

Critical Review

Integrative monitoring strategy for marine and freshwater harmful algal blooms and toxins across the freshwater-to-marine continuum

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Abstract

Many coastal states throughout the USA have observed negative effects in marine and estuarine environments caused by cyanotoxins produced in inland waterbodies that were transported downstream or produced in the estuaries. Estuaries and other downstream receiving waters now face the dual risk of impacts from harmful algal blooms (HABs) that occur in the coastal ocean as well as those originating in inland watersheds. Despite this risk, most HAB monitoring efforts do not account for hydrological connections in their monitoring strategies and designs. Monitoring efforts in California have revealed the persistent detection of cyanotoxins across the freshwater-to-marine continuum. These studies underscore the importance of inland waters as conduits for the transfer of cyanotoxins to the marine environment and highlight the importance of approaches that can monitor across hydrologically connected waterbodies. A HAB monitoring strategy is presented for the freshwater-to-marine continuum to inform HAB management and mitigation efforts and address the physical and hydrologic challenges encountered when monitoring in these systems. Three main recommendations are presented based on published studies, new datasets, and existing monitoring programs. First, HAB monitoring would benefit from coordinated and cohesive efforts across hydrologically interconnected waterbodies and across organizational and political boundaries and jurisdictions. Second, a combination of sampling modalities would provide the most effective monitoring for HAB toxin dynamics and transport across hydrologically connected waterbodies, from headwater sources to downstream receiving waterbodies. Third, routine monitoring is needed for toxin mixtures at the land–sea interface including algal toxins of marine origins as well as cyanotoxins that are sourced from inland freshwater or produced in estuaries. Case studies from California are presented to illustrate the implementation of these recommendations, but these recommendations can also be applied to inland states or regions where the downstream receiving waterbody is a freshwater lake, reservoir, or river. *Integr Environ Assess Manag* 2022;00:1–19. © 2022 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: Cyanotoxins, Environmental monitoring, HAB management, Harmful algal blooms

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INTRODUCTION

Marine and inland toxin-producing harmful algal blooms (HABs) are typically monitored and managed as hydrologically isolated events. Historically, coastal waters have only been monitored for marine algae and their toxins. Increases in the occurrence and severity of HABs have been reported globally in marine and freshwater environments (Brooks et al., 2017; Hallegraeff, 1993; Hudnell, 2008; O'Neil et al., 2012; Paerl & Huisman, 2009). This intensification has been attributed to

increased temperatures, hydrologic events (such as storms, droughts, etc.), and eutrophication (Lehman et al., 2017; Paerl & Otten, 2013; Paerl et al., 2018; Paerl & Paul, 2012), although the biophysical processes controlling intensification in fresh, marine, and coastal waters may vary between water types. In recent years, recognition has increased of the effect that HABs occurring in freshwater can have on ecosystems at the land–sea interface owing to the hydrological interconnection of the watershed and the coastal ocean. Hydrological interconnections across different types of surface waters and watersheds, from inland lakes and reservoirs, streams, and rivers, to estuaries, coastal lagoons, and marine waters, span the freshwater-to-marine continuum. Estuaries and other types of marine outlets therefore face the dual risk from HABs that occur in the coastal ocean as well as HABs originating in inland watersheds (Gibble & Kudela, 2014; Miller et al., 2010; Peacock et al., 2018; Preece et al., 2017; Tatters et al., 2019, 2021). Despite this risk, most HAB monitoring efforts are not designed to account for hydrological connections. This is particularly so for marine HAB monitoring programs at the land–sea interface, which rarely consider the influence of the flows from freshwater environments and the potential HAB toxins they may transport. Awareness has increased that unique monitoring and management approaches are needed for the land–sea interface due to the potential for the discharge of freshwater HABs into the nearshore coastal environment.

Recent studies have underscored the important role of inland waters as sources of cyanobacterial cells and cyanotoxins that can be transported downstream into marine and estuarine waterbodies. The transportation of cyanobacteria and cyanotoxins can range from a few to hundreds of kilometers (Bormans et al., 2019; Bowling et al., 2013; Davis et al., 2014; Graham et al., 2012; Miller et al., 2010; Otten et al., 2015; Preece et al., 2015; Rosen et al., 2018). Cyanobacteria have been shown to survive release from reservoirs through hydroelectric dams and to remain viable and capable of toxin production downstream (Bouma-Gregson et al., 2017; Genzoli & Kann, 2017; Graham et al., 2012; Ingleton et al., 2008; Otten et al., 2015; Williamson et al., 2018). For *Microcystis* specifically, the transport of cells for long distances, as well as bloom development downstream, has been documented by applying molecular methods in several river, lake, and estuarine systems including the Kansas River, the Lower Great Lakes, and the Klamath River and Estuary (Davis et al., 2014; Graham et al., 2012; Otten et al., 2015). Salinity can influence bloom formation and toxin production, and a wide range of salinity tolerance has been documented for different species and strains of cyanobacteria (Lehman et al., 2005; Miller et al., 2010; Preece et al., 2017; Rosen et al., 2018). For example, Rosen et al. (2018) determined Florida strains of *Microcystis aeruginosa* are tolerant of salinities up to 18 psu, whereas *Dolichospermum circinale* does not tolerate salinities greater than 7.5 psu. Preece et al. (2017) reviewed several studies demonstrating variability of the salt tolerance

of *Microcystis* and *Anabaena* spp. Lehman et al. (2005) demonstrated *M. aeruginosa* survived at salinities from 0.1 to 18 for extended periods, and Miller et al. (2010) documented *Microcystis* spp. could survive in seawater from Monterey Bay, California, for 48 h. Studies have also demonstrated that microcystin (MC) production can continue in saline waters and release of intracellular MCs are observed in higher salinity waters (Preece et al., 2017; Rosen et al., 2018).

Most freshwater studies to date have focused on planktonic blooms in lentic systems; however, benthic cyanobacterial mats in rivers and streams have also been reported as sources of cyanotoxins (Bouma-Gregson, Kudela, et al., 2018; Bouma-Gregson, Olm, et al., 2018; Fetscher et al., 2015; Graham et al., 2020; McAllister et al., 2016; Wood et al., 2020; Quiblier et al., 2013). Benthic cyanobacterial mats often form gas-filled vertical spires that can become detached and float to the surface (Bouma-Gregson et al., 2017). Buoyancy is the result of the production of oxygen bubbles in the intracellular mucus, allowing floating cyanobacterial mat clumps to be transported downstream where they often accumulate in quiescent areas (Bouma-Gregson et al., 2017).

It is unclear to what extent emerging environmental, human, and wildlife health issues are associated with the transport of cyanotoxins to downstream receiving waters because routine, integrated watershed monitoring has been lacking. However, cyanotoxins have been shown to accumulate in aquatic food webs (Acuña et al., 2020; Bolotaolo et al., 2020; Lehman et al., 2010; Smith & Haney, 2006) and to bioaccumulate in estuarine and marine shellfish in the USA (Bukaveckas et al., 2018; Garcia et al., 2010; Gibble et al., 2016; Miller et al., 2010; Peacock et al., 2018; Preece et al., 2015; Tatters et al., 2019). Microcystins can be retained in California mussels (*Mytilus californianus*) for up to eight weeks after exposure (Gibble et al., 2016), and their persistence may increase the risk of exposure to wildlife and humans. The exposures of higher trophic level to MCs have been documented in marine birds (Gibble et al., 2017), cetaceans in Southern California and Florida (Brown et al., 2018; Danil et al., 2021), and bull sharks (*Carcharhinus leucas*) in the Indian River Lagoon, Florida (Michelle Edwards, Florida Atlantic University, personal written communication, 2021). Negative effects from exposure including mortality in sea otters (*Enhydra lutris*) in Monterey Bay and toxic and immune health impacts on coastal bottlenose dolphins (*Tursiops truncatus*) in Florida have also been documented (Brown et al., 2018; Miller et al., 2010). A state of emergency was declared in Florida in 2016 due to the transport of *Microcystis* cells from Lake Okeechobee that caused blooms at the mouths of the Caloosahatchee and St. Lucie Rivers (Florida Department of Environmental Protection, 2017; Rosen et al., 2018). The closure of Mississippi beaches from June to August of 2019 was in part the result of the opening of the Bonnet Carre spillway in Louisiana to manage Mississippi River flooding by releasing freshwater to the coast. This issue has also been observed on a smaller scale

in Lake Pontchartrain, Louisiana (Bargu et al., 2010; Parra et al., 2020).

In California, evidence is mounting that cyanotoxins constitute considerable health risks downstream from their freshwater sources. Cyanotoxins have been detected in a wide array of lentic and lotic ecosystems in California including lakes, reservoirs, depressional wetlands, coastal lagoons, estuaries, streams, and rivers (Bouma-Gregson, Kudela, et al., 2018; Drake et al., 2010; Fetscher et al., 2015; Graham et al., 2020; Howard et al., 2017, 2021; Izaguirre et al., 2007; Kelly et al., 2019; Kudela, 2011; Loftin, Graham, et al., 2016; Magrann et al., 2015; Tatters et al., 2017, 2019). Additionally, benthic freshwater cyanobacterial proliferations, including several toxin-producing cyanobacterial species, have been identified throughout California waterways and regionally (Bouma-Gregson, Kudela, et al., 2018; Bouma-Gregson, Olm, et al., 2018; Bouma-Gregson et al., 2019; Fetscher et al., 2015; Kelly et al., 2019; Izaguirre et al., 2007), although understanding of the factors that promote benthic blooms is limited (see review Wood et al., 2020). California Statewide assessments revealed that benthic algae in wadeable streams were a source of cyanotoxin production and that more than 90% of stream kilometers in California supported potential toxin-producing genera and 23% supported toxin-producing species (Fetscher et al., 2015). Cyanotoxin analysis from that assessment detected MCs in 33% of sites statewide, lyngbyatoxin in 21% of sites, and saxitoxin or anatoxin-a in 7% of sites (Fetscher et al., 2015). Watershed-scale surveys of cyanotoxins in Northern California have linked benthic cyanobacterial mats in the Eel River to more than a dozen dog deaths (Bouma-Gregson, Kudela, et al., 2018; Bouma-Gregson, Olm, et al., 2018; Bouma-Gregson et al., 2019; Kelly et al., 2019; Puschner et al., 2008). Loftin, Clark, et al. (2016) conducted a systematic study of cyanotoxins in water from wadeable streams across the southeastern USA and detected MCs in 39% of the streams.

