STATE OF CONNECTICUT
DEPARTMENT OF AGRICULTURE
Bureau of Aquaculture & Laboratory Services

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2020 Connecticut Harmful Algal Bloom Report

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Report Summary

- Biotoxin and harmful algal bloom (HAB) monitoring has been conducted in Connecticut (CT) since 1985 and 1997, respectively, by the CT Department of Agriculture, Bureau of Aquaculture (DABA). In response to increasing HAB events around the world and regionally (but not in CT), the DABA enhanced the program in 2019 to collect semi-quantitative data and survey widespread CT shellfish growing areas at an increased frequency.
- Improved documentation has revealed the presence of many HAB taxa in CT, but threats to human health and ecosystem function have been rare and localized because populations of HAB species remain modest and potentially toxigenic species seldom produce or only produce low concentrations of toxins. Actual impacts of HAB organisms around the world are presented to demonstrate their harmful effects, in relation to cell and toxin concentrations, outside of CT.
- The presence of HAB taxa with the potential to cause public health and/or ecological harm underscores the necessity for continued monitoring and surveillance. HAB monitoring provides an early warning system to guide management decisions and prevent shellfish recalls and illnesses, and also allows widespread surveillance of shellfish growing areas.
- The downstream movement of freshwater cyanobacteria blooms to CT's estuarine environment represent a newly-recognized concern for shellfish safety in some nearshore locations, and is an emerging issue in many national and international coastal areas.

Overview of HABs

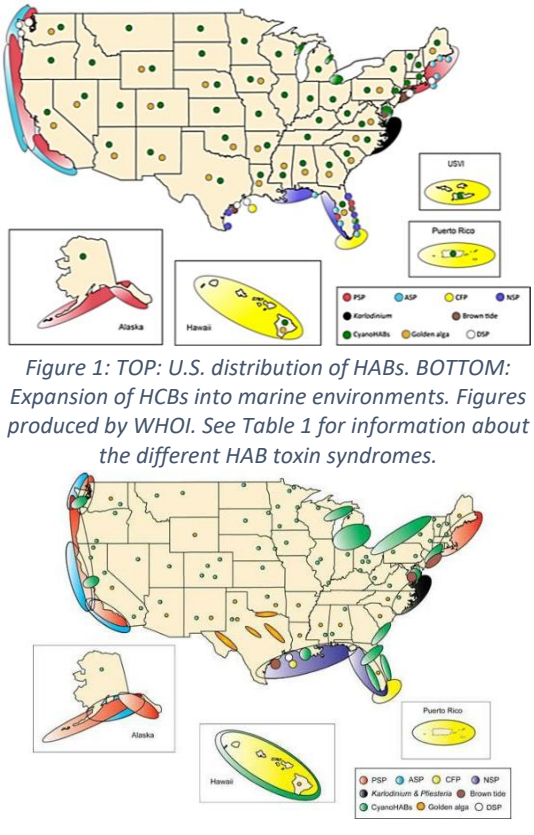

Figure 1: TOP: U.S. distribution of HABs. BOTTOM: Expansion of HCBs into marine environments. Figures produced by WHOI. See Table 1 for information about the different HAB toxin syndromes.

There is a general scientific consensus that harmful algal blooms (HABs) (marine) and cyanobacteria blooms (HCBs (harmful cyanobacteria blooms); blue-green algae) ("freshwater") are increasing in intensity and frequency around the world (e.g. Anderson et al. 2008, 2012; Gobler et al. 2017; Huisman et al. 2018; O'Neil et al. 2012; Paerl and Paul 2012). Some HABs produce toxins that present a public health threat, while others cause environmental disruption (e.g. ecological and habitat degradation, animal kills). Molluscan shellfish are highly efficient filter feeders, and consequently concentrate environmental contaminants like HAB toxins. U.S. shellfish sanitation programs monitor for HABs and their toxins, and close and reopen shellfish growing areas based upon FDA standards to effectively prevent human illnesses. While some cyanobacteria inhabit marine waters, many of the known toxic cyanobacteria genera have caused issues in freshwater environments, such as by contaminating drinking water. Advected freshwater cyanobacteria are being detected in estuarine and marine waters more frequently, and studies have shown that cyanotoxins can accumulate in shellfish (Amorim and Vasconcelos 1999; De Pace et al. 2014; Falconer et al. 1992; Harding 2000; Preece et al. 2015b; Sipia et al. 2002; Van Buynder et al. 2001) and take an extended period of time to depurate from their tissue (e.g. Gibble et al. 2016; Miller et al. 2010). As a result, there

is a movement toward interconnection of management (freshwater-marine continuum). Every U.S. state is now impacted by toxic blooms, and some coastal areas are already concurrently experiencing one, or more, HABs and HCBs (fig. 1).

FDA regulated toxins

The FDA currently regulates five toxins and associated syndromes in shellfish (table 1). Other HAB toxins and cyanobacteria toxins (cyanotoxins) are not yet regulated in shellfish. Representative photos of some HAB organisms are shown (fig. 2).

Table 1. FDA regulated toxins and associated HAB organisms

HAB genus/genera	Toxins, syndrome, & FDA regulatory limit	Symptoms	Notes	Caused a CT closure?
Alexandrium (Approximately half of the 30+ species are toxic)[1,2] *-Pyrodinium bahamense, Gymnodinium catenatum,* some cyanobacteria genera, and most recently *Centrodinium punctatum*[3]	-Saxitoxins (50+ analogues)[1] - **Neurotoxin** -Paralytic shellfish poisoning (PSP) -Regulatory limit: **80 µg saxitoxin equivalent/100 grams shellfish**	-Tingling, numbness, burning in extremities or mouth; lack of coordination/staggering; drowsiness; fever; rash; respiratory difficulty and/or arrest; death -Gastrointestinal symptoms include nausea, vomiting, and diarrhea	-CT's toxin testing trigger is 1,000 cells/L -Some species produce other toxins like goniodomins and spirolides, which are not yet regulated by the FDA	Yes, in Mumford Cove (1985, 1992 & 2020) and Palmer Cove (1992 & 2003), Groton
Pseudo-nitzschia (Approximately half of the 50+ species are toxic)[4] *-Nitzschia navis-varingica, Nitzschia bizertensis,* and *Halamphora coffeaeformis*[4]	-Domoic acid (DA and isomers IA-IE)[4] – **Neurotoxin** -Amnesic shellfish poisoning (ASP) -Regulatory limit: **2 mg DA/100 grams shellfish (20 ppm)**	-Dizziness; disorientation; headache; short-term memory loss; long-term neurological damage, including memory defects and weakening/death of muscles in extremities; seizures; coma; respiratory difficulty; death -Gastrointestinal symptoms include nausea, vomiting, and diarrhea	-CT's toxin testing trigger is 20,000 cells/L, but DABA frequently tests for domoic acid at lower concentrations -DA has never been detected in CT	No, but *P. australis* closed Rhode Island beds in March 2017, and has been a reoccurring issue in northern New England since 2016[4]
Karenia (At least 5 out of the 12 species are toxic)[5]	-Brevetoxins (10+ congeners)[6] – **Neurotoxin** -Neurotoxic shellfish poisoning (NSP)	-Numbness/tingling in the extremities and face, fatigue, disorientation, loss of coordination, partial limb paralysis, slurred speech, headache, pupil dilation,	Not expected or documented in CT.	-No – *Karenia* blooms typically occur in the Gulf of Mexico -Some species have been documented farther north: *Karenia*

	-Regulatory limit: **0.8 mg PbTx-2 eq./kg shellfish (20 MU/100 grams)**	respiratory discomfort/ distress, seizures -Gastrointestinal symptoms include nausea, vomiting, and diarrhea		*mikimotoi* (ichthyotoxic, not an NSP producer) in the Gulf of Maine[7]; *Karenia brevis* and *Karenia papilionacea* (NSP producers) in Delaware[8,9]
Dinophysis (At least 10 out of 120+ species are toxic, but most outbreaks are caused by 6 species)[10] -There are also 2 toxic (out of 50+) *Phalacroma* species[10]	-Okadaic acid and dinophysistoxins (OA, DTX1, DTX2, plus toxin precursors and derivatives)[10] -Diarrhetic shellfish poisoning (DSP) -Regulatory limit: **0.16 mg OA eq./kg shellfish (0.16 ppm)** -Pectenotoxins are not yet regulated by the FDA	-Incapacitating diarrhea, nausea, vomiting, abdominal pain -Recovery typically occurs within 3 days, but can cause dehydration and electrolyte imbalance in severe cases -Potential association with cancer (long-term exposure)	-CT's toxin testing trigger is 30,000 cells/L – DABA has never documented concentrations exceeding (or even near) this limit	No, but *D. acuminata* blooms have caused closures around Long Island[13]
Prorocentrum (At least 9 out of 60 species are toxic, most of which are benthic, epiphytic, and tropical/ subtropical)[11]			Most planktonic *Prorocentrum* spp. do not produce DSP toxins, but some such as *P. minimum* are detrimental to shellfish and environmental health[12]	No – the threat of *Prorocentrum* causing DSP is low[14, 15]
Azadinium & Amphidoma (3/14 *Azadinium* spp. and 1/12 *Amphidoma* sp. (*Amphidoma languida*) are known to be toxic)[16]	-Azaspiracids (at least 26 analogues)[17] -Azaspiracid shellfish poisoning (AZP) -Regulatory limit: **0.16 mg AZA-1 eq./kg shellfish (0.16 ppm)**	-Nausea, vomiting, diarrhea, abdominal cramps -Potential to damage liver, spleen, and intestines	Not documented in CT, and have not caused illness in the U.S.	No – there have been no incidences of AZP in U.S. shellfish; however, azaspiracids (AZA1 and AZA2) were recently detected on the east coast in the Chesapeake Bay[18]

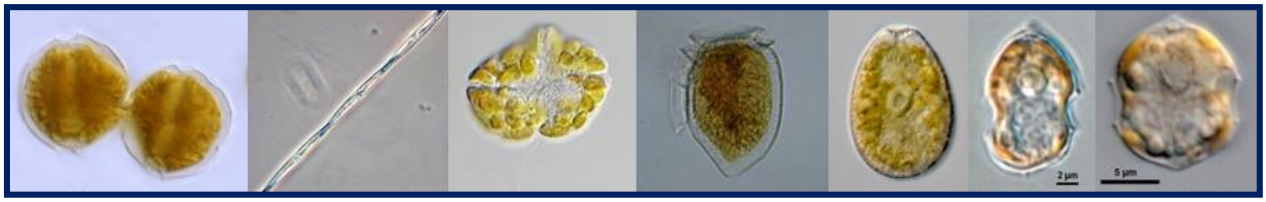

Figure 2: Representative images of the FDA-regulated HAB organisms. Left to right: Alexandrium catenella (previously A. fundyense), Pseudo-nitzschia seriata, Karenia brevis, Dinophysis norvegica, Prorocentrum lima, Azadinium spinosum, and Amphidoma languida. Images from the WoRMS Photo gallery (WoRMS Editorial Board (2021). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2021-03-26. doi:10.14284/170).

Regional Phytoplankton and HAB History

HAB monitoring in CT. The Department of Agriculture Bureau of Aquaculture (DABA) is responsible for shellfish sanitation in Connecticut, and always strives to enhance safety for consumers. The DABA instituted qualitative HAB monitoring in 1997, but effectively began enhancing the program in 2019. The successes were noted by the FDA in the 2019 Annual Program Evaluation Report: "The DABA published a comprehensive 2019 Harmful Algal Bloom Report. In 2019, the DABA revamped the phytoplankton monitoring program to include semi-quantitative sample collection and analysis methods, increased spatial monitoring, and more frequent sampling along the entire coastline. Additional sample stations were added in approved and conditionally approved shellfish growing areas statewide and a town volunteer sample collection program was instituted to assist in monitoring the safety of recreational areas." Given that 2020 was only the second year of routine semi-quantitative analysis, the data will act as baseline information. Samples collected throughout the season illustrated a typical Long Island Sound (LIS) phytoplankton community, with diatom dominance throughout LIS, winter-spring diatom blooms, increased dinoflagellate abundance in the summer, and fall diatom blooms (reviewed in Capriulo et al. 2002; Lopez et al. 2014).

Plankton community structure. LIS has a strong human population gradient between western and eastern LIS, which causes higher nutrient loading, phytoplankton biomass, and primary production in western LIS (reviewed in Lopez et al. 2014). The extreme differences are most notable between the western and eastern ends of LIS, with lower salinities, higher nutrient concentrations, and lower dissolved oxygen levels (annual summer hypoxia) in the western basin (reviewed in Lopez et al. 2014). Multiple studies have demonstrated that LIS phytoplankton communities are diatom dominated, regardless of physical, chemical, and human-induced differences between the regions (Capriulo et al. 2002; Conover 1956; reviewed in Lopez et al. 2014). However, Suter et al. (2014) has documented a reduction in diatom, and corresponding increase in flagellate, abundance (2002-2010). Diatoms remained dominant by the end of the study, but reductions could be linked to nitrogen limitation caused by declining dissolved inorganic nitrogen concentrations (Suter et al. 2014), as the LIS phytoplankton community is nitrogen limited (Gobler et al. 2006). Furthermore, changes have been noted in the zooplankton community, such as copepod species and size abundances due to increasing temperatures related to climate change (Rice et al. 2015; Rice and Stewart 2016). It is still largely unclear how phytoplankton communities will respond to climate change, ocean acidification, and other anthropogenic stressors, and region-specific studies are needed in LIS.

Alexandrium. Alexandrium catenella (formerly *A. fundyense, A. tamarense*) is a HAB organism that produces saxitoxin, which can become concentrated in shellfish tissue and cause paralytic shellfish poisoning (PSP) (table 1). *A. catenella* blooms have also been associated with multiple animal mortality events around the U.S. (reviewed in Anderson et al. 2021), but none specifically in CT. *A. catenella* forms large, widespread blooms that move with the currents in northern New England. Comparatively, *A. catenella* populations are localized in small coves in southern New England, including in Connecticut (Anderson et al. 2005; Richlen et al. 2012). Saxitoxin concentrations in blue mussels have exceeded the regulatory limit (80 µg saxitoxin equivalent/100g shellfish) in Mumford Cove (1985, 1992, and 2020) and Palmer Cove (1992 and 2003) in Groton, CT. *A. catenella* blooms are driven by environmental conditions,

such as season and temperature; anthropogenic influences, such as nutrient loading/eutrophication (e.g. Hattenrath-Lehmann and Gobler 2016); and the presence of hardy cysts that settle in the seafloor sediment and can survive for extended periods of time (e.g. Miyazono et al. 2012), providing an inoculum source for subsequent blooms. As a result, the DABA has monitored for *Alexandrium* spp. and saxitoxin since 1985, with focused monitoring in Groton during late spring – early summer. Past closures have occurred in May (Mumford Cove) and June (Palmer Cove); therefore, the DABA has traditionally structured sampling around these months. Saxitoxin has not been detected outside of Groton, and there have been no PSP illnesses in CT.

Pseudo-nitzschia. Toxic *Pseudo-nitzschia* species produce domoic acid, which can become concentrated in shellfish tissue and cause amnesic shellfish poisoning (ASP) (table 1). Filter-feeding invertebrates and planktivorous fish can accumulate domoic acid and also cause animal mortality events (reviewed in Anderson et al. 2021), but none have been reported in CT. Domoic acid has never been detected in Connecticut, and there consequently have been no ASP closures or illnesses. The first ASP New England (Maine-Massachusetts) closure occurred in fall 2016, when *P. australis* moved into the region and caused substantial impacts on the shellfish industry through closures and shellfish recalls (Bates et al. 2018; Clark et al. 2019). *P. australis* is one of the most toxic species documented (e.g. Bowers et al. 2018), and has been associated with rapid toxin accumulation in multiple Maine shellfish species. Domoic acid was detected in Rhode Island shellfish below the FDA regulatory limit in fall 2016, but *P. australis* was not present (Sterling et al. 2021). However, *P. australis* caused a closure in Rhode Island in March 2017. Therefore, *P. australis* is likely present in Connecticut, in addition to other known toxigenic species (*P. multiseries* (formerly *Nitzschia seriata*)*, P. fraudulenta, P. delicatissima,* and *P. pungens*) that have been documented in local waters (Capriulo et al. 2002; Hargraves et al. 1993; Hargraves and Maranda 2002). *Pseudo-nitzschia* spp. patterns are difficult to predict in Connecticut. Similarly, domoic acid production is difficult to predict, with studies documenting a wide variety of environmental and physiological factors that influence if and when *Pseudo-nitzschia* cells produce DA (Lelong et al. 2012; fig. 3). There are many regional, environmental, and physiological factors that influence *Pseudo-nitzschia* populations, domoic acid production, and bloom toxicity.

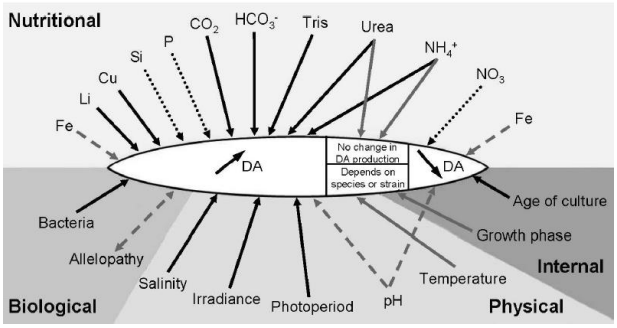

Figure 3: Factors impacting Pseudo-nitzschia domoic acid production, including nutritional, biological, physical, and internal factors (black arrows - effect increases the parameter; black dotted arrows - effect decreases the parameter; grey dashed arrow - conflicting results; grey arrows - result depends on the species or strain, or no change in DA production). Image from Lelong et al. 2012.

Dinophysis and Prorocentrum. Toxic *Dinophysis* spp. and *Prorocentrum* spp. produce okadaic acid and associated toxins, which can become concentrated in shellfish tissue and cause diarrhetic shellfish poisoning (DSP) (table 1). Okadaic acid has never been detected in Connecticut, and there consequently have been no DSP closures or illnesses. DSP events in New England are rare and closure risks are low (e.g. Tong et al. 2015); however, there have been closures in New York, including the largest ever

reported *D. acuminata* bloom in North America in 2011 (Hattenrath-Lehmann et al. 2013; Hattenrath-Lehmann and Gobler 2016). Historic studies have revealed the potential for rare, sporadic DSP illnesses in the surrounding area (Freudenthal and Jijina 1988; Maranda and Shimizu 1987; Staker and Bruno 1978; Staker et al. 1979; Stamman et al. 1987) and Canada (Quilliam et al. 1991; Subba Rao et al. 1993; Todd 1997). *P. lima*, a toxic species, is distributed throughout the New England region, but widespread studies showed that toxin concentrations in shellfish are typically negligible (Maranda et al. 2007a&b).

