We hold these truths to be sometimes hard to measure, that all phytoplankton are not created equal, that they are endowed by their phylogeny with certain limiting factors; that among these are Light, Nutrients and the pursuit of Carbon Dioxide. That to secure these resources, Organelles are instituted among eukaryotes, deriving their just powers from the availability of the ecosystem, that whenever any Form of Environment becomes destructive of these ends, it is the Right of the Phytoplankton to alter it or themselves or to perish, and to institute new Populations, laying its foundation on such principles and organizing its powers in such form, as to them shall seem most likely to affect their Growth and Reproduction.

-MEP

Microcystis in the Cape Fear River, NC
Where, When and Why?

Madison Polera
University of North Carolina at Wilmington
Lawrence Cahoon, Michael Mallin and Patrick Erwin

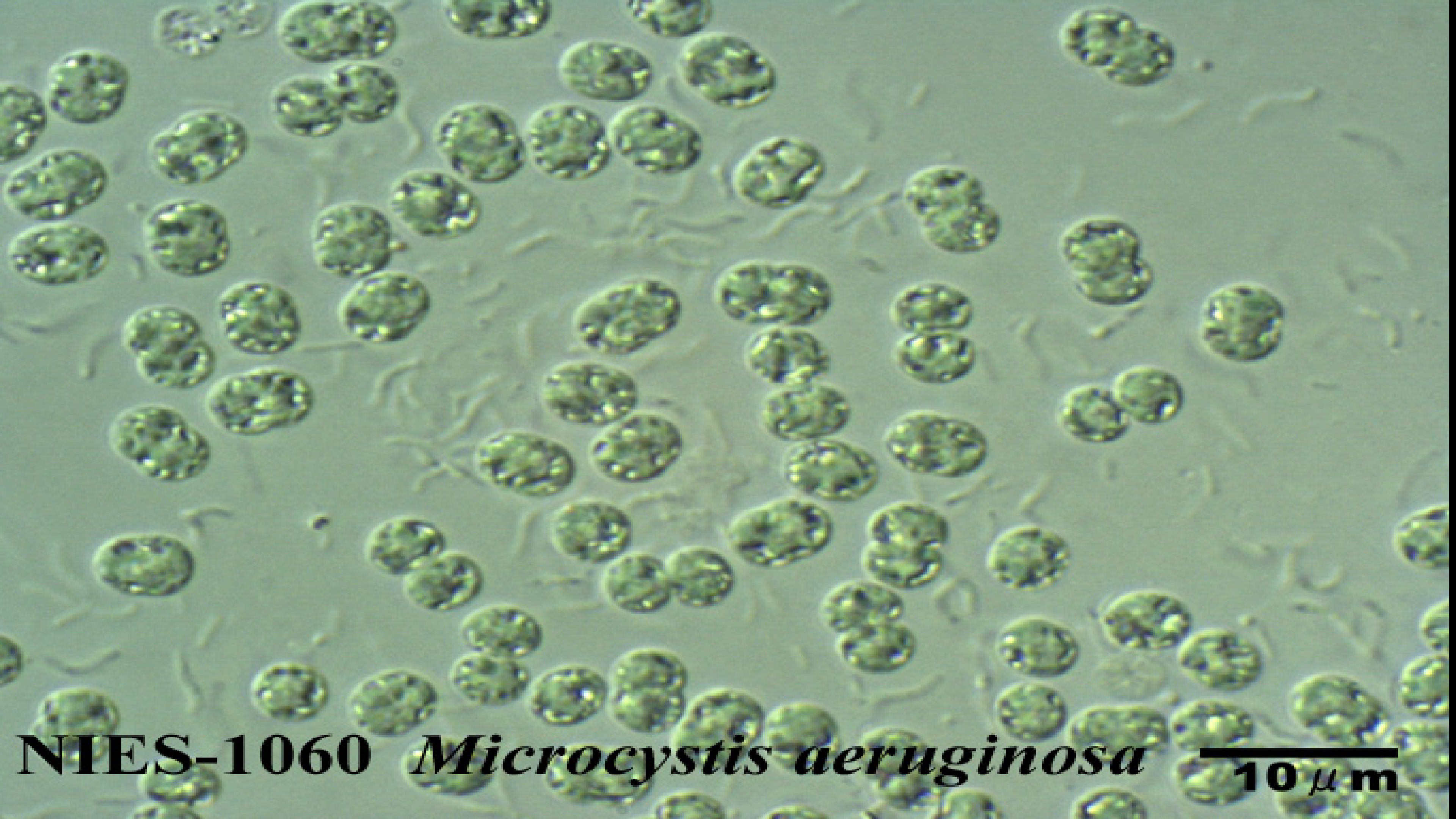
Outline

1. ***Microcystis* and Microcystins**
2. **Cape Fear River Blooms**

-Why in 2009: Monitoring Data and Drivers

-Where and when do we detect *Microcystis* in the CFRB?

-Can we rule out any plausible sources?


NIES-1060 *Microcystis aeruginosa*
10μm

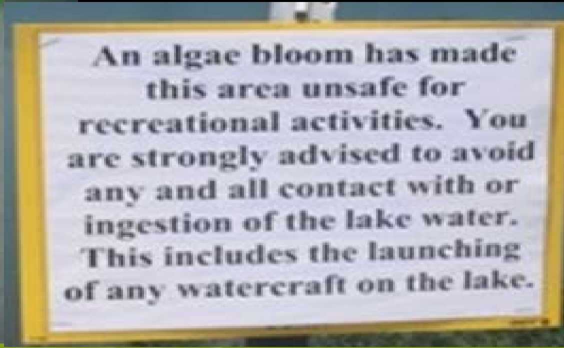

Lake Taihu,
China
Lake Erie Algal Bloom
7 Oct 2007
MODIS imagery
CoastWatch, GLERL
San
Francisco
Estuary
St. Lucie
Estuary,
Florida

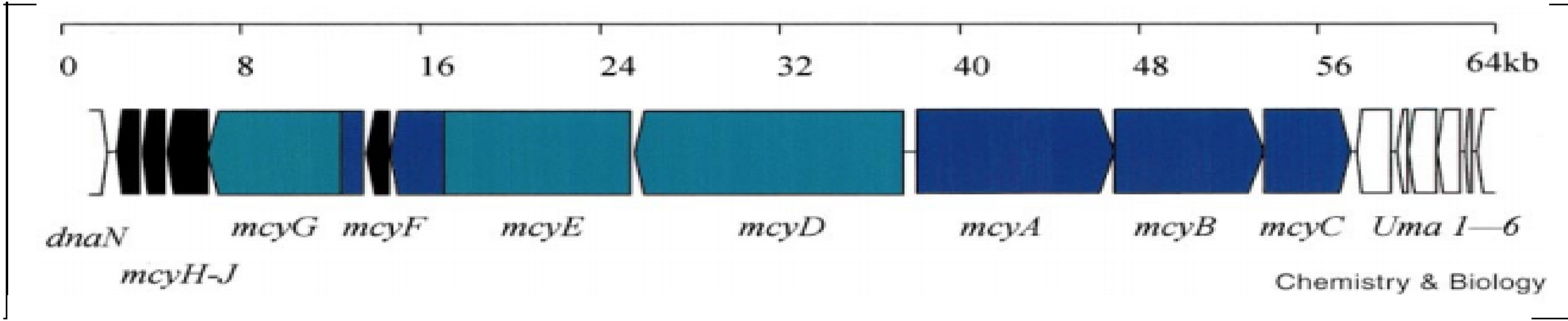
Officials Warning of Blue-Green Algae in Indiana Lakes

by Ray Steele (rsteele@wibc.com)

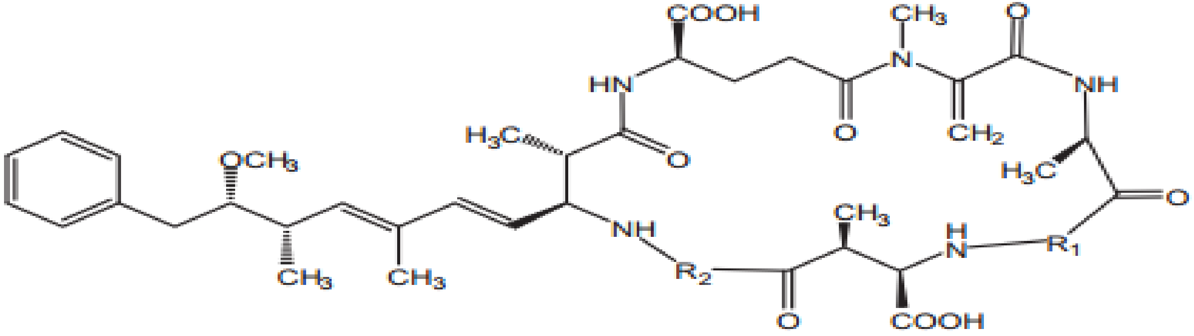
8/1/2012

The blue-green algae showing up in Indiana lakes that has killed at least two dogs in Indiana this summer could also be harmful to humans, though there is little research so far on the subject.



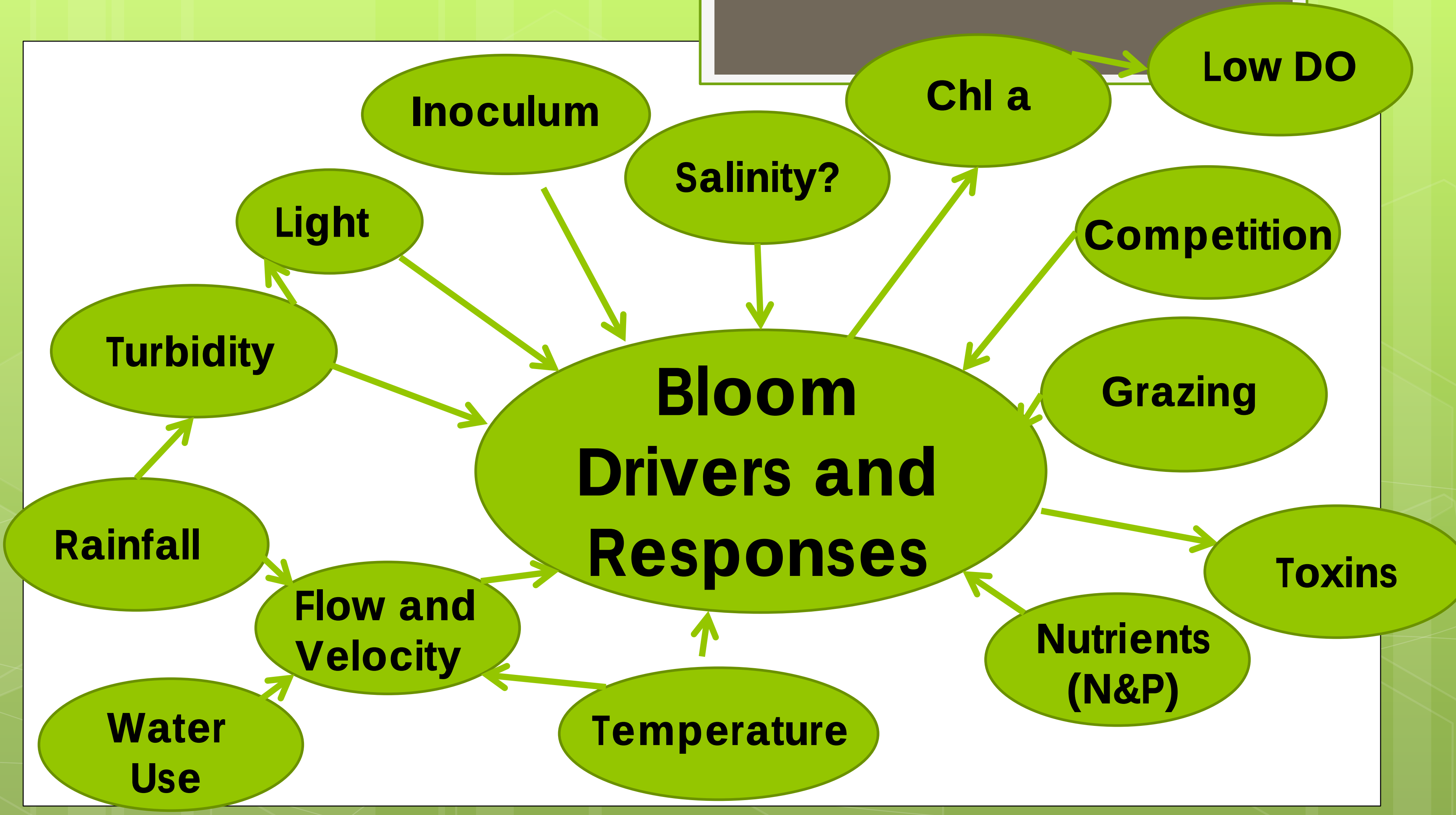


J.D. Isaacs et al. / Harmful Algae 31 (2014) 82–86



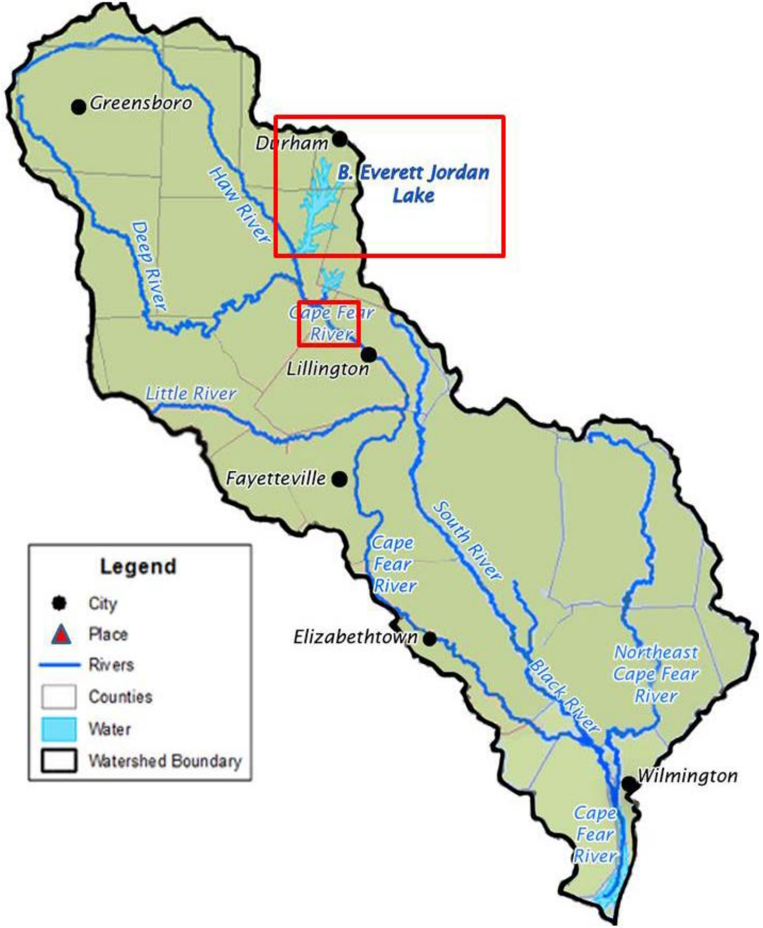
Microcystin	R_1	R_2	MW (Da)
LR	Leu	Arg	994
RR	Arg	Arg	1037

Fig. 1. Chemical structures of microcystins LR and RR found in the Cape Fear River blooms.


