MICROCYSTIS AERUGINOSA BLOOMS IN AN UNLIKELY RIVERINE ECOSYSTEM:

A waste treatment lagoon source

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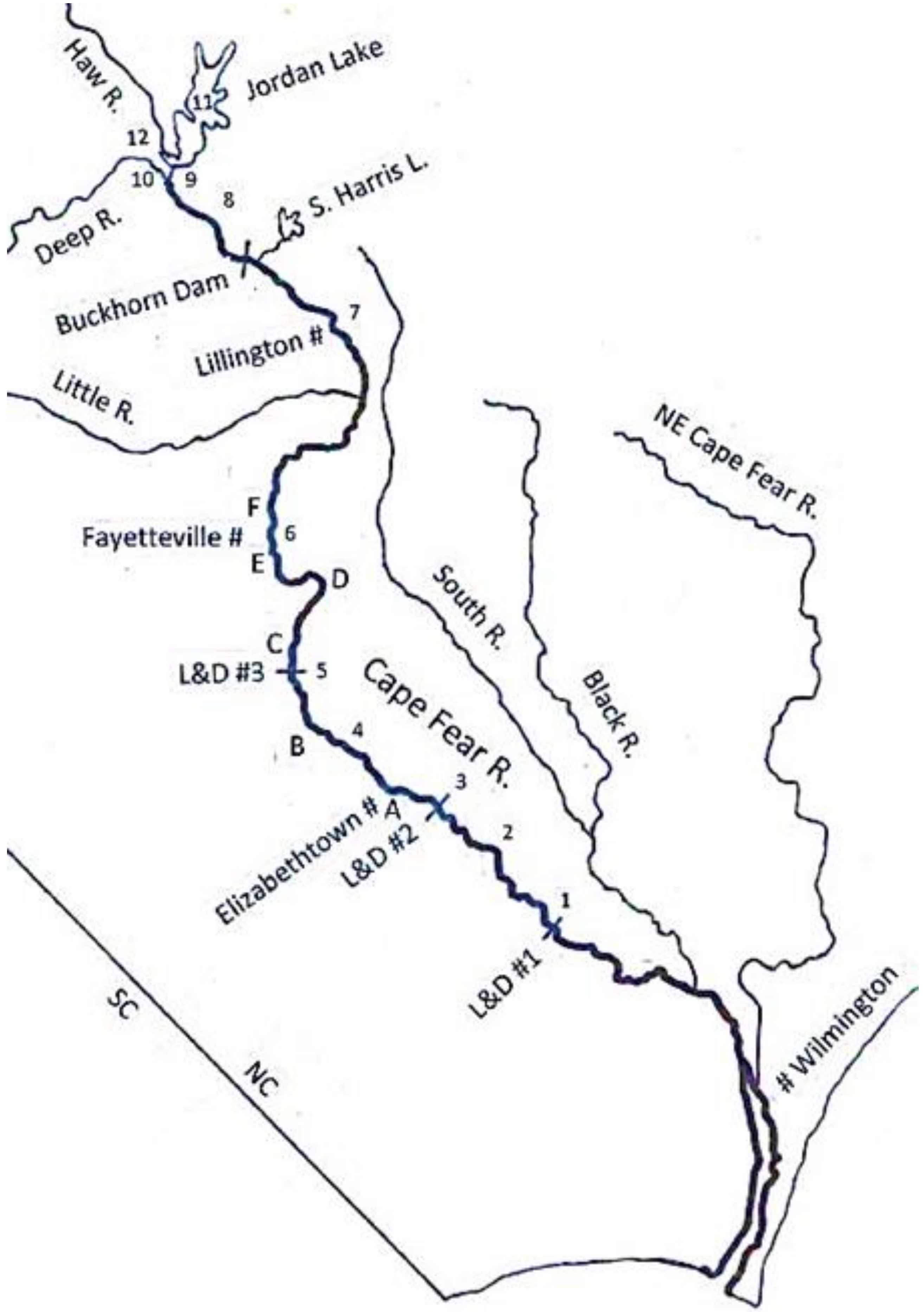
Outline

- Introduction & Problem
- Approach & Methods
- Autochthonous controls
- Allochthonous anthropogenic source



Anomalous *Microcystis* blooms

Almost always associated with proximal lacustrine source or slow-moving lentic conditions

High temperature, low flow, high nutrients, low N:P

Confined to river reach below L&D #3 and above L&D #1

Started and ***<u>stopped</u>*** unexpectedly

Investigation

- Does *M. aeruginosa* **occur** throughout the Cape Fear River basin?
- How did *M. aeruginosa* blooms compare to variations in **phytoplankton biomass through time and space**?
- Did **low flow** conditions support bloom formation?
- Did **high temperatures** favor bloom formation?
- Could variation in **river turbidity** have promoted bloom formation?
- Would **unusual nutrient loading** patterns have driven bloom formation?
- Could *M. aeruginosa* blooms have been seeded from **Jordan Lake**?
- Could there have been an **allochthonous anthropogenic source**?

