Molecular Analysis of Cyanobacteria in Candlewood Lake & Lake Zoar

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Blue-green algae blamed for deaths of thousands of fish at Lake El Reno

Blue-Green Algae Outbreak Kills 32 Cattle in Oregon

Toxic algae bloom kills two dogs in Napa as warnings proliferate

Huge toxic algae bloom sickens more than 100 in Utah amid heatwave

Toxic algae may cause dementia as scientists find higher rates of brain disease near contaminated lakes

Toxin in blue-green algae blooms may increase risk of Alzheimer's and other neurological diseases

Toxic algae becoming severe threat nationwide

Various news sources, 2015–2017

newstimes ☰ Sections

Unwelcome algae blooms in area lakes

Robert Miller Updated 10:44 pm, Thursday, October 24, 2013

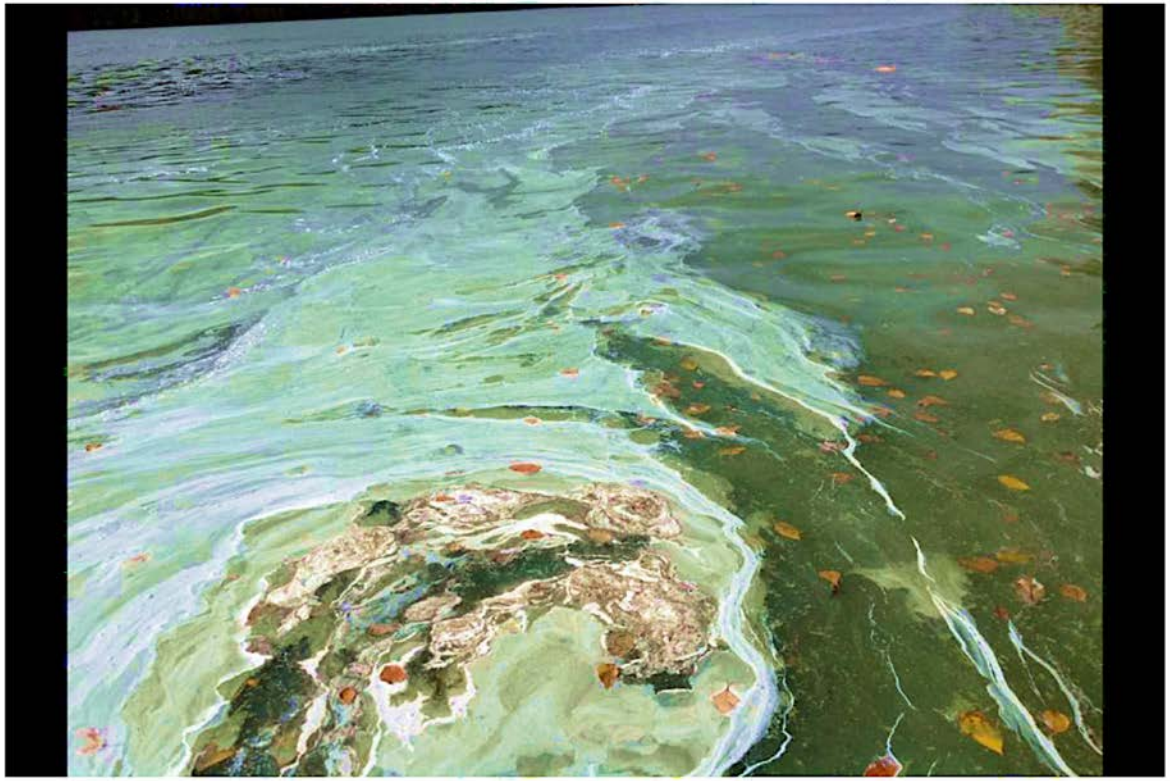
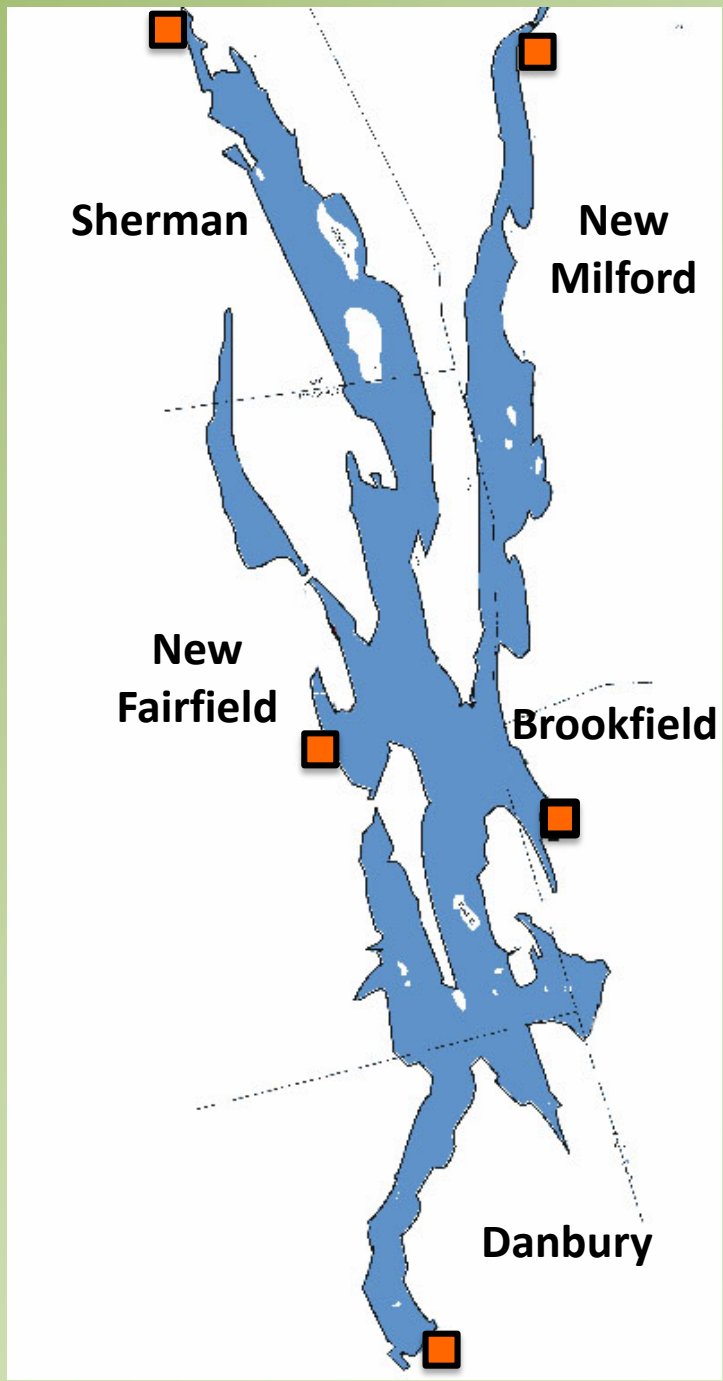


Photo: Contributed Photo/ Candlewood La, Contributed Photo

News-Times (2013)

