University of South Carolina

"Urbanization and Climate Change: A Recipe for Disaster for Ecosystem and Human Health"

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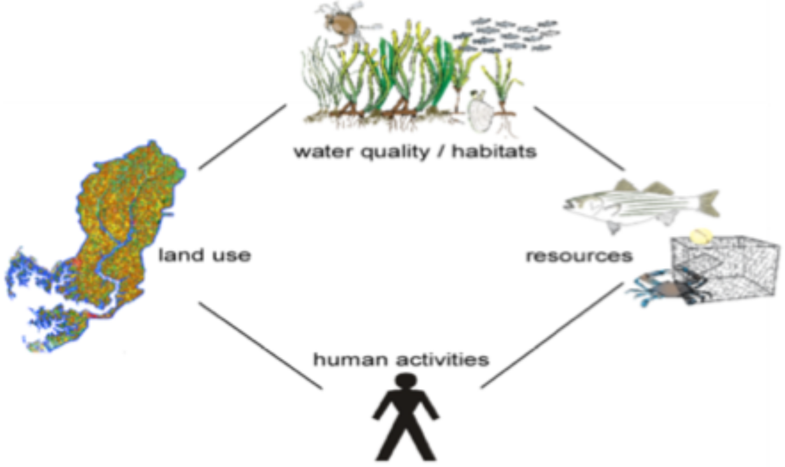
Urbanization in Aquatic Ecosystems

- Globally > 55% of the world's population lives within 50 miles of the coast, 33 of the 50 largest cities in the world are located in coastal areas & more than 80% of world commerce is transported by ships (Dean, 1997)
- Half of the US population (>141 million people) reside within 50 miles of the coast and Great Lakes, which occupies less than 11% of the land area of the lower 48 states (NOAA, 1999; 2005)
- In addition to coastal areas people like to live near freshwater aquatic environments including rivers, lakes and streams
- **PEW COMMSSION:** 25% of all conversion of rural land into suburban/urban land use in the last 300 years for the U.S. occurred in the 15 year period from 1982-1997 (NRI, 2000)

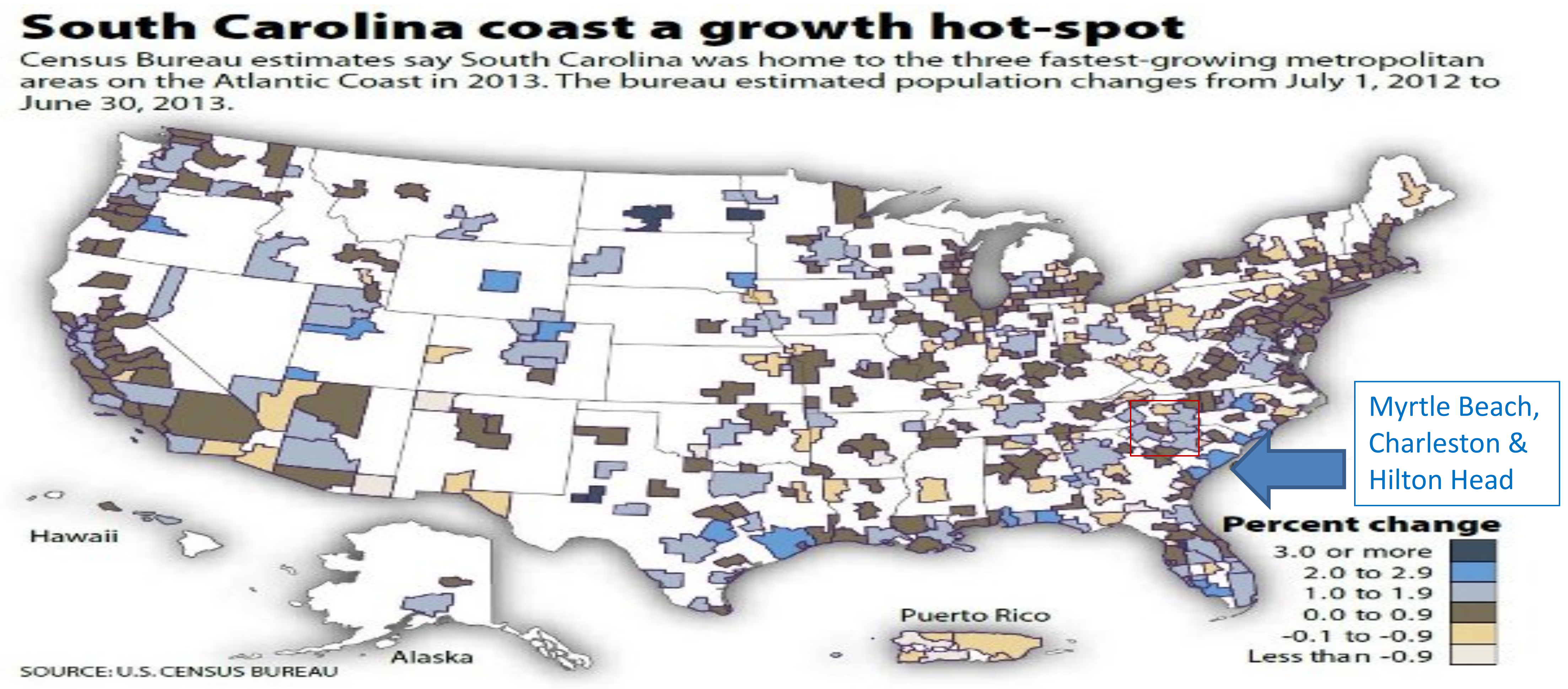
Urbanization in Coastal and Aquatic Ecosystems

- This influx of people, and the associated residential and commercial development of the coastal zone has resulted in significant modification of landscapes such as increased imperviousness.
- Major alterations of the hydrological cycle, which change the transport and delivery of water to coastal watersheds.
- This in turn results in increased discharges of toxic chemicals (pesticides, trace metals, PAHs, personal care products, and pharmaceuticals), nutrients, and microbes.

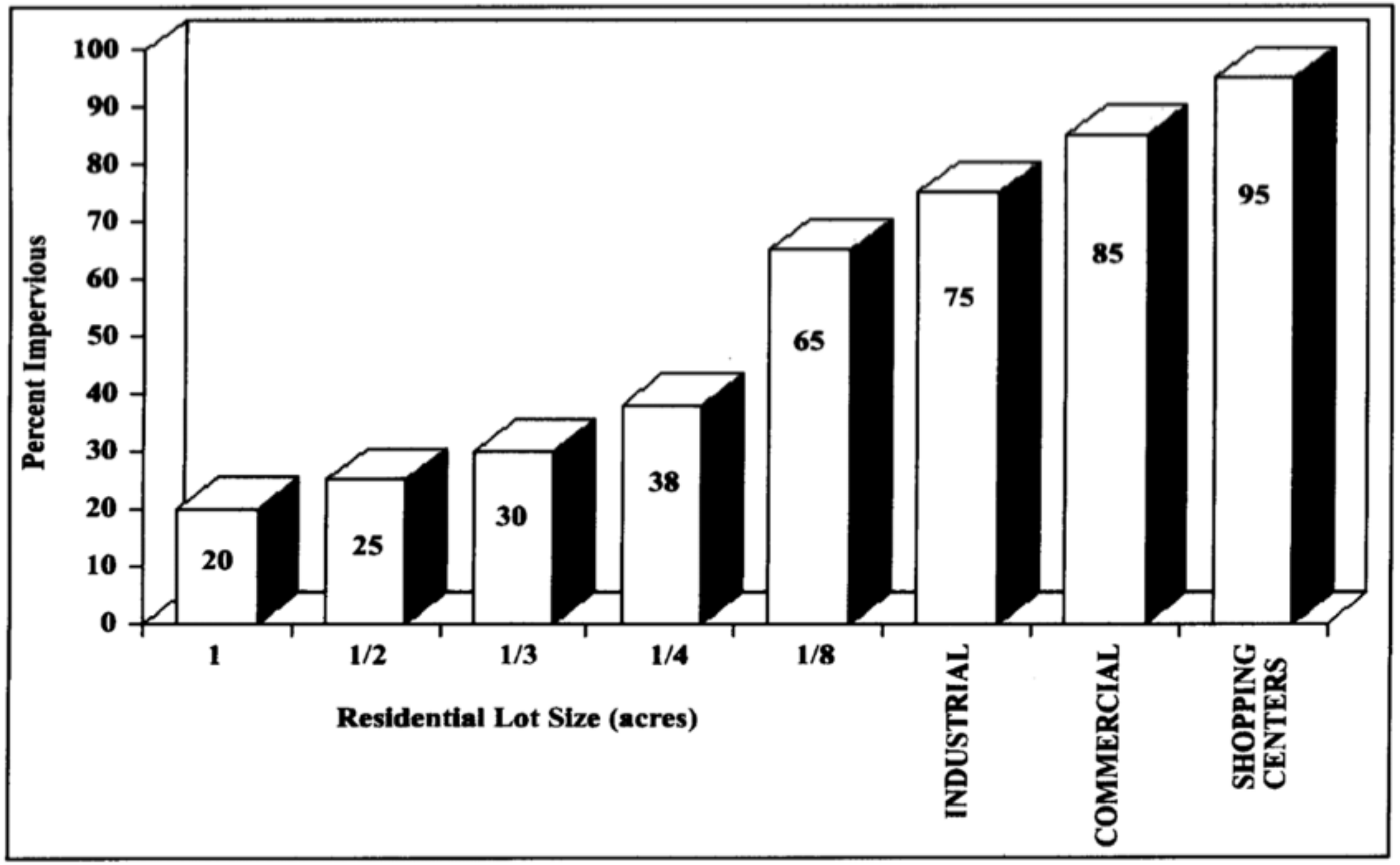


Fastest Growing Municipalities on the East Coast – Myrtle Beach, Charleston and Hilton Head

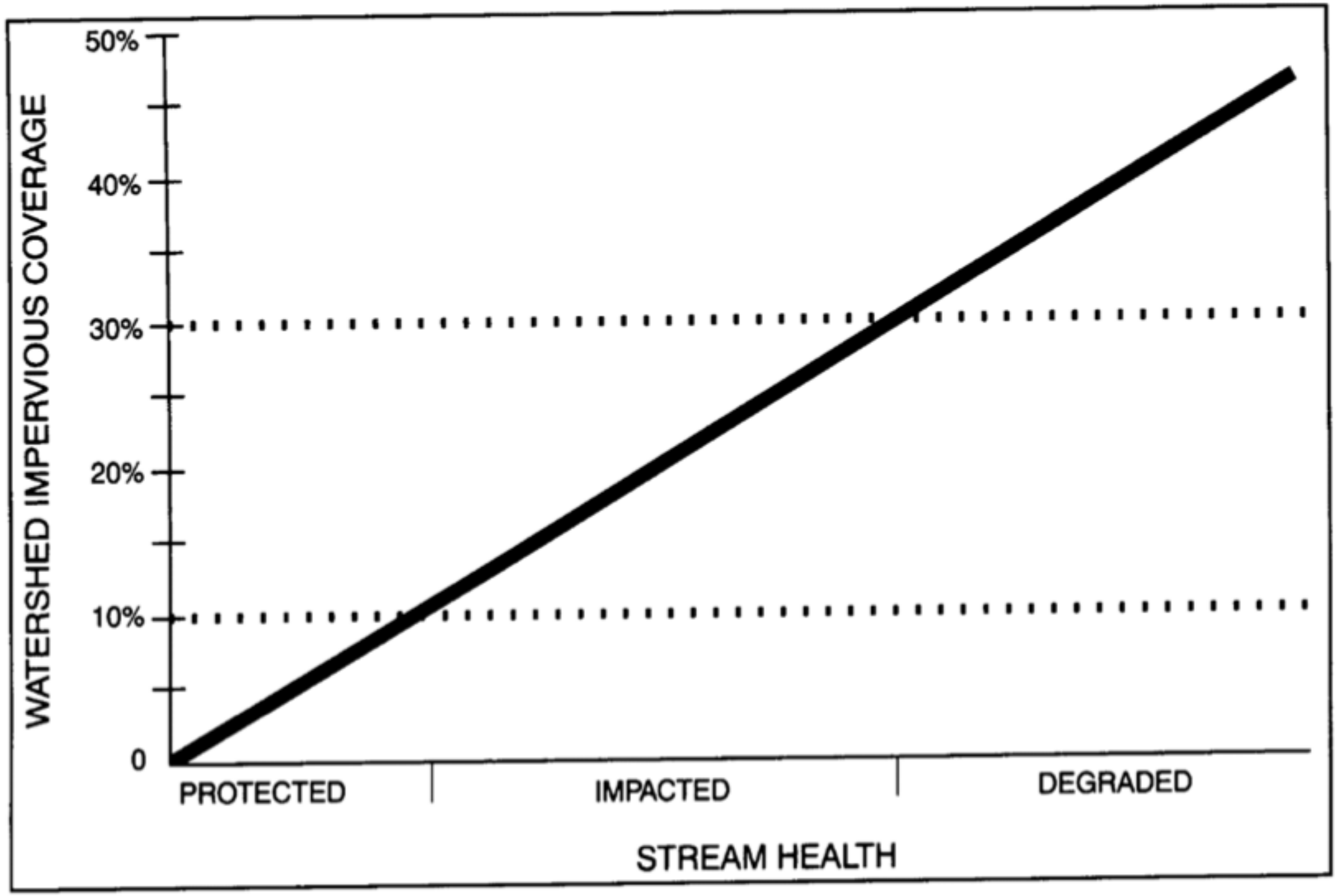
(Charleston Post & Courier 03/27/14)



