Aquat. Living Resour. 19, 257–265 (2006)

DOI: 10.1051/alr:2006026
www.edpsciences.org/alr

Aquatic Living Resources

Depuration of domoic acid from different body compartments of the king scallop *Pecten maximus* grown in raft culture and natural bed

Juan Blanco[1,a], Carmen P. Acosta[1], Carmen Mariño[1], Susana Muñiz[2], Helena Martín[1], Ángeles Moroño[2], Jorge Correa[2], Fabiola Arévalo[2] and Covadonga Salgado[2]

[1] Centro de Investigacións Mariñas, Pedras de Corón s/n, Aptdo. 13, 36620 Vilanova de Arousa, Spain
[2] INTECMAR, Peirao de Vilaxoán, 36611 Vilagarcía de Arousa, Spain

Received 14 February 2006; Accepted 6 July 2006

Abstract – The depuration kinetics of the domoic acid from three body parts (i) digestive gland, (ii) adductor muscle+gonad+kidney+foot and (iii) gills+mantle of the scallop *Pecten maximus* was studied over 154 days. The scallops, which had accumulated the toxins during a *Pseudo-nitzschia australis* outbreak, were obtained from a natural bed and hung from a mussel raft in two locations (front and centre) and at three depths (2, 6 and 10 m). The time course of the depuration of domoic acid (DA), as well as the environmental variables, were monitored throughout the experiment. The whole body depurated the toxin very slowly (*ca* 0.007 day^{-1}) decreasing its concentration from *ca*. 3200 μg DA g^{-1}. Its kinetics was driven mostly by the digestive gland, which accounted for *ca*. 95% of the total toxin burden from the start of the experiment. Suspending the scallops from a raft increased the depuration rate of the whole body and digestive gland (*ca*. 30%) and of the edible tissues (15%). Increases of the depuration rate of domoic acid seem to be related to the pair of covariating variables temperature-salinity. Food amount does not seem to have a significant effect.

Key words: Domoic acid / ASP toxin / Amnesic shellfish poisoning / Depuration / Environmental conditions / *Pecten maximus*

Résumé – Élimination de l'acide domoïque (toxine amnésiante) à partir de différents tissus de la coquille St-Jacques, *Pecten maximus,* élevée sous radeaux et en cantonnements naturels. La cinétique d'élimination de l'acide domoïque à partir : (1) de la glande digestive, (2) de l'ensemble muscle adducteur+gonade+rein+pied et (3) des branchies et du manteau de la coquille St-Jacques *Pecten maximus* a été étudiée durant 154 jours. Les coquilles St-Jacques ayant absorbé des toxines pendant une efflorescence de *Pseudo-nitzschia australis*, ont été collectées sur un banc naturel et suspendues en deux endroits d'un radeau de moules (au bord et au centre) et à trois profondeurs différentes (2, 6 et 10 m). Le temps nécessaire à l'élimination de l'acide domoïque (DA), ainsi que les variables environnementales ont été enregistrés durant l'expérience. Le corps entier élimine très lentement la toxine (environ 0,7 % jour^{-1}) sa concentration était, au début de l'expérience, environ de 3200 μg DA g^{-1}. L'essentiel de la cinétique de décontamination est due à la glande digestive, qui représente environ 95 % du contenu toxinique dès le début de l'expérience. Le fait de suspendre les coquilles St-Jacques à un radeau augmente la vitesse de décontamination du corps entier comme de la glande digestive (de 30 % environ) et des tissus consommables (15 %). L'augmentation de la vitesse de décontamination de l'acide domoïque semble être liée aux co-variables température et salinité. La quantité de nourriture ingérée ne semble pas avoir d'effet significatif.

1 Introduction

Since Amnesic Shellfish Poisoning (ASP) was first recognised in Canada (Bates et al. 1989), this type of toxicity has been recorded in many areas all around the world (Hallegraeff 1993). Exploitable populations of molluscs and crustaceans have been shown to be affected by this toxicity (*Mytilus edulis*, Novaczek et al. 1992; *M. edulis* and *Placopecten magellanicus*; *Volsella modiolus*, Gilgan et al. 1990; *Siliqua patula*, Drum et al. 1993; Whyte et al. 1995, *Mytilus californianus*; Whyte et al. 1995; Lund et al. 1997, *Cerastoderma edule, Venerupis pullastra, Scrobicularia plana, Ensis* spp. Vale and Sampayo 2001; *Ruditapes decussatus* Amzil et al. 2001; Vale and Sampayo 2001), making this toxin increasingly important from both an economical and ecological point of view. The undesirable effects caused by these toxic outbreaks are largely modulated by the depuration rate of the organisms since this is the main process regulating the time period during which they remain toxic for consumers and, consequently, the period during which fish or shellfish cannot be collected for human consumption (Shumway and Cembella 1993).

[a] Corresponding author: jblanco@cimacoron.org

In bivalves, domoic acid depuration time has been shown to be species-specific and to have a wide-ranging variability. Most mytilids, *Mytilus edulis* (Wohlgeschaffen et al. 1992); *M. californianus* (Whyte et al. 1995); and *M. galloprovincialis* (Blanco et al. 2002b), and also other bivalves such as *Mya arenaria* (Gilgan et al. 1990) and *Callista chione* (Fernández et al. 2000), depurate domoic acid very quickly, while *Siliqua patula* (Horner et al. 1993) and *Pecten maximus* (Fernández et al. 2000; Vale and Sampayo 2001; Campbell et al. 2001; Blanco et al. 2002a), may retain the toxin for months.

