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Analysis of the importance of lipid breakdown for elimination of okadaic acid (diarrhetic shellfish toxin) in mussels, *Mytilus edulis*: results from a field study and a laboratory experiment

Susanne Svensson [a,*], Lars Förlin [b]

[a] *Department of Marine Ecology, Tjärnö Marine Biological Laboratory, S-452 96 Strömstad, Sweden*
[b] *Department of Zoology, Göteborg University, Box 463, S-405 30 Göteborg, Sweden*

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Abstract

Okadaic acid (OA) is a lipophilic phycotoxin, which accumulates in the digestive organs of mussels and may cause diarrhetic shellfish poisoning (DSP) in humans. Depuration of toxic mussels is a potential option for the shellfish industry to increase the availability of marketable mussels. To develop cost-effective depuration methods for DSP toxins, knowledge about the environmental conditions and physiological processes regulating the rate of depuration is essential. In this paper, the importance of lipid breakdown for elimination of OA in mussels was investigated by performing a field study and a manipulative laboratory experiment. First, total lipid content and concurrent concentration of OA in the digestive glands of farmed blue mussels, *Mytilus edulis*, was analysed on a monthly basis from January to June 2000. A significant positive correlation between levels of OA and lipid content was observed between January and March, when lipid levels were showing a decreasing trend. This supported a previously proposed model that breakdown of lipid stores may affect the release and elimination of this lipophilic toxin. To test this causal model, a laboratory experiment was performed. Mussels containing OA were exposed to experimental treatments (increased seawater temperature and/or food limitation) for 24 days in order to increase the energy requirements and need to use lipids as an energy source. It was predicted that mussels exposed to these treatments would have a faster elimination rate of OA compared to feeding mussels kept in ambient seawater temperature. The results showed that lipid content was significantly reduced in mussels exposed to an increased water temperature (24 °C) compared to ambient temperature (18 °C). The amount of lipids was not affected by food limitation. Although lipid content was reduced in 24 °C, the rate of depuration of OA was not faster for mussels in this treatment and no correlation was detected between lipid content and OA. Depuration rates were very similar for all treatments and followed an exponential decrease relationship ($t_{1/2} = 8$ days). Thus, the proposed model that lipid breakdown affects the mechanism of elimination of OA was not supported. Nevertheless, the observed rates of depuration provide useful information and a potential predictive tool for large-scale depuration methods of mussels. The difficulties to influence the rate of depuration of this toxin by changing the environmental conditions suggest that processes, insensitive to short-term manipulation of the external environment, regulate depuration of OA.

Keywords: Blue mussel; Depuration; Diarrhetic shellfish toxin; Environmental factor; Lipid; Okadaic acid

* Corresponding author. Tel.: +46-526-68607; fax: +46-526-68678.
E-mail address: susanne.svensson@tmbl.gu.se (S. Svensson).

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1. Introduction

Okadaic acid (OA) and the structurally related Dinophysistoxins (DTX-1, DTX-2 and DTX-3) are the principal toxic compounds causing diarrhetic shellfish poisoning (DSP) in humans (Yasumoto et al., 1985; Kumagai et al., 1986; Carmody et al., 1996). The DSP toxins (DST) are lipophilic polyether molecules produced by dinoflagellates of the *Dinophysis* sp. and *Prorocentrum* sp. genera and accumulate in the digestive system of bivalves when feeding on these plankton species (Yasumoto et al., 1978, 1980; Murakami et al., 1982; Murata et al., 1982). DST occurs in bivalves in all parts of the world and pose a serious threat to both public health and a sustainable aquaculture industry. The toxins accumulate to high levels and usually reside in shellfish for long periods as well as being tumour-promoting agents (Fujiki and Suganuma, 1993; Shumway et al., 1995; Lindegarth, 1997; Vale et al., 1998; Vieites and Leira Sanmartin, 2000). There is an increasing demand from the commercial shellfish industries for management plans to reduce the impacts of DST. Large-scale depuration of toxic mussels is a potential option to increase the availability of marketable mussels (Kollberg, 1999). Whether this will be an economically feasible option depends largely on the rate at which DST can be reduced to acceptable levels in mussels (Shumway et al., 1995). Hence, information about the influences of environmental conditions on depuration rates of DST is of great importance. Equally important is to understand the endogenous physiological processes by which mussels eliminate these compounds.

Temperature is assumed to be a factor regulating depuration rates of toxins in mussels because of its general effect upon basal metabolic rates in poikilothermic organisms (see reviews by Wieser, 1973; Hawkins and Bayne, 1992; Shumway et al., 1995). Observations of mussels in the field also suggest that depuration rates are affected by the availability of non-toxic food (Haamer et al., 1990; Sampayo et al., 1990; Marcaillou-Le Baut et al., 1993; Poletti et al., 1996; Blanco et al., 1999). A physiological process model to explain the relationship between food and the mechanism of depuration of algal toxins was discussed by Morono et al. (1998) and Blanco et al. (1999). They suggested that food, by increasing ingestion rates, affects depuration by increasing the digestive activity and metabolic faecal loss, including faster elimination of DST through faecal deposition. However, this model was not supported in a laboratory depuration experiment performed by Svensson (2003), where blue mussels containing OA were fed different amounts of non-toxic algae. Instead, a strong tendency for lower levels of OA in mussels receiving no food was observed during the last part of the experiment, when the mussels were showing signs of starvation. To explain this enhanced loss of OA during food limitation, an alternative physiological mechanism was proposed. The lipophilic character of the OA molecule together with the relatively high lipid content of the digestive gland (De Zwaan and Mathieu, 1992) indicates that OA may have affinity for lipid-rich cellular and intracellular components such as membranes and lipid droplets. The release of OA would then be dependent on a turnover of such cellular components. Hence, increased usage of lipid storages, which occur during stressed conditions when the demand of maintenance energy exceeds net energy gain (Thompson et al., 1974), could accelerate the rate of elimination. Additional support for this model is that one of the most important factor affecting bioaccumulation of other lipophilic contaminants, such as organic pollutants, are the lipid content of the organism or tissue involved. Accumulation of lipophilic compounds is directly, or indirectly via variability in lipid levels, also affected by reproductive condition and seasonality (Spacie and Hamelink, 1985; Farrington, 1989; Livingstone and Pipe, 1992; Phillips, 1993).

In this paper, the model proposed by Svensson (2003) regarding the importance of lipid breakdown for depuration of OA in *M. edulis* was explored. A field study was performed from January to June 2000, in order to investigate the relationship between lipid content and OA in farmed mussels. We also compiled data from the national monitoring program on OA and plankton concentration in the farm area during this season to study the temporal relationship between depuration of this toxin and blooms of diatoms. It has previously been assumed that the annual spring bloom of diatoms is essential for depuration of OA to occur in mussels along the Swedish and Norwegian coasts. Secondly, a laboratory experiment was performed in August 2000, where mussels containing OA were exposed to different temperature and food conditions

and depurated for 24 days. We predicted that a rise in seawater temperature and/or in combination with starvation would alter metabolic processes and result in a greater demand for energy reserves such as lipids in mussel tissue. We then predicted that mussels with reduced levels of lipids would contain less OA, i.e. a positive correlation between lipid content and OA was expected. The experiment was also performed with the purpose to evaluate the efficiency of manipulating environmental factors for depuration of OA from a management perspective.

