Aquat. Living Resour. 19, 77–84 (2006)
© EDP Sciences, IFREMER, IRD 2006
DOI: 10.1051/alr:2006007
www.edpsciences.org/alr

Aquatic
Living
Resources

Continuous fluorescence recording as a way to improve Pacific oyster (*Crassostrea gigas*) models of paralytic shellfish toxin accumulation

Régis Baron[1,a], Marielle Couedel[2], Camille Joret[2], Pierre Garen[3], Philippe Truquet[2], Pierre Masselin[2], Michèle Bardouil[2] and Patrick Lassus[2]

[1] Ifremer, Department "Marine Food Science and Technology", BP 21105, 44311 Nantes, France
[2] Ifremer, Laboratory "Phycotoxins", BP 21105, 44311 Nantes, France
[3] Ifremer, Laboratory "Pearl oyster domestication", Centre de Tahiti, BP 7004, 98179 Taravao, Polynésie Française

Received 17 October 2005; Accepted 11 January 2006

Abstract – A simple system was used to simulate the effect of alternating toxic (paralytic shellfish poisoning toxins) and non-toxic microalgal diets on oyster feeding behaviors and rates of toxin accumulation. These experimental conditions were meant to reflect, to some extent, the incoming and outgoing fluxes of toxic algae observed at the mouth of the Penzé estuary (Northern Brittany, France). Physiological and toxicological parameters were estimated based on fluorescence measurements recorded continuously at the outlet of each experimental tank, which contained a single oyster. Q_{tox}, this variable describes toxin uptake in oysters, it was used (instead of the toxin ingestion rate): i) in simple graphical analyses, ii) as well as in one- and two-compartment models. Results show that toxin uptake varies widely from one individual to another and is not proportional to the concentration of toxic algae in sea water. A one-compartment model with individual fluorescence recordings as "input" data gave questionable results, however, a two-compartment model was found to effectively describe contamination kinetics in oysters. Limitations of this model as well as possible improvements are discussed.

Key words: PSP / Shellfish toxin / Toxin uptake / Kinetics / Modeling / Fluorescence / *Alexandrium minutum* / *Skeletonema costatum*

Résumé – L'enregistrement en continu de la fluorescence : un moyen pour améliorer les modèles d'accumulation des toxines paralysantes chez l'huître creuse (*Crassostrea gigas*). Un système simplifié est utilisé pour simuler l'effet d'un régime alimentaire alterné toxique et non toxique sur le comportement alimentaire et le taux d'accumulation de toxines chez l'huître. Ces conditions expérimentales ont pour objectif de restituer, autant que possible, les flux entrants et sortants d'algues toxiques dans l'embouchure de la rivière de Penzé (Bretagne Nord, France). Des paramètres physiologiques et toxicologiques sont évalués sur la base de mesures continues de fluorescence à la sortie de chaque bac expérimental contenant une huître. La variable Q_{tox}, est utilisée (et non le taux d'ingestion), elle décrit la prise de toxine prélevée par l'huître dans le milieu pour : i) des analyses graphiques simples et ii) établir des modèles à un et deux compartiments. Les résultats montrent que la prise de toxine varie fortement d'un individu à l'autre et qu'en outre, elle n'est pas proportionnelle à la concentration d'algues toxiques dans l'eau de mer. Un modèle à un compartiment, utilisant les enregistrements individuels de fluorescence comme données « d'entrée », présente des résultats discutables ; tandis qu'un modèle à deux compartiments décrit mieux les cinétiques de contamination des huîtres. Les limites de ce modèle ainsi que ses améliorations possibles sont discutées.

1 Introduction

Accumulation of phycotoxins in marine bioresources is a growing threat to aquaculture, especially bivalve mollusk culture (Shumway and Cembella 1993). The possibility of predicting the duration of harvesting closures, however, can help minimize economic losses. The development of dynamic models that take into account parameters such as the concentration of toxins in algae, environmental conditions, and toxin concentration in bivalves, may therefore prove useful. Useful information on these parameters can be found in the literature, regarding the impact of environmental conditions on the dynamics of mollusk contamination and detoxification (Bricelj and Shumway 1998). Developing models a posteriori based on studies of contamination/decontamination of bivalves in

[a] Corresponding author: rbaron@ifremer.fr

Article published by EDP Sciences and available at http://www.edpsciences.org/alr or http://dx.doi.org/10.1051/alr:2006007

coastal waters exposed to paralytic shellfish toxins (Yamamoto et al. 2003; Blanco et al. 1997; Silvert and Cembella 1995, 1998; Moroño and Blanco 1997, 1998) is a challenge and few data concerning oysters are available so far. It has been shown that good simulations of contamination episodes can be obtained with known concentrations of algae and means to control the environmental parameters that affect mollusk feeding behaviors (temperature, salinity, total seston). Some authors have tried to simulate paralytic toxin uptake (Moroño et al. 2000), while others have focused on the detoxification process, for instance, such studies have been conducted in domoic acid-contaminated shellfish (Blanco et al. 1999, 2002a,b; Douglas et al. 1997). Most of these studies concern mussel contamination and extrapolation to other bivalve species is problematic. Phycotoxin accumulation in bivalves was shown to be species-specific and to vary widely among the species (Blanco et al. 2002a).

