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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; Novaczek 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 (*Nuttallia obscurata*), 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 CL-147 from Caribou Harbour, New Brunswick.

Phytoplankton growth medium (f/2 + Si) was prepared by filtering natural seawater through a 10-μm cloth filter, combining with sodium metasilicate: Na_2SiO_3 (13 mg per liter of seawater), autoclaving in glass flasks, then adding f/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 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 a composite sample of three purple varnish clams, in razor clams from Copalis Beach analyzed by Washington State Department of Health (WSDOH), or in shellfish collected throughout Puget Sound by WSDOH.

For the experiment, bivalves were separated by species and placed into aquaria of natural, unfiltered seawater with air-stone bubblers and submersible water pumps for water circulation. Water temperatures were 8–10 °C. Aquarium volumes ranged from 26 to 84 L, with aquarium size proportional to feeding rate (Dusek Jennings, unpublished data). Clams were fed twice daily over a three-day period, for a total of six feedings. Although clams were in natural seawater augmented with *Pseudo-nitzschia multiseries*, *P. multiseries* contributed the vast majority of the chlorophyll *a* (94% ± 4%) in the aquaria. *P. multiseries* concentration at the beginning of each feeding was 700–3300 cells · ml^{-1}, which is within the range observed during *Pseudo-nitzschia* spp. blooms in Puget Sound (Bill et al., 2006; Trainer et al., 2007) and on the Washington outer coast (e.g., Adams et al., 2000, 2006; Trainer and Suddleson, 2005). Aquaria were cleaned daily by flushing with unfiltered seawater and wiping glass walls with a sponge. Pseudofeces were observed in aquaria of soft-shell clam and razor clam.

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, $DA_t = DA_0 \cdot e^{-rt}$, 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).

DA depuration rates were 0.37·day^{-1} ±0.03 in soft-shell clam, 0.10·day^{-1} ±0.07 in purple varnish clam, 0.44·day^{-1} ±0.02 in Manila clam, and 0.02·day^{-1} ±0.08 in razor clam (Table 1, Fig. 1). Depuration rates for soft-shell clam and razor clam corroborate evidence from the field: in October 1988, soft-shell clam DA concentrations in New Brunswick, Canada declined from 37 mg · kg^{-1} to 3 mg · kg^{-1} over six days (Gilgan et al., 1990), reflecting a depuration rate around 0.40·day^{-1}. In Twin Harbors, Washington, razor clam DA concentrations on December 3, 1991 were 147 mg · kg^{-1}, declining to 70 mg · kg^{-1} in about 20 days (Wekell et al., 1994b). Surf zone samples from November 1991 showed only low densities of unhealthy or dead *Pseudo-nitzschia* cells (Horner and Postel, 1993). Assuming negligible DA accumulation, the depuration rate was about 0.04·day^{-1}, similar to our

Table 1
Experimental parameters, including: clam species, shell length, collection location, maximum DA concentration attained by bivalves, and exponential depuration rate. An estimate of inter-individual variability in DA concentration is provided for each species as the residual standard error (RSE) of the species-specific linear model.

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	*Nuttallia obscurata*	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						
razor clam	*Siliqua patula*	110 (±4)	Copalis Beach, Washington outer coast	3.3	0.02 ± 0.08	1.11

