

Detoxification of Pacific oyster *Crassostrea gigas* fed on diets of *Skeletonema costatum* with and without silt, following PSP contamination by *Alexandrium minutum*

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Abstract – Contamination of shellfish by paralytic shellfish poisoning (PSP) toxins poses an economic threat to shellfish farmers. As contaminated shellfish cannot be harvested for long periods of time, it would be very useful to develop processes to optimise and shorten their detoxification. In this study, Pacific oysters *Crassostrea gigas* were first experimentally contaminated over a period of 13 days with a continuous flow of toxic *Alexandrium minutum* cultures at concentrations ranging from 150 to 200 cell ml⁻¹ (toxin content after 13 days of contamination 438 µg STX equiv. 100 g⁻¹ wet weight). Then, two different detoxification treatments were tested and showed detoxification rates greater than those observed in coastal environments. The first treatment consisted of feeding oysters on *Skeletonema costatum*, at a concentration of 2000 cell ml⁻¹ to speed up detoxification rates. The second detoxification method used the same *Skeletonema costatum* diet, supplemented with silt particles at a concentration of 20 mg L⁻¹. A control was also set up by placing contaminated oysters in seawater with no additional algal food. The detoxification experiment lasted 8 days. Toxin contents were analysed by liquid chromatography with fluorescence detection (LC-FD). The *S. costatum* diet significantly reduced the time needed for oysters to reach the sanitary threshold (80 µg STX equiv. 100 g⁻¹ wet weight), but no effect of the silt supplement could be demonstrated conclusively. These different detoxification methods did not influence toxin biotransformations as observed in oyster tissues, i.e. epimerisation and decarbamylation of gonyautoxins 2 and 3.

Key words: Paralytic shellfish poisoning / PSP / Organic matter / Inorganic matter / Detoxification / *Crassostrea gigas* / *Alexandrium minutum*

Résumé – Décontamination comparée chez des huîtres, *Crassostrea gigas*, contaminées par un dinoflagellé toxique (PSP) *Alexandrium minutum* puis nourries de *Skeletonema costatum* seul ou mélangé à de l'argile. La contamination des coquillages par des toxines paralysantes (PSP) pose des problèmes économiques pour les producteurs. Les coquillages contaminés peuvent rester insalubres et inexploités pendant de longues périodes. Ainsi, il est important de développer et d'optimiser le processus de décontamination. Dans cette étude, les huîtres *Crassostrea gigas* ont été, dans un premier temps, contaminées expérimentalement pendant 13 jours avec un flux continu d'un dinoflagellé toxique, *Alexandrium minutum* (150–200 cell ml⁻¹; le contenu toxinique était de 438 µg équiv. STX pour 100 g de chair au bout des 13 jours de contamination). Afin d'optimiser le taux de décontamination, deux types d'expériences ont été comparés durant 8 jours. Des huîtres ont été nourries, soit de *Skeletonema costatum* (2000 cell ml⁻¹) uniquement, soit de *S. costatum* à la même concentration que précédemment mais en ajoutant des particules d'argile (20 mg L⁻¹). Enfin, un groupe témoin composé d'huîtres contaminées a été placé dans de l'eau de mer, sans apport alimentaire. L'analyse du contenu toxinique a été réalisée par chromatographie en phase liquide (LC-FD). À l'exception du groupe témoin, les taux de décontamination sont plus élevés que ceux observés en environnement côtier. L'alimentation en *S. costatum* réduit significativement le temps nécessaire pour atteindre le seuil sanitaire (80 µg équiv. STX 100 g⁻¹ de chair humide), tandis que l'effet de l'ajout d'argile ne peut pas être démontré de façon décisive. Ces différentes méthodes de décontamination n'influent pas sur les biotransformations observées dans les tissus de l'huître, c'est-à-dire sur l'épimérisation et la décarbamylation des gonyautoxines 2 et 3.

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1 Introduction

France is the largest oyster producer in Europe with an annual output of 115 250 tons (FAO 2004). Like other filter feeders, oysters catch suspended particles, and ingest those in the correct size range. In oysters this range is 2–4 μm (Langdon and Newell 1996), meaning that they may consume algal toxins when these are present among the ingested phytoplankton species. These toxins can then become concentrated in the shellfish tissues. In European coastal waters there are essentially three kinds of phycotoxins: lipophilic toxins (diarrhetic shellfish poisons: DSP; and azaspiracid: AZA), amnesic shellfish poisons (ASP) and paralytic shellfish poisons (PSP). All three of these are known to be present in France.

High levels of paralytic toxins in shellfish tissues can be lethal for human consumers as there is a lack of specific antidotes. PSP toxins consist of a series of approximately 20 naturally occurring substances that vary widely in specific toxicity. The basic molecular structure is that of saxitoxin or STX.

Pacific oyster (*Crassostrea gigas*) contamination by PSP toxins in Europe usually ranges from 150 to 250 μg STX equiv. 100 g^{-1} wet weight (Lassus et al. 2005). As the sanitary threshold is 80 μg STX equiv. 100 g^{-1} wet weight, such toxic levels generally lead to the prohibition of sales from affected oyster farming areas, causing significant economic losses.

Bivalves can detoxify themselves in the sea, although this process can be lengthy. For example, detoxification might take one or two years for some “slow detoxifiers” (Bricelj and Shumway 1998), depending on bivalve species and environmental factors like the ratio of toxic / non-toxic phytoplankton. According to a recent survey, oyster farmers are looking for a detoxification process which could make shellfish safe for the market in less than 6 days (unpublished data). A number of different methods designed to increase detoxification kinetics have already been tested: thermal and osmotic stress, electric shocks, pH decrease, and chlorination (Fernandez et al. 2003). None of these methods produced any meaningful results however. Environmental parameters have also been tested, as has the intrinsic feeding behaviour of bivalves that is thought to consistently influence PSP toxin contamination and detoxification pathways (Blanco et al. 1997; Moróño et al. 2001).

