Degradation of eDNA Materials Associated with Invasive Bigheaded Carp (*Hypophthalmichthys nobilis and H. molitrix*)

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BACKGROUND

1. Environmental DNA (eDNA) surveys experiencing rapid growth in interest and application.
2. Better information = more informed application, more powerful conclusions
3. Need to understand better:
   a. Loading
   b. Form
   c. Dispersion Rates
   d. Dispersion Patterns
   e. Abiotic Degradation
   f. Biotic Degradation
   g. Sorption and Desorption
4. eDNA degradation is a primary mechanism limiting the detection of rare species using eDNA techniques.

BACKGROUND

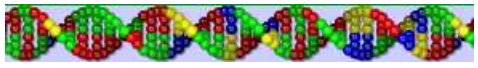
Degradation = Natural decay or destruction of eDNA such that the amount of intact marker DNA is diminished

Abiotic

- UV sunlight
- Harsh environmental chemistry
  - pH
- Heat
- Turbulence

Biotic

- Microbial exonucleases
  - *Affected by*
    - Heat
    - Light
    - pH



Controlled Studies

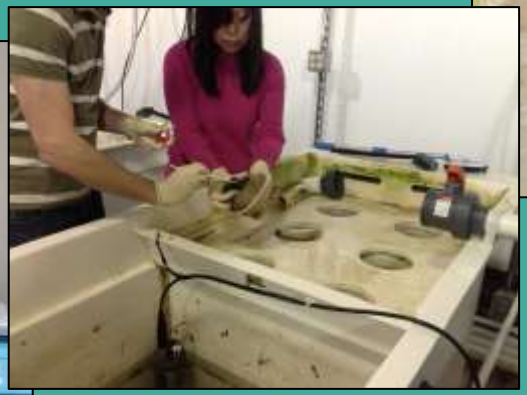
1. Baseline degradation
2. ~~Turbulence~~
3. Temperature
4. pH
5. Microbial load
6. Combined (most vs. baseline vs. least degradative)

In part based on environmental parameters from Chicago Area Waterway System (CAWS)

Baseline Degradation

Basic Protocol

- **Collect bighead carp “slurry”**
- **Dilute 3 g in 50 ml DI water**
  - **Working stock**
- **Add 2 ml working slurry to 12 ml DI water in 15 ml tube**
- **Low shake (66 rpm) at 20° C in dark for 28 days**

