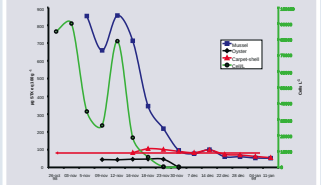


Fig. 6 : *A. tamarense* cells concentration and shellfish toxicity for 3 species according to AOAC mouse test at Bouzigues station.

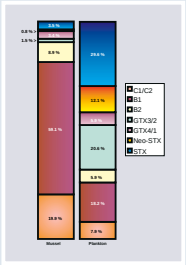


Fig. 8 : Mole percentage of toxin composition of a mussel and plankton extract collected at Bouzigues station.

Material and Methods

Shellfish samples

3 shellfish species were sampled : mussel (*Mytilus edulis galloprovincialis*), Japanese oyster (*Crassostrea gigas*) and carpet-shell (*Ruditapes decussatus*).

Plankton samples

Plankton samples, collected in subsurface water, were examined under inverted microscope using Utermöhl method. Concurrently, large volume of sea-water were collected and filtered on 10µ mesh net. This fraction was stored at 4°C in 0.1N Acetic acid until LC/FD analysis.

Mouse bioassay for PSP toxins

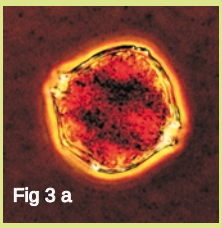
Sample preparation consisted of a boiling acid extraction (0.1 N HCl, 100 ml) of 100 g of whole shellfish tissue and the subsequent injection of 0.5 g tissue equivalents into each 20 g mouse (AOAC International, 1990).

Liquid chromatography-fluorescence detection (LC-FD)

Analysis of the main PSP toxins was performed using the post-column oxidative fluorescence method of Oshima et al. (1995) with slight modifications. Toxins were separated by reversed phase chromatography using a C8-silica column (5 µm Develosil, 4.6 mm i.d. x 250 mm) with a flow rate of 0.8 ml/min.

Toxins were quantified in duplicate with certified toxin standards (PSP-1B) provided by the NRC (Institute for Marine Biosciences, Halifax, Canada).

Two Gonyautoxins (B1, B2) and C-toxins were detected and quantified using acid hydrolysis (HCl 0.4N, 97°C, 5 min.) as indicated by Franco & Fernandez 1993.



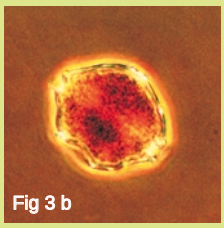


Fig 3 a,b: *A. tamarense* cells of different shape.

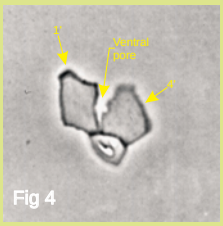


Fig 4 : Ventral pore indenting more apical plate (4') than plate (1')

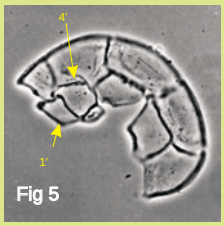


Fig 5: Thecal without visible ventral pore on suture between apical plates 1' and 4'.

Phytoplankton microscopic analysis

Studies to identify phytoplankton species and the toxin profile were performed mainly on samples obtained between November 3 and 12 (mid-bloom). Microscopy studies showed the presence of cells likely to form short chains (2 to 4 cells). The theca of these cells (isolated or in chains) consisted of a collection of plates whose tabular formula corresponded to that of the genus *Alexandrium*.

The shape of the cells varied from rounded to slightly flattened anteroposteriorly (Figs. 3a,b). Their size was heterogeneous (25 to 42 µm in length and width), although the length was often between 30 and 32 µm and the width between 30 and 35 µm.

On the basis of around 30 thecae (both rounded and flattened ones), the details of the tabulation (notably of the pores) were examined in light microscopy. This showed that the plate of the apical pore generally had an ellipsoidal anterior fixation pore (AFP) located between the right edge of the plate and the apical pore, and that the posterior sulcal plate (PSP) had a posterior attachment pore of variable size located in its right half. The ventral pore (VP), independently of thecal shape, was visible on only some of the specimens, being located on the groove between apical plates 1' and 4' and slightly indenting plate 1' (Fig. 4).