Inoculum
Chl a
Low DO
Salinity?
Light
Competition
Turbidity
Bloom Drivers and Responses
Grazing
Rainfall
Toxins
Flow and Velocity
Nutrients (N&P)
Water Use
Temperature


Cape Fear River, NC

	Reported Bloom Date	Location
2009	September 24	Lock and Dam 1
2010	July 15	Lock and Dam1 Downstream to Acme
2011	June 27	Minimal at Lock & Dam 1
	July 7	NC 11 Bridge (Downstream of Lock and Dam 1)
	July 11	Lock and Dam 1
	July 14	Lock and Dam 2 downstream to Sutton Lake Colonies found upstream at Tar Heel Black River
	July 20	Bloom extending downstream to Navassa
2012	May 10	Minimal bloom activity near Lock and Dam 1
	July 3	Above Lock and Dam 1
	July 11	Acme down to Indian Creek Up Black River to Thoroughfare
	July 18	Bloom beginning to break up: elevated flow


Greensboro
Durham
B. Everett Jordan Lake
Haw River
Deep River
Cape Fear River
Lillington
Little River
Fayetteville
Cape Fear River
South River
Elizabethtown
Black River
Northeast Cape Fear River
Wilmington
Cape Fear River
Legend
City
Place
Rivers
Counties
Water
Watershed Boundary

Jordan Lake

Jordan Lake: Nutrient Sensitive Waters

Jordan Reservoir and Haw River Watershed NSW Strategy

Chapter 36 describes the Jordan Reservior stakeholder process, the Clean Water Responsibility Act and the modeling performed to support the nutrient management strategy. Most of the reservoir is Impaired because of chlorophyll *a* violations associated with excess nutrient loading to the reservoir. The nutrient TMDL recommends reductions from both point and nonpoint sources. Chapter 36 provides the framework for making these reductions through a rule-making process.

Chlorophyll *a* levels exceeded the standard in 73 percent of samples in the New Hope River Arm and in 13 percent of samples in mid reservoir. Blooms of blue-green algae associated with taste and odor problems in drinking water were observed in July 2003. The reservoir has been eutrophic since 1982. The Beaver Creek, Parkers Creek and White Oak Creek Arms (2,613.5

Cape Fear Basin Water Quality Management Plan, 2005

Microcystis Blooms: Initiation and Persistence

1. Determine **distribution** of *Microcystis* in the CFR
2. **Investigate** historical monitoring data for patterns that may indicate a **change** in the river's **abiotic ecology**
3. Rule out **Jordan Lake** as a **plausible** source if possible

Monitoring Data Retrieval and Analyses

- **Flow and Nutrient**

North Carolina Division of Water Resources Ambient Monitoring System through the U.S. EPA's STOrage and RETrieval (STORET) warehouse (http://www3.epa.gov/storet/)

- **Temperature and Turbidity**

The Lower Cape Fear Monitoring Program (lcfrp.uncw.edu/riverdatabase/)

- **Jordan Lake Discharge**

The US Army Corps of Engineers database: (http://epec.saw.usace.army.mil/jord.htm)

- **Regressions**
- **One way ANOVA and Tukey's HSD**
- **Significance level: $\alpha = 0.05$**

Site Number	Location	River Miles From Mouth
0	Mouth of the river	0
1	Lock and Dam 1	62
2	Elwell Ferry	70
3	Elizabethtown	85
4	Lock and Dam 2	90
5	Tar Heel Bridge	110
6	Lock and Dam 3	120
7	Cape Fear River at Lillington	150
8	CFR at NC 42	170
9	Haw River at Moncure	176
10	Jordan Lake	200
11	Deep River at Moncure	180
12	Haw River at Bynum	210

DNA Extraction, Amplification and Sequencing

- Bioline MyTaq Extract PCR Kit
  - ITS (Otsuka et al., 1999)
  - mcyB (Kaebernick et al., 2000)
  - mcyD (Ouellette et al., 2006)

Date	Locations Sequenced
2012	Lock and Dam 1
2015	Lock and Dam 1 CFR @ NC 42 Deep River Jordan Lake

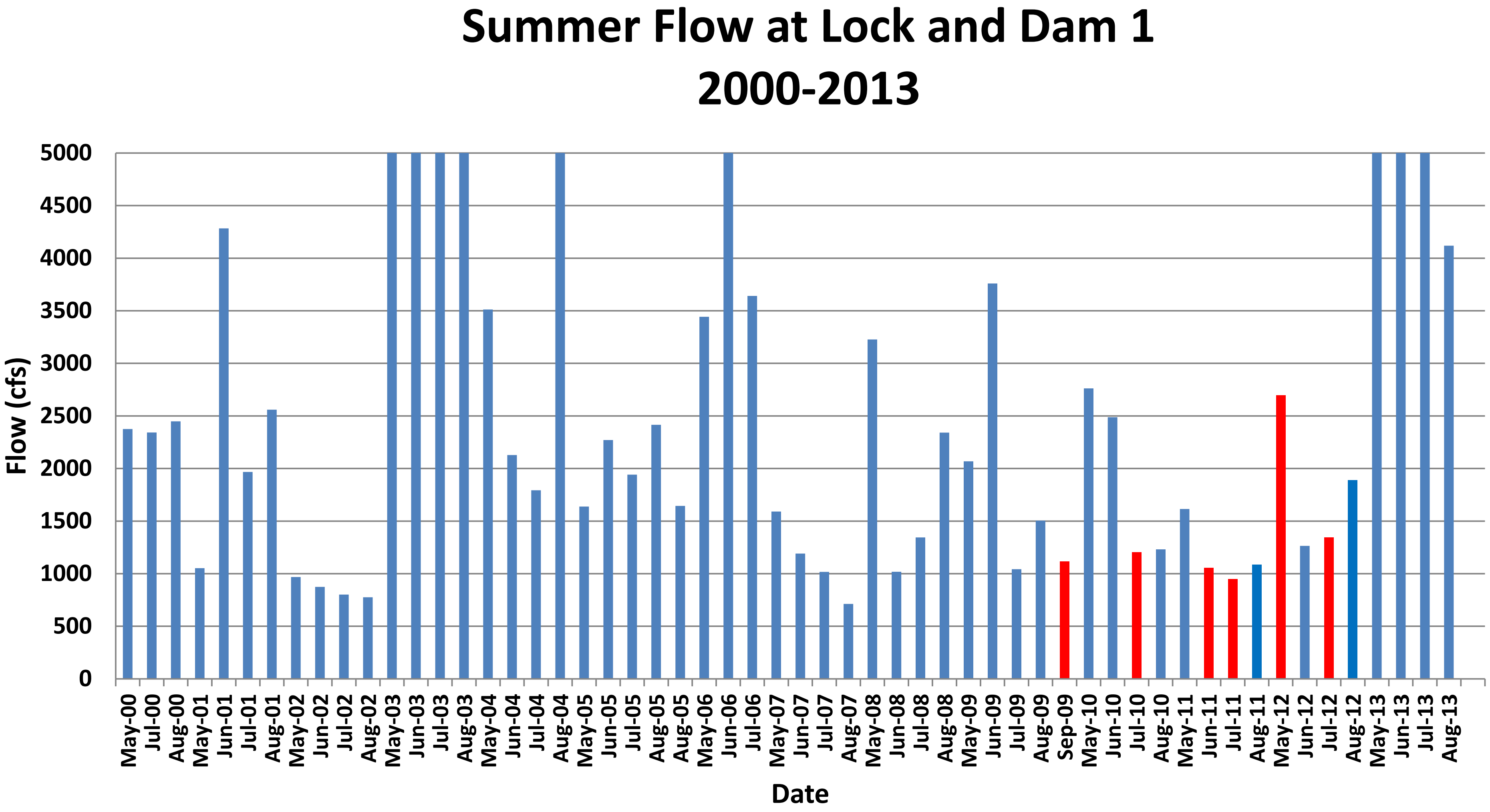
Monitoring Data Results and Analyses

Flow

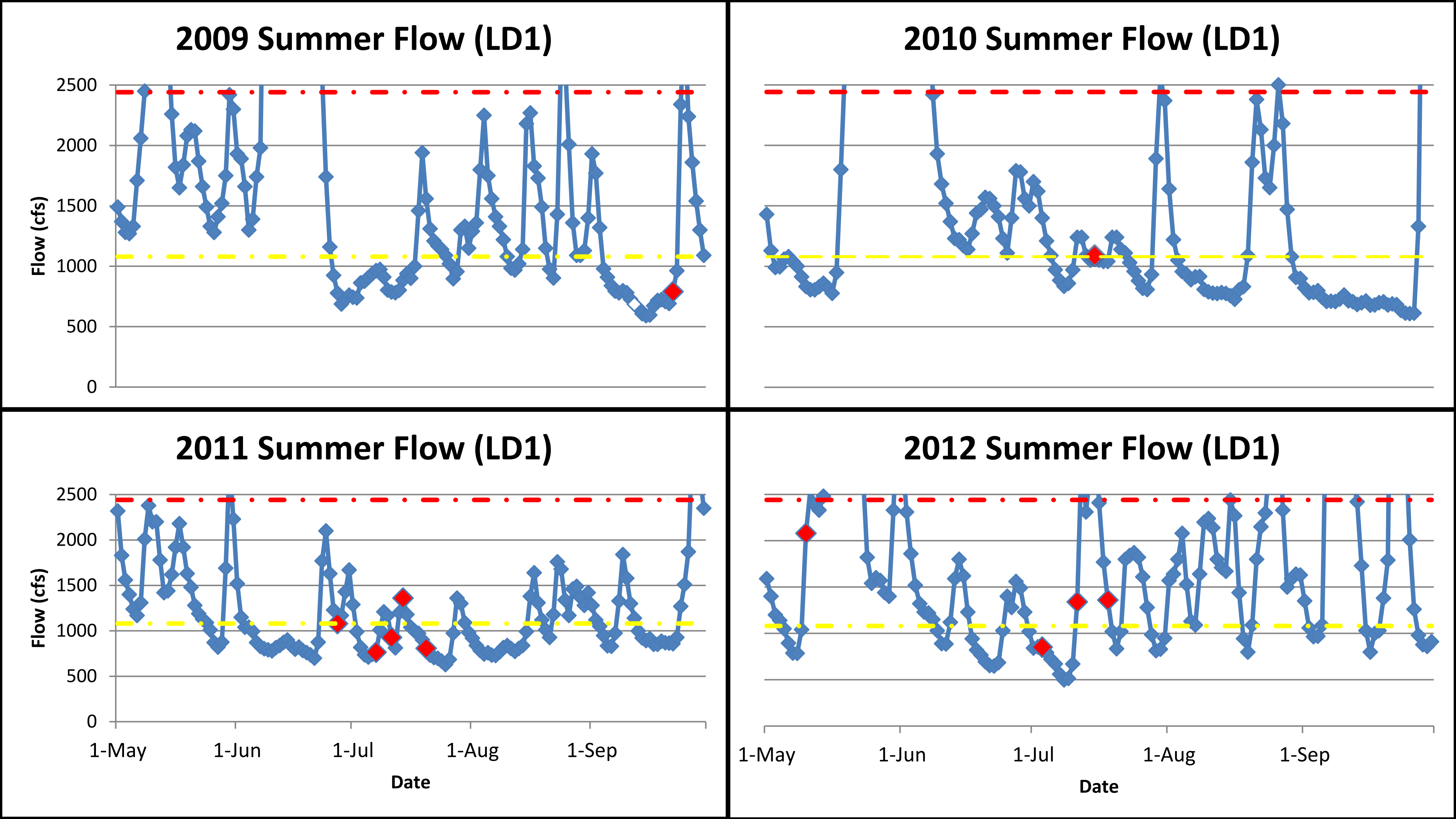
Velocity

Nutrient Concentration and Loads

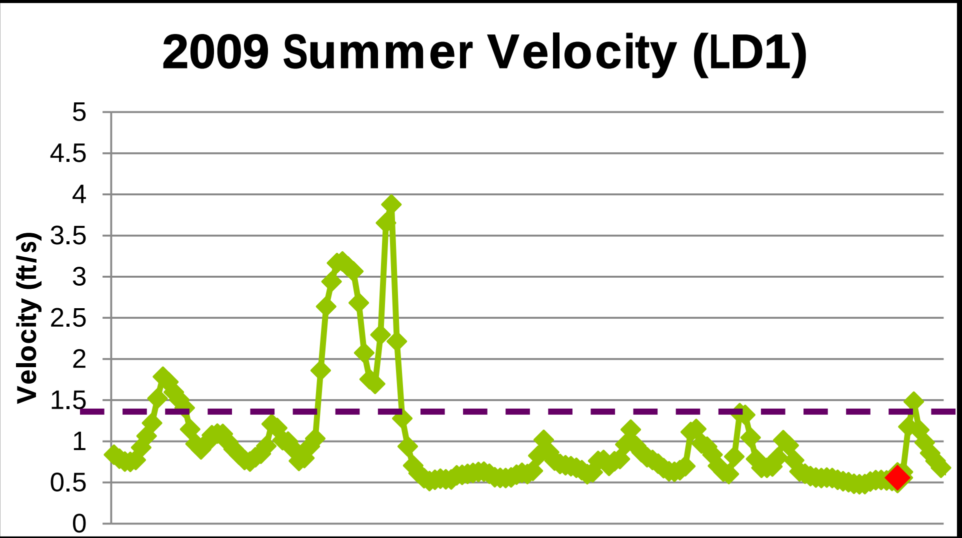
Temperature

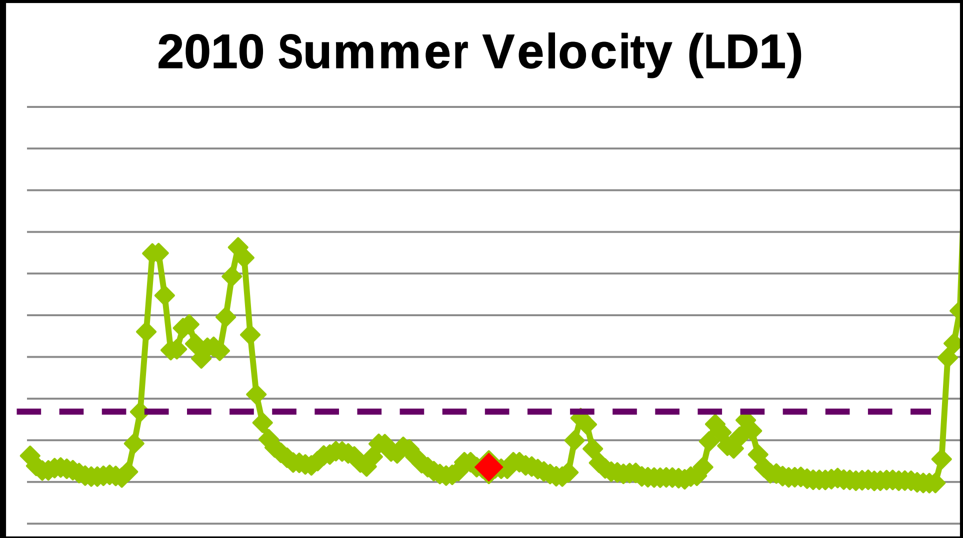

Summer Flow at Lock and Dam 1
2000-2013
Flow (cfs)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
May-00
Jul-00
Aug-00
May-01
Jun-01
Jul-01
Aug-01
May-02
Jun-02
Jul-02
Aug-02
May-03
Jun-03
Jul-03
Aug-03
May-04
Jun-04
Jul-04
Aug-04
May-05
Jun-05
Jul-05
Aug-05
Aug-05
May-06
Jun-06
Jul-06
May-07
Jun-07
Jul-07
Aug-07
May-08
Jun-08
Jul-08
Aug-08
May-09
Jun-09
Jul-09
Aug-09
Sep-09
May-10
Jun-10
Jul-10
Aug-10
May-11
Jun-11
Jul-11
Aug-11
May-12
Jun-12
Jul-12
Aug-12
May-13
Jun-13
Jul-13
Aug-13
Date