Cyanotoxins have been detected in marine outflows and marine ecosystems in California (Gibble et al., 2016; Howard et al., 2017; Magrann et al., 2015; Peacock et al., 2018; Tatters et al., 2017, 2019, 2021), a testament to their prevalence and chemical resilience. Recent studies in California illustrate that cyanotoxins can persist when transported into estuarine and marine waters and can cause direct effects in marine ecosystems (Gibble & Kudela, 2014; Gibble et al., 2016, 2017; Howard et al., 2017; Lehman et al., 2005; Miller et al., 2010; Tatters et al., 2017, 2019, 2021). The first report of marine mammal mortalities linked to watershed flushing of freshwater-sourced cyanotoxins occurred in central California in 2007, when many southern sea otters died after exposure to MCs transported from an upstream lake with chronic MC-producing blooms (Kudela, 2011; Miller et al., 2010). The mortalities were attributed to intoxication of MCs from ingestion of contaminated shellfish (Miller et al., 2010). Subsequent studies conducted in Monterey Bay, California, using passive samplers (see Recommendation 2) revealed that downstream transport of MCs was an issue throughout several

watersheds (not just from a single waterbody), with MCs detected in several outflows into the Monterey Bay National Marine Sanctuary (Gibble & Kudela, 2014). Studies conducted in other areas of California have also detected cyanotoxins at the land–sea interface indicating that these occurrences have existed but have gone largely undocumented (Howard et al., 2017; Magrann et al., 2015; Peacock et al., 2018; Tatters et al., 2017, 2019, 2021).

Historically, cyanobacterial blooms were considered a public health issue solely of freshwater lakes, reservoirs, public water supplies, and rivers. This assumption is reflected in the vast body of scientific literature available on public health risks of cyanotoxin exposure in freshwater habitats and the lack of federal and state regulations addressing cyanotoxin ingestion by commercially harvested marine shellfish. Most HAB monitoring programs are not designed to capture the movement of toxins from headwaters to downstream receiving waters and need to be redesigned to address this important emerging issue. A fundamental requirement is to develop a more holistic approach. Paerl et al. (2018) recommended HAB management and mitigation strategies that focus holistically on the watershed inclusive of all hydrologically interconnected waterways from the headwater sources to the downstream receiving waterbodies. Management and mitigation efforts therefore need to be implemented across the hydrologic interconnected waterways that make up the freshwater-to-marine continuum (Paerl et al., 2018). New strategies are necessary to meet these monitoring challenges across the freshwater-to-marine continuum.

We present a HAB monitoring strategy that builds on the concepts introduced by Paerl et al. (2018), providing more detailed and specific actionable recommendations on HAB monitoring across the freshwater-to-marine continuum that will inform management and mitigation of HABs and address the physical challenges encountered when monitoring in these systems. Case studies from California are provided to define and illustrate the recommendations as these issues have become a focus for California water quality managers due to the impacts of freshwater-sourced cyanotoxins in marine waters. We draw on this knowledge to provide recommendations for HAB monitoring and to address the challenges encountered when monitoring across the freshwater-to-marine continuum.

HAB MONITORING RECOMMENDATIONS AND CASE STUDIES

Several overarching monitoring design principles underpin each of our HAB monitoring recommendations. The HAB monitoring efforts that are driven by clearly defined objectives would serve to address specific information gaps and/or management decisions. The elements of monitoring programs, including the spatial and temporal design, sample types, target analytes, quality assurance, and quality control, warrant thoughtful selection based on needs to achieve the identified objectives. The specificity, accuracy, and precision of selected sample analyses should be evaluated to ensure

that the necessary technical level of support is provided for data interpretation and management decisions.

Based on these criteria, we propose three main recommendations addressing the need for a more holistic monitoring design across the freshwater-to-marine continuum. Case studies developed from published studies, new datasets, and existing monitoring efforts in California are presented to illustrate each recommendation and summarize the advantages and limitations of information gathered by different monitoring approaches.

1. HAB monitoring should be coordinated and cohesive across hydrologically interconnected waterbodies and across organizational and political boundaries and jurisdictions.
2. A combination of sampling modalities would provide the most effective monitoring for HAB dynamics and transport across hydrologically connected waterbodies, from headwater sources to downstream receiving waterbodies.
3. Multiple toxins should be routinely monitored across the freshwater-to-marine continuum, and cyanotoxins should be included in estuarine and marine monitoring programs.

Recommendation 1: HAB monitoring should be coordinated and cohesive across hydrologically interconnected waterbodies and across organizational and political boundaries and jurisdictions

Water quality monitoring programs are often designed based on “artificial” boundaries reflective of political and organizational boundaries, federal, state, county, local agency, and Tribal Nation jurisdictions, requirements or restrictions dictated by funding agencies, individual waterbody management, or somewhat arbitrary distinctions such as the waterbody salinity. To effectively and efficiently monitor, manage, and mitigate HABs, these “artificial” boundaries need to be overcome and replaced with coordinated and cohesive monitoring designs that span hydrologically interconnected waters. Such a design may cross international, state, regional, local, and/or Tribal jurisdictions. A multi-organizational design will require all entities, organizations, government agencies, tribes, and waterbody managers to work collaboratively to organize and implement cohesive monitoring, particularly given that these organizational boundaries are often tied to physical boundaries.

Case Study: A multi-organizational approach documents MCs movement throughout the extensive Klamath River and Estuary Ecosystem. The Klamath River flows approximately 420 km from southern Oregon through California and discharges into the Pacific Ocean in Northern California. Dams within the Klamath Basin and conflicts regarding water management exist among the stakeholders, including Klamath River Native American Tribes, farmers, fishers, private citizens, private interest groups, nonprofit entities, and the

hydroelectric dam owner, PacifiCorp. The Klamath River has multiple impairment listings under the Clean Water Act section 303(d) resulting from excessive concentrations of MCs, nutrients, metals, sediment, temperature, and low dissolved oxygen. Historically, many diverse groups were conducting monitoring in the Klamath Basin but as a result of resource limitations and the range of water quality issues, a coordinated monitoring program was developed. Federal, state, and local agencies; tribal entities; hydroelectric dam operators; and watershed groups collectively implement the Klamath Basin Monitoring Program (KBMP). The KBMP was developed to create a comprehensive and inclusive monitoring strategy, develop an efficient way to use resources, generate a holistic picture of the health of the basin, and expand interagency partnerships (<http://kbmp.net/documents/monitoring-plan>). KBMP developed a sampling and analysis plan and data management and sharing plan to provide methodologically consistent water quality data throughout the Klamath River Basin. That information supports management decisions such as development and implementation of Total Maximum Daily Loads (TMDLs) and the removal of four hydroelectric dams per the Klamath Hydroelectric Settlement Agreement (https://www.waterboards.ca.gov/waterrights/water_issues/programs/water_quality_cert/docs/klamath_ferc2082/040616_executed_fnl_khsa.pdf).

Standard operating procedures (SOPs) have been developed for HAB sample collection, analysis, and quality assurance to ensure data are comparable and able to be collated for data analysis (https://www.waterboards.ca.gov/northcoast/water_issues/programs/tmdl/klamath_river/klamath_river_khsa_monitoring/pdf/090629/app_a/Cyanobacteria_Sampling_SOP.pdf). The sampling schedule is also codified, generally every two weeks during peak bloom season (May through October) and monthly from November to April. Harmful algal bloom monitoring throughout the river and estuary is implemented by different entities in different regions, including the U.S. Geological Survey (Upper Klamath Lake), the U.S. Bureau of Reclamation (Link and Keno reservoirs), E&S Environmental Chemistry, Inc. (on behalf of PacificCorp; JC Boyle to Hatchery Bridge), the Karuk Tribe Department of Natural Resources (Hatchery Bridge to Orleans), and the Yurok Tribe Environmental Program (Weitchpec to the lower estuary outflow; Figure 1A).

