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Paralytic shellfish poison outbreaks in the Penzé estuary: Environmental factors affecting toxin uptake in the oyster, *Crassostrea gigas*

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Abstract – Several experiments using a self-regulated system were conducted to define the factors likely to influence the uptake of paralytic shellfish poison (PSP) by oysters in the Penzé estuary (France, Brittany). Each 4-day experiment was carried out in a recirculated sea water system using 15 Pacific oysters (*Crassostrea gigas*) separated from each other and supplied with unfiltered natural seawater containing alternatively toxic (*Alexandrium minutum*) or non-toxic (*Skeletonema costatum*) algal diets. The food supply and exposure times to toxic diets were determined according to field studies of the upstream and downstream movement of patches containing *A. minutum*. The experimental parameters corresponded roughly to the hydrological conditions generally observed in June when tidal coefficients are lowest and blooms occur: (i) *A. minutum* concentrations in sea water of 200, 5000 and 10 000 cell ml^{-1}; (ii) inorganic matter consisting of 5 and 15 mg L^{-1} of calcinated muddy sediments; and (iii) low and high tide salinities of 25 and 35‰, respectively. Significant experimental contamination (greater than the 80 μg STX equiv. 100 g^{-1} sanitary threshold) occurred after 4 days of exposure for the monospecific *A. minutum* diet (20−200 cell ml^{-1}) and alternated *A. minutum* and *S. costatum* diets (5000 and 20 000 cell ml^{-1}, respectively). Contamination levels were less than the sanitary threshold for alternated *A. minutum*/*S. costatum* diets of 200 and 20 000 cell ml^{-1}, respectively, and for a monospecific *A. minutum* diet (1000−10 000 cell ml^{-1}). In the last case, the accumulation rate was quite low, possibly because of inhibition of the filtration rate related to a lower biodeposit production rate and decreased feeding time activity. The addition of inorganic matter appeared to play a significant role in the observed increase of toxin uptake, whereas salinity was not a determining factor for toxin accumulation rates. These last observations were corroborated by statistical analysis and stepwise multiple linear regressions integrating all or some of the experimental parameters.

Key words: PSP / Toxin / Dinophyceae / Oyster toxicity / Experimental contamination

Résumé – Contamination par des toxines paralysantes en estuaire de Penzé : facteurs environnementaux modifiant l'accumulation des toxines chez l'huître, *Crassostrea gigas*. Afin de définir les facteurs favorisant la bioaccumulation de toxines paralysantes (PSP) par l'huître creuse, en estuaire de Penzé (Bretagne nord) des expérimentations ont été réalisées à partir d'un système automatisé. Chaque expérience de 4 jours, sur 15 huîtres, était effectuée en circuit fermé et en eau de mer naturelle. Les huîtres étaient soumises individuellement à une alternance de régimes alimentaires toxiques (*Alexandrium minutum*) et non toxiques (*Skeletonema costatum*) selon une fréquence et des temps d'exposition simulant les effets de la marée sur le déplacement des lentilles d'eau colorée à *A. minutum*. Les paramètres testés ont été successivement : la concentration cellulaire en *A. minutum* (200, 5000 et 10 000 cell ml^{-1}), les matières minérales (5 et 15 mg L^{-1} de vase ne comprenant plus que les particules inorganiques) et les salinités à basse mer et pleine mer (25 et 35 ‰), soit des conditions correspondant aux épisodes d'eau colorées généralement observées sur site en juin, aux plus faibles coefficients. Les résultats des essais font apparaître une contamination des huîtres, en 4 jours, supérieure au seuil sanitaire (80 μg STX equiv. 100 g^{-1} de chair) pour un régime constitué uniquement d'*A. minutum* à 20−200 cell ml^{-1} et pour un régime mixte constitué d'une alternance : *A. minutum* à 5000 cell ml^{-1} et *S. costatum* à 20 000 cell ml^{-1}. Les bioaccumulations sont inférieures en 4 jours au seuil sanitaire pour une alternance *A. minutum* 200 cellules ml^{-1} et *S. costatum* 20 000 cell ml^{-1} et pour un régime constitué exclusivement d'*A. minutum* à 1000−10 000 cell ml^{-1}. Dans ce dernier cas, il semble que la très faible bioaccumulation soit le résultat d'une inhibition partielle de la consommation, attestée par une production moindre de biodépôts et une activité de filtration (FTA) plus faible. L'ajout de matière minérale aurait un effet positif sur les cinétiques de bioaccumulation tandis que la salinité n'aurait pas de rôle prépondérant dans l'accumulation des toxines. Ces dernières observations ont été vérifiées statistiquement en utilisant un modèle de régression linéaire multiple « pas à pas » intégrant l'un ou l'autre des paramètres testés.

