ELSEVIER

Aquat. Living Resour. 15 (2002) 53–60

Aquatic Living Resources

www.elsevier.com/locate/aquliv

Depuration of mussels (*Mytilus galloprovincialis*) contaminated with domoic acid

Juan Blanco [a,*], Magdalena Bermúdez de la Puente [b], Fabiola Arévalo [b], Covadonga Salgado [b], Ángeles Moroño [b]

[a] *Centro de Investigacións Mariñas, Pedras de Corón s/n, Aptdo. 13, 36620 Vilanova de Arousa, Spain*
[b] *Centro de Control da Calidade do Medio Mariño, Peirao de Vilaxoán, 36600 Vilagarcía de Arousa, Spain*

Received 7 August 2000; accepted 12 September 2001

Abstract

Domoic acid is a neurotoxin responsible for the Amnesic Shellfish Poisoning (ASP). With the aim of determining its depuration kinetics in raft mussels *Mytilus galloprovincialis* and the effects of body weight, salinity and temperature on it, an experiment involving these factors was carried out. Mussels which had incorporated this toxin, to a concentration of 153 $\mu g \cdot g^{-1}$ of soft tissue, during a bloom of the pennate diatom *Pseudo nitzschia australis*, were collected from the Galician Rías and placed at temperatures of 18 and 22 ºC and salinities of 12.5 and 31. Mussel samples were taken at the start of the experiment and daily during the four subsequent days and the domoic acid contents of the soft tissues were analysed. The simple one-compartment kinetics, that is a kinetics in which all toxin depurates at the same rate, was fitted to the data, with good quantitative results. Notwithstanding, the deviations of the model from the actual data were dependent on time, suggesting that such a simple model is not enough to correctly describe the data, and that a more complex kinetics may be more adequate. A two-compartment kinetic model, in which two pools of toxins exist (compartments), each one depurating at different rate, described qualitatively better the depuration but its quantitative contribution to the fit was not statistically significant. The parameters of the model, obtained by least squares fitting, suggest the possibility of a small second compartment of very small or null depuration rate, as detected in other species. This kind of model would explain the reduced quantitative contribution of the second (slowly depurating) compartment. Low salinity was shown to reduce the depuration rate. The two other factors checked, temperature and body weight, nor any interaction, had significant effect on depuration rate.

Resumen

Depuración del ácido domoico de mejillones (*Mytilus galloprovincialis*). Con objeto de determinar la cinética de depuración del ácido domoico, una neurotoxinas productora de toxicidad de tipo amnésico (ASP), se recogieron mejillones de batea *Mytilus galloprovincialis*, que habían incorporado esta toxina durante una floración de la diatomea pennada *Pseudo-nitzschia australis*, hasta alcanzar una concentración de 153 $\mu g.g^{-1}$ de vianda. Los mejillones, procedentes de las Rías Gallegas, se colocaron a 18 y 22º C de temperatura y a 12.5 y 31 de salinidad. Se obtuvieron muestras de dichos mejillones al comienzo del experimento y durante los cuatro dias siguientes y se determinó la concentración de ácido domoico presente en ellos. Una cinética simple, de un compartimento, o lo que es lo mismo, una cinética en la cual toda la toxina se depura a la misma velocidad, se ajustó cuantitativamente bien a los datos obtenidos. Sin embargo, las diferencias entre el modelo y los datos reales resultaron dependientes del tiempo, sugiriendo que un modelo tan simple no es suficiente para describir correctamente la depuración y que, por tanto, una cinética más compleja debe ser más adecuada. Un modelo de dos compartimentos, en el cual parte de la toxina se depura rápidamente y otra parte a mucha menor velocidad, describió cualitativamente mejor la cinética de desintoxicación, pero no produjo una mejora estadísticamente significativa del ajuste. Los parámetros de los modelos, obtenidos por medio de mínimos cuadrados, sugieren la posibilidad de que exista que realmente exista un segundo compartimento constituido por una pequeña porción del ácido domoico que no se depura, lo cual se ha descrito previamente en otras especies. Este tipo de modelo explicaría la pequeña contibución cuantitativa de la inclusión del segundo compartimento. La salinidad baja redujo la tasa de depuración. Ninguno de los otros dos factores probados, temperatura y peso de vianda, ni las interacciones entre cualquiera de ellos resultó

* Corresponding author. Fax: +34 986 506788.
E-mail address: jblanco@cimacoron.org (J. Blanco).

PII: S0990-7440(01)01139-1

tener un efecto significativo en la tasa de depuración.

