Journal of Shellfish Research, Vol. 42, No. 2, 265–279, 2023.

BIOTOXIN UPTAKE, RETENTION, AND DEPURATION TRENDS IN PURPLE-HINGED ROCK SCALLOPS, *CRASSADOMA GIGANTEA* (GRAY 1825)

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ABSTRACT The purple hinged rock scallop, *Crassadoma gigantea* (Gray 1825), is a species of interest for commercial-scale aquaculture in its native range, along the Pacific coast of North America from Baja California, Mexico to southeastern Alaska. One serious, unresolved issue, however, is the lack of information on uptake, retention, and depuration of algal biotoxins in this species. It is known that rock scallops can retain high levels of paralytic shellfish toxins (PST), including saxitoxin and derivatives, within its tissues including the adductor muscle. Paralytic shellfish toxins can pose serious public health risks, including paralytic shellfish poisoning (PSP), which can be lethal in humans. Diarrhetic shellfish toxins (DST) produced by algal species within the genus *Dinophysis* spp. is another suite of marine biotoxins monitored by public health agencies, known to cause diarrhetic shellfish poisoning (DSP) in humans. This is the first study to investigate dynamics of *Dinophysis* spp., and DST in the rock scallop. The present study examined uptake, retention, and depuration of two common toxic algal species and associated biotoxins in Puget Sound, WA: *Alexandrium catenella* (PST) and *Dinophysis* spp. (DST), through multiyear field exposures and controlled laboratory studies. Assessment of PST in rock scallop tissues by receptor binding assay from field and laboratory studies revealed very high and persistent levels of PST in visceral tissue and also PST in adductor muscle tissue beyond the FDA limit (80 µg STX equivalents 100 g^{-1} shellfish tissue) for safe shellfish consumption. An estimate of total depuration time of PST in rock scallop viscera was inconclusive, indicating potentially long depuration times for this species. Toxicity levels varied among individuals of the same cohort, size class, collection time, and location for both visceral and adductor muscle tissues. Laboratory results showed PST levels beyond the FDA limit within adductor muscle tissue during a 6-wk depuration period, indicating a shucked, adductor-only product for this species will require careful testing and management to ensure rock scallops are safe for consumption. More research is needed to decouple the complex interactions of *Dinophysis* spp., DST, water quality, and rock scallop physiology to inform shellfish managers and public health agencies reliably.

KEY WORDS: *Crassadoma gigantea*, rock scallop, saxitoxin, biotoxin, paralytic shellfish toxins, diarrhetic shellfish toxins, *Alexandrium catenella*, *Dinophysis*, depuration, aquaculture

INTRODUCTION

The purple hinged rock scallop, *Crassadoma gigantea* (Gray 1825), has long captured the interest of commercial shellfish farmers in its native range along the Pacific coast of North America from Baja California, Mexico, to southeastern Alaska (Leighton & Phleger 1981, Bourne et al. 1989, Bourne 1991, Leighton 1991, 2003, Chew et al. 1999, Davis 2003, RaLonde et al. 2012, Jackson 2021, Culver et al. 2022). To date, no commercial-scale aquaculture production of this species has occurred. Cultivation of rock scallops presents a potential opportunity to expand sustainable aquaculture practices by diversifying production of a native bivalve and mitigating environmental risks often associated with growing nonnative species (McKindsey et al. 2007, Cook et al. 2008, De Silva et al. 2009, Toledo-Guedes et al. 2014, Lima et al. 2018, Ju et al. 2019). Market-sized scallops can be produced in 2–4 y throughout their range in all existing Pacific coast aquaculture environments, including nearshore and offshore open-ocean locations (Leighton 1991, Chew et al. 1999, RaLonde et al. 2012). Despite the past and ongoing investments to develop this species for aquaculture, critical public health and safety issues remain. Harmful algal blooms (HAB) and the associated toxic algal species pose a serious threat to human health and shellfish aquaculture (Shumway 1990, Shumway & Cembella 1993, Matsuyama & Shumway 2009). This study serves to resolve data gaps related to rock scallop biotoxin uptake, retention, and depuration of two common HAB-forming dinoflagellates in Puget Sound, WA; *Alexandrium catenella* producing paralytic shellfish toxins (PST) and *Dinophysis* spp. producing diarrhetic shellfish toxins (DST). Dynamics of toxin retention and depuration below regulatory limits for safe harvest and consumption are largely unknown for this species. This information is essential for future aquaculture development and management of purple hinged rock scallop.

Paralytic Shellfish Toxins

The toxin-producing dinoflagellate *Alexandrium catenella* is a bloom-forming species on the Pacific coast of North America commonly found in Puget Sound, WA (Trainer et al. 2003). This species produces a suite of marine biotoxins referred to as PST, including the most potent, saxitoxin (STX) and its derivatives.

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DOI: 10.2983/035.042.0209

Symptoms of human poisoning occur soon after ingestion of toxic shellfish resulting in paralytic shellfish poisoning (PSP) with sometimes fatal results, thus the consequences of eating PST burdened shellfish constitutes a major public health risk (Kao 1993). Human health impacts have been well-documented (see Etheridge 2010, Grattan et al. 2018), including human death specifically associated with ingesting PST-contaminated rock scallops (Price et al. 1991). Whole scallop fisheries for pink and spiny scallops (*Chlamys rubida*, *C. hastata*, respectively) have been largely curtailed in Washington state mainly due to the propensity for biotoxin uptake and long-term retention (Washington Administrative Code, WAC 220–52–069). The Washington State Department of Health (WDOH) and other US and Canadian public health agencies regularly test commercially farmed and harvested shellfish species across the region to ensure safe consumption and sale of shellfish. When PST in target shellfish species are found by shellfish authorities to be elevated at or beyond the FDA National Shellfish Sanitation Program defined regulatory limit (80 μg STX equivalents $100\,g^{-1}$ shellfish tissue), the shellfish growing area is closed to harvest. In Washington state, rock scallops are not currently among the species routinely tested for biotoxin contamination, and the standing recommendation from WDOH is for no recreational harvest of rock scallops at any time.

In bivalves, PST may be concentrated in the viscera or more generally distributed in all or most body tissues. Some species of commercially valuable shellfish, including the sea scallop *Placopecten magellanicus* (Haya et al. 2003), butter clam *Saxidomus giganteus* (Beitler & Liston 1990, Kibler et al. 2022), Atlantic surf clam *Spisula solidissima*, and Pacific geoduck *Panopea generosa* (Curtis et al. 2000, Medina-Elizalde et al. 2018), all have the propensity to accumulate PST in specific tissues, and maintain toxicity for long periods of time (Hall 1982, Cembella et al. 1993, White et al. 1993, Shumway et al. 1994, Degrasse et al. 2013, Basti et al. 2018, Álvarez et al. 2019). Unlike Pacific oysters (*Crassostrea gigas*) that tend to curtail feeding during the presence of toxic dinoflagellates and, therefore, do not generally become toxic (Shumway et al. 1990, Bardouil et al. 1993), mussels and scallops do not suspend feeding activities and may become very toxic (Bricelj et al. 1990). Limited data from the State of Washington indicated that PST are not generally distributed in body tissues of rock scallops, but remain concentrated in the viscera, with approximately 10% of the toxins relative to visceral tissue accumulating in the adductor muscle (Beitler 1991), with similar findings from rock scallops collected from California waters (Sharpe 1981). The present study evaluates the temporal uptake, concentration, and retention of PST in different tissues (viscera and adductor muscle) through multiyear sampling of naturally exposed rock scallops at three locations within Puget Sound, WA, from 2017 to 2019, and also examines uptake and depuration of PST from multiple tissue types (digestive gland, adductor muscle, and remaining viscera) under controlled laboratory conditions.

Diarrhetic Shellfish Toxins

The genus *Dinophysis* contains mixotrophic dinoflagellates that produce a suite of toxins, including dinophysistoxins (DTX) and okadaic acid (OA), collectively referred to as DST, that can result in diarrhetic shellfish poisoning (DSP) in humans when DST-contaminated shellfish are consumed. Symptoms include nausea, vomiting, abdominal pain, and diarrhea. Many species of *Dinophysis* have been documented in Washington waters for many years (Horner et al. 1997), but only recently has the toxicity increased to levels of concern to public health. In June 2011, the first confirmed clinical report of DSP illnesses in the United States occurred in the State of Washington, where three people became ill from contaminated blue mussels harvested at Sequim Bay State Park (Lloyd et al. 2013). Following this event, monitoring for *Dinophysis* and DST in Washington state became formalized by public health authorities (Trainer et al. 2013). The WDOH regularly monitors for DST in recreational and commercial shellfish species, closing harvest areas when levels exceed the FDA National Shellfish Sanitation Program threshold of 16 μg DST $100\,g^{-1}$ of shellfish tissue. The present study evaluates comparative temporal uptake and depuration trends of DST in naturally exposed rock scallops and blue mussels from 2017 to 2020 at two locations in Puget Sound, WA, in collaboration with the WDOH as part of their routine marine biotoxin monitoring program.

MATERIALS AND METHODS

Spike Recovery and Extraction Solvent Comparisons

Two STX extraction procedures have been validated for application in seafood safety regulatory settings, 0.1N hydrochloric acid (AOAC Official Method 2011.27) and 1% acetic acid (AOAC Official Method 2011.02). In the present study, given the difficulties in preparing an extract using the hydrochloric acid method, acetic acid was additionally used as an alternative extraction solvent to better reflect the composition of PST present in a sample. The spike recovery study was conducted to determine the relative efficiencies of the two different extraction methods in a rock scallop matrix and their performance in the STX receptor binding assay (RBA) (AOAC Official Method 2011.27).