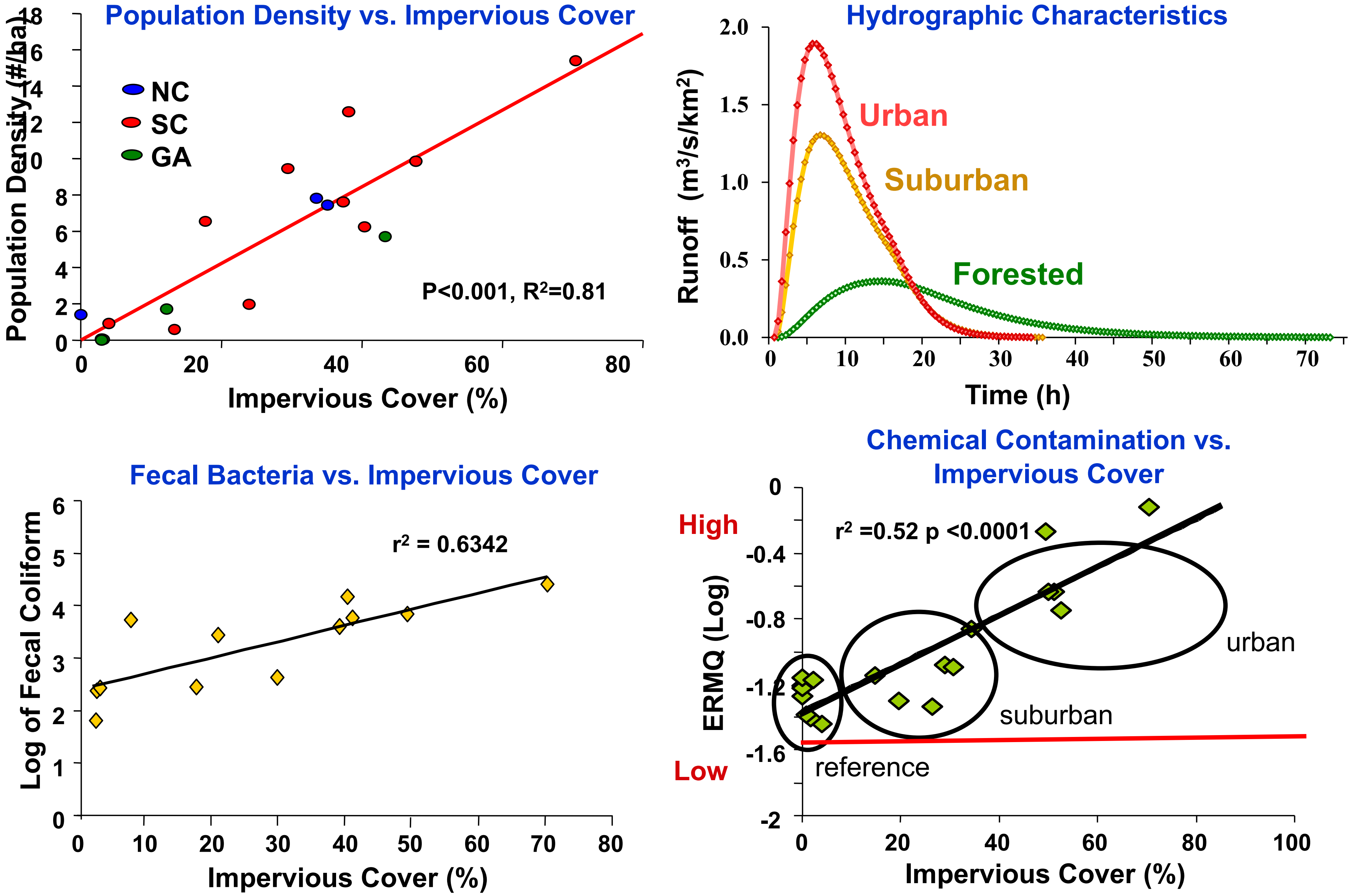
Effects of Lot Size on Imperviousness



Effects of Imperviousness on Water Quality (Schuler et. al. 1992)



Summary: Urbanization Effects



Pollution Effects and Urbanization

Urbanization Effects

- Hydrological Cycle
- Nitrogen Cycle
- Phosphorous Cycle

Climate Change

- Carbon Cycle
- How Will Future Climate Change Effects Interact with Current Known Urbanization Effects?

Hypoxia (lack of oxygen) and Eutrophication (increased nutrients) are Common Results

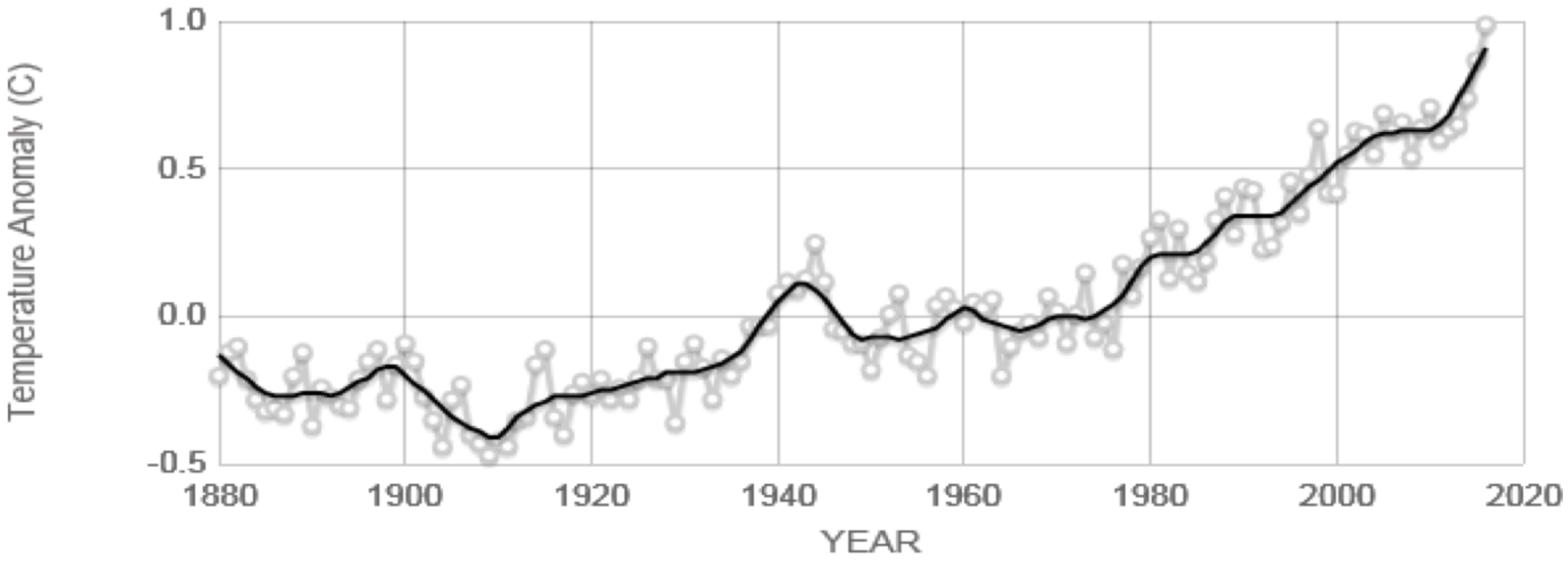
GLOBAL WARMING

- Present level of CO_2 are >400 ppm, which has increased by 25% since the start of the industrial revolution & has increased by 12% since 1960.

- This CO_2 increase has caused a 1 degree F increase in global temperatures during the 20th century.

- Projections are for CO_2 levels to double by 2050 which will increase global temperatures by 1.5 - 4.5 degrees F.

Recent Changes in Global Temperature



Source: climate.nasa.gov

IPCC PREDICTED ECOSYSTEM IMPACTS OF GLOBAL CLIMATE CHANGE

Phenomenon[a] and direction of trend	Likelihood of future trends based on projections for 21st century using SRES scenarios	Examples of major projected impacts by sector: Agriculture, forestry and ecosystems [4.4, 5.4]	Water resources [3.4]	Human health [8.2, 8.4]	Industry, settlement and society [7.4]
Over most land areas, warmer and fewer cold days and nights, warmer and more frequent hot days and nights	Virtually certain[b]	Increased yields in colder environments; decreased yields in warmer environments; increased insect outbreaks			
Warm spells/heat waves. Frequency increases over most land areas	Very likely	Reduced yields in warmer regions due to heat stress; increased danger of wildfire			
Heavy precipitation events. Frequency increases over most areas	Very likely	Damage to crops; soil erosion, inability to cultivate land due to waterlogging of soils			
Area affected by drought increases	Likely	Land degradation; lower yields/crop damage and failure; increased livestock deaths; increased risk of wildfire			
Intense tropical cyclone activity increases	Likely	Damage to crops; windthrow (uprooting) of trees; damage to coral reefs			
Increased incidence of extreme high sea level (excludes tsunamis)[c]	Likely[d]	Salinisation of irrigation water, estuaries and freshwater systems	Decreased freshwater availability due to saltwater intrusion	Increased risk of deaths and injuries by drowning in floods; migration-related health effects	Costs of coastal protection versus costs of land-use relocation; potential for movement of populations and infrastructure; also see tropical cyclones above

[a] See Working Group I Fourth Assessment Table 3.7 for further details regarding definitions.
[b] Warming of the most extreme days and nights each year.
[c] Extreme high sea level depends on average sea level and on regional weather systems. It is defined as the highest 1% of hourly values of observed sea level at a station for a given reference period.
[d] In all scenarios, the projected global average sea level at 2100 is higher than in the reference period [Working Group I Fourth Assessment 10.6]. The effect of changes in regional weather systems on sea level extremes has not been assessed.

- •Increased temperatures (warmer nights and fewer colder days)
- •Increased heat waves
- •Increased Heavy Precipitation Events
- •Increased Periods of Drought
- •Increased Tropical Cyclone Activity
- •Increased Sea Level Rise
- •***Overall increased extreme weather***

Natural Disasters 1950-2012

(Sandifer & Heyward-Walker. 2018. Frontiers in Public Health 6: 373)

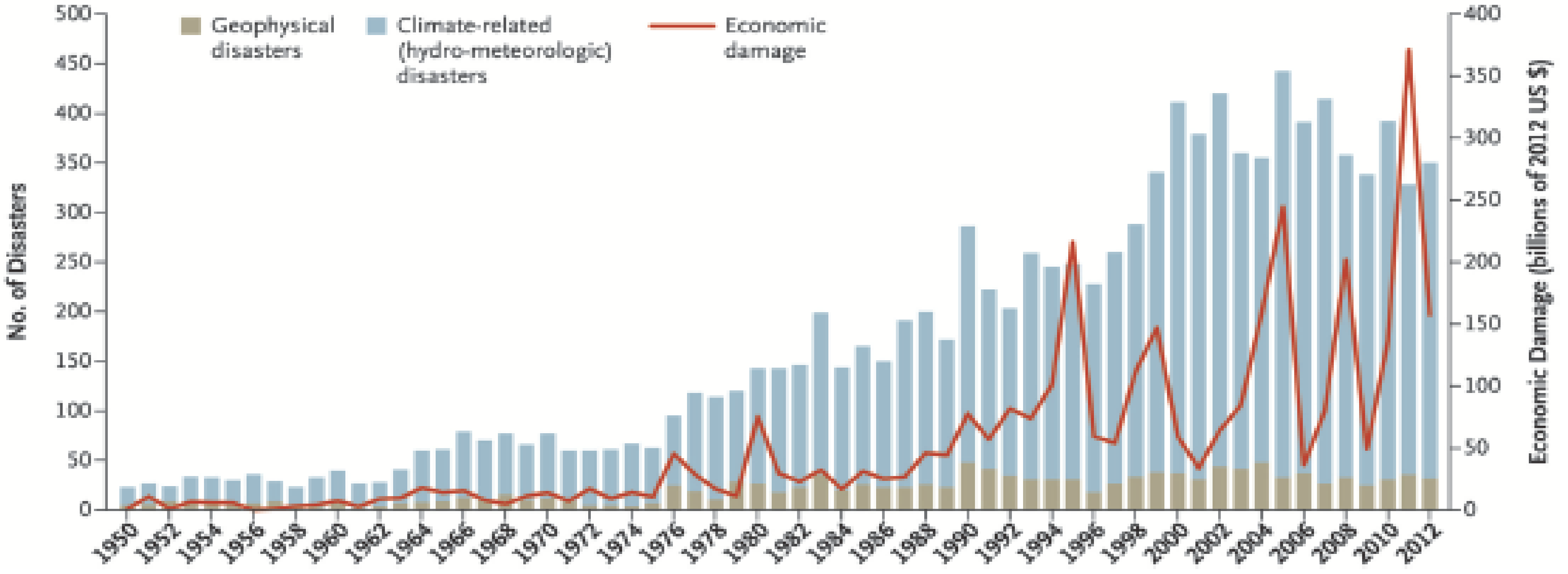


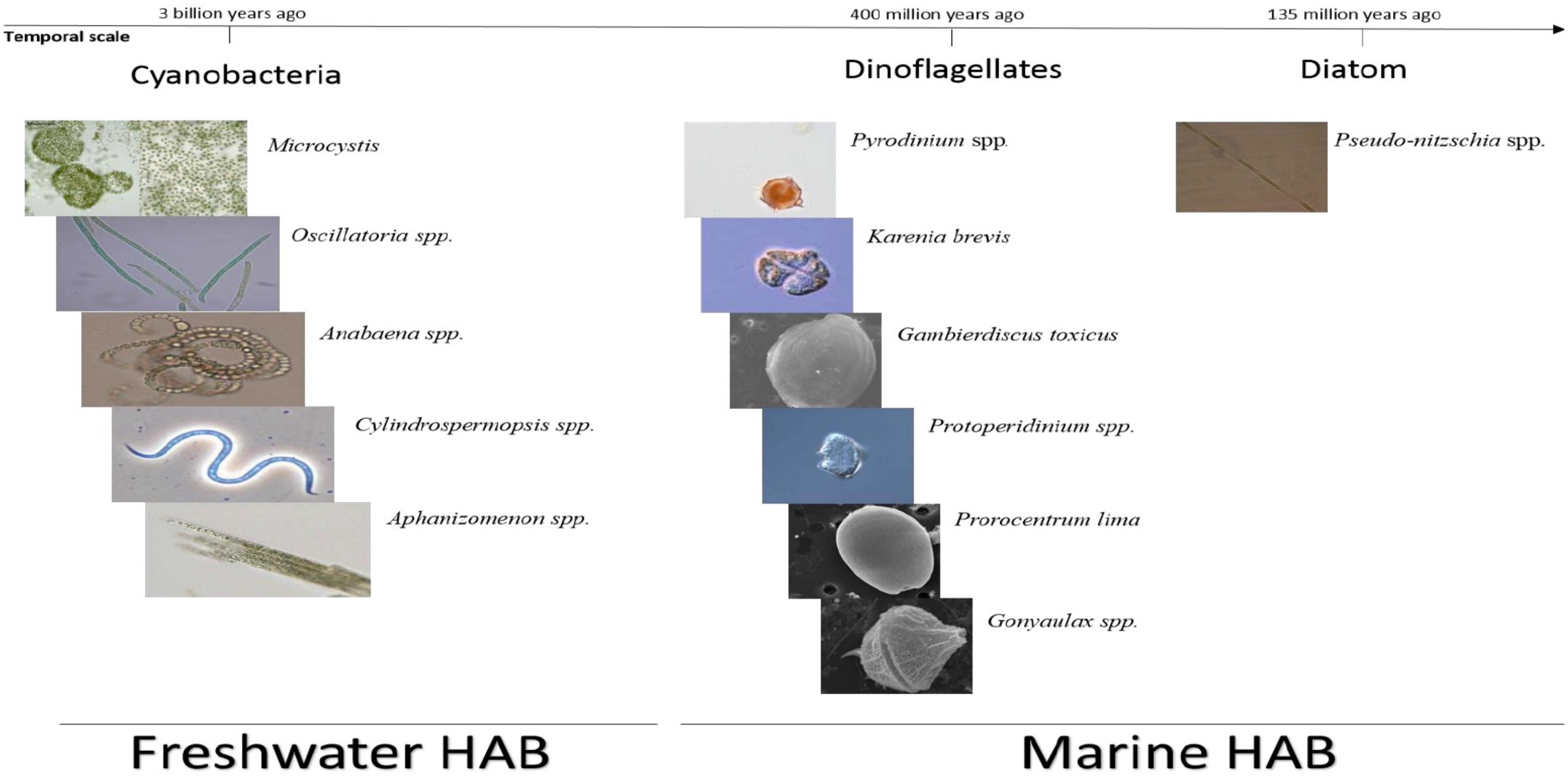
FIGURE 1 | Numbers and types of natural disasters, 1950–2012, not including biological disasters [From Learning and Guha-Sapir (18). Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society].

