

Tracking Taste & Odor Producers and Potential Degradation in a Drinking Water Supply Reservoir

WaterTop COST Meeting
7-9 October 2021, Tartu, Estonia

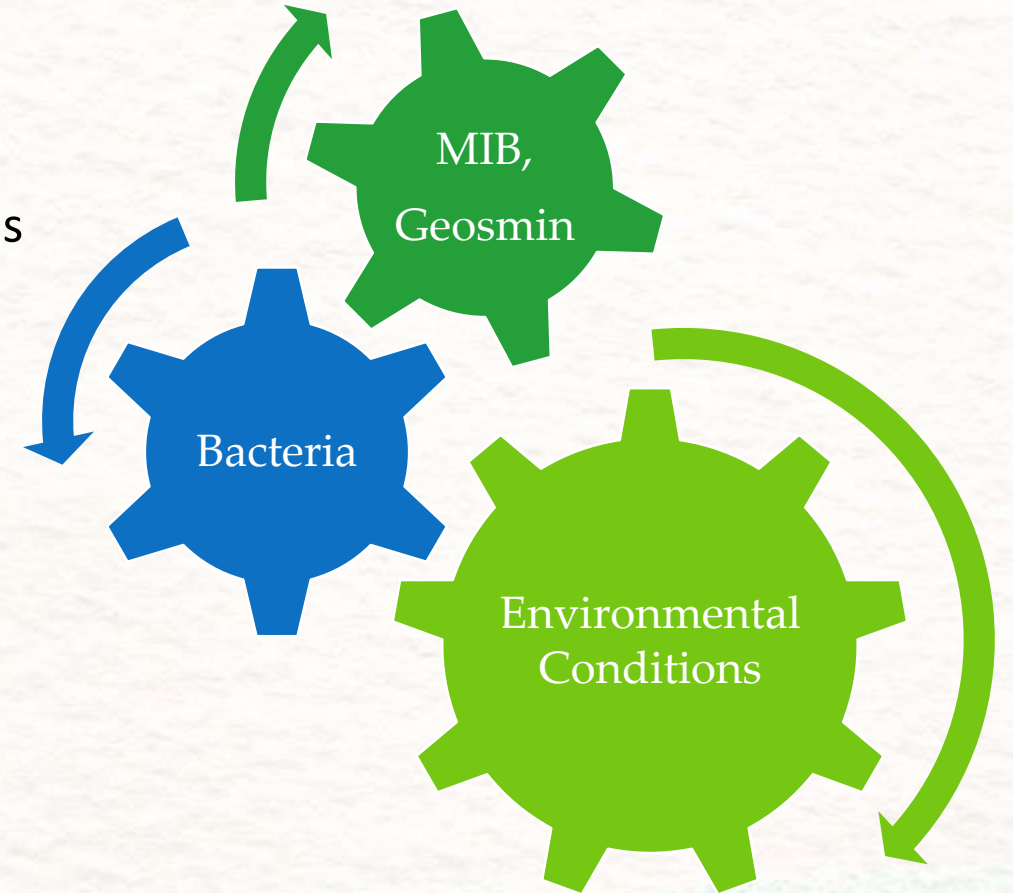
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Taste & Odor Issues

- Taste-and-Odor (T&O) nuisances are reported worldwide
- Major concern for water utilities as T&O outbreaks taint source waters
 - Foul smell;
 - Difficulty of removal with traditional water treatment;
 - Source of numerous customer complaints;
- Aesthetics are the primary criteria that determine consumer confidence in the safety of a water supply
- Volatile metabolites account for the majority of reported T&O
 - with substantial costs to water production and aquaculture industries

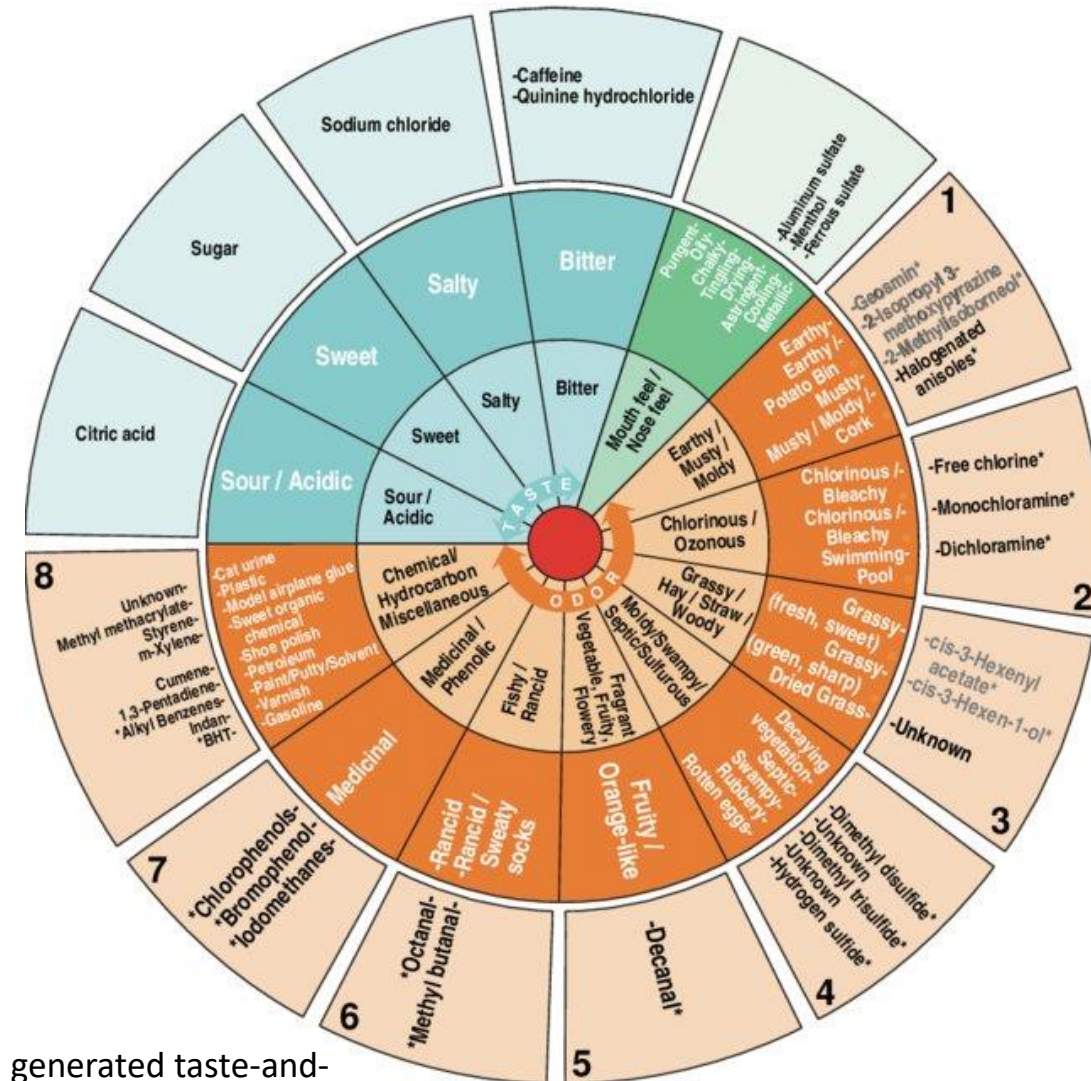
T&O Challenges

- In aquatic ecosystems, it is difficult to link T&O occurrences with specific taxa
 - Diverse sources: planktic, epiphytic or benthic.
- Co-occurring organisms may produce same compounds
 - Cyanobacteria (= *autotrophic* bacteria);
 - Actinobacteria (= *heterotrophic* bacteria);
- Species abundance or dominance may show no apparent relationship with T&O levels
 - Peak production and population density can be asynchronous;
 - Sub-dominant species might be the producers.
- Biosynthesis of each metabolite can vary significantly within and among taxa



Characteristics of Taste & Odors

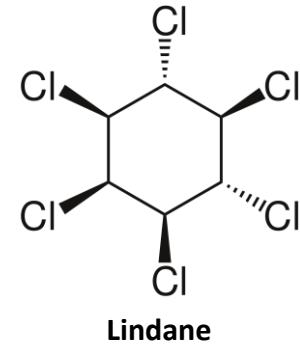
- The terms 'T&O' used in the water industry
- Only four taste sensations:
 - sour, sweet, salty and bitter
- Taste alone is rarely a problem in water supply
- Odors occur due to foreign compounds in water
- Eight odor categories:
 - earthy/musty/moldy;
 - chlorinous/ozonous;
 - grassy, hay-like, woody;
 - decaying/swampy/septic/sulfurous;
 - fruity, flowery, fragrant;
 - rancid/fishy;
 - medicinal/phenolic;
 - chemical/paint-like



Khiari, D. et al (2002). Distribution generated taste-and-odor phenomena. American Water Works Association.

