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Domoic acid uptake and elimination kinetics in oysters and mussels in relation to body size and anatomical distribution of toxin

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ABSTRACT

Toxin accumulation by suspension-feeding qualifier depends on a balance between processes regulating toxin uptake (i.e. ingestion and absorption of toxic cells) and elimination (i.e. egestion, exchange among tissues, excretion, degradation and/or biotransformation) during exposure to toxic blooms. This laboratory study compares the size-specific uptake and elimination kinetics of domoic acid (DA) from *Pseudo-nitzschia multiseries* in two co-occurring bivalves, the oyster *Crassostrea virginica* and the mussel *Mytilus edulis*. Domoic acid concentrations were measured in visceral and non-visceral tissues of different-sized oysters and mussels during simultaneous long-term exposure to toxic *P. multiseries* cells in the laboratory, followed by depuration on a non-toxic algal diet. Mussels attained 7–17-fold higher DA concentrations than oysters, depending on the body size and exposure time, and also detoxified DA at higher rates ($1.4\text{--}1.6\text{ d}^{-1}$) than oysters ($0.25\text{--}0.88\text{ d}^{-1}$) of a comparable size. Small oysters attained markedly higher weight-specific DA concentrations (maximum = $78.6\text{ }\mu\text{g g}^{-1}$) than large, market-sized individuals ($\leq 13\text{ }\mu\text{g g}^{-1}$), but no clear relationship was found between body size and DA concentration in mussels (maximum = $460\text{ }\mu\text{g g}^{-1}$). Therefore, differential DA accumulation by the two species was, on average, ~3-fold more pronounced for large bivalves. An inverse relationship between DA elimination rate and body size was established for oysters but not mussels. Elimination of DA was faster in viscera than in other tissues of both bivalves; DA exchange rate from the former to the latter was higher in oysters. The contribution of viscera to the total DA burden of mussels was consistently greater than that of other tissues during both uptake (>80%) and depuration (>65%) phases, whereas it rapidly decreased from 70–80% to 30–40% in oysters, and this occurred faster in smaller individuals. Residual DA concentrations ($\leq 0.25\text{ }\mu\text{g g}^{-1}$) were detected at later depuration stages (up to 14 d), mainly in viscera of oysters and non-visceral tissues of mussels, suggesting that a second, slower-detoxifying toxin compartment exists in both species. However, a simple exponential decay model was found to adequately describe DA elimination kinetics in these bivalves. The lower capacity for DA accumulation in oysters compared to mussels can thus only be explained by the former's comparatively low toxin intake rather than faster toxin elimination.

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1. Introduction

Suspension-feeding bivalves are important vectors of domoic acid (DA), a neurotoxic amino acid produced by diatoms mainly of the genus *Pseudo-nitzschia* (Trainer et al., 2008), and the causative agent of amnesic shellfish poisoning (ASP). Although *Pseudo-nitzschia* cells can release relatively large amounts of DA in the water column, especially under macronutrient- (Bates, 1998) or

iron-limitation (Maldonado et al., 2002), only minor toxin incorporation (0.3–0.6% of available DA in 5–24 h) has been reported from the dissolved phase by mussels (Madhyastha et al., 1991; Novaczek et al., 1991). Fast photodegradation (Bouillon et al., 2006) may further limit the availability of dissolved DA to marine organisms. Therefore, toxic diatom cells (i.e. the particulate phase) are the main source of DA for suspension-feeding bivalves.

During toxic blooms, accumulation of DA to levels exceeding the regulatory limit (RL) of $20\text{ }\mu\text{g g}^{-1}$ in bivalve tissues thus depends on the density and toxicity of cells in suspension, which can be highly variable even for a single *Pseudo-nitzschia* species (Bates et al., 1998), as well as on the balance between the mechanisms regulating DA uptake and elimination in bivalves. High inter-specific differences in DA accumulation capacity have been reported for

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various bivalve species. Oysters, for instance, accumulate consistently lower DA concentrations than other co-occurring bivalves, and rarely attain the RL (reviewed in Mafra et al., 2009a). During an early spring *Pseudo-nitzschia seriata* bloom in eastern Canada, in April 2002, maximum DA concentrations in oysters (*Crassostrea virginica*) were only $0.9 \mu\text{g g}^{-1}$, whereas mussels (*Mytilus edulis*) accumulated up to $200 \mu\text{g DA g}^{-1}$ (Canadian Food Inspection Agency, CFIA, data). This low DA uptake capacity in *C. virginica* relative to *M. edulis* was confirmed in the laboratory, and attributed to a combination of low clearance rate (CR, the volume of water cleared of particles per unit time) and selective rejection of *Pseudo-nitzschia* cells in pseudofeces by oysters (Mafra et al., 2009a,b). The present study investigates the possibility that the low capacity for DA accumulation in oysters may also be a consequence of more efficient toxin elimination mechanisms.

For water-soluble toxins such as DA and paralytic shellfish toxins (PSTs), elimination mechanisms may include toxin egestion in feces, exchange among tissues, excretion, toxin degradation and/or conversion into non-toxic or less toxic compounds (Bricelj and Cembella, 1995; Bricelj and Shumway, 1998; Lassus et al., 1996). The magnitude of these processes is expected to be inversely related to the toxin's binding affinity or retention in the organs/tissues where it is originally absorbed or secondarily re-allocated, as occurs during the slow elimination of strongly bound PSTs from the siphons of butter clams *Saxidomus giganteus* (Beitler and Liston, 1990). Retention of DA in bivalve tissues is highly species-specific. Mussels (*Mytilus edulis*, *M. californianus* and *M. galloprovincialis*), oysters (*Crassostrea gigas*), and softshell clams (*Mya arenaria*) are able to rapidly eliminate DA, typically purging most of the assimilated toxin within a few days following termination of the toxic exposure (summarized in Blanco et al., 2002a). In contrast, prolonged DA retention occurs in other bivalves such as scallops *Pecten* spp. (Fernández et al., 2000; Blanco et al., 2002a, 2006) and razor clams *Siliqua patula* (Drum et al., 1993).

Two types of toxin elimination kinetics have been identified in DA-contaminated bivalves. In the first category, DA elimination occurs at a constant decay rate, which leads to exponentially decreasing toxin concentrations over the entire depuration period, as indicated for *M. edulis* and *Pecten* spp. (Novaczek et al., 1992; Wohlgeschaffen et al., 1992; Blanco et al., 2002a). In this case, toxin elimination can be described by a single-compartment kinetics model. The second category is associated with an initial phase of rapid DA elimination followed by a period of slower toxin loss. As a result, residual DA concentrations may be retained for prolonged periods, as observed in 10% of the mussels (*M. edulis*) exposed to toxic *P. multiseriatus* in the laboratory by Novaczek et al. (1992), as well as in *S. patula* (Drum et al., 1993; Horner et al., 1993) and the mussels *Volsella modiolus* (Gilgan et al., 1990) and *Mytilus galloprovincialis* (Blanco et al., 2002b). A two-compartment model, with a different elimination rate for each compartment and transfer of toxin from the faster- to the slower-detoxifying compartment, more adequately describes DA elimination kinetics in these cases. To date, however, this has only been successfully demonstrated for *M. galloprovincialis*, revealing the presence of a slowly detoxifying second compartment containing a small amount of DA (Blanco et al., 2002b). In contrast, Douglas et al. (1997) did not find evidence of two-compartment kinetics in DA-contaminated sea scallops, *Placopecton magellanicus*, possibly because high DA concentrations were still present at the end of the 14-d depuration period.

