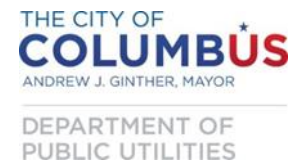


# Assessing Ultrasound as a Source Water Reservoir Management Strategy to Control Cyanobacteria Blooms

C.R. Weaver, Linda Weavers, Zuzana Bohrerova, Jason Cheng, Yousuf  
Yousuf and Alex Viera



Funding:

Ohio Water  
Development Authority

# Can ultrasound in your reservoir ...

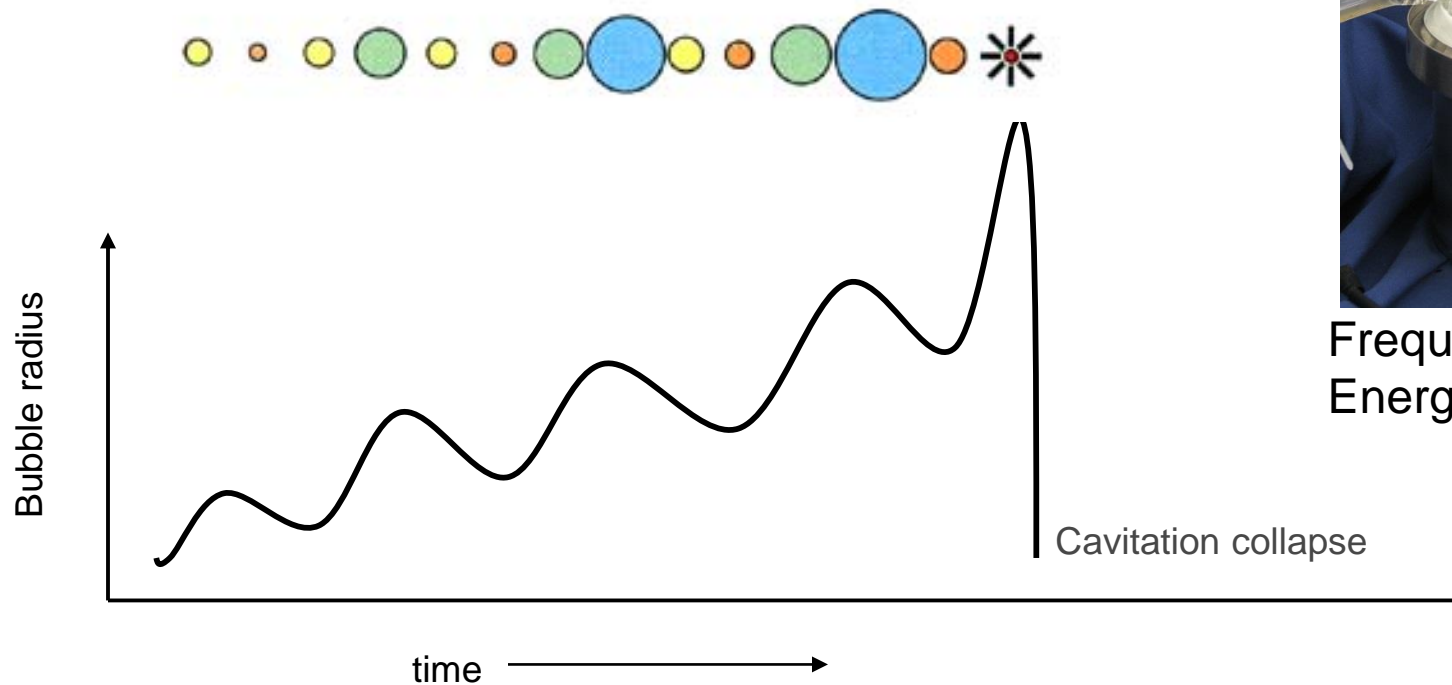
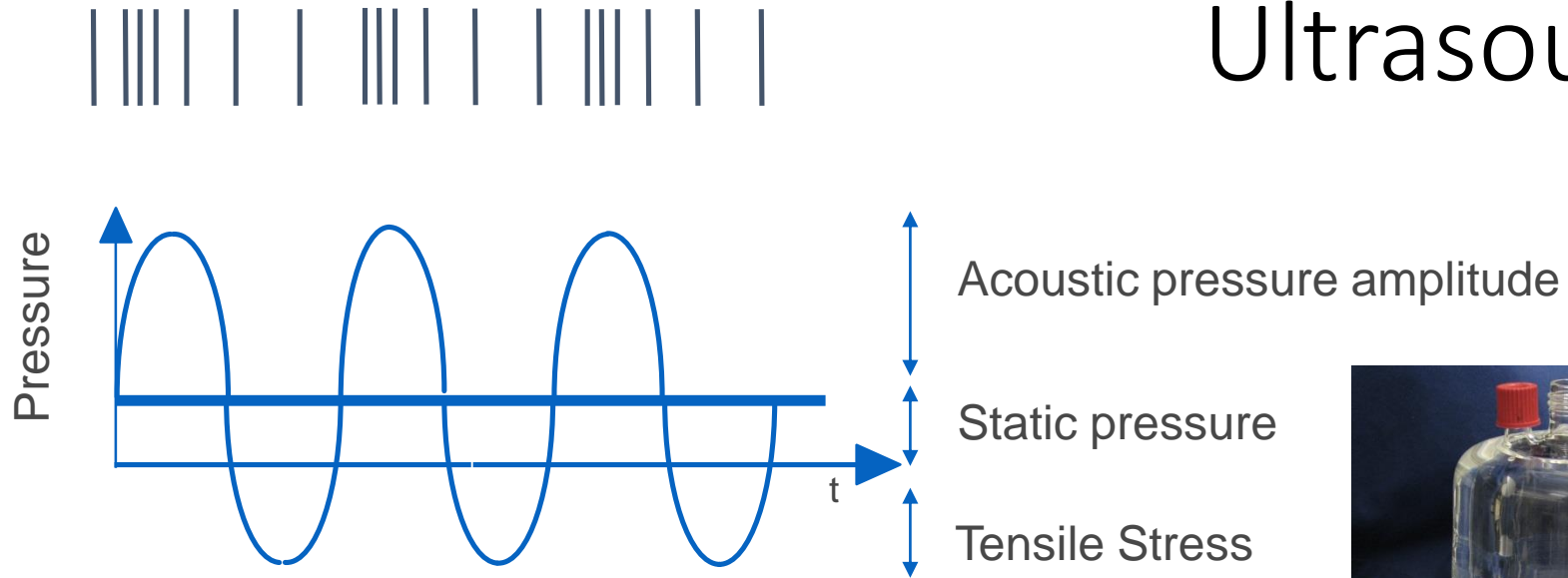
...turn this...



...into this?