2. Materials and methods

2.1. Field study

The field study was conducted between January and June 2000. Blue mussels (7–8 cm shell length) were collected once every month from a long-line mussel farm at Tjärnö in the Northern archipelago of Bohuslän on the Swedish west coast. The mussels were transported to Tjärnö Marine Biological Laboratory where they were immediately frozen and stored in −74 °C until the various analysis were done. At each sampling occasion, the concentration of OA in the digestive gland together with the lipid content were analysed in 6–8 individual mussels as described below. To study the temporal relationship between depuration of OA and spring bloom of diatoms during this year, data from the national monitoring program on concentration of OA (measured by HPLC according to Lee et al., 1987, values in μg OA kg^{-1} mussel meat) in mussels from the farm site from January to June, 2000 was compiled together with total cell concentration of diatoms (cells 10^6 l^{-1}), sampled from 0–10 m in the nearby Kosterfjord.

2.2. Laboratory experiment

The laboratory experiment was performed during 24 days, starting on the 19th of August 2000. Mussels were harvested from a commercial long-line mussel farm 80 km south of Tjärnö Marine Biological Laboratory where the experiment was conducted. The mussels contained 1055 μg OA kg^{-1} mussels at the day of collection, no other DST compounds were present (data from the national monitoring program, hydrolysis not done). In the laboratory, mussels were cleaned from epiphytic growth and sorted to obtain mussels of similar size for the experiment (shell length 70 mm). They were then kept in air at 4 °C overnight until the next day when the experiment was started. A randomly collected sample of mussels were frozen in −80 °C for subsequent analyses of start levels of OA, dry weight, glycogen and lipid contents.

Mussels were placed in plastic tanks (20 l) connected to hoses in a flow-through system (1.2 l min^{-1}). The experimental treatments consisted of two different temperature regimes (18 or 24 °C), with or without the addition of algal food particles (food or no food). The ambient seawater temperature in the mussel farm area was 18 °C when mussels were collected which also was the temperature in the laboratory water system. Immersion heaters were used to achieve 24 °C, which was done by directing the incoming seawater into a large water tank containing adequate numbers of immersion heaters. The warmed water was then redirected to the appropriate experimental tanks. Water flow and temperature of the incoming water was checked daily and, if necessary, adjusted to achieve a constant temperature throughout the experiment.

To the food treatments, algae were added to the tanks from plastic 1.5 l bottles placed upside-down above the experimental set-up. Hoses were connected to the bottles from which the algal suspension of desired flow rate could be supplied into the tanks. A mixture of four different species of microalgae was used as food supply: *Isochrysis galbana var. tahaitian* (T-ISO), *Chaetoceros gracilis* (CHGRA), *Tetraselmis* sp. and *Thalassiosira pseudonana* (3H). The algae were obtained from Reed Mariculture, Inc., Inland Sea Farm, USA, as concentrated algal pastes. Equal volumes of each algal species were mixed and suspended in filtered seawater to desired concentrations. Diluted algal paste corresponding to 4.8×10^9 cells (or 1.0 g dry weight) of each species was added to each food treatment every day during a period of 8 h. Earlier experiments have shown that mussels receiving no extra food experience starvation since the concentration of food particles in the incoming seawater are negligible (Svensson, 2003). As an indirect measure of ingestion, the amount of faecal production (dry weight) was estimated. At the end of the experiment, faecal pellets from each tank were collected on a filter (100 μm) and transferred to pre-weighed vials, followed by drying at 80 °C over night and then weighed.

For each combination of temperature and food, two replicate tanks were used, yielding a total number of eight tanks. Forty mussels were placed in each tank. Sampling was done after 8, 16 and 24 days of depuration. At each sampling occasion, two replicates, each consisting of three pooled individuals, were taken from every tank. These samples were analysed for OA, glycogen concentration and lipid content. An additional four mussels were removed for individual dry weight analysis. Thus, 10 individuals were removed from the tanks at each sampling. In order to keep constant densities in the tanks, removed mussels were replaced by 10 new mussels, which were tagged for identification but never used for analysis. After removal, mussels were immediately frozen and stored in −80 °C pending analyses, then the mussels were thawed and the digestive glands were removed. Total soft tissue and digestive gland wet weights were determined for each pooled replicate sample.

2.3. Measurements

2.3.1. Preparation of samples for analysis of OA, glycogen and lipids

The digestive glands were homogenized for 30 s in ice-cold conditions using an Ultra-Turrax knife homogenizer. One gram of the homogenate was diluted with 4 ml of 80% methanol for analysis of OA. Another 500 mg of the homogenate were transferred into Eppendorff tubes and immediately frozen and stored in −80 °C for subsequent analysis of glycogen (not done in the field study). The remaining homogenate was transferred into pre-weighed test tubes and freeze-dried for analysis of total lipid content.

2.3.2. Analysis of OA in mussel tissue

The methanol extracts were centrifuged in 3000 rpm for 10 min and then cleaned using petroleum ether and chloroform extraction following the sample clean-up protocol for HPLC by Lee et al. (1987). The chloroform extracts were used for detection of OA by protein phosphatase inhibition assay (PPIA) according to Vieytes et al. (1997) with slight modifications described by Godhe et al. (2002). OA concentrations in the samples were calculated using an OA standard curve and expressed as μg OA equivalents g^{-1} digestive gland (μg OA eq. g^{-1}). 10 randomly chosen samples were also analysed by HPLC (Lee et al., 1987) and compared to the results from the PPIA analysis. The chemical method confirmed that OA was the only DST compound present in mussel tissue (hydrolysis not done).

2.3.3. Analysis of glycogen

Glycogen concentrations in the digestive gland tissue were determined spectrophotometrically in a coupled reaction using a commercial kit (Boerhinger Mannheim, kit nr. 207748). Briefly, glycogen in the tissue is hydrolyzed into D-glucose by the enzyme amyloglucosidase. D-glucose is then phosphorylated to glucose-6-phosphate (G-6-P) by hexokinase in the presence of ATP. Finally, G-6-P is oxidized by G-6-P dehydrogenase to D-gluconate-6-phosphate in the presence of NADH, which is converted to NADPH. The amount of NADPH formed is determined by reading the absorbance at 340 nm and is stoichiometrically equivalent to the total amount of D-glucose in the tissue (free D-glucose and D-glucose formed in reaction step 1). For each sample, the amount of free D-glucose in the tissue (sample blank) was measured and subtracted from total D-glucose by omitting the enzyme amyloglucosidase in the first step of the reaction. Homogenized digestive gland tissue was diluted in ice-cold water (1:100) and used in the analysis, which was performed in 96 well plates. A glycogen standard curve (0.2–2 mg ml^{-1}) was used for calculation of glycogen in the samples. Glycogen concentration was expressed as mg g^{-1} wet weight.

2.3.4. Analysis of lipids

Total content of lipids in digestive gland tissue was analysed using the gravimetric method by Gardner et al. (1985) with some modifications. Approximately 1 g (wet weight) of homogenized tissue was freeze-dried in pre-weighed test tubes and then finely grinded. The dry weight of the material was determined. Five millilitre of chloroform–methanol (2:1) was added and the sample was thoroughly mixed, ultrasonicated for 30 s. and then centrifuged for 15 min in 3000 rpm. The supernatant was collected into new, pre-weighed test tubes and 2 ml of chloroform–methanol (2:1) was added to the remaining pellets and mixed. The solution was centrifuged again as above and the supernatant was pooled with the previous supernatant. To this extract, 1.7 ml of KCl (0.88%) was added to remove non-lipid

contamination. The mixture was centrifuged in 300 rpm for 15 min and the overlying aqueous phase was removed by a Pasteur pipette. The organic solutions in the extracts were evaporated in room temperature in a fume cup-board and the test tubes were further dried in 50 °C in a drying cabinet for a couple of hours. The weight of the remaining lipid fraction in the test tubes was determined and lipid content was calculated as % lipids of dry weight.