To accelerate the decontamination process, two steps could be considered as potential targets for investigation: the defecation process, and detoxification within oyster tissues. Defecation is related to the digestive process. Consequently, the presence of organic matter may accelerate this process, but suspended inorganic matter potentially dilutes it, decreasing the overall amount of organic matter filtered (Kjørboe and Møhlenberg 1981). However, silt combined with an artificial algal diet can increase the growth of the bivalve molluscs because organic matter is absorbed better due to a higher clearance rate and a higher level of absorption efficiency (Griffiths 1980; Hawkins et al. 1996).

Another way to accelerate the detoxification process would be via tissue detoxification and biotransformation processes, i.e.: epimerisation, hydrolysis and reduction (Oshima 1995; Blanco et al. 2003; Suzuki et al. 2003). These biotransformations can affect net toxin content by transforming molecules into less toxic analogues, therefore modifying the detoxification kinetics (Silvert and Cembella 1995).

The amount of toxin in different oyster tissues must also be considered since, while this amount is generally lower elsewhere than in digestive gland tissue (Li et al. 2005), it is by no means negligible.

This study focuses on a comparative analysis of the effects of three diets on detoxification kinetics in PSP-contaminated oysters. The oysters, initially contaminated under controlled conditions by feeding with the toxic dinoflagellate *Alexandrium minutum* were then fed according to one of the following treatments: i) starvation, ii) *Skeletonema costatum* (diatom) and iii) *Skeletonema costatum* and silt. The effects of these different diets were observed on toxin levels in either digestive gland and remaining flesh, as were their effects on toxin biotransformation.

2 Methods

2.1 Biological material

Oysters - Pacific oysters (*Crassostrea gigas*) were obtained from a producer in Bourgneuf Bay (Atlantic coast, France), where they had been grown safely, with no history of paralytic toxin contamination. Once in the laboratory, epibionts were removed from their shells and each animal was placed in a sea water tank for a period of 4 days acclimatisation at 16 ± 0.5 °C. The oysters, with a mean flesh weight of 4.9 ± 0.1 g (dry weight of 1.02 ± 0.04 g), were sexually inactive.

Microalgae - *Alexandrium minutum* (AM89BM strain), and *Skeletonema costatum* (PBA855 strain) were cultivated in thermoregulated rooms (16 ± 1 °C) with a diurnal photon density flux of 50 ± 4 $\mu\text{mol photons m}^{-2}$ and 12h:12h light:dark. *A. minutum* was grown in Guillard's F/2 medium, and had a mean toxin content per cell of 1.47 ± 0.17 pg STX equiv. cell⁻¹. The non-toxic species *Skeletonema costatum* was cultivated in Provasoli's ES medium under the same conditions. Silt particles (kaolinite) with a diameter ranging from 2 to 4 μm were added with the *S. costatum* to the appropriate treatment raceway in the experimental circuit.

2.2 Feeding and detoxification experiment

Two 100 L-raceways were used (Fig. 1) during the contamination of the oysters by *A. minutum*, with 64 oysters placed in each raceway (RC2 and RC3). Seawater was recirculated (flow rate of 800 L h⁻¹) in each raceway and buffer tank (40 L), and kept at a constant temperature of 16 ± 0.4 °C. A pump placed in the buffer tank continuously supplied the detection cell of a Turner Design fluorometer equipped with 340–500 nm excitation and 665 nm emission filters, allowing continuous measurement of chlorophyll-*a* fluorescence.

The oysters were fed *Alexandrium minutum* at concentrations ranging from 150 to 200 cell ml⁻¹ for 13 days.

At the end of the contamination phase, 126 of the oysters were divided between three raceways (RC1 to RC3), so that there were 42 in each (Fig. 1). In the first raceway, oysters were fed *Skeletonema costatum* at a concentration of 2000 cell ml⁻¹

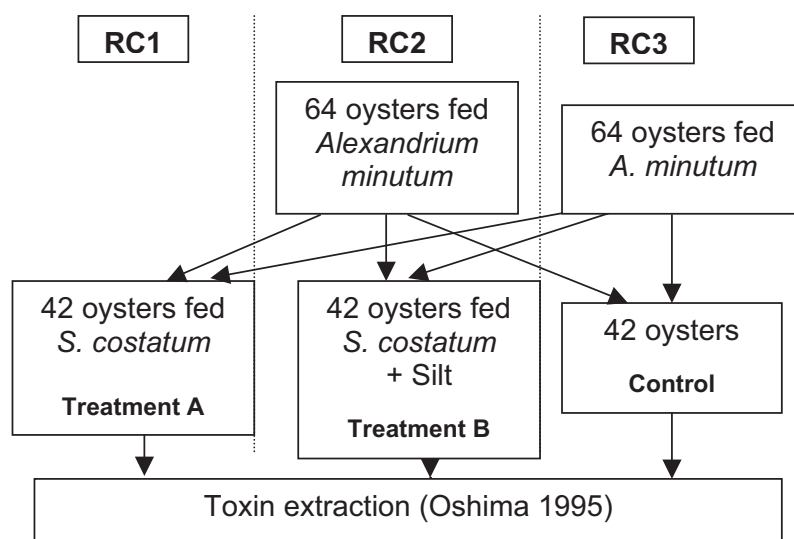


Fig. 1. Diagram showing the experimental steps of contamination and detoxification (RC: raceway)

(equivalent total particulate matter or TPM: 1.60 mg L^{-1} ; treatment A). The second tank, contained both *Skeletonema costatum* at a concentration of $2000 \text{ cell ml}^{-1}$ and silt particles at a concentration of 20 mg L^{-1} (treatment B). The last tank contained unfiltered seawater only (Control). These detoxification conditions were maintained for 8 days.