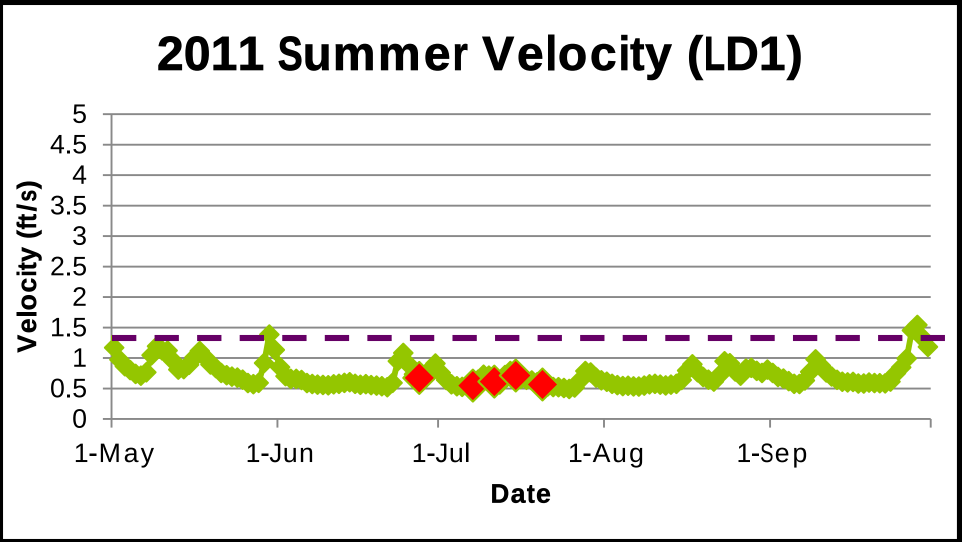
Time	LD3	LD1	State
August 2007	629	713	Non-bloom
August 2002	701	776	Non-bloom
July 2002	699	802	Non-bloom
June 2002	846	874	Non-bloom
July 2011	946	951	Bloom
May 2002	882	968	Non-bloom
July 2007	1001	1017	Non-bloom
June 2008	982	1018	Non-bloom
July 2009	895	1043	Non-Bloom
May 2001	1056	1052	Non-bloom
June 2011	1097	1055	Bloom
August 2011	947	1086	Non-Bloom
September 2009	937	1117	Bloom
June 2007	1093	1191	Non-bloom
July 2010	1109	1205	Bloom
August 2010	1037	1233	Non-Bloom
June 2012	1029	1264	Bloom
July 2008	1398	1345	Non-bloom
July 2012	1235	1346	Bloom
June 2000	1131	1396	Non-bloom
August 2009	1383	1505	Non-Bloom
May 2007	1507	1591	Non-bloom
May 2011	1276	1615	Non-Bloom
May 2005	1478	1638	Non-bloom

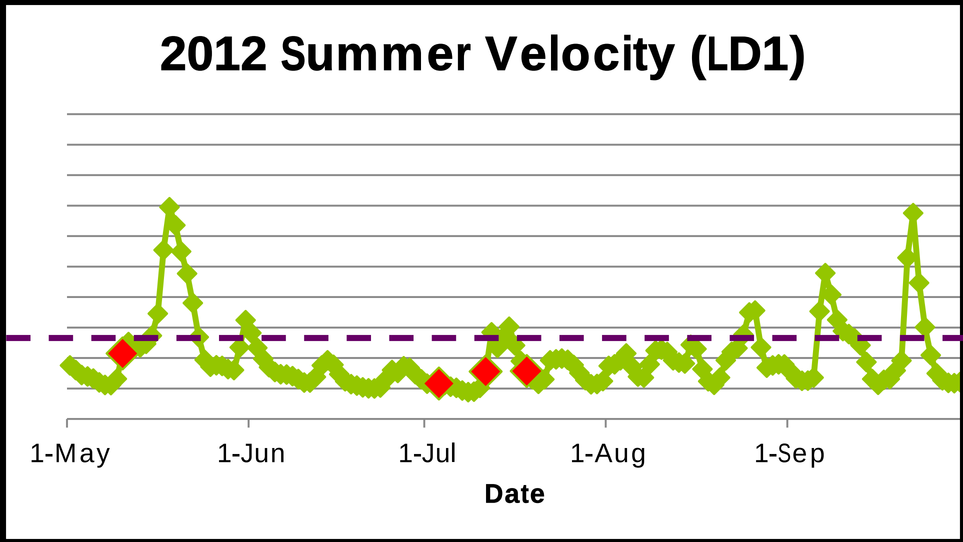

2009 Summer Flow (LD1)
2010 Summer Flow (LD1)
2011 Summer Flow (LD1)
2012 Summer Flow (LD1)
Flow (cfs)
2500
2000
1500
1000
500
0
1-May
1-Jun
1-Jul
1-Aug
1-Sep
Date

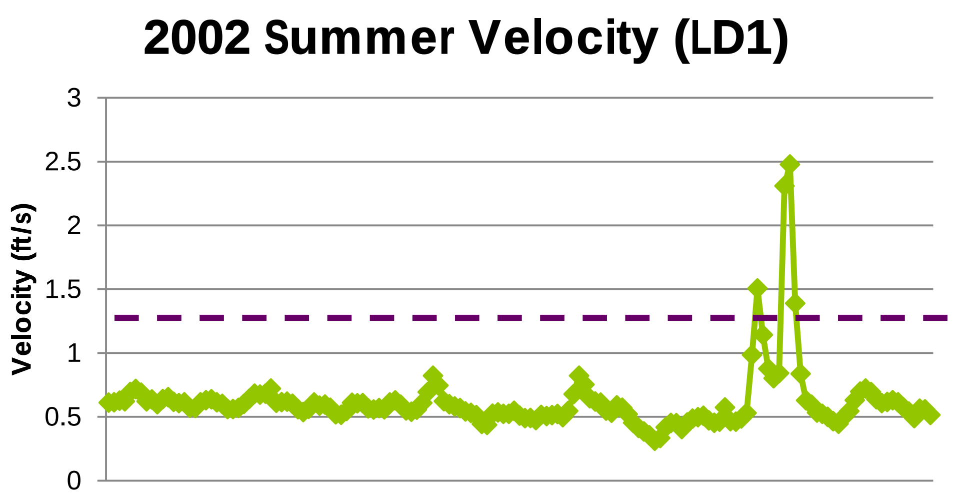
Year	Lock and Dam 1 % Below Threshold Highest reported flow during bloom + 1 s.d. 2400 cfs	Lock and Dam 3 % Below Threshold 2400 cfs	Lock and Dam 1 % Below Baseline Geometric mean of flows during blooms 1080 cfs	Lock and Dam 3 % Below Baseline 1080 cfs
2002	97.4	97.1	83.7	84.2
2007	100.0	100.0	66.7	66.7
2008	67.3	67.3	27.5	27.5
2009	82.4	82.4	33.3	33.3
2010	83.0	83.0	49.0	49.0
2011	100	97.4	52.3	60.8
2012	78.4	78.4	30.7	30.7
2013	33.3	34.0	10.5	10.5
2014	70.6	70.6	16.3	16.3

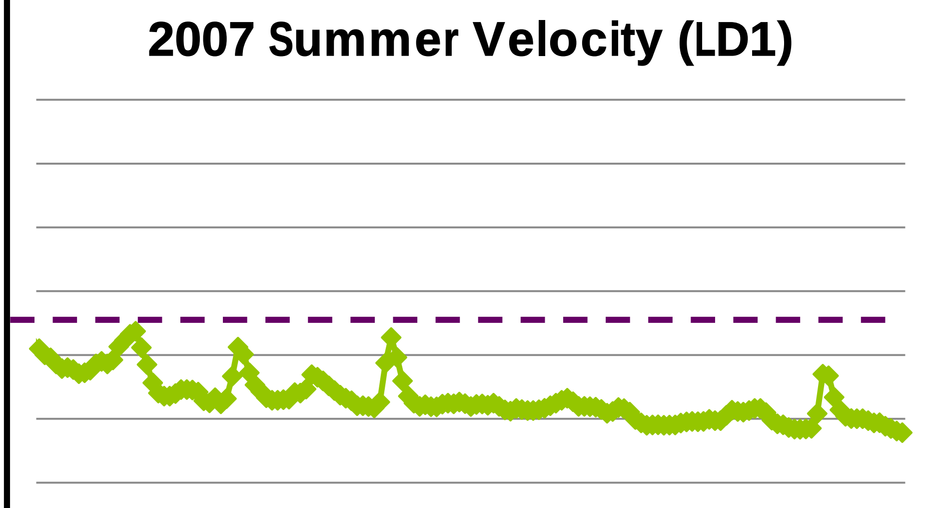

2009 Summer Velocity (LD1)
Velocity (ft/s)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0

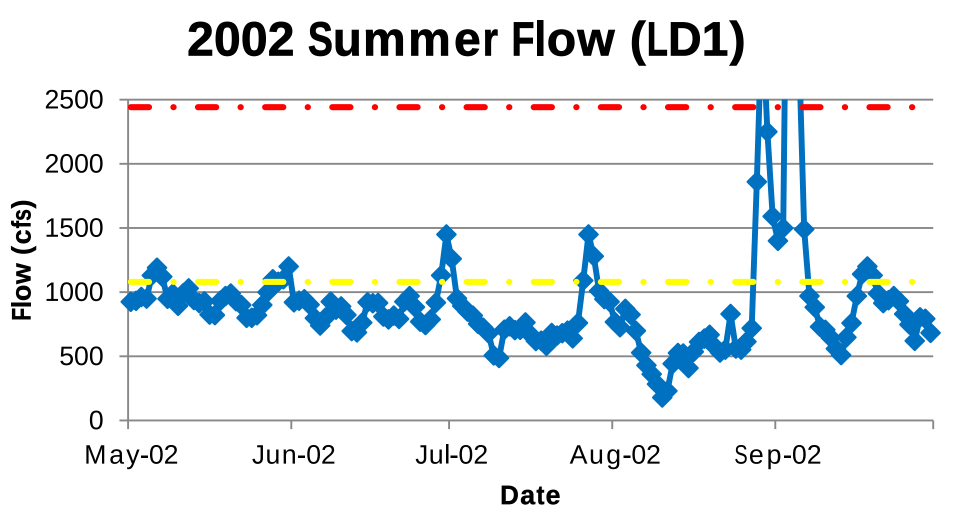

2010 Summer Velocity (LD1)

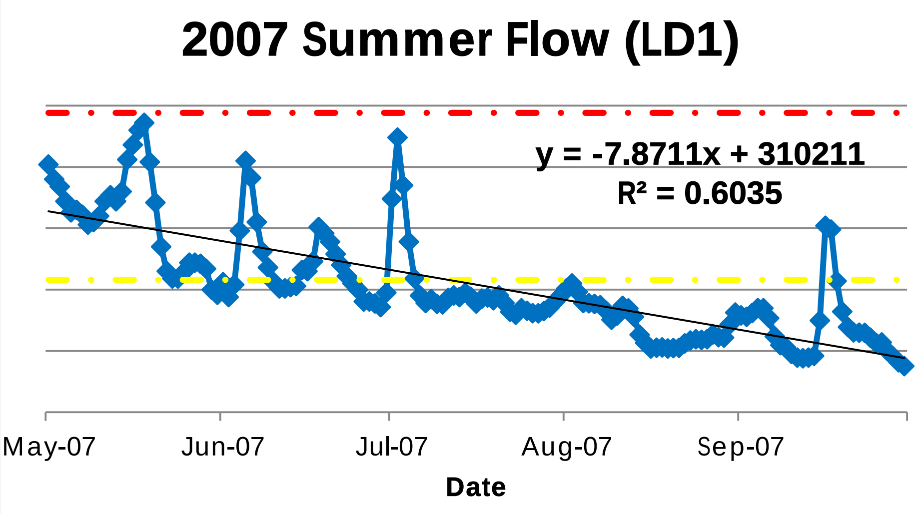

2011 Summer Velocity (LD1)
Velocity (ft/s)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1-May
1-Jun
1-Jul
1-Aug
1-Sep
Date