# Ultrasound



Frequency: 205 kHz  
Energy input: 45 W/L

# Cavitation Bubble

## Thermolytic Center

State: Gaseous

Collapse Temperature: **~3360 K**

Collapse Pressure: **~313 atm**

Resonant Radius: 5  $\mu\text{m}$  - 200  $\mu\text{m}$

Lifetime:  $\sim O(10)$   $\mu\text{s}$

Thermolysis of water vapor and volatile compounds

## Interfacial Region

State: Fluid under extreme conditions

Collapse Temperature: **~1900 K**

Width:  $\sim 200$  nm

Lifetime:  $< 2$   $\mu\text{s}$

$\bullet\text{OH}$  Concentration: **~4 mM**

Oxidation & thermolysis of non-volatile and ionic compounds

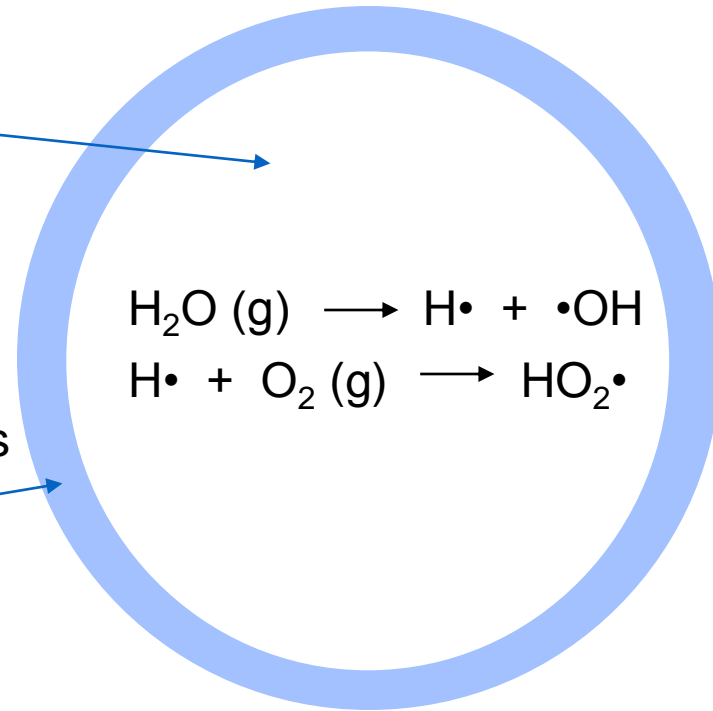
## Bulk Region

State: Liquid

Temperature: Ambient

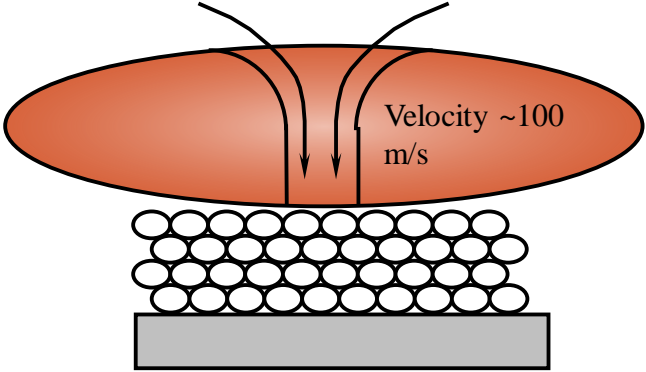
Survived  $\bullet\text{OH}$

Accumulation of  $\text{H}_2\text{O}_2$



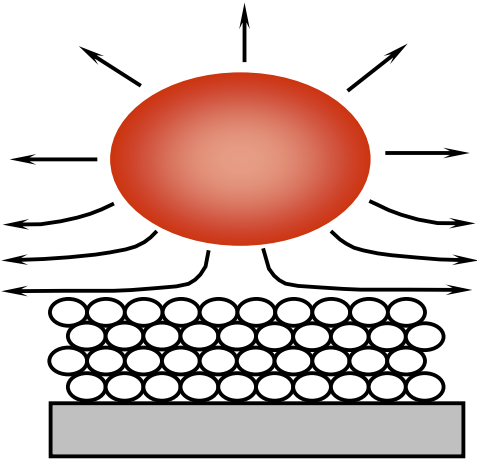
# Physical Mechanisms

## Microjets

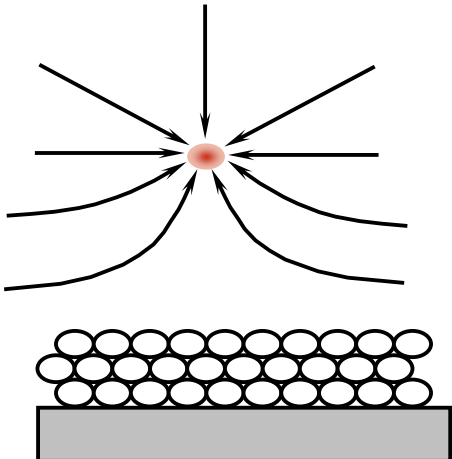


Transient Collapse

## Microstreaming

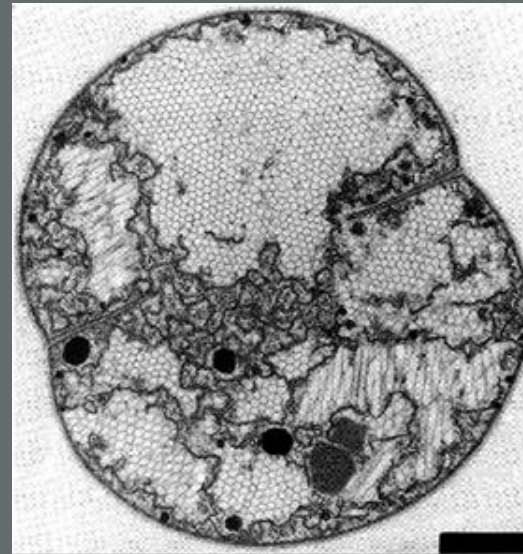
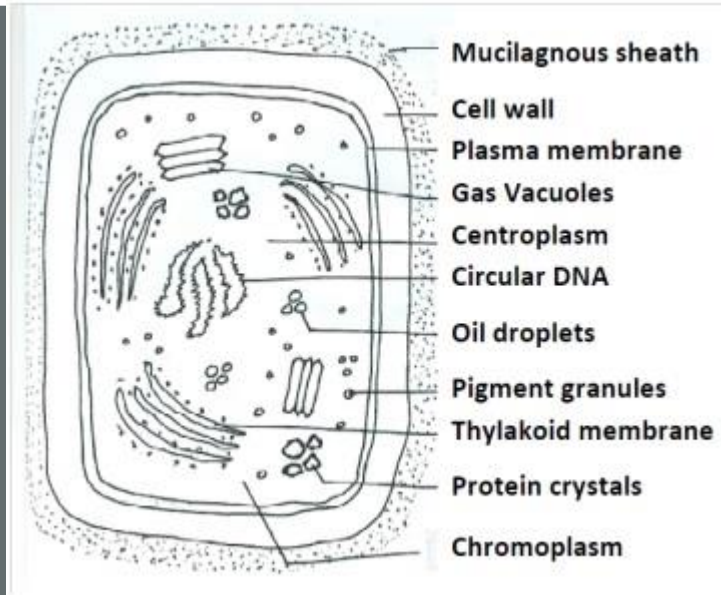


Bubble increases and decreases in size—high shear stress

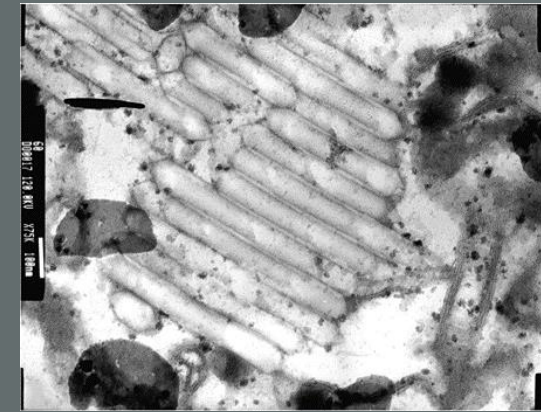


Stable Collapse

# How Can Ultrasound Control Cyanobacteria Blooms?

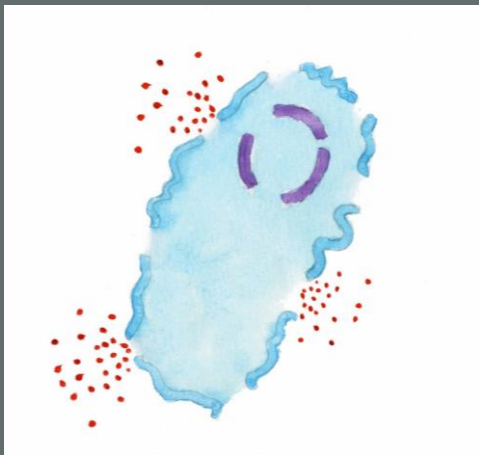


Microcystis Cell

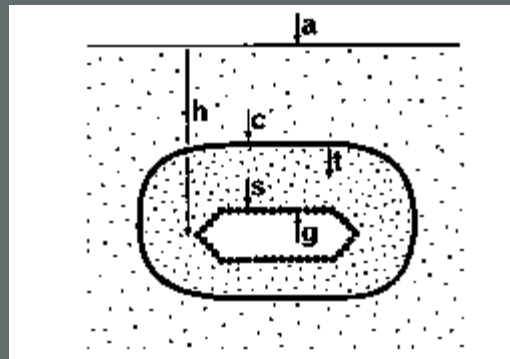


Gas Vesicles in Anabaena

## Lyse Cells



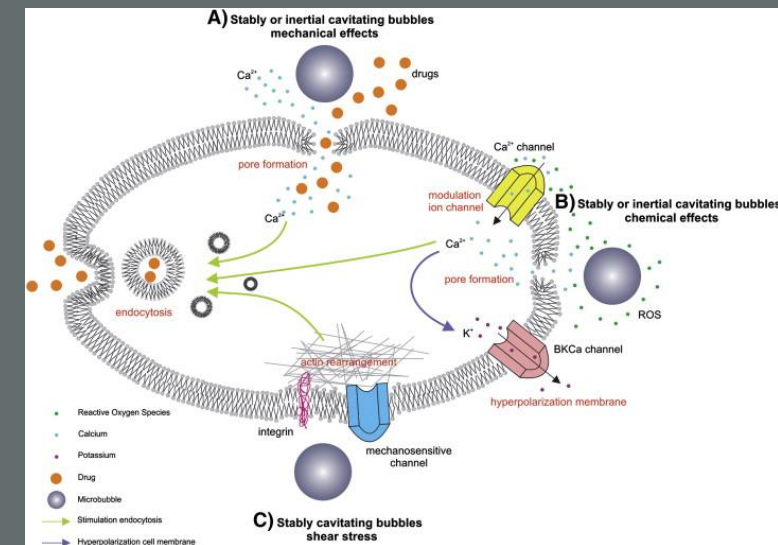
## Collapse Gas Vesicles



a = atmospheric  
 h = hydrostatic  
 c = surface tension cell wall  
 t = turgor pressure of cell  
 s = surface tension vesicle wall  
 g = gas  
 ac = acoustic

$$P_{net} = P_a + P_h + P_c + P_t + P_s - P_g \pm P_{ac}$$

## Sonoporation



# Using Ultrasound to Control Cyanobacterial Blooms

## Ultrasound has been shown to:

- Collapse gas vesicles in cells
- Break filamentous cyanobacteria
- Inhibit growth
- Reduce cell concentrations
- Inactivate cells
- Reduce photosynthetic activity
- Increase sedimentation rates
- Contribute to toxin release
- Negligible or detrimental effect on other organisms
- Decrease cell counts in reservoir

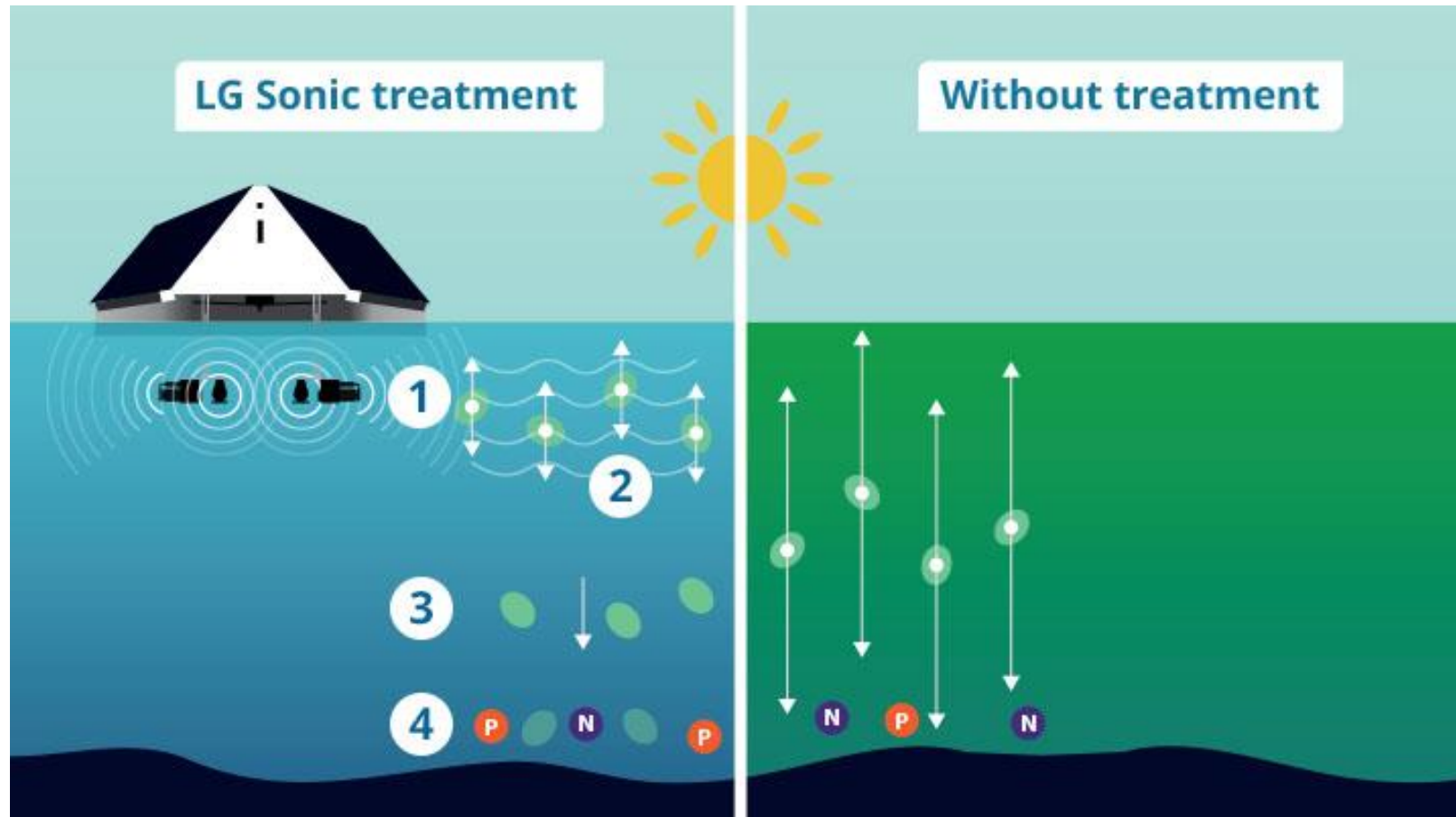
## High Power Ultrasound

- Transient Cavitation present
- Lyse and inactivate cells
- High potential to release toxins

## Low Power Ultrasound

- No cavitation or only stable cavitation present
- Collapse gas vesicles in cells
- Low potential to release toxins

# Common Mechanism Cited by Manufacturers



<https://www.lgsonic.com/ultrasonic-algae-control-technology/>





<https://www.sonicsolutionsllc.com/products/>



<https://www.environmental-expert.com/products/vor-algae-model-xxl-series-controller-248458>



<https://www.toscano.es/en/ultrasound/>



<https://www.lgsonic.com/ultrasonic-algae-control-technology/>

# Ultrasonic Device Manufacturers

- LG Sonic Algae Control
- DUMO Algacleaner
- Ultrasound Algae Killer
- VoR Algae Controller XXL
- Sonic Solutions LLC