Rock scallop samples were parsed as separate adductor and digestive gland tissue, homogenized and spiked at four concentrations. The concentrations chosen were to be near the STX RBA detection limit (10 μg STX·$100\,g^{-1}$), half the FDA limit (40 μg STX·$100\,g^{-1}$), at the FDA limit (80 μg STX·$100\,g^{-1}$), and higher (300 μg STX·$100\,g^{-1}$). Purified FDA STX dihydrochloride standard was added to each sample type, homogenized, and an aliquot prepared for extraction using either 0.1N hydrochloric acid or 1% acetic acid. Extracts were prepared by adding the respective acid to the spiked material, boiling, centrifugation, and collection of the supernatant then analyzed using the STX RBA.

Alexandrium *Culturing and Saxitoxin Determination*

The *Alexandrium* monoculture (NWFSC 458) used for the exposure study was originally established in April 2017 and maintained in filter-sterilized (0.2 micron) natural seawater amended with Guillard's (F/2) Marine Water Enrichment Solution (Sigma # G0154). Cultures were established by single chain, microcapillary isolation of single chain vegetative cells that germinated from sediment (top 0–1 cm) collected from Hood Canal, WA, on January 14, 2017. Sediment was stored in the dark and at 4°C until portions were removed for germination. The culture was maintained in approximately 30 ppt

salinity F/2 enriched seawater at 15°C, approximately 150 µE m^{-2} s^{-1} light level, and on a 12:12 (h) light:dark cycle.

Cultures for the exposure study were grown in 10 L clear polycarbonate carboys (Nalgene) at the same approximate levels of salinity, temperature, and light level as the original inoculum culture. The average cell density for the 10 carboys at the time of transfer to Manchester was 4590 ± 580 cells/mL.

An aliquot of culture from each carboy was taken at the time of transfer for determination of particulate STX levels via ELISA (Gold Standard Diagnostics-Abraxis #52255B). The average particulate STX level for the 10 carboys was 2.65 ± 0.5 ng/mL of culture. Saxitoxin per cell concentrations averaged 0.58 ± 0.12 pg/cell.

Feeding Trial: Uptake of PST from Alexandrium *by Rock Scallops Exposed in the Laboratory*

A laboratory study was carried out at the Pacific Hybreed Hatchery located at the EPA/NOAA facility in Manchester, WA, in September 2018. Hatchery-reared, subadult rock scallops (shell length approximately 40–60 mm) were exposed to cultured *Alexandrium catenella* cells for 4 consecutive days (September 10–13, 2018). Rock scallops were maintained throughout the study in a single, 20-L experimental tank maintained at a temperature of 15°C–16°C and average salinity of 30 ppt. Rock scallops (n = 25) were added to the bottom of the tank containing seawater filtered to 1 micron for 1 h prior to initial feeding on September 10, 2018. Immediately prior to feeding with *A. catenella,* a 10-L volume of filtered seawater was removed from the tank, followed by a 10-L volume of *A. catenella* culture, gently poured into the experimental tank. When *A. catenella* culture was added to the tank, the flow was turned off to enable scallops to feed on all available cells under static conditions, with gentle aeration. This process was completed twice daily, AM and PM, for an average feeding phase of 7 h each day. Relative density of *A. catenella* cells in the experimental tank varied slightly over the 4-day feeding period, with concentrations ranging between 4,500 and 5,200 cells/L per day. After the fourth day of exposure to *A. catenella*, scallops were placed in a flow-through seawater tank and supplied with a hatchery diet of nontoxic diatoms and dinoflagellates. Scallops were subsampled (n = 5) at day 0 (pre-exposure), 1, and 4 days exposure, 7, 14, 28, and 58 days post-exposure. Scallop adductor muscle and digestive gland were removed. Samples were placed in individual vials and frozen at −20°C until extracted. Extracts were analyzed using the STX RBA (AOAC Official Method 2011.27) at NOAA/NOS in Charleston, SC.

Two of the five individual rock scallop extracts from each collection time were additionally analyzed by tandem mass spectrometry coupled to liquid chromatographic separation (LC-MS) at NOAA/NOS to attain the PST congener profile for each tissue type. Sample cleanup and the LC-MS analysis method is described in Hattenrath-Lehmann et al. (2018). Briefly, acidic extracts analyzed by RBA were further prepared for LC-MS analysis by using C18 solid phase extraction cartridges. The LC-MS method used an acidified water/acetonitrile mobile phase and a TSK-gel Amide-80 column, using an Agilent 1100 HPLC coupled to an ABI-SCIEX 4000 Qtrap triple quadrupole mass spectrometer using multiple reaction monitoring (MRM). Multiple reaction monitoring (MRM) transitions were developed using certified PSP standards from the National Research Council of Canada.

Paralytic Shellfish Toxins and Diarrhetic Shellfish Toxins in Field-Exposed Rock Scallops and Blue Mussels

Field Sites

Rock scallops previously produced from Washington broodstock at the Taylor Shellfish Hatchery in Quilcene, WA, were deployed at four sites (Fig. 1A) with a history of biotoxin blooms and at locations routinely monitored by WDOH Sentinel Mussel Monitoring Program. Three sites were in North Puget Sound, WA: Sequim Bay at, John Wayne Marina (JWM), a private residence (PR), and Discovery Bay (DB) (Fig. 1B). Sequim Bay has one of the longest recorded histories of PST in Washington state (Trainer 2002, Trainer et al. 2003) and is considered a hot spot due to frequently elevated levels of STX and its congeners in mussels (Lefebvre et al. 2008). The fourth site was located in South Puget Sound, 90 miles south of the northern sites at West Bay Marina (WBM) in Budd Inlet, Olympia, WA (Fig. 1C). This site was selected based on its history of *Dinophysis* spp. blooms and regular biotoxin monitoring by the WDOH Sentinel Mussel Monitoring Program.

Scallop Deployment to Fields Sites

On February 24, 2017, in partnership with Jamestown S'Klallam Tribe, Pacific Hybreed, and Pacific Shellfish Institute, subadult scallops (shell length approximately 40–60 mm) were deployed at all four sites and maintained until early 2020. Scallops were placed in cages made from polyethylene bags commonly used for bivalve mariculture and supported by PVC frames to provide structure for suspending the cages from floating subtidal dock sites. Scallops were deployed 55 to a cage, four cages to an array, and two arrays per site. Arrays were suspended approximately 3 m below the surface at each site. After 8 mo of grow out in the polyethylene bags, it was evident that gear fouling was a challenge. Heavy fouling in warm summer months restricted water flow and feeding for growing scallops and increased the overall weight of the gear, making the arrays difficult to monitor and manage. Therefore, the rock scallops were transferred in October 2017 to replicate PVC trays commonly used in subtidal aquaculture with larger mesh size and a removable lid for easy maintenance for the duration of the project.

Through a collaboration with two existing biotoxin monitoring programs, the WDOH Sentinel Mussel Monitoring Program and the Jamestown S'Klallam Tribe's Natural Resources Monitoring Program, rock scallops and blue mussels were collected to assess PST and DST contamination. Jamestown S'Klallam Tribe led biotoxin monitoring and shellfish collections at Sequim Bay and Discovery Bay sites, Pacific Shellfish Institute led biotoxin monitoring and rock scallop collections at Budd Inlet, and WDOH collected blue mussels from Budd Inlet as part of their routine monitoring. Standard protocols for the Sentinel Mussel Monitoring Program include growing blue mussels (defined here as *Mytilus* spp. due to difficulty differentiating *M. trossulus* and *M. edulis* in the field) in mesh cages suspended from subtidal dock sites and collection biweekly for PST testing using the mouse bioassay (AOAC Official Method 959.08) at WDOH. Rising PST levels observed by the biotoxin