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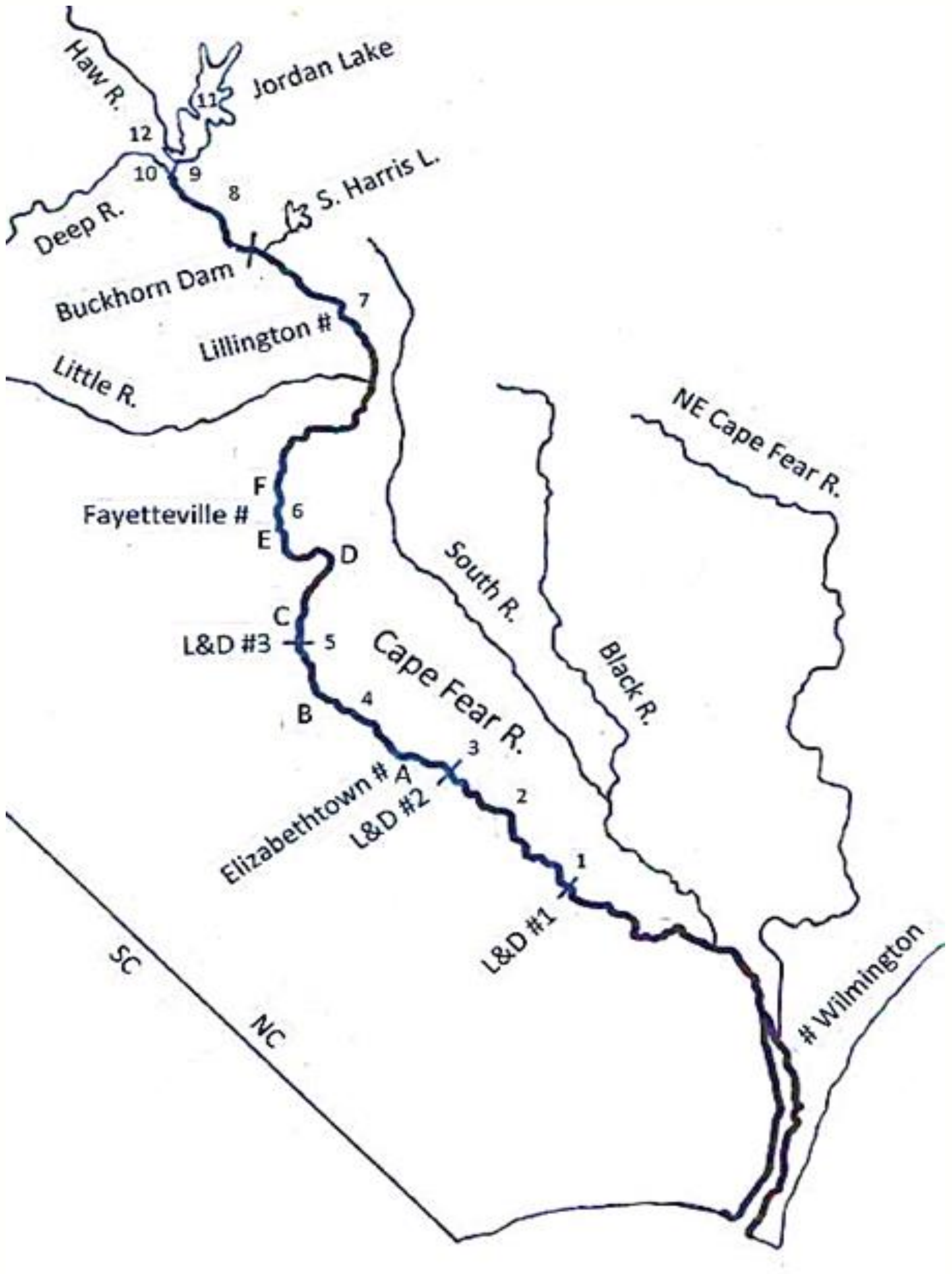
Study Area & Methods

Location	km	Station ID# / USGS Gaging Station
River mouth	0	
L&D #1	100	M B8349000/2105769
Elwell Ferry	113	M B8339000
L&D #2	145	M B8339000
NC 1316	177	M B8305000
L&D #3	193	M B8290000/2105500
Fayetteville	220	-
Lillington	241	M B6370000
NC 42	274	M B6160000
Haw R. @ Moncure	283	U B4080000
Deep R. @ Moncure	290	U B6040300
Jordan Lake outflow	322	-
Jordan Lake @ US 64	-	-
Haw R. @ Bynum	338	U B2100000

ID	km	NPDES #	Monthly Discharge Limit (m^3d^{-1})
A	144	0026671	4,640
B	179	0078344	11,360
C	194	0003573	7,570
D	208	003719	1,890
E	212	00500105	79,840
F	224	0023957	94,620