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1 Introduction

Commercial bivalves along French coasts have been regularly contaminated by ingestion of microalgae that produce diarrhoeic phycotoxins (since 1983), paralytic phycotoxins (since 1988), and amnesic toxins (since 1999). As it is difficult to predict the occurrence of the toxic blooms responsible for contamination, one of the most efficient alternatives is to improve detoxification conditions for molluscs.

Experimental studies carried out between 1996 and 2000 have helped elucidate (i) the feeding response of oysters, the shellfish most physiologically affected by paralytic toxins (Bardouil et al. 1996; Lassus et al. 1996, 1999; Wildish et al. 1998), and (ii) the detoxification kinetics of several types of bivalves as a function of diet (Lassus et al. 1996, 2000; Bougrier et al. 2001).

Modelling trials a posteriori relative to the contamination of bivalves in coastal waters by paralytic shellfish poisoning (PSP) (Blanco 1997; Silvert and Cembella 1995; Moroño and Blanco 1997; Silvert et al. 1998; Moroño et al. 1998) have shown that the toxic episodes observed can be simulated precisely on the basis of a known concentration of algal cells and consideration of the environmental factors involved (temperature, salinity, total seston).

In France, two completely different areas are subject to toxic *Alexandrium* blooms: the North Brittany (English Channel) coasts by summer episodes of *A. minutum*, a slightly toxic species easily ingested by mussels and oysters (Morin et al. 2000); and the Thau Lagoon (Mediterranean Sea) by autumn episodes of *A. catenella*, a fairly toxic species detected recently that contaminates mussels particularly and oysters to a much lesser extent (Masselin et al. 2001). In the first area, shellfish contamination occurs in highly turbid estuarine zones influenced by tidal currents, whereas in the second area the dissemination of algae in different parts of the the lagoon is governed by more complex hydroecological processes.

Existing ecological and toxicological data for the North Brittany site (Masselin et al. 1996; Morin et al. 2000) allowed the general features of summer blooms of *A. minutum* to be defined in the Penzé estuary. Contamination episodes were simulated experimentally relative to the conditions observed, so that the factors likely to modify contamination dynamics (i.e. those in direct relation to bivalve filtration and ingestion activities) could be studied separately. *A. minutum* concentrations during blooms, the load of inorganic matter, and salinity were studied in particular. As data acquired by other teams concerned only daily counts, it was essential to perform hourly monitoring of phytoplankton populations during at least two tidal cycles in order to integrate the variations observed into the programme running the experimental circuit. Thus, the objectives of this study were successively: i) to determine the main features of exceptional blooms occurrences in the Penzé estuary (cell concentrations, Total Particulate Matter (TPM), time sequences), ii) to determine, from experimental approaches, the environmental parameters assumed to stimulate or hamper toxin accumulation process, iii) to set up from these data a simple model which could simulate as precisely as possible the oyster detoxification process.

2 Methods

2.1 In situ sampling

Samples were taken from the Penzé River in June 2001 and 2002 during blooms of *Heterocapsa triquetra* and *A. minutum*. Water samples were obtained every hour for 24 h at a fixed point "Pont de la Corde" in the outer estuary near the first oyster beds (Fig. 1). After fixation in acetic Lugol's solution, the samples were used to count the planktonic microflora (Utermöhl method). Real-time temperature and salinity measurements were performed using a WTW thermoconductance meter, and turbidity was measured in the laboratory on an HACH-2100A turbidimeter. Total fluorescence was estimated continuously with a Turner Design fluorometer.

2.2 Experimental system

The experimental system consisted of three stands on which six individual boxes were placed plus a "control" box containing an empty oyster shell. Water was circulated in a closed system using a pump drawing alternatively (according to an automation programme developed on LabView) seawater alone, or seawater containing algal cultures from *A. minutum* and *Skeletonema costatum* tanks, depending on the different cycles under study. The programme maintained a given cell concentration in the circuit, which was controlled continuously by mean fluorescence determinations in the feeding tank. The successive toxic ("peak" concentrations) and non-toxic algal cycles were regulated on a 2 h/4 h basis corresponding to the most extreme contamination conditions observed in the Penzé estuary (i.e. the most unfavourable conditions in terms of oyster toxicity). The programme also allowed continuous acquisition of the fluorescence signals recorded at the outlet of the three stands. Each box was connected to a measurement device providing fluorescence determinations during one minute. The measurements acquired were recorded on a computer in volts as a function of time.

