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Depuration of Okadaic acid (Diarrhetic Shellfish Toxin) in mussels, *Mytilus edulis* (Linnaeus), feeding on different quantities of nontoxic algae

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Abstract

Depuration of mussels contaminated by Diarrhetic Shellfish Toxins (DST) is a potential option for the shellfish industry to manage the impact of DST. Field observations have suggested that the main factor regulating the rate of depuration of DST is the quantity of nontoxic algae available for the mussels to feed upon. In this paper, the effects of the quantity of food, which mussels feed upon, on the rate of depuration of DST in *Mytilus edulis* L. was tested in a laboratory experiment. Mussels naturally contaminated by the DST okadaic acid (OA) were collected from a mussel farm located on the Swedish west coast during a bloom event. Individual mussels were placed in filtered seawater and given daily rations of a mixture of nontoxic algae as follows: no food, 0.5% and 1.5% of dry weight body mass day^{-1}. Depuration was performed over 1, 2, 4, 8, 16 or 32 days. The levels of OA decreased in all treatments with time, with an average of approximately 50% reduction after 32 days. No significant differences in content of OA among food rations were detected. In contrast to predictions, a trend towards lower levels of toxins in the mussels receiving no food compared to both food treatments was observed after 32 days of depuration. The loss of toxins in mussels that were not feeding correlated with a considerable loss in the mass of the digestive gland between 16 and 32 days. It was concluded that the rate of depuration of OA in mussels is not positively correlated with digestive activity and fecal production. Instead, the lipophilic character of the OA molecule suggests that OA may have affinity for lipid-rich cellular and intracellular components. Increased usage of lipid stores, which occur during starvation, may accelerate the release of OA. This model could explain the observations made during the last part of this experiment. In management of toxic

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mussels, depuration in waters free of toxic algae is not likely to be enhanced by increasing the food supply to mussels; however, long periods of depuration in the absence of food should be avoided because of the negative effects on the condition of the mussels.

Keywords: Mussels; Diarrhetic Shellfish Toxins; Okadaic acid; Depuration; Digestive activity; Management

1. Introduction

Diarrhetic shellfish poisoning (DSP) is one of several seafood poisoning syndromes caused by marine phycotoxins. Although not a life-threatening condition, DSP is a worldwide problem for the bivalve aquaculture and fisheries industries (Hallegraeff, 1993). Closure of shellfish harvest areas for long periods due to the presence of DSP toxins (DST) are common and are causing major economical losses in some countries. For example, farmed blue mussels, *Mytilus edulis*, along the Swedish west coast generally contain levels of DST above the tolerance limit for harvest of shellfish for up to 6 months each year (Lindegarth, 1997). This is the largest impediment to the development of mussel farming in Sweden (Kollberg, 1999).

The toxic compounds mainly responsible for DSP are okadaic acid (OA) and the structurally related DTX-1, DTX-2 and DTX-3 (Yasumoto et al., 1985; Kumagai et al., 1986; Carmody et al., 1996). Except for DTX-3, which is believed to be a metabolic product in the bivalves (Lee et al., 1989), these lipophilic polyether fatty acids are produced by marine dinoflagellates of the genera *Dinophysis* and *Prorocentrum* (Yasumoto et al., 1980; Murakami et al., 1982; Murata et al., 1982). The toxicity of the OA group of compounds comes from their ability to bind and inhibit the activity of protein phosphatases, which in turn causes major effects on signal transduction pathways inside cells (Bialojan and Takai, 1988; Haystead et al., 1989).

Different options for how the negative impacts of DST can be managed and minimised have been proposed (Christophersen and Strand, 1994; Dijkema et al., 1995; Blanco et al., 1999; Kollberg, 1999). In several areas, DST-producing algae have been reported to be more or less permanently absent (Sedmak and Fanuko, 1991; Haamer, 1995; Poletti et al., 1996). Such waters, usually fjords, bays and lagoons characterized by restricted inflow of water from the open sea, can be used permanently for farming but also temporarily for ‘storing’ and depuration of bivalves, which are relocated from other areas.

Another option is to develop methods for the depuration of toxic mussels in natural or controlled systems. However, to achieve cost-effective depuration both in controlled systems and in the natural environment, it is essential to identify whether environmental conditions affect the depuration kinetics of DST. Also important is to understand the physiological mechanisms by which bivalves eliminate DST.

