Domoic acid depuration by intertidal bivalves fed on toxin-producing *Pseudo-nitzschia multiseries*

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ABSTRACT

Domoic acid (DA), a neurotoxin produced by certain species within the diatom genus *Pseudo-nitzschia*, has caused numerous persistent harvest closures for razor clam *Siliqua patula* along the outer coast of Washington State (USA) over the last three decades. In comparison, bivalve harvest closures for DA have only occurred three times in Washington's largest inland estuary, Puget Sound, which has a variety of bivalve species excluding razor clam. While differing bloom dynamics in the two locations are responsible for much of the disparity in shellfish harvest closures, species-specific differences in DA depuration may affect the duration of harvest closures in the two regions. Toxin-producing *Pseudo-nitzschia multiseries* were fed to four species of bivalves, followed by measurement of tissue DA content over time to estimate depuration rate. Experimental species include razor clam and three species of intertidal Puget Sound bivalves: soft-shell clam *Mya arenaria*, purple varnish clam *Nuttallia obscurata* and Manila clam *Ruditapes philippinarum*. Using an exponential decay model, DA depuration rates were estimated as: 0.02 day−1 ± 0.08 for razor clam, 0.10 day−1 ± 0.07 for purple varnish clam, 0.37 day−1 ± 0.03 for soft-shell clam, and 0.44 day−1 ± 0.02 for Manila clam. Puget Sound species depurated DA between five and 22 times as fast as outer coast razor clam. Within Puget Sound species, slow DA depuration rates in purple varnish clam indicate that it may be a good sentinel organism for assessing beach-wide maximum DA concentrations in Puget Sound bivalves.

When blooms of the toxin-producing diatom *Pseudo-nitzschia* are advected over shellfish beds, suspension-feeding bivalves ingest the phytoplankton and can accumulate domoic acid (DA) toxin in their tissues. Clam toxicity during and after a bloom is determined in part by bivalve depuration of DA. DA depuration rates can vary widely between species: razor clams (*Siliqua patula*) require many months to depurate DA from their tissues (Adams et al., 2000; Trainer and Bill, 2004; Wekell et al., 1994a), while blue mussels (*Mytilus edulis*) are capable of depurating DA over hours to days (Krogstad et al., 2009; Novaczeck et al., 1992).

In Washington State, USA, DA accumulation in edible shellfish is a recurring health concern on the outer, oceanic coast where razor clam is the primary suspension-feeding bivalve on sandy beaches. DA concentrations exceeding the US Food and Drug Administration (FDA) regulatory limit of 20 mg·kg−1 have been observed in nearly half of all outer coast razor clam harvest seasons over the last three decades (WDFW, 2019), owing to the long depuration time of razor clams (Drum et al., 1993; Horner et al., 1993; Wekell et al., 1994b) combined with several long-lasting coastal *Pseudo-nitzschia* blooms (Du et al., 2016; Horner et al., 1997; Trainer and Suddleson, 2005). Toxin-producing species of *Pseudo-nitzschia* are present in the inland waters of Puget Sound (Bill et al., 2006; Stehr et al., 2002; Trainer et al., 1998), but only three harvest closures have occurred in Puget Sound (Sept 2003, Sept 2005, Oct 2005) (Bill et al., 2006; Trainer et al., 2007). While *Pseudo-nitzschia* bloom frequencies differ in the two locations, with the outer coast experiencing more frequent (e.g., Adams et al., 2000, 2006; Hickey et al., 2013; McCabe et al., 2016; Trainer et al., 2009) and potentially more toxic (Baugh et al., 2006) blooms compared to Puget Sound (Bill et al., 2006; Trainer et al., 2007), the bivalve communities also differ: the outer coast is dominated by razor clams, whereas Puget Sound’s mixed sand/gravel beaches have a wide variety of bivalves excluding razor clams (Kozloff, 1983).

Abbreviations: DA, domoic acid.
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The contrasting history of DA closures on the Washington outer coast and in Puget Sound raises the question: How do bivalve communities in these locations differ in their ability to depurate DA? We present experimentally-derived estimates of DA depuration rate for three common Puget Sound species: Manila clam (Ruditapes philippinarum), purple varnish clam (Nuttalia obscura), and soft-shell clam (Mya arenaria); and for razor clam from the outer Washington coast.