For chemical analysis during the detoxification, 8 oysters were collected from each raceway on the first, second and third days, then 6 oysters were collected per day on the fourth, fifth and sixth days. For each daily sample, tests were conducted on pooled digestive gland and on the pooled remaining flesh fractions.

2.3 Toxin extraction using liquid chromatography / fluorescence detection (LC/FD)

Digestive gland and remaining tissues were dissected and placed in a Büchner funnel for 1 h to drain off the excess water. At the end of this process both parts were weighed. Drained flesh was then ground in 0.1 N HCl (2 v/w) at $4 \text{ }^\circ\text{C}$. Homogenized flesh was centrifuged for 15 min at $3000g$ and $4 \text{ }^\circ\text{C}$, and pH adjusted to $3.0\text{--}3.5$ with 12 N HCl . Supernatants were then prepared by ultrafiltration (with a molecular weight cut-off of 20 kDa) using a Sartorius Centrafiltration device, and stored at $4 \text{ }^\circ\text{C}$ until analysis.

To quantify the toxin content of phytoplankton cells themselves, 10 ml of toxic algae culture was sampled at the end of the exponential phase (14 days). Tubes were placed in the centrifuge for 15 min at $3000g$ and $4 \text{ }^\circ\text{C}$. The supernatant was kept frozen subsequently at $-20 \text{ }^\circ\text{C}$. Sedimented pellets were collected with $250 \mu\text{l}$ of acetic acid (0.1 N) following homogenisation with a vortex. The tubes were then placed in the freezer at $-80 \text{ }^\circ\text{C}$ and were allowed to thaw naturally at ambient air temperature for at least 1 hour. They were then sonicated for 15 min in the presence of ice. Finally, the tubes were centrifuged for 15 min at $17000g$ and $4 \text{ }^\circ\text{C}$.

Analysis of PSP toxins was performed according to Oshima et al. (1995) with slight adaptations. Toxins were

separated using reversed phase chromatography and a C8 column ($5 \mu\text{m}$ Develosil, $4.6 \text{ mm i.d.} \times 250 \text{ mm}$) with a flow rate of 0.8 ml min^{-1} . Elution pH and/or column temperature were calibrated to optimise the separation of gonyautoxins dc-GTX3/B1/dc-GTX-2. Toxins were quantified using certified standards provided by CNRC-Halifax-Canada. B2 and C-toxins were detected and quantified indirectly following acid hydrolysis ($\text{HCl } 0.4 \text{ N}$ at $97 \text{ }^\circ\text{C}$ for a period of 5 min) (Masselin et al. 2001).

Toxin concentrations in *A. minutum* cultures and contaminated oysters were converted into μg saxitoxin (STX) equiv. 100 g^{-1} wet weight using the conversion factors determined by Oshima (1995).

The analysis was performed three times for each sample.

2.4 Physiological parameters

Physiological parameters were used to compare the feeding behaviour of oysters in the different raceways.

Discrete analyses of dissolved ammonia concentrations in each raceway were performed every morning using the Koroleff (1969) method.

Biodeposits (faeces and pseudofaeces) were collected twice a day, making it possible to calculate both clearance and filtration rates using the formula from Hawkins et al. (1996).

$$CR = \frac{IFR}{PIM} \quad (1)$$

$$FR = CR \times TPM \quad (2)$$

where CR is the clearance rate (L h^{-1}), IFR (inorganic filtration rate) the amount of inorganic matter taken up from the same volume of seawater (mg L^{-1}), PIM the amount of inorganic matter in the seston fraction contained in one litre of seawater (mg L^{-1}), FR the filtration rate (mg L^{-1}) and TPM (total particulate matter) for the amount of matter in one litre.

Diurnal shell valve activity was recorded every hour and expressed as the ratio of the number of oysters that opened their valves to the number of oysters used in the experiment.

2.5 Statistical analysis

Detoxification kinetics were constructed as a one-compartment model as recommended by Bricelj and Shumway (1998) and Silvert et al. (1998). The equation for this model can be expressed as follows:

$$\frac{dC}{dt} = \alpha I - \beta C \quad (3)$$

where C is the toxin concentration expressed as μg STX equiv. 100 g^{-1} of flesh, I is the toxic algal cell-related ingestion rate (cell d^{-1}), α the cell toxin content (μg STX equiv. cell^{-1}), and β the detoxification factor (d^{-1}). During detoxification, the ingestion rate of toxic cells was equal to zero which simplifies the previous equation as follows:

$$\frac{dC}{dt} = -\beta C. \quad (4)$$

The solution for this equation is:

$$c_t = c_0 \exp(-\beta t) \quad (5)$$

where C_0 is the initial toxin concentration.

The coefficient β was determined with the Matlab 6.5 “curve fitting” tool. Prediction bounds of 95% were computed for the “predint” function. The time (t in days) required to detoxify PSP-contaminated oysters from 200 (mean value in Europe) down to $80 \mu\text{g}$ STX equiv. 100 g^{-1} wet weight, could therefore be deduced from the following equation:

$$C = 200 \exp(-\beta t) \quad (6)$$

$$t = \frac{\ln(80/200)}{\beta} = \frac{0.91}{\beta}. \quad (7)$$

3 Results

3.1 Contamination

Oysters in both raceways behaved the same way during the contamination phase: shell valve activity was similar, with the overall trend roughly following the ammonia concentration trends. These variations were cyclic and correlated directly with seawater renewals. The ANOVA test allowed us to confirm that oysters had the same clearance rate in the two tanks: $0.17 \pm 0.16 \text{ L h}^{-1}$.