Some field observations and experiments on depuration of DST in various species of bivalves have been performed (Lindahl and Hageltorn, 1986; Haamer et al., 1990; Sampayo et al., 1990; Marcaillou-Le Baut et al., 1993; Sedmak, 1995; Bauder et al., 1996; Poletti et al., 1996; Blanco et al., 1999). Some of these studies have suggested

that the quantity of nontoxic food resources is the most important factor regulating the rate of depuration. One physiological model to explain how food affects the rate of depuration of DST in mussels is discussed in Blanco et al. (1999). When nontoxic food resources become more abundant, ingestion rates in the mussels increase, which in turn leads to a higher digestive activity and greater metabolic fecal loss. Fecal deposition has been suggested to be the main route for elimination of DST (Bauder et al., 1996; Blanco et al., 1999). Thus, as a consequence of increased feeding rates, it is hypothesized that DST is eliminated at a higher rate in the mussels. In Swedish blue mussels, high levels of OA are generally detected during the autumn and winter period, followed by a fast reduction during early spring. The reduction of OA coincides with the spring bloom of diatoms. This indicates that feeding on nontoxic algae may be important for depuration. The observations of positive correlations between the concentration of algae and the rate of depuration is consistent with, but not evidence for the notion that high rates of ingestion causes high rates of depuration. This has yet to be shown in manipulative experiments.

Here, the model that the supply of nontoxic algae to mussels affects the rate of depuration is tested. It was predicted that the concentration of OA would decrease at a greater rate in mussels, which received larger concentrations of food than in those receiving lower concentrations or no food. A laboratory experiment was performed where blue mussels, naturally contaminated by OA, were supplied with different rations of algae and depurated for up to 32 days.

2. Materials and methods

2.1. Animals and algal food source

Blue mussels containing OA were collected from a long-line mussel cultivation farm on the 23rd of October, 1998, 1 day prior to the start of the experiment. The farm is situated in the vicinity of Tjärnö Marine Biological Station on the west coast of Sweden where the experiment was conducted. Levels of OA above the limit for harvest (160 μg kg^{-1} mussel meat) had been detected for approximately 1 month prior to October 23. Monitoring data from the farm between July 1998 and April 1999 is shown in Fig. 1 and the experimental period is indicated in the graph. Equally sized mussels (age 3–5 years, shell length 85 ± 3.5 mm S.D.) were selected for the experiment and a subsample was taken for determination of soft tissue dry weight (4.6 ± 1.5 g) and OA (2.90 ± 0.90 μg OA g^{-1} digestive gland). The mussels were kept in air at 4 °C overnight.

A mixture of two species of unicelled algae were used as food: *Isochrysis galbana* var. *tahaitian* (T-ISO), and *Thalassiosira pseudonana* (3H), supplied as algal pastes from Reed Mariculture, Inland Sea Farm, USA. The dry mass per volume unit (mg ml^{-1}) was estimated for each paste and based on this, equal amounts of paste were then combined in the proportions four parts of T-ISO to five parts of 3H. The final cell concentration was 5.6×10^9 cells g^{-1} dry mass. The algal pastes were resuspended in filtered seawater by a magnetic stirrer to desired concentrations.