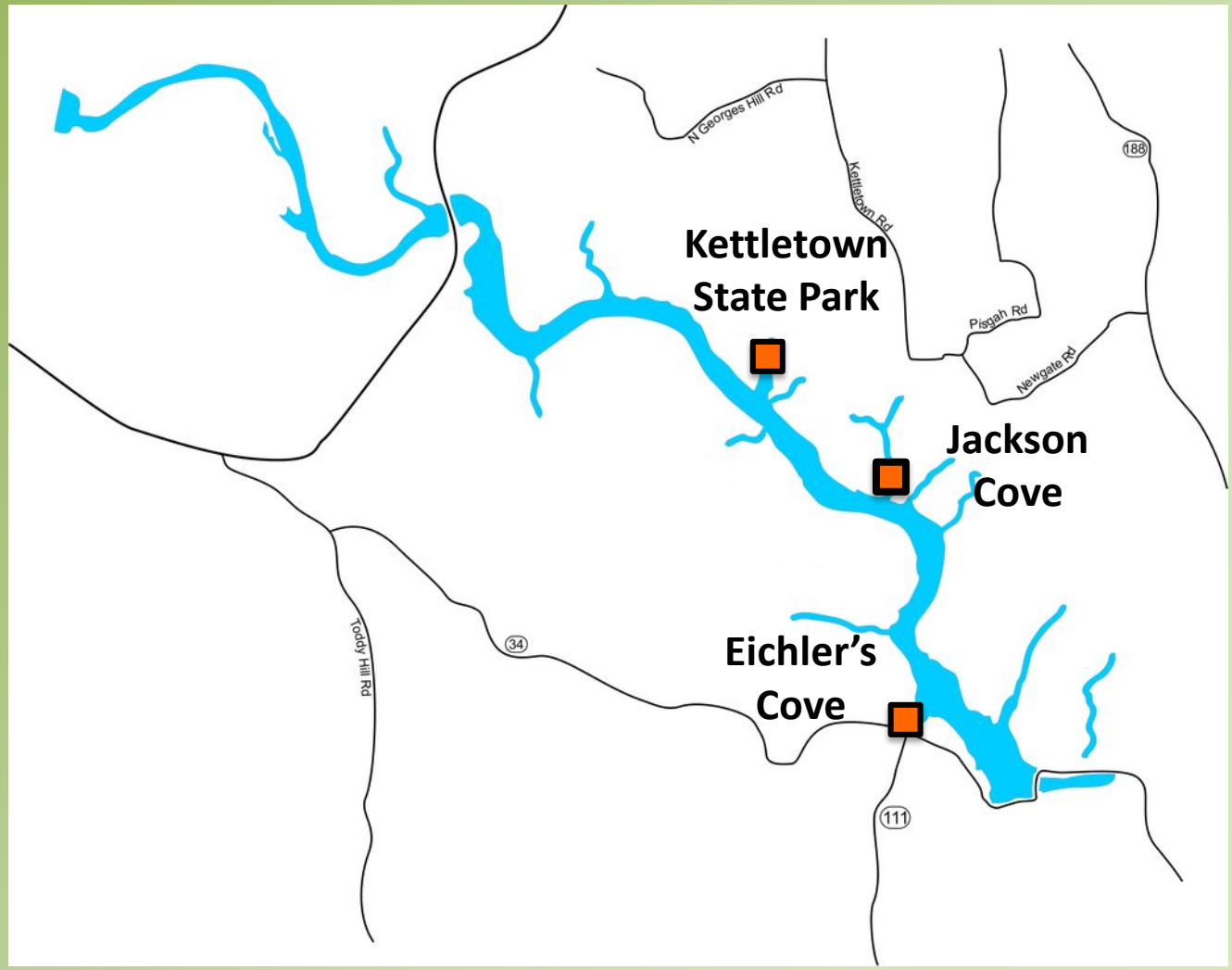
Candlewood Lake

- Beaches

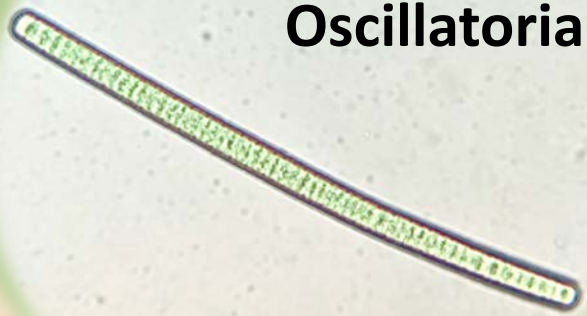


Lake Zoar

- Beaches



Microcystis



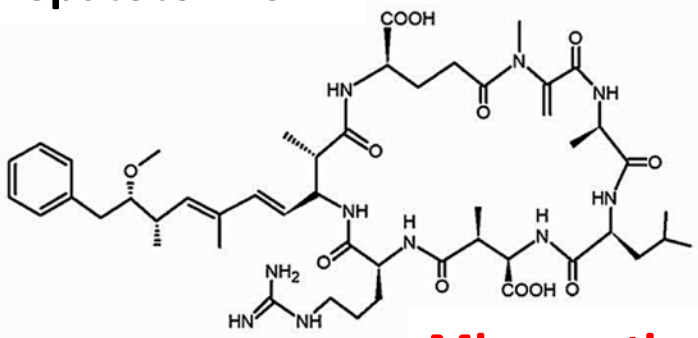
Oscillatoria

Dolichospermum

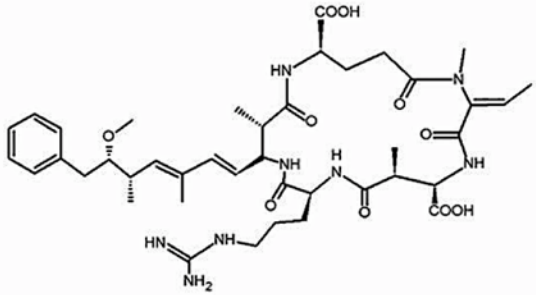
Anabaena

Woronichinia

Hepatotoxins

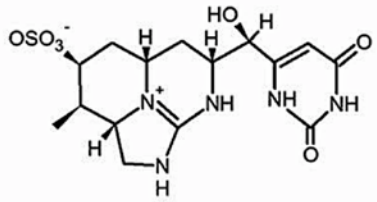


Microcystin



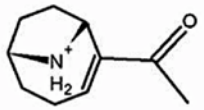
Nodularin

Cytotoxin

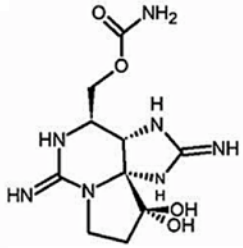


Cylindrospermopsin

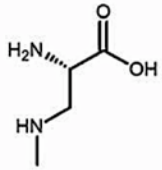
Neurotoxins



Anatoxin

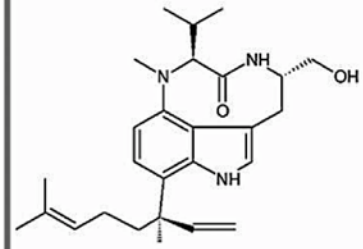


Saxitoxin

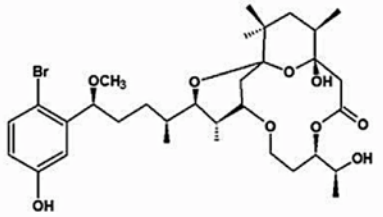


BMAA

Dermatoxins



Lyngbyatoxin A



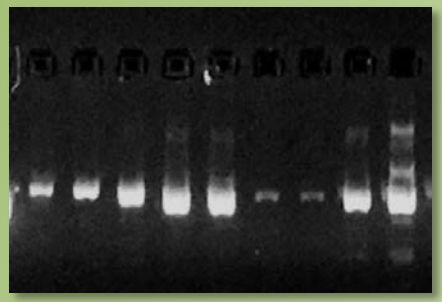
Aplysiatoxin

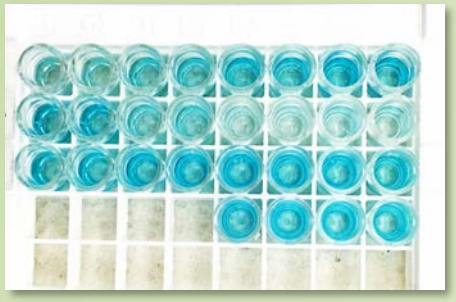
Dittmann, et al. (2013) FEMS Microbiol Rev 37:23–43

