



Freshwater Cyanotoxins: A Threat to Marine Seafood



EPA Webinar, March 22nd, 2022

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Background:

Cyanotoxins, have been found **persistently** at the land-sea interface

Harmful concentrations to humans have been found in invertebrates, fish, mammals, and humans

May disproportionately impact subsistence harvesters

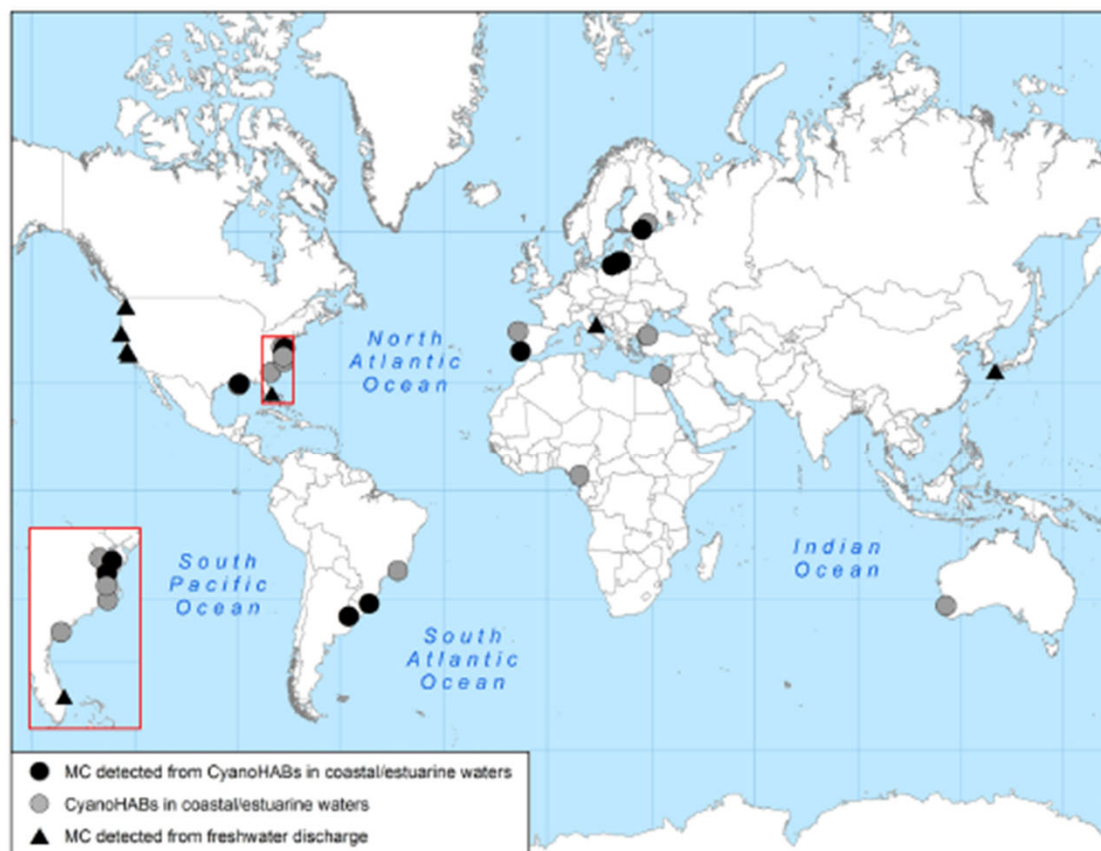


Fig. 3. Location of CyanoHABs and MCs in the world's coastal waters. For references and details, see the text.

How do cyanotoxins enter the estuarine and marine environment?

Cyanobacteria grow in freshwater, but often can be flushed into estuarine and coastal environments

Cyanobacteria and their toxins can enter estuarine and marine waters through high tides, storms, or river input

Brackish and estuarine waters have supported cyanobacteria for centuries, but intrusion into coastal waters is becoming frequent



Cyanotoxins in estuarine and marine environments



Article

First Evidence of the
Article



Microcystin Contamination in Sea Mussel Environmental Research Southern Adriatic journal homepage: www.elsevier.com/locate/envres Bacterial Blooms in

Micro Review

Water A review of microcystin detections in Estuarine and Marine waters:
Environmental implications and human health risk

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The tide turns: Episodic and localized cross-contamination of a California
coastline with cyanotoxins

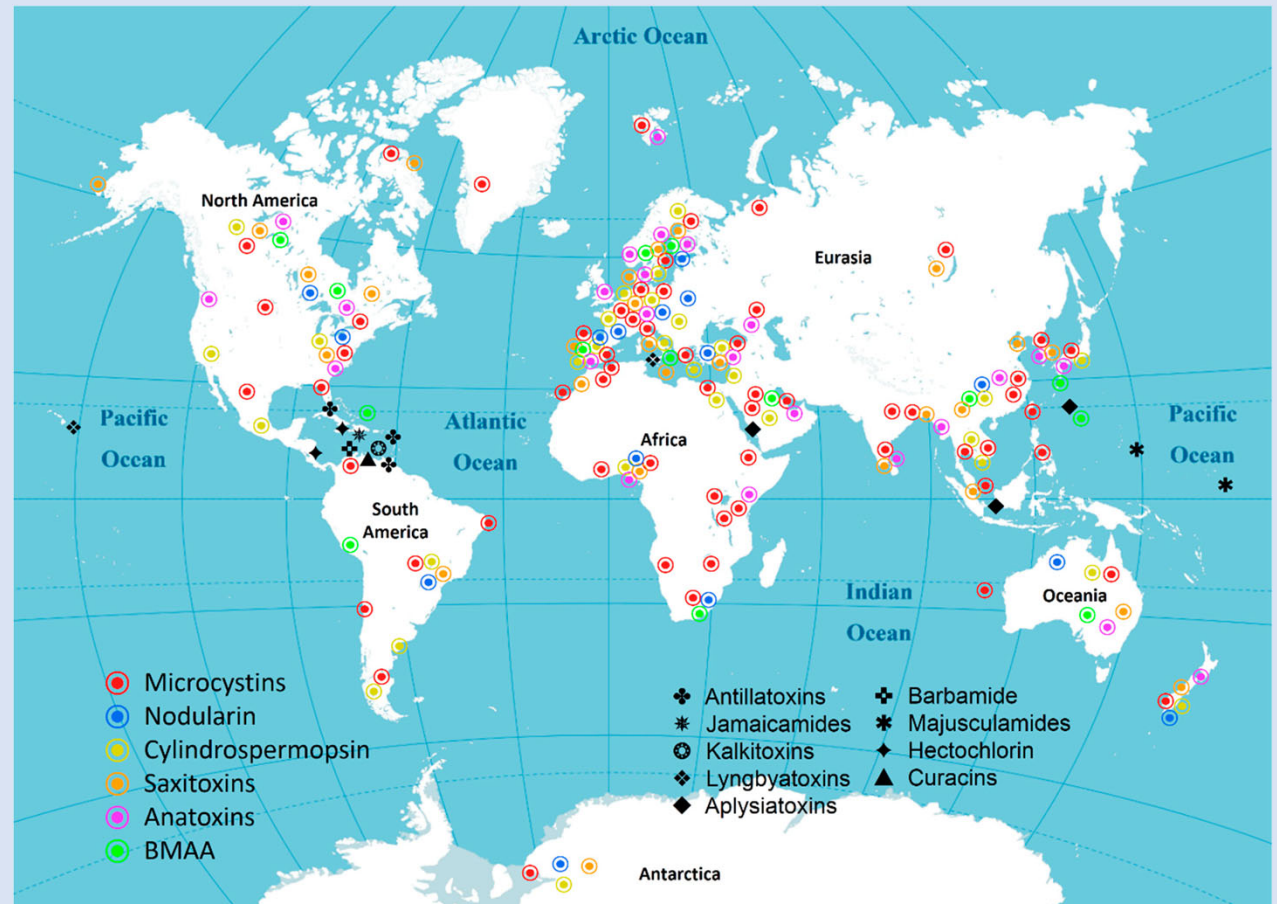
Microcystin in Seafood

Avery O. Tatters^{a,1,*}, Jayme Smith^b, Raphael M. Kudela^c, Kendra Hayashi^c,
Meredith DA. Howard^d, Ariel R. Donovan^e, Keith A. Loftin^e, David A. Caron^f

Microcystin⁴, Andrew Humpage⁵,

Who are the culprits?