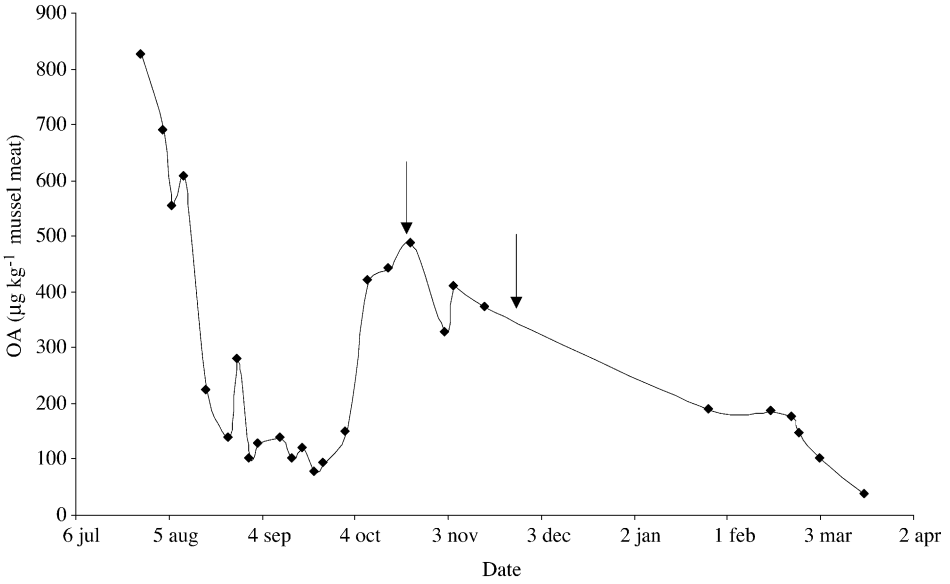

Fig. 1. Data from the national monitoring program on concentration of OA (µg kg^{-1} mussel meat) in mussels from Tjärnö Vattenbruk farm site from July 1998 to April 1999. The experimental period is indicated between the arrows.

2.2. Experimental conditions

The experiment was designed as a two-factor analysis of variance (ANOVA) with food rations and days of depuration as fixed factors. Three levels of food rations were used: no food, 0.5% (ration 1) and 1.5% (ration 2) of dry body mass day^{-1}. The total amount of algae and cell numbers in each food ration is shown in Table 1. These food rations were chosen as moderate and high concentrations and were based on observations on feeding rates for mussels of the size employed in this experiment (Bayne, 1976).

The mussels were depurated for 1, 2, 4, 8, 16 or 32 days. The animals were then sacrificed for subsequent weight measurements and analysis of OA content. Five replicate mussels were included per treatment at each time. Thus, a total of 90 mussels were included in the experiment.

The experiment was conducted between October 24 and November 26, 1998 at 10 °C. This corresponded to the water temperature in the field when mussels were collected.

Table 1
Total amounts of algae in food rations 1 and 2

	A	B	C
Ration 1	0.5	23	129
Ration 2	1.5	69	387

(A) % dry body mass day^{-1}.
(B) mg dry weight day^{-1}.
(C) cell numbers × 10^6 day^{-1}.

Individual mussels were placed in 1l plastic beakers containing 800 ml of filtered natural seawater (0.2 μm Millipore filter) with a salinity of 2.5%. Air was supplemented to each beaker in order to keep the algae in suspension and to avoid oxygen depletion, as well as to reduce ammonia levels. Care was taken not to resuspend any fecal material. Every second day during the experiment, the whole volume of water was replaced by freshly filtered seawater. Prior to changing water, the fecal deposits were collected by a Pasteur pipette into preweighed glass vials and stored in a freezer for determination of total fecal production for each depuration period. The insides of the beakers were cleaned to avoid microbial growth.

Algal particles were added by pipetting 5 ml algal suspension to each beaker 10 times per day. Before adding new algae, the water was visually inspected to verify that the mussels had cleared the cells from the previous addition. To the no-food treatment, 5 ml of filtered seawater was added at each feeding occasion. Ingestion rates were not explicitly measured but the production of faeces was measured and used as an indirect measure of ingestion. Only minor amounts of pseudofaeces were occasionally produced in the high ration level. This was not included in the estimation of fecal production.

2.3. Measurements

When mussels were sacrificed, soft tissue was removed from the shell and drained on paper whereafter the digestive gland was separated from the rest of the tissue. Total soft tissue wet weights and weights of the digestive glands were determined. The digestive glands were then immediately frozen in -20 °C for chemical analysis of OA.

Content of OA and DTX-1 was analyzed in individual digestive glands according to the HPLC method of Lee et al. (1987) with minor modifications. The 1-pyrenyldiazomethan (PDAM, from Molecular Probes, Europe, the Netherlands) was used as a fluorescent labelling agent and for clean-up procedures, silica gel cartridge columns were used.

Faeces produced for each treatment were stored in -20 °C and dry mass was later determined by incubating the glass vials for 12 h in 80 °C. OA content of faeces was not measured.

2.4. Statistical analysis

Differences among treatments were analysed using a two-factor analysis of variance (ANOVA). Data was first checked for homogeneity of variances using Cochran's test. For significant effects, Student–Neuman–Keuls (SNK) a posteriori test for differences among means was applied.

