

EarthTec QZ: Control of Dreissenid Mussels through a more Rational Use of Copper

**David Hammond, PhD and Fred Singleton, PhD
Senior Scientists
Earth Science Labs, Inc.**



Main message of this talk:

There is a new generation of liquid copper products that

- deliver copper entirely as cupric ions, Cu^{++}
- are highly bioavailable,
- are effective at unprecedentedly low doses

Green Chemistry

1. Prevent waste
2. Maximize atom economy
3. Design less hazardous chemical syntheses
4. Design safer chemicals and products
5. Use safer solvents and reaction conditions
6. Increase energy efficiency
7. Use renewable feedstocks
8. Avoid chemical derivatives
9. Use catalysts, not stoichiometric reagents
10. Design chemicals and products to degrade after use
11. Analyze in real time to prevent pollution
12. Minimize the potential for accidents

EarthTec is an example of Green Chemistry:

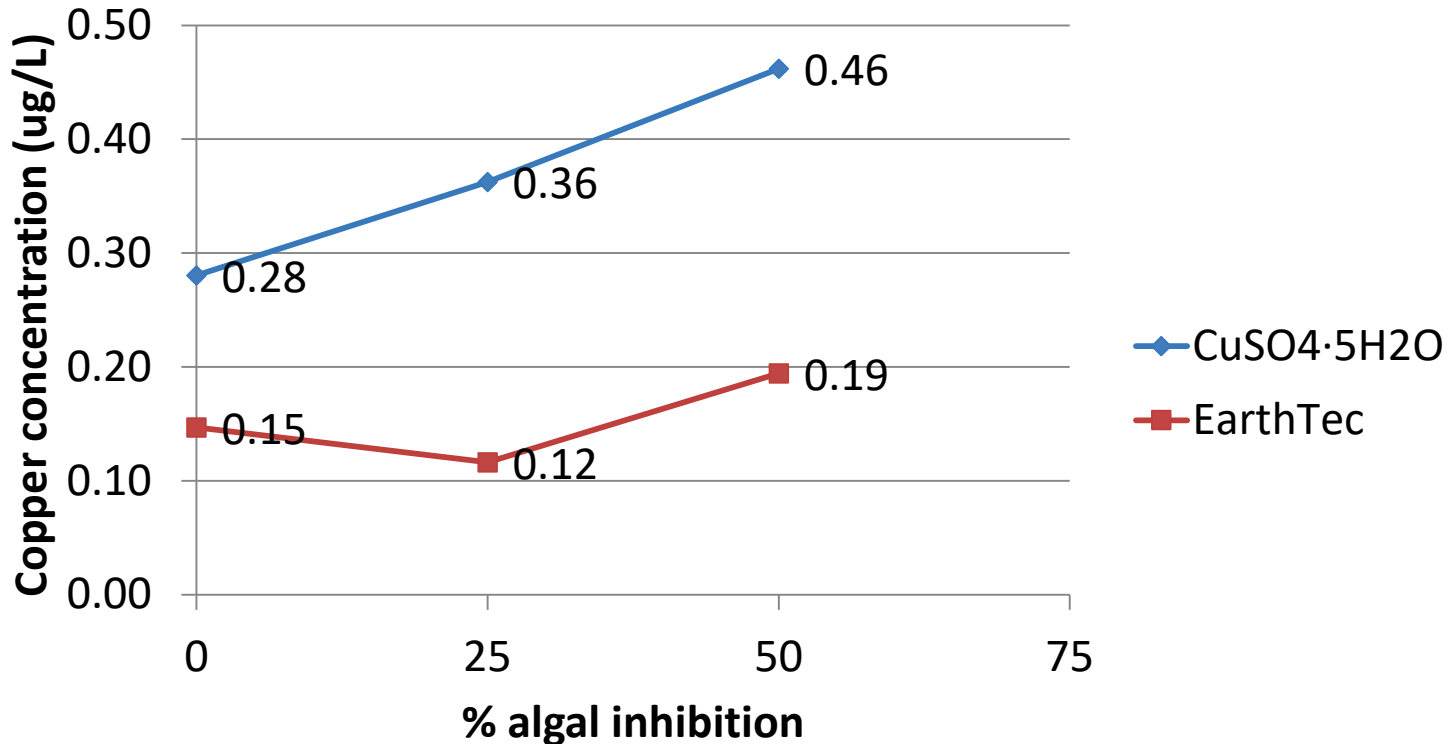
a safer and more efficient formulation that

achieves the desired benefits at lower doses, with less waste



Copper Sulfate vs EarthTec

% Inhibition of Algal Growth after 96h of exposure to copper delivered as conventional copper sulfate vs EarthTec



The copper dose required to achieve a given % inhibition of algae is much lower if applied as EarthTec than if applied as copper sulfate

Tests performed by **Aquatic BioAssay and Consulting Inc.**, against the indicator algal species, *Selenastrum capricornutum*, according to standard bioassays of chronic exposure, 96 hours.

Recommended Dose of EarthTec according to Severity of Cyanobacterial Bloom

Algal bloom conditions	μL/L, as EarthTec	ppm, as copper	ppb, as copper
Preventative dose	0.5	0.030	30
Mild bloom	1	0.060	60
Full bloom	2	0.120	120
Severe bloom	3	0.180	180

EPA max for copper algaecides is 1 mg/L as copper
= 1,000 ppb as copper
(about 8x to 16x the usual dose of EarthTec)

Product Comparison

Copper Sulfate vs EarthTec

Norwalk, OH (2012 vs 2013)

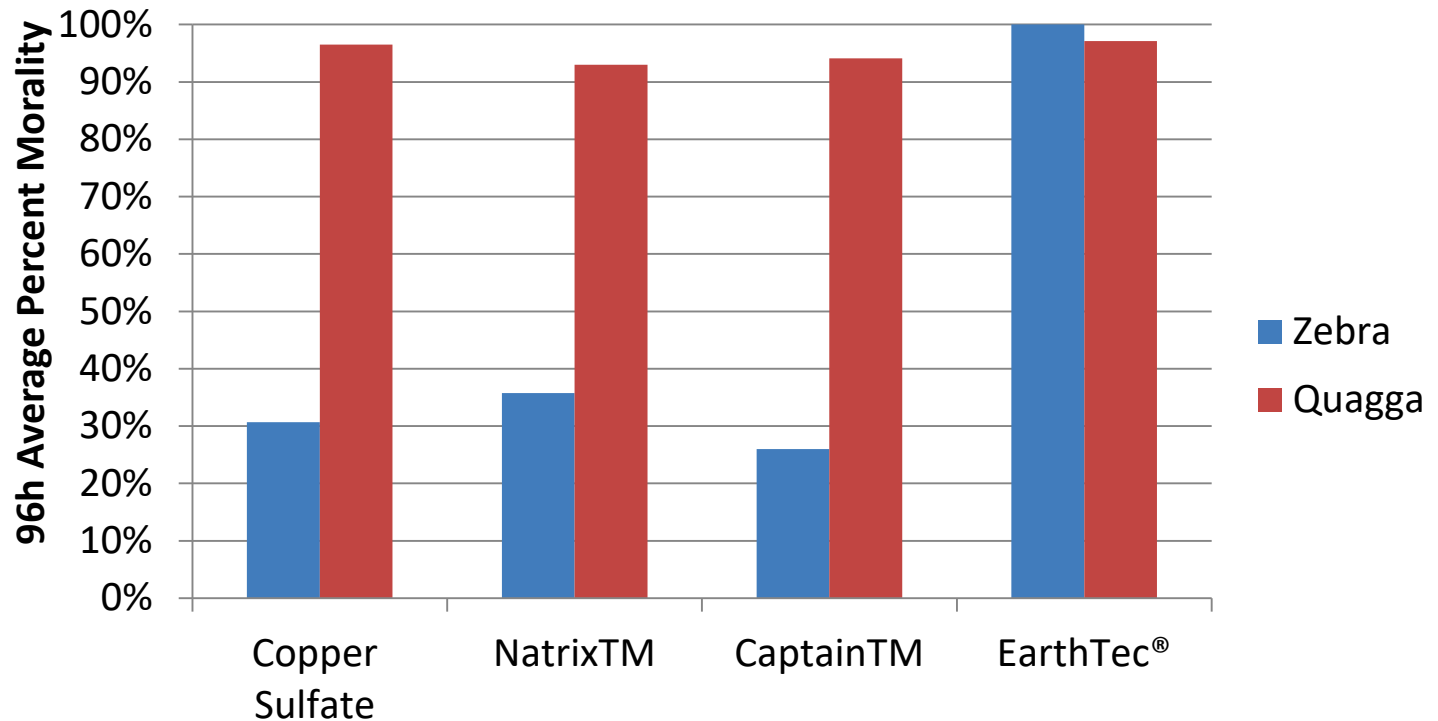
Copper sulfate 2012	EarthTec 2013	
10	8	treatments per year
500	60	lbs or gallons per treatment
	10	lbs, weight per gal of EarthTec
5,000	4,800	lbs per year
25%	5%	fraction that is elemental copper
1,250	240	elemental copper applied, lbs
100%	19.20%	total copper applied, %

When this customer switched from copper sulfate to EarthTec, they had fewer total applications per year, applied only 19% of the elemental copper used in previous years, and their treatment season spanned a longer portion of the year.

Copper Sulfate vs EarthTec

Average percent mortality after 96h of exposure to copper-based algaecides at 0.5 mg/L copper equivalent

0.5 mg/L copper equivalent



Even at equivalent doses of active ingredient, EarthTec is more effective.

And we now know much lower doses of EarthTec are still effective against mussels.

Renata Claudi M.Sc., T.H. Prescott P.Eng., Sergey Mastisky Ph.D. & Heather Coffey M.Sc., "Efficacy of Copper Based Algaecides for Control of Quagga and Zebra Mussels", January, 2014.

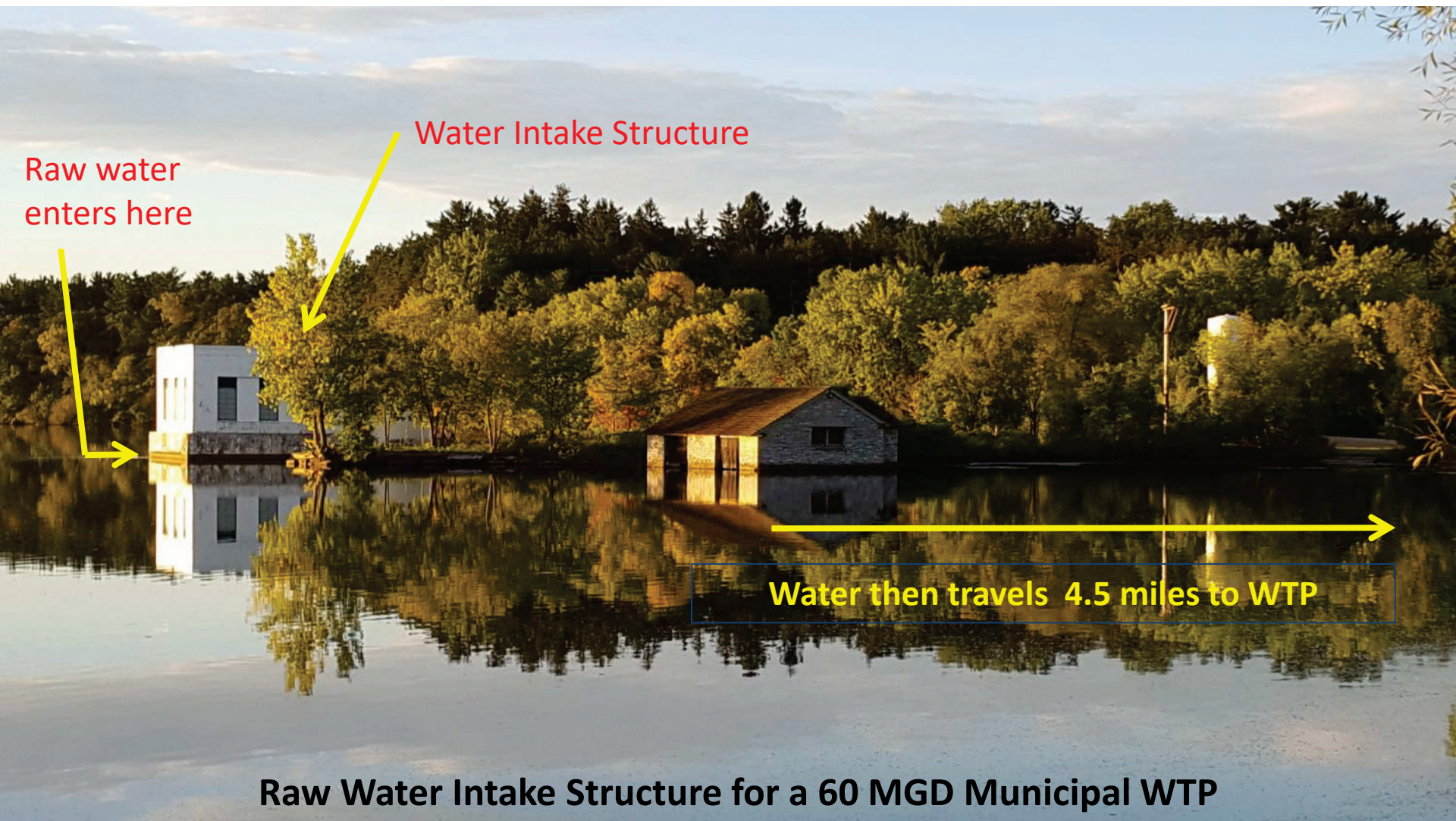


3.5" = 9 cm



Zebra mussels have historically infested the intake structure of a major municipal WTP in the Midwest