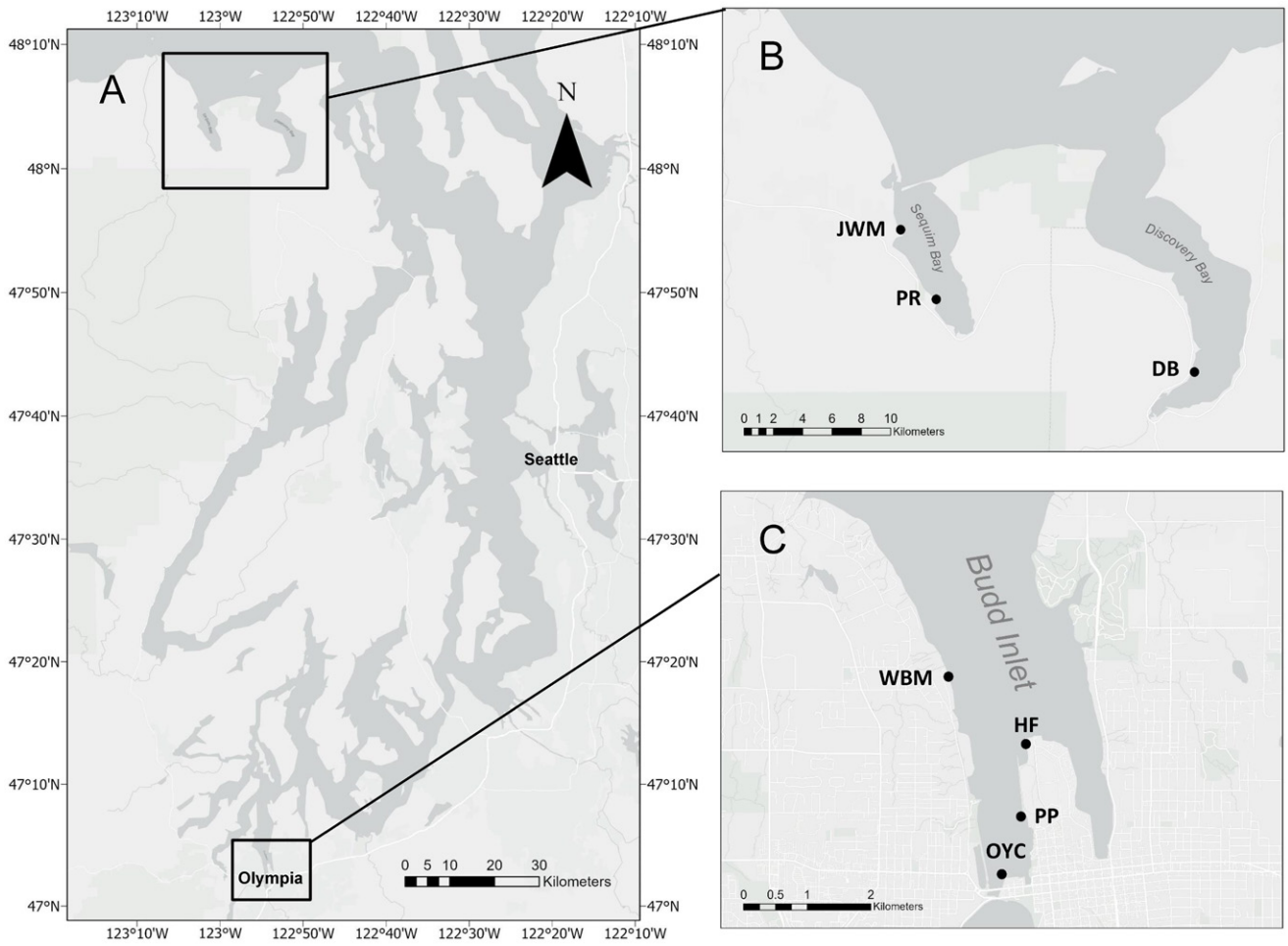

Figure 1. Experimental sites in (A) Puget Sound, WA, including locations of rock scallop outplants for field exposure trials in: (B) John Wayne Marina (JWM) and a Private Residence (PR) in Sequim Bay, and Discovery Bay (DB). Sample sites in (C) South Puget Sound, WA, for DST monitoring: rock scallop collection site at West Bay Marina (WBM), Washington Department of Health (WDOH) Sentinel Mussel site at Olympia Yacht Club (OYC), SoundToxins monitoring sites are at PR, Port Plaza (PP), and Hearthfire (HF).

monitoring programs trigger more targeted and frequent sampling regimens in other shellfish species besides blue mussels. When existing monitoring indicated rising or sustained biotoxin levels at or above the FDA limit for PST (80 µg ·100 g^{-1} tissue) or DST (16 µg 100 g^{-1} tissue), 10 animals were removed from culture cages as soon as feasible and shipped whole on ice overnight to either Sitka Tribe of Alaska Environmental Research Laboratory (STAERL) for PST analysis or WDOH Public Health Laboratory for DST analysis. Ten scallops were sampled each week for the duration of the bloom or until the animals no longer contained toxins above the FDA limit.

The STAERL conducted PST analyses using the STX RBA method (AOAC Official Method 2011.27). Rock scallops were dissected into two component parts (adductor muscle and viscera) for two separate RBA analyses per animal. Whole scallops (n = 10) collected at different times between May and October 2017 were pooled and homogenized in the laboratory and represented a single PST value for a particular component animal part, location, and collection date. From October 2017 through early 2020, each of 10 scallops and component parts (adductor or viscera) were analyzed individually at each time point.

When monitoring programs indicated DST levels in blue mussels were above the FDA limit, scallops were shipped to the WDOH Public Health Laboratory for testing using liquid chromatography-tandem mass spectrometry in whole animals (Braña-Magdalena et al. 2014).

Phytoplankton Collection and Enumeration

Phytoplankton monitoring for HAB species occurred at rock scallop field sites during the time frame of the field exposure trials in Sequim Bay and Budd Inlet, WA, led by Jamestown S'Klallam Tribe and Pacific Shellfish Institute, respectively (Fig. 1B, C). Plankton samples were collected using a vertical net tow of specified distance (d) (meters) according to SoundToxins protocols (SoundToxins manual 2016). The total volume of water sampled (πr^2) (d) was divided by the final volume of the concentrated sample to determine the concentration factor. Cell counts of HAB species present were quantified at 200× magnification using a Palmer-Maloney counting chamber to determine cell counts present in a 0.1 mL aliquot of collected sample water. Cell counts were converted to cells/L and then divided by the concentration factor. *Dinophysis* spp. collected in Budd Inlet during the study period were further identified to species by local expert, Aimee Christy (Pacific Shellfish Institute).

Hatchery Depuration of PST from Field-Exposed Rock Scallops

Toxin concentrations in rock scallops grown subtidally in Sequim Bay, WA, were determined using RBA at the STAERL. The Sequim Bay scallops contained high concentrations of PST (4,567 µg STX equivalents 100 g^{-1} in visceral tissue, and 90 µg STX equivalents 100 g^{-1} in adductor muscles) on September 11, 2017. On September 14, 2017, scallops (n = 105) were transferred from the field location in Sequim Bay (JWM) to the Pacific Hybreed Hatchery in Manchester, WA. Scallops were placed in tanks with filtered seawater under similar temperature and salinity conditions as the field location for a period of 6 wk. Tissue samples were collected as soon as they entered the laboratory (day 0), and then weekly for 6 wk. Sampling consisted of dissecting 15 randomly selected rock scallops. Three tissue types were collected: adductor muscle, digestive gland, and the remaining tissues pooled (gonad, gill, and mantle) from each scallop. Tissue samples were placed in individual vials and maintained frozen at −20°C until extraction. Tissue samples were shipped to the Bigelow Analytical Services, Boothbay Harbor, ME for PST analyses using postcolumn oxidation high-performance liquid chromatography (HPLC) (AOAC Official Method 2011.02) to describe the complete profile of PST congeners present in the samples.

RESULTS

Spike Recovery and Extraction Solvent Comparisons

The recovery of STX from rock scallop matrix was 66%–132% for digestive gland and 63%–133% for adductor muscle (Table 1). No obvious differences were observed when comparing the two acids used, nor across spike concentrations. These values are representative of one homogenate, and replication of extraction would likely improve the observed variability. The observed recovery was similar to other matrix types suggesting that either acid is appropriate for use in preparing rock scallop tissue for analysis by STX RBA.

TABLE 1.

Relative efficiencies (% recovery) of two STX RBA extraction methods, 0.1N HCl and 1% acetic acid, in a rock scallop matrix spiked with purified FDA STX dihydrochloride standard.

	Spike concentration (µg STX/100 g tissue)	Percent recovery (%)	
		0.1N HCl	1% acetic acid
Adductor muscle	0	<LOD	<LOD
–	10	<LOD	124%
–	40	78%	74%
–	80	133%	106%
–	300	108%	63%
–	–	–	–
Digestive gland	0	<LOD	<LOD
–	10	<LOD	<LOD
–	40	85%	132%
–	80	66%	75%
–	300	93%	103%

Rock scallop adductor muscle and digestive gland tissues were analyzed separately at four concentrations of STX (µg STX/100 g tissue). Limit of detection less than 15 µg/100 g tissue (<LOD) in the STX RBA.

Feeding Trial: Uptake of PST from Alexandrium *by Rock Scallops Exposed in the Laboratory*

During the feeding trial with *Alexandrium catenella*, PST concentrations increased in individual adductor muscle tissues beyond the FDA limit (80 µg STX equivalents·100 g^{-1} shellfish tissue) by the 4-day exposure and 7-day post-exposure from initial day 0 (pre-exposure) and 1-day exposure (Fig. 2B). During these two time intervals, three of five adductor muscle samples exceeded the FDA limit. At the end of the 4-day exposure, digestive gland PST concentrations were three times higher than at the start of the experiment (Fig. 2A). Concentrations of PST in the digestive gland were more than an order of magnitude higher than PST in adductor muscle tissue across all time intervals, with adductor muscles being 0.6%–8% of the concentrations of the digestive gland. Complete depuration of PST within either rock scallop tissues, adductor muscle, or digestive gland were not observed at the terminus of the experiment. At 58 days post-exposure, two adductor muscles contained approximately 20 µg STX equivalents 100 g^{-1} and mean PST in digestive gland were above the FDA limit at 296 ± 107 µg STX equivalents 100 g^{-1}.

The LC-MS analysis showed the presence of nearly every one of the 18 PST congeners measured in digestive gland extracts at every time point (Fig. 3A). Specifically, the day 0 (pre-exposure) digestive gland extract contained STX = NEO > GTX2 = GTX3 > GTX1 = C2 > dcGTX2 = dcGTX3 > GTX4 = C1 = M1beta > GTX5 = M5 > GTX6. The PST congeners

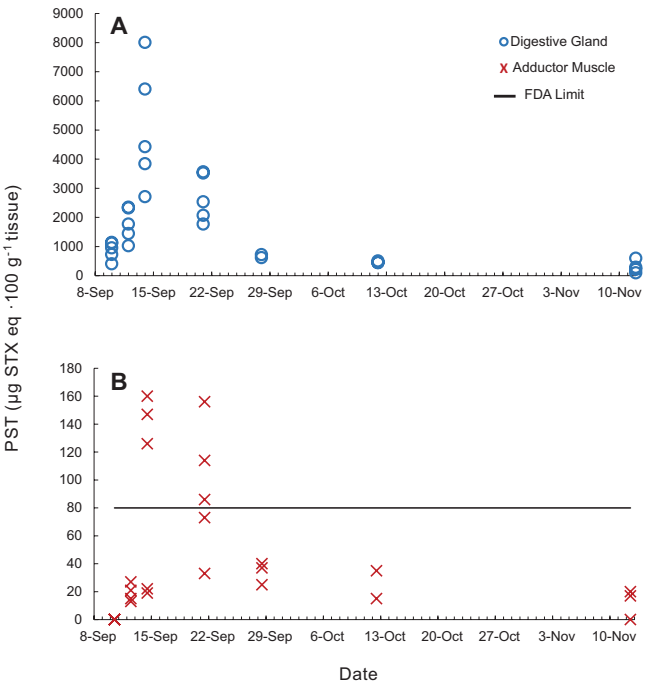

Figure 2. Rock scallops were exposed to bloom concentrations (4,500–5,200 cells/L) of *Alexandrium catenella* cells for 4 consecutive days in a controlled laboratory setting from 10–13 September 2018. Digestive gland (A) and adductor muscle (B) PST values (µg STX equivalents per 100 g of tissue) were determined by RBA. Rock scallops were sampled at day 0 (September 10), 1 day exposure (September 12), 4 days exposure (September 14), and post-exposure at 7 days (September 21), 14 days (September 28), 28 days (October 12), and 58 days (November 12). Zero values were below the limit of detection (<5 µg STX eq·100 g^{-1} tissue).