2.3.5. Statistical analysis

Analysis of variance (ANOVA) was used to test hypotheses about the effects of temperature, food and time on concentration of OA (start value not included). A three factor (fixed effects) orthogonal design was applied (Underwood, 1997). In order to control for any potential "tank effects", the experimental unit (tank) was replicated and included in the statistical analysis as a nested factor within temperature and food. Data was checked for homogeneity of variances using Cochran's test and for significant effects, Student–Neuman–Keuls (SNK) a posteriori test for differences among means was applied. ANOVA was also used to test the effects of treatments on lipid content (tank not included as a factor).

Rates of depuration for each treatment was calculated by fitting the data to an exponential function, $T_t = T_0\, e^{-\lambda t}$ where T_0 is the toxin concentration at the start of depuration (μg OA eq. g^{-1}), λ the exponential decay coefficient (loss per day) and t the time (days). This relationship assumes that the depuration rate is constant over time, which corresponds to a one-compartment model (Spacie and Hamelink, 1985; Silvert and Cembella, 1995). The correlation coefficient value (r^2) indicates how well the data fit the chosen model. Using this equation, the depuration half-lives ($t_{1/2}$), which is the time to reach a 50% reduction in toxin content, were calculated as $t_{1/2} = -\ln(1/2) \times \lambda^{-1}$.

The relationship between lipid content and concentration of OA was tested using correlation analysis (Underwood, 1997).

3. Results

3.1. Field study

Fig. 1 shows the monitoring data on OA concentration (μg kg^{-1} mussel meat) in mussels from the Tjärnö

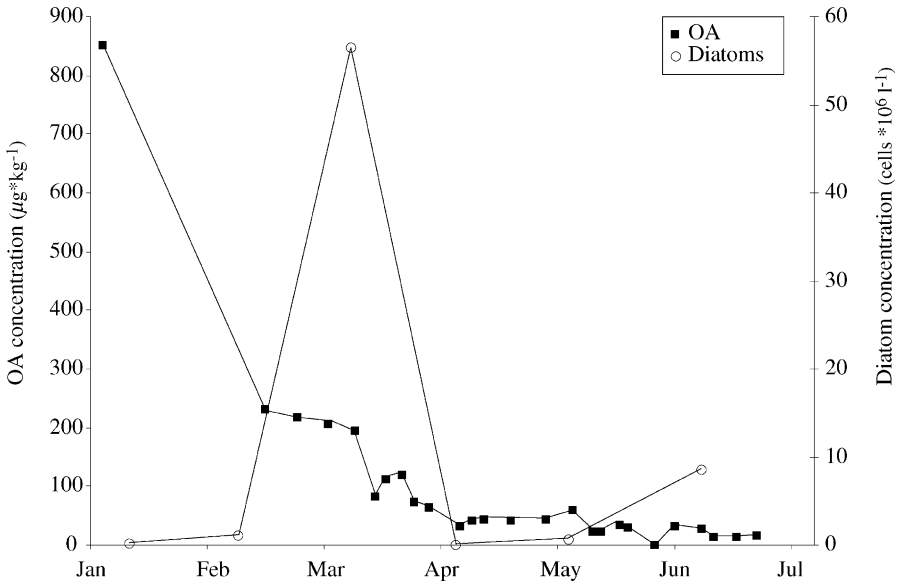

Fig. 1. OA concentration (μg kg^{-1} mussel meat, filled squares) in mussels from Tjärnö mussel farm (primary y-axis) and total diatom concentration (cells $10^6\, l^{-1}$ sea water, 0–10 m depth, open circles) in the Koster Archipelago between January and June 2000 (secondary y-axis). Data are from the national monitoring program.

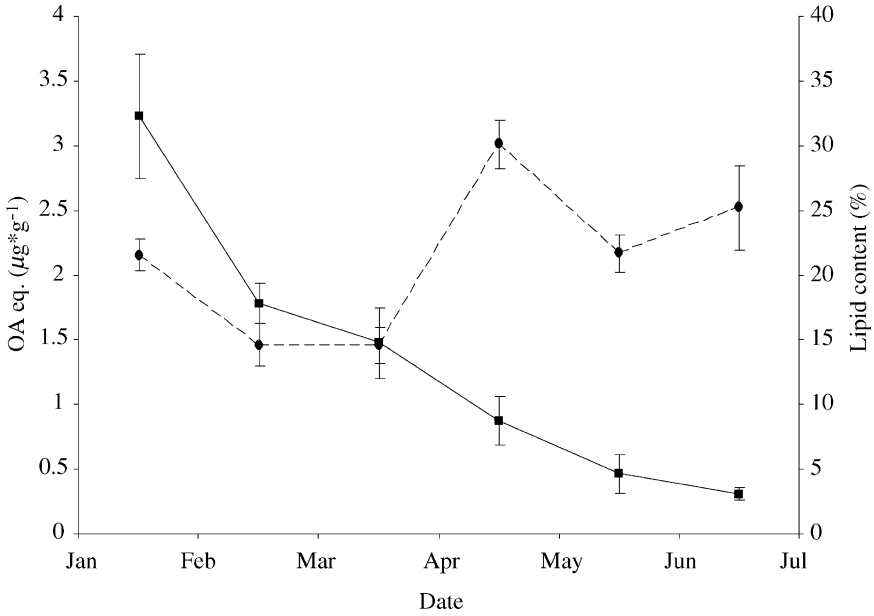

Fig. 2. OA concentration ($\mu g\,g^{-1}$ digestive gland, filled squares, primary *y*-axis) and lipid content (% total lipids of dry weight, filled circles, secondary *y*-axis) in the digestive glands of individual mussels from Tjärnö farm site between January and June 2000. Error bars represent S.E. of means.

farm site and total diatom concentration (cells $10^6\,l^{-1}$) sampled in the nearby Koster Archipelago between January and June 2000. OA was the only DSP toxin detected in mussels during this period (DTX-3 was not analysed). A fast decrease in toxin concentration occurred between the 4th of January ($852\,\mu g\,kg^{-1}$) and the 15th of February ($231\,\mu g\,kg^{-1}$). Low concentrations of diatoms (below 10,000 cells l^{-1}) were observed between January and February. Then, a large increase occurred between 8th of February (9400 cells l^{-1}) and 8th of March (56.5 cells $10^6\,l^{-1}$). The bloom had disappeared in early April. This data indicated that a substantial reduction of OA occurred in mussels before the peak of the diatom spring bloom during winter-spring, 2000, in this area. The same observation was made along the Norwegian coast during this season and also during 2001 and 2002 (T. Aune, pers. comm.). Thus, the diatom spring bloom is not a prerequisite for depuration of DSP toxins to occur in this region, which has previously been assumed.

