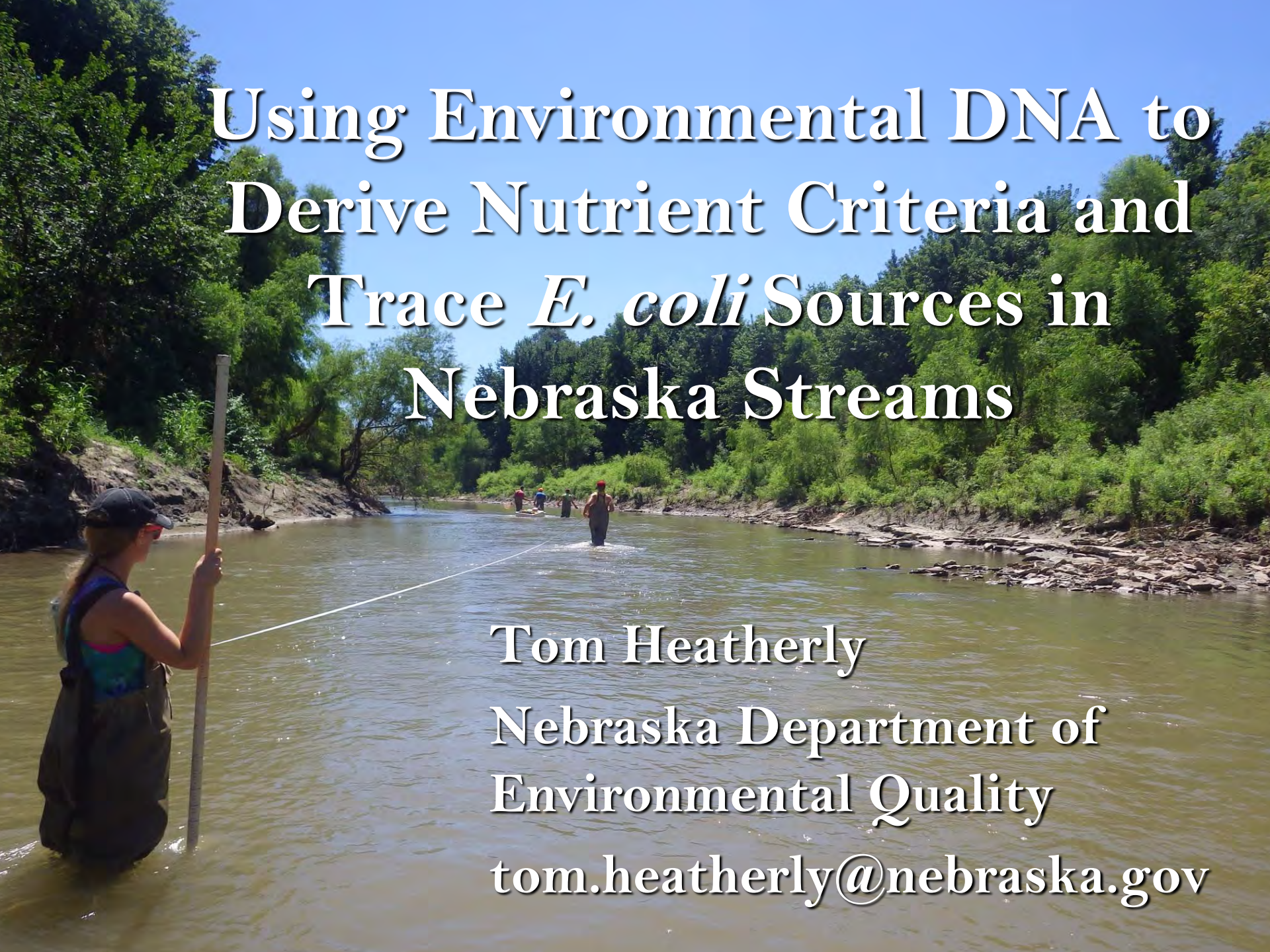


Using Environmental DNA to Derive Nutrient Criteria and Trace *E. coli* Sources in Nebraska Streams

Tom Heatherly

Nebraska Department of Environmental Quality

tom.heatherly@nebraska.gov

Outline

1. **Quick eDNA intro.**

2. **eDNA for local nutrient impacts & nutrient criteria**

3. **eDNA for tracing *E. coli***

Part 1: Introduction to eDNA

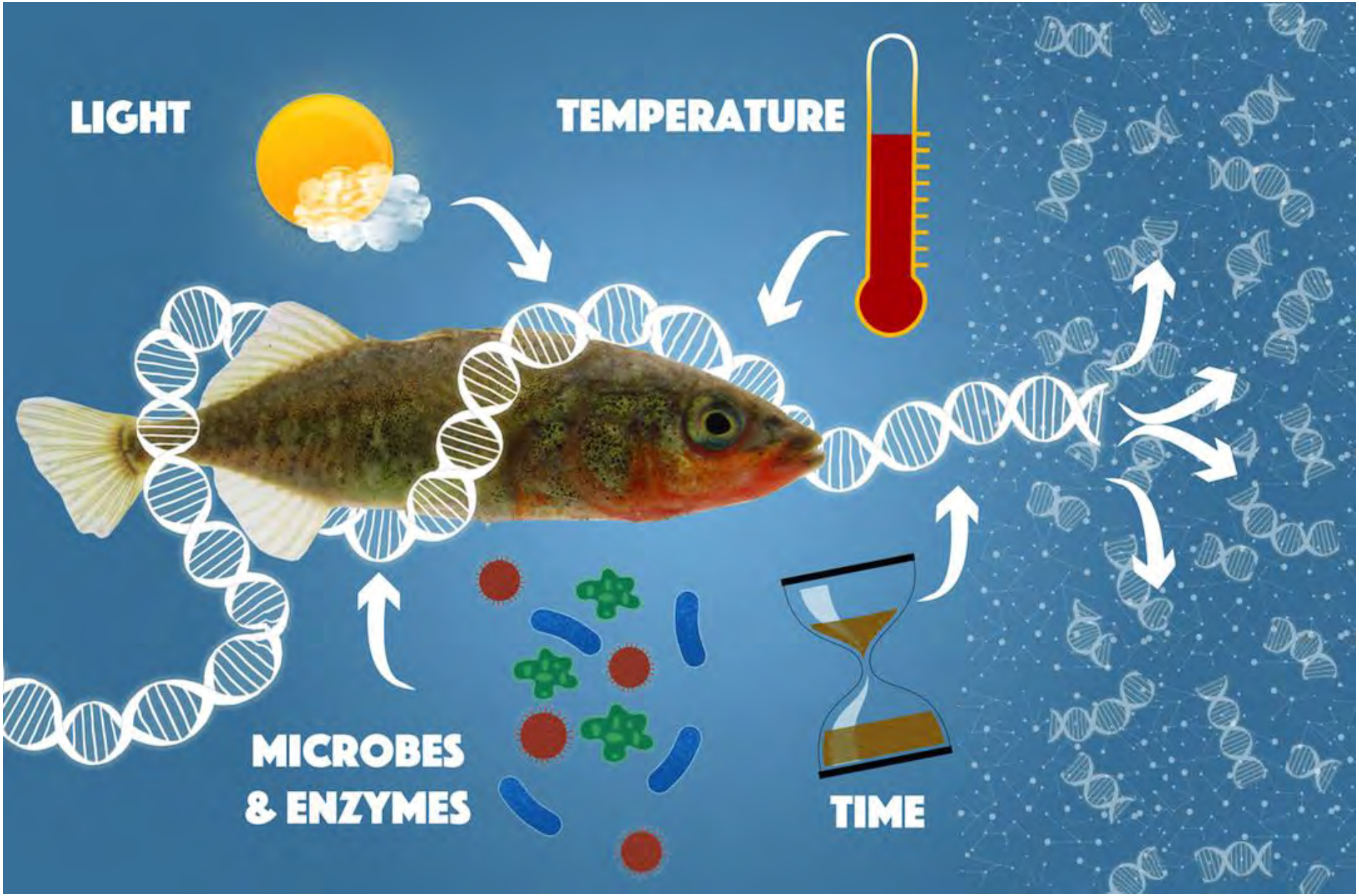
- **Genetic material shed into the environment**
- **Feces & urine are main sources**
- **Also, skin sloughing, decomposition, reproduction, etc.**

BugGuide.net

Some eDNA applications

- **Forensics**
- **Endangered, invasive, elusive species**
- **Community reconstruction**
- **Ecosystem dynamics**

Advantages



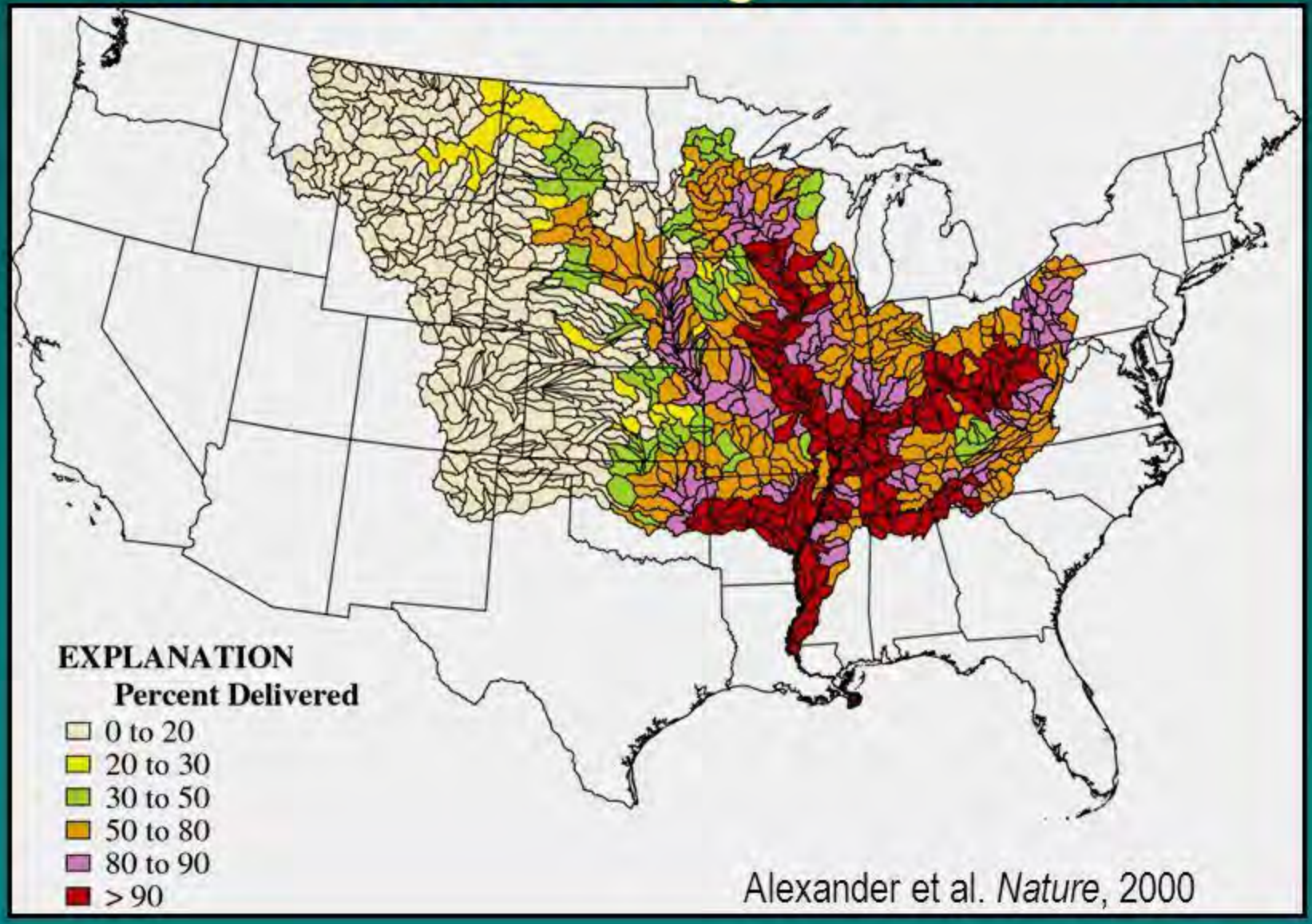
www.fishbio.com

Potential Disadvantages in Streams

- **Detection depends on:**
  - **Volume of water filtered**
  - **Equipment and primers**
  - **Organism size, density, & shed rate**
- **Scale differences**
  - **eDNA shed from watershed**
  - **Scraped algae are local**
- **Longitudinal stream degradation**
- **No positive controls**

