

eDNA Science & Application

Detecting Species Sight Unseen

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DAVID R. ATKINSON CENTER
for a Sustainable Future
Cornell University

Indirect, DNA-based Species Detection









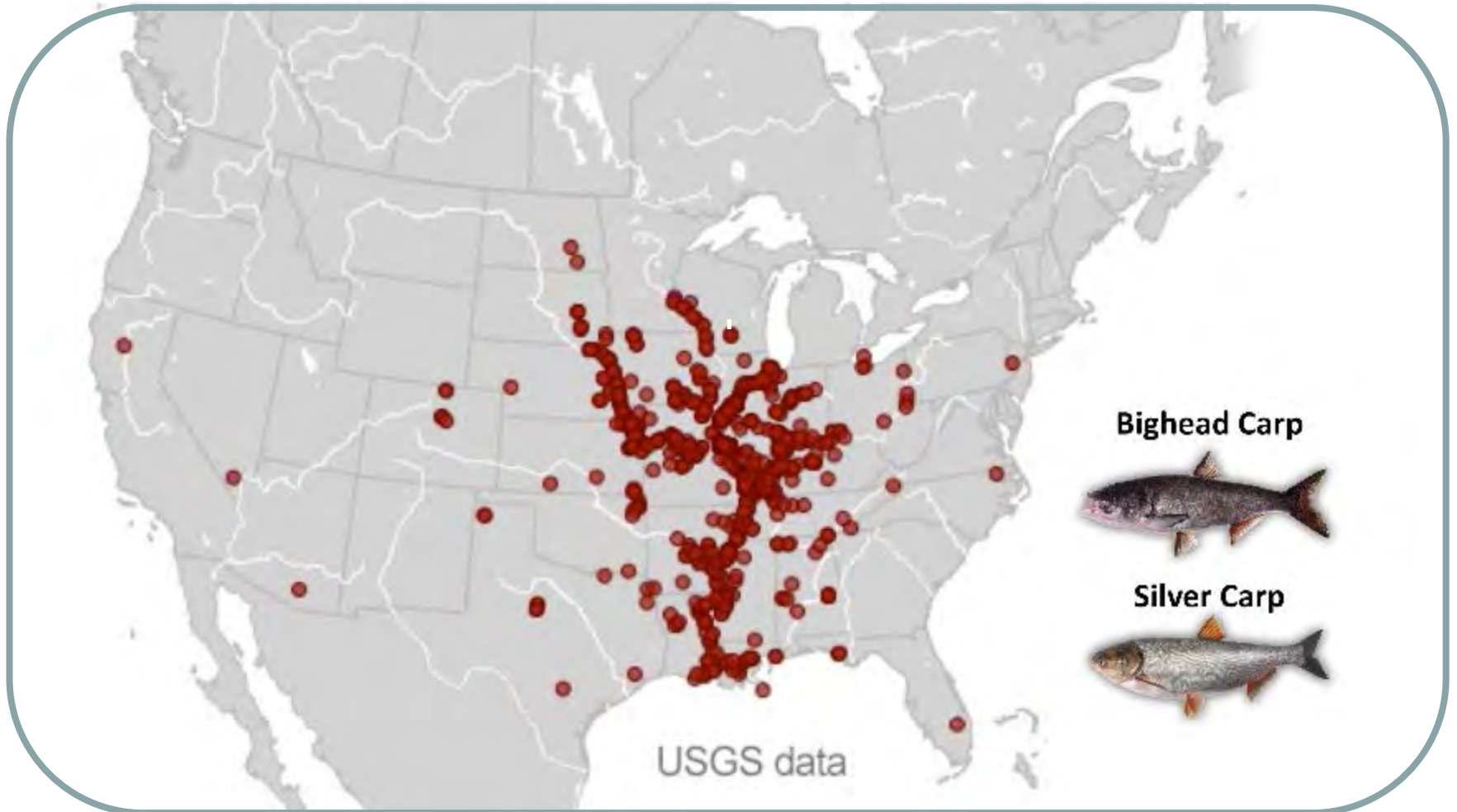
Asian Carps: Imminent Threat to Great Lakes

1975



Asian Carps: Imminent Threat to Great Lakes

2013



Chicago Sanitary & Ship Canal

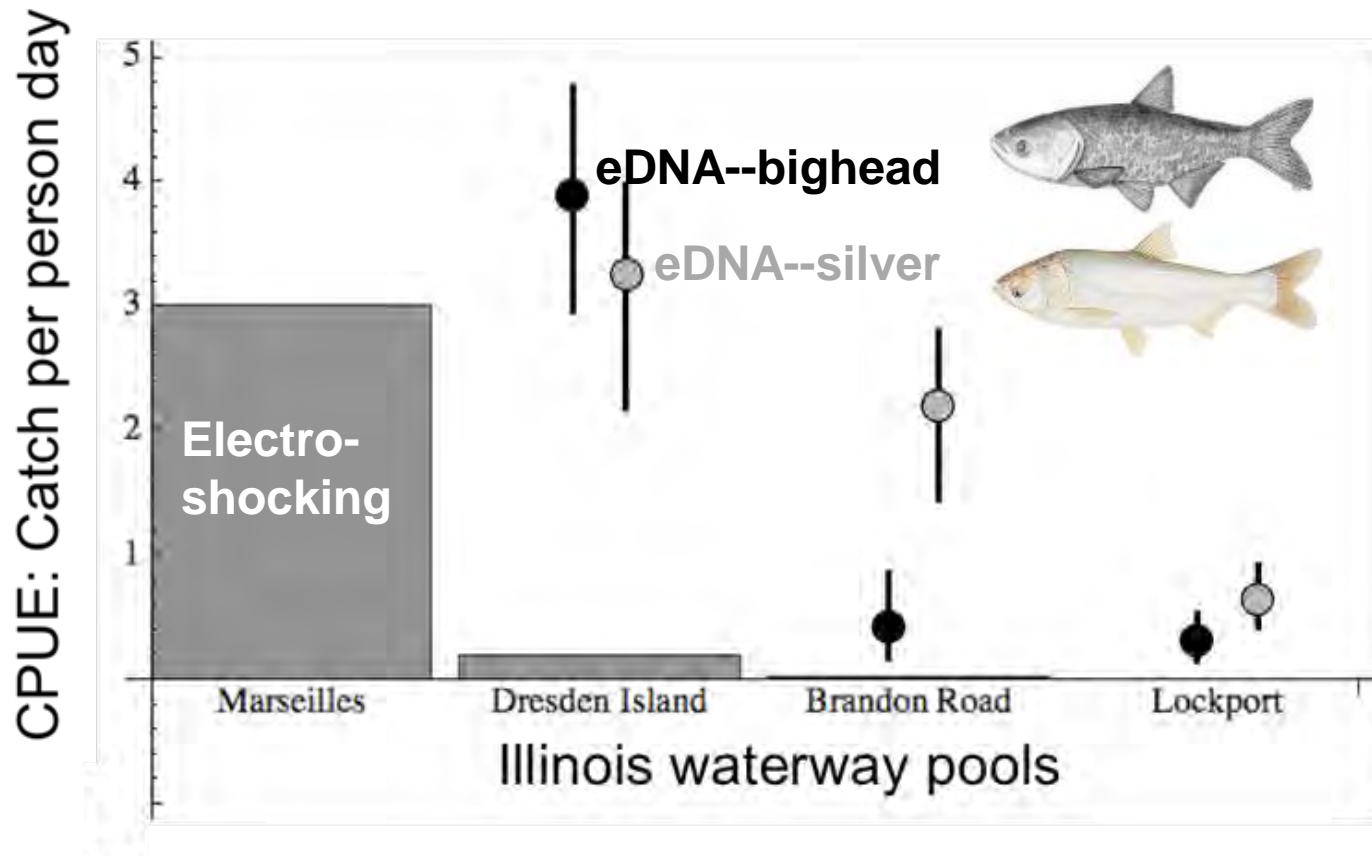
Mississippi River Basin



Canal opened ~1900



Silver and bighead carps CPUE: Electroshocking vs. eDNA



(Jerde et al 2011. Conservation Letters)