water sample

toxin gene PCR assay

toxin ELISA assay





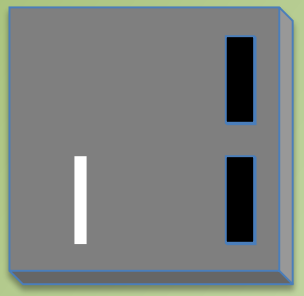
Detecting toxin genes by PCR

Collect cyanobacteria

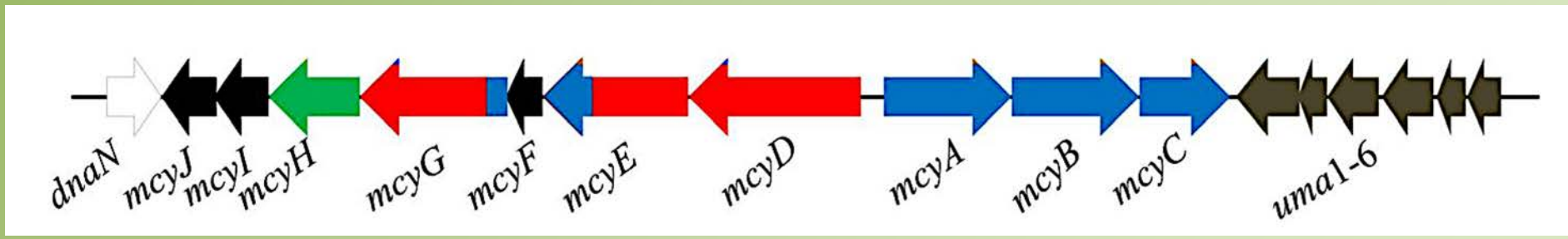
Isolate total genomic DNA

Amplify toxin gene-specific DNA sequences by PCR

Visualize products by gel electrophoresis



Detecting Microcystin genes by PCR
Robert McArthur



Microcystin (*mcy)* gene cluster

Rastogi, et al. (2015) Front. Microbiol. 6:1–22

McyD Gene Screening (2017)

Beaches	June 13	June 20	June 27	Aug 3	Aug 10	Aug 17	Aug 24	Aug 31	Sep 7
Brookfield	+	+	+	+	+	+	+	+	+
New Milford	+	+	+	+	+	+	+	+	+
Sherman	+	+	+	+	+	+	+	+	+

McyD Gene Screening (2017)

Beaches	Jul 13	Jul 20	Jul 27	Aug 03	Aug 10	Aug 17	Aug 24	Aug 31	Sep 07
Danbury	+	+	+	+	+	+	+	+	+
New Fairfield	+	–	+	+	+	+	+	+	+
Squantz Pond	+	+	+	+	+	+	+	+	+

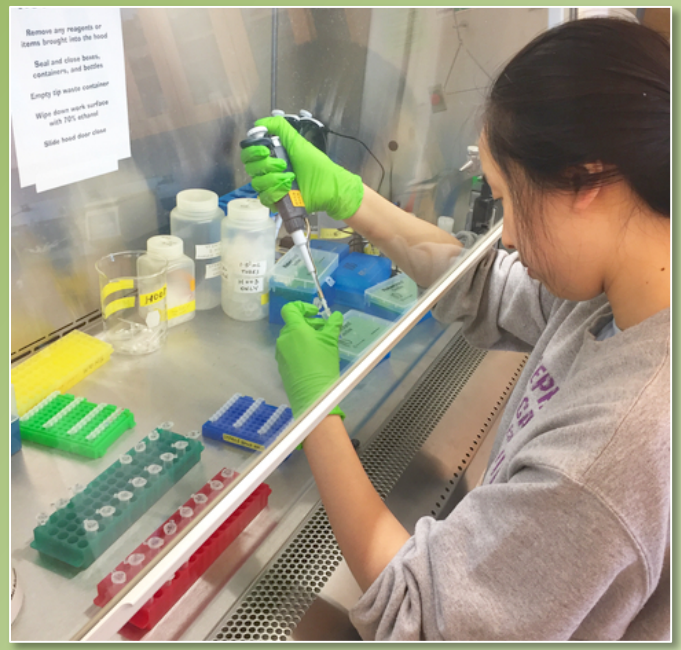
Conclusion: McyD Gene Screening (2017)

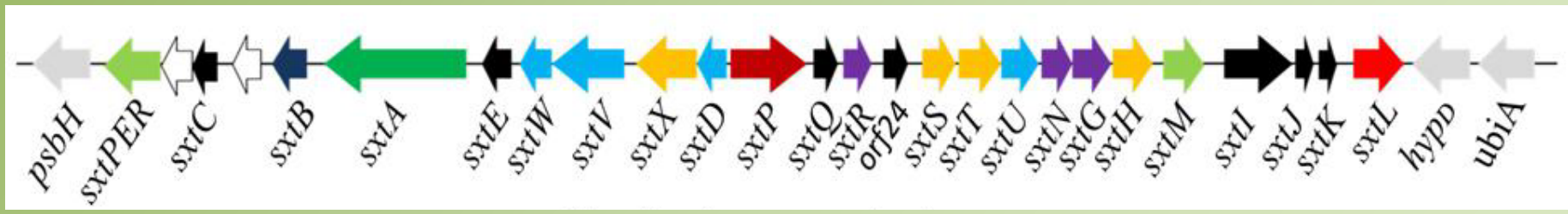
1. **Genetic potential for Microcystin synthesis is present throughout testing season (July – early Sep) at all sites that were sampled.**

2. **Need to quantify the fraction of the cyanobacteria population that carries Mcy genes; new qPCR machine at WCSU will enable that type of analysis.**

