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# Feeding mechanics as the basis for differential uptake of the neurotoxin domoic acid by oysters, *Crassostrea virginica*, and mussels, *Mytilus edulis*

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ABSTRACT

The neurotoxin domoic acid (DA), produced by diatoms *Pseudo-nitzschia* spp., is transferred to humans via consumption of contaminated bivalves. This study examines feeding mechanisms, namely reduced filtration, pre-ingestive rejection and poor absorption, that might explain the comparatively low DA levels commonly found in oysters during toxic *Pseudo-nitzschia* blooms. Clearance rate (CR), absorption efficiency (AE) of organic matter and selective rejection in pseudofeces of oysters (*Crassostrea virginica*) and mussels (*Mytilus edulis*) were investigated in relation to the DA levels accumulated during 2-wk, simultaneous exposure to toxic *Pseudo-nitzschia multiseries*. Effects of temperature and *P. multiseries* cell size were also tested to identify conditions, if any, under which oysters can accumulate unsafe DA levels. Oysters accumulated 3.0–7.5× less DA than mussels from a short-celled *P. multiseries* clone (length = 24 μm) at 12 °C. This was related to the 7.4–8.5× lower CRs determined for oysters relative to mussels at this temperature. Exposure to a longer-celled *P. multiseries* clone (81 μm) resulted in up to 70× lower toxin levels in oysters compared to mussels, which was attributed to differential feeding selectivity. Mussels were unable to discriminate between long- and short-celled *P. multiseries* clones from a mixed suspension, whereas oysters were previously shown to preferentially reject long cells (>70 μm) in pseudofeces. Both bivalves selectively rejected *P. multiseries* cells from mixed suspensions containing a flagellate but not another diatom. AE of organics from *P. multiseries* cells by oysters and mussels was comparably low (42 and 39%, respectively) and thus unlikely to explain their differential DA accumulation. CR and DA uptake by oysters were negligible at ≤4 °C but increased with increasing temperature up to 18 °C, although mean DA levels barely attained the regulatory limit (20 μg g$^{-1}$) when oysters were exposed to long *P. multiseries* cells. The maximum DA levels accumulated by mussels (320 μg g$^{-1}$) and oysters (44 μg g$^{-1}$) exposed to short *P. multiseries* cells in our study support the inter-specific differences in toxicity during *Pseudo-nitzschia* blooms, which are expected to be exacerbated at lower temperatures and when long cells or chains are dominant. Additionally, when alternate, non-diatom phytoplankton species are present, both bivalves can feed selectively and thus accumulate much lower DA levels than those predicted from their overall CRs. Our results provide support for the evaluation of species-specific management of DA-contaminated shellfish and need to be considered in modeling DA toxin kinetics of the two target species.



## 1. Introduction

The neurotoxin domoic acid (DA) is mainly produced by diatoms of the genus *Pseudo-nitzschia* (reviewed in Trainer et al., 2008), especially when environmental stress conditions limit cellular growth and there is free energy available in the form of ATP (see Bates, 1998). Toxic *Pseudo-nitzschia multiseries* blooms were first linked to a human intoxication outbreak in eastern Canada in 1987 (Wright et al., 1989), and subsequently to massive marine faunal mortalities (e.g. Scholin et al., 2000), resulting in economic losses to fisheries and aquaculture. During these events, suspension-feeding bivalve molluscs are able to accumulate high DA levels and suffer only minor (Jones et al., 1995; Liu et al., 2008) or no adverse effects.

Bivalves exhibit marked (up to 100-fold) inter-specific differences in their capacity to accumulate phycotoxins such as diarrhetic shellfish toxins (DSTs) (Reizopoulou et al., 2008) and paralytic shellfish toxins (PSTs) (reviewed in Bricelj and Shumway, 1998). Similarly, oysters consistently accumulate lower DA levels than other co-occurring bivalve species during *Pseudo-nitzschia* blooms

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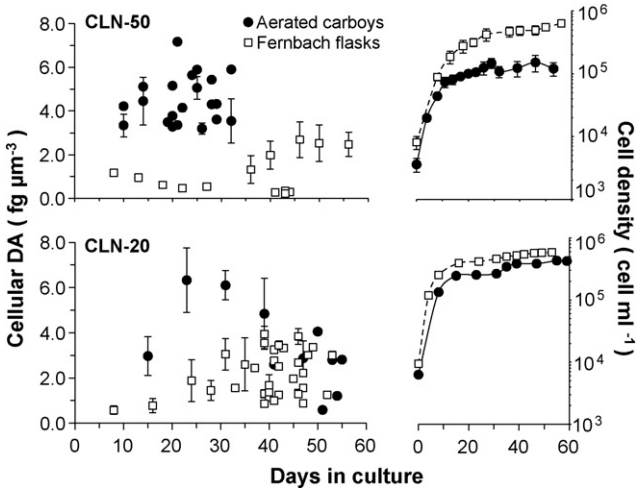


**Fig. 1.** Concentrations of intracellular domoic acid (DA; left panels) and cell density (right panels; mean ± standard error, SE; $n = 2$) in batch cultures of two *Pseudo-nitzschia multiseries* clones: CLN-50 (mean cell length = 64–100 μm) and CLN-20 (25–52 μm). Toxin samples were collected over the growth cycle from successive cultures (over a period of 1–1.5 yr), and cell densities are shown for representative cultures of either manually stirred Fernbach flasks (open squares) or aerated carboys (solid circles).