Potential Applications for eDNA

- 1. Species that are rare**
 - Incipient invasions—early detection-rapid response
 - Threatened, endangered species
- 2. Species that are difficult to sample with traditional tools**
- 3. Species for which handling causes harm**
- 4. Habitats in which traditional tools are difficult to deploy**
 - Limited access for boats and sampling gear
 - Strong currents
 - Interference with navigation, fishing, or other uses
- 5. Where/when “integrative” samples are cost effective**
 - “contaminant” species in bait trade, fish stocks, imports

Environmental DNA Overview

1

Collect
water
sample

2

Filter
water
sample

3

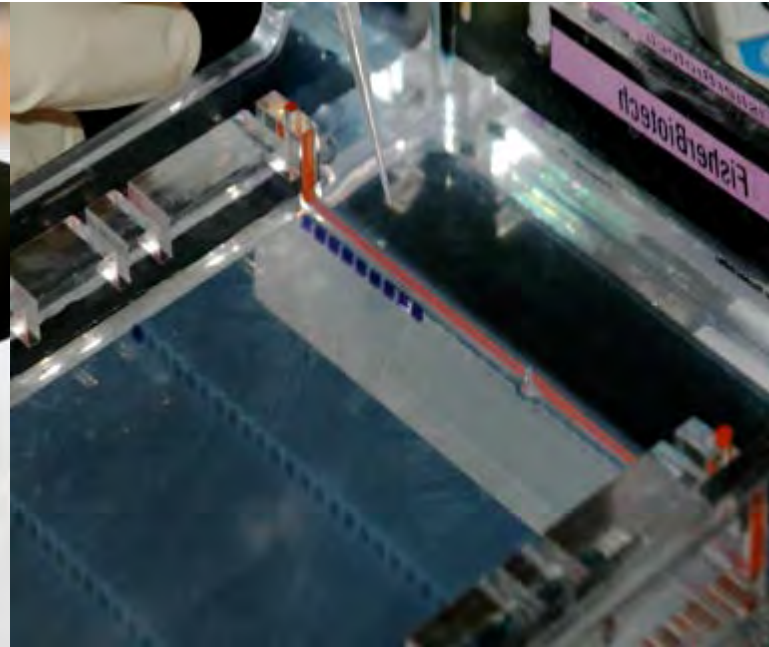
Extract
all DNA

4

Amplify
target
DNA

5

Visualize
DNA
presence



Environmental DNA Overview

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Environmental DNA Overview

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Environmental DNA Overview

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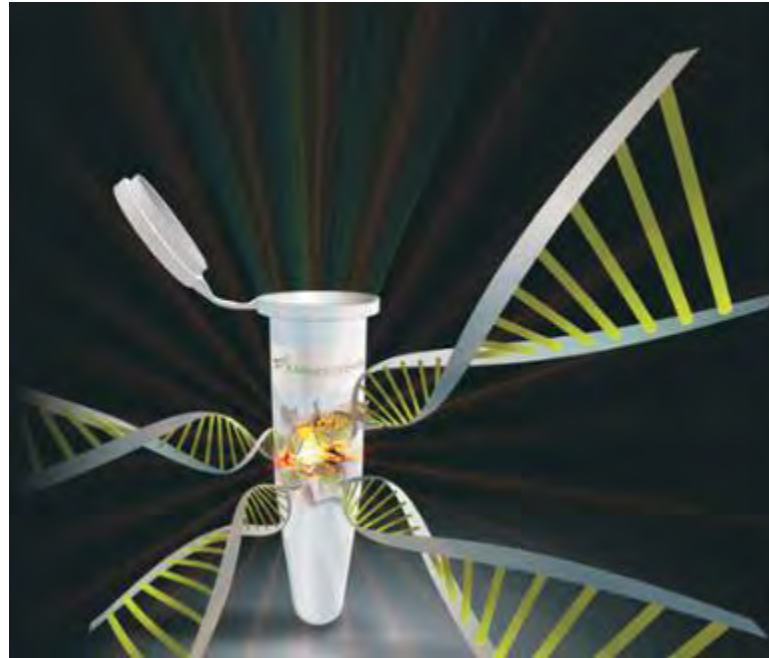
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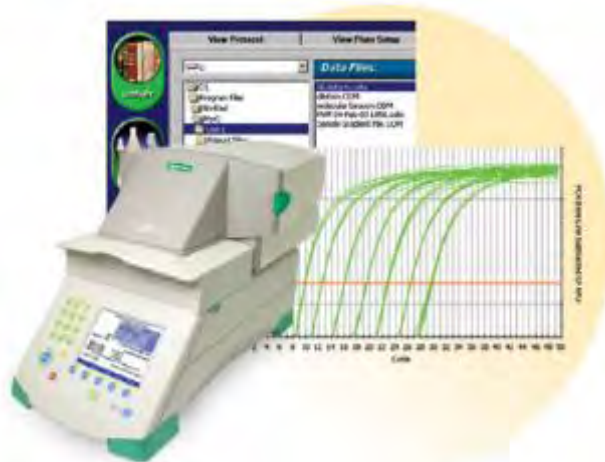
Environmental DNA Overview

- 1 Collect water sample
- 2 Filter water sample
- 3 Extract all DNA
- 4 Amplify target DNA