Note the Significant Increase in Climate Related Disasters since the 1970's

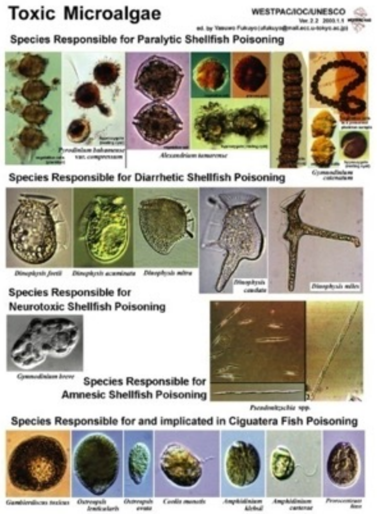
How May Climate Change Affect These Ocean Health Threats?

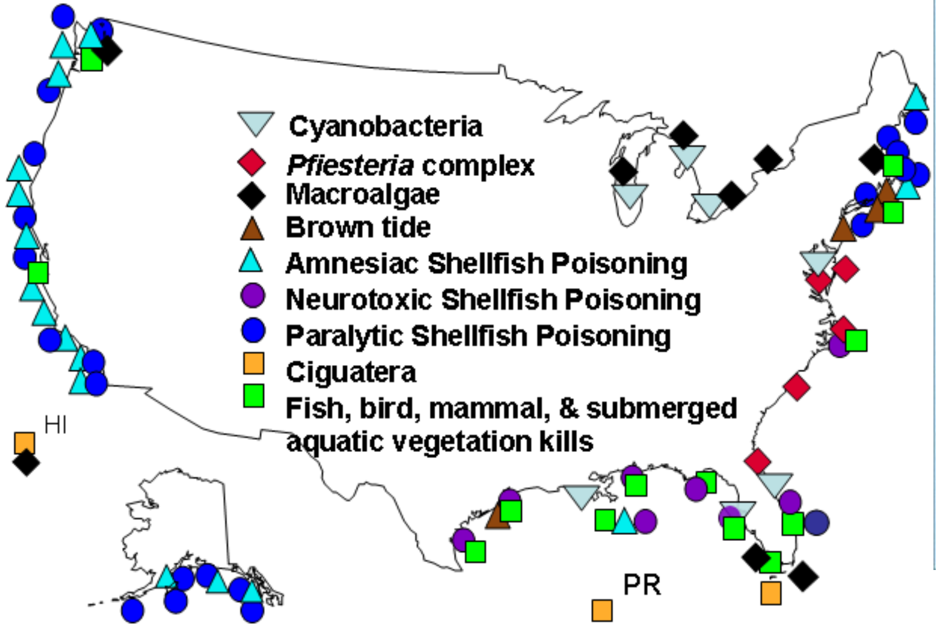
- Climate may directly affect growth, survival, persistence, distribution, transmission, and virulence of disease-causing organisms and harmful algal blooms and distribution and concentrations of chemical contaminants in coastal and ocean waters.
- Climate may also affect the distribution of disease vectors, including marine organisms.
- **Major Climate Factors -** ***Temperature***, Precipitation (and associated drought, flooding, and runoff), Sea Level Rise - Salinity, ***Extreme Weather Events,*** and **Ecological Shifts in Geographical Range of Organisms**.
- ***Potential Interactions of Climate Change and Urbanization***

Major Types of Harmful Algae

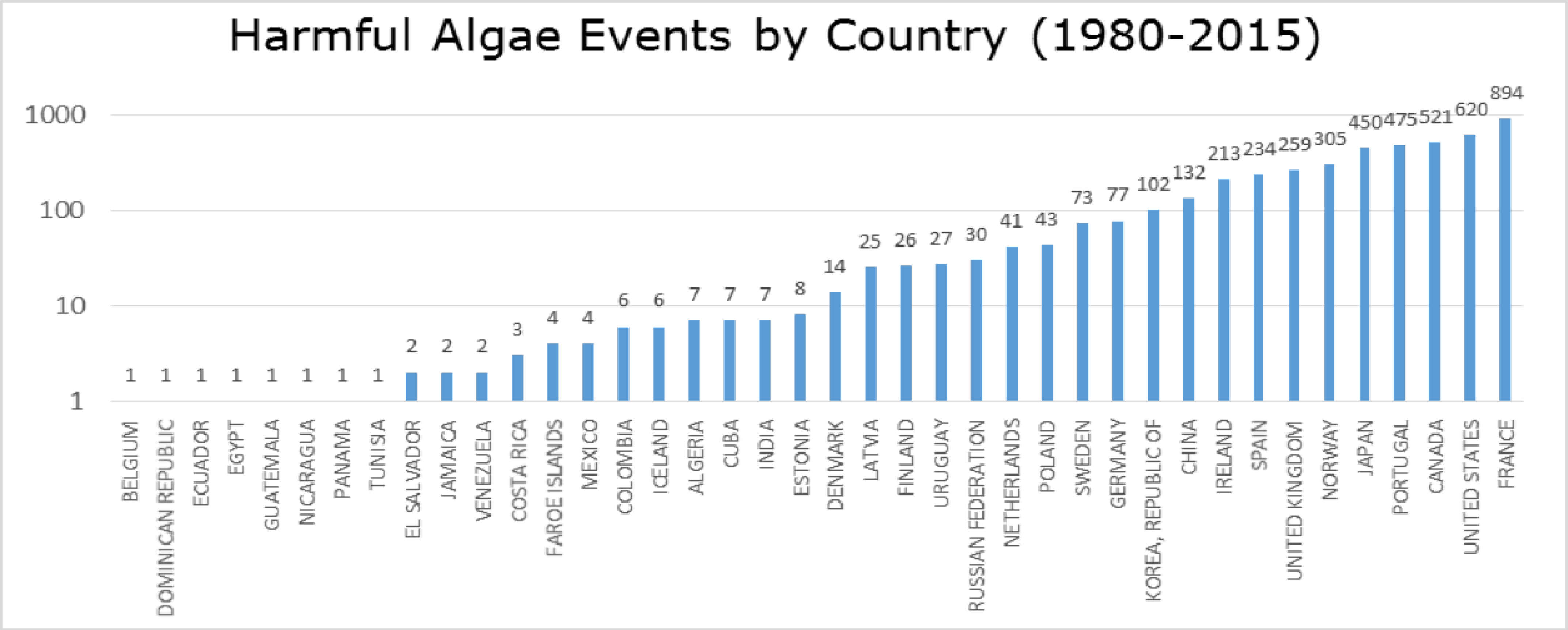


Harmful Algal Blooms (HABs)





Harmful Algal Events Dataset (HAEDAT)



Human Illness Measured in HAB Events in the US : 2007-2011 (HABISS)

Human Illness	Number of Cases (%)
Ciguatera fish poisoning	248 (54)
Rash from unknown organism or toxin	89 (19)
Illness from unknown organism or toxin	49 (11)
Microcystin poisoning	28 (6)
Other cyanobacteria- or algae-related illness not specified in HABISS	27 (6)
Paralytic shellfish poisoning (saxitoxins)	13 (3)
Neurotoxic shellfish poisoning (brevetoxins)	2 (<1)
Anatoxin poisoning	1 (<1)
Amnesic shellfish poisoning (domoic acid)	1 (<1)
Total	458

(**Source:** Backer at al. 2015. *Toxins 7*: 1048-1064)

HABs in the US 2007-2011 Reported by CDC in HABISS

Toxin	Water Type				
	Fresh	Brackish	Marine	Unknown	Total (%)
Anatoxin	243	2	0	1	246 (7)
Azaspiracid	0	0	1	0	1 (<1)
Brevetoxoins	0	3	0	0	3 (<1)
Cylindrospermopsin	4	0	0	0	4 (<1)
Domoic Acid	0	0	31	0	31 (1)
Karlotoxins	0	3	1	0	4 (<1)
Microcytins Total	2629	35	2	10	2676 (81)
Microcytsin LR	21	0	0	0	21 (1)
Okadaic Acid	1	2	0	0	3 (<1)
Saxitoxins	296	1	11	3	311 (9)
Unidentified Toxin	0	1	0	0	1 (<1)
Total	3194	47	46	14	3301

96.7% of HABs occur in Freshwater

(**Source:** Backer et al. 2015. *Toxins* 7: 1048-1064)

Are HABs Becoming the Greatest Inland Water Threat to Public Health

In This Issue:

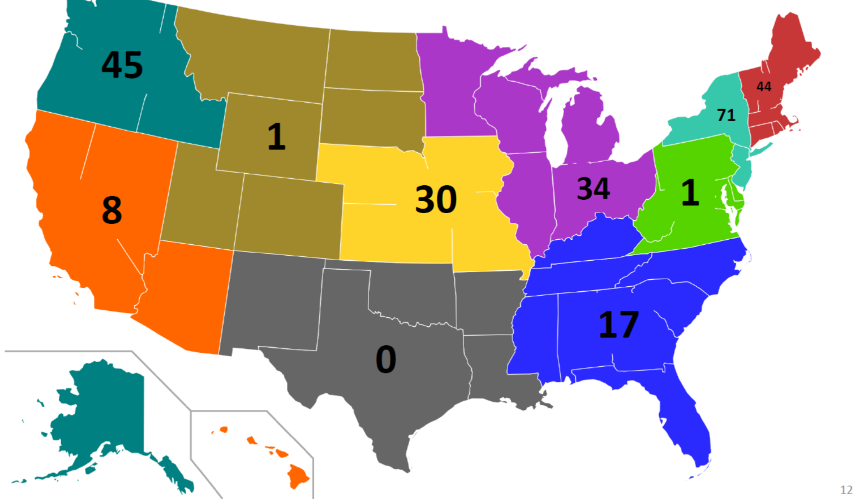
ET&C FOCUS

Focus articles are part of a regular series intended to sharpen understanding of current and emerging topics of interest to the scientific community.

Are Harmful Algal Blooms Becoming the Greatest Inland Water Quality Threat to Public Health and Aquatic Ecosystems?

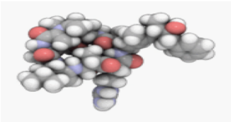
Bryan W. Brooks,*† James M. Lazorchak,‡ Meredith D.A. Howard,§ Mari-Vaughn V. Johnson,|| Steve L. Morton,# Dawn A.K. Perkins,†† Euan D. Reavie,‡‡ Geoffrey I. Scott,§§ Stephanie A. Smith,|||| and Jeffery A. Steevens##

2015 Reported HAB Advisories by Region



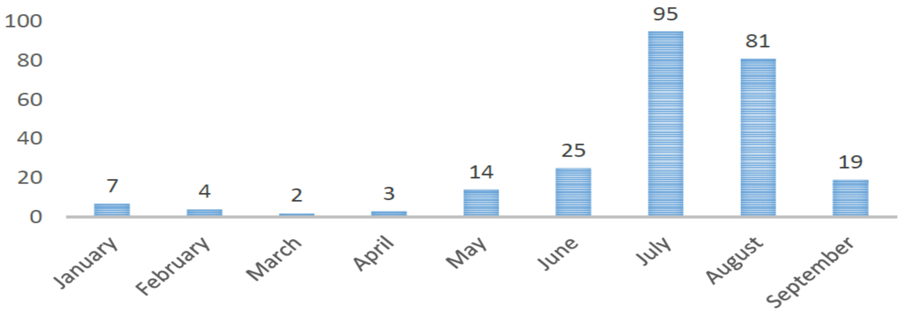
Range of 2015 Reported Microcystin Levels

	Microcystin
Average Cyanotoxin Concentration (µg/L)	627.7 ug/L
Median Cyanotoxin Concentration (µg/L)	20.0 ug/L
Range of Cyanotoxin Concentration (µg/L)	0.2 - 42,000 ug/L



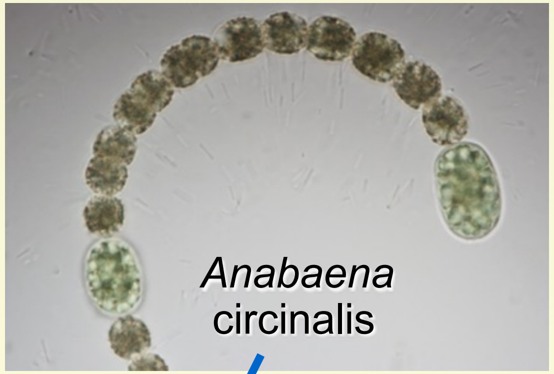
Microcystin

NUMBER OF HEALTH ADVISORIES ISSUED BY MONTH

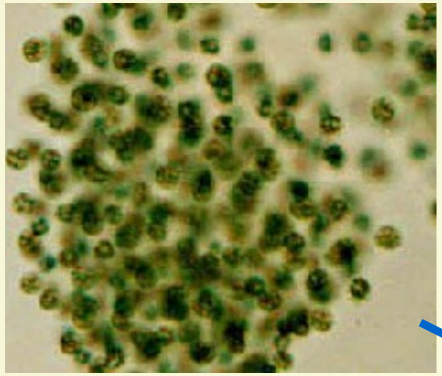


(Sources: Brooks et al. 2015. ETC; Ravencroft, J. 2016. Update on Development of Recreational WQC for Cyanotoxins. EPA Office of Water)

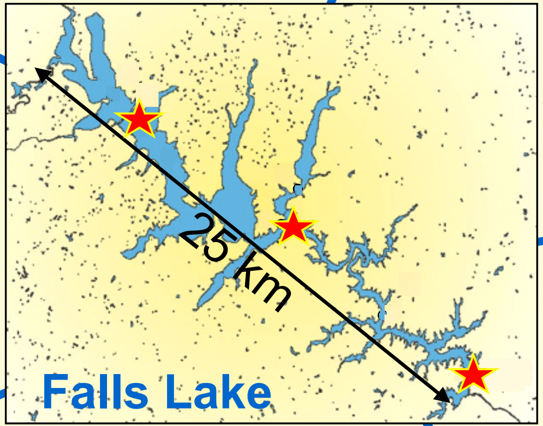
Potentially toxic cyanobacteria (max. abundance)



Anabaena spp.
87,000 cells / mL



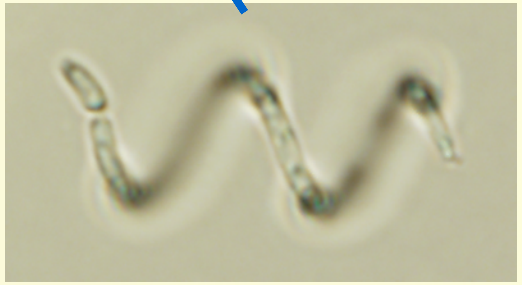
Microcystis aeruginosa
41,000 cells / mL



Drinking water for 0.5 million people

Cylindrospermopsis raciborskii
270,000 cells / mL

Raphidiopsis curvata
1,000 cells / mL



Cylindrospermopsis philippinensis
23,000 cells / mL

LMs: E. Allen

Cyano Harmful Algal Blooms (cyanoHABs)

Mounting evidence indicates global climate changes support increased frequency and geographic extent of HABs.