OA concentration and lipid content in the digestive glands from the monthly field sampling are shown in Fig. 2. Between 18th of January and 16th of February, a large decrease in OA concentration was observed. Mean concentration of OA in the digestive gland was reduced from 3.23 to 1.78 $\mu g\,g^{-1}$. This is in agreement with the data from the monitoring program, where the fastest reduction of OA was observed during the same

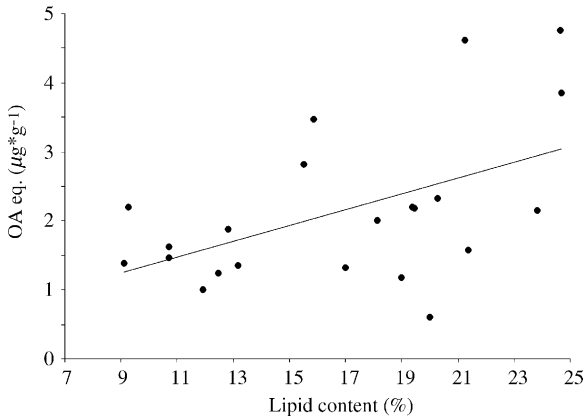

Fig. 3. Correlation between lipid content (% of dry weight) and concentration of OA (μg OA eq. g^{-1}) in digestive glands of individual mussels from Tjärnö farm site, data from January to March 2000. A significant positive correlation was detected ($P < 0.05$, $r^2 = 0.26$).

period. OA was further decreased but at a lower rate during the rest of the period and reached the lowest value in June (0.31 $\mu g\,g^{-1}$). Lipid content in the digestive gland showed the same trend as OA concentration between January and March, with a reduction from 21.6 to 14.6% but in April, a large increase in lipid levels was measured (30.2%). Lipid content remained relatively high throughout the rest of the study. The relationship between lipid content and OA concentration from January to March was analysed using correlation analysis, these results are shown in Fig. 3. There was a significant positive relationship between these variables ($P < 0.05$, $r^2 = 0.26$). This result supported the model that the large reduction in OA content may be due to the breakdown of lipid stores during this period.

3.2. Laboratory experiment

3.2.1. General observations and effects of environmental factors on physiological variables

Differences in ingestion between mussels fed the algal suspension and those not receiving food were confirmed by the results from the faecal production measurements. Mussels supplied with extra food had produced approximately five times as much faeces compared to the no food treatment at day 24 (data not shown). Soft tissue dry weights, glycogen concentration and total lipid content in the digestive glands were measured as general markers of nutritive status and physiological stress (Table 1). During the last days of the experiment, some mortality was observed for feeding mussels kept in 24 °C. Data on mean dry weights showed a decreasing trend for mussels in this treatment, indicating that they were loosing body mass. This was not observed for the other treatments. Thus, mussels in this experimental condition appeared to be highly stressed. The same pattern was not observed for glycogen, where no difference among treatments was apparent (Table 1). It should be noted that the variability among replicates in glycogen concentration was large. Lipid content in the mussel digestive glands was, however, significantly affected by temperature (Table 2, $P < 0.05$ for temperature). Compared to 18 °C, lipid content in mussels subjected to 24 °C was lower already after 8 days of depuration and throughout the rest of the experiment (SNK: 18 °C > 24 °C, Table 2). Although significantly different, the differences in lipid content appeared to be small, about 2% lower in 24 °C on days 8 and 24 and 5% on day 16. However, if calculated as proportional loss, 2% reduction (e.g. from 20 to 18%) represents

Table 1
Soft tissue dry weights (g), glycogen concentration ($mg\,g^{-1}$ wet weight) and total lipid content (% lipids of dry weight) in the digestive glands in the various treatments

Variable	Temperature (°C)	Food/no food	Start	Days of depuration		
				8	16	24
Dry weight (g)						
	18	Food	2.1 ± 0.5 (8)	2.0 ± 0.4 (8)	2.1 ± 0.8 (8)	1.9 ± 0.9 (8)
	18	No food		1.8 ± 0.6 (9)	1.8 ± 0.9 (8)	2.3 ± 0.3 (8)
	24	Food		2.1 ± 0.7 (7)	1.6 ± 0.5 (8)	1.2 ± 0.7 (4)
	25	No food		2.1 ± 0.4 (7)	2.1 ± 0.9 (8)	1.9 ± 0.7 (8)
Glycogen ($mg\,g^{-1}$)						
	18	Food	42.1 ± 31.7 (4)	52.3 ± 11.3 (4)	50.2 ± 22.0 (4)	33.8 ± 10.5 (4)
	18	No food		44.8 ± 5.3 (4)	32.8 ± 5.8 (4)	31.6 ± 10.8 (4)
	24	Food		41.1 ± 15.6 (4)	47.1 ± 32.3 (4)	32.6 ± 2.9 (4)
	24	No food		37.4 ± 10.4 (4)	28.4 ± 10.5 (4)	41.1 ± 3.9 (4)
Lipid content (%)						
	18	Food	23.0 ± 1.6 (2)	18.3 ± 3.5 (3)	18.8 ± 1.8 (4)	18.2 ± 2.7 (2)
	18	No food		20.4 ± 5.8 (3)	19.0 ± 1.5 (4)	18.1 ± 1.8 (4)
	24	Food		19.6 ± 2.1 (3)	13.3 ± 0.5 (2)	17.3 ± 5.9 (4)
	24	No food		17.7 ± 0.7 (3)	15.6 ± 0.9 (3)	15.9 ± 2.1 (4)

Values are means ± S.D. with number of replicates inside parenthesis.

Table 2
ANOVA on the effects of temperature, food supplies and days of depuration (time) on lipid content in the digestive glands (% lipids of dry mass)

Source of variation	d.f.	MS	*F*	*P*	Error term
Temperature	1	40.50	4.53	0.04	Residual
Food/no food	1	0.02	0.00	0.96	Residual
Temp × Food	1	1.46	0.16	0.69	Residual
Time	2	20.36	2.28	0.12	Residual
Time × Temp	2	12.15	1.36	0.27	Residual
Time × Food	2	4.78	0.54	0.59	Residual
Time × Temp × Food	2	8.11	0.91	0.42	Residual
Residual	27	8.94			

Significant effects for differences among means ($P < 0.05$) were analyzed using Student–Neuman–Keuls (SNK) a posteriori test. SNK: 18 °C > 24 °C.

a 10% loss of lipids. Food limitation did not affect lipid content in this experiment (Table 2, $P > 0.05$ for food/no food). Hence, increasing the temperature reduced lipid contents, which was according to the predictions but high temperature in combination with starvation did not further reduce lipid levels.

3.2.2. Effects of environmental factors on elimination of OA

The results from the experiment are shown in Fig. 4. At the start of the experiment, the mussels contained on average 2.89 ± 0.36 S.E. OA eq. g^{-1} digestive gland. The effects of environmental factors on