The main phytoplankton species that produce domoic acid in Spain, and in other areas as well, bloom at least once a year following a strong seasonal pattern, with maxima, in Galicia, occurring preferentially in autumn and/or spring (Pazos et al. 2003; Moroño et al. 2004). This recurrence, combined with the high accumulation and slow depuration capability of *Pecten maximus*, make the scallop populations to remain toxic for years, in some occasions. When the scallops approach allowable levels of domoic acid concentration after a toxic outbreak, they are frequently affected by a new toxic bloom, which renders the scallop populations again useless from a commercial point of view (Salgado et al. 2003). Because of this low depuration rate and its consequent linking of successive banning periods, the situation of the exploitation of this resource has become dramatic in many affected areas (Arévalo et al. 1997; Campbell et al. 2001), and the growers and/or fishermen ask for methods to remove the toxins consisting mainly of accelerated depuration or extracting the most toxic organs. In this context, the EC (Anonymous 2002) and the Galician Authorities (Xunta de Galicia) have recently allowed the exploitation of this resource under a special regime and only when the populations fulfilled two criteria (Fernández et al. 2003b; Salgado et al. 2003). The first limits the domoic acid concentration of the whole body, and the second limits the concentration in the edible part. The capability to exploit this resource still depends on the depuration rate of the different tissues and organs. The enhancement of the depuration rates, particularly of the organs, with a higher toxin content would therefore be of great economical and social interest.

Several possible methods can be used to accelerate the depuration of live scallops, the most important of which are adequate management plans for the natural or cultured populations and the design of specific treatments. Implementing any of these approaches requires a good knowledge of the effect of the environmental conditions. Taking this into account, and for the purpose of evaluating the importance of the environmental conditions in the depuration rate of domoic acid from the scallop, we have designed an experiment in which the differences in depuration between bottom (natural bed) and surface (suspended from a raft) populations, as well as among the different depths and locations in the raft, were studied.

2 Material and methods

The scallops were collected, by means of a net trawl, from a natural bed in the Ría de Arousa (Fig. 1) where they had acquired ASP toxicity from a bloom of *Pseudo-nitzschia australis*, which had reached its maximum two weeks earlier.

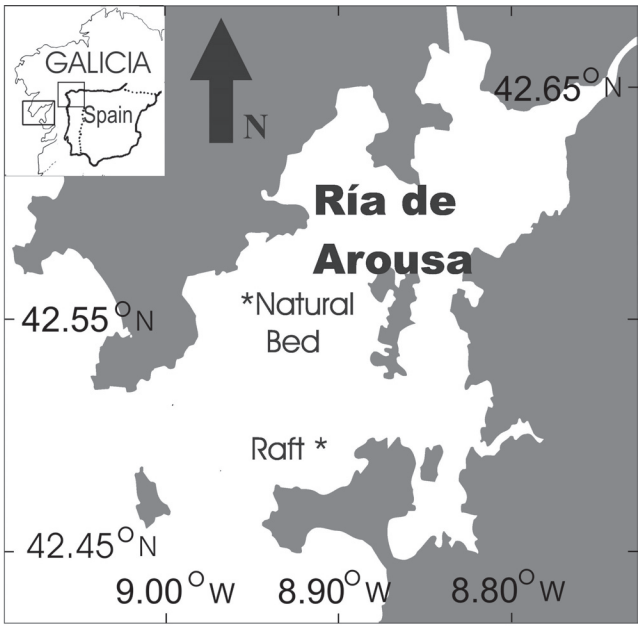

Fig. 1. Location of the natural bed and the raft where the scallops were placed during the experiment, Ría de Arousa, Spain.

They were randomly distributed into 31 groups (corresponding to 3 locations in the raft × 5 sampling dates + 1 initial sample) of 12 individuals, making a total of 372 scallops. The groups were distributed into two locations – the front and the central area – of a mussel raft which contained *ca*. 400 ropes, at three depths in each location (2, 6 and 10 m). The different depths were chosen because they had been associated with different temperatures, salinities and food concentrations. The centre and front of the raft were also expected to have different amounts of food and current velocities (Blanco et al. 1996). On days 25, 61, 110 and 152, one of these groups was collected from each raft location/depth and 12 additional scallops were obtained from the natural bed.

The sampled scallops were dissected into three groups of organs: a) digestive gland (DG), b) the gonad and muscle group (GoMu) including gonad, kidney, adductor muscle, foot (the edible organs plus others that cannot be easily separated), and c) the rest of the tissues which included the gills and mantle (GiMa). The groups of each type were pooled to give four samples containing three groups each.

Each pooled samples was weighed, homogenised and extracted with 50% methanol (MeOH) in a proportion of 4 ml of extraction solvent per gram of tissue. The extracts were analysed with the technique of Quilliam et al. (1989) using a Waters Alliance 2690 HPLC System, with an UV spectrophotometer Waters 996 PDA-UV detector, a Merck LiChrospher 100-RP18 chromatographic column, and domoic acid (DA) reference solutions (DACS-1C, i.e. 100 μg DA ml^{-1}) were obtained from National Research Council (Halifax, Canada).

Temperature, salinity, light transmission and fluorescence were monitored weekly with a Sea-Bird Sealogger 25. The two latter variables were used as indicators of the amount of food available for the scallops since "in vivo" fluorescence is proportional to the amount of phytoplankton in the water, and light

transmission has an inverse dependence on the volume of suspended matter.

Several statistical procedures were used to analyse the data obtained. To compute the depuration rates in the locations under study, a simple exponential decrease model was assumed. The differences of depuration rate between the different locations and depths, and also those of growth rate, were tested by means of regression, with dummy variables (Kleinbaum et al. 1988) of the logarithmically-transformed domoic acid concentration (and therefore no error bar is given in the corresponding figures). General linear ANOVA and MANOVA, best-subsets (including the use of the Mallow's C_p statistic) and stepwise regression, as well as Principal Component Analysis were also used. In all cases, the analyses were carried out using the MINITAB 14 statistical package, following the documentation included in the package.