In experiments to determine the impact of inorganic matter, estuarine mud was introduced (MMA = mineral matter added) into the system after preliminary burning at 450 °C to destroy organic matter. A micropump was used to inject the suspension into the feeding tank.

2.3 Biological material

Oysters (*Crassostrea gigas*) obtained from a producer in Morlaix Bay were raised on racks and controlled as being free of PSP contamination (no detectable levels of PSP toxins by HPLC analysis). After removal of epibionts, the animals (total weight: 51.3 ± 5.1 g) were acclimated for 5 to 6 days in natural seawater at 16.0 ± 0.5 °C before being transferred into experimental units. The steady state cultures of *A. minutum* (AM89BM strain) were developed in 10-L tanks in Guillard's F/2 medium, with a light intensity of 50 ± 4 μmol photons m^{-2} s^{-1} and a 12 h/12 h photoperiod. The mean toxicity, as quantified by ion pairing high performance liquid chromatography (IP-HPLC) (see below), of the

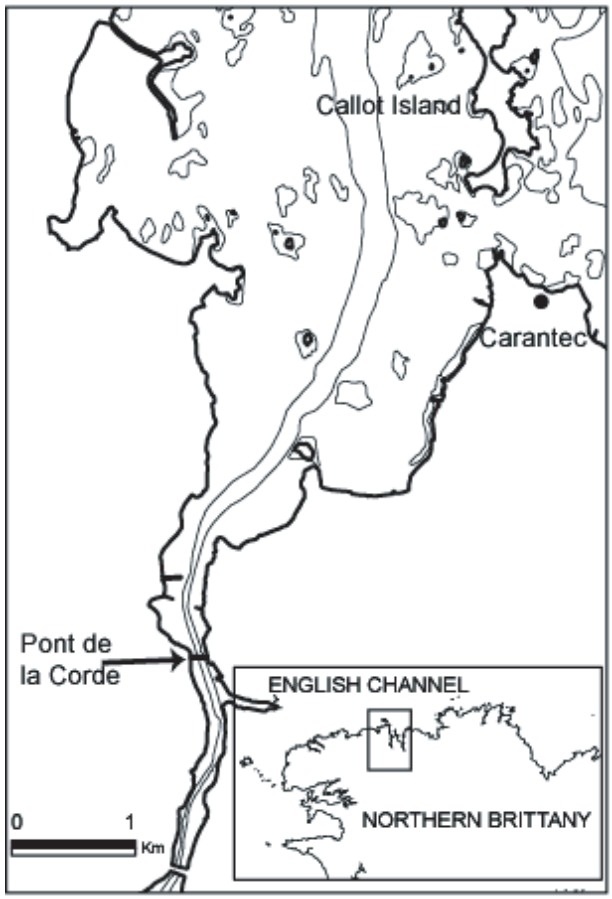

Fig. 1. Penzé river area and sampling station (Pont de la Corde) situation.

culture during experiments ranged from 1.4±0.2 to 1.6±0.3 pg saxitoxin (STX) equiv. per cell (at the end of the exponential growth phase). *A. minutum* typical toxin profile is made of gonyautoxin-2 (GTX2, 30%), gonyautoxin-3 (GTX3, 68%) and traces of C-toxins (<2%). *S. costatum* cultures were developed in 10-L tanks in the same growth conditions, but using Provasoli's enriched sea water medium.

2.4 Chemical analyses

Analyses of paralytic phycotoxins were performed daily by IP-HPLC on each individual during the contamination period according to the method of Oshima et al. (1995a). Total flesh was ground in CH_3COOH 0.1 N at 4 °C on a v/w basis. After centrifugation (3000 × g, 15 min, 4 °C), the pH of extracts was adjusted to 3.0–3.5 with glacial acetic acid. After demi-dilution, the supernatants were ultrafiltrated (20 000 Da, Sartorius Centrisart) and stored at 4 °C until analysis. For *A. minutum* cultures, 10 ml were pipeted at the end of the exponential phase. The cells were counted on hematimetric cells, and the samples were centrifuged (3000×g, 15 min, 4 °C). After removal of the supernatant, acetic acid 0.1 M was added to the pellet and the cells were broken up by a freezing/thawing method (Ravn et al. 1995).

Solutions of each compound of a PSP-1C certified reference material (Marine Analytical Chemistry Standards Programme, NRC-Halifax, Canada) were diluted 1:200 and used as external standards for quantitative detection. Given the dilution factors, the molar concentration was converted into μg STX equiv. 100 g^{-1} of bivalve flesh by using the conversion factors of Oshima (1995b), i.e. 297 μg STX.equiv. μM^{-1} for GTX3, and 168 for GTX2.