The presence of a VP on some thecae allowed us to identify these specimens as *A. tamarense*. However, for some cases it was unclear whether one species or a complex of two toxic species was involved. In fact, thecae without a VP (Fig. 5) might correspond to a variety of *A. tamarense* existing in France, a variety of *A. minutum* without a VP, or *A. catenella* considered as an entirely distinct species.

Discussion

Phytoplankton monitoring

Thau lagoon is a large Mediterranean lagoon, 20 km long and 5 km wide. Its mean depth is 4.5m with a maximum of 10m. Two channels link it to the sea, 80% of exchanges are done by the Sète channel (North of lagoon).

Figure 2 shows *A. tamarense* cells density in 3 sites which, during the toxic event, were sampled for plankton counting.

Angle creek, a small closed embayment at North, produced highest cells numbers (Maximum: 350 000 cellsL^{-1}). Nearby **Bouzigues** with countings fluctuated between 2 and 9.10^4 cellsL^{-1} for almost 10 days ; at the South **Marseillan** presented a time-shifted bloom with lower density. Wind-induced water-mass circulation in the lagoon and exchanges with the sea could explain such heterogeneous distribution between these 3 sites. After November 18th, cells number decreased in conjunction with temperature drop.

Monitoring shellfish toxicity

Bouzigues site is an interesting example of different shellfish contamination patterns, toxicities being evaluated using AOAC mouse tests (Fig. 6) Mussels exhibit highest contamination, closely related to *A. tamarense* cells fluctuation. So high that regulatory level (80 µg STXeq. 100g^{-1} whole meat) is met again, for this specie, one month after cells disappearance. Oysters, reared in the same point, always remain during the bloom far below this Sanitary Threshold . Whereas Carpet-shell toxicity is slightly above it, but this species have a long decontamination period. A slow-detoxifier behaviour and/or some toxin biotransformation, toxin transformed into more toxic analogues, may explain such pattern, as indicated by Bricelj & Shumway 1998.

Toxin content

A complex toxin profile was observed when plankton and mussel samples, collected at Bouzigues site, were analysed (Fig.7a,7b).Acid hydrolysis allowed complete identification. Thus, the increase of STX and Neo-STX revealed the presence of B1 and B2 gonyautoxins respectively. The increase of GTX-3 and GTX-2 indicated the presence of C1 and C2 toxins respectively. Conversely, the lack of increase of GTX-1 and GTX-4 gonyautoxins confirmed the absence of C3 and C4 toxins.

As the toxin composition in the mussel sample was the same as that in the phytoplankton extract, which pointed out that *A. tamarense* was the causative agent.

Plankton toxicity had a mean value, estimated at 26 pg STXeq. cells^{-1}. A major proportion of Beta isomers(C2, GTX-4, GTX-3) was observed in plankton, whereas Alpha isomers were dominant in the mussel sample. This indicates that contamination was recent since the epimerisation of the toxins was not complete.

The molar fraction of the toxins, in both samples, is given in Figure 8. Since sample extraction was different, a direct comparison between the two molar percent is not conclusive. This has already been established in literature. In plankton, highly toxicogenic carbamate toxins (STX, NeoSTX, GTX 1-4) represented up to 60% of total toxins, whereas N-sulfocarbamoyl toxin (B1, C1, C2) are predominant (80%) in mussel extract.

Conclusion

The overall results obtained allowed us to confirm the involvement of *A. tamarense* in this new episode of **autumn** shellfish toxicity on the Mediterranean coast. Its complex and highly toxicogenic toxin profile can now explain high toxicity encountered despite low cell density.

As this bloom reappeared in October 1999, it will probably recur according to an annual cycle. The culturing of isolated toxic species during these episodes will allow more thorough identification studies with molecular biology tools to determine the origin of the strain. Conditions for toxin production will be also evaluated.

The mastery of mass culture of the toxic strain will facilitate future investigations of the contamination-decontamination process through performance of complementary laboratory experiments.

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