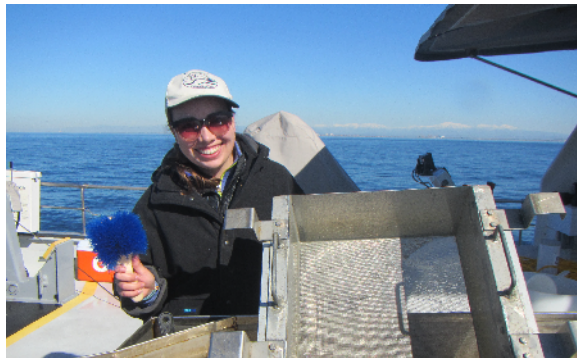




Harmful Algal Blooms

BIGHT '18



Southern California Bight
2018 Regional Monitoring Program
Volume VIII

SCCWRP Technical Report 1170

Southern California Bight 2018 Regional Monitoring Program: Volume VIII. Harmful Algal Blooms

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FOREWORD

The 2018 Southern California Bight Regional Monitoring Program (Bight '18) is part of a collaborative effort to provide a large-scale, integrated assessment of the Southern California Bight (SCB). Bight '18 is a continuation of previous regional monitoring surveys conducted on a five-year cycle since 1994. This collaboration represents the joint efforts of 46 organizations. Bight '18 is organized into five elements: 1) Sediment Quality (formerly Contaminant Impact Assessment/Coastal Ecology), 2) Microbiology, 3) Ocean Acidification, 4) Harmful Algal Blooms, and 5) Trash. This assessment report presents the benthic domoic acid study results which is the main component of the Harmful Algal Blooms element. Copies of this and other Bight '18 reports, as well as workplans and quality assurance plans, are available for download on SCCWRP's Regional Monitoring website: <https://www.sccwrp.org/about/research-areas/regional-monitoring/southern-california-bight-regional-monitoring-program/>.

ACKNOWLEDGEMENTS

This report came to fruition because of the hard work and commitment of many individuals and organizations who share a common goal of improving our understanding of the environmental quality of the Southern California Bight. The authors wish to thank the members of the Bight '18 Harmful Algal Blooms Committee for their assistance with study design, sample analysis, data analysis, and report review. This study would not have been possible without the knowledge and skill of the field sampling personnel from the following organizations: City of San Diego, Orange County Sanitation District, Los Angeles County Sanitation Districts, City of Los Angeles Environmental Monitoring Division, Southern California Coastal Water Research Project, Marine Biological Consulting, and Aquatic Bioassay and Consulting Laboratories. We also wish to thank Ami Latker (City of San Diego) for data interpretation and discussion; Miranda Roethler, Rosaly Castorena, Raeann Iler, Kelcey Chung, Sarah Bowen and Aimee Ellison (SCCWRP) for assistance with benthic infauna collection, sorting and processing. We would also like to thank the National Centers for Coastal Ocean Science (NCCOS) HAB Event Response Program for supporting sampling efforts in 2017. Analysis of domoic acid concentrations was provided by three laboratories: Kudela Laboratory at the University of California, Santa Cruz, Weck Laboratories, Inc., and Orange County Sanitation District, Laboratory, Monitoring & Compliance Division. This document is NCCOS HAB Event Response publication #29.

EXECUTIVE SUMMARY

Harmful algal blooms (HABs) are a persistent and escalating issue in California's coastal and inland waterbodies. In coastal waters, the most observed HAB organisms in the region are species within the diatom genus *Pseudo-nitzschia* spp., several of which produce the neurotoxin domoic acid (DA). Large *Pseudo-nitzschia* spp. blooms have been frequently documented in the Southern California Bight (SCB), and DA associated with these events has been known to contaminate fisheries, creating both human and wildlife health risks. Recent studies have indicated that marine sediments may act as a reservoir for DA, extending the risk of food web contamination long after water column blooms end. Notably, the Dungeness crab fishery was closed for nearly a year after a historic bloom of *Pseudo-nitzschia* in 2015 that spanned the U.S. west coast, resulting in severe ecological and socioeconomic impacts.

The Southern California Bight 2018 Regional Marine Monitoring Program (Bight '18) provided the first ever regional assessment of extent and magnitude of DA in the benthic environment. The Bight '18 HABs component aimed to address three main questions about DA in benthic environments:

1. What is the extent and magnitude of DA in continental shelf sediment?
2. How does the concentration of DA vary over different time scales?
3. How does sediment DA concentration compare to concentrations in benthic infauna?

Approach. Bight '18 undertook three major sampling efforts to address these study questions. To understand the areal extent of DA in SCB sediment, we collected sediments and measured DA concentrations in 90 probabilistically drawn stations within three shelf habitats (inner (0-30 m), middle (30-120 m) and outer (120-200 m) shelf) in the summer of 2018. To assess multi-year spatial and temporal changes of DA and investigate lower ecosystem impacts, we collected sediment and benthic infauna samples from 20 stations in central SCB in the summers of 2017 and 2019. In the summer of 2019, in addition to the revisit of the 20 stations sampled in 2017, 11 additional sediment samples were collected off the coast of San Diego to expand spatial coverage. Lastly, we investigated temporal variation in DA in SCB sediment (n=190) and benthic infauna tissue (n=89) by measuring DA in samples collected monthly for 16 months within the Central Bight between March 2018 and June 2019. For comparison, observations of DA in the water column at four piers in the SCB were collected by the California HABMAP program (<https://calhabmap.org/>) during the study period.

Extent and Magnitude of Sediment Domoic Acid. DA was widespread in continental shelf sediments of the SCB. The toxin was detected in 54% of the total shelf habitat area sampled and was most prevalent in the middle shelf strata (67% of mid-shelf area). In 2018, detectable concentrations ranged from 0.11 ng/g to 1.36 ng/g. The highest concentrations of DA were observed in regions with histories of water column blooms and retentive circulation patterns.

Time-Scales. DA was consistently detected in the sediments during 3 years of field surveys, although the spatial extent of sites sampled varied across years. DA was detected in the sediments annually in 2017, 2018 and 2019, with maximal concentrations of > 150 ng/g observed in 2017. DA concentrations in sediment were commonly below detection or at low

concentrations (< 0.25 ng/g) across monthly times scales, although these observations were spatially limited. The observations of DA in sediments did not always have an apparent water column source.

Domoic Acid in Infauna. DA was consistently detected in benthic infauna tissues on annual and monthly timescales. In most samples, DA was detected in tissues but not in the co-located surface sediments. The FDA safe-to-eat level of 20 ppm was used to assess infauna tissue toxin concentrations that would be considered high in fishery species and relate to risk of bioaccumulation to higher trophic levels. Samples were above the FDA safe-to-eat level in 10% of all the infauna samples collected. Coarse taxonomic sorting of the infauna suggested the DA differentially accumulated in different taxa. DA tissue concentrations were also consistently higher at one monitoring station compared to the other, suggesting that there might be site-specific risks of increased DA body burdens in benthic infauna. The persistent presence of DA in lower ecosystem organisms pose a risk for transfer into higher trophic levels including key benthic fishery species. Further investigation is needed to establish exposure pathways for infauna species and trophic transfer.

Next Steps. We demonstrated the environmental persistence of DA in SCB benthic environments, but the mechanisms mediating DA cycling and persistence are not well understood. Developing a deeper understanding of these processes will allow for characterization of regional and site-specific risk of persistent sediment DA and how long the risk extends beyond bloom events. DA was observed in sediments during periods when detectable water column DA concentration at HABMAP pier stations were rare, however pier monitoring locations only represent very nearshore environments and blooms might occur in offshore or subsurface environments that are difficult to monitor. Better characterization of the spatial and temporal variability of *Pseudo-nitzschia* and DA is needed to understand potential sources of DA to the benthos. Nonetheless, this work suggests that sediments may be a long-term source of DA to higher trophic levels in the benthos; therefore, future studies should consider examining the rate of occurrence of DA in key benthic fishery species. This work highlighted the importance of standardized DA analysis methodology for the extraction and analysis of DA in marine sediments. Future work should include the development of standardized analysis protocols to ensure the comparability of future research and monitoring efforts. Lastly, recent observations have shown that with shifting climate regimes other HAB species and their associated toxins may become increasingly relevant in the Bight over the next few decades. Future studies should work to better characterize the occurrence of these taxa and toxins in the region, as well as assess the environmental factors that support the growth and toxin production of these organisms within the Bight.

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INTRODUCTION

The Southern California Bight (SCB) is a roughly 700-km bend in the coastline of the U.S. west coast that extends from Point Conception, California, south to beyond the U.S. international border with Mexico. The SCB is a unique region of the California coastline, with distinct oceanography from the coastal region north of Point Conception. Curvature of the SCB coastline acts to buffer the Southern California coast from prevailing wind patterns that influence the rest of the north-south trending coastline of the state. Wind events, rainfall and river discharges are highly seasonal and upwelling events are most common during winter and spring. Together with the coastal topography, the presence of the Channel Islands creates complex bathymetry and currents in the region that support a diverse marine ecosystem in the SCB. The SCB provides a habitat to more than 500 species of fish and several thousand species of invertebrates and is also a major migration route for marine bird and mammal populations.

Harmful algal blooms (HABs) and associated algal toxins have been a persistent and escalating issue in California's coastal and inland waterbodies. Globally, HABs have increased in frequency, severity and spatial extent over the past decade, and anthropogenic nutrient inputs and warmer temperatures (i.e., climate change) are considered the most significant factors contributing to these increases (Anderson et al. 2002; Glibert et al. 2005; Hallegraeff 1993; Hallegraeff et al. 2004; O'Neil et al. 2012; Paerl et al. 2011). Blooms of HAB taxa in the urbanized central SCB have increased over the last 15 years (Kim et al. 2009; Schnetzer et al. 2007; Seubert et al. 2013; Shipe et al. 2008; Smith et al. 2018a). However, the causes of this increase have been difficult to tease apart. Elevated concentrations of nitrogen from anthropogenic sources has been proposed as a factor in the apparent increase in HAB events and some evidence suggested that anthropogenic nitrogen sources could either promote the specific growth of or increased toxin production by toxigenic species (Howard et al. 2007; Kudela et al. 2008; Lundholm et al. 2010). Regional studies in the SCB have reported increased chlorophyll *a* concentrations unrelated to upwelling events, implying anthropogenic nitrogen sources may contribute to local phytoplankton blooms (Corcoran et al. 2010; Kim et al. 2009; Nezlin et al. 2012), but they have not been linked to the specific stimulation of HAB events, and more recent measurements taken in 2015-2016 suggested that primary productivity is not elevated in close proximity to these sources (McLaughlin et al. submitted). Furthermore, a long-term study of chlorophyll in the SCB demonstrated the importance of Pacific Basin scale events on bloom development in the region, such as deepening of the nitricline, which may obscure any potential influence of coastal sources (Nezlin et al. 2018).

The most commonly observed HAB organisms in the SCB are species within the diatom genus *Pseudo-nitzschia*, several of which produce the neurotoxin domoic acid (DA) (Smith et al. 2018a). Trophic transfer of DA in the food web can contaminate fisheries, presenting both human and wildlife health hazards. Consumption of contaminated seafood can cause amnesic shellfish poisoning (ASP) in humans, resulting in diarrhea, gastrointestinal pain, disorientation and memory loss, and in extreme cases, death (Bates et al. 1989). Stranding and mortality events in marine mammals and birds have also been attributed to exposure to DA (Fire et al. 2010; Lefebvre et al. 2002; Smith et al. 2018a). Annual blooms of *Pseudo-nitzschia* spp. that produce DA have been documented in California since the 1990s and annually in the SCB since 2003. DA producing blooms have caused major socioeconomic impacts, including prolonged closures of key fish, bivalve and crab fisheries (Lefebvre et al. 2002; McCabe et al. 2016; Scholin et al.

2000). The longevity of these events is typically limited, with events generally lasting a few weeks, although in 2015, a nearly West Coast wide bloom of *Pseudo-nitzschia* persisted for multiple months (Du et al. 2016; McCabe et al. 2016; Ryan et al. 2017). This 2015 bloom resulted in extensive impacts to the food web and DA caused pervasive impacts on multiple species of marine mammals, birds and fish during the bloom (McCabe et al. 2016). The bloom also caused closures of both commercial and recreational Dungeness crab and razor clam fisheries along the U.S. west coast that extended for over a year after the bloom ended due to prolonged contamination with DA (Ekstrom et al. 2020). The long-term impacts of the 2015 bloom renewed questions about the longer-term ecological and economic impacts of blooms, particularly the impacts on the benthos, which are not well characterized in many regions.

To date, few studies have examined the presence or impacts of DA in benthic environments in the SCB, and those that have were limited in time and geographic scope (Schnetzer et al. 2007; Sekula-Wood et al. 2011, 2009; Umhau et al. 2018). A majority of work to date has focused on characterizing DA in the particulate fraction and within shellfish tissues. Monitoring over the last 15 years has shown that DA has been observed in shellfish tissue on a near annual basis in the region (Smith et al. 2018a). Particulate DA concentrations observed during toxigenic blooms of *Pseudo-nitzschia* spp. in the SCB often exceed concentrations of 10 mg/L and some events have even exceeded 50 mg/L (Smith et al. 2018a). Observations of dissolved DA concentrations have been more limited in the region, but recent work has indicated that dissolved DA may be a significant fraction of the total DA (particulate plus dissolved DA) pool with contributions as high as 50% (Umhau et al. 2018).

Previous Southern California Bight Regional Marine Monitoring Program Studies

The Southern California Bight Regional Marine Monitoring Program (Bight Program) is an integrated and collaborative monitoring program designed to provide large scale assessments of the SCB. A key line of investigation for the water quality assessments of the Bight Program has been to understand potential impacts of land-derived nutrients on the SCB on algal bloom development in general, and HABs in particular. More than 20 million people live within an hour's drive of the coast and coastal infrastructure includes 17 wastewater treatment facilities, the nation's two largest commercial ports, more than 20 pleasure craft harbors, and the nation's third-largest naval facility (Lyon and Stein 2009). Additionally, there are 17 major watersheds that discharge largely untreated surface runoff from urban and agricultural land uses to the SCB. Land use in the regions around the SCB vary significantly, with large regions of urbanized coastline, agricultural lands and undeveloped land. This diversity of land use results in unique sub-regional nutrient loading into the coastal zone (Sutula et al. submitted). Collectively, anthropogenic nutrient inputs into the coastal zone appear to constitute a significant nutrient source, particularly in highly urbanized regions of the coast (Howard et al. 2014; Kudela et al. 2008; Reifel et al. 2013). There has been concern that the anthropogenic inputs may stimulate additional primary production, potentially leading to increased occurrence of HABs.

While the Bight Program has tackled extent and magnitude of algal blooms in general, this is the first assessment focused specifically on HABs. The Bight 2008 Program (Bight '08) investigated the relative magnitude of local nutrient sources and nutrients derived from upwelling. This study found that the relative magnitude of nutrient sources was scale dependent. That is, region-wide upwelling provided the greatest magnitude of nitrogen loading to the SCB, but in highly urbanized coastal zones, anthropogenic nutrient inputs were of a similar magnitude (Howard et

al. 2014). Bight '08 also found that the extent of surface algal blooms was shown to have increased over the last decade, with chronic blooms documented in areas of the SCB co-located with major inputs of nutrients and longer residence times of coastal waters (Nezlin et al. 2012). It also provided insight into algal bloom development by documenting the transport of a subsurface *Pseudo-nitzschia* spp. bloom closer to shore and into surface waters during an upwelling event, resulting in bloom intensification (Seegers et al. 2015). The benthic sampling efforts from Bight '08 were also leveraged to collect sediment samples to assess the presence of DA in marine sediments in tandem with a sediment trap study in the Santa Barbara Basin, which was one of the first studies demonstrating the transfer of DA to the benthic environment from surface waters (Sekula-Wood et al. 2011).

The Bight 2013 Program (Bight '13) demonstrated the importance of regional factors in algal blooms in the SCB. This program evaluated decades of water column chlorophyll profiles and found that chlorophyll concentrations and the depth to the chlorophyll maximum is strongly impacted by regional factors such as upwelling and Pacific Basin-scale changes in circulation (e.g., Pacific Decadal Oscillation) (Nezlin et al. 2018). Investigations of primary production and phytoplankton nitrogen uptake demonstrated that there was no difference in the rates of these processes in close proximity to ocean outfalls compared to locations distal from outfalls (McLaughlin et al. submitted).

The Bight Program has served to illuminate the complexity of algal blooms within the SCB. The Harmful Algal Blooms element of the Bight '18 Program builds upon these previous studies with a goal of conducting the first regional assessment of DA presence in the benthic environment of the continental shelf. While the pelagic impacts of DA have been well studied, the fate and persistence of DA has been historically understudied. However, the large socioeconomic impacts in recent years have created interest from California management communities to understand the long-term impacts and fate of DA in benthic habitats. The Bight '18 program will provide a regional assessment of DA in the sediments, as well as a monthly time-series in Newport Beach, to determine how extensive DA contamination is in the SCB and how these concentrations change throughout the year.

Objectives of the 2018 Regional Monitoring Program

The Harmful Algal Blooms element of the Bight '18 Program aimed to address three main questions about DA in benthic environments:

1. What is the extent and magnitude of DA in continental shelf sediment?
2. How does the concentration of DA vary over different time scales?
3. How does sediment DA concentration compare to concentrations in benthic infauna?

Additionally, this element sought to address a second HAB research theme to understand regional impacts of freshwater HAB toxins transported down watersheds to the coast. This study element was piloted during Bight '18. Although results indicated that freshwater toxins were detected in shellfish deployed at the land-sea interface, the study was not implemented region-wide due to a need for increased method sensitivity and standardization. The findings of this pilot study are discussed in Appendix C.

I. METHODS

Program components

This study is divided into three study components. First, to understand the areal extent of DA in SCB sediment, the **2018 Bight Program** collected sediment samples for DA analysis from 90 stations throughout the SCB in the summer of 2018. Second, to assess multi-year spatial and temporal scales of DA in sediment and benthic infauna, the **Central Bight Revisit Study** leveraged samples collected in 2017, as part of National Centers for Coastal Ocean Science (NCCOS) HAB Event Response efforts, sampled and collected sediment and benthic infauna from 20 stations in central SCB in the summer of 2019. Additionally, in 2019, 11 sediment samples were also collected off the coast of San Diego. Third, to assess shorter temporal scale changes in DA in SCB sediment and benthic infauna tissue, the **Monthly Revisit Study** began in spring of 2018 and continued for 16 months. Samples were collected for the analysis of DA in sediment from three stations off the coast of Newport Beach (these stations were also sampled in the Central Bight Revisit Study) and in benthic infauna tissue samples from two of the stations.