3 Results

Environmental conditions

The environmental conditions, as expected, varied with the location where scallops were placed. Temperature decreased, while salinity increased with depth. There were no noticeable differences in this trend between the centre and the front of the raft. Food (as measured by means of fluorescence and light transmission) was least abundant in the natural bed but otherwise it was not dependent on depth. The front of the raft seems also to have more available food than the central area (Fig. 2) but the differences found were only statistically significant when fluorescence was used as index ($p = 0.049$ ANOVA) and not when light transmission ($p = 0.104$, ANOVA) or the co-variation between this variable and fluorescence were used ($p = 0.103$, MANOVA).

Growth

The scallops grew throughout the experiment. On average, the body weight of the wild scallops at the end of the experiment was the lowest out of the seven locations studied (Fig. 3), but differences in growth rate were small (Fig. 4). This relatively small weight of the wild scallops may very likely be due to the sampling method. In the first sampling, there may have been a proportion of scallops that were small enough to pass through the net trawl without being caught, but large enough to be retained in a second sampling, after some growth took place. The scallops suspended from the raft, at the surface layer, in both the front and central locations attained the highest weights and exhibited the fastest growth rates.

Although the raft and wild scallops had similar growth rates, the biomass increase was not distributed evenly among the different tissue groups (Fig. 5). The edible part, GoMu, grew faster in the raft scallops than in the wild ones. On the other hand, GiMa grew faster in the wild population. No obvious differences were found in the growth of the digestive gland, with the exception of the two deepest locations in the front of the raft, where this organ grew at a slower rate than in the other locations.

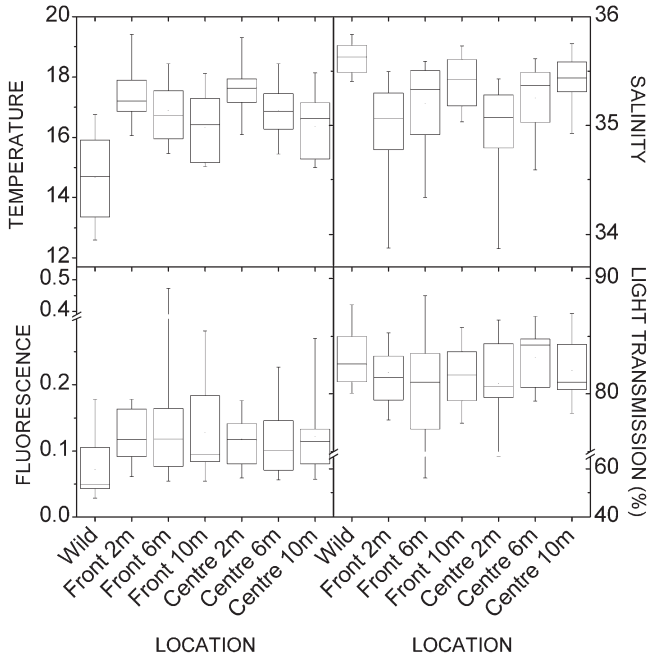

Fig. 2. Temperature, salinity, *in vivo* fluorescence and light transmission recorded at the seven locations used in the experiment. The box-and-whisker plots show the descriptive statistics corresponding to each variable. The dot, inside the box, represents the mean. The horizontal line, in the center of each box, represents the median and the bottom and top limits of the box represent the 25% and 75% quartiles, respectively. The extremes of the whisker (vertical lines) represent the 1 and 99% percentiles.

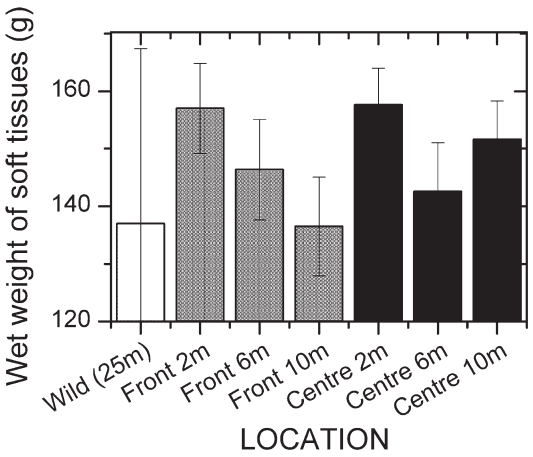

Fig. 3. Weight of the scallop tissues at the end of the experiment in the seven locations of the study. Bars indicate the mean and the vertical lines the standard deviation.

Toxin concentration and depuration rate

Toxin levels

The toxin concentration in the whole body of the scallop was well above the regulatory limit of 20 $\mu g\, g^{-1}$, throughout the experiment. Most of the toxin was accumulated in the digestive gland. The two other groups of organs had much less domoic acid concentration being, in the case of the GoMu

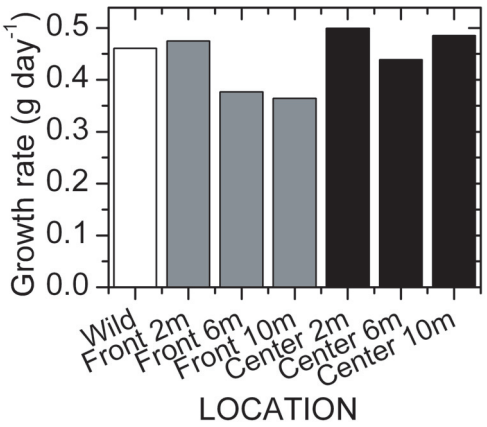

Fig. 4. Growth rate of the scallop soft tissues during the experiment at the different locations (estimated by means of multiple regression with dummy variables).