2012 Summer Velocity (LD1)
1-May
1-Jun
1-Jul
1-Aug
1-Sep
Date


2002 Summer Velocity (LD1)
Velocity (ft/s)
3
2.5
2
1.5
1
0.5
0


2007 Summer Velocity (LD1)

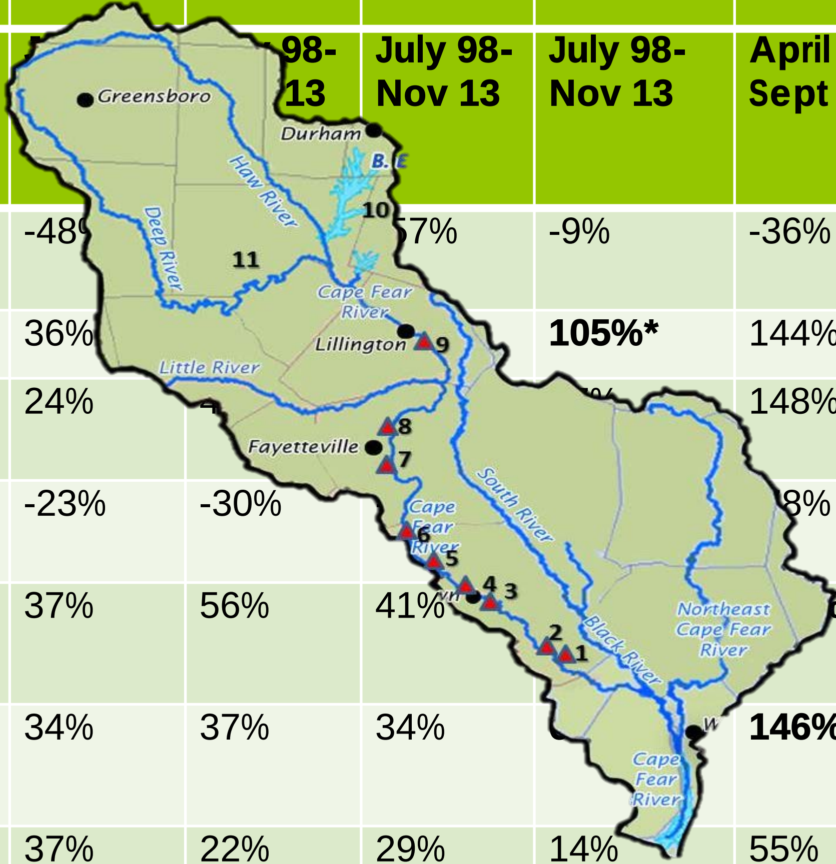

2002 Summer Flow (LD1)
Flow (cfs)
2500
2000
1500
1000
500
0
May-02
Jun-02
Jul-02
Aug-02
Sep-02
Date

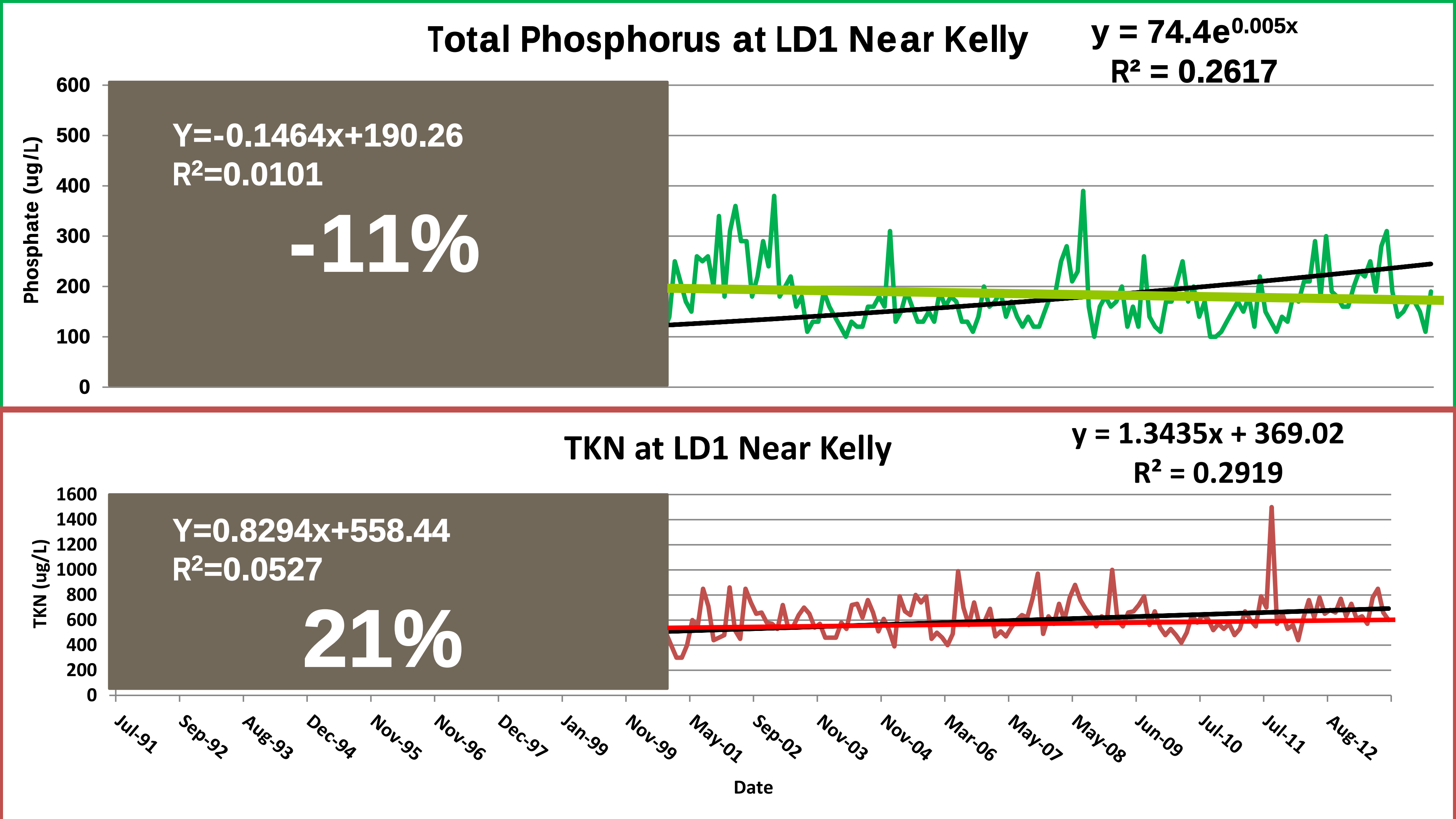

2007 Summer Flow (LD1)
y = -7.8711x + 310211
R² = 0.6035
May-07
Jun-07
Jul-07
Aug-07
Sep-07
Date

Flow and Velocity Summary

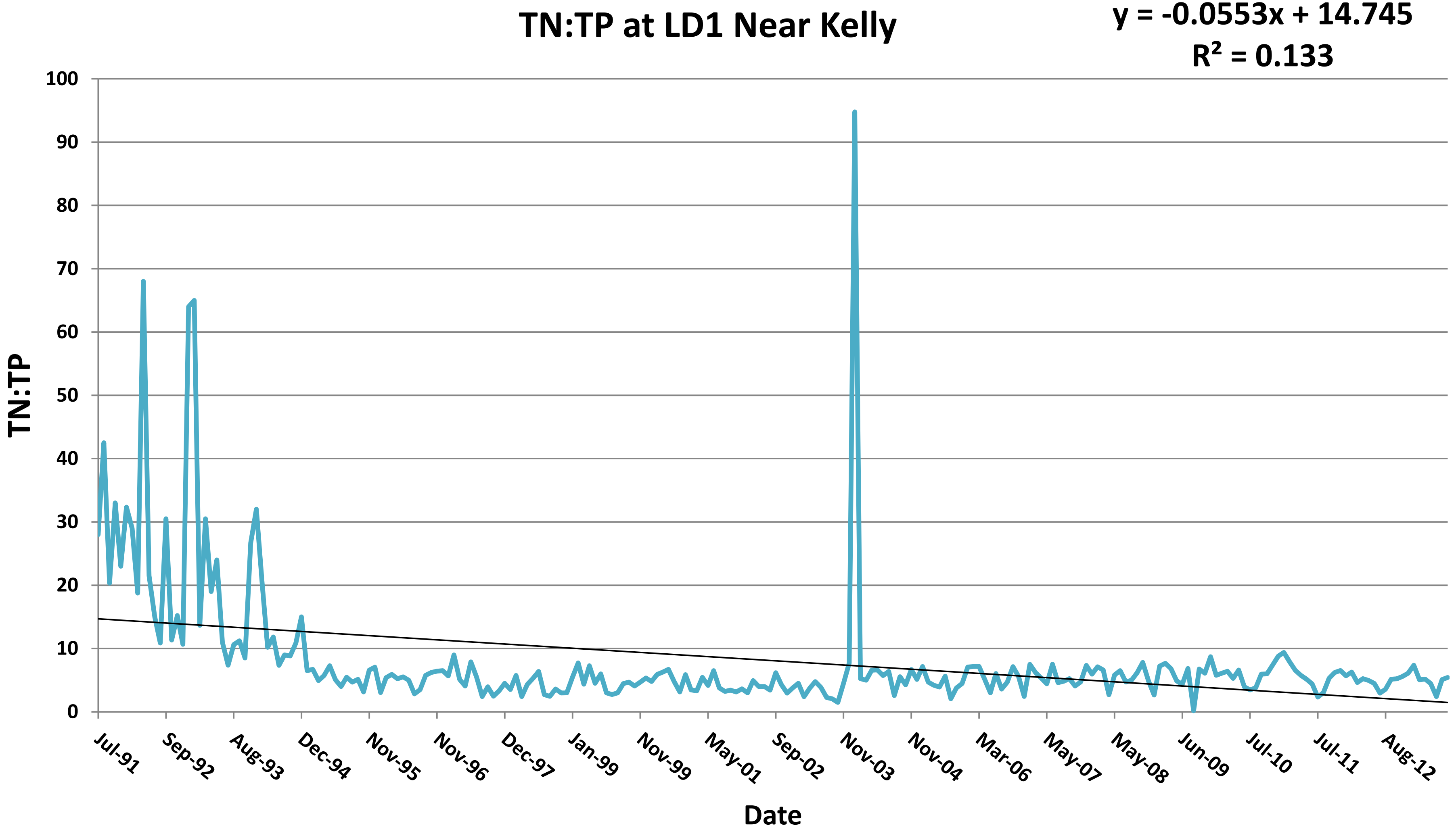
- **Monthly summer averaged 2009-2012** flows and velocity were **not** significantly different than **2002 or 2007**
- **Daily flow in 2009** was significantly **higher** than in **2007** ($F_{(304,1)}$= 15.3271, p<0.0001)
- **Monthly averaged** flow at **Lock and Dam 1** and **Lock and Dam 3** from **2000-2013** were **not** significantly different ($F_{(110, 1)}$=0.4147, p=0.5209).
- **Proportion** of days of low flow below both the **threshold** and **baseline** are **identical** between **Lock and Dam 1** and **Lock and Dam 3.**

[Nutrient]	LD1 Near Kelly	Lock and Dam 1 Upstream at Arcadia	Below LD 2 at RM 70	Above LD2	RM 80	Upstream Smithfield	Lock and Dam 3	Fayetteville	Lillington
Time Span Covered	July 91-Sept 13	July 98-Dec 13	[illegible]	[illegible]	July 98-Nov 13	July 98-Nov 13	April 92-Sept 13	April 92-Sept 13	Mar 92-Sept 13
Ammonia	-56%	-35%	[illegible]	[illegible]	[illegible]	-9%	-36%	-65%	-44%
DIN	44%	32%	36%	[illegible]	[illegible]	**105%***	144%	44%	7%
TKN	**87%***	42%	24%	[illegible]	[illegible]	[illegible]	148%	**160%***	**102%***
Phosphate	**89%***	-24%	-23%	-30%	[illegible]	[illegible]	[illegible]	-20%	33%
TON	**140%***	51%	37%	56%	41%	[illegible]	[illegible]	**72%***	**145%***
TN	**57%***	37%	34%	37%	34%	[illegible]	**146%***	**46%***	48%
TN:TP	**-90%***	61%	37%	22%	29%	14%	55%	**70%***	0%





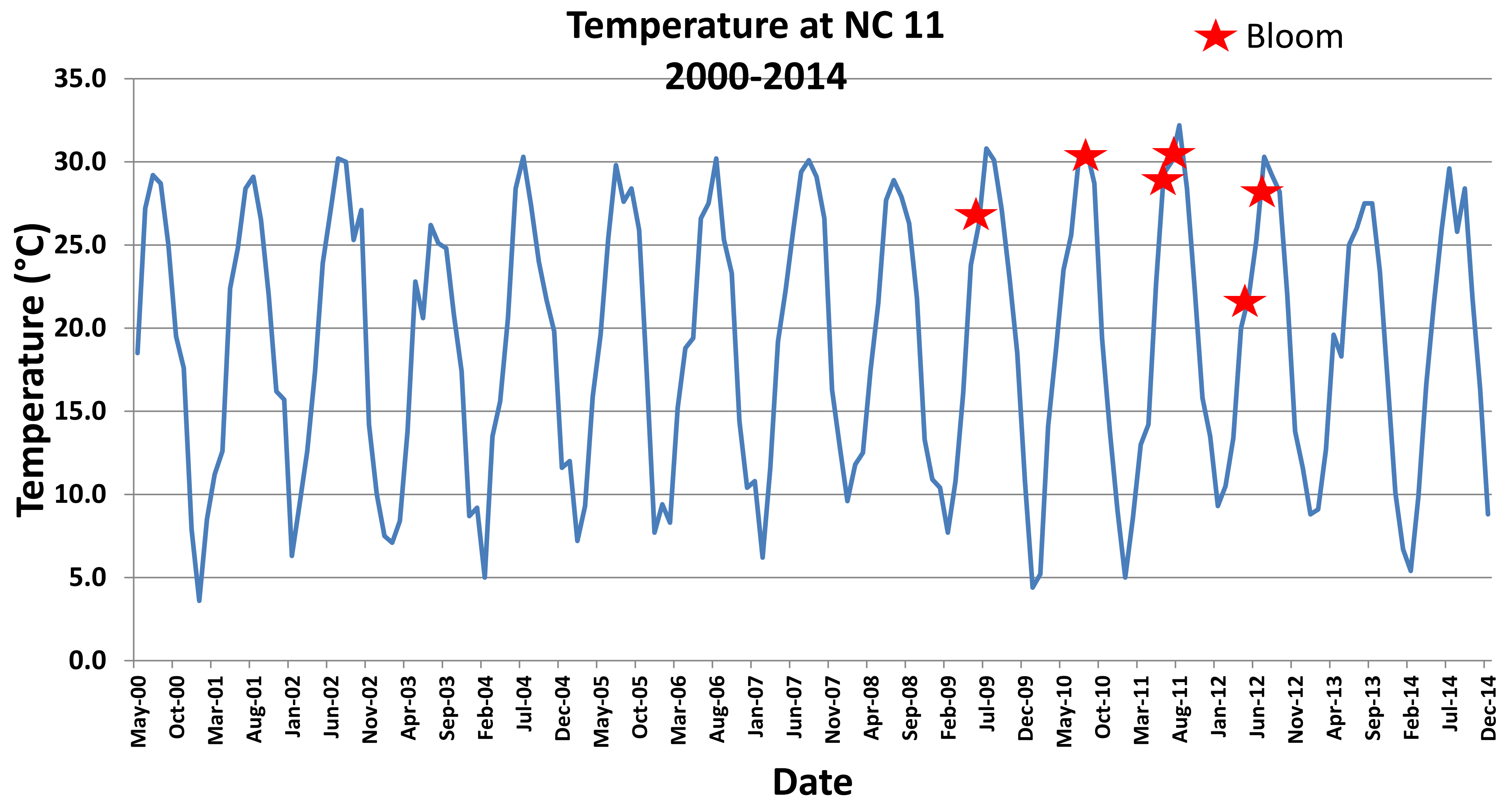
Total Phosphorus at LD1 Near Kelly
y = 74.4e^0.005x
R² = 0.2617
Phosphate (ug/L)
600
500
400
300
200
100
0
Y=-0.1464x+190.26
R2=0.0101
-11%
TKN at LD1 Near Kelly
y = 1.3435x + 369.02
R² = 0.2919
TKN (ug/L)
1600
1400
1200
1000
800
600
400
200
0
Y=0.8294x+558.44
R2=0.0527
21%
Jul-91
Sep-92
Aug-93
Dec-94
Nov-95
Nov-96
Dec-97
Jan-99
Nov-99
May-01
Sep-02
Nov-03
Nov-04
Mar-06
May-07
May-08
Jun-09
Jul-10
Jul-11
Aug-12
Date


TN:TP at LD1 Near Kelly
y = -0.0553x + 14.745
R^2 = 0.133
TN:TP
100
90
80
70
60
50
40
30
20
10
0
Jul-91
Sep-92
Aug-93
Dec-94
Nov-95
Nov-96
Dec-97
Jan-99
Nov-99
May-01
Sep-02
Nov-03
Nov-04
Mar-06
May-07
May-08
Jun-09
Jul-10
Jul-11
Aug-12
Date

Nutrient Loads	Lock and Dam 1 Near Kelly	Lock and Dam 3	Lillington
Time Span Covered	July 91-Sept 13	April 92-Sept 13	March 92- Sept 13
Ammonia Load	-81%	-8%	54%
DIN Load	-30%	-35%	-43%
TKN Load	-12%	-25%	28%
Phosphate Load	-12%	-70%	-12%
TON Load	12%	-15%	-47%
TN Load	-21%	-30%	-13%

Nutrient Summary

- The only **statistically significant** nutrient concentration changes occurred well **before** the bloom period
- The watershed's **base nutrient sources haven't** appreciably **changed** in a way to support unprecedented blooms

