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Improving detoxification Efficiency of PSP-contaminated oysters (Crassostrea gigas Thunberg)

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Shellfish production, and production of marine bivalve molluscs in particular, is the main form of marine aquaculture in the European Union, with a production estimated at 1,200,000 tons in 1999 (Food and Agricultural Organization). Moreover, aquaculture production is rapidly expanding throughout Europe and is becoming a major economic resource in developing areas such as Northern Greece, Ireland, and northwestern Spain.
These shellfish, mainly mussels, oysters, and clams, are produced in a natural environment. Due to variations in their environment, these otherwise edible species may however become unsafe for human consumption.
Potential problems are amplified due to the nature of shellfish themselves: as they are filter feeders, they consume large amounts of algae. Contaminants, which are not harmful to them, therefore tend to concentrate in shellfish. Toxic algae have been a growing threat these last decades and at least three different types of shellfish poisoning, namely, diarrhetic, amnesic, and paralytic shellfish poisoning (DSP, ASP, and PSP), have been observed in Europe so far.

Toxins that cause PSP are lethal compounds and first symptoms in man requires immediate medical attention due to the lack of specific antidotes. Accordingly, the Council of the European Communities have decided that live bivalve molluscs intended for immediate human consumption had to meet the following requirement regarding PSP toxins: "the total Paralytic Shellfish Poison content in the edible parts of molluscs must not exceed 80 microgrammes per 100 g of mollusc flesh" (Council Directives 91/492/EEC and 97/61/EEC). However, several weeks may be required for shellfish to eliminate saxitoxin or other analogs through a natural self-purification process termed depuration. Moreover, it is impossible to estimate the time required to reach regulatory PSP toxin levels in shellfish contaminated by the micro-alga *Alexandrium*, as there is no way to predict the size and duration of the bloom. No practical system exists that is capable of reducing this depuration/detoxification period. PSP toxin levels encountered in Europe typically range from 150 to 250 µg STX eq/100 g. They may, however, reach 1,000 µg STX eq/100 g, and even more in mussels. Although most of the time PSP toxin levels in oysters have been shown to be lower than that in mussels collected in the same area, they have often reached values either just above, or well above the regulatory limit (80 µg STX eq/100 g) and have been generally shown to range from 150-250 µg STX eq/100 g.

Various methods to accelerate the detoxification process, including thermal and osmotic stress, electric shocks, decrease in pH, and chlorination (Shumway et al, 1995) have been tested. None of these methods, however, has proved effective. Biotransformation of phycotoxins by enzymes is still under investigation and ozonization of seawater has been giving contradictory results.
In light of the fact that an increase in algal biomass could affect toxin release from shellfish flesh (Lassus et al, 1994) and that the impact of temperature upon feeding activity would be likely to trigger toxin release, pre-industrial size experiments were carried out to optimize PSP-detoxification in oysters. This study aimed at determining the effects of the non-toxic *Isochrysis galbana* Parke (Tahiti strain) on PSP-detoxification in live Pacific oysters (*Crassostrea gigas* Thunberg) and optimizing conditions to speed up this process. Results were used to establish a standard detoxification model which can be used for the implementation of an optimal industrial-scale detoxification system that meets the European oyster farmers needs, a system that allows detoxification of PSP-contaminated shellfish in less than 6 days (2003 inquiry, data not published). This study is part of a 2003-2005 EU project (SHELLFISH).

Oysters (*C. gigas)* were obtained from a farm in the Bay of Bourgneuf (France, Atlantic coast) with no history of toxic algal bloomsToxic and non-toxic algal strains used throughout the experiments were, respectively, *Alexandrium minutum* Halim (AM89BM) and *Isochrysis galbana* Parke. both cultured in Provasoli's nutrient enriched seawater (Fig 1). Three 100-l flumes ((Fig 2) were used as experimental tanks. Each flume contained 45 oysters (Fig 3) and were continously supplied with seawater by a recirculating circuit (flow rate of 800 l/h). Water was maintained at 15.9 ± 0.4°C. Fluorescence measurement data were integrated using an acquisition and control card connected to a computer. The experimental setting was similar to that described previously (Lassus et al, 1999; 2000). Seawater was totally renewed (130 L) every two days to prevent an increase in ammonia concentration (dissolved ammonia was checked every day using method of Koroleff, 1969).