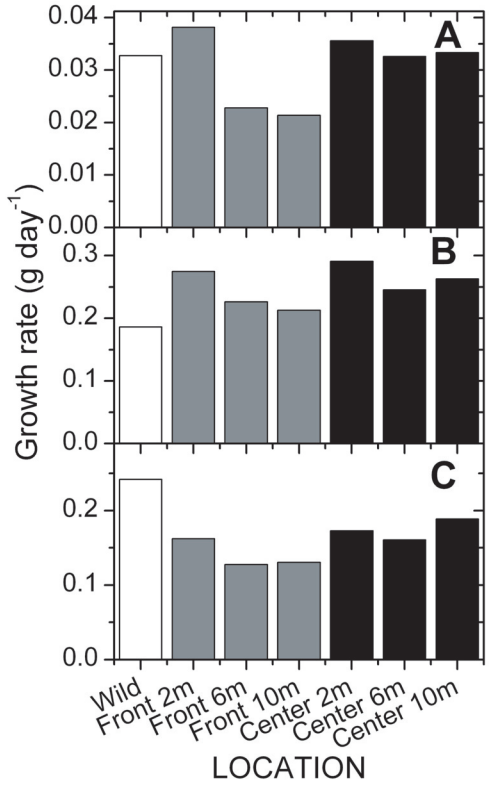

Fig. 5. Growth rate of the three groups of organs of the scallops during the experiment, at the studied locations (estimated by means of multiple regression with dummy variables). A: Digestive gland (*DG*); B: Gonad+Adductor muscle+Kidney+Foot (*GoMu*); C: Gill+Mantle (*GiMa*).

group above the maximum allowable level for evisceration of the European Union, at the beginning of the experiment but below that limit at the end (Fig. 6).

Whole body tissues

The depuration rate of the whole body was very low in all cases (less than 0.011 day^{-1}) (Fig. 7). Affected by the location

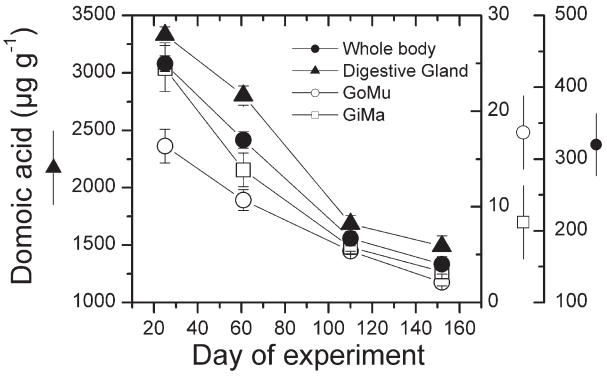

Fig. 6. Domoic acid concentration in the whole body and in the three groups of organs studied, throughout the experiment (*GoMu*: Gonad+Adductor muscle+ Kidney + Foot; *GiMa* : Gill+Mantle).

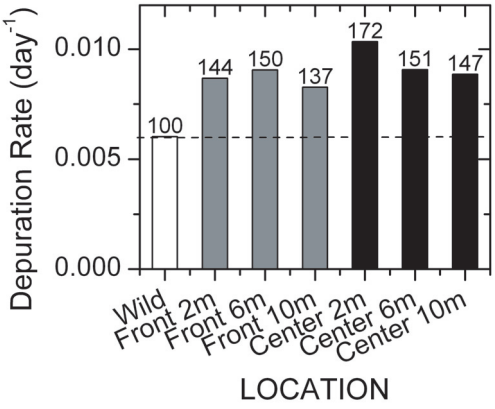

Fig. 7. Depuration rate of domoic acid from the scallops at the different locations used in the study (estimated by means of multiple regression with dummy variables). Numbers at the top of the bars are the depuration rates expressed as percentages of those in wild scallops.

Table 1. Statistical significance (p), obtained by regression with dummy variables, of the differences, in domoic acid depuration, found between the wild scallop population and the scallops placed in the raft, for the whole body and for each of the tissue groups studied; ns: not significant.

Tissue	F	p
Whole body	4.123	0.00096 (***)
Digestive gland (DG)	4.069	0.0011 (**)
Gonad+muscle+kidney+foot (GoMu)	0.870	0.520 (ns)
Gill + mantle (GiMa)	3.883	0.0016 (**)

(Table 1), this rate was found to be especially low in the wild population and particularly high in the surface population suspended from the centre of the raft. Both depth and location in the raft seem to affect the depuration of domoic acid – the former producing a decrease in the rate and the latter an increase (Fig. 8). Nevertheless, the differences inside the raft were found to be small.

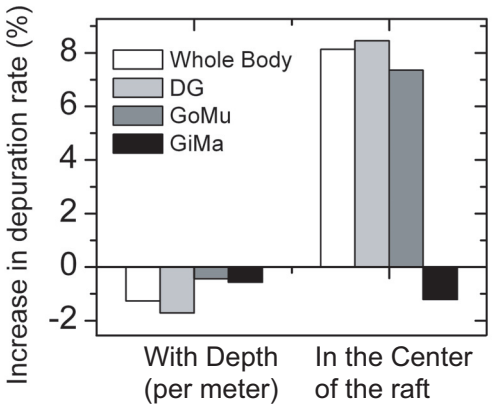

Fig. 8. Effect of depth and horizontal location in the raft on the depuration rate of domoic acid from the scallop tissues (estimated by means of multiple regression with dummy variables). The left group of columns shows, for each group of tissues, the effect of increasing 1 meter the depth at which the scallops are located. The right group shows the effect of placing the scallops at the center of the raft instead of at the front.