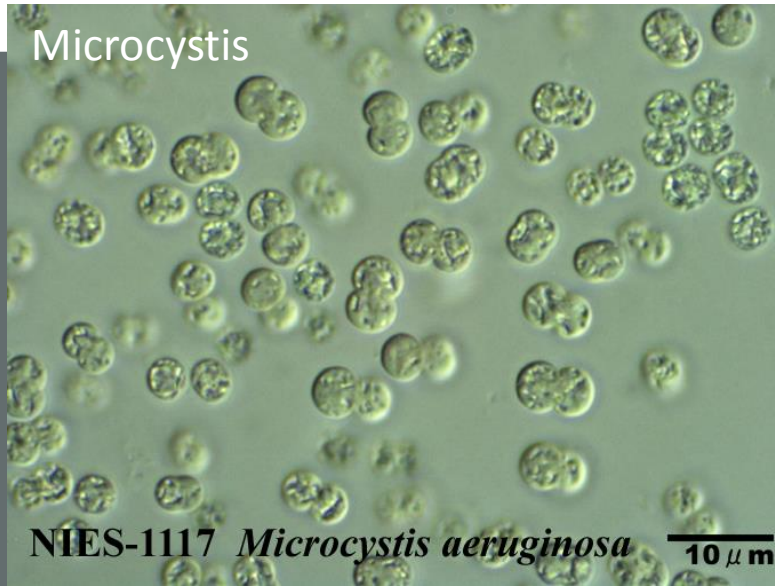
# Challenges

- Concern about toxin release
- Inconsistent results reported due to differences in
  - powers used in studies and
  - experimental design
- Potentially detrimental to other organisms
- Systematic studies in reservoirs lacking

# Objectives of our study

- Provide systematic field study of effectiveness of ultrasound
- Bridging gap between lab studies and field studies
- Assess changes to physiology and numbers of cyanobacteria and non-target organisms
- Assess ability of ultrasound to release intracellular toxins

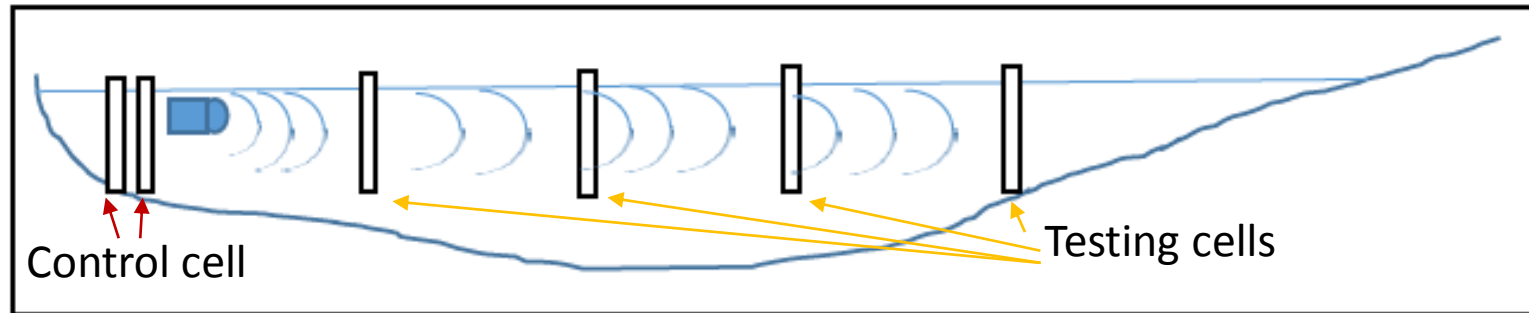
# Cyanobacteria Targeted



- Found in Ohio waters
- Produce Microcystins
- Contain gas vesicles
- Have different critical pressures needed to collapse vesicles

# Initial Experimental Plan

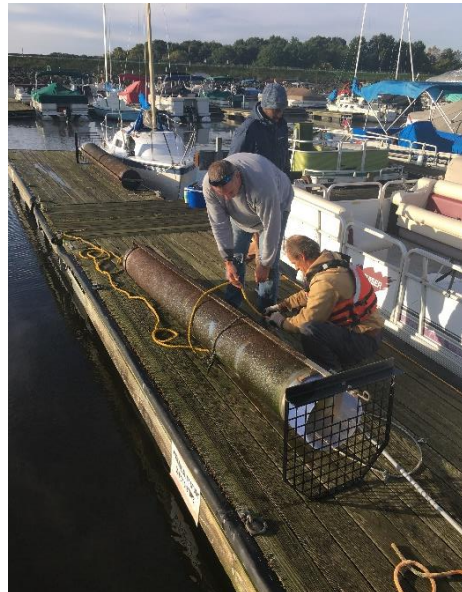
1. Deploy ultrasonic system at drinking water reservoir operated by City of Columbus
2. Characterize sound field by hydrophones
3. Set up control and test cells



4. Run tests with the presence of different organisms  
*Microcystis aeruginosa*, *Anabaena*, and *Planktothrix*, and possibly *Lyngbya*, and *Cylindrospermopsis*.
5. Monitor water quality along water column in each cell

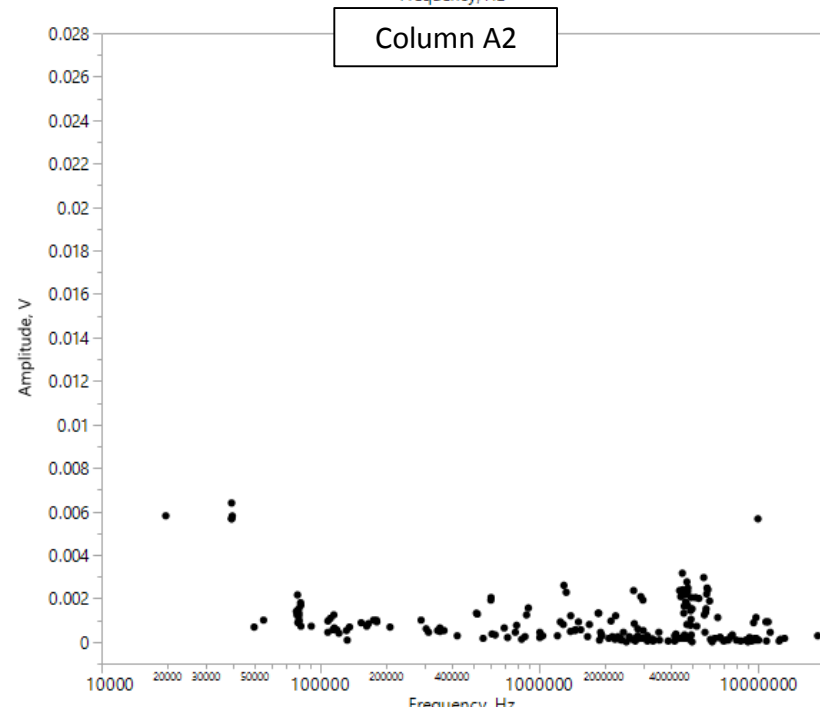
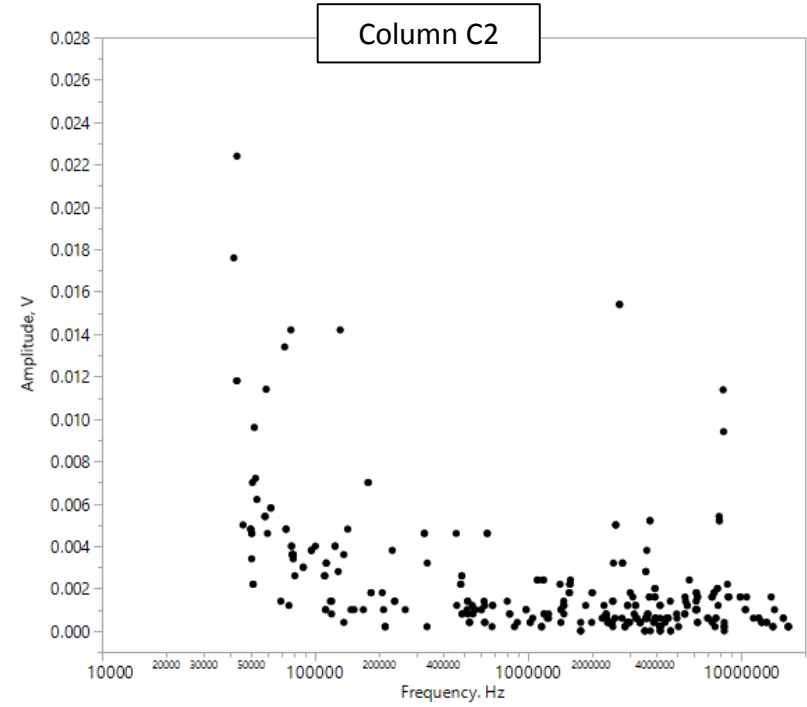
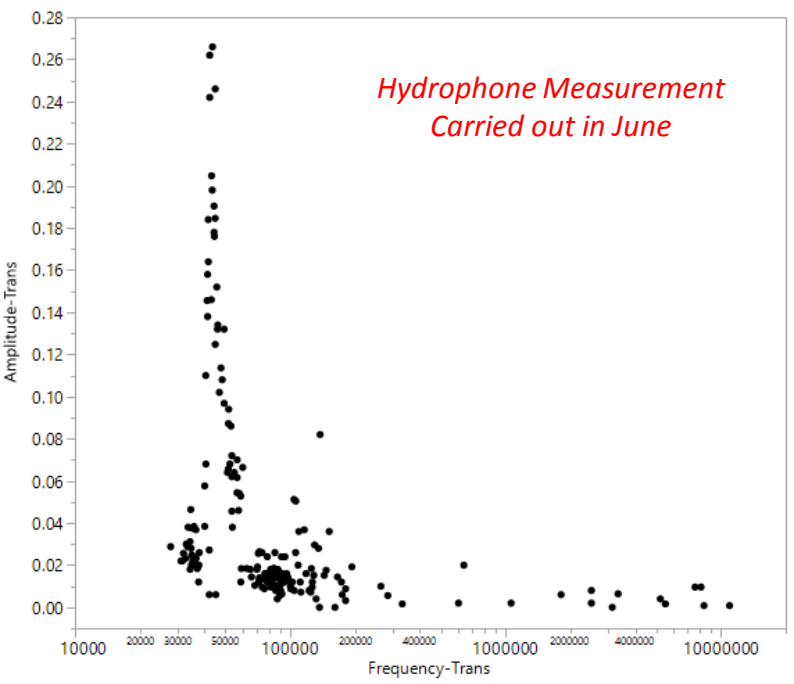
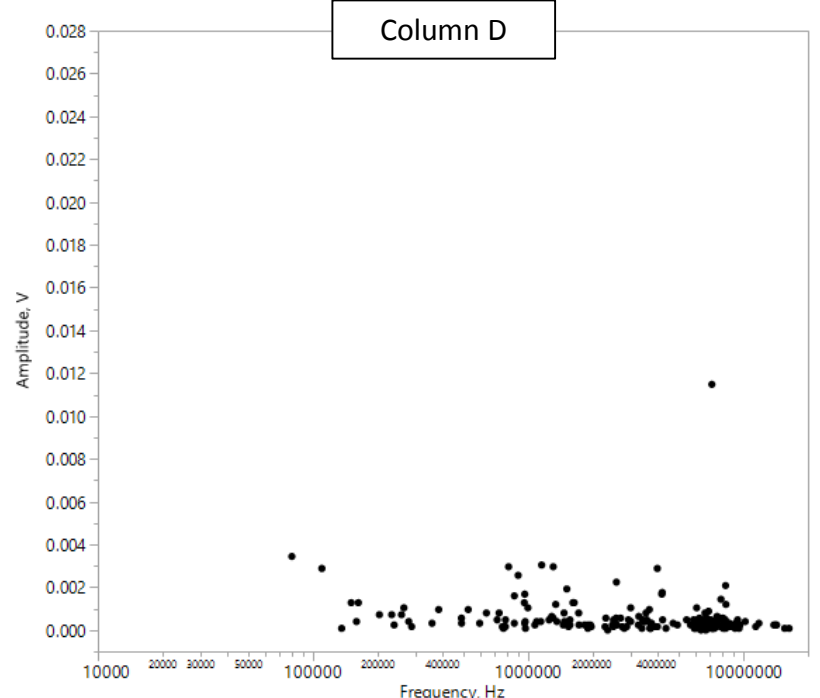
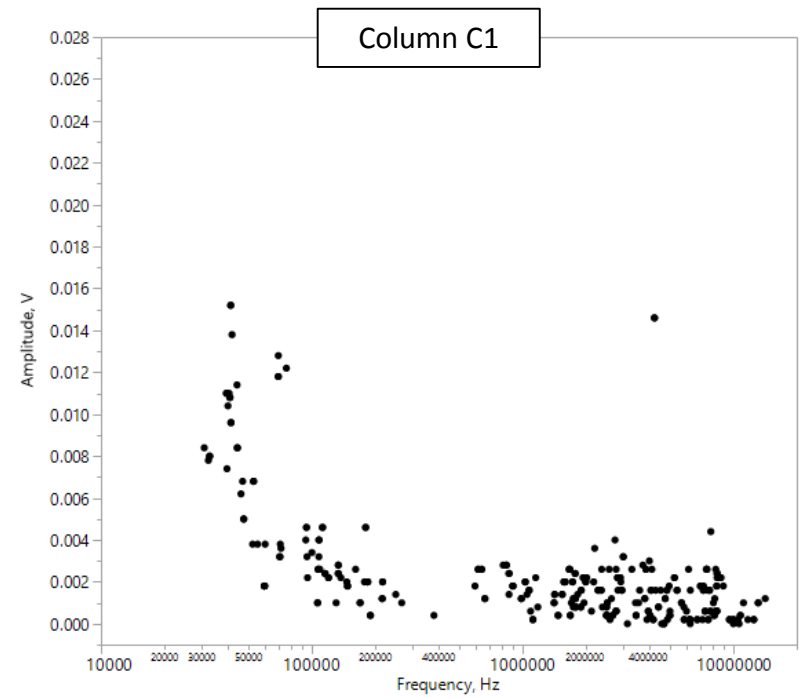
# Hoover Column Experiments Summer 2018