2.5 Physiological analyses

The biodeposit rate (BR), representing the amount of faeces and pseudofaeces produced per unit of time (in mg h^{-1} g^{-1} of dry weight) corresponded to the sum of the rejection rate (RR) and the egestion rate (ER), i.e. BR = RR + ER.

In practice, biodeposit production was used as a suitable indicator of feeding behavior only in "control" experiments involving 2 h of contact with *A. minutum* and 4 h of feeding in saltwater i.e.: a monospecific diet. However, during these experiments and those with mixed diets (*A. minutum*/*S.costatum* in alternation), continuous measurement of the fluorescence differential at the outlet of "study" boxes and "control" boxes enabled us to estimate feeding time activity (FTA), i.e. the percentage of time during which the shellfish consumes microalgae. FTA was only regarded as significant when the retention rate for feeding particles was at least 5% (Bougrier et al. 2001).

2.6 Statistical analysis

A statistical validation phase was necessary to define more precisely the roles of salinity, *A. minutum* cell concentration and the addition of mineral matter on the final toxicity of the oysters. Accordingly, experimental data were analysed using Statgraphics Plus software and stepwise multiple linear regressions.

The principle of this evaluation was to observe the response of oyster toxicity to different environmental factors and to estimate the slopes most descriptive of the bioaccumulation process. The final toxicities of each oyster were considered, and the experimental parameters, as well as the period of oyster presence in the system, were included in the model as independent variables.

3 Results

3.1 Observations in the Penzé estuary (24 h monitoring)

Hydroclimatic conditions at the time discoloured waters were observed in the Penzé estuary in June 2001 were similar to those described by Morin et al. (2000) for the development of *A. minutum* at this site, i.e. water temperature between 14.6 and 16.4 °C, salinities between 18.5 et 32.5‰, and an expanse of discoloured waters at low tide in the outer part of the estuary. These conditions were not encountered in June 2002.

The qualitative and quantitative study of phytoplankton samples, performed in 2001, indicated that the

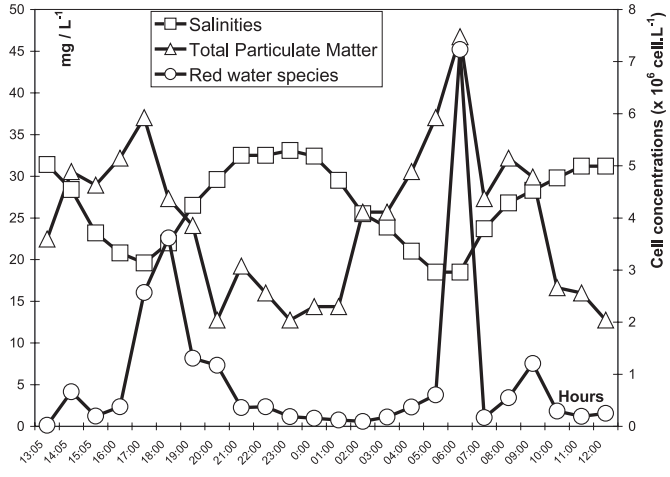

Fig. 2. 24 h monitoring of salinity, total particulate matter (TPM) concentrations and red water species cell concentrations (*Heterocapsa triquetra, Scrippsiella* sp., *Alexandrium minutum*) in the outer part of the Penzé river, in June 2001.

five most common species were: *Heterocapsa triquetra* (50 000 to 6 550 000 cell L^{-1}), *Nitzschia acicularis* (10 000 to 1 500 000 cell L^{-1}), *A. minutum* (8000 to 650 000 cell L^{-1}), *Chaetoceros* spp. (9000 to 225 000 cell L^{-1}) and *Scripsiella* sp. (6000 to 150 000 cell L^{-1}). Samples obtained from the discoloured waters were composed almost exclusively of *H. triquetra*, *A. minutum* and *Scripsiella* sp.

The development of species within discoloured waters in 2001 showed two concentration peaks (Fig. 2) of 3×10^6 and 7.2×10^6 cell L^{-1} situated at low tide +1 h, thus at the time of maximum Total Particulate Matter (TPM) values. The same general pattern was apparent in June 2002, but only involved *H. triquetra* and *Scripsiella* sp. for maximal values of 0.2×10^6 cell L^{-1}. Observations by another team on 13 June 2001 in discoloured waters further downstream showed a dominance of *A. minutum* [5 700 000 cell L^{-1} vs. 840 000 for *H. triquetra*]. These results seem to indicate some heterogeneity concerning specific dominance within the discoloured waters.