Microcystins
Anatoxin-a
Nodularin Toxins
Cylindrospermopsin
Saxitoxins
Saxitoxins
BMAA



Du et al. 2019; *Toxins*

What can happen (from a human health perspective)?

Table 1. Common genera of planktonic cyanobacteria that contain toxin and taste-and-odor producing strains.

[All data included in this table are based on documented production in laboratory cultures; data based on circumstantial evidence, such as co-occurrence of genera and toxin or taste-and-odor compounds in environmental samples, were not included in this table. LYN, lyngbyatoxin-a; APL, aplysiatoxins; LPS, lipopolysaccharides; CYL, cylindrospermopsins; MC, microcystins; NOD, nodularins; ATX, anatoxins; BMAA, β -N-methylamino-L-alanine; NEO, neosaxitoxins; SAX, saxitoxins; GEOS, geosmin; MIB, 2-methylisoborneol]

Cyanobacterial Genera	Dermatoxins			Hepatotoxins			Neurotoxins				Tastes and odors	
	LYN	APL	LPS	CYL	MC	NOD	ATX	BMAA	NEO	SAX	GEOS	MIB
Colonial/filamentous												
<i>Anabaena</i>			X	X	X		X	X	X	X	X	
<i>Anabaenopsis</i>			X		X							
<i>Aphanizomenon</i>			X	X	X		X	X	X	X	X	
<i>Aphanocapsa</i>			X		X							
<i>Cylindrospermopsis</i>			X	X				X		X		
<i>Microcystis</i>			X		X			X				
<i>Nodularia</i>			X			X		X				
<i>Oscillatoria (Planktothrix)</i>	X	X	X		X		X	X		X	X	X
<i>Pseudanabaena</i>			X		X						X	X
<i>Raphidiopsis</i>			X	X			X					

Graham et al. 2008. *USGS SIR 2008-5038*. <http://pubs.usgs.gov/sir/2008/5038/pdf/SIR2008-5038.pdf>

Table 2. Concentrations (mg kg⁻¹ FW)¹ of free, bound or total BMAA and isomers (DAB, AEG) in edible parts of aquatic organisms reported in A graded studies. When concentrations were reported in mg kg⁻¹ DW by the authors, an estimate in mg kg⁻¹ FW was calculated. The concentration ranges are indicated for all the samples analyzed.

Type of Sample	Species	Origins	Concentrations (mg kg ⁻¹ FW) ⁽¹⁾			LOD	LOQ	Reference
			(Number of Positive Samples/Number of Samples)					
			BMAA	AEG	DAB			
Bivalves	Mussels <i>Mytilus galloprovincialis</i>	Thau Lagoon, Mediterranean sea, France	fBMAA ND-0.2 (16/34) tsBMAA max 1.65, mean 0.68 (34/34)	fAEG ND-0.05 (5/34) tsAEG max 0.2 (31/34)	fDAB ND-1.05 (34/34) tsDAB max 1.8, mean 1.22 (34/34)	-	0.15 DW	[24]
	Mussels <i>Mytilus galloprovincialis</i> and <i>Mytilus edulis</i> , Oysters <i>Crassostrea gigas</i>	Channel, Atlantic, Mediterranean Sea, France	tsBMAA 0.07-1.13 (74/74) tsBMAA 0.03-0.41(23/23)	ND ND	Mussels and oysters: tsDAB 0.2-4.84 (97/97)	-	BMAA: 0.45 DW DAB: 0.15 DW	[42]
	Mussels <i>Mytilus</i> sp Oysters <i>Ostrea edulis</i> , <i>Crassostrea gigas</i>	North Atlantic, Sweden west coast, Greece, France	fBMAA 0.08-0.9 (6/6) fBMAA 0.1-0.66 (4/4)			<0.01 ⁽²⁾	<0.01 ⁽²⁾	[50]
	Oysters <i>Crassostrea virginica</i>	Louisiana Mississippi	fBMAA 1.5-8 (12/12) fBMAA 1.2-1.7 (3/3)			0.5 ⁽³⁾	1.7 ⁽³⁾	[52]
	Mussels <i>Mytilus galloprovincialis</i>	Thau Lagoon, Mediterranean Sea, France	fBMAA < 0.34 (4/11) tsBMAA 0.64-2.45 (11/11)	fAEG < 0.08 (3/11) tsAEG 0.1-0.2 (11/11)	fDAB 0.08-1.2 (11/11) tsDAB 0.6-1.6 (11/11)	-	0.15 DW	[23]
	Oysters <i>Crassostrea gigas</i>		fBMAA < 0.08 (1/8) tsBMAA 0.5-1.8 (8/8)	fAEG ND (0/8) tsAEG 0.1-0.3 (8/8)	fDAB 0.03-0.6 (8/8) tsDAB 0.6-1.5 (8/8)	-	0.15 DW	[23]
	Mussels	Western coast of Sweden	fBMAA 0.27-1.6 (4/4)				0.15	[53]
	Mussels <i>Mytilus edulis</i> <i>Mytilus edulis platensis</i> <i>Perna canaliculus</i> Scallops <i>Placopecten magellanicus</i>	Scandinavia South America Australia US	fBMAA ND (0/6) fBMAA 0.28-0.59 (6/6) fBMAA ND-0.38 (5/12) fBMAA 1.69-7.08 (12/12) fBMAA ND-0.38 (1/3) fBMAA 0.55-1.14 (3/3) fBMAA 0.18-0.46 (3/3) fBMAA 1.12-1.46 (3/3)			0.10 ⁽⁴⁾	0.15 ⁽⁴⁾	[54]
	Shrimps <i>Caridea</i> sp Crayfish <i>Astacus leptodactylus</i>	North Atlantic, Sweden Turkey, Sweden	fBMAA 0.11-0.46 (6/6) fBMAA ND (0/6)			<0.01 ⁽²⁾	<0.01 ⁽²⁾	[50]
	Crustaceans	Blue crabs <i>Callinectes sapidus</i>	Florida	fBMAA 1.08-3.02 (5/5)			0.5 ⁽³⁾	1.7 ⁽³⁾
	Crabs <i>Cancer pagarus</i> <i>Portunus haasi</i> Crayfish <i>Procambrus clarki</i> Shrimps <i>Pandalus borealis</i>	Ireland, North Atlantic Vietnam China Greenland, North Atlantic	fBMAA detected, NQ (1/1) fBMAA ND (0/1) fBMAA ND (0/1) fBMAA ND (0/3)			0.10 ⁽³⁾	0.15 ⁽³⁾	[54]
Type of Sample	Species	Origins	Concentrations (mg kg ⁻¹ FW) ⁽¹⁾			LOD	LOQ	Reference
			(Number of Positive Samples/Number of Samples)					
			BMAA	AEG	DAB			
Fish	Plaice <i>Pleuronectes platessa</i> , Herring <i>Clupea harengus</i> Char <i>Saltelinus alpinus</i> Salmon <i>Salmo salar</i> Cod <i>Gadus morhua</i> Perch <i>Perca fluviatilis</i>	North Atlantic Baltic Sea Baltic Sea Sweden Norway Norway North Atlantic Sweden	fBMAA 0.01-0.02 (3/3) fBMAA ND-0.02 (1/3) fBMAA ND-0.01 (1/3) fBMAA ND (0/4) fBMAA ND (0/4) fBMAA ND (0/4)			<0.01 ⁽²⁾	<0.01 ⁽²⁾	[50]
	Atlantic salmon <i>Salmo salar</i> Sea bass <i>Dicentrarchus labrax</i> Sea bream <i>Sparus aurata</i> Whitefish <i>Coregonus</i> sp, Pike perch <i>Sander luciperca</i> , Sea trout <i>Salmo trutta</i>	Norway Italy Greece Sweden Sweden Baltic Sea Sweden Bothnian Sea	fBMAA ND (0/1) fBMAA ND (0/1) fBMAA ND (0/1) fBMAA ND (0/1) fBMAA ND (0/1) fBMAA ND (0/2)			0.10 ⁽⁴⁾	0.15 ⁽⁴⁾	[54]
	Shark cartilage powder, variety of shark species not identified	Commercial food supplements, from 7 manufacturers	(In mg kg ⁻¹ DW) fBMAA ⁽⁵⁾ 74.8-352.2 (15/16)	(In mg kg ⁻¹ DW) fAEG ⁽⁵⁾ 1298.4-1765.1 (16/16)	(In mg kg ⁻¹ DW) etDAB ⁽⁵⁾ 69.2-1483.4 (16/16)	(in µg L ⁻¹) BMAA 1.1 AEG 1.2 DAB 0.8		[55]