Each year the desire to live at lakeside and the reliance on large surface waters for recreation and drinking water puts more people and animals at risk for exposure to HABs and the toxins they can produce.

Lure Bar & Grill, Lake Petenwell, WI

Credits: NOAA

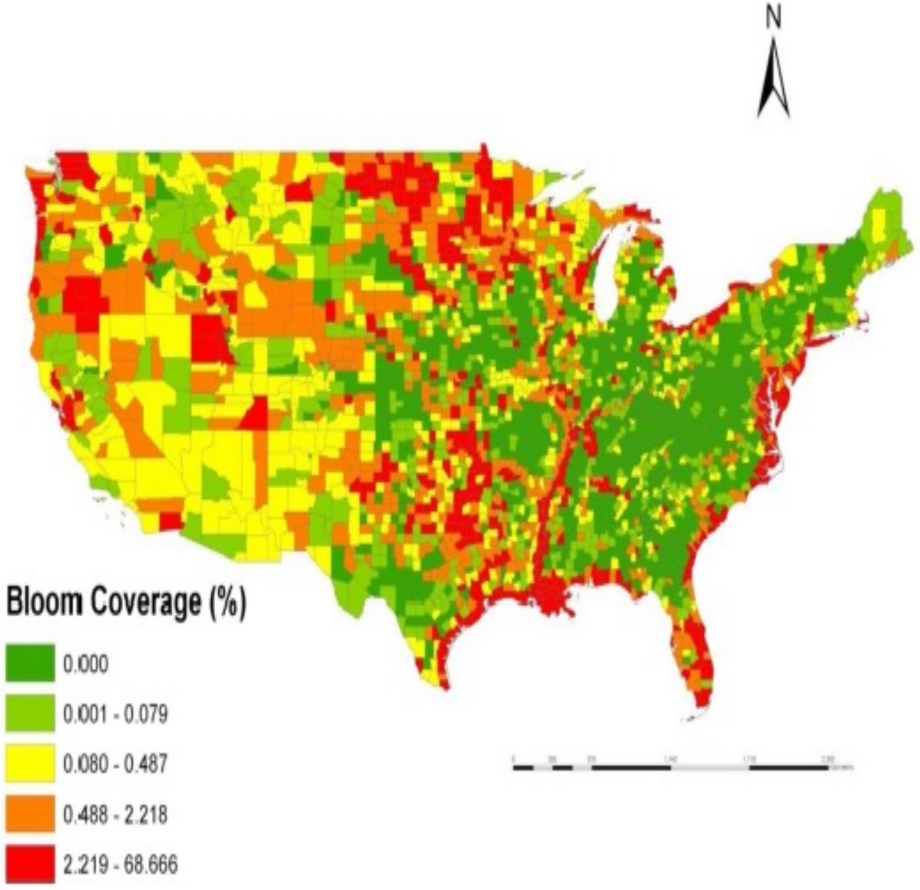
CyanoToxins

Toxins Produced	Type of Toxin	Target Organ	Onset of symptoms
Anatoxin-a Saxitoxins	Neurotoxins	**Nervous System** *Labored breathing, convulsions, numbness, paralysis and death* *Dog deaths caused by Anatoxin-a*	***Minutes to hours***
Microcystins	Hepatotoxin	**Liver** *GI symptoms, elevated liver enzymes in blood, death of cells, destruction of blood vessels*	***Hours to days***
Cylindrospermopsins	Hepatotoxin	**Liver and Kidneys** *Symptoms like food poisoning/Pos. kidney failure*	***Hours to days***

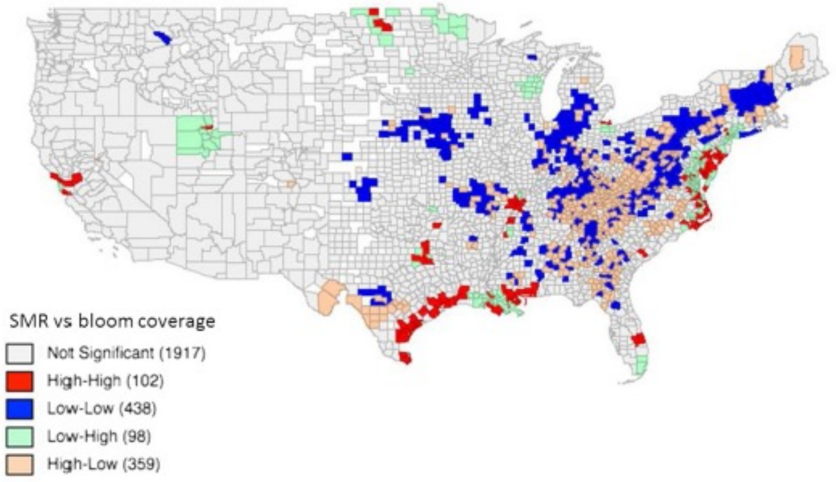
Correlation Between CHAB Blooms and Non-Alcoholic Fatty Liver Disease

(61% of US Counties Have CHABs and for Every 1% increase in CHABs Results in a 0.3% Increase in Non-Alcoholic Fatty Liver Disease)

CHAB Bloom Coverage

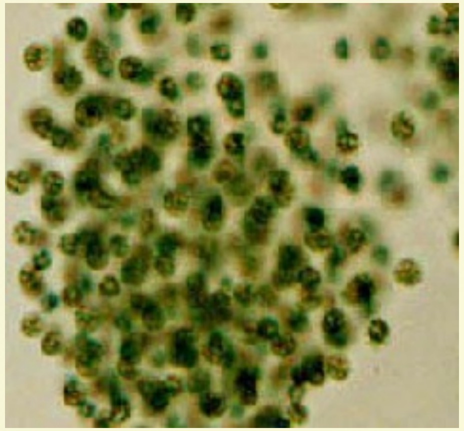


Fatty Liver Disease & CHABs

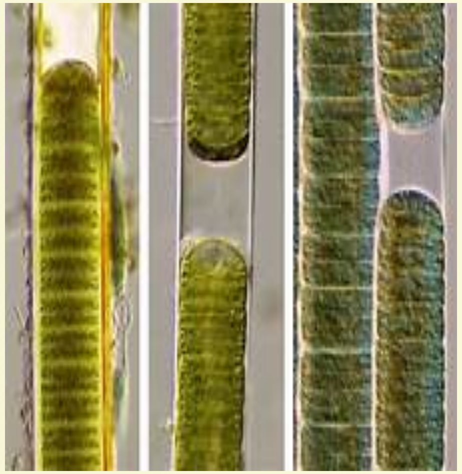


(**Source:** Zhang et al. 2015. Env. Health 14: 41-52)

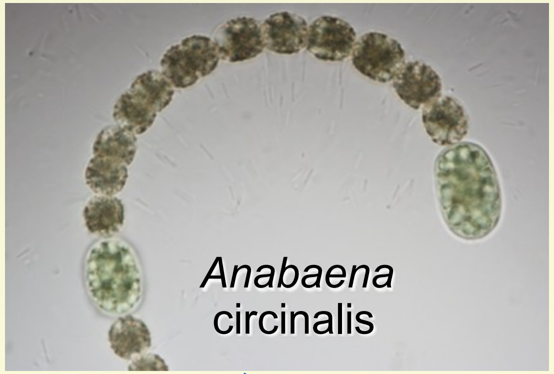
Potentially Toxic Cyanobacteria



Microcystis aeruginosa

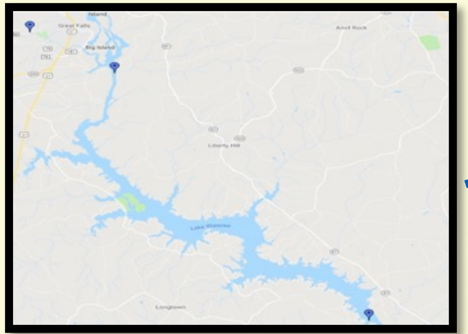


Lyngbya wollei



Anabaena circinalis

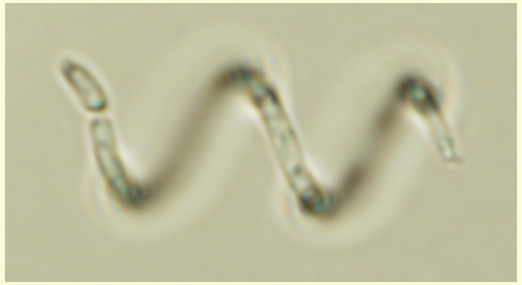
Anabaena spp.