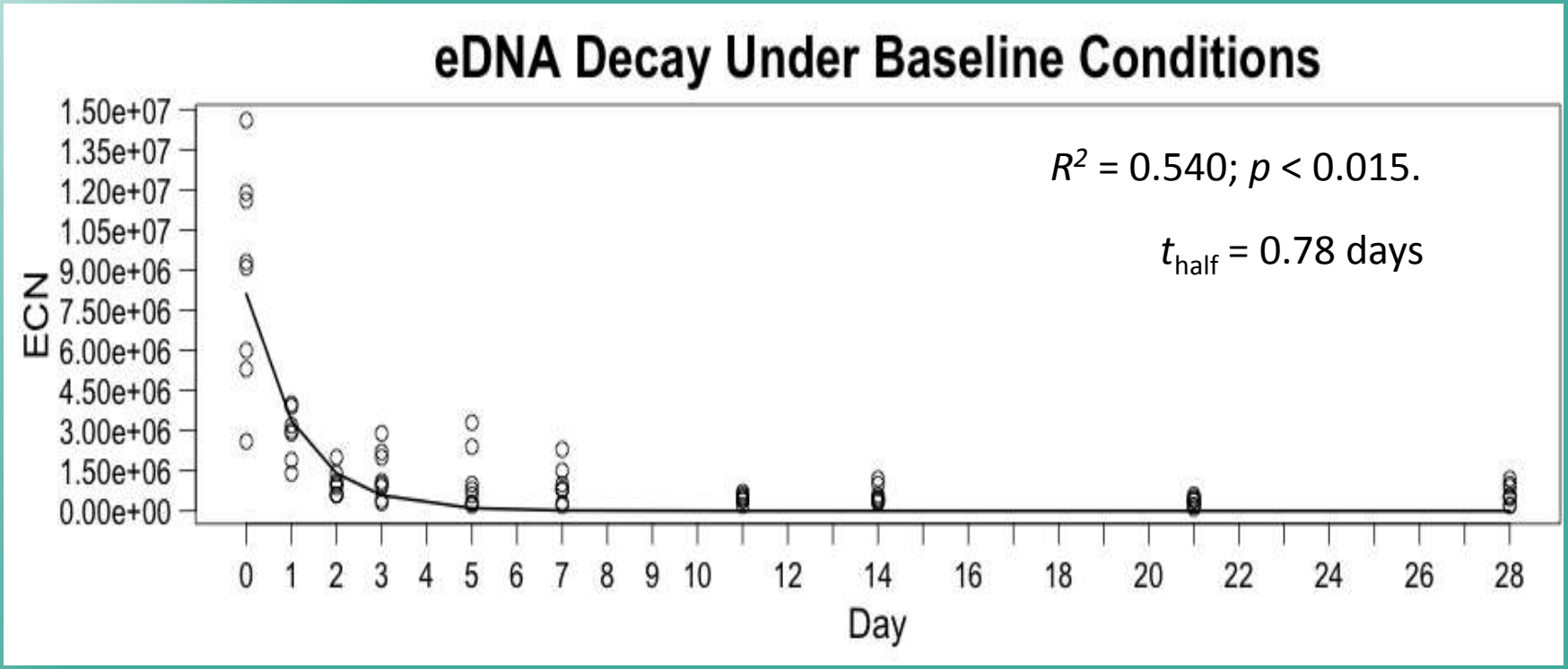


Baseline Degradation

Basic Protocol

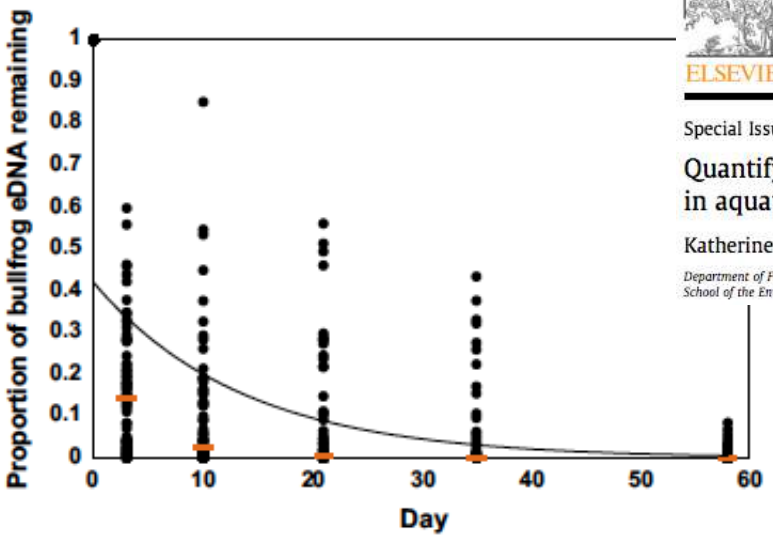
- Randomly selected 8 tubes and 1 water blank tube at Days 0, 1, 2, 3, 5, 7, 10, 14, 21, and 28
- Centrifuged tubes at 4° C for 15 min. and decanted water
- Stored pellet at -20° C
- Extracted DNA with CTAB method, eluted in 100 µl DI water
- Conducted qPCR with TaqMan marker UMESC_HN
- Used TaqMan® Environmental Master Mix 2.0
  - 3 replicate qPCRs per sample and control (different plate each)

Baseline Degradation



ECN = Estimated Copy Number

BACKGROUND (cont.)



Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

ELSEVIER

Special Issue Article: Environmental DNA

Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms

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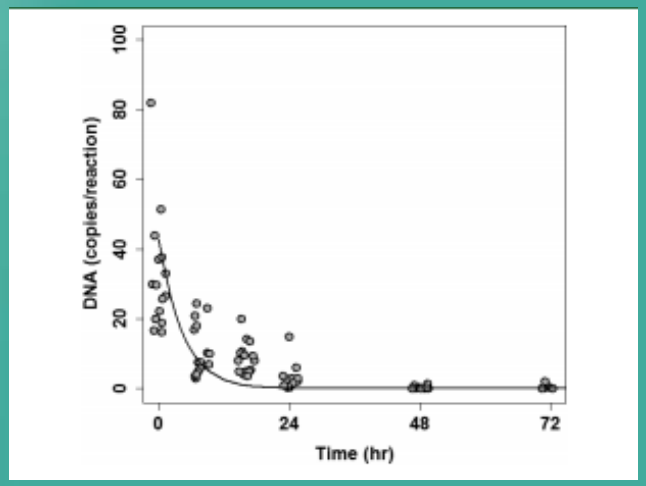
2014