⁽¹⁾ Except for Mondo et al. [55] whose results are reported in mg kg⁻¹ DW. ⁽²⁾ LODs and LOQs determined for a crayfish matrix. ⁽³⁾ LODs and LOQs determined for a sea hare matrix. ⁽⁴⁾ LODs and LOQs determined for a mussel matrix. ⁽⁵⁾ Two methods were used in this study but only samples analyzed by UPLC-MS/MS are considered here. Sb = soluble bound; pb = precipitated bound; f = free; ts = total soluble; t = total; NA = data not available; NQ = not quantified; ND = not detected; DW = dry weight; FW = wet weight.

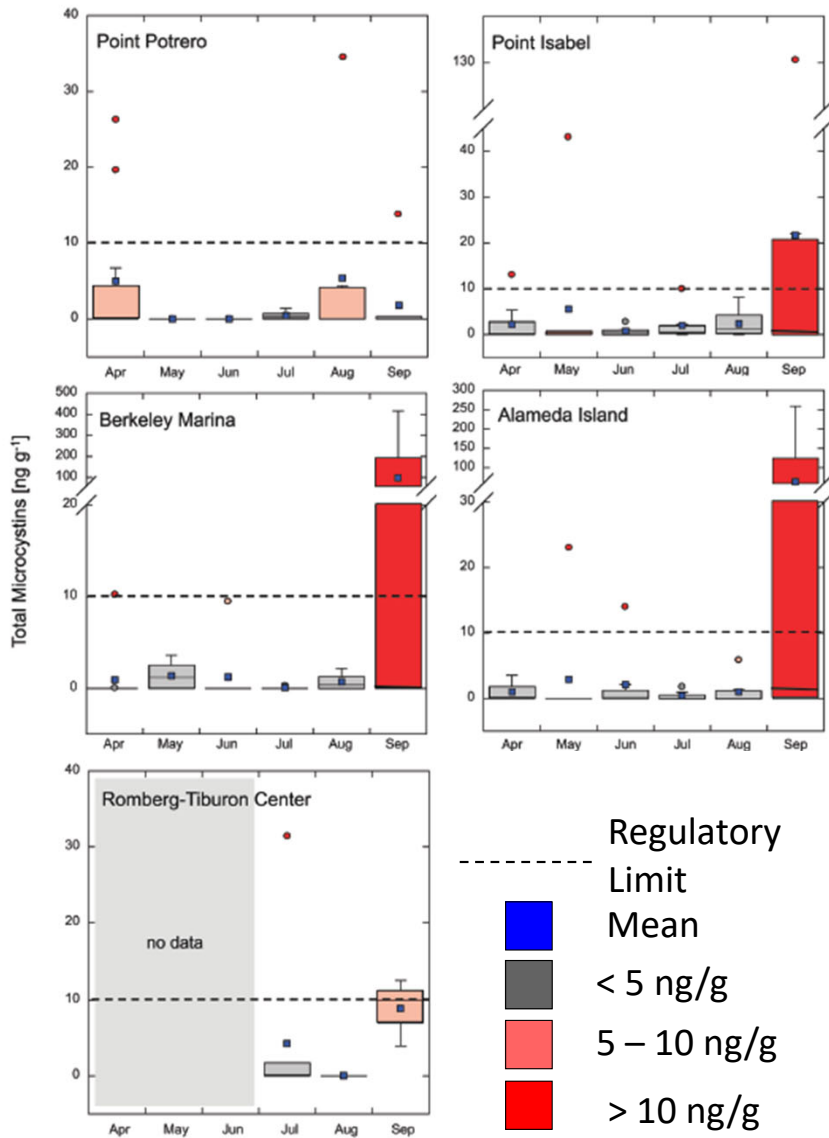
Bioaccumulation in Seafood

There are multiple studies and literature reviews documenting freshwater cyanotoxins found in seafood

Cyanotoxins have been found in bivalves, fish, benthic invertebrates, and mammals at **lethal concentrations**

Often these toxins in seafood are also **co-occurring with multiple cyanotoxins**, or both marine and freshwater toxins