Lake Wateree

Cylindrospermopsis raciborskii

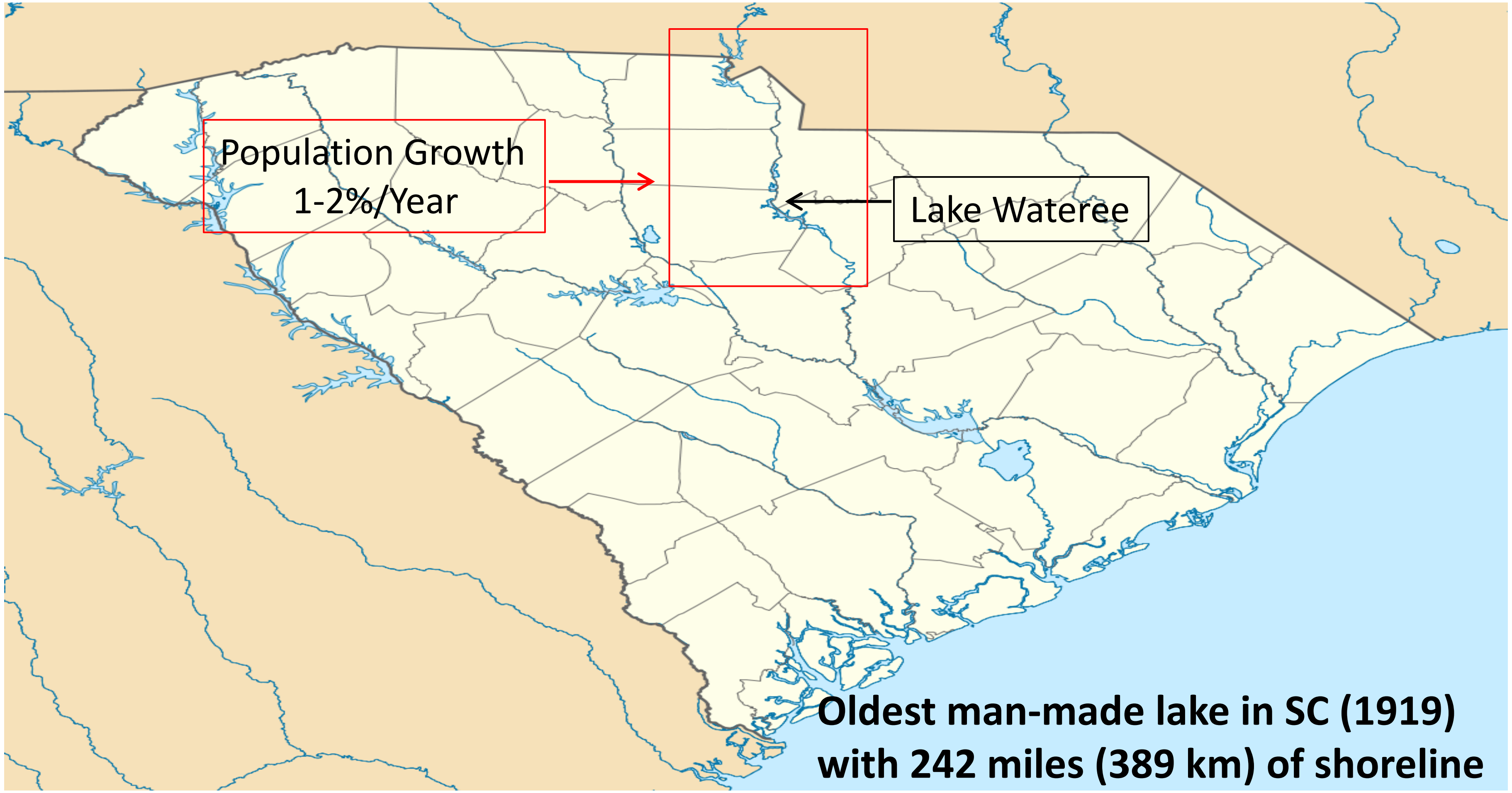
Raphidiopsis curvata



Cylindrospermopsis philippinensis

LMs: E. Allen

Lake Wateree



Lake Wateree

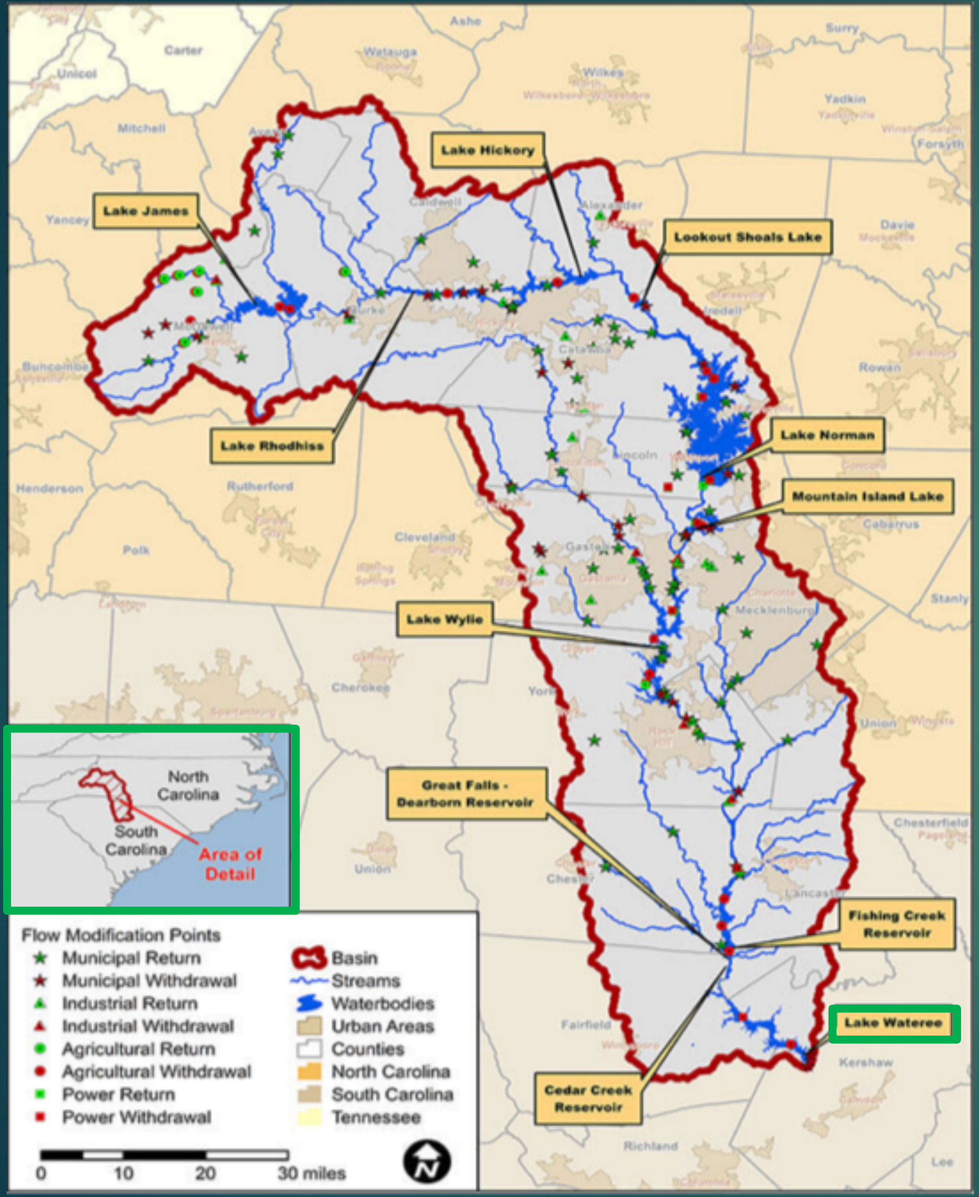
- Owned and managed by Duke Energy (formerly Duke Power), Lake Wateree was created in 1919 when the Wateree River was dammed.
- The Wateree Hydro Station produces 56 megawatts of electricity.
- This created a lake of nearly 14,000 acres (19 square miles or 57 km^2) within three counties.

Lake Wateree

- It is one of the oldest man-made lakes in SC with 242 miles (389 km) of shoreline.

- It includes Lake Wateree State Park, a bird refuge, and Shaw Air Force Base Recreation Center

- It is named after the now extinct Wateree Native Americans, who lived in the area until the time of European settlement.



Source: Duke Energy (2007)

Catawba-Wateree River Basin

- 11 reservoirs, 14 dams and 5000 miles of waterways
- Supplies drinking water to approximately 2 million people.
- Most endangered river *(American Rivers, 2008)*
- 3rd most endangered river in SE U.S. *(Southern Environmental Law Center, 2012)*
- 4th most stressed river in the U.S. from power production *(Union of Concerned Scientists, 2011)*

(Clyburn, K. 2019. Masters Thesis, USC)

The “Recipe” for Healthy Living Things

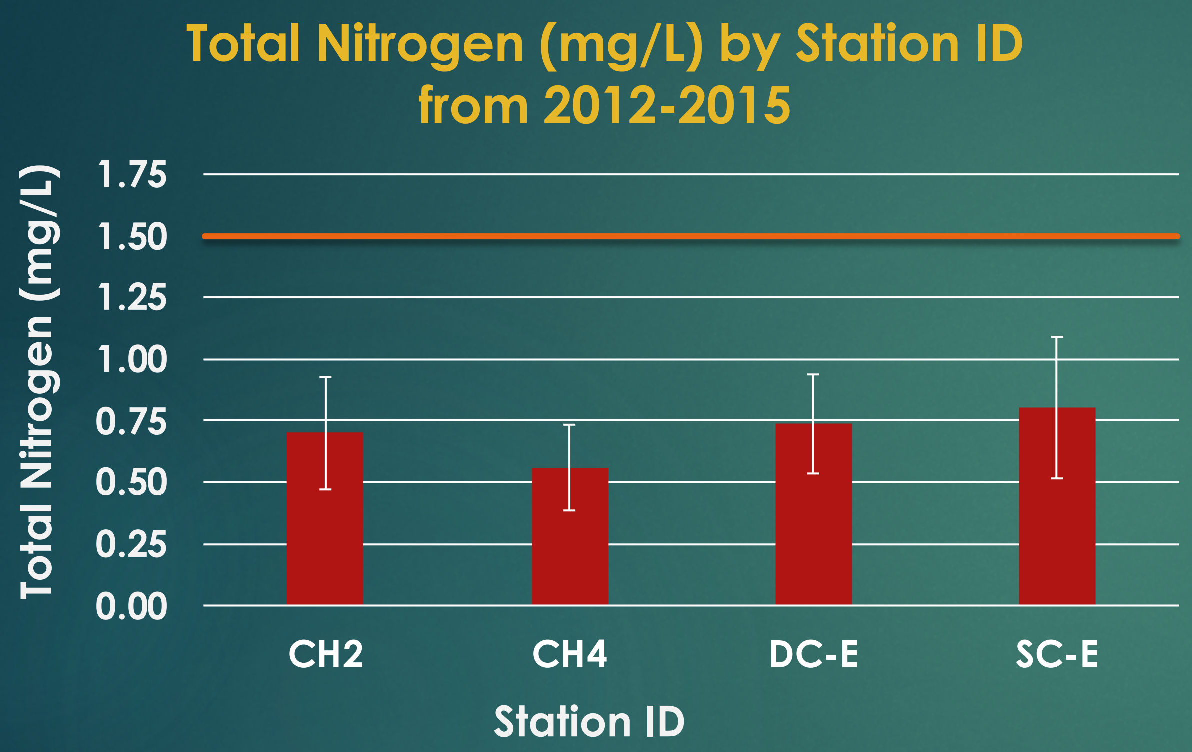
- Scientists have measured the elements that make up DNA-based life. Essentially all living things are:
- 1 part phosphorous
- 16 parts nitrogen
- 106 parts carbon

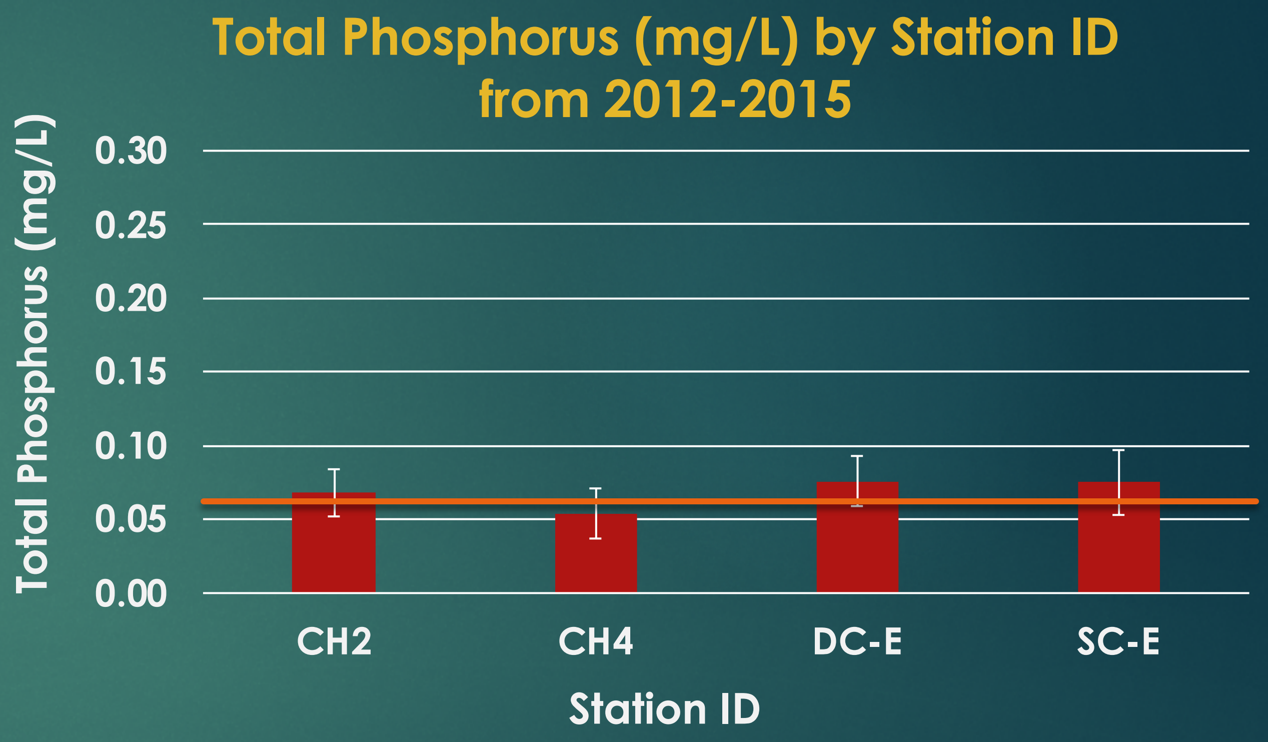
- Fertilizers used by farmers (e.g.10:10:10) have to have

Precise nutrient ratios for healthy plants

In a **Healthy Water Body**, the ratio of these elements limits how things grow. **So if you have one part phosphorous you usually can’t have more than 106 parts carbon.**

Historical Average Total Nitrogen (mg/L) and Phosphorus (mg/L) Concentrations from 2012-2015





- Little variance by location
- TN lower than SCDHEC WQ standards (1.50 mg/L)
- TP higher than SCDHEC WQ standards (0.06 mg/L)

(Clyburn, K. 2019. Masters Thesis, USC)

Nutrient Limitation

Lyngbya wollei

- **The Redfield Ratio:** The molecular ratio of carbon, nitrogen and phosphorus in algae maintains that the balanced system of C:N:P molar ratio is 106:16:1 when nutrients are not limiting.

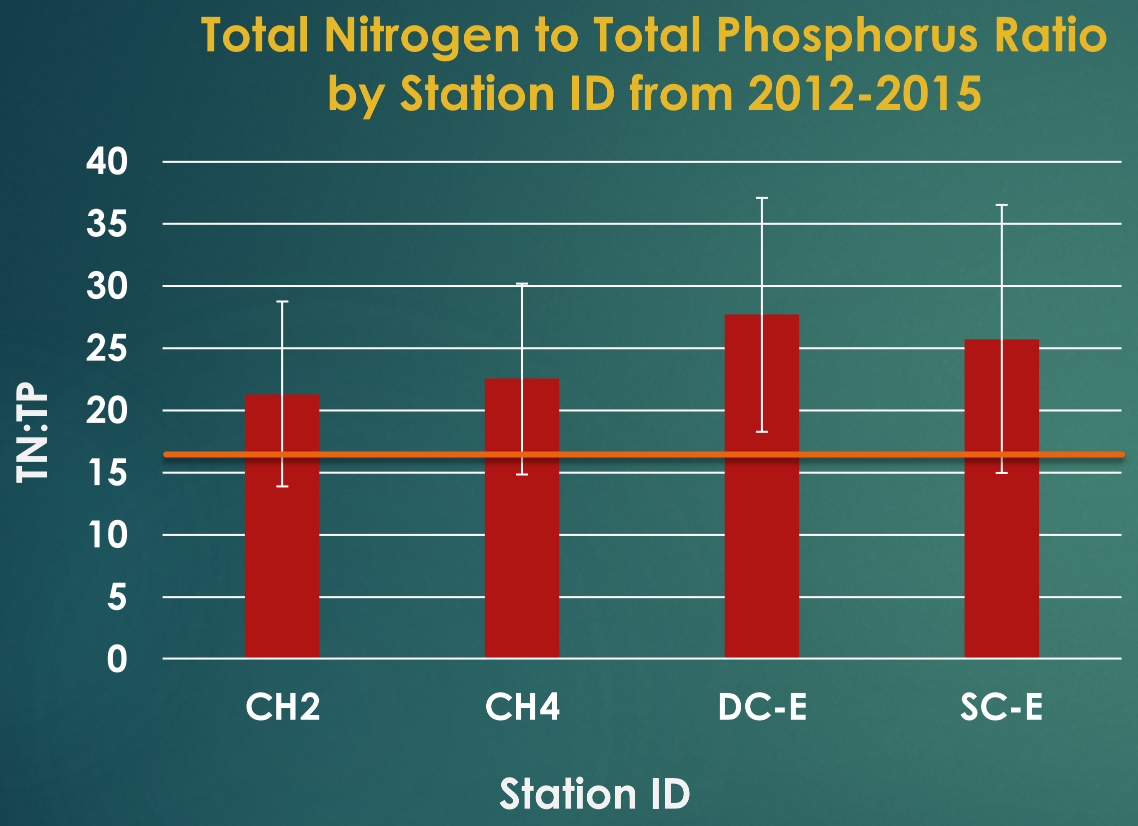
- A N:P molar ratio > 20:1 is typically phosphorus limiting (freshwaters)

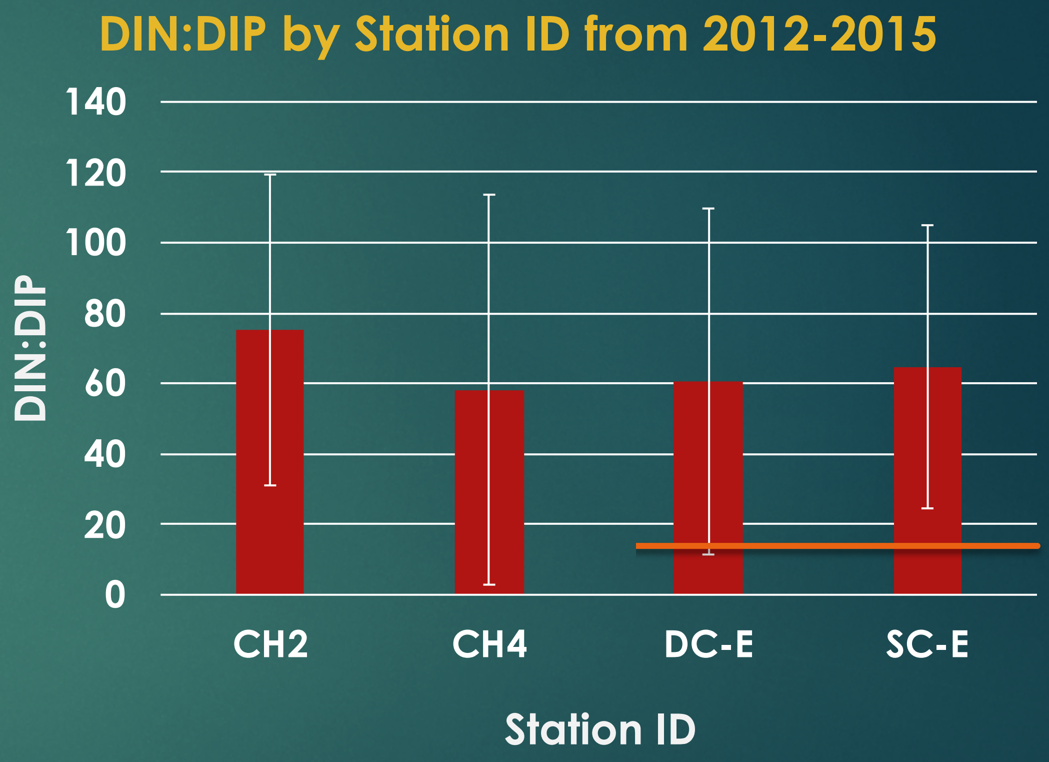
- A N:P molar ratio < 10:1 is usually nitrogen limiting (freshwater or seawater)

- Nutrient supply is relative to algae demand and may in part determine the types of algae observed.