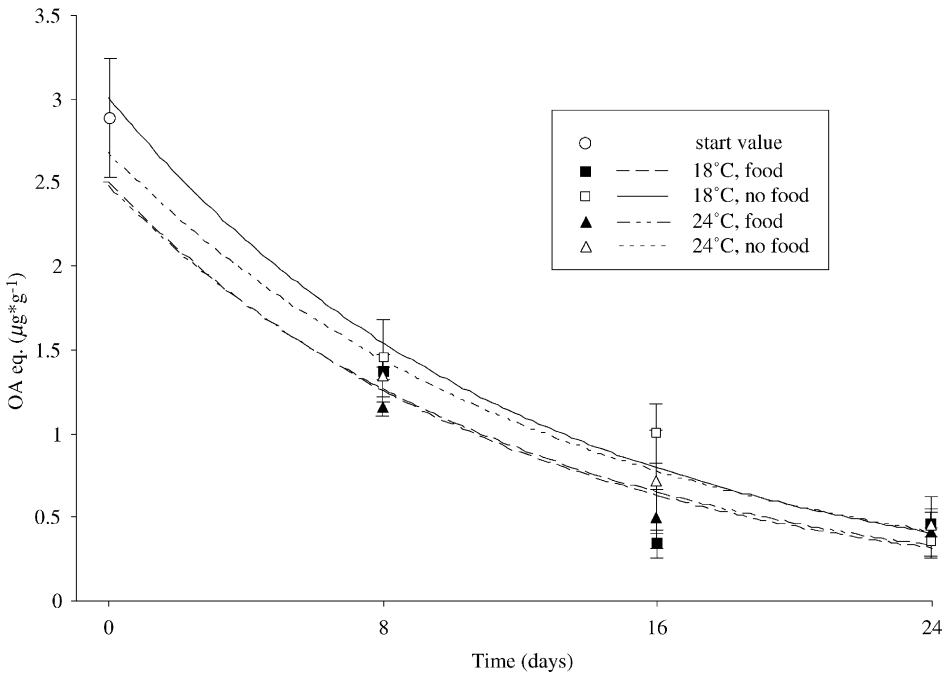

Fig. 4. The effects of temperature and food on the rate of depuration of OA (OA eq. g^{-1} digestive gland). Values are means ± S.E. Depuration curves for each treatment during the experiment were added by fitting data to the exponential decay equation $T_t = T_0\, e^{-\lambda t}$. T_0: toxin concentration at the start of depuration (μg OA eq. g^{-1}), λ: exponential decay coefficient (% per day) and t: time (days). 18 °C food: $T_t = 2.51\, e^{-0.086t}$, $r^2 = 0.82$; 18 °C no food: $T_t = 3.01\, e^{-0.083t}$, $r^2 = 0.96$; 24 °C food: $T_t = 2.48\, e^{-0.084t}$, $r^2 = 0.94$; 24 °C no food: $T_t = 2.68\, e^{-0.077t}$, $r^2 = 0.99$.

Table 3
ANOVA on the effects of temperature, food supply and days of depuration (time) on okadaic acid (μg OA eq. g^{-1} digestive gland), untransformed data

Source of variation	d.f.	MS	F	P	Error term
Temperature	1	0.06	0.71	0.45	Tank (Temp, Food)
Food/no food	1	0.40	4.66	0.10	Tank (Temp, Food)
Temp × Food	1	0.01	0.15	0.72	Tank (Temp, Food)
Tank (Temp, Food)	4	0.08	0.82	0.52	Pooled MS
Time	2	3.61	34.87	0.0001	Pooled MS
Time × Temp	2	0.04	0.36	0.70	Pooled MS
Time × Food	2	0.23	2.21	0.13	Pooled MS
Time × Temp × Food	2	0.11	1.04	0.36	Pooled MS
Time × Tank (Temp, Food)	8	0.07	0.61	0.76	Residual
Residual	24	0.12			
Pooled MS	32	0.10			

Significant interaction effects for differences among means ($P < 0.05$) were analyzed using Student–Neuman–Keuls (SNK) a posteriori test. SNK: 8 days > 16 days > 24 days.

elimination of OA was analysed using ANOVA (Table 3). The experimental unit (Tank) was replicated and included in the statistical analysis as a nested factor within temperature and food (Time Tank (Temp Food)). No "tank" effects were found ($P = 0.76$, Table 3), hence tests of hypotheses about effects of food, temperature, time and their interactions were valid (Underwood, 1997). The concentration of OA was significantly different among times ($P < 0.001$ for Time, Table 3) which was also the only factor affecting this variable in the experiment. Mean concentration of OA was 1.35 ± 0.09 S.E. OA eq. g^{-1} after 8 days of depuration and OA was further reduced to 0.70 ± 0.13 and 0.42 ± 0.07 after 16 and 24 days, respectively. SNK a posteriori test showed that there were significant differences among all levels of time (8 days > 16 days > 24 days, Table 3). Apart from the significant effect of time, there was a trend towards an interaction between time and food treatments (Time Food/no food, $P = 0.13$, Table 3). Compared to the no food treatment, concentration of OA in mussels receiving food tended to be lower at both 8 days (no food: 1.44 ± 0.17, food: 1.28 ± 0.04) and 16 days (no food: 0.90 ± 0.20, food: 0.46 ± 0.12) but not at 24 days of depuration (no food: 0.40 ± 0.10, food: 0.44 ± 0.10). We observed that variability among replicates were larger in the no food treatment compared to mussels receiving food at both 8 and 16 days of depuration.

To obtain rates of depuration, data for each treatment was fitted to the exponential loss equation $T_t = T_0\, e^{-\lambda t}$, fitted curves and equations are shown in Fig. 4. The r^2 values ranged between 0.82 and 0.99, which suggested that the depuration dynamics was accurately described using a one-compartment model approach. Depuration rates were highly similar for all treatments, ranging from 8.4% per day for the 18 °C food treatment to 9.3% per day for the 24 °C food treatment. The average expected half-life ($t_{1/2}$) for all treatments was calculated to 8 days.

3.2.3. Relationship between lipid content and OA

The relationship between lipid content and OA concentration in the digestive glands during the experiment was analysed using correlation analysis (Fig. 5).

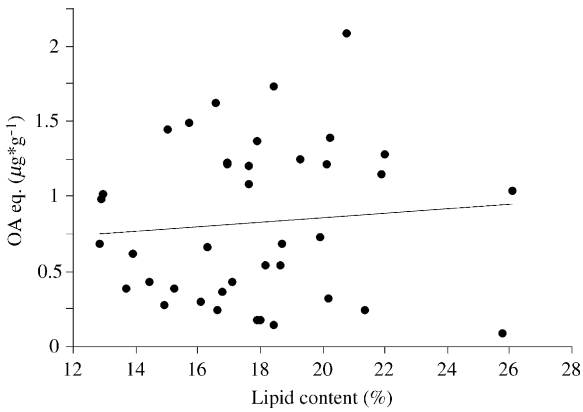

Fig. 5. Correlation between lipid content (% of dry weight) and concentration of OA (μg OA eq. g^{-1}) in digestive glands of mussels, data from the laboratory experiment. No correlation was detected ($P > 0.05$, $r^2 = 0.007$).

According to the predictions, a positive correlation between these variables was expected, however, no trend towards a relationship between lipid content and OA was detected ($P = 0.61$, $r^2 = 0.007$). Data for lipid content ranged between 13 and 26% and OA concentration between 0.2 and 2.2 μg OA eq. g^{-1}.