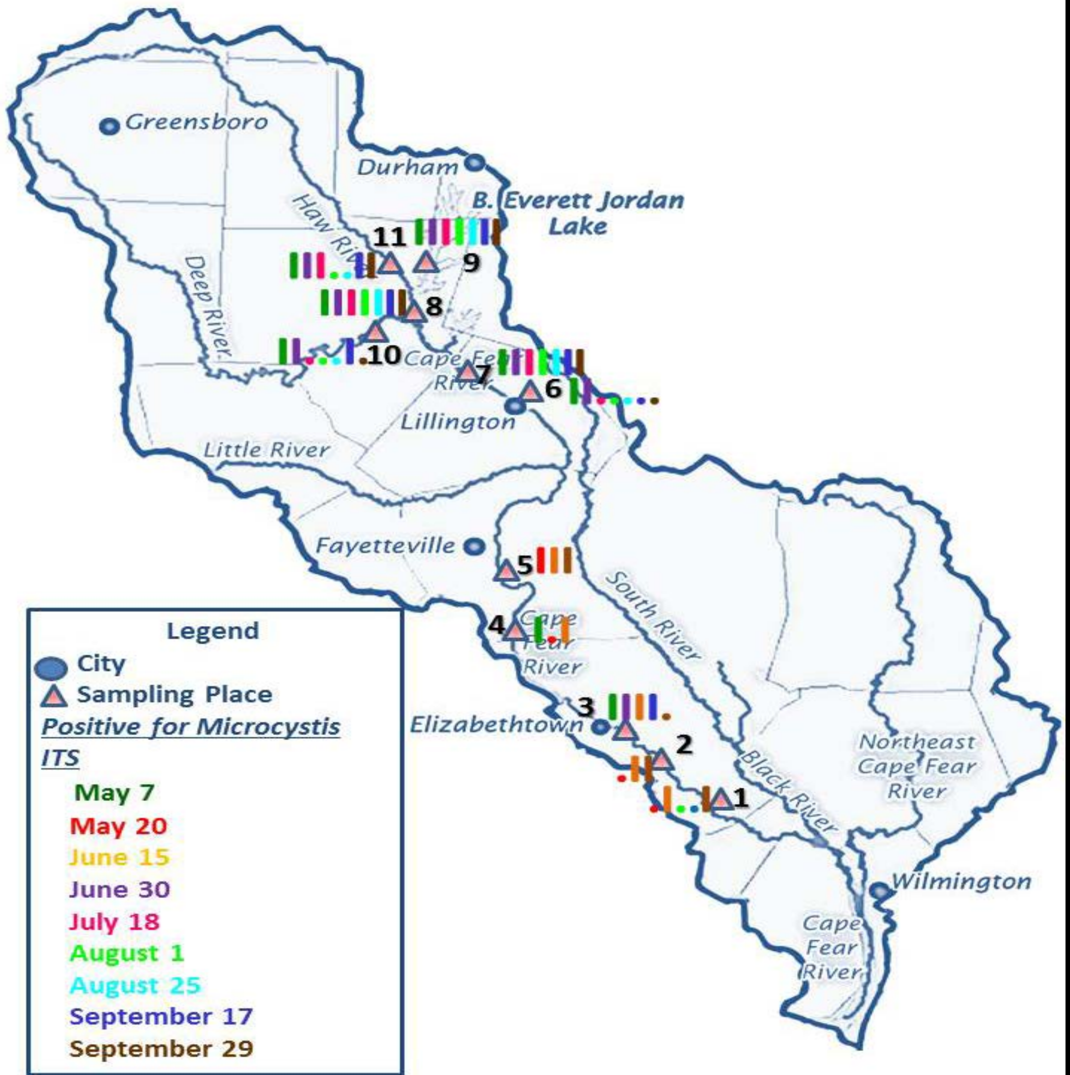


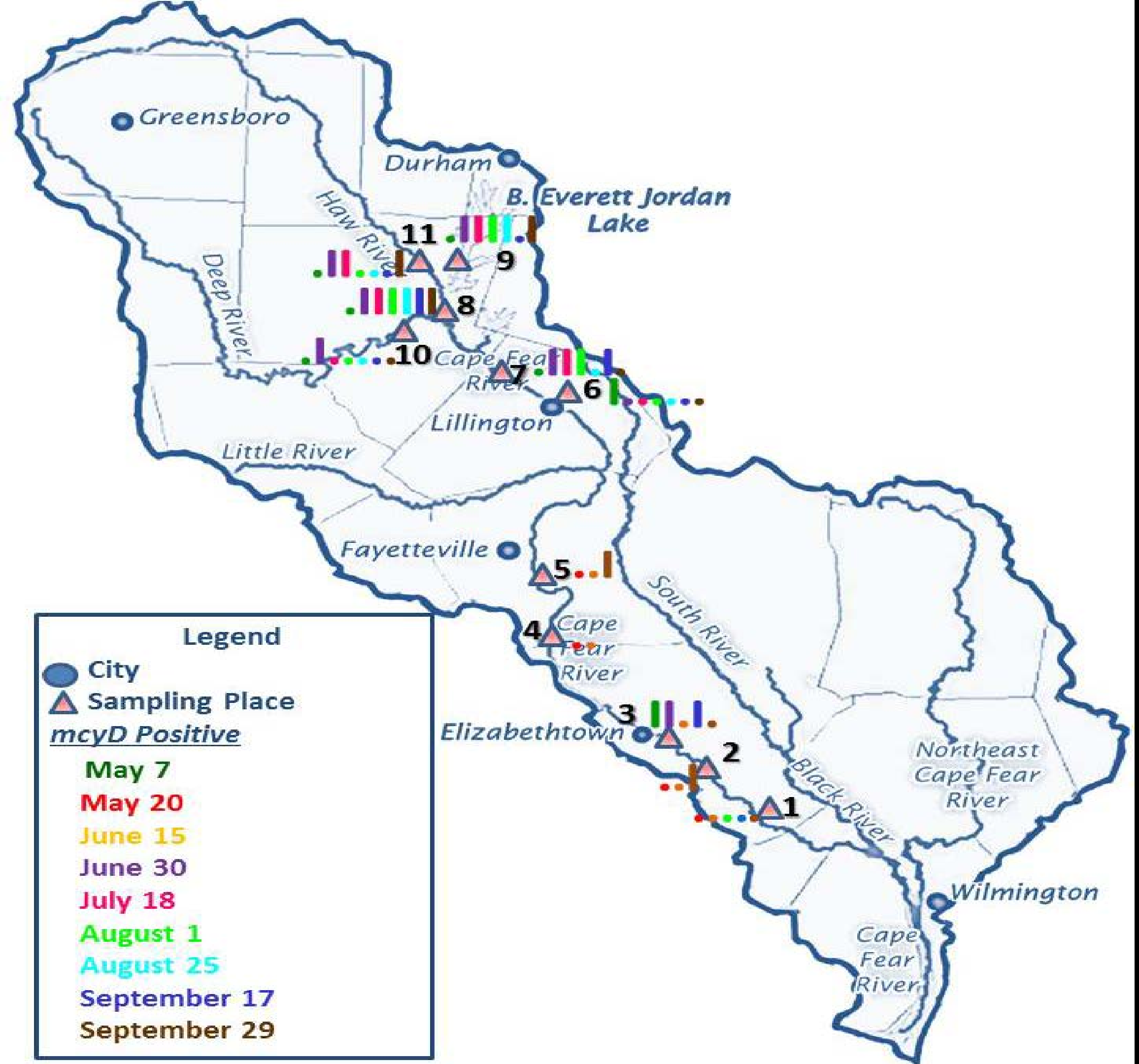
Temperature at NC 11
2000-2014
Bloom
Temperature (°C)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
May-00
Oct-00
Mar-01
Aug-01
Jan-02
Jun-02
Nov-02
Apr-03
Sep-03
Feb-04
Jul-04
Dec-04
May-05
Oct-05
Mar-06
Aug-06
Jan-07
Jun-07
Nov-07
Apr-08
Sep-08
Feb-09
Jul-09
Dec-09
May-10
Oct-10
Mar-11
Aug-11
Jan-12
Jun-12
Nov-12
Apr-13
Sep-13
Feb-14
Jul-14
Dec-14
Date

2015 Cape Fear River Basin Study Results

Chlorophyll *a* Population Mapping

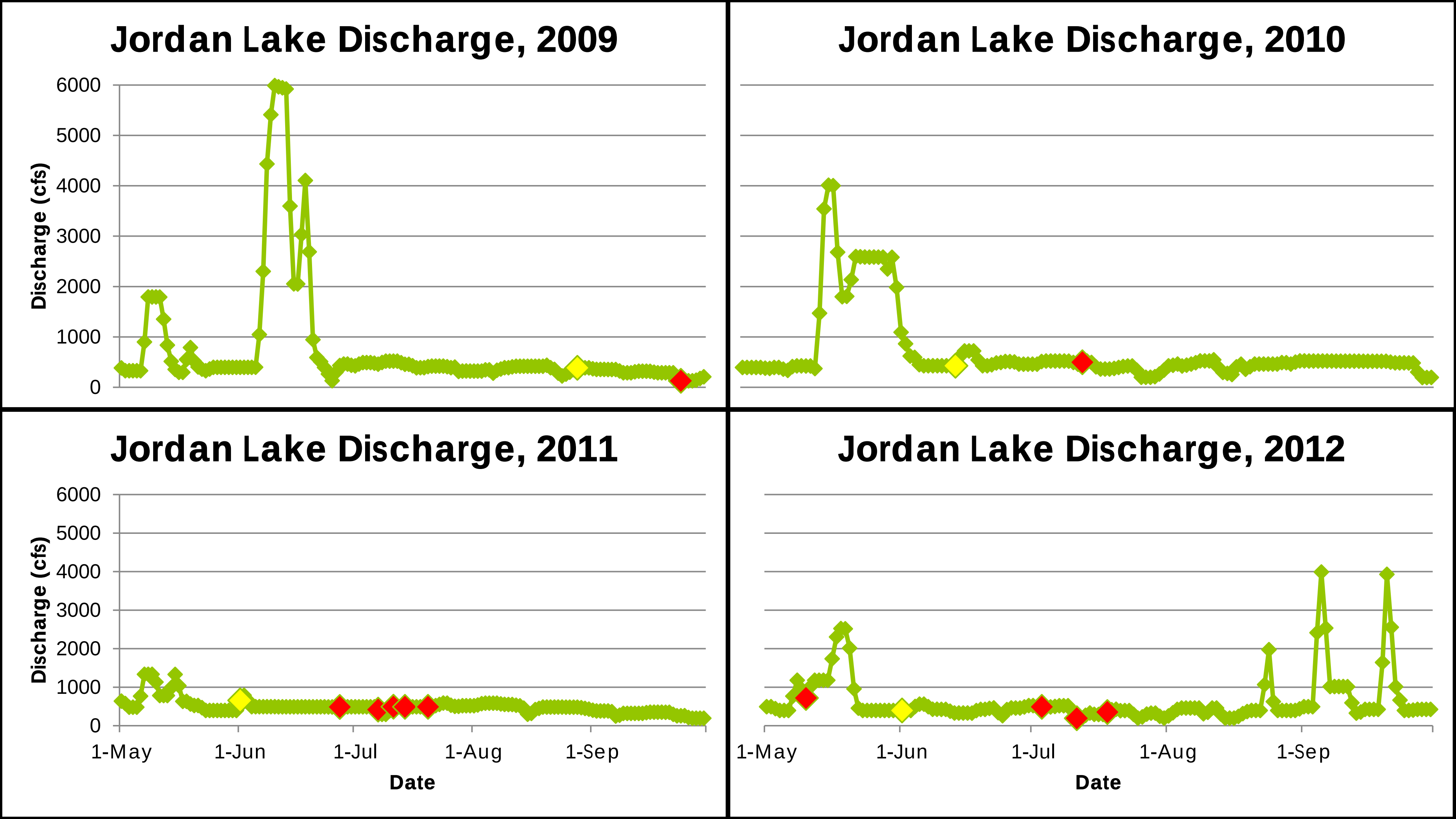
Chlorophyll *a* (ug/L)	7 May, 2015	30 June, 2015	18 July, 2015	1 August, 2015	2010: (NCDWQ, 2011)
Haw River at Bynum	2.5	3.4	2.3	1.4	15.4
Jordan Lake	20.6	20.7	19.7	10.1	
Haw River Downstream of Jordan Lake	4.2	36.3	22.8	18.5	17.0
Deep River at Moncure	1.5	5.3	3.3	2.1	2.9
CFR at NC 42	6.7	21.3	31.2	20.8	34.0
CFR at Lillington	1.6	7.1	2.4	1.6	2.1
Elizabethtown	1.4	9.0	13.3	42.9	18.0


Greensboro
Durham
B. Everett Jordan Lake
Haw River
Deep River
Cape Fear River
Lillington
Little River
Fayetteville
South River
Cape Fear River
Elizabethtown
Black River
Northeast Cape Fear River
Wilmington
Cape Fear River
1
2
3
4
5
6
7
8
9
10
11
Legend
City
Sampling Place
Positive for Microcystis ITS
May 7
May 20
June 15
June 30
July 18
August 1
August 25
September 17
September 29


Greensboro
Durham
B. Everett Jordan Lake
Haw River
Deep River
Cape Fear River
Lillington
Little River
Fayetteville
South River
Cape Fear River
Elizabethtown
Black River
Northeast Cape Fear River
Wilmington
Cape Fear River
1
2
3
4
5
6
7
8
9
10
11
Legend
City
Sampling Place
mcyD Positive
May 7
May 20
June 15
June 30
July 18
August 1
August 25
September 17
September 29

JORDAN LAKE:
DISCHARGE, NUTRIENTS AND PHYTOPLANKTON


Greensboro
Durham
B.
Haw River
Deep River
Jordan Lake
Cape Fear River
Lillington
Little River
Fayetteville
South River
Cape Fear River
Black River
Northeast Cape Fear River
Lock and Dam 1
Cape Fear River


Jordan Lake Discharge, 2009
Jordan Lake Discharge, 2010
Jordan Lake Discharge, 2011
Jordan Lake Discharge, 2012
Discharge (cfs)
6000
5000
4000
3000
2000
1000
0
1-May
1-Jun
1-Jul
1-Aug
1-Sep
Date

Long Term Monitoring: What has **changed**?

- Flow? **NO**
- Nutrient concentration? **Yes, but in the 1990s**
- Nutrient load? **NO**
- Temperature? **NO**
- Turbidity? **NO**

The abiotic ecology of the river has not changed in a way to support unprecedented bloom formation

Jordan Lake, the **logical** upstream source: is it **plausible**?

Why **here** and **now** but not **there** or **then?**

- **Discharge during “windows of opportunity”**
- **No blooms following surges from Jordan Lake**
- **No blooms at Lock and Dam 3 or Buckhorn Dam**
- **Phytoplankton biomass sags between Jordan Lake and Lillington**
- **Outside control on phytoplankton biomass?**
- **ITS results support uncoupling of phytoplankton discharge from Jordan Lake and *Microcystis* blooms downstream**

Microcystis in the Cape Fear River

- Definition and Dimensionality
- Susceptibility and Exposure
- Potential and Opportunity