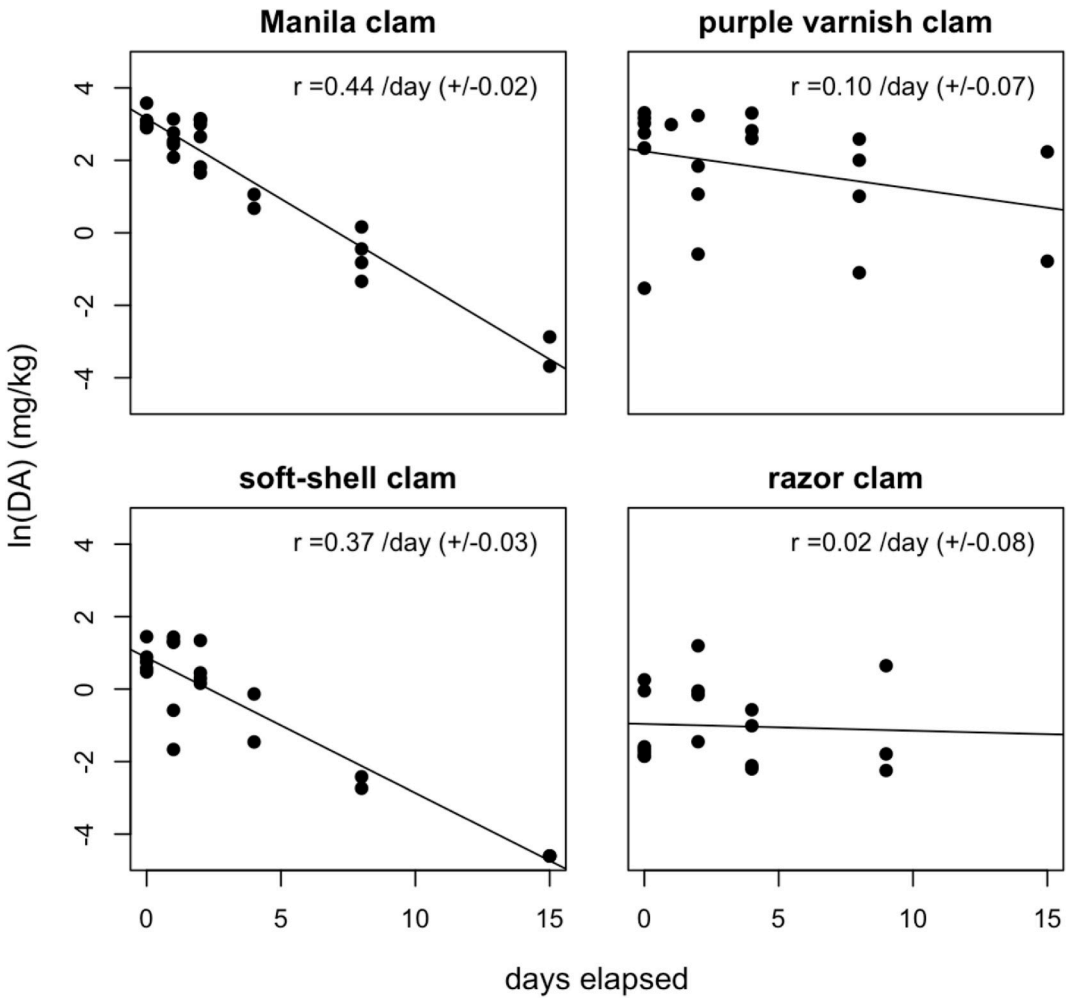

Fig. 1. *Ln*-transformed DA concentrations for individual clams at various time points. For each species, the exponential daily DA depuration rate is the slope of the regression line ($r \pm$ standard deviation). On the x-axis, 0 corresponds to the beginning of depuration, when clams were no longer being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

estimate of 0.02·day^{-1} ±0.08. Our DA depuration rate estimates in Manila clam and soft-shell clam compare to those of Mediterranean mussel *Mytilus galloprovincialis* (0.40·day^{-1}: Blanco et al., 2002) and Atlantic oyster *Crassostrea virginica* (0.25–0.88·day^{-1}: Mafra et al., 2010). All bivalve species tested in this study depurate DA slower than blue mussel *Mytilus edulis* (2.0·day^{-1}: Krogstad et al., 2009; 1.4–1.6·day^{-1}: Mafra et al., 2010).

