HABs in NJ Freshwater Lakes: Monitoring, Detection & Management

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Cyanobacterial Toxins

- ~50 species of cyanobacteria have been shown to produce toxins which are harmful.
- Microcystis, Anabaena, Oscillatoria, Nodularia
- Three main types of cyanotoxins:
  - Neurotoxins,
  - hepatotoxins, and
  - dermatoxins
- Cyanotoxins have no current cure (CDC 2011)

Cyanobacterial Toxins

Seasonal occurrence and toxicity of *Microcystis* spp. and *Oscillatoria tenuis* in the Lebna Dam, Tunisia

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Predicting potentially toxigenic *Pseudo-nitzschia* blooms in the Chesapeake Bay

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Detection of Saxitoxin-Producing Cyanobacteria and *Anabaena circinalis* in Environmental Water Blooms by Quantitative PCR[∇]†

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Cyanobacterial Toxins: Removal during Drinking Water Treatment, and Human Risk Assessment

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Identification of HABs-causing microorganisms (phytoplankton)

- How?
  - Microscopy
  - Polymerase Chain React
  - Flow Cytometry

Develop Rapid PCR methods

- Optimization of the conditions
- Sensitivity study
- Test and design general or specific primers for this study

DNA Extractions

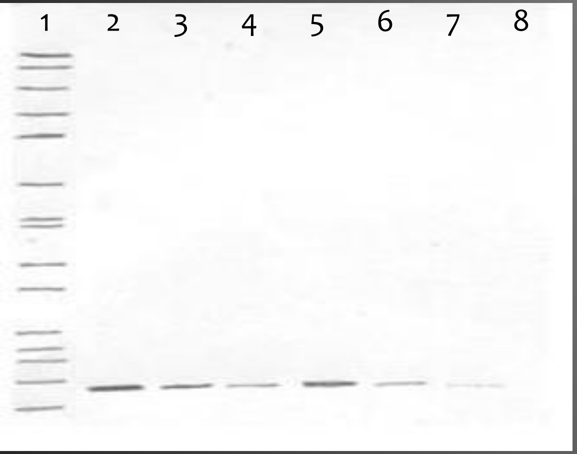
- A modified Chelex® DNA extraction protocol was used to isolate mixed population of DNA from environmental samples.

Primers Tested

No.	Primer name	Primer sequence (5' → 3')	Tm (°C)	Amplicon size (nt)	Gene	Source
1	Uf	GAGAGTTTGATCCTGGTCAG	52-56	791	16s rRNA, phytospecific species	Stiller & McClanahan, 2005. Canavate et al 2014
	PSr	CCCTAATCTATGGGGWCATCAGGA				
2	27FB	AGAGTTTGATCCTGGCTCAG	58	740	16s rRNA of all bacteria, cyanobacteria and phytoplankton	Barkovskii & Fukui, 2004.
	785R	ACTACCRGGGTATCTAATCC				
3	PSf	GGGATTAGATACCCCWGTAGTCCT	50	735	16s rRNA in phytoplankton, general photosynthetic bacteria (Ur)	Stiller & McClanahan, 2005.
	Ur	ACGGYTACCTTGTTACGACTT				
4	CPC1f	GGCKGCYTGYYTRCGYGACATGGA	50	389	β-subunit of phycocyanin gene for cyanobacteria	Stiller
	CPC1r	AARCGNCTTGVGWATCDGC				
5	AN3801f	CAAATCACTCAGTTTCTGG	55	171	DNA polymerase III of *S.* IU 625 & *S. elongatus*	Chu & Rienzo 2013
	AN3801r	CAGTAGCAGCTCAGGACTC				
6	ANAf	GATCTAGCCTCACCTGTTGACTT	55-58	457	Toxin biosynthesis gene cluster of *Anabaena circinalis*	Chu & Rienzo 2013
	ANAr	GGGATCCTTTTTGCTGCGCC				
7	Msf	ATCCAGCAGTTGAGCAAGC	58	1369	*mcyA*	Rillet et al. 2001
	Msr	TGCAGAAAACTCCGCAGTTG				
8	2156f	ATCACTTCAATCTAACGACT	50	973	*mcyB*	Mikalsen et al 2003
	3111r	AGTTGCTGCTGTAAGAAA				
9	PSCf	GCAACATCCCAAGAGCAAAG	58	674	*mcyC*	Ouahid et al 2005
	PSCr	CCGACAACATCACAAAGGC				
10	DINAf	GAATCTGCCCTCAGGAGGGGG	58	2283(syn) 2134(proch)	16S-23S ITS Prochlorococcus & Synechococcus (predominantly marine species)	lavin et al. 2008
	DINAr	GGGTTGCCCCATTCGGAAAT				

No.	Primer name	Primer sequence (5' → 3')	Tm (℃)	amplicon size (nt)	gene	source
1	Pro-psbA-1F	AACATCATYTCWGGTGCWGT	52	773	*psbA*	Chenard & Suttle,2008
	Pro-psbA-1R	TCGTGCATTACTTCCATACC				
2	MCPF5	GTTCCTGGACACCTGAAGCGT	56	350	*mcp*	Baker et. al 2006
	MCPR5	GAT GCC GAC ACA AGC GAT GGT AAG				
3	GSPATG00022332001F	TGGCATTGGATAATGCAGAA	52.3	616	*E1B*	Catania et al 2008
	GSPATG00022332001R	GTAGAGGCAATGGCGTGAAT	55.4			
4	GSPATG00000223001F	TTCCCTGACCGAATAGGATTT	53.4	592	calcium binding protein gene	Catania et al 2008
	GSPATG00000223001R	GGCCATAAGCATCCAAGATT				
5	Syn3	TACGACTTCACCCCAGTCAYCAGCC	50	1362	ssu rDNA	Sanchez-Baracaldo et al 2008
	Syn5	CAGGATGAACGCTGGCGGCYSTGC				
6	16s.19F	AAGCCTGACGGAGCAACGCC	63	362	ssu rDNA	Sanchez-Baracaldo et al 2008
	16S.409R	GGTATCTAATCCCTTTCGCTCC	60			
7	phnD_syn119F	TCGGNGCMATYCCSGATCAGAACCCSG	55-60	616-618	*phnD*	Ilikchyan et al 2009
	phnD_syn734R1	TTGGGCTGSGCGASCCAGTGGTARTC				
8	phnD_pro307F	GTNATWGCTCAAAGAGATATWGAT	50-57	242	*phnD*	
	phnD_pro551R	GTTGCATCATGACTNCCRCTATANCC				
9	PITSANF	CGTAACAAGGTAGCCGTAC	46	839	ITS-1	becker et al 2002
	PITSEND	CTCTGTGTGCCAAGGTATC	45			
10	MICF	ATGTGCCGCGAGGTGAAACCTAAT	55	238	16s rRNA	Hotto et al 2007
	MICR	TTACAAYCCAARRRCCTTCCTCCC				
11	mcyAF	AAAAGTGTTTTATTAGCGGCTCAT	55	297	*mcyA*	Hotto et al 2007
	mcyAR	AAAATTAAAAGCCGTATCAAA				
12	mcyE-F	GAAATTTGTGT(A/C)GAAGGTGC	55	247	*mcyE*	Hotto et al 2007
	mcyE-R	CAATGGGAGCATAACGAG				
13	mcyE-F	GAAATTTGTGTAGAAGGTGC	55	247	*mcyE*	Hotto et al 2007
	mcyE-R	CAATCTCGGTATAGCGGC				
14	mcyE-F	GAAATTTGTGTA/CGAAGGTGC	55	327	*mcyE*	Hotto et al 2007
	mcyE-R	CTCAATCTGAGGATAACGAT				
15	mcyE-F2a	GAAATTTGTGT(A/C)GAAGGTGC	60	247	*mcyE*	Vaitomaa et al 2003
	MicmcyE-R8	CAATGGGAGCATAACGAG				
16	mcyE-F2a	GAA ATT TGT GTA GAA GGT GC	58	262	*mcyE*	Vaitomaa et al 2003
	AnamcyE-12R	CAA TCT CGG TAT AGC GGC				
17	anxgenF	ATGGTCAGAGGTTTTACAAG	52	861	*anaC*	Rantala-Ylinen et al 2011
	anxgenR	CGACTCTTAATCATGCGATC				
18	ana-C-genF	TCTGGTATTCAGTCCCCTCTAT	58	366	*anaC*	Rantala-Ylinen et al 2011
	ana-C-genR	CCCAATAGCCTGTCATCAA				
19	anaC-anabF	GCCCGATATTGAAACAAGT	60	263	*anaC*	Rantala-Ylinen et al 2011
	anaC-anabR	CACCCTCTGGAGATTGTTTA				
20	anaC-oscF	CTCTATTCTCACAAGTTTGGTCT	60	216	*anaC*	Rantala-Ylinen et al 2011
	anaC-oscR	GTTAGTTCAATATCAAGTGGTGGA				

PCR Sensitively Limit



Lane	Sample	Storage Temp (°C)	Starting cell count
1	1 Kb Ladder	------	------
2	AN DNA	-20	4×10^4
3	AN DNA	-20	4×10^3
4	AN DNA	-20	4×10^2
5	AN DNA	-70	4×10^4
6	AN DNA	-70	4×10^3
7	AN DNA	-70	4×10^2
8	Negative control	------	------

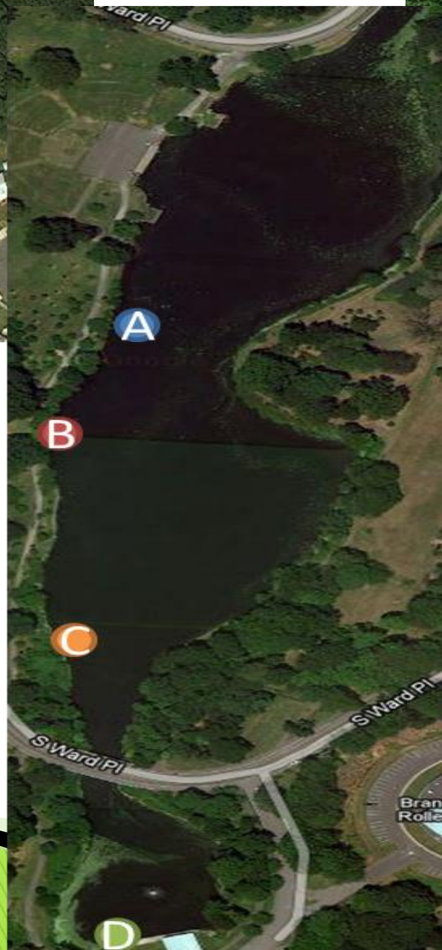
Freshwater Ponds & Lakes

- Clarks Pond – Bloomfield, NJ
- Diamond Mill Pond – Millburn, NJ
- South Orange Duck Pond – South Orange, NJ
- Branch Brook State Park Lake – Newark, NJ
- Verona Lake – Verona, NJ