Environmental Science & Technology

2014

Article

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Environmental Conditions Influence eDNA Persistence in Aquatic Systems

Matthew A. Barnes,*[,†] Cameron R. Turner,[†] Christopher L. Jerde,[†] Mark A. Renshaw,[†] W. Lindsay Chadderton,[‡] and David M. Lodge[†]

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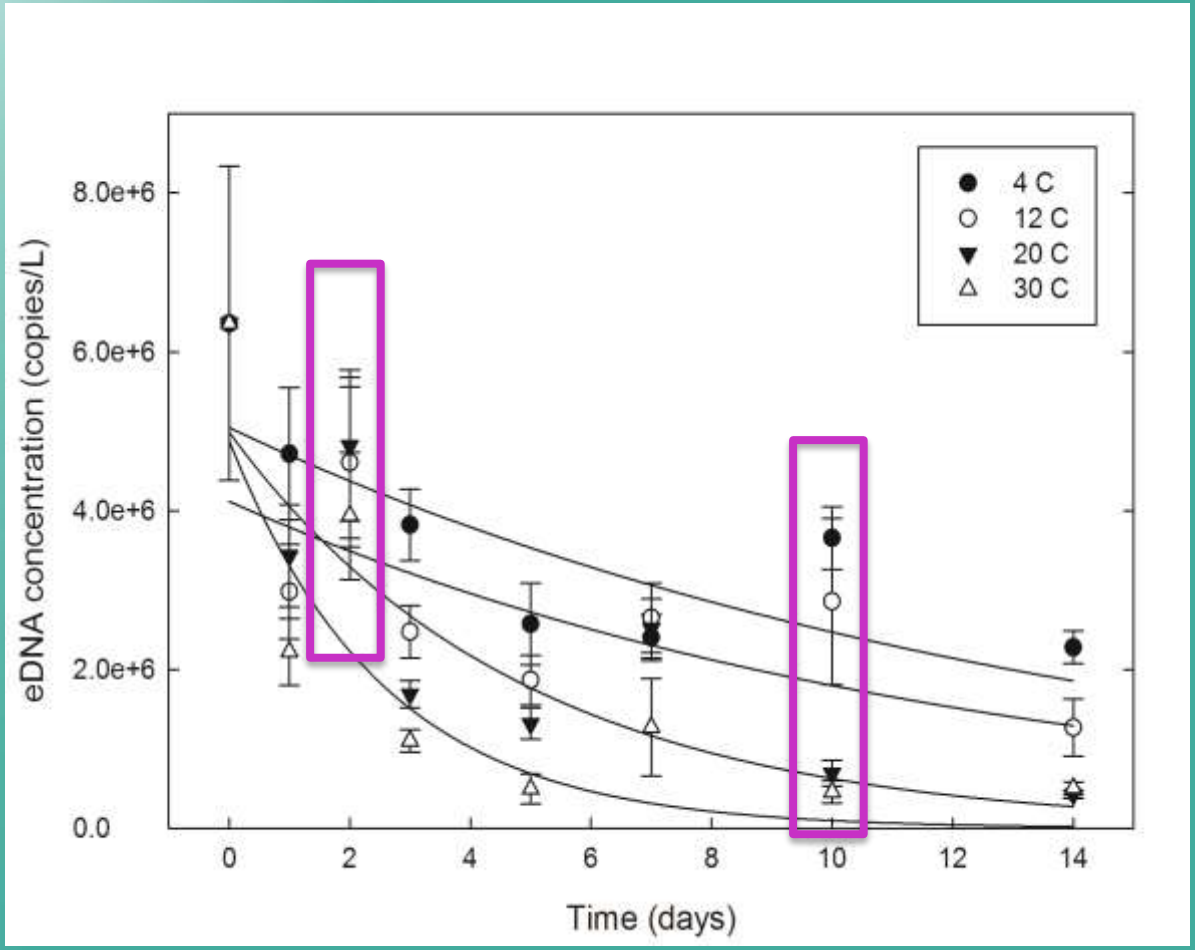
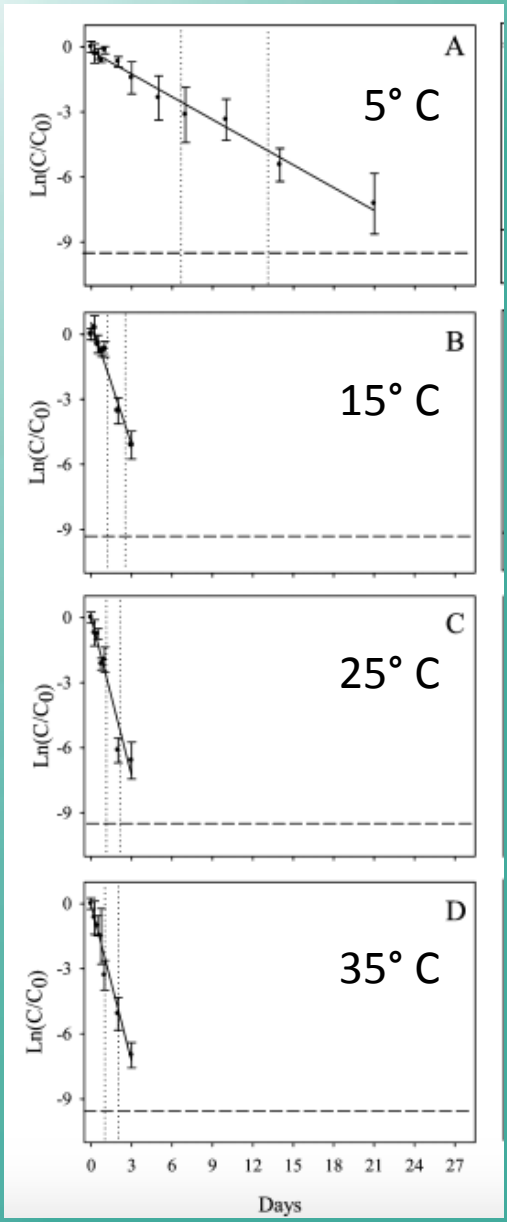
Temperature