Analysis for PSP toxins detection in *Alexandrium minutum* and in PSP contaminated oysters were performed by reverse-phase ion-pairing high-performance liquid chromatography (IP-HPLC) according to the method described by Oshima (1989). Statistical comparison (ANCOVA) was used to determine whether differences between detoxification kinetics were significant. Detoxification trend were assumed to be first order exponential functions as demonstrated by Bricelj and Shumway (1998) for mussel, oyster and other 'fast detoxifier' bivalve species.
Considering that a PSP-toxin concentration in oyster flesh of at least 200 STX eq/100 g must be obtained in order to mimic average PSP contamination levels observed in European oysters, and that a long contamination phase may be detrimental to the overall experimental results, a 10 day-contamination period was therefore considered the best compromise and used for all following experiments.
Toxin content per *A. minutum* cell was monitored daily in each experiment : it ranged from 1.4 to 1.8 pg STX eq/cell.

Different non toxic diet were first tried for detoxification (*Skeletonema costatum, Thalassiosira weissflogii, Tetraselmis suesica,* and *Isochrysis galbana)* but with no significant difference providing initial toxin levels in oyster ranged between 200 and 300 µg STX eq/100 g, temperature was 16° ± 1°C, and the total particulate matter (TPM) concentration in the flume was 0.5 mg/l (Lassus et al, 1999; 2000). All of the detoxification data obtained in these experiments could therefore be combined and used to establish a "standard" PSP detoxification model to be used in experiments performed in similar conditions (Fig 4). As previously shown in other studies (Lassus et al, 1999; 2000), wide individual variations were observed, regardless of contamination time and oyster samples size (9 to 45 animals). Such variations are known to directly and significantly affect oysters feeding activity in experimental as well as natural conditions. As a result, the graphical representation was made using the Excel "Solver" option and the general first order exponential function is

$$C = 191\, e^{-0.22t} \qquad (1)$$

where C is the toxin concentration in µg eq STX/100 g, 0.22, the slope (k value), 191 the initial toxin concentration at t = 0, and t, the time in days. From this equation (1) it can be easily deducted that the time required to detoxify oysters contaminated with 200 to 80 µg STX eq/100g, can be expressed according to equation (2) :

$$t(d) = 0.91/k \qquad (2)$$

According to the "standard" detoxification model, shown in figure 4, t = 4.13 days with an initial toxin level of 200 µg STX eq/100 g, instead of 191 µg STX eq/100 g^{-1}. This duration meets the shellfish farmers' request.

Besides, previous experiments showed a slow detoxification process in PSP-contaminated oysters supplied with seawater only, and a faster detoxification rate when fed *Isochrysis galbana* (T-iso strain) at 16°C with a TPM concentration of 0.5 mg/l, which corresponds to a concentration of 12,000 cells/ml.
Accordingly, three *Isochrysis galbana* concentrations were tested: 12,000, 36,000, and 72,000 cells/ml, which corresponded to, 0.59, 1.16, and 1.95 mg/l TPM, respectively. Initial toxicities ranged from 100 to 200 µg STX eq/100 g^{-1} and the limit of 80 µg STX eq/100 g^{-1} was attained in 2 days with 12,000 cells/ml, and in less than 24 hours with either 36,000 or 72,000 cells/ml. However, statistical comparison of detoxification kinetics showed no significant differences.
Detoxification kinetics data for each tested temperature were fitted in a similar way. A lower initial toxicity was observed for one group (20°C), i.e., 160 µg STX eq/100 g, while toxicity levels for the other groups were close to or higher than the expected value (200 µg STX eq/100 g). This time, the regulatory limit was reached between day 4 and day 5 at 20°C, while at 12°C and 16°C, 6 days were required.

Discussion

Individual variations in the bioaccumulation of PSP toxins are common and have been shown to reach a maximum of 43% (White et al., 1993) in wild populations of several species. Regarding the experimental conditions used in this study, it seems clear that recorded ammonia concentrations cannot be considered as a factor which may have contributed to decrease toxin clearance.

The main objective of this study, i.e : to detoxify PSP-contaminated oysters in less than 6 days and thereby meet the shellfish industry's demand, was clearly achieved. Detoxification should have been, however, as efficient as that predicted by the standard model or improved by changes in the temperature and an increase in non-toxic algae concentration.

It appeared that k values (slopes) of detoxification curves, observed at either 12, 16, or 20°C, were slightly lower than that of the standard curve (k = -0.22), eventhough the initial toxin levels in these 3 experiments were in the same range as the expected initial level. Consequently, longer detoxification times, 5.4 to 6.5 days (calculated from equation 2), were found. Conversely, k values with algal concentrations ranging from 12,000 to 72,000 cells/ml were higher (by a factor of 2) than that obtained with the standard model, which translated into a shorter detoxification time (1.9 to 2.4 days). It seems that some unidentified parameters, different from those considered in this study (temperature and algal concentration) may have a significant effect on the slope of the detoxification curve.