Microcystins in marine mussels, San Francisco Bay, CA

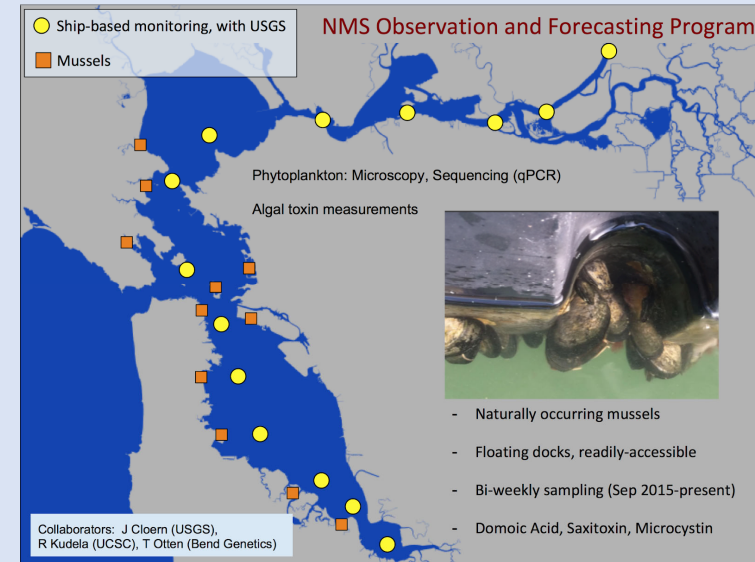


Gibble et al. 2016; *Harmful Algae*

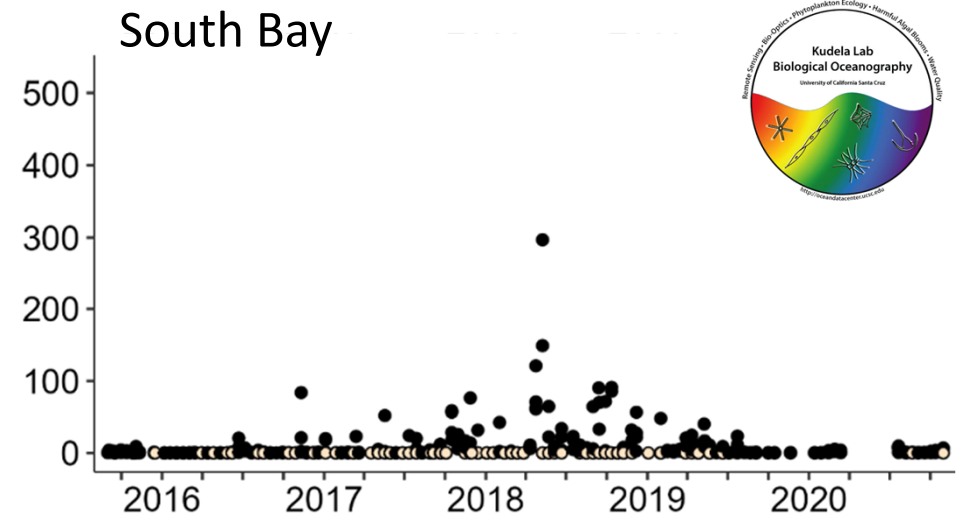
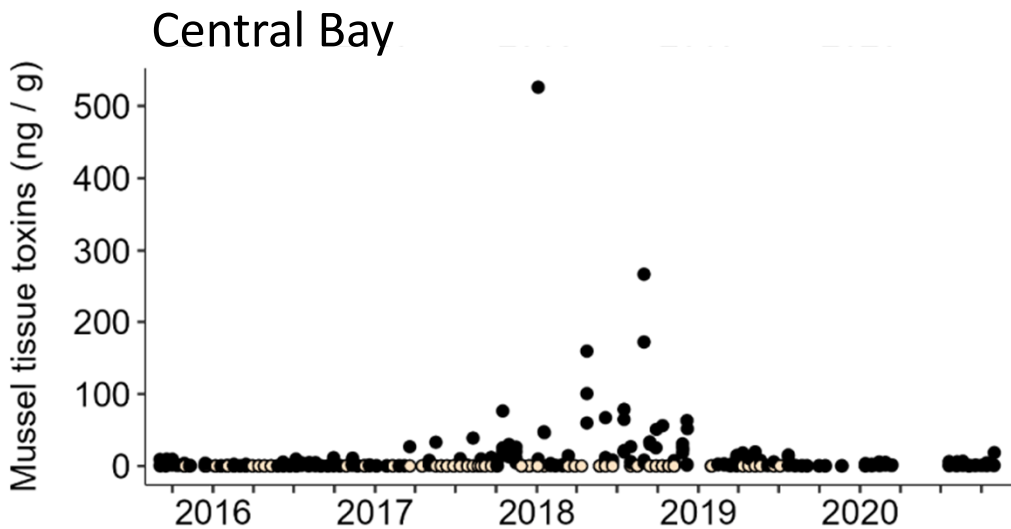
In San Francisco Bay, microcystins were detected in 56% of individual mussels tested in 2015, with **concentrations as high as 350 $\mu\text{g}/\text{kg}$** , greatly exceeding regulatory guidance of 10 $\mu\text{g}/\text{kg}$

Microcystins in marine mussels, San Francisco Bay, CA

A multi-year time series shows **persistent** and occasionally **dangerously high** levels of microcystins in mussels



Kudela et al. in prep



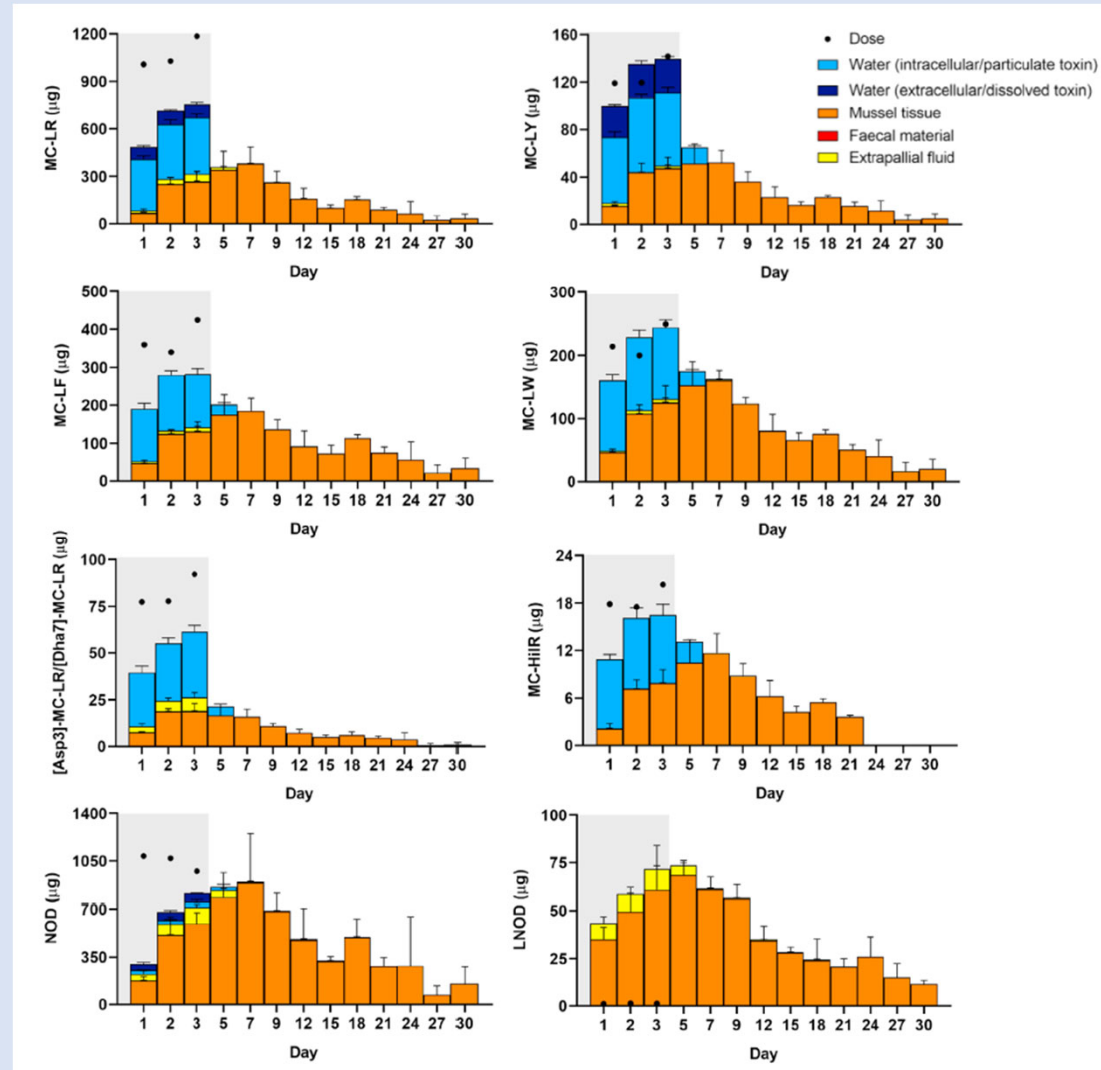
Fast uptake, slow depuration

Microcystins and nodularin toxins **rapidly accumulated** in the common blue mussel

Toxins were **still detected 27-days** post exposure in the mussel tissue

There needs to be a better understanding of toxin profiles to be able to estimate risk to human health

Camacho-Munoz et al. 2021; *Environ Pollution*



Fast uptake, slow depuration

Gibble et al. 2016; *Harmful Algae*

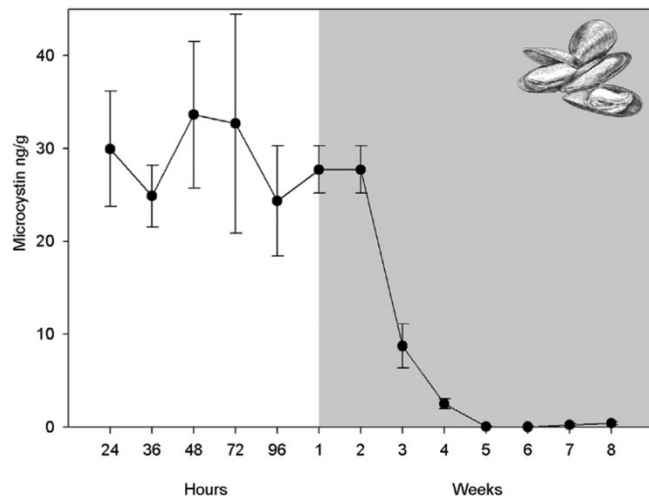


Fig. 2. *M. californianus* and particulate microcystin toxin in high-concentration experimental trials. X-axis begins at 24 h denoting removal from water containing microcystins.