Detecting Saxitoxin genes by PCR

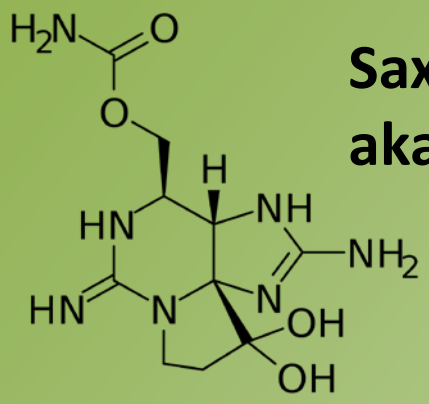
Kayla Zhang



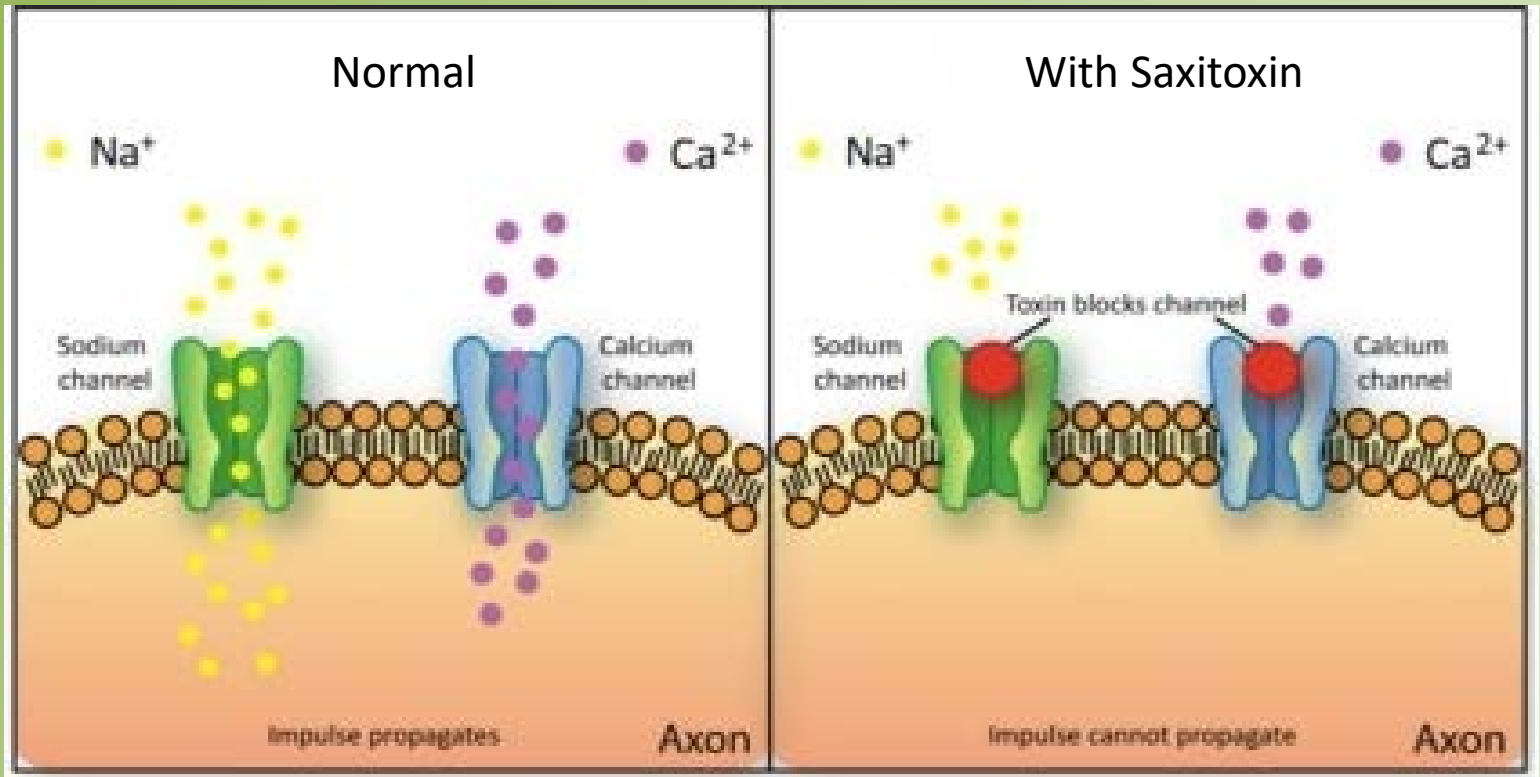


Saxitoxin (*sxt)* gene cluster

Rastogi, et al. (2015) Front. Microbiol. 6:1–22



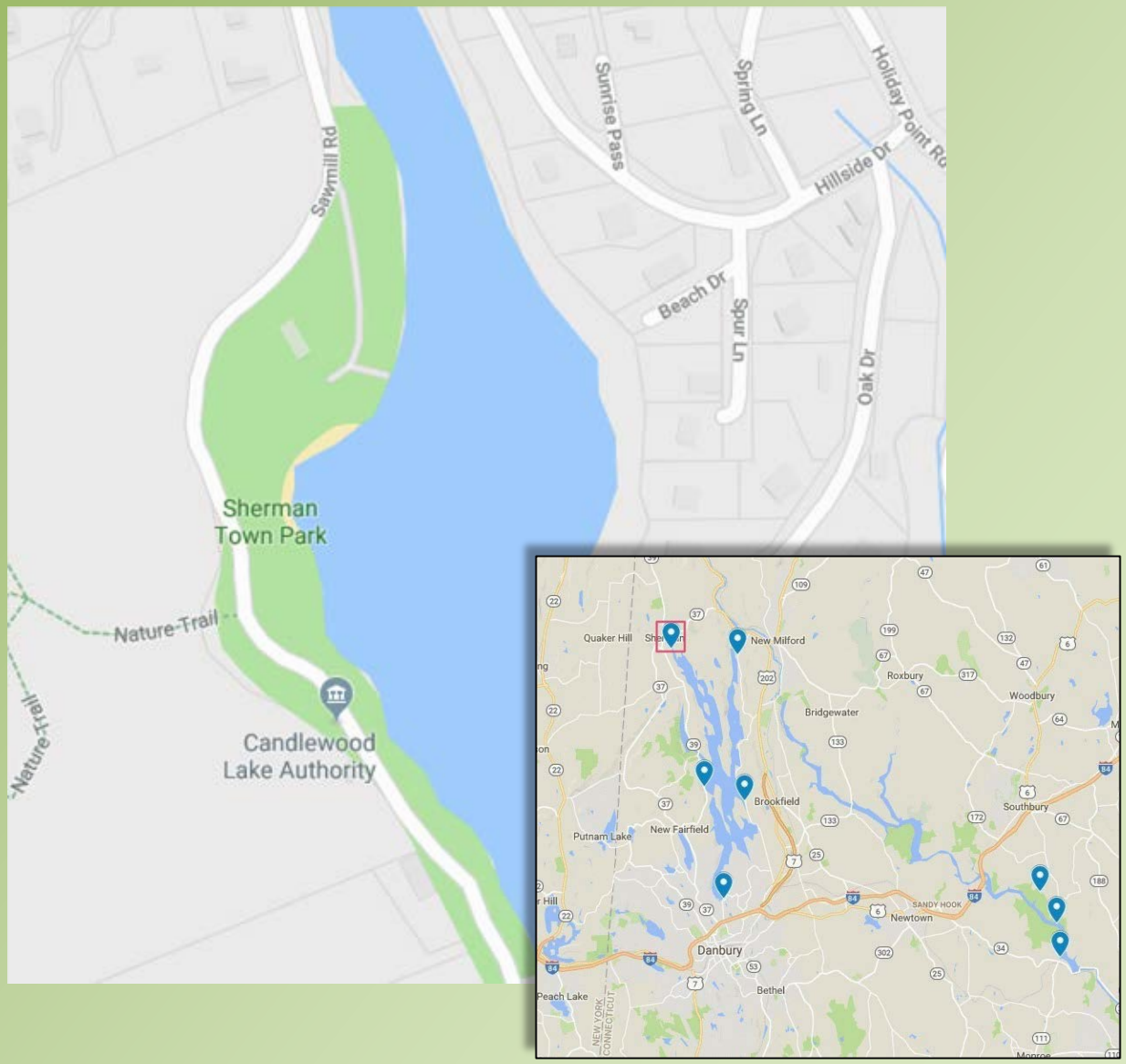
Saxitoxin
aka “paralytic shellfish toxin”



Sherman (2017)