Jordan Lake has been ruled out as the source of Microcystis blooms

Many, Many Thanks

Advisor: Larry Cahoon

Committee: Michael Mallin and Patrick Erwin

CAPE FEAR RIVER PARTNERSHIP

WATERKEEPER® ALLIANCE

USGS science for a changing world

DIVISION OF WATER QUALITY DWQ

U.S. FISH & WILDLIFE SERVICE DEPARTMENT OF THE INTERIOR

UNCW®

The Nature Conservancy

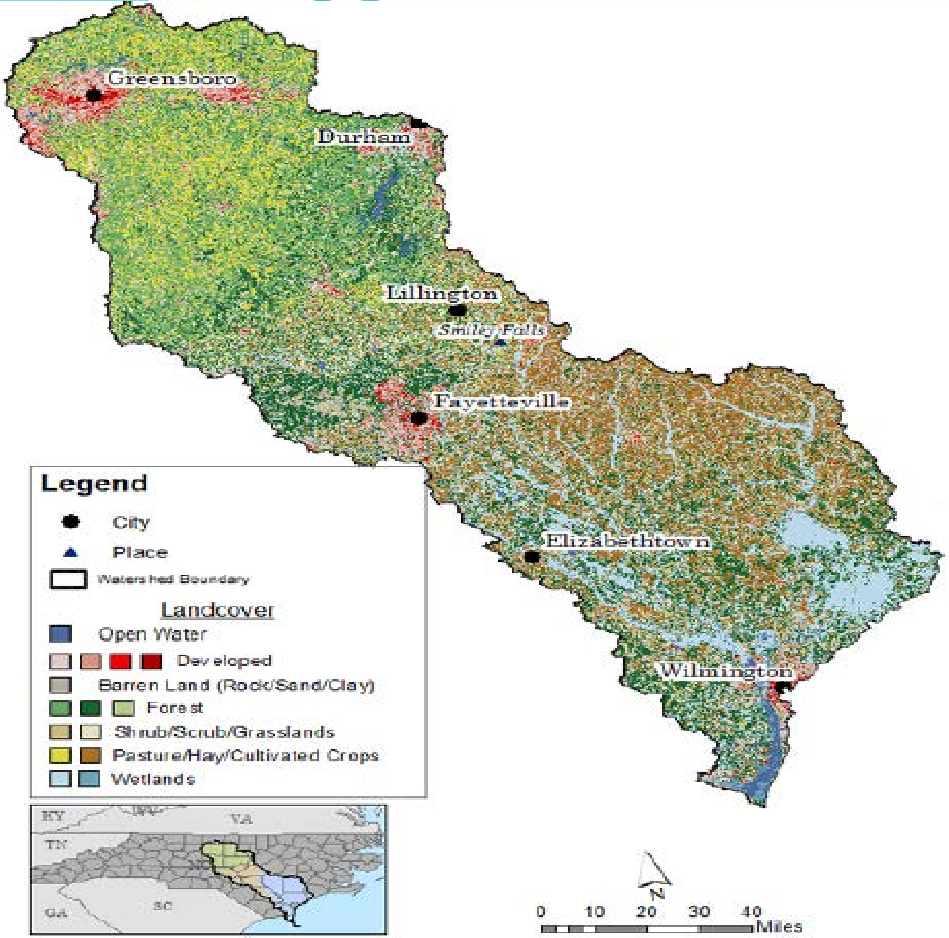
CAPE FEAR RIVER WATCH

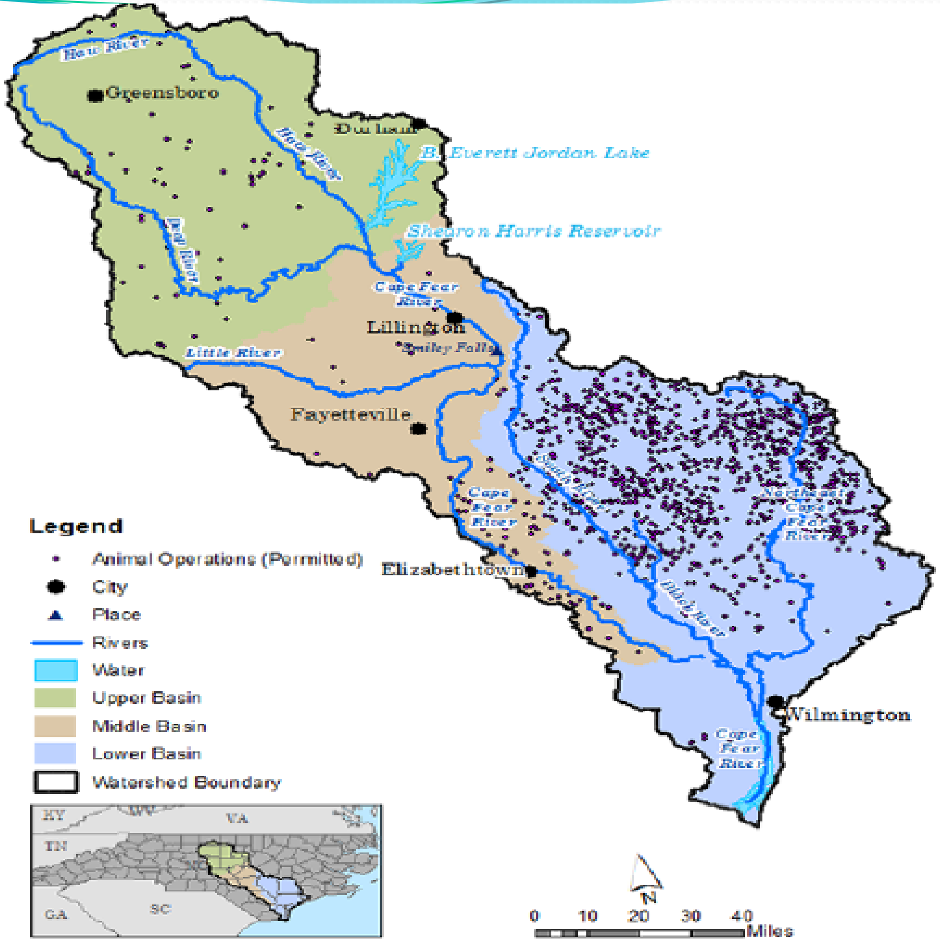

Questions?

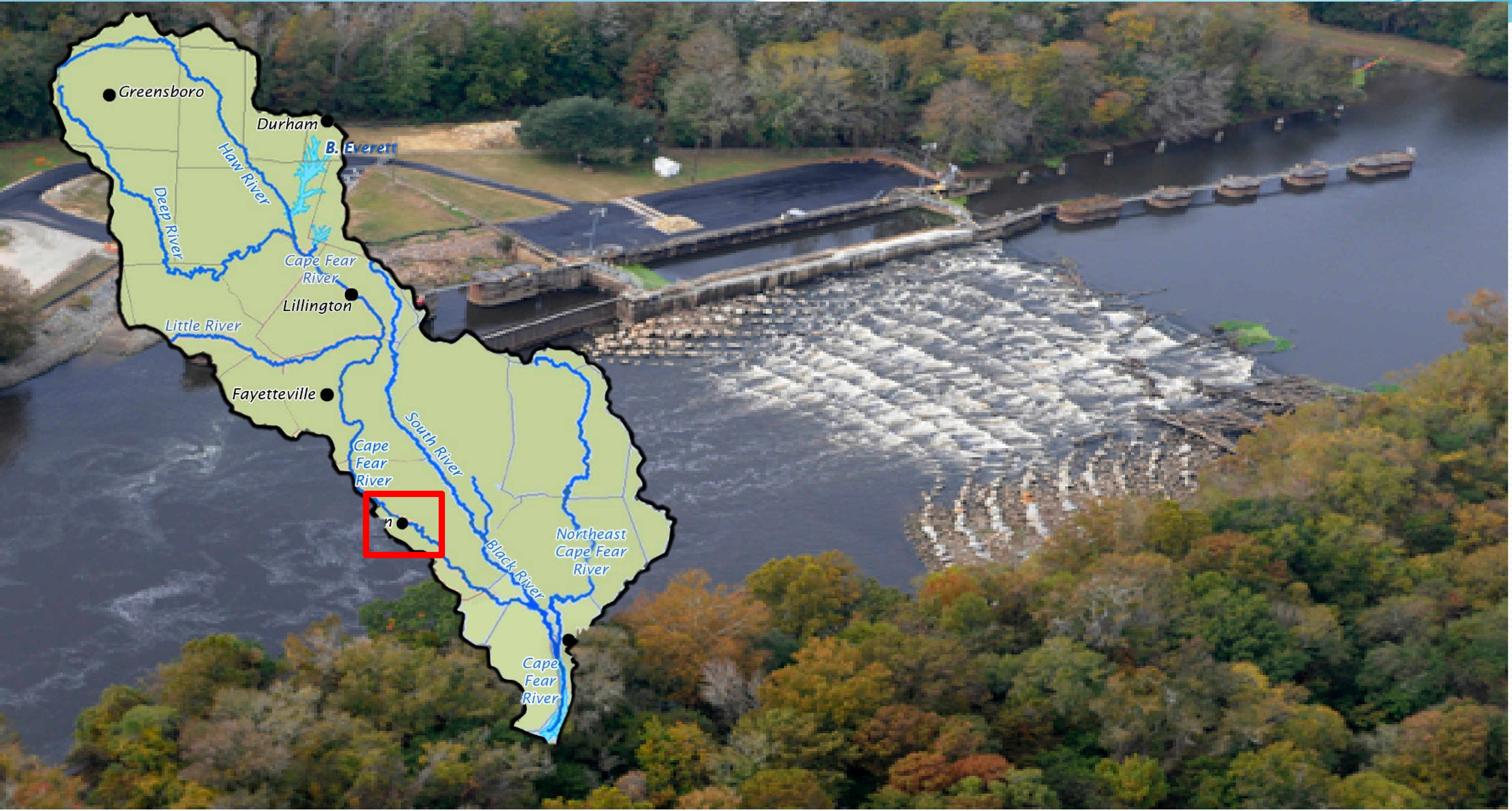
“How often have I said to you that when you have eliminated the impossible, whatever remains, however improbable, must be the truth?”

-Sir Arthur Conan Doyle

Developed, Industrial and Agricultural

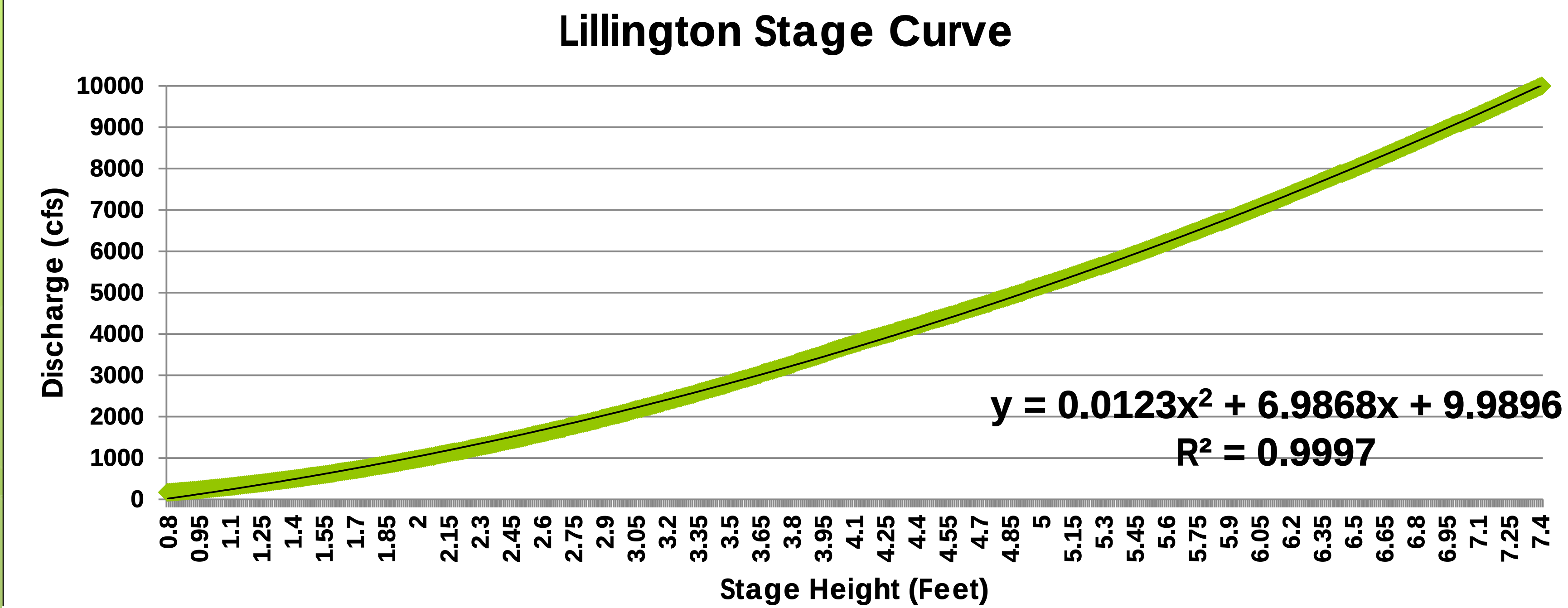





Greensboro
Durham
B. Everett
Haw River
Deep River
Cape Fear River
Lillington
Little River
Fayetteville
South River
Cape Fear River
Black River
Northeast Cape Fear River
Cape Fear River

Available Data: Flow

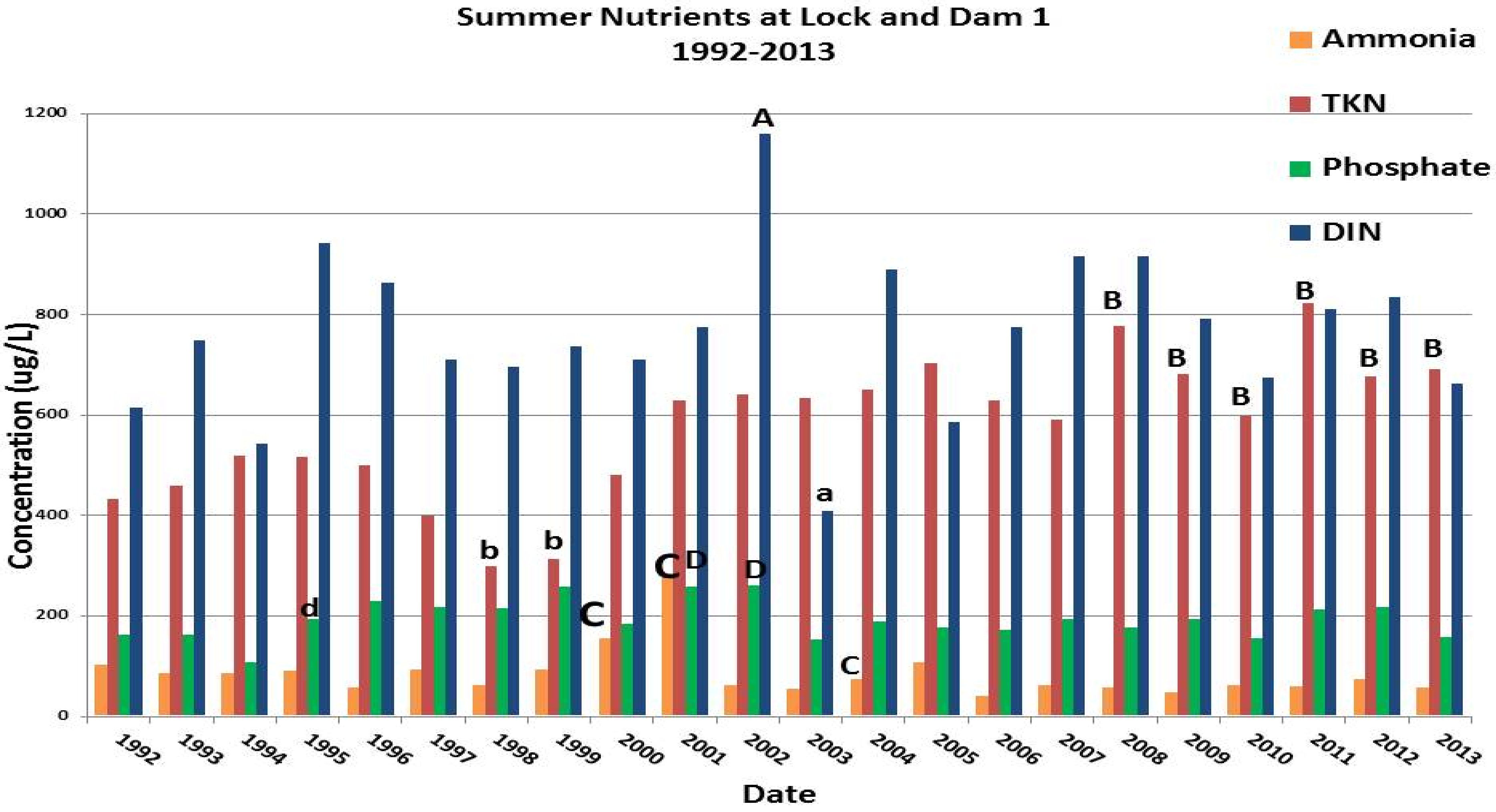
Parameter	Frequency	Time Period	Locations
Flow	Monthly for Summers: May-September	2000-2013	Lock and Dam 1 Lock and Dam 3
	Daily	1991-2015	Lock and Dam 1 Lock and Dam 3 Lillington



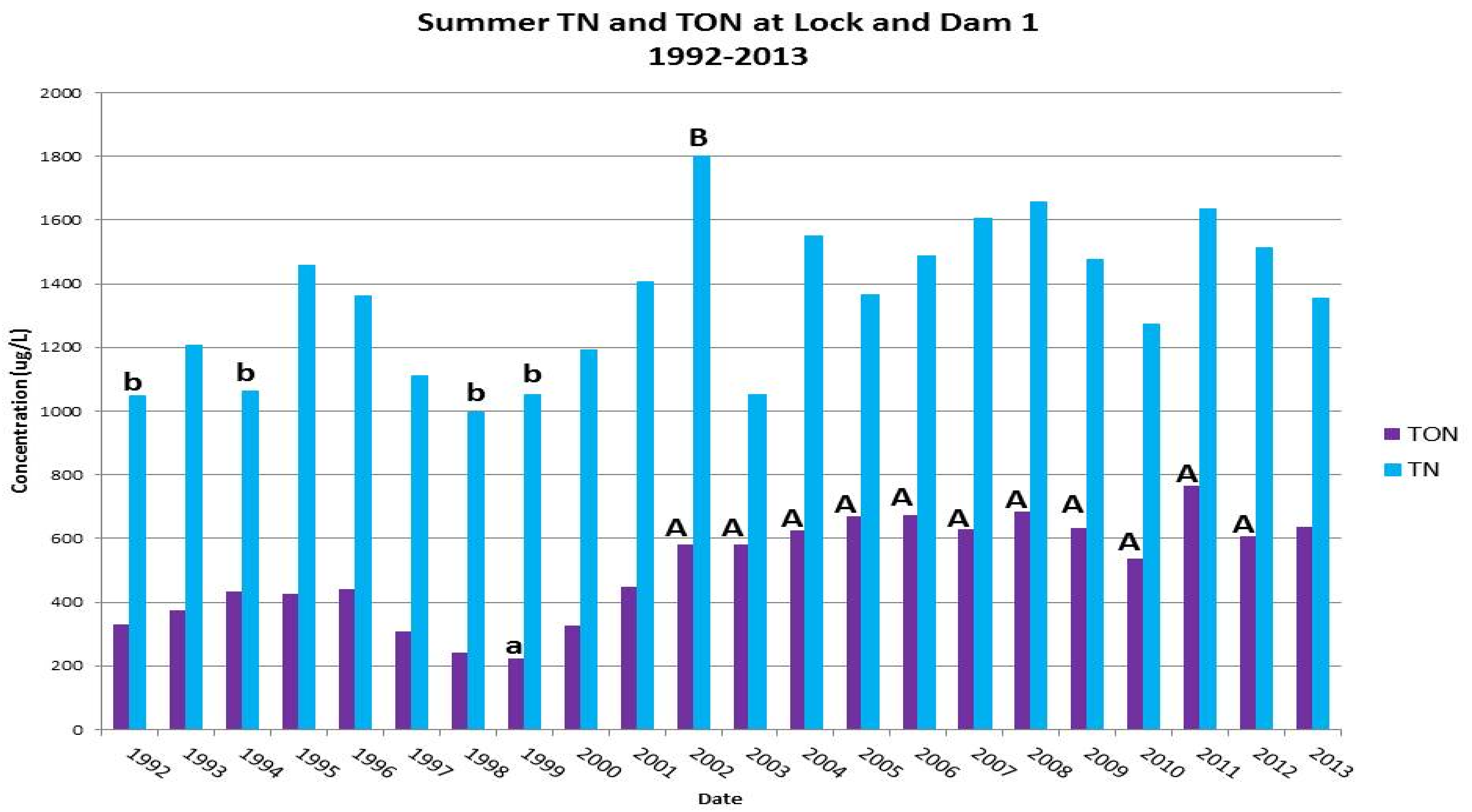
Parameter	Frequency	Time Period	Locations
Velocity	Daily	Summers of 2002; 2007-2014	Lock and Dam 1 Lillington