Different models can be used to describe the relationship between shellfish toxicity and environmental parameters. Models based on uptake and clearance rates are one-compartment models where the variation in toxin concentration over time is equal to the difference between toxin input and output. These models differ in the number of compartments and classification of variables. Models describing toxin kinetics found in the literature essentially belong to the "one-compartment" type and describe the total amount of toxin accumulated per unit of weight. Phycotoxin kinetics within shellfish is the result of a balance between several processes: toxic algae ingestion, digestion, and assimilation, transfer of toxins between organs, transformation of toxins into derivatives or hydrolyzed metabolites, detoxification through various excretion processes (Blanco et al. 2003; Li et al. 2005). A complete kinetic model therefore involves the description and quantification of all of these processes.

Although a general model would constitute a convenient starting point, the fact that a limited amount of biological data is currently available prevents us from estimating a sufficient number of parameters and establishing such a model. Available biological data have most often lead to the use of simplified models with one or two artificial compartments.

Mollusks grown along the French coasts have been contaminated regularly since 1988, by ingestion of micro-algae that contain paralytic phycotoxins. As it is difficult to predict when toxic blooms responsible for shellfish contamination will occur, one of the most efficient alternatives is to encourage conditions that promote detoxification.

Previous experimental studies, carried out between 1996 and 2000, have helped elucidate the physiological response of oysters which one likely to be more or less damaged by paralytic toxins (Bardouil et al. 1996; Lassus et al. 1996, 1999; Wildish et al. 1998) – to contamination with paralytic toxins (Lassus et al. 1996, 2000). In France, two very different areas are subjected to toxic *Alexandrium* blooms: Northern Brittany (English Channel) coasts, with summer episodes of *Alexandrium minutum* (Dinophyceae), a slightly toxic species easily ingested by mussels and oysters (Morin et al. 2000), and Thau Lagoon (Mediterranean Sea), with fall episodes of *A. catenella*, a fairly toxic species which has been detected recently. *A. catenella* mainly contaminates mussels and, to a lesser extent, oysters (Masselin et al. 2001). In Northern Brittany, shellfish culture occurs in highly turbid estuarine waters subjected to tidal currents. Dissemination of toxic algae in the Thau lagoon is, on the other hand, governed by more complex hydro-ecological processes.

The general conditions prevailing during summer blooms of *A. minutum* in coastal waters of Northern Brittany, especially in the Penzé estuary estuary, have been defined based on known ecological and toxicological data (Masselin et al. 1996; Morin et al. 2000). In this paper, toxic episodes were simulated experimentally using the environmental conditions that are most commonly observed, especially the effects of tidal currents on toxic cells concentrations.

A recirculated sea water system was used to ensure continuous or semi-continuous recording of individual physiological parameters and in order to have a closer look at the relationship between the contamination level and individual physiological behaviors. With such an experimental setting the different types of physiological responses can be better identified and characterized.

In this study, several physiological and toxicological parameters were estimated based on continuous fluorescence signals detected at the outlet of each experimental tank which contained a single oyster. These methods enabled us to investigate the kinetics of toxin ingestion and release in a one-compartment model and determine the conditions that ensure optimal toxin ingestion and release.

2 Methods

2.1 Experimental system

Due to the difficulty of rapidly eliminating toxic substances excreted by oysters, such as ammonia, we restricted the number of oysters used.

The experimental system consisted of three stands on which six individual tanks, each containing a live 50 g oyster, and a control box, containing an empty oyster shell, were placed. Water was circulated in this closed system using an automated pump that alternatively drew sea water alone, or sea water containing either *Alexandrium minutum* (toxic) or *Skeletonema costatum* (non-toxic) cells, into the tanks. The automation program was developed in the laboratory and was also designed to keep cell concentration constant within the circuit. Cell concentration was continuously monitored and was determined based on the mean fluorescence detected in the feeding tank.

Oysters were alternatively exposed to a toxic diet for 2 hours and a non-toxic diet for 4 hours, which corresponds to the most extreme contamination conditions observed in the Penzé estuary (i.e., the most unfavorable conditions in terms of oyster toxicity). Fluorescence measurements were expressed as volts and were recorded continuously (Lassus et al. 1999; 2000).

Experimental conditions were as follows. Toxic diets: 5000 ± 500 *A. minutum* cells ml^{-1} with a residual concentration of the non-toxic alga *S. costatum* of 1000 to 3000 cells ml^{-1}. Non-toxic diets: $20\,000 \pm 2000$ *S. costatum* cells ml^{-1} with a residual concentration of the toxic alga *A. minutum* of 300 to 700 cells ml^{-1}. An amount of 0.5 mg L^{-1} TPM (Total

Particulate Matter) is equivalent to 120 cells ml^{-1} *A. minutum* or 1900 cells ml^{-1} *S. costatum* (Lassus et al. 1994). The experiment consisted in repeating cycles of alternated exposure to toxic and "non-toxic" diets, and was done three times at different periods (November 2001, April and November 2002). The experiment was conducted over a period of 4 days each time. A total of 63 oysters were thus tested.

2.2 Biological material

Oysters (*Crassostrea gigas*) were obtained from a producer from the Bay of Morlaix, France. They were grown on racks and controls were done to make sure they were free of paralytic toxin contamination. Epibionts were first removed. Animals (total weight: 51.3 ± 5.1 g) were then acclimated to natural sea water at 16 ± 0.5 °C for 5 to 6 days before being transferred into experimental tanks. *A. minutum* cultures (AM89BM strain) were grown in 10-L tanks using Guillard's F/2 medium, with a light intensity of $50 \pm 4\,\mu$mol photon $m^2\,s^{-1}$ and a 12h/12h L/D photoperiod until steady state was reached (constant cell density). Algal culture toxicity was quantified by ion-pairing high performance liquid chromatography (IP-HPLC), as reported below, and ranged from 1.4 ± 0.2 to 1.58 ± 0.29 pg saxitoxin equivalent (STX equiv.) per cell (at the end of the exponential growth phase) during experiments. *S. costatum* cultures were grown in 10-L tanks under the same conditions with Provasoli's ES medium (1965).