On the basis of these observations, it was decided (i) to maintain contact with the bloom at a fixed point for at least 2 h; (ii) to consider that two contacts per tidal cycle were likely at this point as a function of flood and ebb tides, even if the peak at ebb tide was not systematically observed; and (iii) to consider that changes in the total concentration of species over time, regardless of the relative proportions in discoloured waters, can be attributed to changes occurring in a bloom monospecific for *A. minutum*.

3.2 Simulation of different contamination cycles

The experimental conditions are summarised in Table 1. For practical reasons, all experiments were planned to last four days, i.e. a sufficient period for observation of contamination which was at least above the sanitary threshold (according to the results of feasibility tests).

3.3 Residual concentrations

Cell concentrations of *A. minutum* and *S. costatum* were estimated daily for each contact phase by means of a multisizer and an inverted microscope. Even though the estimated mean time required to rinse the circuit with seawater to reduce the *A. minutum* concentration in contact phase to one-tenth of its original value was around 12 min, it proved nearly impossible to reach a lower value because of various obstacles inherent to the structure of the circuit (tubing, shape of the boxes, bends, etc.) and especially because of the thin layer composed of cysts and slow-moving swimming cells deposited at the bottom of the boxes. This "biofilm" represented a continual potential source of cells for re-circulation in the closed system.

Thus, the mean concentrations of residual *A. minutum* during feeding phases with *S. costatum* ranged from 1 to 10% of the concentration in "peak" phase. If these observations are compared with the counts performed in situ during 24 h monitoring in the Penzé estuary, the mean concentrations preceeding the *A. minutum* peak at 650 000 cell L^{-1} were around 55 000 cell L^{-1}, i.e. about a tenth of this value. Thus, in a certain sense, it would seem that the residual concentration of *A. minutum* inherent to the limits of the experimental system reproduces rather well the conditions of the natural environment.

3.4 Biodeposit production

The production of biodeposit (essentially faeces, except at 10 000 cell ml^{-1} of *A. minutum* where some pseudo-faeces were observed) was recorded cumulatively every week for the monospecific diet, i.e. 2 h of contact with *A. minutum* at the nominal concentration and 4 h of rinsing of the circuit with seawater (presence of residual *A. minutum* representing less than 10% of the nominal value). The concentrations of 5000 and 10 000 cell ml^{-1} can be considered as corresponding to bloom conditions in the Penzé estuary. Over a 4-day period, biodeposit production was around 50 mg for 200 cell ml^{-1} and 60 to 100 mg for 5000 cell ml^{-1}. For 10 000 cell ml^{-1} two subpopulations of oysters reached productions respectively of 60 to 80 mg (inhibition of feeding activity) and 140 mg.

3.5 Feeding time activity (FTA)

Although large individual variations in FTA were observed (Fig. 3), three typical patterns were apparent (FTA individual data obtained from stands a, b, c oyster observations). Oyster-(a) had a cyclical feeding pattern relative to the type of algal diet consumed during the first three days, i.e.: quite low FTA values with *A. minutum* diet and up to 100% FTA with *S. costatum* diet. However, no distinction between diets could be detected on the 4th day. Oyster-(b) showed more varied behaviour, with a less marked decrease in FTA during *A. minutum* cycles. Oyster-(c) made little or no distinction between the two diets with FTA values close to 100%. However, these variations did not run counter to overall FTA, which was generally lower for "*Alexandrium"* cycles and higher for "*Skeletonema"* cycles, when all experimented oysters are considered together (Fig. 4).

Table 1. Concentrations in number of cell ml^{-1} for each diet tested in October/November 2001, April 2002 and November 2002 (MMA: mineral matter added). Alex = *A. minutum*, Skel = *S. costatum*, *S* = salinity. Excepted in November 2002, all experiments were performed at 35‰ salinity.

Dates	Diets/*MMA/S*	*A. minutum* (cell ml^{-1})	*S. costatum* (cell ml^{-1})
Oct. 2001	Alex	200	0
Oct. 2001	Alex/Skel	200	20 000
Nov. 2001	Alex/Skel	5000	20 000
Nov. 2001	Alex	10 000	0
Apr. 2002	Alex	5000	0
Apr. 2002	Alex/Skel	5000	20 000
Apr. 2002	Alex/Skel/5 mg L^{-1}	5000	20 000
Apr. 2002	Alex/Skel/15 mg L^{-1}	5000	20 000
Nov. 2002	Alex/Skel/25‰	5000	20 000
Nov. 2002	Alex/Skel/35‰	5000	20 000