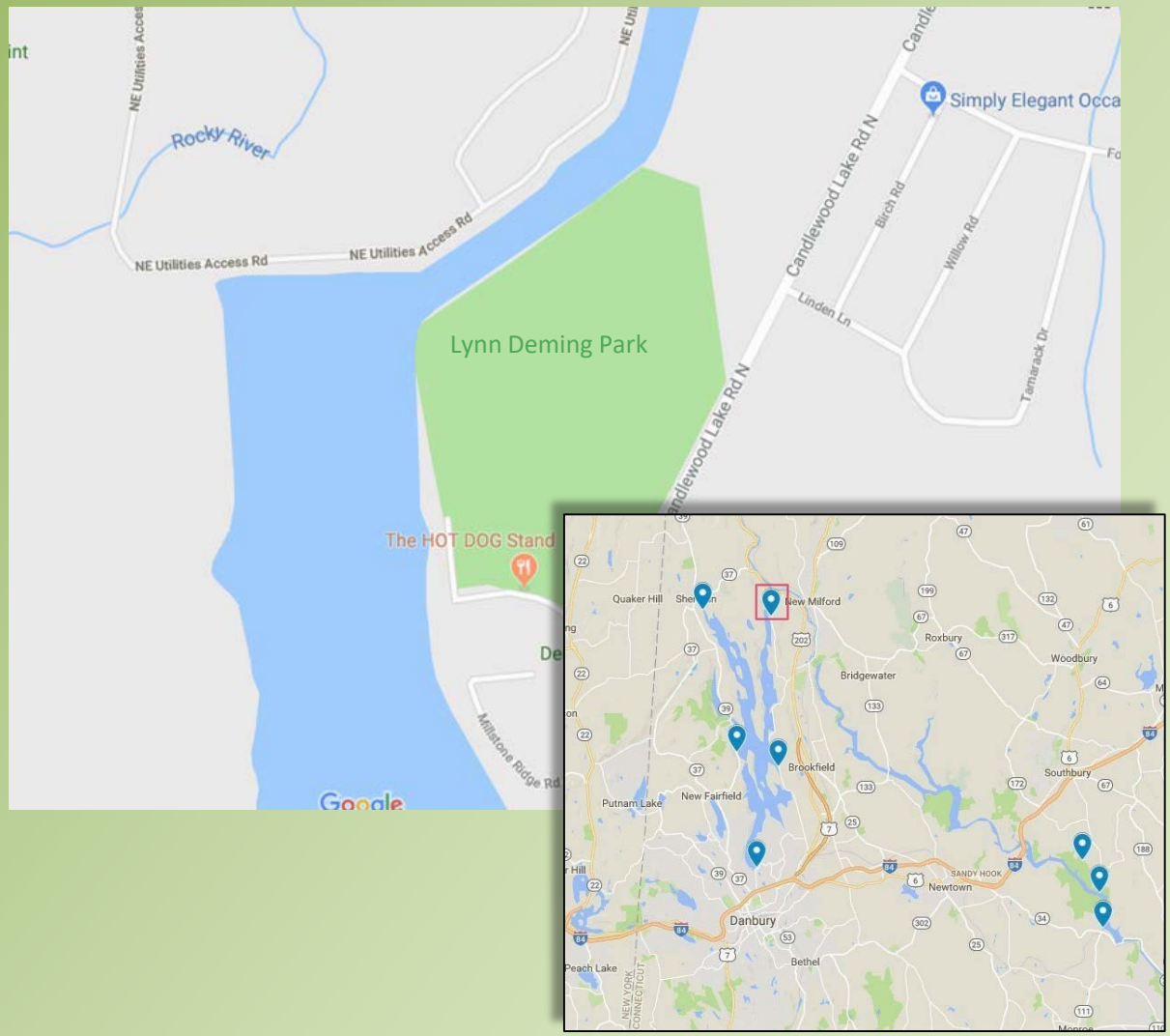
—

Date	SxtA gene
Aug 17	–
Aug 24	+
Aug 31	–



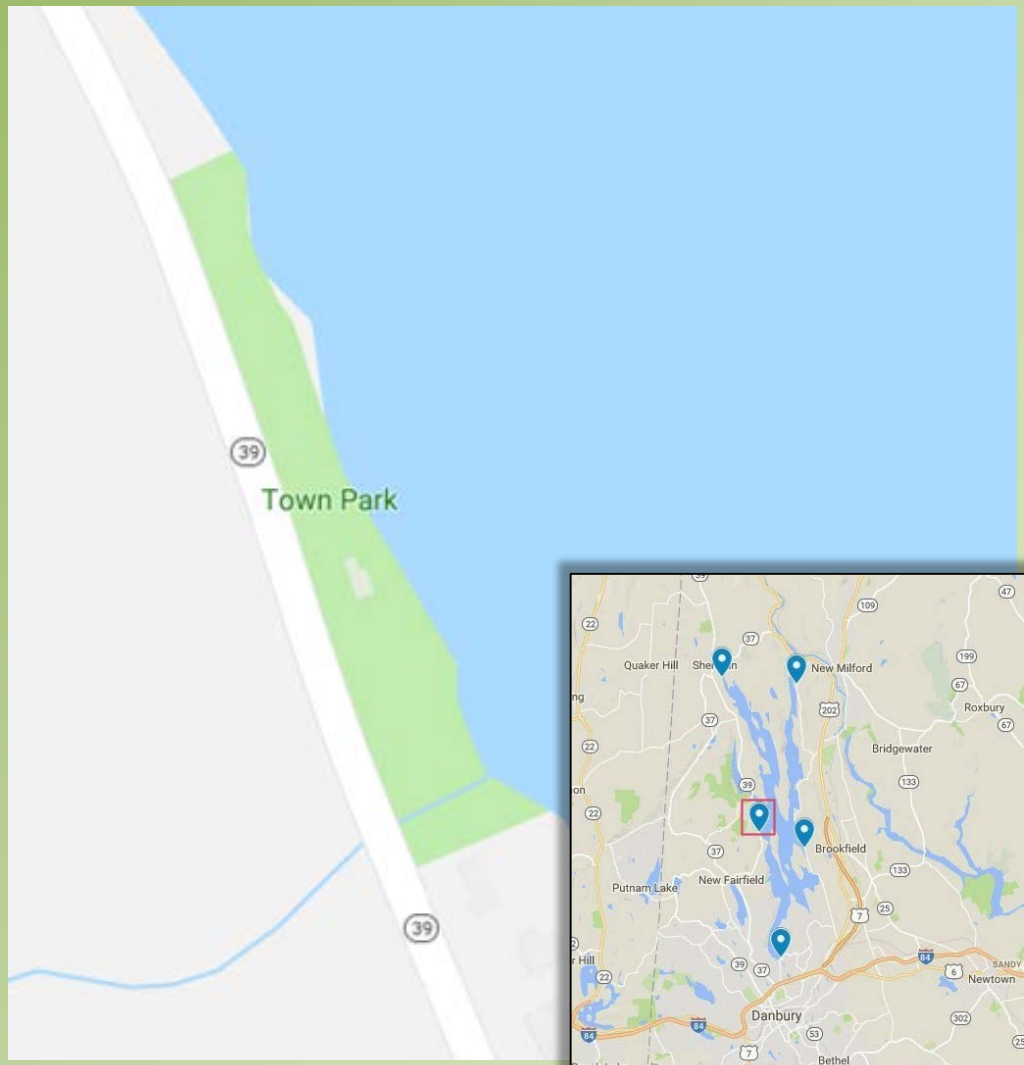
New Milford (2017)