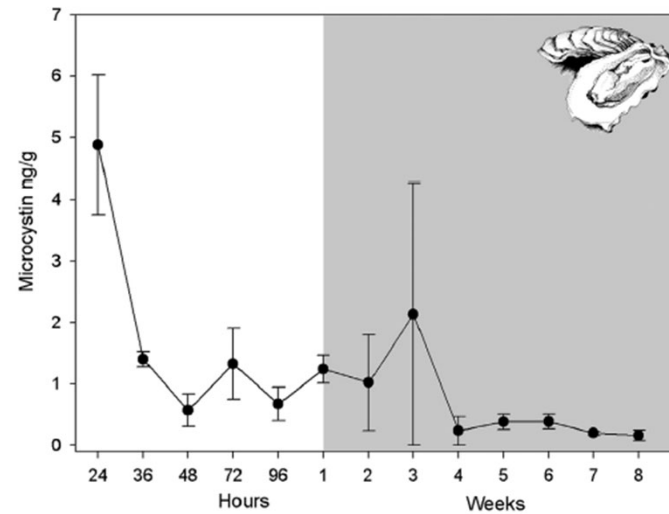


Fig. 5. Commercial oyster and microcystin toxin experimental trials. X-axis begins at 24 h denoting removal from water containing microcystins.

Microcystins **rapidly accumulated** in the common California mussel and oysters

Toxins were still detected **8-weeks** post exposure

Communities that rely on aquaculture and wild-harvest shellfish are at risk

The Salish Sea

Shared state, federal, and tribal waters

Annually has a **\$270 million** shellfish industry

In 2017 more than 431,000 people purchased recreational shellfish harvest licenses

Employs more than 3,200 people annually



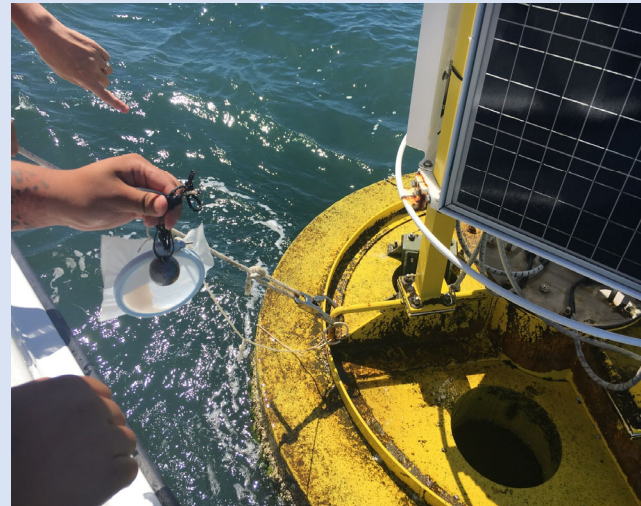
Solid Phase Adsorption Toxin Tracking (SPATT) Sampler

Small bags of resin that can be left in the water to monitor for biotoxins

Can provide continuous monitoring of **both freshwater and marine** biotoxins

Only measures dissolved toxins (not total)

Cheap, easy to use, and already widely monitoring for both freshwater and marine toxins

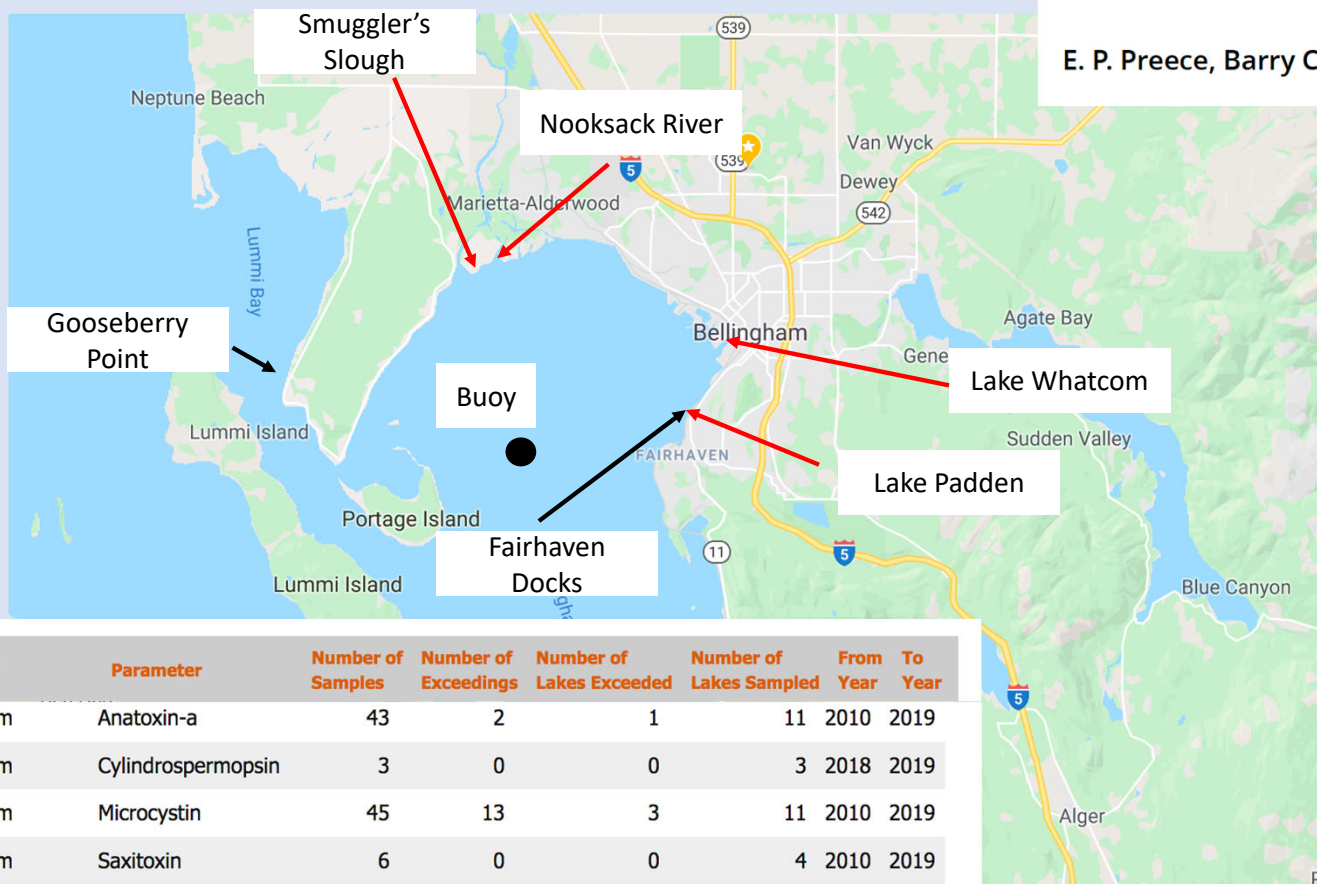


Cyanotoxins in Bellingham Bay

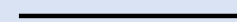
First detection of microcystin in Puget Sound, Washington, mussels (*Mytilus trossulus*)

<https://doi.org/10.1080/10402381.2014.998398>

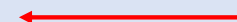
E. P. Preece, Barry C. Moore, F. Joan Hardy & Lee A. Deobald



Monitoring site



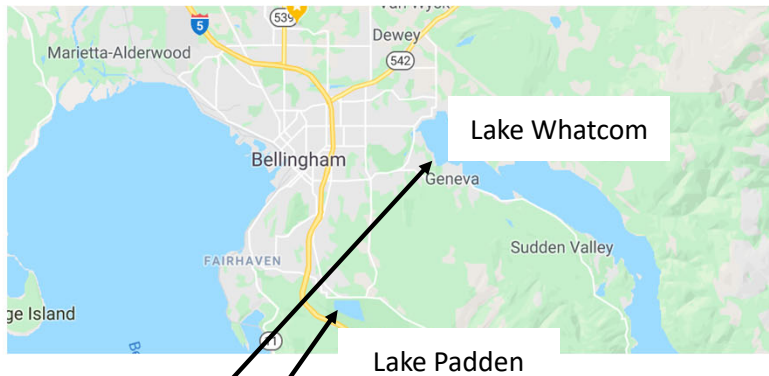
Outflow



County	Parameter	Number of Samples	Number of Exceedings	Number of Lakes Exceeded	Number of Lakes Sampled	From Year	To Year
Whatcom	Anatoxin-a	43	2	1	11	2010	2019
Whatcom	Cylindrospermopsin	3	0	0	3	2018	2019
Whatcom	Microcystin	45	13	3	11	2010	2019
Whatcom	Saxitoxin	6	0	0	4	2010	2019