- Columns installed in July
- Analysis start 9/2 and end 9/20
- Lab Analysis (top column, last sample also bottom)
  - Total chlorophyll (mg/L), spectrometry method
  - OD 610 nm
  - Microcystin – total and extracellular
  - Nitrogen and phosphorous
- Sondes – temperature, DO, conductivity, pH, turbidity, chlorophyll A, phycocyanin

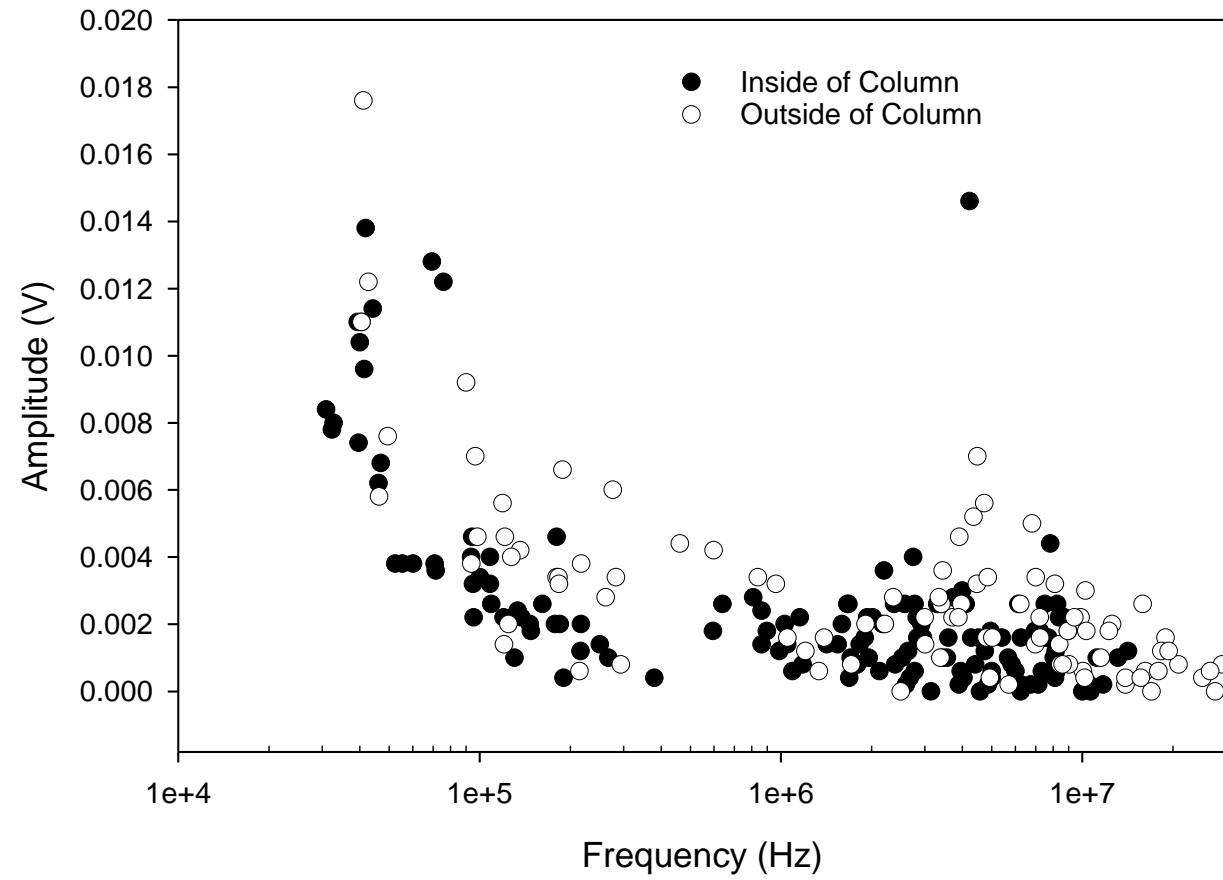




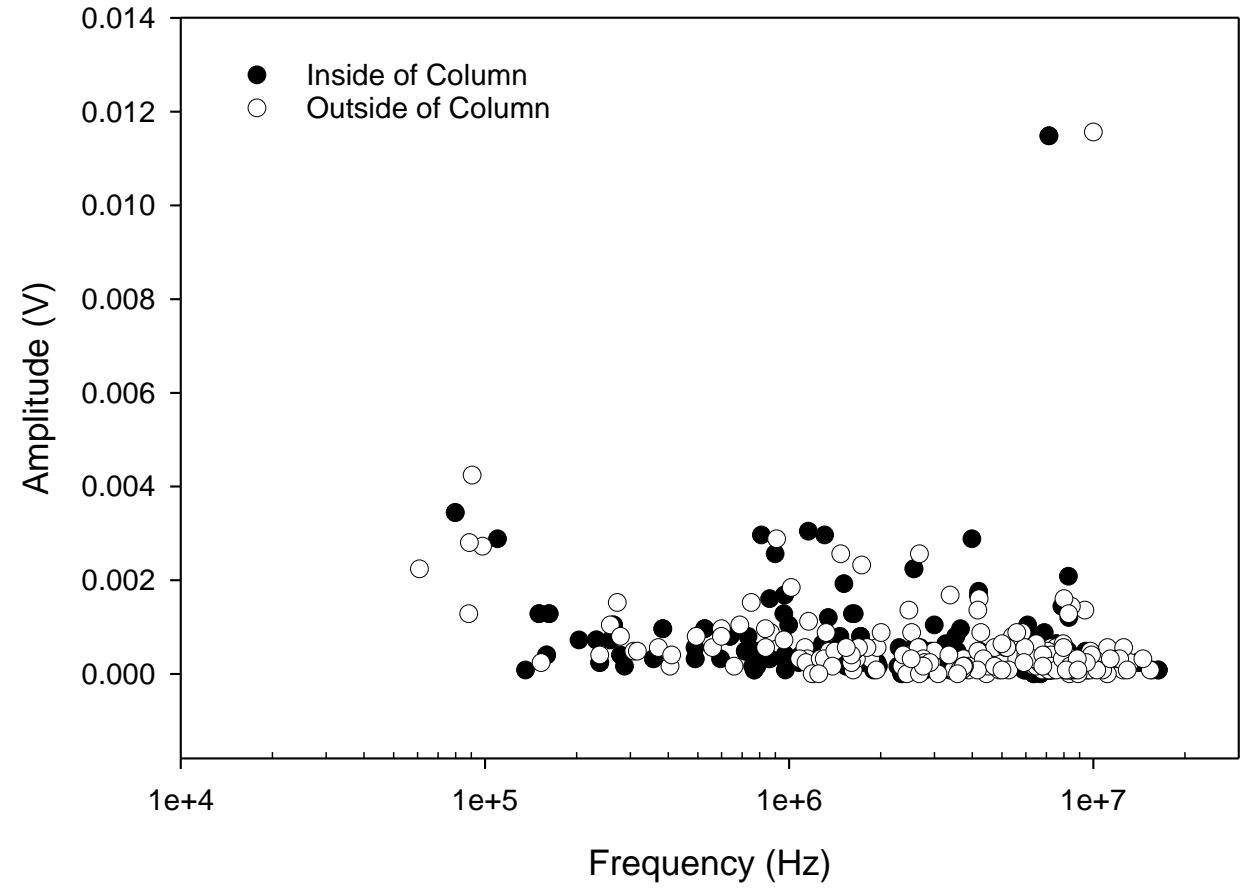
Transducer



Column C1



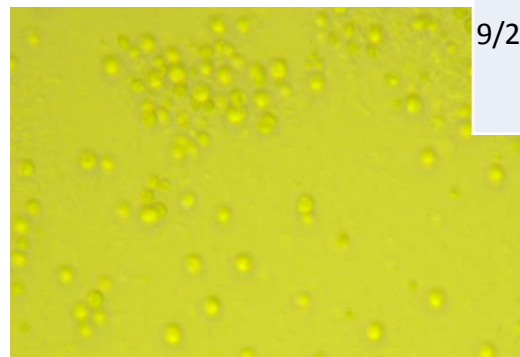
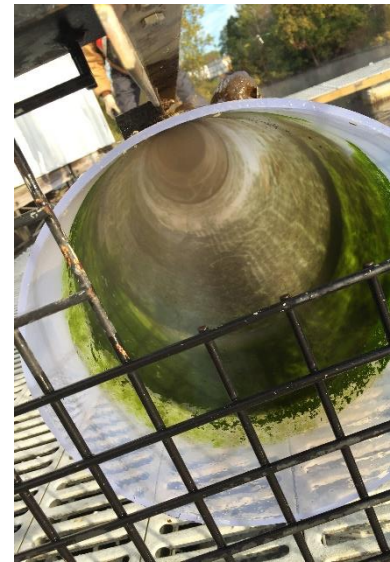
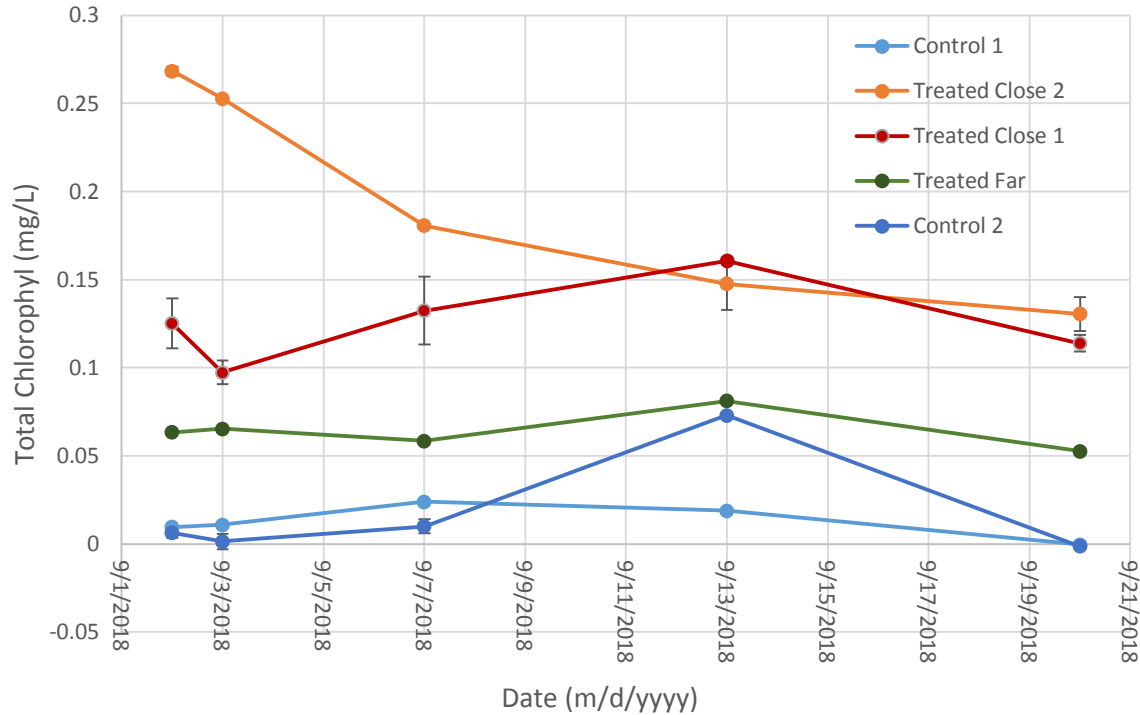
Column D