Date	SxtA gene
Aug 17	–
Aug 24	–
Aug 31	+



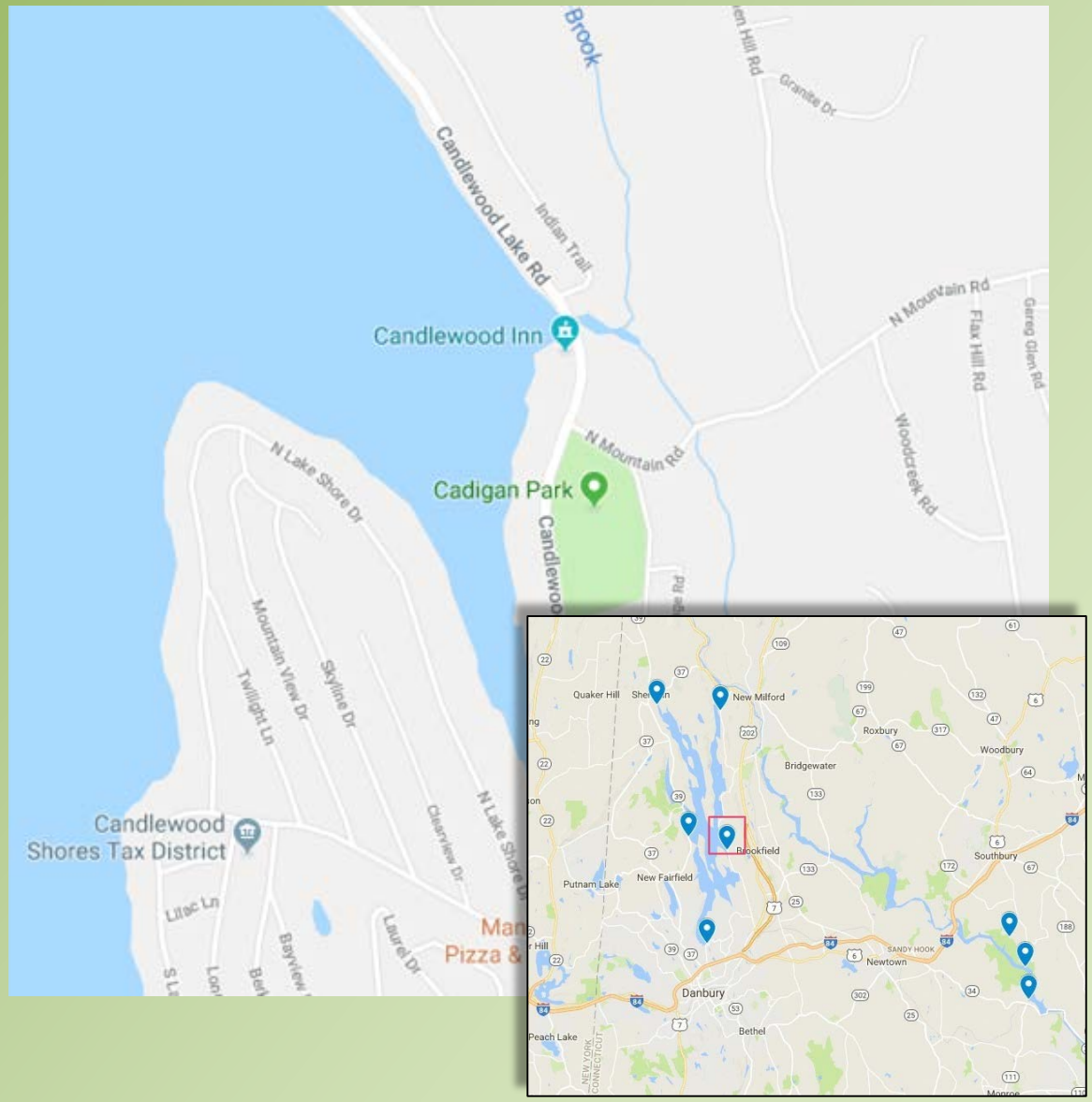
New Fairfield (2017)

Date	SxtA gene
Aug 17	+
Aug 24	+
Aug 31	+



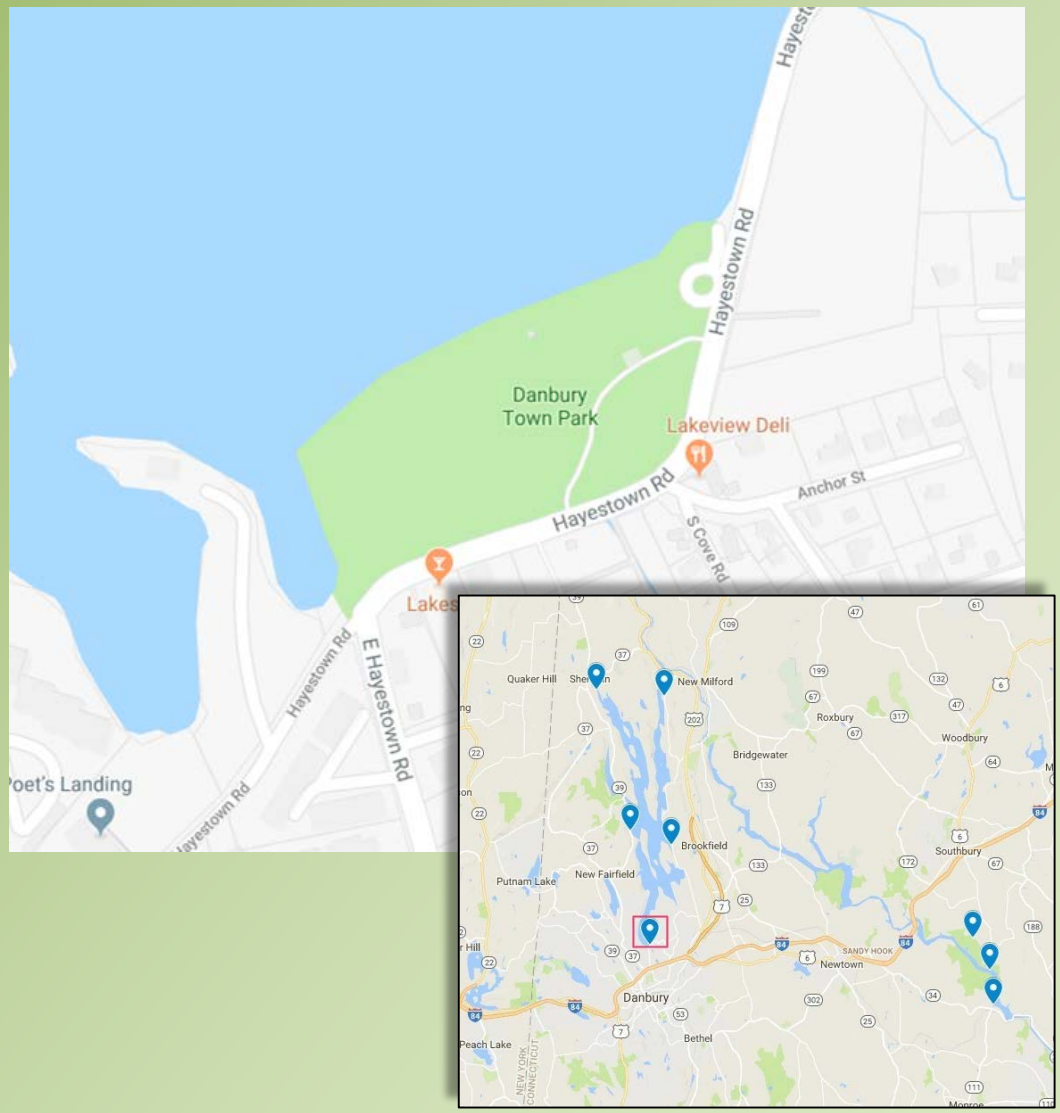
Brookfield (2017)