Keywords: Phycotoxins; ASP; Amnesic shellfish poisoning; Depuration kinetics; Models; Temperature; Salinity; *Mytilus galloprovincialis*

1. Introduction

Since the Amnesic Shellfish Poisoning (ASP) was first recognised in Canada (Bates et al., 1989), this kind of toxicity was recorded in many areas all over the world (Hallegraeff, 1993). Its presence in the Galician Rías (NW Spain) was first detected in cultured mussels (*Mytilus galloprovincialis*) concurrently with a natural bloom of *Pseudo-nitzschia* spp., dominated by *P. australis*, that took place in 1994 in the Ría de Muros (Míguez et al., 1996). In subsequent years, several ASP episodes were again detected, affecting several bivalve species but with a major impact on the king scallop (*Pecten maximus*) fishery and the mussel culture. In the first case due to the long persistence of the toxins in the bivalve and in the second, because the foremost importance of mussel culture in the area (with a production of ca. 200 t·year^{-1}). The episodes in which mussels became affected were, in general, short-lived while those in big scallops, persisted for months or even years (Arévalo et al., 1997).

The ability to accumulate domoic acid, the toxin responsible for ASP, differs greatly between species, as it has been shown in several studies (*Mytilus edulis*, Novaczek et al., 1992; *M. edulis* and *P. magellanicus*, Wohlgeschaffen et al., 1992; *Volsella modiolus*, Gilgan et al., 1990; *Siliqua patula*, Drum et al., 1993; *Mytilus californianus*, Whyte et al., 1995). Although most of these studies were not designed to elucidate the depuration kinetics, two main depuration behaviours can be inferred. One is an exponential decrease in the domoic acid content, that would be easily described by means of a one-compartment model (*Mytilus edulis*, Novaczek et al., 1992; *M. edulis* and *P. magellanicus* Wohlgeschaffen et al., 1992). The other is a bi-phasic depuration, in which the elimination of domoic acid takes place quickly at the initial depuration steps but becomes slower as depuration progresses. The latter kinetics would need, at least, a two-compartment model to be described. This kinetics probably apply to the razor clams (*Siliqua patula*), taking into account the observations made by Horner et al. (1993) suggesting that this species is able to depurate domoic acid in their natural environment but maintain a low toxin level for long periods. This is also the case with the scallop *Placopecten magellanicus* (Gilgan et al., 1990; Douglas et al., 1997) and *Volsella modiolus* (Gilgan et al., 1990).

The only attempts to model the accumulation of domoic acid in bivalves were carried out by Silvert and Subba Rao (1992) in the mussel *Mytilus edulis*, and by Douglas et al. (1997) in the scallop *Placopecten magellanicus*. Both studies tried a mono-compartmental model and they both showed some discrepancies with the actual data that, in the former, the authors linked to a number of different causes, including the possible need for a second compartment.

The effects of environmental conditions and bivalve size on the depuration of domoic acid were the object of even fewer studies, with the factors examined being restricted to temperature (Silvert and Subba Rao, 1992; Novaczek et al., 1992), salinity (Novaczek et al., 1992), amount of food and mussel size (Wohlgeschaffen et al., 1992).

No information is available on the accumulation or depuration kinetics of domoic acid in *Mytilus galloprovincialis*, the most important bivalve species in Galicia and in the Mediterranean Sea, and consequently, one of the most important species world-wide. In this study we have tried to model the depuration kinetics of domoic acid in this species and to quantify the effect of mussel size and two important environmental variables, temperature and salinity, on its the depuration rate.

2. Material and methods

2.1. Experimental and analytical techniques

Mussels, *Mytilus galloprovincialis*, were obtained from a raft in the Ría de Muros (Galicia, NW Spain) where a toxic bloom (that last for approximately two weeks) of *Pseudo-nitzschia australis*, was detected (monitoring run by the Centro de Control da Calidade do Medio Mariño). The domoic acid concentration in mussels is routinely quantified by the Centro de Control de Calidade do Medio Mariño, using the same extracts than those of the standard determination of Paralytic Shellfish Poisoning toxicity by mouse bioassay, and therefore, from each mussel sample obtained, once in the lab, a subsample was taken to give 100 g of soft tissues, and extracted with water acidified with HCl to a pH between 3 and 4. These subsamples were analysed by High Performance Liquid Chromatography with Ultraviolet detection (HPLC-UV) (Lawrence et al., 1991). When the domoic acid concentration reached a level that was considered high enough to be useful for this study, the remaining mussels of the sample were distributed into three groups of 24 mussels. Two of them included mussels from 30 to 96 mm long that were split into six size classes and placed into 120 litre tanks with water at 22 ºC but with different salinities (12.5 and 31). The third group (initially belonging to a different experiment), that included a shorter size range of mussels, was also placed into a 120 litre tank but at 18 ºC