**Design primers for target DNA;
Polymerase Chain Reaction (PCR)**



PCR



**Quantitative
PCR**



**Digital
droplet PCR**

Environmental DNA Overview

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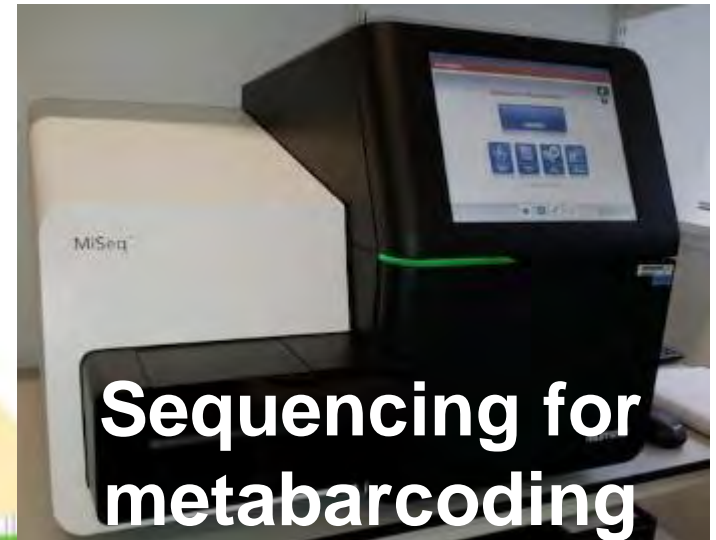
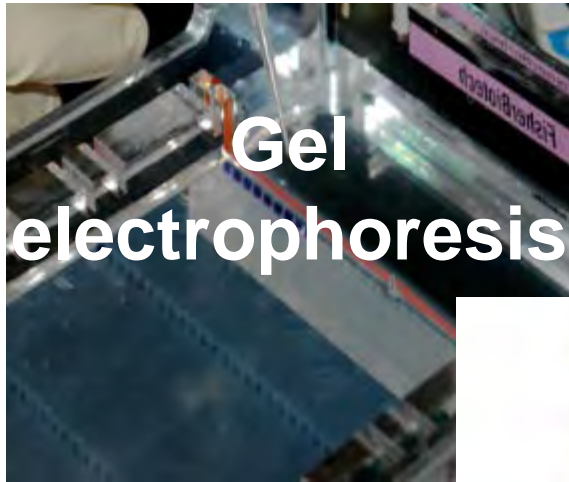
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**Digital readout for qPCR and ddPCR,
Laser Transmission Spectroscopy**

Environmental DNA Overview



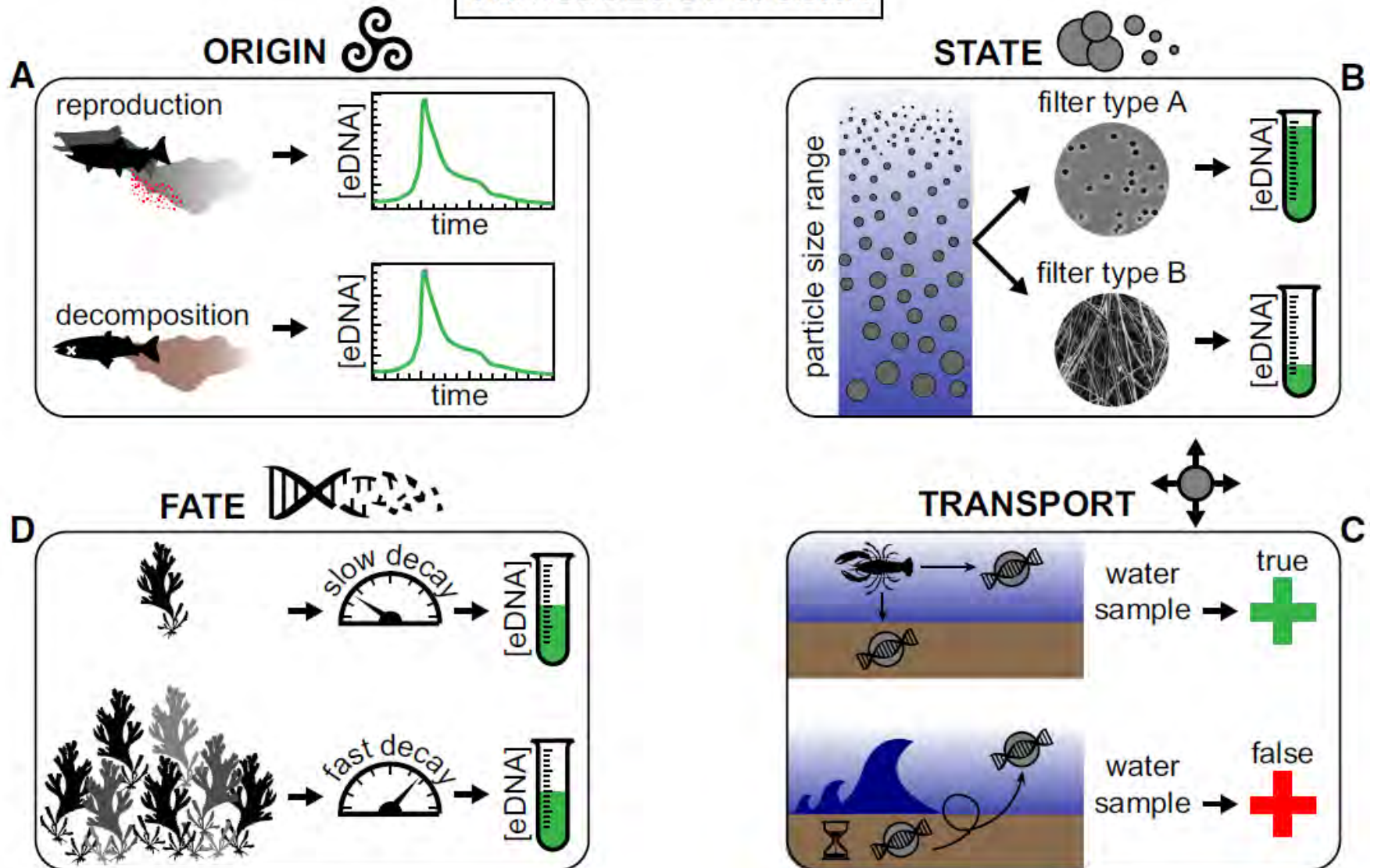
The more sensitive an assay, the greater the risk for false positives from contamination.

Meticulous care is required to reduce risk of contamination at every step in the field and laboratory.

- **Laboratory design to separate different processes**



THE ECOLOGY of eDNA



Sources of Asian Carp DNA in Canal (in the absence of live fish)?

- Ballast or bilge water from barges?**
- Dead fish kicked off of barges?**
- Sewage discharge from carp eating humans?**
- Feces from carp eating birds?**
- Overflow from Chicago ponds?**

None of these possibilities were plausible explanations for the overall temporal and spatial pattern of eDNA results.

Interpretations of eDNA results and implications for management

Strong

Multiple eDNA detections on multiple occasions, invasion pathway exists, historical capture record