2018 Bight Regional Program

a. Sampling design and sample collection

Sediment samples were collected for the analysis of DA concentrations from 90 stations throughout the SCB from Pt. Conception in the north to the U.S.-Mexico international border in the south (Figure 1A). The sampling effort was carried out by multiple agencies that contribute to SCCWRP's Bight Marine Monitoring Program. A stratified random sampling design was selected to ensure an unbiased sampling approach to provide areal assessments of environmental condition (Stevens 1997). Stratification provides an appropriate number of samples (target $n = 30$) to characterize each stratum with adequate precision (90% confidence interval of about $\pm 10\%$ around estimates of areal extent assuming a binomial probability distribution and $p = 0.2$). Three strata were sampled: inner (0-30 m), middle (30-120 m) and outer (120-200 m) continental shelf. One sampling location for the outer-shelf exceeded the outer-shelf maximum target depth of 200 m (actual sample depth was 212 m) and was moved to the upper slope strata for the Bight '18 Sediment Quality Assessment (Du et al. 2020); however, for the purposes of this study, it was included as an outer-shelf sample.

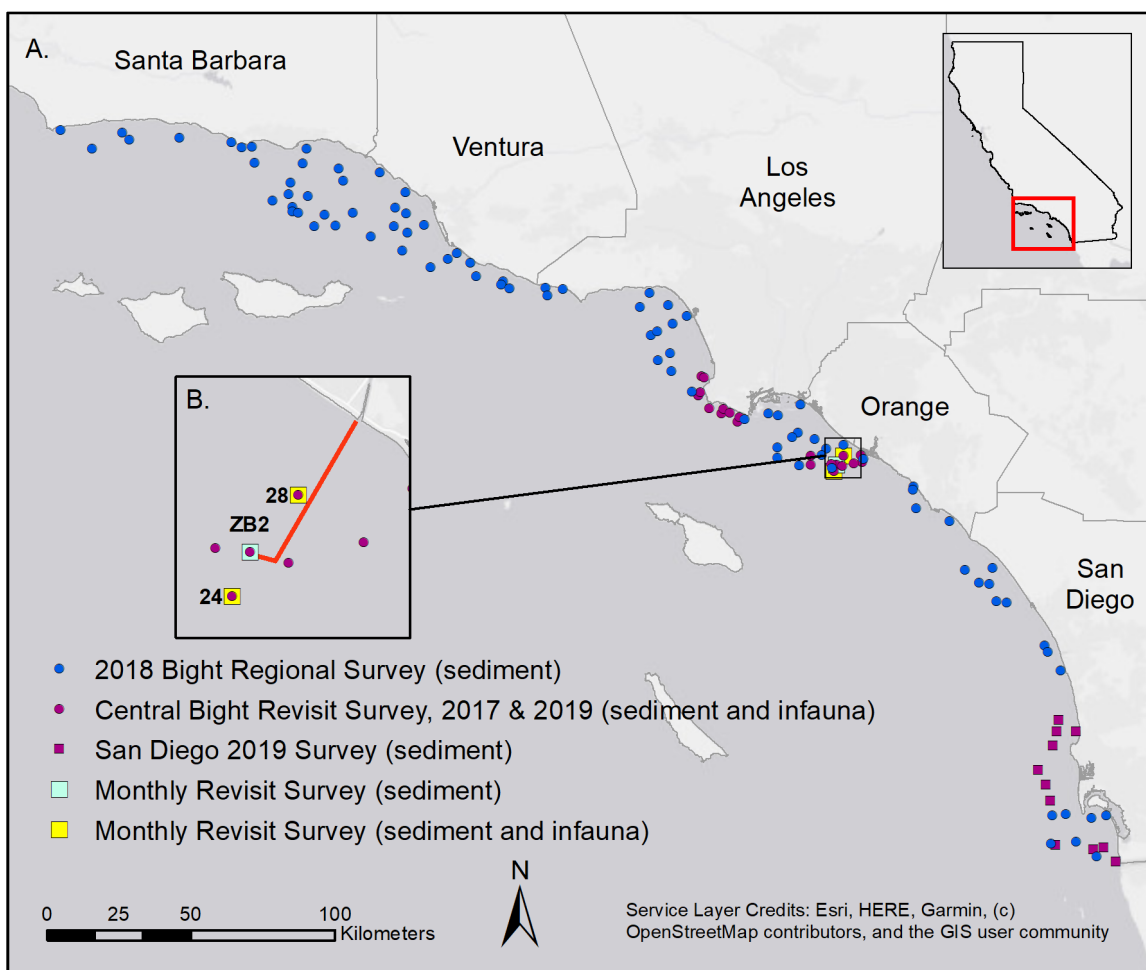


Figure 1. Map of sampling locations for all program components. Panel A shows an overview of the entire study area where blue circles represent the 2018 Bight Regional Program, yellow squares represent the Monthly Revisit Study, purple circles represent the Central Bight Revisit Study stations sampled in 2017 and 2019, and purple squares represent the Central Bight Revisit Study stations sampled in 2019 only. B) Inset map of Monthly Revisit Study stations in relation to the Orange County Sanitation District ocean outfall. Yellow squares represent the revisit stations where both sediment and infauna were collected, and the light blue squares represent the revisit station where only sediment was collected.

b. Sediment DA sample collection and analyses

Sediment samples were collected using a tandem Van Veen grab. Upon recovery, the top 2 cm of sediment was scooped into an amber jar which was kept frozen at -20 °C until analysis. All 90 sediment samples collected were extracted and analyzed by Weck Laboratories. Extractions were conducted according to Sekula-Wood et al. (2011). Briefly, 30 mL of 50% MeOH were added to 5 g sediment wet weight, and samples were left on a shaker table at 4°C for 12-24 hours in the dark, centrifuged at 3800 RPM for 10 minutes, and filtered through a 0.2 µm filter. DA was quantified on an LC-MS/MS according to a modified Wang et al. (2012) protocol using $^{13}\text{C}_3$ -caffeine as an internal standard. Minimum detection limit for these samples ranged from 0.10-0.15 ng/g.

c. Environmental variables

A suite of environmental samples, including grain size, total organic carbon (TOC) and total nitrogen (TN) used to contextualize this study's findings were collected as part of the Bight '18 Sediment Quality element. For grain size sample collection, 100 g of sediment was collected from the top 2 cm of each Van Veen grab and stored in a 4 oz. plastic container at 4°C until analysis. Grain size samples for this study were analyzed by the City of San Diego, Physis and Eurofins with results reported as phi size (% of sample). Phi sizes were then grouped as fine particles ($\Phi \geq 5$), fine sands (Φ 3-4), coarse sands (Φ 1-2) and coarse particles ($\Phi \leq 0$) for analysis. For TN and TOC sample collection, 200 g sediment from the top 2 cm were scooped into an 8 oz amber jar and frozen within 24 hours. Samples were analyzed by City of Los Angeles (CLA), Los Angeles County Sanitation Districts (LACSD), Orange County Sanitation District (OCSD) and City of San Diego (CSD). Further details on sample collection and analysis of TN and TOC, as well as grain size, can be found in both the Sediment Quality Assessment Field Operations Manual and the 2018 Sediment Chemistry Report (Du et al. 2020).

Central Bight Revisit Study, 2017 and 2019

a. Sampling design and sample collection

Sequentially, the first sampling events of this project were conducted in the summer 2017 by the LACSD and OCSD. A total of 20 stations in central SCB was sampled for concurrent sediment and benthic infauna DA. Stations were sampled as a part of a larger, NCCOS funded, event response study (NCCOS 2017). Areal extent of the stations ranged from off the coast of Torrance in the north to off the coast of Newport Beach in the south. The southernmost 10 stations were sampled by OCSD on 7/18/17 and 7/19/17. The northernmost 10 sites were sampled by LACSD on 7/31/17 and 8/1/17. All samples were successfully collected and quantified with the exception of two benthic infauna samples (OCSD Stations 44 and 5) that did not have enough biomass for analysis.

In the summer of 2019, the same 20 stations were resampled by OCSD and LACSD for sediment and benthic infauna. The 10 southernmost central SCB stations were sampled by OCSD on 7/15/19, 7/17/19 and 7/18/19. The 10 northernmost central SCB stations were sampled by LACSD on 7/15/19, 7/22/19 and 7/31/19. In addition to the 20 revisited stations, the City of San Diego collected 11 sediment DA samples on five dates ranging from 7/11/19-7/29/19. Sediment was collected by OCSD and LACSD with a tandem Van Veen grab following the same approach as the Bight '18 Program described above.

b. Sediment DA analyses

In 2017, sediment samples were extracted and analyzed by the Kudela Lab at the University of California, Santa Cruz (UCSC). Samples were extracted and analyzed following the methods described in Sekula-Wood et al. (2011). Briefly, 30 mL of 50% MeOH was added to 5 g sediment wet weight, and samples were left on a shaker table at 4°C for 12-24 hours in the dark, centrifuged at 3800 RPM for 10 minutes, and filtered through a 0.2 μ m filter. Samples were quantified on an LC-MS using an external DA standard (National Research Council Canada,

Halifax, NS, Canada) curve with spike-recovery of a subset of samples to assess matrix effects and recovery. Minimum detection limit for sediment samples was 0.18 ng/g.

In 2019, samples were extracted and analyzed by the Laboratory, Monitoring & Compliance Division at OCSO. The extraction and quantification methods are outlined in Phonsiri et al. (in prep) based on an optimized method developed in-house. Briefly, 10 mL of 10% MeOH was added to 10 g of ideal wet weight (wet weight equivalent to 10 g dry weight sediment) and vortexed for 2 minutes on high. Samples were then centrifuged at 3500 RPM for 10 minutes and the supernatant was collected for analysis on a LC-MS/MS instrument using an external DA standard curve. The minimum detection limit for this method was 0.5 ng/g. The 11 San Diego samples collected were extracted and analyzed by Weck Laboratories. Weck Laboratories extractions were conducted according to Sekula-Wood et al. (2011) and quantified on an LC-MS/MS according to a modified Wang et al. (2012) protocol using $^{13}\text{C}_3$ -caffeine as an internal standard (the same lab and method used for the samples collected for the 2018 Bight Program and Monthly Revisit Study). Minimum detection limit for these samples ranged from 0.10-0.12 ng/g. More information about variation in sediment extraction protocols used throughout the different components of this project and laboratory intercalibration can be found in Appendix B.

c. Benthic Infauna DA sample collection and analyses

Benthic sediment grabs were taken using a tandem 0.12 m² Van Veen grab sampler. A sub-sample (upper 2 cm) was collected for sediment DA analysis from one grab. Sediment from the second grab was placed on a wash tray and rinsed with filtered seawater through a 1.0 mm mesh screen to collect infauna. If insufficient biomass was obtained, additional drops were made to obtain enough biomass for toxin analysis. Samples were kept cool and in the dark until they were brought back to the lab at SCCWRP, where they could be further rinsed to remove remaining sediment, sorted, and stored frozen at -20 °C until analysis.

For extractions, samples were thawed and any organisms with hard parts that would not break down during homogenization were dissected to remove these parts. The material that was not homogenized included urchin tests, gastropod shells and thick bivalve shells. In the majority of bivalves collected, the shells crushed easily with surgical scissors, so these remained as part of the sample. After any needed dissections, all samples were coarsely chopped with surgical scissors to aid in homogenization.

In 2017, all benthic infauna tissues from a given station were homogenized together, yielding a composite sample of all organisms collected. Samples were prepared and analyzed by the Kudela Lab at UCSC following the tissue methods described in Peacock et al. (2018). To extract the composite tissue homogenates, 10 mL of 50% MeOH was added to 1 g of tissue, the sample was homogenized for 30 seconds, centrifuged at 3800 RPM for 20 minutes and passed through a 0.2 µm filter. 3 mL of extract were SPE cleaned using Biotage ISOLUTE 500 mg columns pre-conditioned with 6 mL 100% MeOH, 3 mL MQ and 3 mL 50% MeOH, and the toxin was eluted with 10% acetonitrile and 2% formic acid. Quantification was conducted on an LC-MS using an external DA standard curve.

In 2019, infauna were sorted into groupings defined by broad taxonomic class and presumed feeding strategies to determine if differential accumulation of DA might be occurring. Animals were divided into four groupings. Group 1 was primarily comprised of marine worms

(predominantly taxa such as polychaetes and nemerteans) and rarely non-worm taxa such as holothurians. Since this group was dominated by worm taxa, it is referred to as marine “worms”, Group 2 was primarily comprised of filter feeders (predominantly bivalves, tunicates, barnacles and minor contributions of non-target organisms such as siphonophores and scaphopods), Group 3 was primarily comprised of surface feeders (predominantly urchins, sea stars, snails, shrimp, isopods and amphipods), and Group 4 comprised of brittle stars, also surface feeders. Sorted organisms were stored in 50 mL falcon tubes or wide-mouth HDPE jars, depending on the size and number of organisms collected. The weight of each feeding group was taken, and samples were stored frozen at -20 °C until analysis.

Samples were extracted based on the methods described in Litaker et al. (2008) and Kvitek et al. (2008). For samples with less than 3 g of tissue for a given sorting group, 50% MeOH was added in a 1:10 (tissue:methanol) ratio, then the sample was homogenized using an Omni Tissue homogenizer with hard tissue plastic probe. For samples greater than 3 g, tissue was first homogenized dry using the hard tissue plastic probe. Following this step, 1 g of the homogenate was aliquoted into a 15 mL falcon tube and 10 mL of MeOH was then added (to maintain the 1:10 tissue:methanol ratio). The sample was homogenized and vortexed thoroughly for 1 minute. Samples were then centrifuged at 4,100 RPM for 25 minutes at 4°C. The supernatant was filtered with a 0.45 µm polyethersulfone syringe filter (Litaker et al. 2008) and the extract was stored in the freezer at -20 °C until analysis.

Benthic infauna extracts collected in 2019 were analyzed at SCCWRP with enzyme-linked immunosorbent assays (ELISA; Mercury Science DA Test Kit product #DAK-36) using the methods described in Litaker et al. (2008) for shellfish tissues. All samples were run with at least a 1:10 dilution with the sample diluent provided in the kit to mitigate any matrix effects from the methanol. This method has a minimum detection limit of 0.01 ppm. Although benthic infauna generally are not consumed by humans and are not harvested commercially in the SCB, the FDA safe-to-eat level of 20 ppm was used to contextualize infauna tissue toxin concentrations against concentrations that would be unsafe for commercial fish and shellfish tissues and also provide context for bioaccumulation risk in higher trophic levels. DA concentrations of benthic infauna tissue samples are reported as ppm throughout this study to facilitate an easier comparison with the FDA safe-to-eat level.

d. Binning sorted benthic infauna tissue concentrations

The groupings described above were based on coarse taxonomic identifications that were not refined enough to accurately assess feeding strategies and therefore could not be used to address varying toxin loads between different feeding types as originally intended. Ultimately, the Bight ‘18 HABs committee decided a more appropriate question to ask is: what concentration of DA is found in the benthic infauna as a whole from each station? Because the samples were processed and quantified individually for each sorted group, a weighted average was calculated to bin all benthic infauna data from each sample. This was calculated using the following formula where the subscript denotes each of the four groups:

$$\frac{([DA_1] * wt_1) + ([DA_2] * wt_2) + ([DA_3] * wt_3) + ([DA_4] * wt_4)}{(wt_1 + wt_2 + wt_3 + wt_4)}$$

The concentration from each group (denoted as $[DA_{1-4}]$) was multiplied by the weight of that group (denoted as wt_{1-4}) to give total mass DA. The mass of DA across all four groups was summed and divided by the total weight of benthic infauna in the sample and these numbers are reported as the sample weighted average DA in benthic infauna tissue throughout this report.

Monthly Revisit Study

a. Sampling design and sample collection

Sediment samples were collected from three stations (28, ZB2 and 24) located along the OCSD Ocean Outfall pipe (Figure 1B). Benthic infauna samples were collected from two of those stations (28 and 24). The deepest station, Station 24, had an average sampled depth of 304 m and was chosen to represent a region with high deposition. The shallowest station, Station 28, had an average sampled depth of 32 m, is heavily influenced by the Santa Ana River and was chosen to represent a region with lower deposition. At the end of the 5-mile OCSD effluent outfall, near the center of the diffusers, Station ZB2 had an average sampled depth of 57.6 m. These stations were sampled monthly from March 2018 – June 2019 by OCSD staff aboard the M/V *Nerissa*. In total, there were 16 visits to Stations 24 and ZB2, and 17 visits to Station 28 (on one sample date, Station 28 was successfully sampled before the boat turned back on account of bad weather).

b. Sediment DA sample collection and analyses

Sediment was collected with a tandem Van Veen grab following the same methods described above. All 49 sediment samples collected were extracted and analyzed by Weck Laboratories using the same methods as the 2018 Bight Program. Minimum detection limit for these samples ranged from 0.10-0.25 ng/g.

c. Benthic Infauna DA sample collection and analyses

Benthic infauna samples were collected, sorted, extracted, and quantified at SCCWRP as described in Central Bight Revisit Study methods for 2019 samples above. Benthic infauna tissue concentrations were calculated as weighted averages of DA as described in the Central Bight Revisit Study methods for 2019 samples. In addition, results were calculated as concentrations present in 2 groups: DA concentrations in Group 1 (marine worms) tissues and the weighted average of groups 2-4. Although the grouping of marine worms includes worms with varied feeding strategies and minor contributions from other taxonomic groups, there were consistently high levels of DA detected in this group throughout the timeseries compared to all other benthic organism groupings therefore the DA concentrations of marine worms were compared to the weighted average of the remaining three groupings. This type of analysis was not conducted for the 2019 Central Bight Revisit Study due to the lack of temporal resolution.

Data Analysis

Sediment and tissue DA concentrations were analyzed to determine descriptive patterns in the extent and magnitude of DA concentrations. Statistical analyses throughout this report were conducted in R (R Core Team 2020) and figures were generated using ggplot2 (Wickham 2016; excluding maps which were generated in ArcMap 10.6.1). All data below the method detection limit were treated as zero when data were reported.