(reviewed in Mafra et al., 2009a), and rarely attain the regulatory limit (RL) of 20 μg $g^{-1}$ wet tissue weight. When DA concentrations in any bivalve species reach this RL, however, harvesting closures are often issued for all species in the affected area. This occurred during an unusual early spring bloom of *Pseudo-nitzschia seriata* in eastern Canada, in April 2002, when mussels accumulated up to 200 μg DA $g^{-1}$, whereas the maximum DA level in oysters was only 0.9 μg $g^{-1}$ (Canadian Food Inspection Agency, CFIA, data). At that time, commercial oyster growers requested a study that could provide a scientific basis for the evaluation and potential implementation of species-specific management of DA-contaminated stocks. It was also suggested that oysters might not pose a risk of DA contamination at low temperatures typical of the 2002 outbreak. Species-specific management practices, however, must be preceded by comprehensive understanding of the factors controlling the uptake and loss of toxins by the target species.

In bivalves, contamination by DA is expected to occur primarily via ingestion of toxic diatoms (i.e. the particulate fraction), as poor DA incorporation from the dissolved fraction was shown by Novaczek et al. (1991) in mussels—up to 8.6× lower toxin retention than that from DA-containing particles (3–5 μm liposomes). Therefore, DA accumulation in bivalves is expected to be greatly affected by their clearance rates (CRs; i.e. the volume of water cleared of particles per unit time), as well as by their ability to capture toxic microalgae from suspension, their capacity to select particles prior to ingestion, and the efficiency of organic matter absorption and toxin elimination processes. The pathways and fate of suspended particulates (and thus toxins associated with them) following capture by suspension-feeding bivalves have been outlined by Bayne and Newell (1983) (Figs. 1 and 5).

Oysters and mussels can retain particles >4–5 μm on the gills with ~100% efficiency (Haven and Morales-Alamo, 1970; Palmer and Williams, 1980; Riisgård et al., 1996); thus these two bivalves are not expected to exhibit differential capture of *Pseudo-nitzschia multiseries* cells [size = 4.3–5.2 μm width × 25–81 μm length (present study); maximum = 5.3 μm × 169 μm (Villac, 1996)]. However, the pallial organs of bivalves are able to efficiently sort captured particles prior to ingestion, based on their chemical and/or physical characteristics (e.g. Ward and Targett, 1989; Cognie et al., 2003). Typically, nutritious organic particles are transported to the mouth for ingestion while undesirable particles are rejected in pseudofeces, providing an efficient mechanism for enrichment of the food supply (Ward and Shumway, 2004). Pseudofeces production, along with changes in CR, allows bivalves to regulate the quality and amount of ingested food and thus to maintain a positive energy balance, even from nutritionally poor and/or excessively concentrated suspensions (Bayne and Newell, 1983; Bacon et al., 1998). Since diatoms are often preferentially rejected in pseudofeces of several bivalves (e.g. Bougrier et al., 1997), DA uptake may be limited by pre-ingestive selection against *Pseudo-nitzschia* cells.

Following ingestion, particles may be re-sorted in the alimentary tract and either incorporated in the digestive glands for intracellular digestion, or directed to the intestine for elimination in feces (reviewed in Ward and Shumway, 2004). The bivalve's absorption efficiency (AE), or its capacity to absorb nutrients from ingested particulate organic matter (POM), is thus regulated by the gut retention time and the presence and activity of appropriate digestive enzymes, and may vary with food composition and concentration (Ibarrola et al., 2000). Therefore, DA accumulation in bivalves may also be affected by their AE of ingested, toxic *Pseudo-nitzschia* cells. Ultimately, DA accumulation in the tissues of bivalves will be a balance between the processes governing toxin intake (i.e. filtration, ingestion and digestion of *Pseudo-nitzschia* cells) and the existence of efficient metabolic detoxification mechanisms.

Two prior studies (Mafra et al., 2009a,b) determined important aspects of feeding on *P. multiseries* by the eastern oyster (*Crassostrea virginica*), namely (a) the reduced CR on unialgal suspensions of this diatom, which was independent of *P. multiseries* cell size, growth stage, cell density and DA toxicity over the range tested, and (b) the selective rejection of *P. multiseries* cells in pseudofeces from mixed suspensions with flagellates, which was enhanced for longer (>70 μm) *P. multiseries* cells. Here, we compare the DA accumulation rates from *P. multiseries* cells by *C. virginica* and another co-occurring, commercially important bivalve species, the blue mussel *Mytilus edulis*, under controlled experimental conditions. This study also investigates pre- and post-ingestive feeding processes expected to affect the differential DA uptake from *P. multiseries* cells in unialgal and mixed suspensions. Pre-ingestive particle selection of mussels, as well as the CR and AE of oysters and mussels exposed to the same experimental conditions were quantified and related to the DA levels accumulated by both bivalves during long-term (14 d) toxification experiments. The effects of temperature and *P. multiseries* cell size, known to be highly variable in *Pseudo-nitzschia* spp., were also evaluated to determine whether changes in these variables could lead to accumulation of DA concentrations exceeding the RL in *C. virginica*. As indicated above, results of the present study, in addition to those obtained by Mafra et al. (2009a,b), are of special significance for the establishment of species-specific management of DA-contaminated bivalve stocks as a tool to mitigate the economic losses caused by toxigenic *Pseudo-nitzschia* spp. blooms. Comparative accumulation of DA by oysters and mussels of varying body sizes is the subject of a follow-up study.

## 2. Materials and methods

### 2.1. Algal cultures

Five toxic *Pseudo-nitzschia multiseries* clones, CLN-20, CLN-46, CLN-50, CLNN-16, and CLNN-21, were obtained as offspring from the mating of strains isolated from eastern Canada, following the methods of Davidovich and Bates (1998). Other non-toxic algal species were acquired from CCMP (Center for the Culture of Marine Phytoplankton, ME, USA): the diatom *Thalassiosira weissflogii* (Actin clone, CCMP1336) and the flagellates *Isochrysis galbana* (T-Iso clone, CCMP1324), *Pavlova pinguis* (CCMP609)