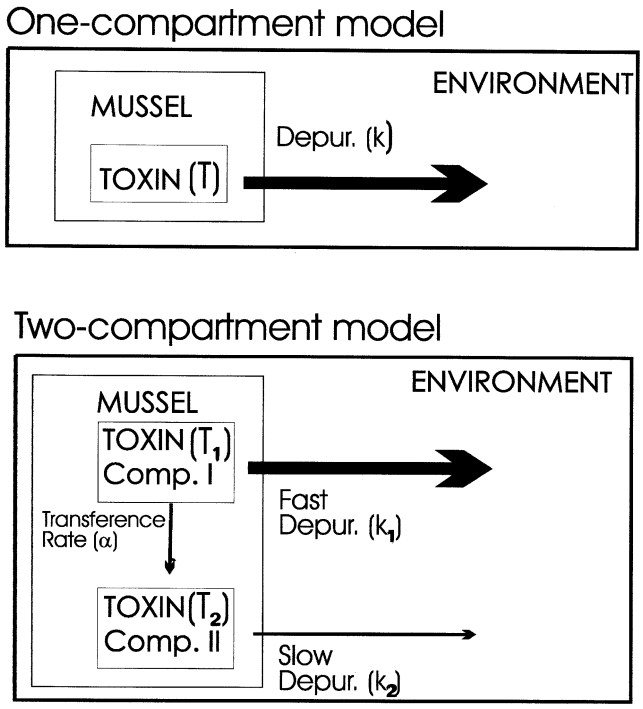

Fig. 1. Conceptual representation of the one-compartment and two compartment models. Bracketed letters correspond to those in the differential equations shown in the Material and Methods section.

and 31. Mussels were not fed during the experiment because two reasons: a) from the monitoring data it was clear that the depuration rate was very high, and b) experiments with *Mytilus edulis* and domoic acid (Wohlgeschaffen et al., 1992), and with *M. galloprovincialis* with other toxins (Blanco et al., 1997; Blanco et al., 1999) showed that the effect of food is small. A six mussel sample was obtained daily by taking one mussel of each size class from the first two tanks and at random from the third one (total number of samples was 72 plus the initial estimate which was shared for all treatments). Mussels were then measured, weighed, the whole mass of soft tissues homogenised, extracted and analysed by HPLC-UV following the procedure proposed by Lawrence et al. (1991).

2.2. Modelling

One- and two-compartment models (Fig. 1) were implemented and fitted to the data, using Matlab, Matlab Simulink and Matlab Optimization Toolbox, using least squares. The models fitted to the data were formulated as follows:

2.2.1. Models with rates independent from the environmental conditions

One-compartment model:

$$\frac{dT}{dt} = -kt$$

Two-compartment model:

$$\frac{dT_1}{dt} = -k_1 T - \alpha T_1$$

$$\frac{dT_2}{dt} = -k_2 T_2 + \alpha T_1$$

$$T = T_1 + T_2$$

T: total domoic acid concentration; T_1 and T_2: domoic acid in compartment 1 and 2, respectively; k: depuration rate; k_1 and k_2: depuration rates of the first and second compartments, respectively; α: transfer rate from compartment 1 to compartment 2.

2.2.2. Models with rates dependent on the environmental conditions

Two models were used with the depuration and transfer rates defined as a linear combination of temperature and salinity. All the rates in these models have therefore the form:

$$\text{rate} = a + b \times \text{temperature} + c \times \text{salinity}\ .$$

All models were implemented with MATLAB 5.3. Fitting was obtained by means of least squares minimisation.

2.3. Data processing

The experimental data were analysed by multiple regression using the statistical package Minitab 12.21. Assuming that all the toxin contained in the mussels has the same depuration kinetics and, therefore, a one compartment depuration kinetics, assuming also a negative exponential curve as depuration model, the parameters of such a model can be obtained by linear regression applied to the logarithmically transformed concentrations of domoic acid in the tissues. Consequently, the original data were transformed logarithmically and the depuration rate estimated by regression. The dependence of the depuration rate (coefficient of depuration time) on temperature, salinity and mussel weight, was checked by including in the multiple regression, as independent variables, the depuration time and its interactions with those factors, using dummy variables (Table 1) (Kleinbaum et al., 1988). To evaluate the possible non-linear response of the domoic acid content to the independent variables, quadratic terms were included in the equations, by including each independent variable centered and squared (calculated by squaring the original variables after substraction of the mean and division by the standard deviation), in the multiple regression.