Purple varnish clam and Manila clam both attained tissue DA concentrations above the 20 mg · kg^{-1} harvest limit, while razor clam and soft-shell clam only accumulated low DA concentrations (<5 mg · kg^{-1}; Table 1). Low DA in razor clam and soft-shell clam was at least partly due to rejection of filtered cells as pseudofeces. In our experiment, many of the cultured *Pseudo-nitzschia multiseries* cells exhibited an unusual stacking configuration of three to five cells adjoined at the girdle. This stacking configuration is not observed in natural assemblages, but can occur when *Pseudo-nitzschia* are maintained in asexually reproducing monocultures for long periods. These larger food particles may have been preferentially rejected by soft-shell clam and razor clam. Indeed, Atlantic oysters preferentially reject larger *P. multiseries* clones relative to smaller ones, and accumulate lower DA by producing pseudofeces (Mafra et al., 2009a, 2009b). Particle ingestion and rejection are species-specific: Manila clam and purple varnish clam were fed on the same cultures but did not produce observable pseudofeces.

Within each species-specific DA depuration model, variance of the *ln*-transformed DA concentrations was fairly constant across time, thus residual standard error (RSE) provides an estimate of inter-individual variability in DA content (Table 1). RSE was highest in purple varnish clam and lowest in Manila clam. High inter-individual variability within a species can result from individual differences in feeding rate, toxin uptake, or depuration, and has also been observed in razor clam (Wekell et al., 2002), blue mussel (Gilgan et al., 1990), Mediterranean mussel (Blanco et al., 2002), and sea scallop *Placopecten magellanicus* (Douglas et al., 1997).

This paper has presented estimates of DA depuration in four species from two Pacific Northwest, USA, intertidal bivalve communities. Razor clam is the predominant bivalve on outer Washington coast sandy beaches, and its slow depuration rate is likely responsible for the long duration of toxicity even after a *Pseudo-nitzschia* bloom diminishes. In contrast, Puget Sound species depurated DA from five to 22 times as fast as razor clam, resulting in a more rapid decline in toxicity. Of the Puget Sound species we tested, purple varnish clam had the slowest depuration rate, with Manila clam and soft-shell clam depurating DA about four times as quickly. This suggests that purple varnish clam may be a good lagging indicator of shellfish toxicity at the end of a bloom, although proper characterization of the high inter-individual variability requires sampling of multiple individuals, as is the practice with razor clam (Wekell et al., 2002).

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. Simenstad:** Conceptualization, Writing - review & editing, Funding acquisition.

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References

Adams, N.G., Lesoing, M., Trainer, V.L., 2000. Environmental conditions associated with domoic acid in razor clams on the Washington coast. J. Shellfish Res. 19, 1007–1015.

Adams, N.G., MacFadyen, A., Hickey, B.M., Trainer, V.L., 2006. The nearshore advection of a toxigenic *Pseudo-nitzschia* bloom and subsequent domoic acid contamination of intertidal bivalves. Afr. J. Mar. Sci. 28, 271–276.

Baugh, K.A., Bush, J.M., Bill, B.D., Lefebvre, K.A., Trainer, V.L., 2006. Estimates of specific toxicity in several *Pseudo-nitzschia* species from the Washington coast, based on culture and field studies. Afr. J. Mar. Sci. 28, 403–407. https://doi.org/10.2989/18142320609504187.

Bill, B.D., Cox, F.H., Horner, R.A., Borchert, J.A., Trainer, V.L., 2006. The first closure of shellfish harvesting due to domoic acid in Puget Sound, Washington, USA. Afr. J. Mar. Sci. 28, 435–440. https://doi.org/10.2989/18142320609504193.

Blanco, J., Bermúdez De La Puente, M., Arévalo, F., Salgado, C., Moroño, Á., 2002. Depuration of mussels (*Mytilus galloprovincialis*) contaminated with domoic acid. Aquat. Living Resour. 15, 53–60. https://doi.org/10.1016/S0990-7440(01)01139-1.