2.3 Chemical analyses

Quantification of paralytic phycotoxins in oyster flesh was performed by IP-HPLC during the exposure period, in accordance with methodology described by Oshima et al. (1995). Total flesh was ground in 0.1 N CH_3COOH (v/w) at 4 °C. Extracts were then centrifuged (3000 g, 15 min, 4 °C) and the pH adjusted to 3.0−3.5 with glacial acetic acid. Supernatants were diluted with one volume of water that had previously been subject to ultrafiltration (20 kDa) with Centrisart filters (Sartorius, Göttingen, Germany), and then stored at 4 °C until analysis. Samples (10 ml) were removed from *A. minutum* cultures at the end of the exponential growth phase. Cells were then counted with a hemocytometer and samples centrifuged (3000 g, 15 min, 4 °C). Supernatants were subsequently removed and 0.1 M acetic acid added to the pellets. Cells were then lysed by freeze / thawe methodology.

A certified PSP toxin standard (certified reference materials CRM-decarbamoyl GTX2&3) which contains gonyautoxin-2 (GTX2) at a concentration of 114 μM and gonyautoxin-3 (GTX3) at a concentration of 32 μM, was obtained from the NRC Institute for Marine Biosciences (Halifax, NS, Canada). The stock solution was diluted 1:200 and used as a standard for quantitative detection. The molar concentration of each compound, i.e. GTX2, GTX3 and C Toxins (Lassus et al. 1994) in either *A. minutum* cultures or in contaminated oysters, was converted into μg saxitoxin (STX) equiv.100 g^{-1} of bivalve flesh using the conversion factors determined by Oshima (1995), i.e., 297 μg STX equiv.μM^{-1} for GTX3, and 168 μg STX equiv.μM^{-1} for GTX2. The values thus obtained were noted C_f in this paper.

2.4 Physiological analyses

Because a destructive method was used for chemical analyses, we were only able to get one toxicity value per individual at the end of the experiment. It is, however, reasonable to assume that contamination affects the animal's physiology, and that the regular monitoring of individual physiological parameters provides an estimation of the contamination kinetics which can be adjusted based on chemical analysis data.

The biodeposition rate (*BR*), i.e., the amount of faeces and pseudofaeces produced per unit of time, is expressed in mg $h^{-1}\,g^{-1}$ dry weight. It corresponds to the sum of the rejection rate (*RR*) and the egestion rate (*ER*). Therefore, $BR = RR + ER$. This parameter may be an indicator of the relative toxin excretion rate when $RR \approx 0$ (see below).

Another important physiological parameter which can be used to estimate contamination kinetics (Model 1) is the toxin ingestion rate (*TIR*), which can be expressed as follows:

$$TIR = \frac{TN_a}{p_a N_a + p_s N_s}(FR - RR) \quad (1)$$

where T is the cellular toxin content of *A. minutum* (1.4 to 1.6 pg STX equiv. per cell in our experiments), N_a, *A. minutum* cell density (cells per ml), N_s, *S. costatum* cell density (cells per ml), p_a, the weight of a cell of *A. minutum*, p_s, the weight of a cell of *S. costatum*, *FR*, the filtration rate, i.e., the amount of food filtered by an oyster per unit of time per dry weight of oyster flesh (mg $h^{-1}\,g^{-1}$), *RR,* the rejection rate, i.e., the amount of pseudofaeces produced per unit of time (mg $h^{-1}\,g^{-1}$). As the amount of pseudofaeces was negligible in all of the experiments carried out during this study, $RR = 0$.

Feeding behavior provides a good indicator of the filtration rate. Discrete monitoring of shell valve activity was first considered (Lassus 1992), however, the percentage of time devoted to feeding (FTA: Feeding Time Activity) and the estimated toxin uptake (based on on-line fluorescence detection of cells) proved more useful, as information on valve activity could not discriminate feeding from oxygen consumption.

The semi-continuous and simultaneous monitoring of the fluorescence measured at the outlet of experimental and control tanks allowed us to estimate the FTA for each oyster, i.e., the percentage of time during which shellfish consumes microalgae, as well as the amount of toxin removed from the medium per unit of time per 100 g of oyster flesh (Q_{tox}).

The FTA was only considered significant when the retention rate of food particles was at least 5% (Bougrier et al. 2001).

Measured fluorescence is strongly dependent on the concentration of *A. minutum* and *S. costatum* in sea water, however, the relationship between these two factors is not linear. This relationship was shown to vary according to the fluorescence recording unit used and the period at which the experiments were carried out (e.g. all R^2 values obtained were higher than 90% from date to date). The equation reflecting the relationship was adapted for each experiment, which enabled us to quantify the toxic algal biomass in the system based on daily assessments of *S. costatum* /*A. minutum* ratios (cell counts). Fluorescence recordings, cell counts, mean algal cell toxicity, and flow rates in each experimental tank were used to quantify

toxin uptake per unit of time for each oyster, according to the following Model (2):

$$Q_{\text{tox}} = 100 \frac{TQ}{P\left(\alpha + \dfrac{\beta}{Y_{A,B}}\right)} (y_i - y) \tag{2}$$

with Q, the flow rate in the experimental tank (ml d^{-1}), P, the wet weight of oyster flesh (g), Y, *A. minutum*/*S. costatum* ratio (A: toxic diet and B: non-toxic diet)[1], α and β, the regression coefficients in the following equations: $y_i = \alpha N_{ai} + \beta N_{si}$ and $y = \alpha N_a + \beta N_s$, where y_i and y represent fluorescence signals and i, the "input" values in the experimental tanks.