Part 2: eDNA and Nutrient Criteria

Fraction of In-Stream Nitrogen Delivered to Gulf

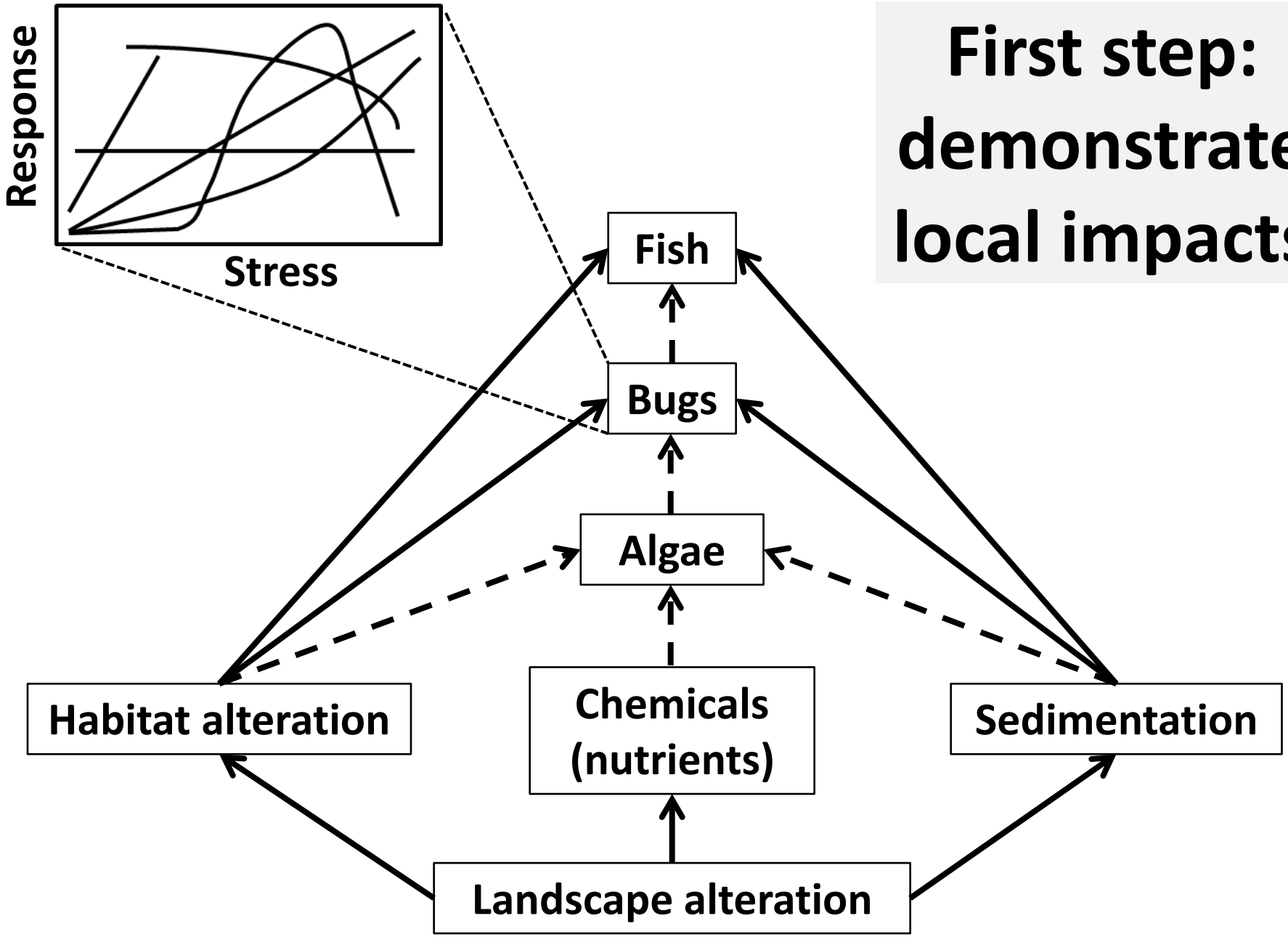


USGS

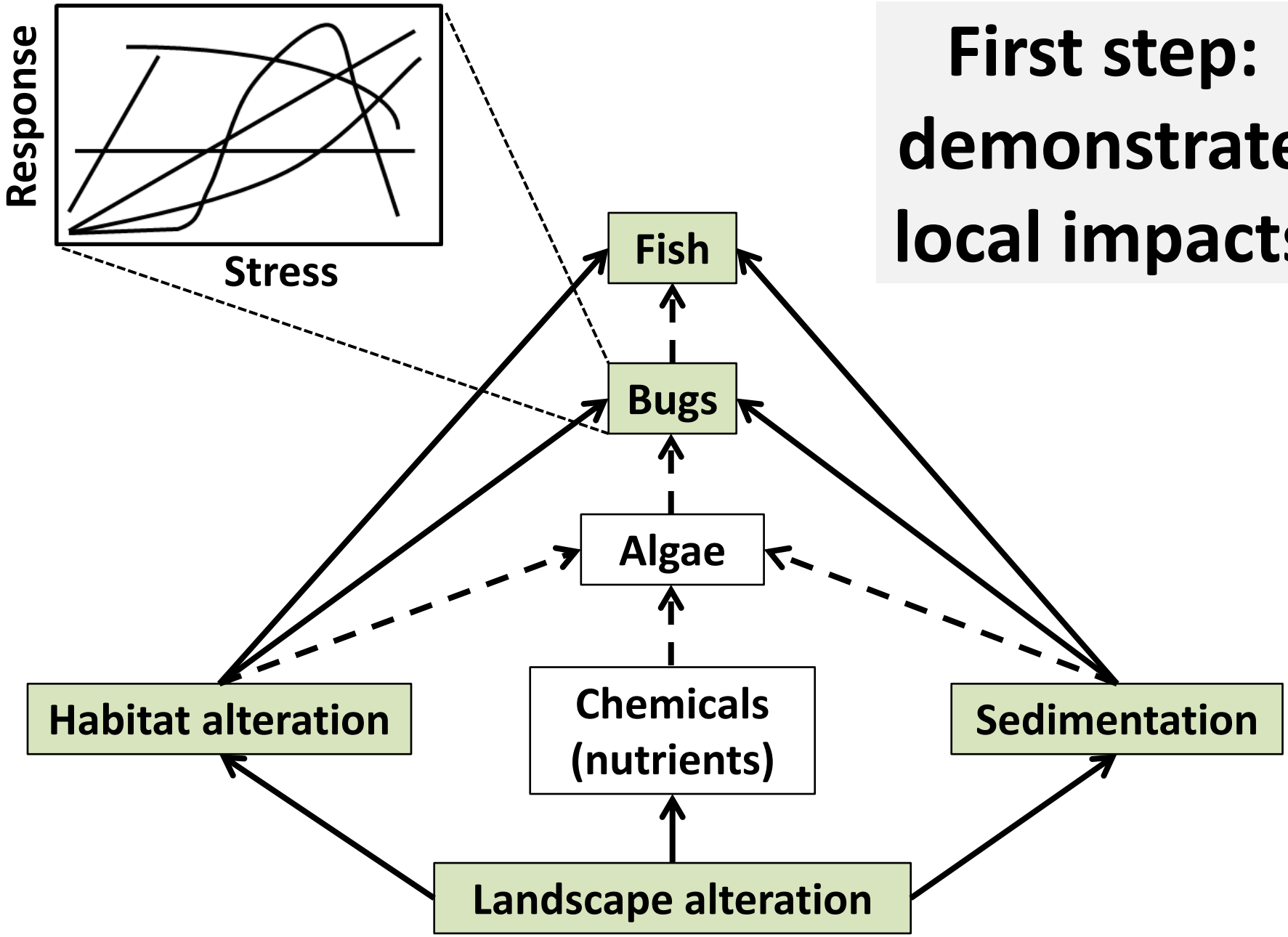
"Numeric nutrient criteria are quantitative expressions of water quality management goals"

- **Uncertain background concentrations**
- **Uncertain local impacts to water quality**
- **Serious ecological & economic consequences**