- R^2 = 0.458-0.795, all $p < 0.001$



- 4° C t_{half} = 9.7 days
- 12° C t_{half} = 8.3 days
- 20° C t_{half} = 3.3 days
- 30° C t_{half} = 1.8 days

N = 64 tubes/ treatment



2016

Environmental Science & Technology

Article

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Effects of Temperature and Trophic State on Degradation of Environmental DNA in Lake Water

Jessica J. Eichmiller,* Sendréa E. Best, and Peter W. Sorensen

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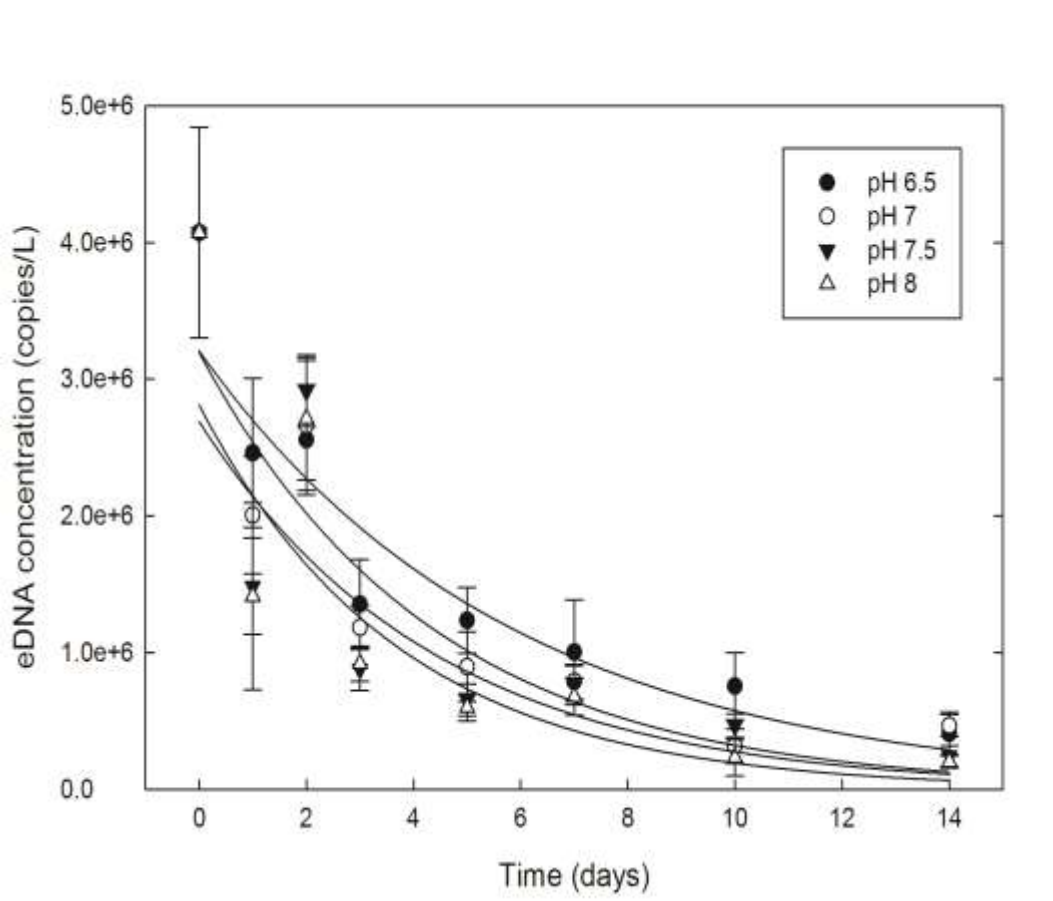
pH