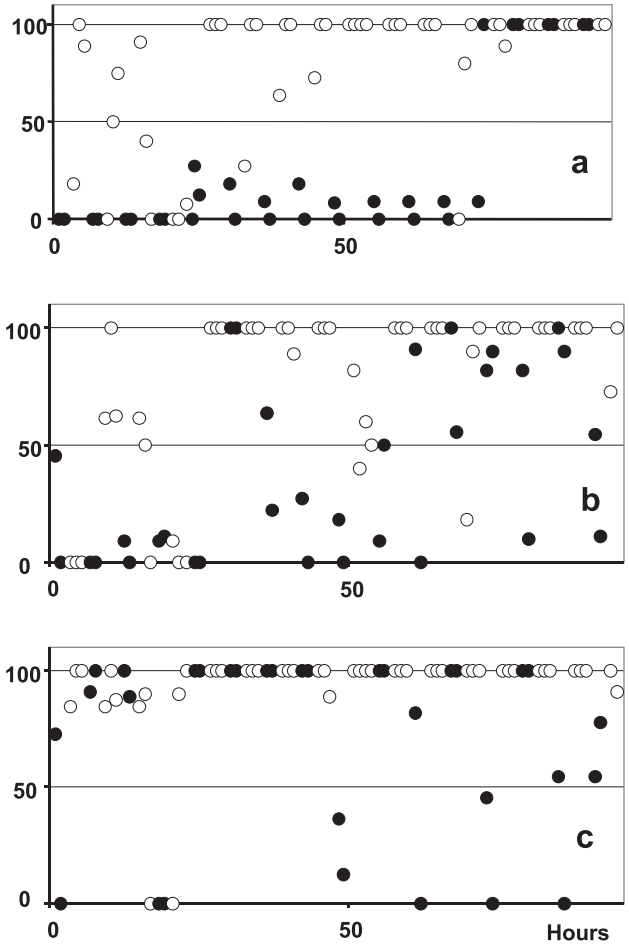

Fig. 3. Typical feeding time activities (FTA, %) of 3 oysters chosen for their different behaviour: (a), (b) and (c). Open circle: *Skeletonema costatum* cycles, Black circle: *Alexandrium minutum* cycles.

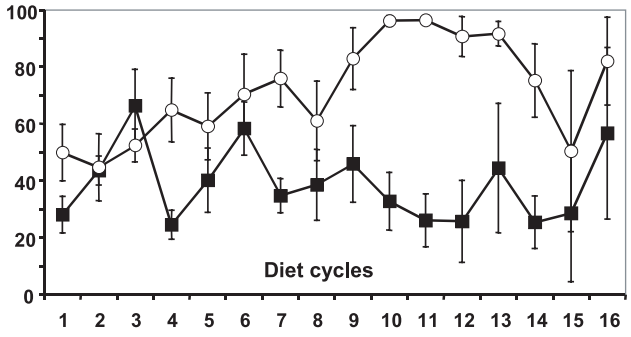

Fig. 4. Mean feeding time activity (FTA) calculated for *A. minutum* (black squares) and *S. costatum* + Mineral matter added, MMA (open circles) cycles during the 4th week of April 2002 experiment, see Table 1.

3.6 Contamination kinetics

Each sample was injected three times into the chromatographic system. To verify that the oysters were not contaminated before the experiment, "control" oyster samples were also injected. The chromatograms obtained after HPLC analysis enabled us to calculate the mean toxicity of the oysters and to plot contamination curves for each experiment (Fig. 5).

A residual amount of some tens of cells per mL was observed with 200 cell ml^{-1} of *A. minutum* after 2 and 4 h in seawater without algal food. In this case, oysters sampled on day 4 showed contamination (192 μg STX equiv. 100 g^{-1}) above the sanitary threshold (80 μg STX equiv. 100 g^{-1}). In all experimented oysters, total toxin content is generally the result of GTX3 analogue pre-eminence (roughly 92% of molar percentages). However, the longer the contamination period, the higher the relative percentage of GTX2 analogue. Oyster toxicity, at the end of the experiment, involving alternation of *A. minutum* 200 cell ml^{-1} and *S. costatum* 20 000 cell ml^{-1}, remained lower than the sanitary threshold. For *A. minutum* 5000 cell ml^{-1} and *S. costatum* 20 000 cell ml^{-1}, the kinetics showed considerable inter-individual variation. Despite an inexplicable reduction in overall toxicity on day 4 of the experiment in November 2001, identical experiments repeated in April and November 2001 showed systematic exceeding of the sanitary threshold after 4 days.