All saxitoxin analogues were analysed through three successive runs, and only GTX3-2, C2-1 and dcGTX3-2 were detected. The toxin content of digestive gland after 13 days of contamination was $1405 \pm 128 \mu\text{g}$ STX equiv. 100 g^{-1} whereas the toxin content of the remaining flesh was much lower ($76.6 \pm 3.3 \mu\text{g}$ STX equiv. 100 g^{-1}). The highest toxin content, equal to 95% of the total toxin body burden was found in the digestive gland.

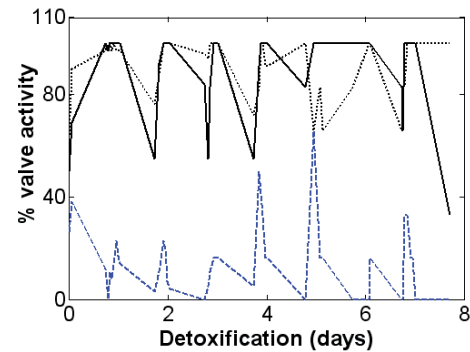


Fig. 2. Valve activities (%) during the detoxification period with different diets: *S. costatum* (—); *S. costatum* + silt (...); Control (unfed oysters - - - in blue).

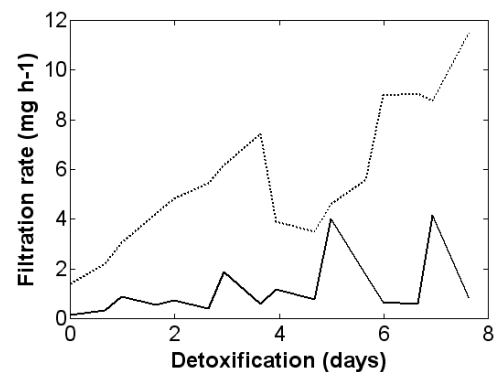


Fig. 3. Filtration rates of oysters fed *S. costatum* (—); *S. costatum* + silt (...).

3.2 Detoxification

3.2.1 Physiological parameters

Individually, oysters displayed different behaviour profiles during detoxification. Shell valve activity was high for oysters fed *S. costatum*, with or without silt: $90 \pm 18\%$ and $92 \pm 11\%$ respectively. On the other hand, oysters in the control raceway containing only seawater exhibited much lower levels of activity ($16 \pm 15\%$, Fig. 2). An ANOVA confirmed that shell valve activities for fed and unfed oysters were significantly different. In the control raceway, the amount of biodeposits detected was very low, thus making it quite difficult to calculate the filtration rate. Filtration rate was fairly steady in the oysters fed *S. costatum*, except for three slightly higher values (Fig. 3). The filtration rate for oysters fed *S. costatum* and silt increased through the detoxification phase (Fig. 3).

3.2.2 Toxin content

When fed non-toxic microalgae (*S. costatum* and *S. costatum* + silt treatments) oysters displayed faster detoxification rates in both their digestive glands (Fig. 4a) and in the remaining flesh (Fig. 4b) compared with unfed oysters. Oysters fed non-toxic microalgae thus had a detoxification factor at least 3.25 times higher for digestive gland, and 2.75 times higher for the remaining flesh. Considering the two *S. costatum* diets,

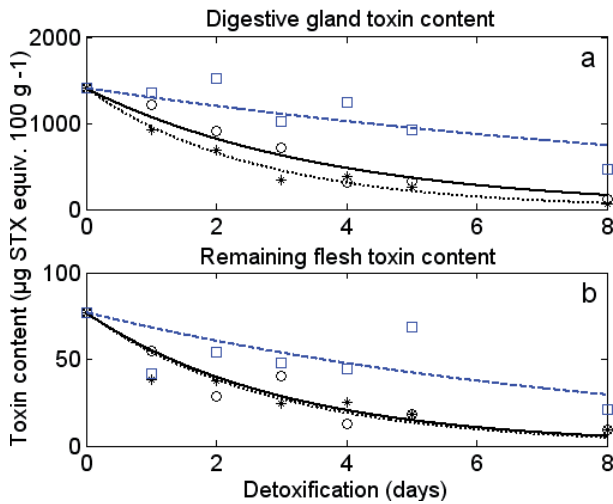


Fig. 4. a) Digestive gland toxin content ($\mu\text{g STX equiv. } 100 \text{ g}^{-1}$) of oysters fed *S. costatum* (—) $y = 1405 \exp(-0.27 t)$; oysters fed *S. costatum* + silt (...) $y = 1405 \exp(-0.38 t)$; Control (- - - in blue) $y = 1405 \exp(-0.08 t)$. b) Detoxification kinetics of flesh without digestive gland for oysters fed *S. costatum* (—) $y = 77 \exp(-0.33 t)$; *S. costatum* + silt (...) $y = 77 \exp(-0.35 t)$; Control (- - -) $y = 77 \exp(-0.12 t)$. All trends redrawn from the same initial toxin content.