Partitioning of toxins among body tissues is of particular concern for bivalve species in which only specific tissues are intended for human consumption. Although this does not apply to oysters and mussels, toxin allocation between tissue pools of these bivalves remains an important consideration to provide an understanding of the processes that may retard toxin loss, and to allow selection of appropriate toxin kinetics models. High-affinity binding sites for

DA may be present in different bivalve tissues, such as the digestive gland of *Pecten* spp. (Fernández et al., 2000; Blanco et al., 2002a, 2006) and non-visceral tissues of *S. patula* (Drum et al., 1993), leading to differential contribution of each tissue to the total DA body burden. The anatomical distribution of DA is also affected by the bivalve's capacity to transport DA across the gastrointestinal membrane after ingestion (Madhyastha et al., 1991) and to transfer substantial proportions of the total toxin from visceral to other tissues via the circulatory system. This capacity was suggested for *C. gigas* (Jones et al., 1995), but is lacking or limited in *Pecten maximus* (Blanco et al., 2002a). Therefore, visceral tissues (including the digestive gland) may account for 94–99% of the toxin burden in DA-contaminated *P. maximus* (Blanco et al., 2002a, 2006; Campbell et al., 2003; Bogan et al., 2007), 93% in *M. edulis* (Grimmelt et al., 1990), but only 70% in *C. virginica* (Roelke et al., 1993) during the toxin uptake phase.

Toxin kinetics may be affected by the body size of contaminated organisms. For diarrhetic shellfish toxins (DSTs) and PSTs, toxin uptake and elimination rates are inversely related to body size, although dilution effects by differential growth must be considered mainly in species or stages that exhibit slow toxin loss and/or fast growth rates (Bricelj and Shumway, 1998; Moróño et al., 2001; Duinker et al., 2007). The influence of bivalve body size on DA uptake and elimination, however, remains controversial. Novaczek et al. (1992) measured higher elimination rates in smaller mussels in the laboratory, and Bogan et al. (2007) found that smaller scallops exhibited faster toxin uptake and depuration in the field. Other studies reported no relationship between body size and DA concentration in various invertebrates, such as *P. maximus* (Arévalo et al., 1998), the sand crab *Emerita analoga* (Powell et al., 2002) and the cuttlefish *Sepia officinalis* (Costa et al., 2005).

In the present study, eastern oysters (*C. virginica*) and blue mussels (*M. edulis*) of varying body sizes were used to test the effects of body mass on DA uptake and elimination, as well as to allow extrapolation of findings previously obtained with juveniles (Mafra et al., 2010) to market-sized individuals. Mussels in the present study exhibited a narrower body size range (16–45 mm) than that of oysters (15–78 mm), but they were both representative of full size ranges commonly reported for natural and farmed populations of these bivalves in temperate waters.

There is limited information on DA elimination kinetics by oysters, and it is derived from short-term (3–5 d) laboratory experiments (Roelke et al., 1993; Jones et al., 1995). Domoic acid elimination has been more extensively studied in mussels (Novaczek et al., 1992; MacKenzie et al., 1993; Whyte et al., 1995; Blanco et al., 2002b), but inter-species comparisons are made difficult by the inconsistent toxin exposure conditions used in different studies. The present study examines DA uptake and elimination kinetics in oysters and mussels simultaneously exposed to toxic *P. multiseriatus* cells under “common-garden”, controlled laboratory conditions. Domoic acid concentrations were quantified in visceral (containing the stomach + digestive gland + intestine) and non-visceral (remaining) soft tissues of individual bivalves. Toxin kinetics models were fitted to the data to determine overall elimination rates and exchange rates between tissue compartments. The hypothesis that inter-specific differences in DA elimination mechanisms contribute to the differential DA accumulation by these two co-occurring bivalves was thus tested.

2. Materials and methods

2.1. Biological material

A toxic clone of *Pseudo-nitzschia multiseriatus*, CLN-50, was provided by S.S. Bates (Fisheries and Oceans Canada, Moncton, NB) and

maintained in batch culture at the Marine Research Station, Institute for Marine Biosciences, National Research Council of Canada, Halifax, NS. Cultures were initially maintained in 2.8 l glass Fernbach flasks filled with 1.5 l of autoclaved, 0.22 μm cartridge-filtered seawater (FSW) enriched with *f/2* medium (Guillard, 1975) at 16 °C, 30 salinity, 140 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ light intensity, on a 14 h light:10 h dark photoperiod. For bivalve experiments, cultures were scaled up in 10–20 l glass carboys with a continuous supply of filtered air (PTFE membrane filters, 0.2 μm particle retention, Pall Co.) and harvested at stationary growth phase, when maximum toxicity was attained. Cell size was determined using a microscope (Leica model DMLB 100S) with a coupled Pulnix camera (Model TMC-7DSP) and image analysis software (Image Pro Plus Version 4.5, Media Cybernetics); cell volume was calculated by the formula described in Lundholm et al. (2004) and simplified as: cell volume (μm^3) = $0.8 \times L \times W^2$, where L and W are the cell length and width, in μm , respectively. Cell density was measured by microscopic counting of diluted samples ($n \approx 400$ cells) on Palmer–Maloney chambers.

Triplicate 15 ml culture samples were gently passed through Whatman GF/F glass microfiber filters (25 mm diameter, 0.7 μm particle retention). The cellular content of *P. multiseriis* was released following disruption of the filters using a Vibracell VC375 sonicator (Sonics & Materials), and re-filtered on Ultrafree-MC centrifugal filters (Durapore PVDF, 0.45 μm particle retention) at 10,000 $\times g$ for 30 s. Particulate DA was then quantified by liquid chromatography with ultraviolet detection (LC–UVD), according to the methods described in Mafra et al. (2009c). The non-toxic flagellates commonly used as bivalve food, *Isochrysis galbana* (T-Iso clone CCMP1324) and *Pavlova pinguis* (CCMP609), were acquired from the Center for the Culture of Marine Phytoplankton (CCMP), ME, USA, and cultivated in semi-continuous, 200 l photobioreactors at 20 °C. Cell density and cellular volume of the flagellates was determined with a Beckman–Coulter Multisizer 3 particle counter.

Bivalve shell size was measured as the maximum long axis from the umbo to the ventral margin of the shell, which represents the shell height (SH) in oysters (Carriker, 1996) but is commonly referred to as the shell length (SL) in mussels (Seed, 1968). Oysters, *C. virginica* (SH = 15–78 mm), were obtained from Bay Enterprises Ltd., Malagash, and mussels, *M. edulis* (SL = 16–45 mm), from Indian Point Marine Farms Ltd., Mahone Bay, NS, Canada in May 2007. Bivalves were maintained in active upwellers (31 cm diameter) inside 1000 l insulated tanks filled with FSW at 12 °C and 30 salinity, and continuously fed a mixture of *P. pinguis* and *I. galbana* at a total cell density equivalent to 30,000 *I. galbana* cells ml^{-1} . Prior to toxification, bivalves were acclimated to the experimental tanks on this same non-toxic algal suspension for ~20 h.