2015-16





This is what the clean screens should look like

Zebra mussels historically fouled the intake screens of the WTP 2015



Screen fouled with
zebra mussels, 2015

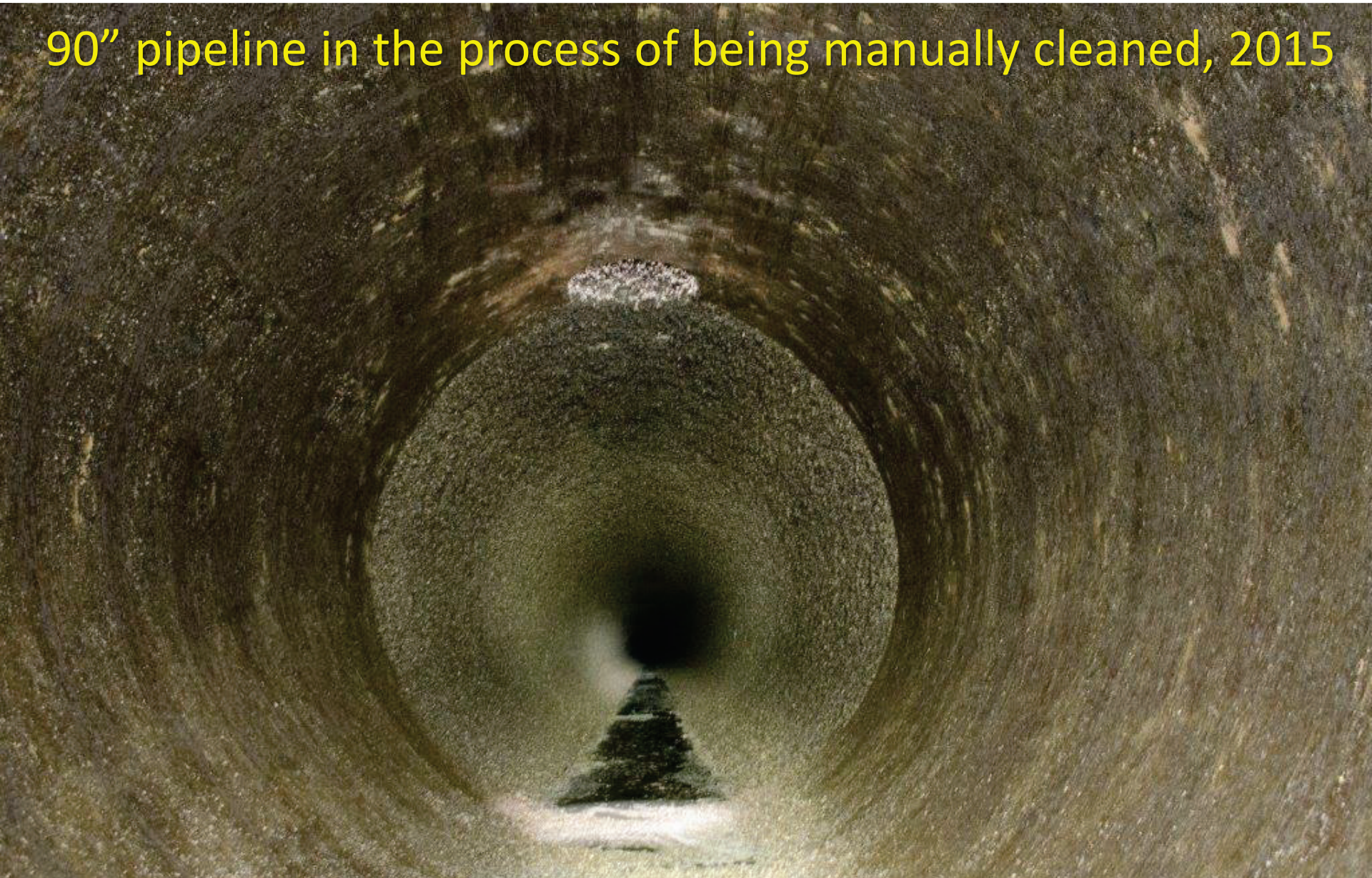


Screens and intake fouled
with zebra mussels, 2015

Zebra Mussels Infesting the 90" Raw Water Pipeline

2015

90" pipeline in the process of being manually cleaned, 2015



Zebra Mussels Infesting the 90" Raw Water Pipeline 2015

Manually scraped mussels to be removed, 1 foot deep, 2015



Zebra mussels being removed from the raw water pipeline 2015

Manual cleaning represents a worker safety hazard, requiring Tyvek suits and respirators



Zebra mussels removed from the pipeline and screens



Mussels are removed by the dumpster load

Zebra Mussel Control using EarthTec QZ

Summer, 2016



Bulk storage tank for EarthTec QZ next to intake -- 5,500 gallons

Results of treatment with 1ppm QZ
Ensured intake screens free of zebra mussels during height of the mussel season
September, 2016



Treatment with 1ppm QZ ensured intake screens are free of zebra mussels
September, 2016



EarthTec QZ successfully prevented biofouling in Summer-Fall of 2016

Treatment with 1ppm QZ ensured pipeline remained free of zebra mussels

September, 2016

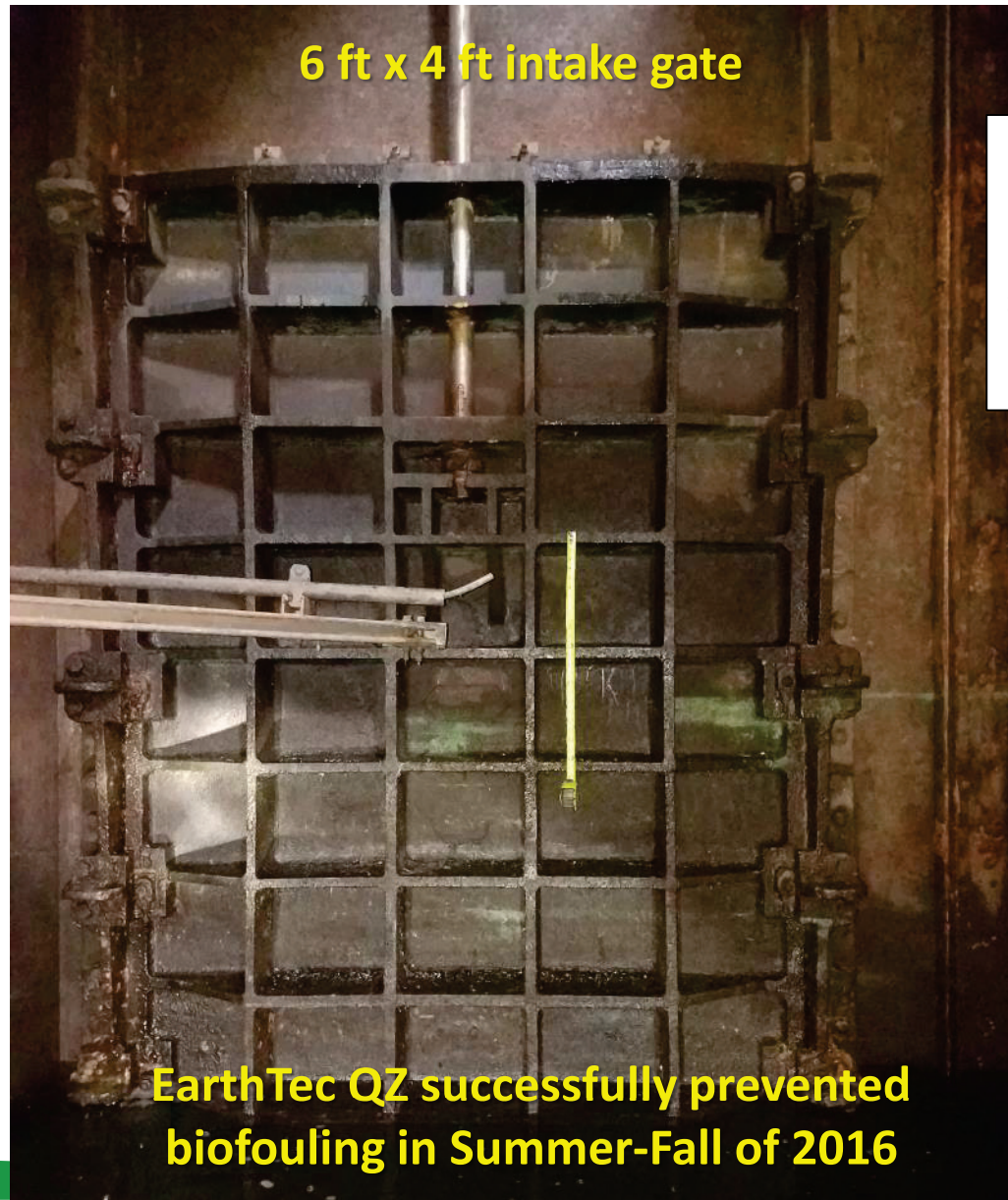


**1 ppm dose as QZ
= 60 ug/L as copper
sufficient to achieve
complete control**

**EarthTec QZ successfully prevented
biofouling in Summer-Fall of 2016**

Treatment with 1ppm QZ ensured intake gates remained free of zebra mussels

September, 2016



6 ft x 4 ft intake gate

**1 ppm dose as QZ
= 60 ug/L as copper
sufficient to achieve
complete control**

**EarthTec QZ successfully prevented
biofouling in Summer-Fall of 2016**

Treatment with 1ppm QZ ensured intake gates remained free of zebra mussels

September, 2016



1 ppm dose as QZ
= 60 ug/L as copper
sufficient to achieve
complete control

Note that mussels
were only able to
colonize a few spots
within eddies of
unmixed water,
such as on the feed
line itself.

Zebra Mussel Control at City of St Paul, Minnesota

**Copper Concentration (ug/L = ppb) in treated water
reaching the St Paul WTP, summer of 2016**

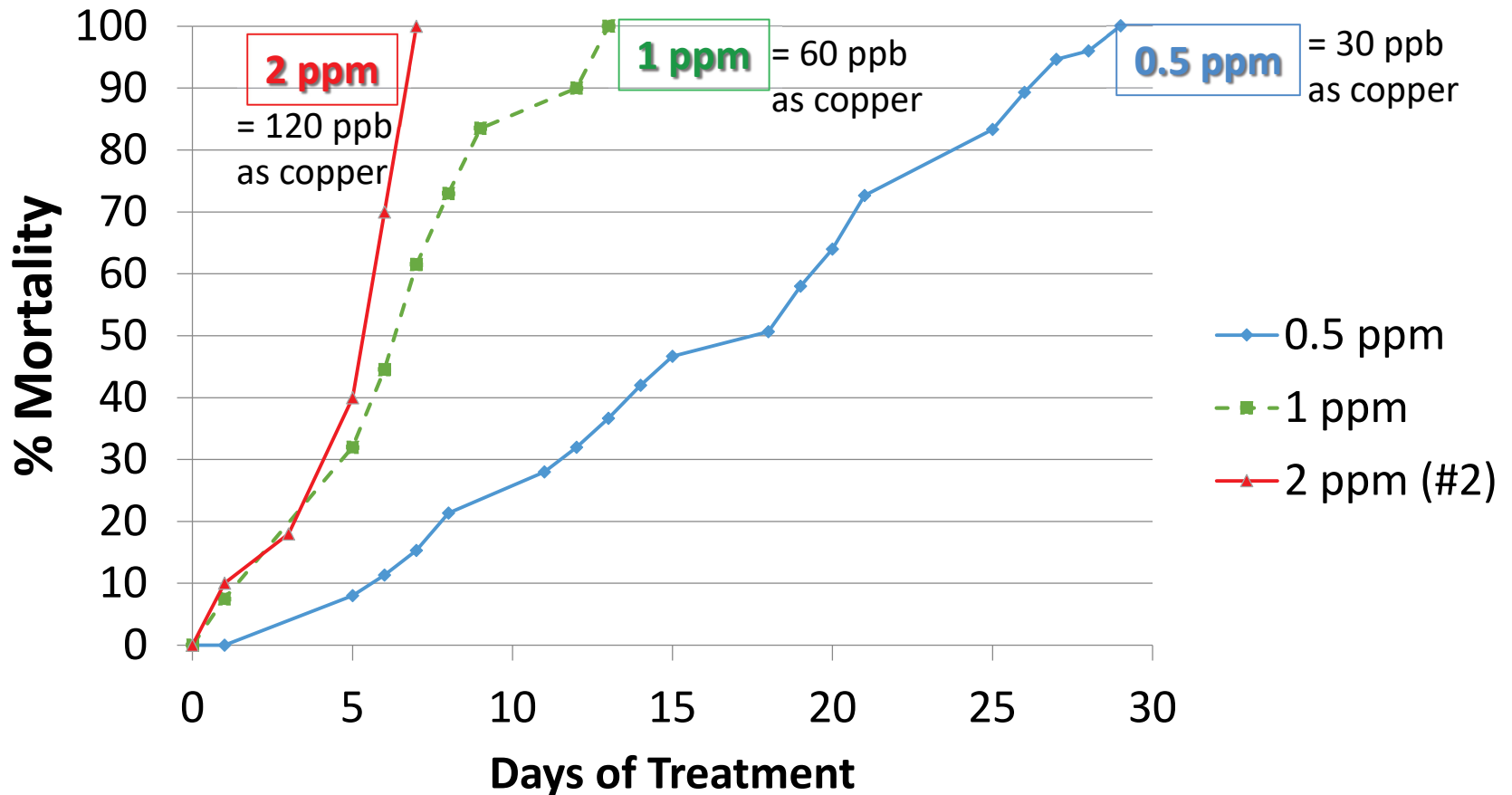
**Dose applied at
pipeline intake
1 ppm as QZ
= 60 ug/L as copper**