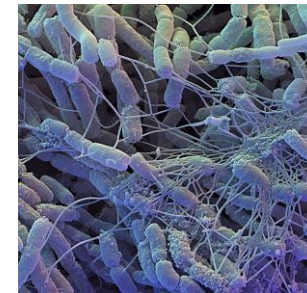
1/2 Sources of T&O

- Man-made
 - Organics
 - Wastes of industries, cities, agriculture
 - Chlorinated phenols and hydrocarbons (pesticides)
 - Inorganics
 - Fertilizers (NPK) -> algal growth
 - Sulfur
 - Metals
 - Treatment & distribution system
 - Free halogens residuals (chlorine, bromide, iodine) produce T&O in potable water

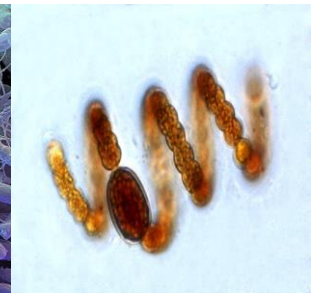


2/2 Sources of T&O

- Natural
 - Algae
 - *Chlorophyceae, Bacillariophyceae, Euglenophyceae, Chrysophyceae...*
 - Alcohols, esters, aldehydes, ketones, and acids
 - Decaying vegetation
 - algae, leaves, macrophytes
 - Phenols, organic matter
 - Bacteria:
 - Actinobacteria (*Streptomyces, Micromonospora, Nocardia...*)
 - Soils, sediments
 - Cyanobacteria (*Dolichospermum, Aphanizomenon, Planktothrix, Pseudanabaena...*)
 - Photic zone, epilimnion
 - Sulfate-reducing bacteria (Desulfobacterales, Desulfovibrionales, Syntrophobacterales)
 - Hydrogen sulfide, H₂S in hypolimnia and aquifers
 - Zooplankton
 - *Cyclops, Daphnia...*
 - Fishy smell



Streptomyces



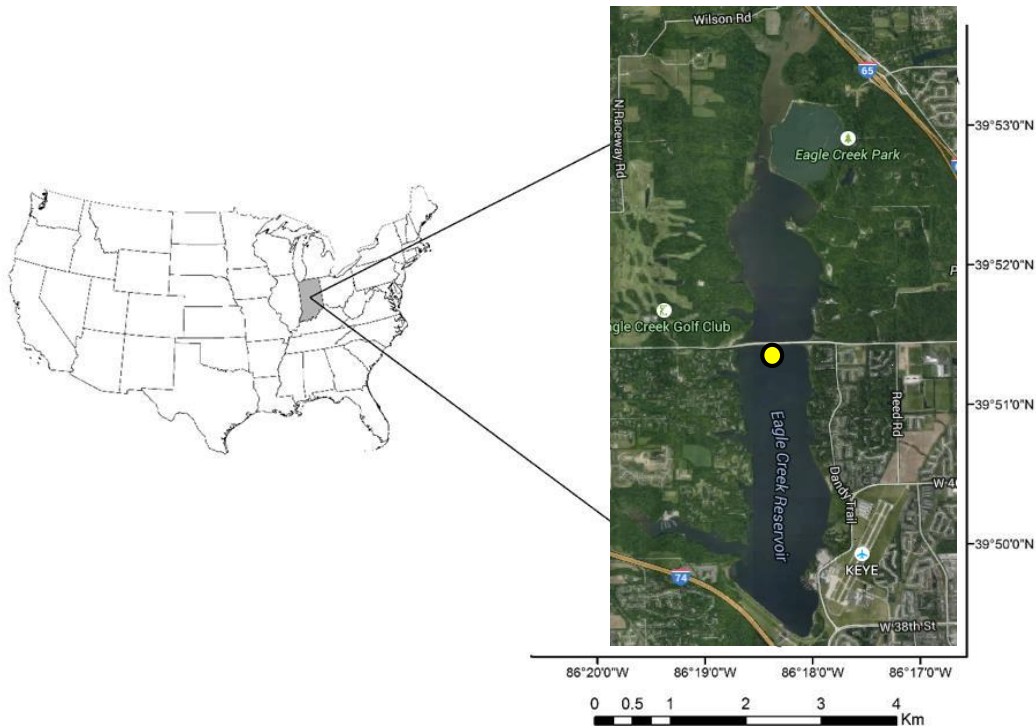
Dolichospermum

Biological T&O Wheel



Watson, S., & Jüttner, F. (2019). Biological production of taste and odour compounds.

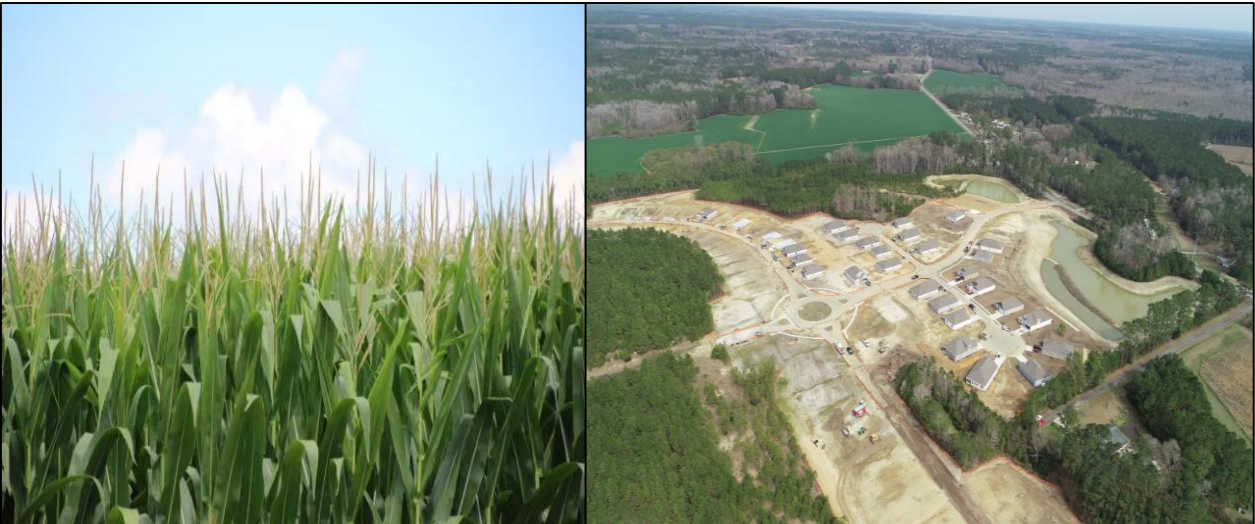
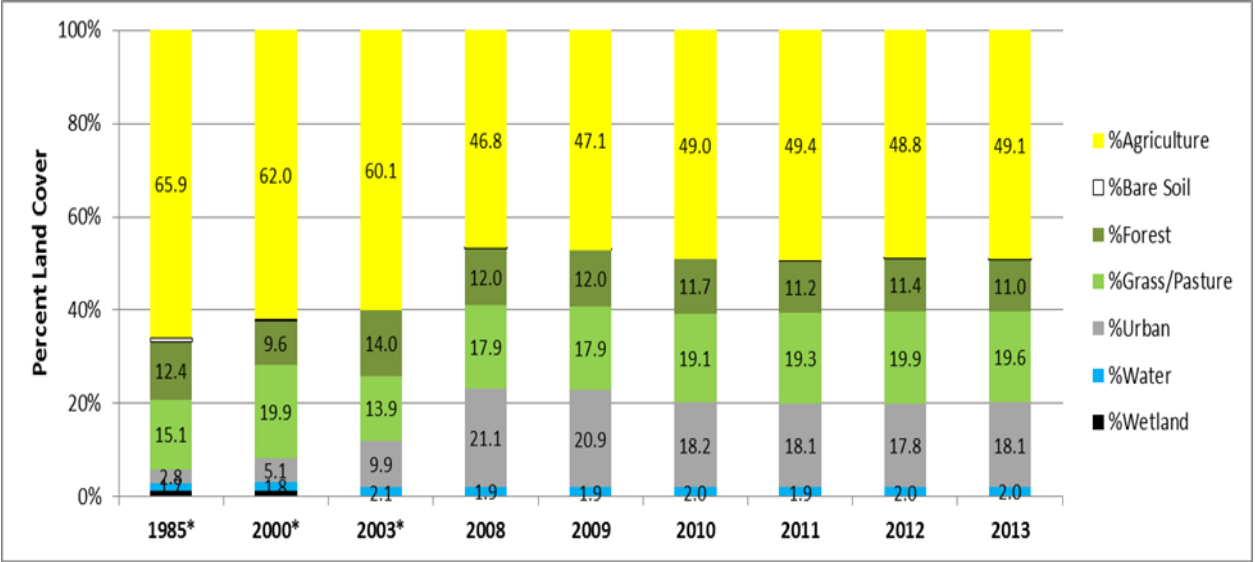
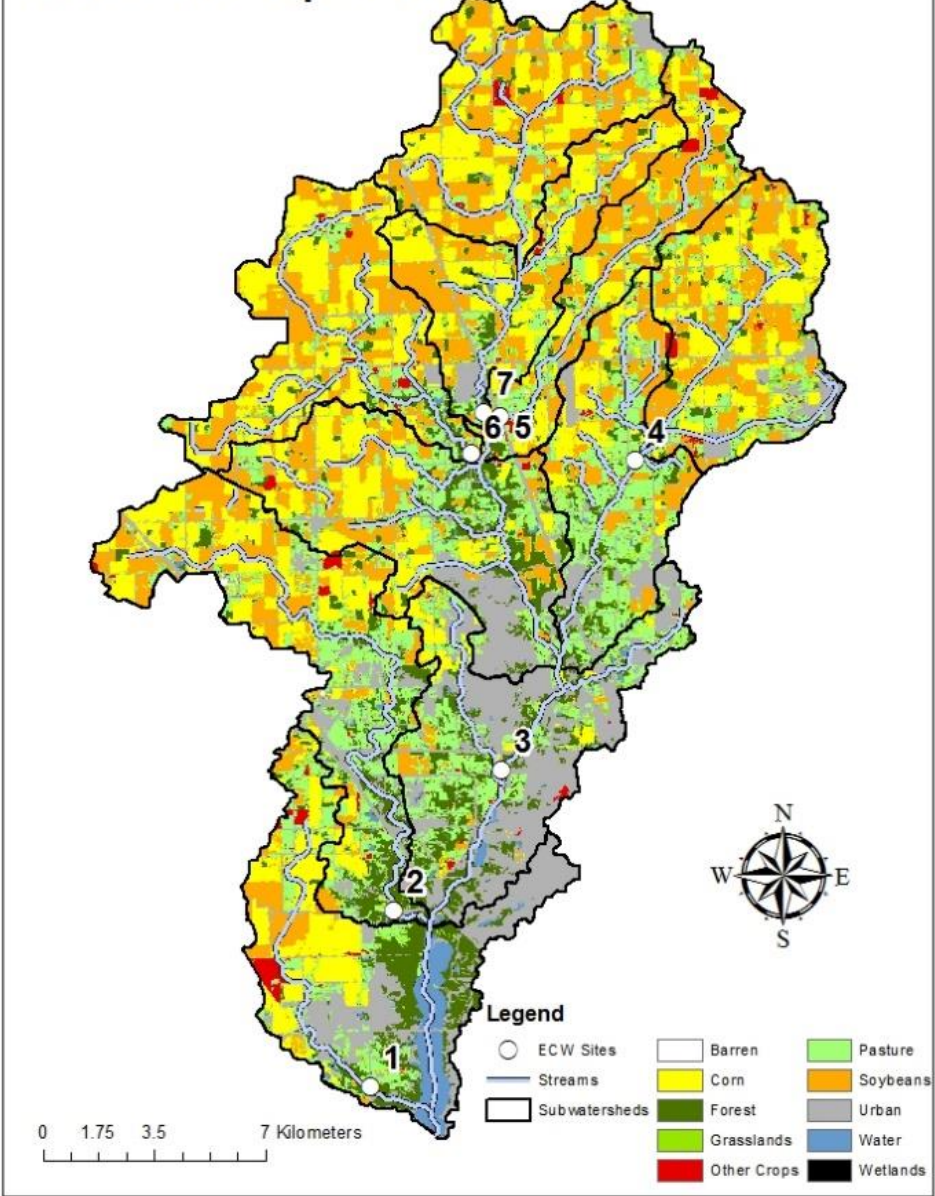
Eagle Creek Reservoir