The Klamath River and Estuary experiences annual toxic HABs dominated by *Microcystis aeruginosa*, originating in the upstream headwater reservoirs (Copco and Iron Gate reservoirs). *Microcystis* cells and MCs are transported downstream and contaminate the river and estuary (Genzoli & Kann, 2017; Kann & Corum, 2009; Kann et al., 2010; Otten et al., 2015). Molecular methods have been used to confirm toxin-producing *Microcystis* blooms originating in Iron Gate Reservoir are the source of downriver *Microcystis* assemblages (Otten et al., 2015). The concentration of *Microcystis* cells and MCs exceeded public health thresholds annually during 2005–2016 in downstream waters (Genzoli & Kann, 2017). Figure 1B illustrates this contamination of the river and estuary

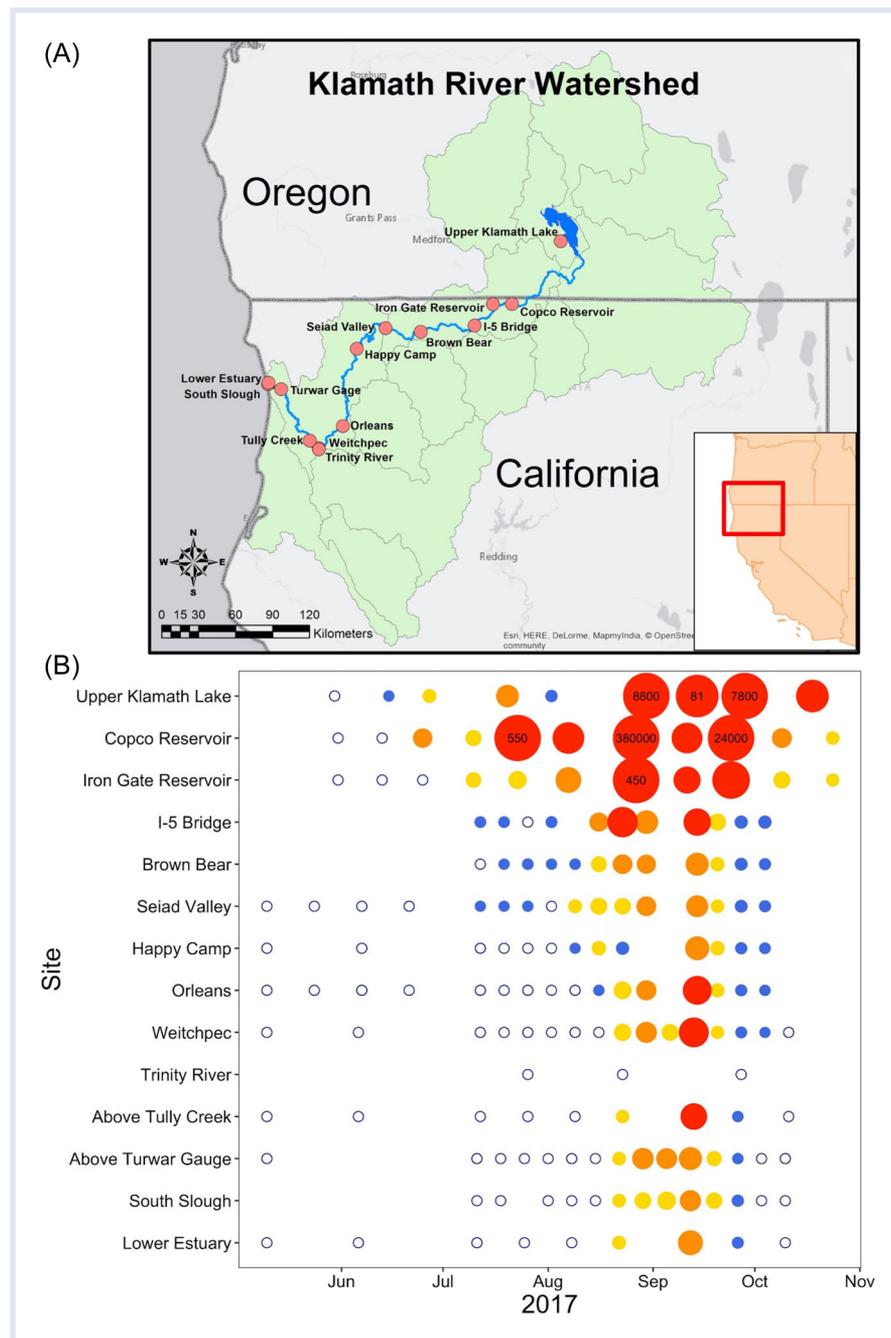


FIGURE 1 Map of a subset of the sites (and location of the area on the west coast of North America in the insert) sampled for the Klamath Basin Monitoring Program (A), and microcystins (MCs) concentrations from water samples collected throughout the Klamath River and Estuarine System during 2017 (B). Sampling sites in (B) on the y-axis are listed from headwaters (top) to downstream river sites (bottom), with the bottom two sites located in the estuary. Colors of symbols and values (when given) indicate the MCs concentrations in $\mu\text{g L}^{-1}$ according to California Recreational Health Thresholds of Caution Action Trigger (yellow: $0.8 \mu\text{g L}^{-1}$), Warning Tier I (orange: $6 \mu\text{g L}^{-1}$), or Danger Tier II (red: $20 \mu\text{g L}^{-1}$; https://mywaterquality.ca.gov/habs/resources/docs/trigger_levels_and_response_decision_tree_for_planktonic_blooms.pdf). Empty circles indicate samples that were below the detection limit for MCs, and filled blue circles indicate samples that were above the detection limit but below the health thresholds

ecosystem and, for the sake of simplicity, shows a subset of sites sampled in 2017 (to coincide with the coastal California dataset collected in 2017 discussed below in Recommendation 3). Microcystins were detected at moderate to high concentrations in Copco and Iron Gate Reservoirs throughout most of summer and autumn, beginning in late June. River samples collected in July and August had

detectable MCs but were below the California recreational health thresholds ($<0.8 \mu\text{g L}^{-1}$). As MCs increased in Copco and Iron Gate Reservoirs in mid- to late summer and autumn, concentrations at the river sites increased substantially. The highest MCs detections in Copco and Iron Gate Reservoirs exceeded the California Danger Tier II threshold ($20 \mu\text{g L}^{-1}$) and coincided with dates when MCs were detected

throughout the Klamath River and Estuary. All sites exceeded California recreational health thresholds ($>0.8 \mu\text{g L}^{-1}$) at that time. Microcystins decreased in Copco and Iron Gate Reservoirs during mid- to late autumn, coinciding with decreases in MCs at the downstream sites. The reservoirs were the sites of HAB origination as they provided ideal habitat for the growth of toxic *Microcystis*, and MCs produced there were transported long distances downriver, more than 300 km, to the estuary (Genzoli & Kann, 2017; Kann & Corum, 2009; Otten et al., 2015).

These insights into bloom origin and transport dynamics have been possible because of the holistic, basin-wide monitoring design and the collaboration of multiple organizations constituting the KBMP. They exemplify how attempts at mitigation of downriver and estuary locations would be ineffective without mitigation in the headwater reservoirs. The coordinated, multi-agency, multi-stakeholder, basin-wide approach to monitoring is key to collecting data and information to make informed management decisions across the freshwater-to-marine continuum. Challenges to the establishment of this approach include a willingness to coordinate among agencies with different jurisdictional boundaries and missions and exertion of effort and resources to initially establish such a program.

Recommendation 2: A combination of sampling modalities are needed to effectively monitor HAB toxin dynamics and transport across hydrologically connected waterbodies, from headwater sources to downstream receiving waterbodies.

Traditional HAB monitoring programs typically rely on discrete sampling (“grab” samples). Grab samples are exceptionally useful for assessing cyanobacterial community composition, toxin concentrations, and for ancillary chemical and physical measurements. However, individual samples provide only “snapshots” of cell abundance or toxin concentration at a single moment in time and space. This approach can miss ephemeral or episodic events, cannot easily characterize spatial and temporal variability, and does not consider that cyanobacteria and toxins within a hydrologically interconnected waterbody may be sourced elsewhere. These issues limit the utility of a grab sample as an observational or predictive tool. In contrast or in addition to grab samples, passive sampling methods provide a cost-effective, continuous, and integrative assessment of dissolved toxin presence in aquatic environments that more readily capture ephemeral or episodic events (see Kudela, 2017 for a review of passive sampling approaches for HAB toxins). Similarly, tissue samples indicate bioaccumulation of toxins and the potential for transfer of toxins to higher trophic levels. Use of these methodologies in concert can provide a powerful approach to characterizing HAB occurrence.

To meet the monitoring challenges across the freshwater-to-marine continuum, multiple sampling modalities, including different sample types and matrices, are required to provide a comprehensive and holistic understanding of toxin dynamics

and transport. We recommend a combination of sample types and matrices fit for purpose, including water samples, passive samplers, and, when present, cyanobacterial mat samples and shellfish or fish tissue. If the goal of the monitoring program includes identifying the source of cells or toxins, then approaches that incorporate genetic methods to determine the relatedness of cyanobacterial populations across hydrologically interconnected waterways are recommended, as applied by Davis et al. (2014) and Otten et al. (2015). Decisions on the specific sample types, sample matrices, and additional water quality measurements to include would be based on the monitoring program objectives, as different combinations may be used based on the goals and information needs identified for the program. Emergence of new information on the importance of dissolved toxins has resulted in new approaches to complementing traditional monitoring methods. Dissolved toxins, however, can be transported long distances and have a relatively ubiquitous distribution in the water column. Monitoring programs have not routinely measured dissolved toxins because these compounds were previously difficult to detect, and the particulate or total fractions have been assumed to be most relevant to public health protection and food web contamination. Recent advances in passive sampling devices have addressed some of the methodological difficulties of measuring dissolved toxins, permitting an enhanced understanding of toxin presence and diversity. For example, recent studies have demonstrated that dissolved domoic acid (dDA) is a significant part of the total domoic acid (DA; particulate plus dissolved DA) present in coastal ecosystems, and dDA is chronically present, even when blooms are not evident (Marquez et al., 2020; Peacock et al., 2018; Umhau et al., 2018; this study, see Recommendation 3 below). Given that persistent low-level exposure to DA has been demonstrated to have serious health consequences (Hiolski et al., 2014; Lefebvre et al., 2017), dDA measurements are increasingly relevant to routine HAB monitoring programs. Cyanotoxins are also ubiquitous in both freshwater and marine receiving waters and evidence is emerging that dissolved cyanotoxins can contaminate the food web through uptake by bivalves (Bouma-Gregson, Kudela, et al., 2018; Gobble & Kudela, 2014; Gobble et al., 2016; Howard et al., 2017, 2021; Peacock et al., 2018; Tatters et al., 2021; Miller et al., 2010). Dissolved MCs can be bioconcentrated by shellfish (Gobble et al., 2016) indicating that, even in the dissolved phase, cyanotoxins pose a health risk. Similarly, contact exposure to cylindropermopsin (Moreira et al., 2013) and lyngbyatoxins or related compounds (Puschner et al., 2017) have been documented in recreational waters.