Study Area & Methods

Monitoring Data Sources

U.S. Army Corps of Engineers
U.S. Geological Survey
Upper CFR Basin Association
Middle CFR Basin Association
Lower CFR Basin Association
N.C. Division of Water Resources
EPA's STORET
Discharge Monitoring Reports

Parameters

Flow and discharge
Dissolved inorganic nitrogen
Total Kjeldahl nitrogen
Total nitrogen
Total phosphorus
Chlorophyll *a*
Temperature
Turbidity

Field Sampling

Targeted 2015-2016
Remote sensing imagery 2016-2019

Extraction, Cloning, Sequencing

mcyB
mcyD
16S-23S rRNA ITS
Next Generation Sequencing

Statistical Analysis

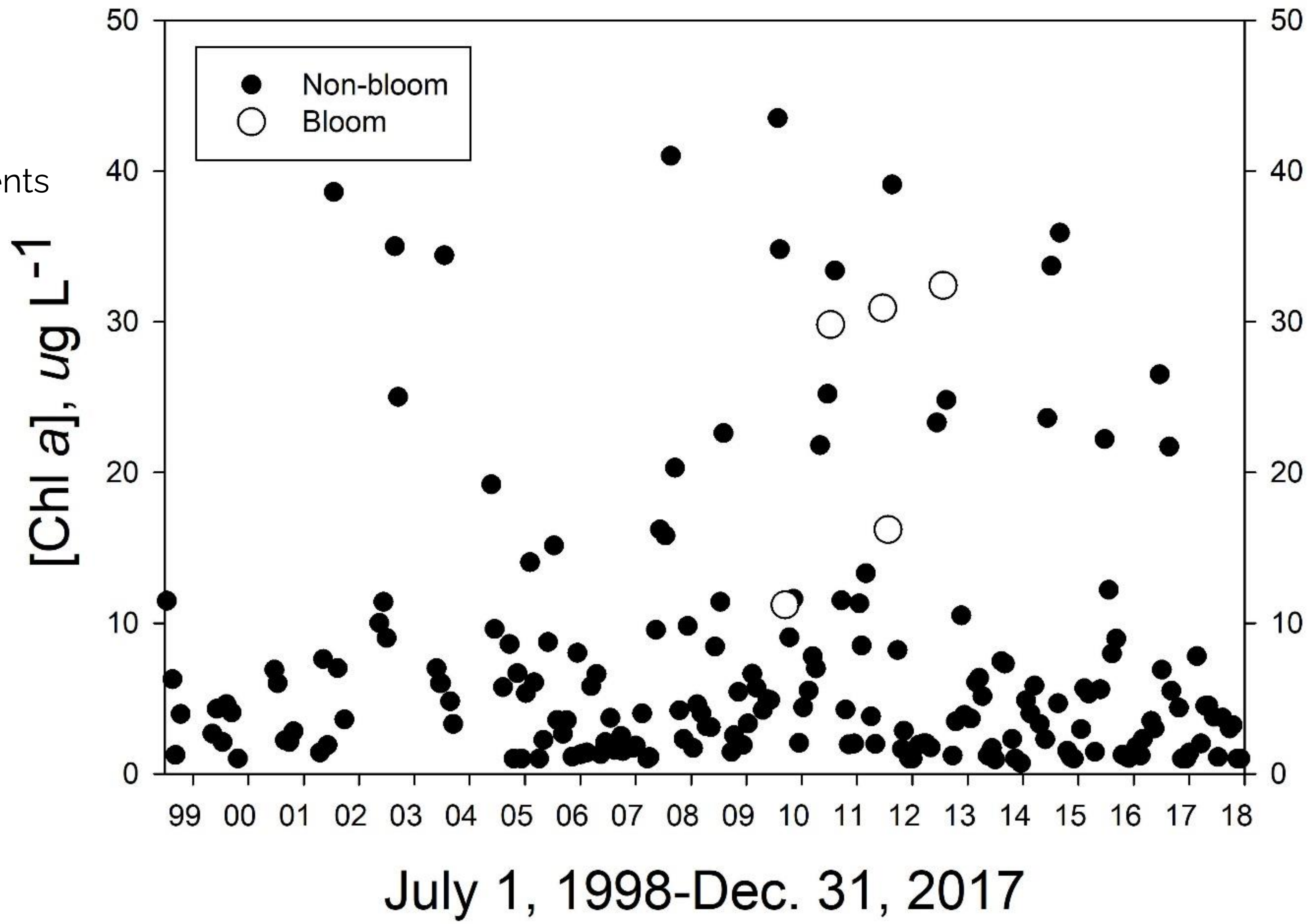
One-way ANOVA
Tukey's HSD
Regression analyses
Linear regression
Multidimensional scaling