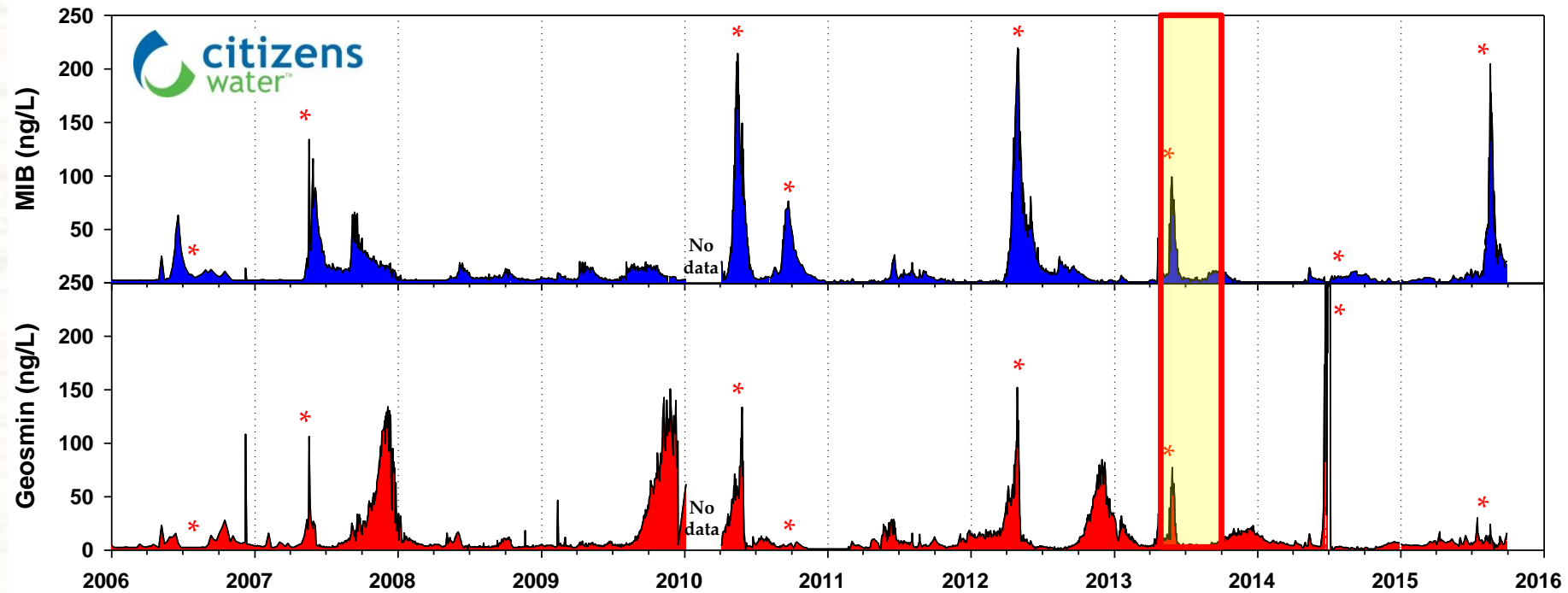
- Built in 1967 to provide flood control and drinking water to Indianapolis, IN
- Dimictic and eutrophic
- Surface area: 5.7 km²
- Capacity: 21 x 10⁶ m³
- Z_{max} = 13 m
- Z_{mean} = 4.2 m
- Watershed area: 419.6 km²
- %Agri. vs. %Urban: 49.1% vs. 18.1%