Date	SxtA gene
Aug 17	+
Aug 24	+
Aug 31	+



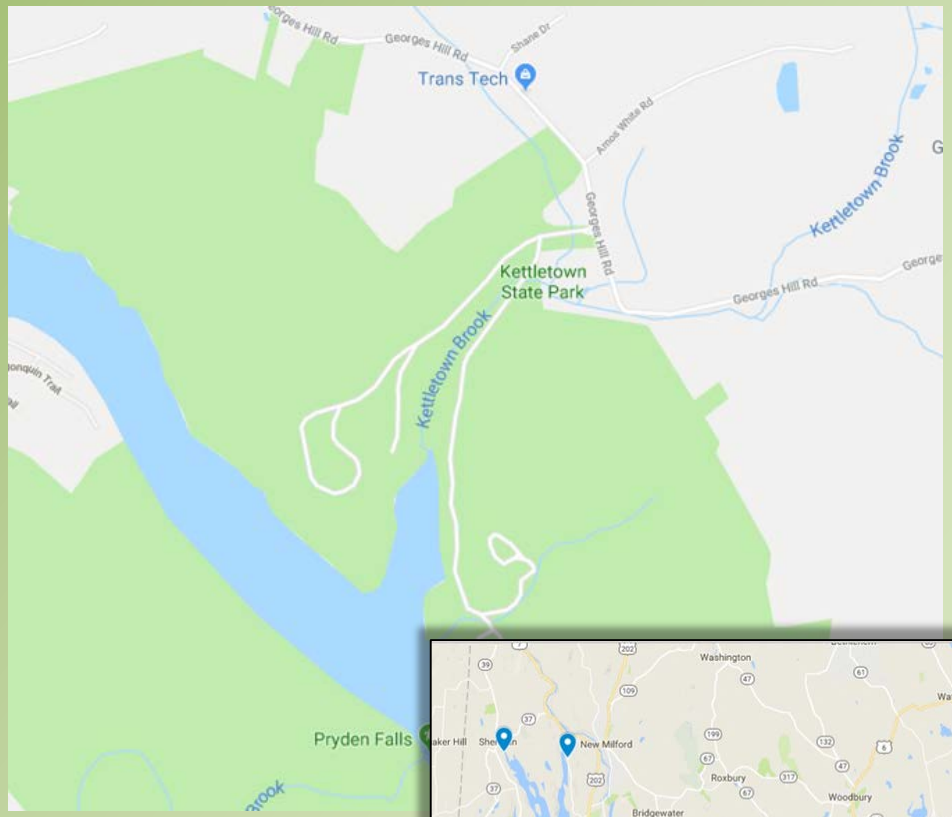
Danbury (2017)

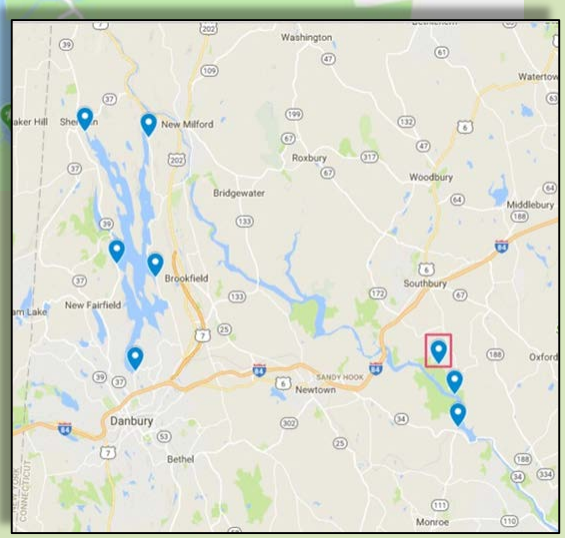
Date	SxtA gene
Aug 17	+
Aug 24	+
Aug 31	+



Kettletown (2016 and 2017)

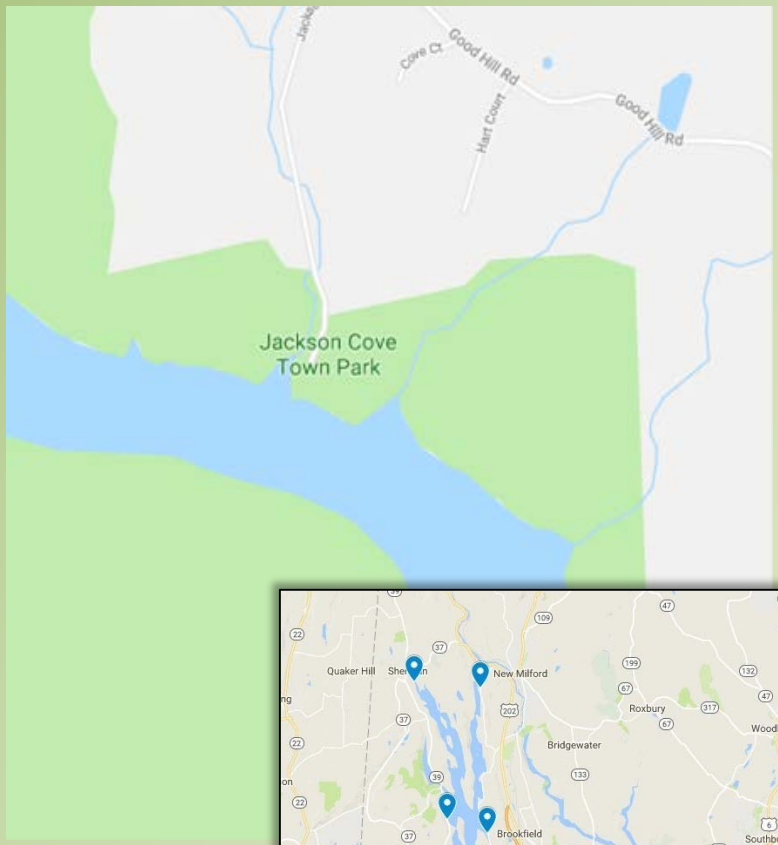
2016 Date	SxtA gene	2017 Date	SxtA gene
Jul 7	–	Jul 13	–
Jul 14	–	Jul 20	–
Jul 21	–	Jul 27	–
Aug 4	+	Aug 3	+
Aug 12	+	Aug 10	–
		Aug 17	+
		Aug 24	+
		Aug 31	+
		Sep 7	–

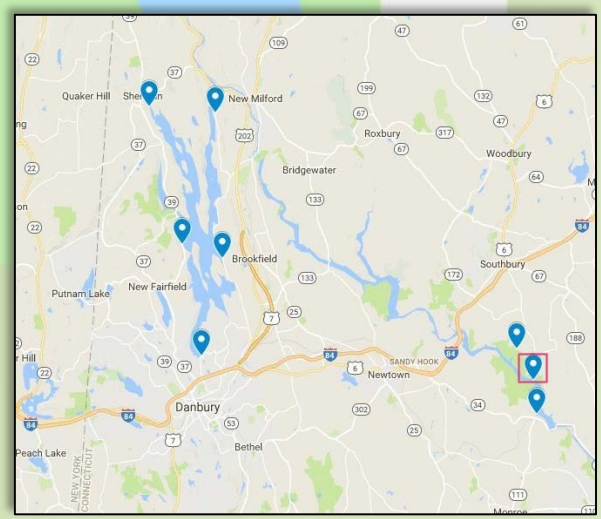




Jackson Cove (2016 and 2017)