Box plots were used to compare DA concentrations in sediments between strata in tissue concentrations between stations. The middle of the box is centered on the median, the lower and upper hinges correspond to the first and third quartiles, and outliers are defined as points further than 1.5 times the inner-quartile range. Data points are plotted over the box using the package beeswarm (Eklund, 2016). For comparisons across 3 or more categories, a Kruskal Wallis (non-parametric) rank sum test was run in R using the package stats (R Core Team 2020) to determine if differences existed between the categories. When a significant difference was found, the FSA package was used to run a post hoc Dunn's comparison test for multiple pairwise comparisons (Dinno 2017; Ogle et al. 2020). The false detection rate was controlled using the Benjamini-Hochberg adjustment and adjusted p values are reported. A Wilcoxon Rank Sum test (non-parametric) was run in R using the package stats (R Core Team 2020) to determine if the two categories compared on each boxplot were significantly different.

Empirical cumulative density functions (ECDFs) were used to visualize the percent of continental shelf area as a function of DA concentration. During the Bight Regional Program's station selection process for the HAB DA component, 30 randomly selected stations were chosen from each continental shelf strata (inner, mid and outer). This statistically allows each station to represent a pre-determined fraction of the Bight continental shelf area, based on the relative area of each strata within the whole continental shelf area. Within the Bight continental shelf, inner-shelf comprises 31% of shelf area (1.03% for each of the 30 stations), mid-shelf comprises 53% of the shelf area (1.77% for each of the 30 stations) and outer-shelf comprises 16% of the shelf area (0.53% for each of the 30 stations). To accommodate the variable weight each station contributes to percent area as described above, empirical cumulative density functions were calculated and visualized in R using 'stat_ecdf' from the package ggplot2 (Wickham 2016) with an added function to allow weighting (https://github.com/NicolasWoloszko/stat_ecdf_weighted).

Spearman correlations are presented for the relationship between percent fine particles and sediment DA concentrations. These were calculated in R using corr.test from the stats package (R Core Team 2020).

Principal components analysis (PCA) was conducted to understand sediment DA concentrations in the context of multiple variables. PCA results are visualized as two biplots presented in Appendix A. Both PCA analyses were conducted and visualized in R using the package factoextra (Alboukadel and Mundt 2020).

II. RESULTS

Extent and magnitude of sediment domoic acid in the 2018 Bight Regional Program

Domoic acid (DA) was detected in over half the area (54%) of SCB continental shelf during the Bight '18 with detectable concentrations ranging from 0.11 ng/g to 1.36 ng/g (Figure 2, Figure 3A). DA was spread geographically throughout the Bight, with the highest relative concentrations located in sediments collected in the Santa Barbara Channel, Santa Monica Bay, and San Pedro Shelf (Figure 2). The highest concentration of 1.36 ng/g DA was collected from an outer-shelf station in the Northern Santa Barbara Channel, approximately 6 km off the coast of Gaviota.

Of the three continental shelf strata sampled, the greatest relative extent of DA was present in mid-shelf sediment, with DA detected in 67% of the mid-shelf area (Figure 3B). The inner and outer-shelf strata had detectable DA in 27% and 40% area, respectively. DA concentration varied significantly among strata with the mid-shelf having a higher median concentration (0.185 ng/g) DA than the inner (median = 0 ng/g) or outer (median = 0 ng/g) shelves (Figure 3C; Kruskal-Wallis test, $H = 9.52$, $df = 2$, $p < 0.01$). The mid-shelf DA concentrations were significantly higher than the inner-shelf (adjusted $p < 0.01$) concentrations, but not significantly different than outer-shelf (adjusted $p = 0.16$) concentrations, and no significant difference was found between the inner- and outer-shelf (adjusted $p = 0.14$; post hoc Dunn's Multiple Comparisons test).

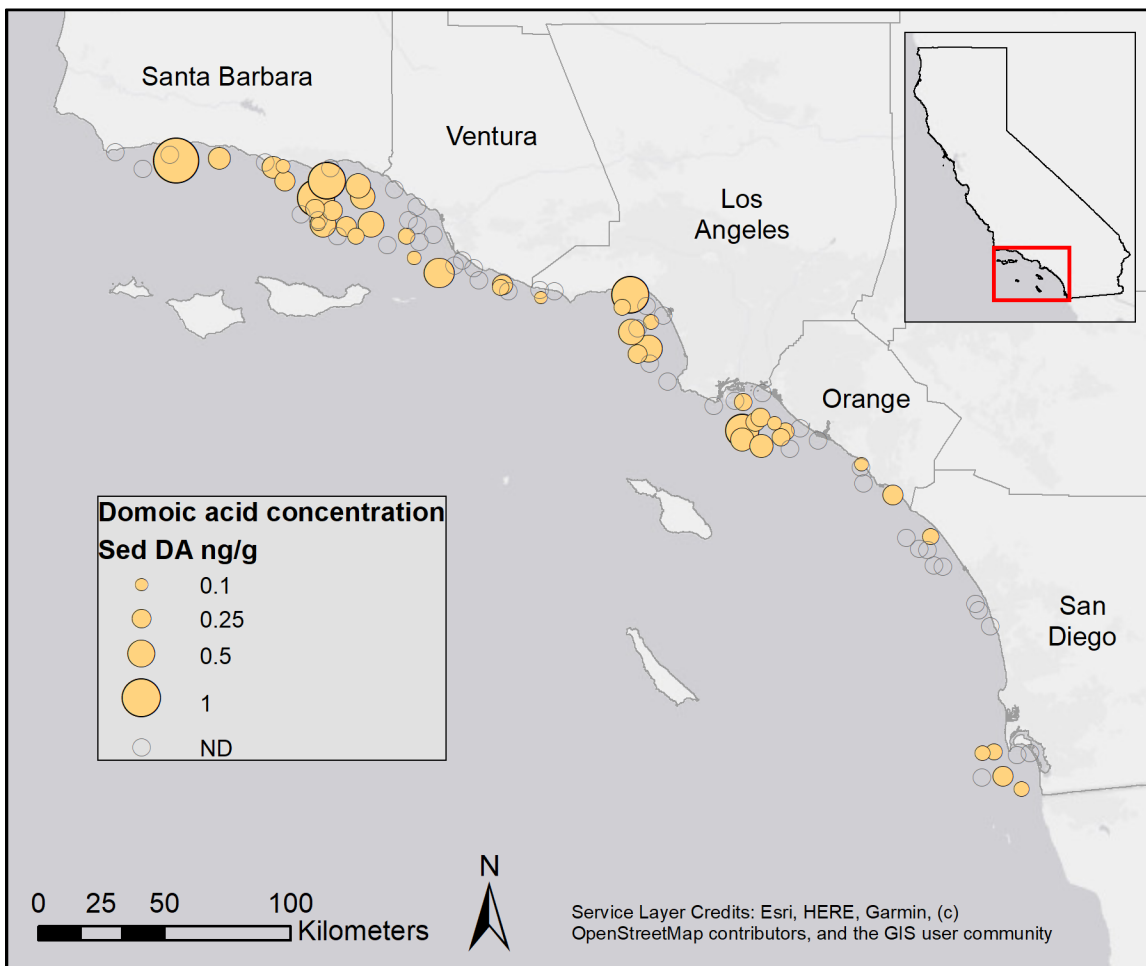


Figure 2. Locations and relative concentration of DA in 90 sediment samples collected in the 2018 Bight Regional Program. An empty circle indicates the location of stations where DA was not detected.

The presence of DA in sampled sediments did not show any strong relationships with any of the observed environmental factors. In samples where DA was detected ($N = 44$), a significant but weak correlation was found between DA concentrations and percent fine particles (Supplemental Figure A1 Spearman correlation: $\rho = 0.34$, $p = 0.025$). When considering all samples ($N = 90$), a weak positive relationship was observed between DA concentrations and percent fine particles,

but it was not statistically significant (Supplemental Figure A1 Spearman correlation: $\rho = 0.15$, $p = 0.167$). No significant relationships were observed between sediment DA concentrations and sediment TN or TOC, station latitude or station depth; although sediment TN and TOC showed similar positive trending relationships to what was observed between sediment DA concentrations and fine particles.

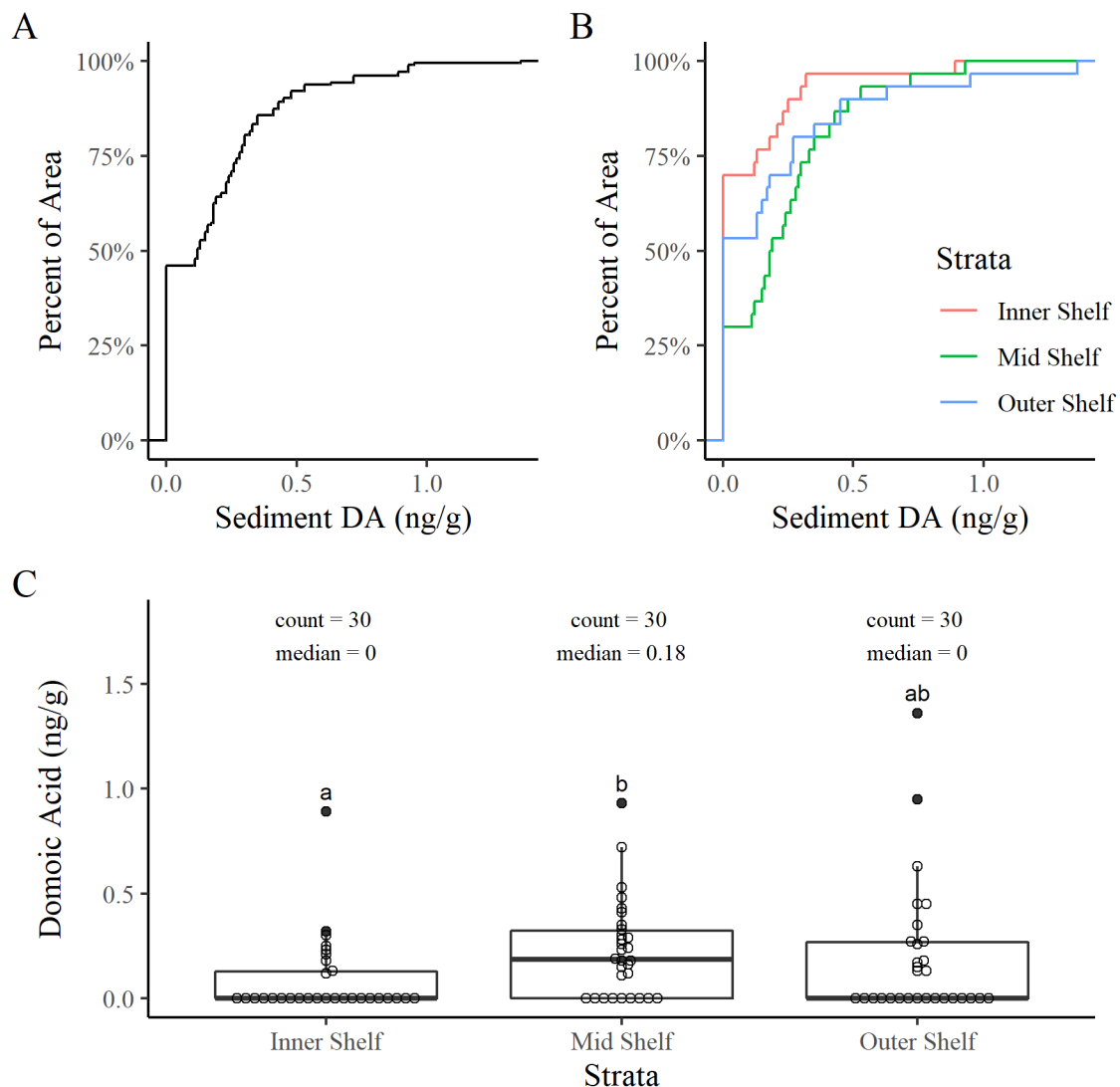


Figure 3. Empirical cumulative distribution frequency of domoic acid concentration in A) all continental shelf sediment samples from the 2018 Bight Regional Program and B) each of the three continental shelf strata sampled during the 2018 Bight Regional Program; C) boxplot of the domoic acid concentrations measured in 30 samples from each continental shelf strata.

Variability of sediment domoic acid in the Central Bight, 2017 and 2019

In the 2017 Central Bight Study, DA was detected in 95% of sediment samples collected (Figure 4), which was likely caused by a significant toxin producing *Pseudo-nitzschia* bloom in the region. Detected sediment DA concentrations ranged from 3.1 to 168.0 ng/g sediment following

the methods of Sekula-Wood et al. (2011). In 2019 when these stations were revisited, DA was detected in 35% of the samples collected (Figure 4). Detected sediment DA concentrations ranged from 0.57-13.2 ng/g sediment following the methods of Phonsiri et al. (*in prep*). Additionally, eleven sediment samples were collected in San Diego in 2019 but all sampled stations were below the detection limit (Supplemental Figure A6). Sediment samples collected in 2017 and 2019 were analyzed using different methodologies and laboratory and method intercalibration results indicated that the concentrations cannot be quantitatively compared across years (See Appendix B). Therefore, spatial relationships within a sampling event can be assessed, but comparisons across survey years cannot.

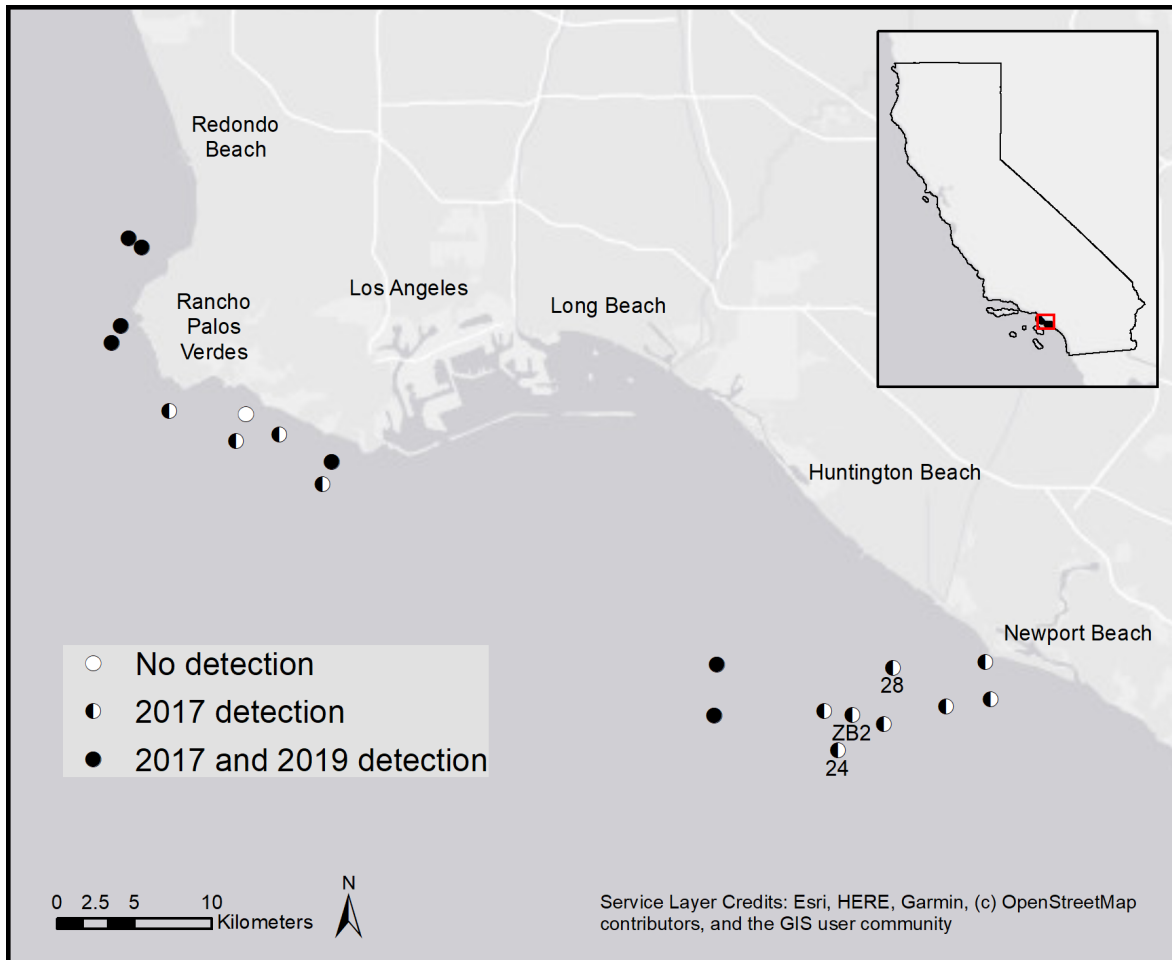


Figure 4: Central SCB map showing stations with detected DA in 2017 and 2019. An empty circle represents no DA detected either year, a half-filled circle indicates that DA detected in 2017 and a filled circle indicates that DA was detected in both 2017 and 2019. No stations had DA detected in 2019, but not 2017. The MDL was 0.18 ng/g in 2017 and 0.5 ng/g in 2019. The three stations selected for the Monthly Revisit Study are labeled with station names.

Variability of benthic infauna tissue domoic acid in the Central Bight, 2017 and 2019

Benthic infauna samples co-located with the previously described sediment samples were collected in both 2017 and 2019. In 2017, DA concentration was measured for a composite sample of organisms collected from each station (this included major taxonomic groups such as crustaceans, polychaetes, echinoderms and mollusks). Of the 20 stations sampled, two stations did not recover enough tissue for analysis. Of the remaining 18 stations sampled, all infauna samples had detectable levels of DA (Figure 5). DA concentration of the composite benthic infauna tissue ranged from 0.1 ppm to 29 ppm.

Similar to 2017, in 2019, DA was detected at every station where benthic infauna tissues were collected and analyzed (N = 20; Figure 5). In 2019, DA was measured in four coarsely sorted taxonomic groups, but was binned together for analysis using a weighted average as described above. The weighted average DA concentration of benthic infauna tissue in 2019 ranged from 0.02 ppm to 26 ppm. Note that benthic infauna samples collected in 2017 and 2019 were analyzed by two separate laboratories using the same extraction method, but different quantification methods. Intercalibration exercises showed high repeatability between methods (See Appendix B), but the variation in quantification methods (2017 is a composite value, 2019 is a calculated weighted average value) should be taken into consideration when comparing across years.