Eagle Creek Watershed

2012 ECW Crop Cover



T&O in Eagle Creek Reservoir

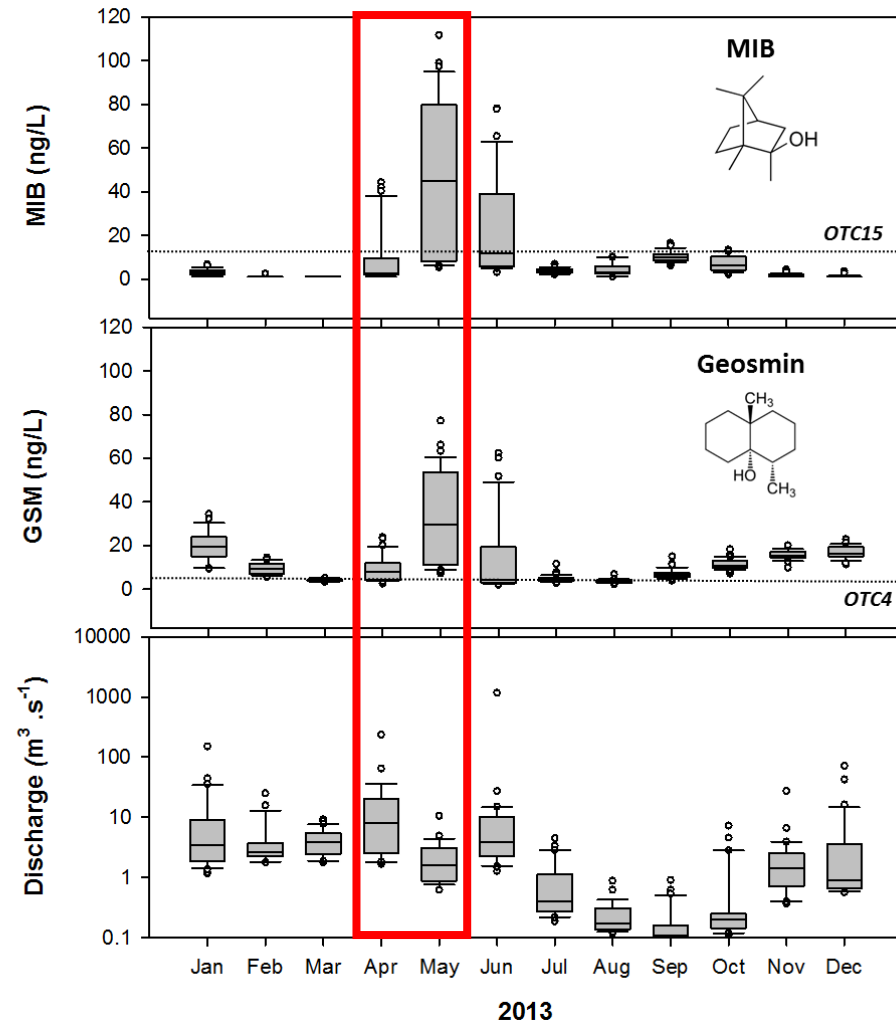


Long-term patterns show that T&O episodes are highly variable in:

- Frequency: 0-4 events/year
- Duration: MIB <3 months; GSM >5 months
- Intensity: 5-15 ppt (>OTC) – several hundreds ppt.

* Algaecide treatment

Peak Discharge (Q) and T&O Occurrences



- Q and T&O are asynchronous

Table 3

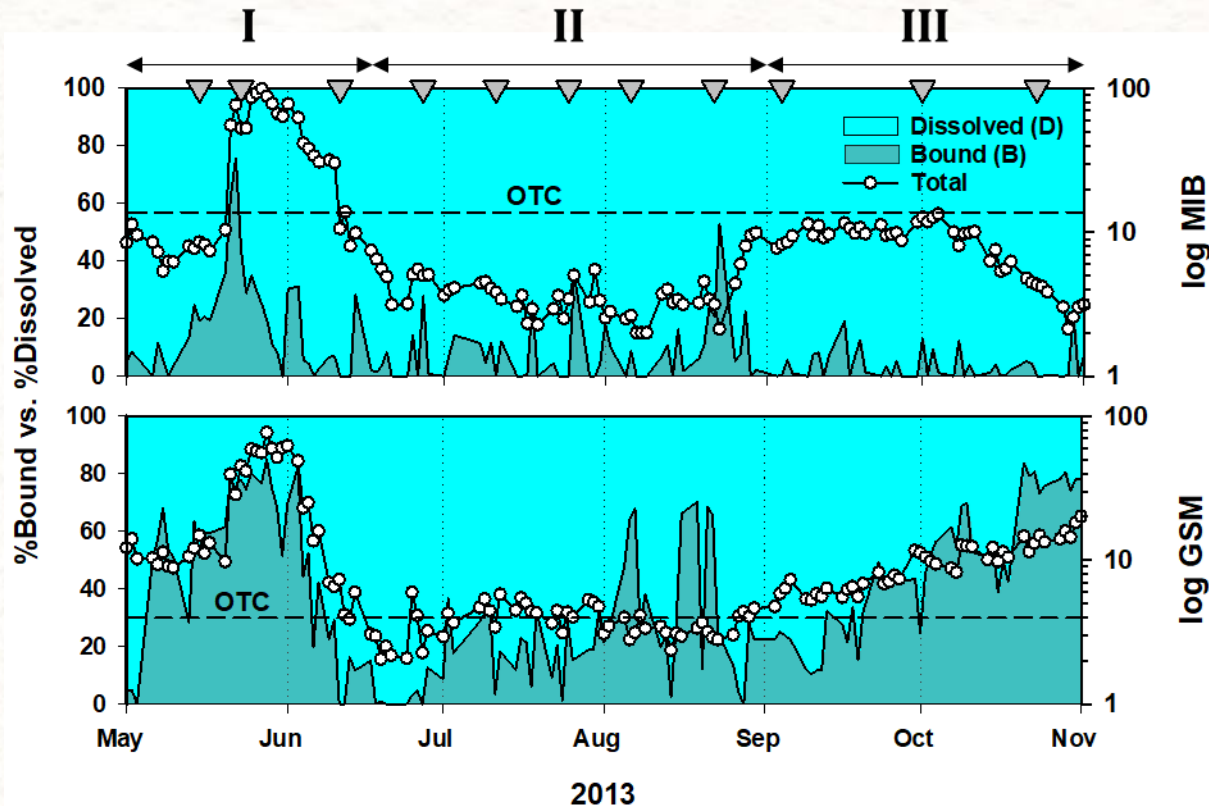
Cross-Correlation Between Off-Flavor Metabolites (2-Methylisoborneol [MIB] and Geosmin [GSM]) Versus Main Tributary Inflow (Q)

MIB			GSM		
Lag (d)	Correlation	<i>p</i>	Lag (days)	Correlation	<i>p</i>
-40	0.414	0.0493	-40	0.423	0.0443
-39	0.412	0.0454	-39	0.516	0.0099
-38	0.529	0.0065	-38	0.402	0.0461
-37	0.582	0.0018	-37	0.665	0.0002
-36	0.402	0.0376	-36	0.386	0.0466
-35	0.233	0.2322	-35	0.249	0.2015
...
-3	-0.107	0.4488	-3	-0.028	0.8457
-2	-0.216	0.1212	-2	-0.141	0.3153
-1	-0.223	0.1047	-1	-0.132	0.3420
0	-0.148	0.2825	0	-0.077	0.5781

Clercín & Druschel (2019). *Water Resources Research*, 55(7), 5413-5430.

- Lag phase: 36-40 days
- Watershed origin:
 - Source of Nitrogen and Actinobacteria

Fractionation – Bound versus Dissolved



Clercín *et al.* (2021). *Journal of Environmental Management*, 297, 113304.

- Phase I (spring): Odorous episode
- Phase II (summer): less or non odorous
- Phase III (fall): Odorous episode

- MIB = mostly **dissolved**
 - 14.2 % > OTC (15 ng L^{-1})
- GSM = mainly **cell-bound** (intracellular)
 - 76.9 % > OTC (4 ng L^{-1})
- T&O problems when compounds are cell-bound!

Water column characterization & sample collection

In situ

- Biweekly (May to Oct. 2013)
 - Water column mixing;
 - Summer stratification.
- YSI sonde profiling for
 - temp, cond, TDS, pH, DO, ORP,
 - Chl + PC fluorescence
- Discrete samples
 - 0m, 3m, 6m and 10m.



In Lab

- Nutrients
 - $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NH}_3\text{-N}$, TN,
 - Ortho-P, TP
- T&O compounds (MIB and geosmin)
 - using HS-SPME-GC/MS
- Microcystins
 - Abraxis kits
- Bacterioplankton
 - 0.22 μm filters

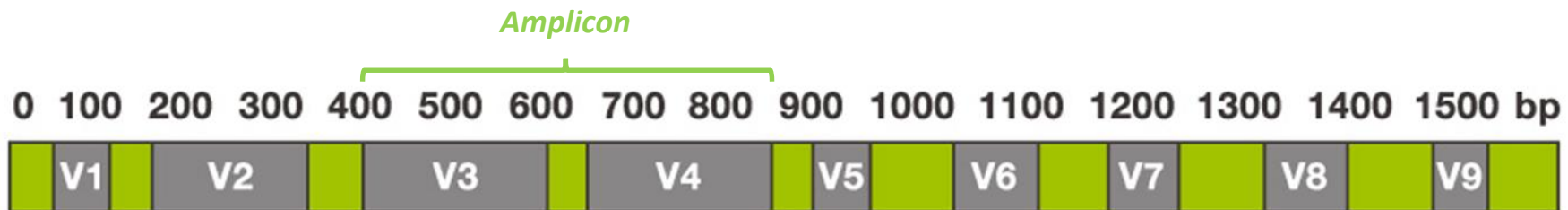
Bacterial Community Identification

Bacterioplankton (MiSeq, Illumina)

- 16S rRNA ~ 1,500 base pairs long
- Primers for V3 and V4 regions
- Amplicon ~ 460 bp