The work has been published in 2013

Clarks Pond

Branch Brook Lake

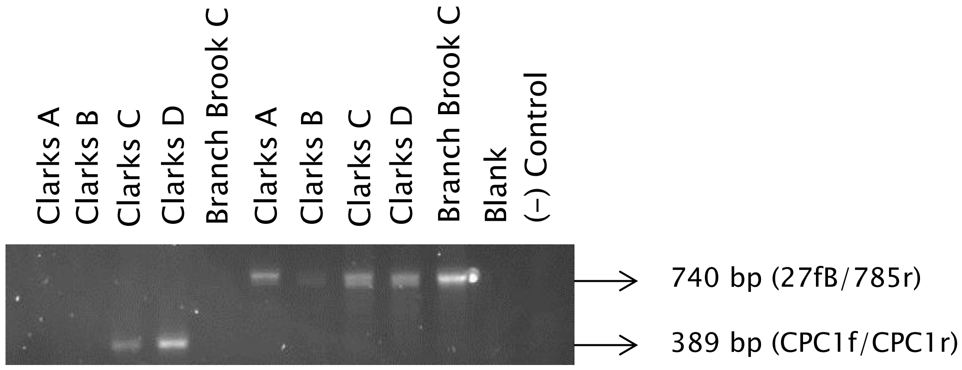


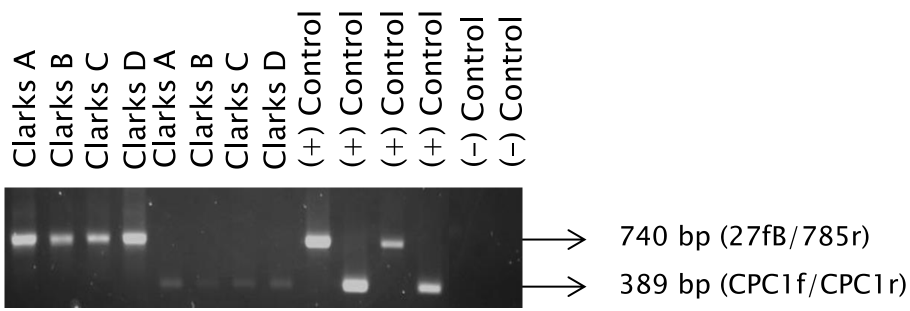
Diamond Mill Pond

Verona Lake

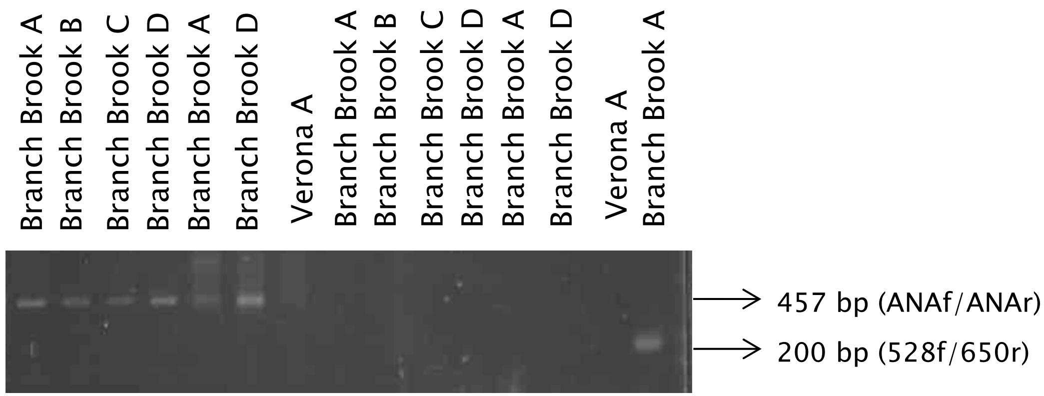
uth Orange Duck Pond

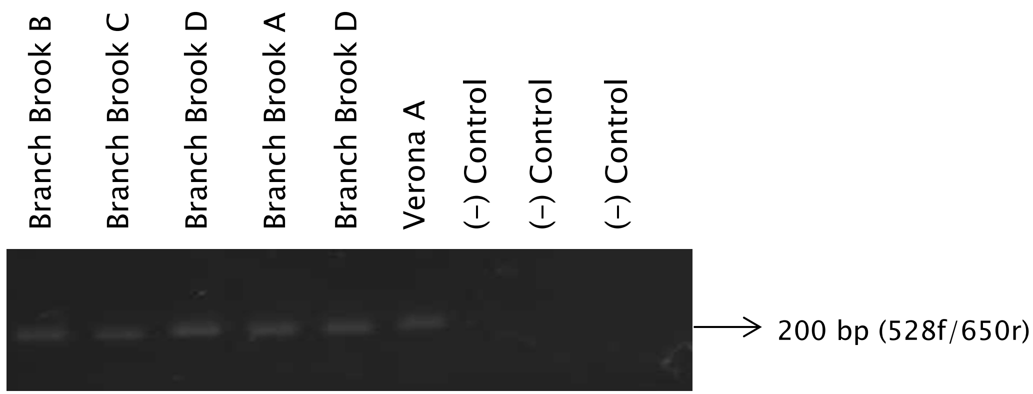
PCR Results (Summer)





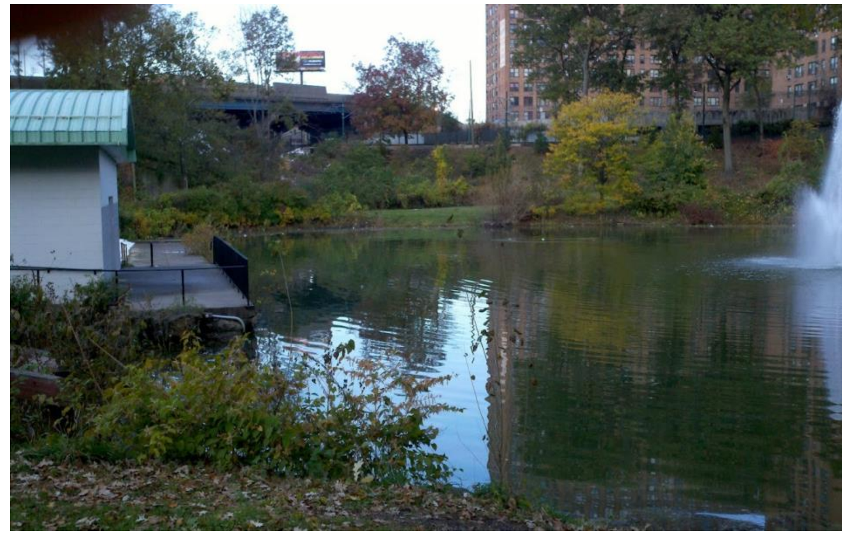
PCR Results (Fall)





Seasonal Eutrophication

Branch Brook State Park Lake
(June 14th, 2011)



Branch Brook State Park Lake
(November 1st, 2011)

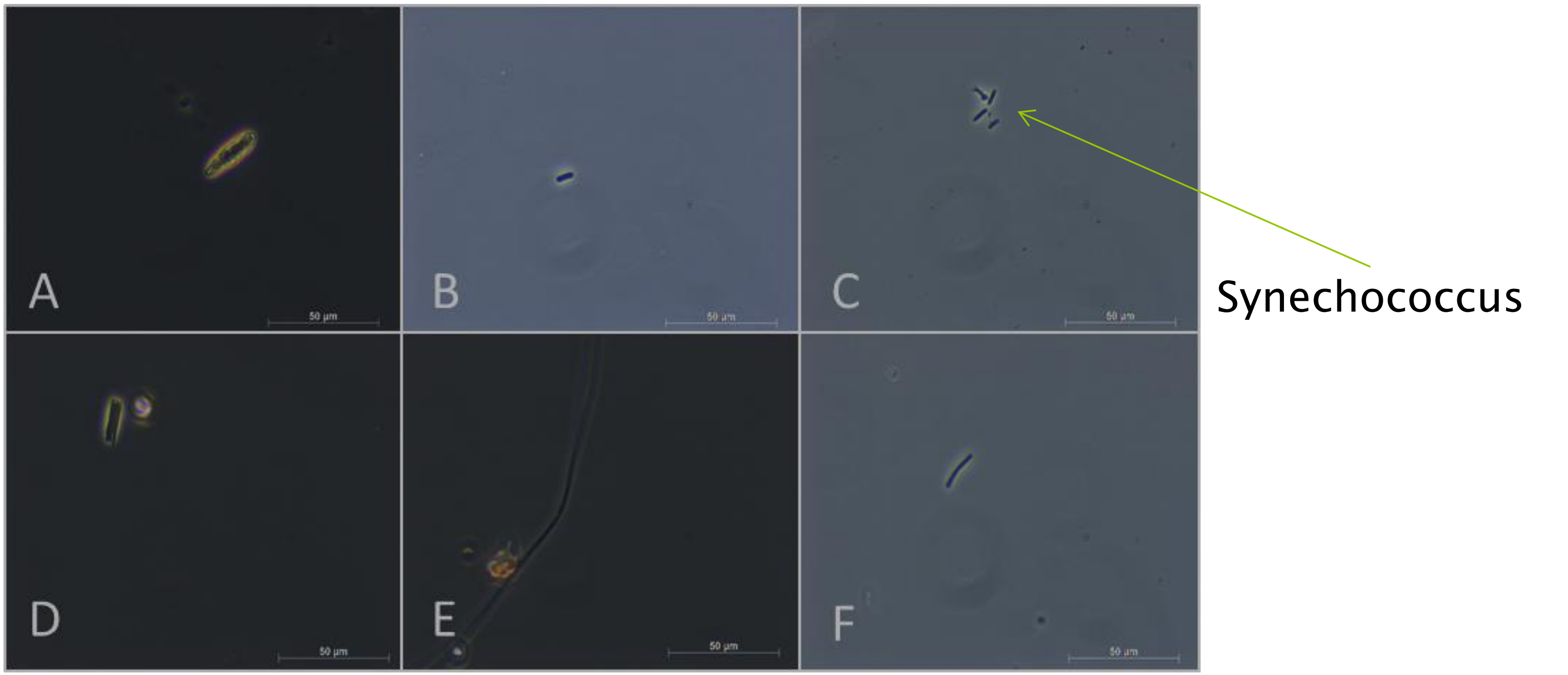
Cyanobacterial PCR Detection Summary – Summer vs. Fall

Water bodies	Sites collected	Summer positive detection	Fall positive detection
Brank Brook State Park	A, B, C, D	A, B, C, D	A, B, C, D
Clarks Pond	A, B, C, D, E	A, B, C, D	ND*
Diamond Mill Pond	A, B, C, D	A, C	ND*
South Orange Duck Pond	A, B, C, D	A, B	ND*
Verona Lake	A, B, C	A	A

Table 3. Summary of cyanobacterial detection in the summer and in the fall among 5 water bodies. ND indicates non-detectable. The results showed the fall water samples contain fewer cyanobacteria.