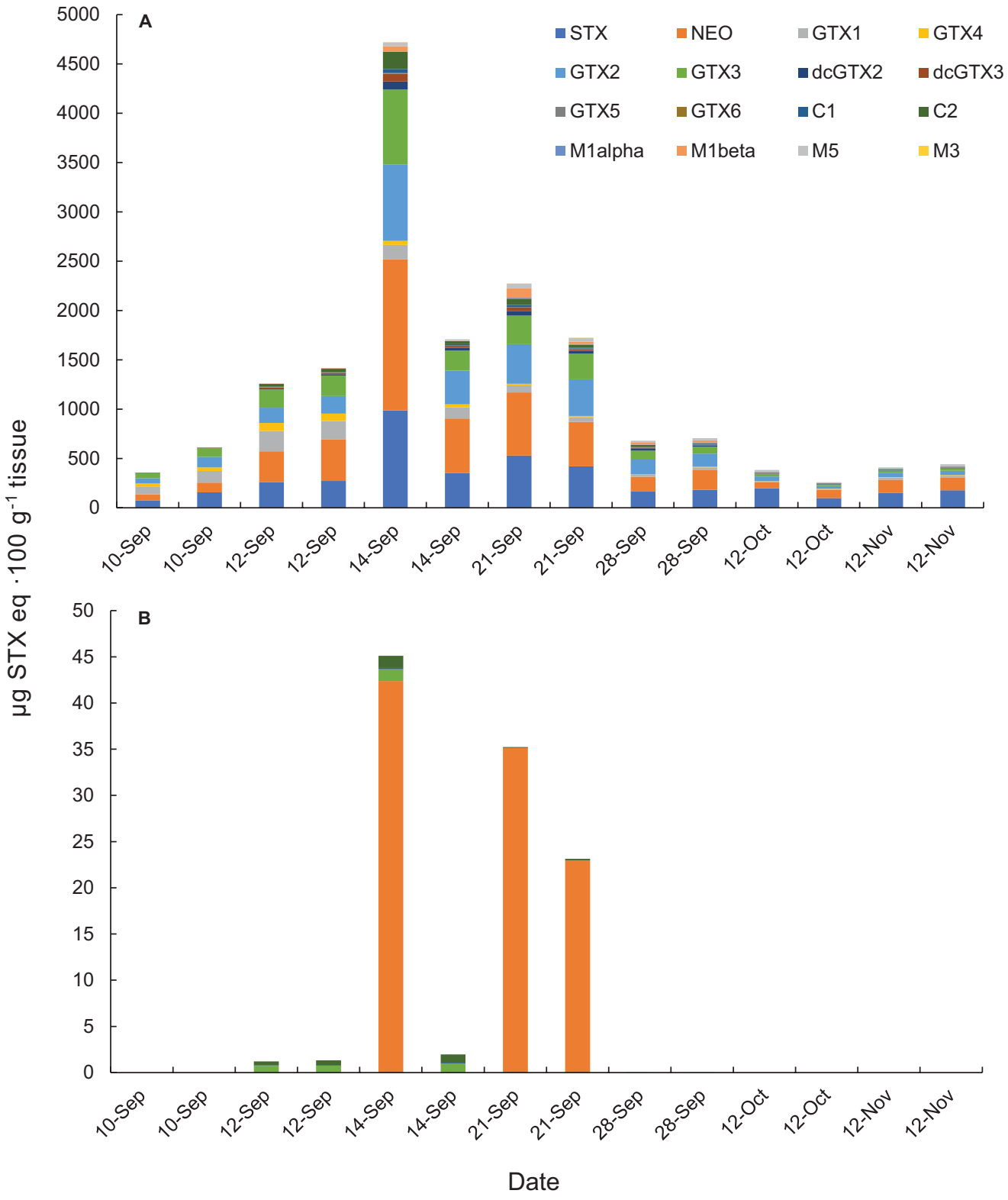

Figure 3. Rock scallops were exposed to bloom concentrations (4,500–5,200 cells/L) of *Alexandrium catenella* cells for 4 consecutive days in a controlled laboratory setting, 10–13 September 2018. Two rock scallops were collected on each date. PST congeners in µg STX equivalents per 100 g of tissue in digestive gland (A) and adductor muscle (B) are shown. Rock scallops were sampled at day 0 (September 10), 1 day exposure (September 12), 4 days exposure (September 14), and post-exposure at 7 days (September 21), 14 days (September 28), 28 days (October 12), and 58 days (November 12). PST congeners analyzed include: STX, NEO, gonyautoxin 1–6 (GTX1, GTX2, GTX3, GTX4, GTX5, GTX6), decarbamoyl gonyautoxin 2 and 3 (dcGTX2, dcGTX3), *N*-sulfocarbamoyl gonyautoxin 2 and 3 (C1 and C2), and M toxins (M1alpha, M1beta, M3, M5). Zero values were below the limit of detection (<5 µg STX eq·100 g^{-1} tissue).

M4/M8, dcGTX2, and dcGTX3 were absent in day 0 (pre-exposure) samples. The LC-MS of adductor muscle (Fig. 3B) showed the presence of trace amounts of C1 and C2 toxin from 1-day exposure through 14-day post-exposure, GTX3 from 1-day exposure through 4-day exposure, and high levels of NEO in some animals from 4-day exposure and 7-day post-exposure. All other PST congeners were absent from adductor muscle including: STX, GTX1, GTX2, GTX4, GTX5, GTX6, dcGTX2, dcGTX3; M1, M3, M5, M7, and M4/M8.

Paralytic Shellfish Toxins and Diarrhetic Shellfish Toxins in Field Exposed Rock Scallops and Blue Mussels

Paralytic Shellfish Toxins

The first PST time series from Sequim Bay (SB) was initiated August 3, 2017, when PST in blue mussels (181 µg STX equivalents·100 g^{-1}) were above the FDA limit, resulting in a shellfish harvesting closure that persisted for 4.5 mo (Fig. 4A). Four days following the shellfish closure, rock scallops tested high in the viscera at both Sequim locations using RBA, SB-PR (297 µg STX equivalents·100 g^{-1}), and JWM (500 µg STX equivalents·100 g^{-1}). In contrast, adductors tested at significantly lower toxin levels, below the FDA limit at both sites, SB-PR (8 µg STX equivalents·100 g^{-1}), and JWM (31 µg STX equivalents·100 g^{-1}). At the end of August, the peak toxicity in rock scallop adductor at JWM (162 µg STX equivalents·100 g^{-1}) was observed with proportionately high toxins in viscera (1,671 µg STX equivalents·100 g^{-1}). In early September, blue mussels reached their peak toxicity (346 µg STX equivalents·100 g^{-1}) for the data series, following peak toxicity in rock scallop viscera 6 days later at both locations, SB-PR (6,450 µg STX equivalents·100 g^{-1}), and JWM (4,567 µg STX equivalents·100 g^{-1}). From October onward, the mean toxicity in rock scallop adductor from both sites (JWM, SB-PR) and blue mussels followed a similar depuration trend, whereas PST in rock scallop viscera remained very high (>400 µg STX equivalents·100 g^{-1}) for the duration of the time series (Fig. 4A, B). Blue mussels depurated all toxins by