MiSeq

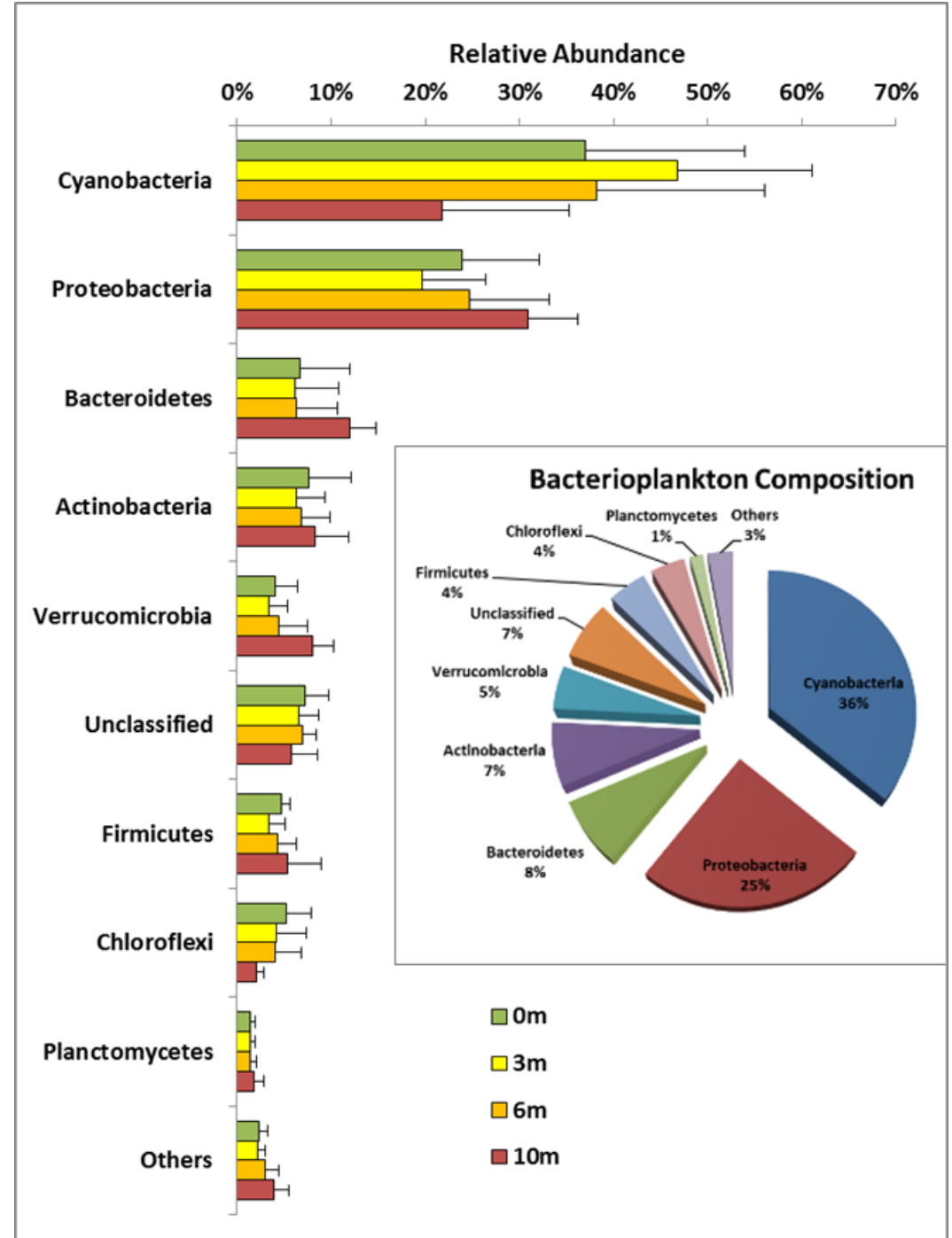


CONSERVED REGIONS: unspecific applications

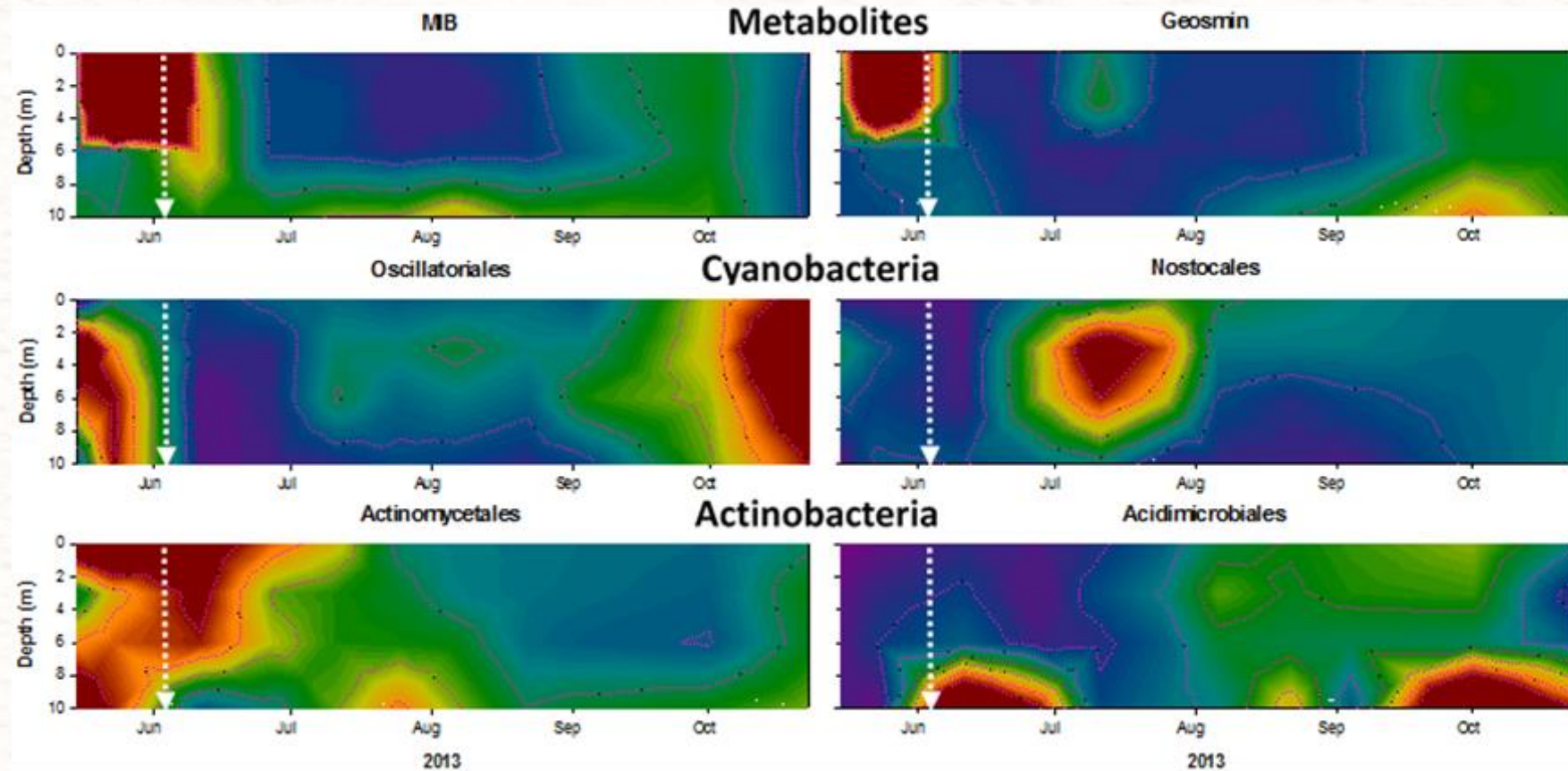
VARIABLE REGIONS: group or species-specific applications

Bacterial Community Composition

- Overall dominance of
 - Cyanobacteria (36%)
 - Upper layers (3m)
 - Proteobacteria (25%)
 - Bottom layers (10m)
- Whereas
 - Actinobacteria (7%)
 - Evenly distributed



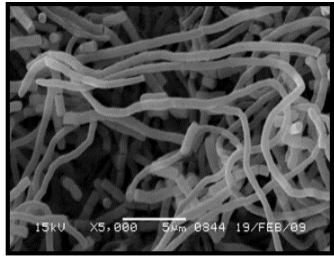
Spatial & Temporal Distribution



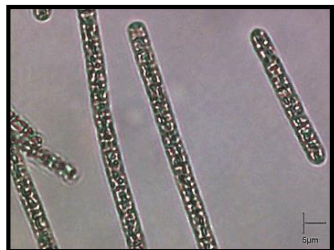
Warmer colors = higher concentrations or abundances

Vertical arrow = algaecide treatment

Spearman's ρ ⁽¹⁾ – Potential Producers



Streptomyces sp.



Planktothrix sp.