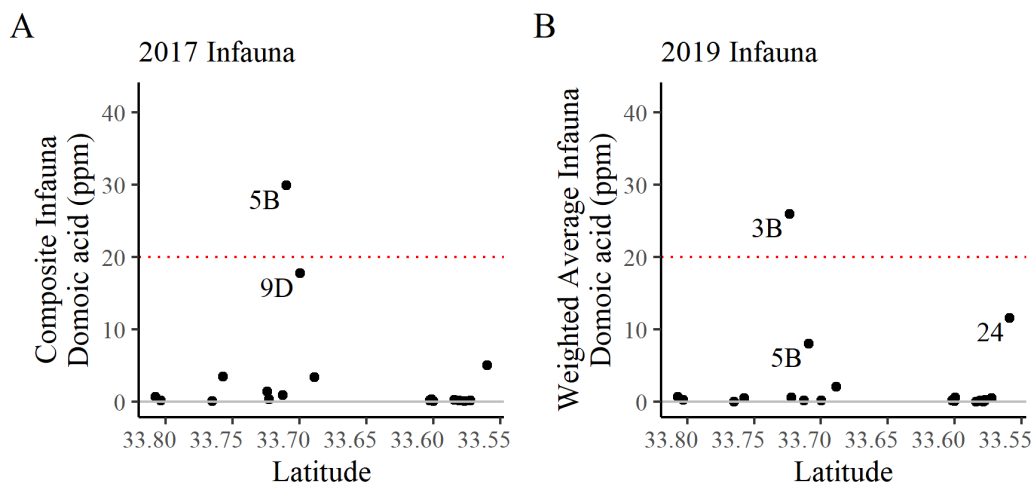


Figure 5. Domoic acid concentration A) in composite infauna samples collected in 2017 and B) as a calculated weighted average of benthic infauna samples at stations revisited in 2019. The dotted red line denotes the FDA safe-to-eat level of 20 ppm DA. Station name is included as a label for samples with concentration of DA greater than 8 ng/g DA. See methods for information on Composite Infauna DA versus Weighted Average Infauna DA.

Temporal and spatial variation in sediment and benthic infauna tissue domoic acid during the Monthly Revisit Study

Concentrations of DA were mostly below (e.g., not detected) or near detection limit (0.10-0.25 ng/g) in the 49 sediment samples collected from three stations off the coast of Newport Beach

over the course of 16 months (Figure 1A, Figure 6). DA was not detected in 88% of sediment samples collected. Of the six samples in which DA was detected, concentrations ranged from 0.16 to 6.9 ng/g with the highest DA sediment concentration being observed at Station 24. During the 16 months of sampling, DA was detected in 6% (1/17) of samples at Station 28, 13% (2/16) of samples at Station ZB2 and 19% (3/16) of samples at Station 24. A majority of samples with detectable DA were collected between March 2018 and June 2018.

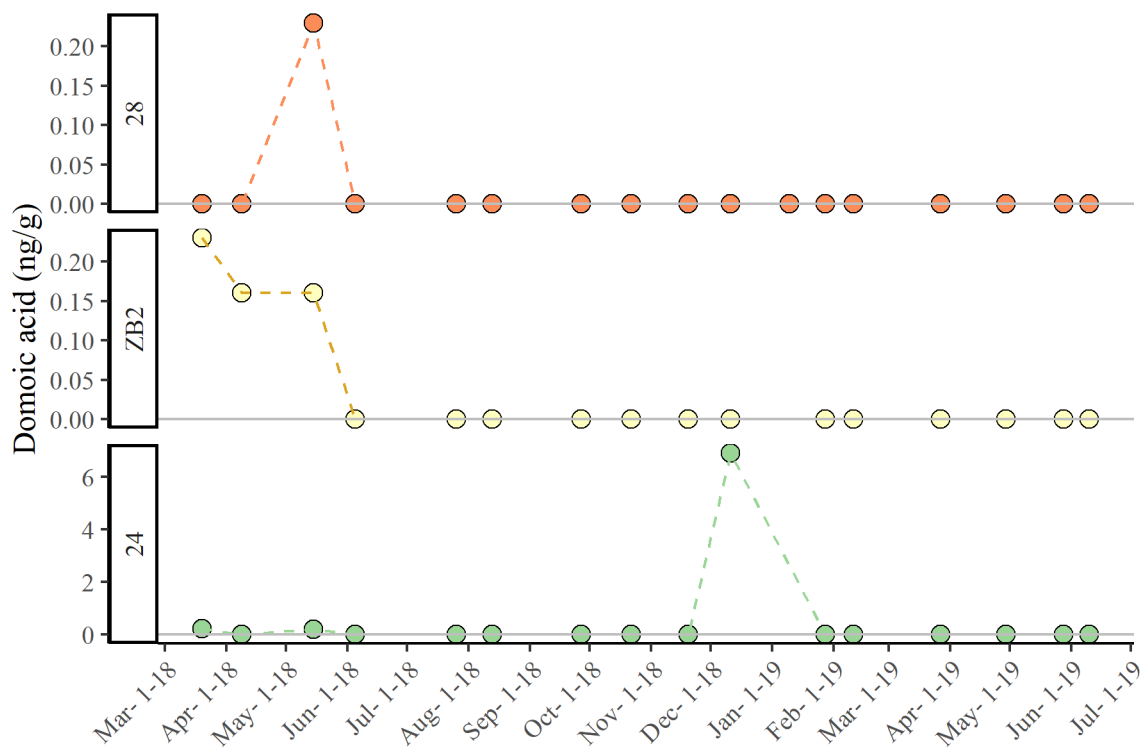


Figure 6. Monthly Revisit Study domoic acid concentrations (ng/g) in sediment samples collected at stations 28 (32 m), ZB2 (58 m) and 24 (204 m) from Mar 2018 – Jun 2019. Note the difference in y-axis scales between facets. Depths provided are the mean station occupation depth rounded to the nearest integer.

In contrast to DA levels in the Monthly Revisit Study sediment samples, DA was detected in all co-located benthic infauna samples at Stations 24 and 28 (Figure 7A). The weighted average of DA concentrations in tissues ranged from 0.07 ppm to 1.8 ppm at Station 28 and from 7 ppm to 70 ppm at Station 24. Weighted DA concentrations in benthic infauna tissue was significantly higher at Station 24 (median 18.44 ppm), compared to the concentrations observed at Station 28 (median 0.29 ppm; Wilcoxon Rank Sum test, $W = 0$, $p < 0.01$). Additionally, the weighted average of DA in benthic infauna tissue exceeded the FDA safe-to-eat level of 20 ppm in 44% of samples collected at Station 24, while the weighted average DA concentration at Station 28 never exceeded 20 ppm.

Among the sorted groups of benthic infauna, Group 1 (marine worms, which included other taxonomic groups as well) had consistently higher body burdens of DA (Figure 8). While groups are binned to a weighted average for the majority of this report, the high levels of DA found in

marine worms as a group warranted individual consideration. At Station 28, DA concentrations in marine worms (median 0.44 ppm) ranged from 0.1-6.5 ppm and was significantly higher than weighted average DA concentration of the remaining organisms (median 0.07 ppm), which ranged from 0.02-0.6 ppm (Figure 8B; Wilcoxon Rank Sum test, $W = 265$, $p < 0.01$). At Station 24, DA concentrations in marine worms (median 64.61 ppm) ranged from 18-221 ppm and was significantly higher than the weighted average DA concentration of the remaining organisms (median 0.12 ppm), which ranged from $< 0.01 - 2.6$ ppm (Figure 8C, Wilcoxon Rank Sum test, $W = 256$, $p < 0.01$). Throughout the time series study, marine worms showed a temporally consistent pattern of having detectable body burdens of DA. At Station 24, marine worm tissues were above the FDA safe-to-eat level of 20 ppm in 94% (15/16) of samples collected during the time series.

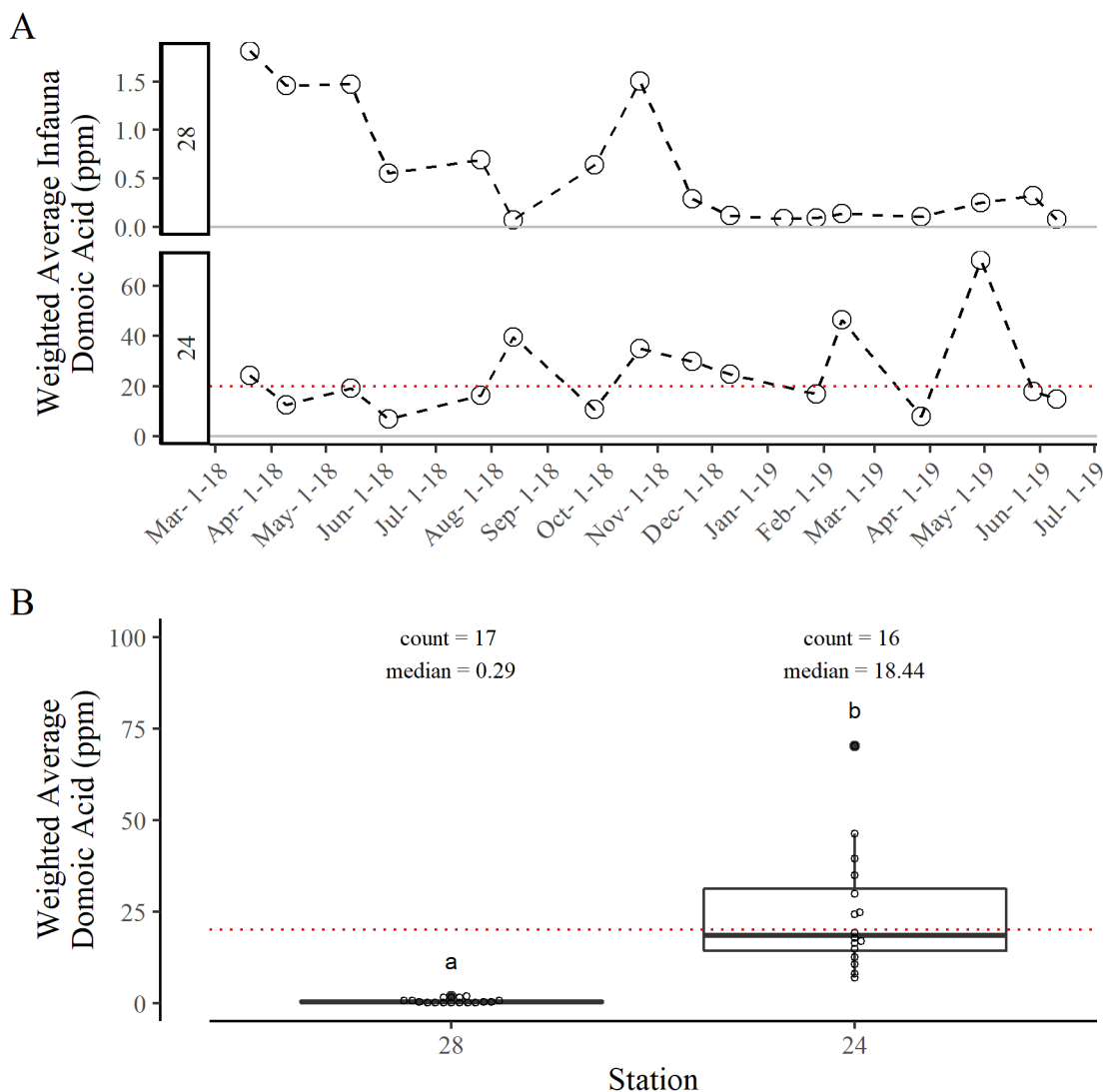
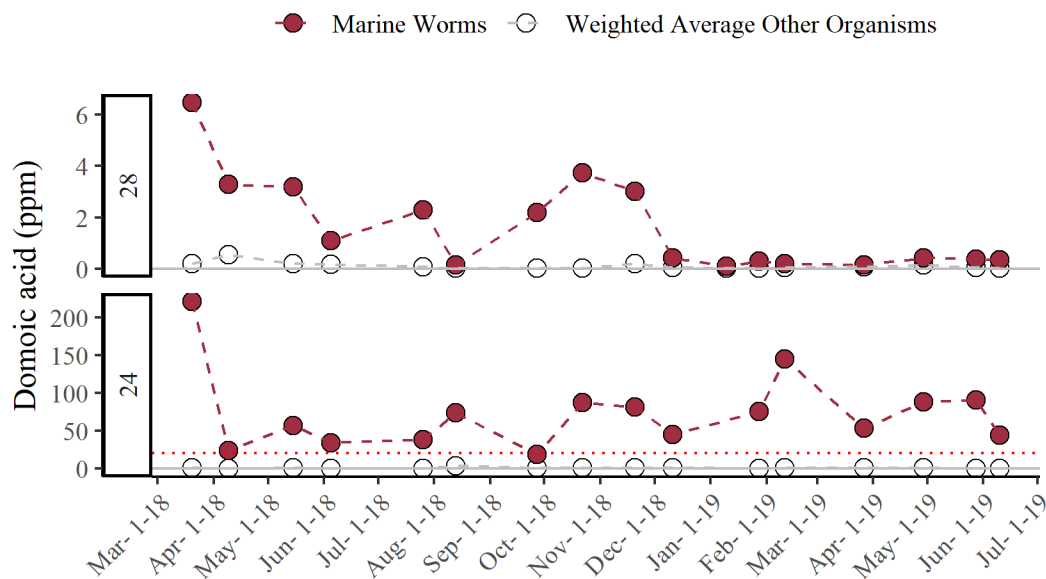
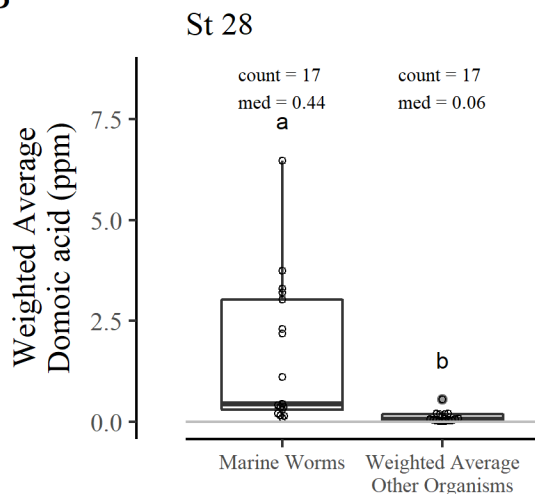


Figure 7. Weighted average domoic acid concentration (ppm) in benthic infauna tissue collected during the monthly revisit Study, Mar 2018 - Jun 2019. A) Time series of DA for each station and B) boxplot comparing weighted average DA concentration between Station 28 and Station 24. Red dashed line indicated the FDA safe-to-eat DA concentration of 20 ppm.

A



B



C

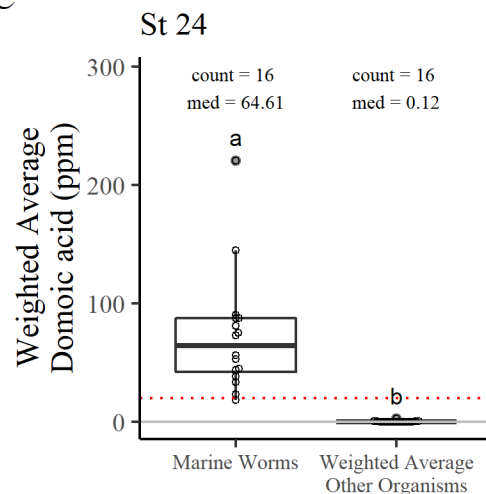


Figure 8. Domoic acid concentration in tissues of marine worms collected during the Monthly Revisit Study compared to the weighted average of all other organisms, Mar 2018 – Jun 2019 presented as A) a time series and (B, C) boxplots for each station.

Relationship between sediment and benthic infauna tissue domoic acid

Throughout different components of this study, measured sediment DA concentration was not found to directly correlate with DA concentration in the tissue of co-located benthic infauna (Figure 9). In a comparison of this relationship across space from the 2017 and 2019 Central Bight samples, there are stations where low sediment DA corresponded to high infauna DA and vice versa, with no strong relationship present (Figure 9A, B). In a comparison of this relationship through time from the monthly revisit samples, DA was routinely detected in benthic infauna, but largely not detected in co-located sediment samples (Figure 9C).

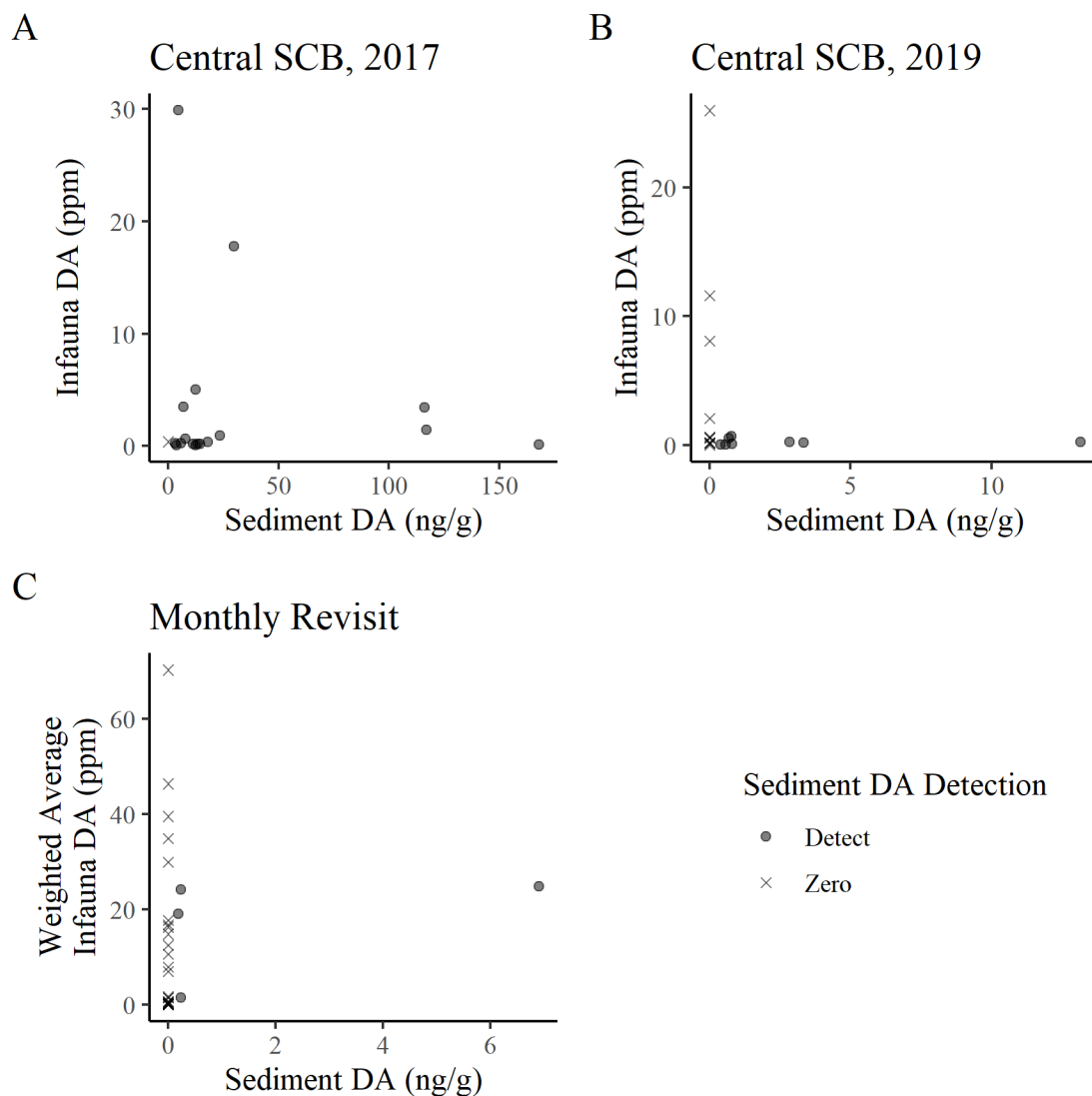


Figure 9. Sediment DA (ng/g) versus infauna tissue DA (ppm) from co-located samples collected during the A) 2017 Central Bight Study, B) 2019 Central Bight Study and C) Monthly Revisit Study.