4. Discussion

Our results from the field study together with the monitoring data from the Tjärnö region during winter-spring, 2000, indicated two things. First, a rapid decrease in OA concentration occurred before the peak of diatoms this season. Together with the data from the Norwegian coast during 2000–2002 (T. Aune, pers. comm.), this observation conflicts the previous opinions that depuration of DSP toxins do not occur until the onset of the spring bloom of diatoms in the Skagerack region. The observed loss of OA is likely to be explained by a reduced uptake rate of toxic *Dinophysis* sp. by the mussels. Since the accumulation of foreign compounds in an organism is a function of both uptake and depuration rates (Spacie and Hamelink, 1985), a reduced uptake rate results in a net loss of toxins, even if the rate of depuration remains constant. Alternatives which could result in a reduced ingestion rate of toxins in mussels include: (1) the absolute concentration of toxic dinoflagellates decreases or disappears from the water column, filtration rate is maintained in the mussels; (2) the relative abundance of accompanying non-toxic species increases which alters the filtration and ingestion rates of the mussels; (3) *Dinophysis* populations change from toxic to non-toxic strains. The importance of these mechanisms for elimination of DSP toxins should be explored in future studies. Secondly, a large reduction in OA between January and March coincided with a decrease in lipid content in the digestive glands. The significant positive correlation which we detected between these variables supported the theory that breakdown of lipid stores may affect the elimination of the lipophilic OA. The reduction in lipid content occurred during the winter season when food is known to be limited, indicating that mussels have to depend on energy storage products such as glycogen and lipids to maintain vital processes. The digestive gland is together with the mantle the major storage organ for lipids in *Mytilus* sp. (De Zwaan and Mathieu, 1992). Thompson et al. (1974) found seasonal changes in the amount of lipids in *M. edulis* with maximum levels in July (>30% of dry weight) and minimum in late winter (11%). We found that total lipid content in the digestive glands changed considerably during the study period, for example, the content doubled between March (14%) and April (30%). These values are within the range of those that Thompson et al. (1974) reported. The observed increase in April is most likely a response to the algal spring bloom, when mussels were able to replenish their lipid stores. Since an increase in lipid levels occurred between April and June and at the same time, OA concentration was further reduced, this implies that the elimination of this toxin is not exclusively dependent on a net breakdown of lipids.

Following on the observations of a positive correlation between lipid and OA content in the field, we wanted to test this causal model in a laboratory experiment. A prerequisite for this was to obtain variability in lipid content among individuals or groups of toxic mussels. Therefore, factors that are likely to influence the energy requirements and need to metabolise lipids were considered. In poikilothermic organisms such as mussels, the surrounding seawater temperature strongly regulates the energy requirements for maintenance metabolism (see reviews by Wieser, 1973; Hawkins and Bayne, 1992). Living in a high temperature is associated with higher energy requirements compared to low temperature. When mussels are transferred from low to high temperature, this is known to result in modulation of biochemical and physiological rate processes in order to acclimate to the new condition (Hawkins et al., 1987). This acclimation period may last for days or even weeks and is associated with an extra energy cost (Bayne et al., 1973; Widdows and Bayne, 1971). If acclimation is incomplete, the reduction in net energy gain signifies a ‘stressed condition’ (Bayne, 1985) and material from the digestive gland is rapidly utilized (Thompson et al., 1974). Hence, we predicted that exposing mussels to a raise in temperature would increase the need to utilize lipids as an energy source compared to mussels maintained in the ambient seawater temperature. Accordingly, significantly lower levels of lipids were observed in mussels exposed to 24 °C compared to 18 °C at all sampling occasions. The relative differ-

ences in mean lipid content among treatments were small but variability among individual samples during the experiment ranged from 13 to 26%, hence we could test the relationship between lipid content and OA concentration. We found no correlation between these variables in the experiment, which was in contrast to the significant positive correlation observed in the field study.

A reduced lipid content was also expected for non-feeding compared to mussels receiving food as a response to starvation, following the observations made by Svensson (2003). In *Mytilus* sp., metabolic rate processes and usage of body energy reserves are highly regulated by the availability of food (Hawkins and Bayne, 1992). However, our results showed that lipid content remained unaffected by food treatment in this experiment. Bayne et al. (1973) and Widdows (1973) reported a decline in metabolic rates in *M. edulis* in response to starvation. Thus, it is possible that non-feeding mussels in our experiment had reduced their metabolic rates and energy requirements and thus the need to use lipids. The increased mortality and loss of body mass which was observed towards the end of the experiment for feeding mussels kept in 24 °C indicated that mussels were highly stressed in this treatment and were using their body energy reserves for survival. This was not observed for non-feeding mussels in 24 °C, which suggested that the energy requirements were lower during food limitation, possibly as a result of a general depression of metabolic rates as discussed above. Alternatively, the quantity or the quality of the microalgae, supplied to the mussels in the food treatment, may have been inadequate for maintenance, producing a similar effect on lipid content as for non-feeding mussels. Interestingly, there was no significant effect of temperature on glycogen concentration, which would be expected if glycogen reserves are used as the primary energy source before lipids as suggested by Zaba and Davies (1984). It should be noted that variability among samples was large, indicating that the difference among treatments needs to be large to detect statistical differences.

Even though temperature reduced lipid content in mussel digestive glands, we found no effect of environmental conditions on the rate of depuration of OA. The reduction rate of OA was highly similar for all treatments. This is in contrast to previous beliefs. It has been assumed that depuration is faster in higher temperatures due to a generally higher metabolic activity (Shumway et al., 1995). This was, however, not observed in an early experiment performed by Lindahl and Hageltorn (1986), who compared depuration efficiency of DST between mussels (*M. edulis*) kept in ambient temperature (0–2 °C) and in heated seawater (10 °C). They found no effect of temperature on the rate of elimination of these toxins. In this paper, we exposed mussels to a direct increase of 6 °C and compared depuration rates with those kept in ambient seawater temperature. A different approach to experimentally test the effects of temperature is to gradually adjust mussels to a higher temperature for some time and then compare depuration rates to mussels acclimated in lower temperature. This would abolish the stress effects and cost of acclimation and perhaps represents the "true" effects of temperature, comparable to long-term seasonal temperature changes in the sea. However, naturally intoxicated mussels cannot be used in this kind of study. We expected complete acclimation to the new temperature after 14 days in our experiment, following the results by Widdows and Bayne (1971) and Hawkins et al. (1987). This suggested that any effect of temperature should have been detected after 24 days of depuration in our experiment but this was not seen. Also, feeding on non-toxic algae has been assumed to affect the mechanism for elimination of DST and thus increase rate of depuration (Morono et al., 1998, Blanco et al., 1999) but this was not experimentally supported by Svensson (2003). Our results further indicate that depuration is not accelerated in feeding mussels compared to non-feeding mussels.

By fitting the data to the exponential loss equation, we could calculate the rate constant for depuration of OA and depuration half-lives ($t_{1/2}$). The average half-life was calculated to 8 days for all treatments in our experiment. This is considerably faster compared to laboratory results obtained by Svensson (2003) who found a 50% reduction of OA in *M. edulis* after 16 days. Lindahl and Hageltorn (1986) observed a 50% reduction after 1.5 months and Marcaillou-Le Baut et al. (1993) after 1 month of depuration in the laboratory. Similar depuration rates was observed by Marcaillou-Le Baut et al. (1993) ($t_{1/2}$ = 12 days), Fernández et al. (1998) ($t_{1/2}$ = 11–12 days), Blanco et al. (1999) and Morono et al. (2003) ($t_{1/2}$ = 7–8

days) for *M. galloprovincialis*. Croci et al. (1994) observed very high rates of depuration of DST in *M. galloprovincialis* ($t_{1/2} = 3$ days).