Measured in June.



# Column Study Results



C-Date	C-Time	Collector	Nitrate	Ammonia	TP	o-P	Sample C-Comment
7/16/2018	5:00 AM	C.R. Weaver	<0.2	<0.02	0.09	<0.02	D dock
8/2/2018	5:00 AM	C.R. Weaver	0.96	0.04	0.11	0.05	D dock
8/14/2018	5:00 AM	C.R. Weaver	2.68	0.03	0.16	0.09	D dock
9/7/2018	12:00 PM	C.R. Weaver	12.35	<0.02	0.72	0.63	US D1
9/20/2018	12:30 PM	C.R. Weaver		<0.02	0.88	0.82	RSCR US D1a

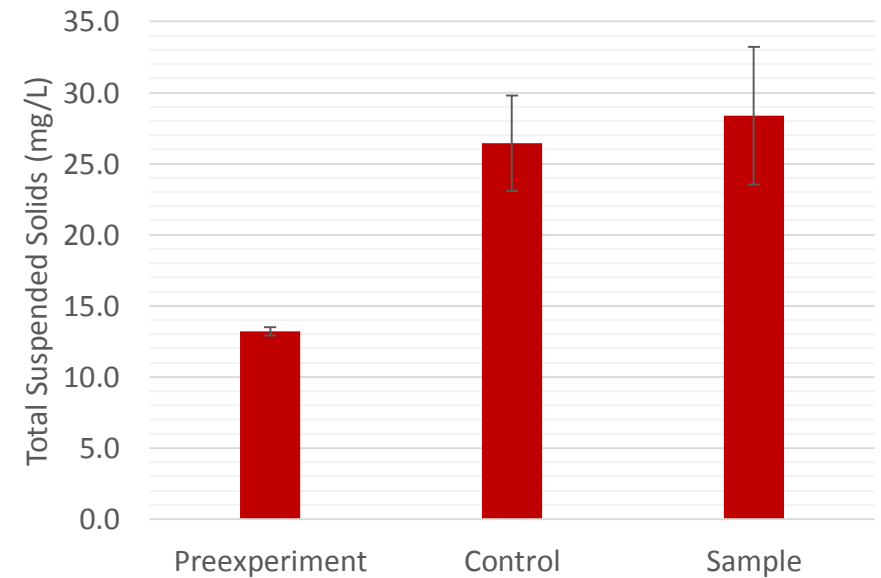
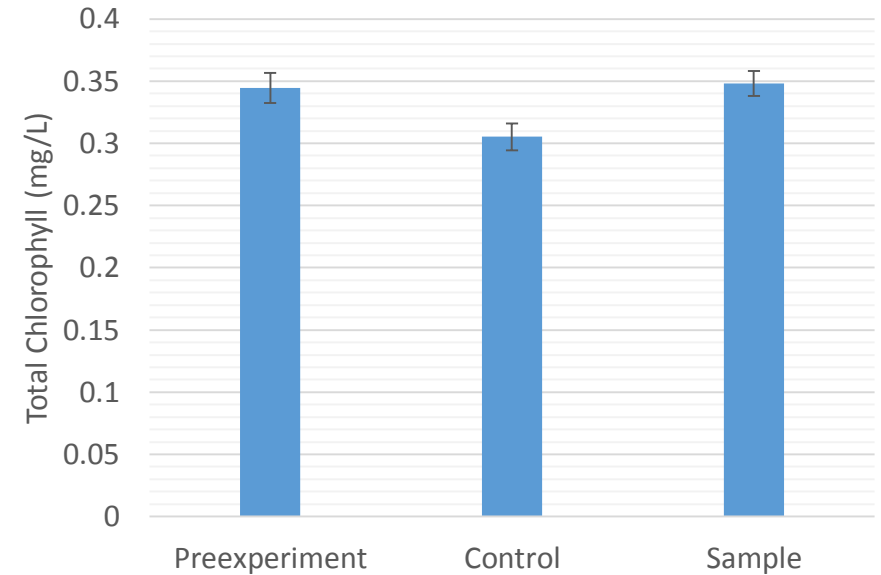
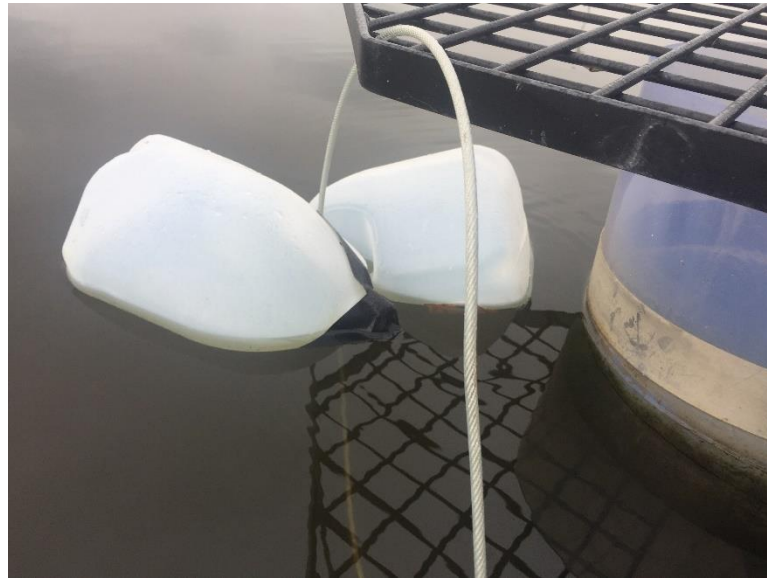
Column sample became dominated by *Chlorella* sp.

- Hard to control large volume homogeneity
- Difficult to encourage cyanobacterial growth (other green algae and bacteria compete for growth)
- Difficult to control nutrients and rain/evaporative conditions
- Sondes maintenance and regular calibration needed

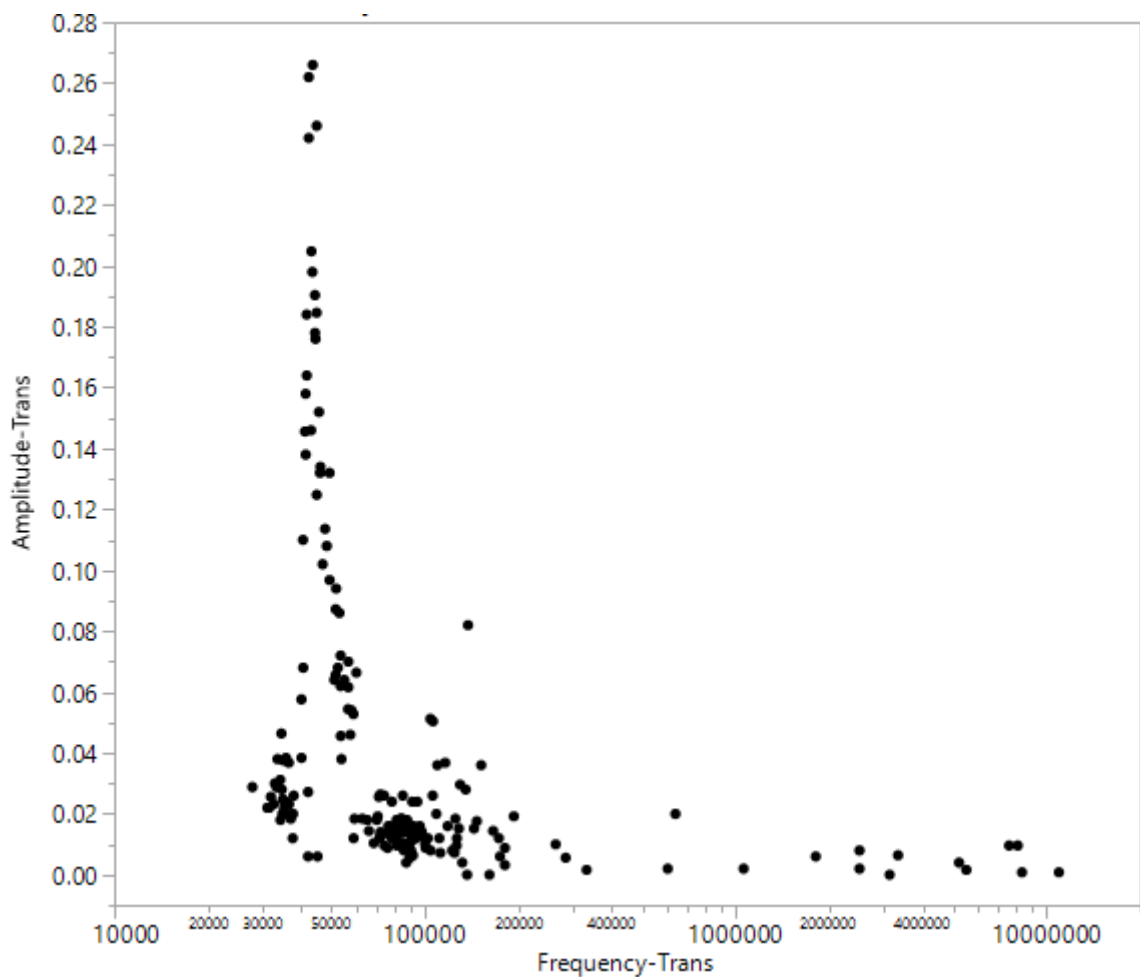
# Controlling our system - jugs

- Analysis start 9/27 and end 10/04 (week)
- Lab Analysis
  - Total chlorophyll (mg/L), spectrometry method
  - OD 610 nm
  - Microcystin – total and extracellular
  - Total Suspended Solids
  - pH, Conductivity, Turbidity

- Easier to achieve homogeneity
- Closed system (light penetration?) – possible direct use of cyanobacteria
- Controlling predation/competition of other organisms?