The most common passive samplers include Solid Phase Micro-Extraction (Ouyang & Pawliszyn, 2006), the Polar Organic Chemical Integrated Sampler (POCIS; Alvarez et al., 2004), Low Density Polyethylene (LDPE), Polydimethylsiloxane (PDMS) strips (Zendong et al., 2014), organic-diffusive gradients in thin films (o-DGT; D'Angelo, 2019; Yao et al., 2019), and Solid Phase Adsorption Toxin Tracking (SPATT; MacKenzie et al., 2004). The most

widely used type of passive sampler for HAB toxins is SPATT, with the number of annual peer-reviewed citations on SPATT increasing 14-fold during the past decade (Kudela, 2017). SPATT is relatively inexpensive, can be constructed using a variety of resins and configurations based on the users' needs (Kudela, 2017), and can measure multiple marine and freshwater HAB toxins simultaneously (Bouma-Gregson, Kudela, et al., 2018; Howard et al., 2018, 2021; Peacock et al., 2018). SPATT samplers can be deployed in multiple ways, including on moorings or buoys (Smith et al., 2019), in ship flow-through systems (Peacock et al., 2018), on autonomous vehicles (Berdal et al., 2014), on a pier or dock (Howard et al., 2021; Kudela, 2011; Lane et al., 2010; Smith et al., 2019), on deployed sondes and flowmeters (Asarian & Higgins, 2018; Howard et al., 2017), on metal pipes (Bouma-Gregson, Kudela, et al., 2018), or on a weighted line and secured by a stake near the water's edge (Gibble & Kudela, 2014; Howard et al., 2017; Tatters et al., 2019).

The adsorption and desorption characteristics of passive samplers make them limited for regulatory uses at this time because of the difficulty of relating toxin concentrations to health thresholds. As with any deployed equipment, vandalism, destruction by debris in flowing systems (e.g., after storms), and fouling by bacteria and sediment can complicate recovery of passive sampling devices or potentially affect the interpretation of recovered toxins. Nonetheless, a combined monitoring approach using SPATT as a complement to water samples has been integrated into many water quality monitoring and assessment studies throughout California as a result of the usefulness of SPATT as a sentinel tool and the ability to capture transient pulses of cyanotoxins (Gibble & Kudela, 2014; Howard et al., 2017, 2021; Peacock et al., 2018; Tatters et al., 2019, 2021).

Marine filter-feeding bivalves have long been used as sentinels for marine toxins, and more recently for freshwater toxins, because they often bioaccumulate HAB toxins. Microcystins in marine bivalves have been reported at concentrations more than 100-fold greater than concentrations in the surrounding water, and both particulate and dissolved toxins can be concentrated by shellfish (Gibble et al., 2016; Miller et al., 2010). Miller et al. (2010) provided the first evidence of bioaccumulation of freshwater-sourced MCs in marine shellfish but a growing number of studies in recent years have documented similar findings along all major US coastlines, with highest concentrations ranging from 15 to 415 $\mu\text{g kg}^{-1}$ (Bukaveckas et al., 2018; Garcia et al., 2010; Gibble et al., 2016; Miller et al., 2010; Peacock et al., 2018; Preece et al., 2015; Tatters et al., 2019, 2021; Christopher Gobler, Stony Brook University, personal written communication, 2020). California's Office of Environmental Health and Hazard Assessment (OEHHA) set a guidance level for MCs in fish tissue for human consumption at 10 $\mu\text{g kg}^{-1}$ (regardless of waterbody type), and the World Health Organization (WHO) has set a provisional tolerable daily intake value for chronic exposure to MC-LR of 0.04 $\mu\text{g kg}^{-1}$ body weight (Chorus & Bartram, 1999; OEHHA, 2012). These studies have documented MCs in shellfish that

exceed OEHHA's guidance level for human consumption of fish tissue, indicating a human health risk confounded by the lack of monitoring or regulation for MCs in commercially harvested shellfish.

The rapid bioaccumulation and retention of MCs in marine mussels and oysters increases the risk of exposure for wildlife and humans and the transfer of toxins to higher trophic levels. Gibble et al. (2016) examined rates of toxin uptake and depuration for both particulate and dissolved fractions of MCs in California mussels and oysters (*Crassostrea* sp.). Particulate MCs were retained in California mussels for up to eight weeks after exposure, whereas oysters purged MCs relatively quickly (within days), but low concentrations were retained for several weeks. Dissolved MCs were taken up and purged relatively quickly (within days) in California mussels. These studies highlight an unrecognized risk to human health because MCs and other cyanotoxins are not included in most biotoxin shellfish monitoring programs and no regulatory guidelines or thresholds currently exist for MCs in commercially harvested shellfish.

Case Study: Multiple sampling modalities in San Francisco Bay, California, characterize multiple toxins in the plankton and their risks to human health. One of the most extensive time-series datasets of both marine algal toxins and cyanotoxins has been collected in San Francisco Bay Estuary (SFB) in California. Freshwater flow into SFB comes from the Sacramento and San Joaquin Rivers that drain 40% of California's landscape, including agricultural, urban, and stormwater runoff. The SFB consists of six sub-embayments, Suisun Bay (closest to major freshwater inflows), San Pablo Bay, Central Bay (closest to marine inflows), South-Central Bay, and South Bay (longest residence time; Figure 2; also see Figure 5 for California map showing SFB). Peacock et al. (2018) used a combination of sampling modalities, including SPATT, particulate water samples, and mussel samples in the first study to report the simultaneous detection of both freshwater and marine algal toxins in mussels. The combined results of this study revealed ubiquitous and year-round toxins throughout SFB with the potential for both acute and chronic risk of effects to wildlife and humans.

Particulate toxins analyzed from water samples were collected monthly from November 2011 to June 2016 at a series of stations throughout all six sub-embayments. SPATT samples were obtained during cruises using a flow-through underway system, with a different SPATT sampler deployed in each of the six sub-embayments. California mussels were deployed in cages throughout SFB during 2012 and 2014.

Detectable MCs were observed in 50% of the particulate water samples, 11% had detectable DA, 3% had both toxins, and there was no correlation between the presence of potential toxin-producing phytoplankton and cyanobacteria. By contrast, MCs were detected in 76% of the SPATT samples, 97% had detectable DA, and 73% had both toxins. Microcystins were detected in 82% and 100% of mussels in 2012 and 2014, respectively, with concentrations up to 18.9 $\mu\text{g kg}^{-1}$. Domoic acid was detected in all mussels

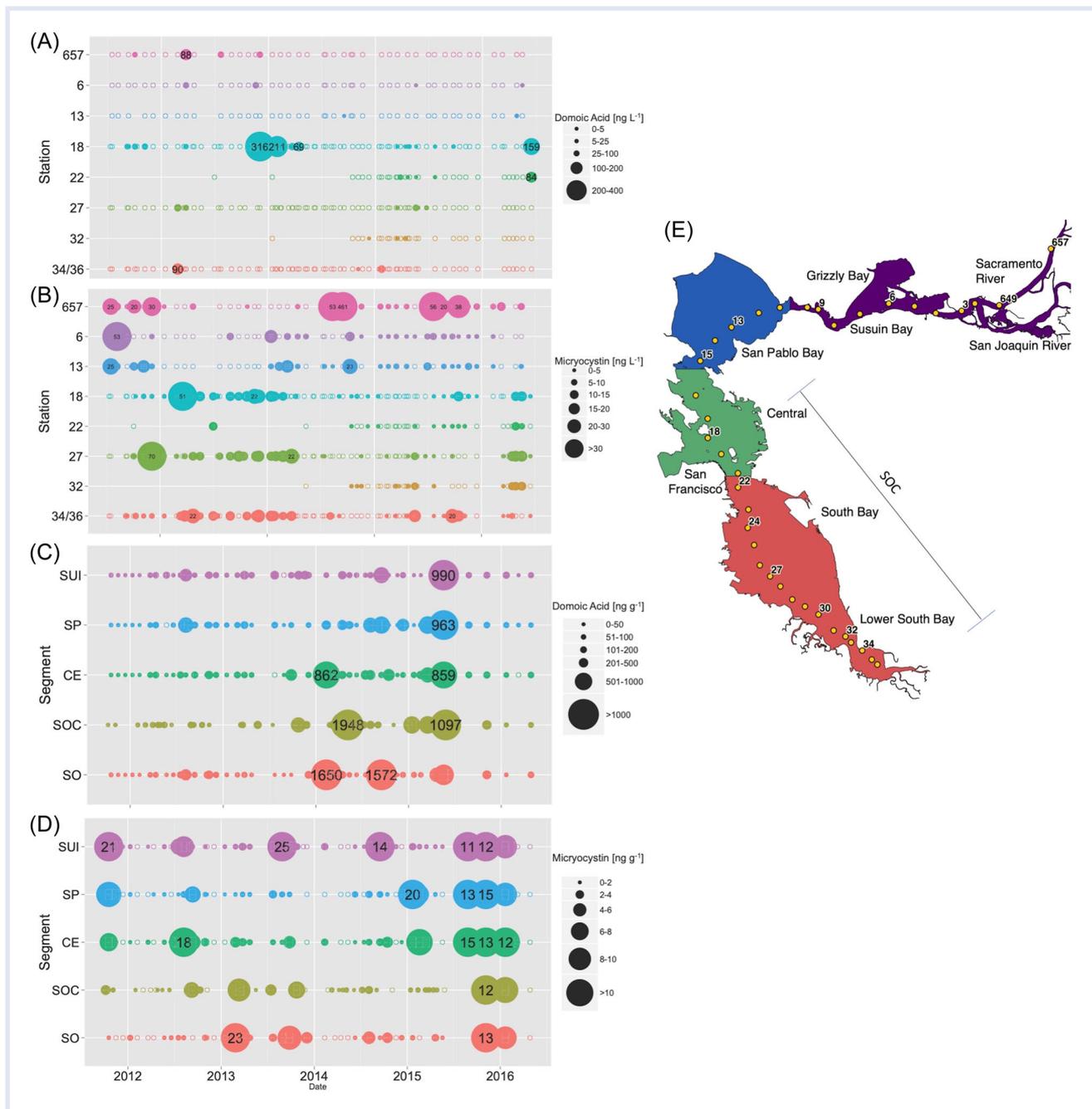


FIGURE 2 Particulate water sample results for domoic acid (DA) (A) and microcystins (MCs) (B), Solid Phase Adsorption Toxin Tracking (SPATT) results for DA (C) and MCs (D) from San Francisco Bay, reproduced from Peacock et al. (2018). Map of stations (E) that correspond to sampling locations for particulate water samples indicated by yellow circles and SPATT results from different areas of SFB indicated by colors and labels Suisun (SUI; purple), San Pablo (SP; blue), Central (CE; green), South Central (SOC; bracket), and South Bay (SO; red), which progresses from zero to full salinity (roughly SUI to CE)