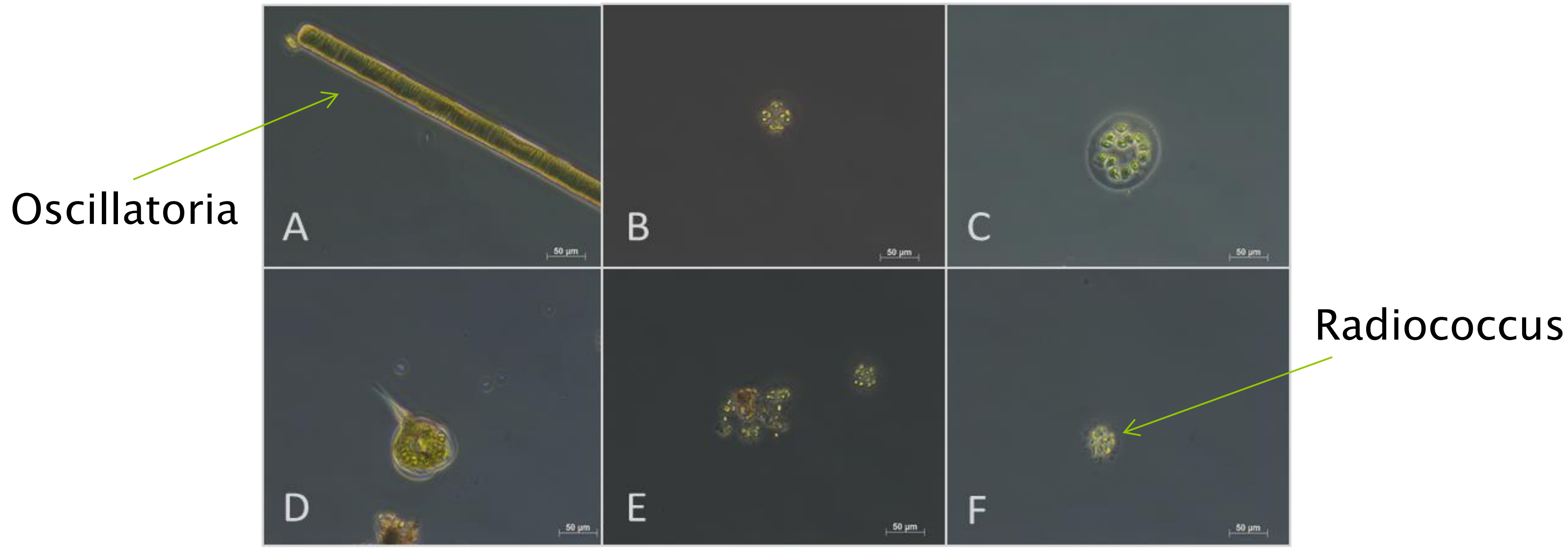
Microscopic Analysis

Cyanobacteria from Diamond Mill Pond



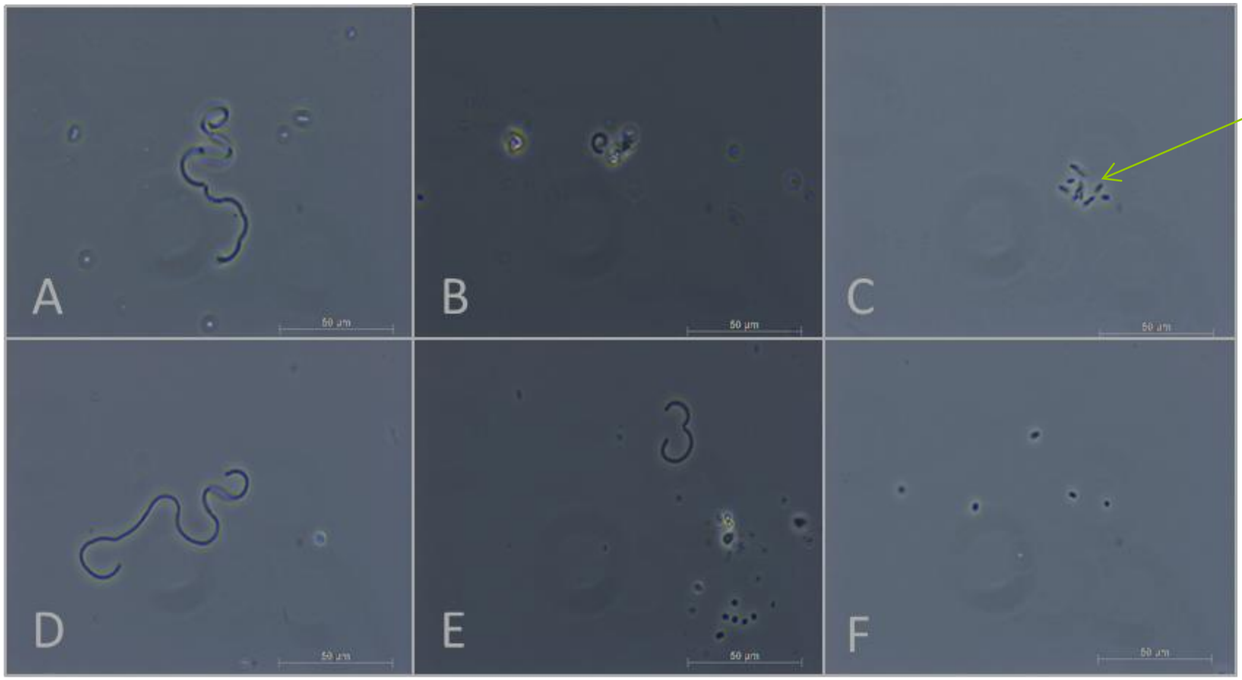
Microscopic Analysis

Cyanobacteria from Branch Brook Park



Microscopic Analysis

Cyanobacteria from South Orange Duck Pond

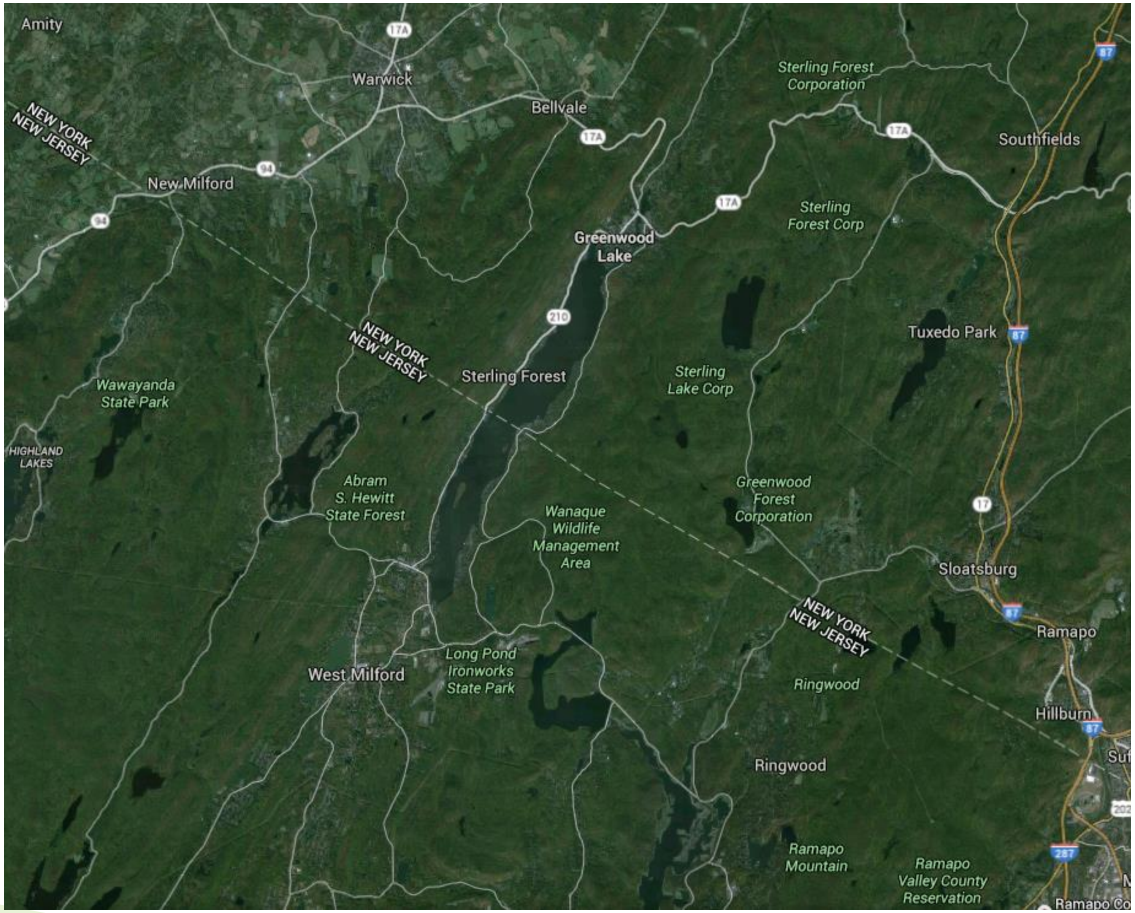


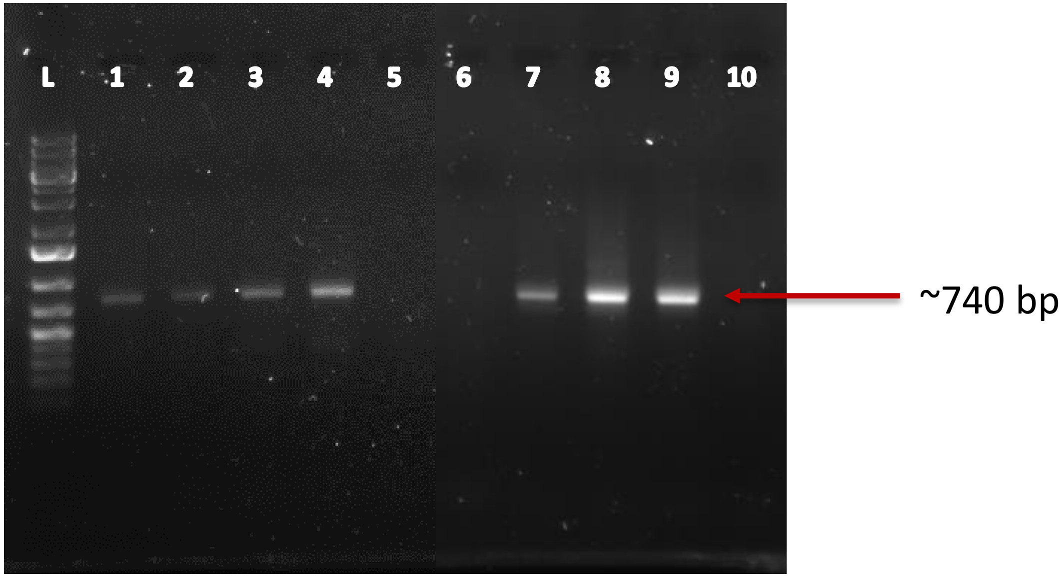
Water Body	Site	Diatoms	Cyanobacteria	Photosynthetic Bacteria
Branch Brook Lake	A	PCR & MI	PCR & MI	PCR & MI
	B	PCR & MI	PCR	PCR & MI
	C	PCR	PCR	PCR & MI
	D	PCR	PCR	PCR & MI
Clarks Pond	A	MI	MI	PCR & MI
	B	MI	MI	PCR & MI
	C	MI	MI	PCR & MI
	D	MI	MI	PCR & MI
Diamond Mill Pond	A	MI	MI	PCR & MI
	B	MI	MI	PCR & MI
	C	MI	MI	PCR & MI
	D	MI	MI	PCR & MI
	E	MI	MI	PCR & MI
South Orange Duck Pond	A	MI	MI	PCR & MI
	B	MI	MI	PCR & MI
	C	MI	MI	PCR & MI
	D	MI	MI	PCR & MI
Verona Lake	A	MI	PCR	PCR & MI
	B	MI	MI	PCR & MI
	C	MI	MI	PCR & MI

Table 6. The correlation between microscope findings and PCR findings from fall collections is depicted.