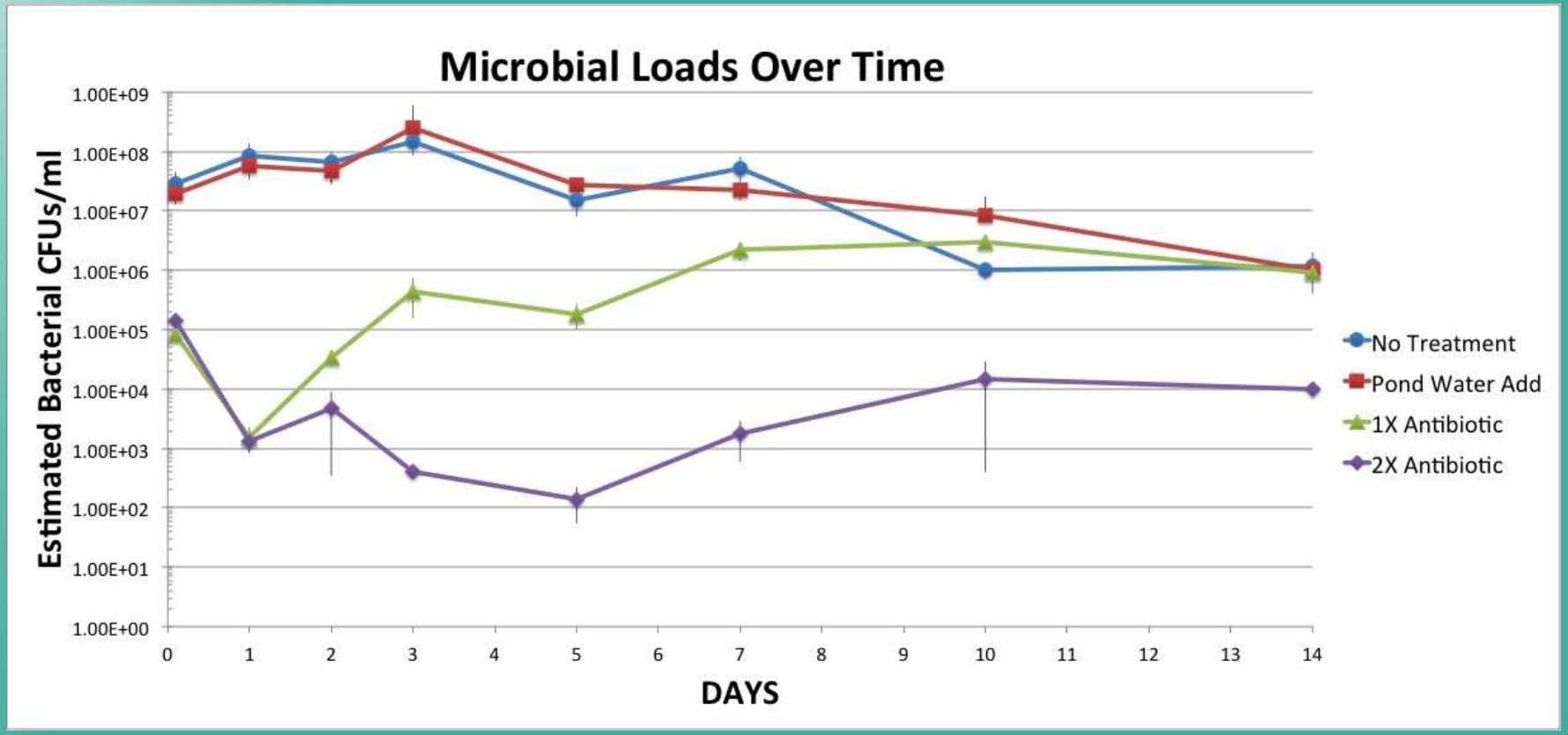
- R^2 = 0.694-0.890, all $p < 0.02$



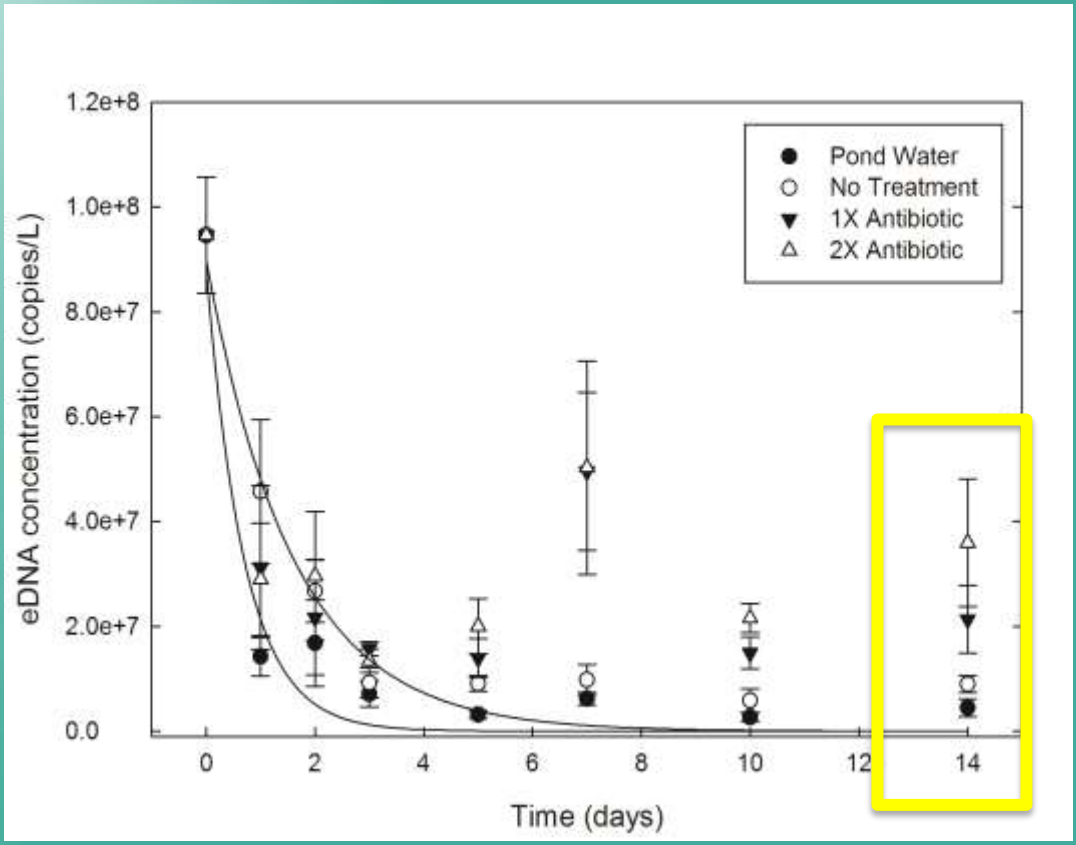
- **pH 6.5 t_{half} = 4.0 days**
- **pH 7.0 t_{half} = 3.0 days**
- **pH 7.5 t_{half} = 3.0 days**
- **pH 8.0 t_{half} = 2.6 days**