Phylum	Order	Genera	Habitat	MIB	GSM	RTRM	Q
Actinobacteria	Actinomycetales	<i>Arcanobacterium</i>	Soil	0.27	-0.15	0.23	0.81**
		<i>Cryobacterium</i>	Soil	0.44*	-0.05	-0.05	0.64**
		<i>Demequina</i>	Soil	0.33	-0.24	0.20	0.69**
		<i>Georgenia</i>	Soil	0.40*	-0.15	0.13	0.75**
		<i>Mycobacterium</i>	Soil	0.10	-0.20	0.22	-0.21
		<i>Nocardia</i>	Soil	0.15	0.17	-0.22	-0.66**
		<i>Rhodococcus</i>	Soil	-0.06	-0.32	0.23	0.51**
		<i>Saccharomonospora</i>	Soil	0.45*	-0.11	0.06	0.69**
		<i>Saccharopolyspora</i>	Soil	-0.24	-0.13	-0.11	0.31
		<i>Sanguibacter</i>	Soil	0.41*	-0.06	0.02	0.78**
		<i>Streptomyces</i>	Soil	0.42*	-0.13	0.16	0.17
		<i>Streptosporangium</i>	Soil	0.23	0.08	-0.16	-0.37
Cyanobacteria	Chroococcales	<i>Chroococcus</i>	Planktic	-0.48**	0.07	-0.20	0.02
		<i>Cyanobacterium</i>	Planktic	-0.27	0.37	-0.46**	-0.74**
		<i>Microcystis</i>	Planktic	-0.58**	-0.18	0.23	-0.54**
		<i>Prochlorococcus</i>	Planktic	-0.59**	-0.48**	0.71**	-0.10
		<i>Snowella</i>	Planktic	-0.23	0.16	-0.26	-0.81**
		<i>Microcoleus</i>	Planktic	-0.17	0.45*	-0.62**	-0.21
	Oscillatoriales	<i>Oscillatoria</i>	Benthic	-0.57**	-0.07	0.01	-0.41*
		<i>Phormidium</i>	Benthic	-0.15	0.42*	-0.61**	-0.31
		<i>Planktothrix</i>	Planktic	-0.17	0.53**	-0.67**	-0.31
		<i>Leptolyngbya</i>	Epiphytic	-0.41*	0.06	-0.13	-0.63**
	Pseudanabaenales	<i>Limnothrix</i>	Planktic	-0.54**	0.07	-0.07	-0.30
		<i>Prochlorothrix</i>	Planktic	-0.46*	-0.01	0.05	-0.82**
		<i>Pseudanabaena</i>	Planktic	-0.59**	0.04	-0.04	-0.16
		<i>Aphanizomenon</i>	Planktic	-0.60**	-0.16	0.23	-0.55**
	Nostocales	<i>Calothrix</i>	Epiphytic	-0.31	0.32	-0.35	-0.23
		<i>Cylindrospermopsis</i>	Planktic	-0.45*	-0.02	-0.06	-0.67**
		<i>Dolichospermum</i>	Planktic	-0.75**	-0.37	0.50**	-0.12
		<i>Nostoc</i>	Benthic	-0.22	0.44*	-0.58**	-0.17

Actinobacteria

- **N.S with GSM**
- **(+) with MIB**
 - *Streptomyces* ($p < 0.01$)
- **Strong (+) with high Q!**
 - *For most genera*

Cyanobacteria

- **Strong (-) with MIB**
- **(+) with GSM**
 - *Planktothrix* ($p < 0.001$)
- **Strong (-) with RTRM!**
 - *(+) Mixing conditions!*

⁽¹⁾ Significant correlation in bold $p < 0.05$; * $p < 0.01$; ** $p < 0.001$

Q: Discharge

RTRM: Relative Thermal Resistance to Mixing

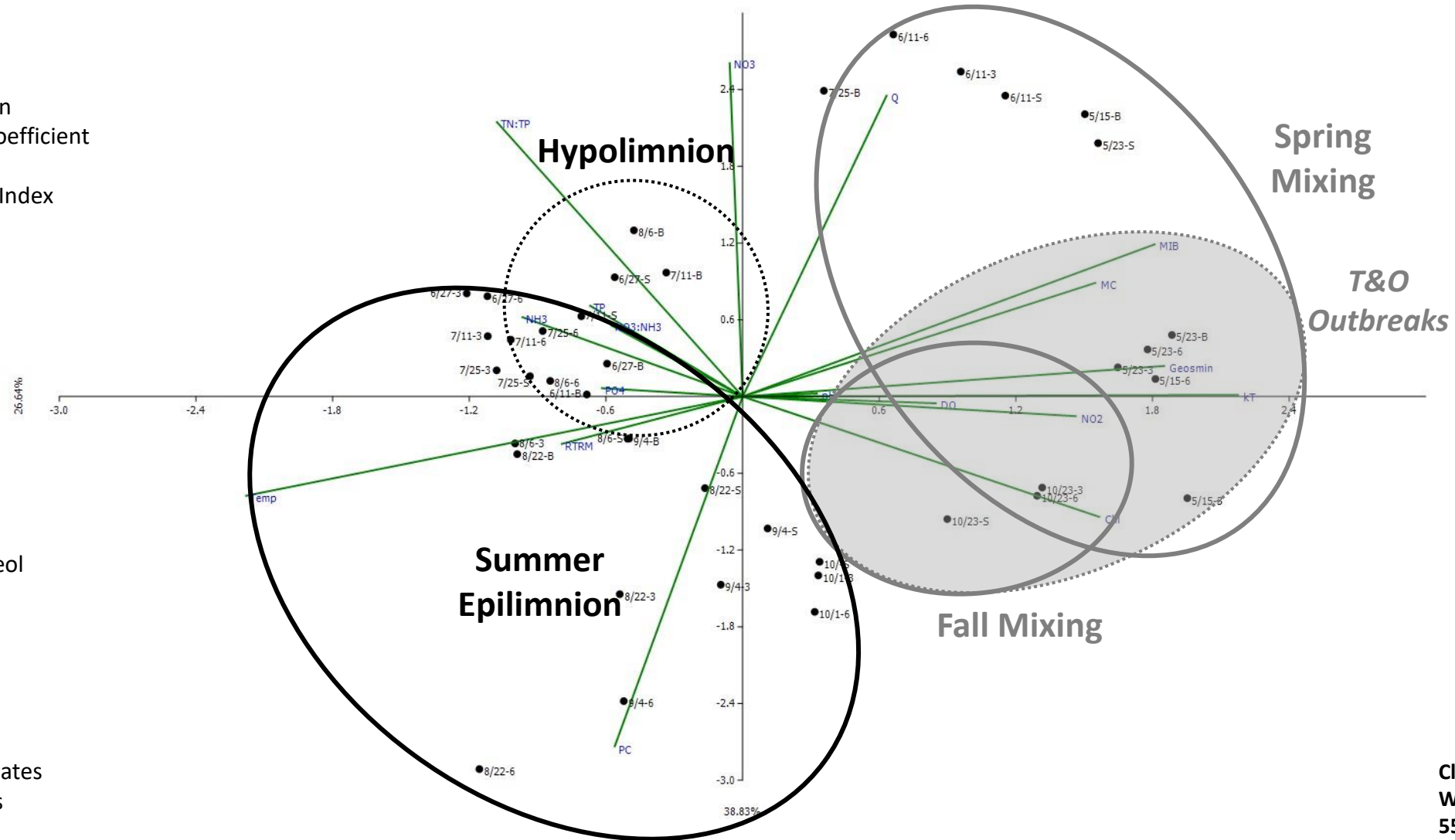
CCA - Temporal Distribution of Samples

Physical:

- DO = Dissolved Oxygen
- k_T = Light extinction coefficient
- Q = Discharge
- RTRM = Stratification Index
- Temp = Temperature

Chemical:

- Chl = Chlorophyll a
- GSM = Geosmin
- MC = Microcystins
- MIB = Methylisoborneol
- NH_3 = Ammonia
- NO_2 = Nitrites
- NO_3 = Nitrates
- PC = Phycocyanin
- Q = Discharge
- TN = Total Nitrogen
- OPO_4 = Ortho-Phosphates
- TP = Total Phosphorus