in both years with concentrations ranging from 20.5 to 565 $\mu\text{g kg}^{-1}$. Paralytic shellfish toxins (PSTs) and *Dinophysis* shellfish toxins (DSTs) were also detected in 45% (2012) and 83% (2014) of mussels, and 91% (2012) and 100% (2014) of mussels, respectively. Detectable levels of all four toxins were present in nearly half of the mussel samples. Additionally, endemic mussel populations were sampled during 2015 at four locations within the Central Bay (closest to the marine inflows). Microcystins were detected in 61% of

samples, ranging up to 416 $\mu\text{g kg}^{-1}$, whereas DA, PSTs, and DSTs were detected in 98%, 59%, and 71% of mussel samples, respectively (Gibble et al., 2016). The mussel results were considerably higher than the OEHHA established guidance for fish tissue for human consumption.

Chronic, system-wide toxins were present and multiple co-occurring toxins resulted in substantial contamination of mussels. The combination of sampling modalities used in SFB provided a much more comprehensive picture of,

and insights into, toxin patterns and human and wildlife health risk than would have been apparent using only a single sampling modality. The synergistic effects of exposure to multiple toxins are poorly known (see Recommendation 3 below). Monitoring and management practices need to be redesigned for HAB toxins at the land–sea interface because traditional monitoring approaches would not capture the toxin dynamics, patterns, and health risks in SFB.

Case Study: Multiple sampling modalities in Eel and Russian Rivers, Northern California, provide an assessment of human and animal exposure to benthic cyanobacterial mats. Benthic cyanobacterial mats in rivers and streams can be a source of cyanotoxin production (Bouma-Gregson, Kudela, et al., 2018; Fetscher et al., 2015; McAllister et al., 2016; Puddick et al., 2021; Quiblier et al., 2013; Wood et al., 2020), and several recent studies have focused on identification of toxin-producing benthic cyanobacterial taxa

(see Introduction; Bouma-Gregson, Olm, et al., 2018; Kelly et al., 2019; Wood et al., 2020). The Eel River is the third largest river in California and drains 9546 km². The river flows north, with the headwaters originating in Mendocino County and terminating in the Pacific Ocean (Power et al., 2015; Figure 3A). Precipitation occurs mostly from October through March followed by little to no rainfall in summer, creating optimal conditions for cyanobacterial growth as flow diminishes (Power et al., 2015). Cyanotoxin poisoning in the Eel River has been linked to more than a dozen dog deaths since 2000 in Northern California, prompting several watershed-scale surveys and assessments (Asarian & Higgins, 2018; Bouma-Gregson, Kudela, et al., 2018; Bouma-Gregson, Olm, et al., 2018; Kelly et al., 2019; Puschner et al., 2008).

Bouma-Gregson, Kudela, et al. (2018) conducted watershed-scale surveys of the Eel River in 2013 and 2014 using a combination of sampling modalities that included water samples, cyanobacterial mat samples, and SPATT

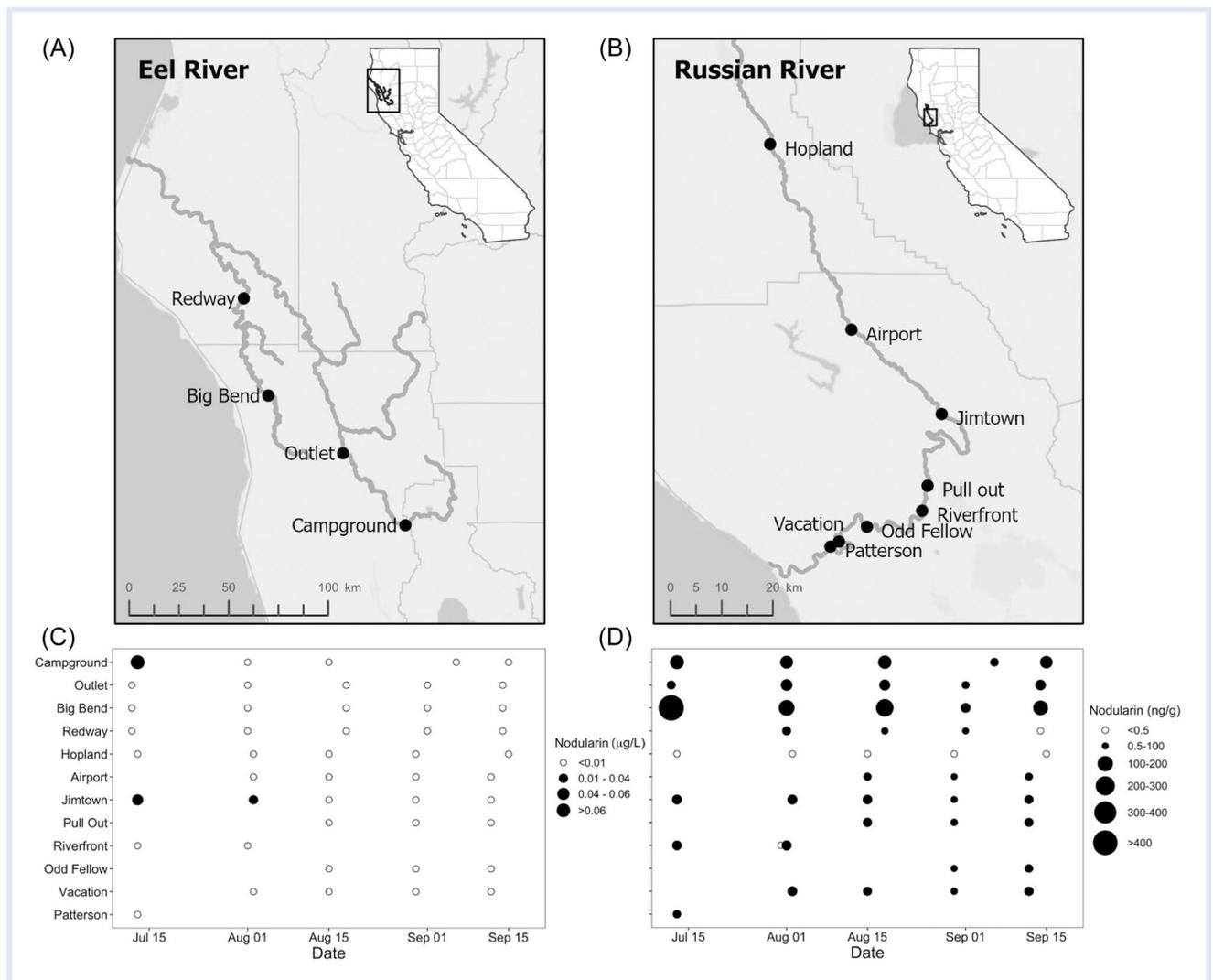


FIGURE 3 Map of the Eel River (A) and Russian River (B) with sampling locations noted by circles. Nodularin concentrations detected from water (C) and Solid Phase Adsorption Toxin Tracking (D) samples during 2016. The sampling sites are on the y-axis organized from headwaters (top) to downstream river sites (bottom). The first four sites from the top are located in the Eel River and all other sites are located in the Russian River

samplers. The survey documented widespread distribution of anatoxin-a and MCs via all three sampling modalities and was the first study to document watershed-wide distributions of anatoxin-a. The Eel River Recovery Project, a nonprofit group of volunteers that organizes many watershed health and restoration projects throughout the Eel River, continued the cyanotoxin monitoring starting in 2015 and developed a monthly sampling program in coordination with Bouma-Gregson, University of California, Berkeley, the North Coast Regional Water Quality Control Board (NCRWQCB), and Sonoma County Department of Health Services. Adjustments in the number of sampling sites, the groups carrying out the sampling, and the lengths of SPATT deployments make it difficult to directly compare all data collected throughout 2013–2017; however, anatoxin-a was generally observed at sites with warmer water than sites with the highest MC concentrations (Asarian & Higgins, 2018).

In 2016, the NCRWQCB conducted a more intensive field survey of benthic cyanobacterial mats and cyanotoxins in both the Russian and Eel Rivers (Figure 3A,B) using a combination of sampling modalities including whole water samples, SPATT samplers, and cyanobacterial mat samples. Nodularin concentrations between water and SPATT samples were markedly different; therefore, relying on one solely, without the use of both metrics, paints a very different picture of toxin patterns in these rivers. Detailed information on sample collection and analysis are provided in Supporting Information: Supplementary Materials and Methods. A total of four and eight sites were sampled in the Eel and Russian Rivers, respectively, during 2016 (Figure 3C,D). Nodularin was detected in the water samples at only two sites, Campground (Eel River) and Jimtown (Russian River), whereas nodulin was detected in SPATT samples at all sites throughout the sampling period in both rivers except at the Hopland site in the Russian River.