High r^2 values for all treatments ($r^2 = 0.82$–0.99) suggested that the depuration kinetics of OA was accurately explained using a simple one-compartment model, where depuration rate is constant over time (Spacie and Hamelink, 1985; Silvert and Cembella, 1995). This was also observed by Croci et al. (1994) and Blanco et al. (1995). A two-step depuration curve for DST, corresponding to a two-compartment model with an initial high depuration rate during the first days of depuration followed by a lower rate, has previously been observed by Bauder et al. (2001) in the digestive gland of scallops, *Agropecten irradians*, and in mussels *M. galloprovincialis* (Fernández et al., 1998; Marcaillou-Le Baut et al., 1993) and *M. edulis* (Svensson, 2003). Blanco et al. (1999) investigated the effects of environmental conditions on depuration rates of DST in *M. galloprovincialis* in the field and used both a one- and two-compartment model approach. They concluded that the most accurate model describing depuration kinetics was dependent on whether the effects of environmental variables were included or not. A recently published study by Morono et al. (2003) used both a one- and two-compartment approach to model the accumulation and depuration of DSP toxins in *M. galloprovincialis*. These authors also included analysis of acylated (DTX-3) and conjugated (diol-esters, DTX-4) forms of OA and they estimated conversion and detoxification rates for these compounds in their modelling. They found that a one-compartment model was sufficient to explain the intoxication-detoxification processes. Very low acylation and detoxification rates of DTX-3 compared to the rate of detoxification of OA were found, which suggested that acylation of OA in the digestive glands of mussels is of little importance in this species. This was also concluded by Fernández et al. (1998) who compared the detoxification kinetics between OA/DTX-2 and DTX-3 in *M. galloprovincialis*. Several other studies have observed that Mytilus sp. generally contains only low amounts of DTX-3 of the total DST present in tissue (Marr et al., 1992; Fernández et al., 1996; Fernández et al., 1998; Suzuki and Mitsuya, 2001; Vale and Sampayo, 2002). So far, there is no published data on the occurrence and magnitude of DTX-3 in blue mussels from northern Europe. Data from the monitoring of DST in Norwegian mussels show that approximately 50% of total toxin burden may constitute of DTX-3 (T. Aune, pers. comm.). In Swedish blue mussels, DTX-3 is currently not included in monitoring program for DST. A preliminary analysis of DTX-3 in subsamples from this current experiment showed that the proportion of acylated OA ranged between 25 and 74% (data not shown). Hence, significant amounts of DTX-3 may be present in Swedish and Norwegian mussels and analysis of these acylated forms needs to be included in future studies on depuration of DST. Because acylated OA is assumed to rapidly hydrolyze to OA in the human stomach, large amounts of DTX-3 also imply an additional risk for consumers of mussels and have to be accounted for in monitoring of DST.

5. Conclusions

Since we could not experimentally verify the significant relationship between lipid content and OA concentration observed in the field, the model that lipid breakdown affects elimination of OA was not supported. However, on a seasonal basis, lipid content in the digestive glands may play a role in determining the levels of bioaccumulation of the lipophilic DST. Our results together with the results from other studies on depuration of DST also emphasize the difficulties to accelerate the mechanisms of depuration by short-term manipulation of external factors. This suggests that endogenous processes, insensitive to immediate changes in the surrounding environment, regulate the mechanism of depuration of the DST. We propose that seasonal changes in the physiological status of mussels may account for some of the variability in depuration rates of DST observed in the field. Especially for mussel populations in temperate latitudes, the physiological status is related to the annual reproductive cycle and is associated with marked seasonal changes in both biochemical composition and physiological rate processes (Hawkins and Bayne, 1992). Thus, season, or time of the year, should be relevant to consider when uptake and elimination of DSP toxins are studied. Ideally, a physiological variable, which could be used to predict the outcome of depuration of DST in mussels, may exist.

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References

Bauder, A.G., Cembella, A.D., Bricelj, V.M., Quilliam, M.A., 2001. Uptake and fate of diarrhetic shellfish poisoning toxins from the dinoflagellate *Prorocentrum lima* in the bay scallop *Agropecten irradians*. Mar. Ecol. Prog. Ser. 213, 39–52.

Bayne, B.L., 1985. Responses to environmental stress: tolerance, resistance and adaptation. In: Proceedings of the 18th European Marine Biology Symposium, pp. 331–349.

Bayne, B.L., Thompson, R.J., Widdows, J., 1973. Some effects of temperature and food on the rate of oxygen consumption by *Mytilus edulis* L. In: Wieser, W. (Ed.), Effects of Temperature on Ectothermic Organisms. Springer-Verlag, Berlin, pp. 181–193.

Blanco, J., Fernández, M., Marino, 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., Fernández, M.L., Míguez, A., Morono, 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.

Carmody, E.P., James, K.J., Kelly, S.S., 1996. Dinophysistoxin-2: the predominant diarrhoeic shellfish toxin in Ireland. Toxicon 34 (3), 351–359.

Croci, L., Toti, L., De Medici, D., Cozzi, L., 1994. Diarrhetic shellfish poisoning in mussels: comparison of methods of detection and determination of the effectiveness of depuration. J. Int. Food Microbiol. 24, 337–342.

De Zwaan, A., Mathieu, M., 1992. Cellular energy metabolism in the Mytilidae: an overview. In: Gosling, E. (Ed.), The Mussel Mytilus: Ecology, Physiology, Genetics and Culture. Elsevier, Amsterdam, pp. 223–307.

Farrington, J.W., 1989. Bioaccumulation of hydrophobic organic pollutant compounds. In: Levin, S.A., Harwell J.R., Kelly J.R., Kimball K.D. (Eds.), Ecotoxicology: Problems and Approaches. Springer-Verlag, New York, pp. 279–313.

Fernández, M.L., Míguez, A., Cacho, E., Martínez, A., 1996. Detection of okadaic acid esters in the hexane extracts of Spanish mussels. Toxicon 34 (3), 381–387.

Fernández, M.L., Míguez, A., Morono, A., Cacho, E., Martínez, A., Blanco, J., 1998. Detoxification of low polarity toxins (DTX-3) from mussels *Mytilus galloprovincialis* in Spain. In: Reguera, B., Blanco J., Fernández M.L., Wyatt T. (Eds.), Harmful Algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, pp. 449–452.

Fujiki, H., Suganuma, M., 1993. Tumor promotion by inhibitors of protein phosphatase 1 and 2A: the okadaic acid class of compounds. Adv. Canc. Res. 61, 143–194.

Gardner, W.S., Frez, W.A., Cichocki, E.A., 1985. Micromethod for lipids in aquatic invertebrates. Limnol. Oceanogr. 30 (5), 1099–1105.

Godhe, A., Svensson, S., Rehnstam-Holm, A.-S., 2002. Oceanographic settings explain fluctuations in *Dinophysis* spp. and concentrations of diarrhetic shellfish toxin in the plankton community within a mussel farm area on the Swedish west coast. Mar. Ecol. Prog. Ser. 240, 71–83.

Haamer, J., Andersson, P.-O., Lange, S., Li, X.P., Edebo, L., 1990. Effects of transplantation and reimmersion of mussels *Mytilus edulis* L. on their contents of okadaic acid. J. Shellfish Res. 9 (1), 109–112.

Hawkins, A.J.S., Bayne, B.L., 1992. Physiological interrelations, and the regulation of production. In: Gosling, E. (Ed.), The Mussel *Mytilus*: Ecology, Physiology, Genetics and Culture. Elsevier, Amsterdam, pp. 171–222.

Hawkins, A.J.S., Wilson, I.A., Bayne, B.L., 1987. Thermal responses reflect protein turnover in *Mytilus edulis* L. Funct. Ecol. 1, 339–351.

Kollberg, S., 1999. Beskrivning av den svenska musselnäringen (In Swedish). Vattenbrukarnas Riksförbund, 68 pp.

Kumagai, M., Yanagi, T., Murata, M., Yasumoto, T., Kat, M., Lassus, P., Rodriguez-Vazquez, J.A., 1986. Okadaic acid as the causative toxin of diarrhetic shellfish poisoning in Europe. Agric. Biol. Chem. 50 (11), 2853–2857.