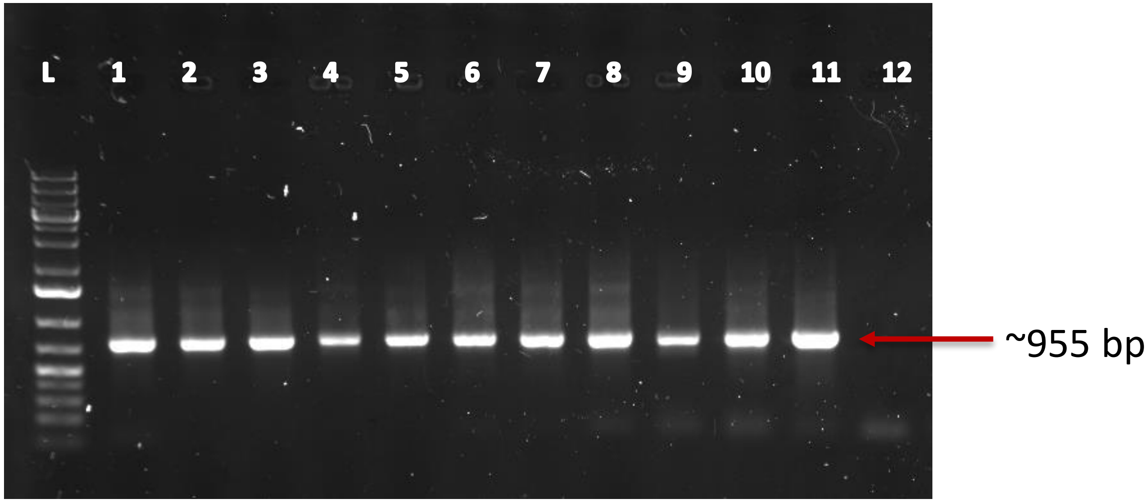
Summer 2015

- Greenwood Lake

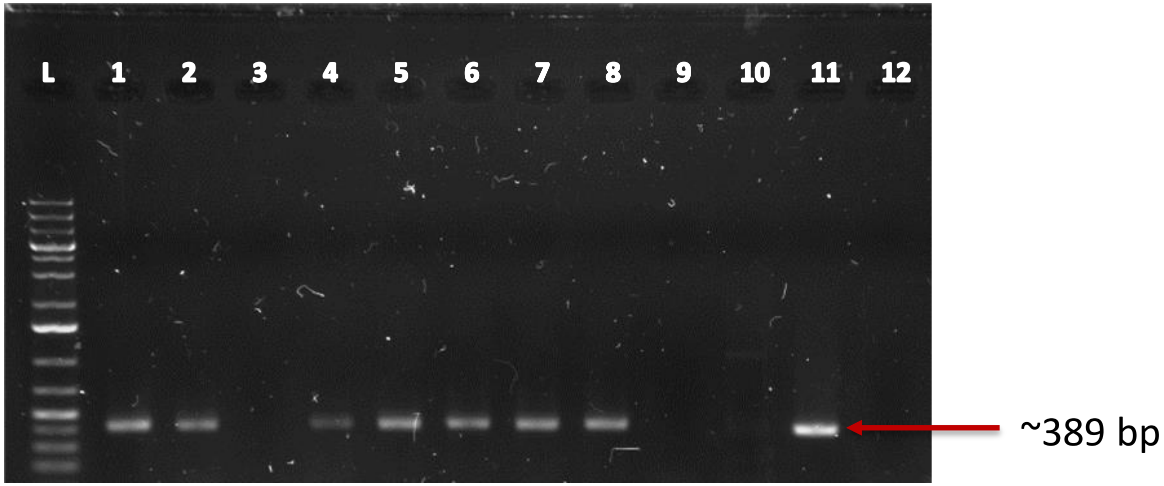




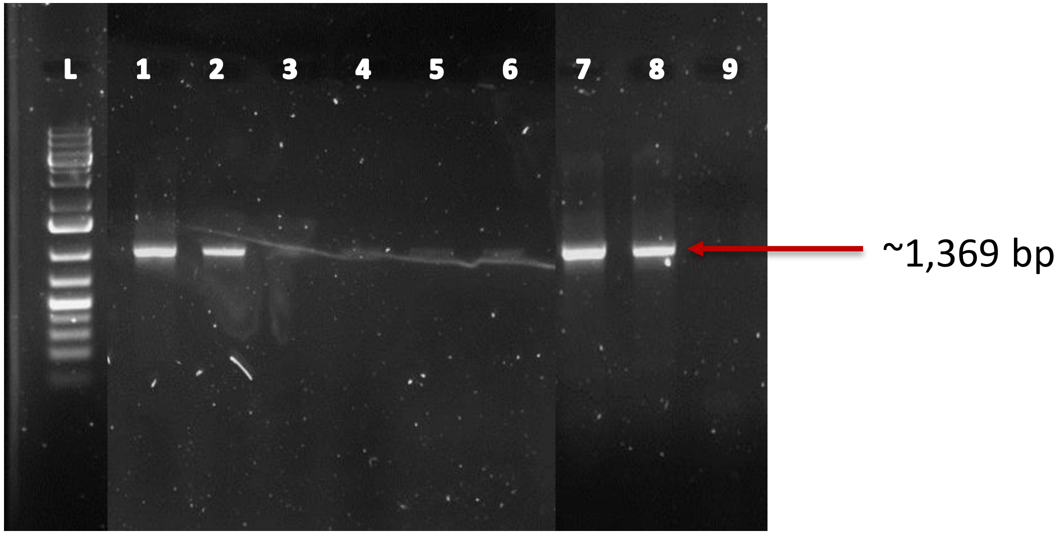
Lane	Template	Primer Set	Tm (°C)	Amplicon (nt)
1	WS1-1	27FB + 785R	58	740
2	WS1-2	27FB + 785R	58	740
3	WS1-3	27FB + 785R	58	740
4	WS1-4	27FB + 785R	58	740
5	NC	27FB + 785R	58	740
6	WS1-B1-1	27FB + 785R	58	740
7	WS1-B1-2	27FB + 785R	58	740
8	WS1-B1-3	27FB + 785R	58	740
9	WS1-B1-4	27FB + 785R	58	740
10	NC	27FB + 785R	58	740



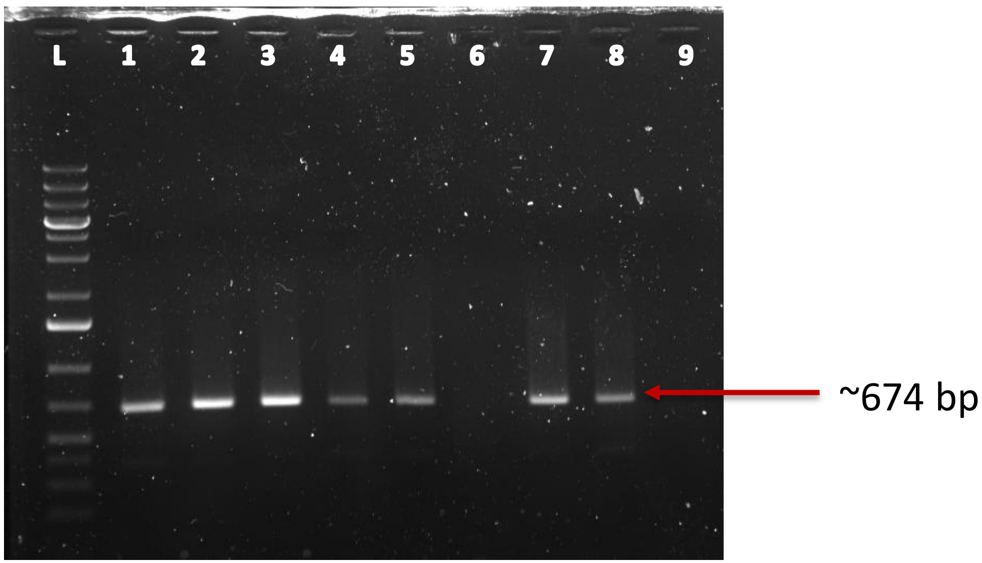
Lane	Template	Primer Set	Tm (°C)	Amplicon (nt)
1	WS1-1	PSf+Ur	50	955
2	WS1-2	PSf+Ur	50	955
3	WS1-3	PSf+Ur	50	955
4	WS1-4	PSf+Ur	50	955
5	WS1-B1-1	PSf+Ur	50	955
6	WS1-B1-2	PSf+Ur	50	955
7	WS1-B1-3	PSf+Ur	50	955
8	WS1-B1-4	PSf+Ur	50	955
9	Mixed1	PSf+Ur	50	955
10	Mixed2	PSf+Ur	50	955
11	S.IU625	PSf+Ur	50	955
12	NC	PSf+Ur	50	955



Lane	Template	Primer Set	Tm (°C)	Amplicon (nt)
1	WS1-1	CPC1f+CPC1r	50	389
2	WS1-2	CPC1f+CPC1r	50	389
3	WS1-3	CPC1f+CPC1r	50	389
4	WS1-4	CPC1f+CPC1r	50	389
5	WS1-B1-1	CPC1f+CPC1r	50	389
6	WS1-B1-2	CPC1f+CPC1r	50	389
7	WS1-B1-3	CPC1f+CPC1r	50	389
8	WS1-B1-4	CPC1f+CPC1r	50	389
9	Mixed1	CPC1f+CPC1r	50	389
10	Mixed2	CPC1f+CPC1r	50	389
11	S.IU625	CPC1f+CPC1r	50	389
12	NC	CPC1f+CPC1r	50	389