Table 1
Equations fitted to estimate the effects of the studied factors, their corresponding explained sum and mean squares and the significance of the different treatments. A and A_0 are the natural logarithms of domoic acid concentration and initial domoic acid concentration, respectively, t is time (in days). Z_S and Z_T are the dummy variables which describe the treatments, and Z_S t and Z_T t are their interactions with time (i.e. their effects on the depuration rate). All k are the regression coefficients. The significance of the effect of each factor on the toxin contents of the bivalves were estimated by comparing the explained mean squares by two equations, one containing and other not containing it (significance of the progressive inclusion of variables in the regression). Time, salinity and temperature ranges are 0-4 days, 12.5-31 and 18 °C-22 °C, respectively. Weight (g): wet tissue of mussel

Sources of variability	Regression equation A =		Sum of Squares	Mean Squares
Time	$A_0 - k\,t$	[1]	15.12	
Time and salinity	$A_0 - k\,t + k_S\,Z_S\,t$	[2]	21.23	0.559
Time and temperature	$A_0 - k\,t + k_T\,Z_T\,t$	[3]	18.18	0.607
Time and salinity and temperature	$A_0 - k\,t + k_S\,Z_S\,t + k_T\,Z_T\,t$	[4]	21.46	0.564
Time and salinity and temperature and weight	$A_0 - k\,t + k_S\,Z_S\,t + k_T\,Z_T\,t + k_w\,W$	[5]	22.30	0.560

Significance of the progressive inclusion of variables in the regression	Equations compared	F ratio	Significance level
Salinity	[2] with [1]	10.92	**
Temperature	[3] with [1]	5.04	*
Temperature after including the effect of salinity	[4] with [2]	0.41	NS
Weight after including the effects of temperature and salinity	[5] with [4]	1.52	NS

NS: not significant

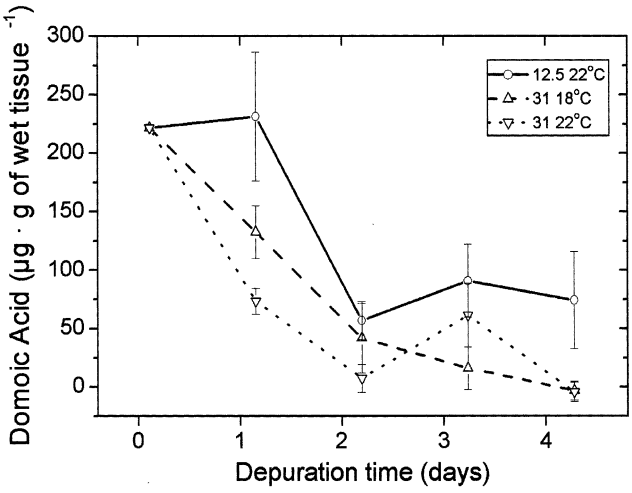

Fig. 2. Means of domoic acid concentration during the experiment (symbols) and their correspondent standard errors (vertical lines).

3. Results

Domoic acid concentration in mussels decreased drastically during the experiment. Depending on the conditions, the reduction was between 56 and 86% (Fig. 2).

When the multiple regression was fitted, only depuration time and its interaction with salinity were found to have significant effect on domoic acid concentration (Table 1). The remaining variables –temperature and body weight- had no significant effect on domoic acid concentration neither did any quadratic term. When the effects of salinity and temperature are considered together, it can be observed that this later factor had a small negative effect on the estimated depuration rate (Table 2). Notwithstanding, even when the join effect was found to be significant, the inclusion of temperature after salinity in the equation was not, showing that temperature had not a real effect (Table 1). The significance of the interaction between time and salinity

Table 2
Estimated regression equations relating domoic acid concentration: A = ln(domoic acid concentration), with time (t) and dummy variables (Z) when only salinity and when both salinity and temperature are considered to be significant. The parameters of the regression are given for the equations including the dummy variables (general) and for each level of the studied factors, after the substitution of each dummy variable by its corresponding value

Only salinity significant	Equation A =
General	$4.805 - 0.486\,t + 0.238\,Z_S\,t$
Low salinity	$4.805 - 0.248\,t$
High salinity	$4.805 - 0.486\,t$
Salinity and temperature significant	**Equation A =**
General	$4.803 - 0.455\,t + 0.208\,Z_S\,t - 0.054\,Z_T\,t$
Low salinity, Low temperature	$4.803 - 0.301\,t$
High salinity, Low temperature	$4.803 - 0.509\,t$
Low salinity, High temperature	$4.803 - 0.247\,t$
High salinity, High temperature	$4.803 - 0.455\,t$