Long Island. This report focuses solely on the Connecticut portion of Long Island Sound. It is important to distinguish between the Connecticut and New York (Long Island) borders of Long Island Sound. Note that Long Island can be broken up into separate regions, including the north shore, which is part of Long Island Sound; the Peconic estuary, the eastern inlet; and the south shore bays (fig. 4). Multiple types of HABs occur in the Peconic estuary and south shore bays (fig. 4), which are not directly connected to Long Island Sound. Long Island has dealt with HAB issues dating back to the 1980s, which have severely impacted shellfish production and environmental health (reviewed in Hattenrath-Lehmann and Gobler 2016). Suffolk County is one of the most heavily HAB-impacted counties in the U.S., and some harmful blooms have become annual, widespread occurrences (fig. 4) (reviewed in Hattenrath-Lehmann and Gobler 2016). The increased frequency, intensity, and (in some cases) toxicity of multiple HABs impacting Long Island have been associated with nitrogen pollution (e.g. Gobler et al. 2012; Hattenrath et al. 2010; Hattenrath-Lehmann et al. 2015). From 2005-2010, ~1.1 million (out of 7.56 million) people on Long Island lived in unsewered areas and relied on onsite septic systems (USGS 2021). The Connecticut and New York ecosystems and HAB prevalences are very different, as HAB events in Connecticut are rare, more localized, and substantially less severe than those documented in New York.

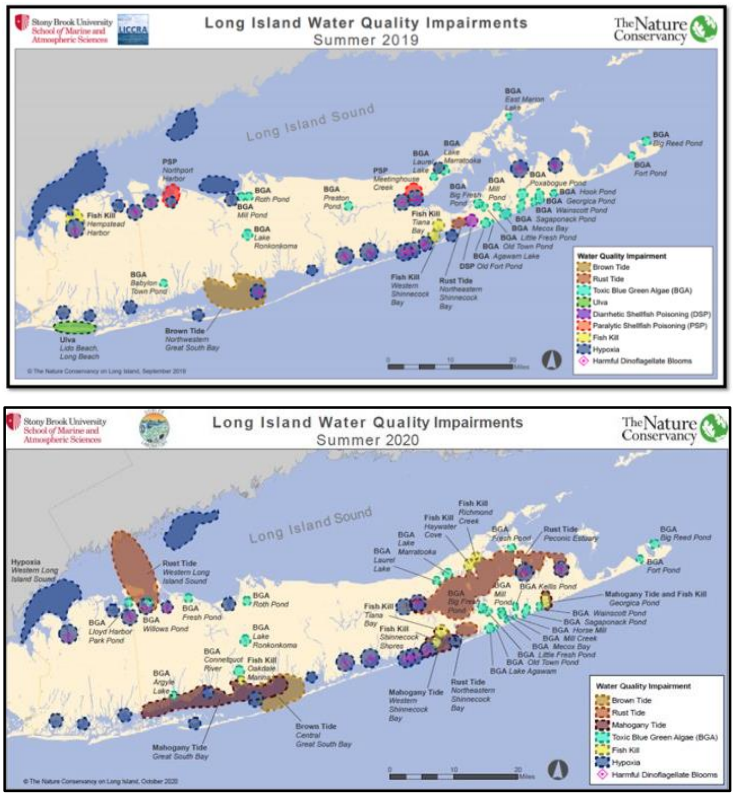

Figure 4: 2019 (top) and 2020 (bottom) Long Island Water Quality Impairment Maps. The maps show HAB, hypoxia, and fish kill events. Maps produced by the Gobler Laboratory (Stony Brook University).

Brown tide is caused by the picoplankton Aureococcus anophagefferens, rust tide is caused by the dinoflagellate Margalefidinium polykrikoides, blue green algae is a synonym for cyanobacteria, ulva is a type of macroalgae (seaweed), diarrhetic shellfish poisoning events were caused by Dinophysis acuminata, paralytic shellfish poisoning events were caused by Alexandrium catenella, mahogany tide is caused by the dinoflagellate Prorocentrum minimum

Methods

Despite the challenges of COVID-19 and multiple HAB events, the DABA successfully managed and continued to advance the HAB and biotoxin monitoring programs throughout the year. The goal of the enhanced phytoplankton monitoring program is to collect at least one monthly sample from every town with an active shellfish program – additional samples are collected if elevated HAB concentrations are detected. Routine phytoplankton monitoring was conducted from March – October. Overall, the program was a success in 2020, with a total of 226 samples covering all of the major growing areas from Greenwich to Stonington (Table 2).

Table 2: 2020 total phytoplankton samples per town by month

Town (town code)	January	February	March	April	May	June	July	August	September	October	November	December
Branford (14)			1	1	1	1	1	1	1	1	1	
Clinton (27)		2	2	2	2	2		2	2	4	2	2
Darien (35)			1	1	1	1	1	1	1	1	1	
East Lyme (45)			1	1	1	3	1	1	2			
Fairfield (51)			1	1	1	1	1	1	1	1	1	
Greenwich (57)	2		3		2	2	6	2		4		2
Groton (59)			5	7	9	8	7	1	1	1	1	
Guilford (60)			1		1	3	1	1	1			
Madison (76)			1		1	3	1	2	1	1	1	
Milford (84)			1	1	1	1	2	1	1	1	1	
Norwalk (103)			1	1	1	1	1	1	1	1	1	
Stamford (135)			1	1			1		1			1
Stonington (137)		1	2	1	3	4	4	2	3	1	1	
Stratford (138)			1	1	1	1	1	1	1	1	1	
Waterford (152)			1		1	3	1	1	1			
West Haven (156)			1	1	1	1	1	1	1	1	2	
Westport (158)			1	1	1	2	1	1	1	3	1	
Number of towns with 1+ samples	1	2	17	13	16	16	16	16	16	13	12	3
Total samples/month	2	3	25	20	28	37	31	20	20	21	14	5

HAB and biotoxin monitoring are essential program components required by the FDA. HAB monitoring methods are established separately by each shellfish sanitation program, but all generally concentrate HAB organisms with phytoplankton nets or sieves and analyze the samples with light microscopy. Biotoxin testing has stricter rules, as the FDA validates procedure efficacy, and establishes approved and approved limited use methods (see National Shellfish Sanitation Program (NSSP) model ordinance (MO) (https://www.fda.gov/food/federalstate-food-programs/national-shellfish-sanitation-program-nssp)).

HAB monitoring. Briefly, the DABA collects semi-quantitative samples using a 20µm phytoplankton net, equipped with a flowmeter, at routine locations chosen to represent heavily harvested locations in shellfish growing areas (fig. 5). An interactive Aquaculture Mapping Atlas is available, and shows the shellfish classifications, shellfish beds, and all DABA sampling stations (the majority of which are solely for fecal coliform analysis): http://cteco.uconn.edu/viewer/index.html?viewer=aquaculture. Vertical net tows have the benefits of concentrating large volumes of water and sampling the entire water column, which are important for monitoring HAB organisms in coastal shellfish growing areas. The samples are concentrated to ≤100mL; fixed with Lugol's iodine; delivered on ice; analyzed in duplicate samples using a PhycoTech nannoplankton chamber with a light microscope; and cell concentrations are calculated using cell counts, flowmeter counts, and concentrated sample volumes. The standard operating procedure (SOP) is described in detail in the DABA's 2021 HAB Net Tow Collection SOP.

The DABA established a recreational phytoplankton sample collection program in 2019, where shellfish commissions collect 500mL grab samples, which are fixed with Lugol's iodine and delivered on ice to the DABA laboratory for analysis. Samples are concentrated using a 20µm sieve to ≤10mL; analyzed in duplicate samples using a PhycoTech nannoplankton chamber with a light microscope; and final cell concentrations are calculated using cell counts, and total and concentrated sample volumes. The SOP is described in detail in the DABA's 2021 HAB Grab Sample Collection SOP.

143 semi-quantitative net tows and 53 grab samples were collected in 2020. The remaining samples were non-metered tows or grab samples of various volumes when 500mL bottles were not available.

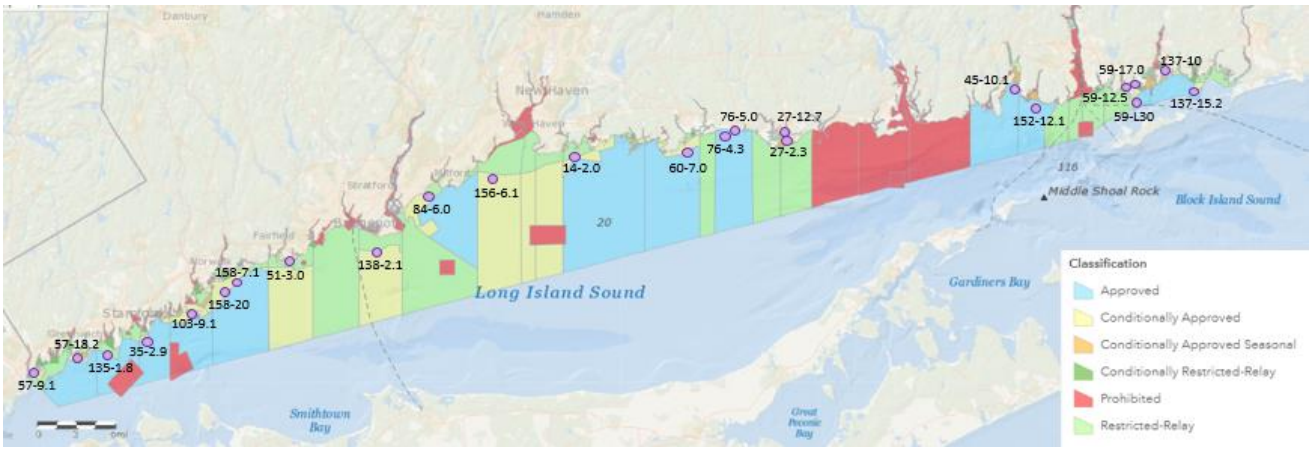

Figure 5: Map of routine DABA phytoplankton stations in CT, showing shellfish classifications in the background. The stations are located in approved and conditionally approved areas, where shellfish are directly harvested for consumption.

Biotoxin monitoring. In accordance with the NSSP MO, routine toxin testing is conducted in areas that previously had biotoxin closures. PSP testing has annually been conducted in Groton, CT since 1985, as this is the only area with a history of biotoxin closures. The DABA uses HAB monitoring as an early warning system for potential biotoxin threats, and conducts toxin testing in shellfish in response to elevated HAB concentrations. The DABA maintains in-house qualitative (positive/negative) tests for PSP and ASP, and a quantitative (toxin concentration in shellfish) test for PSP. As needed, quantitative testing for all other toxins are shipped out to affiliated laboratories. 20 PSP, 11 ASP, and 16 microcystin (cyanotoxin) and 2 associated (cyanobacteria) PSP toxin samples were analyzed in 2020.

Alexandrium

Alexandrium spp. are armored dinoflagellates, some of which produce saxitoxin (table 1). The DABA has monitored for *Alexandrium* spp. and saxitoxin since 1985, with focused monitoring in Groton during late spring – early summer. Since initiating an effort to identify *Alexandrium* to the species level in 2019, the DABA has identified 4 species: *A. catenella*, *A. ostenfeldii, A. pseudogonyaulax,* and *A. margalefii* (fig. 6).

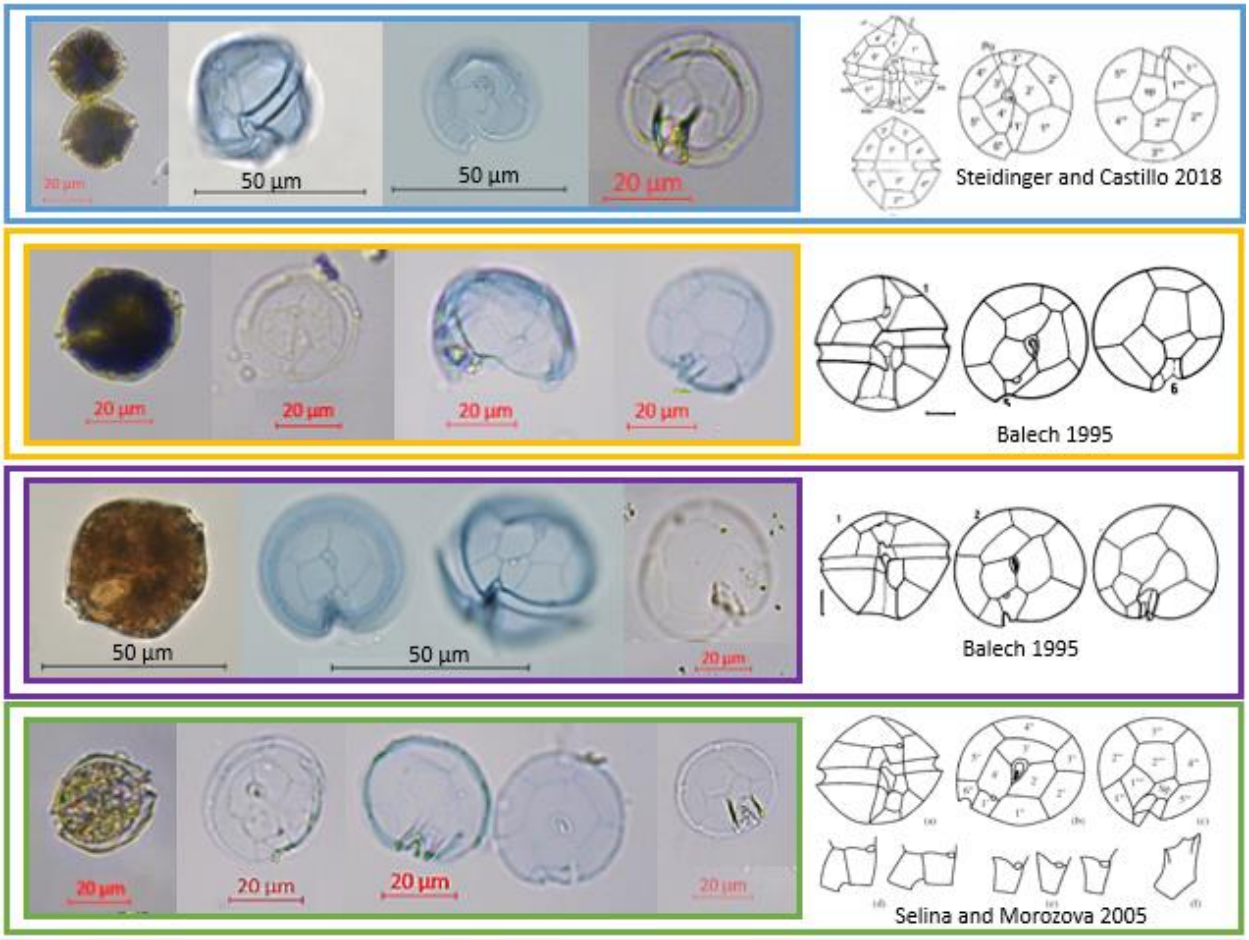

Figure 6: The number and pattern of thecal plates (tabulation), along with the apical pore complex (comma shaped, located on apex) are unique to the Alexandrium genus, but thecal plate shapes are unique to each Alexandrium species. The only diagnostic characteristic using light microscopy is thecal plate orientation. Top to bottom: A. catenella (formerly A. fundyense, A. tamarense), A. ostenfeldii (synonym A. peruvianum), A. pseudogonyaulax, and A. margalefii. Photos of cells and thecal plates, taken of CT species, are shown for comparison purposes. The drawings were taken from the respective references. Thecal plates appearing blue were stained with Trypan blue. A. catenella is typically found as single cells in CT; a rare chain of 2 cells is shown above. No chains of the other Alexandrium species have been observed in CT. Note that all Alexandrium species identified in CT thus far have a ventral pore (Vp) connected to the 1' plate. For reference, Balech 1995 reported the following cell dimensions: A. catenella (20-50µm length, 22-50µm width), A. ostenfeldii (33-56µm length, 33-57µm width), A. pseudogonyaulax (41-70µm length, 58-70µm width), A. margalefii (27.5-39µm length, 27.5-35µm width).

Saxitoxin producing species. *A. catenella* produces saxitoxin, and is present in isolated populations in Groton, CT. *A. catenella* cyst beds were historically documented in Mumford Cove and Palmer Cove (Anderson et al. 1982; Anderson 1986; WHOI 1999), which provide an inoculum source for future spring blooms. *A. ostenfeldii* (synonym *A. peruvianum* (Kremp et al. 2014)) was identified for the first time in CT in 2020, but this is not a surprising finding due to the prevalence of *A. ostenfeldii* along the east coast

(e.g. Borkman et al. 2012; Gribble et al. 2005; Steidinger and Castillo 2018; Tomas et al. 2012). *A. ostenfeldii* strains from Narragansett Bay produced saxitoxins, spirolides, and a gymnodimine (Borkman et al. 2012). Spirolides and gymnodimines are neurotoxins that are not yet regulated by the FDA, but Munday et al. (2012) showed that spirolides have a much lower toxicity when administered orally compared to other methods (e.g. intraperitoneal injection). Gulf of Maine *A. ostenfeldii* isolates produce spirolides, but do not produce saxitoxins (Gribble et al. 2005; Kremp et al. 2014). Atlantic Canadian isolates mainly produce spirolides (Cembella et al. 2000), but low saxitoxin concentrations have recently been reported (Qiu et al. 2018).

Species that do not produce saxitoxin. *A. pseudogonyaulax* does not produce saxitoxin, spirolides, or gymnodimines; however, it does produce goniodomin A (GDA; Zmerli Triki et al. 2016), which is toxic to fish and other animals. GDA is not yet regulated by the FDA. Other states are currently dealing with GDA-producing *Alexandrium* spp., such as annual summer blooms of *A. monilatum* that can reach high concentrations (millions of cells per liter) in Chesapeake Bay and cause widespread ecological disruption (e.g. Harding et al. 2009; May et al. 2010). *A. pseudogonyaulax* blooms have also been associated with skin discomfort in Norwegian swimmers (Karlson et al. 2021). *A. margalefii* is not known to produce any toxins (Anderson et al. 2012), and there were no documented negative impacts from a *A. margalefii* bloom in California (Tiffany et al. 2007). *A. margalefii* has been identified throughout the world, but rarely in the U.S. There are a few reports of *A. margalefii* on the U.S. west coast (Band-Schmidt et al. 2003; Morquecho and Lechuga-Deveze 2003; Tiffany et al. 2007) and the Gulf of Mexico (Steidinger and Castillo 2018), but no records were found for the east coast, potentially making this the first report.