Multiple eDNA detections on multiple occasions, invasion pathway exists

Multiple eDNA detections from one sample event, invasion pathway exists

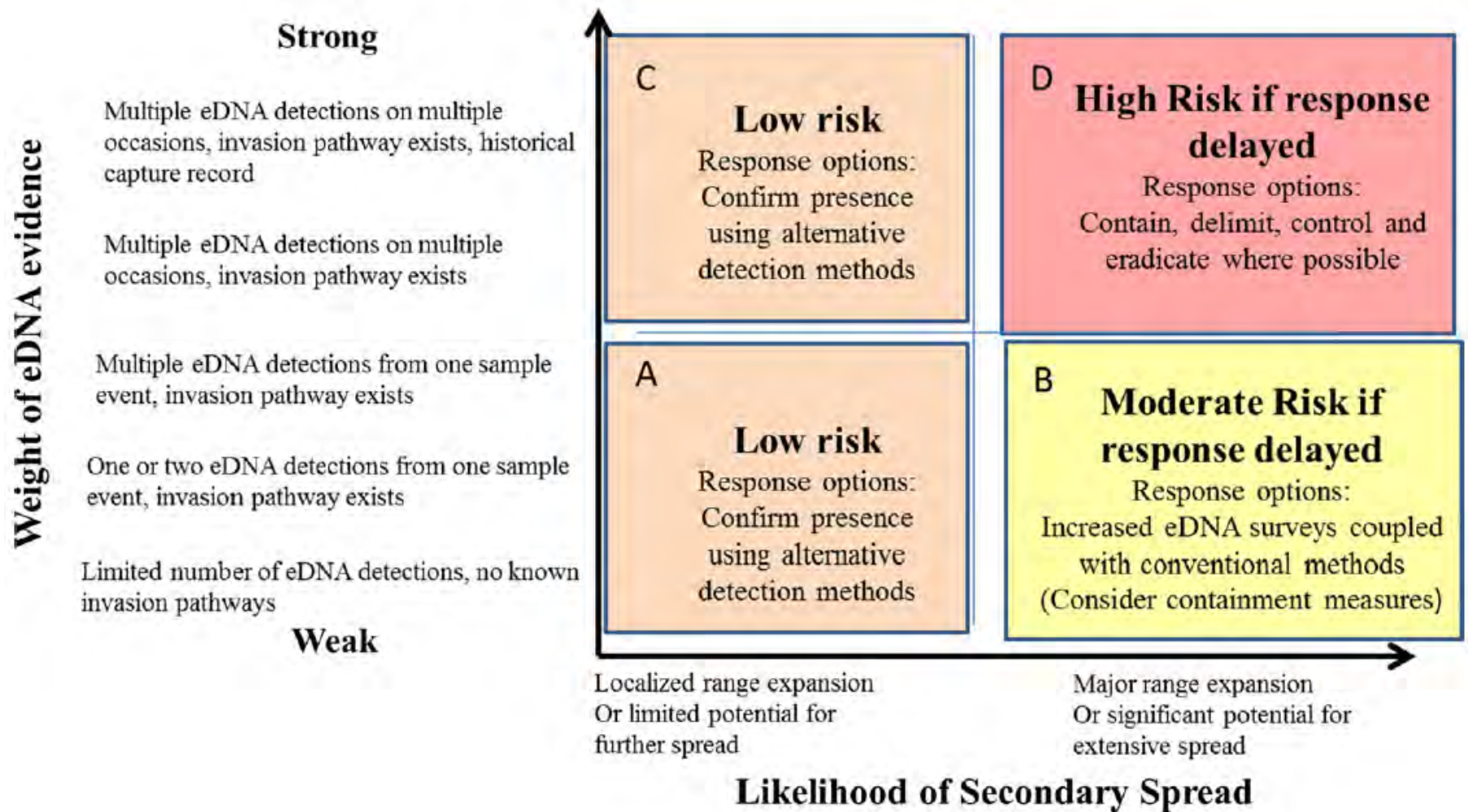
One or two eDNA detections from one sample event, invasion pathway exists

Limited number of eDNA detections, no known invasion pathways

Weak

Weight of eDNA evidence

Interpretations of eDNA results and implications for management



Current & Future eDNA R&D

1. Degradation rates of eDNA
2. Assay sensitivity & capture efficiency of eDNA
3. Vertical transport of eDNA
4. Horizontal transport of eDNA
5. Differences among taxa in ecology of eDNA
6. eDNA concentration re population size
7. Intrapopulation variation (population genetics)
7. Metabarcoding for community detection
9. Faster, portable, automated analysis

Automated sampling, detection



S. Howard (ND)
Portable PCR









END

Future general directions for eDNA R&D

1. Degradation rates of eDNA
2. Assay sensitivity & capture efficiency of eDNA
3. Vertical transport of eDNA
4. Horizontal transport of eDNA
5. Differences among taxa in ecology of eDNA
6. eDNA concentration re population size
7. Intrapopulation variation (population genetics)
7. Metabarcoding for community detection
9. Faster, portable, automated analysis

Current/future management applications?

1. Statewide fisheries surveys (e.g., more efficient, cheaper than electrofishing & nets?) to prioritize systems for follow up.
2. Statewide invasive or imperiled species surveillance (e.g., pathway hotspots like ports).
3. Post management monitoring program for invasive (e.g. hydrilla) or imperiled species (e.g. hellbender, cisco).

Tension points at research-management interface

1. More humility often needed from academics.
 - Be open to questions from management;
 - Be sensitive to limited resources, political context, rapidly changing priorities;
 - Communicate clearly about uncertainty.
2. More understanding of academic environment often needed from managers.
 - Creativity and cheap labor come with slower, episodic progress;
 - Interest in novelty in addition to problem solving.
3. Data management, sharing protocols essential.
4. Communications protocols are essential.