Organs and groups of tissues

All organs and groups of tissues depurated domoic acid at slow rates, with the fastest rate recorded being 0.025 day^{-1} (GiMa, in the wild population). The digestive gland was the organ that retained the toxin most efficiently, in both wild and raft scallops. The fastest depurating tissue, however, was dependent on the location. In the case of raft scallops it was the GoMu group while in wild scallops it was the GiMa tissues (Fig. 9).

Generally speaking, the location did affect the depuration of all the tissue groups studied. The digestive gland and GoMu lost toxicity faster in the raft than in the natural bed, while the opposite was true for GiMa, which coincided with the effect of these locations on the growth rate of these body parts.

In the raft, the effects of both depth and location were similar to those found for the whole body (Fig. 8). However, some differences can be also observed: a) the effect of depth was smaller in the GoMu group and GiMa, than in the digestive gland (but not statistically significant, Table 2), b) the effect on the GiMa group was the opposite of the effect on the other fractions.

Relationship to growth and environmental variables

No significant effect of weight increase on the depuration rate was detected when taking into consideration either the whole body or any of the three fractions studied. Nevertheless, the fact – which was pointed out in the previous section – that there was a coincidence between the locations with the fastest depuration rate and those with the fastest growth rate, suggests the possibility of a real, albeit minor, effect masked by the experimental error.

Depuration seems to be affected by environmental conditions. Notwithstanding, due to the co-variation that exists among these conditions, in this study, it was not possible to identify which variables had a real effect.

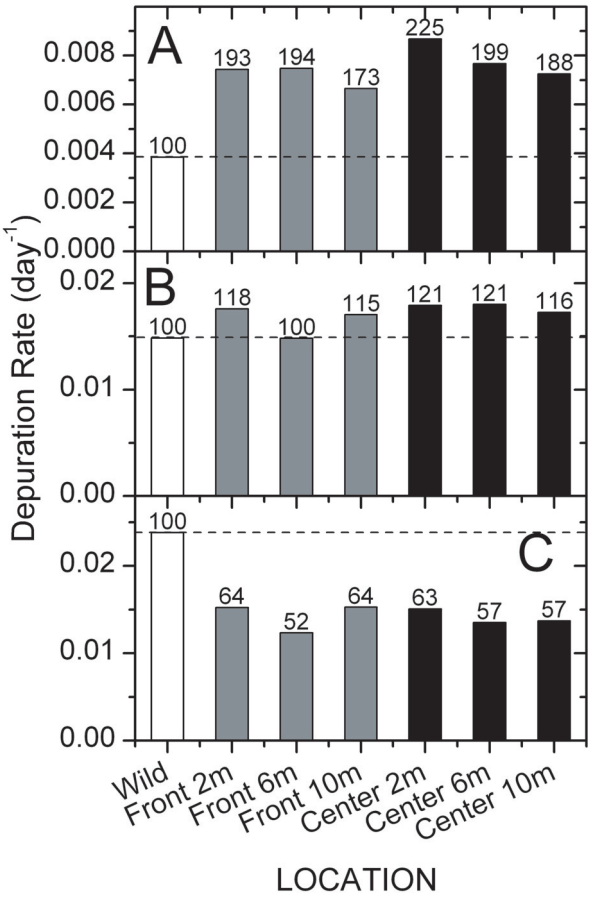

Fig. 9. Depuration rate of domoic acid from the three groups of organs of the scallops at the different locations used in the study (estimated by means of multiple regression with dummy variables). A: Digestive gland (*DG*); B: Gonad+Adductor muscle+Kidney+Foot (*GoMu*); C: Gill+Mantle (*GiMa*).

After testing the effect of each individual variable and their combinations, temperature appeared to be the most important variable. Nevertheless, the high Mallows C_p statistic of each regression in the analysis indicates that the estimates of the regression coefficients are highly imprecise, making it impossible to distinguish the true effects of the environmental variables (Table 3). In an attempt to reduce this mathematical effect of the co-variation, we have substituted the original variables with their co-variation. Each of the two pairs of variables, that were closely interrelated, were replaced by their co-variation estimated by means of the scores of the first principal component extracted by the principal component analysis (PCA) of each pair. Depuration rates were then regressed against the new variables obtained. Even though, the two new variables were still interrelated, they were independent enough to result in a much lower Mallows C_p number, and consequently their effects can be efficiently estimated (Table 4). In this analysis, it was found that most of the effect was due to temperature-salinity, and little or no effect can be attributed to the pair of variables describing food availability (fluorescence-light transmission) because their inclusion in the regression equation produces a decrease in the explained variance (Table 4).

Table 2. Statistical significance (p), coefficients (Coef.) and their standard error (SE Coef.), of the effects of depth and horizontal location of the scallops in the raft on the depuration of domoic acid. The effects were estimated by regression, using as predictor variables the interactions Depth × Day, and Horizontal location × Day.

Tissue	Predictor variables	Coef. × 10^{-6}	SE Coef. × 10^{-6}	p
Whole body	Depth × Day	118	53	0.028 (*)
	Horiz. Location × Day	−762	345	0.030 (*)
Digestive gland	Depth × Day	138	53	0.012 (*)
	Horiz. Location × Day	−677	349	0.055 (ns)
Gonad+ muscle+ kidney+ foot (GoMu)	Depth × Day	74	157	0.638 (ns)
	Horiz. Location × Day	−1245	1028	0.229 (ns)
Gill+ mantle (GiMa)	Depth × Day	84	126	0.507 (ns)
	Horiz. Location × Day	179	825	0.829 (ns)

Table 3. Best-subsets regression models of the depuration rate of the whole body tissues using temperature (T), salinity (S), *in vivo* fluorescence (Fluo.) and light transmission (LightTr.) as predictors. R^2 (adj): the explained percentage of variance corrected in function of the number of variables in the regression. An X in the column of T, S, Fluo. or LightTr., indicates that the variable was included in the regression. The high Mallows C_p indicates that the imprecision of the estimated coefficient is large.