2.2. Size-specific domoic acid uptake in mussels and oysters

Bivalves were divided into three size classes – “small”, “medium” and “large” – described by the following size ranges: oysters SH = 20–29 mm (mean $\pm$ standard error, SE = 23.8 ± 0.3 mm; $n = 60$), 33–49 mm (39.4 ± 0.5 mm), and 52–78 (62.9 ± 0.8 mm); mussels SL = 21–25 mm (mean $\pm$ SE = 22.8 ± 0.2 mm; $n = 48$), 28–33 mm (30.7 ± 0.2 mm), and 36–42 (39.0 ± 0.2 mm), respectively. Thirty oysters and 24 mussels of each size class were placed in each of two shared glass aquaria containing 140 l of FSW to assure identical toxification conditions, and were fed toxic *P. multiseriis* cells at 3100 ± 100 cells ml^{-1} (mean $\pm$ SE) for 4–7 d. The aquaria were held in a temperature-controlled, walk-in environmental chamber at 12 °C. Each species/size group was enclosed in plastic cages (mesh size = 0.5 mm), and suspended from the bottom to facilitate sampling and periodic removal of biodeposits. Aeration was provided via air stones placed at opposite corners of the aquaria and by two recirculating aquarium pumps mounted

externally, which also ensured mixing and maintenance of the cells in suspension. Cell density of *P. multiseriis* was kept nearly constant by delivering fresh culture with a peristaltic pump at a rate similar to the bivalves' total consumption. Cell density was assessed every ~30 min (except overnight), and, if different from the desired value by >10%, corrected by adjusting the pump delivery rate and/or by bulk addition of culture or dilution with FSW. Aquaria were cleaned and fresh suspension added every 1–2 d.

Six oysters of each size class were sampled from each tank after 0.25, 1, 2, 4, and 7 d of exposure to toxic *P. multiseriis* cells; the same number of mussels was simultaneously collected, except at the last sampling time when no mussels were available. Sampled bivalves were placed on ice and immediately dissected. Soft tissues were removed, rinsed with cold FSW, and carefully separated into visceral and non-visceral (remaining) pooled tissues. Tissue pools from each bivalve were weighed after removing excess water with absorbent paper, and stored at –80 °C. Analysis of extracted DA was performed within 1–2 months, following methods of Quilliam et al. (1995). Briefly, DA was extracted by sonication of the tissues in 50% aqueous MeOH (4:1, v/v) for 3 min at 50% duty cycle, followed by filtration on centrifugal filters at 10,000 $\times g$ for 30 s, and the extracts were analyzed by LC–UVD.

The effects of exposure time and body size on DA accumulation by oysters and mussels were tested by two-way ANOVAs ($\alpha = 0.05$). Particulate DA concentrations available in each tank were calculated every ~30 min (except overnight) and compared by paired Student's *t*-test ($\alpha = 0.05$). These and all other statistical tests were performed on SYSTAT® 12 software.

Net DA accumulation efficiency (DA_{AE}) was calculated at each sampling time as the proportion of DA incorporated in bivalve whole tissues (DA_{AC}) relative to that ingested from suspension (DA_{ING}), as follows:

$$DA_{ING}(\mu\text{g DA g}^{-1}) = \frac{(CR \times C \times tox \times t)}{(WW \times 10^6)} \quad (1)$$

$$DA_{AE}(\%) = \frac{DA_{AC}}{DA_{ING}} \times 100 \quad (2)$$

where CR is the weight-standardized clearance rate per individual ($\text{ml min}^{-1} \text{ind}^{-1}$), as scaled from juveniles (Mafra et al., 2010) using an allometric equation with exponent = 0.616 (Bayne and Newell, 1983); C is the average cell density in the tank (cells ml^{-1}); tox is the cellular toxicity of the stock culture (pg DA cell $^{-1}$); WW is the average wet soft tissue weight of the bivalves (g ind^{-1}) sampled at a given time t (min); and DA_{AC} is the DA concentration measured in bivalve tissues ($\mu\text{g DA g}^{-1}$). Based on a previous experiment on *P. multiseriis* filtration by juvenile oysters (Mafra et al., 2009a), as well as on observations made in the present study, pseudofeces production was negligible at the cell density used, such that ingestion rate = $CR \times C$.

2.3. Size-specific domoic acid elimination in mussels and oysters

Oysters ($n = 150$) and mussels ($n = 150$) were placed together in each of two 140-l glass aquaria with FSW and exposed to toxic *P. multiseriis* cells at ~1000 cells ml^{-1} for 2 d (i.e. toxification period). After this period, all bivalves were transferred to suspended upwellers inside a common 1000 l insulated tank, and received non-toxic *I. galbana* and *P. pinguis* at an ~constant cell density of 30,000 cells ml^{-1} for 21 d (i.e. depuration period). The depuration tank was continuously replenished with FSW at 40 l h^{-1} and maintained at 12 °C. In addition, bivalves were transferred to an alternate tank filled with fresh, non-toxic algal suspension every 24 h to minimize re-filtration of toxic biodeposits.

Bivalves were divided into three size classes – “small”, “medium” and “large” – with size ranges as follows: oysters SH = 19–33 mm (mean $\pm$ SE = 25.7 ± 0.5 mm; $n = 50$), 29–47 mm (40.4 ± 0.5 mm), 50–85 mm (63.8 ± 0.8 mm); mussels SL = 16–26 mm (mean $\pm$ SE = 20.2 ± 0.2 mm; $n = 50$), 25–34 mm (28.6 ± 0.2 mm) and 33–45 mm (38.1 ± 0.3 mm). Twelve bivalves of each size class were sampled after 0, 0.12, 1, 2, 4, 7, 14, and 21 d of depuration on the non-toxic diet. Individual visceral and non-visceral soft tissues were dissected and processed as described in Section 2.2. Extracts were analyzed by LC-UVD according to the methods of Quilliam et al. (1995), except those from bivalves collected at or after Day 7 of depuration. These later samples were analyzed using a more sensitive LC-UVD method for determination of trace DA concentrations in seawater (Mafra et al., 2009c), which was adapted to bivalve samples following 3-fold dilution of the extracts. Initial DA concentrations (depuration time = 0) were compared among size classes of oysters and mussels by one-way ANOVAs ($\alpha = 0.05$). Toxin elimination rates were calculated by fitting models to the measured DA concentrations.

2.4. Modeling

Different one-compartment DA elimination models that assume a first-order decay of toxin were fit (using Matlab Version 7.4, R2007a) to the absolute DA amounts (μg) in whole and partitioned soft tissues, which were obtained by multiplying the toxin concentration by the WW of the corresponding tissue. Domoic acid concentrations equivalent to zero were replaced with the detection limit of the analytical method (0.2 ng g^{-1}). In addition, a two-compartment model similar to that used in Blanco et al. (2002b) was fit to the bivalve tissue pools in which residual DA amounts were detected, but this is not described in detail because it did not result in improved fits or reduced uncertainty.

2.4.1. Simple exponential decay

The simple one-compartment model,

$$\frac{\partial T}{\partial t} = -kT \quad (3)$$

where k is the DA elimination rate (d^{-1}), and T is the toxin burden ($\mu\text{g DA}$) after a depuration time t (d), represents the exponential decay of DA over time. The analytical solution to Eq. (3) is

$$T(t) = T_0 \times e^{-kt} \quad (3.1)$$

where T_0 is the initial toxin burden ($\mu\text{g DA}$). The parameter k was obtained from Eq. (3.1) by non-linear least-squares fitting using the Matlab function `nlinfit`, and represents the exponential loss of DA per unit time relative to the initial DA concentration, expressed in units of $\mu\text{g d}^{-1} \mu\text{g}^{-1}$, or simply d^{-1} . This model was applied to the DA burden of whole bivalves, and to visceral and non-visceral tissues to obtain the respective toxin elimination rates. Confidence intervals (95% CI) were calculated for each rate for statistical comparisons using the Matlab function `nlparci`. It was confirmed that the residuals for the obtained model fits were random without systematic patterns.