When biodeposits exclusively consist of faeces ($RR = 0$), which was the case most of the time, toxin ingestion rate (*TIR*) may be expressed as follows: $TIR = 1 \times 10^{-5} \times Q_{\text{tox}} \times (1 - \text{H}/100)$, with H, water content of oyster flesh (%).

2.5 Estimation of contamination kinetics

Based on previous observations, individual contamination kinetics can be estimated using this one-compartment model (Model 3):

$$\frac{\mathrm{d}C(t)}{\mathrm{d}t} = Q_{\text{tox}} - kC(t) \tag{3}$$

with $C(t)$, the estimated toxicity per 100 g of oyster flesh. The k value, a depuration rate coefficient, is adjusted for each oyster tested in order to reach the reference toxicity value at the time of sampling, i.e., the value determined based on the chemical analysis or $C(t_f) = C_f$.

The same model (Model 3) can be used to estimate the average contamination kinetics using mean Q_{tox} and mean C_f values to adjust the k value.

A two-compartment model, described by a system of equations (4), can also be considered to estimate the average contamination kinetics. In these equations C_1 and C_2 represent toxin concentration in the first and second compartment, C the global toxicity per 100 g of oyster flesh, respectively, k_1 and k_2, depuration rate constants in the first and second compartment, respectively, and $k_{1,2}$, the transfer rate of toxin between the first and the second compartment.

$$\begin{aligned}
\frac{\mathrm{d}C_1(t)}{\mathrm{d}t} &= Q_{\text{tox}} - (k_1 + k_{1,2})C_1(t),\\
\frac{\mathrm{d}C_2(t)}{\mathrm{d}t} &= k_{1,2}C_1(t) - k_2C_2(t),\\
C(t) &= C_1(t) + C_2(t).
\end{aligned} \tag{4}$$

3 Results

Preliminary studies of the FTA (data not shown) have clearly shown that the FTA$_{\text{ske}}$, corresponding to FTA during *S. costatum* diet, plays an important role in the contamination process, however, the FTA only reflects indirectly oyster ingestion and / or contamination rates and exploiting this parameter is definitely not an easy task. Observed feeding behaviors are, on the other hand, more closely related to Q_{tox}, a more accurate parameter than the semi-quantitative FTA. Therefore, we decided to focus on Q_{tox} in this paper.

Q_{tox} data clearly revealed high individual variations. Under the same experimental conditions, all oysters did not actively feed on algae the same way: some oysters fed during the toxic-diet cycle, others during the "non-toxic" diet cycle (i.e., with a low concentration of toxic *A. minutum*), and others, during both types of cycles.

When looking at individual oyster's toxicity by plotting both the Q_{tox} values obtained with toxic and non-toxic feeds (Fig. 1), we observed that individual Q_{tox} values obtained with the non-toxic diet were not negligible although most individual oysters Q_{tox} values were higher with the toxic diet. This indicates a significant effect of the non-toxic diet on toxin uptake. The same pairs of data were used to compare the lowest and highest mean Q_{tox} values for each animal (we simply used the arithmetic mean of values obtained with the non-toxic or toxic feeds for each oyster). Besides, the lowest and highest mean Q_{tox} values on a day varied by a factor of 40, which indicates that under identical experimental conditions, some oysters are able to exhibit a high toxin uptake whereas others only exhibit moderate or low toxin uptake.

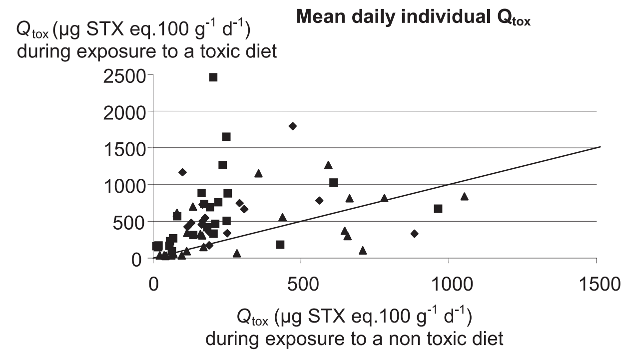
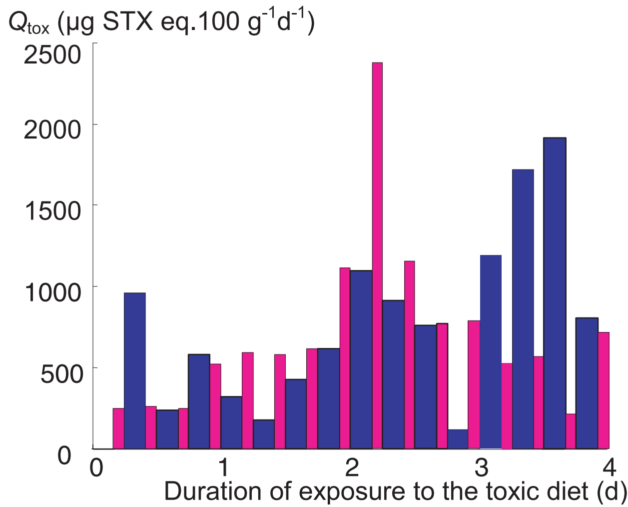


Fig. 1. Relationship between mean daily individual Q_{tox} (toxin uptake in the water) values recorded during exposure to toxic (x axis) or non-toxic (y axis) diets.