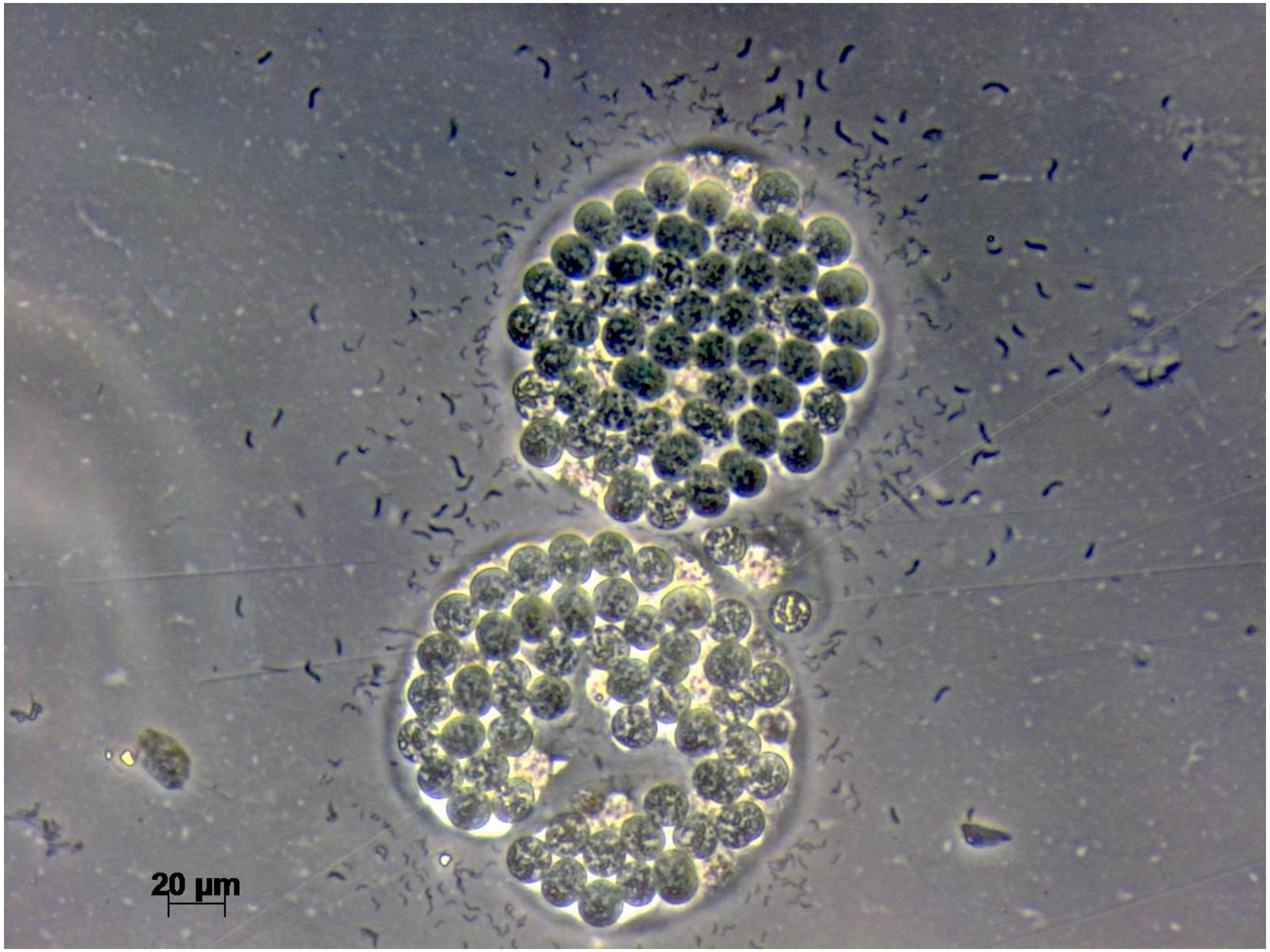


Lane	Template	Primer Set	Tm (°C)	Amplicon (nt)
1	WS1-1	MSf + MSr	58	1369
2	WS1-2	MSf + MSr	58	1369
3	WS1-3	MSf + MSr	58	1369
4	WS1-4	MSf + MSr	58	1369
5	WS1-B1-1	MSf + MSr	58	1369
6	WS1-B1-2	MSf + MSr	58	1369
7	WS1-B1-3	MSf + MSr	58	1369
8	WS1-B1-4	MSf + MSr	58	1369
9	NC	MSf + MSr	58	1369



Lane	Template	Primer Set	Tm (°C)	Amplicon (nt)
1	WS1-1	PSCf + PSCr	58	674
2	WS1-2	PSCf + PSCr	58	674
3	WS1-3	PSCf + PSCr	58	674
4	WS1-4	PSCf + PSCr	58	674
5	WS1-B1-1	PSCf + PSCr	58	674
6	WS1-B1-2	PSCf + PSCr	58	674
7	WS1-B1-3	PSCf + PSCr	58	674
8	WS1-B1-4	PSCf + PSCr	58	674
9	NC	PSCf + PSCr	58	674

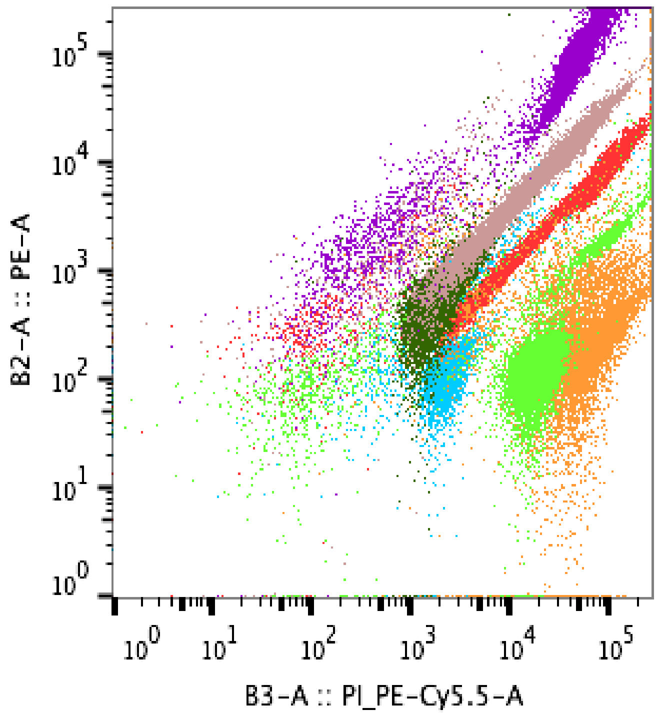
Microscopic Analysis

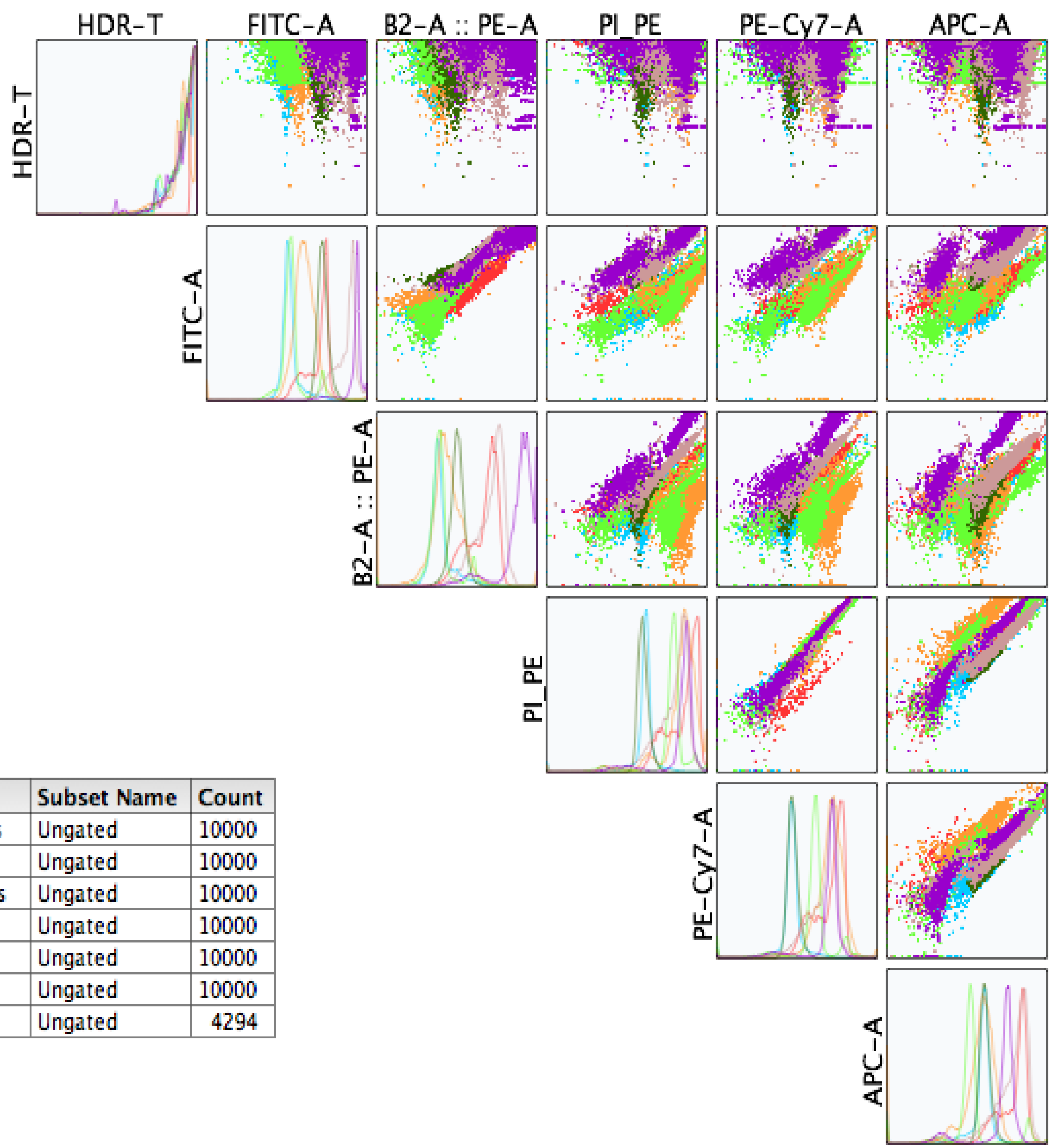


Cyanotoxin Detection with Flow Cytometer

- Several commercial available methods such as ELISA
- Field test kit: immunochromatographic strip test
- Protein phosphatase inhibition assay