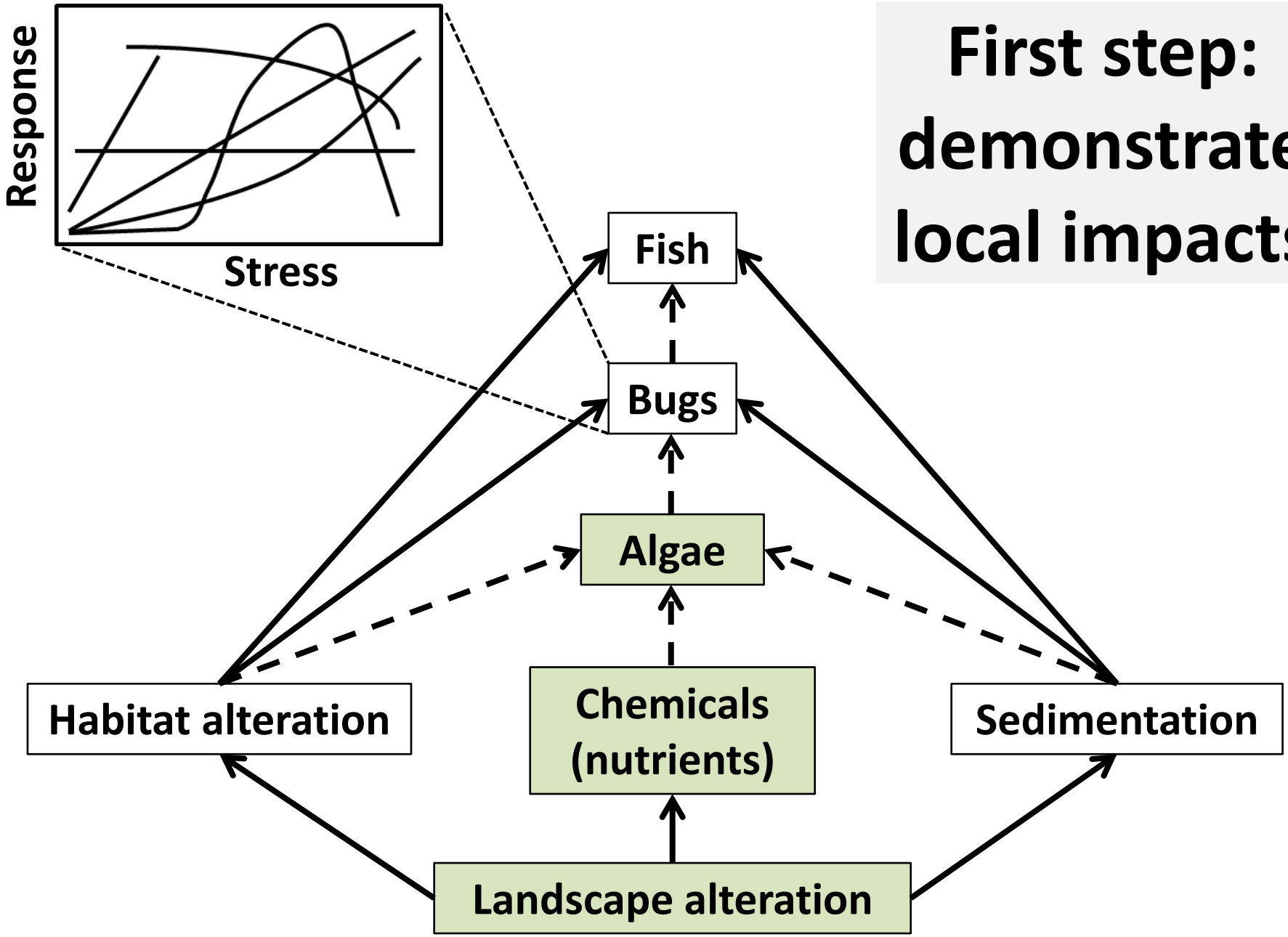
First step: demonstrate local impacts

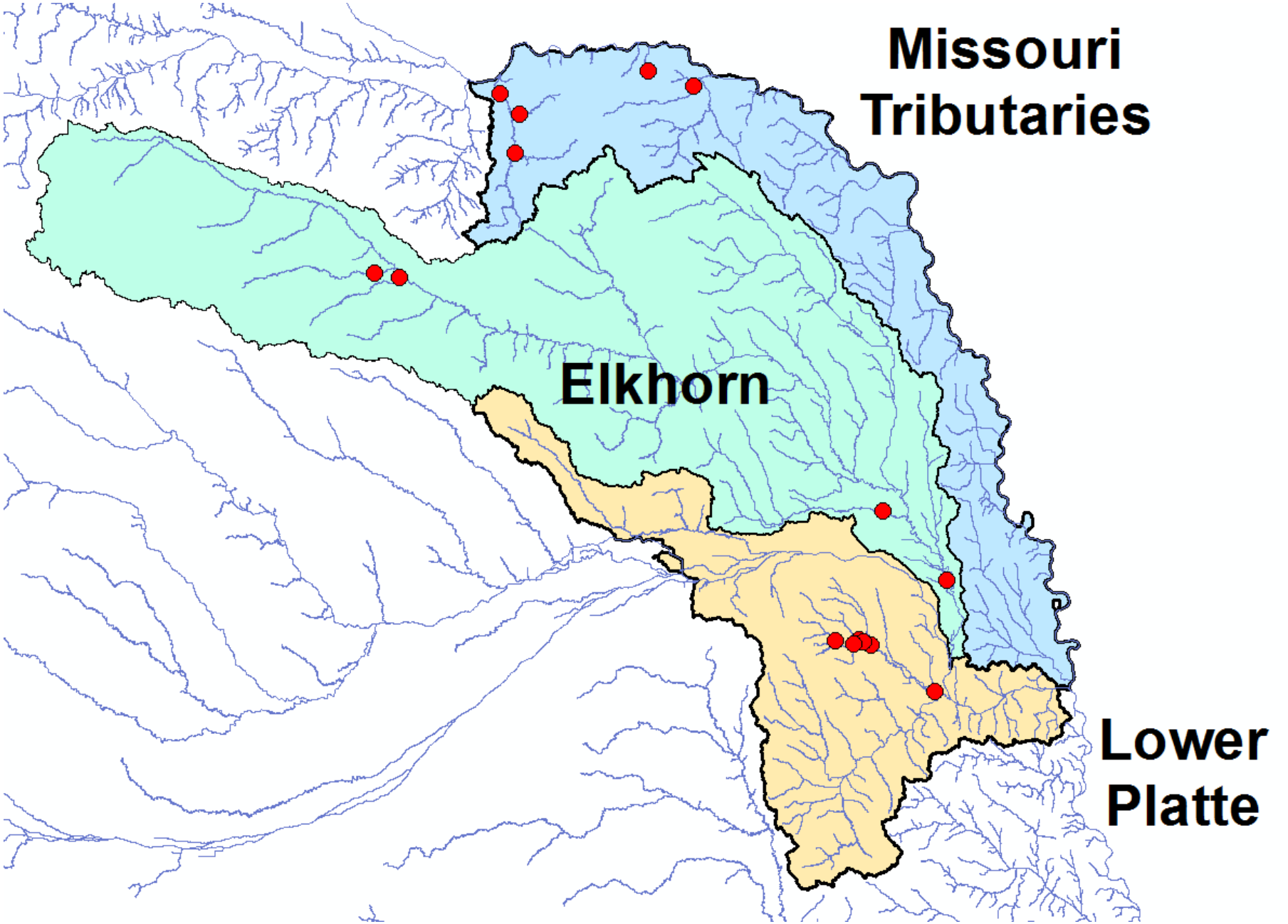


First step: demonstrate local impacts



First step: demonstrate local impacts




Missouri
Tributaries
Elkhorn
Lower
Platte

Missouri Tributaries

Missouri Tributaries

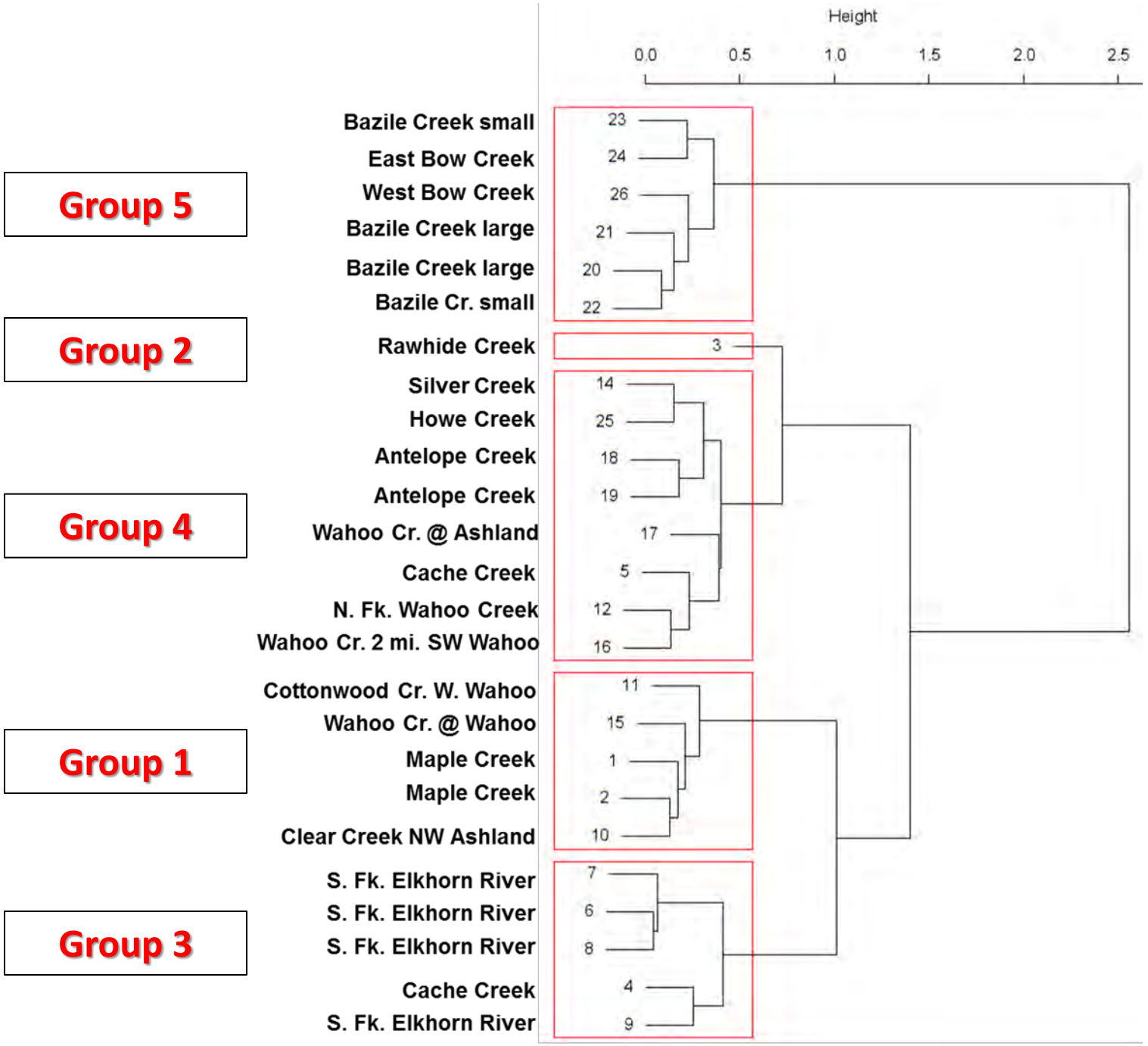
er

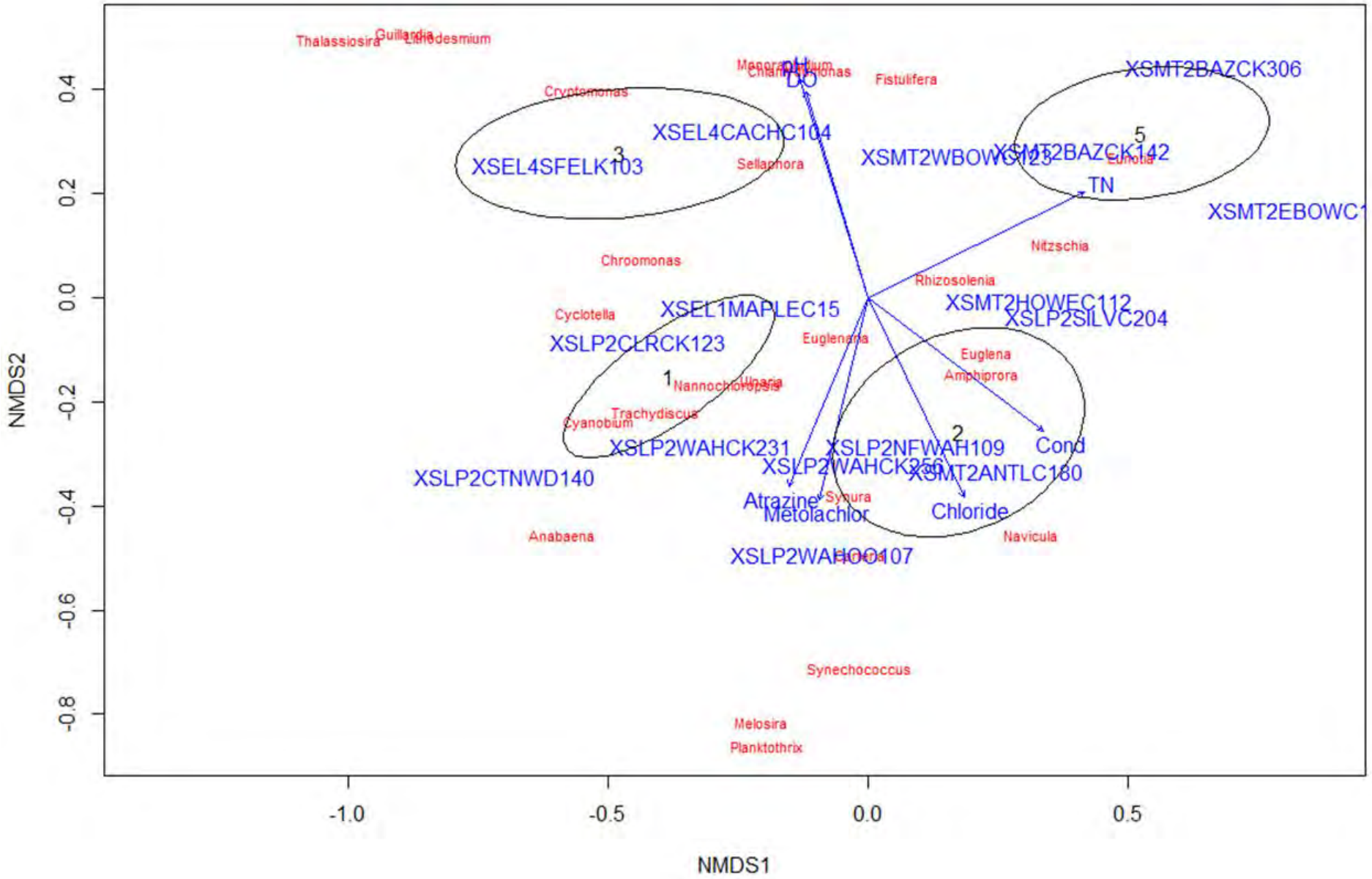
te

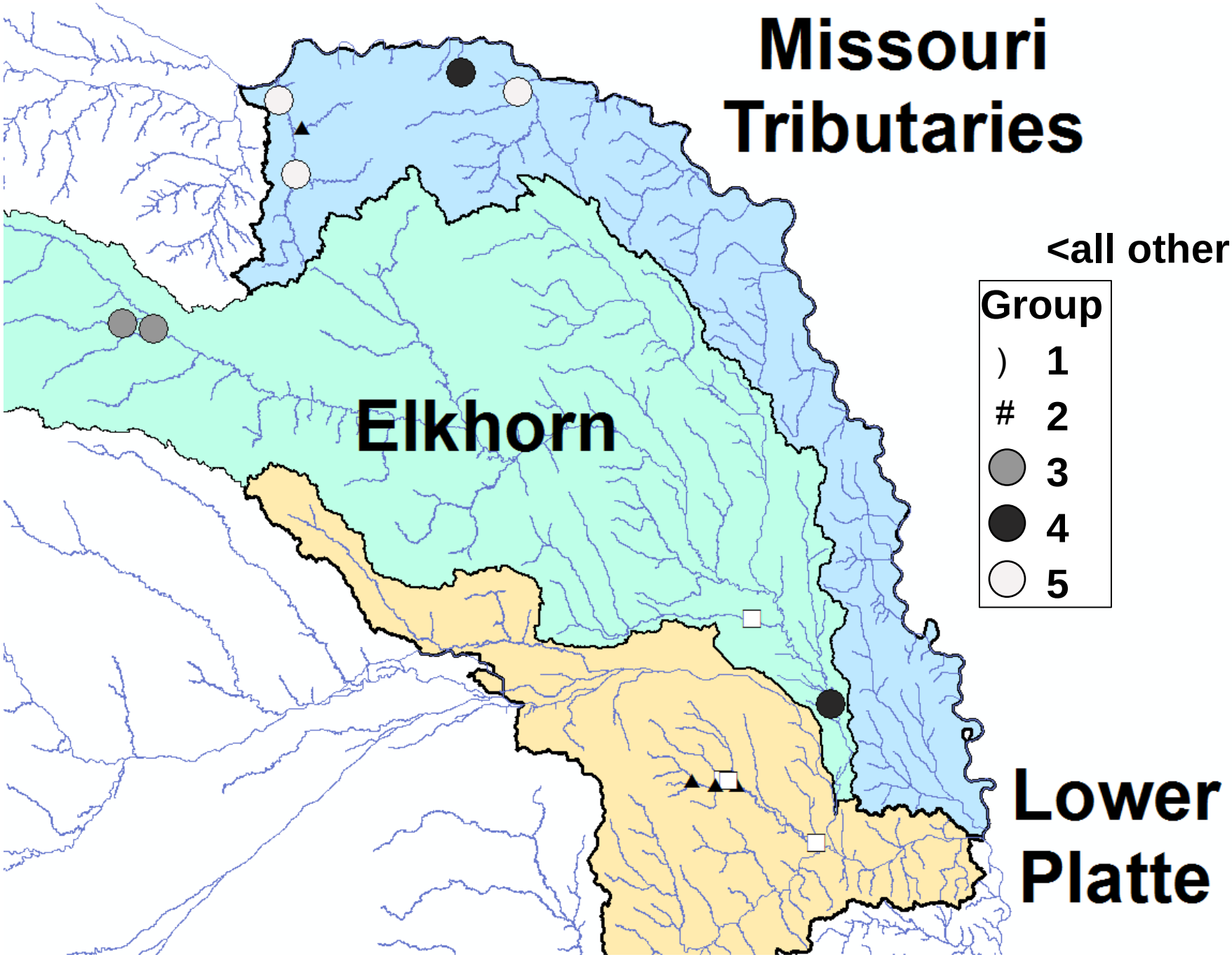
Missouri Tributaries

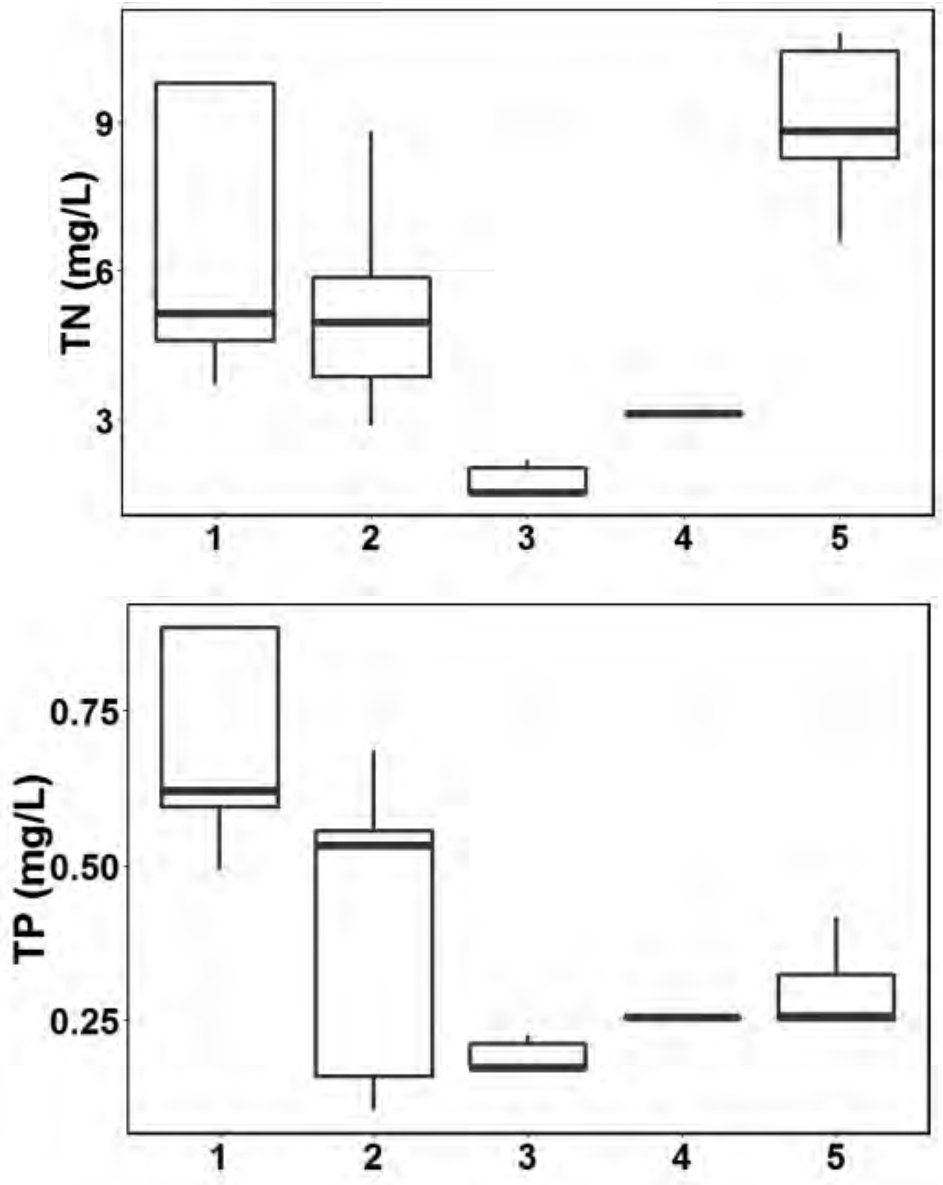
Lower Platte

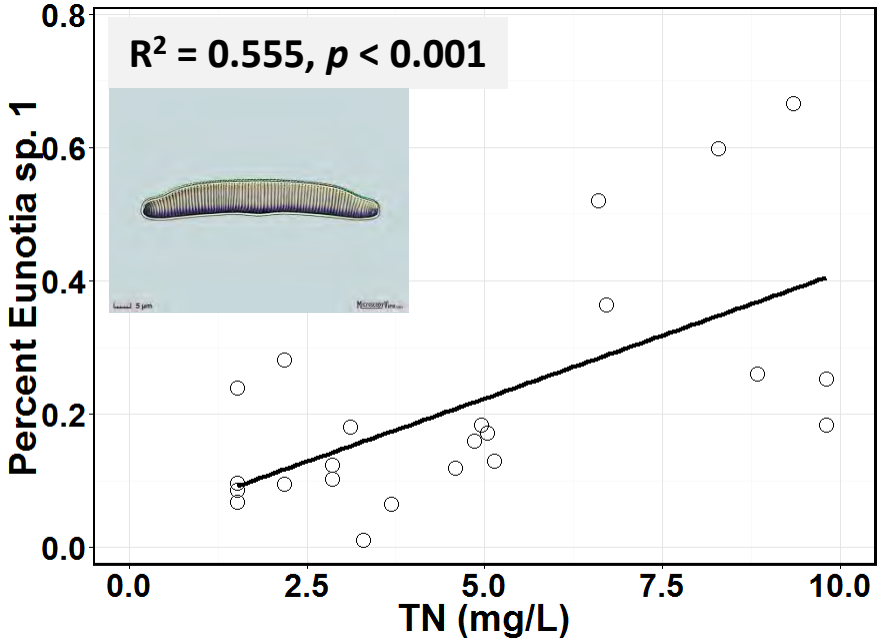
www.fishbio.com

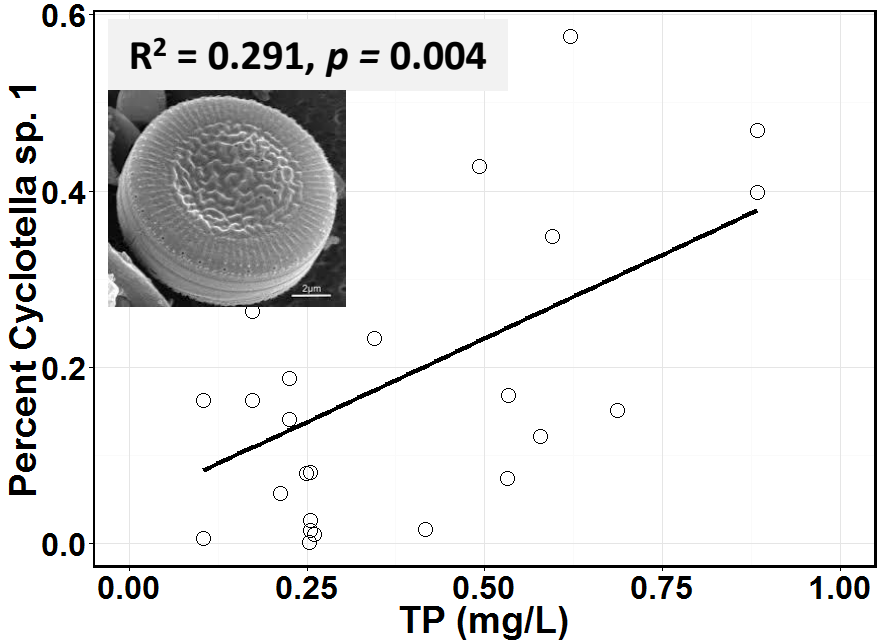

Height
0.0
0.5
1.0
1.5
2.0
2.5
Group 5
Group 2
Group 4
Group 1
Group 3
Bazile Creek small
East Bow Creek
West Bow Creek
Bazile Creek large
Bazile Creek large
Bazile Cr. small
Rawhide Creek
Silver Creek
Howe Creek
Antelope Creek
Antelope Creek
Wahoo Cr. @ Ashland
Cache Creek
N. Fk. Wahoo Creek
Wahoo Cr. 2 mi. SW Wahoo
Cottonwood Cr. W. Wahoo
Wahoo Cr. @ Wahoo
Maple Creek
Maple Creek
Clear Creek NW Ashland
S. Fk. Elkhorn River
S. Fk. Elkhorn River
S. Fk. Elkhorn River
Cache Creek
S. Fk. Elkhorn River
23
24
26
21
20
22
3
14
25
18
19
17
5
12
16
11
15
1
2
10
7
6
8
4
9