Date	WTP
6/14/2016	0
6/23/2016	2
6/30/2016	0
7/7/2016	3
7/14/2016	4
7/21/2016	1
7/28/2016	0
8/11/2016	0
8/18/2016	1
8/25/2016	0
8/31/2016	0
9/15/2016	0
Average:	0.92

**Copper is consumed by
background demand
in the pipeline**

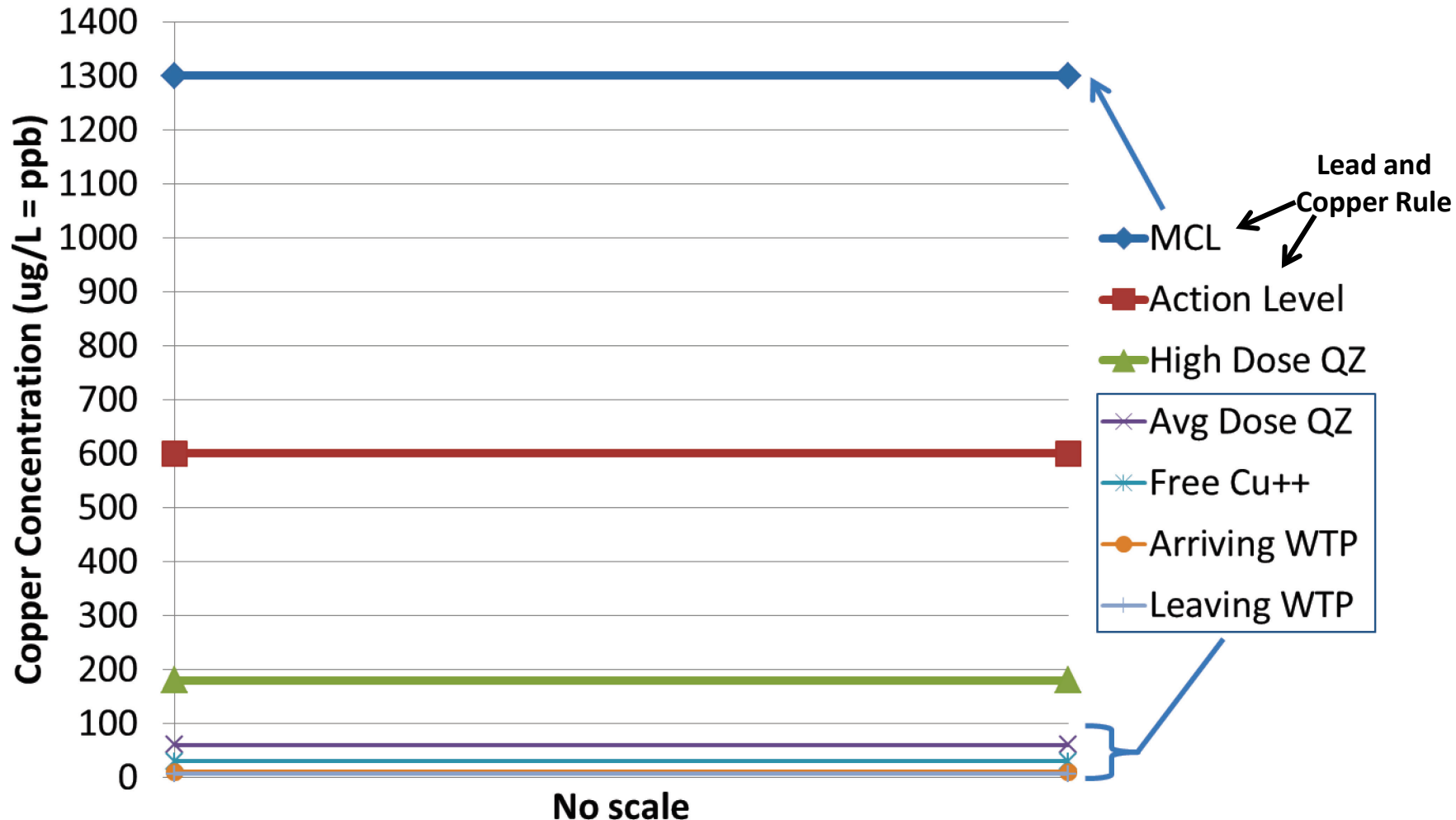
Ohio WTP

Avg Mussel Mortality to QZ Concentration Applied in Pipeline

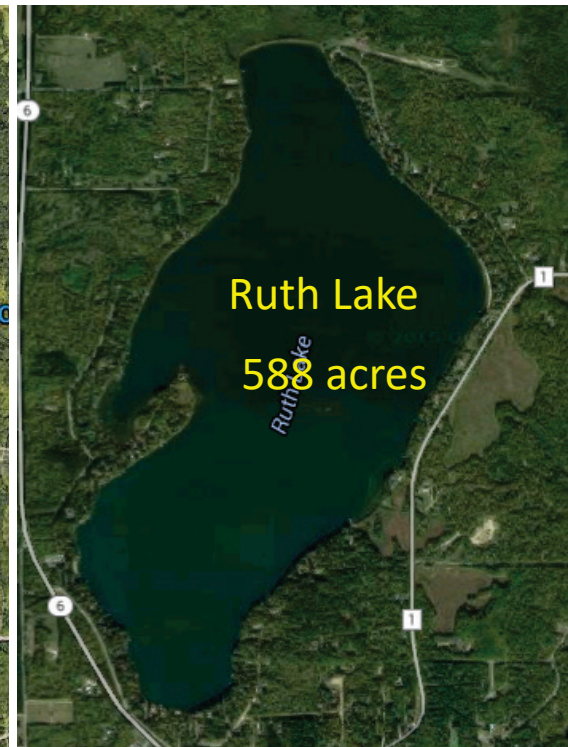


100% mortality in 6 days at 2 ppm, in 12 days at 1 ppm, in 28 days at 0.5 ppm

Copper Concentrations in Drinking Water



Recent Treatments to Control Mussels using EarthTec QZ in Open Waters of Lakes



*Work performed by PLM, Inc.,
out of Brainerd MN*

Control of Mussels with EarthTec QZ in the Open Waters of Lakes Independence Lake, MN



Control of Mussels with EarthTec QZ in the Open Waters of Lakes Independence Lake, MN





Recent Efforts to Control Mussels Using QZ in the Open Waters of Lakes

Lake Name	Christmas	Independence	Ruth Lake
	Lake	Lake	
State	Minnesota	Minnesota	Minnesota
Size (acres)	265	844	588
Treatment area (acres)	0.64	0.4	2.8
Treatment start date	2014/11/3	2014/11/3	2015/10/12
Water Temperature	39.8 F	41.9 F	56.4 F
Duration of Treatment (days)	8	8	6
Number of Doses	4	7	6
Avg Copper Conc during treat (ppb)	780	762	760
Target Organism	Zebra mussel	Zebra mussel	Zebra mussel
Outcome	Eradicated in Treatment Area	70% mortality in Treatment Area	Eradicated in Treatment Area
Chemical Cost	\$1,200	\$2,000	\$4,910

Work performed by PLM, Inc., out of Brainerd MN

Lessons: Warmer = Better, Rapid Response must be Rapid!

Recent Efforts to Control Mussels Using QZ in the Open Waters of Lakes



Last day of treatment at Independence Lake

Lessons: Warmer = Better, Rapid Response must be Rapid!

Public Perception:

“In 25 years, in hundreds of lakes and rivers across North America, it has been proven again and again that eradication does not exist...”

“Zebra and quagga mussels, once in a lake or river, cannot be stopped.”

Okanagan filmmaker Brynne Morrice.

Quoted October 21, 2015, in *The Osoyoos Times*, “Failure to eradicate mussels on Lake Winnipeg should be lesson for B.C., filmmaker tells Polak”

True or False? Should we just give up?

A thick, solid green line with a wavy, undulating shape that spans the width of the slide at the bottom.

Use of EarthTec QZ for Invasive Mussel Eradication in Open Waters

Zebra Mussels discovered in Lake Minnewashta, Minnesota

August 18, 2016

Mussel eradication led by:
**Minnehaha Creek
Watershed District**

Supported by:

- **Lake Preservation Association**
- **Carver County**

EarthTec QZ applied by:
PLM Lake and Land Mgt

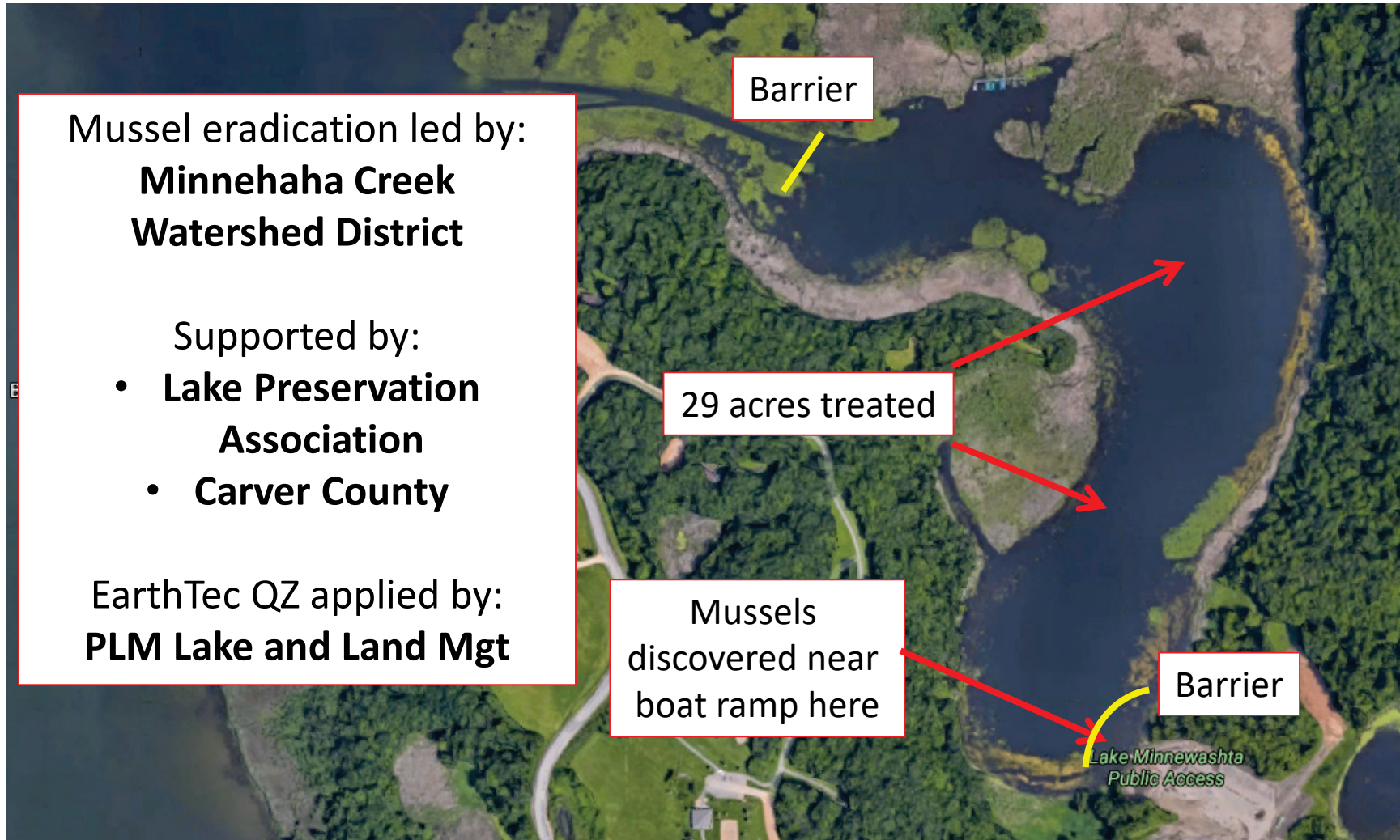
Barrier

29 acres treated

Mussels
discovered near
boat ramp here

Barrier

Lake Minnewashta
Public Access



Eradication of Zebra Mussels from Lake Minnewashta, Minnesota

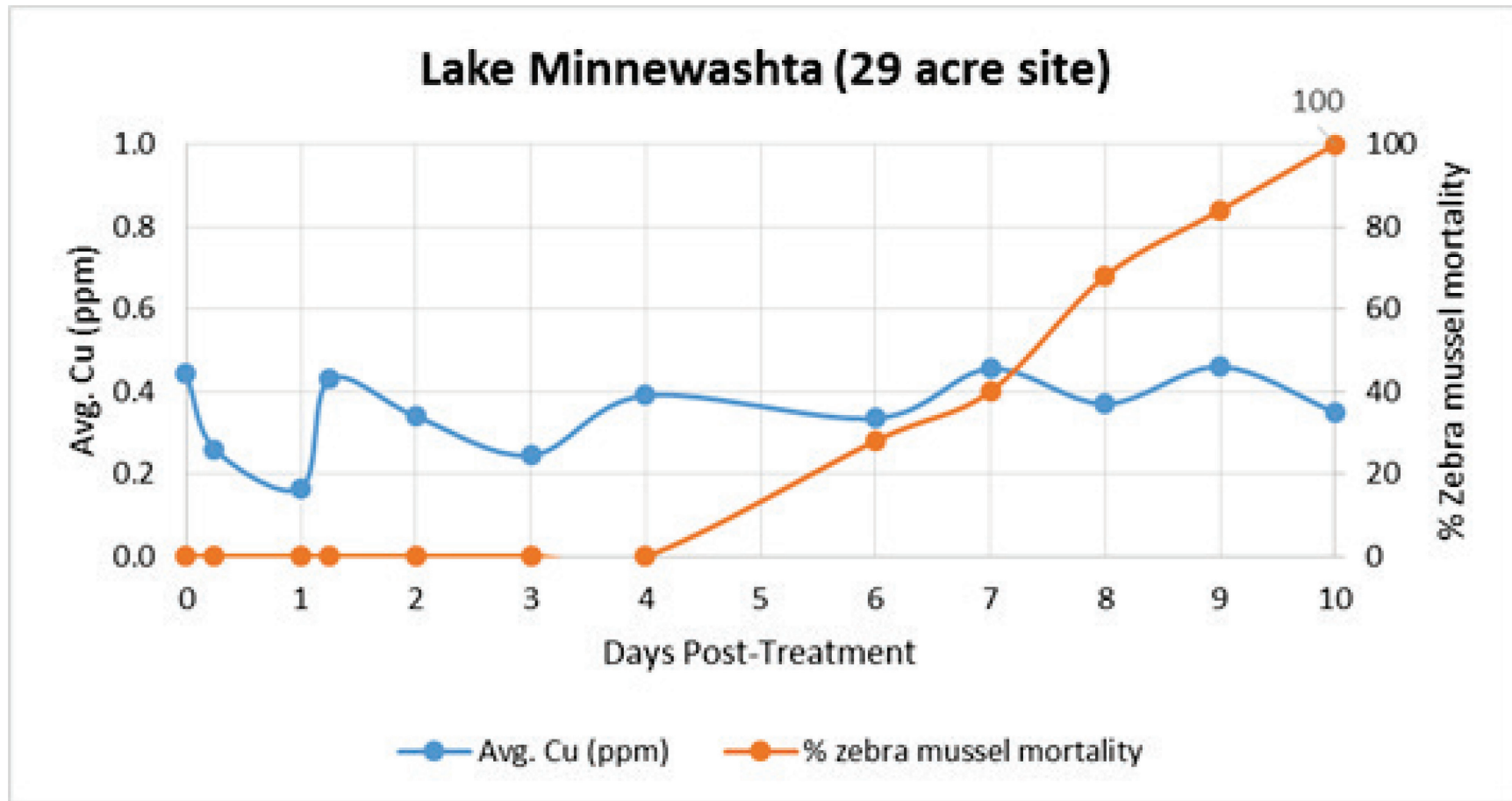
Sept 13-23, 2016



Eradication of Zebra Mussels from Lake Minnewashta, Minnesota

Sept 13-23, 2016

Water Temperature 19°C = 66°F

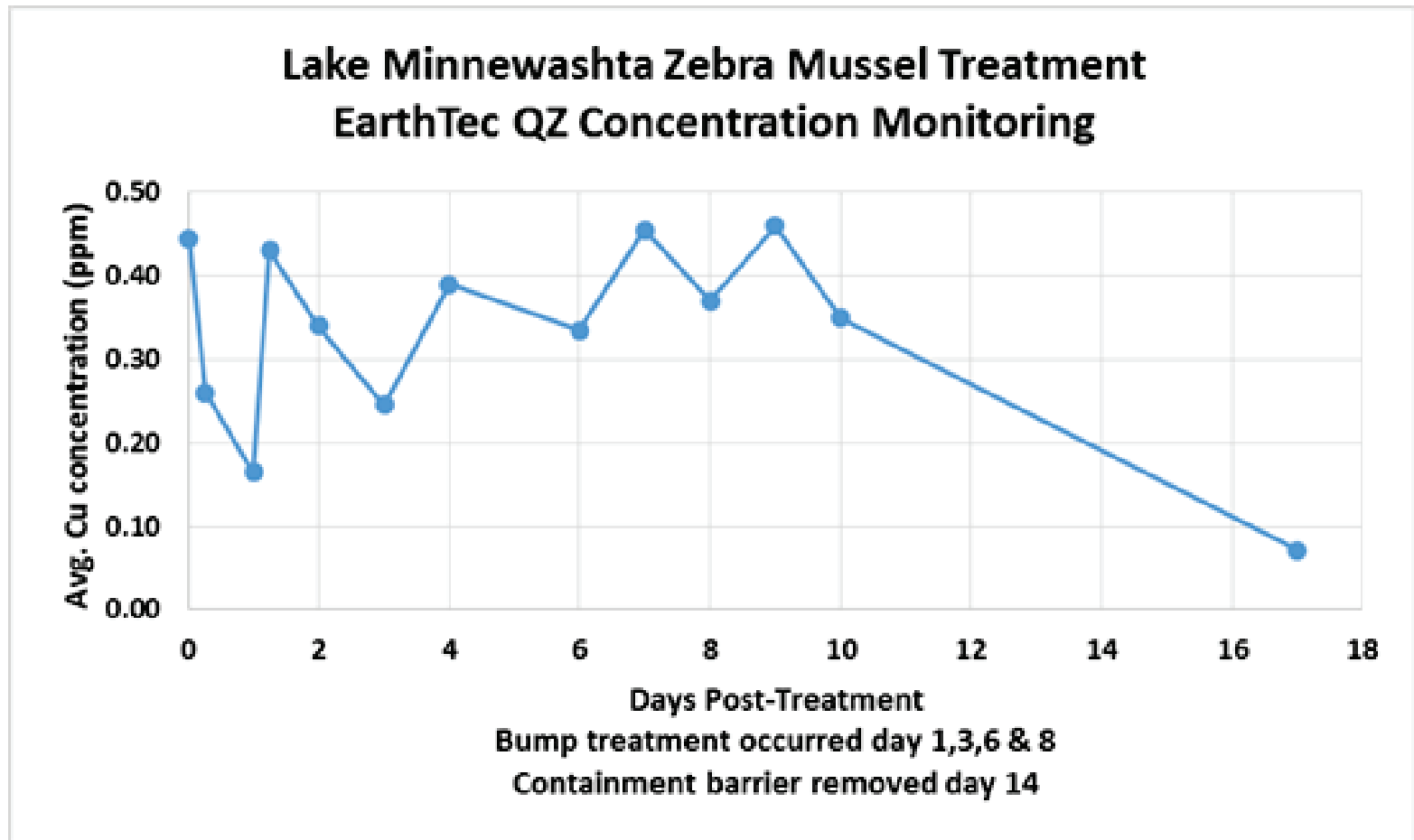


Source: Eric Fieldseth and Jill Sweet, Minnehaha Creek Watershed District

Residual copper during eradication of Zebra Mussels from Lake Minnewashta, Minnesota

Sept 13-23, 2016

Water Temperature 19°C = 66°F



Source: Eric Fieldseth and Jill Sweet, Minnehaha Creek Watershed District

Eradication of Zebra Mussels from Lake Minnewashta, Minnesota

Sept 13-23, 2016

Summary of QZ Dosing and Costs for Eradicating Invasive Mussels from Open Waters

Lake size, acres	29
Avg depth	9 ft
# of Applications	5
Treatment period	10 days
Sum of copper applied (5 doses)	1.06 mg/L
Chemical cost	\$22,500
Cost per acre	\$776
Cost per million gallons	\$265

Work performed by PLM Lake and Land Management, Inc.



Unique to the EarthTec QZ Label

- **Repeat applications are permissible** if needed to maintain lethal concentrations of copper for sufficient time period.
When re-applying, **do not exceed a resulting concentration of 1.0 mg/L** of metallic copper (background + applied copper) in the treated water.
- Pipelines are included

What about the fish?

What about the fish?

- **EarthTec has 15-year history of use in fish farms.**
 - **Start at shoreline and move outward in bands, allowing fish to move to untreated areas.**
 - **Apply at dose that fish tolerate, but mussels don't**
 - **Salmonids are about 10x more sensitive, so try to isolate by time and/or space.**
- 
- A decorative green wavy line at the bottom of the slide.

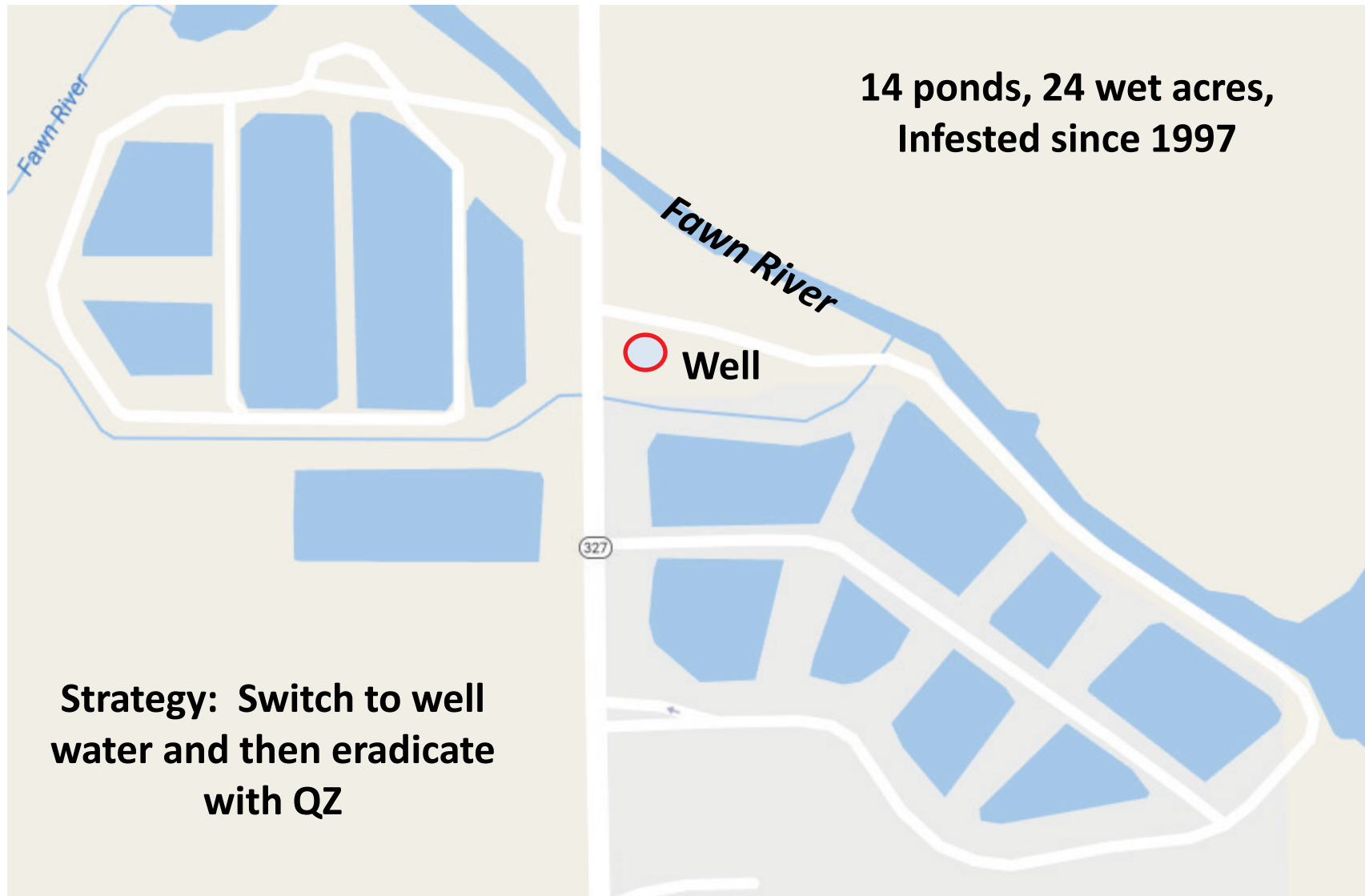
Eradication of Zebra Mussels from Fawn River Fish Hatchery, Indiana