Number of variables	R^2	R^2 (adj)	Mallows C_p	Predictor variables T	S	Fluo.	LightTr.
1	83.8	80.6	108.7	X			
1	65.5	58.6	235.4		X		
2	87.2	80.8	87.5	X	X		
2	84.6	76.9	105.5	X		X	
3	88.0	76.1	83.6	X	X	X	
3	87.9	75.7	84.9	X	X		X

Table 4. Best-subsets regression of the depuration rate of the whole body tissues on the co-variation (scores of the principal components analysis of the variables in the pair) of the temperature-salinity (T−S) and fluorescence- light transmission (Fluo.-LightTr.) pairs. Other entries as in Table 3. C_p values are much smaller than those in Table 3 and indicate a substantially better precision in the estimation of the regression coefficients.

Number of variables	R^2	R^2 (adj)	Mallows C_p	Predictor variables Fluo. - LightTr.	T−S
1	76.4	71.7	1.5		X
1	44.1	33.0	7.6	X	
2	78.9	68.3	3.0	X	X

The three groups of organs studied were not affected by the environmental conditions in the same way. The depuration of the digestive gland mimics the response of the whole body, as would be expected, owing to its great contribution to the pool of toxins (more than 95% on average). The depuration of domoic acid from the GoMu group, the edible part of the scallop, was the least affected by the environmental conditions. In fact, none of the two pairs of variables had a significant effect. The GiMa group was negatively affected by fluorescence-light transmission (Table 5).

Table 5. Parameters of the regressions of the depuration rates on the co-variation (scores of the principal components analysis of the variables in the pair) of the temperature-salinity (T−S), and fluorescence-light transmission (Fluo.- LightTr.) pairs, for each group of tissues used in the experiment (*GoMu* = Gonad + adductor muscle + kidney + foot; *GiMa* = Gill + Mantle). R^2 (adj) is the explained percentage of variance corrected in function of the number of variables in the regression.

Tissue	Constant ×10^{-4}	Coefficients of the Predictor variables T−S ×10^{-4}	Fluo. -LightTr. ×10^{-4}	R^2 (adj)
Whole body	86	11.5		71.7
Digestive gland	70	13.7		78.7
GoMu	-	-	-	-
GiMa	156		−30.0	54.7

4 Discussion

Depuration rate

The depuration rates measured in this study are consistent with previous observations made on this species of scallop from Galicia (Blanco et al. 2002a), but slower than what was reported by Fernández et al. (2000) for the same species in Andalucía (S Spain). Other scallop species, *Placopecten magellanicus*, depurate faster (Douglas et al. 1997) and, among other bivalves, the differences seem to be even greater than in pectinids. Mussels, for example, depurate very quickly, (Gilgan et al. 1990; Novaczek et al. 1992; MacKenzie et al. 1993; Whyte et al. 1995; Blanco et al. 2002b) and the razor clam *Siliqua patula* (Horner et al. 1993; Drum et al. 1993), on the other end of the depuration range, does so even more slowly than *Pecten maximus*.

Effect of location on the depuration rate

Placing scallops containing domoic acid in rafts is a good way to accelerate their depuration. The time required to reach toxin concentrations below the legal limit can be reduced by more than 30%. Although this reduction of the duration of the banning period is important in itself, it would likely have

greater repercussions on the harvesting activities because it increases the probability of attaining a low toxin concentration (below the regulatory ban limit for harvesting, for direct consumption or for selective evisceration) between two successive toxic outbreaks. This 30% reduction could help to mitigate the effects of the usual coupling between successive intoxication processes due to seasonal toxic phytoplankton outbreaks, which is one of the processes responsible for the long duration of banning periods.

The depuration of the edible tissues (GoMu group) was also accelerated when scallops were suspended from the raft, but this acceleration was substantially lower than those of the whole body and digestive gland. In any case, it is possible to obtain an approximate reduction of 15% in the time needed to reach toxin concentrations below the legal threshold for harvesting without selective evisceration.

Of the non-edible tissues, digestive gland depuration was affected by location in the same way as the whole body, as was to be expected, since it is responsible for more than 95% of the total toxin contents. The GiMa group, on the other hand, was affected by the relocation of scallops in the raft, by slowing down the depuration process.

If selective evisceration were carried out, according to EU Decision 206/2002 (Anonymous 2002), eliminating the most toxic and the non-edible tissues to render the scallops fit for human consumption, then the benefit obtained by relocation in rafts would correspond to the reduction of depuration time estimated for the edible tissues (*ca.* 15%). Notwithstanding, if, for authorisation, the entire process of evisceration necessitates an additional requirement whereby the whole tissues must not surpass a certain threshold to minimize the risk involved in processing highly toxic organisms, as stipulated by the European Union (Fernández et al. 2003a; Fernández et al. 2003b), then the reduction in depuration time would be substantial (over 30%), and the associated economical benefits, of considerable importance. Just to illustrate the possible advantages obtained, let us assume that a population of scallops has accumulated 1000 μg of domoic acid g^{-1} of tissues during the spring toxic outbreak. It would take the wild populations nearly 8 months to attain 250 $\mu g\, g^{-1}$ of tissues (the established threshold to allow harvesting for evisceration in the EU), while that period would be reduced to 5 months for the scallops located in the raft. In the first case, the scallops might very well become re-intoxicated during an autumn outbreak, if one should occur. However, in the second case, it is highly likely that a major part of the population would be harvested and processed before the next toxic outbreak took place.