END



Arroyo Toad

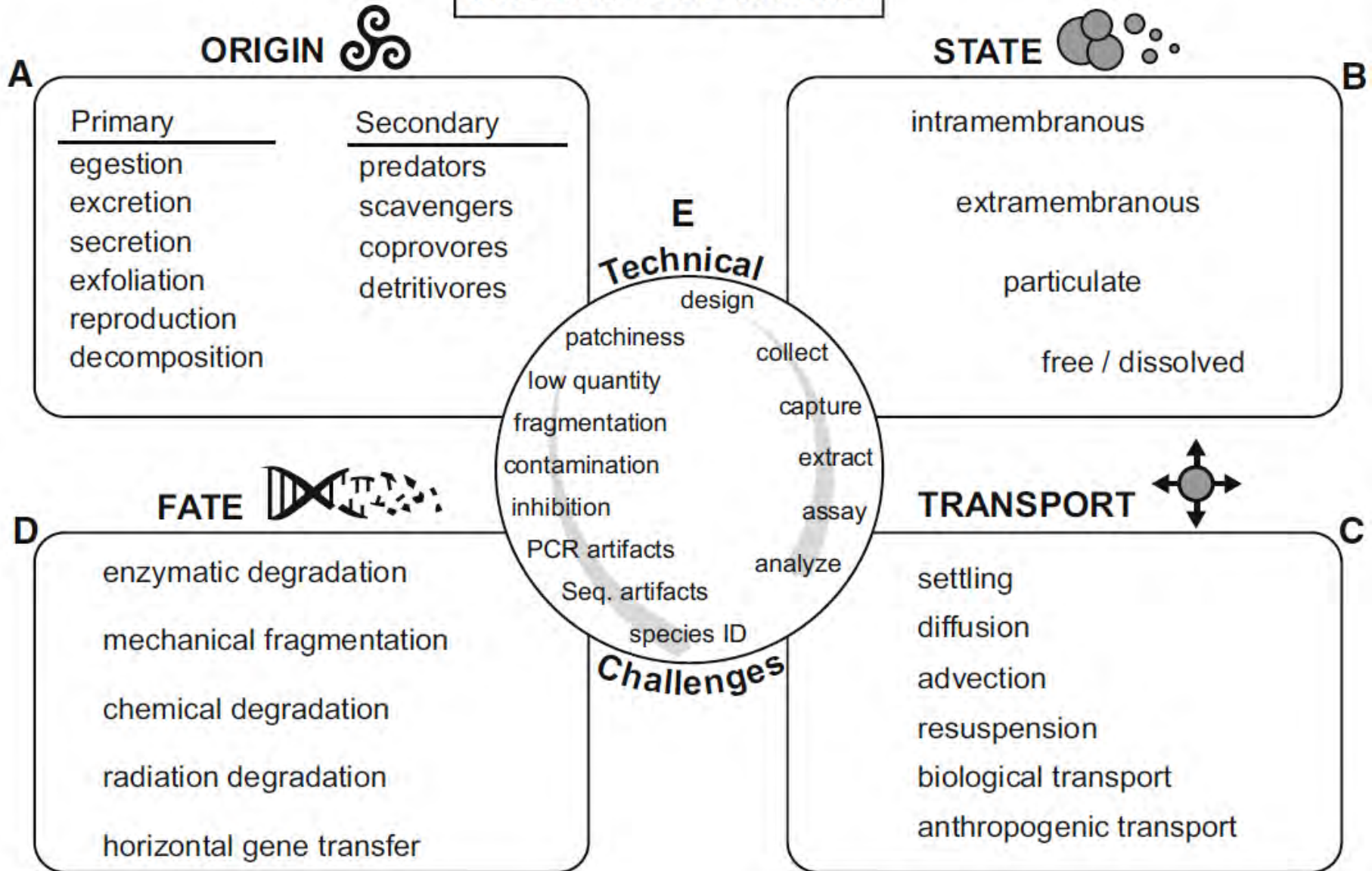


Bull Trout

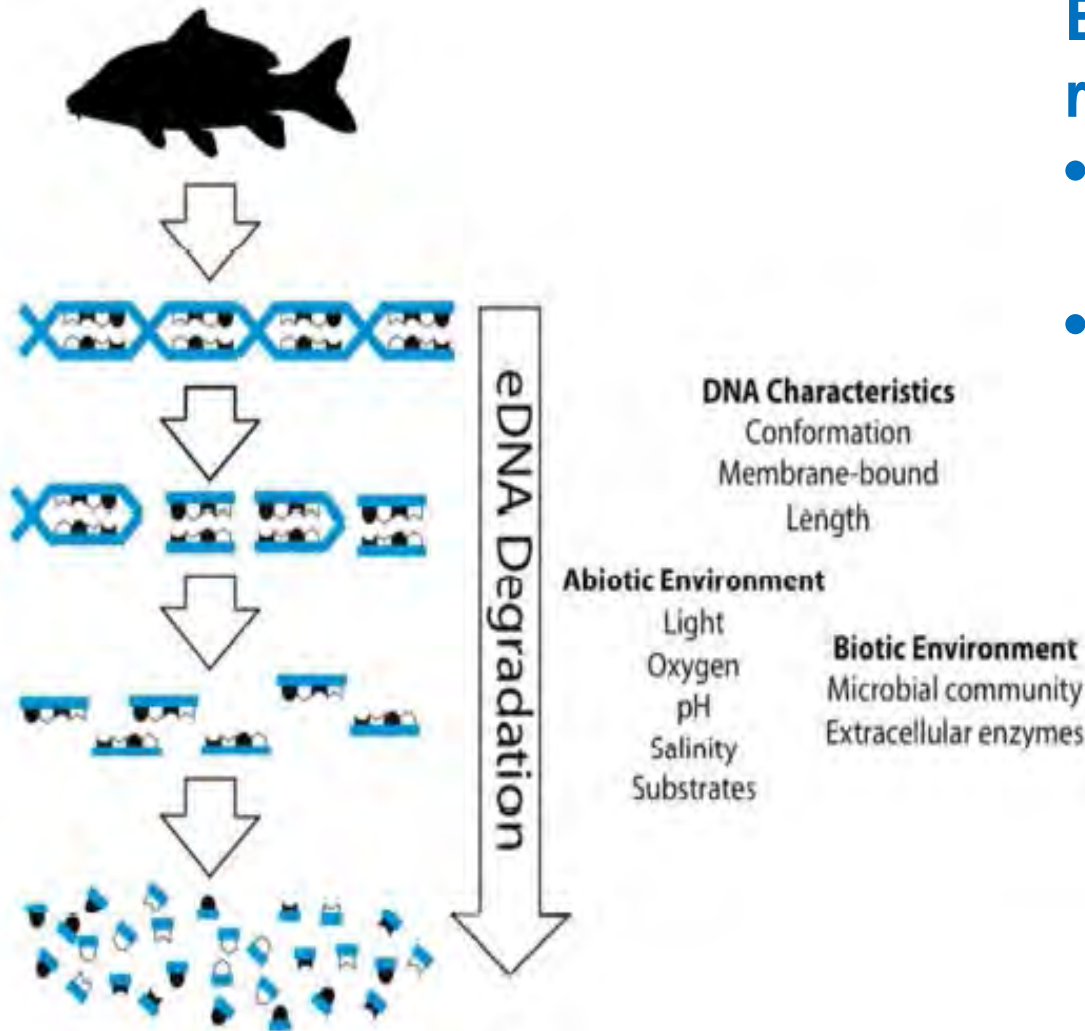


Hellbender

THE ECOLOGY of eDNA



Degradation of eDNA: How long ago was the animal present?