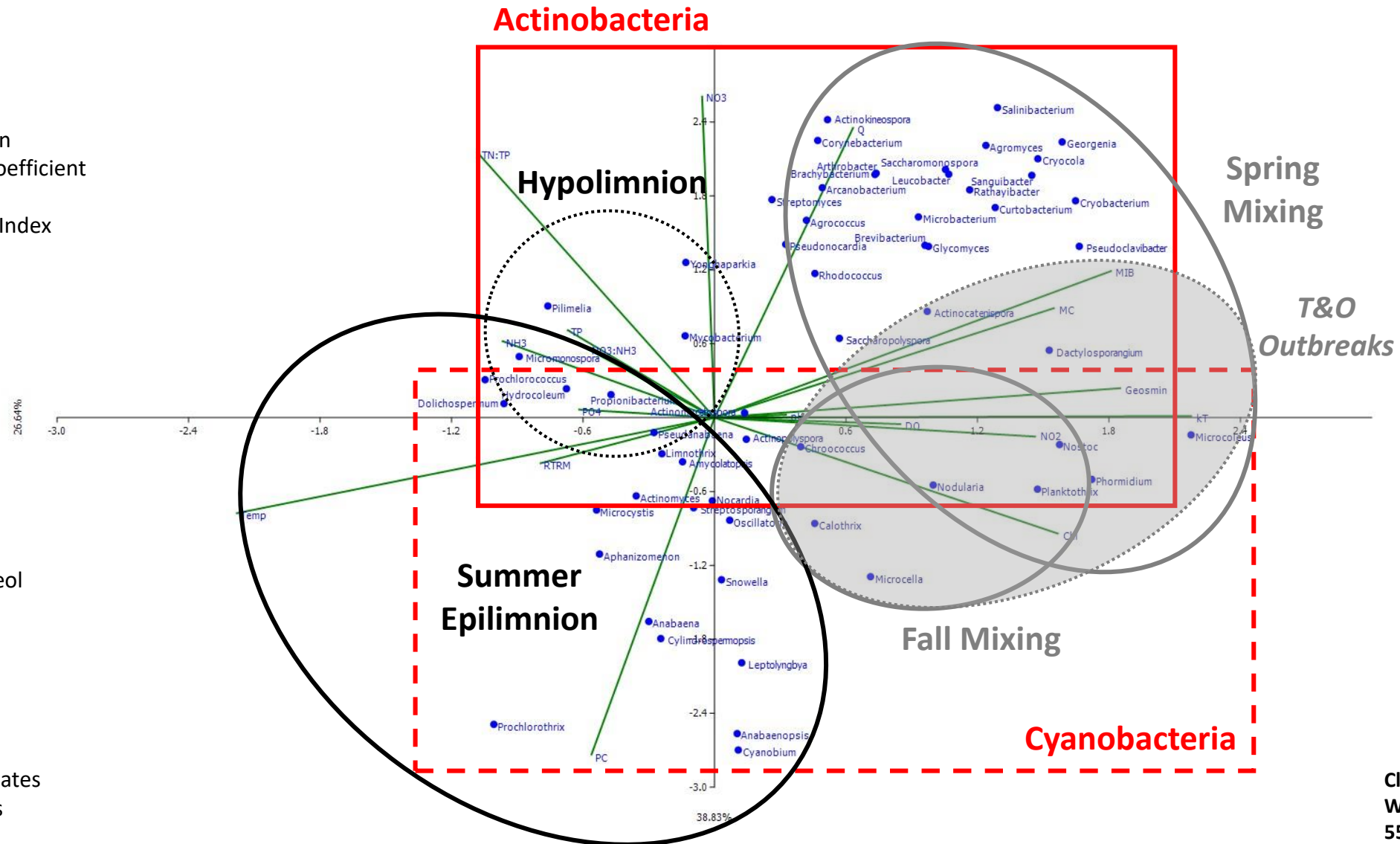
CCA - Temporal Distribution of Bacteria

Physical:

- DO = Dissolved Oxygen
- k_T = Light extinction coefficient
- Q = Discharge
- RTRM = Stratification Index
- Temp = Temperature

Chemical:

- Chl = Chlorophyll *a*
- GSM = Geosmin
- MC = Microcystins
- MIB = Methylisoborneol
- NH₃ = Ammonia
- NO₂ = Nitrites
- NO₃ = Nitrates
- PC = Phycocyanin
- Q = Discharge
- TN = Total Nitrogen
- OPO₄ = Ortho-Phosphates
- TP = Total Phosphorus

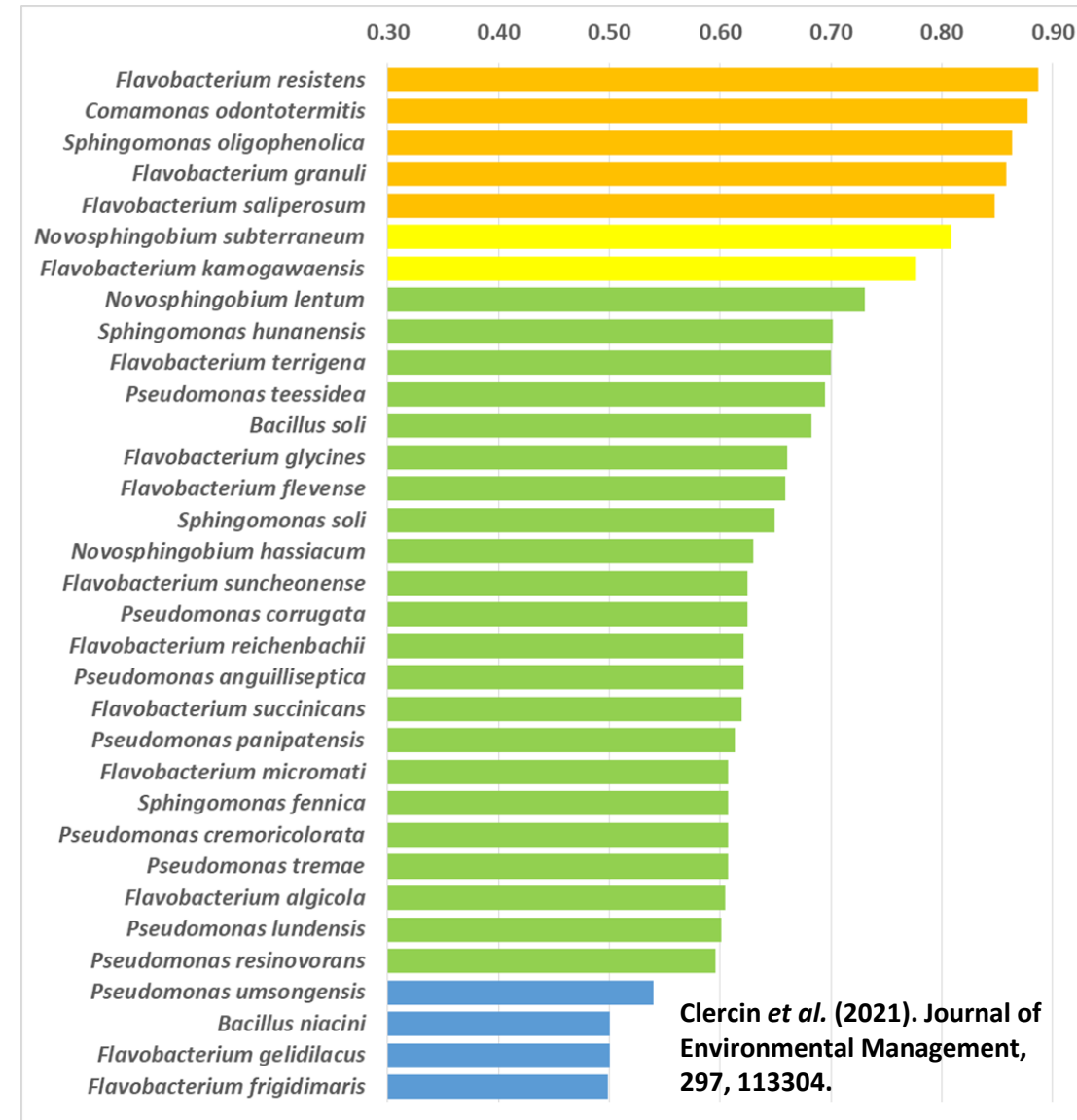


Identification of Potential MIB-Degrading Bacteria

A total of 138 OTUs potentially capable to degrade either MIB (n=96) or GSM (n=42) were identified

- Potential MIB-degraders: Bacteroidetes, Firmicutes and γ -Proteobacteria
- Potential GSM-degraders: α - and β -Proteobacteria