Experiments were conducted at Friday Harbor Laboratories on San Juan Island, Washington, between March and May 2009. Bivalves received a diet of DA-producing Pseudo-nitzschia multiseries for three days, then purged DA from their tissues in unfiltered seawater prior to lethal sampling at specific time intervals. A toxin-producing strain of P. multiseries, CLNN-17, was used as a source of DA. CLNN-17 is an F2 generation from Stephen Bates and Claude Leger; isolates crossed to obtain the F1 generation were CL-143 from Little Harbour, Nova Scotia, and for razor clam from the outer Washington coast.

Phytoplankton growth medium (f/2 + Si) was prepared by filtering natural seawater through a 10-μm clothe filter, combining with sodium metasilicate: Na₂SiO₃ (13 mg per liter of seawater), autoclaving in glass flasks, then adding 3/2 solution (Fritz Industries, Inc) to the cooled medium. Growth medium was decanted into twelve clean, 18-L polypropylene carboys and inoculated with pure Pseudo-nitzschia multiseries culture. Carboys were kept at 13 °C under bright 24-hr fluorescent light with constant magnetic stirring and moderate aeration. Carboys of cultured P. multiseries were fed to bivalves after they had been in stationary growth phase for at least three days, to increase the probability of high cellular DA. Prior to each feeding, 10 ml of culture were filtered onto 0.45-μm HA filters (Millipore) and stored at ~20 °C. Particulate DA was extracted from filters into 10 ml of ultrapure distilled water (Milli-Q; MilliPore), and samples were analyzed for DA using ELISA direct competitive enzyme-linked immunosorbent assay (ELISA; Biosense Laboratories) following the Biosense protocol (Kleivdal et al., 2007). DA per P. multiseries cell was 9.3–71.3 pg · cell⁻¹.

For each bivalve species, 27–34 individuals of similar size were collected from Whidbey and Orcas Islands in Puget Sound, and from the outer Washington coast (Table 1). Puget Sound bivalves were collected 21 days prior to the experiment. Razor clams were collected three days prior to the experiment, to prevent degeneration that occurs within weeks in the laboratory. Bivalves were placed into containers of sand by species and held in flow-through aquaria of natural (unfiltered) seawater. At the time of shellfish collection, no DA was present in the aquaria.

Table 1

| Common Name                     | Latin Name                           | Shell Length (mm) | Collection Location          | Maximum bivalve DA (mg kg⁻¹) | Exponential DA Depuration Rate (day⁻¹ ± sd) | Residual Standard Error of the linear model |
|--------------------------------|--------------------------------------|-------------------|-----------------------------|-------------------------------|--------------------------------------------|---------------------------------------------|
| Puget Sound Species            |                                      |                   |                             |                               |                                            |                                             |
| Manila clam                    | Ruditapes philippinarum              | 50 (±2)           | Cultus Bay, Whidbey Island  | 35.9                          | 0.44 ± 0.02                             | 0.53                                         |
| purple varnish clam            | Nuttalia obscura                     | 45 (±1)           | Crescent Beach, Orcas Island | 27.5                          | 0.10 ± 0.07                             | 1.52                                         |
| soft-shell clam                | Mya arenaria                         | 77 (±3)           | Cultus Bay, Whidbey Island  | 4.3                           | 0.37 ± 0.03                             | 0.79                                         |
| Washington Outer Coast Species | Silula pataula                       | 110 (±4)          | Cephalis Beach, Washington outer coast | 3.3                           | 0.02 ± 0.08                             | 1.11                                         |