Lee, J.S., Yanagi, T., Kenma, R., Yasumoto, T., 1987. Fluorometric determination of diarrhetic shellfish toxins by high pressure liquid chromatography. Agric. Biol. Chem. 51, 877–881.

Lindahl, O., Hageltorn, M., 1986. Detoxification experiments of DSP in blue mussels. In: Proceedings of the 15th Nordic Veterinary Congress, pp. 463–466.

Lindegarth, M., 1997. Sammanställning och tolkning av de kontinuerliga mätningarna av okadasyrahalter längs den svenska västkusten (In Swedish). Länsstyrelsen Västra Götaland, 17 pp.

Livingstone, D.R., Pipe, R.K., 1992. Mussels and environmental contaminants: molecular and cellular aspects. In: Gosling, E. (Ed.), The Mussel Mytilus: Ecology, Physiology, Genetics and Culture. Elsevier, Amsterdam, pp. 425–464.

Marcaillou-Le Baut, C., Bardin, B., Bardouil, M., Bohec, M., Le Dean, L., Masselin, P., Truquet, P., 1993. DSP depuration rates of mussels reared in a laboratory and an aquaculture pond. In: Smayda, T.J., Shimizu Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier, pp. 531–535.

Marr, J.C., Hu, T., Pleasance, S., Quilliam, M.A., Wright, J.L.C., 1992. Detection of new 7-O-acyl derivatives of diarrhetic shellfish poisoning toxins by liquid chromatography–mass spectrometry. Toxicon 30 (12), 1621–1630.

Morono, A., Fernández, L.M., Franco, J.M., Martínez, A., Reyero, I.M., Míguez, A., Cacho, E., Blanco, J., 1998. PSP and DSP 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 Intergovernmental Oceanographic Commission of UNESCO, pp. 445–448.

Morono, A., Arévalo, F., Fernández, M.L., Maneiro, J., Pazos, Y., Salgado, C., Blanco, J., 2003. Accumulation and transformation of DSP toxins in mussels *Mytilus galloprovincialis* during a toxic episode caused by *Dinophysis acuminata*. Aquat. Toxicol. 62, 269–280.

Murakami, Y., Oshima, Y., Yasumoto, T., 1982. Identification of okadaic acid as a toxic component of a marine dinoflagellate *Prorocentrum lima*. Bull. Jpn. Soc. Sci. Fish. 48, 69–72.

Murata, M., Shimatani, M., Sugitani, H., Oshima, Y., Yasumoto, T., 1982. Isolation and structural elucidation of the causative toxin of the diarrhetic shellfish poisoning. Bull. Jpn. Soc. Sci. Fish. 48, 549–552.

Phillips, D.J.H., 1993. Bioaccumulation. In: Calow, P. (Ed.), Handbook of Ecotoxicology. Blackwell Scientific Publications, pp. 378–398.

Poletti, R., Viviani, R., Casadei, C., Lucentini, L., Funari, E., Draisci, R., 1996. Decontamination dynamics of mussels naturally contaminated with diarrhetic toxins relocated to a basin of the Adriatic sea. In: Yasumoto, T., Oshima Y., Fukuyo Y. (Ed.), Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO, pp. 429–432.

Sampayo, M.A., Alvito, P., Franca, S., Sousa, I., 1990. *Dinophysis* spp. toxicity and relation to accompanying species. In: Granéli, E., Sundström B., Edler L., Anderson D.M.M. (Eds.), Toxic Marine Phytoplankton. Elsevier, New York, pp. 215–220.

Shumway, S.E., van Egmond, H.P., Hurst, J.W., Bean, L.L., 1995. Management of shellfish resources. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A. (Eds.), Manual on Harmful Marine Microalgae. UNESCO, Paris, pp. 433–462.

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.

Spacie, A., Hamelink, J.L., 1985. Bioaccumulation. In: Rand, G.M., Petrocelli, S.R. (Eds.), Fundaments of Aquatic Toxicology. Hemisphere Publ. Corp., New York, pp. 495–525.

Suzuki, T., Mitsuya, T., 2001. Comparison of dinophysistoxin-1 and esterified dinophysistoxin-1 (dinophysistoxin-3) content in the scallop *Pactinopecten yessoensis* and the mussel *Mytilus galloprovincialis*. Toxicon 39, 905–908.

Svensson, S., 2003. Depuration of okadaic acid (diarrhetic shellfish toxin) in mussels, *Mytilus edulis* (Linnaeus), feeding on different quantities of non-toxic algae. Aquaculture 218, 277–291.

Thompson, R.J., Ratcliffe, N.A., Bayne, B.L., 1974. Effects of starvation on structure and function in the digestive gland of the mussel (*Mytilus edulis* L.). J. Mar. Biol. Ass. U.K. 54, 699–712.

Underwood, A.J., 1997. Experiments in Ecology; Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge, UK.

Vale, P., Sampayo, M.A., Quilliam, M.A., 1998. DSP complex toxin profiles relation with *Dinophysis* spp. occurrence and domoic acid confirmation by LC–MS in Portugese bivalves. In: Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T. (Eds.), Harmful Algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, pp. 503–506.

Vale, P., Sampayo, M.A.M., 2002. Esterification of DSP toxins by Portugese bivalves from the Northwest coast determined by LC–MS—a widespread phenomenon. Toxicon 40, 33–42.

Vieites, J.M., Leira Sanmartin, F., 2000. Incidence of marine toxins on industrial activity. In: Botana, L.M. (Ed.), Seafood and Freshwater Toxins. Pharmacology, Physiology and Detection. Marcel Dekker, New York, pp. 741–760.

Vieytes, M.R., Fontal, O.I., Leira, F., Baptista de Sousa, J.M.V., Botana, L.M., 1997. A fluorescent microplate assay for diarrheic shellfish toxins. Anal. Biochem. 248, 258–264.

Widdows, 1973. Effects of temperature and food on the heart beat, ventilation rate and oxygen uptake of *Mytilus edulis*, Mar. Biol. 20, 269–276.

Widdows, J., Bayne, B.L., 1971. Temperature acclimation of *Mytilus edulis* with reference to its energy budget. J. Mar. Biol. Ass. U.K. 51, 827–843.

Wieser, W., 1973. Temperature relations of ectotherms: a speculative view. In: Wieser, W. (Ed.), Effects of Temperature on Ectothermic Organisms. Springer-Verlag, Berlin, pp. 1–24.

Yasumoto, T., Oshima, Y., Yamaguchi, M., 1978. Occurence of a new type of shellfish poisoning in the Tohoku district. Bull. Jpn. Soc. Sci. Fish. 44 (11), 1249–1255.

Yasumoto, T., Oshima, Y., Sugawara, W., Fukuyo, Y., Oguri, H., Igarashi, T., Fujita, N., 1980. Identification of *Dinophysis fortii* as the causative organism of diarrhetic shellfish poisoning. Bull. Jpn. Soc. Sci. Fish. 46, 1405–1411.

Yasumoto, T., Murata, M., Oshima, Y., Sano, M., Matsumoto, G.K., Clardy, J., 1985. Diarrhetic shellfish toxins. Tetrahedron 41, 1019–1025.

Zaba, B.N., Davies, J.I., 1984. Glycogen metabolism and glucose utilisation in the mantle tissue of *Mytilus edulis*. Mol. Physiol. 5, 261–282.