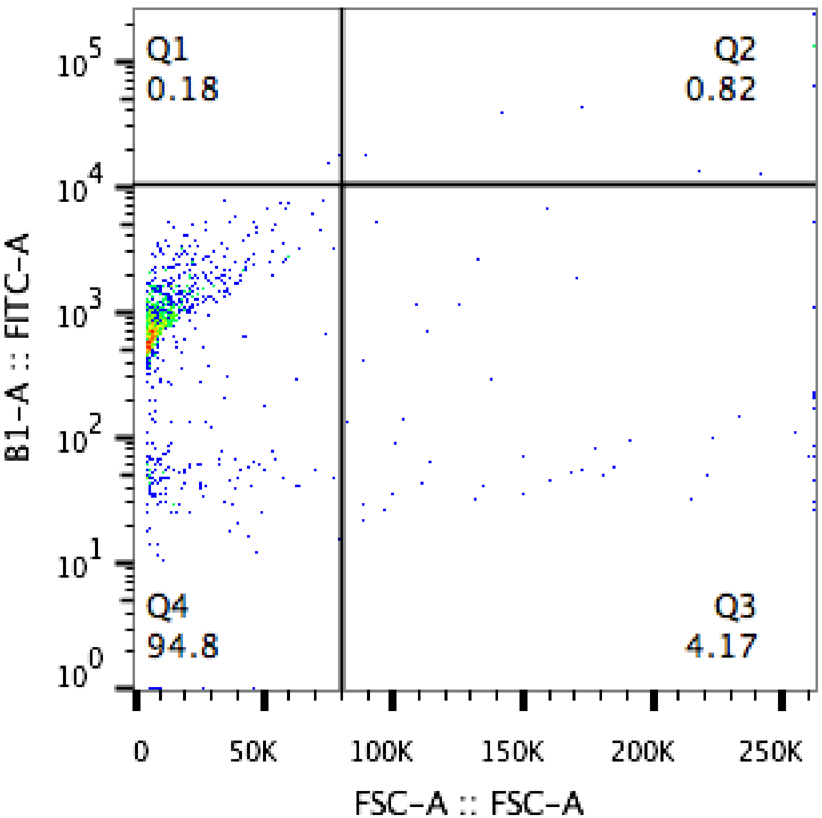
Cost, accuracy, time??



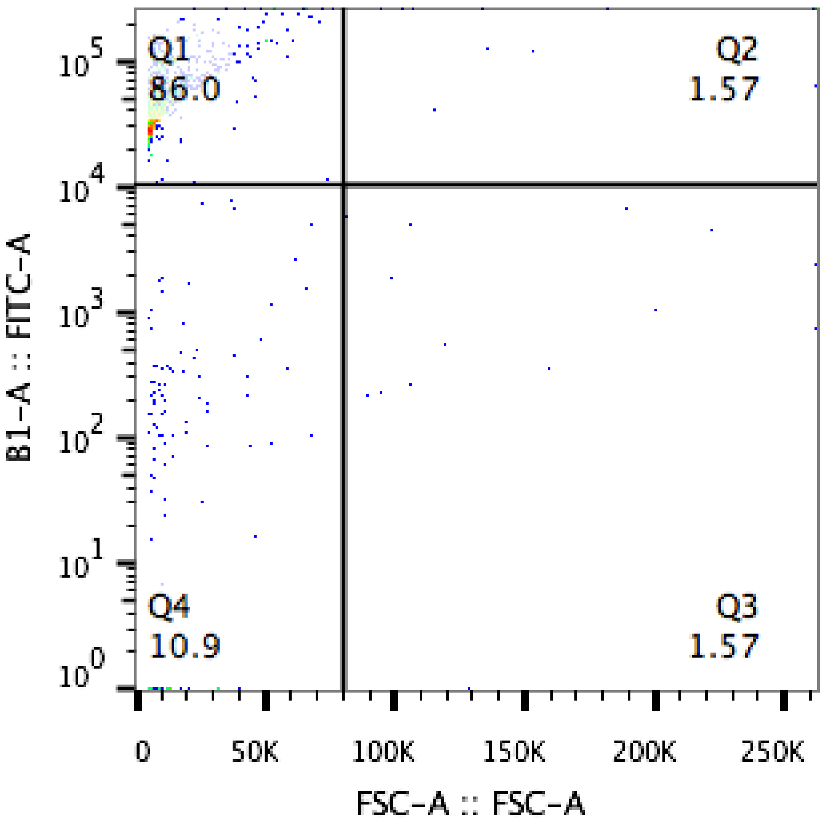


	Sample Name	Subset Name	Count
	TC2015-09-14gleotrichia_OldSettings_1000.001.fcs	Ungated	10000
	TC2015-09-14cyl_OldSettings_1000.001.fcs	Ungated	10000
	TC2015-09-14Microcystis_OldSettings_1000.001.fcs	Ungated	10000
	Oscil2.006.fcs	Ungated	10000
	Nostoc.003.fcs	Ungated	10000
	Anabeana.002.fcs	Ungated	10000
	SIU625.009.fcs	Ungated	4294