Thalassiosira
Guillardia
Limnodesmium
Cryptomonas
Chroomonas
Cyclotella
Nannochloropsis
Trachydiscus
Cyanobium
Anabaena
Synechococcus
Melosira
Planktothrix
Fistulifera
Sellaphora
Nitzschia
Rhizosolenia
Euglenaria
Euglena
Amphiprora
Navicula
Synura
DO
pH
TN
Cond
Atrazine
Metolachlor
Chloride
XSMT2BAZCK306
XSMT2BAZCK142
XSMT2EBOWC1
XSMT2WBOWC123
XSEL4CACHC104
XSEL4SFELK103
XSEL1MAPLEC15
XSLP2CLRCK123
XSMT2HOWEC112
XSLP2SILVC204
XSLP2NFWAH109
XSLP2WAHCK231
XSLP2CTNWD140
1
2
3
5
NMDS1
NMDS2
-1.0
-0.5
0.0
0.5
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8


Missouri
Tributaries
Elkhorn
Lower
Platte
<all other valu
Group
1
2
3
4
5


TN (mg/L)
9
6
3
1
2
3
4
5
TP (mg/L)
0.75
0.50
0.25
1
2
3
4
5
Stream group





Genus	Percentage
Eunotia	28.87
Cyclotella	20.82
Navicula	8.97
Amphiprora	7.03
Synechococcus	6.18
Lithodesmium	4.88
Cryptomonas	3.88
Melosira	3.10
Planktothrix	2.76
Sellaphora	1.38

Photos: microscopyView.com, nordicmicroalgae.org

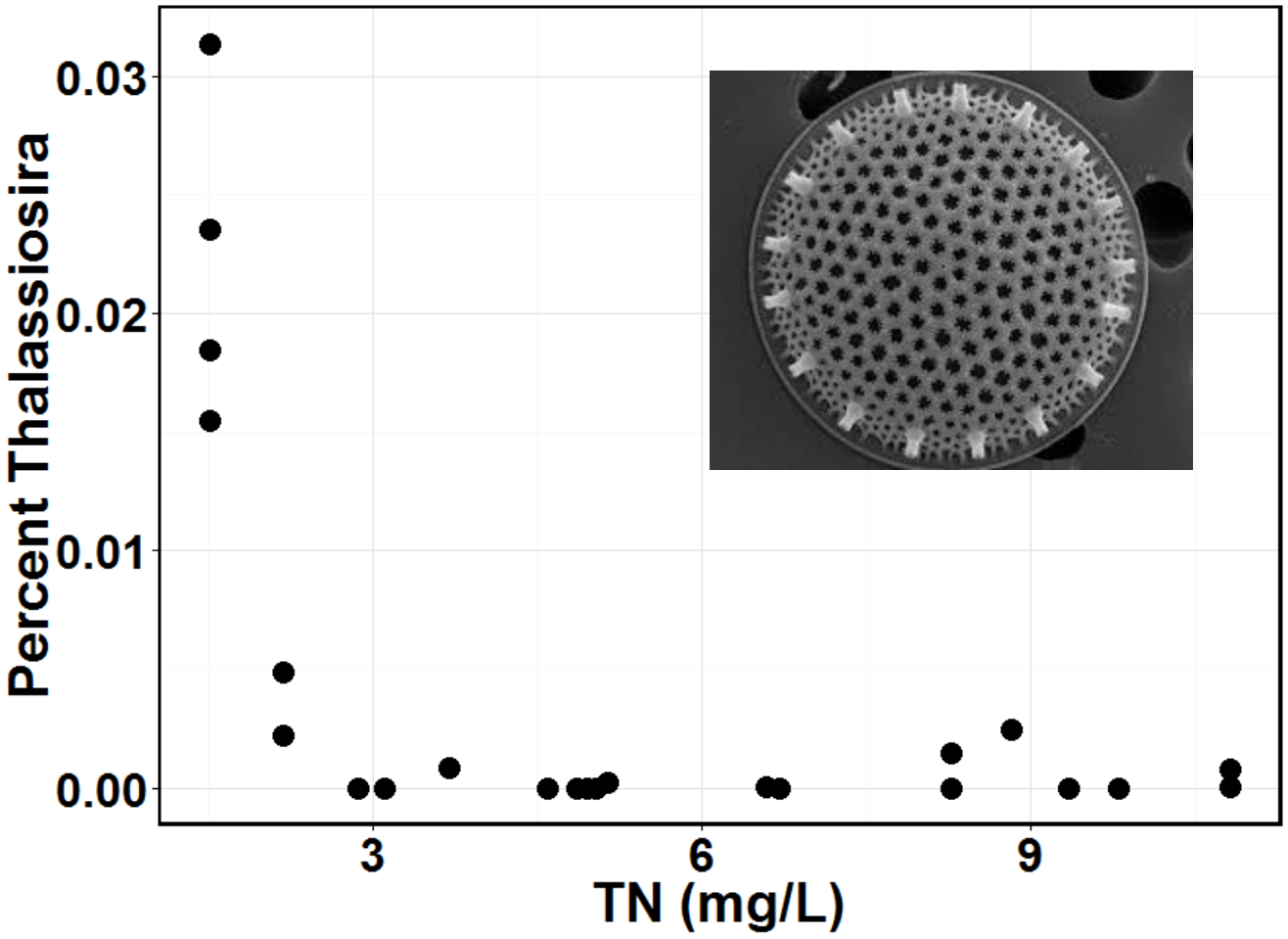
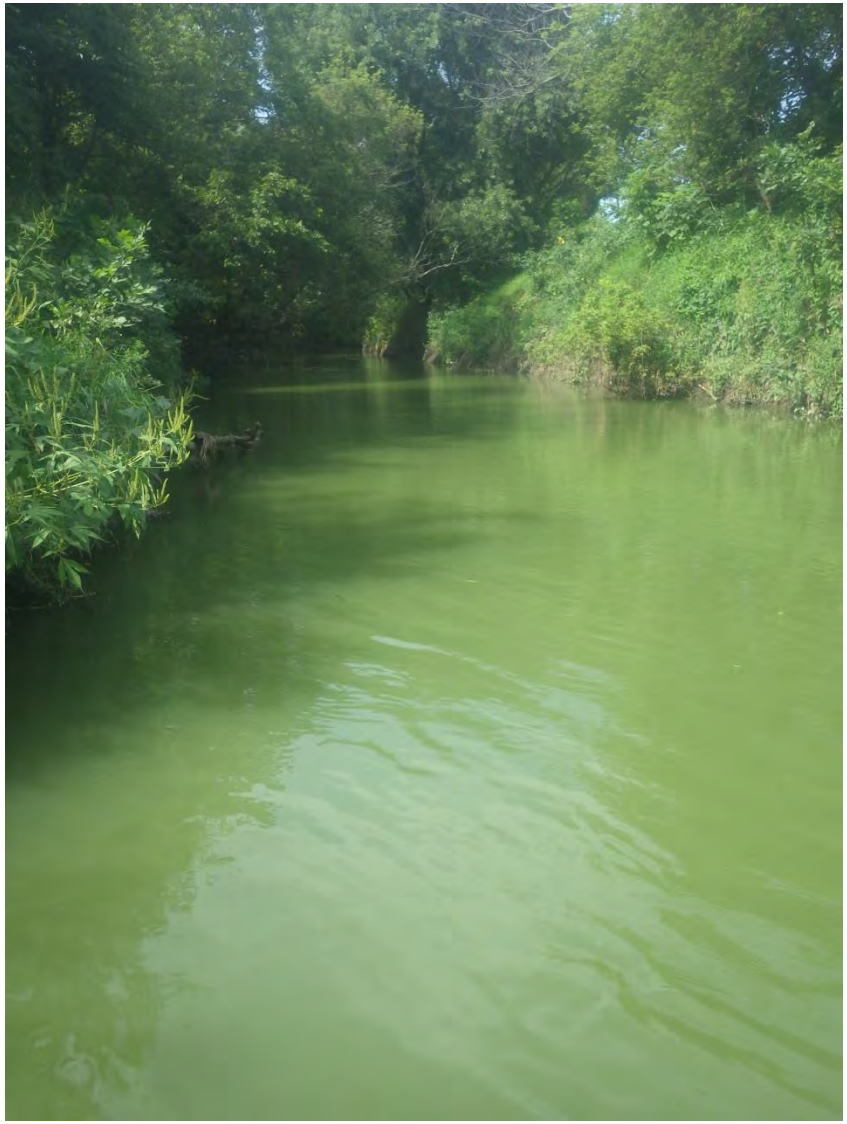