Available Data: Nutrient Concentration

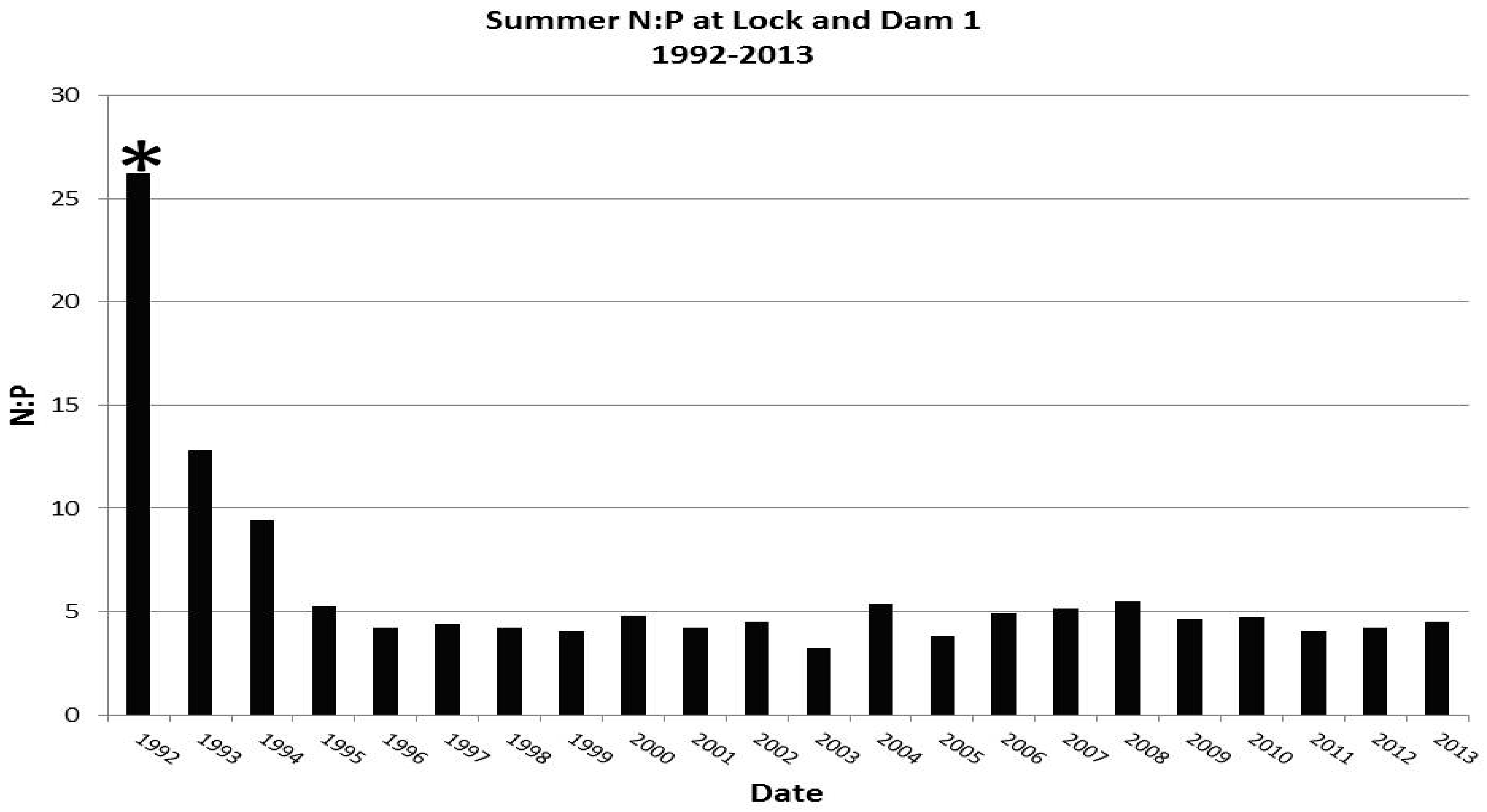
Site Number	Location	DWQ monitoring station
1	Lock and Dam 1 Near Kelly	B8350000
2	Above Lock and Dam 1 Near Arcadia	B8349000
3	Below Lock and Dam 2 at River Marker 70	B8340130
4	Lock and Dam 2	B8339000
5	River Marker 80 Near Ruskin	B8306000
6	Upstream Smithfield Foods	B8302000
7	Lock and Dam 3	B8301000
8	Fayetteville	B7600000
9	Lillington	B6370000

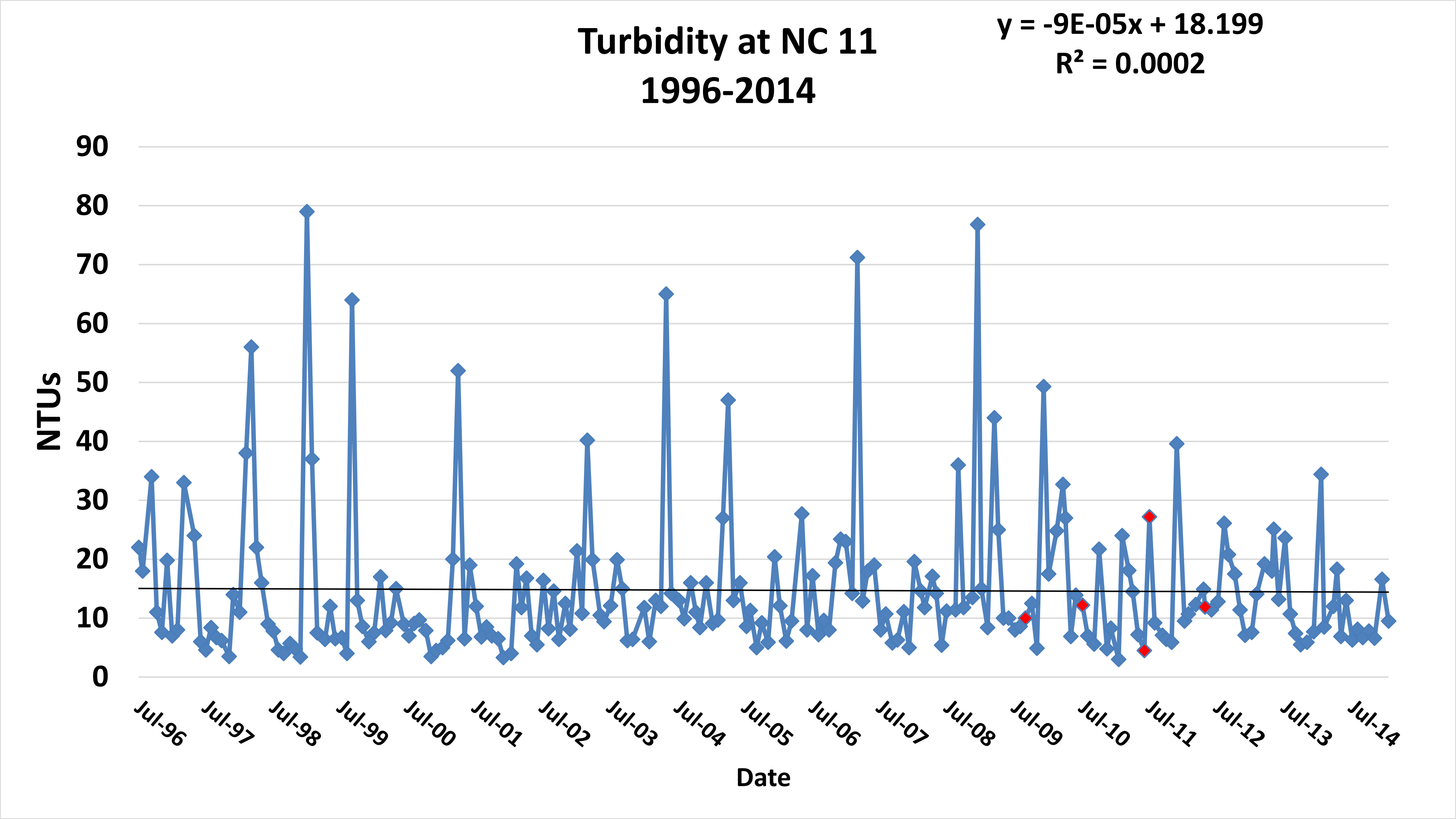


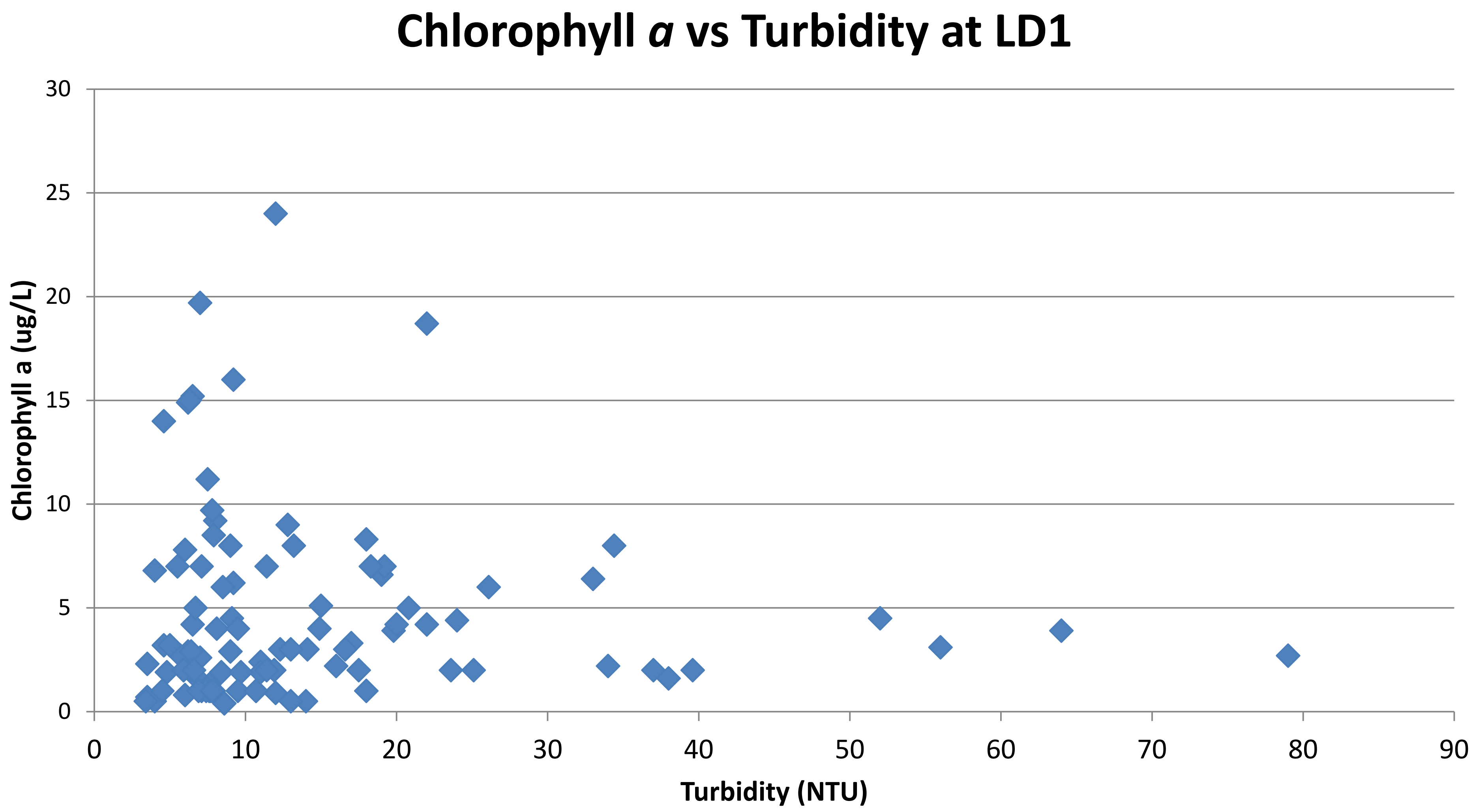
Summer Nutrients at Lock and Dam 1
1992-2013
Ammonia
TKN
Phosphate
DIN
Concentration (ug/L)
0
200
400
600
800
1000
1200
A
B
B
B
B
B
B
a
b
b
C
C
C
D
D
d
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
Date



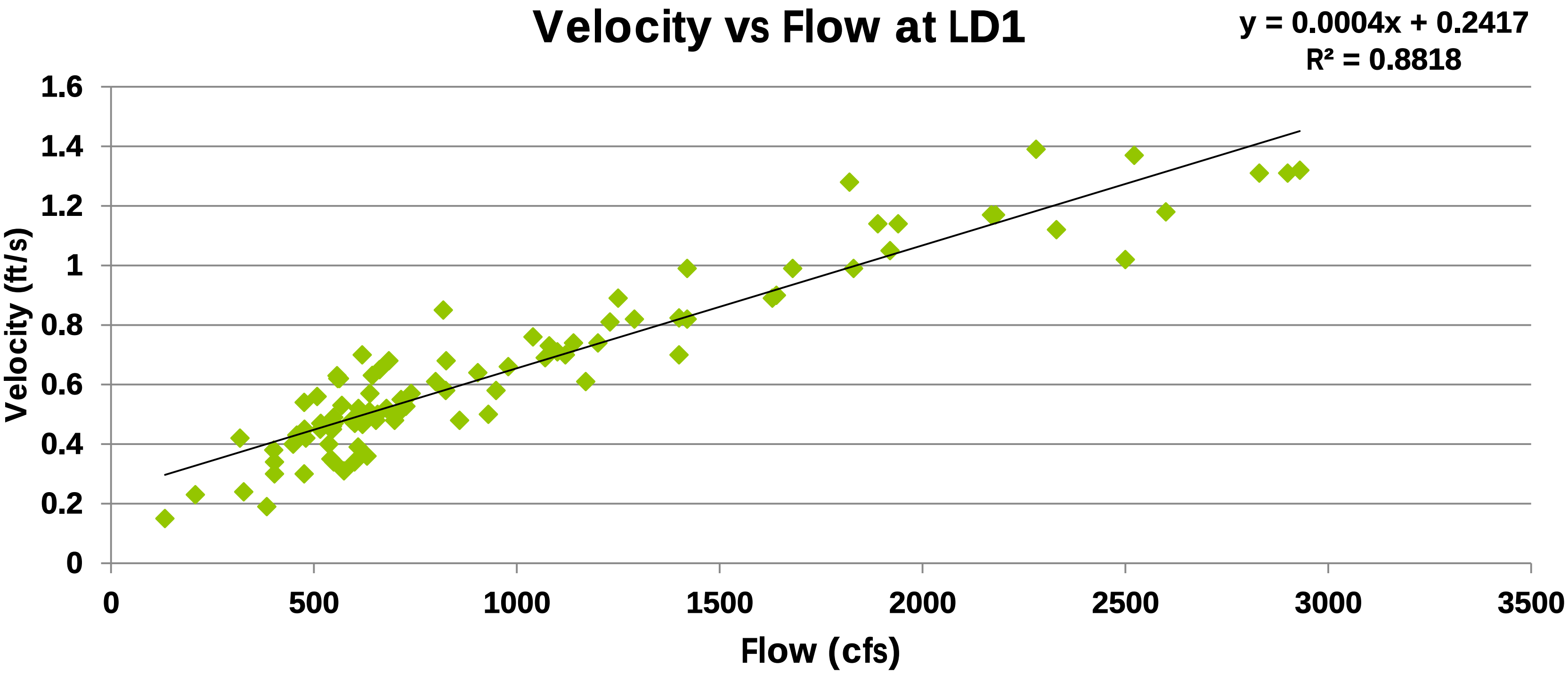
Summer TN and TON at Lock and Dam 1
1992-2013
Concentration (ug/L)
Date
TON
TN
0
200
400
600
800
1000
1200
1400
1600
1800
2000
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
b
b
b
b
B
a
A