2020 findings. *Alexandrium* was identified in 49 samples (21.6%) from March-October (5.9-25.8℃, 24.3-31.4 ppt). The maximum concentrations recorded in 2020 were: *A. catenella* (370 cells/L on 4/6 at 59-12.5 (Groton)), *A. pseudogonyaulax* (809 cells/L on 7/15 at 137-16.2 (Stonington)), *A. margalefii* (721 cells/L on 7/15 at 152-12.1 (Waterford) (mix of *A. margalefii* and *A. pseudogonyaulax*)), and *A. ostenfeldii* (24 cells/L on 6/2 at 137-15.2). *A. pseudogonyaulax* was the most common *Alexandrium* spp. (72% of *Alexandrium* samples), was present from May-October 2020, and is distributed throughout LIS coastal waters. *A. pseudogonyaulax and A. margalefii* were identified at higher maximum concentrations in 2019 (1,298 cells/L on 8/19/19 at 84-6.0 (Milford) and 1,254 cells/L on 6/25/19 at 59-6.0 (Groton), respectively). PSP scotia rapid test kits are sometimes used as a secondary confirmation when non-toxic *Alexandrium* spp. are present at high concentrations, and have always confirmed the absence of saxitoxin.

Regional findings. Comparatively, large *A. catenella* blooms have been reported around Long Island, with multiple locations having at least one sample containing >1,000 cells/L from 2007-2016 (Northport Bay and Mattituck Inlet (north shore Long Island); Sag Harbor Cove, James Creek, and Meetinghouse Creek (Peconic Estuary); and Shinnecock Bay (South Shore)) (reviewed in Hattenrath-Lehmann and Gobler 2016). At the same time, Gobler and Hattenrath-Lehmann (2011) conducted a 3-year survey in Connecticut and only reported *Alexandrium* spp. at non-detectable levels to <100 cells/L, and similarly low toxin quotas (maximum <10pmol STX eq./L in CT vs. >300 pmol STX eq./L in NY). The largest *A. catenella* bloom recorded on Long Island occurred in 2008 in Northport Bay when the density exceeded 1 million cells/L, saxitoxin concentrations in the water exceeded 20,000 pmol STX eq./L, and deployed

mussels and soft shell clams became highly toxic (maximum 1,400 and 600 µg STX eq./100g shellfish, respectively) (Hattenrath et al. 2010). Additionally, large blooms of *A. ostenfeldii* have been reported in Rhode Island, with a maximum concentration of 14,352 cells/L in 2009 in Wickford Cove (Borkman et al. 2012).

2020 Mumford Cove closure. Past PSP closures, due to *A. catenella,* have occurred in May (Mumford Cove) and June (Palmer Cove); therefore, the DABA has structured sampling around these months. 2020 is the first time that Mumford Cove was set to be open from November 2019 – April 2020 since it was upgraded to a conditionally approved seasonal area. The Groton recreational areas closed on 3/25 due to rainfall over 1 inch. As part of the reopening procedure, blue mussels were collected from sentinel cages and phytoplankton samples were collected to monitor *A. catenella* concentrations on 4/6. *A. catenella* cells were present at 370 cells/L and saxitoxin concentrations were recorded at 128.5 µg STX/100g (fig. 7). This is the first year since 2003 that there has been a mandatory PSP closure in CT, and the first time since 1992 that Mumford Cove had a mandatory closure. Mumford and Palmer Coves remained closed for the remainder of the spring and summer seasons. Due to COVID-19 restrictions, phytoplankton samples were not collected for most of April, but blue mussels contained 186.1 µg STX/100g on 4/20 (fig. 7). By 4/28, blue mussel concentrations dropped to 165.44 µg STX/100g (fig. 7). Softshell clams (steamers) from Mumford Cove on 5/5 were weakly positive using a positive/negative screening method, but negative by the quantitative method, suggesting that saxitoxin concentrations could have been between 20-38.3 µg STX/100g based on the limits of detection for each method. Oysters from lot 30 (off Morgan Point in Groton approved waters) were collected on 5/5 to assess the potential movement of toxin, and were negative for saxitoxin. Blue mussels from Mumford Cove on 5/12 were negative for saxitoxin (fig. 7), and saxitoxin was not detected again in Mumford Cove and was never detected in Palmer Cove in 2020. Interestingly, *A. margalefii* (non-toxic) was detected in both Mumford Cove and Palmer Cove by mid-May, highlighting the importance of species-level identification for all samples containing *Alexandrium* cells.

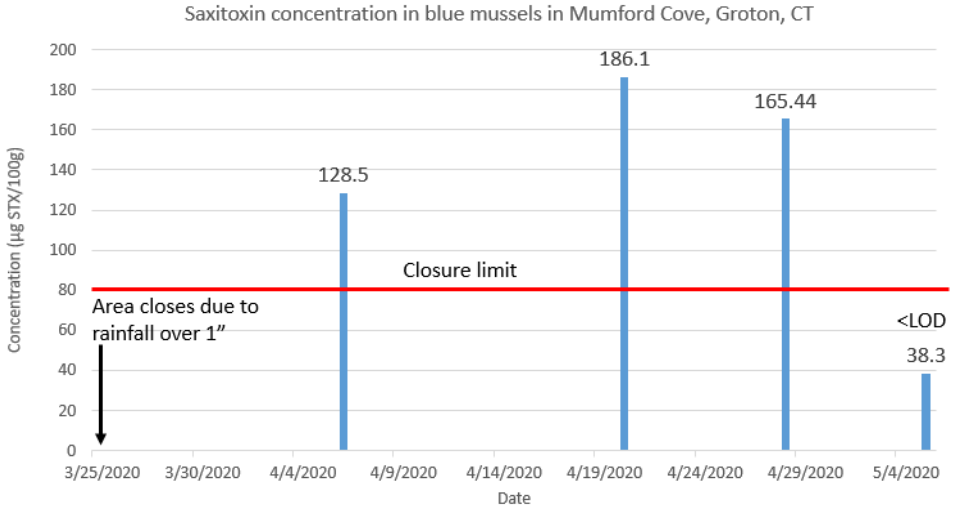

Figure 7: 2020 Mumford Cove, Groton, CT saxitoxin concentrations in blue mussels. The FDA regulatory closure limit is 80µg STX/100g shellfish, shown by the red line. Saxitoxin concentrations in blue mussels are shown as blue bars. Blue mussel saxitoxin concentrations tested above the regulatory limit from 4/6-4/28. The area was closed for the entire graphed time period.

2020 marks the earliest saxitoxin has ever been detected in Mumford Cove (and Connecticut in general), suggesting the need to lengthen the PSP monitoring season. Additionally, the saxitoxin concentrations detected in blue mussels in 2020 were the highest in Mumford Cove since 1985. Mumford Cove also closed in 1992, but saxitoxin concentrations only reached 96.3 µg STX/100g, approximately half of the maximum concentration detected in 2020. *Alexandrium catenella* strains in southern New England have lower cellular toxin quotas than northern New England strains; therefore, the high saxitoxin concentrations in 2020 suggests that a large, persistent bloom formed in April. In addition, saxitoxin was detected in Palmer Cove (under the regulatory closure limit) in 2019, the first time since the 2003 closure. Given that *Alexandrium catenella* formed blooms in 2019 and 2020, new cyst beds were likely laid in the surface sediments of both coves, providing an inoculum source for future blooms.

Of note, blue mussels are the standard biotoxin monitoring shellfish species and typically accumulate and depurate toxins rapidly, mimicking toxin concentrations in the water, but wild mussels are not present in Mumford and Palmer Coves. Saxitoxin was below the limit of detection in soft shell clams and oysters (n=2) in 2020, which corresponds to the most recent CT PSP closure in 2003 when oysters and hard clams were tested (n=12) and saxitoxin was always below the limit of detect (DABA, unpublished data).

Environmental factors. From 4/6-4/28, water temperatures in Mumford Cove remained relatively stagnant. While the water typically warms rapidly during the PSP season, the cold evenings kept Mumford Cove ~8℃ for the entire month. *Alexandrium* cysts can germinate and enter the water column ~5℃, rapid growth for New England isolates has been documented at 10-15℃, and bloom termination occurs at ~20℃ (Anderson 1998; Etheridge and Roesler 2005; Hattenrath et al. 2010; Schrey et al. 1984). However, even before the coves hit these warmer temperatures, a bloom clearly occurred in Mumford Cove. Therefore, PSP monitoring must be initiated in CT before the coves hit 8℃ in the future. The coves reached ~10℃, 10.5℃, 15.7℃, and 17℃ by 5/5, 5/12, 5/26, and 6/9, respectively. Temperature data is not available for the early *A. catenella* blooms (1980-90s); however, the trends documented in Mumford Cove in 2020 do not correlate with trends documented in the 2003 bloom in Palmer Cove. Despite the close proximity of the coves, the *A. catenella* bloom in Palmer Cove in 2003 initiated and persisted at higher temperatures (16-18℃). Rainfall is also known to be an important factor in HAB regulation, as it carries nutrients into the marine environment via runoff, increases freshwater input, and can disturb the sediment either directly or indirectly. There were 3 significant rain events (0.95, 1.54, and 0.81 inches on March 19, 23, and 29, respectively), prior to the first saxitoxin detection (4/6) in Mumford Cove. Additionally, there were 3 significant rain events in April (0.83, 1.42, and 1.5 inches on April 9, 13, and 30, respectively), as saxitoxin concentrations remained elevated, hit the maximum concentration (186.1 µg STX/100g) on 4/20, and stayed over the regulatory limit (165.44 µg STX/100g) on 4/28.

Of note, Fort Hill Brook, which feeds Mumford Cove, is an emergency outfall for the Groton town waste water treatment plant, but nutrient concentrations are not routinely monitored to the knowledge of this author. Hattenrath et al. (2010) showed that nitrogen loading can increase the intensity and toxicity of *A. catenella* blooms on Long Island. However, the abundance and toxicity of *Alexandrium catenella* cells and blooms in Connecticut has consistently been lower than those documented around Long Island (DABA data; Gobler and Hattenrath-Lehamann 2011).

Pseudo-nitzschia

While many HABs are caused by dinoflagellates, *Pseudo-nitzschia* is one of the most prominent harmful diatom genera, as some species produce domoic acid (table 1). *Pseudo-nitzschia* has become increasingly difficult to predict in New England, following the unprecedented appearance of *P. australis* and ASP closures starting in 2016. *Pseudo-nitzschia* has caused closures in Maine in September-January, Rhode Island in March, and most recently, New Hampshire and Maine in June. Domoic acid has never been detected in CT shellfish, but the recent emergence of *Pseudo-nitzschia* as a serious threat to New England shellfisheries, unpredictable *Pseudo-nitzschia* blooms, and unforeseen impacts from global climate change support the need for continued vigilance. The limitations of light microscopy prohibit the DABA from identifying *Pseudo-nitzschia* to the species-level; species-level identification requires advanced microscopy and/or molecular techniques. Therefore, the DABA uses the popular method of characterizing *Pseudo-nitzschia* by cell width size: cells are either large (≥3µm) or small (<3µm), as established by Hasle as the "seriata" and "delicatissima" complexes, respectively (e.g. Hasle et al. 1996).

2020 findings. *Pseudo-nitzschia* species were identified in 123 samples (54.2%), of which 90 were from central to eastern LIS (West Haven – Stonington) and 33 were from central to western LIS (Greenwich – Milford). *Pseudo-nitzschia* was present from February-December (4.9-25.6℃, 26.1-31.7ppt). Overall, higher concentrations were detected in eastern LIS (45 and 5 samples ≥1,000 cells/L in eastern and western LIS, respectively; max concentrations: 134,203 cells/L in Waterford (6/2) vs. 18,357 cells/L in Greenwich (10/6)). Interestingly, 4 out of the 5 highest western *Pseudo-nitzschia* cell concentrations were from Greenwich, while nearby Darien, Norwalk, and Westport always had low to non-detectable *Pseudo-nitzschia* concentrations. Statewide, 81 samples contained large (≥3µm width) and 66 contained small (<3µm width) cells (some samples contained a mixture of both large and small cells). Some images of small and large *Pseudo-nitzschia* spp. from LIS are shown below (fig. 8). A more extensive list of select cell concentration and environmental data are provided in **supplemental table 1**.

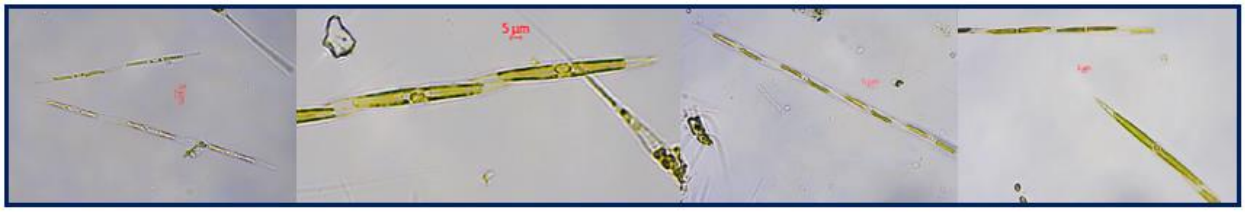

Figure 8: Photos of LIS Pseudo-nitzschia. Left to right: Small Pseudo-nitzschia cells (152-12.1, 6/2), Large cells (137-16.2, 7/15), Large cells (152-12.1, 9/13), Two types of large cells (14-5.2, 9/22).

2019 & 2020 "bloom" trends. *Pseudo-nitzschia* remained at low concentrations throughout LIS in spring 2019. The highest *Pseudo-nitzschia* concentration detected in 2019 was 14,317 cells/L at 137-15.2 (Stonington) in September. Overall, there was a trend toward higher *Pseudo-nitzschia* concentrations in offshore eastern LIS waters (East Lyme – Stonington).

These trends were somewhat consistent in 2020. *Pseudo-nitzschia* spp. were present at low to undetectable levels throughout LIS until late May – early June, when concentrations reached a maximum of ~135,000 cells/L at 152-12.1 (Waterford) on 6/2 (fig. 9). The bloom was dominated by small *P. delicatissima*-like cells (fig. 8). Blue mussels were preferentially sampled as sentinel species and

oysters were preferentially sampled from heavily harvested commercial areas. All mussel and oyster samples were negative for domoic acid (fig. 9). The highest July (7/15) concentration was 12,041 cells/L at 137-16.2, which is located in Stonington Harbor, while offshore Stonington waters were only at 3,498 cells/L (see supplemental table 1). At this point, large *Pseudo-nitzschia* cells were predominant (fig. 8). An oyster sample from the surrounding area was negative for domoic acid. Concentrations were low in August, but increased again in September and October. The maximum eastern concentration was 15,748 cells/L at 152-12.1 (Waterford) on 9/13 (fig. 10). Concentrations in central LIS remained low until September, when 14-5.2 (Branford) reached 15,824 cells/L on 9/22 (fig. 10). Western LIS had low concentrations in September and October, with the exception of Greenwich, which reach a maximum of 18,357 cells/L on 10/6 (fig. 10). All fall samples were dominated by large *Pseudo-nitzschia* spp. (fig. 8). Oyster samples from five towns were negative for domoic acid (fig. 10).

June Pseudo-nitzschia bloom. 7 samples collected on 5/27 or 6/2 exceeded the DABA toxin testing trigger of 20,000 cells/L for *Pseudo-nitzschia* spp. (fig. 9). *Pseudo-nitzschia* concentrations were elevated from Madison – Stonington (fig. 9; 43,364 cells/L recorded at 76-4.3 (Madison) (supplemental table 1)). All samples tested, including phytoplankton and multiple shellfish species, were negative for domoic acid (fig. 9). None of the samples contained monospecific blooms; *Pseudo-nitzschia* spp. were dominant (134,203 cells/L), but there were over 10 other diatom species in Waterford on 6/2. Coincidently, New Hampshire was detecting *Pseudo-nitzschia delicatissima* concentrations reaching 1-2 million cells/L at the same time that the DABA detected elevated concentrations of small *Pseudo-nitzschia* cells. However, states between New Hampshire and Connecticut were not reporting elevated concentrations, suggesting these blooms were separate populations. Lower concentrations of large *Pseudo-nitzschia* spp. were also present in some of the samples. Follow up samples were collected over the next two weeks, and showed a rapid decrease in *Pseudo-nitzschia* cell concentrations (see supplemental table 1). 152-12.1 was added as a phytoplankton monitoring station in 2020 to increase spatial monitoring; therefore, there is no historic data available for this location, but it appears to be a "hot spot" for *Pseudo-nitzschia.* The Millstone power plant discharges warm water near 152-12.1, and large toxic *Pseudo-nitzschia* blooms have been associated with unusually warm water on the U.S. west coast (e.g. McCabe et al. 2016) and during the 2016 *P. australis* bloom in New England (Clark et al. 2019).

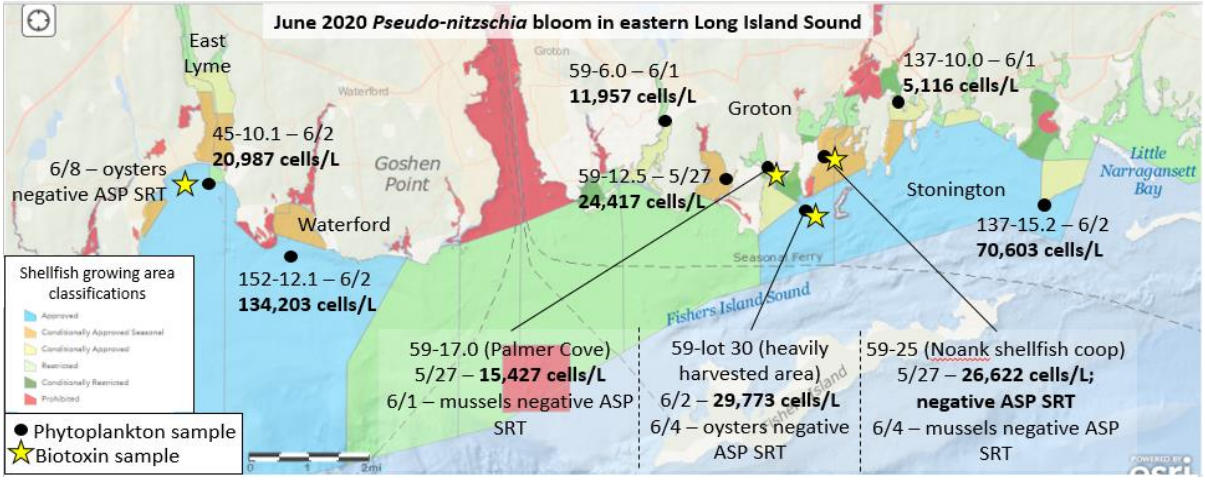

Figure 9: Elevated Pseudo-nitzschia concentrations were detected in eastern LIS in late May/early June. Domoic acid was not detected in mussel or oyster samples.