July, 2016



Eradication of Zebra Mussels from Fawn River Fish Hatchery, Indiana

July, 2016



Eradication of Zebra Mussels from Fawn River Fish Hatchery, Indiana

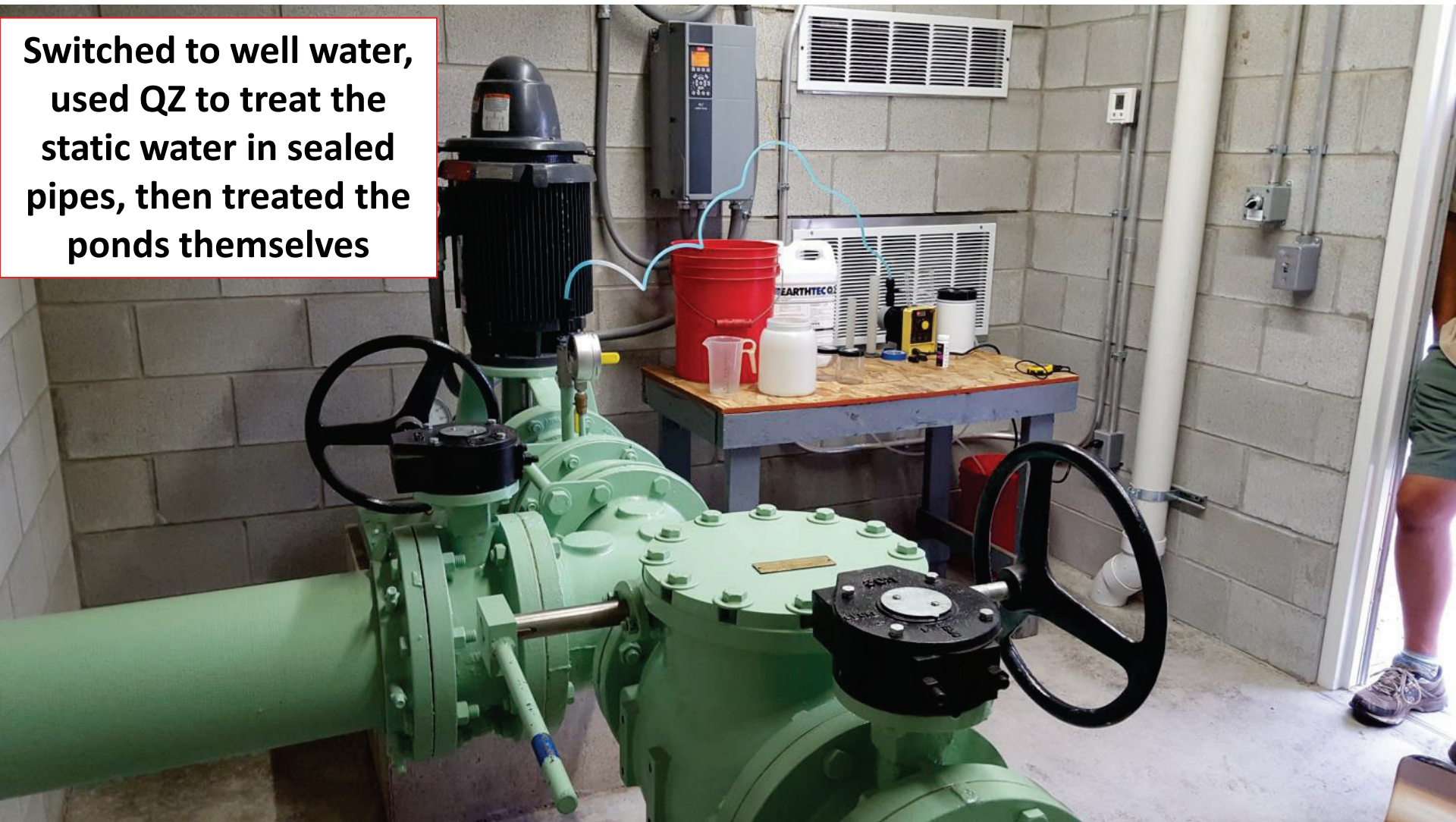
July, 2016



Eradication of Zebra Mussels from Fawn River Fish Hatchery, Indiana

July, 2016

Switched to well water,
used QZ to treat the
static water in sealed
pipes, then treated the
ponds themselves



Eradication of Zebra Mussels from Fawn River Fish Hatchery, Indiana

July, 2016

Results:

- Now monitoring for presence of veligers
- Hope to be removed from the “mussel positive” list in 2018.



Future Research

- **Dose-Response relationship for veligers**
 - **Other application sites (e.g. boats, power plants)**
 - **Other invasive species**
- 
- A solid green decorative wave shape at the bottom of the slide, starting from the left, dipping down, and then rising towards the right.

Quagga Mussel and Colonial Hydroid Control in Colorado River Water

Sept, 2016





Quagga Mussel and Colonial Hydroid Control in Colorado River Water

Sept, 2016





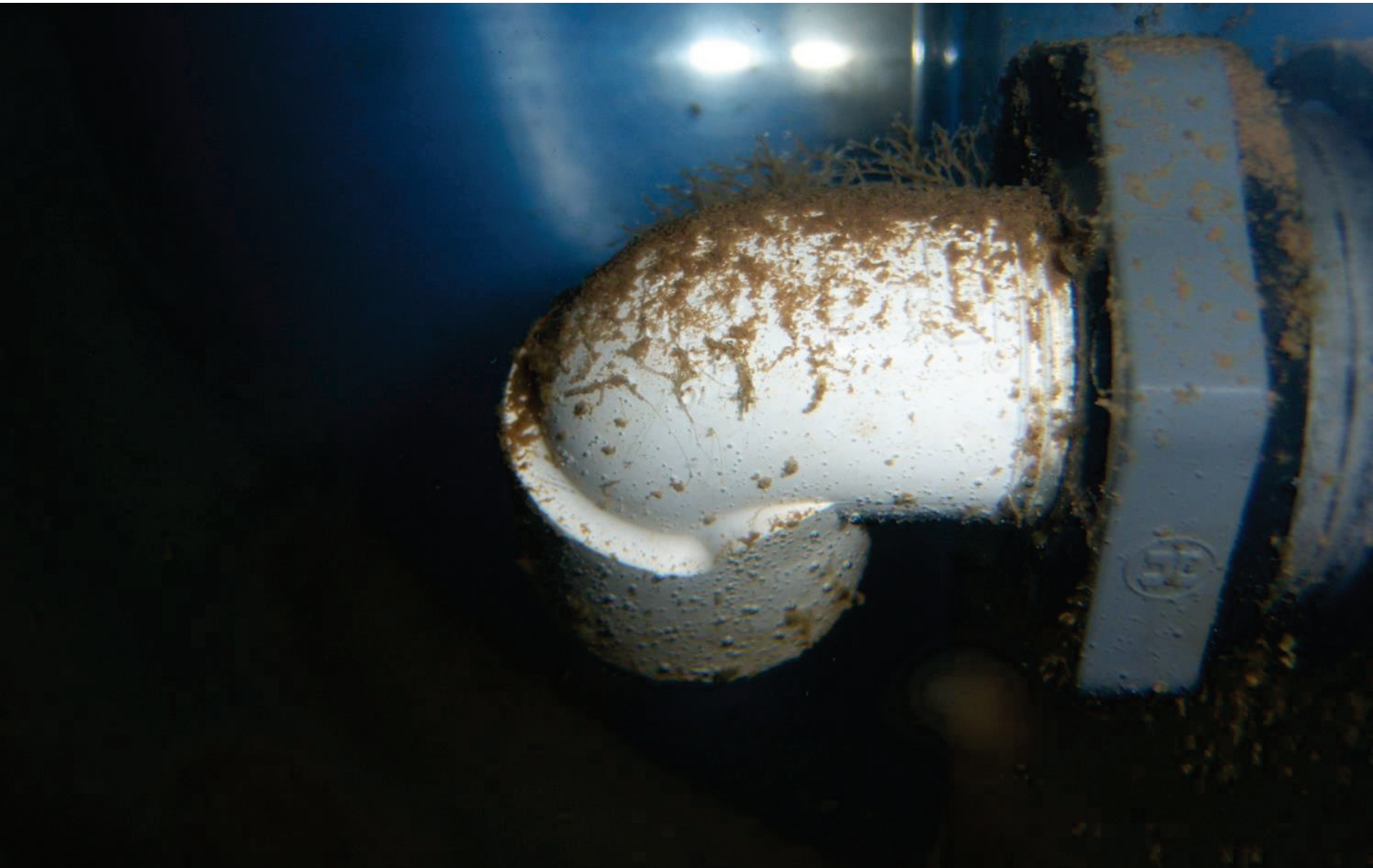


Quagga Mussel and Colonial Hydroid Control in Colorado River Water



Quagga Mussel and Colonial Hydroid Control in Colorado River Water

Sept, 2016



Quagga Mussel and Colonial Hydroid Control in Colorado River Water

Sept, 2016



What about Harmful Algal Blooms?

HABs in the News

Environment, US News

Toxic Algae Rampant in California, Also Spreading Nationwide

Drought has helped cr

By Tara MacIsaac, Epoch Times

for algae's growth

SCIENTIFIC AMERICAN

English Cart

Toxic Algae Blooms Are One Health Harm

The causes include incr

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™
CDC launches updated Harmful Algal Bloom System OHHABS as hot weather b



Harmful Algal Blooms and microcystins

HAB in Herriman, Utah in Aug, 2015



Herriman responded by immediately applying 4 ppm EarthTec and two weeks later a follow-up dose of 2 ppm

Harmful Algal Blooms and microcystins

Herriman city officials say Blackridge Reservoir again safe for swimming

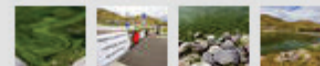
By Tori Jorgensen
For the Deseret News

Published: Wednesday, Sept. 2 2015 5:45 p.m. MDT

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The Blackridge Reservoir was closed due to an algae bloom in Herriman on Friday, Aug. 7, 2015.

Stacie Scott, Deseret News

Summary

Herriman city officials say Blackridge Reservoir is now safe

HERRIMAN — City officials say Blackridge Reservoir is now safe for swimming after chemical algaecide treatment decreased contamination levels.

Harmful Algal Blooms and microcystins

HAB in Norwalk, OH 2014



Monday morning

Microcystin = 12 -22 ug/L

Treated with EarthTec Monday afternoon



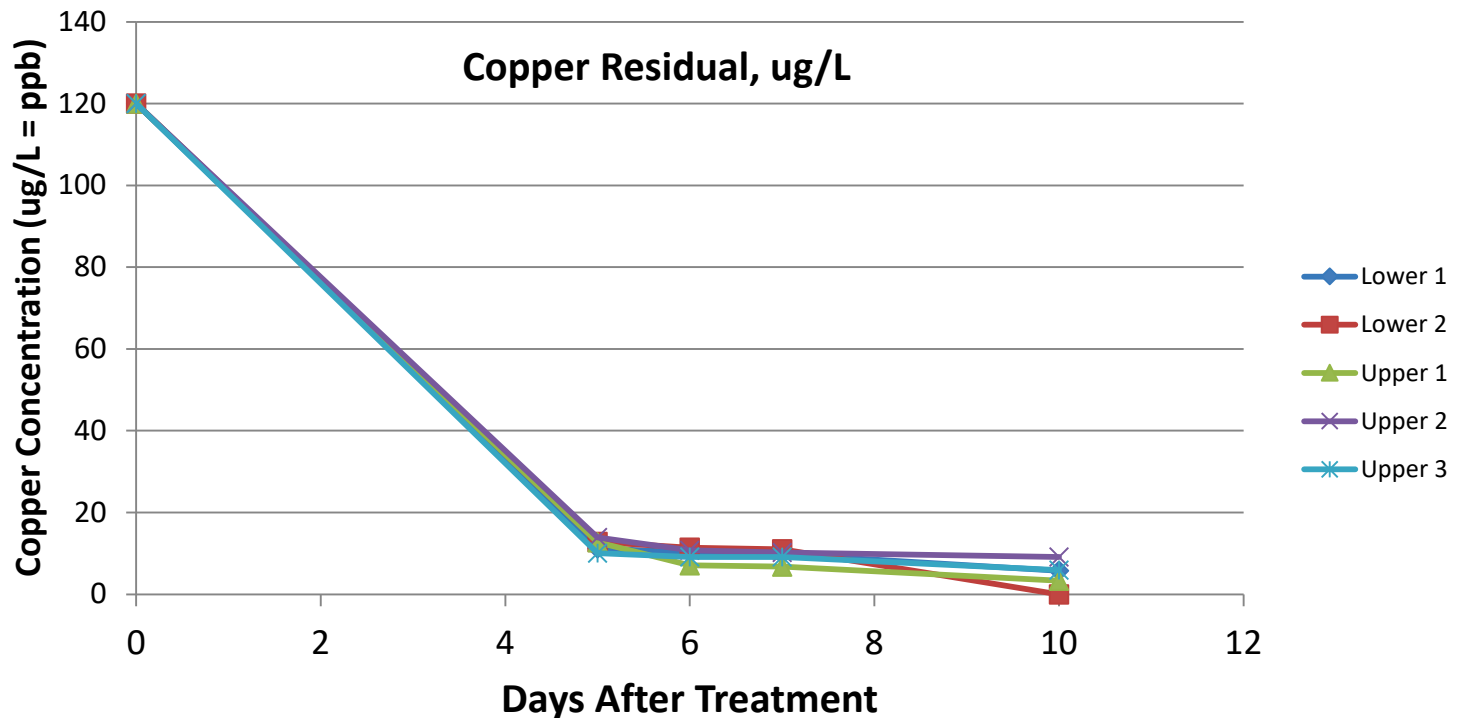
Wednesday morning

36 h post-treatment.

Microcystin <1 ug/L by Friday

Ohio EPA commended Norwalk on their appropriate management of the bloom.