Results: Occurance

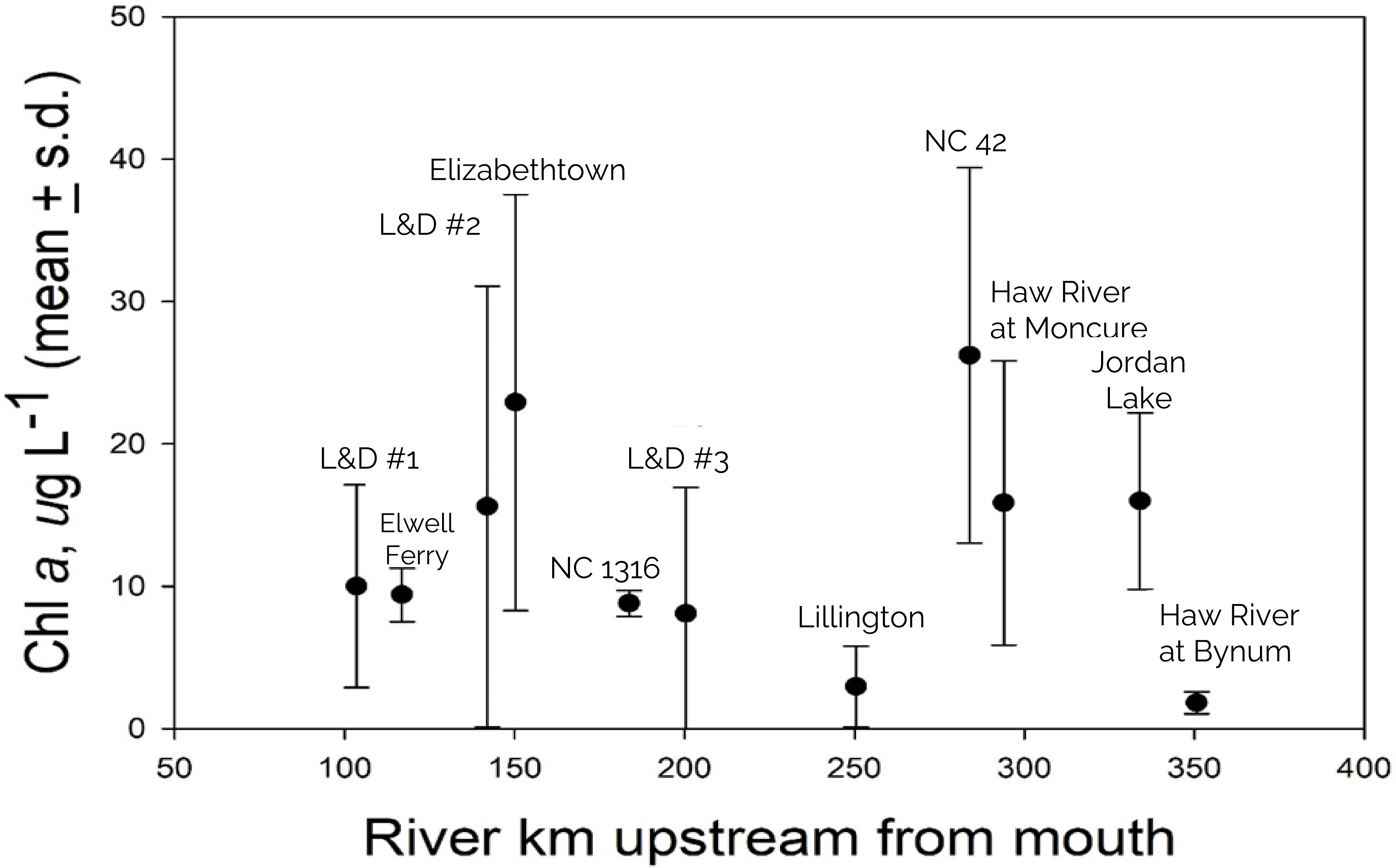
Site Name (# in Fig. 1)	ITS (#pos/#tot)	mcyD (#pos/#tot)	Top BLAST Match	Reference
Haw R @ Bynum (12)	**5/7**	**3/7**	-	-
Jordan L @ US 64 (11)	**7/7**	**5/7**	*M. wesenbergii* NIES44*	Otsuka et al. (1999)
			M. aeruginosa MP07B7	Sabart et al. (2014)
Haw R @ Moncure (9)	**7/7**	**6/7**	-	-
Deep R @ Moncure (10)	**3/7**	**1/7**	*M. wesenbergii* VN484	Nguyen et al. (2012)
CFR @ NC 42 (8)	**7/7**	**4/7**	*M. sp.* Clone CTL 2122	Xu et al. (2011)
CFR @ Lillington (7)	**2/7**	**1/7**	-	-
CFR @ Fayetteville (6)	**3/3**	**1/3**	-	-
CFR @ L&D #3 (5)	**2/3**	**0/2**	-	-
CFR @ L&D #2 (3)	**4/5**	**3/5**	-	-
CFR @ L&D #1 (1)	**2/5**	**0/5**	*M. wesenbergii* VN484, NIES44*	Otsuka et al. (1999), Nguyen et al. (2012)