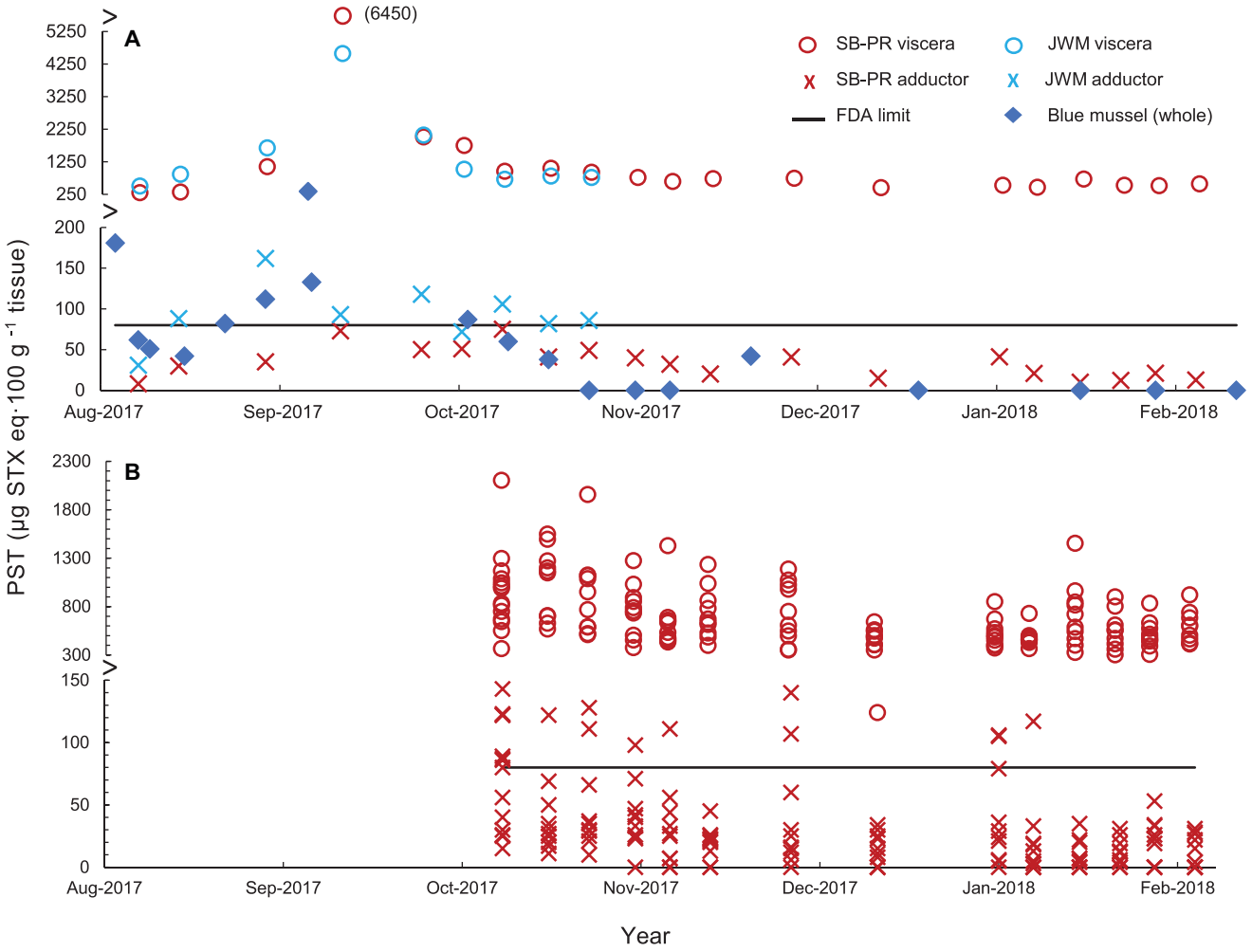

Figure 4. PST in rock scallop adductor and viscera tissue collected from two Sequim Bay (SB) sites, a Private Residence (PR) and John Wayne Marina (JWM), determined by receptor binding assay, August 2017 to February 2018. (A) Each data point from August to October 2017 represents a composite sample from multiple individuals to achieve a total of 100 g of tissue for adductor and 100 g of tissue for viscera. Data from October 2017 to February 2018 represents the mean of 10 individuals. Whole blue mussel PST data from Sequim Bay State Park were provided by Washington Department of Health (WDOH). (B) PST in individual scallop adductor and viscera from October 2017 to February 2018 collected from Sequim Bay (SB-PR). Individual scallops (n = 14 10/9/17 only, n = 10 all others) from each sample date are represented. FDA limit = 80 µg STX eq·100 g^{-1} shellfish tissue is shown as a horizontal line.

the end of the year. The WDOH reported Sequim Bay open for blue mussel harvest on December 14, 2017. Rock scallops, however, persisted with high PST in the viscera, well above the FDA limit with minimal depuration for the duration of the 6-mo time series (Fig. 4A). Two additional time series from Discovery Bay (2017, 2019–2020), indicated similarly high and persistent toxicity levels within rock scallop viscera, showing no apparent trend of depuration (Fig. 5A, B). In contrast, adductor muscle tissue remained relatively low in toxicity throughout the time series, except for the initial shellfish closures (June 2017, October 2019). Mean adductor toxin levels eventually fell below the FDA limit for PST in late 2017 and late 2019. Mean PST in rock scallop viscera during the 2019 period (1,248 ± 793 µg STX equivalents·100 g^{-1}) was more than twice the mean PST in 2017 rock scallop viscera (540 ± 295 µg STX equivalents·100 g^{-1}), whereas mean PST in adductor muscles were very similar between the 2 y: 2017 (41 ± 27 µg STX equivalents·100 g^{-1}) and 2019 (43 ± 48 µg STX equivalents·100 g^{-1}), with comparatively less variability than in viscera.

Table 2 compares PST values in rock scallop adductor muscle and viscera tissue as determined through RBA. Across all time series and locations, mean PST in viscera tissue (861 ± 582 SD µg STX equivalents·100 g^{-1} tissue) was more than 16 times greater than mean PST in adductor muscle tissue (53 ± 46 SD µg STX equivalents·100 g^{-1} tissue) for the study period.

Diarrhetic Shellfish Toxins

Two time series were collected for Budd Inlet, WA, 2017–2018 and 2019–2020 tracking DST in rock scallops, blue mussels, and *Dinophysis* spp. cell counts (Fig. 6A, B). The first time series begins in August 2017 after blue mussels tested over the FDA limit for DST (16 µg DST·100 g^{-1} tissue) on August 24, 2017, (19 µg DST·100 g^{-1}) prompting a shellfish harvesting closure for all species by WDOH (Fig. 6A). Rock scallops (n = 10) were sampled weekly during the closure until the Bay was opened to all species on March 7, 2018. Budd Inlet was only opened briefly with another DST closure 2 wk later, on March 22, 2018, when blue mussels tested again over the FDA limit (19 µg DST·100 g^{-1}). The last rock scallops collected for this early spring period were sampled on April 3, 2018. When sample collection resumed in July 2018, both rock scallops and blue mussels tested below the FDA limit,

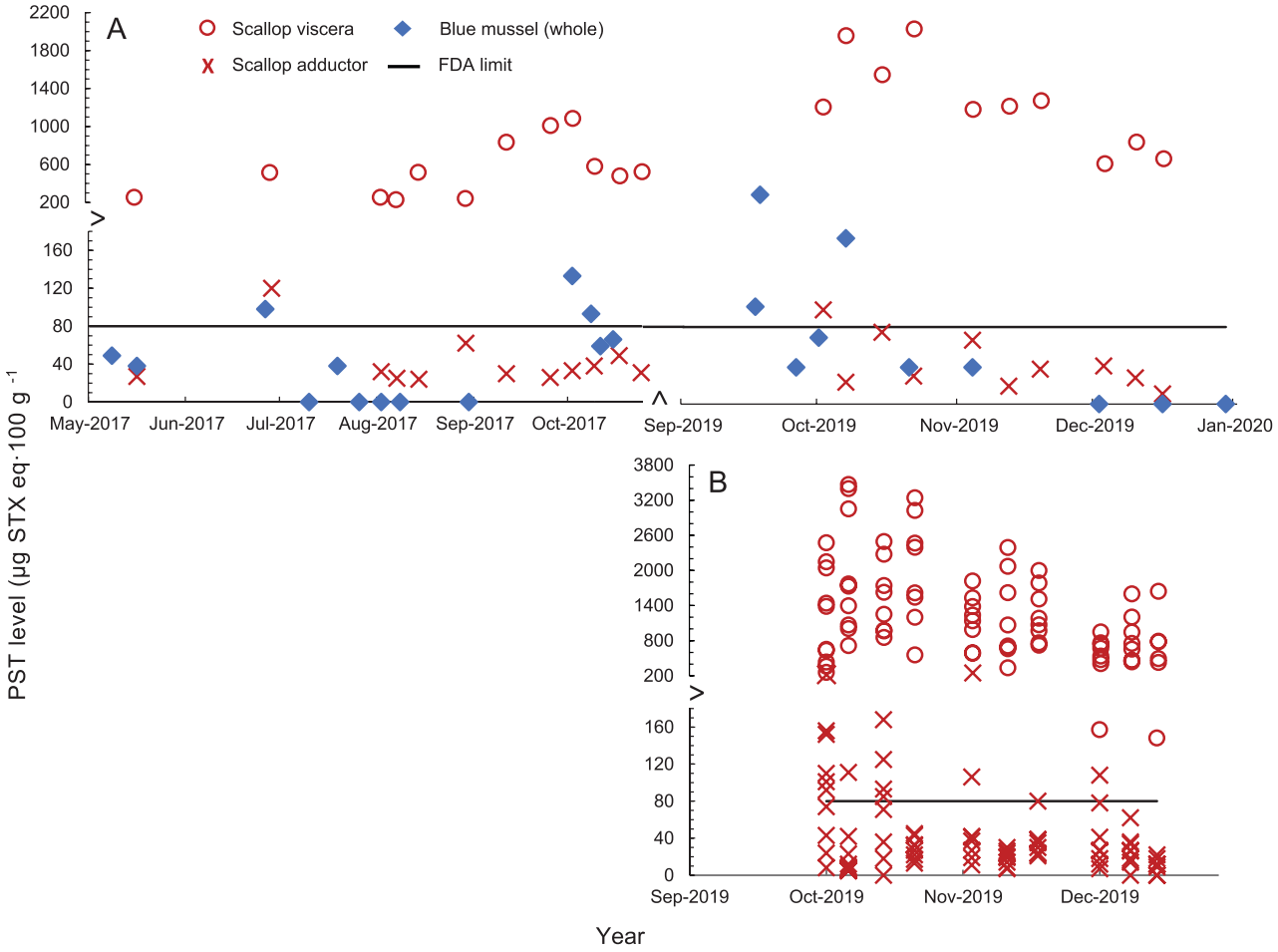

Figure 5. PST in rock scallop adductor and viscera tissue from Discovery Bay (DB), determined by receptor binding assay. (A) Each data point from May to October 2017 is from a composite sample from multiple individuals to achieve a total of 100 g of tissue for adductor and 100 g of tissue for viscera. Data points from September 2019 to January 2020 represent the mean of 10 individuals. Whole blue mussel PST data from the same sample location in DB were collected by Washington Department of Health (WDOH). (B) PST in adductor and viscera from individual scallops (n = 10) shown from DB, October 2019 to December 2019. FDA limit = 80 µg STX eq·100 g^{-1} shellfish tissue is shown as a horizontal line.