Douglas, D.J., Kenchington, E.R., Bird, C.J., Pocklington, R., Bradford, B., Silvert, W., 1997. Accumulation of domoic acid by the sea scallop (*Placopecten magellanicus*) fed cultured cells of toxic *Pseudo-nitzschia multiseries*. Can. J. Fish. Aquat. Sci. 54, 907–913. https://doi.org/10.1139/cjfas-54-4-907.

Drum, A.S., Siebens, T.L., Crecelius, E.A., Elston, R.A., 1993. Domoic acid in the Pacific razor clam *Siliqua patula* (Dixon, 1789). J. Shellfish Res. 12, 443–450.

Du, X., Peterson, W., Fisher, J., Hunter, M., Peterson, J., 2016. Initiation and development of a toxic and persistent Pseudo-nitzschia bloom off the Oregon coast in spring/summer 2015. PloS One 11. https://doi.org/10.1371/journal.pone.0163977.

Gilgan, M.W., Burns, B.G., Landry, G.J., 1990. Distribution and magnitude of domoic acid contamination of shellfish in Atlantic Canada during 1988. In: Graneli, E., Sundstrom, B., Edler, L., Anderson, D.M. (Eds.), Toxic Marine Phytoplankton: Proceedings of the Fourth International Conference on Toxic Marine Phytoplankton. Elsevier, New York, pp. 469–474.

Hickey, B.M., Trainer, V.L., Kosro, P.M., Adams, N.G., Connolly, T.P., Kachel, N.B., Geier, S.L., 2013. A springtime source of toxic *Pseudo-nitzschia* cells on razor clam beaches in the pacific northwest. Harmful Algae 25, 1–14. https://doi.org/10.1016/j.hal.2013.01.006.

Horner, R.A., Garrison, D.L., Plumley, F.G., 1997. Harmful algal blooms and red tide problems on the U.S. West Coast. Limnol. Oceanogr. 42, 1076–1088.

Horner, R.A., Kusske, M.B., Mynihan, B.P., Skinner, R.N., Wekell, J.C., 1993. Retention of Domoic acid by Pacific razor clams, *Siliqua patula* (Dixon 1789): preliminary study. J. Shellfish Res. 12, 451–456.

Horner, R.A., Postel, J.R., 1993. Toxic diatoms in western Washington waters (U.S. West Coast). Hydrobiologia 269/270, 197–205.

Kleivdal, H., Kristiansen, S., Nilsen, M.V., Briggs, L., 2007. Single-laboratory validation of the Biosense direct competitive enzyme-linked immunosorbent assay (ELISA) for determination of domoic acid toxins in shellfish. J. AOAC Int. 90, 1000–1010.

Kozloff, E.N., 1983. Seashore Life on the Northern Pacific Coast: an Illustrated Guide to Northern California, Oregon, Washington and British Columbia. University of Washington Press, Seattle.

Krogstad, F.T.O., Griffith, W.C., Vigoren, E.M., Faustman, E.M., 2009. Re-evaluating blue mussel depuration rates in "Dynamics of the phycotoxin domoic acid: accumulation and excretion in two commercially important bivalves.". J. Appl. Phycol. 21, 745–746. https://doi.org/10.1007/s10811-009-9410-4.

Mafra, L.L., Bricelj, V.M., Fennel, K., 2010. Domoic acid uptake and elimination kinetics in oysters and mussels in relation to body size and anatomical distribution of toxin. Aquat. Toxicol. 100, 17–29. https://doi.org/10.1016/j.aquatox.2010.07.002.

Mafra, L.L., Bricelj, V.M., Ouellette, C., Léger, C., Bates, S.S., 2009a. Mechanisms contributing to low domoic acid uptake by oysters feeding on *Pseudo-nitzschia* cells. I. Filtration and pseudofeces production. Aquat. Biol. 6, 201–212. https://doi.org/10.3354/ab00121.