For an alternation of *A. minutum* 10 000 cell ml^{-1} and seawater alone (but with a residual amount of 100 to 1500 cell ml^{-1}), the sanitary threshold was never exceeded

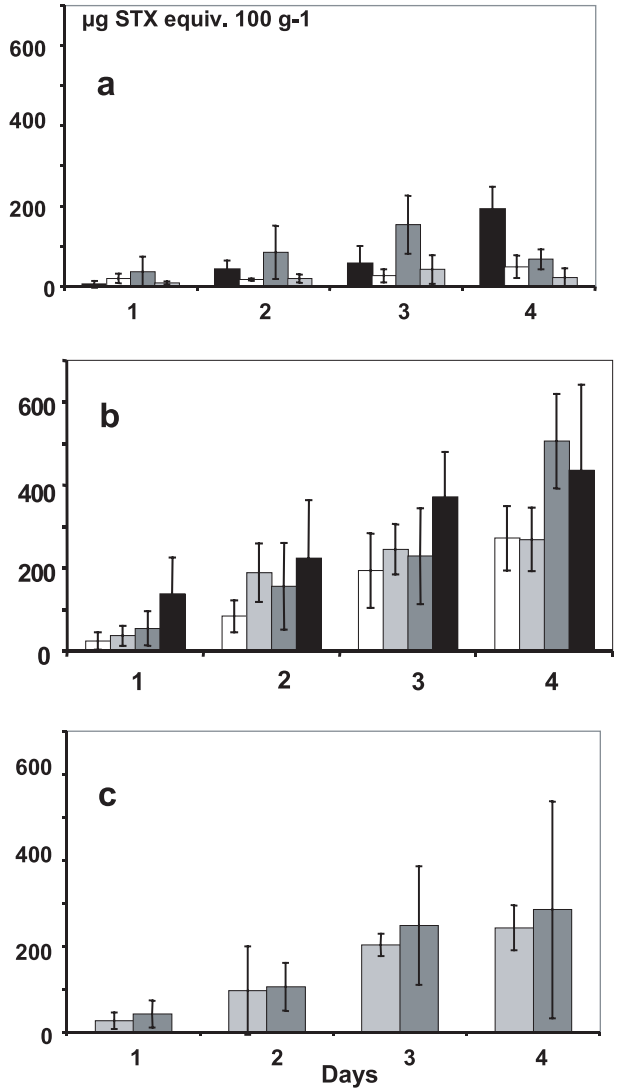

Fig. 5. Every day mean toxin contents and standard deviations (μg STX equiv. 100 g^{-1}) in oysters fed alternatively *A. minutum* and *S. costatum* or *A. minutum* and no diet. Samples size = 6. (**a**) *A. minutum/S. costatum*. From left to right: 200/0, 200/20 000, 5000/20 000, 10 000/0; (**b**) *A. minutum/S. costatum/MMA* concentrations. From left to right: 5000/0/0, 5000/ 20 000/0, 5000/20 000/5 mg l^{-1}, 5000/20 000/15 mg l^{-1}; (**c**) *A. minutum/S. costatum*/salinity. From left to right: 5000/20 000/25‰ and 5000/20 000/35‰.

during the 4 days, despite a cell concentration corresponding to that of discoloured waters observed in the Penzé estuary.

The comparison of bioaccumulation kinetics for 4 days relative to *A. minutum*/seawater and *A. minutum*/*S. costatum* diets (each involving 5000 cell ml^{-1} of the toxic dinoflagellate) showed no significant differences. However, the addition of mineral matter seemed to intensify the bioaccumulation process. This observation can be related to the high production of pseudofaeces in the presence of MMA, which is probably the result of both increased clearance rates and enhanced sorting of mineral and organic particles by the labial palps.

Finally, because of the large individual variations, there was no significant difference between toxin bioaccumulation at 25 or 35‰.

To discriminate between influent experimental parameters taken into account on oyster toxicity, a simple stepwise multiple regression model was tested (Table 2). This model ensure a high adjusted correlation coefficient = 80.26%, and *A. minutum* cell concentration as well as mineral matter added (MMA) related to toxin contents are pointed out as significant parameters.

On the basis of these results, it seems that MMA and *A. minutum* cell concentration would affect PSP bioaccumulation rates in the oysters. However, this was apparently not the case for salinity (p-value > 0.1 for all models in which this parameter was involved). *Skeletonema* concentration was also included in the stepwise regression, but was not a significant parameter and was rejected.