Fall Pseudo-nitzschia blooms. Elevated *Pseudo-nitzschia* concentrations were detected in some towns in September and October (fig. 10). All fall samples contained mostly large *Pseudo-nitzschia* cells. The maximum concentrations for eastern LIS concentration was 15,748 cells/L at 152-12.1 (Waterford, 9/13), central LIS was 15,824 cells/L at 14-5.2 (Branford, 9/22), and western LIS was 18,357 cells/L at 57-18.2 (Greenwich, 10/6). 5 oyster samples were negative for domoic acid by HPLC-UV analysis (samples were sent prior to collection of the Greenwich sample).

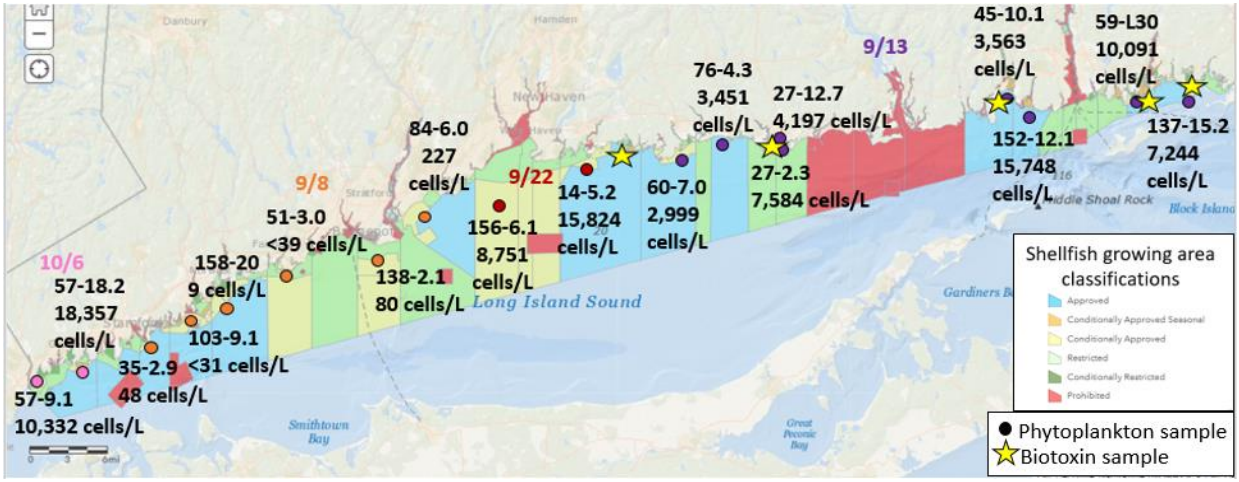

Figure 10: Elevated Pseudo-nitzschia concentrations were detected throughout LIS in September and October 2020. Concentrations of all towns sampled are shown to present the wide variation in Pseudo-nitzschia concentrations throughout LIS over a short period of time. Domoic acid was not detected in oyster samples collected throughout LIS shellfish growing areas.

DNA fingerprinting. Preliminary *Pseudo-nitzschia* DNA fingerprinting analysis (e.g. Hubbard et al. 2014; Clark et al. 2019) was performed by the Florida Fish and Wildlife Conservation Commission-Fish and Wildlife Research Institute, led by Dr. Kate Hubbard, through her collaborative research for the Woods Hole Center for Oceans and Human Health. The analysis provides results in percentages of each species, as detected by the amount of DNA present. *P. delicatissima* and *P. plurisecta* were dominant during June (fig. 11). However, lower concentrations of *P. pungens* and *P. australis* were present in Stonington and Waterford in June (fig. 11). The *P. australis* DNA sequence was the right size, but additional samples are necessary for confirmation; therefore, *P. australis* is currently considered a putative identification. All June samples were dominated by small *Pseudo-nitzschia* cells, but lower concentrations of large *Pseudo-nitzschia* cells were present in Stonington and Waterford. By July 15, large cells made up a majority of the *Pseudo-nitzschia* composition throughout the state, which corresponds to *P. pungens* dominance in July and September (fig. 11). While domoic acid was not detected during 2020, these results support the need for continued monitoring due to the prevalence of multiple *Pseudo-nitzschia* species that are known to produce toxins. This is the first year that CT DABA has had access to *Pseudo-nitzschia* species-level identification; therefore, additional data is necessary to understand species assemblages, seasonality and patterns, and confirm the presence of *P. australis* in Connecticut.

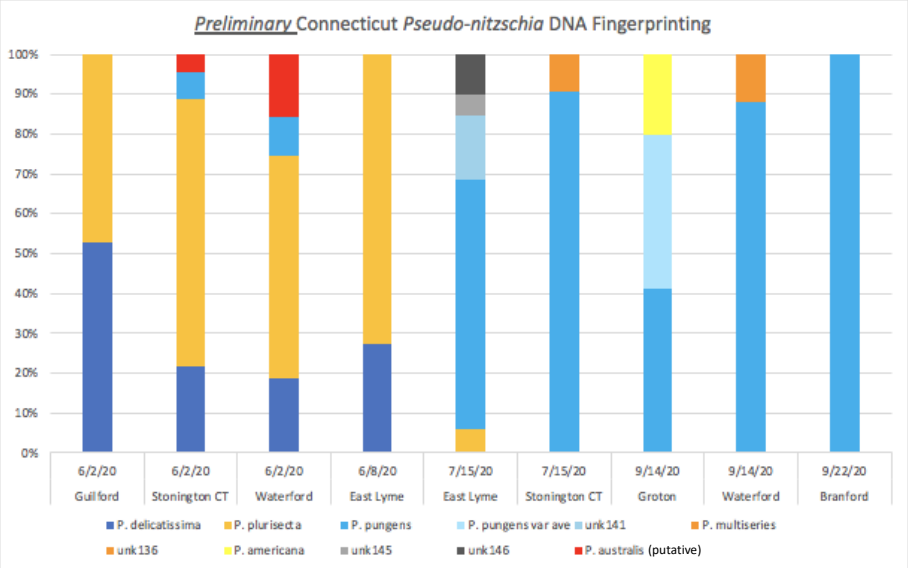

Figure 11: Preliminary Pseudo-nitzschia DNA fingerprinting results for select samples from Connecticut 2020. The results are shown as percentages of each species based on the amount of DNA present; therefore, the results do not necessarily indicate relative cell prevalence due to differences in cell sizes. There are currently multiple unknown Pseudo-nitzschia species, as this technique requires optimization for each region. P. australis is considered a putative identification until further analyses are completed. This work was conducted by Florida Fish and Wildlife Conservation Commission-Fish and Wildlife Research Institute as part of a collaborative study for the Woods Hole Center for Oceans and Human Health.

The preliminary Connecticut results largely align with findings from Maine and Rhode Island, where consistent *Pseudo-nitzschia* species assemblage monitoring has been conducted (Chadwick et al. 2021; Clark et al. 2019; Sterling et al. 2021). Contrary to northern New England where *P. australis* has occurred annually in the fall since 2016, *P. australis* was potentially present in June but was not detected in September in Connecticut. However, it is possible *P. australis* was present in Connecticut fall samples that were not sent for sequencing. Additionally, *P. australis* caused Rhode Island's only mandatory domoic acid closure in March 2017, suggesting that it potentially is a threat in southern New England in the spring. There was a clear shift in New England in 2016 when *P. australis* first appeared in the region, but these patterns have not been studied in Connecticut. Long-term monitoring (1999-present) in Rhode Island has shown that *Pseudo-nitzschia* concentrations regularly exceed the action threshold of 20,000 cells/L (which triggers toxin testing), even exceeding 1 million cells/L a few years, without causing closures in the region (Sterling et al. 2021). Long-term qualitative monitoring in Connecticut by DABA parallels Rhode Island's data, as domoic acid has never been detected in shellfish, despite samples where *Pseudo-nitzschia* was recorded as "common" (20-50% sample) and "abundant" (>50% sample) (DABA, unpublished data). 2017-2019 data from Rhode Island showed the highest domoic acid concentrations occurred in May, June, September and October (not including the March 2017 closure) (Sterling et al. 2021). In Rhode Island in 2017-2019, *P. australis* was present in 23% of samples, and was detected in the fall, winter, and spring (Sterling et al. 2021). There are over 15 *Pseudo-nitzschia* species in Maine (Bates et al. 2018 and references therein). The highest particulate domoic acid concentrations in Maine shellfish have been associated with *P. australis* and *P. plurisecta* from 2013-2020, but closures have only been associated with *P. australis* in the fall of 2016-2019 (Chadwick et al. 2021). *P. delicatissima* can form abundant blooms, most often in the spring, in Maine and New Hampshire, but they were not associated with domoic acid accumulation or shellfish closures (Chadwick et al. 2021).

Dinophysis

Global DSP impacts. Dinophysis spp. are armored dinoflagellates, some of which are associated with the production of diarrhetic shellfish toxins (table 1). DSP is a major health concern in Europe and around the world (reviewed in Reguera et al. 2014). Recently, a massive *Dinophysis acuminata* bloom in Brazil was associated with okadaic acid bioaccumulation throughout the entire food web (Mafra et al. 2019). The authors reported the highest okadaic acid concentrations ever recorded in oysters worldwide, over 22 times the regulatory closure limit, and the highest mussel sample was over 48 times the regulatory limit (Mafra et al. 2019). Lower levels of okadaic acid were detected in zooplankton (phytoplankton consumers); gastropods (e.g. snails); and novel toxin vectors like sand dollars, ghost shrimp, and pelagic fish species (*Chaetodipterus faber, Mugil liza*) (Mafra et al. 2019). Okadaic acid was even detected in the liver of dolphins (*Sotalia guianensis*) and penguins (*Spheniscus magellanicus*) (Mafra et al. 2019).

DSP in New England. More than 10 *Dinophysis* species have been identified in Long Island Sound and the surrounding area (Fredudenthal and Jijina 1988). The risk of a significant DSP event occurring in New England or Canada was rated as low-moderate, due to the relatively low concentrations of diarrhetic shellfish toxins detected from *D. acuminata* isolates (Tong et al. 2015). The risk is further reduced because Connecticut commercial operations do not harvest mussels, which have been linked to many DSP outbreaks around the world (e.g. Reguera et al. 2014). However, *D. acuminata* has caused closures on Long Island, with a 2011 bloom in Northport Bay (north shore) that peaked at ~1.3 million cells/L, and blue mussels, ribbed mussels, and soft shell clams all exceeded the regulatory limit (blue mussels reached almost 8 times the regulatory limit) (Hattenrath-Lehmann et al. 2013).

2020 findings. Dinophysis acuminata and *Dinophysis norvegica* are the most common *Dinophysis* species in Connecticut (fig. 12), and both are known to produce toxins. *Dinophysis* was present in 111 (48.9%) samples from March-September (4.9-26℃, 23.2-31.4 ppt), all of which contained *D. acuminata. D. norvegica* was only present in 20 samples. The highest concentration of *D. acuminata* was 2,199 cells/L from Stonington (137-10.0) on 6/15, and there were only 9 samples with concentrations ≥1,000 cells/L (all of which only contained *D. acuminata*). These concentrations are significantly lower than those reported for DSP closures around Long Island. The highest concentration of just *D. norvegica* was 173 cells/L on 6/8 in East Lyme (45-10.1). *D.* cf. *fortii* was only identified in 1 sample in 2020 (78 cells/L (mixed with *D. acuminata*) at 137-15.2 (Stonington) on 9/14).

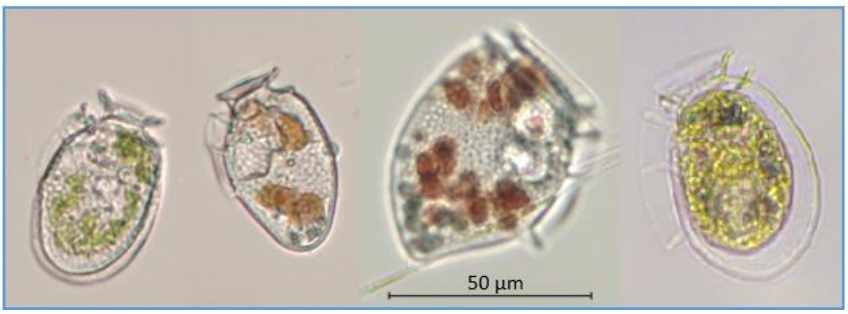

Figure 12: Dinophysis species in Connecticut. Left to right: Dinophysis acuminata (two cells), Dinophysis norvegica, Dinophysis cf. fortii. Scale bar applies to all photos.

Prorocentrum

Prorocentrum spp. are armored dinoflagellates, some of which are associated with the production of diarrhetic shellfish toxins (table 1). The CT DABA has documented the follow *Prorocentrum* spp.: *P. lima, P. micans, P. minimum, P. scutellum,* and *P. triestinum* (fig. 13), all of which are planktonic species except for *P. lima. P. lima* is a known diarrhetic shellfish toxin producer. *P. micans, P. scutellum,* and *P. triestinum* are non-toxic species. There are conflicting results about *P. minimum* toxicity, but evidence suggests that blooms (called mahogany tides) are harmful to ecosystems and shellfish, and that at least some strains produce tetrodotoxin (see below).

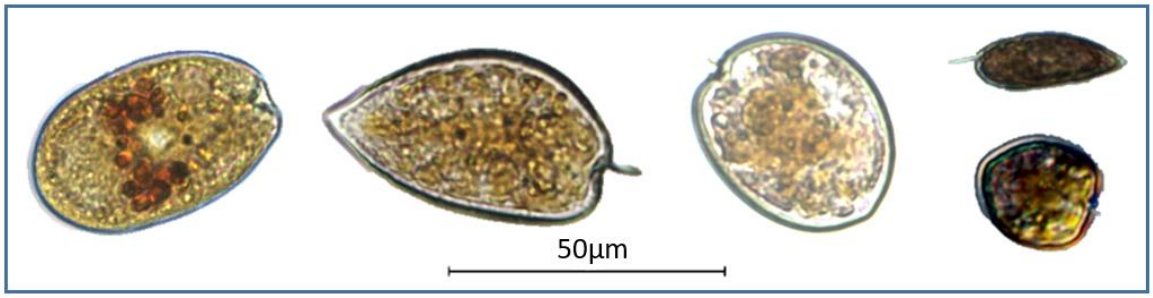

Figure 13: Prorocentrum species in Connecticut. Left to right: P. lima, P. micans, P. scutellum, P. triestinum (top), and P. minimum (bottom). In addition to unique cell shapes, the planktonic Prorocentrum spp. have distinct apical spines. Scale bar applies to all photos.

Prorocentrum lima. P. lima is present throughout New England and is known to produce diarrhetic shellfish toxins; however, the risk of DSP from *P. lima* has been assessed and determined to be low (Maranda et al. 2007a&b). Since *P. lima* is a benthic species, it is not typically detected during phytoplankton monitoring. The DABA uses vertical net tows to monitor the entire depth of the water column, which increases the likelihood of collecting *P. lima* that have entered the plankton following sediment disruption. However, planktonic concentrations of *P. lima* do not necessarily reflect toxin concentrations in shellfish (Levasseur et al. 2003; Morton et al. 2009), making it a difficult species to monitor. Most DSP events have been attributed to *Dinophysis* spp.; however, *Prorocentrum lima* has been identified as the causative organism of DSP toxins in shellfish or DSP events in Maine (Morton et al. 1999); Nova Scotia, Canada (Lawrence et al. 2000; Levasseur et al. 2003); Argentina (Gayoso et al. 2002); England (Nascimento et al. 2005; Foden et al. 2005); and the Black Sea (Moran et al. 2009). Similar to *Dinophysis* spp. DSP illnesses, most of the cases were associated with mussel consumption (Gayoso et al. 2002; Lawrence et al. 2000; Levasseur et al. 2003; Morton et al. 1999, 2009).

2020 findings. Prorocentrum spp. were detected in 149 (65.6%) samples, of which 90 had *P. scutellum,* 72 had *P. micans*, 51 had *P. triestinum,* 23 had *P. minimum,* and 3 had *P. lima. Prorocentrum* spp. were detected from February-December, with *P. micans* dominance in the spring, followed by *P. minimum* in the summer, and *P. scutellum* and *P. triestinum* in the summer-late fall. The highest concentrations corresponded to non-toxic species (*P. triestinum* reached a maximum of 131,738 cells/L on 8/2 in Greenwich (57-9.1); *P. scutellum* reached a maximum of 2,750 on 8/13 in West Haven (156-6.1); *P. micans* reached a maximum of 732 cells/L on 8/17 in Groton (59-L30)). *P. minimum* was most prevalent in June and July (max. 11,936 cells/L on 6/15 in Stonington (137-10.0)). *P. lima* was only detected in

shallow coves (Palmer and Mumford Coves, Groton; Clinton Harbor) at low concentrations (max. 136 cells/L, which was detected 1 day after >0.5 inches rain). Morton et al. (2009) also reported *P. lima* detection in the water column following rainfall. *P. lima* was detected in April, May, and October, which corresponds to regional research (Rhode Island populations reached the highest concentrations in spring-fall) (Maranda et al. 2007a).

Prorocentrum minimum. There have been proposed taxonomic revisions for *P. minimum*, some authors give preference *to P. cordatum* (Velikova and Larsen 1999); however, *P. minimum* is used herein until there is a formal taxonomic revision. The toxicity of *P. minimum* has been debated for decades. While it has been shown that *P. minimum* does not produce diarrhetic shellfish toxins, human poisonings via consumption of contaminated shellfish have historically been associated with *P. minimum* in Japan, Portugal and Norway (reviewed in Heil et al. 2005 and Vlamis et al. 2015). *P. minimum* also causes negative environmental impacts: mortality in scallops; growth inhibition in hard clams (Wikfors and Smolowitz 1993); mortality and feeding reduction in juvenile oysters (Luckenbach et al. 1993); and alterations in the immune system of juvenile oysters, scallops (Hegaret and Wikfors 2005), blue mussels (Galimany et al. 2008), and hard clams (Hegaret et al. 2010).