Results: Phytoplankton biomass through time

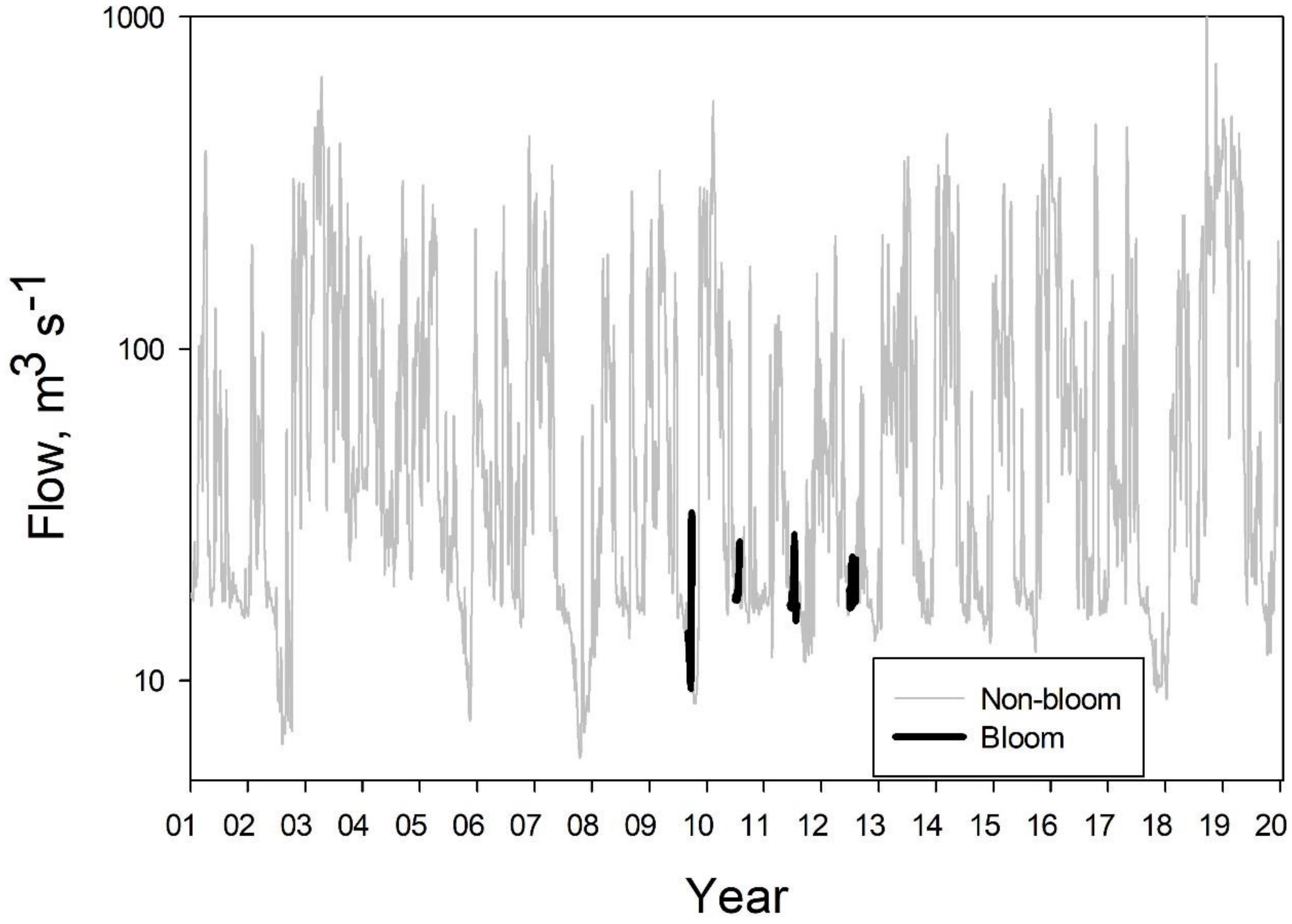
- Biomass **significantly higher** during bloom events
- High chl *a* during non-blooms
- **No relationship** with turbidity