Copper residual concentration following treatment of algal bloom July 1, 2016 using EarthTec



Copper residuals do not persist

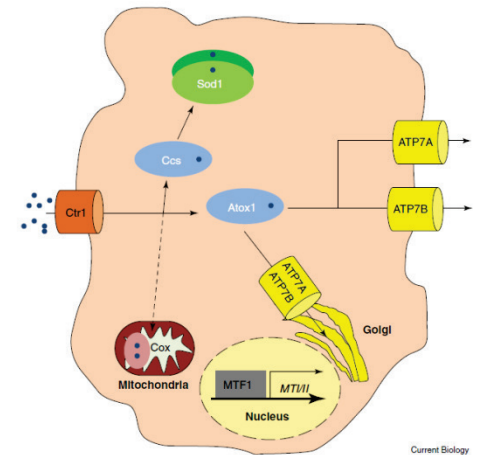
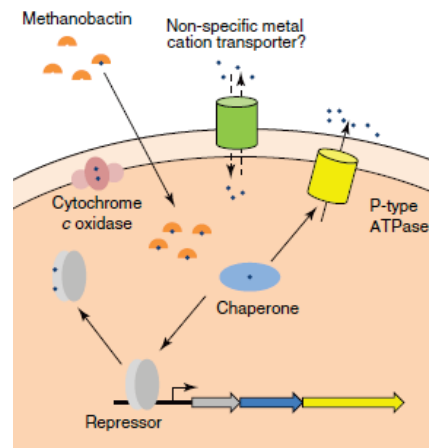
Figure: Residual free copper in the days following algaecide application by boat of 2 gallons EarthTec per surface acre on two different reservoirs of the Norwalk, Ohio WTP, Upper and Lower. Samples taken at 6" depth at different locations around the reservoir shoreline.

Primer

Copper: An essential metal in biology

Richard A. Festa and Dennis J. Thiele*

Life on Earth has evolved within a complex mixture of organic and inorganic compounds. While organic molecules such as amino acids, carbohydrates and nucleotides form the backbone of proteins and genetic material, these fundamental components of macromolecules are enzymatically synthesized and ultimately degraded. Inorganic elements, such as copper (Cu), iron and zinc, once solubilized from the



Current Biology

Table 1. Examples of Cu-dependent proteins and Cu homeostasis proteins.

Protein	Function	Bacteria	Fungi	Animals	Plants
Transcriptional regulators					
Ace1	Transcriptional activation in high Cu conditions		X		
CopY	Bacterial Cu metalloregulatory repressor	X			
CsoR	Bacterial Cu metalloregulatory repressor	X			
Mac1	Transcriptional activator in low Cu conditions		X		
CueR	Bacterial Cu metalloregulatory repressor	X			
Mtf1	Metalloregulatory transcription factor			X	
Sp17	Transcriptional activator responding to Cu deficiency				X
Chaperones/storage					
Atox1	Metallochaperone delivering Cu to P-type ATPases		X	X	X
Ccs	Delivers Cu to the Cu/Zn SOD1		X	X	X
CopZ	Bacterial Cu chaperone	X			
Metallothionein	Low molecular weight, cysteine-rich metal-binding and detoxification	X	X	X	X
Cell surface/secretory compartment transporters and receptors					
P1B-type ATPases	Cu ²⁺ -exporting proteins	X		X	X
Ctr	Cu ⁺ -importing proteins		X	X	X
Ethylene receptor	Uses Cu as a cofactor for ethylene signaling				X
Oxidoreductases					
Ascorbate oxidase	Reduction of L-ascorbate			X	
Dopamine-monooxygenase	Tyrosine metabolism			X	
Galactose oxidase	Reduction of galactose		X		
Amine oxidase	Oxidation of diamines	X	X	X	X
Electron transfer/energy production/blue Cu proteins					
Cytochrome c oxidase	Necessary for the last step of respiration	X	X	X	X
Plastocyanin	Electron transfer during photosynthesis	X			X
NADH dehydrogenase	Electron transfer from NADH to coenzyme Q	X	X	X	X
Nitrite reductase	Reduces nitrite to nitric oxide	X			
Amicyanin	Electron-accepting intermediate in the conversion of methylamine to formaldehyde and ammonia	X			
Free radical scavenging					
Cu/Zn SOD	Free radical scavenging	X	X	X	X
Oxidase					
Laccase	Melanine production	X	X	X	X
Lysyl oxidase	Catalyzes the formation of collagen and elastin precursors, extracellular			X	
Ceruloplasmin	MultiCu oxidase			X	
Hephaestin	Transmembrane ferroxidase, transports iron from the intestine to the circulatory system			X	
Multicopper ferroxidase	Cu-dependent iron uptake		X	X	X
Monoxygenase					
Methane monooxygenase	Oxidizes C-H bond in methane	X			
Phenylalanine hydroxylase	Hydroxylation of the aromatic side chain of phenylalanine to generate tyrosine			X	
Tyrosinase	Monophenol monooxygenase, catalyzes the oxidation of phenols, melanin synthesis	X	X	X	X

Harmful Algal Blooms and common perception on cell lysis

The Lysed Cell (or Leaky Cell) Hypothesis:

Cyanobacteria contain microcystin internally, and upon treatment with algaecides the cells are lysed and release microcystin into the water, consequently posing increased threats to organisms.

Implication:

If you've got a cyanobacterial bloom, you better not treat (and especially not with copper sulfate) because you'll make a bad problem a lot worse!

**How accurate and relevant is this depiction?
In what situations should this thinking carry the day?**

Source: Modified from West M. Bishop, Brenda M. Johnson, John H. Rodgers, Jr., Clemson University, Clemson SC
"Microcystin concentrations following treatments of harmful algal blooms"

Harmful Algal Blooms and cyanotoxins

WHO's Provisional Guideline for Microcystin Risk in Drinking Water:

- Total microcystin-LR (free plus cell-bound) is 1 µg/L

EPA 10-Day Drinking Water Health Advisories:

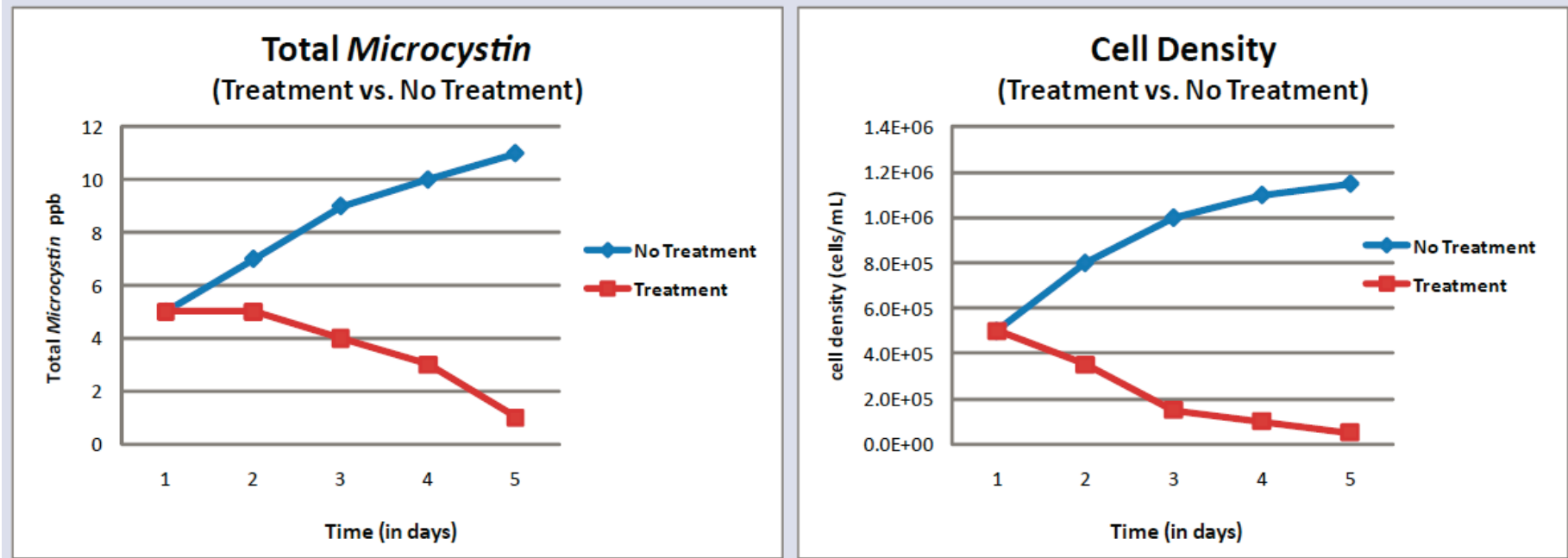
- Microcystin – 0.3 ug/L in children, 1.6 ug/L in adults
- Cylindrospermopsin – 0.7 ug/L in children, 3.0 ug/L in adults

There is no distinction made between the toxicity of intracellular and extracellular cyanotoxins in drinking water. They're equally toxic, whether inside the cell or outside the cell.

The question of intracellular vs extracellular is relevant primarily in the context of a WTP's capacity to remove those cyanotoxins.

A green decorative wave graphic at the bottom of the slide.

Harmful Algal Blooms and Microcystin



The result of treatment with algaecide is a far lower concentration of total cyanotoxin as compared to taking no action

Source: West M. Bishop, Brenda M. Johnson, John H. Rodgers, Jr., Clemson University, Clemson SC
"Microcystin concentrations following treatments of harmful algal blooms"

Harmful Algal Blooms and microcystins

The Lysed (or Leaky) Cell Hypothesis:

Cyanobacteria contain microcystin internally, and upon treatment with algaecides the cells are lysed and release microcystin into the water, consequently posing increased threats to organisms.

The Rebuttal:

- If the source of the toxin is controlled (i.e. cyanobacteria) no more toxin can be produced.
- Any cyanotoxin present begins to degrade (bacteria help!)
- Treatments do not have to lyse the cell for cell death to occur.
- A decision to take “no action” often results in increased toxin and consequent risk.

Source: Modified from West M. Bishop, Brenda M. Johnson, John H. Rodgers, Jr., Clemson University, Clemson SC
“Microcystin concentrations following treatments of harmful algal blooms”

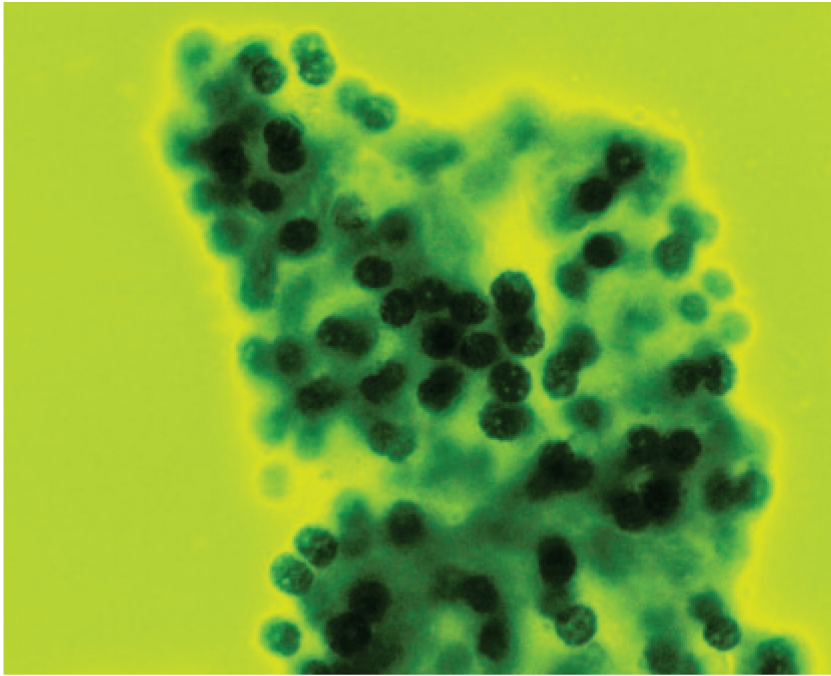
EarthTec study against a severe cyanobacterial bloom in Florida Fred Singleton, Ph.D.



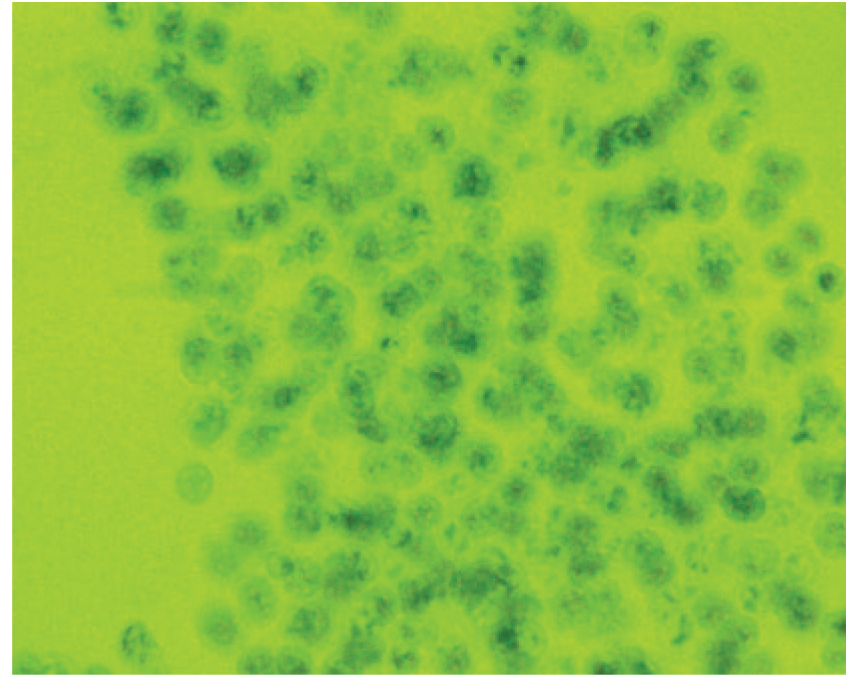
Location: Central Marine Stuart, Stuart, FL (July 2016)

Severity: Significant – Biomass mat was *ca.* 8" thick. Primarily *Microcystis*.