2.4.2. Exponential decay with transfer between tissues

A more elaborate one-compartment model with transfer of DA from visceral to non-visceral tissues, similar to that used by Blanco et al. (2002a), employed two coupled equations:

$$\frac{\partial X}{\partial t} = -aX - bX = -(a + b)X = -cX \quad (4)$$

$$\frac{\partial Y}{\partial t} = -dY + bX \quad (5)$$

where X and Y are the DA burden (μg) in visceral and non-visceral tissues, respectively; c is the DA elimination rate (d^{-1}) in viscera and is the sum of direct elimination, a , and transfer to non-visceral tissues, b ; d is the DA elimination rate in non-visceral tissues (d^{-1}). Eq. (4), which describes the change of X in time, is independent of Y . Thus c can be obtained as described in Section 2.4.1 above. Eq. (5), which describes the change of Y in time, depends on X as well, but can be reformulated by inserting the solution to Eq. (4) into Eq. (5):

$$\frac{\partial Y}{\partial t} = -dY + b(X_0 \times e^{-ct}) \quad (5.1)$$

Here X_0 is the initial DA burden (μg) in viscera. Eq. (5.1) is no longer dependent on X and can be solved analytically:

$$Y(t) = \left[\frac{bX_0}{(c-d)} \right] e^{-ct} + \left\{ Y_0 - \left[\frac{bX_0}{(c-d)} \right] \right\} e^{-dt} \quad (5.2)$$

Since X_0 and Y_0 are defined as the mean DA burdens at $t = 0$, and c was previously calculated from Eq. (4), b and d can be determined by non-linear fitting as described in Section 2.4.1. Then $a = c - b$ can be calculated.

Fig. 1. Accumulation of domoic acid (DA, mean $\pm$ standard error, SE) in whole tissues of oysters (*Crassostrea virginica*, upper panel) and mussels (*Mytilus edulis*, central panel) during 4–7 d of exposure to toxic *Pseudo-nitzschia multiseries* (cell length = $64 \mu\text{m}$; toxicity = $3.8\text{--}8.7 \text{ pg DA cell}^{-1}$) at 12°C . Concentrations of particulate DA (pDA) in suspension (mean $\pm$ standard deviation of 2 tanks) are shown in the lower panel. Bivalve size classes were based on shell height (SH) for oysters = 20–29 mm (small), 39–49 mm (medium), 52–78 mm (large); and shell length (SL) for mussels = 21–25 mm (small), 28–33 mm (medium) and 36–42 mm (large). Horizontal line defines the regulatory limit (RL) for shellfish harvesting. Statistical results of between size-class comparisons (one-way ANOVA) are shown above the bars. ns: non-significant difference; * $p \leq 0.05$, ** $0.001 < p \leq 0.01$ ($n = 6\text{--}12$ individuals).

Fig. 2. Oysters, *Crassostrea virginica* (left panels; SH = 20–78 mm) and mussels, *Mytilus edulis* (right panels; SL = 21–42 mm). Relationship between domoic acid (DA) concentration in whole tissues ($\mu\text{g g}^{-1}$ of soft tissue wet weight) and bivalve body size after 0.25, 1, 2, and 4 d of exposure to toxic *Pseudo-nitzschia multiseries* (cell length = 64 μm ; toxicity = 3.8–8.7 pg DA cell $^{-1}$) at 12 °C (n = 24–36 individuals per sampling). r^2 : coefficient of determination of the fitted power equations. Note different Y-scales for each species.

3. Results

Cellular toxicity of *P. multiseries* cultures used in the DA uptake experiment ranged from 3.8 to 8.7 pg DA cell $^{-1}$, and cell length from 54 to 71 μm (mean $\pm$ SE = 64 $\pm$ 0.8). Bivalves were

exposed to these cultures at a mean cell density of 3100 cells ml $^{-1}$. Particulate DA concentrations were highly variable over time (2.7–36.6 ng DA ml $^{-1}$; time-average = 19.2 ng DA ml $^{-1}$), but not statistically distinguishable between the two experimental tanks (p = 0.87, paired Student's t -test) (Fig. 1, lower panel). Bivalves sam-

Table 1

Net domoic acid accumulation efficiency [%; mean and standard deviation, SD (in parentheses)] by oysters, *Crassostrea virginica*, and mussels, *Mytilus edulis*, calculated as the proportion of toxin accumulated relative to the amount ingested from suspension during continuous exposure to *Pseudo-nitzschia multiseries* (mean cell length = 64 μm ; toxicity = 3.8–8.7 pg DA cell $^{-1}$) at 12 °C. Bivalve size classes as defined in Fig. 1; inter-specific comparisons (Student's t -test) were performed for each size class (n = 10–12 individuals).

Exposure time (h)	Oysters			Mussels		
	Small	Medium	Large	Small	Medium	Large
6	21 (8.8)	32 (15)	43 (28)	32 (5.2)**	39 (7.1) ^{ns}	42 (12) ^{ns}
24	13 (6.7)	21 (15)	23 (15)	35 (4.9)***	44 (12)**	43 (16)*
48	28 (25)	8.9 (5.0)	14 (6.6)	37 (17) ^{ns}	46 (15)***	60 (19)***
96	6.6 (3.9)	7.8 (4.6)	10 (7.5)	8.4 (4.2) ^{ns}	11 (4.1) ^{ns}	16 (5.2) ^{ns}
168	1.3 (0.8)	2.7 (1.9)	2.1 (1.2)	n.d.	n.d.	n.d.

ns: non-significant difference; n.d.: not determined.

* $p \leq 0.05$.

** $0.001 < p \leq 0.01$.

*** $p \leq 0.001$.

pled from both tanks were thus treated as replicates. There was no significant change in the mean body mass of bivalves collected over the course of the toxification period ($p=0.11$ – 0.93 and 0.09 – 0.45 for oysters and mussels of various size classes, respectively; one-way ANOVA). The mean ($\pm$ SE; $n=50$) WW of small, medium and large bivalves was 0.13 ± 0.01 g, 0.51 ± 0.02 g and 1.83 ± 0.07 g in oysters, and 0.31 ± 0.01 g, 0.70 ± 0.02 g and 1.16 ± 0.03 g in mussels, respectively.

3.1. Inter-specific differences in DA uptake and elimination

During the longer (4–7 d) toxification period, mussels attained 8–17-fold higher DA concentrations (max. $460 \mu\text{g g}^{-1}$) than oysters (max. $78.6 \mu\text{g DA g}^{-1}$), depending on the size class and exposure time (Figs. 1 and 2). Similarly, mussels of different size classes attained 5.6–7.7-fold higher DA concentrations than oysters (maximum = 179 and $29.5 \mu\text{g g}^{-1}$, respectively) at the end of the shorter (2 d) toxification period that preceded the depuration experiment. In addition, mussels exhibited consistently higher net DA accumulation efficiencies (DA_{AE}) than oysters of a comparable size, although the difference was not always significant (Table 1).

Toxin elimination rates of mussels (1.4 – 1.6 d^{-1}) were generally 2–4-fold higher than those of oysters (0.25 – 0.88 d^{-1}) of a comparable size (i.e. 26-mm oysters and 20-mm mussels; 40-mm oysters and 38-mm mussels) during the initial 14 d of depuration (Fig. 3a), as calculated from a simple one-compartment DA elimination model. Improved fits of the model were achieved for mussels ($r^2=0.48$ – 0.88) relative to oysters ($r^2=0.06$ – 0.59) (Table 2). Samples collected on Day 21 of depuration were not analyzed since DA concentrations were already mostly below the detection limit of the method (0.2 ng g^{-1}) by Day 14.

3.2. Effect of body size on DA uptake and elimination

Weight-specific DA accumulation was significantly affected by body size in oysters ($p<0.001$), but not in mussels ($p=0.37$) (two-way ANOVA) during the longer (4–7 d) toxification period. Additionally, DA concentrations attained by both bivalves changed significantly with the duration of the exposure to toxic cells ($p=0.02$ and <0.001 , respectively), and the interaction between exposure time and body size was significant for oysters ($p=0.002$) but not for mussels ($p=0.36$). The effects of body size and exposure time on DA accumulation by oysters remained significant ($p=0.005$ and 0.007 , respectively) even after removing the markedly higher values obtained by smaller individuals at 48 h (Fig. 1) from the analysis (two-way ANOVA). When weight-specific DA concentrations were compared in oysters of different body sizes at each exposure time (Fig. 1), however, differences were only significant at 48 h exposure ($p=0.007$; one-way ANOVA), due to high individual variability of DA concentrations within each size class [coefficient of variation (CV) = 42–76% (small oysters), 32–67% (medium), 47–77% (large)].