Flow Cytometry Results

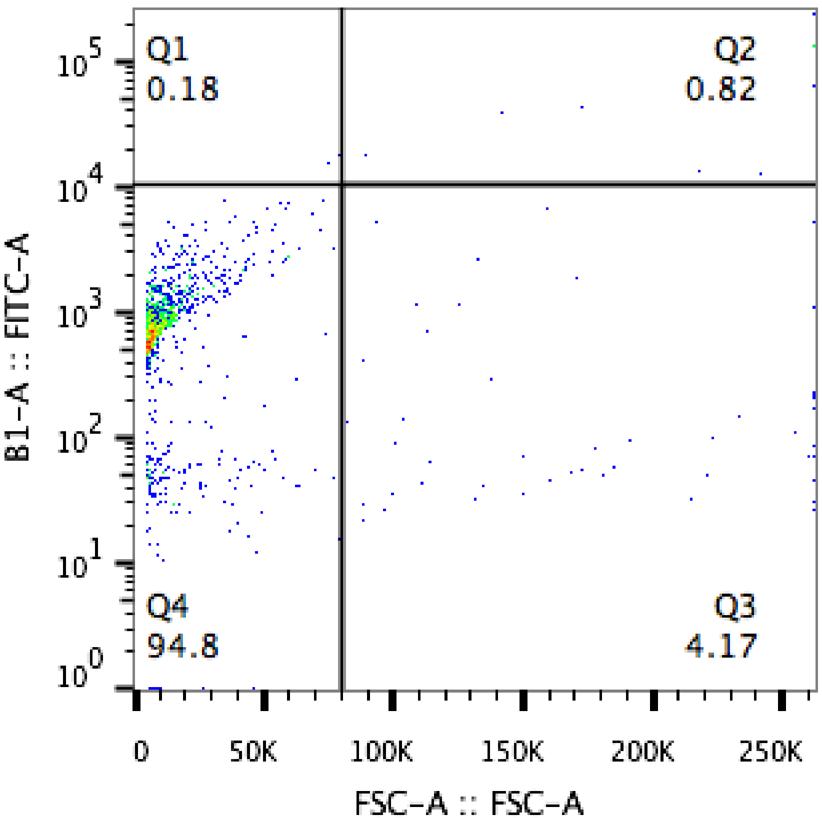


TC2015-09-14beadsonly.003.fcs
Ungated
1102

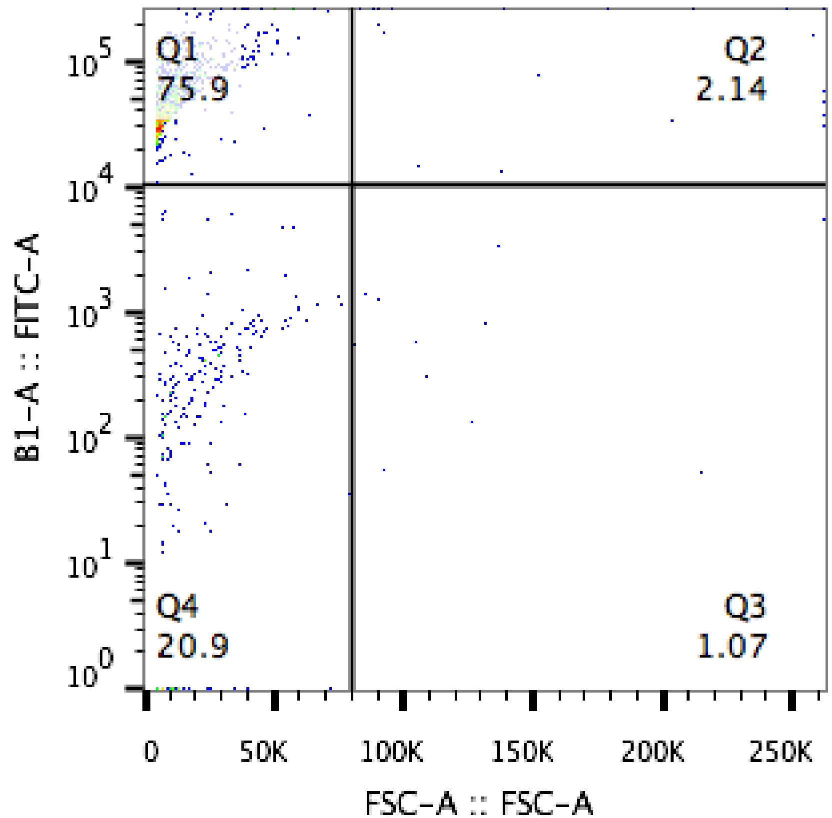


TC2015-09-14_5ppb_beads_ab_2.001.fcs
Ungated
1027

Flow Cytometry Results

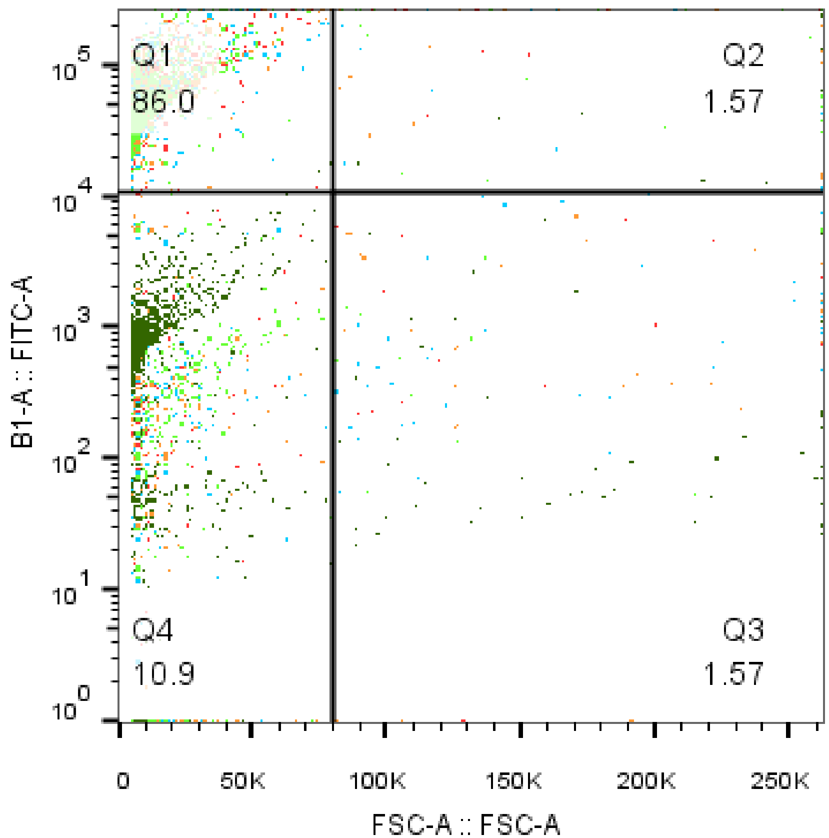


TC2015-09-14beadsonly.003.fcs
Ungated
1102

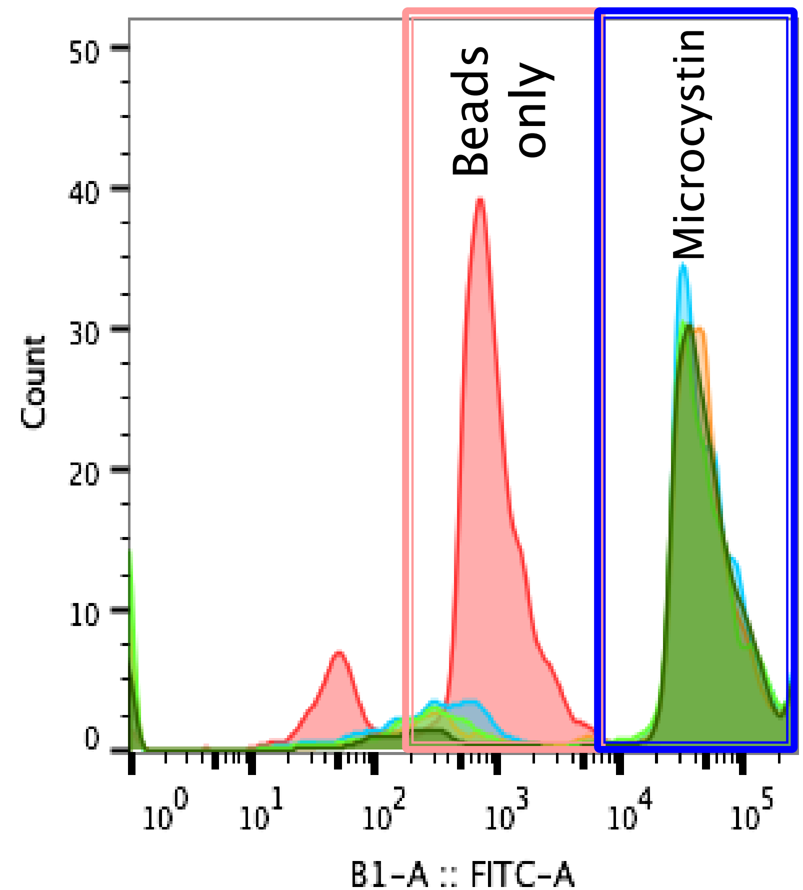


TC2015-09-14_0_1ppb_beads_ab_2.002.fcs
Ungated
890

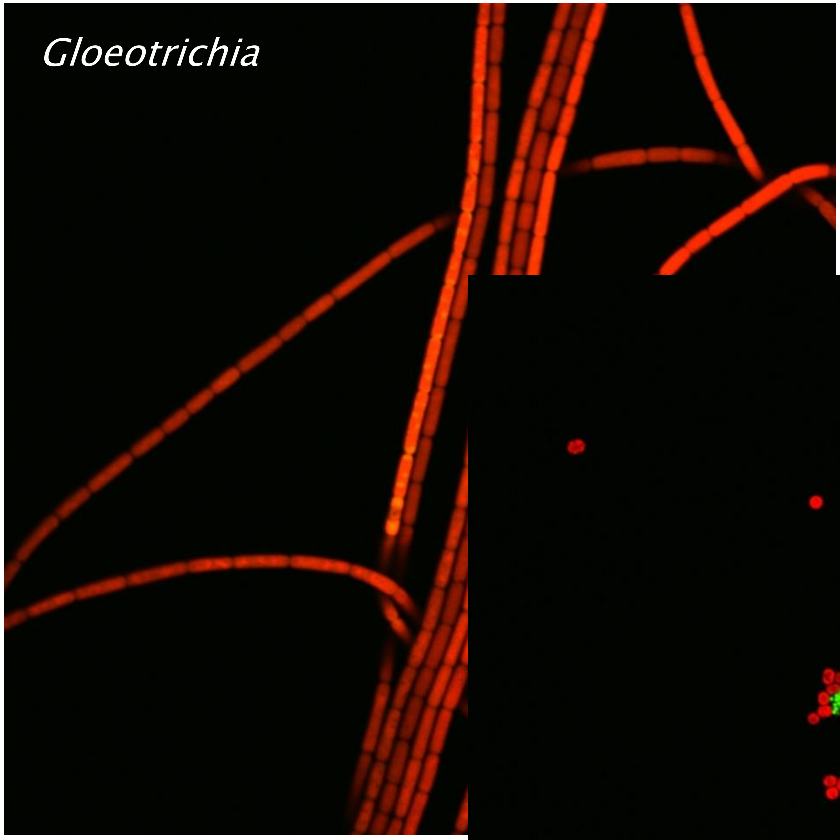
Flow Cytometry Results

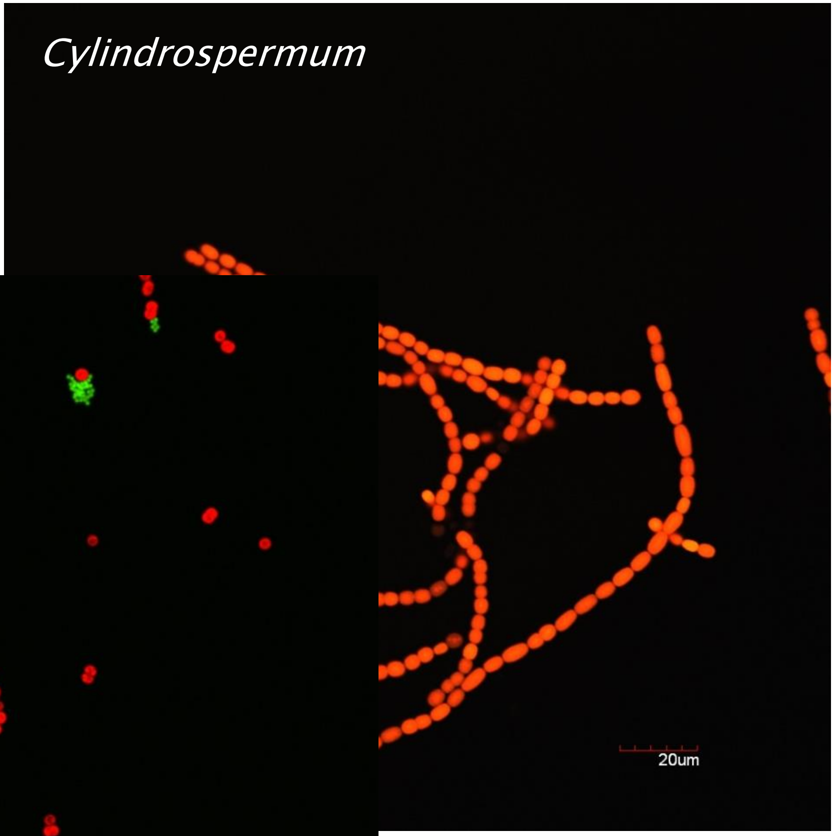


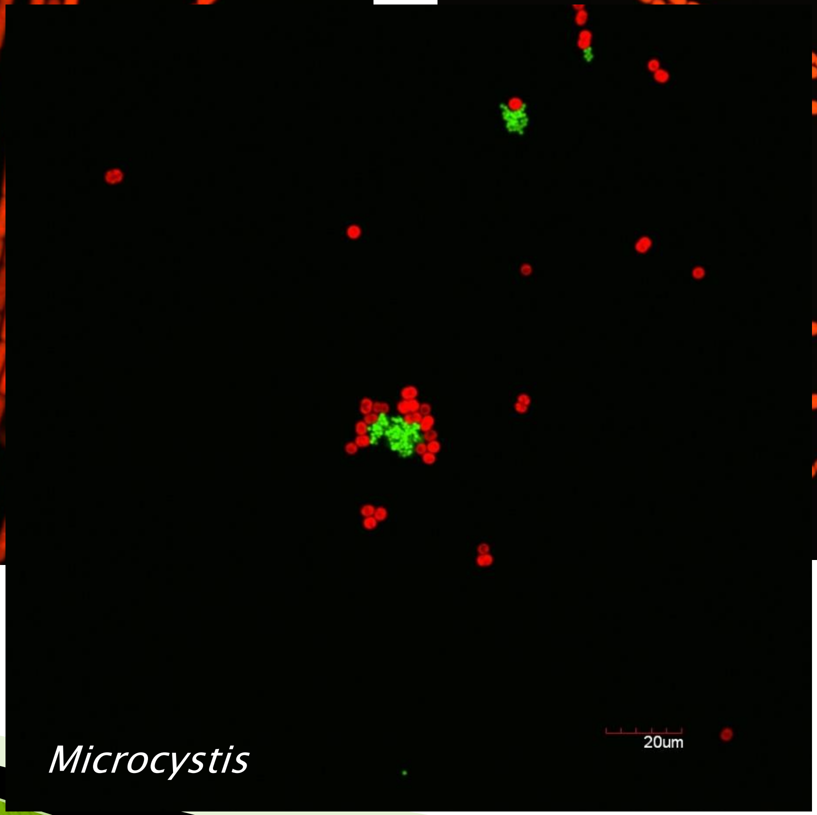
	Sample Name	Count
(dark green)	TC2015-09-14beadsonly.003.fcs	1102
(light green)	TC2015-09-14_5ppb_beads_ab_2.001.fcs	1027
(orange)	TC2015-09-14_1ppb_beads_ab_2.001.fcs	930
(blue)	TC2015-09-14_0_5ppb_beads_ab_2.001.fc	934
(red)	TC2015-09-14_0_1ppb_beads_ab_2.002.fc	890