***Microcystis* sp. + 120 ppb Cu (EarthTec), T = 0**



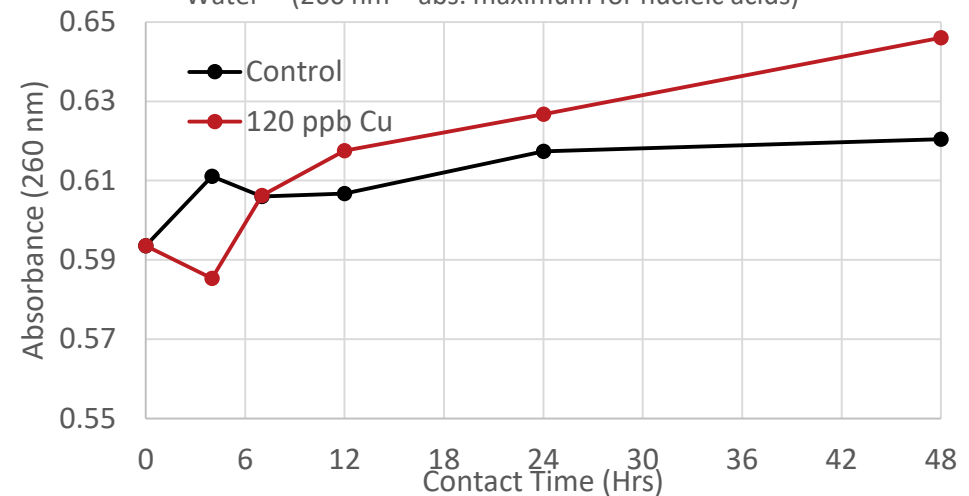
***Microcystis* sp. + 120 ppb Cu (EarthTec), T = 24 hr**



OBSERVATIONS

- Mass of cell clusters greatly reduced
- EarthTec does NOT cause immediate or extensive lysis of *Microcystis* cells
- EarthTec treatment results in cells slowly leaking their cytoplasmic materials into the surrounding water
- EarthTec does NOT inhibit non-target microorganisms that consume leaked cytoplasmic materials

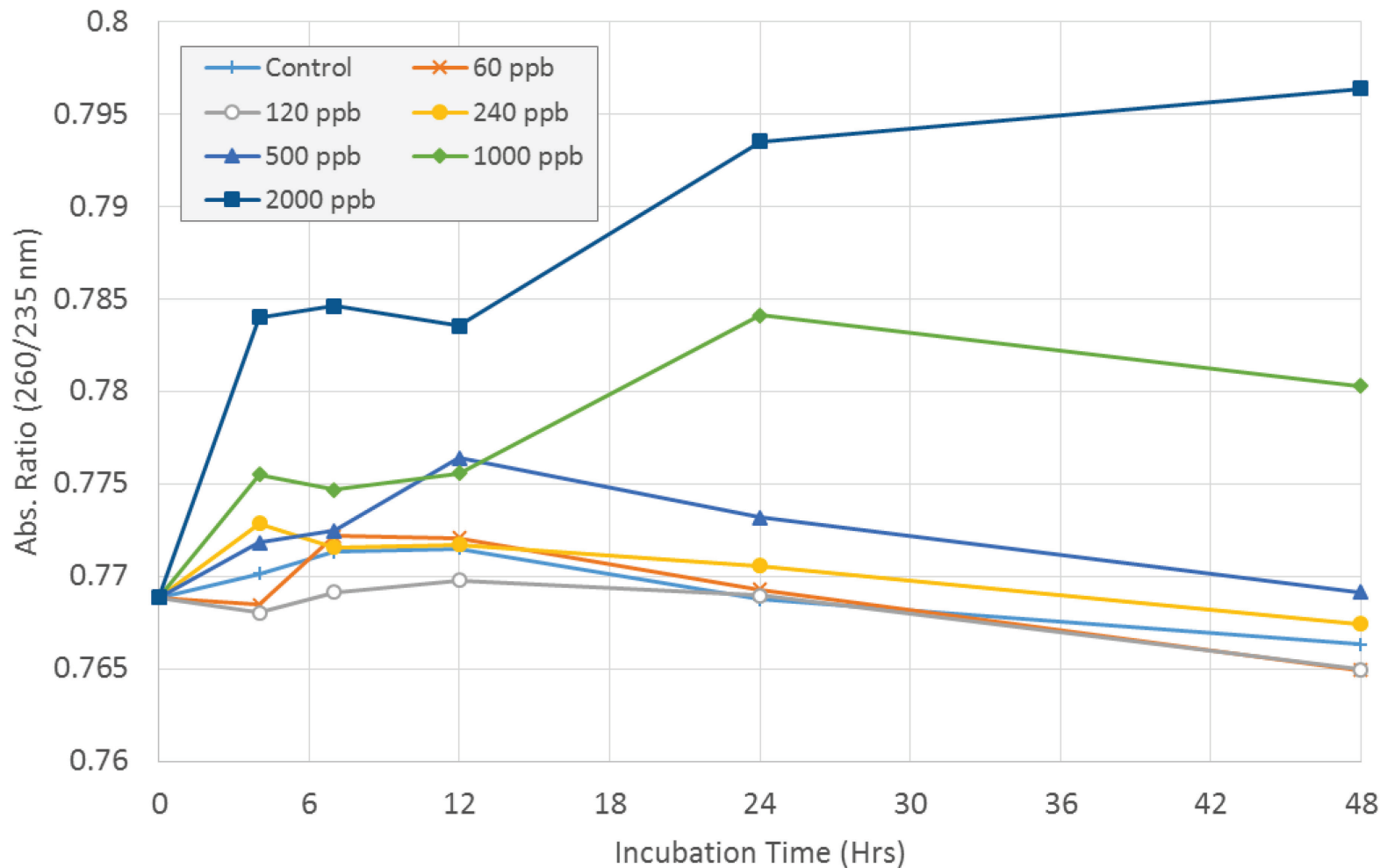
Membrane Leakage of Organics from *Microcystis* sp. in Marina Water -- (260 nm = abs. maximum for nucleic acids)



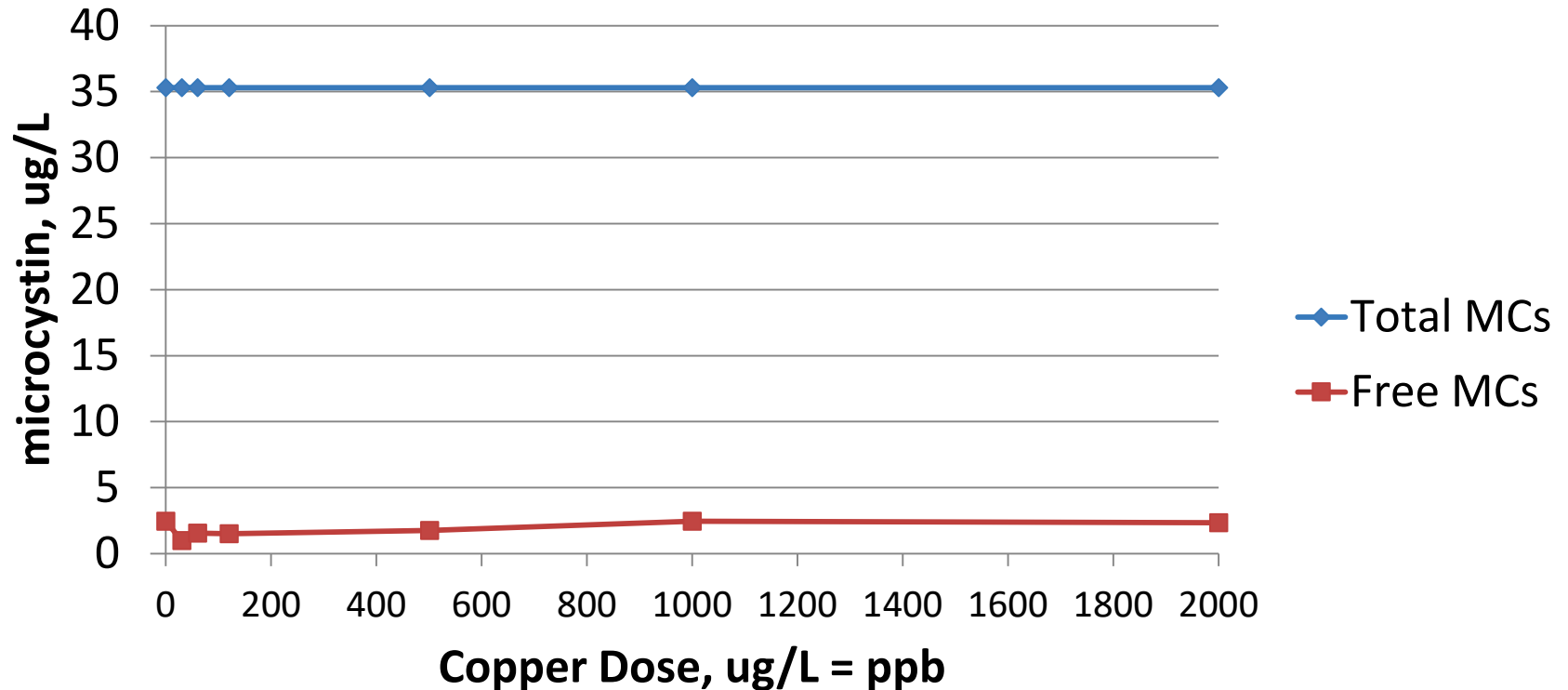
Change in relative concentrations of dissolved organics in marina water (collected during a heavy *Microcystis* bloom) treated with various concentrations of Cu (as EarthTec).

Wavelengths = 260 and 235 nm as indices of nucleic acids and carbohydrates, respectively.

Significant changes occur only at doses of 500 ppb and higher.



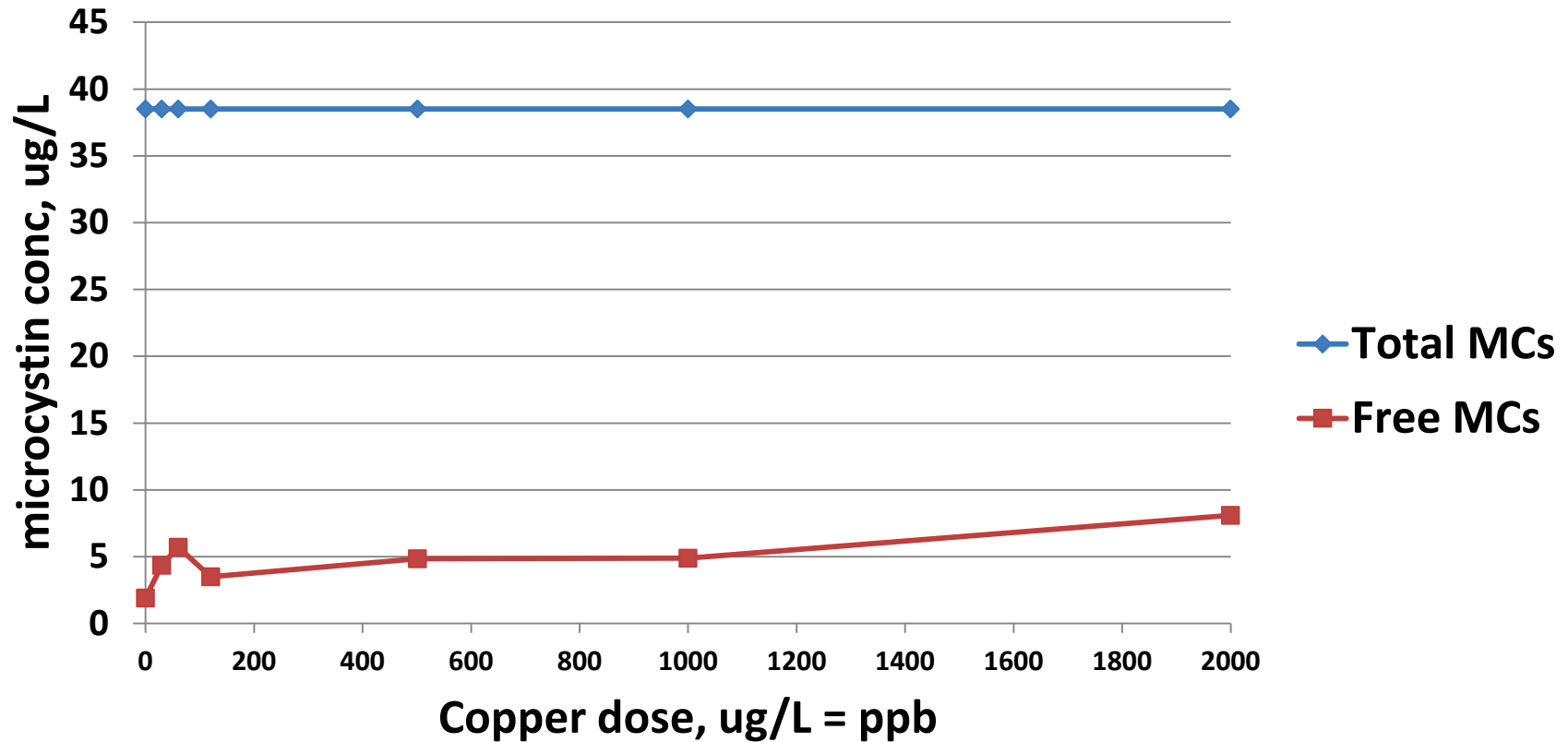
Total vs Free microcystins (MCs) following different doses of copper as EarthTec, T= 1 hr