Despite feeding on relatively high *P. multiseriis* cell densities and toxicities, larger oysters (SH > 55 mm) never accumulated

DA concentrations exceeding the RL of $20 \mu\text{g g}^{-1}$ in whole tissues; maximum DA concentrations in market-sized individuals (SH > 65 mm) were $\leq 13 \mu\text{g g}^{-1}$ (Fig. 1). Smaller oysters consistently attained higher weight-specific DA concentrations than larger individuals (Fig. 1), and reached maximum concentrations at 48 h of exposure perhaps related to fluctuations in CRs over time, which were not measured in this experiment but previously reported for juvenile individuals (Mafra et al., 2010). In the present study, an inverse functional relationship between oyster SH and DA concentrations could be established at 48 h of exposure (Fig. 2). In contrast, weight-specific DA concentrations in mussels were less variable at a given exposure time [CV = 19–49% (small mussels), 20–34% (medium), 28–35% (large)] and generally unrelated to body size (Figs. 1 and 2), except at 24 h of exposure when the difference between size classes was marginally significant ($p=0.033$; one-way ANOVA).

Similar patterns were found during the 2-d toxification period that preceded the depuration experiment. Larger oysters (SH > 52 mm) attained lower DA concentrations (1.5 – $14.6 \mu\text{g g}^{-1}$) than smaller individuals (3.0 – $29.5 \mu\text{g g}^{-1}$), whereas no clear relationship between DA concentration and body size could be established for mussels. The differences among size classes, however, were not significant at this time for either mussels or oysters [$p=0.54$ and 0.20 , respectively (one-way ANOVA)].

Larger mussels exhibited higher DA_{AE} values than smaller individuals, incorporating in their tissues 16–60% and 9–37% respectively of the available particulate DA in suspension, depending on the exposure time (Table 1). Oysters followed a similar trend, except at 48 h of exposure, when DA_{AE} of small oysters was higher than that of larger animals (Table 1). However, values were not statistically different among size classes.

During the 14-d depuration period on a non-toxic algal diet, smaller oysters eliminated DA at higher rates than larger ones, whereas elimination rates of mussels were not related to body size (Fig. 3a) as calculated using a simple one-compartment model (Table 2). It must be noted, however, that the overall size range of oysters was greater than that of mussels. While the mean body mass of oysters did not change significantly over the depuration period ($p=0.47$ – 0.61) within each size class [overall means = 0.17 g (small), 0.60 g (medium) and 2.05 g (large)], mussels exhibited a significant weight gain (40–69%) after 14 d of depuration ($p<0.001$; one-way ANOVA), growing from 0.18 to 0.30 g (small), 0.46 to 0.66 g (medium) and from 0.85 to 1.20 g (large).

3.3. Anatomical distribution of domoic acid

When the same simple one-compartment model was applied to the DA content of each tissue pool individually, elimination rates in the viscera were generally higher than those of non-visceral tissues of both oysters and mussels, although differences were non-significant for some size classes (Fig. 3b). The highest overall elimination rate was measured in the viscera of large mussels (mean $\pm 95\%$ CI = $2.06 \pm 0.15 \text{ d}^{-1}$) and the lowest one in non-visceral tissues of large oysters ($0.16 \pm 0.11 \text{ d}^{-1}$). Considering all size classes of both bivalves, the simple model fit the visceral toxin burden ($r^2=0.21$ – 0.88) better than non-visceral tissues ($r^2=0.06$ – 0.70).

The inclusion of a parameter to estimate DA transfer from viscera to non-visceral tissues (i.e. one-compartment model with toxin transfer) slightly improved the model fit in most cases (1–9% increase in r^2). In two out of six cases (small oysters and medium mussels), the one-compartment model with toxin transfer provided an adequate fit to the data allowing calculation of the DA transfer rate (Table 3). This equalled 0.35 d^{-1} in small oysters; overall, 26% of the toxin eliminated from the viscera of these

Table 2

Coefficients of determination (r^2) of the simple one-compartment, exponential decay model fitted to the experimental data of DA elimination by oysters and mussels of the three size classes, by tissue type. Elimination rates (exponents of these equations) are plotted in Fig. 3.

	Oysters			Mussels		
	Small	Medium	Large	Small	Medium	Large
Whole tissues	0.556	0.140	0.163	0.937	0.877	0.663
Visceral tissues	0.590	0.476	0.109	0.802	0.881	0.767
Non-visceral tissues	0.302	0.209	0.065	0.992	0.705	0.671

Table 3

Domoic acid (DA) elimination parameters, as estimated by fitting a one-compartment model with toxin transfer (see Section 2.4.2) to the total DA burden in viscera and non-visceral tissues of oysters (*Crassostrea virginica*) and mussels (*Mytilus edulis*) over 14 d of depuration on a non-toxic algal diet. Initial DA burden (μg), DA elimination rate (d^{-1}), and rate of DA transfer (d^{-1}) from viscera to non-visceral tissues are compared in two selected bivalve size classes (r^2 : coefficient of determination of the fitted model). The relative contributions of direct DA elimination to the external medium and transfer to non-visceral tissues, to the total DA loss from viscera are indicated. Size classes as defined in Fig. 3; $n = 12$ individuals per sampling time.

Bivalve	Non-visceral tissues			Viscera			
	Initial DA	DA elimination rate	r^2	Initial DA	DA elimination rate	Transfer rate	r^2
Small oysters	1.106	0.831	0.328	1.266	0.992 (74%)	0.346 (26%)	0.590
Medium mussels	4.820	1.744	0.759	30.45	1.319 (89%)	0.159 (11%)	0.881

bivalves was transferred to non-visceral tissues, while the rest was directly lost from the organism, presumably via degradation, egestion and/or excretion. Medium mussels exhibited slower transfer rates (mean = 0.16 d^{-1}), and the fraction of DA transferred to non-visceral tissues was only 11% of the total loss of visceral DA (Table 3). In all other cases (small mussels, medium oysters, and large individuals of both species), however, model parameters could not be adequately calculated due to violations of primary assumptions, as described below. Poor model fits and, in some cases, degenerate parameters such as negative transfer rates resulted from increasing toxin content during initial depuration stages, as found in both tissue pools of oysters and mussels (Fig. 4a–c and f), as well as from highly variable DA content among individuals of the same size class (Fig. 4g) at a given sampling time (CV = 34–342% and 15–202% for oysters and mussels, respectively). Even after exclusion of one or more sampling times (i.e. depuration starting at maximum whole tissue DA values), DA transfer rates could not be calculated in these four cases.

In mussels, the contribution of visceral tissues to the total DA burden was consistently greater than that of other tissues during both uptake and depuration phases, whereas it was rapidly reduced below that of non-visceral DA in oysters. During the first 2 d of exposure to toxic *P. multiseriis* cells (i.e. uptake phase), viscera

accounted for 80–90% of the total DA burden in mussels of different size classes, gradually decreasing to 65–70% during the depuration phase, as measured from separate experiments (Fig. 5). In contrast, the proportion of visceral DA decreased from 70 to 80% to <50% after only ~36 h of toxin exposure in small oysters and ~72 h in larger oysters, reaching 30–40% in all size classes within four days of depuration (Fig. 5). After the 4th day, toxin elimination to undetectable levels was achieved by some individuals of both species, such that partitioning of DA between tissue pools could not be calculated beyond this time point. Only small amounts ($\leq 0.25 \mu\text{g g}^{-1}$) of slow-detoxifying DA were detected at that time, mainly in viscera of oysters and non-visceral tissues of mussels.