TABLE 2.

Mean PST values ± SD (µg STX eq·100 g^{-1} tissue) in rock scallop viscera and adductor muscle tissues during the project period, 2017–2019 at Sequim Bay and Discovery Bay, WA.

Location	Year	Site	Viscera	*N* =	Adductor	*N* =
Sequim Bay	2017–2018	PR	740 ± 590	149	32 ± 34	149
		JWM	920 ± 746	36	95 ± 65	36
Discovery Bay	2017	Condos	537 ± 195	40	41 ± 36	40
	2019	Condos	1248 ± 793	83	43 ± 48	83
		Total Means	**861 ± 582**	**308**	**53 ± 46**	**308**

prompting the WDOH to open Budd Inlet to all species on July 12, 2018. The second time series from November 2019 to April 2020 (Fig. 6B) identifies a bloom of *Dinophysis* spp. on November 19, 2019 (2,113 cells/L), coincided with increased toxicity in blue mussels (76 µg DST·100 g^{-1}), prompting a DST closure by WDOH. Rock scallops (n = 10) were sampled 6 days later on November 25, 2019, and tested above the FDA limit (42 µg DST·100 g^{-1}). Scallops were tested weekly following the closure. At the date of maximum toxicity, December 3, 2019 (46 µg DST·100 g^{-1}), rock scallops depurated the toxins over 9.5 wk, falling below the FDA limit at the end of January 2020. DST in blue mussels was above the FDA limit from November 2019 through the end of the time series in May 2020, resulting in the DST shellfish closure of Budd Inlet to extend beyond the study period.

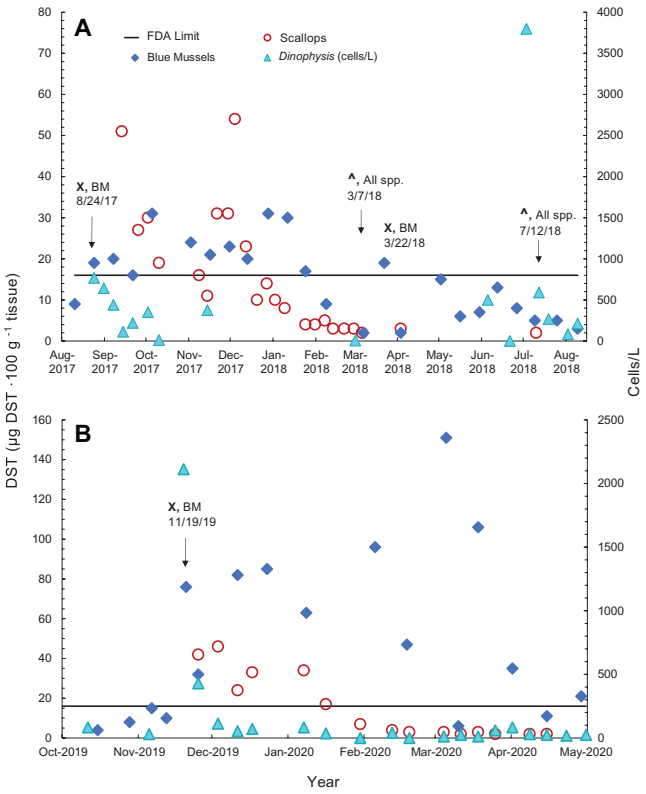

Figure 6. DST in whole rock scallops and whole blue mussels sampled from Budd Inlet, WA, in south Puget Sound from (A) August 2017 to August 2018 and (B) November 2019 to April 2020. *Dinophysis* spp. cell counts are from concentrated vertical plankton net tows at the scallop sampling location, West Bay Marina (WBM). Plankton were collected on 11/5/19, 11/19/19 at Port Plaza (PP), and 4/23/20, 5/1/20 collected at Hearth Fire (HF) (see map, Figure. 1 for all abbreviations.). FDA limit = 16 µg DST·100 g^{-1} tissue, shown as a horizontal line. Blue mussel data provided by Washington Department of Health (WDOH). WDOH shellfish closure (X) and open dates (^) for blue mussels (BM) or all harvestable shellfish species (All spp.).

A limited number of blooms and subsequent DST-related closures occurred during the project period for the northern Puget Sound sites; however, data were available from 2017 to early 2018 for Sequim Bay (PR), and are illustrated in Figure 7. Presence of toxic Manila clams, *Ruditapes philippinarum*, prompted the DST shellfish harvesting closure on October 4, 2017. Blue mussels remained below the FDA limit at that time, whereas whole rock scallops tested well over the FDA limit 13 days after the closure (59 µg DST·100 g^{-1}). High cell counts of *Dinophysis* spp. in SB coincided with high toxicity in rock scallops during the time series in October and November 2017. Peak toxicity of DST in whole rock scallops (108 µg DST·100 g^{-1}) occurred on November 1, 2017, which was seven times the equivalent toxicity observed in blue mussels (15 µg DST·100 g^{-1}) on the same collection date. In general, DST in blue mussels remained low, whereas rock scallops were above the FDA limit, finally depurating toxins below the FDA limit 2 mo after the initial closure.

Hatchery Depuration of PST from Field Exposed Rock Scallops

Results for the hatchery depuration study of PST from rock scallop tissues are illustrated in Figure 8. Total mean toxin load of PST (µg STX equivalents·100 g^{-1}) in the digestive gland decreased from 6,002 ± 1,907 SD µg STX equivalents·100 g^{-1} at day 0 to 985 ± 362 SD µg STX equivalents·100 g^{-1} after 6 wk. Mean PST in adductor muscle on day 0 was 63.8 ± 28 SD µg STX equivalents·100 g^{-1}, below the FDA limit of 80 µg STX equivalents·100 g^{-1}. After 6 wk of depuration in a hatchery tank, mean PST in adductor muscle increased above the FDA limit to 149 ± 112 SD µg STX equivalents·100 g^{-1}, a 2.3-fold increase in toxicity on a per gram basis from the initial time point. The total toxin load in the remaining tissues on day 0 was 150 ± 65 SD µg STX equivalents·100 g^{-1}, after 6 wk, mean PST declined to 116 ± 62 SD µg STX equivalents·100 g^{-1}, remaining above the FDA limit of 80 µg STX equivalents·100 g^{-1}.

Toxicity levels for 12 PST congeners determined by post-column oxidation HPLC (AOAC Official Method 2011.02) in adductor muscle, digestive gland, and remaining tissues at initial and final end points (week 6) are shown in Figure 9A–C. Mean toxicity of neosaxitoxin (NEO) in adductor muscle increased from 42 ± 20 SD µg STX equivalents·100 g^{-1} to

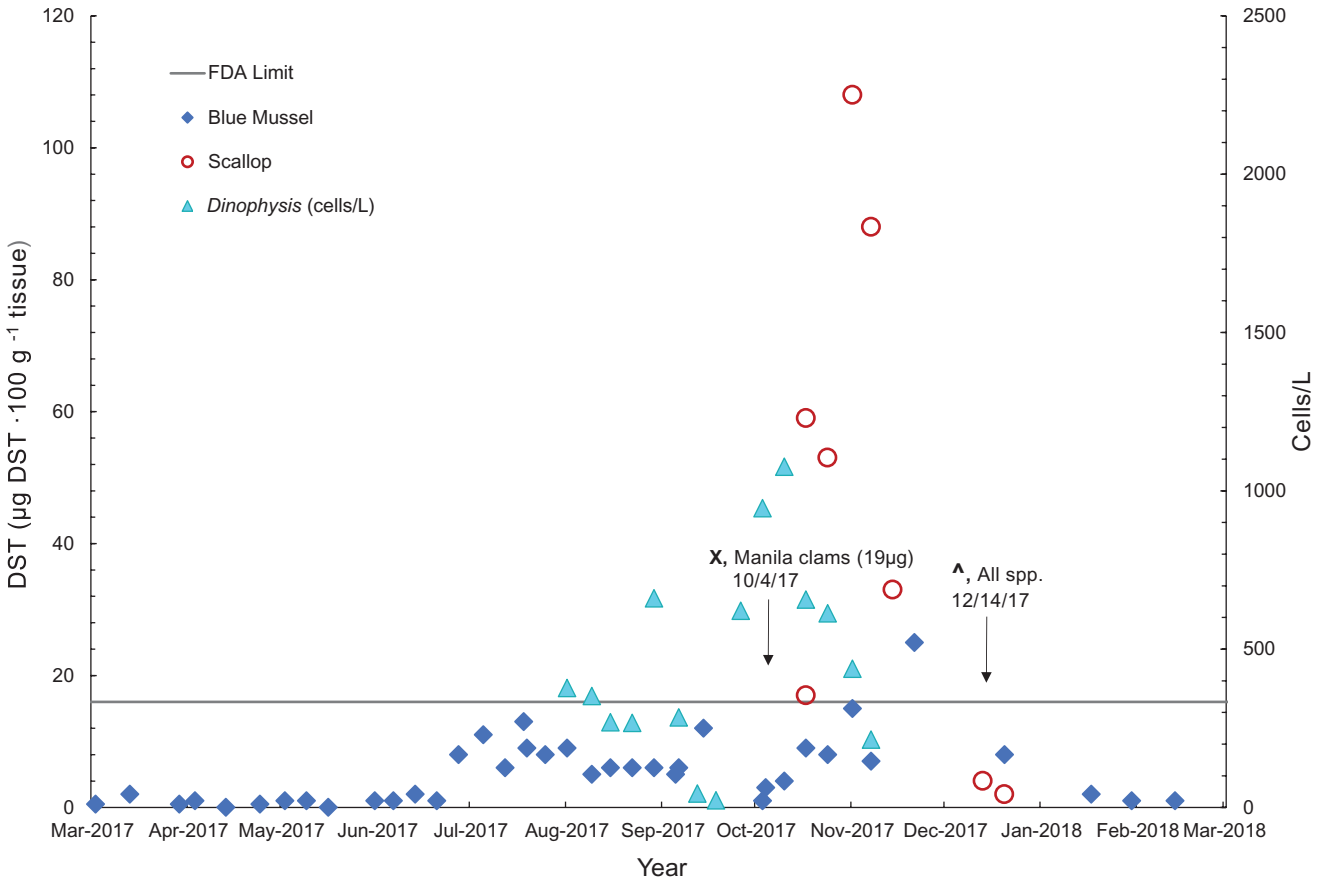

Figure 7. Time series of DST in whole blue mussels and whole rock scallops (n = 10) from Sequim Bay, at a private residence (PR), over a 12-mo period from March 2017 to March 2018. Blue mussel data were provided by Washington Department of Health (WDOH). *Dinophysis* spp. cell counts were provided by SoundToxins from Sequim Bay (PR). FDA limit = 16 µg DST 100 g^{-1} tissue is shown as a horizontal line. WDOH shellfish closure (X) and open dates (^).