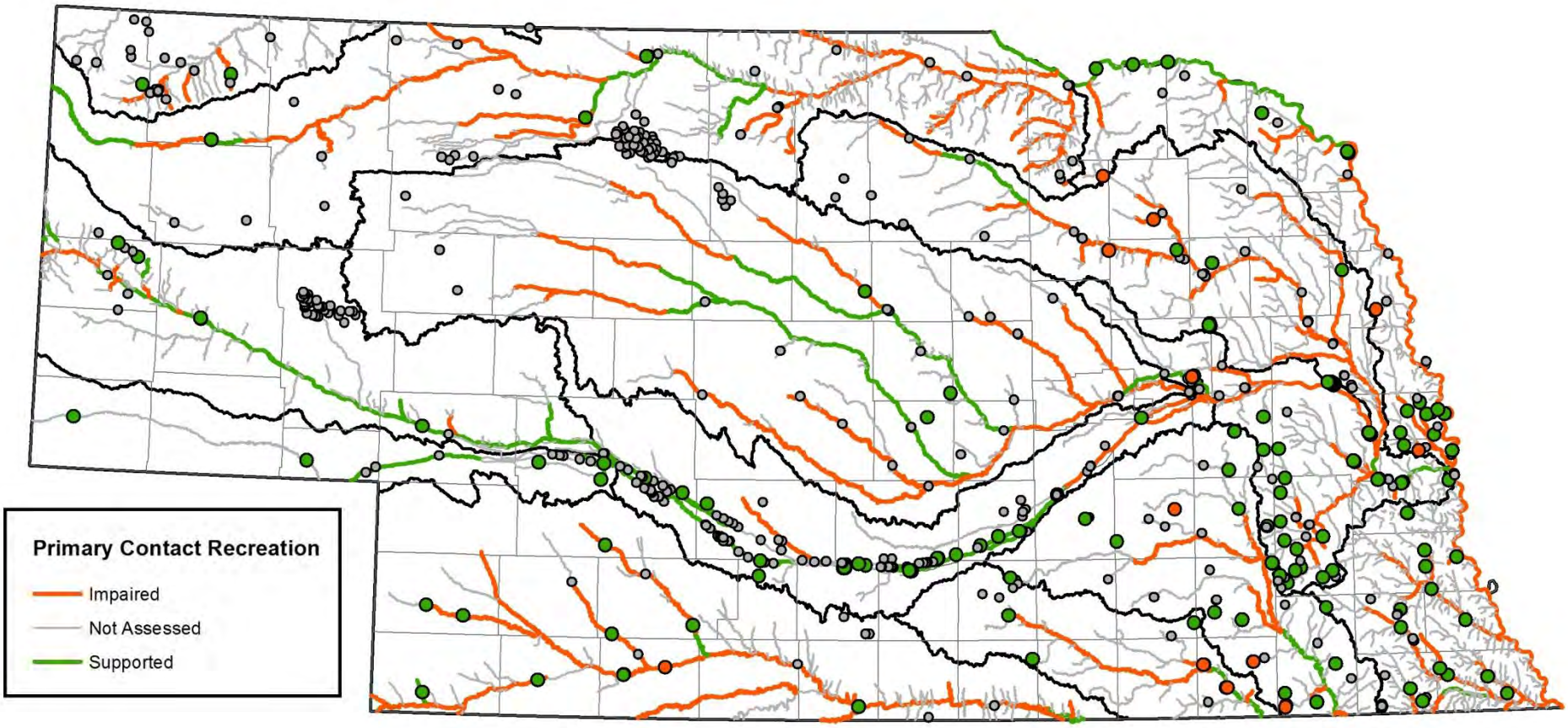
Photo: inaturalist.org

Part 2 Summary

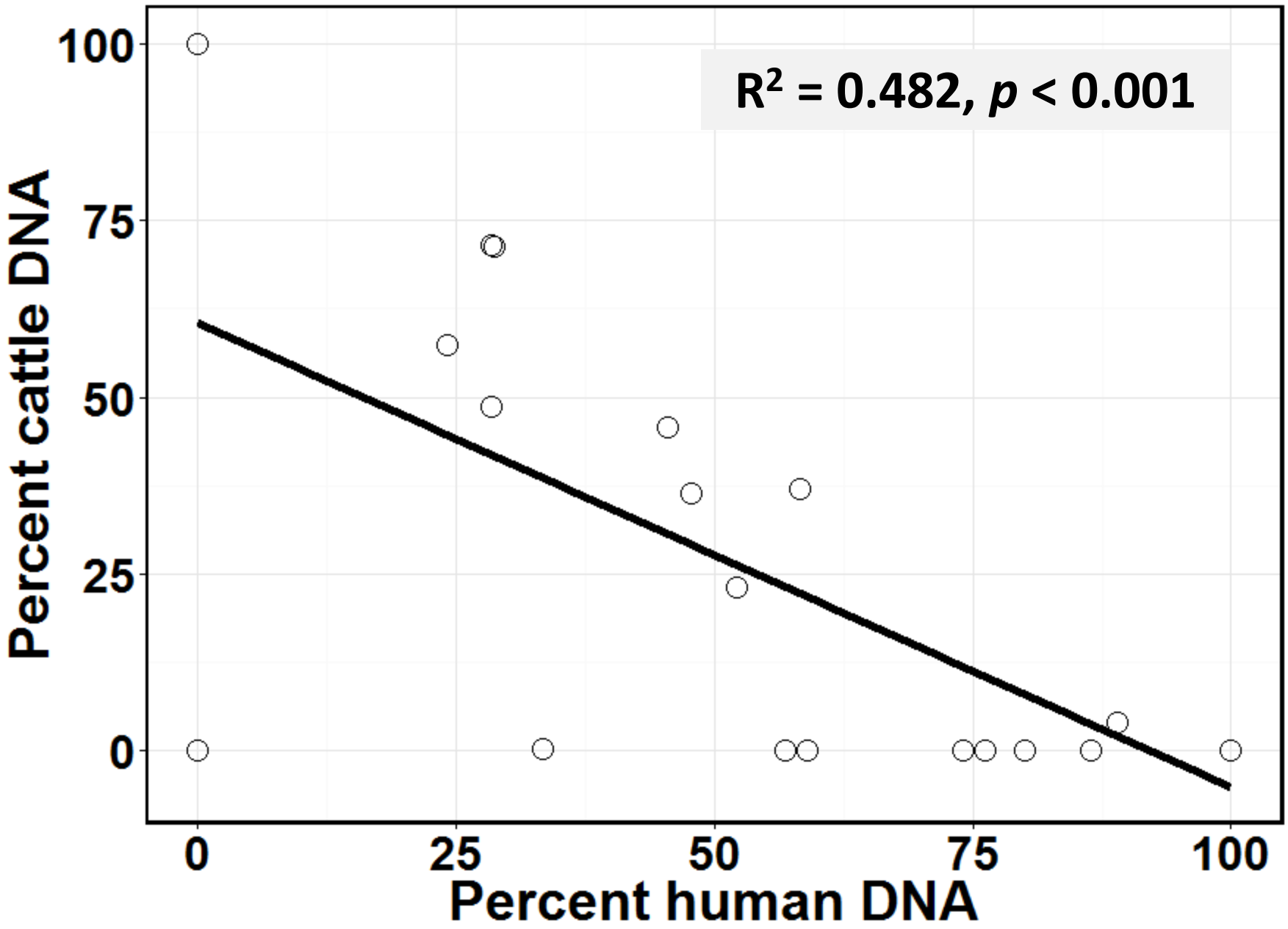
- eDNA sampling was fast and cheap
- Not prone to burial, herbivores, etc.
- Apparent community & population responses to N & P