Even at high doses, copper as EarthTec shows no catastrophic release of microcystins

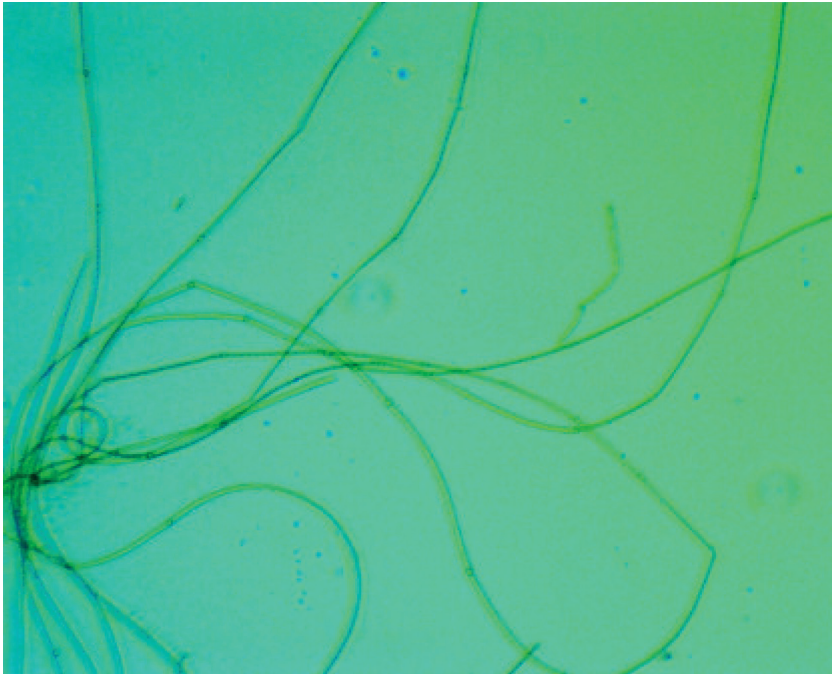
Total vs Free microcystins (MCs)

following different doses of copper as EarthTec, T=24 hr

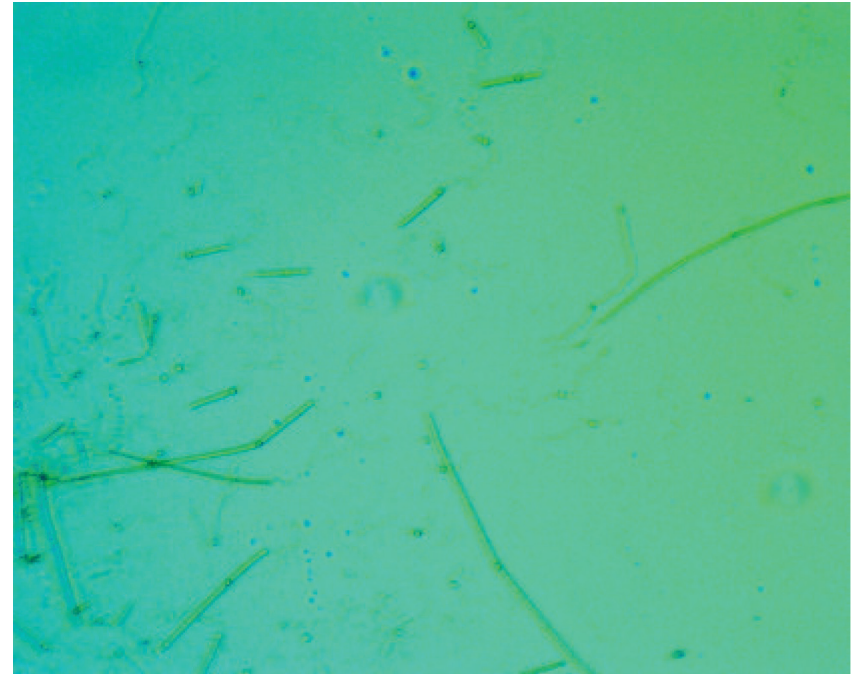


Even at high doses, copper as EarthTec shows no catastrophic release of microcystins

***Anabaena*, 120 ppb Cu (EarthTec), T = 0**



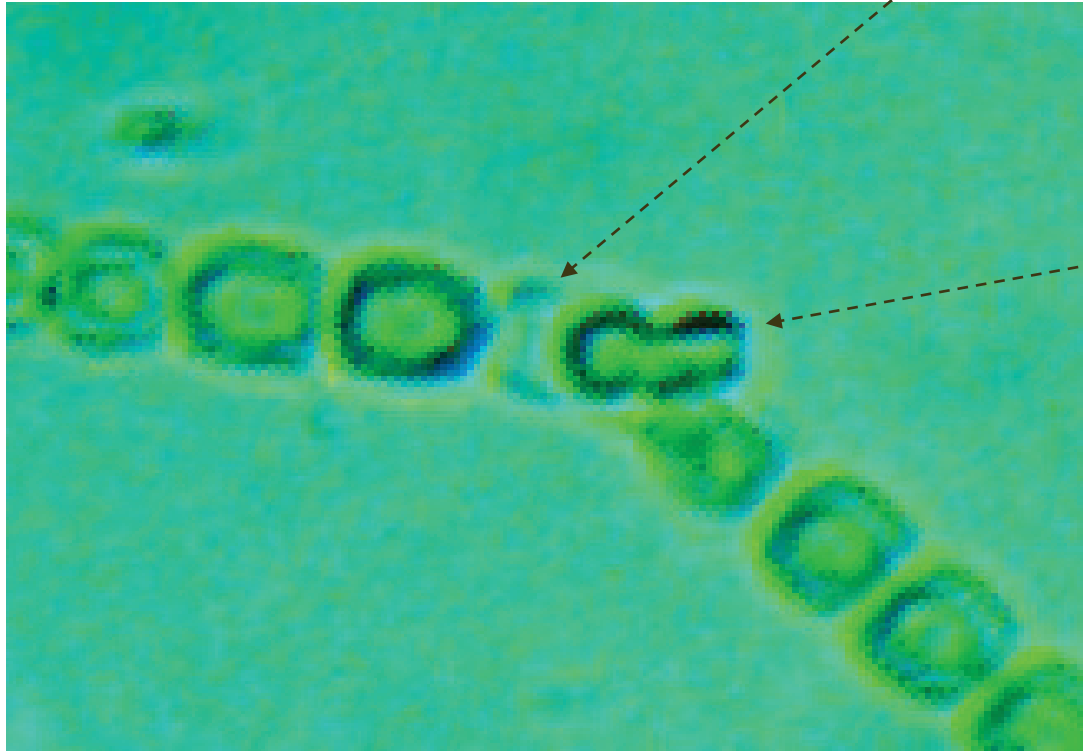
***Anabaena*, 120 ppb Cu (EarthTec), T = 8 hr**



- Release of cells from filaments during 8 hr contact
- Many of the photosynthetic cells remained structurally intact
- Heterocysts remained structurally intact

Filamentous *Anabaena* treated with 120 ppb Cu as EarthTec

Remnant of sheath/gelatinous layer

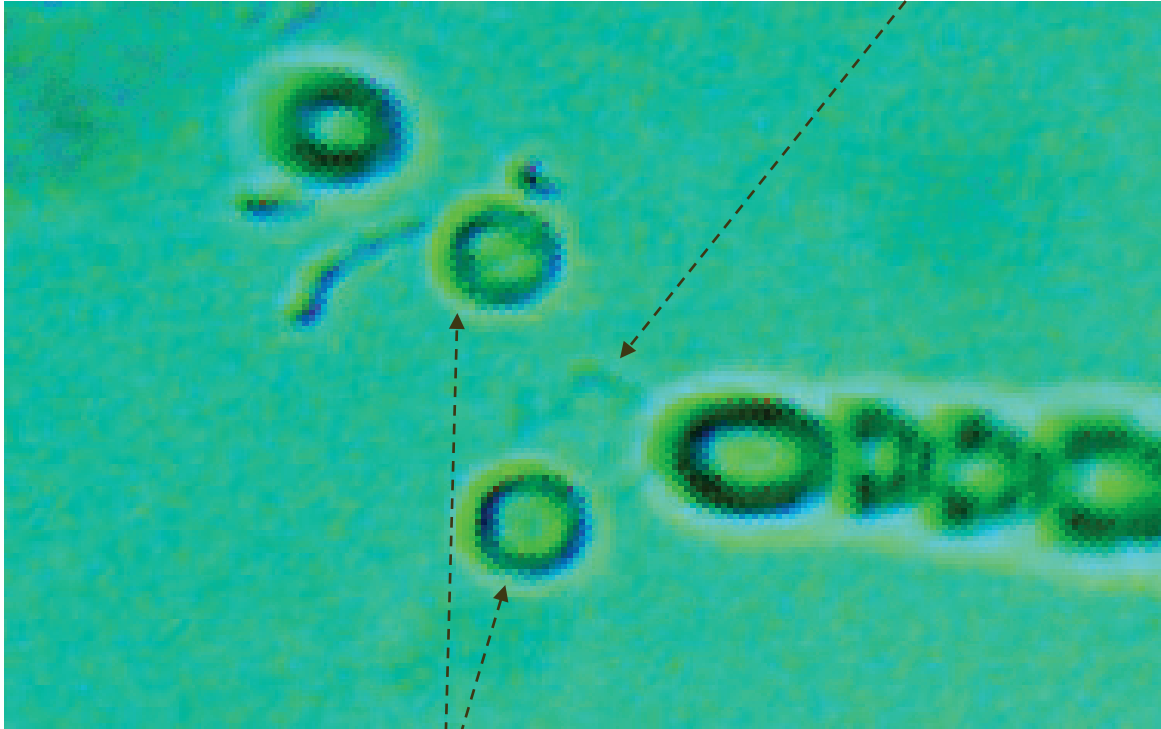


Cell being
released
from filament –

Cell is intact
(no lysis)

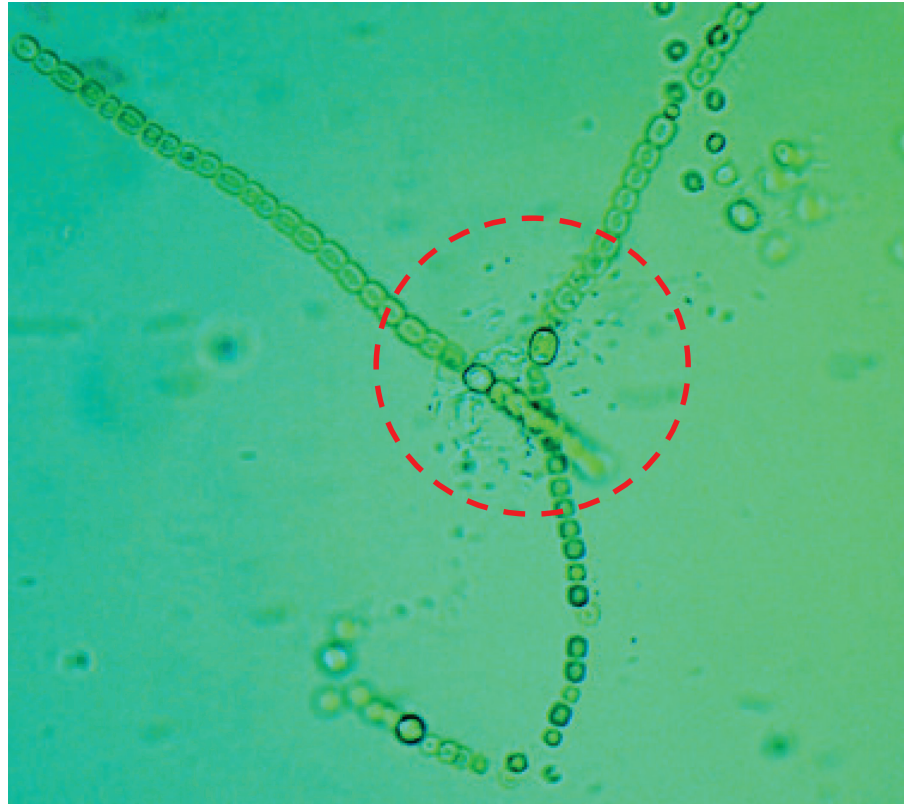
Anabaena, 120 ppb Cu as EarthTec

Remnant of sheath/gelatinous layer



Cells immediately after separation from filament

Anabaena, 120 ppb Cu as EarthTec, T = 14.5 hr



- Heterotrophic bacteria feeding on materials released from cyanobacterial cells –
- Illustrates non-target organisms are not affected by EarthTec copper
- Most of the photosynthetic cells remained structurally intact
- Heterocysts remained structurally intact

Percarbonate added to cyanobacterial culture, T = 0 to 30 mins

Percarbonate (strong oxidizer) is wide-spectrum, killing everything.

EarthTec added to cyanobacterial culture, T = 48 hrs

Amoebas happily feeding on cytoplasm leaking through cyanobacterial cell wall

In Summary, Regarding the Cell Lysis Conversation

Copper is relatively slow-acting, on various metabolic functions

EarthTec copper is somewhat selective against cyanobacteria

EarthTec doses relevant to HAB management don't have to lyse the cell

There is slow leaking of cytoplasmic materials at a pace compatible with bacterial decomposition

If you're treating a location that is days away from the intake of a WTP, algaecidal treatment will stop production of more toxin and allow existing toxins to begin degrading

At or near the WTP, your protocol should be customized to the situation, but low doses of copper are a valuable part of the toolbox



Thank you!

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