N = 64 tubes/ treatment

Microbial Load



Microbial Load



No Treatment

$R^2 = 0.519$

$p < 0.02$

$t_{half} = 1.1$ days

Pond Water Added

$R^2 = 0.506$

$p < 0.02$

$t_{half} = 0.5$ days

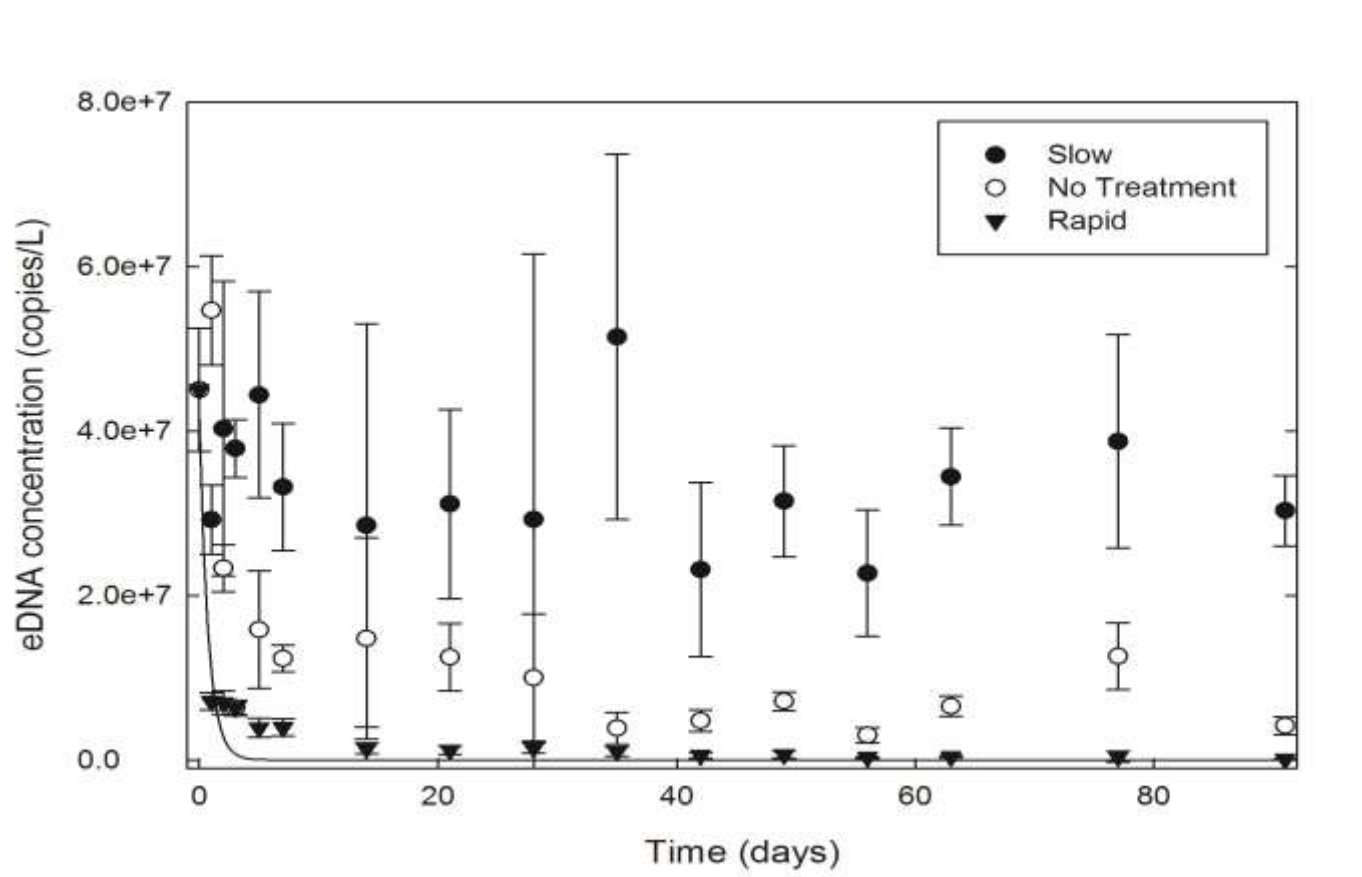
N = 64 tubes/ treatment

Slow vs. Rapid Degradation

- 91 day trial
- 3 Treatment Classes
  - Slow degradation: 4° C, pH = 6.5, 2X Antibiotics
  - Baseline: 20° C, pH unregulated, no antibiotics
  - Rapid degradation: 30° C, pH = 8, pond water added
- Sampling points:

  Days 0, 1, 2, 3, 5, 7, 14, 21, 28, 35, 42, 49, 56, 63, 77, 91

Slow vs. Rapid Degradation



Lack of fits to exponential decay

Rapid Decay

$R^2 = 0.647$

$p < 0.003$

$t_{half} = 12$ hours

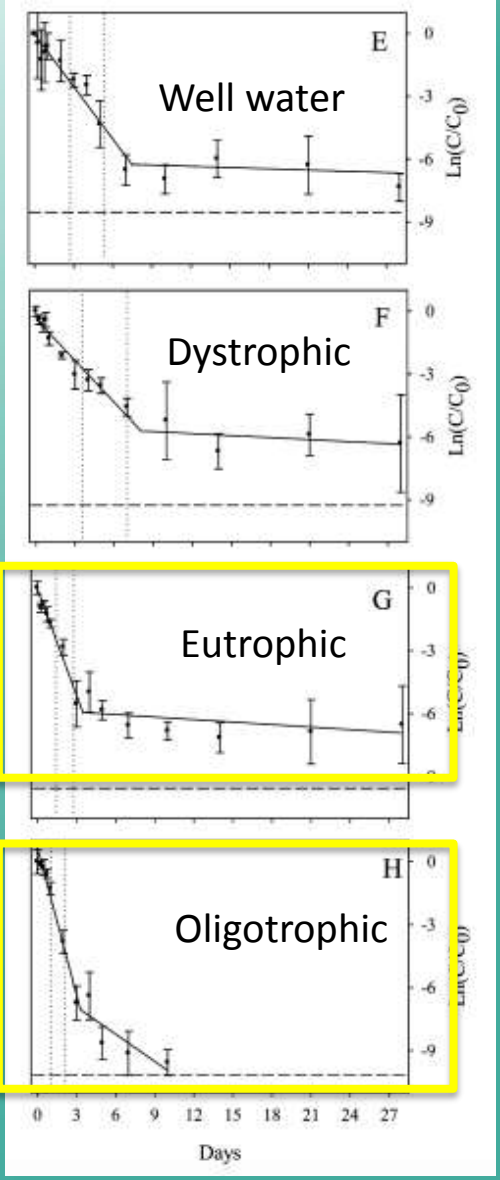
- **Slow decay samples lost about 30% of eDNA at 91 days**
- **Untreated samples lost about 90% of eDNA at 91 days**
- **Rapid decay samples lost 98-100% of eDNA at 91 days**

Conclusions

- Turbulence had no effect
- Temperature differences had strong effect
  - Relatively very slow decay at 4° C
  - Relatively very rapid at 30° C
  - Alters microbial activity?
    - Seems to be consensus
- Microbial load had strong, if messy, effect
  - 1X and 2X treatments much reduced degradation
- pH (CAWS range) had small effect

Conclusions (cont.)

- Rapid decay in first 24-48 hours
- Potential for long-term persistence of some eDNA?
  - How to discriminate persistent fraction from low abundance?
- Lack studies of absence following long-term residence



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Effects of Temperature and Trophic State on Degradation of Environmental DNA in Lake Water

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Department of Fisheries, Wildlife, and Conservation Biology, Minnesota Aquatic Invasive Species Research Center, University of Minnesota, Twin Cities, Saint Paul, Minnesota 55108, United States

- Role for sorption
  - And desorption
- Where is the eDNA flotsam?
  - Floating?
  - Sinking?
  - Both?
- How big is the eDNA bank?
- What layers, microhabitats to sample?

Conclusions (cont.)

- Rapid decay in first 24-48 hours
- Potential for long-term persistence of some eDNA?
  - How to discriminate persistent fraction from low abundance?
- Lack studies of absence following long-term residence

Acknowledgments

Special appreciation for Cathy Richter
USGS CERC

CERC & Univ MO	***ERDC***
Duane Chapman	**Eli Navarro**
Craig Paukert	**Christine Edwards**
Nathan Thompson	**Denise Lindsay**
Marie Pope	**Alan Katzenmeyer**
Michelle Anderson	**Jan Hoover**
Haley Chapman	**Jack Kilgore**

Funded by: Great Lakes Restoration Initiative
via the Asian Carp Regional Coordinating Committee