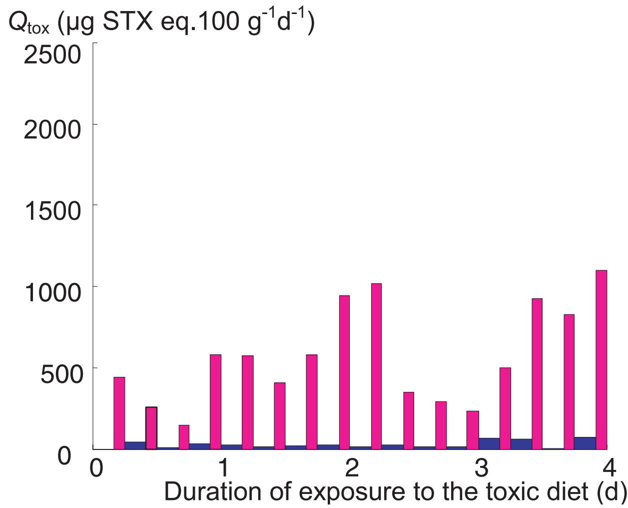
Calculation of the mean Q_{tox} with each type of diet highlighted the significant effect of *A. minutum* when oysters were fed non-toxic algae: most of the oysters tested seemed to have bioaccumulated paralytic shellfish toxins whatever the diet (Fig. 2a). A small number of oysters, showed a specific increase of their Q_{tox}, i.e., only when fed with the toxic diet (Fig. 2b).

The experiment was repeated three times, each time at a different period, under the same experimental conditions (Figs. 3a,b,c). In November 2001, the Q_{tox} value was shown to decrease on day 3 of the experiment. Such a decrease was also identified by HPLC (C_f) (Lassus et al. 2004). Once again, although experimental conditions were identical, slight variations in the overall trend of Q_{tox} were observed from one experiment to another. Q_{tox} values were lower than 400 μg STX equiv. 100 g^{-1} (Fig. 3a) in November 2001

[1] This ratio is considered constant during a given diet and is frequently checked by discrete cell counts. In such experimental conditions, comparison between sea water and biodeposits indicated a lack of pre-ingestion sorting.



(a)

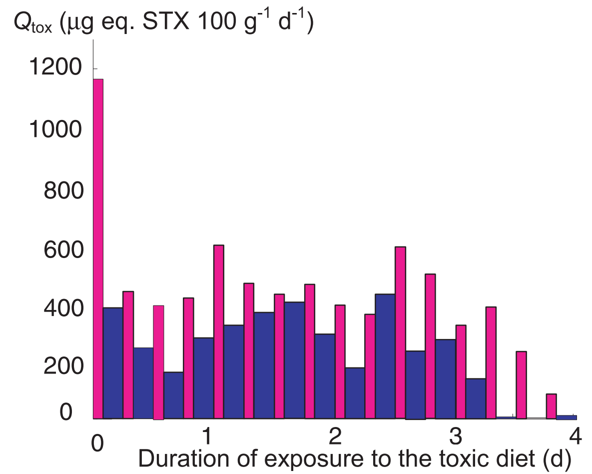


(b)

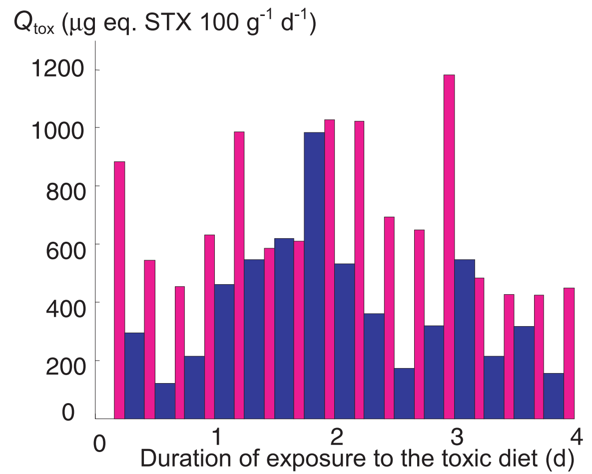
Fig. 2. (a) Oyster for which toxin uptake occurred during exposure to both the toxic and non-toxic diets. (b) Oyster for which toxin uptake mostly occurred during exposure to the toxic diet. Experimental conditions – ■ Toxic exposure phases corresponding to *A. minutum* (4000 to 6000 cells ml^{-1}) and *S. costatum* (500 to 3000 cells ml^{-1}); ■ Non-toxic exposure phases corresponding to *A. minutum* (300 to 800 cells ml^{-1}) and *S. costatum* (18 000 to 22 000 cells ml^{-1}). Frequency and successive exposure durations: every 6 hours, i.e. 2 h-exposure for toxic phase and 4 h- for non-toxic phase.

and April 2002, however, a peak (800 μg STX equiv.100 g^{-1}) was observed on day 2 in April 2002 (Fig. 3b). However, in November 2002, however, mean Q_{tox} values were a little higher, on the order of 500 μg STX equiv.100 g^{-1} (Fig. 3c), but all those variations could be considered as slight variations.