104 ± 86 SD µg STX equivalents·100 g^{-1} at 6 wk, above the FDA limit (Fig. 9A). Mean toxicity of STX in adductor muscle also increased from 8.5 ± 4 SD µg STX equivalents·100 g^{-1} to 40 ± 25 SD µg STX equivalents·100 g^{-1} at 6 wk. Mean toxicity of five congeners: GTX1, GTX3, GTX2, NEO, and STX were above the FDA limit in digestive gland tissue at 6 wk (Fig. 9B). Mean toxicity of NEO in the remaining tissues decreased slightly from above the FDA limit, 90 ± 45 SD µg STX equivalents·100 g^{-1} to 72 ± 44 SD µg STX equivalents·100 g^{-1}, at or just above the FDA limit depending on the individual scallop (Fig. 9C).

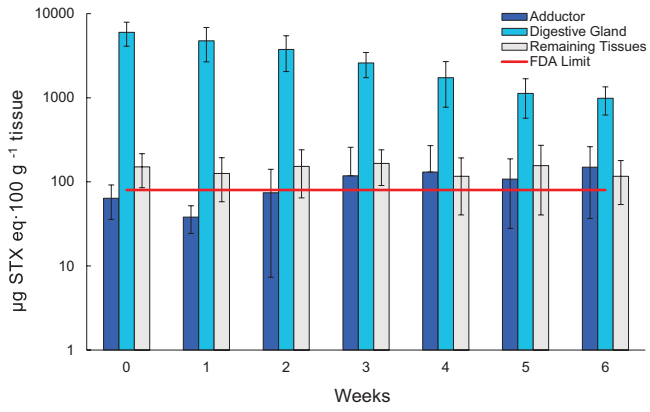

Figure 8. Rock scallops were exposed to natural bloom conditions of *Alexandrium catenella* in Sequim Bay, before being harvested and transported to controlled hatchery conditions for a 6 wk depuration period. Sampling began immediately (week 0) and then weekly for 6 wk. Data represent total mean PST concentration in µg STX equivalents per 100 g tissue plus standard deviation (n = 15) for each tissue type: adductor, digestive gland and remaining tissues (pooled gill, gonad and mantle), determined by high-performance liquid chromatography (HPLC). FDA limit = 80 µg STX eq·100 g^{-1} shellfish tissue is shown as a horizontal line.

DISCUSSION

Paralytic Shellfish Toxins

Rock scallops contained persistently high levels of PST over the 3-y study period, 2017–2020, from Sequim Bay and Discovery Bay, WA. Rock scallop visceral tissue, tested across all time series, years, and bays, remained highly toxic, and did not depurate toxins below the FDA limit for PST (80 µg STX equivalents·100 g^{-1} tissue) over the experimental period. Results from the 6-wk hatchery depuration study further illustrated persistent PST toxicity within whole rock scallops and individual tissues. Although the highest levels of PST were consistently found in the viscera or digestive gland, toxicity levels above the FDA limit were also found in rock scallop adductor muscles. High persistent PST toxicity in scallop species (Shumway et al. 1988, Beitler 1991, Shumway & Cembella 1993, Kaga et al. 2015) and

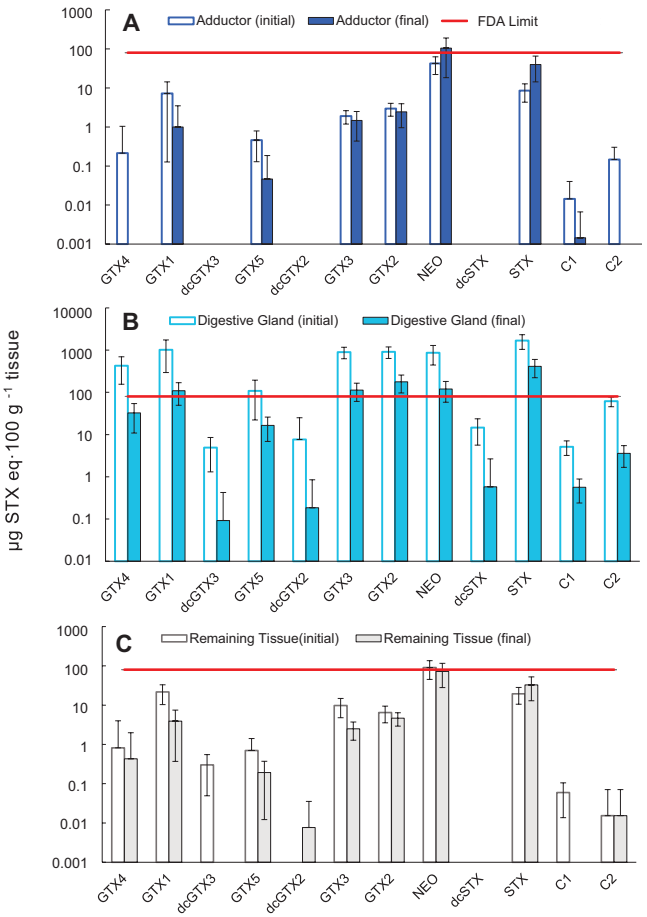

Figure 9. Rock scallops were exposed to a natural bloom of *Alexandrium catenella* in Sequim Bay, before being harvested and transported to controlled hatchery conditions for a 6 wk depuration period. Data represent mean toxin load plus standard deviation of PST congeners in rock scallop (µg STX equivalents per 100 g of tissue), determined by high-performance liquid chromatography (HPLC) in (A) adductor, (B) digestive gland, and (C) remaining tissues (pooled gill, gonad and mantle) at the initial time point and at week 6, the final time point (n = 15 individuals/tissue type). PST congeners analyzed: gonyautoxin 1–5 (GTX1, GTX2, GTX3, GTX4, and GTX5), decarbamoyl gonyautoxin 2 and 3 (dGTX2, dGTX3), NEO, decarbamoyl STX (dcSTX), and *N*-sulfocarbamoyl gonyautoxin 2 and 3 (C1 and C2). FDA limit = 80 µg STX eq·100 g^{-1} shellfish tissue is shown as a horizontal line.

other bivalves has been reported previously (Bricelj & Shumway 1998), including long-term toxicity of butter clams, *Saxidomus gigantea* (Beitler & Liston 1990, Kibler et al. 2022). Paralytic shellfish toxins in rock scallop adductor muscle persisted in some individuals above the FDA limit for up to 4 mo during the field exposures (Figs. 4 and 5) and increased in total toxicity after 3 wk of depuration and proceeded to remain toxic above the FDA limit for 3 additional weeks during the hatchery depuration study (Fig. 8). These findings indicate that the marketable product (adductor muscle) for this species may remain high in PST for weeks and potentially months after initial exposure to an *Alexandrium* spp. bloom, significantly restricting the harvest window and sale of this species for safe human consumption. Given the high level of PST in the viscera at the end of each time series from all field and laboratory exposures, and relatively slow depuration rate in this component and species in general, it is reasonable to discern that depuration of toxins may extend for many months in the viscera, quite possibly a year or longer if scallops still high in PST encounter the following spring bloom season. This species-specific response within rock scallops is in contrast to other commercially available scallop species, including the Atlantic sea scallop, *Placopecten magellanicus,* that does not retain significant PST in adductor muscle tissue specifically (Bourne 1965, Shimizu & Yoshioka 1980, Jamieson & Chandler 1983, Shumway et al. 1988, Shumway & Cembella 1993).

The hatchery depuration study, further supported by the *Alexandrium catenella* feeding experiment, demonstrated that PST congener profiles changed over the depuration phase, with a measurable increase in congeners NEO (feeding study and hatchery depuration) and STX (hatchery depuration only) in rock scallop adductor muscle tissue. Both the NEO and STX are the most toxic carbamate-based neurotoxins of the known PST congeners (Cembella et al. 1993). Changes in the toxin profile in bivalves during both toxification and depuration phases are well-known (Shimizu & Yoshioka 1980, Bricelj et al. 1990, Bricelj & Laby 1996, Choi et al. 2003, Yu et al. 2005, Contreras et al. 2012, Marsden et al. 2015, Liu et al. 2020). Generally, changes occur because of biotransformation processes that include congener conversion in the presence of natural reductants *in vitro,* or resulting from epimerization, the change in physical form from one congener to its chiral counterpart (Cembella et al. 1993, 1994, Bricelj & Shumway 1998).