(Clyburn, K. 2019. Masters Thesis, USC)

Historical Nutrient Ratios from 2012-2015





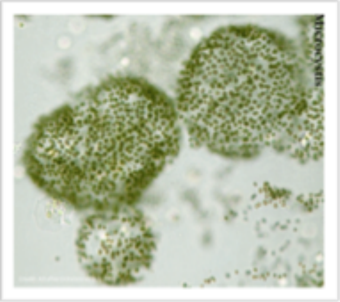
- (Clyburn, K. 2019. Masters Thesis, USC)

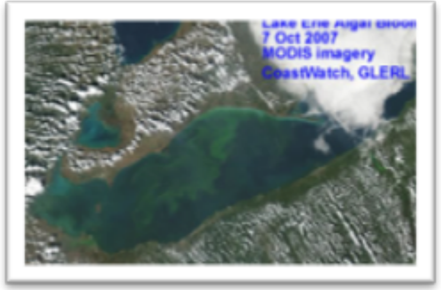
Cyanobacterial Harmful Algal Blooms (cyanoHABs)

Cyanobacteria, formerly called blue-green algae, are a type of photosynthetic bacteria.

Beneficial in that they can produce oxygen for the water and atmosphere.

When environmental conditions are ideal, cyanos can grow rapidly, or ‘**bloom**’, forming thick surface scum layers.

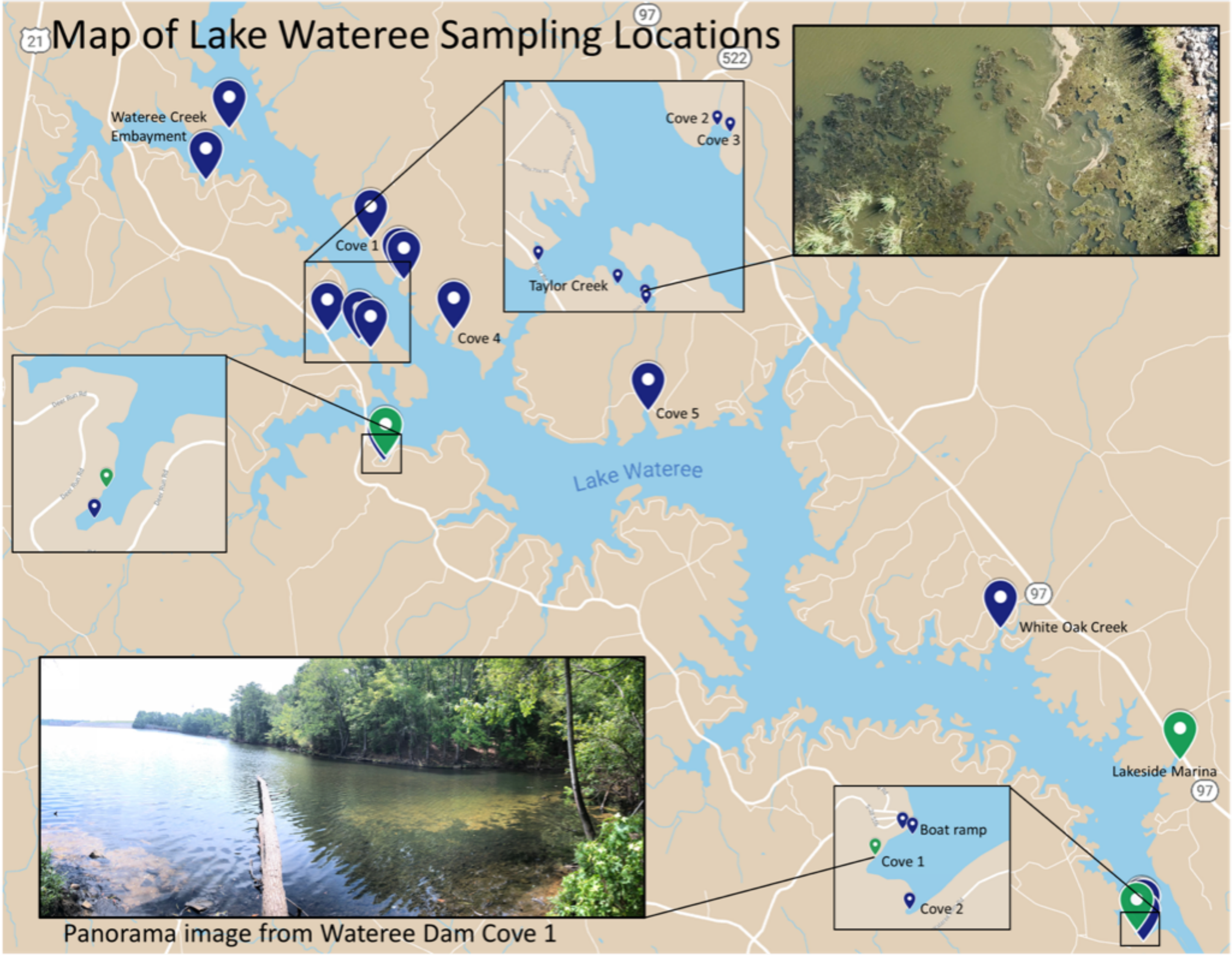




Lyngbya wollei is a Cyanobacteria Growing Throughout Lake Wateree

Lyngbya: When it's growing off your shoreline it looks like (or old, emerging growth):

Map of Lake Wateree Sampling Locations



Panorama image from Wateree Dam Cove 1

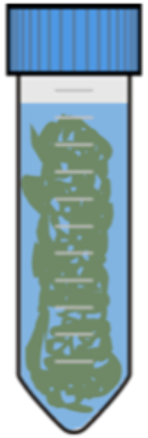
Procedures (abbreviated)

Live *L. Wollei* floating at the surface

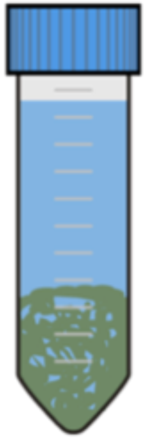
L. Wollei collected in a net and pulled from the water

Freeze dried *L. Wollei*, 82% mass loss from drying

Freeze dried algae was placed into a tube and 10 mL of 0.1 M acetic acid was added

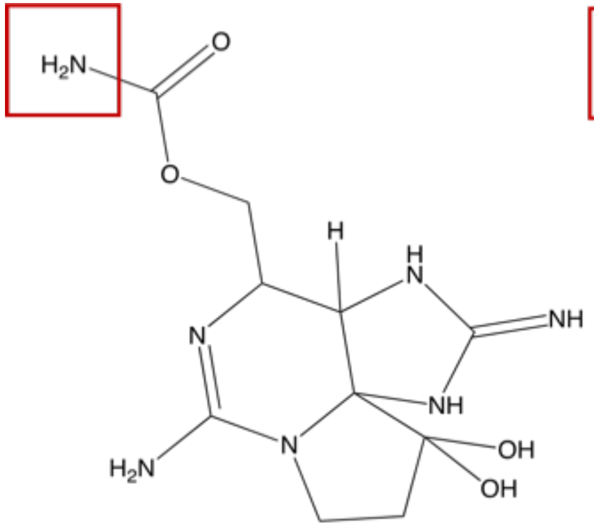


The sample was sonicated for 15 mins and then centrifuged

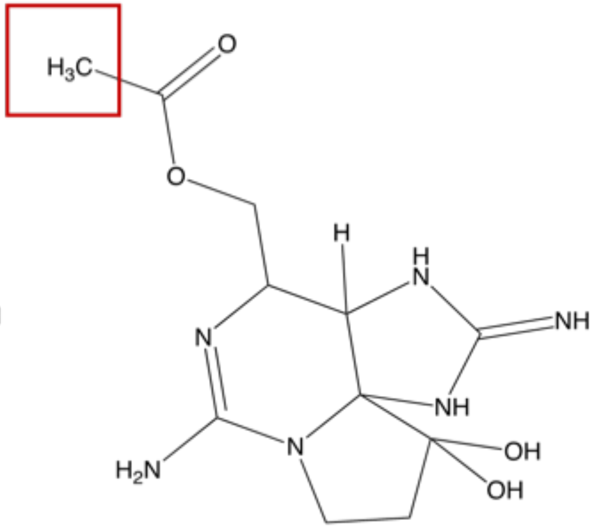


An aliquot was filtered using a nylon 0.45-mircron membrane analyzed by UPLC-MS/MS

Toxin ID is ongoing (qualification and quantification)



Structure of saxitoxin

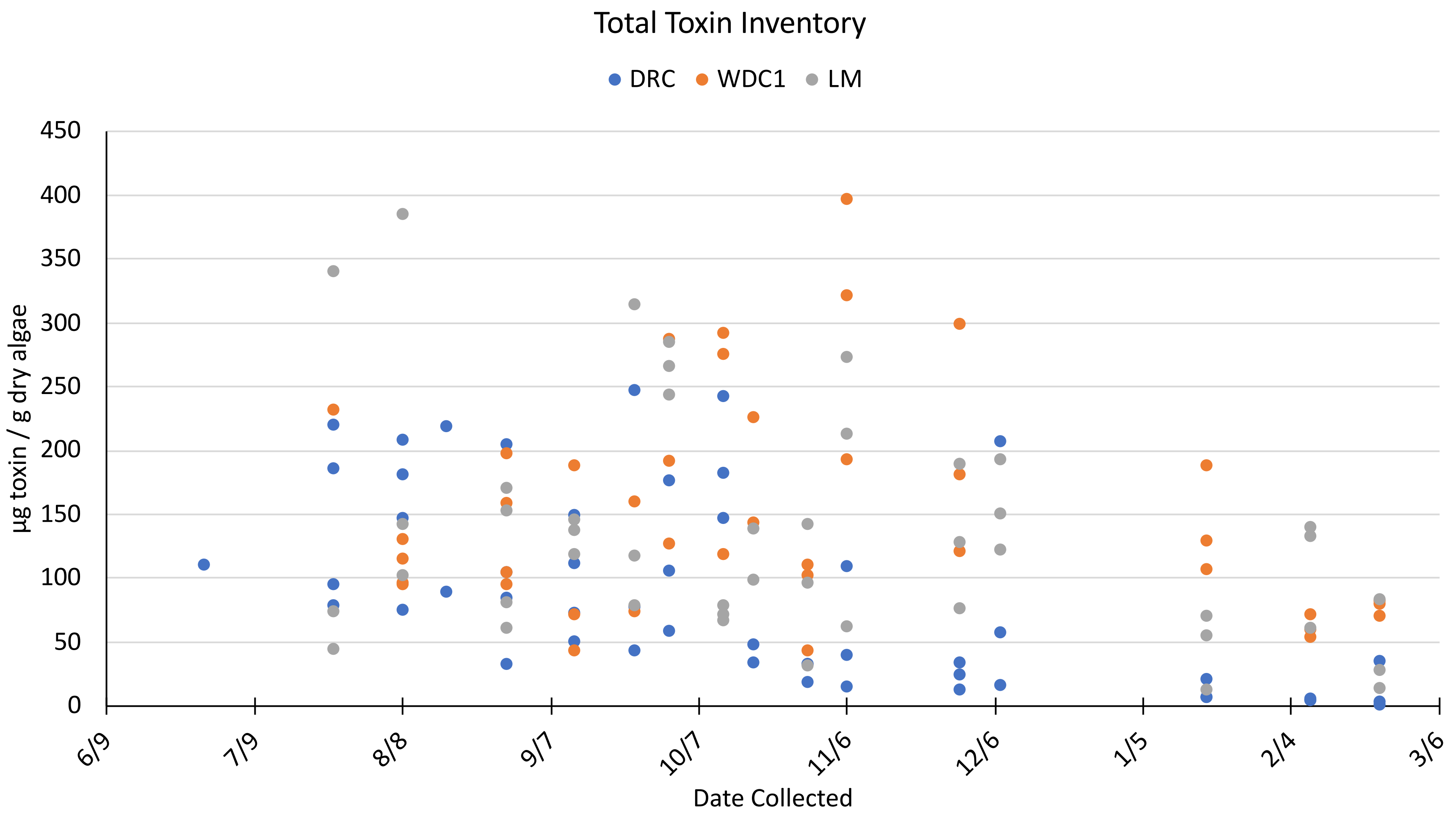


Structure of *Lyngbya Wollei* toxin 5 (LWT5)

In lieu of a reference standard of LWT5, a reference standard of saxitoxin was used for quantitation of LWT5. Structurally, they are similar (replacement of N with C) therefore, their response on the UPLC-MS/MS is assumed to be similar.