This way of acceleration of the depuration has, notwithstanding, the possible drawback that, if a new outbreak occurs, the toxin ingestion of the suspended scallops can be substantially higher that that of the wild scallops. In fact, this happened after the period studied. Although the vertical distribution of toxic phytoplankton during the recorded bloom may not hold for other blooms, this is clearly a risk of using this technique to accelerate the depuration.

There were minor differences in depuration rate between the scallops placed in different locations in the raft. The surface populations located in the central area of the raft depurated faster than the others. However, from a practical point of view, the surface locations should probably be avoided if long depuration periods are expected, because the mortality of the scallops may be higher than in deeper locations where the salinity is higher; the temperature lower and the variations in these two factors are dumpened. In fact, some months after the experiment had ended, all the scallops remaining at the surface of the raft died after a period of heavy rainfall.

Environmental control of depuration

The scallops in the different locations were also subjected to different environmental conditions (Fig. 1). The depuration rate was related to the measured environmental variables, but, although this study was designed to minimise the co-variation between variables, this objective was not fully achieved. As a consequence, it is not possible to determine the individual effect of each of the environmental variables measured.

During our study, the depuration rate of the whole body, and of each of the body tissues studied, increased with the increase of temperature and the decrease of salinity. These two factors were very interrelated (negatively), showing a high covariation, thus making it impossible to statistically discriminate their individual effects. Notwithstanding, some facts suggest that temperature is the main responsible for the regulation of the depuration rate.

Temperature has been assumed to accelerate the depuration of several toxins in different bivalves as it accelerates their metabolism (Bayne and Newell 1983) and increases the rate of several activities of pectinids (Bricelj and Shumway 1991; Laing 2004; Heilmayer et al. 2005), although the capability to acclimatise of bivalves for this factor is, in general, important, and it has interactions with other factors (Navarro et al. 2000). Additionally, in at least some bivalve species such as *Dreissena polymorpha*, nitrogen excretion increases with temperature to a greater degree than respiration, indicating an increased protein catabolism as well (Aldridge et al. 1995), thus suggesting the possibility of acceleration of the elimination of the domoic acid together with other amino acids. To our knowledge, the only studies on domoic acid that involved temperature, dealt with mussels and did not demonstrate the effect of this factor. Silvert and Subba Rao (1992) included temperature in a model of domoic acid accumulation in *Mytilus edulis*, assuming that it has some effect. Blanco et al. (2002b) did not find a clear effect of temperature on the elimination of these toxins from another mussel species (*Mytilus galloprovincialis*). Mytilids depurate domoic acid at a much higher rate than *Pecten maximus* making it probably difficult to estimate precisely the effect of temperature on depuration in the scallop.

On the other hand, although low salinity could be expected to increase the depuration rate, since the excretion of amino acids is involved in the regulation of the osmotic pressure (Burton 1983), it has been shown to have a negative effect on the depuration of domoic acid from *Mytilus galloprovincialis* (Blanco et al. 2002b).

Consequently, it seems unlikely that salinity could play an important role in domoic acid depuration, and temperature seems to be the most important factor of the pair.

The two variables related to food and/or suspended matter, have been suggested to have some importance in the depuration of PSP toxins, another group of hydrosoluble phycotoxins (Blanco et al. 1997). Nevertheless, in this study, they seem to have little effect on the depuration of domoic acid from the whole body and the digestive gland, little or no effect on the GoMu group, and some effect on the GiMa group. In the case of digestive gland, food seems to contribute to depuration (positive regression coefficient) but the opposite occurs in the other tissues studied, where it appears to slow down the process of elimination of domoic acid. This could be caused by the transfer of toxins with food resources from the digestive gland – the organ in charge of acquiring them – to other organs or tissues. This speculation is supported by an apparent transfer from the digestive gland to the gonad. Several other environmental conditions were not measured in this study, but presumably they would differ among the experimental locations, and consequently their effect cannot be precluded. This is true for turbulence, for example, more intense in the front of the raft than in the centre, or light, which decreases with depth. Light has been shown to have an effect on some molluscs (Andreu 1960; Nielsen and Strömgren 1985) and the increased turbulence at the front of the raft could also have had some effect on reducing the activity of the scallops. This, however, seems unlikely, as it has not had any noticeable effect on the growth rate (Fig. 2). The results obtained should, therefore, be interpreted with caution from a causal point of view.

Acknowledgements. This research was funded by the Plan Galego de Investigación e Desenvolvemento Tecnolóxico, through the Project PGIDT-99-PXI50101.

References

Aldridge D.W., Payne B.S., Miller A.C., 1995, Oxygen consumption, nitrogenous excretion, and filtration rates of *Dreissena polymorpha* at acclimation temperatures between 20 and 32 °C. Can. J. Fish. Aquat. Sci. 52, 1761-1767.

Amzil Z., Fresnel J., Le Gal D., Billard C., 2001, Domoic acid accumulation in french shellfish in relation to toxic species of *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima*. Toxicon 39, 1245-1251.

Andreu B., 1960, Ensayos sobre el efecto de la luz en el ritmo de crecimiento del mejillón (*Mytilus edulis*) en la Ría de Vigo. Bol. R. Soc. Esp. Hist. Nat. 58, 217-236.

Anonymous, 2002, Commission Decision 2002/226/EC of 15 March 2002 establishing special health checks for the harvesting and processing of certain bivalve molluscs with a level of annesic shellfish poison (ASP) exceeding the limit laid down by Council Directive 91/492/EEC. Off. J. Eur. Communities 16/03/02, L75, 65-66.