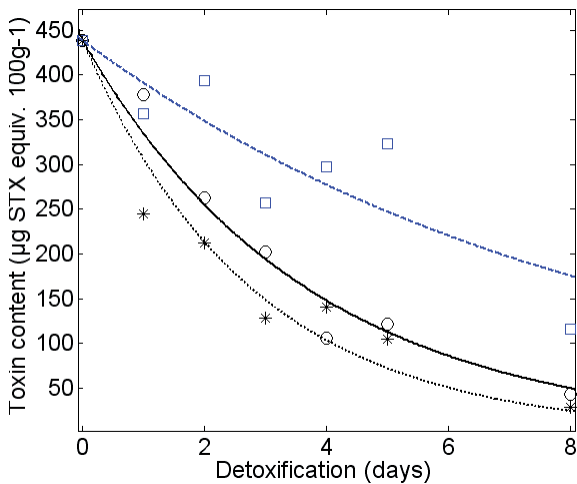


Fig. 5. Overall detoxification kinetics ($\mu\text{g STX equiv. } 100 \text{ g}^{-1}$ flesh) of oysters fed *S. costatum* (—) $y = 438 \exp(-0.27 t)$; *S. costatum* + silt (...) $y = 438 \exp(-0.36 t)$; Control (- - - in blue) $y = 438 \exp(-0.12 t)$. All trends redrawn from the same initial toxin content.

the presence of silt did not affect the detoxification kinetics a great deal. In digestive gland, the detoxification factor observed with *S. costatum* + silt was 1.5 times higher than the detoxification factor obtained with *S. costatum* alone, but the curves representing 95% confidence levels overlap slightly.

The AOAC official method measures the amount of toxin contained within 100 g of shellfish flesh. From the relative percentage of digestive gland and remaining flesh in the oysters' soft parts, it was therefore possible to calculate and express toxin content for 100 g wet flesh. The toxin content of the experimental oysters at the end of the contamination process was therefore $438 \mu\text{g STX equiv. } 100 \text{ g}^{-1}$ of wet weight. Overall detoxification rates were faster for oysters fed *S. costatum*

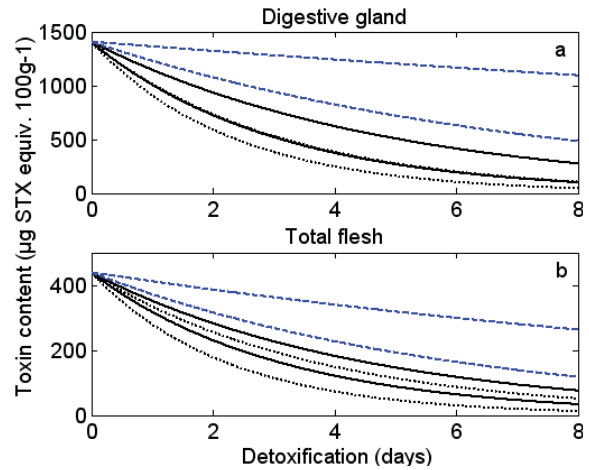


Fig. 6. a) The prediction bounds with a 95% confidence level for detoxification kinetics of the digestive gland for the *S. costatum* diet (—); *S. costatum* + silt (...); Control (- - - in blue). All trends redrawn from the same initial toxicity value. b) The prediction bounds with a 95% confidence level for detoxification kinetics of the total flesh of oysters fed *S. costatum* (—); *S. costatum* + silt (...); Control (- - -). All trends redrawn from the same initial toxin content.

Table 1. Theoretical calculation of the time (t in days) needed to detoxify *Crassostrea gigas* to below the sanitary threshold when the initial toxin content level is: $200 \text{ STX equiv. } 100 \text{ g}^{-1}$ wet weight. Minimal and maximal times correspond to prediction bounds with 95% confidence levels.

	Toxin concentration	Detoxification time (d)		
		mean	min	max
<i>S. costatum</i>	$200 \exp(-0.29 t)$	3.3	2.5	4.3
<i>S. costatum</i> + Silt	$200 \exp(-0.33 t)$	2.8	2.1	4.3
Control (sea water)	$200 \exp(-0.12 t)$	7.6	5.1	18.2

and silt, than for oysters fed *S. costatum* alone, and slower for unfed control oysters (Fig. 5). Detoxification factors for oysters fed *S. costatum* and silt or only *S. costatum* were respectively 3.3 and 2.5 times higher than for unfed oysters. Feeding accelerates detoxification significantly, but silt does not significantly affect the detoxification factor for the total flesh, in contrast to the observations obtained for the digestive gland (Fig. 6). These different effects on digestive gland and total flesh can be explained by the smaller weight of the digestive gland, and differences in toxin body burden.

However, when initial toxin content was equivalent to, or adjusted to, $200 \mu\text{g STX equiv. } 100 \text{ g}^{-1}$ wet weight, the calculated time (Eq. (5)) required to reduce toxin content below the sanitary threshold was not negligible (Table 1). The mean time needed to detoxify unfed oysters was more than twice the time needed for oysters fed microalgae and/or silt. Adding silt improved the detoxification by reducing the time needed to reach the sanitary threshold by half a day. The time needed to detoxify oysters ranged from 2.1 to 4.3 d for oysters fed *S. costatum* and silt, 2.5 to 4.3 d for oysters fed *S. costatum*, and 5.1 to 18.2 d for unfed oysters (prediction bounds with a 95% confidence level).

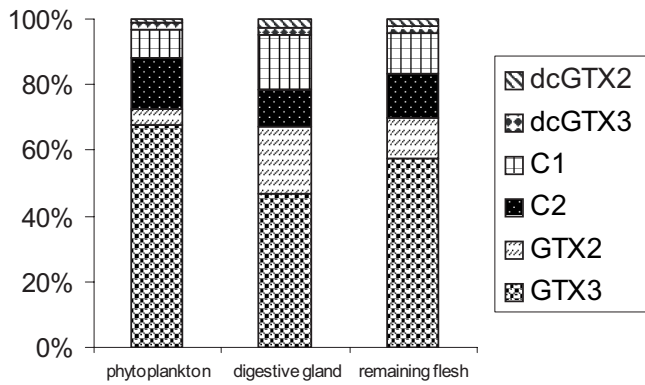


Fig. 7. Phytoplankton toxin profiles (%) and oyster toxin profiles (%) in the digestive gland and remaining flesh at the end of contamination.