Results: Phytoplankton biomass through space



Results: Low Flow

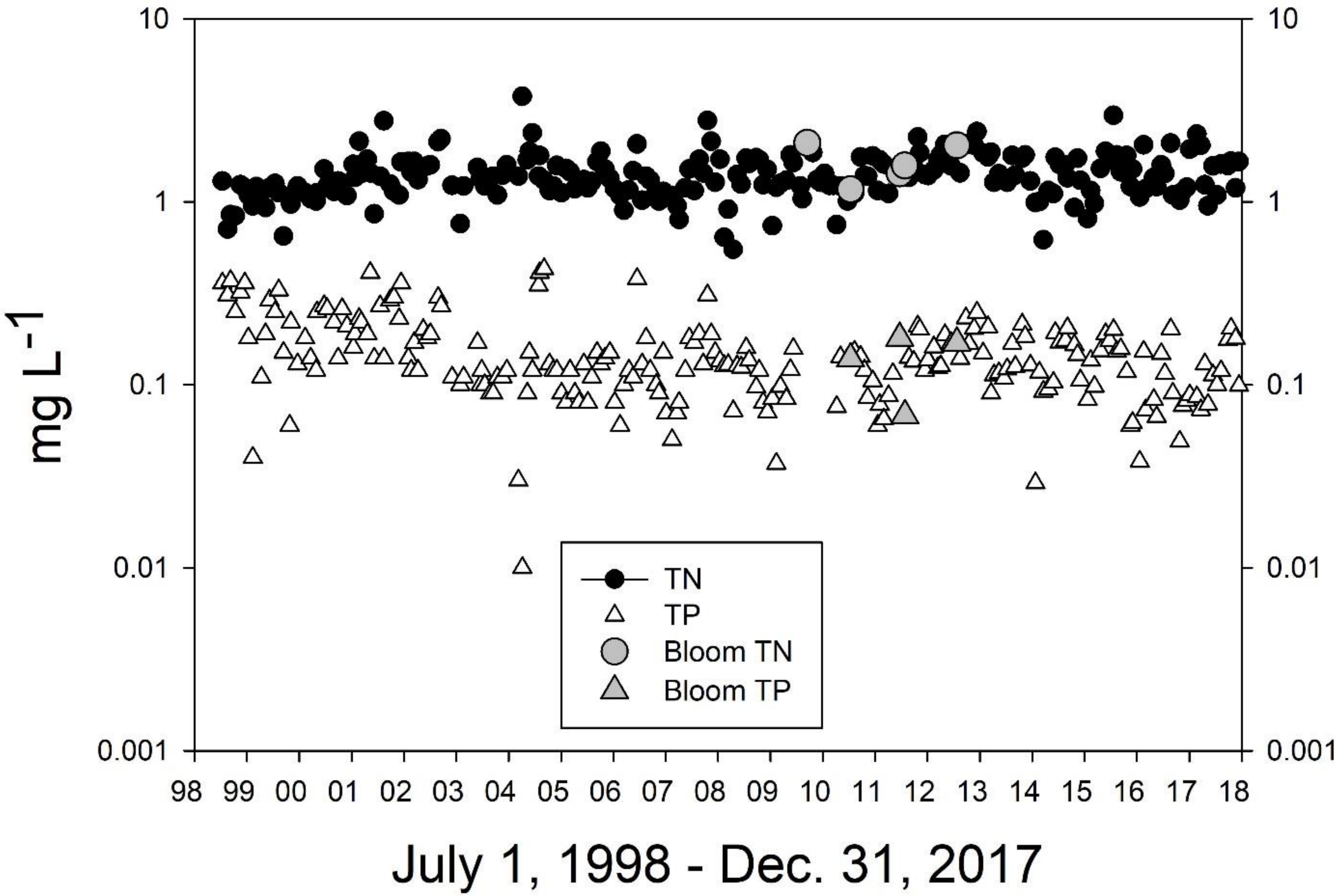


Results: Temperature and turbidity

- All summertime blooms, average 27.2 °C (s.d. = 2.4) at L&D #3
- **No significant trend** of increasing temperatures in the Cape Fear River

- Average turbidity of 10.9 NTU (s.d. = 1.9)
- Increased turbidity with high flow
- Turbidity during bloom events was **not significantly different** from turbidity for non-bloom sample days in summer months