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.

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.

Bayne B.L., Newell R.C., 1983, Physiological energetics of marine molluscs. In: Saleuddin A.S.M., Wilbur K.M. (eds) The Mollusca. Vol. 5. Physiology part 1. Academic Press, New York, pp. 407-515.

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

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. Progr. Ser. 158, 165-175.

Blanco J., Zapata M., Moroño A., 1996, Some aspects of the water flow through mussel rafts. Scient. Mar. 60, 275-282.

Bricelj V.M., Shumway S.E., 1991, Physiology: Energy acquisition and utilisation. In: Shumway S.E. (ed) Scallops: Biology, Ecology and Aquaculture. Elsevier, New York, pp. 305-346.

Burton R.F., 1983, Ionic regulation and water balance. In: Saleuddin A.S.M., Wilbur K.M. (Eds.) The Mollusca. Vol. 5. Physiology part 2. Academic Press, New York, pp. 291-352.

Campbell, D.A., Kelly, M.S., Busman, M., Bolch, C.J., Wiggins, E., Moeller, P.D.R., Morton, S.L., Hess, P., Shumway, S.E., 2001, Amnesic shellfish poisoning in the king scallop, Pecten maximus, from the west coast of Scotland. J. Shellfish Res. 20, 75-84.

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, L., Marco, J., Moreno, O., Santamaría, M., 2000, Ensayos de desintoxicación de ASP en Vieiras (*Pecten* spp.). In: Ildefonso Márquez (Coord.) VI Reunión Ibérica sobre Fitoplancton Tóxico y Biotoxinas. Junta de Andalucía, Sevilla, pp. 175-181.

Fernández M.L., Shumway S.E., Blanco J., 2003a, Management of shellfish resources. In: Hallegraeff G.M., Anderson A.D., Anderson D.M. (Eds.) Manual on Harmful Marine Microalgae. UNESCO Publishing, Paris, pp. 657-692.

Fernández M.L., Míguez A., Cacho E., Martínez A., 2003b, European approaches to marine toxin control. Towards harmonisation. In: Villalba A., Reguera B., Romalde J.L., Beiras R. (Eds.) Molluscan Shellfish Safety. Xunta de Galicia and IOC-UNESCO, Santiago de Compostela, pp. 150-167.

Gilgan M.W., Burns B.G., Landry G.J., 1990, Distribution and magnitude of domoic acid contamination of shellfish in Atlantic Canada during. 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.

Heilmayer O., Honnen C., Jacob U., Chiantore M., Cattaneo-Vietti R., Brey T., 2005, Temperature effects on summer growth rates in the Antarctic scallop, *Adamussium colbecki.* Polar Biol. 28, 523-527,

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

Laing I., 2004, Filtration of king scallops (*Pecten maximus*). Aquaculture 240, 369-384.

Lund J.A.K., Barnett H.J., Hatfield C.L., Gauglitz E.J., Wekell J.C., Rasco B., 1997, Domoic acid uptake and depuration in Dungeness crab (*Cancer magister* Dana, 1852). J. Shellfish Res. 16, 225-231.

MacKenzie A., White D.A., Sim P.G., Holland A.J., 1993, Domoic acid and the New Zealand Greenshell mussel (*Perna canaliculus*). In: Smayda T.J., Shimizu Y. (Eds.) Toxic phytoplankton blooms in the sea. Elsevier Sci. Publ. B.V, Amsterdam., pp. 607-612.

Moroño A., Pazos Y., Doval M.D., Maneiro J., 2004, Floraciones algales nocivas y condiciones oceanográficas en las rías gallegas durante los años 2001 y 2002. In: Norte M., Fernández J.J. (eds) VIII reunión Ibérica sobre fitoplancton tóxico y biotoxinas. Instituto de Bio-orgánica., La Laguna. Spain, pp. 195-210.

Navarro J.M., Leiva G.E., Martinez G., Aguilera C., 2000, Interactive effects of diet and temperature on the scope for growth of the scallop *Argopecten purpuratus* during reproductive conditioning. J. Exp. Mar. Biol. Ecol. 247, 67-83.

Nielsen M.V., Strömgren T., 1985, The effect of light on the shell length growth and defaecation rate of *Mytilus edulis* (L.). Aquaculture 47, 205-221.

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.

Pazos Y., Moroño A., Miranda M., Maneiro J., 2003, Evolución de las condiciones oceanográficas y fitoplancton tóxico/nocivo en los años 1999-2000 en las Rías Gallegas. Actas de la VII Reunión Ibérica sobre fitoplancton tóxico y biotoxinas. Consellería de Agricultura, Pesca y Alimención. Generalitat de Valencia, Valencia, pp. 195-210.

Quilliam M.A., Sim P.G., McCulloch A.W., McInnes A.G., 1989, High-performance liquid chromatography of domoic acid, a marine neurotoxin, with application to shellfish and plankton. Internat. J. Environ. Anal. Chem. 36, 139-154.

Salgado C., Maneiro J., Correa J., Pérez J.L., Arévalo F., 2003, ASP biotoxins in scallops: the practical application in Galicia of Commision Decision 2002/226/EC. In: Villalba A., Reguera B., Romalde J.L., Beiras R. (Eds.) Molluscan Shellfish Safety. Xunta de Galicia and IOC-UNESCO, Santiago de Compostela, pp. 169-177.

Shumway S.E., Cembella A.D., 1993, The impact of toxic algae on scallop culture and fisheries. Rev. Fish. Sci. 1, 121-150.

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.

Vale P., Sampayo M.A.M., 2001, Domoic acid in Portuguese shellfish and fish. Toxicon 39, 893-904.

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. Technique et documentation-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.