A large *P. minimum* bloom (reaching 56 million cells/L) was associated with the accumulation of an unidentified neurotoxin in wild mussels in the French Mediterranean, and laboratory experiments showed that shellfish became toxic when fed *P. minimum* cultures (Denardou-Queneherve et al. 1999). Mussels and *P. minimum* cultures had neurotoxic effects via bioassay, and data supported that the toxicity was associated with bacteria in the cultures (Grzebyk et al. 1997). Recent evidence supports the association of *P. minimum* blooms and shellfish contamination with tetrodotoxin (TTX), a potent neurotoxin. While TTX is commonly associated with pufferfish, it is actually found in phylogenetically distinct groups (bacteria, ribbon worms, arthropods (crabs, horseshoe crabs, copepods), echinoderms, molluscs (bivalves, gastropods, and cephalopods), amphibians, and fish (reviewed in Bane et al. 2014)), leading to the hypothesis that certain bacteria could be the ultimate source of TTX (reviewed in Magarlamov et al. 2017). Vlamis et al. (2015) documented the detection of an unresolved neurotoxin in Greek mussels from 2006-2012, and the co-occurrence of *P. minimum.* TTX concentrations reached 22.9 µg/kg (28% of the regulatory closure limit for saxitoxin, a related toxin) in 2012, but these authors did not directly isolate toxin from *P. minimum* cultures (Vlamis et al. 2015). Rodriguez et al. (2017) isolated TTX-like compounds from *P. minimum* strains (Ecuador; Johor Strait, between Singapore and Malaysia), but not the Sarasota, Florida strain.

Tetrodotoxin is an emerging public health threat, and there is still much to be learned about the causative organism(s), accumulation throughout foodwebs, associated environmental conditions, and global distribution. To date, TTX has been detected in 21 bivalve and gastropod species in 10 different countries, including parts of Europe, Asia, and Oceania (reviewed in Biessy et al. 2019). 3,032 TTX human intoxications have been reported, with 88.6% from Asia (Guardone et al. 2020). There were only 23 cases in North America (0.8%), all due to imported pufferfish (Guardone et al. 2020). The cases cover 5 continents, with 60% of cases due to fish, followed by gastropods (20.9%), arthropods (16.2%), and cephalopods (2.9%) (Guardone et al. 2020). The no observable adverse effect level (NOAEL) was calculated as 44 µg TTX equivalents/kg shellfish, based on a large shellfish portion size of 400g, by the

European Food Safety Authority (EFSA 2017). TTX and saxitoxin are both neurotoxins that inhibit sodium channels, and recent data shows that TTX and saxitoxin have additive properties, suggesting that they should be regulated together (Finch et al. 2018). It is unclear if TTX is a potential threat in the U.S., but human intoxications from TTX-contaminated bivalves have not been reported (Guardone et al. 2020). As a result, the FDA has not yet established closure guidelines for TTX in shellfish. Large "mahogany tides," caused by *P. minimum*, periodically occur on Long Island (fig. 4) and historically occurred in CT, but toxins have not been detected during these events.

Cyanobacteria and *Microcystis*

Overview. Toxic freshwater cyanobacteria are being detected in estuarine and marine waters more frequently around the world (reviewed in Preece et al. 2017), including the U.S. (reviewed in Anderson et al. 2021) (see fig. 1). In particular, Long Island (reviewed in Hattenrath-Lehmann and Gobler 2016), the Chesapeake Bay region (Bukaveckas et al. 2017, 2018; Onofrio et al. 2021; Tango and Butler 2008), Florida (Kramer et al. 2018), California (Miller et al. 2010; Peacock et al. 2018; Tatters et al. 2017), and Mississippi (Bargu et al. 2019; Conrad et al. 2021) have all documented substantial impacts in coastal regions. Studies have shown that cyanotoxins can accumulate in marine shellfish (Amorim and Vasconcelos 1999; De Pace et al. 2014; Falconer et al. 1992; Harding 2000; Preece et al. 2015b; Sipia et al. 2002; Van Buynder et al. 2001) and take an extended period of time to depurate (e.g. Gibble et al. 2016; Miller et al. 2010). The CT Department of Energy and Environmental Protection (DEEP) Water Quality Monitoring Program is primarily responsible for responding to cyanobacteria blooms, releasing information about beach closures for public safety, and maintaining a bloom reporting system that received over 200 reports in 2020. However, DABA responds to cyanobacteria blooms that have the potential to impact coastal areas and shellfish growing areas due to the emergence of cyanotoxins in shellfish as a potential public health threat.

Figure 14: Cyanobacteria bloom photo taken by an unaffiliated citizen scientist on 7/2 at Stanwich Pond

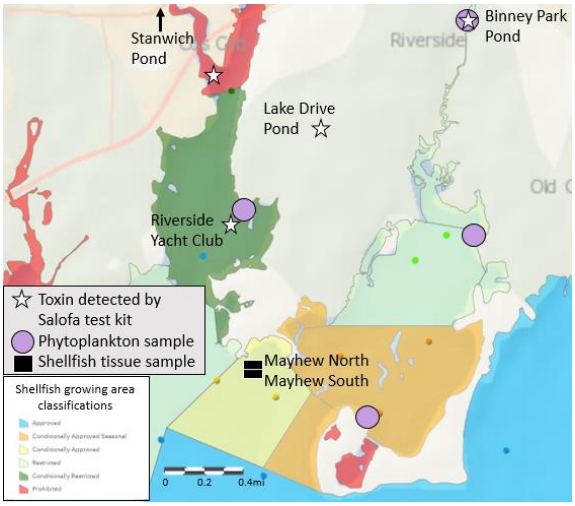

Figure 15: Greenwich microcystin map, showing locations of reported positive toxin results, and DABA phytoplankton and toxin (oyster tissue) samples

Greenwich microcystin bloom. The DABA received a report on 7/16 that an independent citizen scientist had observed suspected cyanobacteria blooms in ponds near/feeding Long Island Sound in Greenwich (fig. 14), knew of one pet illness potentially associated with the bloom, and had positive toxin test results from kits purchased on Amazon. Cyanobacteria blooms were only evident in ponds, not in estuarine waters. The unaffiliated citizen scientist notified the DABA that she had positive toxin results

from water samples taken at Lake Drive Pond, Binney Park Pond, Stanwich Road Pond, and Cos Cob Harbor (Mianus River Boat & Yacht Club and Riverside Yacht Club) (fig. 15) using Salofa Oy (Finland) BlueGreenTest kits, which provide a positive/negative result for microcystin analogues (MC-LR, dm-MC-LR, MC-RR, dm-MC-RR, MC-LA, MC-LY, MC-LF, MC-YR, MC-WR, MC-LW) and nodularin (Nod-R) in water samples. The DABA must use approved testing or screening methods, and the efficacy of the BlueGreenTest is unknown. While blooms were not observed in estuarine waters, there was a clear potential impact on shellfish safety. The blooms were apparently observed the last week of June and the citizen conducted the toxin testing the first week of July. The DABA was not notified until 7/16, and promptly advised potentially impacted shellfish growers to not harvest until a full investigation could be completed.

DABA bloom investigation and findings. Based on the delayed notice, an emergency sample run was conducted on 7/17. Binney Pond, an apparent bloom point source, was investigated, in addition to areas around Greenwich Cove and Cos Cob Harbor. No blooms were found, and phytoplankton samples did not contain high concentrations of cyanobacteria. Phytoplankton samples from Greenwich Cove revealed sparse, small *Microcystis* colonies (fig. 16). The colonies looked stressed, with distorted edges and potentially lysed cells (fig. 16). Individual, non-colonized, small cells were also sporadically observed in samples (not shown), similar to the NOAA photo (fig. 16), but these could not be confirmed as *Microcystis* with light microscopy. The *Microcystis* composition was estimated at ≤5-10% of the estuarine phytoplankton samples. Dr. Dianne Greenfield (City University of New York) also confirmed the presence of *Microcystis aeruginosa* in samples she subsequently collected.

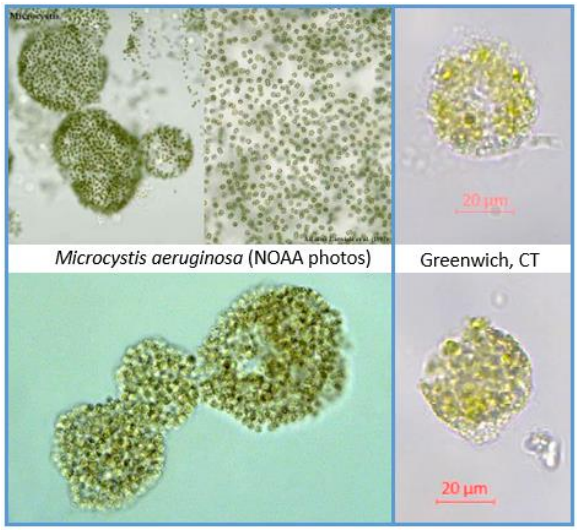

Figure 16: Left (top and bottom): Microcystis aeruginosa colonies and single cells (photos from NOAA); Right (top and bottom): suspected Microcystis colonies from estuarine waters in Greenwich, CT

While *Microcystis* spp. can have a wide salinity tolerance (Preece et al. 2017), multiple studies have documented that cyanobacteria bloom concentrations decrease from the point freshwater source to estuarine and marine environments, due to cell lysis and bloom dilution (Bormans et al. 2019; De Pace et al. 2014; Lehman et al. 2005; Miller et al. 2010; Preece et al. 2015b, 2017; Tango and Butler 2008). Therefore, observed cell concentrations in downstream environments are not sufficient for monitoring the movement of cyanobacteria blooms, and must be used in conjunction with toxin testing. The surface temperature was similar between Binney Pond and the estuarine sample locations (23.1-23.4℃); however, the salinity in Binney Pond was only 0.7 ppt vs the estuarine samples at 25.4-26.4 ppt.

Cyanotoxins in shellfish are an emerging issue and are not yet regulated by the FDA; therefore, the most appropriate option is to use a validated AOAC, BAM, or EPA method. The UConn Center for Environmental Sciences and Engineering laboratory had an established method for testing seawater and shellfish samples by UPLC-MS/MS. Therefore, the DABA shucked and homogenized oyster samples; screened for saxitoxin in-house using scotia rapid test kits; and sent samples to UConn for analysis of

microcystins, anatoxin A, and cylindrospermopsin. Samples were prepared according to the FDA standard biotoxin methods, such that at least 100g oyster was homogenized per sample (12-15 oysters/sample), and frozen until analyzed or immediately delivered on ice. Saxitoxins are produced by marine HABs and some cyanobacteria (see table 1), but were not detected in the oyster samples. Oyster samples from Greenwich Cove (shellfish beds "Mayhew North and South") on 7/20 had microcystin-RR (MC-RR) concentrations of 3.2 and 3.0 ng/g (ppb), respectively (table 3). Out of an abundance of caution, Greenwich Cove was precautionarily closed due to the presence of MC-RR, and the FDA was consulted on how to proceed. While microcystin was not detected in oysters from Mayhew North in subsequent samples, MC-RR persisted in subsequent samples from Mayhew South (table 3). Areas were reopened once MC-RR was no longer detected, and the seasonal recreational area in Greenwich Cove opened in December (on schedule) once multiple shellfish species tested negative for microcystin and other cyanotoxins.

Microcystin guidelines. The World Health Organization (WHO) has defined the tolerable daily intake (TDI) for MC-LR as 0.04 µg MC-LR/kg body weight/day (WHO 1998). Due to the lack of data for many MC congeners, the TDI applies to all microcystins. The EPA has suggested acute and chronic TDIs of 0.006 and 0.003 µg MC-LR/kg/day, respectively, based on additional data since the WHO TDI was established and to incorporate the risk of chronic health effects (USEPA 2006). Ibelings and Chorus (2007) used previous toxicology studies to derive acute (2.5 µg MC-LR/kg b.w./single exposure) and seasonal (0.4 µg MC-LR/kg b.w./day) tolerable intake levels.

Table 3: Microcystin concentrations in Greenwich, CT 2020

	Date	**7/20**	**7/28**	**8/10**	**9/29**	**11/3**	**12/7**
Microcystin concentration in oysters, unless specified otherwise (ng/g, ppb)	Mayhew North	3.2	<1.5	<1.5		<1.5	
	Mayhew South	3.0	4.5	3.8	5.7	<1.5	
	Greenwich cove recreational area						<1.5 (oyster, ribbed mussel, hard clam)
Microcystin in water (ng/mL)	Binney Pond (feeds Greenwich Cove)		<0.5				

The California Environmental Protection Agency has adopted an action level of 10 ng/g (eq. to µg/kg) for microcystins in fish and shellfish for a portion of 32 g/day (OEHHA 2012). The WHO has apparently set a guideline of 24 µg/kg in shellfish and fish (referenced within Gibble et al. 2016; Preece et al. 2017). Ibelings et al. (2021), on behalf of the WHO, stated "that while in many cases concentrations in foods are low, some field observations and laboratory experiments found concentrations that would lead to a dose in the range of – or above – that which would be acceptable for up to 2 weeks for an adult consuming 2 L of drinking-water per day, using the short-term WHO GVs (guideline values) for drinking-water. Trends that can be discerned are that consuming molluscs and crustaceans collected from

environments with blooms might cause higher risks, particularly because they are eaten with the viscera which can contain large amounts of toxic cyanobacteria. In contrast, the edible portions of higher trophic-level organisms (e.g., muscle tissue of fish), excluding viscera, have less chance of containing a large amount of free toxin."

The maximum concentration of 5.7 ng/g detected from Greenwich oysters is below the WHO TDI at 0.0095 µg/kg/day for a 100g portion (~12 oysters, depending on size) and 60kg individual. While this exceeds the EPA acute and chronic TDIs, these guidelines have been established for MC-LR, not MC-RR. Given the variability of public health guidelines for MC and variable toxicity among MC congeners, it is difficult to determine the actual public health threat of these low MC concentrations. Concentrations detected in shellfish around the world and in laboratory studies are typically substantially higher than those reported herein (tables 4&5). All MC-RR concentrations in Greenwich oysters remained below the OEHHA action level (10 ng/g), short-term WHO GV (12 ng/g), and supposed WHO guideline for shellfish (24 ng/g).

Greenwich appears to be one of the highest risk coastal towns in Connecticut for future cyanobacteria blooms due to the higher population density and nutrient inputs (e.g. Lopez et al. 2014). For example, visible cyanobacteria blooms were also identified in July 2019 near the Greenwich border in Milton Harbor and Larchmont Harbor, NY (estuarine locations) (Peter Linderoth (Save the Sound), personal communication, 2/12/20), but DABA was notified after-the-fact and consequently could not investigate the causative species or presence of toxins.

Background information on microcystin in the estuarine/marine environment. There are more than 250 MC congeners (Spoof and Catherine 2017), of which MC-LR is one of the most common and potent. Cyanotoxins have the potential to contaminate entire marine foodwebs, and have been linked to the death of sea birds (Carmichael and Li 2006) and sea otters in California (Miller et al. 2010) and bottlenose dolphins in Florida (Brown et al. 2018). The sea otter deaths were directly linked to the consumption of microcystin-contaminated shellfish (Miller et al. 2010).

MC-RR was the only congener detected in Greenwich oysters. Each congener has unique structural and chemical properties, and MC-RR is more hydrophilic, which is why it is believed to easily incorporate and persist in animal tissues (reviewed in Diez-Quijada et al. 2019 and Ibelings and Chorus 2007). MC-RR has been detected in bivalves, crustaceans, fish, and plants (Diez-Quijada et al. 2019), with the highest concentrations detected in freshwater bivalves in a controlled bloom exposure experiment (up to ~30,000 ng/g in muscle tissue and ~300,000 ng/g in digestive tissue (Kim et al. 2017)). MC-RR was detected in 19/21 wild sea otters that likely died from hepatotoxic shellfish poisoning (1999-2008; maximum concentration 104.46 ng/g) (Miller et al. 2010). Despite the noted predominance of MC-LR during the 2007 *Microcystis* bloom in California, which was associated with the death of 11/21 analyzed sea otter carcasses, MC-LR was only detected in 2 sea otters (Miller et al. 2010).

Studies documenting naturally-contaminated marine shellfish are presented below (table 4). There are many additional studies on microcystin concentrations in freshwater bivalves, but those are not included. Nodularins are structurally related to microcystins, and are consequently included below

(table 4); however, other cyanotoxins are not included in this review. Microcystins are one of the most prevalent and well-studied cyanotoxins; therefore, nodularins are held to similar standards as microcystins, but additional nodularin-specific studies are necessary to understand their public health threat.

Available microcystin shellfish data and testing methods. Preece et al. (2015a) found that ELISA methods frequently overestimate MC results and give false-positives in shellfish samples, relative to chemical analytical methods. In particular, the MC ADDA ELISA, which targets the ADDA structure (unique to MCs), was found to overestimate concentrations and result in false positives (Preece et al. 2015a). To further complicate matters, MCs become bound to tissues in organisms that consume the toxins and can accumulate in the liver when exposed to sub-acute doses (Greer et al. 2018). It is unclear if bound MCs pose a health threat via transfer up the foodweb, but some have made cases that bound MCs do pose a threat (e.g. Mohamed et al. 2018; Smith et al. 2010). Additionally, analysis of bound vs. unbound MCs can drastically change the reported MC concentrations (Greer et al. 2018; Mohamed et al. 2020; Williams et al. 1997). See Testai et al. (2016) for an in-depth review of different analytical methods and their reliability.