Changes in the toxin profile of shellfish tissues may result from selective retention, elimination of individual toxins, epimerisation or biotransformation, such as reduction, hydrolysis at low pH and enzymatic conversion. The appearance of toxins in the bivalve tissues that were not detected in toxic dinoflagellates highlights the metabolic interconversion of toxins (Bricelj and Shumway 1998). In the present study, the toxin profile of *Alexandrium minutum* was: GTX3 (68%), C2 (15%), C1 (9%), GTX2 (5%), dcGTX3 (2%) and dcGTX2 (1%, Fig. 7). Toxin profiles in contaminated oysters were different: GTX3 was the major compound but in lesser proportions than for the *A. minutum* profile. GTX2 and C1 were present at higher proportions (20% and 16% in digestive gland; 12.5% and 12% in remaining flesh). C2 was present in lesser quantities in oysters than in *A. minutum* cells. This would mean that biotransformation occurred during accumulation, with at least two epimerisation processes (GTX3 converted into GTX2, C2 converted into C1). The behaviour of the C2/C1, GTX3/CTX2 and dcGTX3/dcGTX2 ratios enabled us to observe the epimerisation process. The behaviour of toxin types during the detoxification process was the same in oysters from the three raceways: ratios of C2/C1 and of dcGTX3/dcGTX2 were constant; however, the GTX3/GTX2 ratio decreased over time. The proportion of GTX2 increased in comparison with the proportion of GTX3 (Fig. 8). C1/GTX2 and C2/GTX3 (hydrolysis) remained constant. GTX2/ dcGTX2 and GTX3/dcGTX3 (decarbamylation) increased, indicating that decarbamylation had occurred.

4 Discussion

This study complements the information already available regarding detoxification process of paralytic-toxin-contaminated oysters (Lassus et al. 2000, 2005; Yamamoto et al. 2003). The first important result concerns the role of organic matter in the detoxification process. The second result concerns the effect of the inorganic matter which is not clearly demonstrated but is seemingly associated with faster detoxification rate. Adding silt particles enhanced pseudofaeces production by oysters, which is not surprising since this behaviour is usually observed as soon as TPM exceeds 4.6 mg l⁻¹

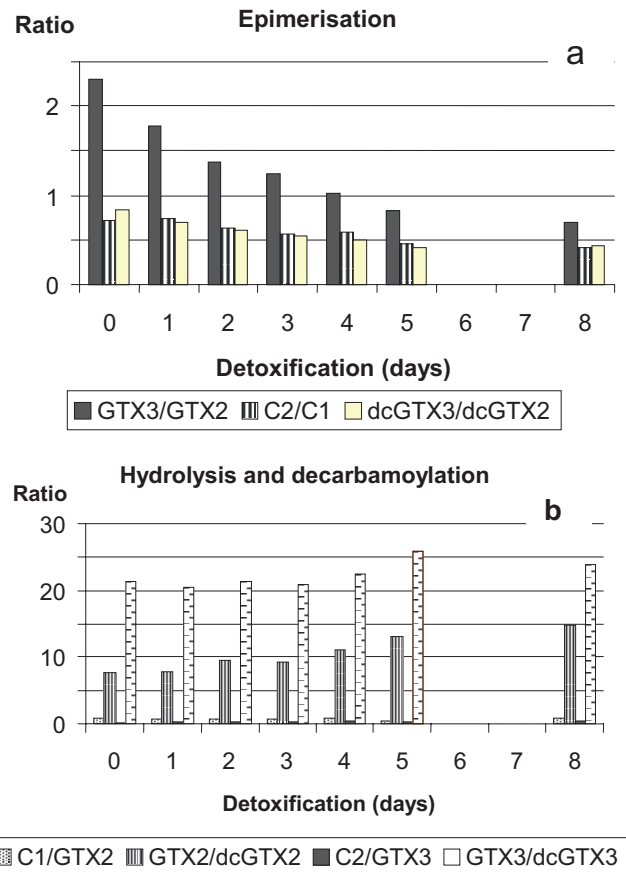


Fig. 8. a) Epimerisation as a result of GTX3/GTX2, C2/C1 and dcGTX3/dcGTX2 ratio evolution during detoxification. b) Hydrolysis (C1/GTX2 and C2/GTX3 ratios) and decarbamylation (GTX2/dcGTX2 and GTX3/dcGTX3 ratios) during detoxification.

(Deslous-Paoli et al. 1992). Increased pseudofaeces production generally indicates increased pre-ingestive selection, thus allowing maintenance of a high organic fraction and maximum absorption efficiency (Méléder et al. 2001). Finally, there was little biotransformation in the oysters which means that the toxin content decrease observed was most probably due to toxin elimination instead.

Detoxification factors obtained in this study ranged from 0.33 to 0.12 d⁻¹ (Table 1). These values are in agreement with those of Lassus et al. (2005) who calculated a detoxification factor of 0.22 d⁻¹ and Yamamoto et al. (2003) who calculated a detoxification factor of 0.31 d⁻¹ for the same oyster species.

The contamination phase conditions were adapted to produce a level of contamination higher than the regulation sanitary threshold (80 µg STX equiv. 100 g⁻¹ wet weight) and therefore corresponded to the range of values observed in European coastal areas (Van Egmond et al. 1993). Feeding behaviour profiles for oysters in the two raceways were similar as far as shell valve activities and biodeposition rates were concerned. The clearance rate was 0.17 ± 0.16 L h⁻¹. Laabir and Gentien (1999), observed a clearance rate ranging between 0.02 and 0.13 L h⁻¹ for the toxic algae *Alexandrium minutum*, *A. tamarense* and *Gymnodinium mikimotoi*. Bardouil et al. (1993), found a clearance rate equal to 0.32 L h⁻¹ for oysters

feeding on *A. minutum*. The clearance rate values observed here therefore fell within the range of values found in the literature for oysters fed *A. minutum*.