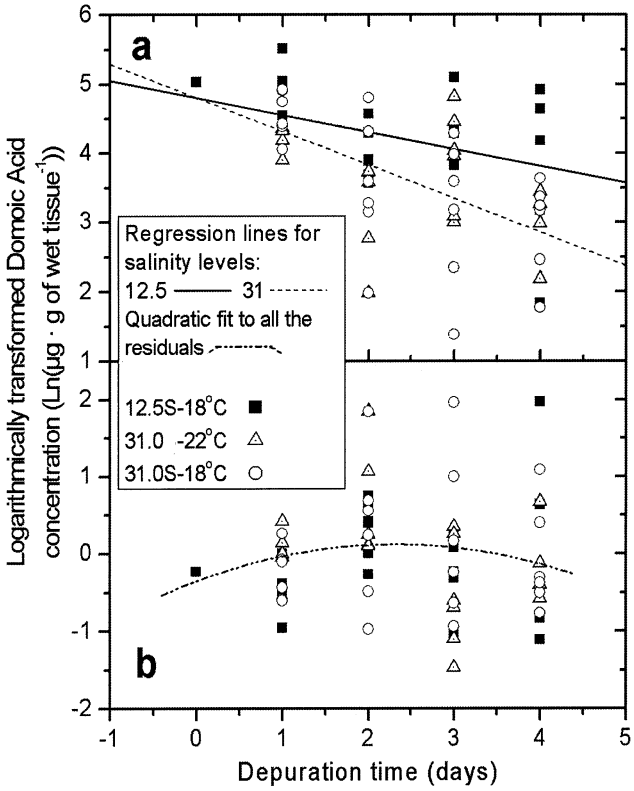

Fig. 3. Regression lines of the logarithmically transformed domoic acid concentration data on time and their residuals. **(a)** Real data (symbols) and fitted lines corresponding to the two salinities. No significant difference was detected between the two temperatures and consequently only a common line is represented. **(b)** Residuals of the two fits (deviations of the data from each corresponding line, symbols) and their quadratic (polynomial of second order) regression on time (showing that they are dependent on time, line).

means that the depuration rate is only significantly affected by salinity. The lack of significance of the quadratic terms of all the variables suggest that the assumed model (exponential decrease) is adequate to describe the domoic acid depuration in this species. Notwithstanding, a careful analysis of the residuals (Fig. 3) showed that they did not distribute randomly, as expected from a correct model fitting, but following a convex curve, which means that mussels depurated faster than expected at the beginning of the experiment and more slowly at the end, and therefore that the model is not fully correct. The inclusion of the quadratic term of time in the regression did only produced a small, non significant, quantitative fit improvement (adjusted R^2 increased from 0.30 to 0.31) but the resulting residuals were, in this case, randomly distributed, showing that the new model is more adequate than the previous one (Fig. 3).

The large unexplained fraction of the variance seems to be mainly due to interindividual variation as only a small part seems to be due to an imperfect linear relationship between the transformed domoic acid concentration and the depuration time, and there is no evidence of interactions between salinity, temperature and weight.

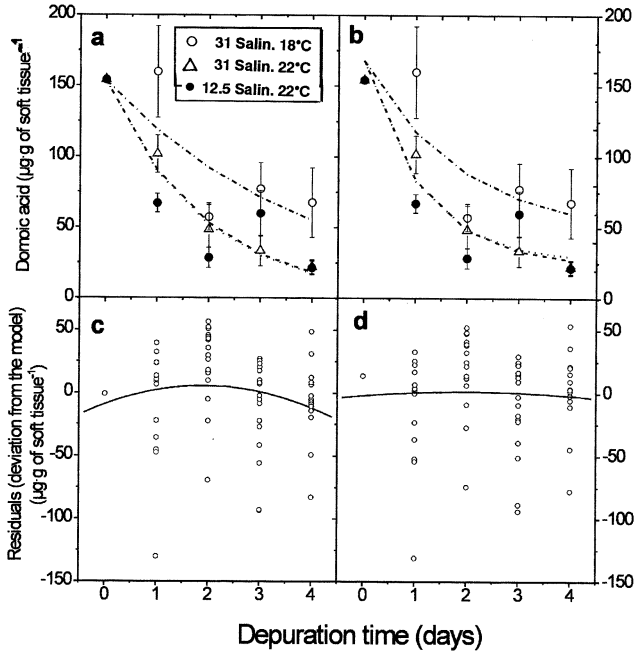

Fig. 4. Fits of the one- **(a)** and two-compartment **(b)** models and their resulting residuals (**c** and **d**, respectively). In **c** and **d**, circles represent the deviations of the data from the model (residuals), and the lines are the quadratic (polynomic of second order) regression of these residuals with time. It can be observed that the inclusion of a second compartment made the residuals more independent from time.

As pointed before, a one compartment model (simple exponential decrease) was not enough to correctly fit the data, and consequenly we tryed a two compartment one (Fig. 1). To compare the ability of one- and two- compartment models to describe the obtained data, we fitted the first two models described in Material and Methods. As expected from the results of the regression analysis, there was little quantitative difference between the fittings of the two types of models, but the two-compartment yielded residual deviations independent from time (Fig. 4). This qualitative difference makes this later type of model more adequate to describe the data. The fitted parameters are shown in Table 3.