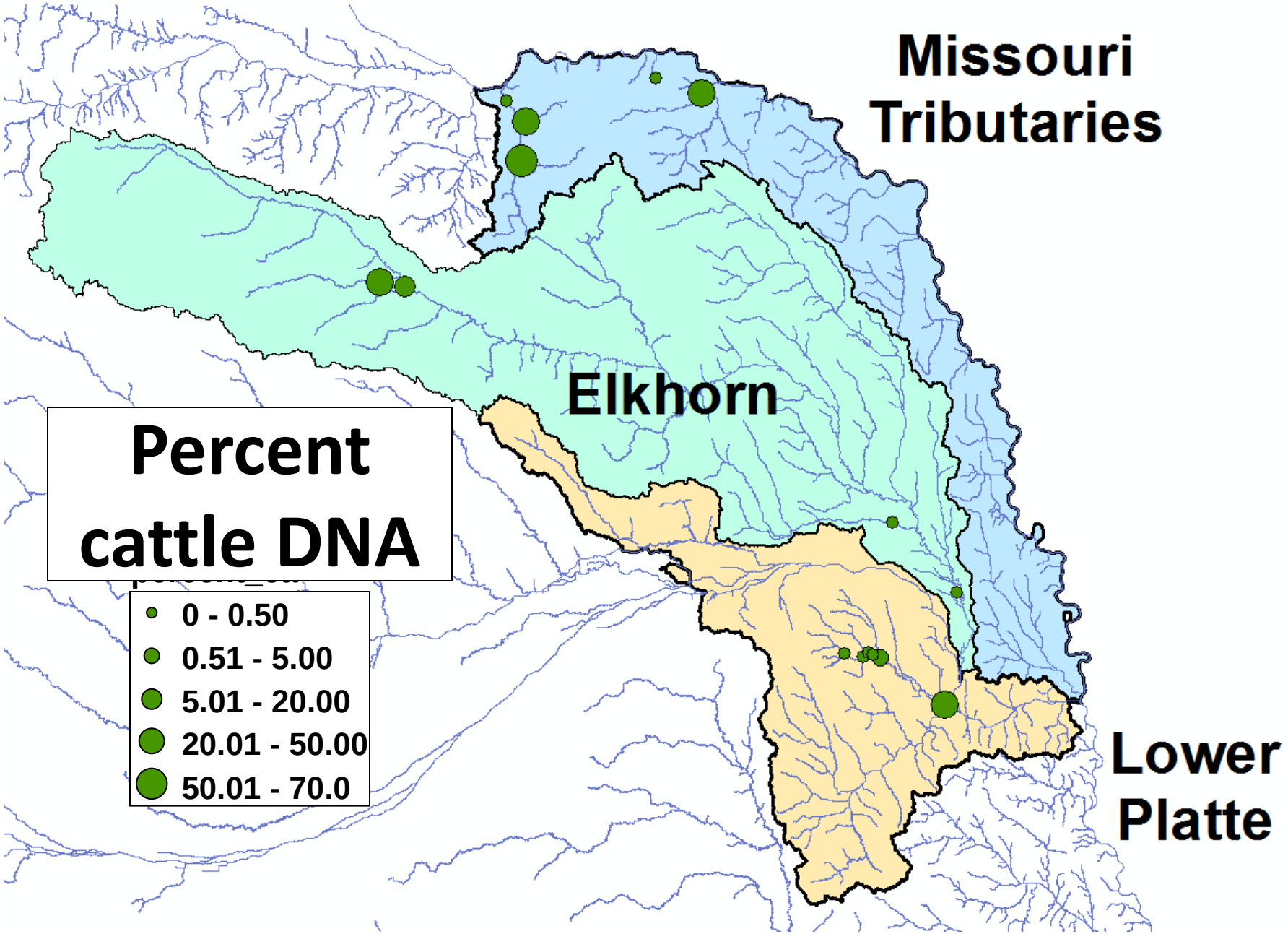


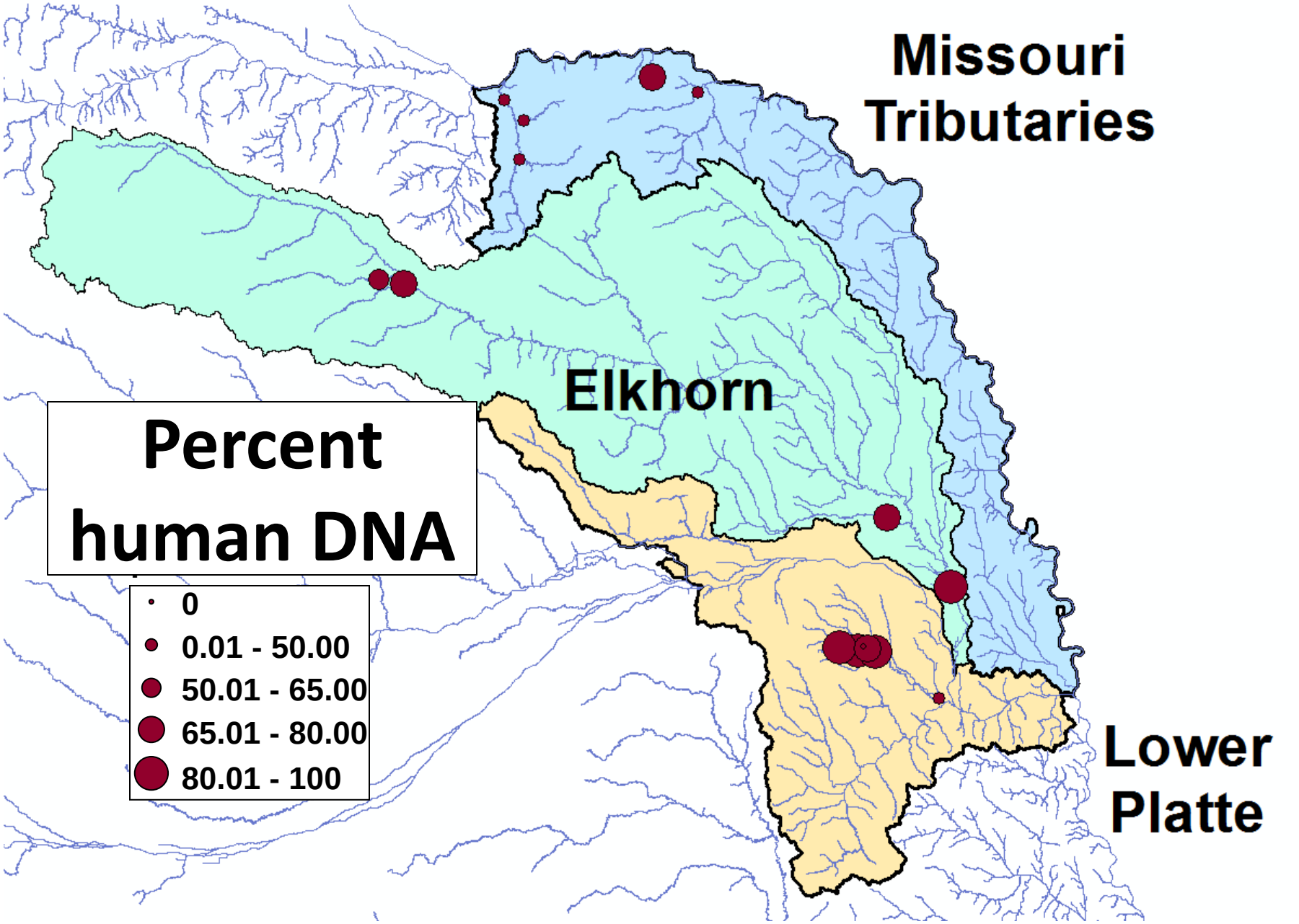
Part 3: eDNA as a Bacterial Tracer

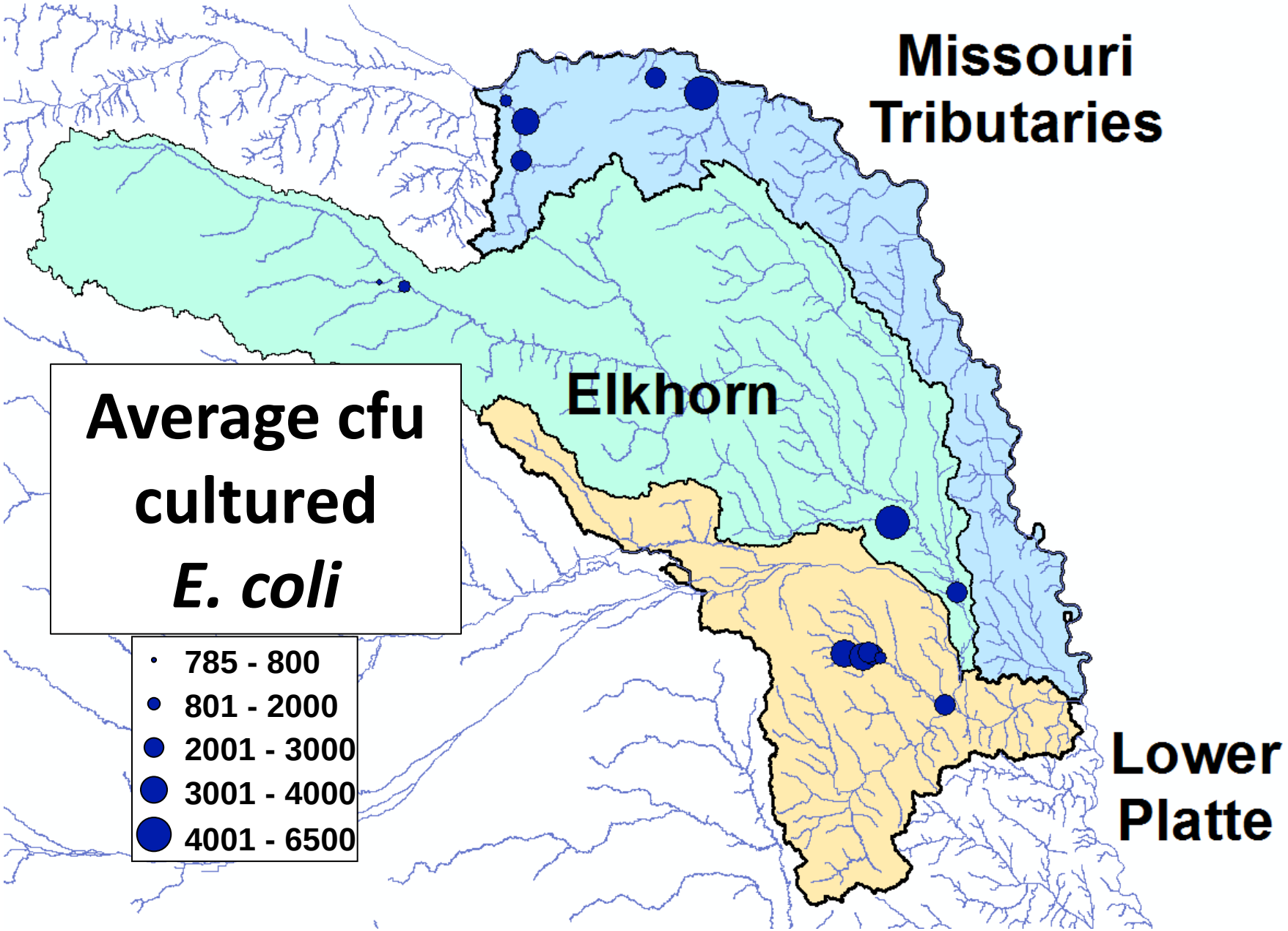


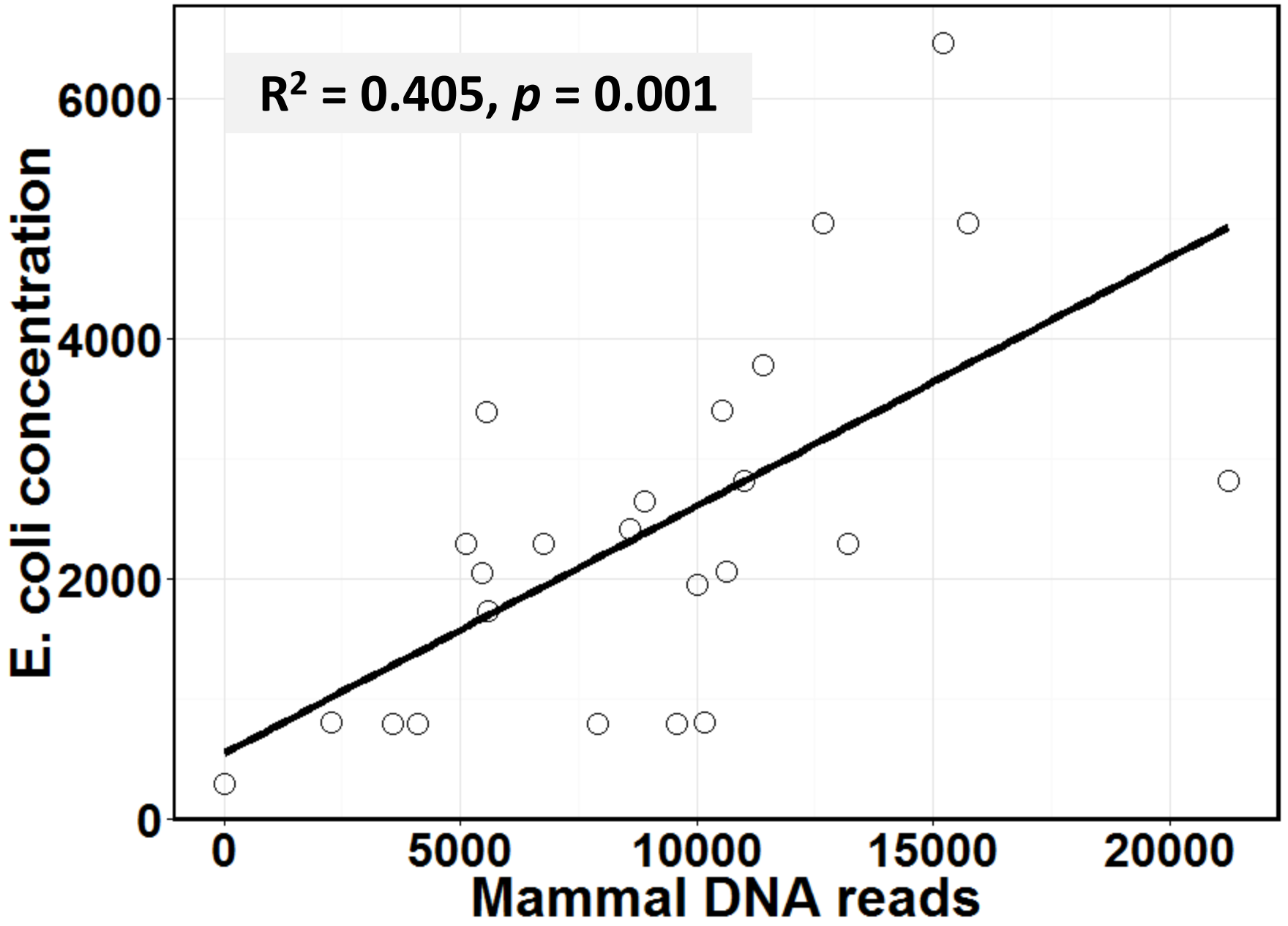
www.fishbio.com

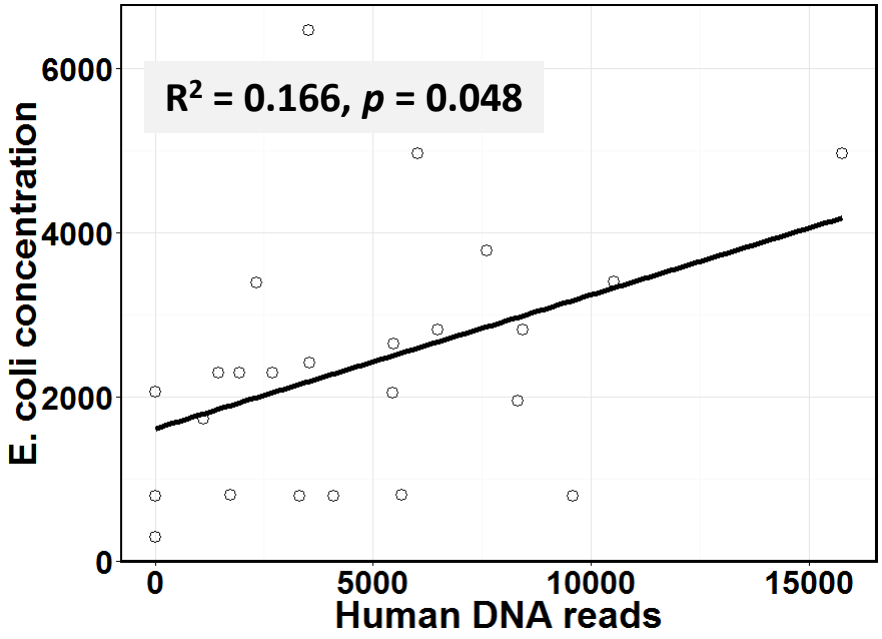

$R^2 = 0.482$, $p < 0.001$
Percent cattle DNA
100
75
50
25
0
0
25
50
75
100
Percent human DNA

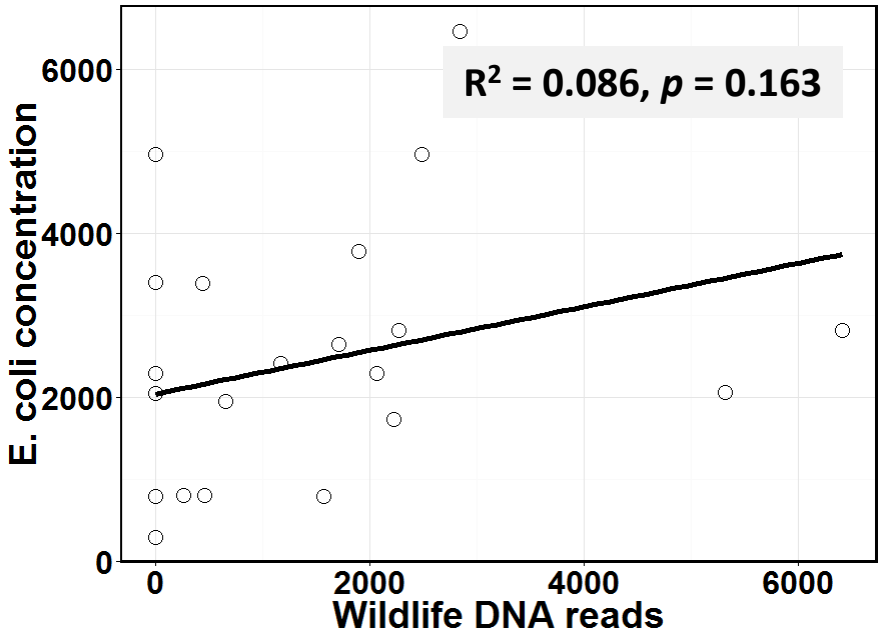

Missouri Tributaries
Elkhorn
Lower Platte
Percent cattle DNA
0 - 0.50
0.51 - 5.00
5.01 - 20.00
20.01 - 50.00
50.01 - 70.0