The *S. costatum* diet doubled the detoxification rates (Table 1). This confirmed the assertions made by Bricej and Shumway (1998) that “molluscs fed non-toxic algae during the detoxification phase may be able to detoxify PSP-toxins faster than those held in filtered, particle-free seawater: active feeding is likely to accelerate gut evacuation rates and overall metabolism (degradation, excretion) of toxins”. In the same way, Blanco et al. (1997) showed that detoxification rates depended in particular on phytoplankton concentration (as measured by transmittance and fluorescence) during mussel detoxification. The presence of food during detoxification seems important for the detoxification kinetic, although Chen and Chou (2001) have found that the detoxification kinetics for the digestive glands of fed or unfed clams were similar. However they found a difference between tissues, as the toxin content of tissues other than digestive gland tissue decreased faster (36 days) in fed than in unfed (42 days) clams, in contrast to the present study where the presence of *S. costatum* optimised oyster detoxification kinetics for both digestive gland and remaining flesh. The digestive gland was the tissue that contained the largest proportion of toxins (95% in our study). This result agrees with those obtained by Chen and Chou (2001) for other bivalve species, especially the purple clam *Hiatula rostrata*, and results by Sagou et al. (2005) on the sweet clam *Callista chione*. However, the digestive gland has also been shown to be the organ which has the highest rate of detoxification (three times higher than other tissues in the clam), followed by the gills, then the mantle, foot, siphon and finally the adductor muscle (Bricej and Shumway 1998). In the present study on oysters though, detoxification rates for the digestive gland (0.38 d^{-1}) and for the remaining flesh (0.34 d^{-1}) were similar.

Similar toxin profiles were found in toxic phytoplankton and contaminated oysters. These data corroborate previous findings by Samsur et al. (2006) on PSP-contaminated *Tapes japonica*. However, along the Moroccan coast, *Acanthocardia tuberculatum* presents a very different toxin profile (there are 6 to 10 times more dcSTX) from that of the dinoflagellate *Gymnodinium catenatum*, and even from other shellfish profiles (*Callista chione*, *Venus gallina* and *Donax trunculus*; Taleb et al. 1996).

Toxin epimerisation ($\text{GTX3} \leftrightarrow \text{GTX2}$) and decarbomoylation ($\text{GTX2} \rightarrow \text{dcGTX2}$ and $\text{GTX3} \rightarrow \text{dcGTX3}$) were observed during our detoxification process. This result is in agreement with those found by Ichimi et al. (2001) on mussels and by Silvert et al. (1998) on surfclam *Spisula solidissima*. Reduction was not observed during our experiments, unlike in the purple clam *Hiatula rostrata* (Chen and Chou 2001), and the oysters did not transform the toxin, in contrast to *Spisula solida* (Artigas et al. 2007).

Silt seems to have an effect on feeding physiology, causing a higher filtration rate (Kjørboe and Møhlenberg 1981) and, might also increase toxin detoxification rates. The difference in detoxification time between oysters fed with silt (2.8 days) and without it (3.3 d) was only one day, though this could have an economic significance. The role of silt has not yet been clearly

established. It would probably be necessary to test different silt concentrations, and to compare detoxification kinetics between oysters kept in particle-free seawater and in seawater with silt but no algae. It would be worth finding out whether the effect of *S. costatum* on detoxification rates could be improved by using other microalgae species as complementary food sources. Lassus et al. (2000) used *Isochrysis galbana*, *S. costatum*, *Thalassiosira weissflogii* and *Tetraselmis suecica* as candidate algal diets to ensure accelerated detoxification rates in PSP-contaminated Pacific oysters. Apart from a slightly significant difference with the *I. galbana* diet, no real differences were observed on detoxification kinetics. The question therefore remains as to whether mixed diets could be used to further accelerate detoxification.

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References

- Artigas M.L., Vale P.J.V., Gomes S.S., Botelho M.J., Rodrigues S.M., Amorin A., 2007, Profiles of paralytic shellfish poisoning toxins in shellfish from Portugal explained by carbamoylase activity. *J. Chromatogr. A* 1160, 99-105
- Bardouil M., Bohec M., Cormerais M., Bougrier S., Lassus P., 1993, Experimental study of the effects of a toxic microalgal diet on feeding of the oyster *Crassostrea gigas* Thunberg. *J. Shellfish Res.* 12, 417-422.
- Blanco J., Moroño A., Franco J., Reyero M.I., 1997, PSP detoxification kinetics in the mussel *Mytilus galloprovincialis*. One- and two-compartment models and the effect of some environmental variables. *Mar. Ecol. Prog. Ser.* 158, 165-175.
- Blanco J., Reyero M.I., Franco J., 2003, Kinetics of accumulation and transformation of paralytic shellfish toxins in the blue mussel *Mytilus galloprovincialis*. *Toxicon* 42, 777-784.
- Bricej V.M., Shumway S.E., 1998, Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. *Fish. Sci.* 6, 315-383.
- Chen C.Y., Chou H.N., 2001, Accumulation and depuration of paralytic shellfish poisoning toxins by purple clam *Hiatula rostrata* Lightfoot. *Toxicon* 39, 1029-1034.
- Fernandez M.L., Shumway S., Blanco J., 2003, Management of shellfish resources. In: Hallegraeff G.M., Anderson M., Cembella A.D. (Eds.). *Manual on Harmful Marine Microalgae*. IOC Manuals and Guides, UNESCO, pp. 657-692.
- Griffiths R.J., 1980, Natural food availability and assimilation in the bivalve *Choromytilus meridionalis*. *Mar. Ecol. Prog. Ser.* 3, 151-156.
- Hawkins A.J.S., Smith R.F.M., Bayne B.L., Héral M., 1996, Novel observations underlying the fast growth of suspension-feeding shellfish in turbid environments: *Mytilus edulis*. *Mar. Ecol. Prog. Ser.* 131, 179-190.
- Ichimi K., Suzuki T., Yamasaki M., 2001, Non-selective retention of PSP toxins by the mussel *Mytilus galloprovincialis* fed with the toxic dinoflagellate *Alexandrium tamarense*. *Toxicon* 39, 1917-1921.
- Kjørboe T., Møhlenberg F., 1981, Particle selection in suspension-feeding bivalves. *Mar. Ecol. Prog. Ser.* 5, 291-296.
- Koroleff C., 1969, Direct determination of ammonia in natural waters as indophenol blue. *ICES, CM* 9, 4 p.