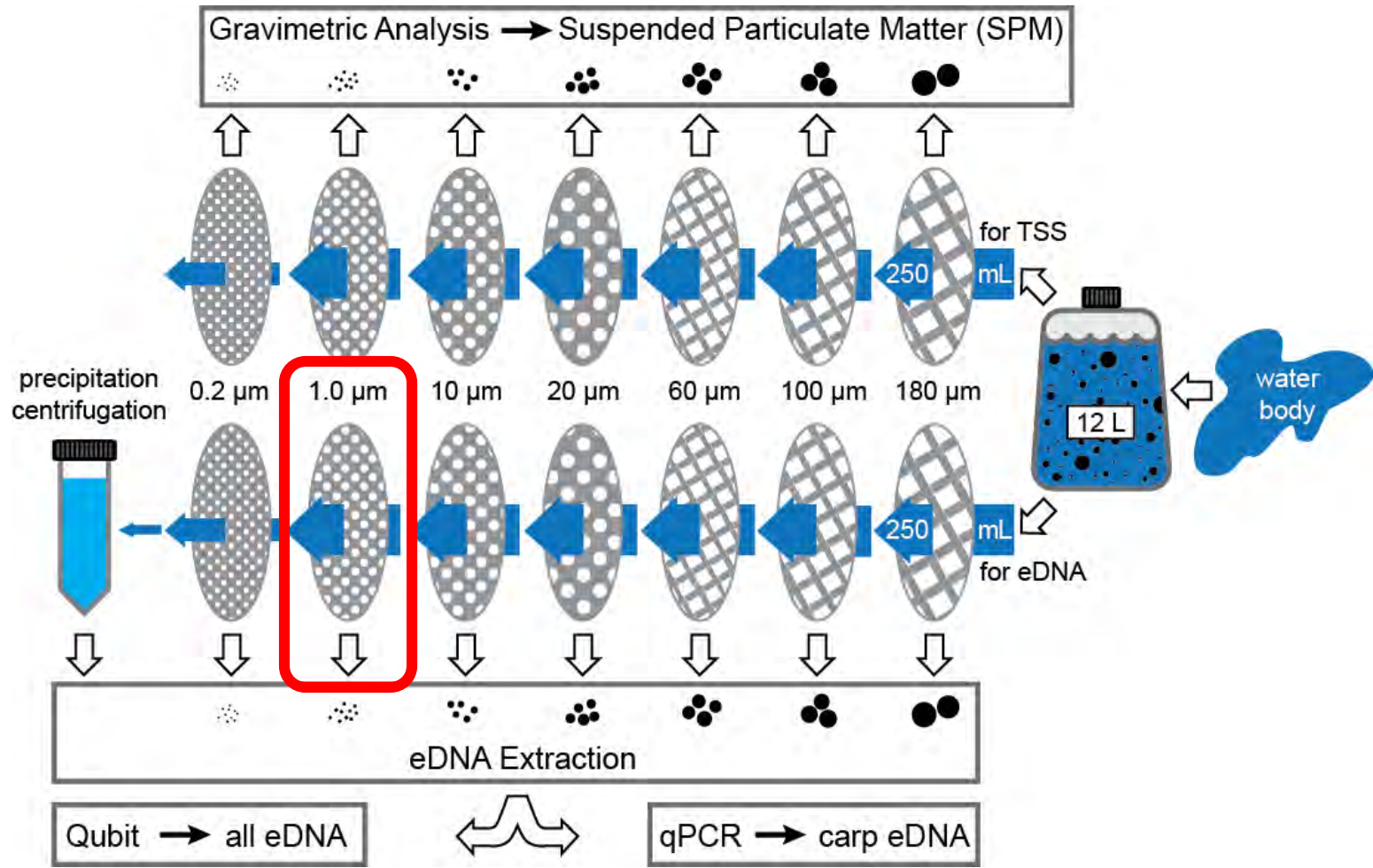


Experimental results:

- eDNA undetectable after hours-days
- Degradation affected by biological activity

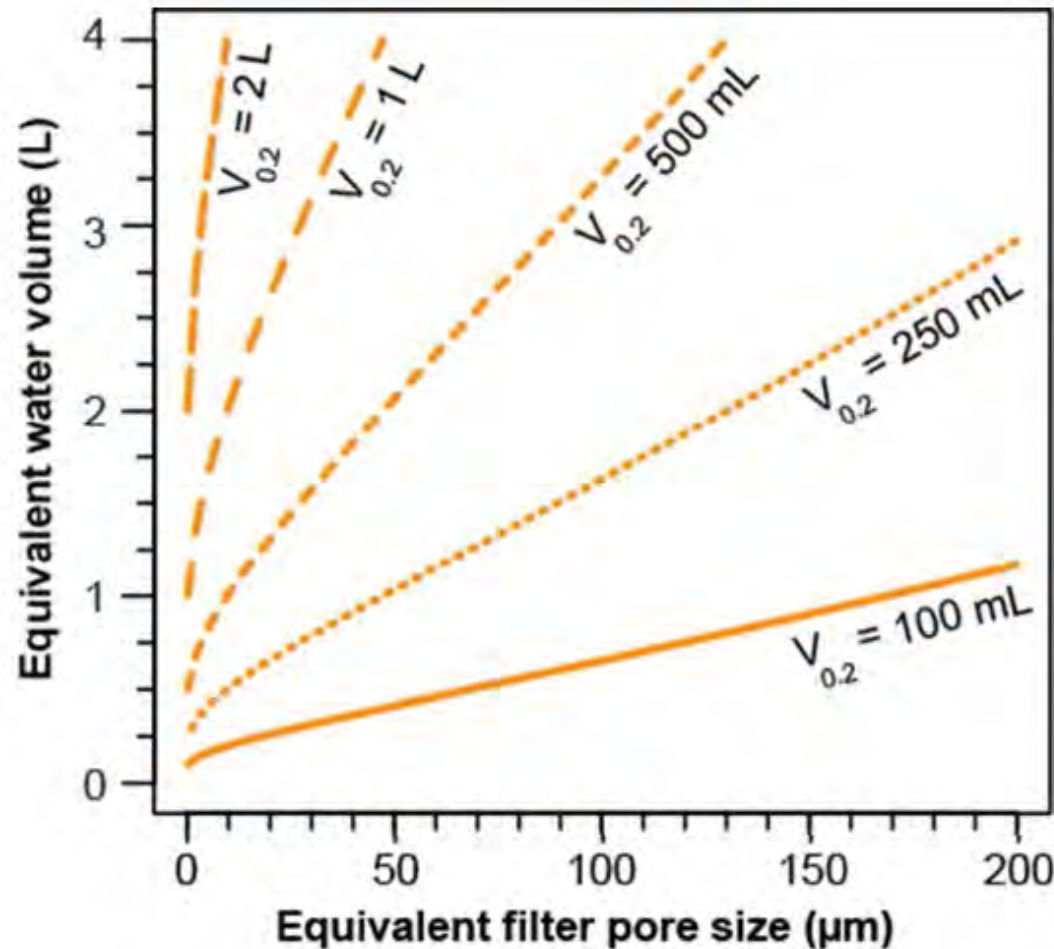
(Barnes et al 2014. Environmental Science & Technology)

eDNA collection:
sequential filtration of field water samples



(Turner et al. 2014 *Methods in Ecology & Evolution*)

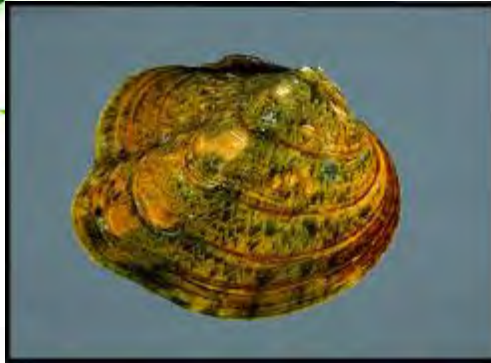
Common carp eDNA collection: sequential filtration of field water samples



For optimal eDNA capture:

- 0.2 μm filtration or
- a combination of larger pore size and larger water volume that captures the same amount of eDNA (i.e., exceeds the 0.2 μm isocline)

Differences among taxa?