Spatial and temporal patterns of MCs obtained from the water samples, SPATT samples, and benthic cyanobacterial mat samples were also unique among the three sample types, indicating that a combination of sampling modalities provided a more comprehensive picture of toxin dynamics, patterns, and the potential for downstream transport of toxins and health risks to humans, canines, and wildlife (Figure 4). Benthic cyanobacterial mats were sources of MCs at all sites in both rivers except at Riverfront and Oddfellow sites in the Russian River. Mats present in August and September were mostly toxin-producing for MCs (Figure 4C). Microcystins were low or below detection from water and SPATT samples in the Russian River throughout the field survey (Figure 4A,B), contrary to the benthic cyanobacterial mat results. Most of the water samples were below the California health thresholds ($0.8 \mu\text{g L}^{-1}$) except at Hopland and Airport sites on 15 August 2016 and the Outlet site on 15 September 2016. The Eel River results indicated dissolved MCs throughout the field survey at most sites (Figure 4B).

Visual observations of cyanobacterial species or genera within mats were not necessarily representative of toxin

presence and concentrations caused by within-mat variability of both toxic and non-toxic strains (Bouma-Gregson, Olm, et al., 2018; Kelly et al., 2019; Wood et al., 2012; Wood et al., 2020). Composite samples were more likely to capture the spatial variability in both the location of cyanobacterial mats and the toxin content within the mats. Several sampling assessments have utilized composite sample methodology, including the California statewide stream assessment and Eel River survey (Bouma-Gregson, Kudela, et al., 2018; Fetscher et al., 2015). Fetscher et al. (2009) developed an SOP for the collection of benthic mats in California monitoring programs that details this recommended sample collection methodology. As stated in Recommendation 1, the use of SOPs for consistent sample collection and quality assurance was critical to ensure multiple datasets could be collated and compared across regions and states.

Recommendation 3: Multiple toxins should be monitored routinely across the freshwater-to-marine continuum, and cyanotoxins should be included in estuarine and marine monitoring programs

Multiple cyanotoxins have been detected from a single sample or location in studies both within and outside the USA, resulting from either co-occurring species or the fact that many species of cyanobacteria can produce multiple toxins (Bouma-Gregson, Olm, et al., 2018; Gkelis & Zaoutsos, 2014; Graham et al., 2010; Howard et al., 2017, 2021; Pekar et al., 2016; Sabart et al., 2015; Tatters et al., 2017, 2019; Wiltsie et al., 2018). The relative frequency of co-occurring cyanotoxins is still poorly defined because the number of comprehensive toxin surveys that measures multiple cyanotoxins is limited. Similarly, multiple toxin-producing algal species can coexist in marine ecosystems; therefore, mixtures of marine algal toxins can also occur (Capper et al., 2013; Fire et al., 2011; Peacock et al., 2018; Smith et al., 2019).

A relatively new finding is the co-occurrence of marine algal toxins and cyanotoxins (Peacock et al., 2018; Tatters et al., 2019; this study). The SFB case study documented the simultaneous detection of four classes of toxins composed of both marine algal toxins and cyanotoxins in multiple sampling matrices. The detection of toxin mixtures highlights an important gap in our understanding of the toxin dynamics at the land–sea interface.

Case Study: California Coastwide Survey documents toxin mixtures across the freshwater-to-marine continuum. The US West Coast has experienced an increase in the frequency and severity of marine HAB events in the past few decades, with the largest documented effects being caused by blooms of *Pseudo-nitzschia*. Domoic acid resulting from blooms of *Pseudo-nitzschia* were first reported as a recurring issue in northern and central California during the 1990s and then on an annual basis in southern California beginning in 2003 (Lane et al., 2010; Lewitus et al., 2012; Schnetzer et al., 2007; Schnetzer et al., 2013; Smith et al., 2018;

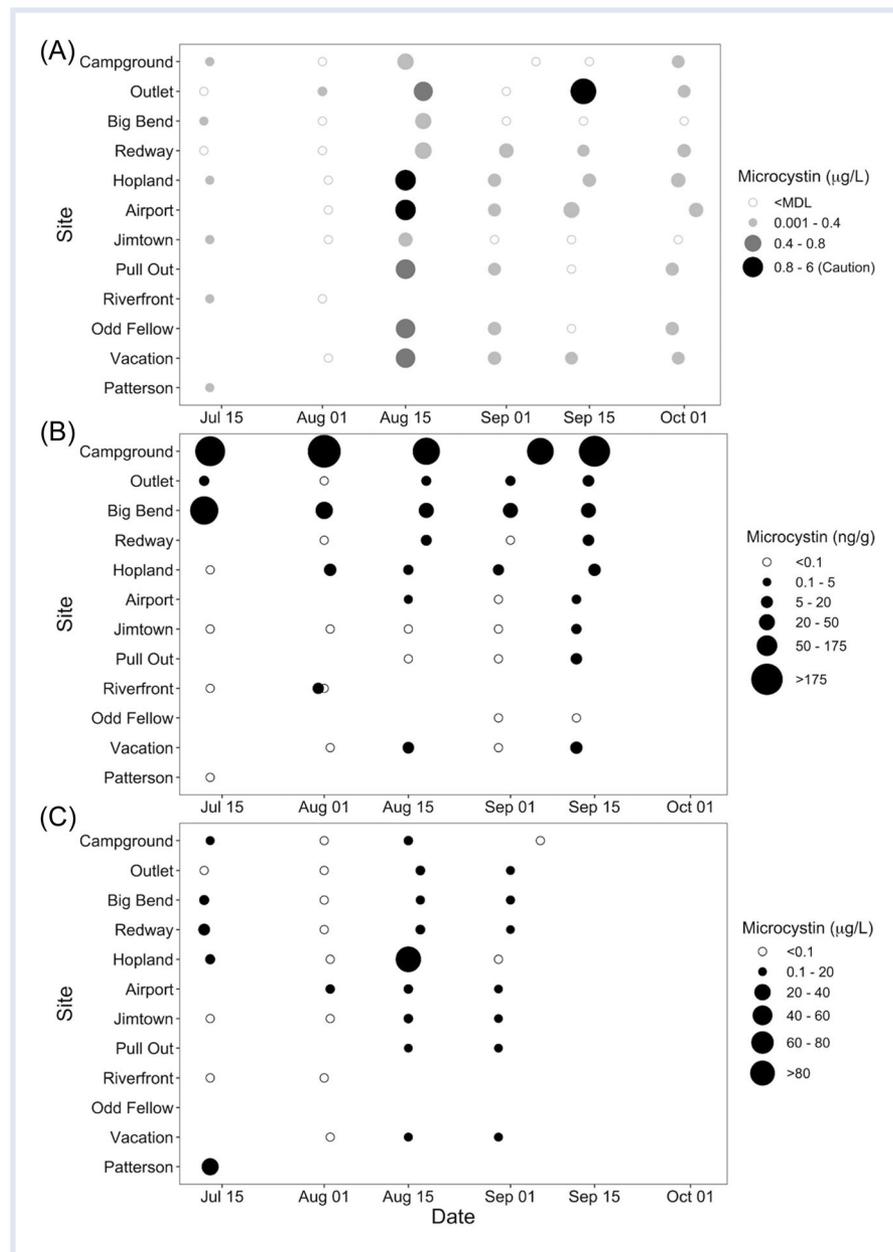


FIGURE 4 Microcystin concentrations from water (A), Solid Phase Adsorption Toxin Tracking (B), and cyanobacterial mat (C) samples collected from the Eel and Russian Rivers during 2016. The sampling sites are listed on the y-axis organized from headwaters (top) to downstream river sites (bottom). The first four sites from the top are located in the Eel River and all other sites are located in the Russian River

Trainer et al., 2000). Paralytic shellfish toxins (e.g., saxitoxins and related compounds) are also prevalent in California; therefore, a Biotxin Monitoring Program (BMP) for DA and PSTs was established by the California Department of Public Health. Other HAB toxins including yessotoxins and DSTs have been detected by research programs along the California coast (DeWit et al., 2014; Howard et al., 2008; Shultz et al., 2019; Smith et al., 2019), although these latter toxins are not routinely monitored by the BMP because they are not regulated (DA and PSTs are regulated by the US Food and Drug Administration).

Most coastal watersheds in northern and central California experience a heavy agricultural influence, whereas watersheds

in southern California are generally more influenced by urban runoff inputs, indicating that different nutrient sources may drive inland and coastal blooms along the vast expanse of the California coast. Precipitation and flushing event patterns also vary throughout California with higher rates of precipitation in the northern and central regions than in the south. To examine these influences, a yearlong survey of eleven locations at watershed termini was conducted in California to assess the presence of toxin mixtures (marine algal toxins and cyanotoxins) and to further refine monitoring tools and approaches to address the challenges of monitoring across the land-sea interface (Figure 5). All locations were sampled monthly from January 2017 to December 2017. Multiple sampling