3. Results

3.1. Concentration of OA in the digestive gland

The effects of food rations and days of depuration on concentration of OA are shown in Fig. 2a. In general, the concentration of OA was reduced in all rations

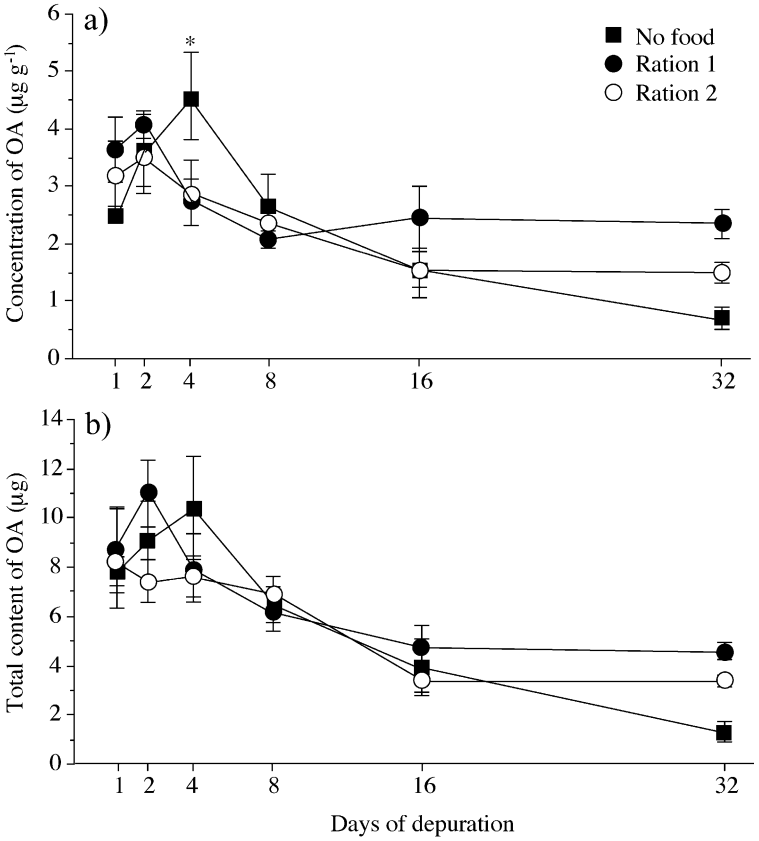

Fig. 2. The effects of food rations and days of depuration on levels of OA. (a) Concentration of OA, expressed as µg g^{-1} digestive gland. (b) Total content of OA, expressed as µg in the digestive gland. Error bars represent S.E.

during the experiment from 3.1 µg OA g^{-1} digestive gland (dg) on average at day 1 to 1.51 µg OA g^{-1} dg at day 32. Between the first 2 days, there was a trend towards an increase in the toxin concentration for all treatments. In mussels feeding on rations 1 and 2, the concentration of OA was then reduced after 4 days, whereas in the mussels receiving no food, there was a further increase. At day 4, the toxin concentration in mussels receiving no food was significantly higher compared to rations 1 and 2 ($p < 0.05$ for food ration × depuration days, Table 2). This was the only day where a significant difference was observed between treatments. Although not statistically different, the second largest effect among experimental treatments was observed after 32 days of depuration. At this time, the lowest concentration of OA was observed in mussels receiving no food. This result was qualitatively contradictory to the predicted effects of food concentration, since it seemed to indicate that mussels receiving no food might have a faster reduction of OA.

Table 2
ANOVA on the effects of food ration and days of depuration on concentration of okadaic acid content (μg OA g^{-1} digestive gland), total content of OA in individual digestive glands (μg OA) and fecal production (g dry weight), untransformed data

Source of variation	*df*	Concentration of OA			Total content of OA			Fecal production		
		MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Food Ration	2	1.32	1.28	0.28	7.46	1.11	0.34	0.10	244.1	0.0001
Days	5	11.96	11.62	0.0001	98.86	14.69	**0.0001**	0.09	212.9	0.0001
Ration × Days	10	2.28	2.21	**0.03**	7.46	1.11	0.37	0.03	65.4	**0.0001**
Residual	72	1.03			6.73			0.0004		
SNK		day 4: no food>ration 2 = ration 1			day 2 = day 4 = day 1 = day 8>day 16 = day 32			day 8, 16 and 32: no food < ration 1 < ration 2		

For significant effects ($p < 0.05$), Student–Neuman–Keuls (SNK) a posteriori test for differences among means was applied.