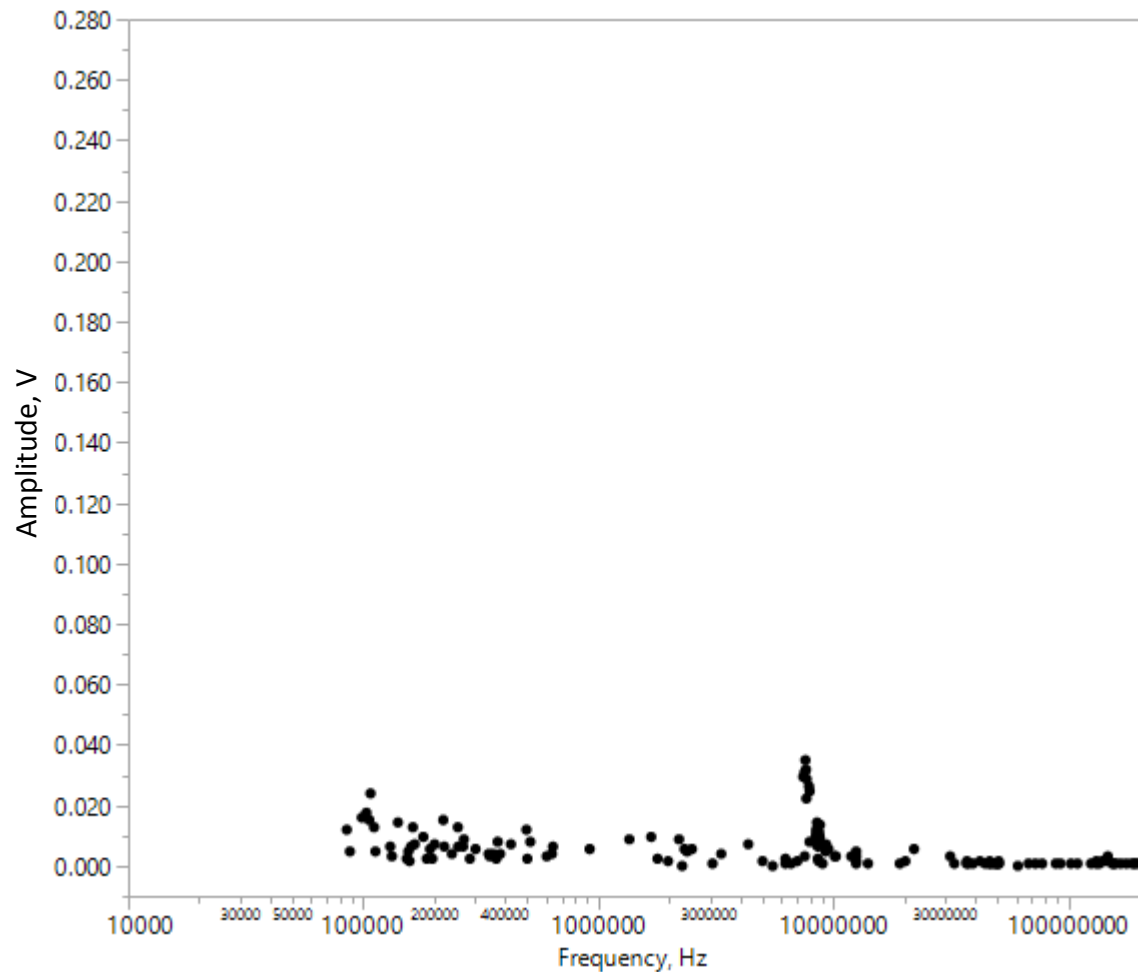


Transducer



Measured in June

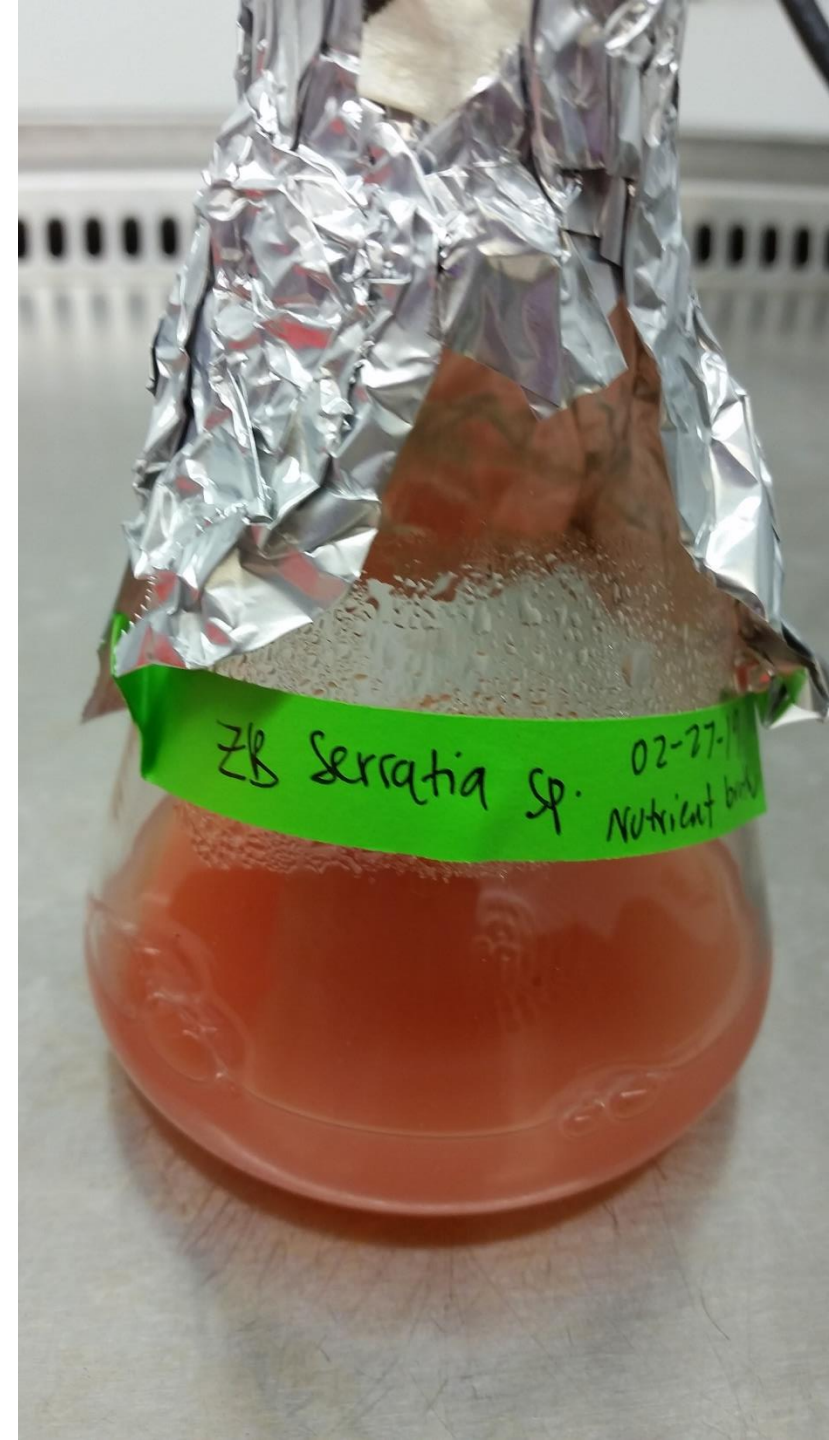
Transducer



Measured in September

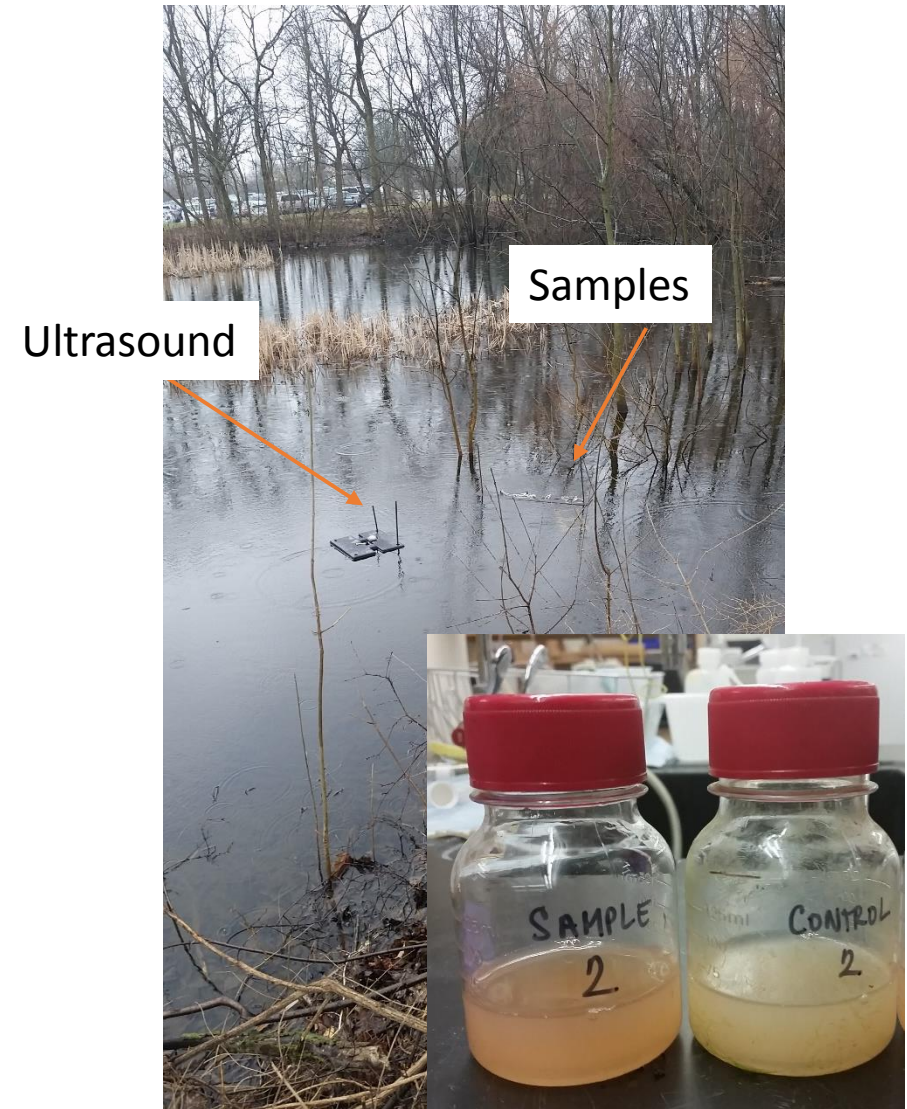
# Cyanobacteria surrogate

- *Serratia species* BSL-1, ATCC 3009
- Freshwater non pathogenic bacteria
- Easy to grow and enumerate on general nutrient media
- Fast growth rate
- Gas vesicles with similar collapse pressure sensitivity as Microcystis (Serratia 0.4334 MPa, Microcystis sp. 0.468 MPa) – Tashiro et al. 2016