Table 4: Microcystin concentrations in naturally-contaminated shellfish

	Species	**Maximum microcystin concentration**	**Method(s)**	**Microcystin congener(s) in shellfish**	**Max. water bloom/toxin concentration (causative organism)**	**Location**	**Reference**
Mussels	Mussels	600 ng MC-LR eq./g	Liquid chromatography linked protein phosphatase bioassay, reverse phase liquid chromatography			Quatsino Sound, British Columbia, Canada	Chen et al. 1993
	Blue mussel (*Mytilus edulis*)	2 ng MC-LR & 0.2 ng nodularin/g				Prince Edward Island, Canada	
	Blue mussel (*Mytilus edulis*)	-63,400 ±11,300 ng/g (covalently bound) -22 ng/g (free MC)	GCMS		*Microcystis aeruginosa*	Campbell River, British Columbia, Canada	Williams et al. 1997

	-Mediterranean mussels (*Mytilus galloprovincialis)* -Black mussels (*Choromytilus meridionali)*	22,000 ng/g	HPLC	MC-LR and MC-YR	200-1,500 µg/g freeze-dried cyanobacterial cells	South Africa	Harding 2000
	Mussels	2,500 ng/g	HPLC-MS/MS	Nodularin	40,000 cells/mL (*Nodularia spumigena*)	Australia	Van Buynder et al. 2001
	Blue mussels (*Mytilus edulis*)	1,490 ng/g	ELISA*	Nodularin (confirmed with LC-MS/MS)		Gulf of Finland, Baltic Sea	Sipia et al. 2002
	Mediterranean Mussel *(Mytilus galloprovincialis)*	141.5 ng/g	ELISA*	MC-LR and MC-YR **in water** (HPLC/MS)	19.8 ng/L	Greece	Vareli et al. 2012
	Mediterranean Mussel *(Mytilus galloprovincialis)*	256 ng/g	ELISA*		-298.7 µg/L in lake water -0.61 µg/L in sea water	Italy	De Pace et al. 2014
		39 ng/g	LC-MS/MS	desMe-MC-RR			
	Pacific blue mussel *(Mytilus trossulus)*	6.5 ng/g	ELISA*	MC-LA (LC-MS/MS confirmatory analyses)	-2,700 ug/L source lakes -0.34 ug/L in marine waters	Puget Sound, WA	Preece et al. 2015b
	Mytilus spp.	416.23 ng/g	LC-MS	Tested for MC-LR, RR, YR, and LA		San Francisco Bay, California	Gibble et al. 2016
	Mediterranean Mussel *(Mytilus galloprovincialis)*	0.6±0.5 ng/g	ELISA*	Only tested for MC-LR	0.75 ng/L Lagoon water	Italy	Baralla et al. 2017
		MC-LR not detected	LC-MS/MS		MC-LR not detected		
	California mussels (*Mytilus californianus)*	18.9 ng/g	LC-MS	Tested for MC-LR, RR, YR, and LA	-Sacramento River 461.6 ng/L (max) -San Francisco Bay opening 51.1 ng/L (max)	San Francisco Bay, California	Peacock et al. 2018

	Blue mussels (*Mytilus edulis*)	70-230 ng MC-LR eq./g	*ELISA (MC ADDA ELISA)	Nodularin	130,000 cells/mL, 0.3-6 µg/L (*Nodularia spumigena)*	Oresund strait, via the Baltic Sea	Carlsson and Rita 2019
	California mussels (*Mytilus californianus)*	232 ng/g	LC-MS	MC-RR and MC-dmLR predominant -MC-YR, MC-LR, and MC-LA detected sporadically	11 different toxic cyanobacteria genera, including *Microcystis* and *Planktothrix* (MC in coastal lagoon reached 6,236 ng/g)	Santa Clara River Estuary, California	Tatters et al. 2021
Clams	*Macoma balthica*	130 ng/g (Nodularin)	*ELISA	Nodularin (confirmed with LC-MS/MS)		Gulf of Finland, Baltic Sea	Sipia et al. 2002
	Clams	20 ng/g	HPLC	Demethyl MC-LR and MC-LR **in water**	42.4±5 µg/L	San Francisco Bay, CA	Lehman et al. 2005
	Chamelea gallina	2.3 ng/g	ELISA*		-298.7 µg/L in lake water -0.61 µg/L in sea water	Italy	De Pace et al. 2014
	Tapes decussatus	0.85 ng/g MC-LR not detected	ELISA* LC-MS/MS	Only tested for MC-LR		Italy	Sedda et al. 2016
	wedge clams (*Rangia cuneata*)	25±2 ng/g	ELISA (MC ADDA ELISA)*		279±19 ng/L	James River Estuary, Virginia	Bukaveckas et al. 2017
	Glauconome sp.	1,900 ng/g	HPLC-MS/MS	MC-LR, MC-YR, MC-LW, MC-RR, MC-LA, MC-LF	Estuary dam: 235 µg/L particulate MC, 139,828 cells/mL (*Microcystis* sp.	South Korea	Kim et al. 2021

					and other toxic cyanobacteria)		
Oysters	Pacific oysters (*Crassostrea gigas)*	370 ng/g	ELISA (MC ADDA ELISA)*		-18 µg/L reservoir water -1.9 µg/L reservoir water discharged to Isahaya Bay (*Microcystis aeruginosa* with other toxic cyanobacteria)	Isahaya Bay, Japan	Takashashi et al. 2014
	Crassostrea sp.	3.42±2.24 ng/g	LC-MS	Tested for microcystin-LR, RR, YR, and LA	*Microcystis* (concentrations not documented)	Tomales Bay, California	Gibble et al. 2016
	Eastern oyster (*Crassostrea virginica)*	9.8 ng/g, 2.33 ng/g (12 samples tested)	HPLC-MS/MS	**MC-RR, MC-LR** (Tested for MC-RR, -YR, -LR, -LA, -LF, -LW, -LY)	Market testing – did not study exposure conditions	China	Cui et al. 2018
Scallops	Yesso Scallops (*Patinopecten yessoensis)*	4.3 ng/g (12 samples tested)		**MC-LR** (Tested for MC-RR, -YR, -LR, -LA, -LF, -LW, -LY)			
Abalone	*Haliotis discus*	<0.6 ng/g (12 samples tested)		(Tested for MC-RR, -YR, -LR, -LA, -LF, -LW, -LY)			

*ELISA (Enzyme Linked Immunosorbent Assay) methods have given false positives and overestimations of microcystin concentrations in shellfish tissues (Preece et al. 2015a).
Abbreviations: LC (Liquid Chromatography), HPLC (High Performance Liquid Chromatography), MS (Mass Spectrometry)
Concentrations have been converted to ng/g to standardize results.

Studies documenting microcystin (and other cyanotoxin) depuration from marine shellfish are outlined below (table 5). Note the wide range of conditions (shellfish species, exposure conditions/ concentrations, analytical method, etc.) among these studies (table 5). **Please note the above disclaimer about results obtained by different analytical methods and bound/unbound microcystin.** As expected, shellfish typically steadily accumulate microcystin during exposure, and concentrations

decrease during depuration. However, multiple studies have shown that microcystin concentrations do typically increase at least once, and then continue to decrease, during depuration (see fig. 17). From the DABA findings and studies reviewed below, it is clear that complete depuration takes an extended amount of time.

Gibble et al. 2016.

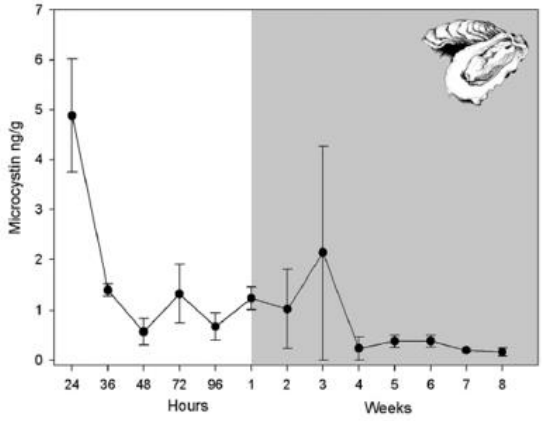

Fig. 5. Commercial oyster and microcystin toxin experimental trials. X-axis begins at 24 h denoting removal from water containing microcystins.

Camacho-Munoz et al. 2021

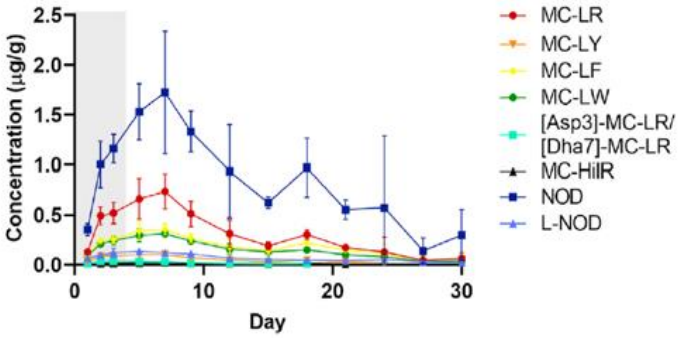

Fig. 3. Temporal evolution of the concentration (µg/g) of cyanotoxins detected in *M. edulis* tissue over the 3 day exposure period (grey area) to a combined culture of *M. aeruginosa* and *N. spumigena* followed by a 27 day depuration period (white area). Data is presented as mean and SD of n = 3.

Figure 17: Studies showing Crassostrea sp. MC depuration over 8 weeks, with a spike in concentration at week 3 (left, Gibble et al. 2016), and Mytilus edulis MC and NOD depuration over 30 days, again with a spike in toxin concentrations during depuration (right, Camacho-Munoz et al. 2021)

Table 5: Laboratory studies on microcystin accumulation and depuration in marine shellfish						
Species		**Exposure conditions**	**Maximum concentration in shellfish**	**Method/ Microcystin congener(s)**	**Depuration timeframe**	**Reference**
Mussels	Blue mussels (*Mytilus edulis*)	*M. aeruginosa* (3 days) (305 mg of microcystins/100 g freeze-dried algal cells)	336,900 ng/g (covalently bound) 204 ng/g (free)	-GCMS -Targeting ADDA	4 days – 11,300 ng/g covalently bound MC 53 days – 14 ng/g free MC	Williams et al. 1997
	Mediterranean mussels (*Mytilus galloprovincialis*)	10^5 cells/mL *M. aeruginosa* (4 days) (3.4 mg MC/10^7 cells)	16,000 ng/g	-ELISA* -MC-LR	Still detectable at ≤5,000 ng/g after 14 days	Amorim and Vasconcelos 1999
	Blue mussels (*Mytilus edulis*)	10.6 µg/mL (ppm) MC-LR (4 days)	979 ng/g	-LC-MS/MS -MC-LR	>21 days	Miller et al. 2010
	California mussels (*Mytilus californianus*)	5.6 µg/L particulate microcystin (24 hours)	~10 ng/g microcystin	-LCMS -Tested for MC-LR, YR, RR, and LA -Presented data as total MC	-Over 8 weeks to depurate completely -Concentration dropped ~3 weeks post-exposure	Gibble et al. 2016
		26.65 µg/L particulate microcystins (24 hours)	39.11 ng/g			Gibble et al. 2016
		7.74 µg/L dissolved microcystin (24 hours)	20.74 ng/g		Complete depuration within 36 hours post-exposure	Gibble et al. 2016
	Mediterranean mussels (*Mytilus galloprovincialis*)	-10^5 cells/mL *M. aeruginosa* and *Chrysosporum ovalisporum* cylindrospermopsin (CYN)) (15 days) -Calculated exposure: 1,150 pg/mL (1.15 µg/L) MC-LR and 392,700 pg/mL (392.7 µg/L)	-14.17 ng/g MC-LR -92.08 ng/g CYN	-LC-MS/MS -MC-LR	After 15 days, 1) CYN concentrations were significantly lower, but still detectable, and 2) MC-LR concentrations were not significantly lower and still detectable	Oliveira et al. 2020

	Blue mussels (*Mytilus edulis*)	*M. aeruginosa* (~85 µg MCs/L) and *Nodularia spumigena* (~47 µg NOD/L) (3 days)	Peaked at 3,400 ng/g combined toxins (1,720 ng/g NOD, 740 ng/g MC-LR, and lower concentrations of other MC congeners) 4 days post-exposure	-UPLC-MS/MS -Tested for and detected microcystins (MC-LR, MC-LF, MC-LW, MC-LY, [Asp3]-MC-LR/[Dha7]-MC-LR, MC-HilR) and nodularins (NOD cyclic and linear)	Toxins were still detectable after 27 days of depuration (490 ng/g MC/NODs)	Camacho-Munoz et al. 2021
Clams	Manila clams (*Tapes semidecussatus*)	10.6 µg/mL MC-LR (4 days)	1,324 ng/g	-LC-MS/MS -MC-LR	>14 days	Miller et al. 2010
Oysters	Pacific oysters (*Crassostrea gigas*)	10.6 µg/mL MC-LR (4 days)	373 ng/g	-LC-MS/MS -MC-LR	>21 days	Miller et al. 2010
	Crassostrea sp.	7.71 µg/L (24 hours)	~6 ng/g	-LCMS -Tested for MC-LR, YR, RR, and LA -Presented data as total MC	-Over 8 week to completely depurate -Concentration dropped at 36 hours and 4 weeks post-exposure	Gibble et al. 2016
Snails	*Tegula* spp.	10.6 µg/mL MC-LR (4 days)	175 ng/g	-LC-MS/MS -MC-LR	Data not provided in article	Miller et al. 2010

*ELISA (Enzyme Linked Immunosorbent Assay) methods have given false positives and overestimations of microcystin concentrations in shellfish tissues (Preece et al. 2015a).

Abbreviations: LC (Liquid Chromatography), HPLC (High Performance Liquid Chromatography), MS (Mass Spectrometry)

Concentrations have been converted to ng/g to standardize results.

July statewide trends

Phytoplankton composition was comparable across LIS in July, as 19 samples were collected from Greenwich-Stonington during the same week (July 13-17). Sea surface temperatures ranged from 20.2-24.4℃ and salinities from 25.4-31ppt. There was an obvious increase in salinity from western to eastern LIS, but no obvious trend in temperature, partly due to variable water column depth between sample locations.

Multiple studies have documented that LIS is typically dominated by diatoms, except for during the summer when dinoflagellates have some competitive advantages, particularly in shallow, eutrophic areas (reviewed in Lopez et al. 2014). Stonington (137-15.2) had the highest diversity with 34 phytoplankton genera, including 26 diatom genera. However, areas in central and western LIS also had high diversity (e.g. Stamford had 32 phytoplankton genera, including 24 diatom genera). Even areas within towns had variable diversity, notably Greenwich, which ranged from low diversity (57-9.1; 9 phytoplankton genera, 5 diatom genera) to high diversity (57-18.0; 29 phytoplankton genera, 21 diatom genera). However, not surprisingly, the lowest diversity was recorded for grab (500mL) samples, which represent significantly smaller sample sizes than net tows.

Eastern LIS (Guilford-Stonington) was dominated by diatom species, while central and western LIS (Greenwich-Branford) were more likely to be dominated by a mixture of zooplankton, dinoflagellates, and (in some towns) diatoms. Regardless of community dominance, diatom genera were present in every samples (5-26 genera). *Alexandrium pseudogonyaulax* and *Pseudo-nitzschia* concentrations were lower from Greenwich-East Lyme vs. Waterford-Stonington (maximum concentrations in Stonington (137-16.2) at 809 cells/L and 12,041 cells/L, respectively). *Dinophysis acuminata* was present at all locations, but the highest concentrations were in Fairfield-Branford (maximum 1,065 cells/L in Fairfield (51-3.0)). *D. norvegica* was also present at low concentrations in 6 samples (Darien, Branford, Waterford, Groton, and Stonington). *Prorocentrum* spp. were detected at every location, and included *P. micans, P. minimum, P. scutellum,* and *P. triestinum* (not all species at all locations). Greenwich (57-18.2) had the highest concentration at 2,829 cells/L, which included *P. minimum* and *P. triestinum.*

Other HABs

Some HABs do not produce toxins, but are detrimental to the environment and can cause animal, including shellfish, kills. Additionally, some HABs produce toxins that are not yet regulated by the FDA.

Yessotoxin-producing species (Gonyaulax spinifera, Lingulodinium polyedrum, Protoceratium reticulatum)

Gonyaulax spinifera, Lingulodinium polyedrum and *Protoceratium reticulatum* (fig. 18) are known to produce yessotoxins and occur in New England (Hargraves and Maranda 2002; Mudie et al. 2002; Pospelova et al. 2002, 2004). The Gonyaulax spinifera complex/group includes three species with similar morphologies, *G. spinifera, G. digitale, and G. diegensis* (reviewed in Rochon et al. 2009). Rhodes et al. (2006) first showed that *Gonyaulax spinifera* produces yessotoxins, and *G. spinifera* is now know to be

20x and 600x more toxic than *P. reticulatum* and *L. polyedrum*, respectively (Paz et al. 2004, 2008; Caron et al. 2010).

Yessotoxin has been associated with potent physiological impacts when injected intraperitoneally, including cardiac muscle, liver, pancreas, and brain damage in mice, with a lethal level of 100 µg/kg (reviewed in Paz et al. 2008). However, mice were essentially unaffected by 10mg/kg oral administration, suggesting oral toxicity is significantly lower (reviewed in Paz et al. 2008). The European Union (EU) has been regulating yessotoxin since 2002, and increased the maximum level from 1mg YTX eq./kg shellfish to 3.75 mg YTX eq./kg in 2013 (European Union 2013). No YTX human intoxications have been reported, despite concentrations exceeding the EU limit around the world (Karlson et al. 2021); therefore, the actual threat is still undetermined.

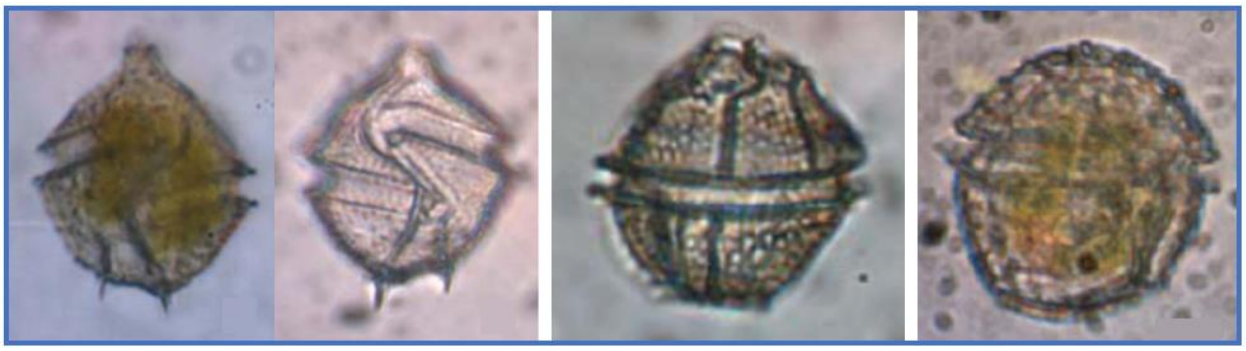

Figure 18: Yessotoxin-producing dinoflagellates: Gonyaulax spinifera (left 2 images), Lingulodinium polyedrum (center), Protoceratium reticulatum (right). Photos from Garate-Lizarraga et al. 2014.