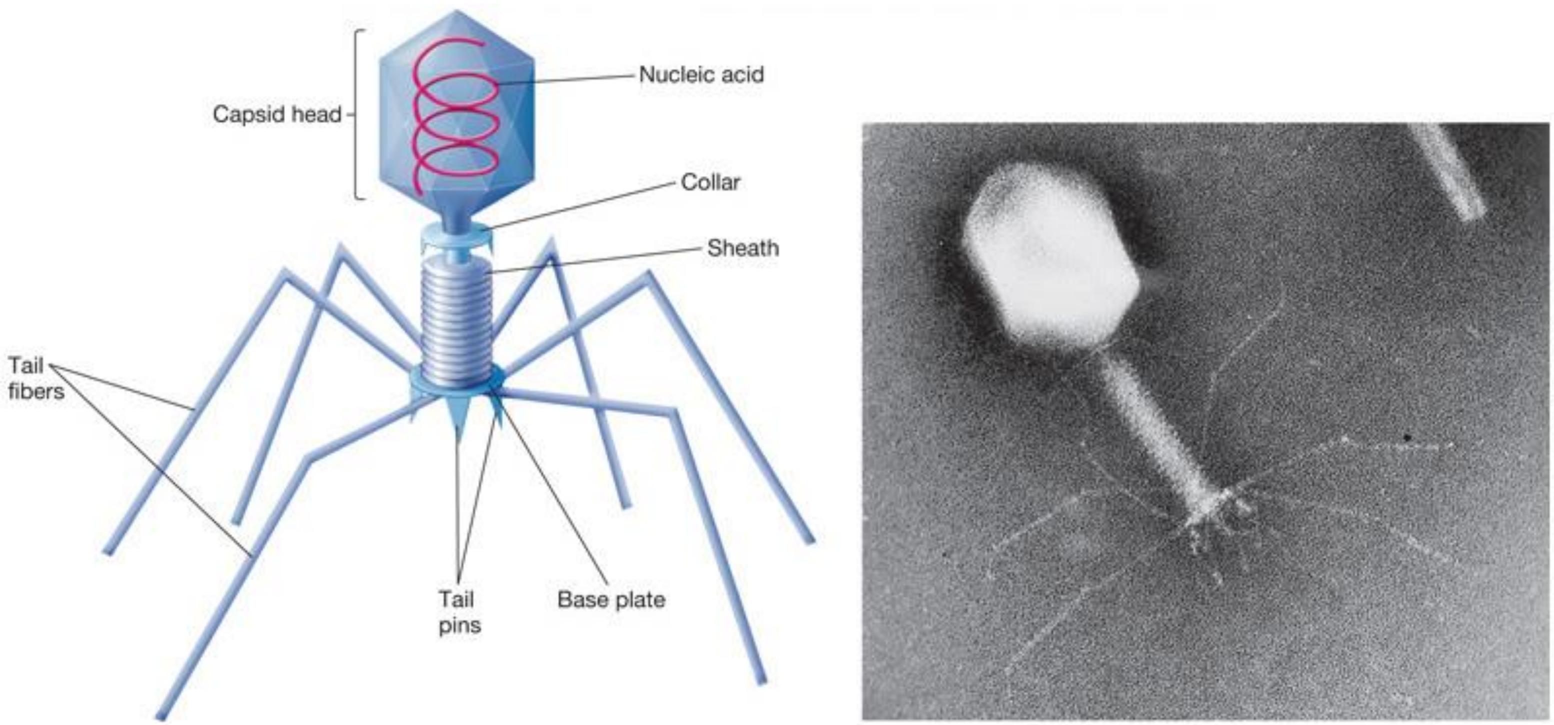
Confocal Fluorescence Microscopy







Cyanophage



(a) (b)

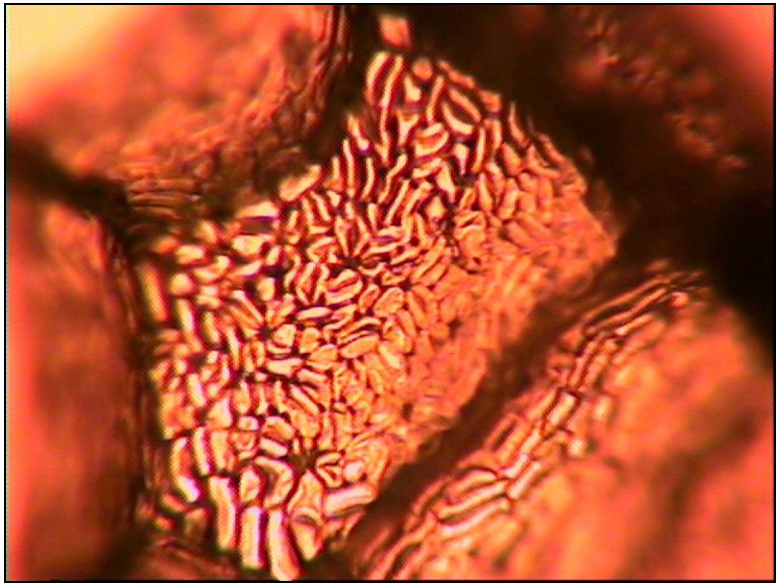
b: © Harold Fisher

Ultrasound

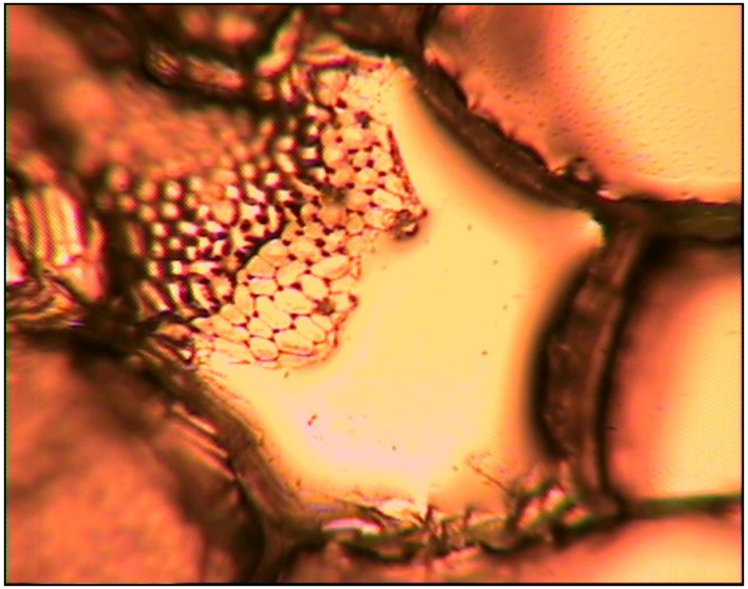
Ultrasound:

- sound wave
- above the audible frequency range for humans
- two major bioeffects:
  - thermal effect: energy is converted into heat
  - acaustic cavitation: cause bubbling activities

Damage Cellular Structure

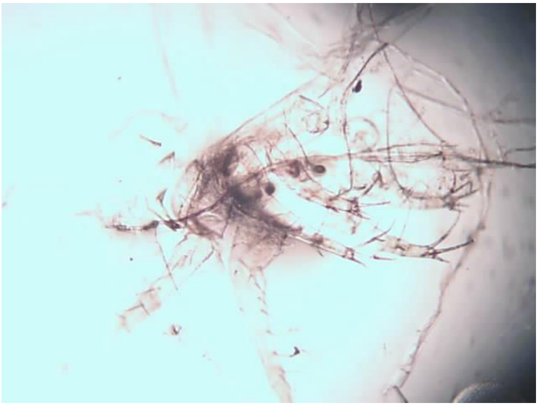


Control



Treatment

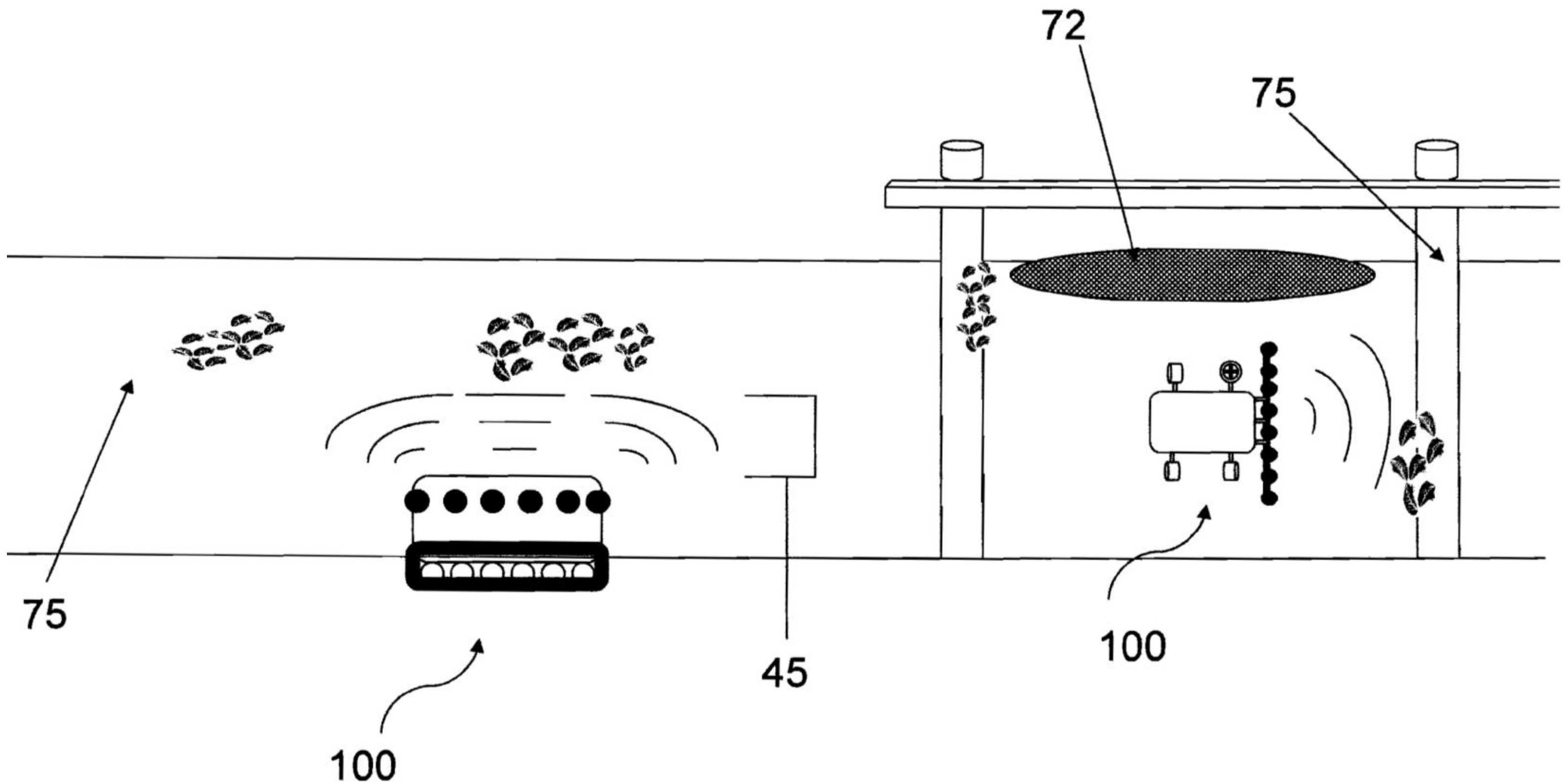
Ultrasonic Control



effectively control *E. coli*, daphnia, copepods & jellyfish (mortality rates > 99

Treatment of Ship Ballast Water

U.S. Patent 7799233 & 8062587B2 and 61/428,479


72
75
75
45
100
100
100

Thank You!