4. Discussion

4.1. Inter-specific differences in DA uptake and elimination

The present study confirms the lower capacity of oysters (*C. virginica*) to accumulate DA from toxic *P. multiseriis* cells relative to that of mussels (*M. edulis*) of comparable body size, as previously reported for juveniles (SH < 29 mm) (Mafra et al., 2010). The inter-specific difference in DA accumulation was even greater (by >3-fold), in larger, market-sized bivalves than that found for juveniles in both this (Fig. 1) and the previous study. Maximum individual toxin concentrations measured during the 4–7-d toxification period ($78.6 \mu\text{g DA g}^{-1}$ in oysters and $460 \mu\text{g DA g}^{-1}$ in mussels) are the highest ever attained in the laboratory, and comparable to the maximum values reported for each bivalve genus during natural *Pseudo-nitzschia* spp. blooms: $30.0 \mu\text{g g}^{-1}$ for *C. gigas* (Trainer et al., 2007) and $790 \mu\text{g g}^{-1}$ for *M. edulis* (Bates et al., 1989). In addition, comparable to higher DA concentrations have been found in whole tissues of slow-depurating bivalves, such as the oyster *Spondylus versicolor* [$147 \mu\text{g g}^{-1}$ (Dao et al., 2009)], the clam *S. patula* [$308 \mu\text{g g}^{-1}$ (Trainer et al., 2000)] and the scallop *P. maximus* [$1569 \mu\text{g g}^{-1}$ (Campbell et al., 2001)]. High DA concentrations in natural bivalve populations (up to $70 \mu\text{g g}^{-1}$) usually occur during massive blooms of highly toxic *Pseudo-nitzschia* spp. cells (water column particulate toxin concentrations up to 14.4 ng ml^{-1}) (e.g. Trainer et al., 2007), conditions that were reproduced in the laboratory during the present study.

Domoic acid accumulation efficiency (DA_{AE}), a balance between concurrent DA uptake and elimination processes, was greater in mussels (32–60%) relative to oysters (10–40%) during the first 48 h of toxification. These values are comparable to that calculated for sea scallops (*P. magellanicus*) after 14 d (51%, Douglas et al., 1997), and higher than those of juvenile *M. edulis* (8–20%) and *C. virginica* (2–6%) during 24 h of toxic exposure at 12°C (Mafra et al., 2010). Significant inter-specific differences in DA_{AE} between these two bivalves (Table 1) can be explained by the reduced capacity of oysters to ingest *P. multiseriis* cells >68 μm (see Mafra et al., 2009b). Although the mean cell length of the *P. multiseriis* cells used in the present study was only 64 μm , some cells exceeded the 68- μm size threshold for selective rejection by oysters, and this threshold may be further reduced due to gill contraction

Fig. 3. Toxin elimination rates (mean $\pm$ 95% confidence interval) of domoic acid-contaminated oysters (*Crassostrea virginica*, left panels) and mussels (*Mytilus edulis*, right panels) during 14 d of depuration on a non-toxic algal diet, as calculated by a simple one-compartment model from: (a) whole bivalves or (b) separate tissue pools (see Section 2.4.1). Bivalve size classes described as follows: oysters = 19–33 mm SH (small), 29–47 mm (medium), 50–85 mm (large); mussel = 16–26 mm SL (small), 25–34 mm (medium) and 33–45 mm (large). Rates were compared across all size classes of both bivalves (a) and between tissue pools of each size class (b). Different capital letters indicate statistically significant differences ($\alpha = 0.05$); ns: non-significant difference; * $p \leq 0.05$, *** $p \leq 0.001$ ($n = 6$ –12 individuals or tissue pools).

Fig. 4. Percent of initial domoic acid (DA) content in oysters (*Crassostrea virginica*, left panels) and mussels (*Mytilus edulis*, right panels) of different size classes during 14 d of depuration on a non-toxic algal diet following DA uptake: (a, d) whole tissues, (b, e) viscera and (c, f) non-visceral tissues (mean, $n = 10\text{--}12$ per size class as defined in Fig. 3). Inset (g) shows DA concentrations in whole tissues of individual large oysters. Zero values were replaced with the analytical detection limit ($0.0002 \mu\text{g DA g}^{-1} \times \text{bivalve wet weight}$, in g) in all cases. Half-time of DA (i.e. time to eliminate 50% of the initial toxin burden) in whole tissues, as calculated from a simple exponential decay model: oysters = 0.8 d (small), 1.6 d (medium), 2.8 d (large); mussels = 0.4 d (small), 0.5 d (medium), and 0.4 d (large).

during suspension-feeding (Mafra et al., 2009b). Relatively high DA_{AE} values attained by both bivalves in the present study are likely associated with their exposure to greater particulate toxicities (time-average = $19.2 \text{ ng DA ml}^{-1}$) than in prior experiments [$5.9\text{--}8.7 \text{ ng DA ml}^{-1}$ (Mafra et al., 2010)].

Domoic acid elimination rates in whole tissues ranged from 0.25 to 0.88 d^{-1} in oysters and from 1.4 to 1.6 d^{-1} in mussels in the present study. These rates are comparable to those measured for the fastest DA-detoxifying bivalves, such as the mussels *Mytilus californianus* [$0.3\text{--}0.5 \text{ d}^{-1}$ (Whyte et al., 1995)], *M. galloprovincialis* [$0.4\text{--}0.6 \text{ d}^{-1}$ (Blanco et al., 2002b)], *Perna canaliculus* [2.0 d^{-1} (MacKenzie et al., 1993)] and *M. edulis* [$0.5\text{--}1.0 \text{ d}^{-1}$ (Novaczek et al., 1992); 2.0 d^{-1} (Krogstad et al., 2009 with data from Wohlgeschaffen et al., 1992)].

This study demonstrated that mussels eliminate DA at consistently higher rates than oysters of a comparable size (Fig. 3a). The hypothesis that fast elimination could explain the lower DA concentrations attained by oysters relative to those of mussels during toxic *Pseudo-nitzschia* blooms must thus be rejected. Contrary to oysters, however, mussels exhibited a significant (1.4–1.7-fold) gain in body mass over the 14-d depuration period. In some cases, differential growth can explain most of the differences in toxin elimination by bivalves, as reported in a long-term (73 d) field study in which DST elimination was greater in faster-growing, smaller *M. edulis* than in larger individuals [(SL = 28 and 46 mm, respectively (Duinker et al., 2007)]. This is caused by dilution of toxin content due to body mass gain, as also reported for PSTs in *M. edulis* and *S. solidissima*

(Bricelj et al., 1990; Bricelj and Cembella, 1995) and for DA in crabs *Cancer magister* (Lund et al., 1997). In the present study, however, elimination rates were calculated based on the total toxin burden of individual bivalves (i.e. DA concentration $\times$ body weight), such that inter-specific differences in DA loss may have been partially underestimated rather than enhanced due to growth. This effect is expected to be minimal (if present) however, since only <3% of the initial DA load remained in mussels after 4 d of depuration, when gain in body mass started to be significant.