As part of a 7 year risk assessment in Canada (both Atlantic and Pacific coasts), YTXs were detected in 65% and 2% of Pacific and Atlantic samples, respectively (Rourke and Haigh 2020). Mussels (57.8%), clams (27.5%), scallops (8.4%), oysters (5.4%), and whelks (1%) were all analyzed on the Atlantic coast (Rourke and Haigh 2020). The highest concentration for the Pacific and Atlantic coasts were 12 and 1.6 mg YTX eq/kg, respectively, and were associated with the highest annual sea surface temperatures (Rourke and Haigh 2020). No human illnesses were reported during the study (Rourke and Haigh 2020). Howard et al. (2008) previously showed that YTX on the U.S. west coast in water and shellfish (max. 0.1 µg/g (eq. to mg/kg)) samples was associated with *L. polyedrum* and *G. spinifera* (Howard et al. 2008). A *G. spinifera* strain isolated from Maine did not produce YTX during their study period (Howard et al. 2008). Therefore, data suggests that YTX is a greater threat to the U.S. west coast, and has remained below the EU regulatory limit when detected on the east coast. YTX-contaminated shellfish have also been found in Africa, Chile, Europe, Japan, and New Zealand (reviewed in Chikwililwa et al. 2019; EFSA 2008; Paz et al. 2008; Howard et al. 2008).

Due to the *G. spinifera* complex, there is a large amount of genetic diversity associated with this group, and phylogenetic analyses with different regional strains may help to elucidate toxic and non-toxic strains (reviewed in Chikwililwa et al. 2019). Using phylogenetic analyses, Chikwililwa et al. (2019) showed that YTX producing *G. spinifera* strains formed a cluster, while nontoxic strains, including New

England strains, were distinct, highly divergent, and grouped with other *Gonyaulax* species. Analyses included nontoxic Maine, Massachusetts, and Rhode Island strains (Chikwililwa et al. 2019).

A *Gonyaulax spinifera* bloom (maximum 611,000 cells/L) in South Africa was associated with the production of yessotoxin and the death of millions of abalone (Pitcher et al. 2019). *Lingulodinium polyedrum* was also present (maximum 160,000 cells/L), but did not produce YTXs in culture (Pitcher et al. 2019). Maximum toxin concentrations of 1.64 mg YTX eq./kg were reported (Pitcher et al. 2019). Abalone gill degeneration and necrosis was observed in a land-based South African aquaculture farm from direct toxin contact (Pitcher et al. 2019). Abalone stopped feeding, crawled out of water, were unresponsive to sunlight and touch, and were unable to right themselves (Pitcher et al. 2019). Larvae failed to settle and had disproportionately high mortality, but all size classes experienced mortality (Pitcher et al. 2019). Wild invertebrates (e.g. sea urchins, top snails, turban snails, limpets) also died and washed up on nearby beaches (Pitcher et al. 2019).

In summary, yessotoxin in shellfish is not yet regulated by the FDA, the EU increased their regulatory limit in 2013, there are no known cases of human intoxication, and tested New England HAB strains and shellfish have had low to non-detectable YTX concentrations. Therefore, the overall YTX risk in New England is low. However, the DABA records YTX producing species during routine monitoring. The *G. spinifera* "complex" has been observed the most frequently and at the highest concentrations of the YTX producing species. The *G. spinifera* complex was detected in 77 samples, of which only 10 exceeded 1,000 cells/L. The *G. spinifera* complex reached a maximum of 5,634 cells/L on 8/2 in Greenwich (57-9.1) during a *Chattonella* sp. (169,873 cells/L) and dinoflagellate bloom (*P. triestinum* (131,738 cells/L), *Heterocapsa* (not quantified), and *Akashiwo sanguinea* (16,034 cells/L)). The next highest concentration of *G. spinifera* was 3,282 cells/L on 10/27 in Greenwich (57-9.1), which also coincided with a high concentration of *Chattonella* sp. (48,785 cells/L).

Margalefidinium polykrikoides

Margalefidinium polykrikoides (formerly *Cochlodinium polykrikoides* (Gomez et al. 2017)) is a harmful unarmored dinoflagellate that forms blooms commonly referred to as "rust tide" because high cell densities can cause a purple-red water discoloration (Fig. 19). Blooms occur in late summer – early fall, and have coincided with temperatures of 20-25℃ and salinities of 22-30 ppt in New York (Gobler 2010). Areas with low flow (high residency times; e.g. coves, embayments) and sources of nitrogen enrichment are ideal environments for *M. polykrikoides* blooms (Tomas and Smayda 2008; Gobler et al. 2008). *M. polykrikoides* has caused fish mortalities around the world, including with caged fish aquaculture operations (Anton *et al.* 2008; Azanza *et al.* 2008; Dorantes-Aranda *et al.* 2010; Gárate-Lizárraga *et al.* 2004; Richlen *et al.* 2010; Whyte *et al.* 2001). Globally, *Margalefidinium polykrikoides* blooms have resulted in fisheries losses of hundreds of millions of dollars, as well as widespread environmental harm (reviewed in Berdalet et al. 2016). Larval and metamorphosing shellfish, as well as larval and juvenile fish, are more susceptible to *M. polykrikoides* blooms (Gobler and Tang 2009). The mechanism causing fish and shellfish mortalities is not fully understood – a variety of hypotheses have been tested including the production of reactive oxygen species (ROS), toxins and mucus (reviewed in Tang and Gobler 2009). Although rare, a few reports of marine mammal deaths have been associated with widespread *M.*

polykrikoides blooms in the Arabian gulf region (Berktay 2011; Richlen *et al.* 2010). A *M. polykrikoides* strain from the Gulf of California was shown to have hemolytic activity in human and fish red blood cells (Dorantes-Aranda *et al.* 2009), suggesting that *M. polykrikoides* may negatively impact human health. A separate study showed that *M. polykrikoides* extracts produced ROS and resulted in oxidative stress and apoptosis in rat hepatocytes (Shahraki *et al.* 2013).

Figure 19: Margalefidinium polykrikoides cells under the microscope (left) collected from the bloom (rust tide) in CT, September 2018 (right)

The first documented bloom of *M. polykrikoides* in the southern New England region was the summer of 1981 when *M. polykrikoides* reached maximum densities exceeding 3.4 million cells/L in Rhode Island (Tomas and Smayda 2008). *M. polykrikoides* was first documented in New York waters in 2002, and dense blooms began to appear by 2004; New York now experiences near-annual blooms (Gobler *et al.* 2008). Gobler (2010) has reported cell densities exceeding 100,000 cells/mL, with bloom patches covering up to 1 km^2. There was a massive soft shell clam mortality event in 2005, and clams were shown to have hemorrhaged digestive tracts containing *M. polykrikoides* cells (Gobler *et al.* 2008). Bay scallop populations have yet to recover since the 1980-1990s, partially as a result of recurrent *M. polykrikoides* blooms (Gobler *et al.* 2008). A mass bay scallop mortality event occurred in Little Peconic Bay and Noyack Bay, Long Island in 2009 due to a *M. polykrikoides* bloom (Gobler 2010). Exposure to *M. polykrikoides* blooms was shown to cause inflammation and hemorrhage in the gills and guts, increased mortality, and decreased growth in bay scallops; cell death in the digestive glands and gills, and increased mortality in American oysters; and gill lamellae fusion in multiple fish species (Gobler *et al.* 2008). Mortalities were reported among fish held in pound nets in the Peconic Estuary and Shinnecock Bay, Long Island during *M. polykrikoides* blooms in 2008 and 2009 (Gobler 2010).

M. polykrikoides blooms are difficult to study because they can form "patches" on the sea surface (with patches and ambient water potentially containing cell concentrations that differ in up to two orders of magnitude), and the bloom samples tend to aggregate (clump), sink, and die rapidly once collected (Gobler 2010). Furthermore, *M. polykrikoides* utilizes diel vertical migration to maximize photosynthesis during the day and nutrient acquisition at night (Gobler et al. 2012). Therefore, rust tides also have a vertical component, with patches appearing on the surface during the day, breaking up late in the day, and sometimes not returning to the surface until the next morning (reviewed in Hattenrath-Lehmann and Gobler 2016).

While *M. polykrikoides* causes near-annual blooms and widespread environmental harm across Long Island, it typically forms localized blooms in CT. *M. polykrikoides* has sporadically been reported in Connecticut waters, and notably caused an extensive, visible bloom that stretched from Darien to Milford, CT in September 2018 (Fig. 19). *M. polykrikoides* could be negatively impacting shellfish and fish health and/or recruitment, but no studies have specifically focused on CT. In 2020, there were multiple

rust tides reported in CT, most notably Stamford Harbor, where the bloom persisted for over 1 week, and followed multiple sewage treatment plant discharges of partially-treated or untreated sewage (fig. 20). Due to the environmental and ecological challenges noted above, the results presented herein do not necessarily represent the highest actual *M. polykrikoides* concentrations in CT. DABA always focuses sampling near/around shellfish growing areas and does not have the staff capacity to consistently monitor blooms that do not have the potential to close shellfish beds (do not pose a public health threat); therefore, these results represent single points in time (fig. 20). Response to bloom reports is often conducted on land, which also limits DABA staff's ability to collect denser patches of *M. polykrikoides* blooms.

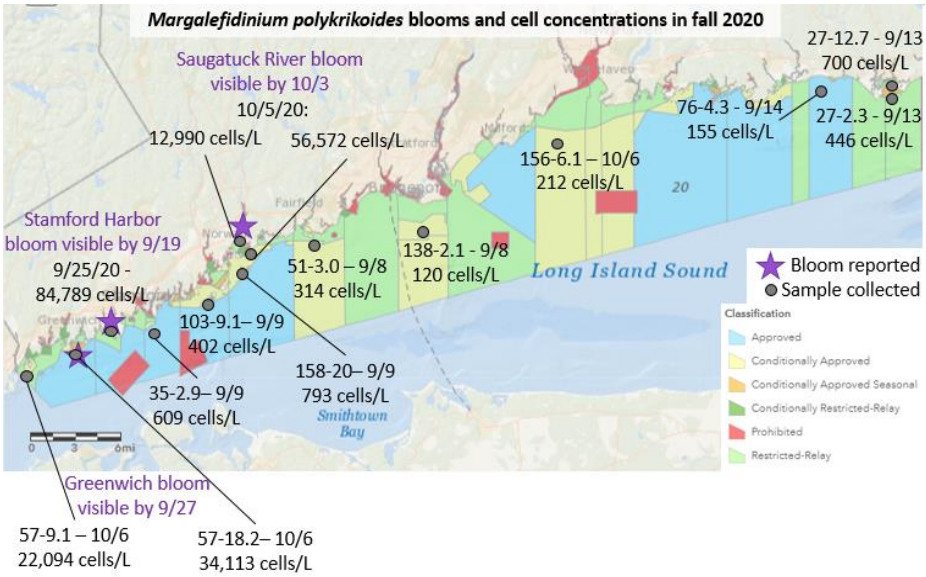

Figure 20: 2020 Margalefidinium polykrikoides prevalence in CT, including reported bloom locations and bloom concentrations. These do not necessarily represent the highest concentrations of M. polykrikoides for each location, as samples were taken opportunistically and should be interpreted as single points in time.

Akashiwo sanguinea

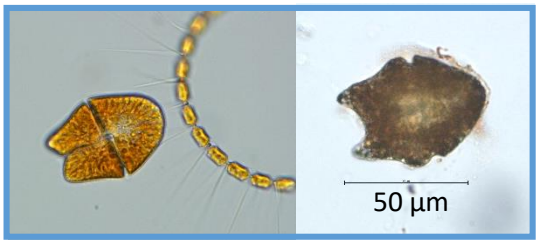

Figure 21: Live Akashiwo sanguinea cell with Chaetoceros in the background (left); A. sanguinea cell fixed with lugol's iodine

Akashiwo sanguinea is an unarmored dinoflagellate that forms blooms associated with animal kills (Cardwell et al. 1979; Harper and Guillen 1989; Shumway 1990). While the mechanism is not fully understood, recent data supports that *A. sanguinea* produces a surfactant (Jessup et al. 2009; Jones et al. 2017), but blooms do not cause a public health threat. An *A. sanguinea* bloom (1.5-15.5 x 10^5 cells/L) in Washington caused the largest HAB-associated seabird mortality ever recorded (Jones et al. 2017). The bloom occurred in the fall (September-October), and killed an estimated 10,500-12,500 birds, consisting of over 10 different species (Jones et al. 2017). The bloom coincided with the peak timeframe for seabird feather molting (Jones et al. 2017). A smaller, but still significant, seabird mortality event occurred in 2007 in California (Jessup et al. 2009), Oregon and Washington (Du et al. 2011; White et al.

2014). The birds during these blooms likely died from hypothermia, as *A. sanguinea* surfactants were hypothesized to disrupt their insulation capabilities (Jessup et al. 2009; Jones et al. 2017). As a result of loss of insulation, the birds died of poor nutritional conditions, hypothermia, anemia, and loss of fat and muscle tissue (Jessup et al. 2009). Severe *A. sanguinea* blooms on the U.S. west coast have been associated with stratification followed by upwelling, which carries nutrient-rich waters to the surface; a prior diatom fall bloom; conditions that transport the bloom nearshore; and wave action that lyses *A. sanguinea* cells and creates a surface layer of surfactants (Du et al. 2011; Jones et al. 2017). Additionally, there is evidence that aerosolized compounds from the bloom caused oxidative stress, as half of the birds showed signs of lung hemorrhage and/or fibrin deposition in air sacs of dead birds (Jessup et al. 2009). Badylak et al. (2014) also documented the production and extrusion of mucus from cells in a Florida bloom. Xu et al. (2017) reported that *A. sanguinea* strains have hemolytic activity, with higher toxicity noted for Chinese strains than the Chesapeake Bay, USA strain. *A. sanguinea* killed 20-100% of shrimp, shellfish, and fish during a 72-hour laboratory exposure experiment (Xu et al. 2017). The highest toxicity was noted at 20℃ and 35 ppt, the ideal growth conditions for *A. sanguinea* (Xu et al. 2017). Increased nutrient supply correlated with increased toxicity, suggesting that eutrophication could result in more severe *A. sanguinea* blooms (Xu et al. 2017).

In Connecticut in 2020, *A. sanguinea* was documented from June-November (13.9-25.8℃, 23.2-31.7ppt) in 62 samples (27.4%). Overall *A. sanguinea* concentrations remained low in areas monitored by DABA. 11 samples had *A. sanguinea* concentrations ≥1,000 cells/L, 7 of which occurred in August (as well as, 1 in June, 1 in September, and 2 in October) (fig. 22). The highest concentrations occurred in early August. The highest three concentrations documented were 16,034 cells/L in Greenwich (57-9.1, 8/2/20, 23℃), which occurred during a dinoflagellate (*Heterocapsa* spp., *Prorocentrum triestinum*) and *Chattonella* sp. "bloom;" 15,625 cells/L in Stonington (137-10, 8/3/20, 25.6℃); and 9,251 cells/L in Branford (14-5.2, 8/13/20, 25.2℃) (fig. 22). While *A. sanguinea* can form "red tides," no blooms or associated animal kills were documented in 2020, and concentrations remained below the "harmful" levels outlined above.

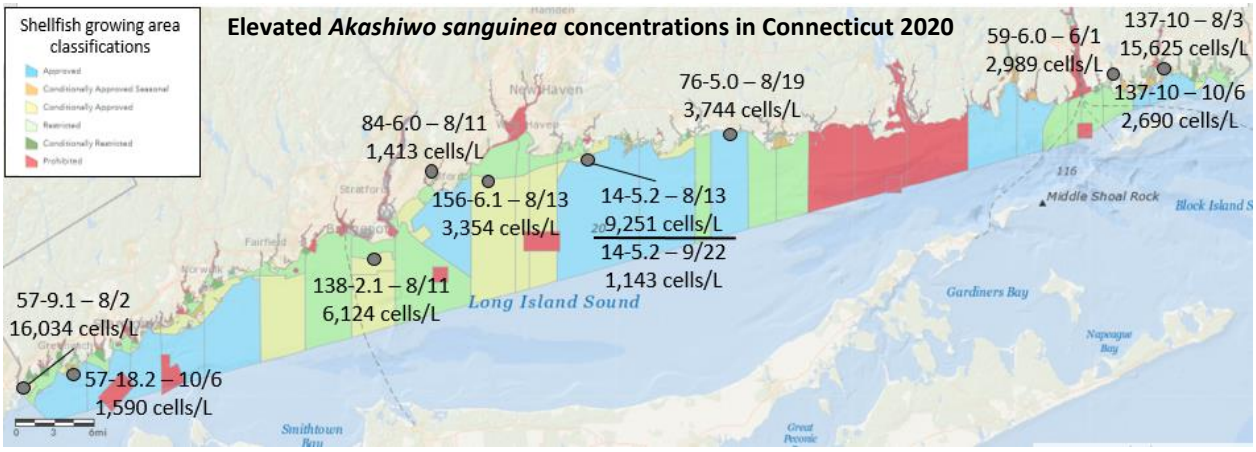

Figure 22: Distribution of Akashiwo sanguinea (only samples with concentrations >1,000 cells/L) in Connecticut in 2020. 7 of the 11 samples containing elevated A. sanguinea concentrations occurred in August.

Amphidinium

Amphidinium spp. are benthic, unarmored dinoflagellates with uniquely small crescent to triangular-shaped epicones (fig. 23). As with *Prorocentrum lima*, *Amphidinium* spp. can enter the water column after sediment disruption (e.g. storm turbulence) (Fukuyo 1981). *Amphidinium* spp. are known to produce toxins largely referred to as amphidinols, which are a group of many different types of secondary metabolites that typically have antifungal and hemolytic properties (e.g. Wellkamp et al. 2020). *Amphidinium* spp. have been associated with fish kills, as have karlotoxins (produced by *Karlodinium* spp.), which are structurally similar to amphidinols (e.g. Van Wagoner et al. 2010).