- Laabir M., Gentien P., 1999, Survival dinoflagellates after gut passage in the Pacific oyster *Crassostrea gigas* Thunberg. J. Shellfish Res. 18, 217-222.
- Langdon C.J., Newell R.I.E., 1996, Digestion and nutrition in larvae and adults. In: Kennedy V.S., Newell R.I.E., Eble A.F. (Eds.) The eastern oyster *Crassostrea virginica*. Maryland Sea Grant College.
- Lassus P., Bardouil M., Baron R., Bérard J.B., Masselin P., Truquet P., Pitrat J.P., 2005, Improving detoxification efficiency of PSP-contaminated oysters (*Crassostrea gigas* Thunberg). Aquaculture Europe, June 2005.
- Lassus P., Bardouil M., Masselin P., Naviner M., Truquet P., 2000, Comparative efficiencies of different non-toxic microalgal diets in detoxification of PSP-contaminated oysters (*Crassostrea gigas* Thunberg). J. Nat. Toxins 9, 1-12.
- Li A.M.Y., Yu P.K.N., Hsieh D.P.H., Wang W.X., Wu R.S.S., Lam P.K.S., 2005, Uptake and depuration of paralytic shellfish toxins in the green-lipped mussel, *Perna viridis*: a dynamic model. Environ. Toxicol. Chem., 24, 129-135.
- Masselin P., Amzil Z., Abadie E., Carreras A., Chiantella C., Le Bec C., Nézan E., Truquet P., 2001, Paralytic shellfish poisoning on the French Mediterranean coast in autumn 1998: *Alexandrium tamarense* complex (Dinophyceae) as causative agent. In: Hallegraeff G.M., Blackburn S.I., Bolch C.J., Lewis R.J. (Eds.). Manual on Harmful Marine Microalgae. IOC Manuals and Guides, UNESCO, pp 26-29.
- Méléder V., Barillé-Boyer A.L., Baud J.P., Barillé L., Cognie B., Rosa P., 2001, Modélisation de l'affinage de l'huître *Crassostrea gigas* alimentée avec la diatomée *Skeletonema costatum*. Aquat. Living Resour. 14, 1-16.
- Moroño A., Franco J., Minanda M., Reyero M.I., Blanco J., 2001, The effect of mussel size, temperature, seston volume, food quality and volume-specific toxin concentration on the uptake rate of PSP toxins by mussels (*Mytilus galloprovincialis*). J. Exp. Mar. Biol. Ecol. 257, 117-132.
- Oshima Y., 1995, Postcolumn derivatization liquid chromatographic method for paralytic shellfish poisoning in Tohoku district. J. AOAC Int. 78, 528-532.
- Sagou R., Amanhir R., Taleb H., Vale P., Blaghen M., Loutfi M., 2005, Comparative study on differential accumulation of PSP toxins between cockle (*Acanthocardia tuberculatum*) and sweet clam (*Callista chione*). Toxicon 46, 612-618.
- Samsur M., Yamaguchi Y., Sagara T., Takatani T., Arakawa O., Noguichi T., 2006, Accumulation and depuration profiles of PSP toxins in the short-necked clam *Tapes japonica* fed with the toxic dinoflagellate *Alexandrium catenella*. Toxicon 48, 323-330.
- Silvert W., Bricelj M., Cembella A., 1998, Dynamic modelling of PSP toxicity in the surfclam (*Spisula solidissima*) multicompartmental kinetics and biotransformation. In: Reguera B., Blanco J., Fernandez M.L., Wyatt T. (Eds.), Harmful Algae, Xunta de Galicia IOC-Unesco, pp. 473-440.
- Silvert W.L., Cembella A.D., 1995, Dynamic modelling of phyco-toxin kinetics in the blue mussel, *Mytilus edulis*, with implications for other marine invertebrates. Can. J. Fish. Aquat. Sci. 52, 521-531.
- Suzuki T., Ichimi K., Oshima Y., Kamiyama T., 2003, Paralytic shellfish poisoning (PSP) toxin profiles and short-term detoxification kinetics in mussels *Mytilus galloprovincialis* fed with the toxic dinoflagellate *Alexandrium tamarense*. Harmful Algae 2, 201-206.
- Taleb H., Vale P., Jaime E., Blaghen M., 2001, Study of paralytic shellfish poisoning toxin profile in shellfish from the Mediterranean shore of Morocco. Toxicon 39, 1855-1861.
- Van Egmond H.P., Aune T; Lassus P., Speijers G.J.A, Waldock M., 1993, Paralytic and diarrhoeic shellfish poisons occurrence in Europe toxicity, analysis and regulation. J. Nat. Toxins 2, 41-83.
- Yamamoto T., Flynn K.J., Takayama H., 2003, Application of atwo-compartment one-toxin model to predict the toxin accumulation in Pacific oysters in Hiroshima Bay, Japan. Fish. Sci. 69, 944-950.