Summer N:P at Lock and Dam 1
1992-2013
N:P
30
25
20
15
10
5
0
*
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
Date


Turbidity at NC 11
1996-2014
y = -9E-05x + 18.199
R² = 0.0002
NTUs
0
10
20
30
40
50
60
70
80
90
Jul-96
Jul-97
Jul-98
Jul-99
Jul-00
Jul-01
Jul-02
Jul-03
Jul-04
Jul-05
Jul-06
Jul-07
Jul-08
Jul-09
Jul-10
Jul-11
Jul-12
Jul-13
Jul-14
Date

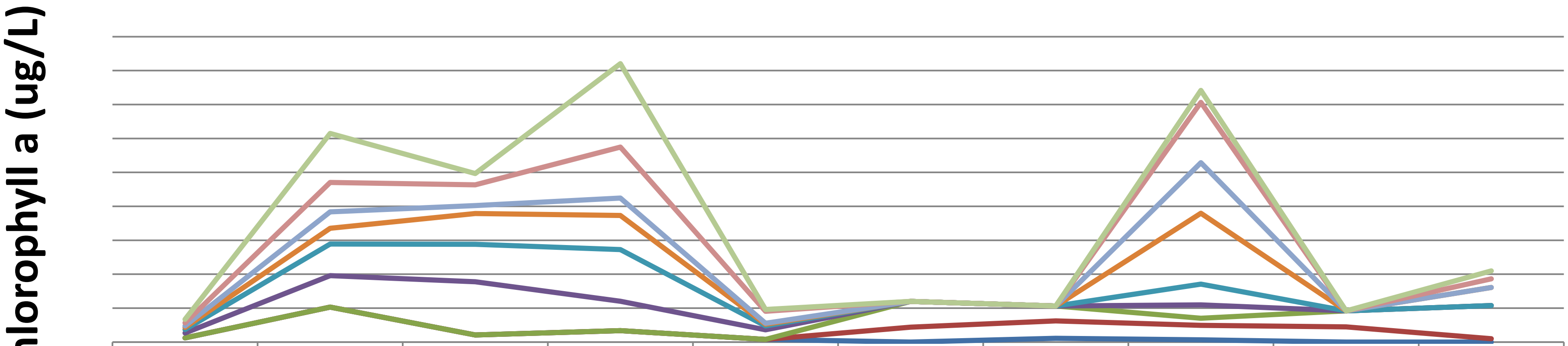

Chlorophyll a vs Turbidity at LD1
Chlorophyll a (ug/L)
Turbidity (NTU)
0
5
10
15
20
25
30
0
10
20
30
40
50
60
70
80
90

Velocity

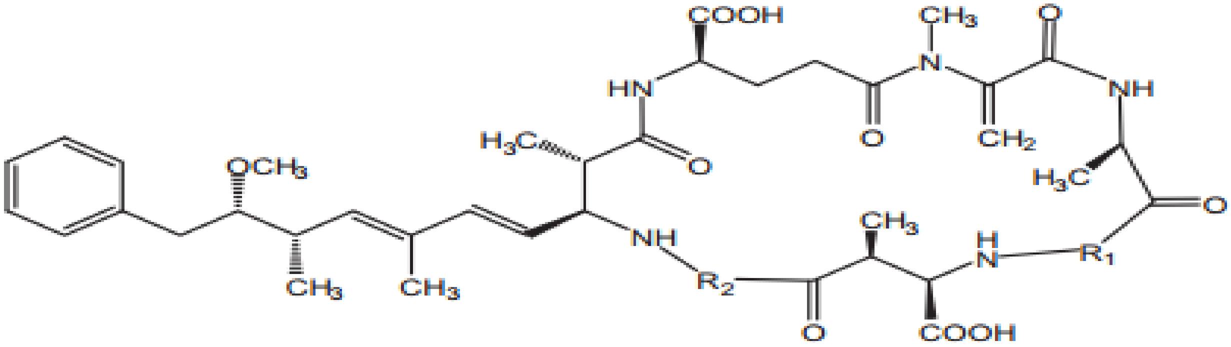


Velocity vs Flow at LD1
y = 0.0004x + 0.2417
R² = 0.8818
Velocity (ft/s)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
500
1000
1500
2000
2500
3000
3500
Flow (cfs)

Chloropyll *a*
Summer 2015

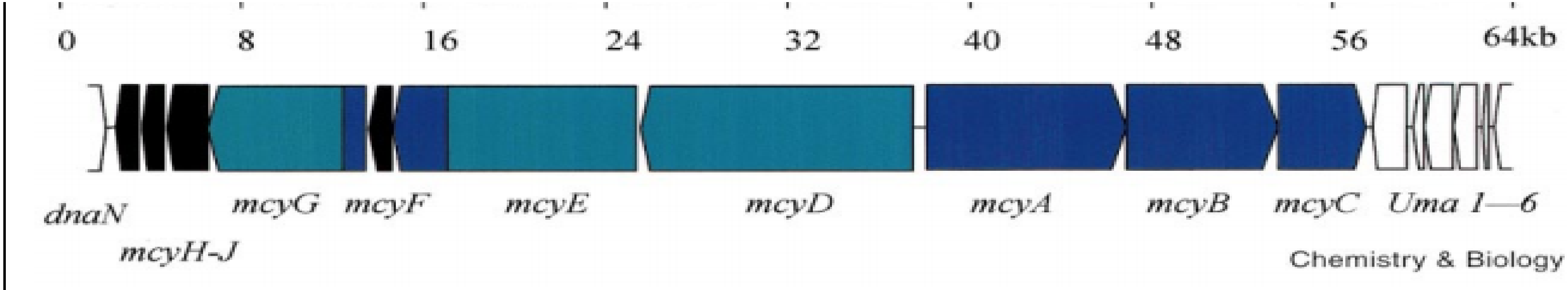


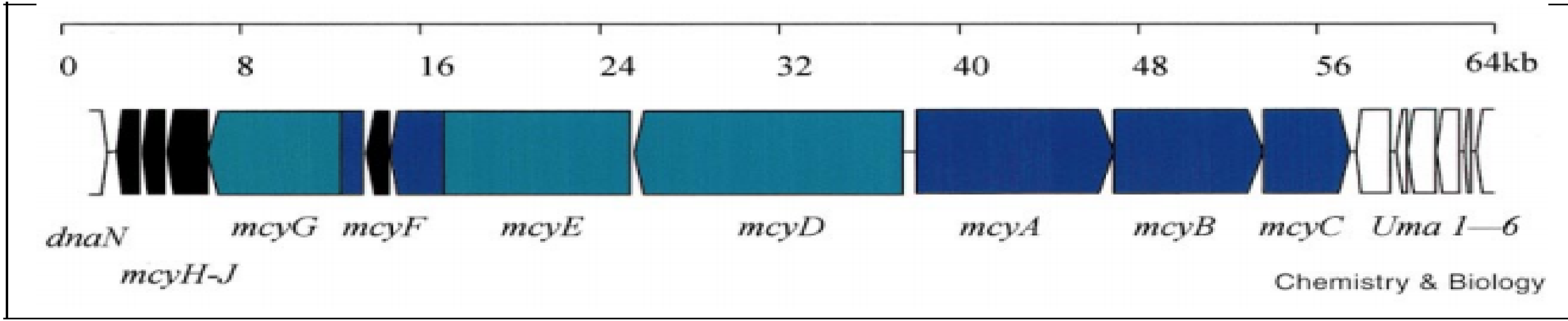
	210.00	200.00	176.00	170.00	150.00	120.00	110.00	90.00	70.00	62.00
29-Sep	2.0	28.9	6.7	49.1	1.1			7.1		4.7
17-Sep	1.9	17.3	12.2	30.1	7.0			35.5		5.1
25-Aug	0.9	9.7	4.7	10.2	0.7			29.8		
1-Aug	1.2	9.3	18.1	20.1	1.0			41.8		10.7
18-Jul	2.0	18.6	22.0	30.5	2.3			12.2		
30-Jun	2.9	18.5	31.4	17.3	5.6			7.9		
15-Jun						15.2	8.8	4.2	9.4	19.5
20-May						8.8	10.3	8.5	9.0	2.0
7-May	2.5	20.6	4.2	6.7	1.6		2.2	1.4		

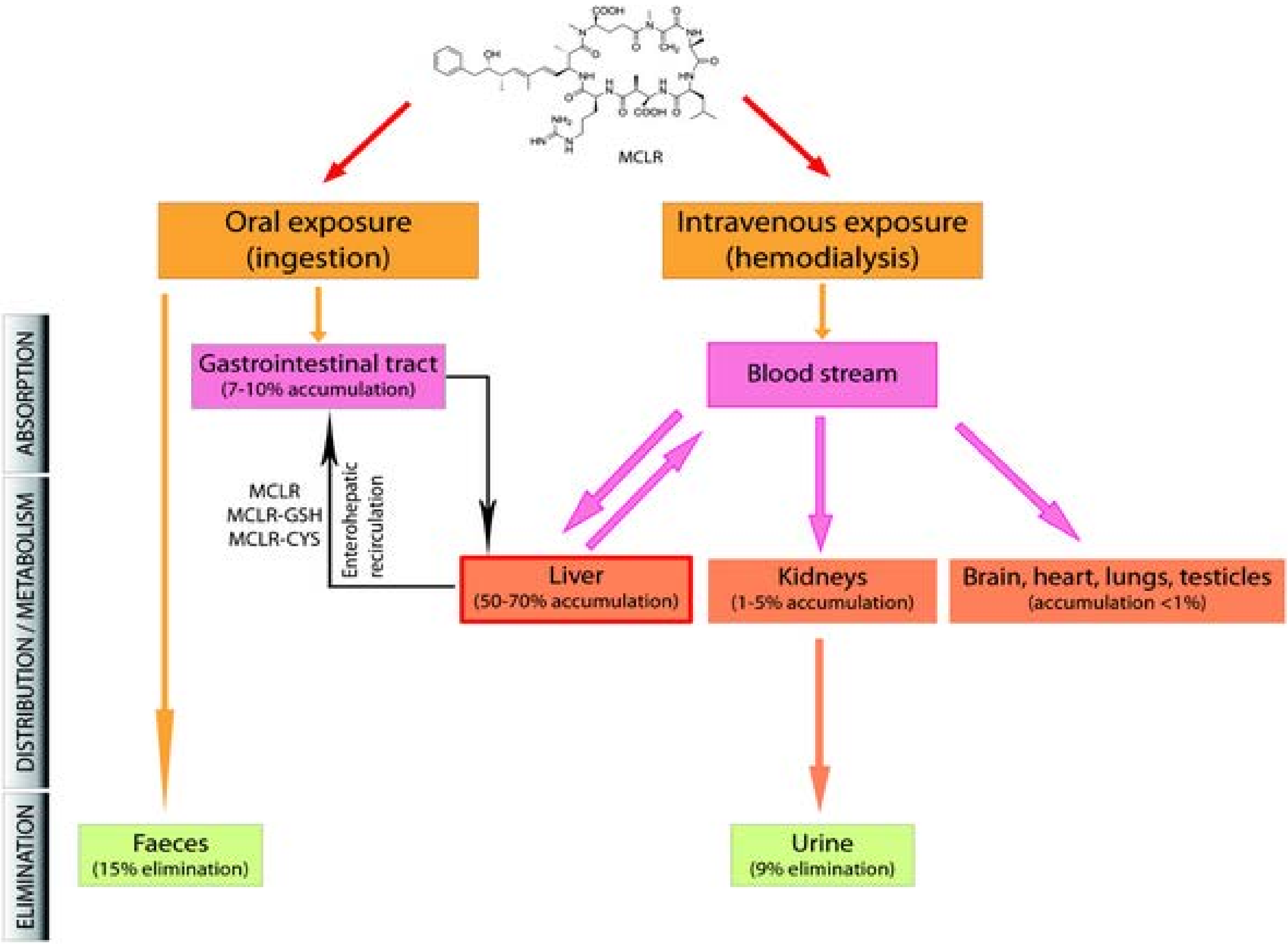


Microcystin	R_1	R_2	MW (Da)
LR	Leu	Arg	994
RR	Arg	Arg	1037

Fig. 1. Chemical structures of microcystins LR and RR found in the Cape Fear River blooms.




0
8
16
24
32
40
48
56
64kb
dnaN
mcyH-J
mcyG
mcyF
mcyE
mcyD
mcyA
mcyB
mcyC
Uma 1—6
Chemistry & Biology



MCLR
Oral exposure (ingestion)
Intravenous exposure (hemodialysis)
ABSORPTION
DISTRIBUTION / METABOLISM
ELIMINATION
Gastrointestinal tract
(7-10% accumulation)
Blood stream
MCLR
MCLR-GSH
MCLR-CYS
Enterohepatic recirculation
Liver
(50-70% accumulation)
Kidneys
(1-5% accumulation)
Brain, heart, lungs, testicles
(accumulation <1%)
Faeces
(15% elimination)
Urine
(9% elimination)

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- A sterilized hole punch was used to cut a sample (area = 28.27 mm^2) from the glass fiber filters. DNA was extracted using Bioline MyTaq Extract PCR Kit following the manufacturer's instructions.
- Genomic DNA of cyanobacterial samples were initially examined by conventional PCR to demonstrate the presence of *Microcystis aeruginosa* using the specific PCR primer set targeting the ITS region (Otsuka et al., 1999). To establish cyanobacterial toxicity, primer sets designed for the MC synthetase genes, mcyB and mcyD, were used to detect MC+ *Microcystis* (Kaebernick et al., 2000; Ouellette et al., 2006).
- Amplifications were carried out in 25 µL volumes in an Eppendorf Mastercycler. Reactions contained 1 µL of DNA extract, 1 µL primer, 12.5 µL 'MyTaq HS Red Mix, 2x' and 9.5 µL PCR water. The following cycling parameters were used: initial denaturation at 95°C for 3 minutes followed by thirty five cycles of denaturation at 95°C for 15 seconds, annealing at 50°C for 15 seconds and extension at 72°C for 20 seconds. Aliquots of PCR reaction products were electrophoresed in 1% agarose gels and captured digitally on a Biospectrum AC Imaging System.

Cultures

Pure cultures of MC+ *Microcystis* LB 2385 were obtained from Eve Wright at the UNCW Marine Biotechnology Laboratory. Triplicate 150 mL of each medium BG-11 and B3N were inoculated with 8 mL LB 2385 and incubated at room temperature with access to natural light and dark patterns. These were used to extract DNA for PCR positive controls.


Energizer