3.2. Total content of OA in the digestive gland

The increase in the concentration of OA during the first days of depuration was somewhat confusing because no toxin-producing algae are added to the mussels. This increase can be explained if the digestive gland mass is reduced and at the same time, the content of OA remains constant or decreases at a lower rate compared to digestive gland mass. Because concentration of OA is calculated as micrograms per gram digestive gland, this could then render higher concentrations of OA. This explanation is supported by the observations of the weights of the digestive glands (Table 3). The increased concentration of OA at day 4 in the mussels, which were starved, coincides with a lower weight of the digestive glands compared to the other food treatments. Therefore, an additional analysis was done on the total content of OA (concentration of OA multiplied by the mass of the digestive gland), which is illustrated in Fig. 2b. The mean content of OA was reduced in all treatments from 8.3 μg on day 1 to 3.1 μg OA on day 32. Toxin content was significantly

Table 3
Soft tissue wet weights and digestive glands wet weights for each food ration and depuration period during the experiment

	Days of depuration					
	1	2	4	8	16	32
Soft tissue wet weight (g)						
No food	18.2 ± 1.6	16.8 ± 4.0	15.7 ± 4.2	16.5 ± 1.7	17.3 ± 3.7	14.4 ± 3.1
Ration 1	16.6 ± 1.9	16.5 ± 3.6	18.9 ± 1.4	19.8 ± 2.2	13.5 ± 2.5	15.5 ± 2.9
Ration 2	17.7 ± 2.3	17.5 ± 3.4	15.7 ± 4.9	19.2 ± 2.2	15.8 ± 1.0	16.6 ± 1.8
Digestive gland wet weight (g)						
No food	3.1 ± 0.5	2.5 ± 0.8	2.2 ± 0.5	2.6 ± 0.6	3.0 ± 0.5	1.7 ± 0.4
Ration 1	2.3 ± 0.2	2.7 ± 0.5	2.9 ± 0.4	2.9 ± 0.4	2.0 ± 0.6	2.0 ± 0.3
Ration 2	2.6 ± 0.5	2.3 ± 0.6	2.9 ± 1.0	3.0 ± 0.5	2.3 ± 0.4	2.4 ± 0.5

Values are means ± S.D. ($n = 5$).

lower after 16 days of depuration and thereafter ($p < 0.0001$, Table 2). No significant differences between mussels fed on the different food rations after 4 days of depuration were detected, which indicated that reduction in the mass of the digestive glands in mussels receiving no food contributed to the significant increase in the concentration of OA in the previous analysis. Even so, a trend towards an increase in content of OA for both ration 1 and the no-food treatment during the first days of depuration remained. The difference, although not statistically significant, between mussels receiving no food on one hand and mussels feeding on rations 1 and 2 was further pronounced after 32 days. Mean total content in the mussels, which did not receive any food, was 1.3 μg OA compared to 4.6 and 3.4 μg OA in rations 1 and 2, respectively.

3.3. Fecal production

Total fecal production (g dry weight) for each treatment during the experiment is shown in Fig. 3. The amount of faeces produced differed significantly among treatments after 8 days and thereafter (Table 2). Ration 2 had a higher production compared to ration 1, which in turn produced more faeces, compared to the starved mussels. In the no-food treatment, only a minor increase in total fecal production was observed during the whole experiment, indicating that no further ingestion occurred. The small increase in fecal production towards the end of the experiment is probably due to metabolic fecal loss. Overall, the differences in fecal production among treatments indirectly confirmed that mussels were feeding and ingesting food according to what was intended in the experimental design.

3.4. Soft tissue and digestive gland mass

Average mass of soft tissues and digestive glands for each treatment during the experiment are presented in Table 3. Some temporal variability among treatments was

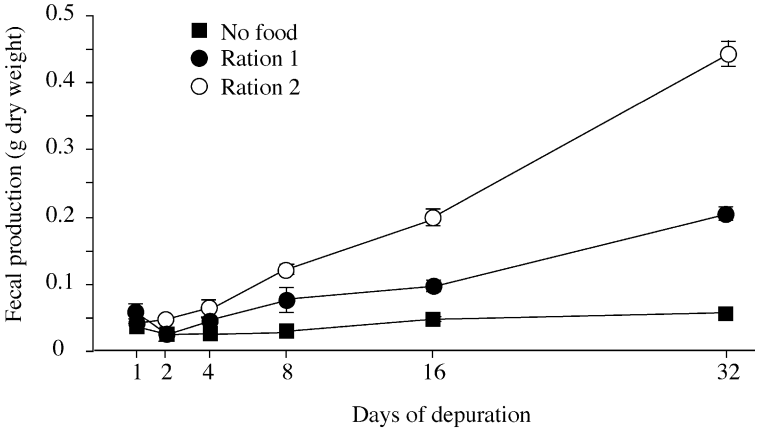

Fig. 3. Total fecal production for each treatment, expressed as g dry weight. Error bars represent S.E.