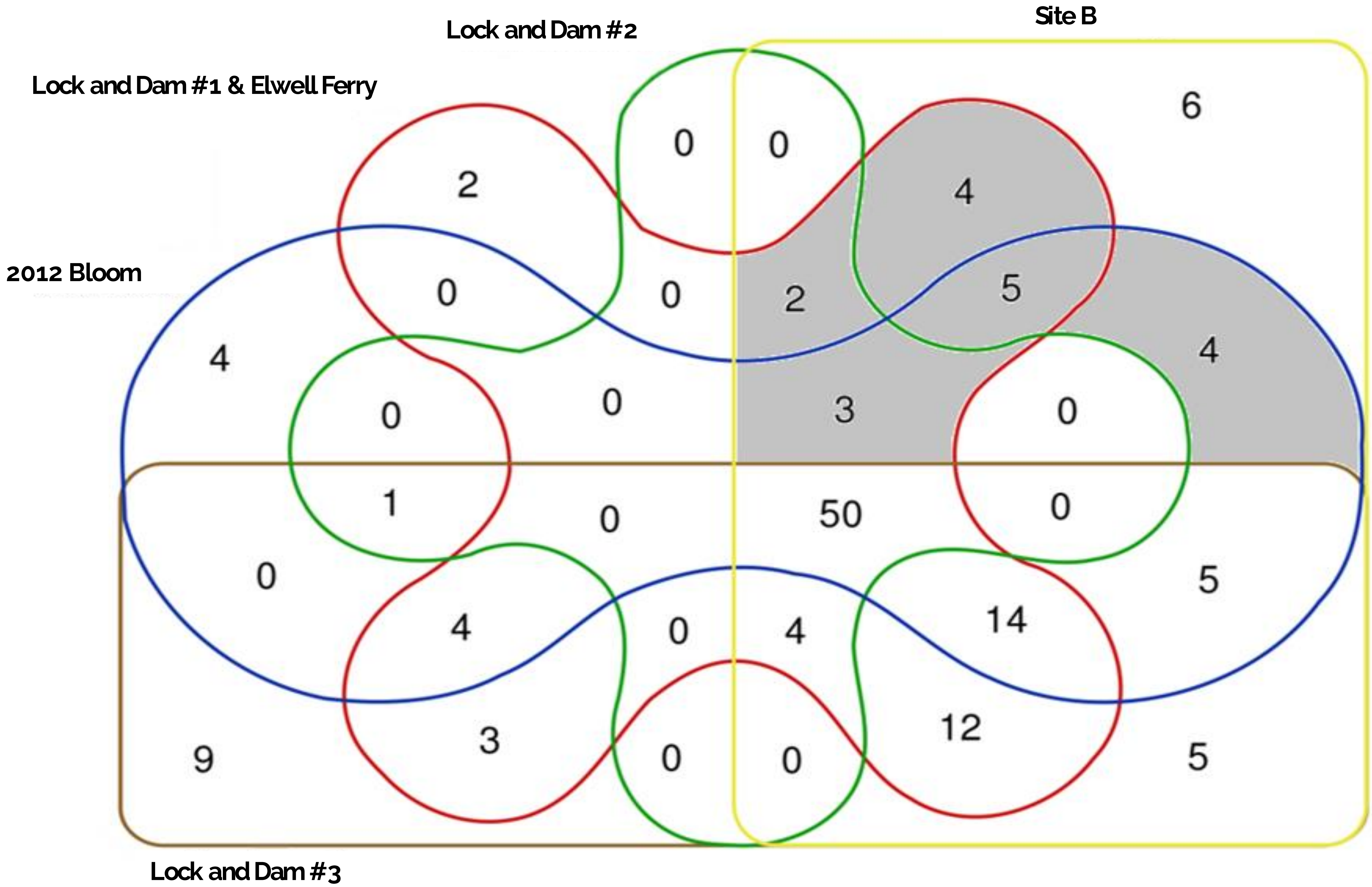
Results: Usual nutrient loading



Results: Allochthonous anthropogenic source

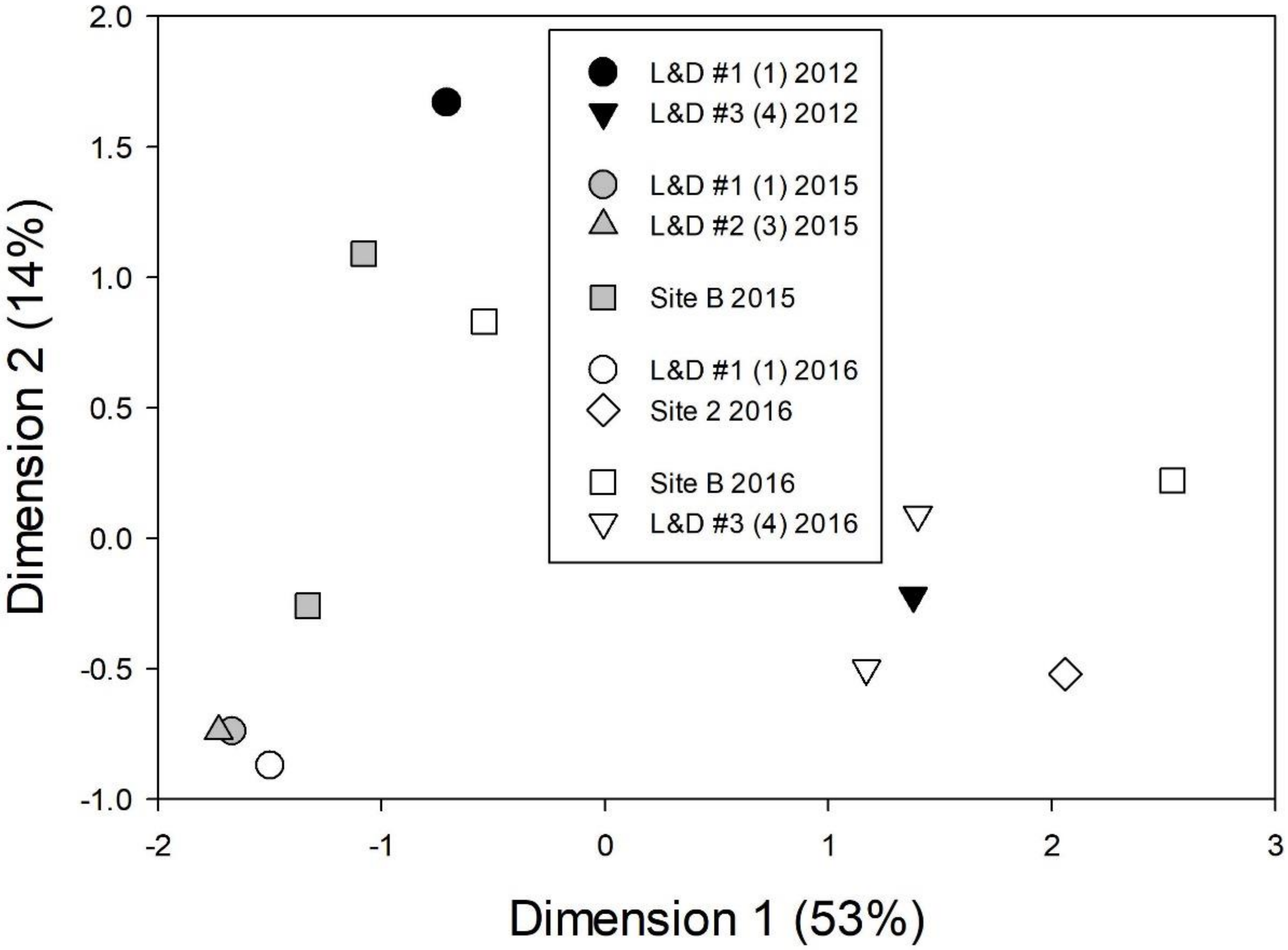
- **No significant changes** over time at wastewater treatment plants (two in Fayetteville, one in Elizabethtown)
- **Negligible nutrient** input from two other major industrial point source dischargers
- Site B:
  - ~340,000 m^3 waste lagoon
  - Large nitrogen and phosphorus loads immediately upstream of reach
  - Average 5.0 °C (s.d. = 2.34) warmer than river temperature
  - Chl *a* 11.1-36.0 µg/L
  - Phycocyanin 3.97 µg/L
  - Neither chlorophyll *a* nor phycocyanin were significantly different between lagoon and river samples
  - *Microcystis* present in waste lagoon and discharge in 2015 (90%) and 2016 (80%)

Results



Lock and Dam #2
Site B
Lock and Dam #1 & Elwell Ferry
2012 Bloom
Lock and Dam #3
0
0
6
2
4
0
0
2
5
4
4
0
0
3
0
1
0
50
0
0
5
4
0
4
14
9
3
0
0
12
5

Results



L&D #1 (1) 2012
L&D #3 (4) 2012
L&D #1 (1) 2015
L&D #2 (3) 2015
Site B 2015
L&D #1 (1) 2016
Site 2 2016
Site B 2016
L&D #3 (4) 2016
Dimension 2 (14%)
Dimension 1 (53%)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-2
-1
0
1
2
3

Results: Allochthonous anthropogenic source

- Nutrient concentrations and loadings to the river provide nutrients necessary to promote microalgal growth
- Total nitrogen from the discharge was ~100x that of the river upstream at L&D #3
- Total phosphorus from the discharge was ~260x that of the river upstream at L&D #3