Lyngbya wollei toxin 1,4,5, and 6 have been qualified and quantified vs saxitoxin

Trends vs Time, ΣLWT



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Qualitatively we have identified the following toxins in Lake Wateree *Lyngbya*:

Toxin	Mouse units (MU) (μmol^{-1})	Relative Toxicity
[a]STX	2483	1.00
[a]dcSTX	1274	0.51
[a]dcGTX2	1617	0.65
[a]dcGTX3	1872	0.75
[a]LWT1	<10	<0.004
[a]LWT2	178	0.07
[a]LWT3	52	0.02
[a]LWT4	<10	<0.004
[a]LWT5	326	0.13
[a]LWT6	<10	<0.004

From: Characterization of paralytic shellfish toxins from Lyngbya wollei dominated mats collected from two Florida springs (Foss and co-workers, 2012)

HAB Standards for Contact Recreation and Drinking Water

A. Contact Recreation

Microcystins	Cylindrospermopsin
8 µg/L	15 µg/L

B. Drinking Water

Cyanotoxin	Drinking Water Health Advisory (10-day)	
	Bottle-fed infants and pre-school children	School-age children and adults
Cylindrospermopsin	0.7 µg/L	3.0 µg/L
Microcystins	0.3 µg/L	1.6 µg/L

South Carolina Lakes and Rivers: Lake Greenwood and Lake Wateree

Lake Greenwood

- Lake Greenwood is a man-made reservoir which was constructed between 1935 and 1940 by the construction of the Buzzard's Roost Dam near Chappels, SC, a hydroelectric dam across the Saluda River.
- The lake spans about The Reedy River and joins the Saluda River at Lake Greenwood.
- Lake Greenwood contains 212 miles of shoreline and 11,400 acres of lake habitat.
- Lake Greenwood is governed by Greenwood County and is not an Army Corp Of Engineer Lake.
- Private docks and seawalls are allowed (with a permit) on most property except for those few in Habitat Restricted areas.
- Lake Greenwood is great for freshwater fish including Largemouth Bass, Striped Bass, Blue Gill, Crappie, Perch, and Catch Fish

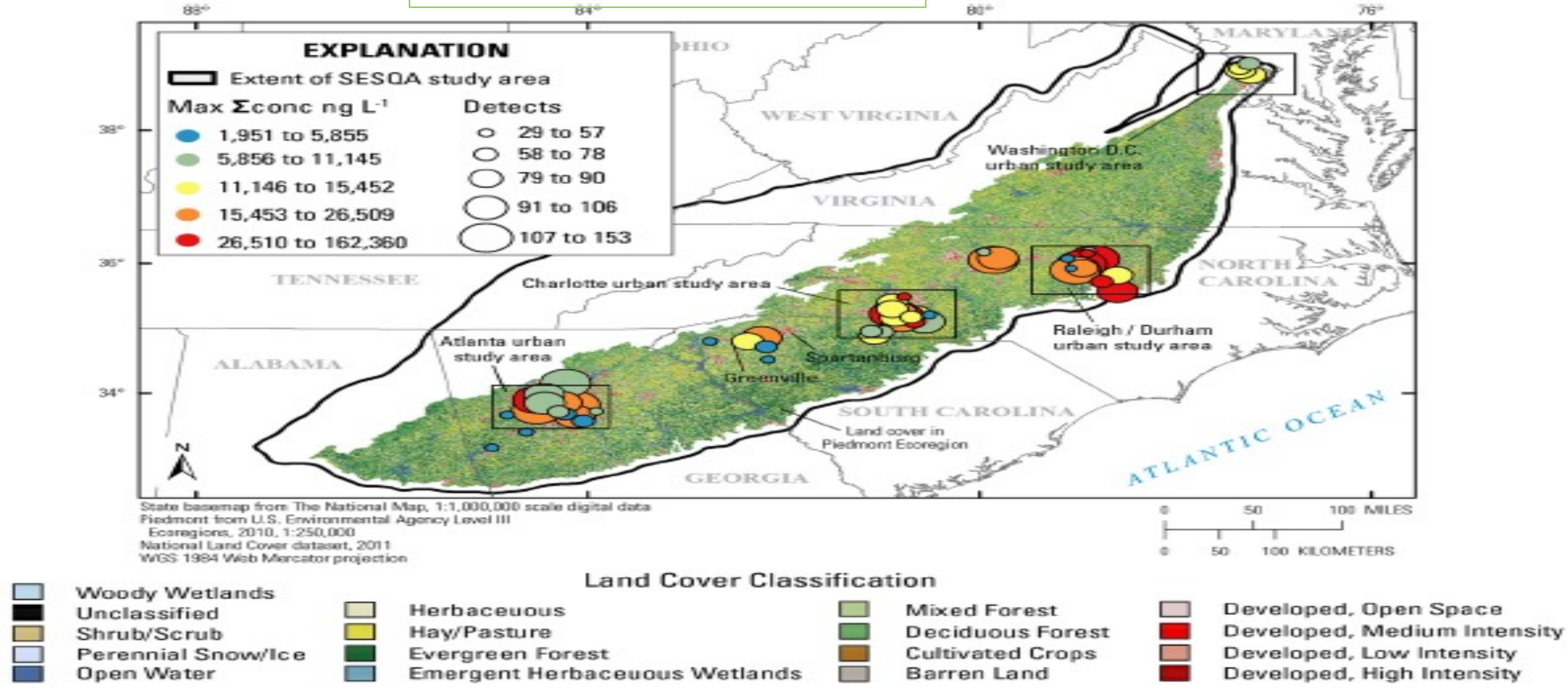
Mixed-chemical exposure and predicted effects potential in wadeable southeastern USA Streams

(Bradley et al. 2019. Science of the Total Env. 655: 70-83)

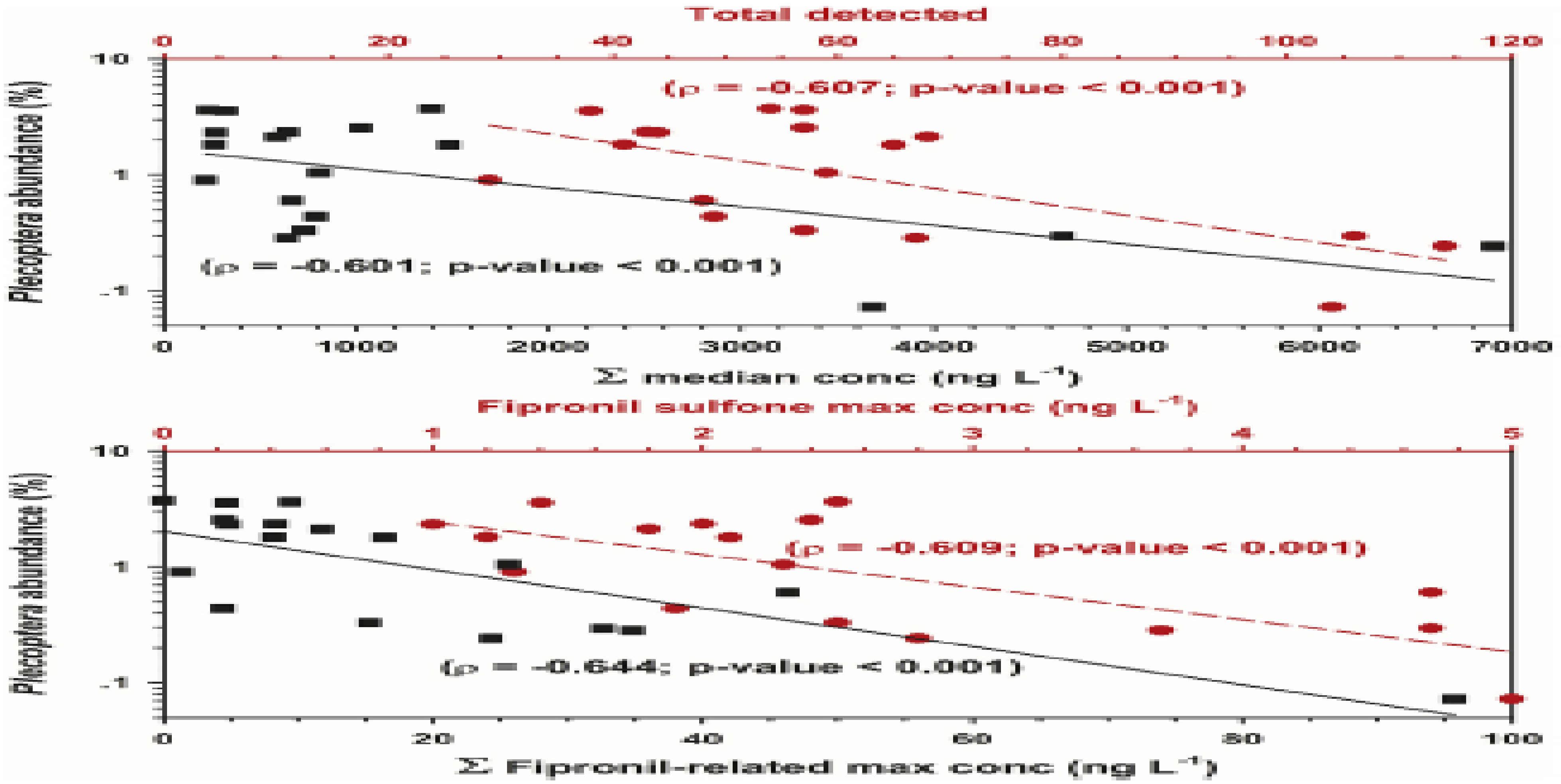
- In 2014, the United States Geological Survey (USGS) sampled 54 Piedmont streams over ten weeks and measured 475 unique organic compounds (Contaminants of Emerging Concern = CECs) using five analytical methods.
- Approximately 56% (264) of the 475 compounds were detected at least once across all sites. Cumulative maximum concentrations ranged 1,922–162,346 ng L^{-1} per site.
- ***Chemical occurrence significantly correlated to urban land use but was not related to presence/absence of wastewater*** **treatment facility discharges**.
- Pesticides and Pharmaceutical and Personal Care Products (PPCPs) were compounds of greatest concern

Detection of CECs in SE Streams

Colored Circles = Concentrations
Circle Size = % Detects



Effects of Fipronil on FW Invertebrates



Nutrient enrichment in wadeable urban streams in the piedmont ecoregion of the southeastern United States

(Journey et al., 2018. Env. Science 4 (11): https://doi.org//10.1016/j.heliyon.2018.e00904)

- The U.S. Geological Survey (USGS) Southeastern Stream Quality Assessment (SESQA) collected weekly samples for nitrogen and phosphorus in 76 wadeable streams in the urbanized Piedmont ecoregion of the Southeastern United States, during April–June 2014.
- Over 50% of TP concentrations in Greenville, Charlotte, Raleigh and CAFO-influenced streams exceeded the EPA guideline and reference location mean concentrations, indicating phosphorus-nutrient enrichment.
- Urban land use, permitted point sources, and soil infiltration metrics best predicted TN exceedances. Elevated TN and $NO_3 + NO_2$ concentrations in urban streams during low flow were consistent with reduced in-stream dilution of point-source or groundwater contributions.

Lake Greenwood: Historical Issues with HABs

- In the past there have been issues with HABs from sources upstream from the lake, but currently there are no HAB Issues affecting contact recreation or drinking water.
- Taste and odor issues at drinking water plants serve as an early warning system to alert officials to enhance treatment processes to reduce these taste and odor issues and in the process provide an additional margin of safety for toxic HABs is they were to occur.
- Toledo, OH Drinking Water Issue was caused by a virus naturally occurring in the Microcystin, which lysed the cell wall to release the toxin. Thus it is important to know that lysing of cell wall can release certain HAB toxins.

What Can You Safely Do About It?

- **Reduce the phosphorous and other nutrients entering the lake!**
- Remove animal waste from the lakeside
- Reduce or eliminate use of fertilizers near the lake
- Maintain Septic Systems
- Restore riparian zones to the lakeshore to reduce erosion as most nutrients are attached to sediments

Next Step: 2019

- Development of a Phytoplankton Monitoring Network Site(s) in Greenwood in partnership with NOAA
- Development of a surface water monitoring volunteer program

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NATIONAL CENTERS FOR COASTAL OCEAN SCIENCE

Science Serving Coastal Communities

PHYTOPLANKTON MONITORING NETWORK

PMN)

To educate the public on harmful algal blooms (HABs) while expanding the knowledge of phytoplankton that exist in coastal waters through research based monitoring.

- **PMN started in 2001 as part of Marine Biotoxins Program in Charleston, SC**
- **Over 100 active sites in 14 coastal states**

PHYTOPLANKTON MONITORING NETWORK (PMN)

NATIONAL CENTERS FOR COASTAL OCEAN SCIENCE

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Cherry Creek Reservoir, Denver, CO June 2016
Dolichospermum bloom with low MC levels

- **CyanoHAB monitoring started in 2015 as part of an EPA Office of Water grant**
- **22 active sites in 9 states**
- **Used previously established PMN methods with modifications for freshwater habitats.**

P[illegible]LANKTON MONITORING NETWORK

NATIONAL CENTERS FOR COASTAL OCEAN SCIENCE

Science Serving Coastal Communities

To educate the public on harmful algal blooms (HABs) while expanding the knowledge of phytoplankton that exist in coastal waters through research based monitoring.

Train citizen scientists to:

- *Collect samples on weekly or bi-weekly basis*
- *Identify potential harmful algal/cyano species*

NOAA scientists can then:

- *analyze water samples for HAB toxins*
- *Together can identify temporal and geographic HAB trends*

Monitoring Benefits

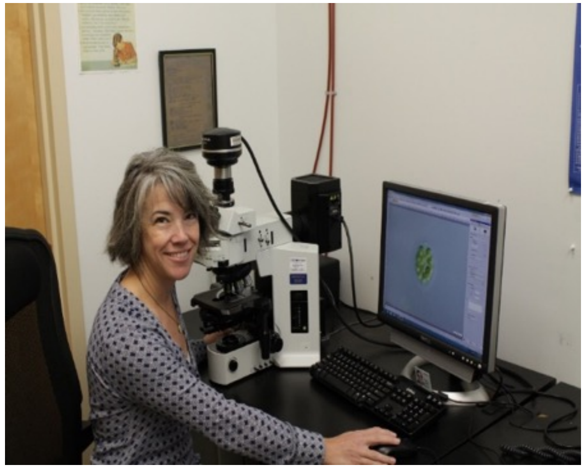
- **Allows for an ‘early warning system’**
  - e.g. Can close shellfish beds/recreational waters and help prevent people from getting sick

- Monitor and maintain an extended survey area along coastal & fresh water bodies throughout the year
- Create a comprehensive list of harmful algal/cyano species inhabiting marine and fresh waters (establish baseline)
- Identify general trends where HABs are more likely to occur
- Promote an increased awareness and education to the public on HABs
- Create a working relationship between volunteers and researchers

Tools for Cyano HAB Training & Algae Identification

WebEx training sessions

- Done remotely to make training more convenient when on-site is not possible
- Allows NOAA scientists to observe real samples online with volunteers



Phytoplankton Monitoring Network

Volunteer Requirements:

1) ***Collect sample*** at least once every two weeks during the sampling season (Ice Out – Ice In)
2) ***Analyze sample*** identifying target algae
3) ***Take*** digital pictures to send into the PMN
4) ***Input*** data into the PMN database
5) ***Preserve*** sample and ship to PMN when water is visibly green

Next Steps: Identify Nutrient Sources Outside of Lake Greenwood

- **Development and/or Continuation of a Volunteer Surface Water Monitoring Program is Important within Lake Greenwood**
- **Critical in understanding nutrient loadings within the Lake and Basin**