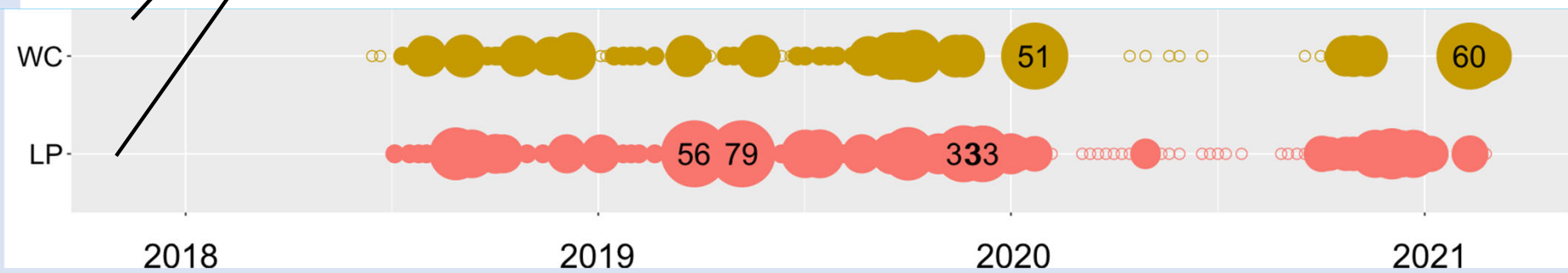
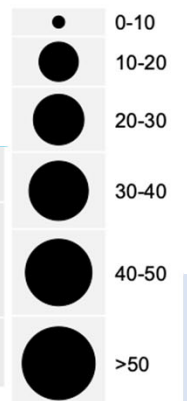
Washington Dept. of Ecology freshwater toxin monitoring program

Microcystins are persistent in two freshwater lakes



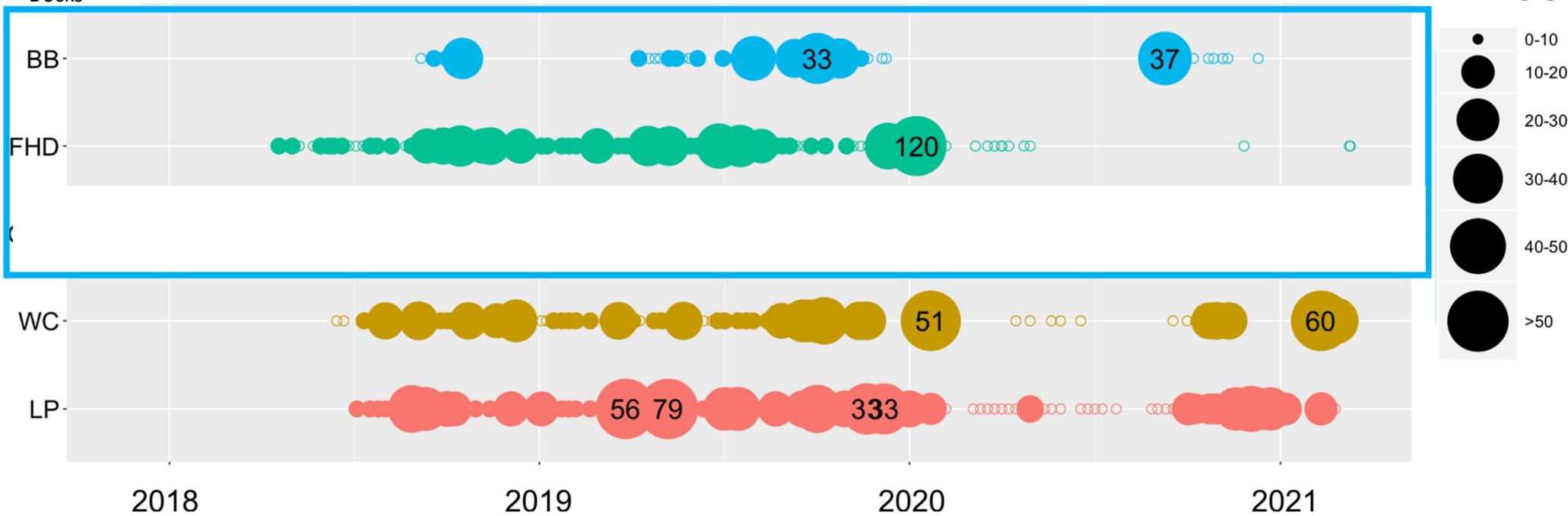
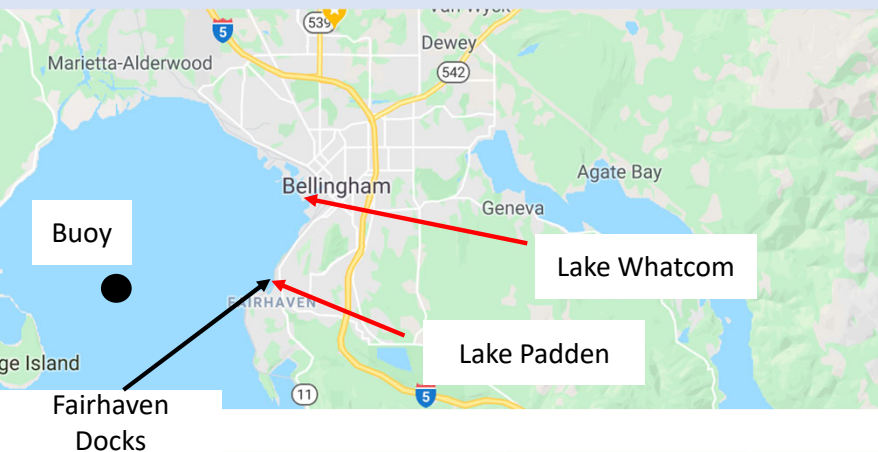
Peacock et al. in prep