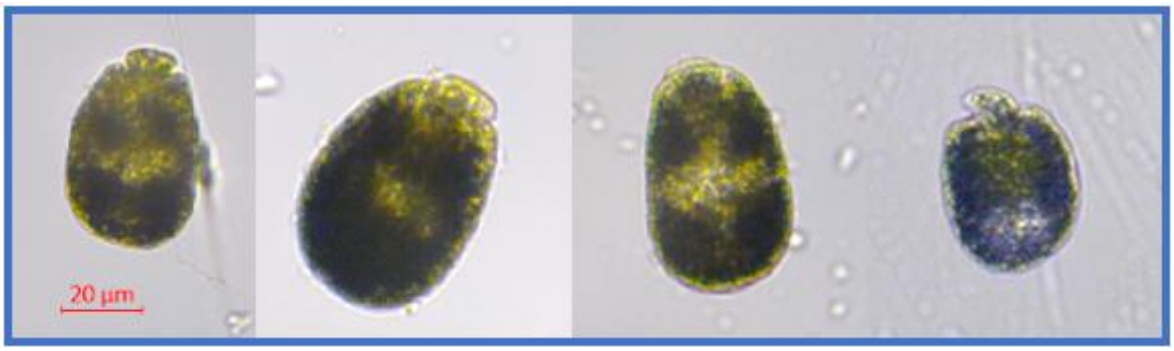

Figure 23: Amphidinium *spp. from Long Island Sound (left to right: Fairfield (51-3.0), Fairfield (51-3.0), Waterford (152-12.1), Groton (59-25.0)). Scale bar applies to all images.*

Based on available data, *A. operculatum* (Woods Hole, MA), *A. steinii* (New Jersey and Maryland), *A. massartii* (Rhode Island), *A. carterae* (cosmopolitan species) (Murray et al. 2004) could all be transient or established species in LIS. *A. operculatum, A. massartii, A. carterae* are all closely related, and have been grouped into the Operculatum Clade (Karafas et al. 2017).

Due to morphological variabilities within and among species (Karafas et al. 2017; Murray et al. 2004), and the absence of critical genetic analysis for these samples, species-level identification was not attempted. *Amphidinium* spp. were identified in 7 samples in Fairfield (avg. depth 7 ft), Groton (depth ~6 ft), and Waterford (avg. depth 37 ft) (fig. 22). Detection was sporadic from March-October (1 in March, 1 in April, 2 in May, 2 in June, 1 in October), and was associated with rainfall (0.1-1.9" 1-5 days prior to sample collection). For example, the 2020 Fairfield sample was collected after 1.02" (cumulative) rainfall 3-5 days prior, and the only Fairfield sample from 2019 with *Amphidinium* occurred on 6/25/19 while it was raining (0.28" same day). The highest concentration was 64 cells/L in Fairfield (51-3.0) on 6/10 (bottom temperature 14.5℃); however, planktonic samples underestimate benthic species concentrations.

Multiple *Amphidinium* spp., particularly *A. carterae*, have been associated with toxicity in bioassays (Baig et al. 2006; Ismael et al. 1999; Jeong et al. 2001; Lee et al. 2003; Moreira-Gonzalez et al. 2019; Nakajima et al. 1981; Nayak et al. 1997; Pagliara and Caroppo 2012; Rhodes et al. 2010; Zimmermann 2006). While typically benthic, an *A. carterae* bloom (~1.8 x 10^5 cells/mL) occurred in a shallow Australian lagoon, and was associated with the death of over 300 fish (Murray et al. 2015). *Anguilla reinhardtii* (longfin eel) carcasses had damaged epithelial and gill epithelial cells (Murray et al. 2015). Subsequent

assays of a fish gill cell line and Luteophanol A, a compound isolated from *Amphidinium*, showed a loss of 27-35% cell viability at low concentrations (0.1-10 µg/mL) and 87% cell viability at high concentrations (100 µg/mL) in 6 hours (Murray et al. 2015). Fish death was associated with the presence of Luteophanol A-like compounds and/or low dissolved oxygen levels (Murray et al. 2015). The bloom was preceded by high nutrient concentrations (Murray et al. 2015). *A. carterae* can acclimate to and use different nitrogen sources (Molina-Miras et al. 2020), suggesting that eutrophication could favor blooms. A *Amphidinium* sp. tolerated wide temperature (13.6-32.9℃), salinity (19-50 ppt), pH (6.45-9.5), and nutrient (NO_3 0.1-10mM, NH_4 0.9-2.8mM, P 50->500µM) conditions (Lee et al. 2003).

Chattonella

Chattonella spp. are a type of flagellate classified as Raphidophytes. *Chattonella* spp. are harmful because they form high biomass and toxic blooms that have been associated with fish kills around the world. *Chattonella* spp. produce reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and hydroxyl radicals (Oda et al. 1997). *C. marina* produces superoxide at levels up to 100x higher than most other algae (Marshall et al. 2002a). Additionally, multiple potentially harmful fatty acids and sterols have been isolated from *Chattonella* spp., including eicosapentaenoic acid (EPA) (Marshall et al. 2002b). Ichthyotoxicity is believed to be related to a combination of ROS and fatty acids, some of which have hemolytic properties (Aquino-Cruz et al. 2020; Marshall et al. 2003). Brevetoxin-like compounds have also been isolated from *Chattonella* (Band-Schmidt et al. 2012), but damselfish mortality was associated with EPA and superoxide rather than brevetoxin in a bioassay experiment (Marshall et al. 2003). In a historic study, fish gill epithelia displayed hypertrophy and edema proportional to the length of duration and cell concentrations of *C. marina* (Endo et al. 1985). *Chattonella* spp. blooms have caused widespread aquaculture fish kills and economic losses, including US$0.5 billion in 1972 in Japan (reviewed in Berdalet et al. 2016). *Chattonella* blooms have also impacted China, Australia, India, and Florida (reviewed in Berdalet et al. 2016).

Chattonella spp. inhabit tropical to temperate estuaries and oceans, salinity ranges of 20-30ppt, and optimal temperatures vary from 20-30℃ depending on the species (Imai and Yamaguchi 2012). *Chattonella* spp. form hardy cysts; emerge from cysts in the spring/early summer; and are present at low cell densities in the water column but can become dominant under ideal conditions, particularly in eutrophic areas (reviewed in Imai and Yamaguchi 2012).

Chattonella are often distorted and can lyse in preserved samples, and analyses have revealed that there is high genetic similarity between *C. antiqua, C. marina,* and *C. ovata* (*Chattonella marina* complex) (Demura et al. 2009). Therefore, *Chattonella* are simply grouped as the *Chattonella marina* complex by the DABA. However, the elongated cell shape and presence of long posterior tails suggests that observed CT strains are *C. marina* var. *antiqua* (fig. 24) (see Demura et al. 2009; Imai and Yamaguchi 2012). The highest concentration of *Chattonella* sp. (169,873 cells/L) coincided with elevated concentrations of dinoflagellates (*P. triestinum* (131,738 cells/L), *Heterocapsa* (not quantified), *Akashiwo sanguinea* (16,034 cells/L), *Gonyaulax spinifera* (5,634 cells/L, highest concentration in 2020)) at 57-9.1 (Greenwich) on 8/2/2020 (23℃). The next highest concentration was 48,785 cells/L *Chattonella* sp. at

57-9.1 (Greenwich) on 10/27 (estimated ~15-16℃). *Chattonella* sp. was also documented in 137-10 (Stonington) at <500 cells/L and 27-12.7 (Clinton) at a maximum of 4,849 cells/L on 10/20/20.

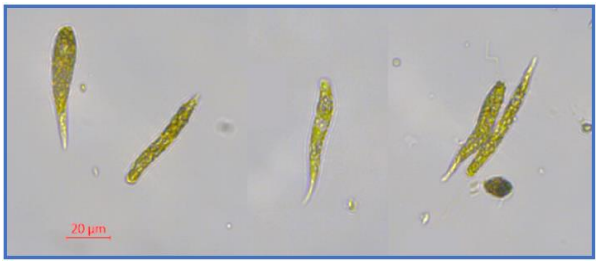

Figure 24: Chattonella sp. from Greenwich (57-9.1, 8/2/20).

Conclusions

In 2020, *Alexandrium catenella* caused the earliest paralytic shellfish poisoning (PSP) closure ever in CT, suggesting the need to expand the PSP monitoring season. However, PSP closures have been isolated to two small coves in Groton, and have been sporadic and rare. The highest *Alexandrium* concentrations in CT have been associated with species that do not produce saxitoxin. *Pseudo-nitzschia* species, predominantly *P. plurisecta* and *P. delicatissima*, were blooming in late May-early June, reaching ~135,000 cells/L. While domoic acid was not detected, *P. australis* (putatively) and *P. plurisecta* were both detected in CT in June, and are associated with domoic acid accumulation in Maine shellfish. In July and September, *P. pungens* became the dominant species, but did not exceed 20,000 cells/L in any towns. Given the low cell concentrations of toxigenic *Dinophysis* spp. and *Prorocentrum* spp. documented by DABA and the absence commercial mussel operations, these species are not currently expected to close CT shellfish beds. Multiple other HAB species were documented in 2020, but were only detected at low concentrations and not all produce toxins. Notably, when HAB events occurred in 2020, no blooms were monospecific. The closest to a monospecific bloom was *Margalefidinium polykrikoides* at 84,789 cells/L in Stamford Harbor, which occurred following multiple sewage treatment plant issues that resulted in the release of untreated or partially-treated sewage. The cyanobacteria toxin, microcystin, was detected in CT oysters for the first time, indicating the need to keep a close eye on cyanobacteria blooms that have a potential to impact Long Island Sound in the future. Given the close proximity of documented HCBs around Long Island, these common freshwater toxic blooms are expected to continue to impact the area in the future. Given that neighboring states have documented shellfish growing area closures (ASP in Rhode Island; DSP & PSP in New York); the documented expansion of HABs around Long Island; and the presence of multiple environmentally disruptive HABs (*Margalefidinium polykrikoides, Aureococcus anophagefferens* (brown tide), *Prorocentrum minimum* (mahogany tide)) around Long Island, CT continues to advance the HAB and biotoxin monitoring programs. In addition to the human health threats, HABs have a major impact on water quality and environmental and ecosystem health. Coastal ecosystems and shellfish are already experiencing, and will continue to increasingly experience, co-stressors, like HABs, climate change, ocean acidification, eutrophication, and hypoxia (Griffith and Gobler 2020), highlighting the importance of this work.

Supplemental Table

Supplemental Table: Summary of select *Pseudo-nitzschia* data and environmental conditions							
Date	**Town (station)**	***Pseudo-nitzschia* (cells/L)/type** (small <3µm width; large >3µm)	**Net change in concentration**	**Temperature (surface/ bottom) (℃)**	**Salinity (surface/ bottom) (ppt)**	**Tide**	**Domoic acid (if applicable)**
5/26/20	Groton (59-25.0)	26,622 cells/L Mostly small, some large	Increase (from 5/12/20, 223 cells/L, data not shown)	13	30.5	Flood	Conc. phyto. and wild blue mussels negative by scotia rapid test (SRT)
	Groton (59-17.0)	15,427 cells/L	Increase (from 5/12/20, 150 cells/L, data not shown)	15.8	29	Ebb	Negative SRT (blue mussels)
	Groton (59-12.5)	24,417 cells/L	Increase (from 5/12/20, 33 cells/L, data not shown)	15.6	29.3	Ebb	
6/1/20	Groton (59-6.0)	11,957 cells/L		16.1		Ebb	
	Stonington (137-10.0)	5,116 cells/L Small and large	Increase (from 5/14/20, data not shown)			Ebb	
6/2/20	Stonington (137-15.2)	70,603 cells/L Mix of small and large	Increase (from 5/5/20, 2,227 cells/L, data not shown)	12.1/12.1	30.8/30.9	Ebb	
	Groton (59-L30)	29,773 cells/L Mix of small and large	Increase (from 5/5/20, 394 cells/L, data not shown)	12.8/12.9	30/30.2	Ebb	Oysters negative by SRT
	Waterford (152-12.1)	134,203 cells/L Mix of small and large	Increase (from 5/5/20, 1,399 cells/L, data not shown)	13.2/12.7	29.5/29.6	Ebb	
	East Lyme (45-10.1)	20,987 cells/L	Increase (from 5/5/20, 220	13.6/13.6	29.3/29.3	Ebb	

		Mostly small, some large	cells/L, data not shown)				
	Guilford (60-7.0)	7,540 cells/L Small	Increase (from 5/5/20, <25 cells/L, data not shown)	13.5/13.1	27.3/27.4	Ebb	
	Madison (76-4.3)	43,364 cells/L Small	Increase (from 5/5/20, 212 cells/L, data not shown)	13.4/13	27.6/27.8	Ebb	
6/8/20	Stonington (137-15.2)	6,733 cells/L small	Decrease (from 6/2/20)	12.5/12.5	31.3/31.3	Flood	
	Groton (59-L30)	6,858 cells/L mix of small and large		13.9/13.9	30.6/30.4	Flood	
	Waterford (152-12.1)	9,743 cells/L small		13.5/13.4	29.7/29.7	Flood	
	East Lyme (45-10.1)	15,630 cells/L mix of small and large		14.9/13.9	29.5/29.6	Flood	Oysters negative by SRT
	Guilford (60-7.0)	1,878 cells/L small		15.3/14.9	27.3/27.3	Flood	
	Madison (76-4.3)	3,706 cells/L small		15.2/14.9	26.2/27.6	Flood	
6/15/20	Stonington (137-15.2)	62 cells/L small	Decrease (from 6/8/20)	15.3/14.3	30.5/31.1	Ebb	
	Stonington (137-10.0)	105 cells/L small				Ebb	
	Groton (59-L30)	464 cells/L small		15.6/15.8	30.1/29.7	Ebb	
	Waterford (152-12.1)	6,529 cells/L Mostly small, some large		18.3/15	29.6/29.7	Ebb	
	East Lyme (45-10.1)	5,963 cells/L Mostly small, some large		18.8/15.9	28.9/29.3	Ebb	
	Guilford (60-7.0)	573 cells/L small		17.1/16	27.8/28	Ebb	
	Madison (76-4.3)	6,466 cells/L small	Increase (from 6/8/20)	17.9/16.9	28.1/28.1	Ebb	

7/6/20	Stonington (137-10.0)	<266 cells/L	Decrease (from 6/15/20)	22.6		Ebb	
7/15/20	Stonington (137-16.2)	12,041 cells/L Large		21.6/19.6	31/31.2	Ebb	
	Stonington (137-15.2)	3,498 cells/L Large	Increase (from 6/15/20)	20.2/19.5	30.9/31.1	Ebb	
	Groton (59-L30)	5,212 cells/L Large		20.6/20.6	30.5/30.4	Ebb	Oysters negative by SRT
	Waterford (152-12.1)	3,301 cells/L Large	Decrease (from 6/15/20)	20.9/20	29.9/30	Ebb	
	East Lyme (45-10.1)	229 cells/L Large		22.4/21.4	29.7/29.9	Ebb	
	Guilford (60-7.0)	<19 cells/L		21.6/21.3	28.2/28.2	Ebb	
	Madison (76-4.3)	<34 cells/L		21.5/21.4	28.4/28.5	Ebb	
7/22/20	Stonington (137-10.0)	936 cells/L Large	Increase (from 7/6/20)	24.4		Ebb	
8/3/20	Stonington (137-10.0)	919 cells/L Large	Decrease (from 7/22/20)	25.6		Ebb	
8/17/20	Stonington (137-15.2)	<18 cells/L	Decrease (from 7/15/20)	21.4/21	31.3/31.4	Ebb	
	Groton (59-L30)	108 cells/L Small		21.3/21.4	31/31	Ebb	
	Waterford (152-12.1)	77 cells/L Large		21.3/20.7	30.6/30.6	Ebb	
	East Lyme (45-10.1)	<17 cells/L		21.7/21.3	30.5/30.6	Ebb	
	Guilford (60-7.0)	<8 cells/L		22.8/22.7	28.7/28.7	Ebb	
	Madison (76-4.3)	244 cells/L Large	Increase (from 7/15/20)	23/22.7	29/29.1	Ebb	
8/19/20	Madison (76-5.0)	<234 cells/L				Ebb	
9/13/20	Clinton (27-2.3)	7,584 cells/L Large	Increase (from 8/24/20, <304 cells/L, data not shown)				Oysters negative by HPLC-UV (<0.8 ppm)
9/14/20	Stonington (137-15.2)	7,244 cells/L	Increase (from 8/17/20)	20.5/19.8	31.4/31.8	Ebb	

		Mix of large and small					
	Stonington (137-WB#1)						Oysters negative by HPLC-UV (<0.8 ppm)
	Groton (59-L30)	10,091 cells/L Mix of large and small	Increase (from 8/17/20)	20.9/20.9	31.2/31.2	Ebb	Oysters negative by HPLC-UV (<0.8 ppm)
	Waterford (152-12.1)	14,551 cells/L Mix of large and small		21.2/21.1	30.8/30.8	Ebb	
	East Lyme (045-10.1)	4,018 cells/L Mix of large and small		21.9/21.9	30.7/30.7	Ebb	Oysters negative by HPLC-UV (<0.8 ppm)
	Guilford (060-7.0)	2,999 cells/L Large		22.6/22.6	29/29	Ebb	
	Madison (076-4.3)	3,451 cells/L Mix of large and small		22.3/22.3	29.2/29.3	Ebb	
9/22/20	West Haven (156-6.1)	8,751 cells/L Large, some small	Increase (from 8/13/20, <24 cells/L, data not shown)	19.1/19.1	28.6/28.5	Ebb	
	Branford (14-5.2)	16,800 cells/L Large	Increase (from 8/13/20, <23 cells/L, data not shown)	18.6/18.6	28.6/28.7	Ebb	Oysters negative by HPLC-UV (<0.8 ppm)
9/24/20	Stonington (137-10.0)	5,521 cells/L Large	Increase (from 8/3/20)	15.9		Ebb	
10/6/20	Stonington (137-10.0)	<336 cells/L	Decrease (9/24/20)	17.5		Ebb	
	Madison (76-5.0)	607 cells/L Large	Increase (8/20/20)			Flood	
	West Haven (156-6.1)	4,229 cells/L Large	Decrease (from 9/22/20)	19.2/19.2	28.5/28.5	Ebb	
	Branford (14-5.2)	4,624 cells/L Large, a few small		19.2/19.2	28.4/28.4	Ebb	

	Greenwich (57-18.2)	18,357 cells/L Large	Increase (from 8/2/20, <284 cells/L data not shown)			Ebb	
	Greenwich (57-9.1)	10,332 cells/L Large	Increase (from 8/2/20, <433 cells/L, data not shown)			Ebb	
10/27/20	Greenwich (57-18.2)	478 cells/L Small and large	Decrease (from 10/6/20)			Ebb	
	Greenwich (57-9.1)	656 cells/L Small and large				Ebb	
11/24/20	West Haven (156-6.1)	132 cells/L Large	Decrease (from 10/6/20)	11/11.1	28.4/28.3	Ebb	
	Branford (14-5.2)	405 cells/L Large		9.8/9.9	28.1/28.1	Ebb	

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