4.2. Effect of body size on DA uptake and elimination

Whereas oysters consistently maintained a slightly inverse relationship between body size and DA content throughout the toxification phase, this relationship was reversed in mussels, becoming null or slightly positive during late toxification (Fig. 2). Inverse relationships between weight-specific toxin concentrations and body size have been previously reported during laboratory and field exposure of bivalves to DA, PSTs and DSTs (Novaczek et al., 1992; Morono et al., 2001; Duinker et al., 2007). In contrast, Roelke et al. (1993) found no relationship between body weight and DA concentration in *C. virginica*, although only low toxin concentrations ($1\text{--}2 \mu\text{g g}^{-1}$) were attained by oysters in their laboratory study. Higher toxin concentrations in smaller bivalves may be a result of faster toxin uptake by small individuals and/or higher elimination rates by large ones (Strohmeier et al., 2005; Bogan et al., 2007). Although unlikely in our study, differential toxin dilution due to

Fig. 5. Percent contribution (mean $\pm$ SE, $n = 6-12$) of viscera and non-visceral tissues to the total body burden of domoic acid (DA) present in different-sized oysters, *Crassostrea virginica* (left panels) and mussels, *Mytilus edulis* (right panels), during 2 days of exposure (i.e. uptake phase) to toxic *Pseudo-nitzschia multiseries* (cell length = 64 μm ; toxicity = 3.8–8.7 pg DA cell $^{-1}$) or 4 days of depuration on a non-toxic algal diet at 12 °C (separate experiment). Small, medium and large size classes as defined in Figs. 1 and 3 for the uptake and depuration phases respectively.

faster growth of smaller individuals must also be considered in some other cases (Bricelj and Cembella, 1995; Duinker et al., 2007), as discussed above.

Small oysters exhibited significantly higher (2–3.6-fold) DA elimination rates than large ones in the present study (Fig. 3a). This is consistent with the fact that metabolic rate per unit mass is inversely related to body size (Bayne and Newell, 1983; Schmidt-Nielsen, 1984), but contrasts with the comparable (this study) to slightly (1.07-fold) higher (Novaczek et al., 1992) elimination rates reported in smaller mussels relative to larger individuals. Such inter-specific difference may be associated with the narrower size range used for mussels in both studies compared to that of oysters in the present study, although no relationship between body size and DA elimination rate was found in Mediterranean mussels (*M. galloprovincialis*) of a wider size range [(SL = 30–96 mm (Blanco et al., 2002b)].

Since oyster body weight remained unchanged during DA toxification, and large oysters eliminated DA at significantly lower rates than smaller individuals (Fig. 3a), the inverse relationship described between DA concentration and body weight can only be explained by higher DA uptake rates of small oysters. In suspension-feeding bivalves, uptake of organic matter (OM) from phytoplankton cells is regulated by several mechanisms: (a) their clearance rates (CRs), (b) particle retention efficiency on the gills, (c) the capacity for pre- and post-ingestive particle sorting and (d) absorption efficiency (AE) (Fig. 6). Neither retention efficiency and sorting (Mafra et al., 2009b), nor AE (Kesarcodi-Watson et al., 2001; Mondal, 2006) are expected to vary with oyster body size. Although the size of the gills and labial palps involved in particle sorting increases with increasing body size, particle selection efficiency in post-metamorphic oysters is not dependent on the absolute size of the palps or gills, but rather on the relative size of the palps and gills, which is not expected to vary with body size of co-occurring oysters.

Greater toxin accumulation by smaller oysters as described herein must therefore result from higher weight-specific CRs, as observed in several bivalves (e.g. Navarro and Winter, 1982; Mondal, 2006). In mussels, faster toxin uptake by smaller individuals has been related to the relatively greater contribution of digestive gland (Novaczek et al., 1992) and thus gut content (Navarro and Winter, 1982) to the total body weight of smaller individuals. Although the viscera (including digestive gland) of small oysters made a greater contribution to the total body weight compared to larger individuals (31% vs. 22% respectively) in the present study, only 30–50% of the total DA burden was present in viscera after 48 h of toxification, contrasting with ~90% in mussels. Differences in the relative size of viscera are thus unlikely to fully explain the greater DA accumulation found in smaller oysters. The relationship between body mass and DA concentration in tissues followed a similarly weak, negative trend in *M. edulis* during the first 24 h of toxin exposure, becoming null or slightly positive thereafter (Fig. 2). Comparable inversion of the relationship between body size and DA concentration was found in *P. maximus* over 1 yr of field-sampling (Bogan et al., 2007), and assumed to result from faster toxin elimination by smaller scallops at later depuration stages. The lack of a significant relationship for mussels in the present study may be likewise related to the comparable elimination rates measured in different-sized individuals.

Domoic acid concentrations were highly variable among individual bivalves within a single size class, sometimes resembling a bi-modal distribution (Fig. 4g) that suggests the occurrence of slow-feeding and/or fast-depurating bivalves in the test population. Individual variability was not related to body size, and was greater in oysters than in mussels (mean CV = 57% vs. 30%, $n = 36$ during uptake; and 129% vs. 67%, $n = 36$ during DA elimination, respectively). This high individual variability probably explains why the relationship between body size and DA concentrations in

Fig. 6. Flow diagram of energy balance and pathways of particulate matter (toxic algal cells in this case) in a typical suspension-feeding bivalve. Lightning bolts represent possible mechanisms (1–7) preventing or reducing toxin uptake and accumulation from toxic cells. Although mechanisms 6 (Mafra et al., 2010) and 7 (this study) are present in *Pseudo-nitzschia*-fed oysters, *C. virginica*, only 4 and 5 (Mafra et al., 2009a,b) can explain their lower capacity for domoic acid accumulation relative to co-occurring mussels, *M. edulis*, since 6 and 7 also occur in the latter. The dashed feedback loop indicates possible toxin-related, motor or neurological feeding impairment, elicited upon initial cell ingestion and toxin release into the digestive tract. *Pseudofeces are only produced above a threshold cell density (C) (Mafra et al., 2009a). CR , FR , IR and Pf : clearance, filtration, ingestion and pseudofeces production rates, respectively. Modified from Malouf and Bricelj (1989).

oysters and mussels was not always significant in the present study. Such high variability has been extensively reported for bivalves (e.g. Blanco et al., 2002b; Bogan et al., 2007), and may be attributed to spatial or tidal toxicity gradients in temperature, salinity (Blanco et al., 2006) and seston quality (Guéguen et al., 2008) in field studies. Under controlled laboratory conditions, however, intrinsic bivalve mechanisms must be invoked to explain individual variability in toxin accumulation. In *C. gigas* this has been associated with their intermittent feeding pattern (i.e. periodic and non-synchronous shell closure) (Bougrier et al., 2003; Baron et al., 2006). It may also be related to genetic differences such as differential enzymatic activity, or differential prevalence of bacterial strains capable of DA degradation in the bivalve gut flora (Stewart et al., 1998; Hagström et al., 2007).

Larger bivalves exhibited slightly but consistently higher DA_{AE} values during toxification, except for oysters at 24 h (Table 1). Increased DA_{AE} with increasing body size was consistent with the slower DA elimination in larger oysters. In contrast, since elimination rates in mussels were independent of weight, increased DA_{AE} of larger mussels could be a result of more efficient absorption of OM, although no relationship between body size and AE has been found in mussels so far (e.g. Navarro and Winter, 1982). Alternatively, CR (and thus toxin uptake) of larger mussels, calculated from juveniles using an allometric equation with exponent = 0.616 (Bayne and Newell, 1983), may have been underestimated.

4.3. Anatomical distribution of domoic acid

Domoic acid was not evenly distributed among bivalve tissues during both uptake and elimination phases. After 6 h of exposure to toxic cells, the viscera contained 70–80% of the total DA burden in oysters and 80–90% in mussels (Fig. 5). Considering the relatively small contribution of these tissues to the total body wet weight (25–35%), this 2–3-fold toxin accumulation factor confirms that the viscera are the primary site for DA uptake in both bivalves. Similarly, during toxification, the viscera may account for 94–99% of the DA burden in *P. maximus* (Blanco et al., 2002a; Campbell et al., 2003), 93% in *M. edulis* (Grimmelt et al., 1990) and 70% in *C. virginica* (Roelke et al., 1993), as well as 80–90% of the PST content in several bivalves (reviewed by Bricelj and Shumway, 1998) and >99% of the DST burden in *M. galloprovincialis* (Blanco et al., 2007).