Table 3
Estimated parameters of the one- and two-compartment models of the depuration kinetics.

	1-Compartment	2-Compartments
Initial domoic acid concentration ($\mu g.g^{-1}$)		
in Comp. 1	149	163
in Comp. 2		0.74
Transfer rate from Comp. 1 to Comp. 2		0.13
Depuration rate of Comp. 1	0.40	0.58
Depuration rate of Comp. 2		0

4. Discussion

Mytilus galloprovincialis depurates domoic acid very quickly being, in this way, very similar to *Mytilus edulis* (Gilgan et al., 1990; Wohlgeschaffen et al., 1992; Novaczek et al., 1992) and to other species of marine bivalves as *Mytilus californianus* (Whyte et al., 1995) and *Mya arenaria* (Gilgan et al., 1990). The depuration rate is notably higher than that of the scallop *Placopecten magellanicus* (Douglas et al., 1997) that is about 10% day^{-1} (Douglas et al., 1997) and it is also higher than the depuration of the razor clam *Siliqua patula* (Horner et al., 1993). This characteristic is consistent with the short persistence of ASP toxicity in the organisms of this species in Galicia, as reported by the Centro de Control da Calidade do Medio Mariño (Arévalo et al., 1997). The observed depuration is very fast as compared to that corresponding to other toxin types (Blanco et al., 1997; Blanco et al., 1999), and it seems also too fast to be assimilated and then excreted, but, on the other hand, it is quite slow, compared to gut evacuation of a similar species (Hawkins et al., 1990), suggesting that a retention mechanism exists. The actual mechanism involved in this depuration remains unknown, but there are several possible hypothesis that can explain its characteristics. One is the possibility that the ingestion of domoic acid decreases the egestion rate and, therefore, the gut evacuation and the toxin elimination from the gut lumen. In such a case, the velocity of egestion of the gut content would be reduced in relation to its usual values and it can, therefore match, the domoic acid depuration rate. This mechanism was suggested by Novaczek et al. (1992) to explain the changes in accumulation rate of domoic acid, and by Silvert and Subba Rao (1992), as one of the mechanisms that might explain the lack of fit of a domoic acid accumulation model to their data. In our experiment we did not observed the drastic reduction in egestion that would be needed to produce a depuration rate so low. Another possibility, also taken into account by Silvert and Subba Rao (1992), would be the uptake of domoic acid by different tissues/organs of the mussels followed by a fast release. This hypothesis seems unlikely because a very fast uptake and release from the organs should be needed to account for the, also fast, depuration in *Mytilus galloprovincialis.* A third possibility would be a weak, reversible binding of domoic acid to the gut walls. Madhyastha et al. (1991) studied the binding capabilities of the cellular fractions of the mussel (*M. edulis*) digestive gland cells, finding a very low binding capacity of the membranes. Even when this fact does not seem to support our latter hypothesis, a weak binding to the membranes would not have been detected under the Madhyastha et al. (1991), experimental conditions.

In our experiment, low salinity reduced the depuration rate. In the only case in which the effect of this factor on the depuration of domoic acid was studied, Novaczek et al. (1992) did not found any effect on *Mytilus edulis*. Both, body weight (Wohlgeschaffen et al., 1992; Novaczek et al., 1992) and temperature (Novaczek et al., 1992), that were shown to affect to *Mytilus edulis*, have no significant effect in this study. The significance levels of these two variables were very low and, therefore, its lack of significance cannot be attributed only to the high interindividual variation detected. Additionally, no interaction between variables was found, so it cannot be argued to be responsible for the non-significance of the effects of these factors.

The obtention of a model of the depuration kinetics is mandatory for the prediction of the depuration time. Several one- and two- compartment models have been used to describe phycotoxins depuration (Blanco et al., 1995; Silvert and Cembella, 1995; Blanco et al., 1997; Moroño et al., 1998; Fernández et al., 1998; Blanco et al., 1999) and, in the case of domoic acid, it seems that both groups of models may be valid for different species. In our study a one-compartment model was sufficient to describe adequately, from a quantitative point of view, the depuration of domoic acid in *Mytilus galloprovincialis*. Nevertheless, a slight dependency of the residuals on the depuration time, suggests the possibility of a very small second compartment, that probably would be put into evidence if the time of exposure of the mussels to the toxins would be long. The fitted simulation models support this supposition. In the two-compartment model, there is a small second compartment with zero depuration rate. This second compartment represents the assimilated toxin, that is slowly released from the mussels which in our experiment, in sight of the depuration rate estimated for it (0), constituted a fraction of the toxin contents that is residual at the time scale of the experiment (it probably would be higher that 0 if the estimates were made using a longer depuration period). Even when the contribution of the inclusion of the second compartment was not statistically significant, it is supported by the analysis of the residuals, as detailed in the results section, and this means that the estimated rates already commented probably represent real toxin fluxes. Several authors found that one compartment models were enough to describe the depuration kinetics of *Mytilus edulis* (Novaczek et al., 1992) and *Placopecten magellanicus* (Douglas et al., 1997). Silvert and Subba Rao (1992) and Douglas et al. (1997), in concordance with our results, suggested the possible need of including a second compartment and observed that, in the soft tissues other that the digestive gland, the depuration was, at first, very rapid but then it became zero or nearly zero until the end of the experiment. A similar case was recorded by Novaczek et al. (1992) in a study of *Mytilus edulis*, where they found a residual domoic acid after the depuration period and where a slight curvature of the response to the depuration can be observed. Some other studies show or suggest a biphasic depuration, that should be described by two compartment models. This was the case for the razor clam *Siliqua patula* that maintains a low level of the toxins for long periods (Horner et al., 1993) and also for the red mussel *Volsella modiolus* and in some cases for *Placopecten magellanicus* (Gilgan et al., 1990).