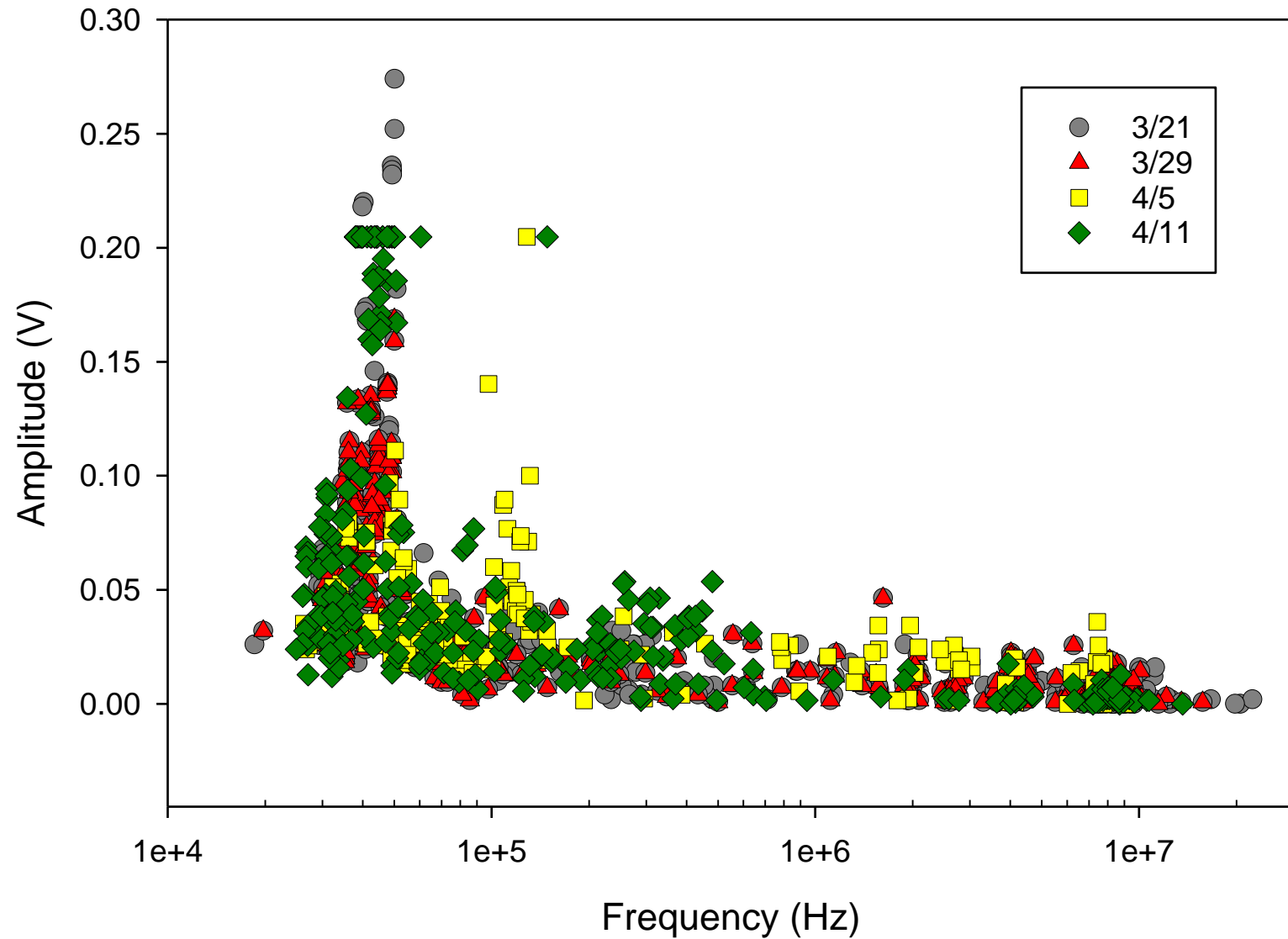


# Serratia sp. Spring 2019 – vernal pool

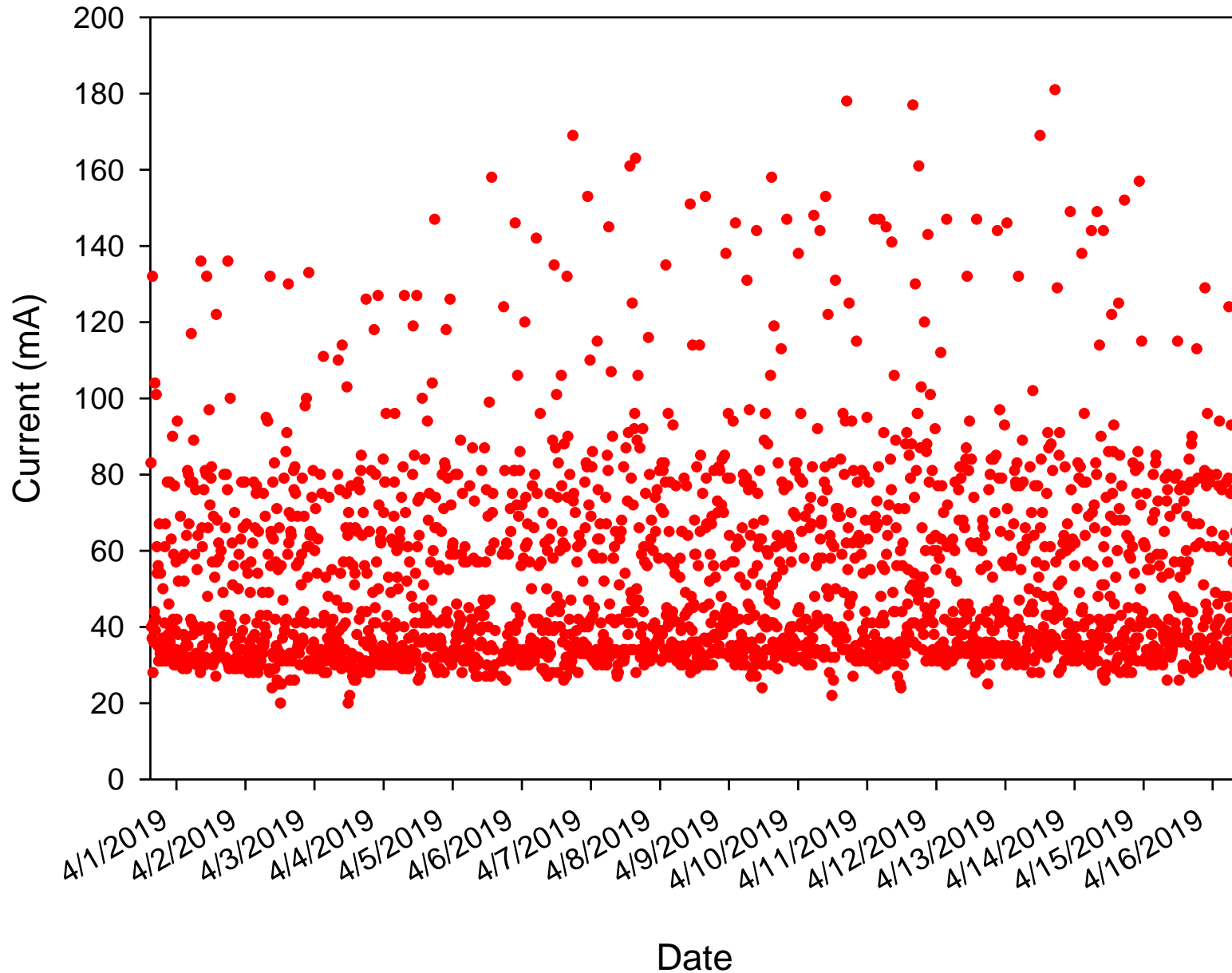
- Experiment start 4/2 and end 4/8 (week)
- Samples in vernal pool and controls in adjacent stormwater pond
- Sample time 0, time 1 (1 day), time 7 (7 days)
- Lab Analysis
  - Enumeration (spot plate nutrient agar) – measure of growth and/or decay
  - OD 610 nm
  - Live/dead stain – measure of membrane integrity
  - TEM – qualitative analysis of gas vesicles (presence and absence)



# Hydrophone Measurement

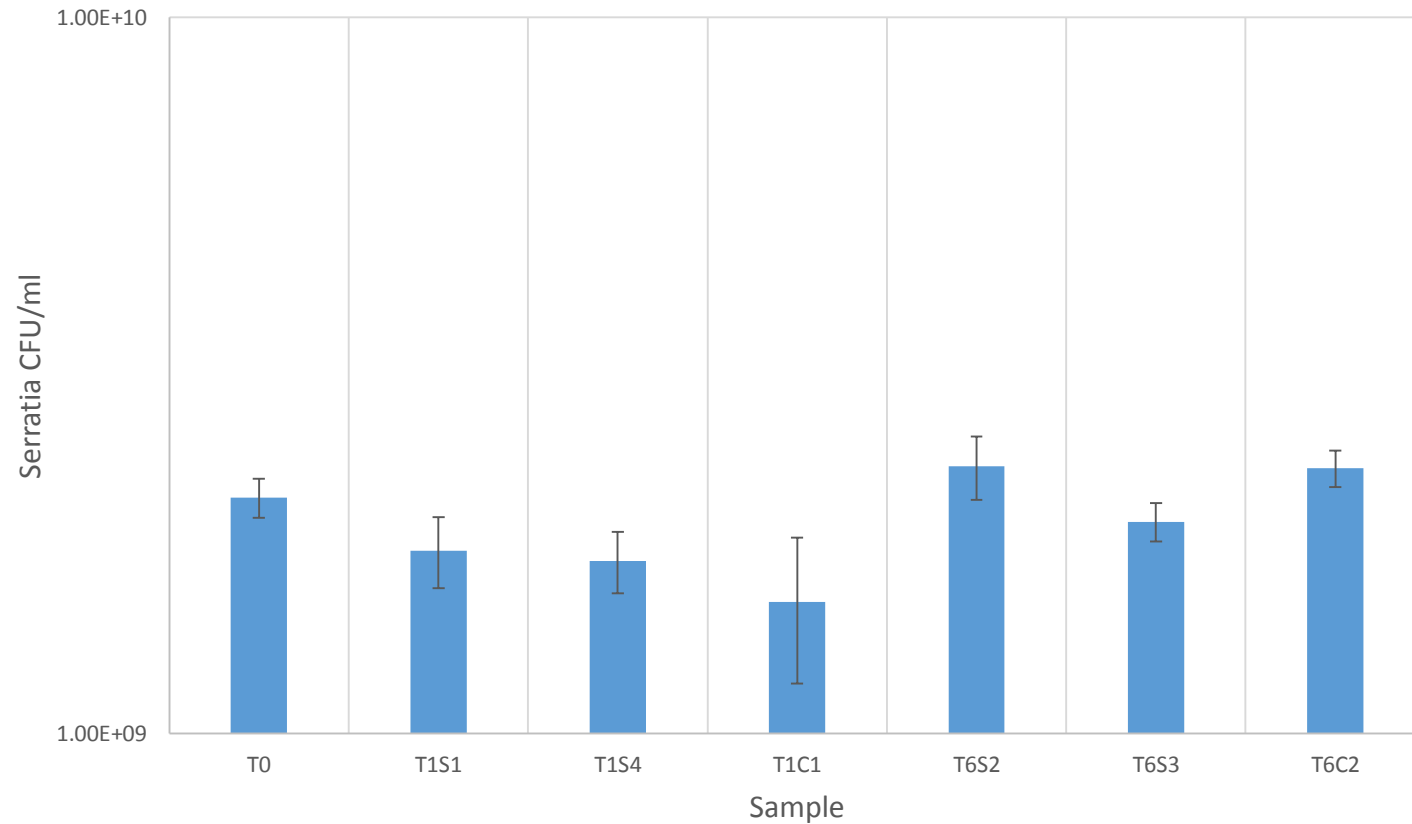


# Operation of Transducer



- A microcontroller was installed to measure and record the current pulled from the power supply by the transducer.
- Combining with the results from hydrophone measurements, the current monitoring indicate that the transducer maintained at operating condition throughout the testing period.