The contribution of viscera to total DA toxicity remained high (>80%) throughout the toxification period in mussels, but decreased rapidly in oysters (Fig. 5). The crossover point, when toxin amounts in visceral and non-visceral tissues were equivalent, ranged from ~36 h in small oysters to ~72 h in larger individuals. The proportion of DA in viscera continued to decrease gradually in both bivalves during the depuration phase, reaching 30–40% in oysters and 65–70% in mussels after 4 d, regardless of body size. For PSTs, crossover points were reported much later in species that exhibit relatively slow toxin elimination, such as *Saxidomus gigas*.

teus (~12 d), *S. solidissima* (~32 d) and *P. magellanicus* (~90 d), and never occurred in those that detoxify more rapidly, i.e. *Mercenaria mercenaria* and *M. edulis* (Bricelj and Shumway, 1998). Examination of temporal changes in the anatomical distribution of toxins has been proposed as a valuable indicator of the timing of bloom initiation and termination (Bricelj and Shumway, 1998), and is also useful in the development of predictive toxin kinetics models (e.g. Blanco et al., 2002a; Li et al., 2005).

Elimination of DA from the viscera of both oysters and mussels (elimination rates = $0.4\text{--}1.3\text{ d}^{-1}$; $1.5\text{--}2.1\text{ d}^{-1}$, respectively) occurred faster than in non-visceral tissues ($0.2\text{--}0.6\text{ d}^{-1}$; $0.9\text{--}1.4\text{ d}^{-1}$) in the present study (Fig. 3b). Faster toxin loss in viscera relative to other tissues has been previously reported for DA-contaminated *C. gigas* [Jones et al. (1995), but see Guéguen et al. (2008)] and PST-contaminated *M. arenaria* (Bricelj and Cembella, 1995), likely due to a combination of egestion (i.e. direct loss in feces) and rapid toxin transfer to other tissues. In *C. gigas*, exchange of DA between tissues was supported by continuously increasing toxin concentrations in non-visceral tissues during a period of persistent shell closure (Jones et al., 1995), which was likely caused by exposure to excessively high cell densities and prevented further DA intake.

In the present study, DA transfer to other tissues was the main factor responsible for the faster toxin elimination in the viscera of small oysters and medium mussels (transfer rate = 0.35 and 0.16 d^{-1} , respectively), as concluded from the modeling work. The addition of a term to account for DA transfer from viscera to non-visceral tissues, however, provided only slightly improved model fits compared to a simpler (no transfer) exponential decay model. Nevertheless, episodes of simultaneous decrease in visceral DA content and increase in that of non-visceral tissues, which were especially noticeable in small- and medium-sized oysters during early depuration (Fig. 4b and c), provide strong evidence for toxin exchange between tissues of this bivalve. Additionally, the calculated transfer rates may be underestimated in the present study, since they only reflect the toxin exchanged during depuration. A large proportion of the DA transfer may have already occurred during the toxification phase, especially in oysters (Fig. 5). Whether DA exchange was important in mussels is not clear. Exchange of DA and DSTs between tissues was found to be negligible in *P. maximus* (Blanco et al., 2002a) and *M. galloprovincialis* (Blanco et al., 2007) respectively, whereas considerable transfer of PSTs from the viscera to four other tissues occurred in green mussels (*Perna viridis*) during toxification and, to a lesser extent during the depuration phase (Li et al., 2005).

Although an increase in non-visceral DA content (as a % of total toxin body burden) can be attributed to transfer from the viscera, an increase in the viscera and/or in whole bivalves (e.g. Fig. 4a and b) can only be explained by *de novo* production. This supports the hypothesis of a microbial or internal mechanism leading to DA production within the digestive tract of mussels, as suggested by Silvert and Subba Rao (1992) to explain divergences between observed field data and model predictions. Furthermore, the present results indicate that post-consumption DA production by *Pseudo-nitzschia* cells, as proposed by Stewart (2008), may actually occur in the bivalve's alimentary tract during early depuration. This finding may need to be considered in the design of depuration experiments and the development of predictive DA kinetic models, since it violates a primary assumption of most models (i.e. exponential decay of toxin over time). If confirmed and quantified, post-consumption production could be added to the initial DA content to correct for possible underestimation of the toxin elimination rate, an essential parameter to accurately predict the time period during which bivalves will remain unsafe for consumers following toxic outbreaks.

Small, residual DA amounts were detected during late depuration (7 d) in both oysters and mussels, suggesting that a second,

slower-detoxifying toxin compartment is present. The anatomical localization of this residual DA pool ($\leq 0.25\text{ }\mu\text{g g}^{-1}$), however, differed between the two bivalves: non-visceral tissues in mussels and viscera in oysters. Likewise, DA is retained for comparatively longer periods in the digestive glands of *S. patula* (Horner et al., 1993) and *P. maximus* (Blanco et al., 2002a), and in non-visceral tissues of *P. magellanicus* (Douglas et al., 1997). It has been suggested that slow-detoxifying, residual DA pools are transported into intracellular compartments following uptake across the gastrointestinal walls (Novaczek et al., 1991). Intracellular DA was described as mostly composed of a soluble, cytosolic form that undergoes rapid excretion via kidney and epithelial tissues, and a minor (<20%) proportion of potentially insoluble, longer-lasting DA pool, which is apparently saturated at relatively low DA concentrations [$\sim 6\text{ ng g}^{-1}$ (Novaczek et al., 1991)].

Despite indications of a very small second DA compartment in *M. edulis* and *C. virginica* in the present study, a simple exponential decay (i.e. one-compartment) model is likely sufficient to quantitatively describe DA elimination by these two fast-detoxifying bivalves, since a two-compartment model resulted in comparable fits, but a higher degree of uncertainty. The only previous attempt to fit a two-compartment DA elimination model, conducted by Blanco et al. (2002b) using *M. galloprovincialis*, led to similar conclusions. Bi-phasic DA elimination, however, has been strongly suggested for *S. patula* (Gilgan et al., 1990), *P. magellanicus* (Horner et al., 1993) and *V. modiolus* (Douglas et al., 1997), and should be examined by means of modeling in these and other bivalve species characterized by relatively slow depuration.

5. Conclusions

Oysters attained significantly lower weight-specific DA concentrations from toxic *P. multiseriis* cells than mussels of a comparable size. In addition, smaller oysters accumulated DA to a greater extent than larger ones due to their comparatively higher toxin uptake rates. The relationship between body size and DA concentration was less clear in mussels and, as a result, differences in weight-specific DA concentrations attained by these two bivalves were more pronounced for larger, market-sized individuals. Large oysters (SH > 55 mm) never accumulated DA concentrations exceeding the regulatory limit. Domoic acid elimination was faster in the viscera of both bivalves probably due to transfer of toxins to non-visceral tissues, which was more evident in oysters. Small oysters eliminated DA at faster rates than larger ones, but elimination rates were always lower than those of mussels of a comparable size. Therefore, faster DA elimination in *C. virginica* can be ruled out as a contributing factor explaining the low capacity of this species to accumulate DA from toxic *Pseudo-nitzschia* blooms, relative to that of co-occurring *M. edulis*. Overall, results of this study suggest that species-specific management of market-sized, DA-contaminated, co-occurring *C. virginica* and *M. edulis* stocks may be a viable practice during toxic outbreaks.

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