The large inter-individual variation in domoic acid contents seems to be a characteristic of most bivalves, as it was detected in several studies on *Placopecten magellanicus* (Gilgan et al., 1990; Douglas et al., 1997), *Mytilus edulis* (Gilgan et al., 1990) and *Pecten maximus* (Arévalo et al., 1998). The same kind of large variation was found by Lobel and Marshall (1988) for Zinc accumulation in mussels *Mytilus edulis*. They attributed it to the presence of a low molecular weight substance in some individuals but not in others, but this mechanism would unlikely affect to a rapid depuration process as that of domoic acid, because it requires the assimilation of the compound. Alternative explanations would be the different degradation of domoic acid in gut lumen by the action of the intestinal flora (Stewart et al., 1998) or different sensitivities of the digestive system to the effect of the toxins, in case that the Novaczek's hypothesis would be correct. The observed inter-individual variation has strong repercusion on the precision at which means of domoic acid concentration in mussels can be estimated and therefore it has to be taken into account when designing sampling protocols for experimental or monitoring purposes.

5. Conclusion

It can be stated that *Mytilus galloprovincialis* is a fast domoic acid depurator, in which most of the toxin depurates following a simple exponentially decreasing kinetics (a one-compartment model). It seems that a small amount of domoic acid depurates very slowly, causing the total depu ration to follow a two-compartment kinetics. Low salinity slowed the depuration process and neither temperature nor body weight had any significant effect. Finally, there is a large inter-individual variation in the depuration of this toxin which has to be taken into account when designing new experiments or estimating domoic acid concentrations for monitoring purposes.

Acknowledgements

This work was partially funded by Project PGIDT-99-PXI50101. We would like to thank M.C. Mariño, M. Miranda, S. Muñiz and J. Correa by their technical assistance.

References

Arévalo, F., Bermúdez de la Puente, M., Salgado, C., 1997. Seguimiento de biotoxinas marinas en las Rías Gallegas: control y evolución durante los años 1995-1996. In: Vieites, J., Leira, F (Eds.), V Reunión Ibérica de Fitoplancton Tóxico y Biotoxinas. ANFACO-CECOPESCA, Vigo, pp. 90–101.

Arévalo, F.F., Bermúdez, M., Salgado, C., 1998. ASP toxicity in scallops: individual variability and tissue distribution. In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T (Eds.), Harmful microalgae. Xunta de Galicia and the IOC of UNESCO, Santiago de Compostela, pp. 499–502.

Bates, S.S., Bird, C.J., de Freitas, A.S.W., Foxall, R., Gilgan, M., Hanic, L.A., Johnson, G.R., McCulloch, A.W., Odense, P., Pocklington, R., Quilliam, M.A., Sim, P.G., Smith, J.C., Subba Rao, D.V., Todd, E.C.D., Walter, J.A., Wright, J.L.C., 1989. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island. Canada. Can. J. Fish. Aquat. Sci. 46, 1203–1215.

Blanco, J., Fernández, M., Mariño, J., Reguera, B., Míguez, A., Maneiro, J., Cacho, E., Martínez, A., 1995. From *Dinophysis* spp. toxicity to DSP outbreaks: A preliminary model of toxin accumulation in mussels. In: Lassus, P., Arzul, G., Erard, E., Gentien, P., Marcaillou, C (Eds.), Harmful marine algal blooms. Lavoisier-Intercept Ltd, Paris, pp. 777–782.

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., 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.

Douglas, D.J., Kenchington, E.R., Bird, C.J., 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.

Drum, A.S., Siebens, T.L, Crecelius, E.A., Elston, R.A., 1993. Domoic acid in the Pacific razor clam *Siliqua patula* (Dixon, 1789). J. Shellfish Res. 12, 443–450.