DA depuration rates were calculated for each species using a one-compartment exponential decay model, $DA_t = DA_0 e^{-rt}$, where $DA_t$ is DA concentration after $t$ days, $DA_0$ represents DA concentration at the end of the feeding phase, $r$ is the depuration rate, and $t$ is days elapsed. $DA_0$ and $r$ were estimated using linear regression after ln-transformation. A straight-line relationship of ln-transformed DA concentrations indicates that depuration rates were constant over the course of the experiment (Fig. 1). Twelve hours after the last feeding, aquaria were connected to the flow-through seawater system. All clams remained in natural seawater for 2 h to purge or digest phytoplankton on their gills, then six to seven clams per species were lethally sampled for DA analysis, representing time-point $T = 0$ days. Subsequently, two to seven clams were lethally sampled on days 1, 2, 4, 8, and 15 for Puget Sound species; and on days 2, 4, and 9 for outer coast razor clam. Razor clams were sampled at fewer time points because of their slow DA depuration rate (Adams et al., 2000; Trainer and Bill, 2004; Wekell et al., 1994a), and their propensity to degenerate in the laboratory. At each time point, selected bivalves were placed onto clean towels for 30 min to drain excess seawater, then bagged and frozen for up to 14 days. Each bivalve was analyzed individually for DA content.

In preparation for DA analysis, frozen clams were thawed and dissected to remove soft tissues and hemolymph, which were blended together to a fine homogenate. Shellfish homogenate was analyzed using ELISA (Biosense Laboratories) test kits following the Biosense methodology (Kleivdal et al., 2007). DA results are the average of two replicates from the ELISA analysis, where the duplicate CV < 0.3.

The rate of DA depuration from shellfish tissue was calculated for each species using a one-compartment exponential decay model, $DA_t = DA_0 e^{-rt}$, where $DA_t$ is DA concentration after $t$ days, $DA_0$ represents DA concentration at the end of the feeding phase, $r$ is the depuration rate, and $t$ is days elapsed. $DA_0$ and $r$ were estimated using linear regression after ln-transformation. A straight-line relationship of ln-transformed DA concentrations indicates that depuration rates were constant over the course of the experiment (Fig. 1).
Aim:

To estimate the daily depuration rates of domoic acid (DA) in Manila clam (Veneridae sp.) and soft-shell clam (Mya arenaria) from Puget Sound. The study compares these rates to those of Mediterranean mussel (Mytilus galloprovincialis) and Atlantic oyster (Crassostrea virginica).

Methods:

- The experiment was conducted in a laboratory setting.
- DA depuration rates were estimated using a log-transformed ln (mg/kg) approach.
- Species-specific DA depuration models were developed based on individual clam data.
- Variance in DA concentrations was analyzed to understand inter-individual variability.

Results:

- The estimated DA depuration rate for Manila clam was 0.44 ± 0.02 day⁻¹.
- Soft-shell clam had a depuration rate of 0.37 ± 0.03 day⁻¹.
- Razor clam had a depuration rate of 0.02 ± 0.08 day⁻¹.
- Purple varnish clam had a depuration rate of 0.10 ± 0.07 day⁻¹.

Discussion:

- All species tested depurated DA at a slower rate than blue mussel (Mytilus edulis).
- Purple varnish clam and Manila clam attained tissue DA concentrations above the 20 mg kg⁻¹ harvest limit, while razor clam and soft-shell clam accumulated lower DA concentrations.
- Low DA in razor clam and soft-shell clam was at least partly due to rejection of filtered cells as pseudofeces.
- The stacking configuration of Pseudo-nitzschia multiseries cells in soft-shell clam and razor clam was unusual and not observed in natural assemblages.

Conclusion:

- This study provided estimates of DA depuration in four species from two intertidal bivalve communities.
- The depuration rate of razor clam is responsible for the long duration of toxicity after a Pseudo-nitzschia bloom diminishes.
- Puget Sound species depurated DA faster than razor clam, resulting in a more rapid decline in toxicity.

Fig. 1. Ln-transformed DA concentrations for individual clams at various time points. For each species, the exponential daily DA depuration rate is the slope of the regression line (r ± standard deviation). On the x-axis, 0 corresponds to the beginning of depuration, when clams were no longer being fed toxin-producing Pseudo-nitzschia multiseries.
Declaration of competing interest

None.

CRediT authorship contribution statement

Eva Dusek Jennings: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Visualization, Funding acquisition. Micaela S. Parker: Conceptualization, Methodology, Writing - review & editing. Charles A. Simonstad: Conceptualization, Writing - review & editing, Funding acquisition.

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