VI. DISCUSSION

Domoic acid extent and persistence in sediments and linkages to surface blooms

Bight '18 sampling indicated geographically widespread presence of DA throughout the SCB, with detectable DA in the sediments in 54% of the continental shelf area. Prior to this study, observations of DA in sediments have been geographically limited to a few stations within the Santa Barbara Basin and the San Pedro Basin. Between 2001 and 2005, 11 surficial sediment samples (0-2 cm) collected from 2 stations in the Santa Barbara Basin and San Pedro Basin had concentrations ranging from 17 to 38 ng/g of DA in dried sediment (Sekula-Wood et al. 2009). A 28-station survey of surficial sediment samples (0-2cm) was conducted in the Santa Barbara

Basin as a part of the Bight '08 Program. Of the 28 stations sampled, DA was present in 8 of the stations and observed concentrations ranged from 1.2 to 8.0 ng DA per gram sediment (Sekula-Wood et al. 2011).

Survey results indicate that there are shelf strata where DA concentrations and presence is higher relative to the continental shelf area as a whole. DA was most prevalent in the mid-shelf strata (67% of the mid-shelf area) compared to the spatial extent observed in inner (27% of inner-shelf area) and outer-shelf strata (40% of outer-shelf area). DA concentrations were also higher in the mid-shelf strata than in the inner-shelf strata. These observations, for the most part, align with horizontal patterns in DA distributions observed during blooms. Multiple studies have reported offshore concentrations of DA are generally higher than those observed at nearshore monitoring stations (Smith et al. 2018b, 2018a; Umhau et al. 2018). This partly accounts for the reduced observations of DA in the inner-shelf, although given this dynamic it might be expected that the spatial extent of DA observed in the mid-shelf and outer-shelf strata might be more comparable. The reduced spatial extent in the outer-shelf sediments may be a result of increased horizontal transport at the outer-shelf stations compared to the mid-shelf stations.

The results of the 2018 program also suggest that there may be geographic benthic hotspots that generally correspond with regions with more intensive bloom activity in overlying waters (Figure 2). While blooms of *Pseudo-nitzschia* have been observed throughout the Bight, some sub-regions of the Bight have been identified as regions where DA concentrations and *Pseudo-nitzschia* cell abundances tend to be elevated, generally due to retentive circulation patterns. These regions include the Santa Barbara Channel (Anderson et al. 2009, 2006; Umhau et al. 2018), the Santa Monica Bay (Seubert et al. 2013; Shipe et al. 2008), the San Pedro Shelf (Schnetzler et al. 2013, 2007; Seubert et al. 2013; Smith et al. 2018b, 2018a) and San Diego (Busse et al. 2006). Like many diatoms, toxin containing cells of *Pseudo-nitzschia* form aggregates, and are readily transported from surface waters to the benthos, providing a source of DA to these environments (Schnetzler et al. 2017; Sekula-Wood et al. 2011, 2009; Thornton 2002; Umhau et al. 2018). Sediment trap studies have indicated that transport of surface cells to depth is relatively rapid, with transport rates between 50 to > 100 m per day (Schnetzler et al. 2017; Sekula-Wood et al. 2009). Experimentally, *Pseudo-nitzschia* has been demonstrated to readily form marine snow aggregates that retain DA, mechanistically explaining observations of rapid transport of the toxin to the benthos (Schnetzler et al. 2017). Given the rapid transport rates of *Pseudo-nitzschia* cells demonstrated in the literature, it is probable that regions with intensified water column blooms may also have increased prevalence and concentrations of DA in the sediments. Additional sampling over time is needed to fully resolve these dynamics, as spatial patterns may ultimately vary on an annual basis.

The present study investigated the temporal dynamics of DA in sediments across varying scales. On an annual basis, DA was detected in the sediments of the SCB annually between 2017 and 2019. Within the Central Bight, the magnitude of DA in 2017 and 2019 cannot be quantitatively compared due to differences in DA extraction and analysis methodologies. Qualitatively, DA was more commonly detected in marine sediments in 2017 (95% of sampled stations) than in 2019 (40% of sampled stations), which generally corresponds with the spatial extent and magnitude of observations of particulate DA in the water column during those years. Ambient monitoring for particulate DA is conducted on a weekly basis at four pier locations within the Bight as a part of the California HABMAP program (<https://calhabmap.org/>; Kudela et al. 2015).

Observations from these stations indicate that a significant Bight-wide toxin producing bloom of *Pseudo-nitzschia* occurred in 2017 that was clearly observed at all pier locations (Figure 10) as well as at multiple offshore locations in April through June of 2017 (Smith et al. 2018a). This bloom was also associated with unusual bird mortality events in Santa Barbara, Ventura and Los Angeles counties and an influx of sea lions to marine mammal rescue centers were attributed to DA poisoning (Smith et al. 2018a). In 2019, the Central Bight showed a generally reduced occurrence of DA in the same stations where DA was present in 2017. This reduction generally aligns with the observation of reduced surface water bloom activity in 2019 (Figure 10).

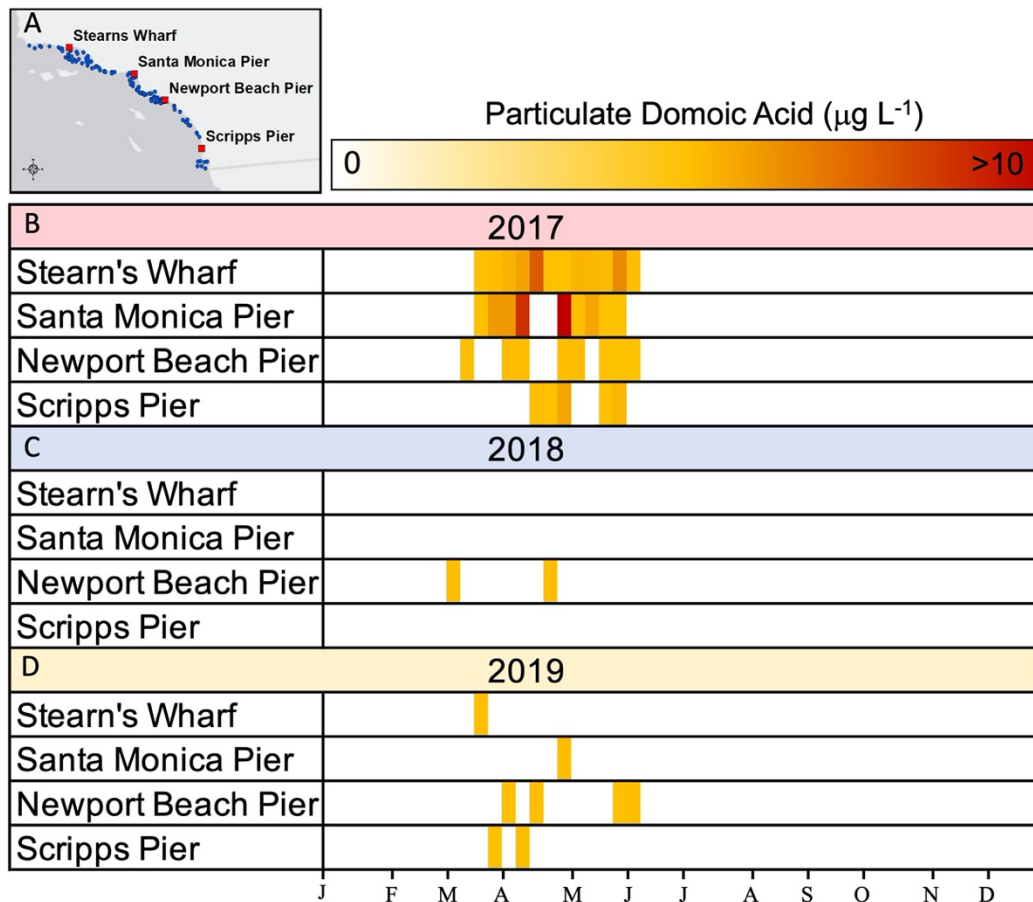


Figure 10. Particulate domoic acid concentrations at the weekly pier monitoring stations in the Southern California Bight. Locations relative to sediment sampling locations are shown in panel A. The particulate domoic acid concentrations at each station are shown during 2017 (B), 2018 (C), and 2019 (D).

The persistence and stability of DA in marine sediments is not well understood, and it is possible that DA occurrence in sediments is related to both near term sources and historical deposition. Compared to 2017, minimal bloom activity was detected at pier monitoring locations throughout the Bight in 2018 (Figure 10C). Despite this, DA was still prevalent across the Bight-wide survey in 2018 (44% of samples and 54% of shelf area). Therefore, it is possible that the distribution patterns observed Bight-wide in 2018 were still strongly influenced by the water column bloom observed in 2017. Previous work has indicated that DA can adsorb to sediments

and clays with adsorption varying based on the composition (Burns and Ferry 2007), which could result in longer term presence of DA in the sediments. Here, a weak positive trending relationship between DA concentration and percent of fine particles was observed (Supplemental Figure A1), but more work is needed to better understand if occurrence patterns are influenced by sediment composition.

Interestingly, the presence of DA was more irregular across shorter monthly timescales during the 16 months of observations in the Central Bight (March 2018 to June 2019). Concentrations of DA were generally low in samples collected from March 2018 through June 2019 and DA was generally not observed at these stations after the summer of 2018. The time-series observations leading up to the Bight-wide survey in the summer of 2018 may highlight the degradation of high DA flux to the benthos in 2017 followed by a period of reduced flux. This is not fully possible to resolve however, since there are no observations of the sediment DA concentrations between July 2017 and March 2018. Additionally, water column sources of DA were not fully characterized given that the water column observation collected at the pier locations only capture very nearshore dynamics and are unable to detect any offshore or subsurface blooms of *Pseudo-nitzschia* that may provide a source of DA to these locations. Overall, the linkage between water column DA concentrations and benthic environment is complex and requires further study to resolve since multiple factors likely contribute to observed patterns.

Persistent domoic acid concentrations in benthic infauna and linkages to the food web

DA was consistently observed in the tissues of benthic infauna organisms across the duration of the study, even when DA was not observed in sediments. Weight averaged DA concentrations exceeded the FDA safe-to-eat level of 20 ppm across multiple years and in 3 of the 22 stations where infauna tissues were collected, which presents an increased risk for transfer to higher trophic levels. The sources of DA to these organisms are unclear given the poor relationship between DA in the sediments and in the co-located infauna (Figure 9). In 2017 and 2019, DA was detectable at all stations where sufficient infauna biomass was collected for analysis; however, a large range of concentrations were observed across the Central Bight. Similarly, significant spatial and temporal variability was observed in the infauna tissues collected over 16 months in the Central Bight. Significantly higher infauna tissue concentrations were observed at Station 24 compared to Station 28. These stations represent two different environments with Station 24 situated more offshore in a region with higher deposition rates, while Station 28 is a more nearshore station with lower deposition and increased terrestrial influence from the Santa Ana River. The differences in the infauna DA concentration are almost certainly a reflection of these differences between these stations, but the dynamics require further study to be resolved to better understand which regions might be more at risk for higher exposure to DA.

Potential sources of DA to the infauna are multiple. Sources may include sub-nanogram concentrations of DA in the sediments that are below the detection limits of the methods used in this study. The dissolved pool of DA at the sediment-water interface may represent another source. Recent work in Monterey Bay found that DA was routinely present in solid phase adsorption tracking samplers (SPATT, a type of passive sampler) deployed at sediment-water interface when co-located sediment samples generally had undetectable concentrations of DA (Zicarelli 2014). Another alternative is that toxigenic *Pseudo-nitzschia* cell populations offshore or subsurface may provide a routine source of DA to the benthos that is not reflected in the

sediments. Given that the uptake and depuration rates of DA for most organisms are not known, the observations of this study may reflect long term retention of DA by multiple taxa.

When infauna DA concentrations were examined according to their coarse sorting groups, the marine worms had temporally consistent DA present in tissues. The marine worms also had the highest concentrations compared to bulk weighted average of all organisms and the other grouping indicating they might be a major repository for DA in the benthic environment. Although the taxonomic resolution of the infauna sorting was very granular, a number of studies indicate that benthic infauna organisms may differentially uptake and retain *Pseudo-nitzschia* cells and DA. A study in the Gulf of Mexico proposed the polychaete *Paraprionospio pinnata* was a major vector of DA after finding elevated abundances of *Pseudo-nitzschia* cells in the guts of collected organisms (Baustian et al. 2018). Observations in Monterey Bay demonstrated presence of DA in infauna tissue long after blooms in overlying waters subsided, similar to this study, and particularly high concentrations were observed in innkeeper worms (*Urechis caupo*) over multiple years (Kvitek et al. 2008). DA was also prevalent in benthic-feeding flatfish compared to pelagic feeding species caught at the same time in Monterey Bay. Curlfin turbot (*Pleuronichthys decurrens*) had the highest observed concentrations of DA of all the species sampled, and these fish feed primarily on polychaetes which suggests these organisms are a potential vector of toxin transfer (Vigilant and Silver 2007). Together the observations of the present study and others suggest that additional investigation into the uptake and depuration rates of specific infauna species could lend a greater understanding of cycling of DA in the benthos and increase the understanding of the routes of transfer to higher trophic levels.

VII. CONCLUSIONS

The Bight '18 Program provided the first ever regional assessment of DA extent in the benthic habitats on the continental shelf. Based on the results of this study, the Harmful Algal Bloom Technical Committee concluded that:

- **DA was detected in the majority of the regions' sediments, with the greatest relative extent in sediments from the mid-shelf strata.**

DA was present in sediments from 54% of continental shelf area in 2018 and the greatest relative extent of DA was observed in the sediments of the mid-shelf strata. The highest concentrations of DA were observed in regions with histories of water column blooms and retentive circulation patterns.

- **DA was detected in sediments every year, but not at every site during our 3-year time series.**

DA was detected in sediments in 2017, 2018 and 2019, indicating DA was present in benthic habitats on an annual basis. DA was not found to be consistently present at all sites. DA was commonly below or near the detection limit for most months during the 16-month time series conducted in the Central Bight. The relationship between sediment DA observations and those in the water column monitoring stations show a general relationship over an annual basis, although observations indicate that DA in the sediment may persist long after blooms end. Comparisons of magnitude for toxin in the sediments across years are confounded by methodological issues.

- **DA appears to be persistent in infauna despite lack of detectable DA in most co-located surface sediments.**

The sources of DA to the infauna are unclear but the consistent presence of DA in infauna may pose a risk for DA transfer to higher trophic levels. Coarse taxonomic sorting of samples also indicated that DA might accumulate differentially in taxa and that different taxa may pose different risks for transfer to higher trophic levels.

VIII. RECOMMENDATIONS

Based on the results of the Bight '18 study, the Harmful Algal Bloom Technical Committee agree on the following recommendations to follow up on current survey results and to improve the next regional survey implementation.

- **Increase our understanding of DA cycling and persistence in sediments and infauna tissues.**

This recommendation resulted from the consistent observations of DA in infauna tissue compared to co-located sediment samples, highlighting a lack of understanding of DA cycling in the benthos. Further investigations of the rates and mechanisms of DA degradation is needed. Additional work is needed to identify which benthic taxa have the greatest and most persistent tissue DA concentrations. Quantification of uptake and depuration rates of key taxa is also needed to inform bioaccumulation risk.

- **Measure DA in tissues of important Southern California benthic fishery species.**
Longevity of infauna tissue DA concentrations indicates a potential route of exposure to higher trophic levels, including important benthic fishery species in the Bight. An extension of the first recommendation, future studies in the region should address the prevalence of DA in these species which represent a potential source of toxin to humans and marine mammals.
- **Develop standard methods for analysis of DA in benthic sediments and tissues to increase comparability and quality of future monitoring efforts.**

The current Bight '18 sediments results were not readily comparable across years due to differences across methodologies identified in the intercalibration study (Appendix B). Standardized methods for extraction and analysis are needed for future studies.

- **Investigate the occurrence of emerging HAB taxa and toxins to develop a better understanding of the drivers and impacts of these events in the Bight.**

Domoic acid has caused the majority of impacts in the Bight over the last two decades, therefore research has focused primarily on the occurrence and drivers of toxic *Pseudo-nitzschia* blooms. Recent research has shown that with shifting climate regimes other species may become increasingly relevant in the Bight over the next few decades (Smith et al. 2018a; Trainer et al. 2020). Indeed, a recent bloom of *Lingulodinium polyedra* in the spring of 2020 has already demonstrated that other HABs species can have significant impacts in the Bight. Less is known about other HAB taxa and toxins beyond some limited descriptions in the region. Future study in the Bight should focus on better characterizing the occurrence of these understudied taxa and toxins in the region, as well as assessing the environmental factors that support the growth and toxin production of these organisms.