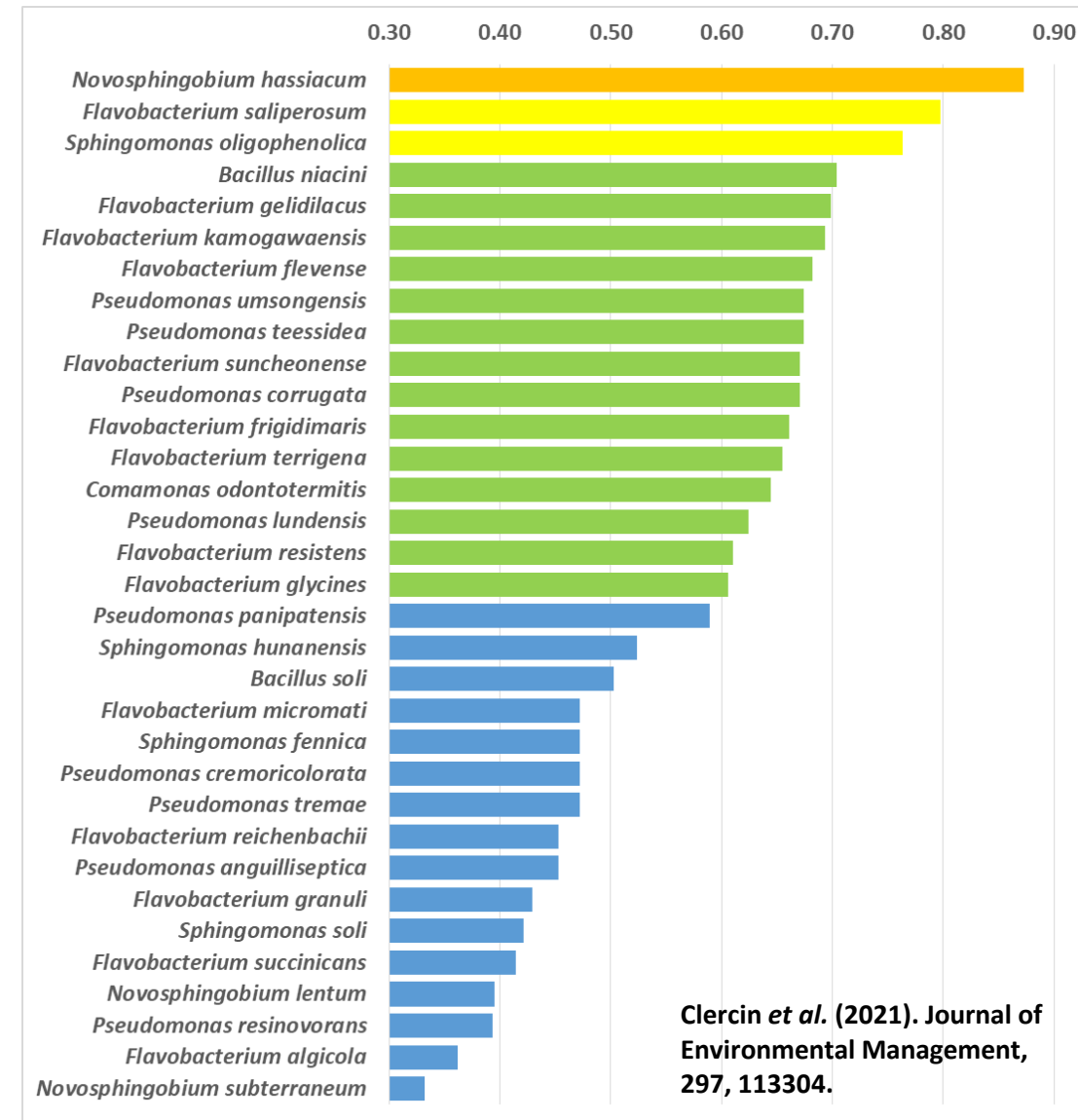
- MIB-degrading bacteria – 29 OTUs with (+) correlation
 - 12 OTUs from Bacteroidetes; genus *Flavobacterium*
 - *F. resistens*, *F. granuli*, *F. saliperosum* (p < 0.001)
 - *F. kamogawaensis* (p < 0.01)
 - 8 *Flavobacterium* (p < 0.05)
 - 8 OTUs from γ -Proteobacteria; genus *Pseudomonas* (p < 0.05)
 - *P. teessidea*, *P. corrugate*, *P. anguilliseptica*, *P. panipatensis*
 - 1 OTU from Firmicutes; *Bacillus soli* (p < 0.05)
- 8 other OTUs not known as capable of degrading MIB
 - *Comamonas odontotermitis* (β -Prot; p < 0.001),
 - Seven α -Proteobacteria: *Sphingomonas oligophenolica* (p < 0.001), *S. hunanensis*, *S. soli*, *S. panipatensis* (p < 0.05), *Novosphingobium subterraneum* (p < 0.01), *N. lentum* and *N. hassiacum* (p < 0.05).



rho > 0.59: blue (N.S), green (p < 0.05), yellow (p < 0.01) and orange (p < 0.001).

Identification of Potential GSM-Degrading Bacteria

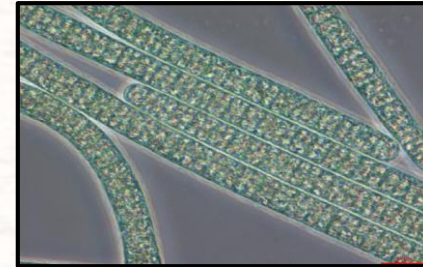
- **GSM-degrading bacteria** – 17 OTUs with (+) correlation
- Only three of them belong to phyla of known degraders
 - 2 OTUs from α -Proteobacteria
 - *Novosphingobium hassiacum* ($p < 0.001$)
 - *Sphingomonas oligophenolica* ($p < 0.01$)
 - 1 OTU from β -Proteobacteria
 - *Comamonas odontotermitis* ($p < 0.05$)
- Potential degraders but not documented
 - 9 OTUs from Bacteroidetes; genus *Flavobacterium*
 - *F. saliperosum* ($p < 0.01$) + 8 others with $p < 0.05$
 - 1 OTU from Firmicutes; *Bacillus niacini* ($p < 0.05$)
 - 4 OTUs of *Pseudomonas* (γ -Proteobacteria):
 - *P. umsongensis*, *P. teessidea*, *P. corrugata* and *P. lundensis* ($p < 0.05$)



rho > 0.59: blue (N.S), green ($p < 0.05$), yellow ($p < 0.01$) and orange ($p < 0.001$).

Conclusions

- Both MIB and GSM occur during the reservoir mixing periods (Spring/Fall)
 - Low detections during summer stratification
- Some Cyanobacteria, strongly correlated to GSM and mixed water columns
 - *Planktothrix* spp. ($p < 0.001$), *Microcoleus* spp. ($p < 0.01$)
- GSM degradation is mostly performed by β -Proteobacteria
 - *Novosphingobium hassiacum* ($p < 0.001$), *Sphingomonas oligophenolica* ($p < 0.01$)
- Actinobacteria, strongly correlated to MIB and high Q
 - *Streptomyces* spp. ($p < 0.01$); *Saccharomonospora* sp. ($p < 0.01$);
 - Potential origin from upstream locations.
- MIB-degrading bacteria belong to Bacteroidetes and γ -Proteobacteria
 - *Flavobacterium* spp. ($p < 0.001$); *Pseudomonas* spp. ($p < 0.05$)



***Planktothrix
rubescens***



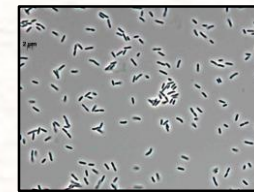
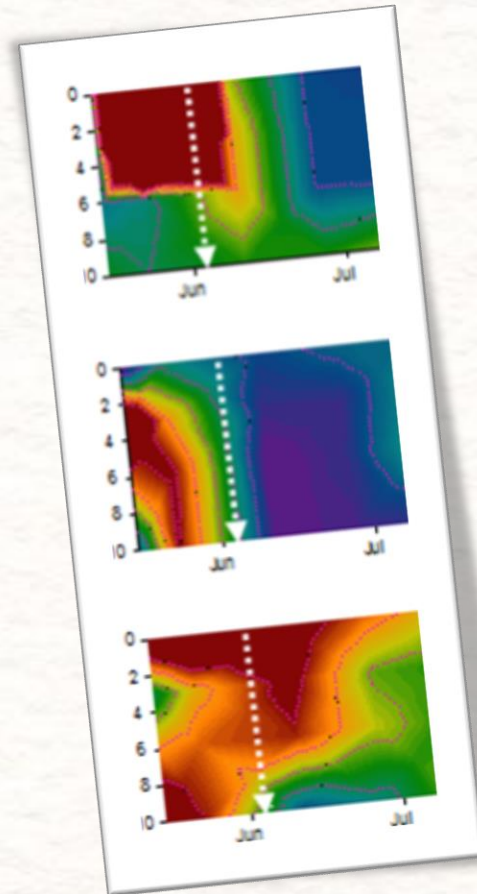
***Microcoleus* sp.**



***Streptomyces* sp.**

General Observations

- Algaecide treatment:
 - Very effective against Cyanobacteria;
 - Terminates associated GSM signal;
 - Less effective against Actinobacteria;
 - Does not terminate MIB.
- Investigations of chemical behaviors:
 - GSM mostly particle-bound;
 - Produced within the epilimnion;
 - Associated with cyanobacterial growth.
 - MIB mainly dissolved;
 - Water column under mixed conditions;
 - In the bottom sediments;
 - Mostly produced by Actinobacteria.



GSM-degrading
Sphingomonas



MIB-degrading
Flavobacterium

Indiana Star – July 2009