2016 Date	SxtA gene	2017 Date	SxtA gene
Jul 7	–	Jul 20	–
Jul 14	–	Jul 27	–
Jul 21	–	Aug 3	+
Aug 12	+	Aug 17	+
		Aug 24	+
		Aug 31	+
		Sep 7	–





Eichler's Cove (2016 and 2017)

2016 Date	SxtA gene	2017 Date	SxtA gene
Jul 7	–	Jul 13	–
Jul 14	+	Jul 20	+
Jul 21	–	Jul 27	–
		Aug 3	–
		Aug 17	+
		Aug 24	+
		Aug 31	+
		Sep 7	+

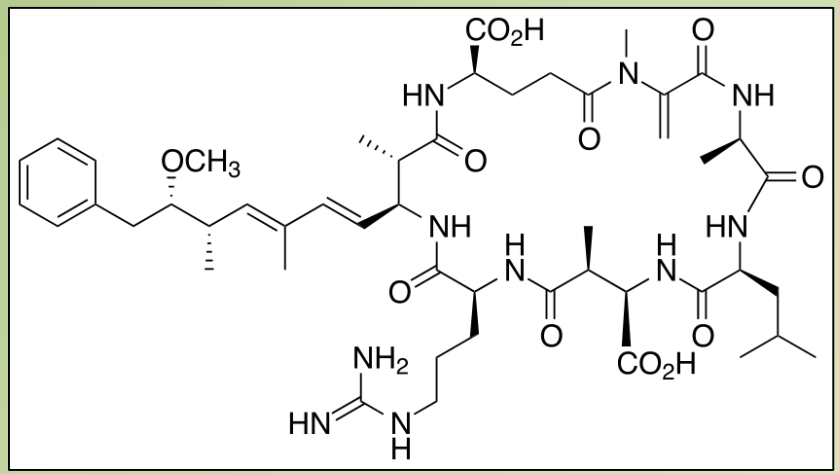
Conclusion: SxtA Gene Screening

1. In Candlewood Lake samples from August 2017, southern beaches (DA, BR, NF) were more likely to contain SxtA genes throughout the month.

2. The presence of Sxt genes suggests that testing for saxitoxin may be warranted.

Detecting Microcystin by ELISA Assay

Ghada Salah Hafez, Ph.D.



Microcystin

What are the Regulations for Cyanotoxins?

U. S. Environmental Protection Agency (EPA)
2016 draft water quality criteria for Microcystin

Recreational water :

4 µg/L (ppb)

	1	2	3	4
A	0.012	1.788	0.058	0.030
B	<0.000	1.863	0.024	0.042
C	0.162	>5.250	0.056	0.047
D	0.135	3.970	0.037	0.071
E	0.381	0.608	0.021	
F	0.427	0.723	0.004	
G	1.016	0.044	0.029	
H	1.034	0.061	0.026	

After: https://www.epa.gov/sites/production/files/2016-12/documents/draft-hh-rec-ambient-water-swimming-factsheet.pdf

Overview summary of Microcystins (ppb) in Candlewood and Zoar lakes Summer 2016 (6/24-9/9)

Date	Danbury	Brookfield	Candlewood Lake Club	Candlewood Point	Eichler Cove	Jackson Cove	Kettletown	Lynn Deming	New Fairfield	Sherman	Squantz
24-Jun	0.0525	0.041	N/A	N/A	N/A	N/A	N/A	0.0465	0.0275	0.036	0.059
7-Jul	0.22	0.104	N/A	N/A	0.0625	0.076	0.181	0.219	0.275	0.35	0.13
14-Jul	0.166	0.733	N/A	N/A	0.182	0.133	0.124	0.277	0.301	0.216	0.153
21-Jul	0.152	0.261	0.224	0.241	0.215	0.144	0.374	0.338	0.255	0.137	0.135
28-Jul	0.037	0.0485	0.0715	0.1895	0.0165	0.071	0.0525	0.0955	0.0855	0.084	0.007
4-Aug	0.258	0.188	0.232	0.211	0.145	0.281	0.829	0.348	0.227	0.602	0.286
11-Aug	0.14	0.063	0.123	0.114	0.076	0.573	0.232	0.231	0.192	0.486	0.212
18-Aug	0. 141	0. 119	0. 080	0. 090	0. 134	0. 533	0. 486	0. 177	0. 144	0. 077	0.105
25-Aug	0. 128	0. 143	0. 120	0. 127	0. 117	0. 312	0. 135	0.159	0. 160	0. 245	0.13
1-Sep	0. 118	0. 122	0.129	0.15	0.107	0. 168	0.478	0.153	0. 136	0.018	0
8-Sep	0.029	0.021	0.005	0.002	0.022	0.039	0.573	0. 026	0.028	0.083	0.026

Conclusion: all samples << 1 ppb

Overview summary of Microcystins (ppb) in Candlewood and Zoar lakes Summer 2017 (6/29-9/8)

Date	Danbury	Brookfield	CandleWood Lake Club	Candlewood point	Eichler's Cove	Jackson Cove	Kettletown	Lynn Deming	New Fairfield	Sherman	Sherm. bloom	Squantz
29-Jun	0.526	0.34	0.337	0.2	0.373	0.3515	0.283	0.416	0.398	0.322	4.69	0.2155
6-Jul	0.442	0.697	0.29	0.591	0.657	0.241	0.519	0.367	0.3	0.457	N/A	0.423
13-Jul	0.122	0.345	0.24	0.361	1.101	0.305	0.419	0.227	0.354	0.442	N/A	0.172
20-Jul	0.39	0.461	0.464	0.269	0.304	0.494	0.573	0.434	0.433	0.605	N/A	0.176
27-Jul	0.181	0.369	0.721	0.457	0.425	0.431	1.965	0.596	0.643	0.515	N/A	0.522
3-Aug	0.884	0.667	0.759	0.715	1.405	2.959	>5.25	0.753	0.645	0.487	N/A	0.641
10-Aug	0.416	0.376	0.304	0.328	>5.25	>5.25	>5.25	0.515	0.36	0.338	N/A	0.163
17-Aug	0. 800	0.613	0.525	0.433	13.8	22.9	18.73	0.597	0.709	0.764	N/A	0.183
24-Aug	0. 42	0.315	0.582	0.367	4.40	5.0	8.15	0.433	0.339	0.605	N/A	0.381
31-Aug	0.325	0.232	0.4	0.345	>5.25	>5.25	>5.25	0.268	0.345	0.293	N/A	0.566
7-Sep	0.334	1.554	0.814	0.555	>5.25	4.084	>5.25	0.48	0.489	0.578	N/A	0.41

Conclusion: Microcystin assays

1. Microcystin levels increased considerably between 2016 to 2017 at all sites sampled.

2. Several sites — specifically at Lake Zoar in August — exhibited Microcystin levels far above the federal limit of 4 ppb. The public was warned not to use the recreational waters during these periods.

3. The factors driving this increase in toxin concentrations are not clear, though nutrient levels and climate may play a role.

Cyanobacteria
Cyanotoxins
TOXIC
No swimming
No drinking

Acknowledgments

Wong Lab (WCSU): Ghada Salah Hafez, Robert McArthur, Kayla Zhang, Shane Peterson

Larry Marsicano

Candlewood Lake Authority

Health Departments of Danbury, New Fairfield, Sherman, New Milford, Brookfield, Newtown, Oxford

Dean of the Macricostas School of Arts & Sciences and the Office of University Relations, WCSU