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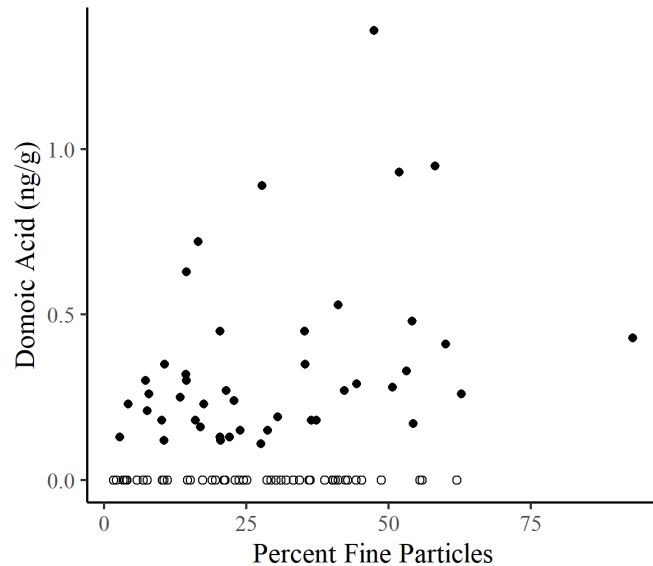
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APPENDIX A: SUPPLEMENTAL FIGURES

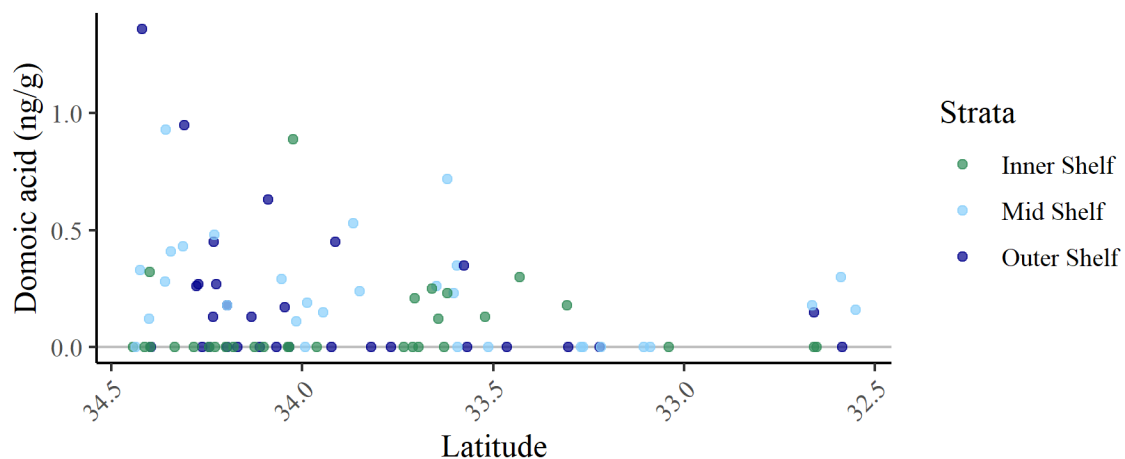
2018 Bight Regional Program



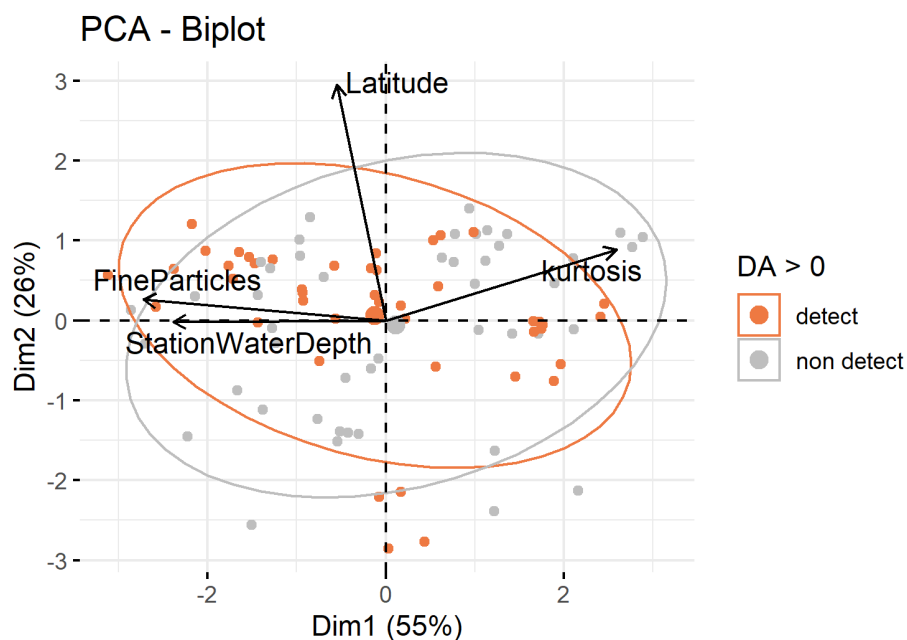
Supplemental Figure A1. Scatter Plot of domoic acid concentration (ng/g) versus percent fine particles in sediment samples collected from the 2018 Bight Regional Program. Solid points represent samples with detected DA while hollow points represent samples where DA was not detected. When considering all samples, there is no significant correlation between sediment DA concentration and percent fine particles (Spearman correlation: $\rho = 0.15$, $p = 0.167$). When considering DA detect samples only, there is a significant but weak correlation between sediment DA concentration and percent fine particles (Spearman correlation: $\rho = 0.34$, $p = 0.0253$).

Summer 2018 Bight Areal Sampling

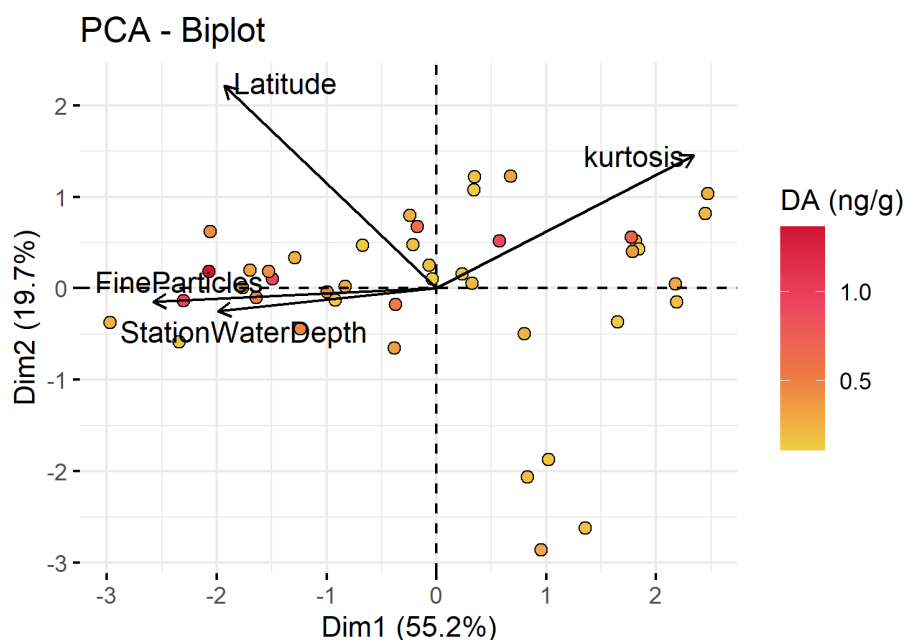
44 Detects, 46 Non-Detects



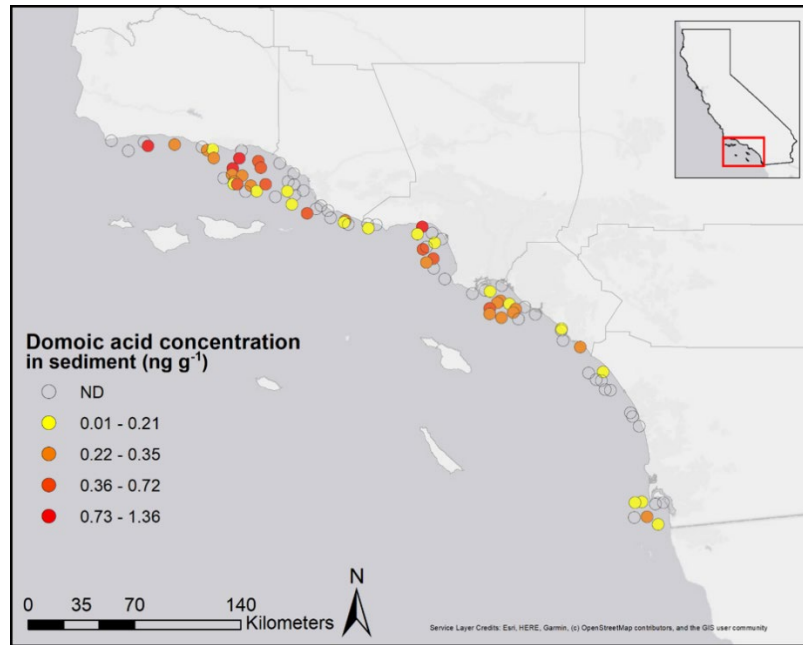
Supplemental Figure A2. Scatter plot of domoic acid concentration (ng/g) versus latitude of sampling station from the 2018 Bight Regional Program.



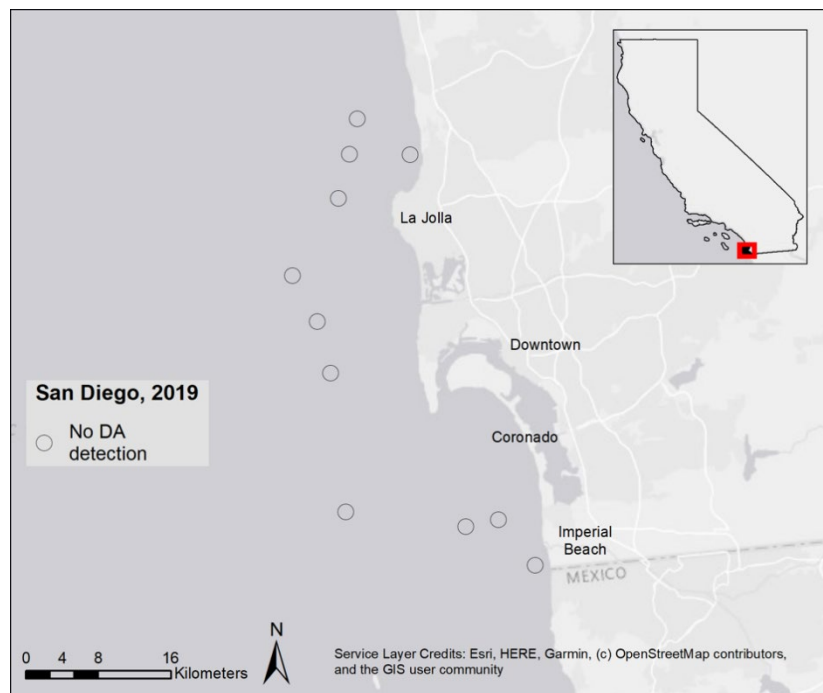
Supplemental Figure A3. PCA biplot of environmental variables that may affect DA presence in the sediment. Points (N = 90) correspond to each Bight 2018 Regional Program sample collected and are colored based on whether or not DA was detected. Concentration ellipses are drawn with a normal probability level of 0.85.



Supplemental Figure A4. PCA biplot of environmental variables that may affect DA concentration in the sediment. Points (N = 44) correspond to each Bight 2018 Regional Program sample with detected DA.



Supplemental Figure A5. Locations and concentration of domoic acid in 90 sediment samples collected in the 2018 Bight Regional Program. Warmer colors indicate higher concentrations of domoic acid while cooler colors indicate lower concentrations. Empty circles indicate the location of stations where DA was not detected.



Supplemental Figure A6. Locations of sediment samples that were collected in San Diego in the summer of 2019. All samples were below the method detection limit of 0.10-0.25 ng/g.

APPENDIX B: DOMOIC ACID INTERCALIBRATION STUDY

Background

The Bight '18 HABs project included three major sampling efforts from 2017 through 2019 to determine the spatial extent and temporal persistence of domoic acid (DA) in marine sediments and benthic infauna tissue. A total of five laboratories contributed to the sample analyses. Three labs conducted sediment DA analyses via analytical approaches (either LC-MS or LC-MS/MS) and two labs conducted the analysis of benthic infauna tissues via analytical (LC-MS) or immunological (ELISA) approaches.

To address any potential variance introduced by the different labs, two laboratory intercalibration efforts were conducted based on the sample matrices, one for the marine sediments and one for benthic infauna tissues. Unlike most laboratory intercalibration exercises, each of the intercalibration studies was conducted after most of the field sampling efforts were completed because some of the participating laboratories had not yet developed and onboarded their methodologies at the onset of the project.

Sediment LC-MS Intercalibration

Study Design

Sediment samples throughout the project were analyzed by three laboratories: UC Santa Cruz, Weck Laboratories and OCSD. All laboratories used either an LC-MS or LC-MS/MS instrument. Two main components likely contribute to variance in DA measurements between labs – (1) variance in the efficiency of extracting DA from the sediment and (2) variance in quantification of DA in the extract, which includes the specifics of how each instrument is tuned as well as each lab's choice of standard. In this appendix, we refer to each unique combination of extraction method and quantification method as an “approach”. Information on each approach is outlined in Table B1. Three approaches were used to measure DA in the various components of the environmental study (Q1-Q3; Table B2) and two additional approaches (Q4 & Q5) were added to the intercalibration work to provide additional method comparisons. This section of the report will compare the four approaches using LC-MS or LC-MS/MS instruments to quantify DA. The fifth approach using ELISA is discussed in Section 4.

Two DA extraction methods were used to measure DA concentrations in sediments for this project. The first method, Sekula-Wood et al. (2011), is currently the only published method for analysis of DA in sediment. The second, Phonsiri et al. (*in prep*), is a DA sediment extraction method developed by OCSD during the time this study was taking place. Three separate quantification methods were used to analyze the data that contributed to this study. Adapted versions of the methods of Wang et al. (2012) were used in approaches 1 and 2, while approaches 3 and 4 used two different quantification methods developed by Phonsiri et al. (*in prep*).

Table B1. The five approaches for measuring DA concentration in sediment used in this intercalibration study. Each approach is a unique combination of extraction method (E1-E2) and quantification method (Q1-Q5).

Approach	Extraction		Quantification		MDL/RL (ng/g)
	ID	Method	ID	Method (and Standard)	
A1	E1	Sekula-Wood	Q1	Wang; External curve	0.18
A2	E1	Sekula-Wood	Q2	Wang; Internal caffeine ¹³ C ₃	0.10-0.25
A3	E2	Phonsiri	Q3	Phonsiri; External curve	0.5
A4	E2	Phonsiri	Q4	Phonsiri; Internal isotope-dilution	0.5
A5	E2	Phonsiri	Q5	ELISA	1.0

Table B2. The approaches defined in Table B1 that were used for each component of the Bight study. Note that approaches 4 and 5 are not included in this table because they were used for research purposes in the intercalibration study only.

Approach	Extraction ID	Quantification ID	Bight Study Components	MDL/RL (ng/g)
A1	E1	Q1	2017 Central Bight	0.18
A2	E1	Q2	2018 Bight Program, 2018-2019 Temporal & 2019 San Diego	0.10-0.25
A3	E2	Q3	2019 Central Bight	0.5

Methods

Sediment samples collected in 2019 from the Central Bight study component were used for intercalibration. The study was designed to compare both the extraction methods and quantification methods of contributing labs.

Each participating lab was sent eleven DA extracts and six sediment samples for the intercalibration experiments. The extracts were prepared by OCSD in January 2020 and consisted of two blanks, two sand spikes, two matrix spikes and 5 extracts from environmental samples. The samples were blinded before being sent to each laboratory. Spikes were prepared with DA certified calibration standards purchased from Certified Reference Materials Program in Canada. The two sand spikes had an expected value of 0.2 ng/mL and the matrix spikes had an added value of 0.2 ng/mL. The environmental samples were extracted using the methods of Phonsiri et al. (*in prep*) and extracts were split by OCSD lab personnel for analysis among labs. Unextracted sediment samples were homogenized and split by SCCWRP lab personnel in January 2020 for use by each participating laboratory.

Splits of the prepared extract were meant to examine the comparability of each of the different quantification methods without the artifact of variance cause by extraction efficiency. The extracts were quantified using methods Q1, Q2, and Q3 in Table B1. These results were

compared to results for Q4, which was included in the analytical intercalibration work to provide an additional method comparison.

Splits of 6 environmental samples were prepared and analyzed by each of the 3 participating laboratories. Each laboratory prepared and analyzed these samples using the procedures used for environmental samples described in the main body of the report. Of the 6 sediment samples provided, 5 were samples that were also prepared as environmental extracts by OCSD, as described above. This was designed to allow for distinction between impact of the quantification method versus impact of the extraction method. The remaining sample was one that was chosen because it was below the limit of detection in the 2019 Central Bight Study, which was intended to determine if a negative result from an environmental sample would be replicated across laboratories.

Results

Each laboratory group was provided with 3 samples where DA concentrations were not expected to be detected. All laboratories reported the sample blank extracts and negative environmental sample as zero, indicating that false positives were not generally occurring among laboratory groups.

All laboratories showed a general agreement on detection but not concentration in split extract aliquots of sample spikes and spiked environmental extracts (Figure B1). The sand spike samples showed that each laboratory detected relatively low levels of DA (sand was spiked with 0.2 ng/mL) although the concentrations reported differed by almost an order of magnitude in some cases (Figure B1A). In the environmental matrix spikes, each lab was able to detect the increase in DA between the environmental sample extract (Station 44) and the environmental matrix spike replicates (Spike R1 and Spike R2), however, there was poor overall agreement in the absolute concentrations detected by each laboratory (Figure B1B).

There was poor agreement in DA concentrations in the environmental sample extracts that were quantified by the individual lab participants (Figure B2). Method Q4 was an experimental method that an isotopically labelled surrogate is added to the samples prior to extraction and used to correct any analyte loss during the extraction process; thus, Q4 showed consistently higher concentrations than the other 3 quantification methods. More experiment is needed to determine whether or not its usage is appropriate for future samples. (Figure B2A). Of the quantification method used for the study, Q1 generally resulted in the highest concentrations and Q2 had the lowest. Additionally, there were 2 instances (Station 0C and Station C2) where DA was detected using 1 method but not by the other methods. There was 1 instance (Station 44) of a DA detection by 2 methods, but not the third (Figure B2B).