From a practical point of view, this means that in order to meet the shellfish industry's demand and implement such an industrial-scale detoxification pilot-program, a number of points must be considered to ensure its feasibility, especially in terms of cost.
An increase in either algal or TPM concentration does not seem to drastically affect detoxification efficiency. Consequently, feeding oysters with non-toxic algae, an thereby increasing TPM concentration to 0.5 mg/l, may be a way to increase detoxification efficiency at low cost. Similarly, comparison of the

detoxification curves obtained in experiments in which the initial toxin level was of 200 µg STX eq/100 g, showed that the temperatures in the range 12 – 20°C does not significantly affect the k value. As a result, temperatures close to those observed in environmental conditions might be considered suitable for an efficient detoxification.
When toxin content in oyster flesh is close to 200 µg STX eq/100 g, the detoxification process should follow a simple standard model, a first order exponential curve with a k value close to 0.22, which should ensure detoxification in 4 to 5 days.
Ammonia levels lower than 20 µgatoms/l and a shell valve activity ranging from 80 to 100% during industrial-scale post-harvest detoxification treatment would also ensure better efficiency.

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Figure 1 : Thermoregulated culture room and mass culture devices (300 L Skobalits and 10 L glass vessels)

Figure 2 : General view of experimental raceways and recirculating water device used for the experiments

Figure 3 : Pacific oysters placed on rows, on the bottom of experimental tanks.

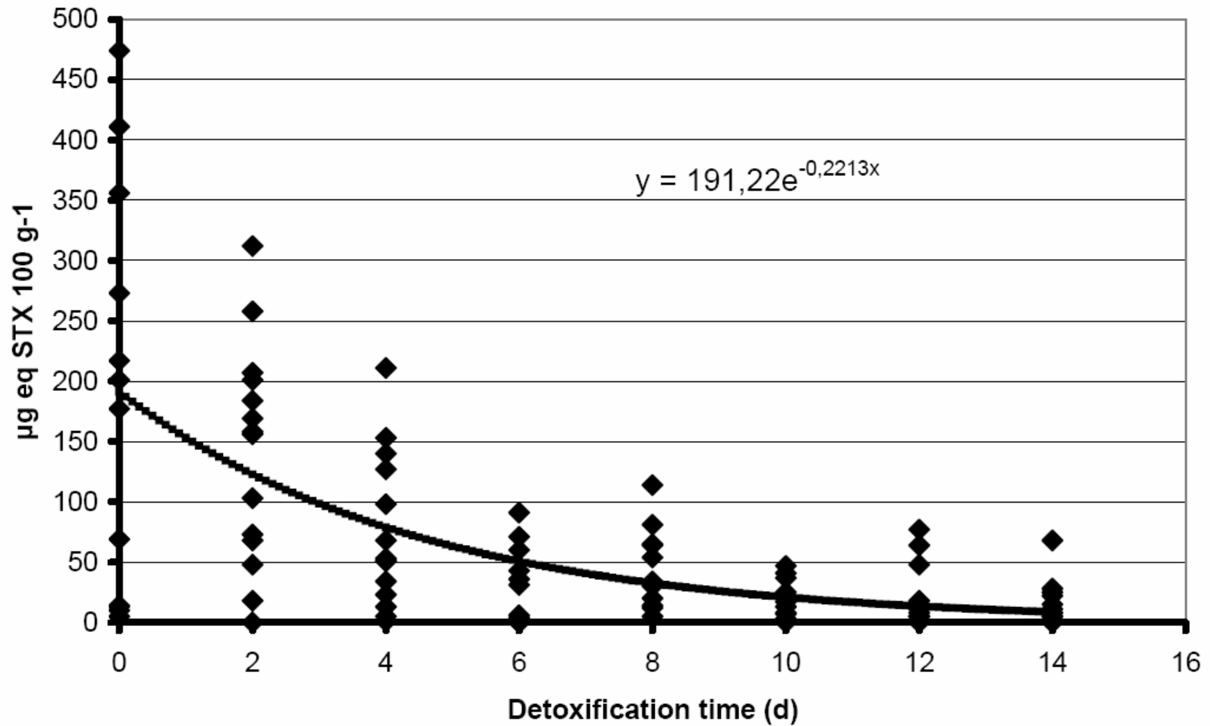

Figure 4. Exponential trend obtained using the Excel "Solver" option. Experimentally PSP-contaminated oysters were detoxified in recirculated seawater at 16°C. TPM concentration was 0.5 mg/l. All data from 4 detoxification experiments were combined.