MC ng g⁻¹

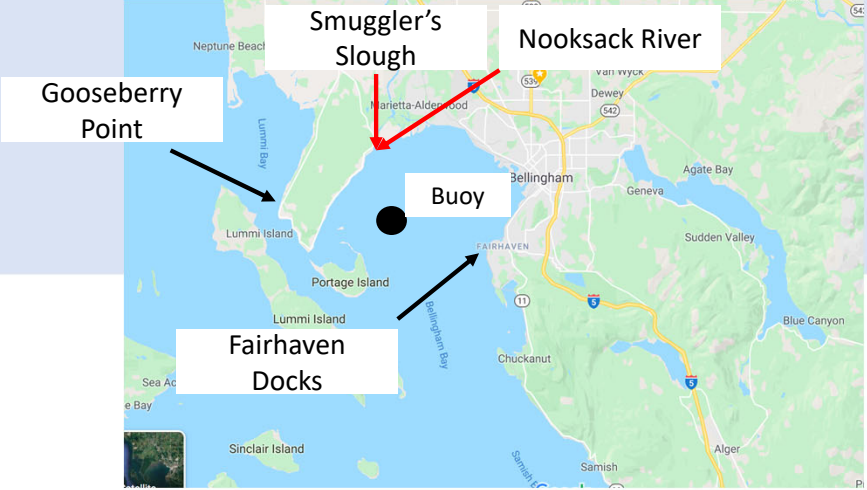


Transfer of freshwater toxins to Bellingham Bay, WA

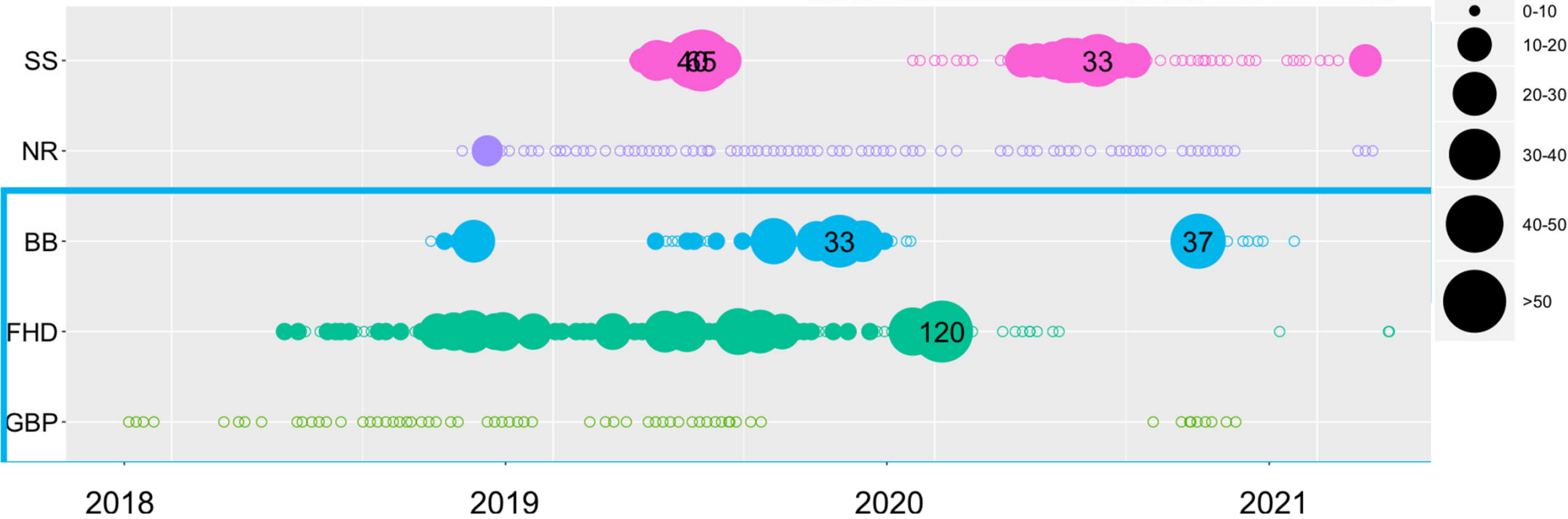
Peacock et al. in prep



Cyanotoxins in Bellingham Bay



Peacock et al. in prep



Microcystins in the marine food web

Butter Clams



Olympia Oysters



Cockles



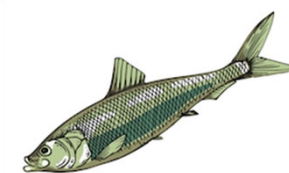
California Mussel



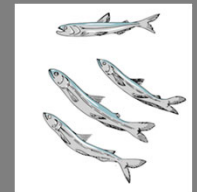
Barnacles



Anchovies



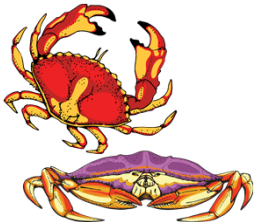
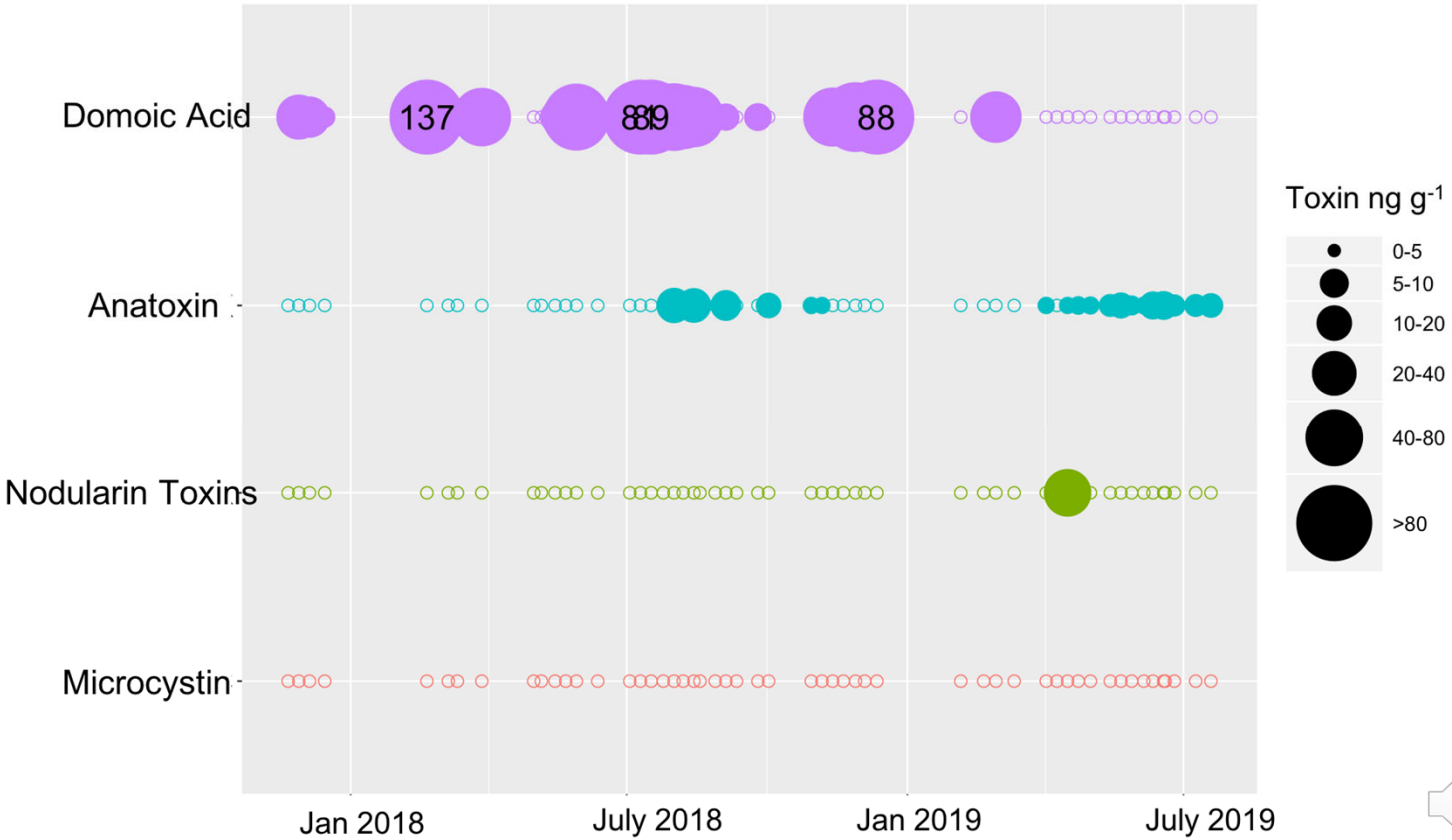
Longfin Smelt