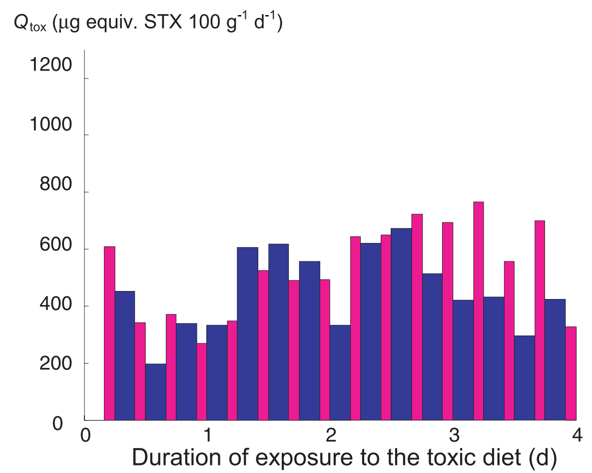
Based on observations made on oysters sampled on day 4 in all three experiments, the one-compartment model (Model 3) revealed that the average trend was a gradual contamination of oysters which is slightly less important between day 3 and day 4 (Fig. 4a). The one-compartment model, on the other hand, with Q_{tox}, and C_f values established based on



(a)



(b)



(c)

Fig. 3. Amount of toxin removed from the medium per unit of time, per 100 g of oyster flesh (Q_{tox}) in all three experiments. (a) November 2001, (b) April 2002, (c) November 2002. Experimental conditions – ■ Toxic phases corresponding to *A. minutum* (4000 to 6000 cells ml^{-1}) and *S. costatum* (500 to 3000 cells ml^{-1}); ■ Non-toxic phases corresponding to *A. minutum* (300 to 800 cells ml^{-1}) and *S. costatum* (18 000 to 22 000 cells ml^{-1}).

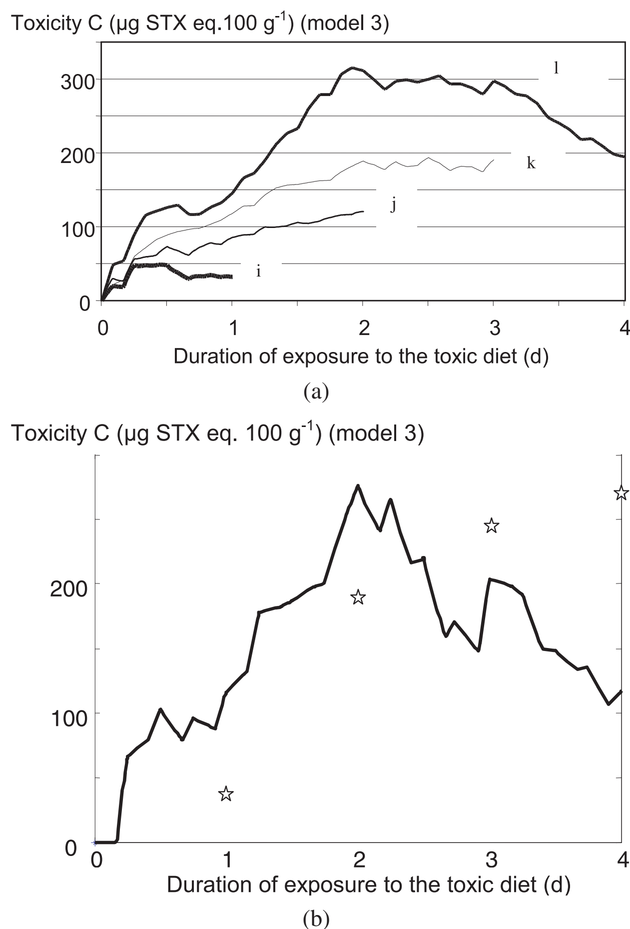


Fig. 4. One-compartment model (Model 3). (a) Mean toxification kinetics of all Nov. 2001, April 2002, Nov. 2002 experiments (i: mean kinetics of oysters extracted after one day of exposure, j: after two days of exposure, k: after three days of exposure, l: after four days of exposure); (b) Model using k an average depuration rate coefficient, $k = 5$. Q_{tox} and C_f (final toxicity per 100 g of oyster) values based on data obtained in April 2002. Black stars represent mean C_f values.

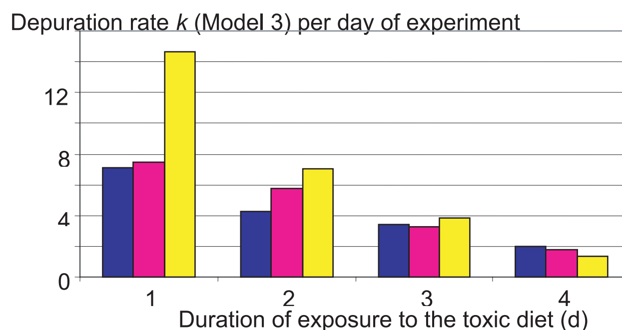


Fig. 5. Mean k depuration rate coefficient values based on measurements made on oysters sampled on day 1, 2, 3, and 4 in all three experiments – November 2001, April 2002, November 2002.