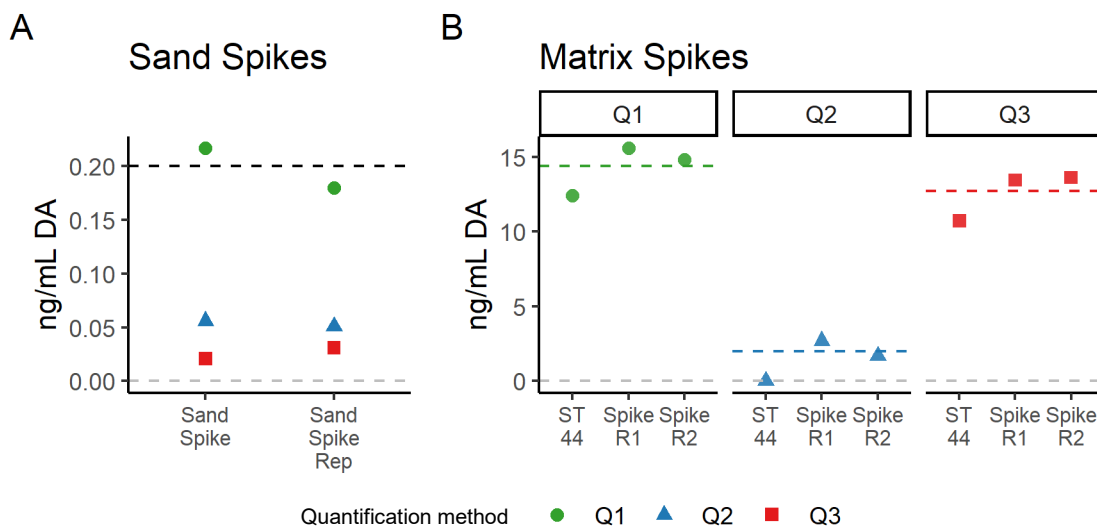


Figure B1. Results from split extract samples of A) sand spikes with the dashed line indicating the spike amount and B) environmental sample (St 44) and environmental matrix spike replicates (Spike R1 and Spike R2) analyzed by each quantitation method utilized for true Bight '18 samples (Q1-Q3). The results are faceted by quantitation method in panel B and the colored dashed lines indicate the average concentration of domoic acid detected in spiked samples using each method.

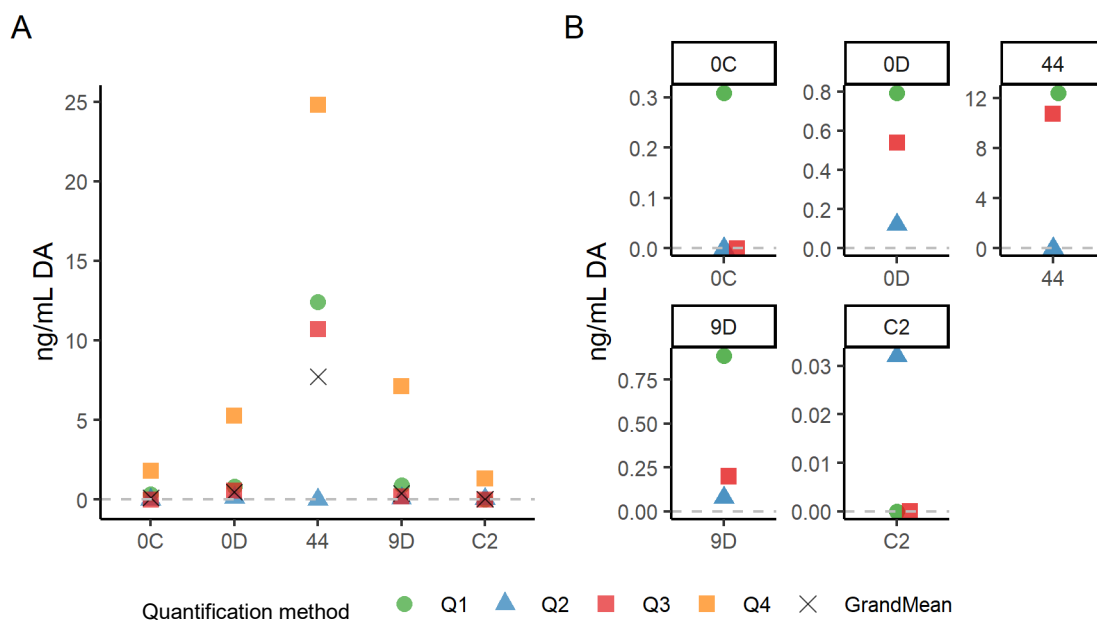


Figure B2. Results from split extract samples analyzed by each laboratory. Panel A) show the results of extract analysis using quantifications methods 1-4 described in Table B1 and panel B) shows the results of approaches Q1, Q2 and Q3 which were the methods utilized for true Bight '18 samples. The results are faceted by station. Samples reported as below the method detection limit are indicated as zeros which is indicated by the grey dashed line.

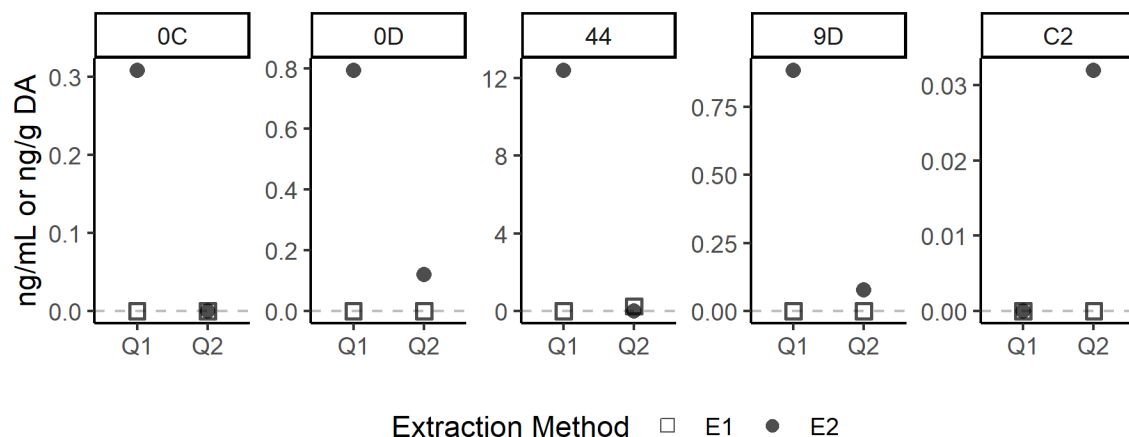


Figure B3. Comparisons of the results of the split sample extracts prepared by a single lab with extraction method 2 (E2, black circle) and the split sediment samples prepared by extraction method 1 (E1, open square) across labs using quantification method 1 and quantification method 2 (Q1 and Q2 on x-axis). Results are faceted by environmental station and y-axis is scaled to the range of concentrations reported from samples for each station.

In addition to analytical method, differences in extraction methods appeared to increase the variability between reported DA concentrations (Figure B3). For the labs that utilized either quantification method 1 or quantification method 2, a general trend was observed that the analytical approach detected DA in samples prepared using extraction method 2 (black circles in Figure B3), while not detecting DA in extracts of the same samples prepared using the currently published method (E1).

Benthic Infauna Intercalibration

Study Design

Benthic infauna samples were collected in the 2017 and 2019 Central Bight Revisit Study and in the 2018-2019 Monthly Revisit Study. The initial 2017 samples were processed by the Kudela Lab at UCSC and subsequent samples processed by the Biogeochemistry group at SCCWRP. Both UCSC and SCCWRP used the same initial extraction protocols, but UCSC conducted an SPE clean up step and quantified the DA extract using LC-MS while SCCWRP quantified the extract using ELISA and did not use an SPE clean up step for extracts. The purpose of the benthic infauna intercalibration experiment was to determine that the extraction and quantification methods yielded comparable results (Table B3).

The preparation of tissue homogenates prior to extraction differed between SCCWRP and UCSC. In the samples analyzed by SCCWRP, collected organisms were sorted coarsely based on taxonomy and estimated feeding strategies, then processed and analyzed as individual sorting groups. A weight averaged DA tissue concentration of sorting groups was then calculated for the purposes of reporting. In contrast, samples analyzed by UCSC were not sorted prior to tissue homogenization and represent a bulk composite DA concentration for benthic infauna tissue in a given sample. The comparability of the weight averaged DA tissue concentrations generated by SCCWRP and the composite concentrations by UCSC are assumed to be variable given that the

different infauna groups were found to have variable body burdens of DA. In a composite tissue homogenate, the signal of organisms of low abundance and high body burdens of DA (e.g., worms) could be diluted by the tissues of organisms of higher abundances and low or no DA tissue concentrations. It was assumed that this reduction effect is minimized when sorted groups were analyzed for tissue DA concentrations individually, although this assumption was not tested in the intercalibration study. The calculation of a weight averaged DA tissue concentration of sorting groups likely increases the comparability between these two approaches, but the overall improvement is not known. To be conservative, samples analyzed by SCCWRP were not quantitatively compared to those analyzed by UCSC since this effect is unknown.

Table B3. Approaches used to quantify the DA concentrations in infauna tissues for each component of the Bight '18 field study.

<i>Tissue Prep</i>	<i>Extraction</i>	<i>Quantification</i>	<i>Bight Study Components</i>	<i>MDL/RL (ng/g)</i>
Homogenize composite	50% MeOH + SPE clean up	LC-MS	2017 Central Bight	0.18
Sort into groups, homogenize groups	50% MeOH	ELISA	2019 Central Bight, 2018-2019 Temporal	10

Methods

A total of 13 intercalibration samples were prepared at SCCWRP using a tissue homogenate from all four sorting groupings. SCCWRP extracted the samples according to the protocol outlined in the methods section of the main body of this report using 50% methanol and a 1:10 tissue mass to methanol volume ratio. One aliquot of sample extract was filtered and analyzed via ELISA. A second aliquot of sample extract SPE cleaned and then analyzed via LC-MS. This exercise was conducted twice, first with 5 samples prepared in the summer of 2018 and the second with 8 samples prepared in the summer of 2019.

Results

General agreement was observed between the samples analyzed via ELISA and LC-MS, as shown in Figure B4. In general, samples were near or above the 1-to-1 line, indicating agreement between methods. The samples quantified via LC-MS generally resulted in higher concentrations than those quantified via ELISA. The percent difference between ELISA and LC-MS results ranged between 1% - 67% for samples below 5ppm ($n = 9$) and between 5% - 83% difference for samples greater than 5 ppm ($n = 4$).

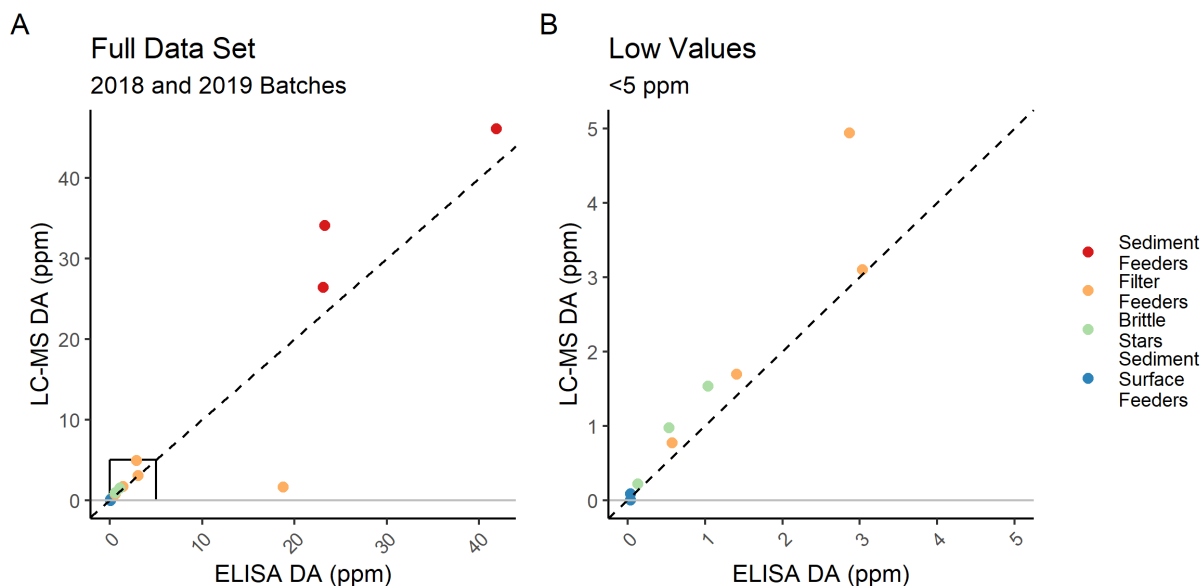


Figure B4. Comparisons of the results of tissue sample extract prepared and analyzed via ELISA at SCCWRP and prepared and analyzed via LC-MS at UCSC. Dashed line indicates the 1-to-1 line of comparison between approaches.

ELISA vs LC-MS/MS Comparison

Study Design

An exploratory exercise was conducted to compare analytical and immunological approaches for the analysis of marine sediments. There was general interest in the development of an ELISA based screening approach for the analysis of marine sediments. This effort leveraged the analyses already conducted on the environmental samples collected from Station 24 and Station 28 for the field study with a re-preparation and analysis of the archived samples via ELISA. This allowed for the comparison of results using Approaches 1, 2, 3 and 5 in Table B1.

Methods

A total of 37 environmental samples were prepared for analysis at SCCWRP in the summer of 2020. Samples from Station 28 and Station 24 collected during the Central Bight Study in 2017 ($n = 2$) and 2019 ($n = 2$), and the Monthly Revisit Study between 2018 and 2019 ($n = 33$) were prepared for analysis via ELISA. Sediment extraction methods followed those of Phonsiri et al. (*in prep*). Briefly, 10 mL of 10% MeOH was added to 10 g of ideal wet weight (wet weight equivalent to 10 g dry weight sediment) and vortexed for 2 minutes on high. Samples were then centrifuged at 3500 RPM for 10 minutes and the supernatant was collected for analysis via ELISA.

Sediment extracts were analyzed via ELISA (Mercury Science DA Test Kit product #DAK-36) using the methods described in Litaker et al. (2008) for water samples. All samples were run with at least a 1:10 dilution with the sample diluent provided in the kit to mitigate any matrix

effects from the methanol and sediment. This method has a minimum detection limit of 1.0 ng DA/g sediment due to the dilution step.

Results

Sediment extracts that were analyzed via ELISA did not show strong agreement with the analytical results reported in the main study. There was agreement between detections in 5% of the samples analyzed, although the concentrations of DA by ELISA were about 3 times lower than the concentrations determined via LC-MS. DA was detected in 8% of samples analyzed via ELISA where analytical approaches indicated DA was below the detection limit. Conversely, 11% of samples analyzed using an analytical approach had detectable DA that was not detected via ELISA. DA was not detected in 76% of samples using both approaches.

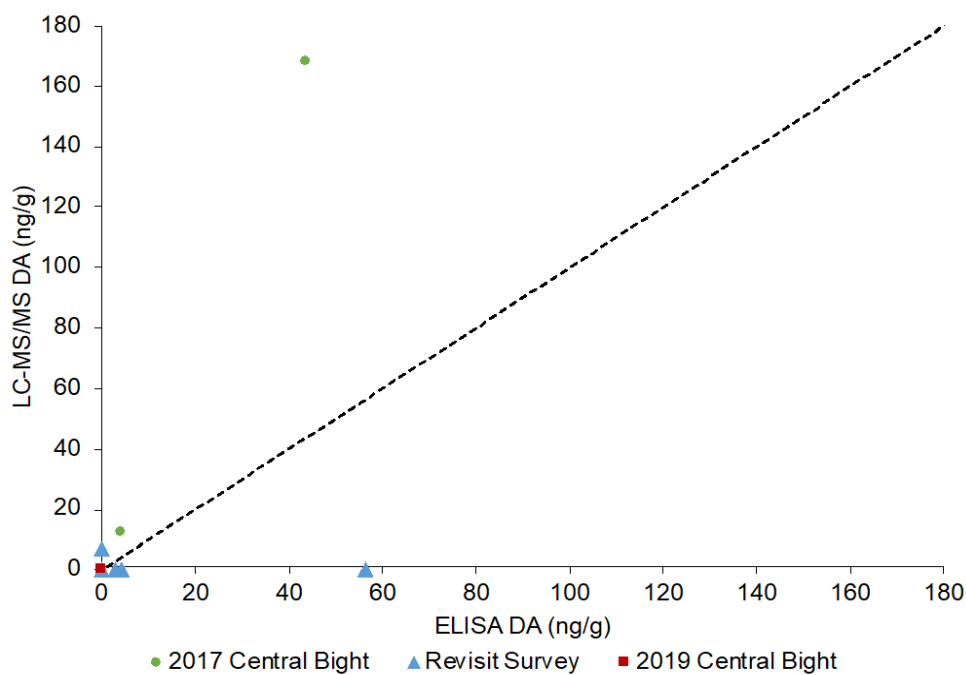


Figure B5. Comparisons of the results of sediment sample extract from the Monthly Revisit Survey prepared following Approach 5 (Table B1) and analyzed via ELISA and samples analyzed via Approaches 1 – 3 (Table B1). Dashed line indicates the 1-to-1 line of comparison between approaches.

Conclusions and Recommendations

The results of this intercalibration study indicated a low level of comparability across DA quantification approaches in marine sediments. The variance between analysis approaches was due to both the variance in the efficiency of extracting DA from the sediment and variance in quantification of DA in the extract. The sample size for this intercalibration study was relatively small but the results of this intercalibration point to some preliminary conclusions about the performance each method. Of the two methods for extraction used in this study (Table B1), extraction method 2 (E2) appeared to provide higher recovery than extraction method 1. Of the quantification methods employed (Table B1), methods utilizing an external standard curve (Q1 and Q3) appeared to perform better than the other methods. Additional optimization and

replication of both extraction and quantification methods for marine sediments is needed to confirm the performance and repeatability of these candidate analytical methods. From these efforts, a standardized approach for extracting and quantifying DA in sediments should be recommended. Furthermore, additional inter-comparison and intercalibration exercises using the standardized approach should be conducted prior to future regional studies involving multiple laboratories.