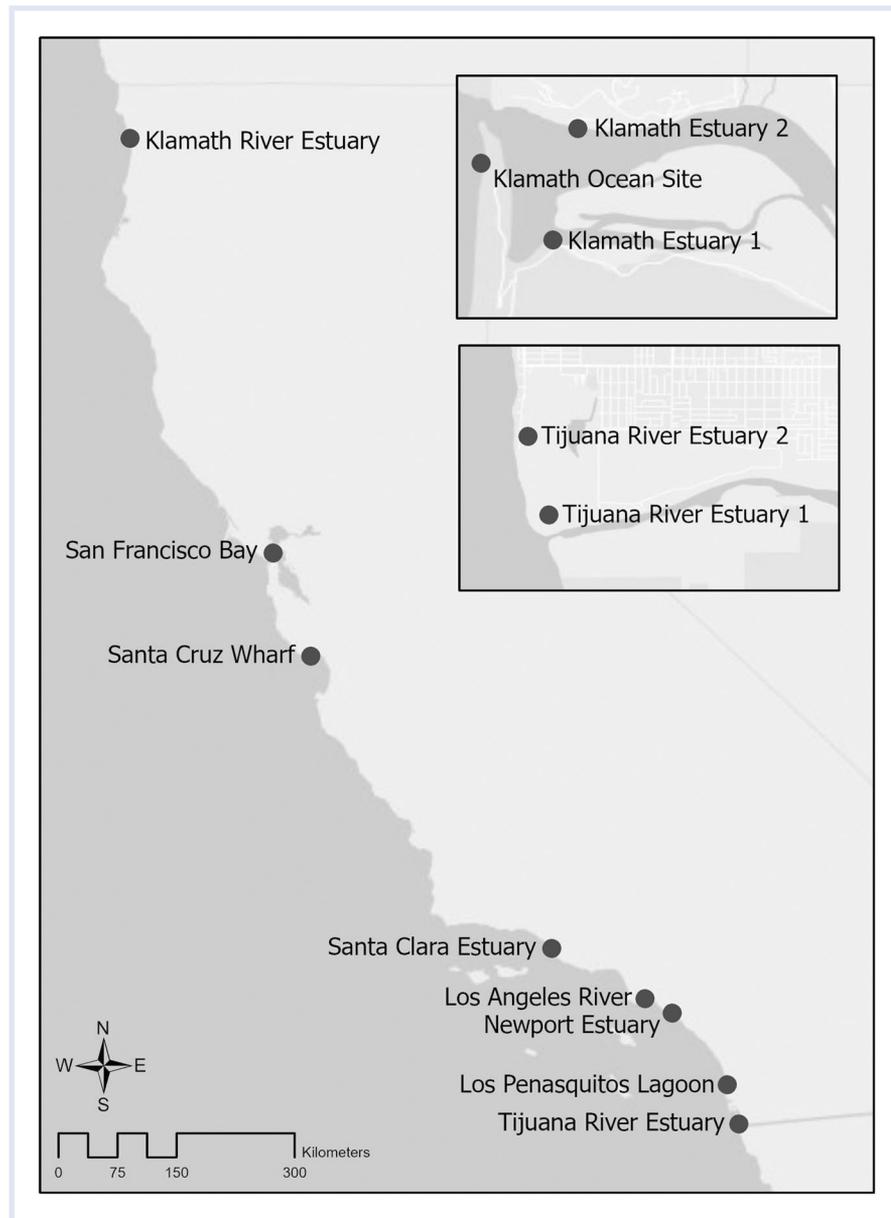


FIGURE 5 Map of the California Coastwide Survey showing all locations, with insets showing three Klamath sites or two Tijuana Estuary sites at higher spatial resolution

modalities were used to characterize the presence of toxin mixtures including water samples and SPATT and analyzed for DA, okadaic acid, dinophysistoxin-1, dinophysistoxin-2, anatoxin-a, nodularin, cylindrospermopsin, and multiple congeners of MCs (see Supporting Information: Supplementary Materials and Methods and Figures 1 and 2; Figure 6). A toxin diversity index (TDI) was calculated for each month at each monitoring location (Figure 6A) using the Shannon–Wiener index based on the number of distinct toxin compounds detected in SPATT samples using the method described in Mantzouki et al. (2018) and described in Supporting Information: Supplementary Materials and Methods.

Multiple toxins (at least two or more) were detected by SPATT simultaneously at all ten monitoring locations throughout California during some portion of the year

(Figure 6, Supporting Information: Figures 1 and 2). The diversity of toxins detected at each location increased in the second half of the year as indicated by the warmer colors and higher TDI (Figure 6A). The TDI exhibited higher toxin diversity at the Los Angeles River station and northward starting in July, with the highest TDIs occurring during autumn at the Los Angeles River, Santa Cruz Wharf, and San Francisco Bay. The TDI also exhibited a significant linear positive correlation between the day of year in which the sample was collected (Supporting Information: Figure 3). The seasonal increase in TDI was less pronounced at the Newport Estuary and southward. The summed concentration of all detected toxins indicated by the size of the circle in Figure 6A revealed an overall increase during summer and autumn, similar to the pattern observed in the

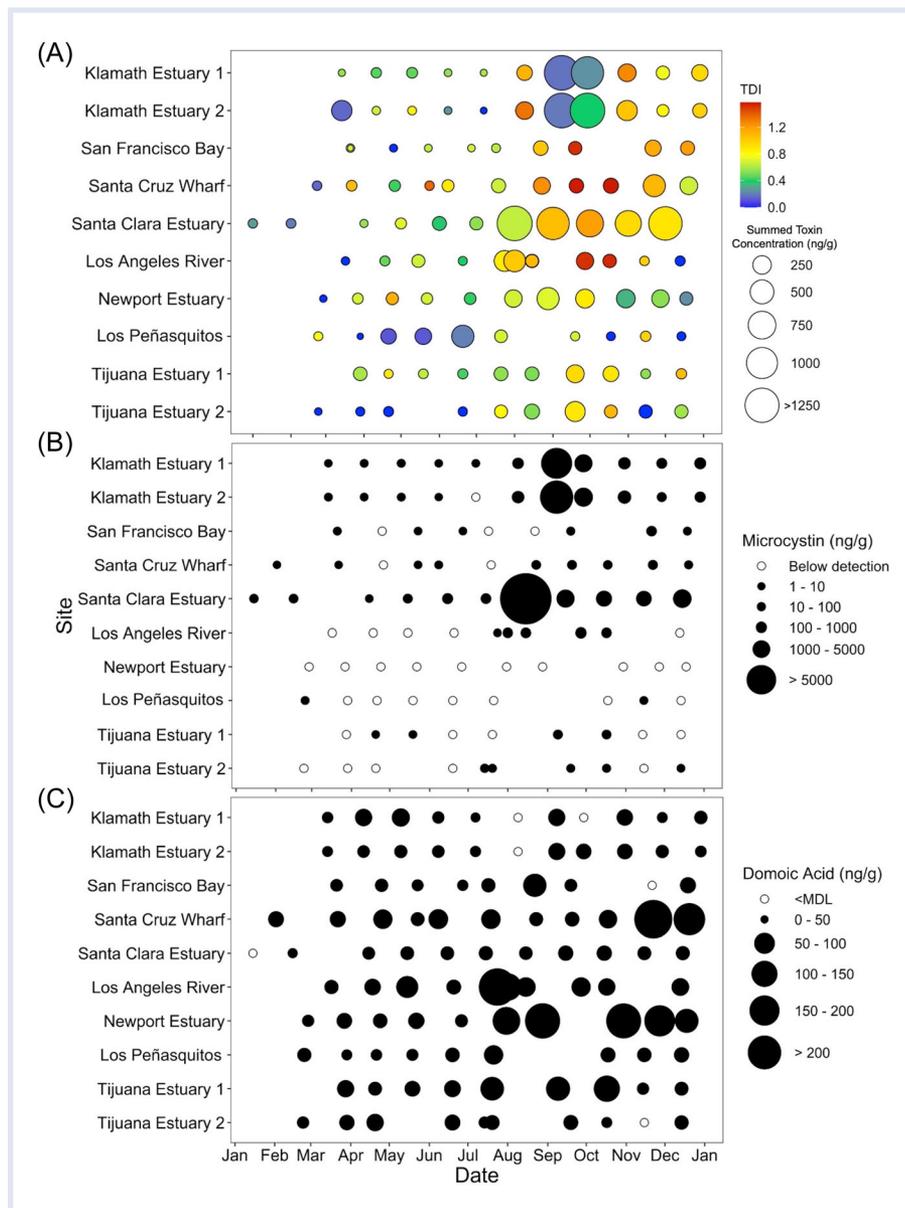


FIGURE 6 The calculated toxin diversity index (TDI) (A) for each month of monitoring during 2017 at each of the ten monitoring locations in the California Coastwide Survey (Figure 5) based on toxins revealed by Solid Phase Adsorption Toxin Tracking (SPATT). Stations are organized from north (Klamath Estuary) to south (Tijuana Estuary) on the y-axis. The TDI is shown with a heat map with warmer colors representing greater diversity of toxins measured in SPATT extracts. The size of the circle corresponds to the summed concentration of all toxins measured from the SPATT resin in ng/g. SPATT measurements of microcystins (B) and domoic acid (C) collected from the California Coastal Survey sites during 2017

TDI. Interestingly, the sites with the highest TDI had lower toxin concentrations than sites with medium to low toxin diversity. The sites with the lower TDI were intermittently open (Klamath, Santa Clara, and Tijuana Estuaries, and Los Peñasquitos Lagoon), and sites with the highest TDI were constantly open and flowing (SFB, Santa Cruz Wharf, and Los Angeles River).

Microcystins were more prevalent in northern and central California based on the SPATT results and were detected at low concentrations throughout most of the year whereas they were detected sporadically in southern California monitoring locations (Figure 6B). A notable exception to this

trend was at the Santa Clara River Estuary, where MCs were observed throughout the year (100% of SPATT samples) and at higher relative concentrations during the second half of the year (Figure 6B). Nodularin was also detected in SPATT samples at least once at seven of the ten sampling locations (Supporting Information: Figure 1A). Anatoxin-a was commonly observed throughout the survey from SPATT samples at least once, and consistently at all sites from July through September (Supporting Information: Figure 1B). Compared with SPATT, MCs were less routinely detected in water samples and were only observed at the Klamath

Estuary sites and the Santa Clara River Estuary (Supporting Information: Figure 4A). Nodularin and anatoxin-a were not detected in any water samples. Similarly, total dinophysistoxins (sum of dinophysistoxin-1 and dinophysistoxin-2) and okadaic acid were detected in 24% and 29% of SPATT samples, respectively (Supporting Information: Figure 2), but were never detected in water samples.