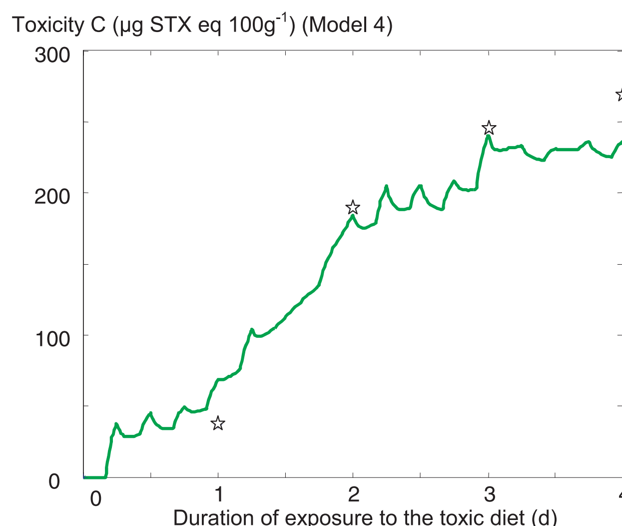


Fig. 6. Two-compartment model (Model 4), Q_{tox} and C_f values were based on data obtained in April 2002, with $k_1 = 16$, $k_{12} = 4.5$, $k_2 = 0.2$.

data collected in April 2002 and a depuration rate coefficient k of 5 (Fig. 4b), seemed inappropriate, as demonstrated by the comparison with mean C_f values.

This concurs with the analysis of the k values obtained based on the analysis of samples harvested at different times during all three experiments (Fig. 5). The detoxification coefficient was shown to decrease with time.

A two-compartment model, such as that expressed by Model 4, with a detoxification coefficient adjusted based on mean C_f values, would better reflect contamination / decontamination kinetics as shown in Fig. 6, where the model reflects observed C_f values obtained in April 2002.

4 Discussion

This work underlines the importance of individual fluorescence signals, and appropriate treatment. Q_{tox} values calculated based on continuous recordings in experimental settings where oysters are supplied with alternated feeds, closely resembling field conditions during PSP outbreaks in the Penzé estuary. These Q_{tox} values can provide useful information on individual toxin ingestion rates. Our results show clearly individual variability in feeding behaviors, which partly explains the difficulty to obtain a model that accurately describes contamination / detoxification kinetics in oysters.

In particular, the observed high individual variability in Q_{tox} value might explain variability of toxin contents reported frequently in the literature for different shellfish species (Lassus et al. 2004; Bricelj and Shumway 1998; Brijcel et al. 2000). These results therefore suggest that the FR can vary greatly based on the type of microalgae available in the environment or their concentration. With a concentration of 5000 algal cells ml^{-1}, the feeding activity of oysters as well as the FR were shown to decrease. However, Q_{tox}, remained high due to the large amount of toxin contained in the ingested algae. Conversely, while exposed to the non-toxic diet, oysters were also shown to feed actively but were less selective (as reflected by the steady Y value, cf. Eq. (2), throughout each

diet cycle), which means that ingestion of residual toxic algae was significant. In these cases, the Q_{tox} value remained high. The mean *FR* calculated, based on the mean Q_{tox} for all of the oysters tested, when fed toxic and non-toxic diets was close to 0.05 mg h^{-1} g^{-1} and 0.125 mg h^{-1} g^{-1}, respectively. These values are consistent with the biodeposition rates recorded in other studies of contamination of oysters exposed to monospecific toxic diets (Lassus et al. 1999, 2000).

Models of toxin ingestion developed based on experiments using toxic and non-toxic algae-based diets or diets consisting of particulate mineral matter (as main variables) should be investigated more extensively to allow for the development of toxin uptake/elimination models. Our results clearly demonstrate that models, such as most of those reported in the literature, in which the filtration rate *FR* is considered to be directly proportional to the clearance rate (CR) and/or the concentration in toxic algae, are unlikely to describe this process properly.

However, all *k* values were relatively high compared to the detoxification rates observed under different experimental conditions (longer periods of time and monospecific diets) such as those used in previous experiments (Lassus et al. 1999, 2001). It was shown they were decreasing with time.

Adjustment of a one-compartment model based on Model 3 also demonstrated that the depuration rate decreases with time. Fitting these models based on data obtained with oysters that have been exposed to contaminated feeds for 1 day, provides higher detoxification rates than those derived from models based on data obtained with oysters that have been exposed to contaminated feeds for 4 days. A two-compartment model obviously fits better with the observed values, as demonstrated (Fig. 6) and suggested by Blanco (pers. comm.).

Finally, taking into account toxin distribution and biotransformation processes in oyster tissues may help characterize bioaccumulation pathways.

References

Bardouil M., Bohec M., Bougrier S., Lassus P., Truquet P., 1996, Feeding responses of *Crassostrea gigas* (Thunberg) to inclusion of different proportions of toxic dinoflagellates in their diet. Oceanol. Acta. 19, 177-182.

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., Fernandez M.L., Miguez A., Moroño A., 1999, Okadaic acid depuration in the mussel *Mytilus galloprovincialis*: one- and two-compartment models and the effect of environmental conditions. Mar. Ecol. Prog. Ser. 176, 153-163.

Blanco J., Acosta C.P., Bermudez de la Puente M., Salgado C., 2002a, Depuration and anatomical distribution of the amnesic shellfish poisoning (ASP) toxin domoic acid in the king scallop *Pecten maximus.* Aquat. Toxicol. 60, 111–121.

Blanco J., Bermudez de la Puente M., Arevalo F., Salgado C., Moroño A., 2002b, Depuration of mussels (*Mytilus galloprovincialis*) contaminated with domoic acid. Aquat. Living Resour. 15, 53-60.

Blanco J., Reyero M.I., Franco J.M., 2003, Kinetics of accumulation and transformation of paralytic shellfish toxins in the blue mussel *Mytilus galloprovincialis*. Toxicon 42, 777-784.

Bricelj M., Shumway S., 1998, Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics and biotransformation. Rev. Fish. Sci. 6, 315-383.