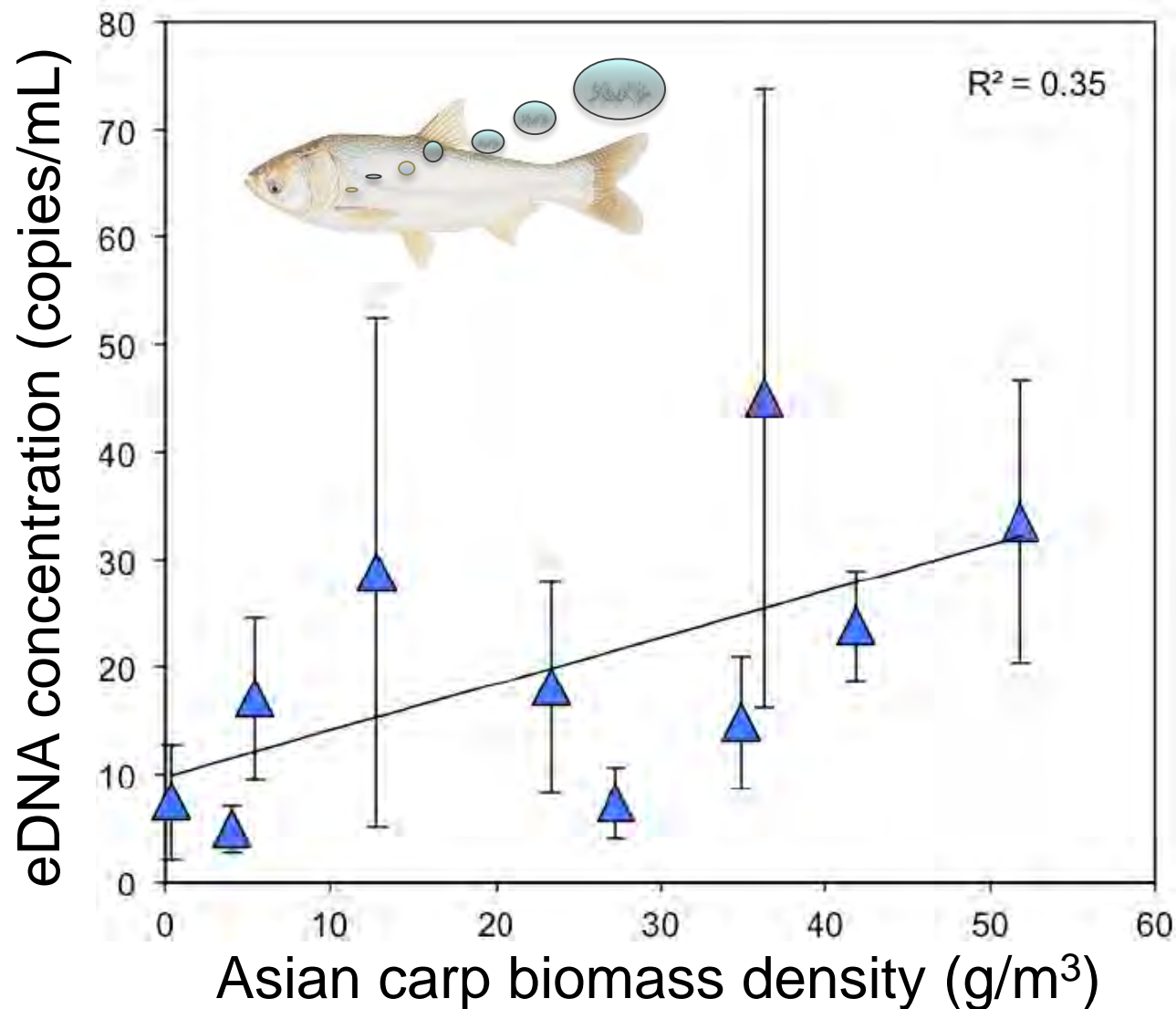


Differences among taxa in:



































- Production rate?
- Degradation rate?
- Particle size?
- Location in water column?
 - Vertical transport?
 - Horizontal transport?



eDNA concentration correlates to pop size

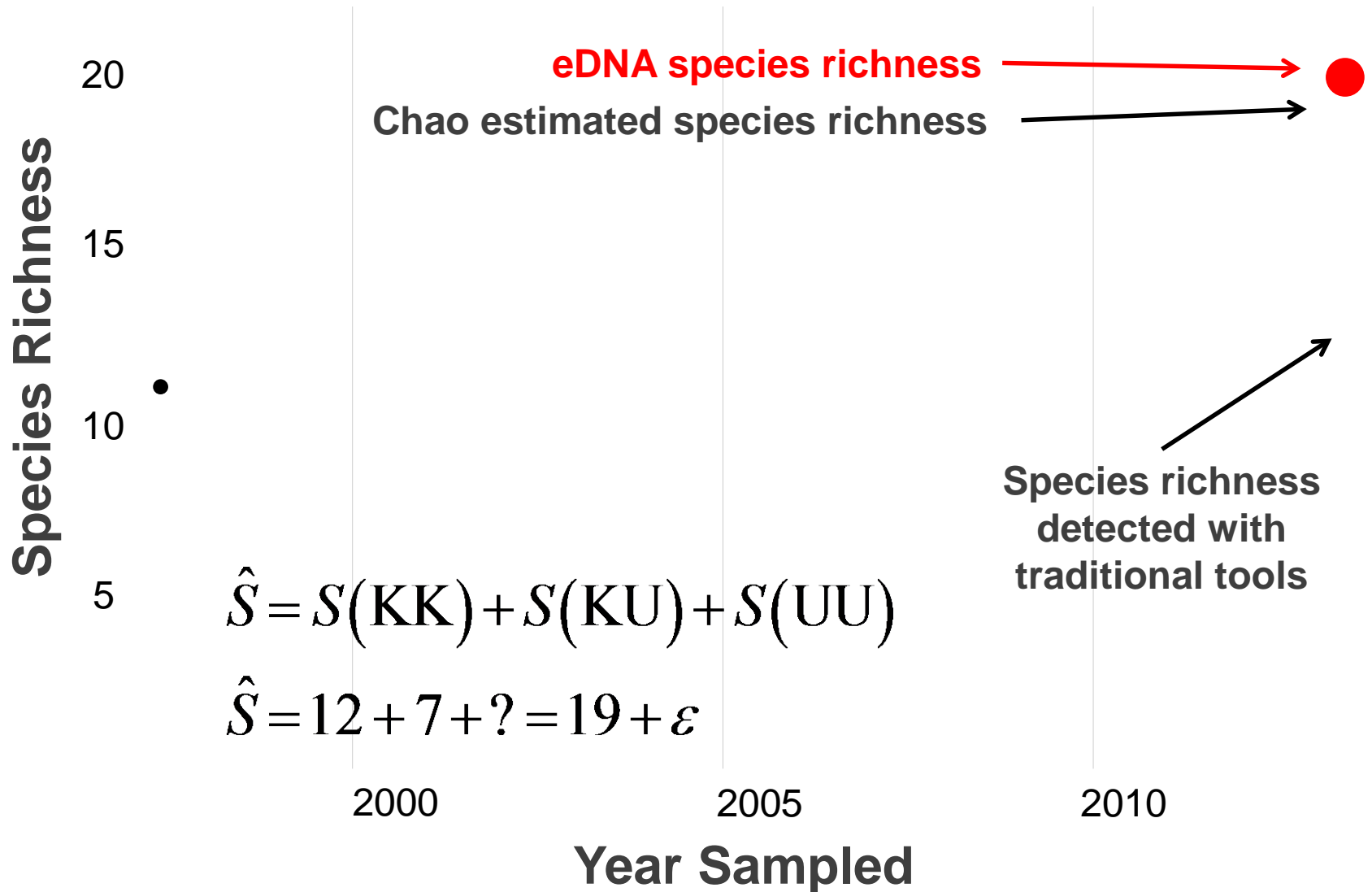


Metagenetic Analysis (Ultrasequencing): Kansas ponds

Grass carp					
Common Carp					
Silver carp					
Bighead carp					
Bluegill					
Redear sunfish		No Reference			
White crappie		No Reference			
Bullfrog					
	Cytb (464 bp)	12S (270 bp)	16S (392 bp)	16S (366 bp)	16S (250 bp)