Finally, the negative coefficients assigned to two parameters in the descriptive model of Table 2 characterising respectively *A. minutum* cell concentration and MMA concentration, indicate that they would have a limiting effect on bioaccumulation for given threshold values. For example, depending on whether MMA = 0 or 5 mg L^{-1}, Cf (toxin concentration) at the 4th day contamination increases from 251 to 410 and from 37 to 356 μg STX equiv. 100 g^{-1} respectively for 5000 and 10 000 cell ml^{-1} of the toxic dinoflagellate.

4 Discussion

Observations performed over 24 h in the Penzé estuary indicate that oysters situated in the outer part of the estuary could come in contact with a toxic dinoflagellate (*A. minutum*) bloom for a maximum period of 2 h. The maximum number of dinoflagellates likely to produce a bloom is generally detected at low tide + 1, i.e. at the very beginning of flood tide. The discoloured waters concentrated upstream in the river at high tide are apparently scattered when carried downstream by the ebb tide.

The experimental system developed here allows simulation of alternating contact phases between toxic and non-toxic phytoplankton in order to evaluate the respective impacts of different features of the system. The results, accredited by p-values of Table 2, show that concentrations lower than those of blooms (200 cell ml^{-1} of *A. minutum*) can cause contamination of bivalves at values above the sanitary threshold in only 4 days, provided that there is no other food source. With a mixed diet including a concentration of non-toxic phytoplankton comparable to that observable in the Penzé estuary in periods of discoloured waters, a detoxification process seems to reduce the level of final toxicity.

Very high concentrations (10 000 cell ml^{-1}) of *A. minutum* might also have an inhibitory effect on biodeposit production (and thus on consumption). By extension, this effect could influence bioaccumulation. This hypothesis, which has already been advanced by Vila et al. (2001) relative to PSP contamination of mussels by *A. catenella* in the Mediterranean Sea, seems corroborated by the limiting role played by cell concentration in the model, see Table 2.

Table 2. Stepwise multiple regression, with forward selection, testing whether the dependent variable (*Cf*) is related to more than one independent variable (N_{alex}, N_{skel}, *t, MMA*, *S*). The coefficients weighting each variable were determined by the software Statgraphics plus 5.1. *t* = Time (days), N_{alex} = Mean *Alexandrium minutum* concentration (cell ml^{-1}) ≈ peak *Alexandrium minutum* concentration / 3 (cell ml^{-1}), N_{skel} = Mean *Skeletonema costatum* concentration (cell ml^{-1}) ≈ peak *Skeletonema costatum* concentration / 3 (cell ml^{-1}), *MMA* = Mineral matter added (mg ml^{-1}), *S* = salinity (‰), *Cf* = Toxin concentration in the oyster at the time of sampling (μg STX equiv. 100 g^{-1}).

Analysis of variance			
	Sum of squares	Degrees of freedom	*p*-value
Model	7.57×10^6	4	0.0000
Residual	1.82×10^6	200	
Total	9.4×10^6	204	
Multiple Regression Analysis			
Independent variables	Estimate	Standard Error	*p*-value
N_{alex}, *t*	7.25×10^{-2}	0.47×10^{-2}	0.0000
N_{alex}, *t*, *MMA*	6.14×10^{-3}	1.39×10^{-3}	0.0000
N_{alex}, *t*, MMA^2	-2.71×10^{-4}	0.94×10^{-4}	0.0044
N^2_{alex}, *t*	-2.09×10^{-5}	0.20×10^{-5}	0.0000
R^2: 80.55%			
R^2 (adjusted for degrees of freedom): 80.26%			
Number of independent variables tested: 7(N_{alex}, *t*; N^2_{alex} *t*; N_{alex}, *t*, *MMA*; N_{alex},*t*, MMA^2; N_{alex}, *t*, N_{skel}; N_{alex}, *t*, *S*; N_{alex}, t^2)			
Number of step for the regression: 8			
Model: $Cf = N_{alex}\, t(7.25 \times 10^{-2} \times 6.14 \times 10^{-3}\, MMA - 2.71 \times 10^{-4}\, MMA^2 - 2.09 \times 10^{-5} N_{alex})$			

Likewise, the amount of MMA involved could stimulate or limit toxin bioaccumulation. However, salinity, which is quite variable in a tidal estuary, does not seem to play any role in the shellfish contamination process.

Nonetheless, due caution is required when interpreting the coefficients weighting each variable. These results were obtained on the assumption of mean continuous feeding on *A. minutum*, which was not the case during our experiments and occurs only rarely in the natural environment.

Finally, individual cases show "tolerant" behaviour, i.e. identical high FTA for toxic and non-toxic cycles, or "sensitive" behaviour, i.e. high FTA in the non-toxic cycle and low FTA in toxic cycles. However, these feeding patterns, which demonstrate the rapid rate of response of the bivalves, could change over time.

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