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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 (1.4–1.6 d^{-1}) than oysters (0.25–0.88 d^{-1}) of a comparable size. Small oysters attained markedly higher weight-specific DA concentrations (maximum = 78.6 $\mu g\, g^{-1}$) than large, market-sized individuals ($\leq$13 $\mu g\, g^{-1}$), but no clear relationship was found between body size and DA concentration in mussels (maximum = 460 $\mu g\, g^{-1}$). 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 $\mu g\, g^{-1}$) 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.

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 $\mu g\, g^{-1}$ 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 $\mu g\,g^{-1}$, whereas mussels (*Mytilus edulis*) accumulated up to 200 μg 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 reallocated, 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. multiseries* 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, *Placopecten 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; Moroño 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 officianalis* (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. multiseries* 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 multiseries*, CLN-50, was provided by S.S. Bates (Fisheries and Oceans Canada, Moncton, NB) and