exceeding regulatory guidance of 10 $\mu\text{g}/\text{kg}$

Testing for multiple toxins using LC-MS/MS

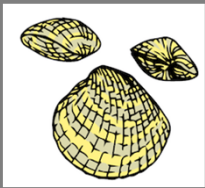
Gooseberry Point



Peacock et al. in prep

Biotoxins in the marine food web

Butter Clams



YTX
PST
DST
DA
MC

Olympia Oysters



YTX
PST
DST
MC

Cockles



YTX
PST
DST
MC

California Mussel



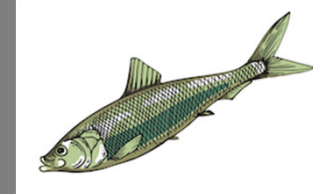
YTX
PST
DST
DA
MC
CYN

Barnacles



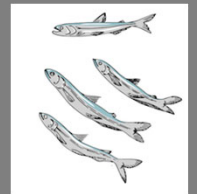
MC

Anchovies



DA
MC

Longfin Smelt



MC

YTX = Yessotoxin

PST = Paralytic Shellfish Toxins

DST = Diarrhetic Shellfish Toxins

DA = Domoic Acid

MC = Microcystins

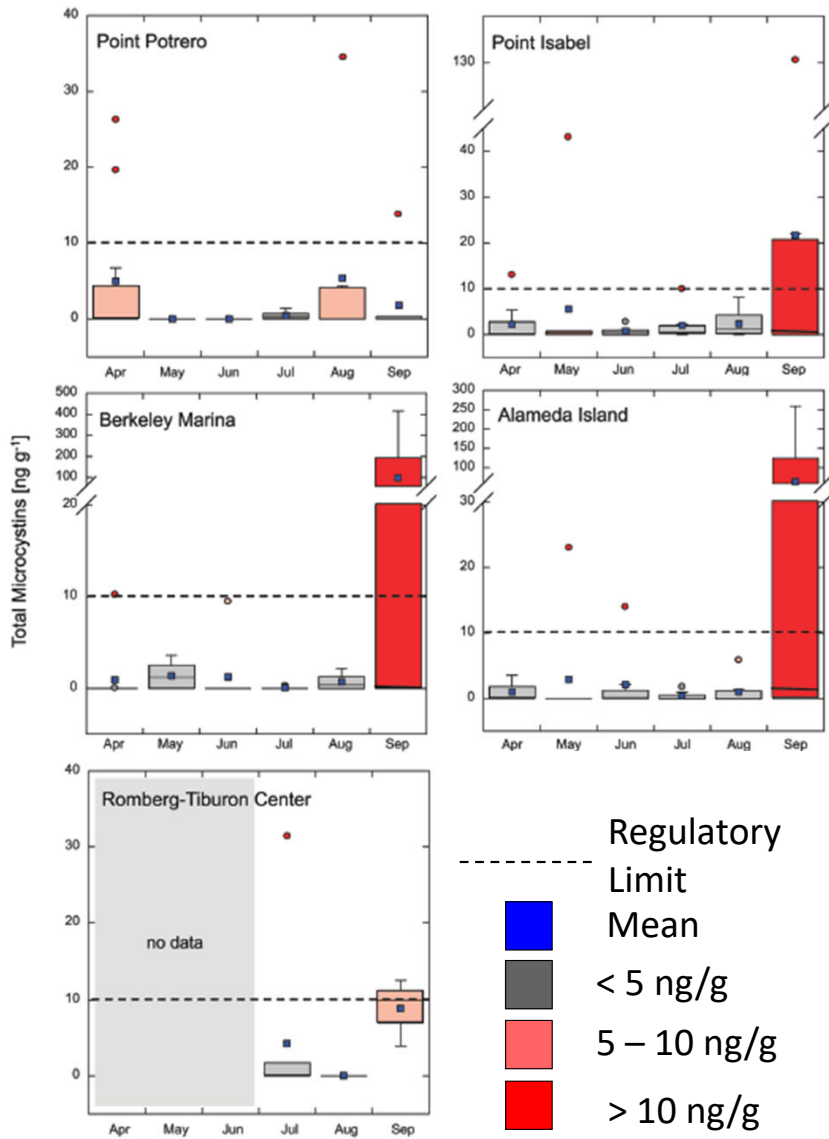
ANA = Anatoxin-a

NOD = Nodularin Toxins

CYN = Cylindrospermopsin

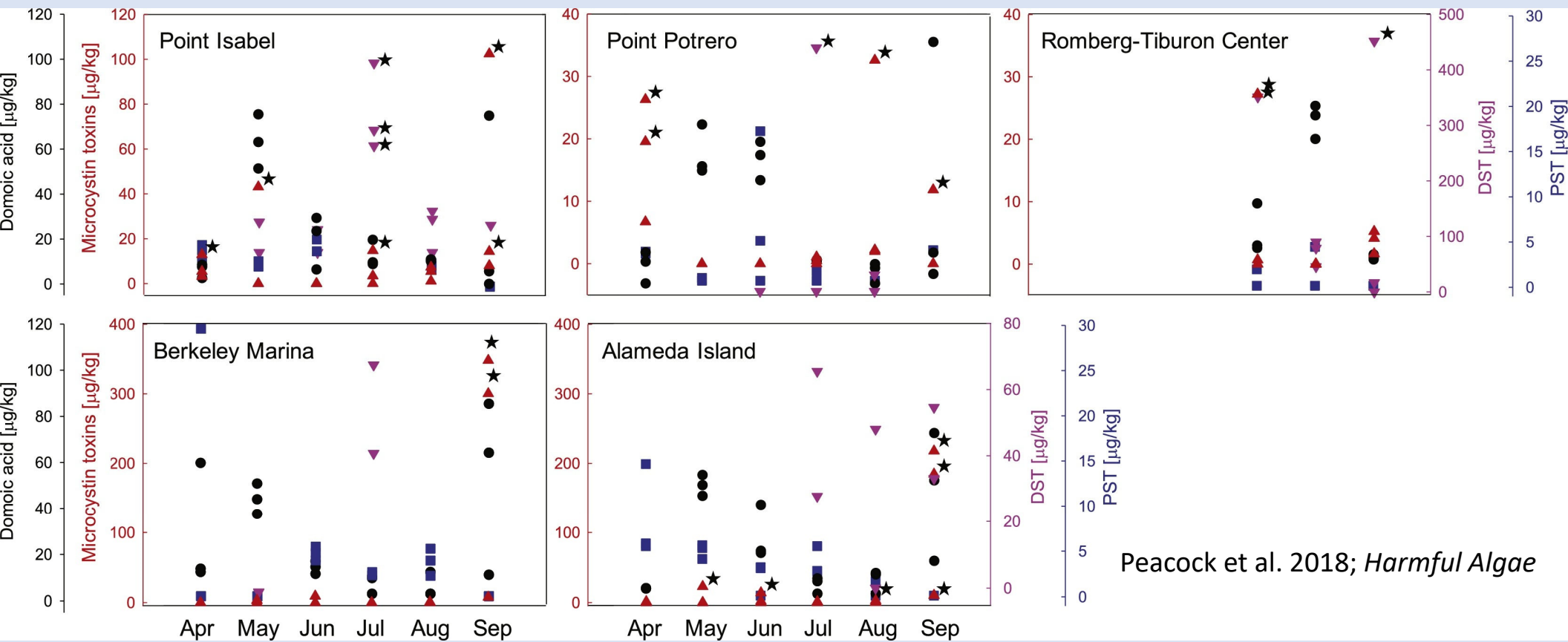
Peacock et al. in prep

Microcystins in marine mussels, San Francisco Bay, CA



Gibble et al. 2016; *Harmful Algae*

Biotoxins in marine mussels, San Francisco Bay, CA



Peacock et al. 2018; *Harmful Algae*

Determining Tolerable Daily Intake (TDI) of cyanotoxins in seafood

Table 6. Health guideline values for cyanobacterial toxins in seafood (based on consumption by 2–16 year age group).

Toxin	Health guideline value ($\mu\text{g}/\text{kg}$ of whole organism sample)		
	Fish	Prawns	Mussels or Molluscs
Cylindrospermopsin and deoxyCYN	18	24	39
Microcystin-LR* or equivalent toxins, incl. Nodularin	24	32	51
Saxitoxins	800	800	800

* The guideline value represents the sum value of all microcystins and nodularin present.

Mulvenna et al. 2012; *Int. J. Environ. Res. Public Health*

Currently in California and Washington, there is **no routine monitoring** of cyanotoxins in marine seafood

Subsistence and cultural harvesting

“You know our old people were conscious of pollutants, like for example, the Soxwe, the butter clams. You had to cut that black nose off because the pollutants are all in the black part of the nose, you have to cut it off, every time they are all cut off, you don’t eat that part. If you cut the noses off and clean the bellies out of the clams you can eat them.” - Chief Tsi’li’xw Bill James

(Lummi Natural Resources Shellfish Survey 2012)

Subsistence and cultural harvesters have increased consumption of seafood, increasing the likelihood of **exceeding TDI for cyanotoxins**



Clam garden, Gulf Island, BC; Ian Reid, Parks Canada



Lummi Nation biotoxin closure signs

Hy'shqe (Thank You)

Special thanks to Rosa Hunter, Mikale Milne, Shelbi Madera, Megan Hintz, Dana Manalang, Rachael Mallon, Brandi Kamermans, Thayne Yazzie, Rachel Arnold, Ben Starkhouse, Karl Mueller, Jan Newton, Teri King, Vera Trainer, Devin Flawd, Jeff Solomon, Roberta Hall, Dave Oreiro, Raphe Kudela, Kendra Negey