observed. In general, the temporal changes for mussels feeding on rations 1 and 2 were very similar. For example, the mass of soft tissue and digestive gland seemed to increase slightly from day 1 to day 8 and then decrease between 8 and 16 days of depuration. After 32 days, tissue and digestive gland mass were more or less similar to the initial values at day 1. For mussels that received no food, a trend towards a decrease in tissue and digestive gland mass was observed during the first 4 days and was followed by a slight increase up to 16 days. Both tissue and digestive gland mass then decreased again and was lower at day 32 compared to day 1. The tissue was also visually inspected when the mussels were dissected. Mussels that were feeding on rations 1 and 2 appeared to have healthy tissues and digestive glands with a dark colour and firm texture throughout the experiment. Mussels receiving no food were showing signs of starvation, such as pale flesh colour and digestive glands as well as water-filled, soft tissues. This was most pronounced at the end of the experiment. Care should always be taken when interpreting data on tissue wet weights since differences in water content in the tissue could mask real changes in tissue mass. For starved mussels, the loss in body mass was probably larger than what was indicated by measuring wet weights. To summarise, the overall observations on total tissue and digestive gland mass indicated that mussels receiving no food behaved differently to those feeding on nontoxic algae.

4. Discussion

The availability of nontoxic food has been proposed by several authors as the main factor affecting depuration of DST in mussels (Haamer et al., 1990; Sampayo et al., 1990; Marcaillou-Le Baut et al., 1993; Poletti et al., 1996; Blanco et al., 1999). A physiological mechanism to explain how food affects rate of depuration of DST was proposed by Blanco et al. (1999) where increased ingestion rates leads to a higher digestive activity and greater metabolic fecal loss. Since fecal deposition has been suggested to be the main route for elimination of DST by Blanco et al. (1999) and Bauder et al. (1996), the toxins are eliminated at a higher rate during high ingestive activity. Field depuration by relocating mussels from toxic to nontoxic environments has been performed by Haamer et al. (1990), Marcaillou-Le Baut et al. (1993), Poletti et al. (1996) and Blanco et al. (1999). Blanco et al. (1999) found in a multivariate analysis of the effects of environmental conditions that indirect measures of particle concentration (fluorescence and light transmission) appeared to increase the rate of depuration. Marcaillou-Le Baut et al. (1993) transplanted toxic mussels to an oyster culture pond and compared depuration with mussels depurated in the laboratory. They assumed that the faster rate observed for mussels in the oyster pond was due to a higher supply of natural phytoplankton compared to the laboratory condition. However, differences in several other factors between the oyster pond and the laboratory environment may also have contributed to this result. Both Haamer et al. (1990) and Poletti et al. (1996) observed reduction in DST content when mussels were transplanted to less toxic environments; however, no measures of food content in the water was performed. Depuration experiments in the laboratory has also been done by Haamer et al. (1990) and Croci et al. (1994). Haamer et al. (1990) compared depuration of mussels in basins with or without the addition of yeast particles. Toxin content was reduced after 1

week in both treatments but the authors observed more variability among replicates in mussels not receiving yeast. Croci et al. (1994) observed a high rate of depuration in two out of three samples in ozonised artificial seawater without any food added. These two studies suggest that depuration can occur even though no food particles are added to the system.