Domoic acid was ubiquitously observed in SPATT extracts from all monitoring locations throughout California (95% of samples) and concentrations were relatively consistent over time (Figure 6C). A substantial spring DA producing bloom event occurred in California in 2017, which was observed in the water samples collected concurrently from the Santa Cruz Wharf and southward to the Tijuana Estuary (Supporting Information: Figure 4B; Donovan et al., 2021). Domoic acid was detected in water samples at most locations at least once after the bloom event in the second half of the year, although not as persistently as in the SPATT extracts (Supporting Information: Figure 4B). Collectively these findings, along with Peacock et al. (2018) and others (Marquez et al., 2020; Umhau et al., 2018), support the growing body of research indicating that dDA is routinely present at low levels in marine and estuarine environments in California and represents an understudied route of contamination into the food web. This finding became apparent from using a multiple modality approach and would not have been apparent from the analysis of water samples alone.

Case Study: Mechanisms of toxin transport into the marine environment from intermittent estuaries were revealed through time-course analysis of multiple toxins. Multiple mechanisms can transport freshwater toxins into the coastal zone. Resolving these dynamics is important to improving our understanding of when and how coastal environments may be at greatest risk for the co-occurrence of toxins of freshwater origin. This is particularly relevant in regions with intermittent estuaries that tend to open seasonally to the coast. These systems are typically separated from the ocean for most of the year by berms, which open to the coast following a hydrologic flushing event or purposeful breaching of berms. Tatters et al. (2021) tested the hypothesis that these intermittent estuaries accumulate cyanotoxins when not flowing and deliver substantial amounts of cyanotoxins to marine waters when flow occurs. Their time-series study of the Santa Clara River Estuary indicated the presence of multiple toxins within and near the lagoon both before and after the breach of the berm by a flushing event (a king tide in December 2017). A combination of SPATT, mussels, and water samples indicated that both MCs and DA were detected inside and outside the estuary before and after the flushing event. Concentrations detected in SPATT following the breach of the berm were considerably higher at all coastal sites, and mussels contained MCs in the week before the breach of the lagoon, but also in the three weeks following the breach. The presence of MCs in mussels outside the Santa Clara Estuary before breaching of the berm indicates other freshwater influences may have

affected the coastline immediately seaward of the Santa Clara River Estuary, because the beach barrier remained intact for nearly a year before the December 2017 King Tides.

Collectively, the findings of the CA Coastal Survey and the intensive field survey in the Santa Clara River Estuary indicate that contamination of the coastal zone with cyanotoxins might be a chronic condition for some coastal ecosystems, and that mixtures of both cyanotoxins and marine algal toxins can occur across many different coastal systems with diverse hydrologic influences. These results highlight the importance of regular monitoring for multiple toxin classes and underscore the value of using multiple sampling modalities to fully characterize toxin presence and dynamics. These studies also indicate that, in some regions, there are multiple and currently uncharacterized sources of cyanotoxins and mechanisms of delivery to the coastal environment, resulting in a more consistent delivery of cyanotoxins to the marine environment than previously hypothesized, consistent with previous geographically limited studies (Gibble & Kudela, 2014; Howard et al., 2017; Tatters et al., 2019).

Currently, the effects of acute and chronic exposure to mixtures of multiple toxins on the health of human and aquatic life are poorly understood. Mixtures of toxins often contain toxins with multiple modes of action (Carmichael & Boyer, 2016); therefore, monitoring efforts that target only a single toxin presumably underestimate the risk to human and ecosystem health. Additionally, most recreational and drinking water health thresholds are based on exposure to a single toxin, not multiple toxins. Therefore, the usefulness of existing health thresholds should be re-examined when considering exposure to toxin mixtures with different mechanisms of toxicity that could have synergistic effects.

APPLICABILITY OF HAB STRATEGY TO INLAND STATES AND WATERSHEDS

The HAB strategy presented here focuses on the freshwater-to-marine continuum of hydrologically interconnected waterbodies with the terminus being the ocean. However, this strategy is applicable in inland regions or in land-locked states where the terminal downstream receiving water may be a lake, reservoir, or river. As an example, Graham et al. (2012) documented the transport of cyanobacteria and associated cyanotoxins from an upstream reservoir, Milford Lake, to a 173-mile reach of the Kansas River during planned reservoir water releases. The development of cyanobacterial blooms in the western basin of Lake Erie has been perceived to be seeded internally or to enter Lake Erie through the Maumee River; however, Davis et al. (2014) identified a third source from Lake St. Clair via the Detroit River. The long-distance transport of toxic *Microcystis* in several interconnected waterways of the Lower Great Lakes is an example of an inland system for which our proposed strategy and recommendations could be utilized.

CONCLUSIONS

Management and mitigation efforts are often aimed at individual waterbodies or segments along the freshwater-to-marine continuum and fail to provide the comprehensive approach necessary to develop effective mitigation strategies. The HAB monitoring strategy recommendations presented here provide a cohesive, comprehensive approach to monitoring across the freshwater-to-marine continuum that is necessary to properly inform management decisions and mitigation approaches to HABs. These recommendations can also be applied to inland regions and states where the terminal downstream receiving water may be a lake, reservoir, or river.

The effective and efficient management and mitigation of HABs requires the removal of “artificial” boundaries, reflecting political and organizational boundaries, and implementation of coordinated and cohesive monitoring designs that span hydrologically interconnected waters. The Klamath River and Estuary Ecosystem has successfully created a comprehensive monitoring program (KBMP) that spans multiple states, includes federal, state, and local agencies, and Tribal Nations. The information provided by the KBMP has provided holistic, basin-wide insights into bloom origin and transport dynamics of both cyanotoxins and cyanobacteria cells across the freshwater-to-marine continuum. This information exemplifies how attempts at mitigation in downriver and estuary locations would be ineffective without mitigation in the headwater reservoirs, as recommended by Paerl et al. (2018). Although the initial efforts to establish this type of multi-organizational approach were extensive, the resulting program has provided important insights and critical information for management decisions.

In addition to the multi-organizational approach, a combination of multiple sample types and matrices should be included in HAB monitoring programs to address the monitoring challenges of hydrologically interconnected waterbodies across the freshwater-to-marine continuum. The specific indicators, tools, and matrices to be included can be determined based on the monitoring program objectives, as different combinations may be used based on the goals and information needs identified for the program. For example, if the protection of public health is the monitoring objective, then toxin measurements across a range of matrices based on exposure routes would be the most critical to include in the design. However, if the monitoring objective is identification of HAB drivers, then water quality measurements could also be prioritized (such as salinity, temperature, nutrient concentrations) in addition to toxin concentrations and biomass measurements.

Recommendation 3 and the case studies presented highlight the importance of regular monitoring for multiple toxin classes and underscore the value of using multiple sampling modalities to fully characterize toxin presence and transport. There are multiple sources of cyanotoxins and mechanisms of consistent delivery to the coastal

environment and recent studies have begun to focus on these previously uncharacterized sources (Gibble & Kudela, 2014; Howard et al., 2017; Peacock et al., 2018; Tatters et al., 2021). Most recreational and drinking water health thresholds are based on exposure to a single toxin, not multiple toxins making their utility limited to determine health risks.

Although the recommendations and case studies presented here highlight the importance of cohesive monitoring approaches across the freshwater-to-marine continuum, the same principle applies to the development of models that help support the management of these dynamic systems. As with monitoring efforts, most current models relevant to HABs represent the land and ocean separately or include transport of biogeochemical properties in one direction (i.e., nutrient eutrophication in coastal marine models; Glibert et al., 2018); therefore, coupling multiple models together becomes necessary at the land–sea interface (Ward et al., 2020). Mechanistic models are not well defined for biogeochemical processes in the coastal environment (Ward et al., 2020), which limits the ability to use modeling to inform transport mechanisms relevant to HABs to inform management decisions. More fundamentally, there are nearly as many modeling frameworks as harmful species of interest, each with particular emphasis or simplifications (Anderson et al., 2015). A clear goal, in line with the monitoring recommendations presented here, is to better integrate existing modeling efforts providing detailed hydrological, biogeochemical, and (ultimately) predictive power for HAB effects at the land–sea interface, but to date there are few if any examples of such coupled model systems.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Meredith D. A. Howard, David A. Caron, Raphael M. Kudela, Jayme Smith, Avery Tatters, and Keith Loftin secured the funding, developed the recommendations, provided data analysis and visualization, and manuscript writing. Jacob Kann, Susan Fricke, Rich Fadness, Susanna Theroux, and Miranda Roethler provided recommendation development, data analysis, and writing.

DATA AVAILABILITY STATEMENT

Total and dissolved-toxin measurement data for discrete water samples measured in samples collected in California coastal study samples can be obtained at <https://www.sciencebase.gov/catalog/item/5fdbddf8d34e30b9123d290f> (Donovan et al., 2021; <https://doi.org/10.5066/P9TEYRNC>). The remaining data is available at: <https://github.com/pseudonitz1987/fresh-marine-strategy>.

SUPPORTING INFORMATION

Figure S1. Solid Phase Adsorption Toxin Tracking measurements of nodularin (A) and anatoxin (B) collected from the California Coastal Survey sites during 2017. Stations are organized from north (Klamath Estuary) to south (Tijuana Estuary) on the y-axis.

Figure S2. Solid Phase Adsorption Toxin Tracking measurements of dinophysistoxins (A) and okadaic acid (B) collected from the California Coastal Survey sites during 2017. Stations are organized from north (Klamath Estuary) to south (Tijuana Estuary) on the y-axis.

Figure S3. Linear regression model showing the significantly positive relationship between day of year and toxin diversity index based on toxins in Solid Phase Adsorption Toxin Tracking samples. The correlation coefficient is 0.363 and the p value = 9.65×10^{-5} .

Figure S4. Microcystins (A) and domoic acid (B) in water samples collected from the California Coastal Survey sites during 2017. Stations are organized from north (Klamath Estuary) to south (Tijuana Estuary) on the y-axis.

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