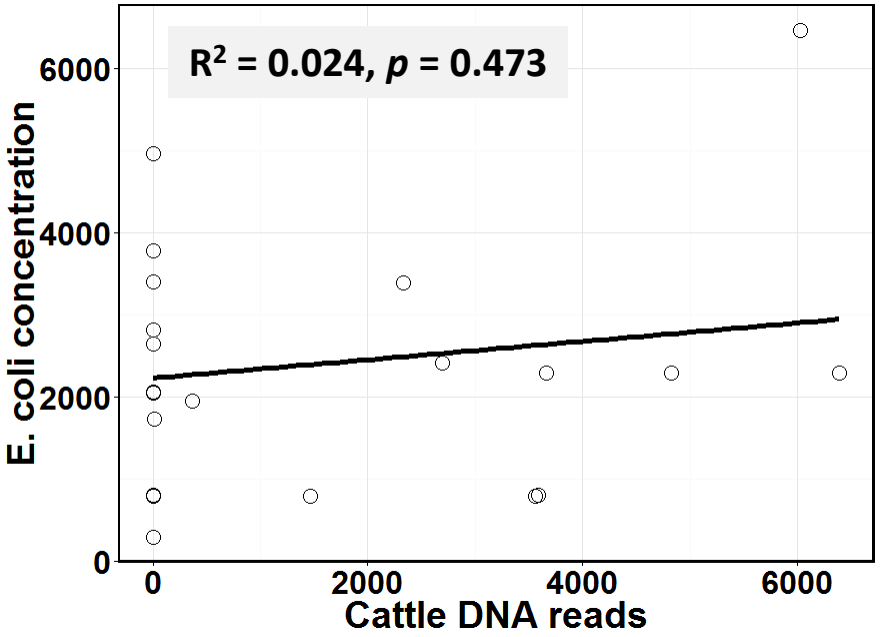

Missouri Tributaries
Elkhorn
Lower Platte
Percent human DNA
0
0.01 - 50.00
50.01 - 65.00
65.01 - 80.00
80.01 - 100


Missouri Tributaries
Elkhorn
Lower Platte
Average cfu cultured *E. coli*
785 - 800
801 - 2000
2001 - 3000
3001 - 4000
4001 - 6500

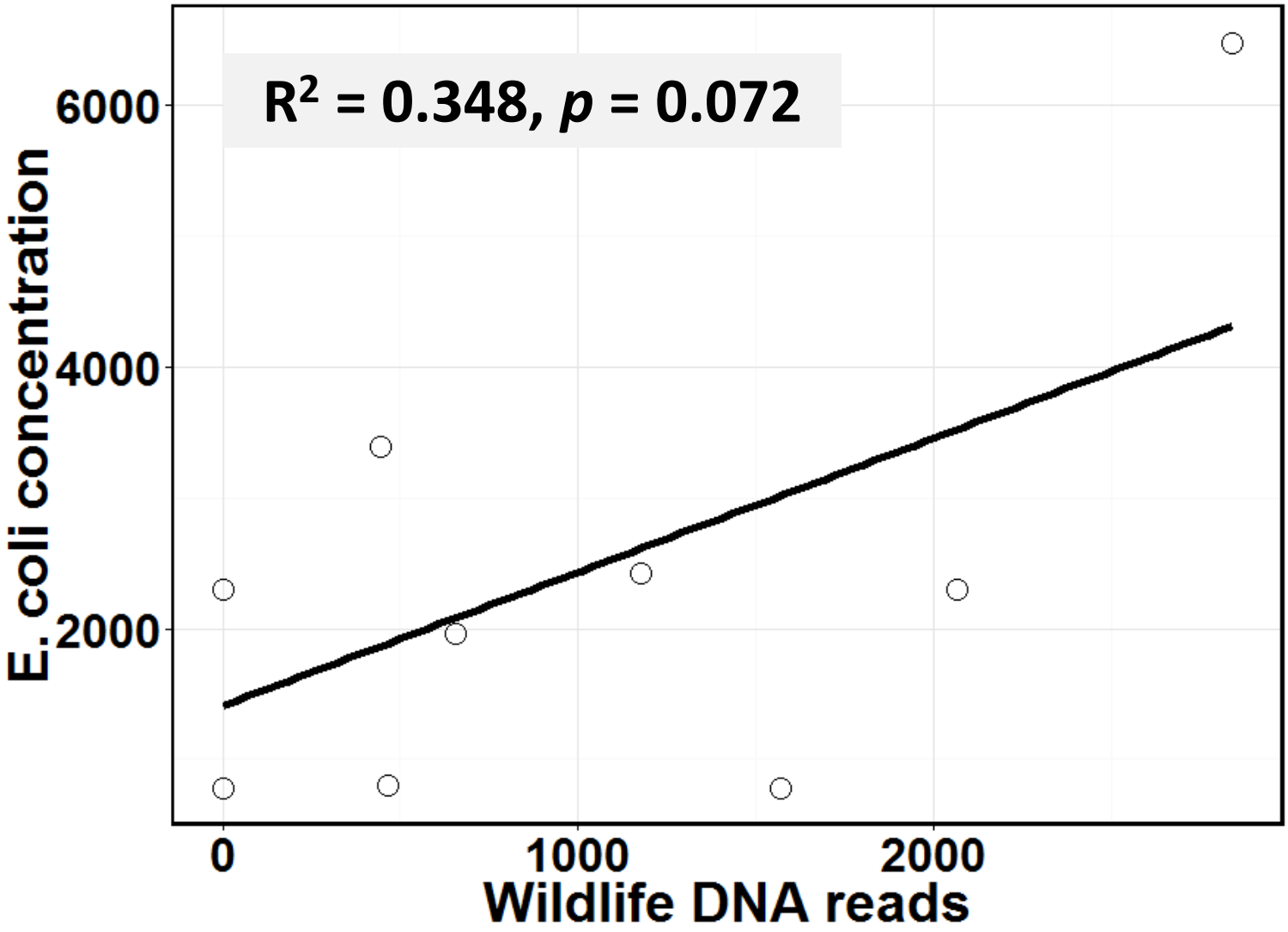

R^2 = 0.405, *p* = 0.001
E. coli concentration
6000
4000
2000
0
0
5000
10000
15000
20000
Mammal DNA reads


R² = 0.166, p = 0.048
E. coli concentration
Human DNA reads
0
2000
4000
6000
0
5000
10000
15000


R² = 0.086, p = 0.163
E. coli concentration
Wildlife DNA reads
0
2000
4000
6000
0
2000
4000
6000


R² = 0.024, p = 0.473
E. coli concentration
Cattle DNA reads
0
2000
4000
6000
0
2000
4000
6000

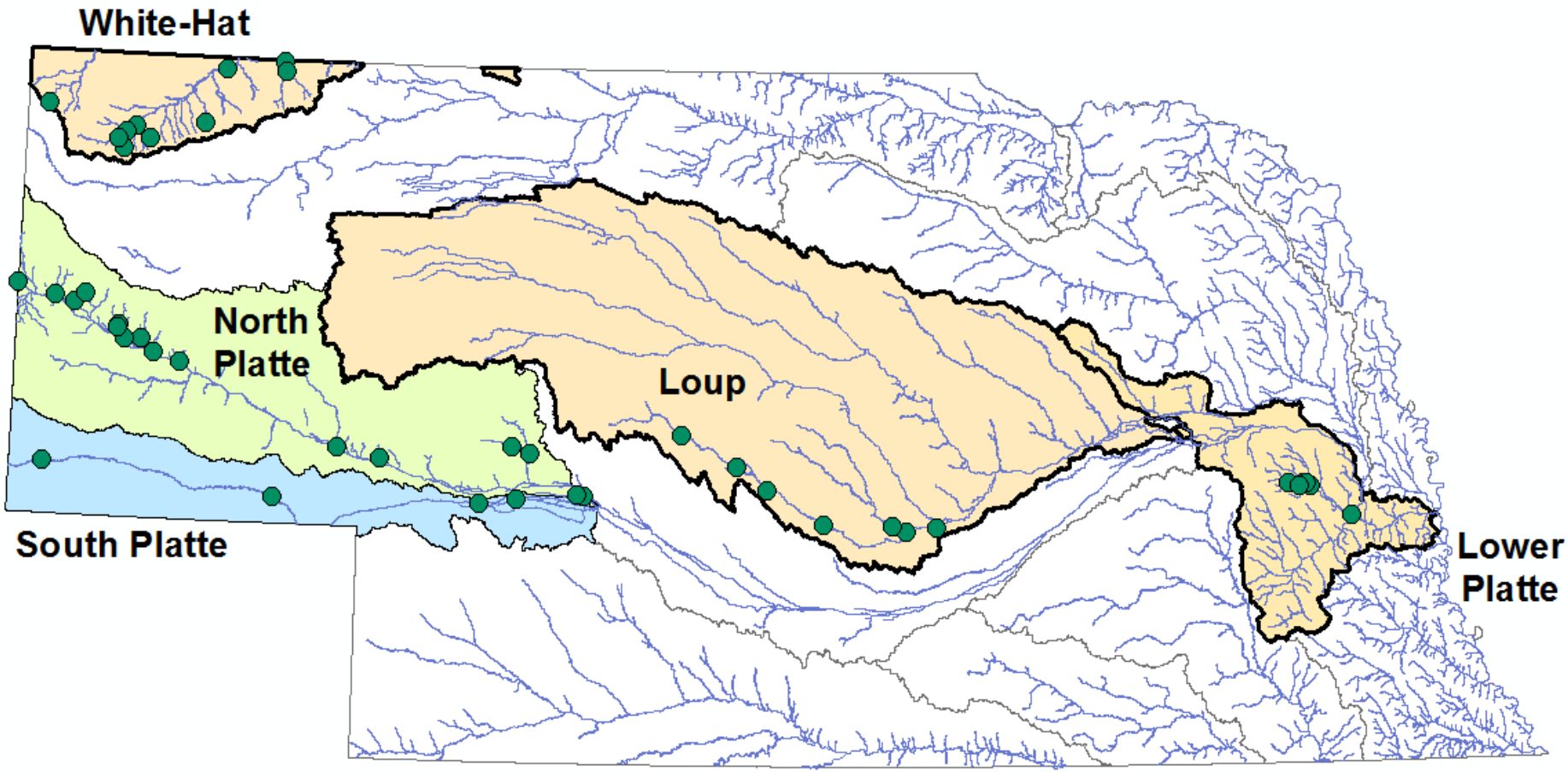
Only the “cattle” sites



Part 3 Summary

- More *E. coli* in streams with more human DNA
- Those streams do not have wastewater inputs

2017 eDNA Sample Locations



2017 Sample Design

- More sites, more gloves
- Mix of spatial and temporal sampling
- Sampling during rain events
- Mix of land use: crops, pastures, feed lots, parks

outdoornebraska.gov

Questions?