Brijcelj M., MacQuarrie S., Twarog M., 2000, Differential sensitivity and uptake of PSP toxins within and between softshell clam (*Mya arenaria*) populations from Atlantic Canada. In: Proc. 9th Int. Conf. Harmful algal blooms, 7-11 Febr. Hobart, Tasmania, abstract book, 9.

Douglas D., Kenchington E., Bird C., Pocklington R., Bradford B., Silvert W., 1997, Accumulation of domoic acid by the sea scallop (*Placopecten magellanicus*) fed cultured cells of toxic *Pseudo-nitzschia* multiseries. Can. J. Fish. Aquat. Sci. 54, 907-913.

Lassus P., 1992, Accumulation des biotoxines par les coquillages. In: Coquillages et santé publique : du risque à la prévention. Lesne (Ed.), ENSP, Rennes, pp. 79-98.

Lassus P., Ledoux M., Bardouil M., Bohec M., Erard-Le Denn E., 1994, Kinetics of *Alexandrium minutum* Halim toxin accumulation in mussels and clams. Nat. Toxins 2, 329-333.

Lassus P., Wildish D., Bardouil M., Martin J.L., Bohec M., Bougrier S., 1996, Ecophysiological study of toxic *Alexandrium* spp effects on the oyster *Crassostrea gigas.* In: Yasumoto, T., Oshima, Y., Fukuyo, Y. (Eds.), Harmful and Toxic Algal Blooms, IOC-UNESCO, 409-412.

Lassus P., Bardouil M., Beliaeff B., Masselin P., Naviner M., Truquet P., 1999, Effect of a continuous supply of the toxic dinoflagellate *Alexandrium minutum* Halim on the feeding behavior of the Pacific oyster (*Crassostrea gigas* Thunberg). J. Shellfish Res. 18, 211-216.

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.

Lassus P., Baron R., Garen P., Truquet P., Masselin P., Bardouil M., Leguay D., Amzil Z., 2004, Paralytic shellfish poison outbreaks in the Penzé estuary: Environmental factors affecting toxin uptake in the oyster, *Crassostrea gigas*. Aquat. Living Resour. 17, 207-214.

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., Le Saux J.C., Le Gal D., Lassus P., 1996, Dosage par CLHP des toxines paralysantes de coquillages contaminés par *Alexandrium minutum* en rivières de Morlaix et de la Penzé en 1994. Rapp. Interne Ifremer Nantes DEL/96.04.

Masselin P., Amzil Z., Abadie E., Carreras A., Chiantella C., Le Bec C., Nézan E., Truquet P., 2001, Paralytic shellfish poisoning on French Mediterranean coast in the autumn of 1998: *Alexandrium tamarense* as the causative agent. In: Hallegraeff G.M., Blackburn S.I., Bolch C.J., Lewis R.J. (Eds.), Harmful Algal Blooms 2000. IOC-UNESCO, 26-29.

Morin P., Erard-Le Denn E., Maguer J.F., Madec, C., Videau, C., Legrand, J., Macé, E., 2000, Étude des causes de prolifération de microalgues toxiques en mer : cas d'*Alexandrium*. Final AELB Rep. Convention 7.98.9476.

Moroño A., Blanco J., 1997, Modelling the kinetics of PSP toxin uptake and release in mussels. Harmful Algae News, IOC-UNESCO, 16, 10-12.

Moroño A., Maneiro J., Pazos Y., Blanco J., 1998, Modelling the accumulation of PSP toxins in Galician mussels: results and perspectives. In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T. (Eds.), Harmful Algae, Xunta de Galicia, IOC-UNESCO, 441-443.

Moroño A., Franco J., Miranda 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* Lmk). J. Exp. Mar. Biol. Ecol. 257, 117-132.

Oshima Y., 1995, Chemical and enzymatic transformation of paralytic shellfish toxins in marine organisms. In: Lassus P., Arzul G., Erard E., Gentien P., Marcaillou C. (Eds.), Harmful Marine Algal Blooms. Lavoisier, Paris, pp. 475-480.

Shumway S.E., Cembella A.D., 1993, The impact of toxic algae on scallop culture and fisheries. Rev. Fish. Sci. 3, 65-105.

Silvert W., Cembella A.D., 1995, Dynamic modelling of phycotoxin kinetics in the blue mussel, *Mytilus edulis*, with implications for other marine invertebrates. Can. J. Fish. Aquat. Sci. 52, 521-531.

Silvert W., Bricelj M., Cembella A.D., 1998, Dynamic modelling of PSP toxicity in the surfclam (*Spisula solidissima*): multicompartmental kinetics and biotransformation. In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T., (Eds.), Harmful Algae, Xunta de Galicia IOC-UNESCO, pp. 437-440.

Vila M., Delgado M., Camp J., 2001, First detection of widespread toxic events caused by *Alexandrium catenella* in the Mediterranean Sea. In: Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J. (Eds.), Harmful Algal Blooms 2000. IOC-UNESCO, pp. 8-11.

Wildish D., Lassus P., Martin J., Saulnier A., Bardouil M., 1998, Effect of the PSP-causing dinoflagellate, *Alexandrium* sp., on the initial feeding response of *Crassostrea gigas*. Aquat. Living Resour. 11, 35-43.

Yamamoto T., Flynn K., Takayama H., 2003, Application of a two-compartment one-toxin model to predict the toxin accumulation in Pacific oysters in Hiroshima Bay, Japan. Fish. Sci. 69, 944-950.