The use of ELISA for the analysis of marine sediments appears to be a viable approach, particularly for use as a screening tool, but may not yield highly comparable results to analytical approaches. A confounding factor in the experiments conducted in this study is the age of the environmental samples (2-3 years) which could have contributed to discrepancies in results between the ELISA approach and the analytical approaches. ELISA approaches for sediment also provide less sensitivity than analytical approaches due to the need to dilute sample extracts to lower the MeOH concentration to prevent assay matrix effects. Additional experiments are needed with reference materials to gain a better understanding of the performance of ELISA with marine sediment samples and the comparability of the approach to analytical methods.

The intercalibration study showed a higher level of comparability between analytical and immunological approaches for infauna tissues, with LC-MS results typically higher than the ELISA results. Either of these approaches could be utilized in future study involving tissues, although inter-comparison and intercalibration exercises should be conducted with both certified reference materials and environmental samples to ensure data quality.

APPENDIX C: CYANOTOXIN TRANSFER AT THE LAND-SEA INTERFACE

Introduction

Cyanobacterial HAB species produce toxins in the freshwater and estuarine environments, which can be transported downstream through hydrological interconnections and cause issues in estuarine and marine waters. These toxins, called cyanotoxins, have caused direct impacts in the marine environment such as the mortality of over 30 threatened marine California Sea Otters (*Enhydra lutris*) due to ingestion of contaminated shellfish (Miller et al. 2010). Watershed studies in Monterey Bay have shown that this downstream transport of microcystins is a persistent and prevalent issue throughout the watershed (Gibble and Kudela 2014), and that cyanotoxins are prevalent throughout freshwater and estuarine environments in the SCB (Fetscher et al. 2015; Howard et al. 2017; Tatters et al. 2017). Cyanotoxins have been shown to bioaccumulate in marine shellfish in CA and WA (Miller et al. 2010; Kudela 2011; Gibble and Kudela 2014; Preece et al. 2015a, 2015b; Gibble et al. 2016; Peacock et al. 2018). Due to the recognition that both marine and freshwater toxins are present in marine waters, recent studies have detected multiple freshwater and marine toxins simultaneously in marine shellfish in central CA (Peacock et al. 2018). To date, marine shellfish have not been investigated for the presence of cyanotoxins in the SCB.

Due to the transport of freshwater toxins into estuarine and marine environments, there is a new recognition that management and mitigation of HABs need to occur cohesively across the freshwater to marine continuum due to the hydrologic interconnections and toxin impacts downstream of the bloom event origin (Paerl et al. 2016; Paerl et al. 2017; Paerl et al. 2018).

Cyanotoxins produced in fresh and estuarine waterbodies have been shown to be transported downstream of their origin, and to cause direct impacts in the marine environment, such as the mortality of threatened marine California Sea Otters. Recent studies have shown that cyanotoxins can bioaccumulate in marine shellfish, posing a human and wildlife health risk since cyanotoxins are not currently included in the California Marine Biotxin Monitoring Program.

The Bight '18 HABs program will determine the concentration of microcystins in caged mussels at the end of the dry weather season and during the first major storm of the wet season, called “first flush”.

Human health guidelines have been developed for microcystins in food (WHO 2003; Ibelings and Chorus 2007; Mulvenna et al. 2012; OEHHA 2012). The World Health Organization (WHO) established a tolerable daily intake (TDI) of 0.04 µg/kg/day for microcystin-LR (WHO 2003). Based on this TDI, several international groups have set guidance levels. Australian health guideline values were established for the No Observed Adverse Effect Level (NOAEL) of 40 µg/kg/day, and an acceptable daily limit of 51 µg/kg of mussel was set (Mulvenna et al. 2012). Ibeling and Chorus (2007) determined a seasonal daily exposure TDI for microcystins in seafood for adults (300 µg/kg/day) and children (40 µg/kg/day). In California, there is regulatory guidance for microcystins in fish tissue that has been established by the Office of Environmental Health and Hazard Assessment (OEHHA) of 10 ng microcystins per gram of fish (OEHHA 2012) and other studies have used this guidance value to provide context for microcystin concentrations detected in mussels (Gibble et al. 2016; Peacock et al. 2018).

Pilot Study Rationale

A cyanotoxin pilot study was conducted in the fall of 2018 to determine the feasibility of mobilizing a Bight-wide project using the proposed study design. The main goals of this pilot project were to:

1. Establish field methods for mussel deployment and collection that result in high survival rates of test organisms.
2. Develop laboratory methods for cyanotoxin extraction and quantification via ELISA.

The field methods were tested and verified, and a detailed SOP was developed for mussel deployment and collection. The laboratory methods are an ongoing project at SCCWRP. The sections below outline methods, preliminary results and lessons learned from the December 2018 cyanotoxin pilot study.

Methods

Mussel Deployment and Collection

Sunset Marina in Huntington Beach (33.727136, -118.076406) was chosen as the single site for the pilot project. This site is located in between Bolsa Chica Channel to the north and East Garden Grove Westminster Channel to the South at the terminus of the Anaheim Bay Watershed in Orange County, California, and receives intermittent freshwater influence.

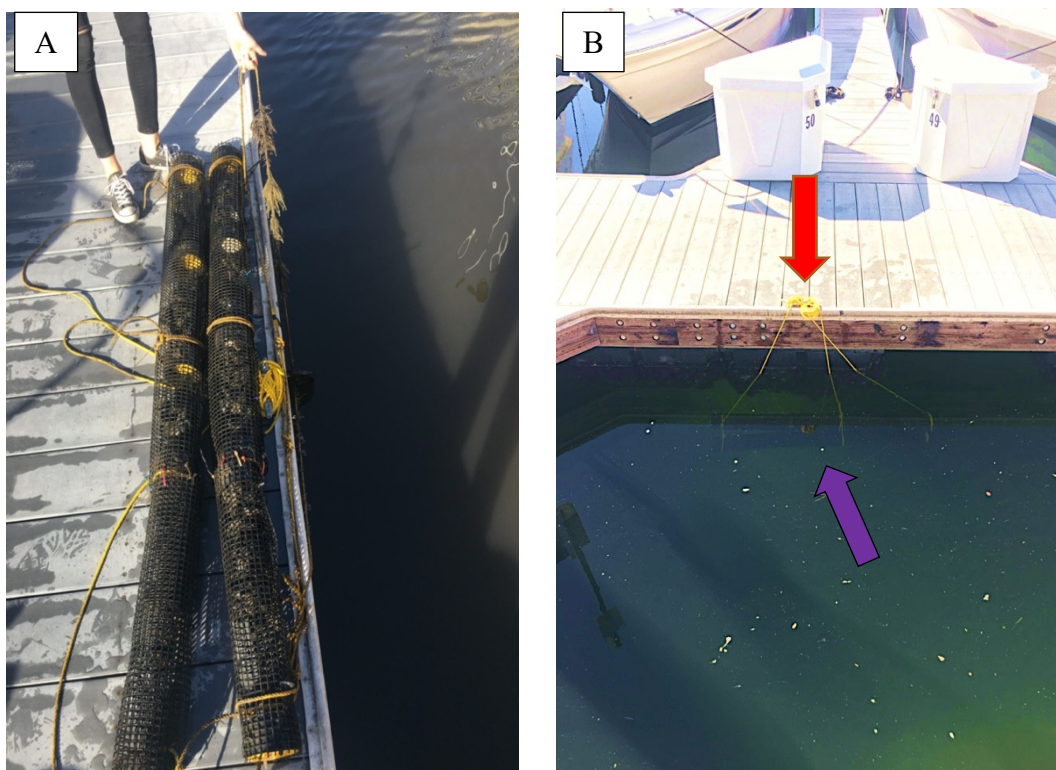


Figure C1. Mussel deployment apparatus. Panel A) shows the assembled cage loaded with mussels and panel B) shows deployed cage (purple arrow) on a single cleat (red arrow) on the floating dock in Huntington Harbor.

To house the mussels, cylindrical cages were built from rigid plastic mesh netting. Four-inch PVC pipe and PVC caps were used as cage endcaps as well as partitions/supports in the middle of the cages. The plastic netting was secured to the end caps, partitions, and itself with zip ties (Figure C1A). Flaps to access the mussels were cut into each partition, then sealed with zip ties to allow for access to the mussels during sampling time points. A line that ran from the bottom of one end of the cage to the other end held a single weight used to steady the cage against tides and currents. Two additional lines, each one secured at one end to the top of either end of the cage and loose on the other end, were used to hang the cage from a single cleat along the walking ramp in the marina (Figure C1B).

Cultured mussels (*Mytilus galloprovincialis*) were used in this study and were provided by Catalina Sea Ranch. Before collection by the Sea Ranch and deployment at Sunset Marina, mussels were living on ropes 5 miles offshore of San Pedro. Initially ~275 mussels were picked up from Catalina Sea Ranch, stored in a cooler with wet paper towels overnight, and deployed in Sunset Marina on 10/19/2018 (T0). A second batch of mussels (n = ~70) clearly marked as separate from the first batch using nail polish were picked up seven weeks later the following week and added to the cages on 12/4/2018.

The pilot experiment ran for 61 days from set-up and deployment on 10/19/2018 through 12/17/2018. Mussels were collected weekly during dry weather and then more frequently 1 – 10 days following precipitation events. Three precipitation events occurred during the pilot study, 1 small event on 11/22 (0.41 in) and larger events on 11/29-11/30 (0.9 in) and 12/6-12/7 (1.71 in). Mussels were collected more frequently following precipitation events (Long Beach Airport Weather Station).

Ten mussels were collected during each site visit. Prior to the collection of mussels from the cage, temperature and salinity were measure with a YSI Professional Plus handheld multiparameter meter at the surface of the water and visual observations of turbidity, weather, and tide were noted. To collect the mussels, the cage was pulled out of the water and the access hatch was opened to remove the mussels. A bucket was used to rinse any sediment off the outside of the mussels with ambient water. The cage was examined for any dead mussels (dead mussels are visually identifiable as they have open shells and have no remaining flesh inside the shell) and all dead mussels were removed and counted. To prevent excessive fouling blocking the water flow through the cages, the cages were scrubbed with a heavy duty green scouring pad before being returned to the water. Initially, mussels had been deployed with groups of ten in small mesh bags for easy recovery of ten individuals; however, it was determined these bags were suffocating the mussels and they were removed.

Upon return to the lab, the mussels were shucked immediately, and then homogenized in a food grade blender. A subset of mussels were frozen whole and shucked and homogenized for later analysis to test feasibility of shucking and homogenizing after being stored frozen.

Laboratory methods for cyanotoxin extraction and quantification

No standardized method exists for the extraction and analysis of cyanotoxins from mussel tissues. Two extraction approaches were tested for their suitability for downstream analysis via ELISA using recovery experiments. The candidate extraction approaches are described in Preece et al. (2015b) and Gobble et al. (2016).

Briefly, tissue from undeployed mussels was homogenized in the same manner as the experimentally deployed mussels. The tissue homogenate was spiked with microcystin-LR standard (Abraxis). Recovery experiments were conducted at two concentrations: 0.6 ppb and 1.2 ppb, mimicking the experimental design described in Preece et al. (2015b). Additional unspiked tissue was also processed as a control. All 3 tissue preparations were extracted according to the candidate extraction approaches (Table C1).

Table C1. Summary of extraction methods used to pilot with Microcystin DM ELISA. Table adapted from Preece et al. (2015b).

Step	Method 1 (Gibble et al. 2016)	Method 2 (Preece et al. 2015b and Wilson et al. 2008)
	Shuck and homogenize mussel tissue	
1	To spike samples, MC stock solution is added to yield final concentrations of 0.60 and 1.2 ppb	Add 1 g of lyophilized mussel tissue to glass centrifuge tubes
2	Weigh out 2g of tissue, add 10mL of 90:10 MeOH:MQ, with 0.1% TFA (Acid MeOH)	Homogenize samples in 8 mL solvent
3	Bath sonicate for 10min	To spike samples, MC stock solution is added to yield final concentrations of 0.60 and 1.2 ppb
4	Centrifuge 10 min at max speed	Vortex samples
5	Pour off supernatant into glass vial.	Add 2 mL of solvent to rinse sides
6	Dilute MeOH from sample to < 5% MeOH by adding 5mL sample + 95mL MQ with 0.1% Formic Acid, 0.05% Trifluoroacetic Acid (Acid Water)	Incubate 2 h at 4°C in dark environment
7	Condition column by adding 10 mL 100% MeOH, vacuum thru*	Centrifuge 10 min~7000 rpm
8	Rinse column by adding 10 mL Acid Water, vacuum thru*	Remove supernatant to 50-mL tubes
9	Load sample (diluted in step 6) by pouring diluted sample into column	Add 8 mL 75 % MeOH+ 0.002 % AA
10	If sample > 1% lipid content, add 5 mL 10% MeOH	Incubate 24 h at 4 °C in dark environment
11	Dry 5 min under vacuum	Centrifuge 10 min~7000 rpm
12	Add 2x 1mL Acid MeOH to elute, store until analysis	Remove supernatant to same tubes
13	-	Decant to 20-mL syringe, 0.45-µm filter
15	-	Evaporate to dryness under N2 at 60 °C
16	-	5 mL 75% MeOH, store until analysis

The extracts from the spike recovery experiments were analyzed via a direct monoclonal (DM) Total Microcystins ELISA kit (Abraxis, LLC). Extracts were diluted using kit provided diluent to reduce MeOH concentrations to < 20% to avoid matrix effects, as recommended by manufacturer guidelines.

An additional 5 samples from environmentally deployed samples were also prepared according to the methods of Gobble et al. (2016) for analysis via LC-MS. Samples from 10/26/2018, 11/23/2018, 11/30/2018, 12/7/2018 and 12/9/2018 were prepared and analyzed at UCSC according to the analytical methods described in Gobble et al. (2016).

Results

ELISA

Method 1 yielded a high rate of false negatives (100%), while Method 2 yielded a high rate of false positives (100%; Figure C2). Method 2 also did not show a signal response to the spike MC concentrations between the 0.6 ppb and 1.2 ppb spikes.

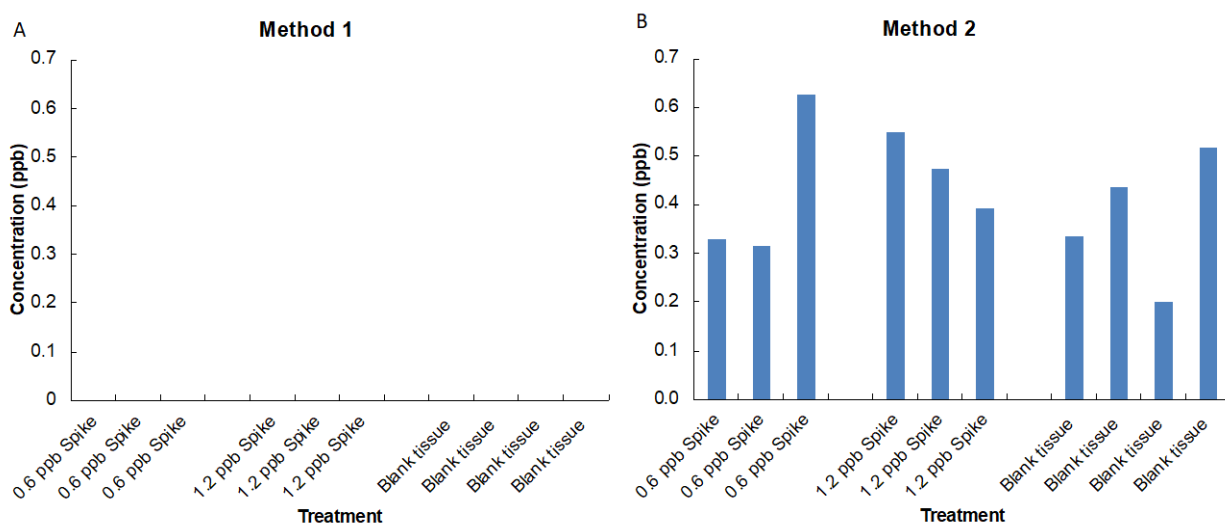


Figure C2. Results from microcystin spike-recovery experiments using a microcystin DM ELISA kit following the extraction methods described in Table C1.

LC-MS

The selected tissue samples analyzed via LC-MS from environmental deployments (Table C2) indicated that microcystins were present in Huntington Harbor and were accumulated in deployed mussels. The results indicate that microcystins were present in the environment early on in the pilot study and then again following a large precipitation (~1.7 inches) event.

Table C2. Tissue samples from environmental deployments that were prepared and analyzed via LC-MS.

Sample	Date	Description	Total Microcystin Concentration (ng/g)
1	10/26/2018	7-days after deployment	15.6
2	11/23/2018	1-day post precipitation event 1	BD
3	11/30/2018	1-day post precipitation event 2	BD
4	12/7/2018	1-day post precipitation event 3	BD
5	12/9/2018	3-days post precipitation event 3	122

Conclusions and Recommendations

The pilot study successfully determined methods to deploy live mussels for an extended period without significant loss of study organisms. The two most likely issues for the mussels during deployment were determined to be suffocation and predators. Mussels can also suffocate if they become buried in the sediment, the cage becomes excessively fouled or if organisms are packed too tightly in the cage. When lowering the cage into the water, the cage should sit below the tidal range to assure they remain in the water, and the cage should hover at least a few feet above the bottom of the channel, to assure the mussels do not get buried with sediment.

Checking that the cage is sitting in the water parallel to the water surface/channel bottom (rather than hanging at an angle) will ensure the mussels are at an ideal depth and prevent them from all ending up clumped on one side of the cage segment. Cages require maintenance on a 7-10-day basis to ensure fouling does not block water-flow and suffocate organisms. Lastly, organisms should not be packed too tightly in the cages. Depending on the location, predators such as crabs may try to get into the cages. This is addressed by securing cage components and cutouts with zip ties and inspecting these connections during each visit to the site.

The pilot study indicated that microcystins were present and accumulated in mussel tissues at Huntington Harbor. This study also highlighted that significant effort is required to develop a mussel tissue extraction protocol that would yield sensitive and reliable results. The pilot relied on testing two candidate methods for use with ELISA and both showed that significant work will be needed to develop an appropriate extraction protocol. Ultimately, these efforts were decided to be outside of the scope of the Bight project and therefore the recommendation is to conduct a future study relying on analytical methods, which is a more commonly used approach for microcystins in tissues within the scientific literature. A project led by Chris Gobler (Stony Brook University) and Raphael Kudela (UC Santa Cruz) to standardize analysis approaches for microcystins in shellfish tissue was recently funded by NOAA and it is recommended that the results of these efforts be leveraged in a future study.