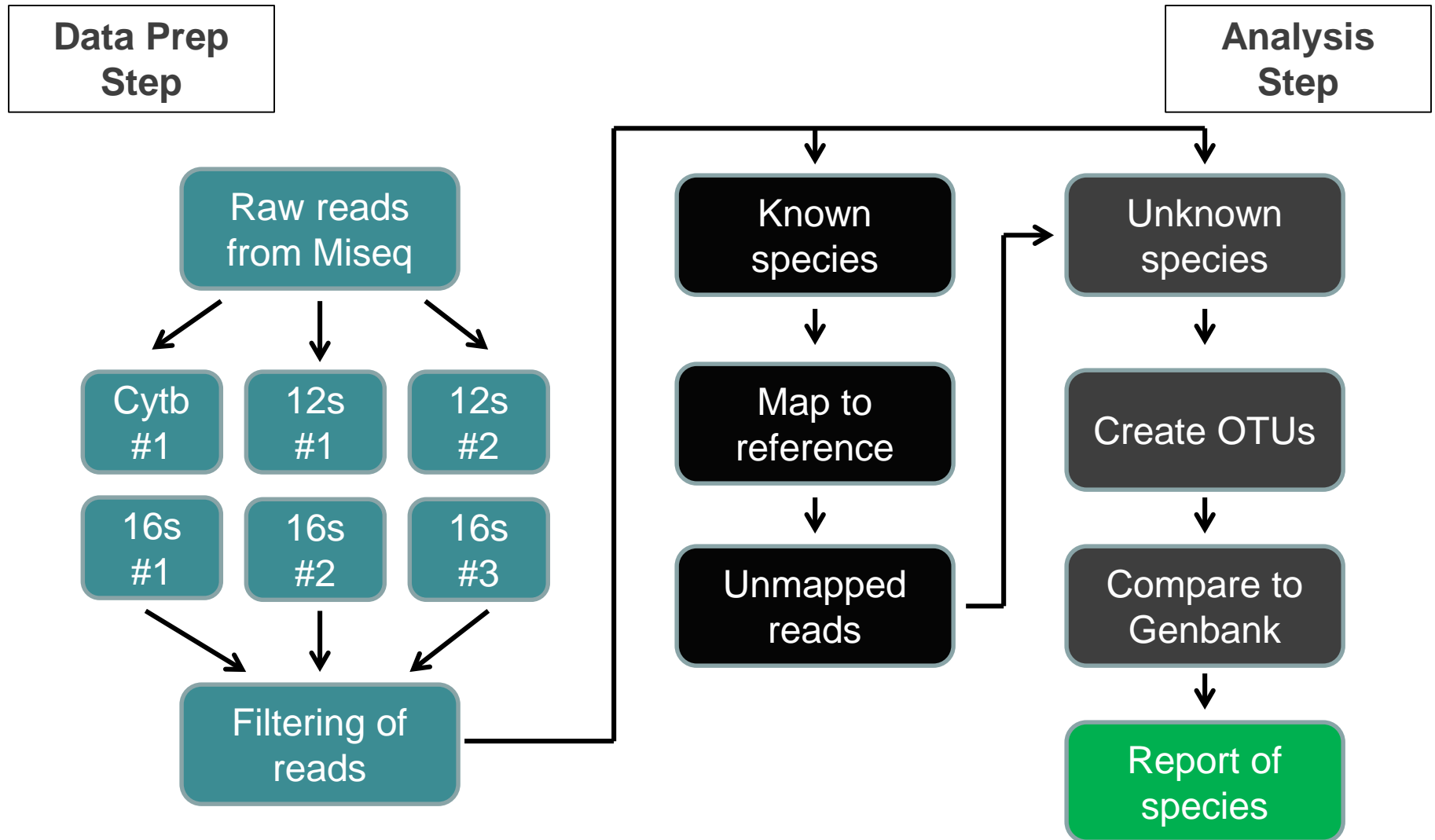
(Olds et al. unpublished)

Metagenetic Analysis (Ultrasequencing): Juday Creek, Notre Dame

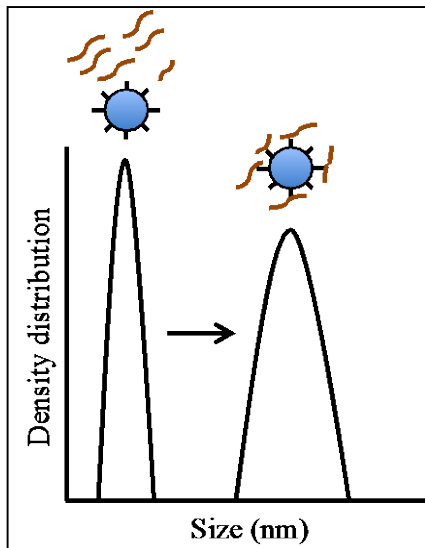
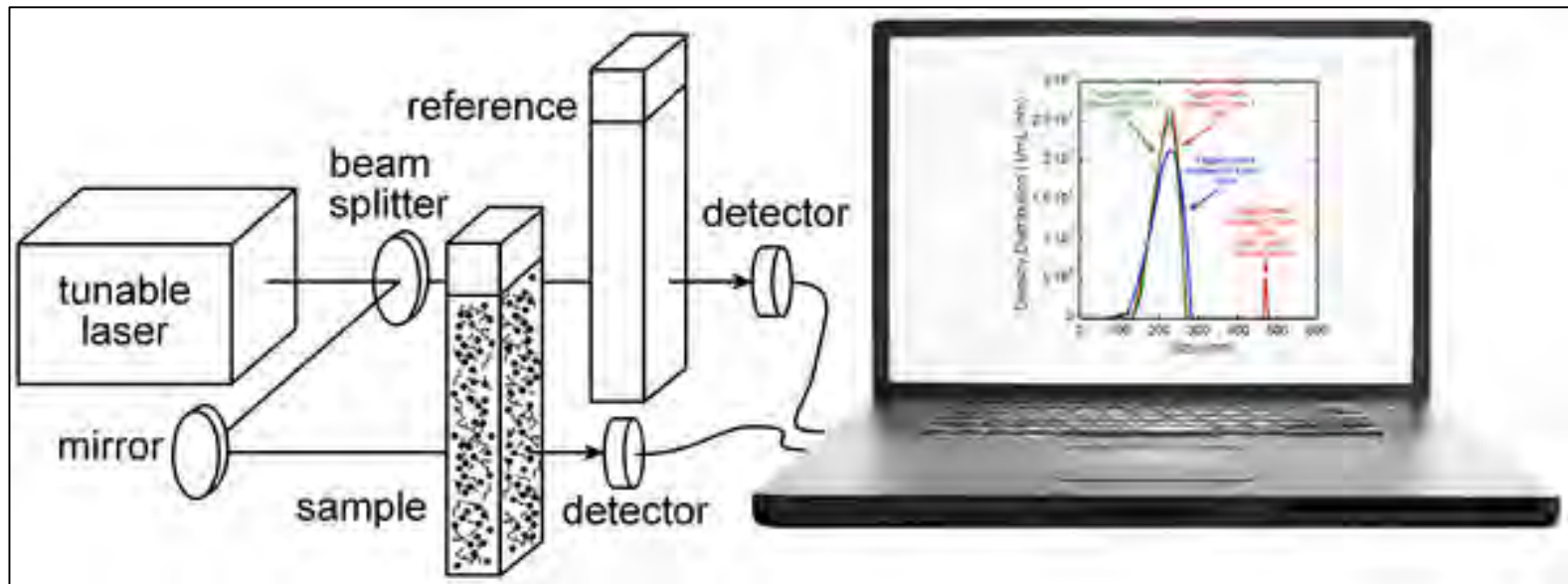


(Jerde et al. unpublished)

Example of bioinformatics workflow



eDNA detection using Laser Transmission Spectroscopy (LTS)



Recent LTS results:

- Very high sensitivity (picomolar range)
- Detects invertebrate species in “ballast” water with 100% accuracy, and no false positives (Egan et al. 2013. *Conservation Letters*; Mahon et al. 2013. *Proc. Roy. Soc. Interface*)

LightSprite (<http://www.m3dev.com/>)