Mafra, L.L., Bricelj, V.M., Ward, J.E., 2009. Mechanisms contributing to low domoic acid uptake by oysters feeding on *Pseudo-nitzschia* cells. II. Selective rejection. Aquat. Biol. 6, 213–226. https://doi.org/10.3354/ab00122.

McCabe, R.M., Hickey, B.M., Kudela, R.M., Lefebvre, K.A., Adams, N.G., Bill, B.D., Gulland, F.M.D., Thomson, R.E., Cochlan, W.P., Trainer, V.L., 2016. An unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions. Geophys. Res. Lett. 43 (10), 366–376. https://doi.org/10.1002/2016GL070023. Received, 10.

Novaczek, I., Madhyastha, M.S., Ablett, R.F., Donald, A., Johnson, G., Nijjar, M.S., Sims, D.E., 1992. Depuration of domoic acid from live blue mussels (*Mytilus edulis*). Can. J. Fish. Aquat. Sci. 49, 312–318.

Stehr, C.M., Connell, L., Baugh, K.a., Bill, B.D., Adams, N.G., Trainer, V.L., 2002. Morphological, toxicological, and genetic differences among *Pseudo-nitzschia* (Bacillariophyceae) species in inland embayments and outer coastal waters of Washington State, USA. J. Phycol. 38, 55–65. https://doi.org/10.1046/j.1529-8817.2002.01106.x.

Trainer, V., Suddleson, M., 2005. Monitoring approaches for early warning of domoic acid events in Washington state. Oceanography 18, 228–237. https://doi.org/10.5670/oceanog.2005.56.

Trainer, V.L., Adams, N.G., Bill, B.D., Anulacion, B.F., Wekell, J.C., 1998. Concentration and dispersal of a *Pseudo-nitzschia* bloom in Penn Cove, Washington, USA. Nat. Toxins 6, 113–126.

Trainer, V.L., Bill, B.D., 2004. Characterization of a domoic acid binding site from Pacific razor clam. Aquat. Toxicol. 69, 125–132. https://doi.org/10.1016/j.aquatox.2004.04.012.

Trainer, V.L., Cochlan, W.P., Erickson, A., Bill, B.D., Cox, F.H., Borchert, J.A., Lefebvre, K.A., 2007. Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways. Harmful Algae 6, 449–459. https://doi.org/10.1016/j.hal.2006.12.001.

Trainer, V.L., Wells, M.L., Cochlan, W.P., Trick, C.G., Bill, B.D., Baugh, K.A., Beall, B.F., Herndon, J., Lundholm, N., 2009. An ecological study of a massive bloom of toxigenic *Pseudo-nitzschia cuspidata* off the Washington State coast. Limnol. Oceanogr. 54, 1461–1474. https://doi.org/10.4319/lo.2009.54.5.1461.

Washington Department of Fish and Wildlife, 2019. WDFW Latest Domoic Acid Levels [WWW Document]. URL. https://wdfw.wa.gov/fishing/basics/domoic-acid/levels. accessed 5.23.19.

Wekell, J.C., Gauglitz, E.J., Barnett, H.J., Hatfield, C.L., Eklund, M., 1994a. The occurrence of domoic acid in razor clams (*Siliqua patula*), Dungeness crab (*Cancer magister*), and anchovies (*Engraulis mordax*). J. Shellfish Res. 13, 587–593.

Wekell, J.C., Gauglitz, E.J.J., Barnett, H.J., Hatfield, C.L., Simons, D., Ayres, D., 1994b. Occurrence of domoic acid in Washington state razor clams (*Siliqua patula*) during 1991-1993. Nat. Toxins 2, 197–205.

Wekell, J.C., Trainer, V.L., Ayres, D., Simons, D., 2002. A study of spatial variability of domoic acid in razor clams: recommendations for resource management on the Washington coast. Harmful Algae 1, 35–43. https://doi.org/10.1016/S1568-9883(02)00004-5.