Even though most of the observations made by these authors suggest that food availability affects the rate of depuration of DST in mussels, the results are inconclusive and lack of replicates and controls for the effects of other factors indicate that it is still not clear whether this factor affects depuration of these toxins. Thus, the experiment presented in this paper was conducted in order to test the effects of food on depuration during controlled conditions. It was found that mussels that were fed with a high ration of algae had the highest amount of fecal production compared to mussels fed with a lower ration and starving animals. This confirmed that ingestion rates differed among treatments according to the predictions and in this way the experiment was successful. However, there was no significant difference in content of OA among food treatments over time. Depuration was achieved in all treatments with an average of 50% reduction after 32 days. Somewhat faster rates of depuration have been found by Marcaillou-Le Baut et al. (1993), Poletti et al. (1996) and Blanco et al. (1999). In these studies, depuration was observed in the field where the naturally occurring seston was available for the mussels to feed upon. The laboratory environment is likely to be less favourable and more stressful for mussels, which may have influenced the overall performance and rendered the relatively lower rates of depuration. Also, only two species of algae were used and the nutritional value of these species might not have been adequate for the physiological needs of the mussels. This imply that not only the quantity of food but the composition and phytoplankton species present in the food may be important aspects to consider for rate measurements in mussels.

Visual observations during the experiment in combination with measures of fecal production did, however, confirm that the mussels were filtering and ingesting algae, indicating that the water quality was acceptable for the mussels. Also, recontamination was avoided by removing fecal pellets together with water changes continuously during the experiment. The different rates of depuration observed in other studies could be explained by seasonal variations in the physiological status of mussels. Particularly in temperate latitudes, mussels undergo annual reproductive cycles that are associated with marked seasonal changes in biological composition and physiological rate processes (Hawkins and Bayne, 1992). Since the experiment reported here was performed in November at a low temperature, it is likely that general physiological rates were slow in the mussels, which resulted in the relatively low rate of depuration.

The significant increase in the concentration of OA for mussels receiving no food compared to the other treatments after 4 days of depuration was abolished by correcting for the decrease in digestive gland mass. Similar results where toxin concentration has been found to increase rather than decrease during the first days of depuration were observed by Haamer et al. (1990) and Marcaillou-Le Baut et al. (1993), who suggested that this phenomenon might be due to transport stress causing an increased metabolic rate and reduction of digestive gland. Hence, for the interpretation of the results in depuration experiments, it is important to monitor such changes in tissue mass. Even so, a trend towards increased levels of OA for ration 1 and the no-food treatment remained after

correction for changes in digestive gland mass. High variability in content of DST among individuals of naturally contaminated mussels (Edebo et al., 1988) may contribute to the variability observed during the first days of depuration where levels seem to fluctuate in different directions between days. It is also possible that hydrolysis of esterified OA to OA by esterases in the mussel digestive gland contributed to the initial rise in OA. Recent papers have indicated that mussels are capable of transforming OA, DTX-1 and DTX-2 to 7-*O*-acyl ester derivatives (so-called DTX-3) by attaching a fatty acyl group to the corresponding parent toxin (Marr et al., 1992; Fernández et al., 1996,1998; Suzuki and Mitsuya, 2001). The esterified DSTs exhibit low polarity and are not detected in standard HPLC analysis because a hydrolysis step to convert the esterified DST to its respective parent compound must be included in the analysis. Hence, a bioconversion of esterified OA to OA in the mussel tissue might have occurred, which could explain the increased, although not statistically different levels of OA during the first days of depuration. Biotransformation of OA to 7-*O*-acyl OA is likely to be a route for detoxification of OA in mussels since esterified OA exhibits lower toxicity, i.e. it is a less potent inhibitor of protein phosphatases (Yasumoto et al., 1989).

It was observed that the rate of depuration for feeding mussels was faster during the first 2 weeks and then slowed down during the last part, indicating biphasic depuration kinetics. This suggests that the toxins are distributed into two compartments with different depuration kinetics. Similar patterns have also been observed by Marcaillou-Le Baut et al. (1993), Fernández et al. (1998) and Blanco et al. (1999) for blue mussels and also for scallops by Bauder et al. (1996). Bauder et al. (1996) found that the rapid loss of toxins during the first 3 days of depuration coincided with the evacuation of toxin-producing algae from the viscera. Fecal deposition of recently ingested toxic algae and toxin not already incorporated into the tissue may hence be important for the release of DST during the first days of depuration. It seems possible that this process could be increased by adding food to the system. This was also observed in our experiment where content of OA in mussels feeding on the high ration was slightly lower on day 2 compared to the other treatments.