Fernández, M.L., Míguez, A., Moroño, A., Cacho, E., Martínez, A., Blanco, J., 1998. Detoxification of low polarity toxins (DTX3) from mussels *Mytilus galloprovincialis* in Spain. In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T (Eds.), Harmful Algae. Xunta de Galicia and Int. Oceanographic Comm. of UNESCO, Santiago de Compostela, pp. 449–452.

Gilgan, M.W., Burns, B.G., Landry, G.J., 1990. Distribution and magnitude of domoic acid contamination of shellfish in Atlantic Canada during 1988. In: Granéli, E., Sundstrom, B., Edler, L., Anderson, D.M (Eds.), Toxic marine phytoplankton. Elsevier Sci. Publ. Co., Inc., New York, pp. 469–474.

Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32, 79–99.

Hawkins, A.J.S., Navarro, E., Iglesias, J.I.P., 1990. Comparative allometries of gut-passage time, gut content and metabolic faecal loss in *Mytilus edulis* and *Cerastoderma edule*. Mar. Biol. 105, 197–204.

Horner, R.A., Kusske, M.B., Moynihan, B.P., Skinner, R.N., Wekell, J.C., 1993. Retention of domoic acid by Pacific razor clams, *Siliqua patula* (Dixon, 1789): preliminary study. J. Shellfish Res. 12, 451–456.

Kleinbaum, D.G., Kupper, L.L., Muller, K.E., 1988. Applied regression analysis and other multivariable methods, 2nd Edn. Duxbury Press, Belmont, California.

Lawrence, J.F., Charbonneau, C.F., Ménard, C., 1991. Liquid chromatographic determination of domoic acid in mussels, using AOAC analytic shellfish poison extraction procedure: collaborative study. J. Assoc. Offic. Anal. Chem. 74, 68–72.

Lobel, P.B., Marshall, H.D., 1988. A unique low molecular weigth zinc-binding ligand in the kidney cytosol of the mussel *Mytilus edulis*, and its relationship to the inherent variability of Zinc accumulation in this organism. Mar. Biol. 99, 101–105.

Madhyastha, M.S., Novaczek, I., Ablett, R.F., Johnson, G., Nijjar, M.S., Sims, D.E., 1991. In vitro study of domoic acid uptake by digestive gland tissue of blue mussel (*Mytilus edulis* L). Aquat. Toxicol. 20, 73–82.

Míguez, A., Fernández, M.L., Fraga, S., 1996. First detection of domoic acid in Galicia, NW of Spain. In: Yasumoto, T., Oshima, Y., Fukuyo, Y (Eds.), Harmful and toxic algal blooms. Intergov. Oceanogr. Comm. UNESCO, Paris, pp. 143–145.

Moroño, A., Fernández, M.L., Franco, J.M., Martínez, A., Reyero, I., Míguez, A., Cacho, E., Blanco, J., 1998. PSP and DSP detoxification kinetics in mussel, *Mytilus galloprovincialis* : Effect of environmental parameters and body weight. In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T (Eds.), Harmful Algae. Xunta de Galicia and IOC of UNESCO, Santiago de Compostela, pp. 445–448.

Novaczek, I., Madhyastha, M.S., Ablett, R.F., Donald, A., Johnson, G., Nijjar, M.S., Sims, D.E., 1992. Depuration of domoic acid from live blue mussels (*Mytilus edulis*). Can. J. Fish. Aquat. Sci. 49, 312–318.

Silvert, W., Subba Rao, D.V., 1992. Dynamic model of the flux of domoic acid, a neurotoxin, through a *Mytilus edulis* population. Can. J. Fish. Aquat. Sci. 49, 400–405.

Silvert, W.L., 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.

Stewart, J.E., Marks, L.J., Gilgan, M.W., Pfeiffer, E., Zwicker, B.M., 1998. Microbial utilization of the neurotoxin domoic acid: blue mussels (*Mytilus edulis*) and soft shell clams (*Mya arenaria*) as sources of the microorganisms. Can. J. Microbiol. 44, 456–464.

Whyte, J.N.C., Ginther, N.G., Townsend, T.D., 1995. Accumulation and depuration of domoic acid by the mussel, *Mytilus californianus*. In: Lassus, P., Arzul, G., Erard, E., Gentien, P., Marcaillou, C (Eds.), Harmful marine algal blooms. Lavoisier-Intercept Ltd, París, pp. 531–537.

Wohlgeschaffen, G.D., Mann, K.H., Subba Rao, D.V., Pocklington, R., 1992. Dynamics of the phycotoxin domoic acid: accumulation and excretion in two commercially important bivalves. J. Appl. Phycol. 4, 297–310.