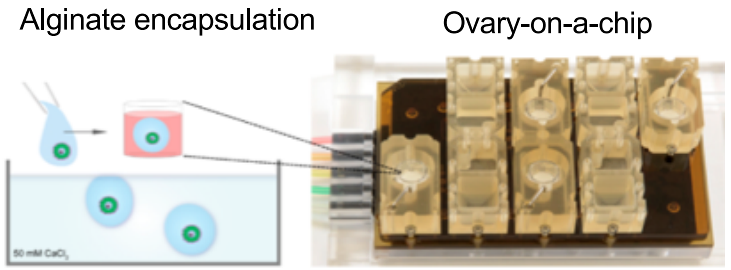
Saluda- Reedy River Basins

OHHC^{2}I

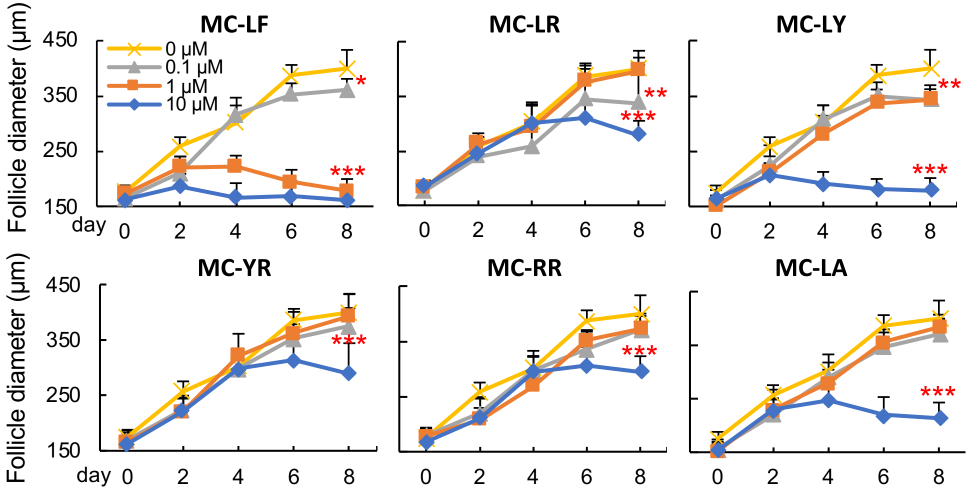
NIEHS Center for Oceans and Human Health on Climate Change Interactions at the University of SC

- **Focus:** **OHHC^{2}I** ’s main purpose will be to assess the effects of ocean health-related illness and disease and then to use this information to develop prevention strategies against ocean-related illness and disease to better protect public health.
- Our specific focus is on climate change-related factors that may enhance the presence, abundance and virulence of ***Vibrio Bacteria*** and ***Freshwater Harmful Algal Blooms***

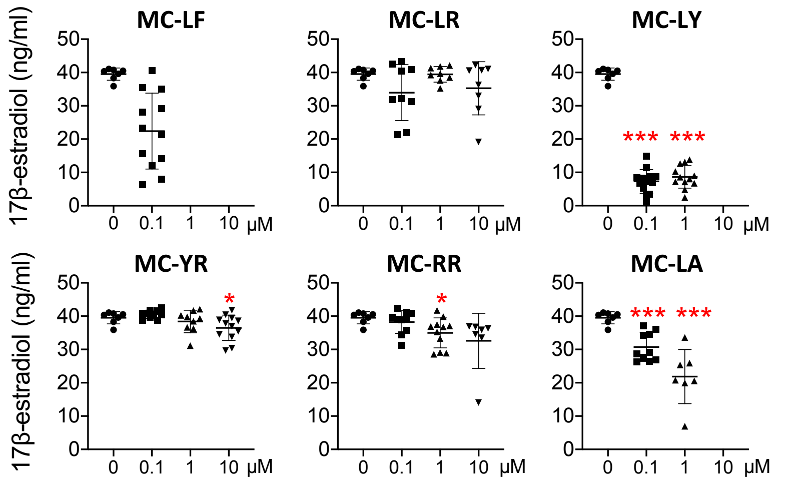
Use of a novel ovary-on-a-chip model to screen for the female reproductive toxicity of microcystins



Follicle growth and development

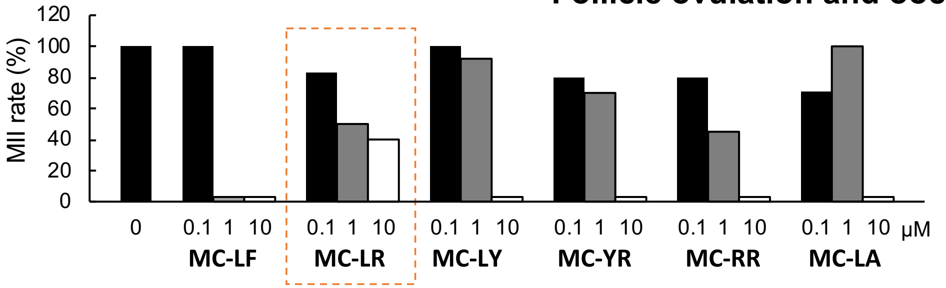


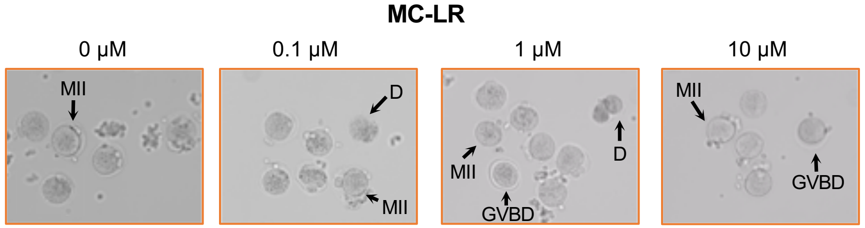
Ovarian hormone secretion



*$p<0.05$, **$p<0.01$, ***$p<0.001$

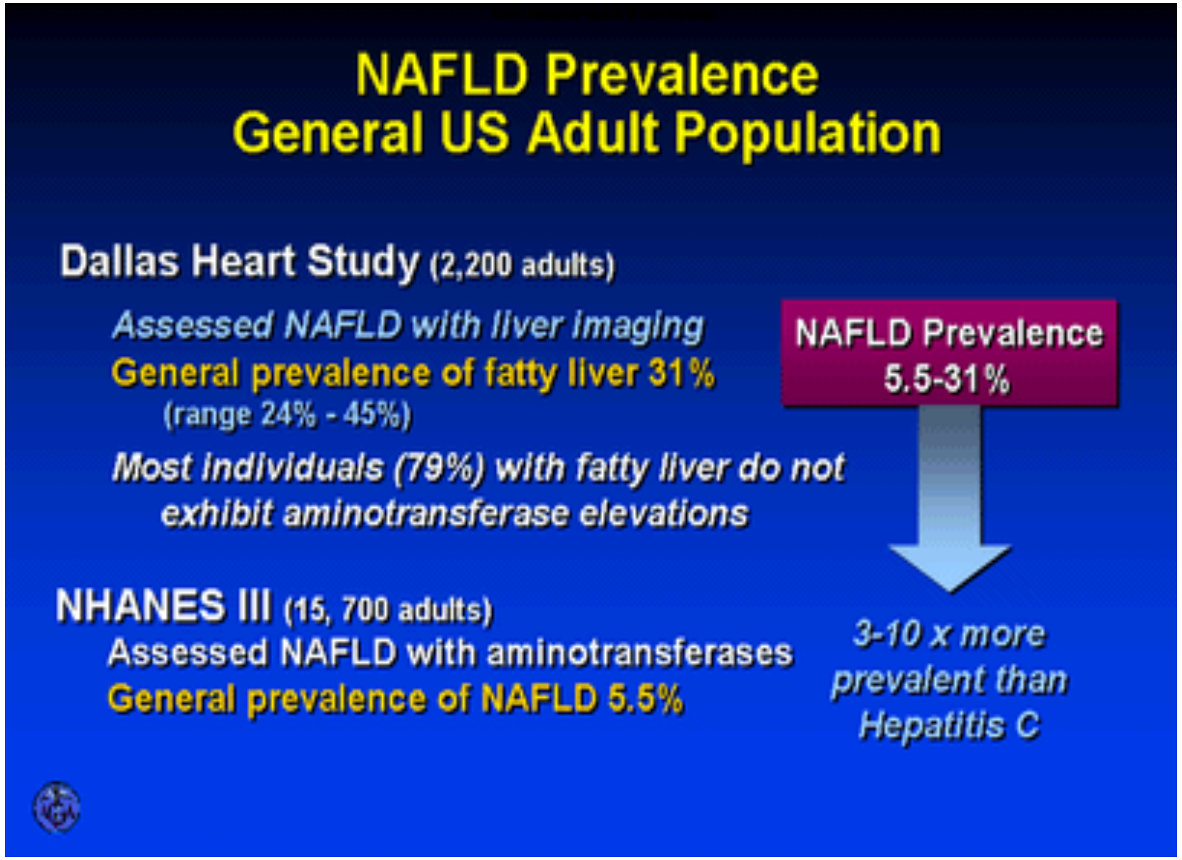
Follicle ovulation and oocyte meiotic maturation

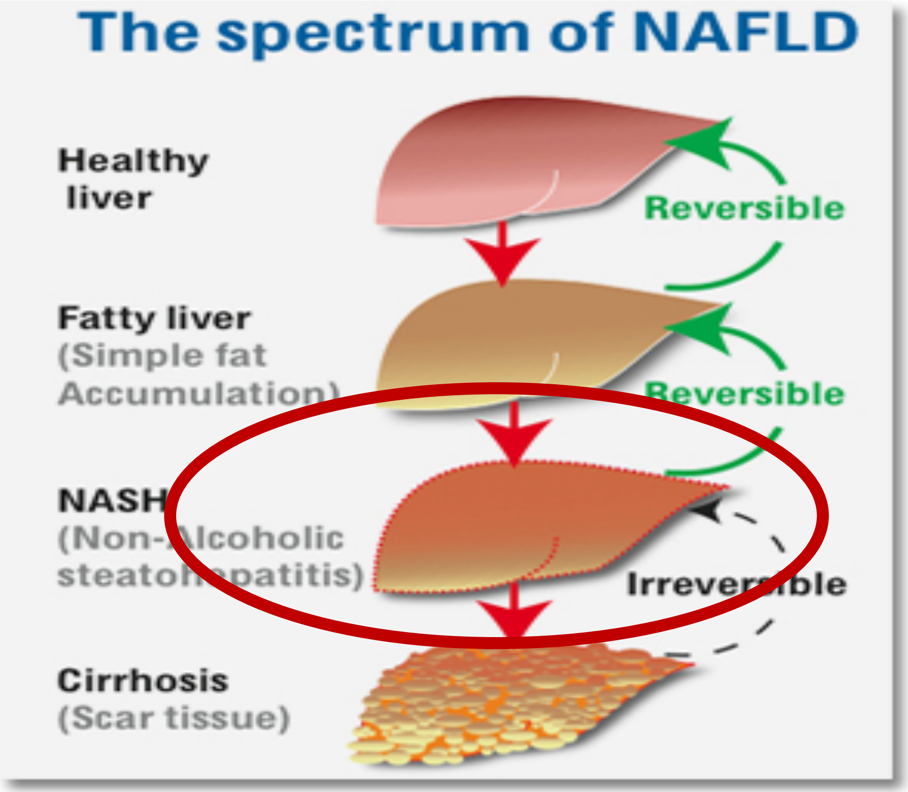




Xiao et al unpublished

NAFLD: Hepatic Manifestation of Metabolic Syndrome



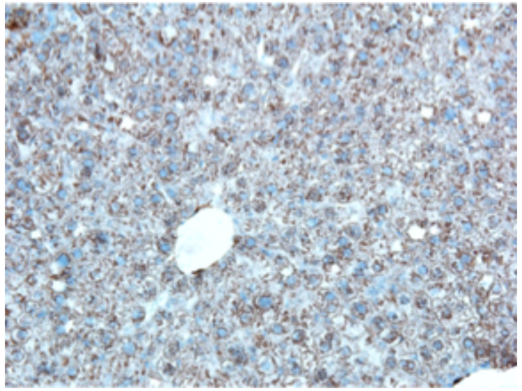


NAFLD develops from simple steatosis to steatohepatitis (NASH), and then cirrhosis. Cirrhosis is no more reversible.

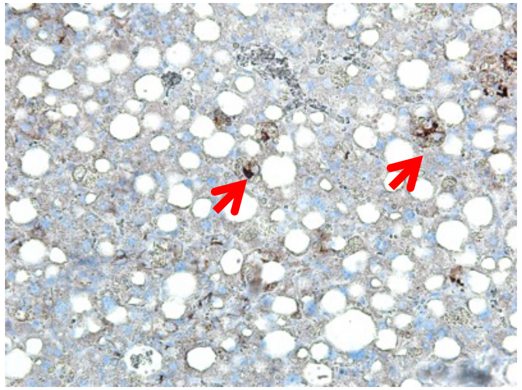
Source: American Gastroenterology Association, 2014

Microcystin-Exposure in NAFLD Mice Leads to Stellate Cell Activation in the Liver (Pre Fibrotic Stage)

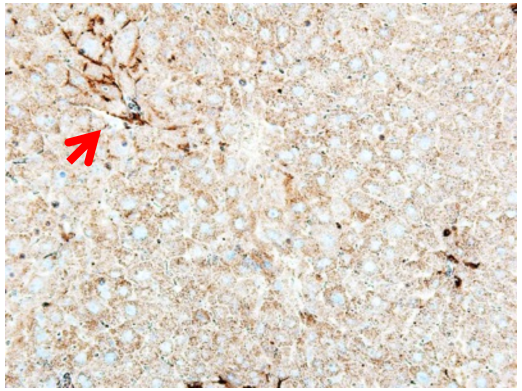
Alpha-Smooth Muscle Actin (marker for activated stellate cells)



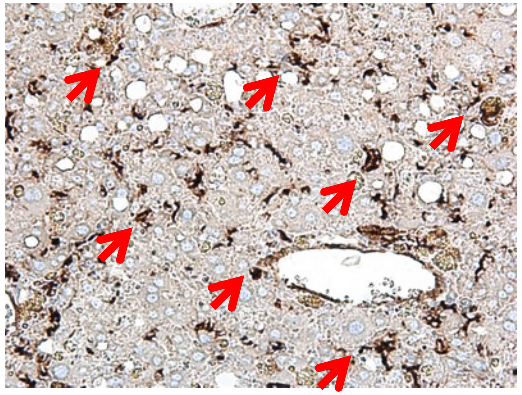
Healthy Controls



NAFLD



Healthy+ Microcystin



NAFLD + Microcystin

Microcystin exposure Leads to Increased Glomerular Inflammation in NAFLD-kidney

Alpha-SMA is a marker for mesangial cell activation crucial for glomerular inflammation