A pool of toxins more tightly bound to the tissue also seem to exist. The distribution and affinity of DST in the digestive gland tissue is likely to be governed to some degree by the physicochemical properties of the toxins. Following the pattern observed for xenobiotics such as organic contaminants, the degree of bioaccumulation depends mainly on the hydrophobicity of the contaminant together with the lipid content of the organism (Phillips, 1993). Similar pattern may be predicted for DST. The DST is lipophilic (Yasumoto et al., 1978) and the digestive gland is a major site for lipid storage in mussels (De Zwaan and Mathieu, 1992). The DST may thus have affinity for lipid-rich cellular and intracellular components such as lipid droplets or membrane structures. The release of DST from this compartment would then be dependent on a turnover of such cellular components. The route for elimination of DST from this compartment is probably through metabolic fecal loss as suggested by Blanco et al. (1999) but increasing the digestive activity by feeding the mussels may not result in a higher loss of DST. Instead, increased usage of lipid storages, which occur during late stages of starvation, might enhance the rate of depuration. This model could explain our observation that mussels given no food had a tendency to loose more toxins at the end of the experiment compared to the feeding

mussels. In future experiments, the relationships between lipid content and levels of toxin during depuration should be explored. To understand the depuration mechanisms, it is also important to identify more precisely where in the digestive gland tissue the DST are localised during uptake and depuration.

Studies on the effects of food on depuration of other types of toxins and contaminants in mussels have been investigated. Novaczek et al. (1992) and Wohlgeschaffen et al. (1992) found no difference in depuration rate of the hydrophilic neurotoxin domoic acid in feeding compared to starving *M. edulis*. Multivariate analysis of the effects of environmental factors on depuration of paralytic shellfish toxins (PST) in *Mytilus galloprovincialis* by Blanco et al. (1997) found that environmental variables, including phytoplankton concentration, seemed to be unimportant for the detoxification process. Chen and Chou (2001) observed that the depuration efficiency of PST in the purple clam, *Hiatula rostrata*, was similar for clams fed with nontoxic algae and starvation. Depuration of three congeners of hydrophobic polychlorinated biphenyls (PCB) in blue mussels was not affected by different algal concentrations (Björk and Gilek, 1997). In summary, depuration of various exogenous substances, including OA in our experiment, seems to be unaffected by food availability and occur regardless of whether the mussels are feeding or not.

In the natural field situation, depuration is likely to occur in mussels when the ingestion of toxic algae per time unit is reduced. This happens if the concentration of toxic algae decreases or disappears from the water column but filtration is maintained at a constant rate in the mussels. Also, an increase in the relative abundance of nontoxic species accompanying *Dinophysis* spp. may enhance depuration of DST. This was observed by Sampayo et al. (1990) in Portuguese waters. They suggested that a high relative abundance of nontoxic phytoplankton caused reduction in the filtration activities of the mussels in order to regulate their physiological needs for food. The intake of *Dinophysis* per time unit would thus decrease. This model is supported by several studies reviewed by Hawkins and Bayne (1992), where mussels have been found to maintain relatively constant rates of nutrient acquisition by adjusting rates of ingestion against their needs for maintenance and growth. This could also explain the increased rates of depuration of DST in mussels in Sweden during the onset of the spring bloom of diatoms. Usually, *Dinophysis* is still present in the water column during this period. This model together with the results from this paper suggest that the quantity of nontoxic food affects the mechanism of accumulation of DST rather than directly affecting the mechanism of depuration. Similar results concerning the rate of accumulation of PST in *M. galloprovincialis* was published by Morono et al. (2001) who found that accumulation of toxins was dependent on the volume-specific toxin concentration (VOSTOC) in a mixture of food particles.

5. Conclusions

Depuration can be an alternative for management of toxic mussels in order to ensure a continuous supply of mussels to the market. The results from this study have shown that depuration occurs even without the presence of algae in the system and that levels of OA can be reduced to approximately 50% of initial toxin content within 16 days. Depuration systems for mussels containing DST could hence be developed without the addition of

extra food, which reduces the cost for such systems. Long periods of depuration during conditions where no food is available is, however, not recommended since it has negative effects on the condition of the mussels, resulting in a loss of the commercial value. For depuration to be successful, i.e. to reach levels below the limit for marketing of mussels, it should be started before the mussels have accumulated high levels of toxin since depuration seems more effective during the first weeks. In order to optimise the conditions for depuration of DST in mussels, more information about seasonal variability as well as the effects of other environmental factors such as salinity and temperature on the rate of depuration is needed. The influence of phytoplankton composition in the food should also be of interest in future studies. These should also include the analysis of the DST ester derivatives and take into account their additional toxicity as well as evaluating the importance of biotransformation for detoxification of DST in mussels.

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