- TN:TP during bloom years was 110.6:40.1 indicating strong N-limitation vs Redfield
- Total nitrogen declined from **2008-2013**, reflective of denitrification and decrease in permitted [TN] from 200 mg TN/L to 100 mg TN/L in **2009**
- Further changes to the facility's operation included more frequent removal of sludge from anaerobic digesters starting in **2014**

Results: Allochthonous anthropogenic source

- Assume low flow <50 m^3/s, downstream velocity of ~0.3 m/s
- Travel time from facility to L&D #1 on the order of 3 days
- Optimal specific growth rate μ_{max} = 3.4/day (but let's assume half that at 1.7/day)
- Discharge of 30 µg/L chl *a* in discharge volume of 8,520 m^3/day

$$[\text{chl } a]_{day3} = [\text{chl } a]_{day0} * e^{1.7*3} = 10\ \mu g/L \text{ dispersed}$$

- Assume half initially discharged population was *Microcystis* and 80% of resulting population floated within 0.1 m of surface

M. aeruginosa-chl *a* could exceed 190 µg/L in that surface layer

Interpretation

- Does *M. aeruginosa* **occur** throughout the Cape Fear River basin? **YES**
- Did **low flow** conditions support bloom formation? **Necessary but not sufficient**
- Did **high temperatures** favor bloom formation? **Necessary but not sufficient**
- Could variation in **river turbidity** have promoted bloom formation? **No**
- Would **unusual nutrient loading** patterns have driven bloom formation? **No**
- Could *M. aeruginosa* blooms have been seeded from **Jordan Lake**? **No**
- Could there have been an **allochthonous anthropogenic source**? **Unable to rule out**