# Results vernal pool – *Serratia* sp.

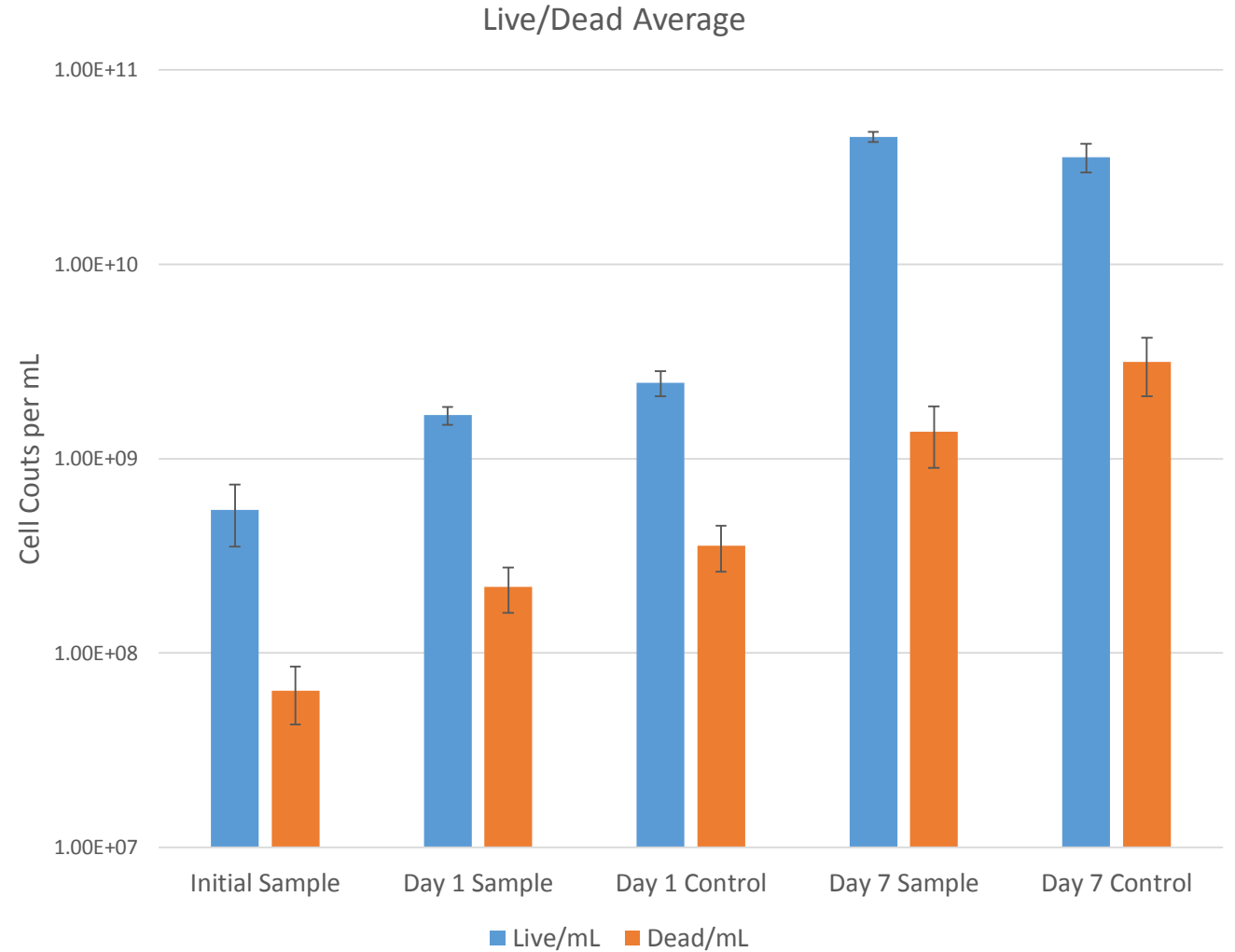


No significant difference  
in *Serratia* sp. growth  
between control and  
sample

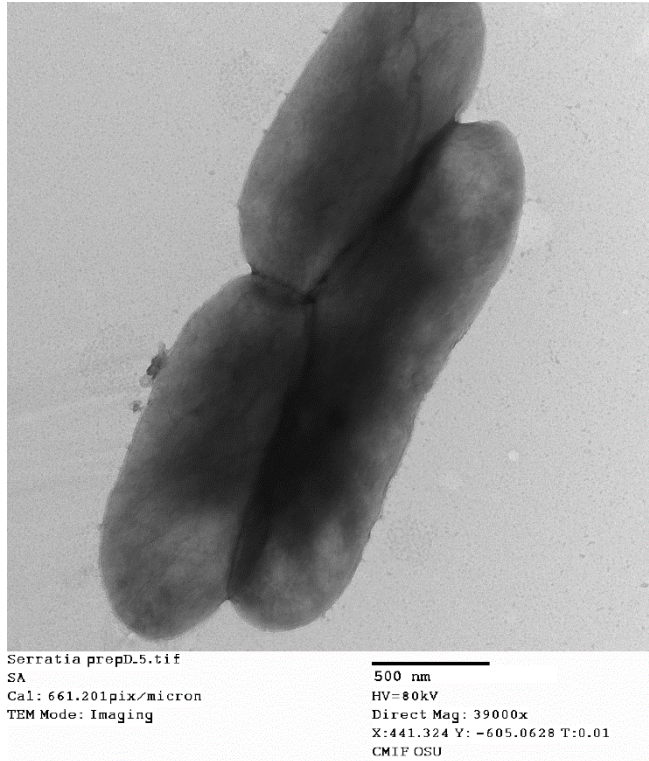


# Results vernal pool – *Serratia* sp.

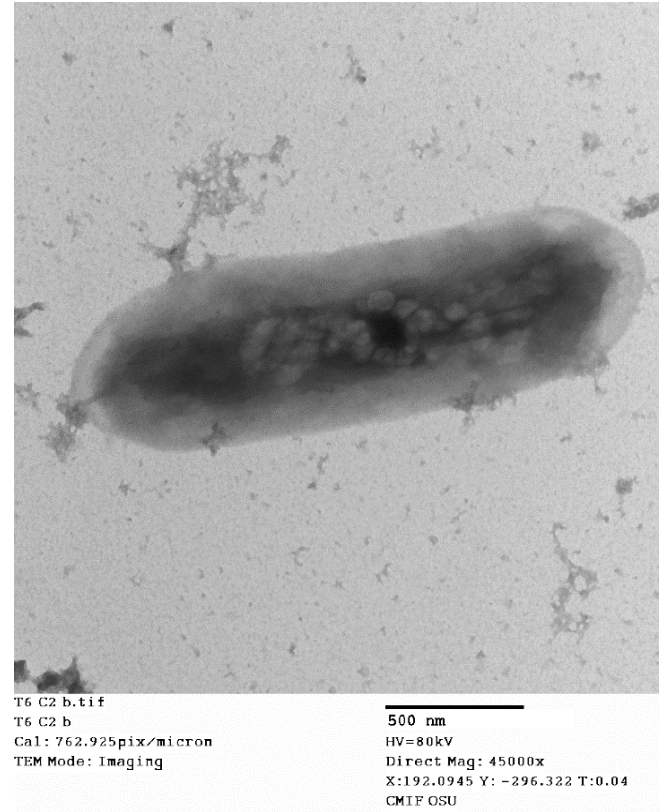
No difference between  
Live/dead cells (membrane  
integrity changes)



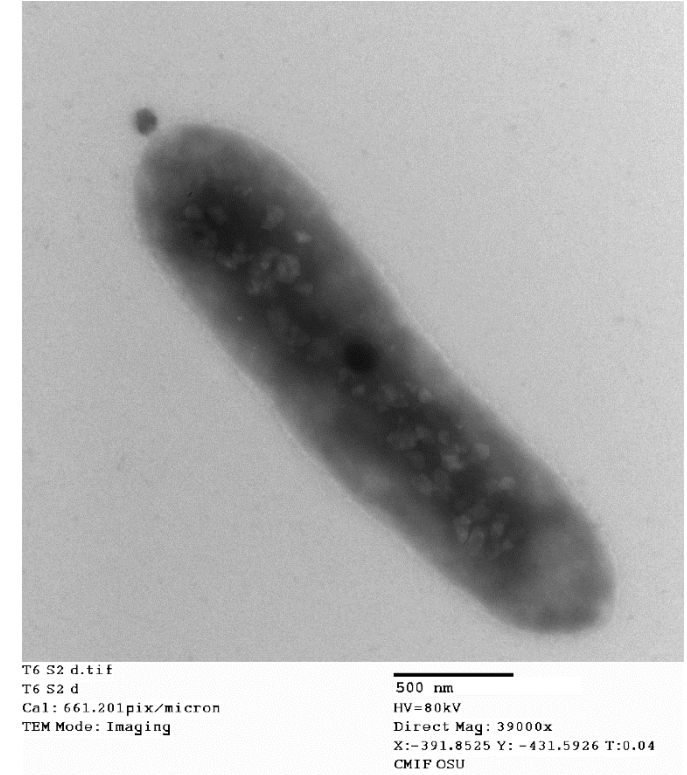
# Results vernal pool – Serratia sp.



TEM of *Serratia* sp. without vesicles



TEM of *Serratia* sp. not exposed to ultrasound 1 week



TEM of *Serratia* sp. exposed to ultrasound 1 week

No observable difference in gas vesicles under TEM

# Continuing Work

- Repeat *Serratia* experiments to confirm results
- More controlled conditions – jugs with more pressure sensitive species

116 WALSBY

MICROBIOL. REV.

- Work with reservoir.

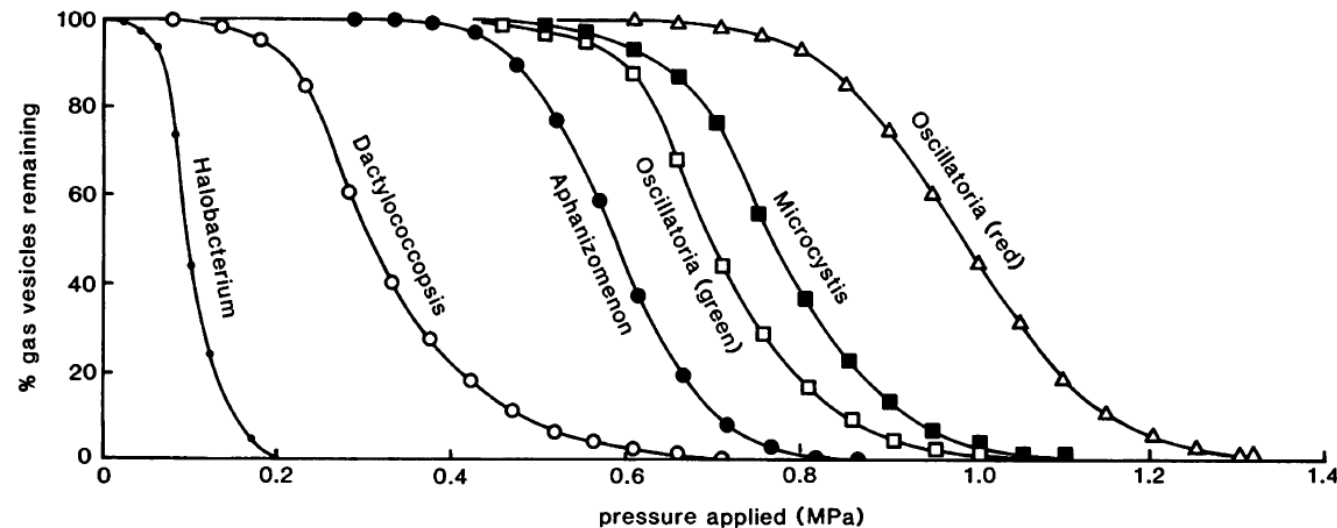


FIG. 20. Critical pressure distributions of gas vesicles from various species of cyanobacteria and a halobacterium (230). Modified from Walsby and Bleything (254) with permission from the publisher.



# So, can ultrasound in your reservoir ...

...turn this...



...into this?



*...it is not an easy question to answer*



- At present, we do not have evidence that ultrasound collapses gas vesicles or changes growth between exposed and control samples
- We did not look for all possibilities of effects of ultrasound. Possible other mechanisms related to sonoporation may be occurring.