Significant time lags of toxin uptake within scallop adductor muscles appear in naturally exposed field and hatchery depurated scallops. First, rock scallop viscera concentrate high levels of PST after a reported biotoxin shellfish harvest closure, followed by a gradual increase of toxicity in adductor muscle tissue. In the 2017 field trials, shortly after a shellfish closure for SB in August, toxins in visceral tissue (500 µg STX equivalents·100 g^{-1}), were well above the FDA limit, whereas adductor muscle tissue gradually increased from low toxicity (31 µg STX equivalents·100 g^{-1}) to peak toxicity (162 µg STX equivalents·100 g^{-1}) over a 3-wk period (Fig. 4A). During the PST shellfish harvest closure in DB in October 2017, visceral tissue from rock scallops tested at very high toxin concentrations (1,082 µg STX equivalents·100 g^{-1}), whereas adductor muscle tissues gradually increased in toxicity over a 15-day period reaching peak toxicity levels of 49 µg STX equivalents·100 g^{-1} (Fig. 5A). Extensive testing of adductor muscles for PST on a time frame that accommodates potential changes in toxin load following toxification of whole scallop tissues will be vital for any future shucked, adductor-only market for rock scallop.

Natural field exposures showed high variability of PST among individuals sampled from the same cohort, size class, time period, location, and grow out cage. Individual adductor muscles from subsampled rock scallops did not aggregate around a mean toxicity level at any specific time points when PST were elevated in the whole animal (Fig. 4B, 5B). This finding will make it difficult to predict or extrapolate mean toxicity levels for rock scallops to aid in the regulation and safe consumption of this species, even at relatively small spatial scales. Depuration rates of PST in adductor muscles across individual rock scallops through time were highly variable. Throughout the time series, 10%–20% of individual adductor muscles from subsampled pools remained above the FDA limit for months, whereas others tested at subregulatory levels of PST or below the limit of detection (Fig. 4B, 5B). The high variability of PST among individuals at single time points and throughout the

time series appeared across two different bays and years (Fig. 4B, 5B). Pooling subsamples of rock scallops to derive a mean toxicity of adductor muscles for regulatory purposes of this species should be approached with caution, given the natural variability of toxin uptake, metabolism, retention, and depuration among individual scallops (Fig. 4B, 5B). Variation of toxicity levels among individuals is not uncommon in bivalves or scallops specifically (White et al. 1993), and may be due to one or more of the following factors: varied feeding and metabolic rates, differences in physiological responses (Contreras et al. 2011, 2012, Borcier et al. 2017, Neves et al. 2021), including intracellular biotransformation rates of PST among tissues types (Bricelj & Shumway 1998), density of toxic algal cells present and ingested prior to sampling (Sekiguchi et al. 2001), and the spatial distribution of toxic algal cells in the water column (Sullivan et al. 2003, Kudela et al. 2010, Li et al. 2020). Some or all of these factors may interact to produce rock scallops with highly variable levels of PST.

Diarrhetic Shellfish Toxins

Results from field exposures at Sequim Bay and Budd Inlet from 2017 to 2020 for DST in whole rock scallop and blue mussels showed significant variability in timing of uptake, peak toxicity, and depuration rates between mussels and scallops sampled from the same waterbody at comparable depth and relatively close proximity (<2.5 km). No distinct relationship or trends were apparent between the two shellfish species across all years at both southern and northern Puget Sound sites (Figs. 6 and 7). For example, rock scallops from Sequim Bay (2017 time series, Fig. 7), elicited a strong species-specific response to high cell counts with comparably high levels of DST within whole animal tissue samples. During the same time frame from the same sample location as the rock scallops, blue mussels tested below the FDA limit and demonstrated low toxicity in general (Fig. 7). Shellfish sampled from Budd Inlet in late 2019—early 2020 showed high levels of DST after an initial algal bloom, followed by low cell counts and 6 mo of prolonged toxicity in blue mussels (Fig. 6B). The differences of DST concentrations in blue mussels and rock scallops during this time frame could be due to a number of physiological and environmental factors, including the distribution and availability of *Dinophysis* spp. cells among the two locations (WBM and OYC), where shellfish were sampled. Seasonal shifts and species composition of *Dinophysis* may influence the amount of DST produced by individual cells and subsequent toxicity within shellfish tissues. These factors may also affect the spatial and temporal variability of DST within and among different shellfish species growing in Puget Sound, WA. Species of *Dinophysis* can exist in thin water layers and, when mixed through storms and currents, can become spatially patchy throughout the water column, even on small spatial scales (Reguera et al. 2012). This may have contributed to the observed differences in uptake and toxicity levels in blue mussels and rock scallops sampled from the same or adjacent locations during the field trials. In general, the time series of DST levels for rock scallops and blue mussels indicate high levels of toxicity in shellfish from September to January with one notable exception of blue mussels in spring 2020. Blue mussels (*Mytilus edulis*) studied on the southern coast of Norway showed the highest concentrations of DST in this species to occur from November to February when phytoplankton productivity was lowest (Dahl & Johannessen 2001), similar to findings reported here. Seasonality of species composition of *Dinophysis* spp. may be an important factor affecting toxicity of cells present. During the Budd Inlet shellfish closures for fall–winter months (2017–2018, 2019–2020), the dominant species present was *D. fortii*, shifting toward a composition of *D. fortii* and *D. acuminata* in spring months. During the summer 2018 algal bloom in Budd Inlet, the dominant species present was *D. norvegica* and did not result in a DST shellfish closure.

For field exposures described here, cell counts of *Dinophysis* spp. preceding two DST closures in Budd Inlet on August 4, 2017 (768 cells/L), and November 19, 2019 (2,113 cells/L), and one DST closure of Sequim Bay on October 4, 2017 (945 cells/L), indicates that high cell counts may lead to elevated DST toxicity in blue mussels and rock scallops in both Budd Inlet and Sequim Bay. One data point for Budd Inlet on July 3, 2018, indicated bloom conditions of *Dinophysis* spp. (3,798 cells/L) collected from WBM did not lead to elevated toxicity in rock scallops (2 µg DST·100 g^{-1}) at the same location or blue mussels (5 µg DST·100 g^{-1}) at OYC (Fig. 6A). The dominant *Dinophysis* species at that time point was *D. norvegica*. A recent study detailing the toxin profile of *D. norvegica* samples from Budd Inlet, WA, found only pectenotoxin-2 (PTX2) and no OA/DTX present (Ayache et al. 2023). Pectenotoxins are lipophilic toxins not considered to cause DSP in humans (Miles et al. 2004) and, therefore, not currently part of the routine biotoxin monitoring program in Washington state. Previous studies of *Dinophysis* cell counts and shellfish toxicity have found no significant relationship between the two variables (Dahl & Johannessen 2001, Shultz et al. 2019). A 10-mo study from Budd Inlet, WA, during 2019 found no significant relationship between DST levels and *Dinophysis* cell counts (*D. norvegica*, dominant species), citing species-specific variation of toxicity for *Dinophysis* may occur in Budd Inlet, WA, that may not lead to elevated DST in shellfish (Estrada-Packer 2019).

CONCLUSIONS

It has not been determined from any of the data presented in the present study, field exposure, or laboratory experiments, how long rock scallops need to depurate PST completely from all tissues. Given the high level of PST measured in the viscera at the end of each time series and relatively slow depuration rate, it is reasonable to discern that complete depuration of toxins may extend for many months. It is also possible that toxins can be bound in the tissues for periods of years as seen in other species, for example, the surf clam *Spisula solidissima* (Bricelj & Shumway 1998, Shumway et al. 1994). This aspect of research will need to be explored further with future monitoring efforts to calculate depuration rates. This data gap aside, it is clear that very high, persistent levels of PST in rock scallop viscera will likely preclude this species from safe, whole product consumption. Any consideration of this species as a shucked, adductor-only product will require careful scrutiny of the spatial and temporal variability of shellfish toxicity, variable toxicity among individual scallops from the same cohort and collection time, and as laboratory results showed, biotransformation rates and toxicity of congeners among tissue types throughout the depuration period. Comprehensive PST testing of rock scallop adductor muscles at harvest time

will be critical prior to commercial sale and consumption of this species.

To predict DST-related closures reliably for monitoring and regulation of commercially produced rock scallops, it is clear that more research is needed to decouple the complex interactions of *Dinophysis*, DST, rock scallop physiology, and water quality. Specifically, variability of toxicity among *Dinophysis* species, their seasonal shifts, and the relation to DST in shellfish are in need of further investigation.

ACKNOWLEDGMENTS

This study was supported by National Sea Grant, grant No. 2606753. We thank Jamestown S'Klallam Tribe Natural Resource staff, including Elizabeth Tobin, Neil Harrington, and Chris Burns, for their regional knowledge, topic expertise, and assistance with field trials in Sequim and Discovery Bays, WA. Neil Harrington provided plankton data included in the DST field trials in Sequim Bay, WA. We acknowledge the SoundToxins program for their leadership in phytoplankton data collection throughout Puget Sound, including Sequim Bay and Budd Inlet, and for sharing the data from these sites. We thank Jerry Borchert and Tracie Barry for advising on Washington state shellfish DST concentrations and contributing the blue mussel DST datasets collected by the WDOH Shellfish Program. The WDOH Public Health Laboratories provided valuable DST analysis and the Sitka Tribe of Alaska Environmental Research Lab provided PST analysis of rock scallop tissue from the Sequim and Discovery Bay field trials. Ashley Lockhart and Kelsey Donahue provided assistance with laboratory trials at the Pacific Hybreed Hatchery in Manchester, WA; Zhihong Wang at NOAA/NOS Marine Biotoxins Program in Charleston, SC, provided PST analysis of rock scallop tissue samples from the laboratory feeding trials; Mary Middleton, Natalie Sahli, and Isabel Platten assisted with field trials. Thank you to Pacific Shellfish Institute Senior Scientist, Daniel Cheney for early manuscript review and counsel.

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