EPA
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Environmental Protection
Agency

Spatial comparison of eDNA vs physical fish data from a complex freshwater estuary

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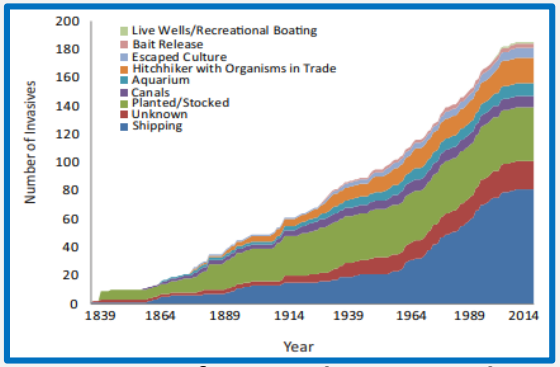
Topic: eDNA as Great Lakes AIS monitoring tool



Source: State of Great Lakes 2017 tech rept.

AIS continue to arrive in Great Lakes.

- *GL WQ Agreement* & *Restoration Initiative* call for multi-species early detection monitoring.
- Enables discovery of new AIS; informs ecological assessment and management response.

Testing eDNA monitoring in St. Louis River estuary

- Largest GL port by ship & cargo
- Known AIS introduction hotspot
- Spatially & hydrologically complex
- Strong fish monitoring history

We expect diffs between eDNA & physical surveys

Physical survey can miss taxa:

- Hidden/not vulnerable to gear (design problem)
- Lack features, keys, AIS awareness (morph ID problem)

eDNA can miss taxa:

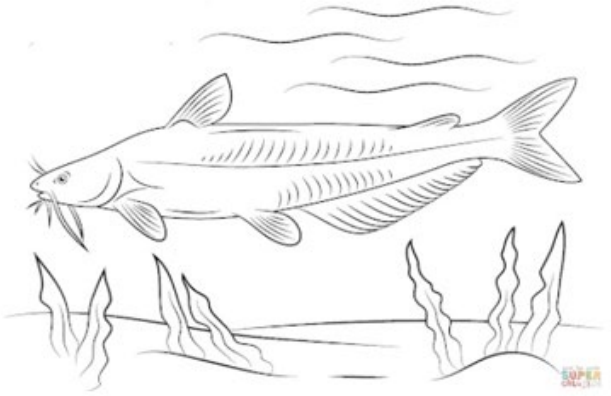
- DNA not released (life history, etc)
- DNA degraded/inhibited (hydro/chem)

Both survey types can miss taxa:

- Lack barcode or distinct marker (DNA ID problem)
- Rare relative to sample size (effort problem)

eDNA can wrongly 'find' taxa:

- Outside DNA carried in (water, predator)

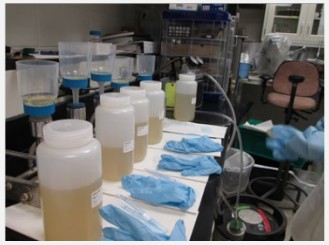


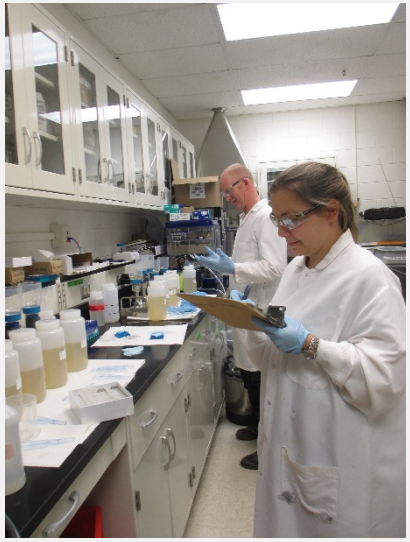
Likely wider dispersal of eDNA than fish. How do spatial patterns compare?

2016 eDNA survey design and methods

Field:

- 120 stations in June, again in October
- Randomized locations locally refined (e.g., windrow, lee of pier, over veg beds)
- 1L surface samples (+ reps & controls)
- Clean protocols throughout





Lab:

- Vacuum filter onto polycarb membrane
- Longmires lysis buffer
- DNA extracted w/ chloroform-isoamyl protocol
- 12S & 16S fish markers amplified w/ PCR thermocycling
- Metabarcoded on Illumina MiSeq

Bioinformatics:

- Raw sequences converted to OTUs
- Cluster, de-noise, remove chimeras
- OTUs to species via BLAST
- Remove false positives based on plate-wide error rate

2016 physical survey design and methods

Larval fish

EPA & U.S. Fish Wildlife Service:

- Impetus: AIS early detection
- 75 loc's, June
- Neuston net, tucker trawl, tow sled
- Sorted into 5mm size classes
- DNA amplification/ sequencing/ bioinformatics as for eDNA
- 15% of samples also morph-ID'd

Adult/juvenile fish

1854 Treaty Authority:

- Impetus: fish pop trends, AIS monitor
- 40 loc's August, 40 loc's October
- 16-foot bottom trawl

MN Dept Natural Resources:

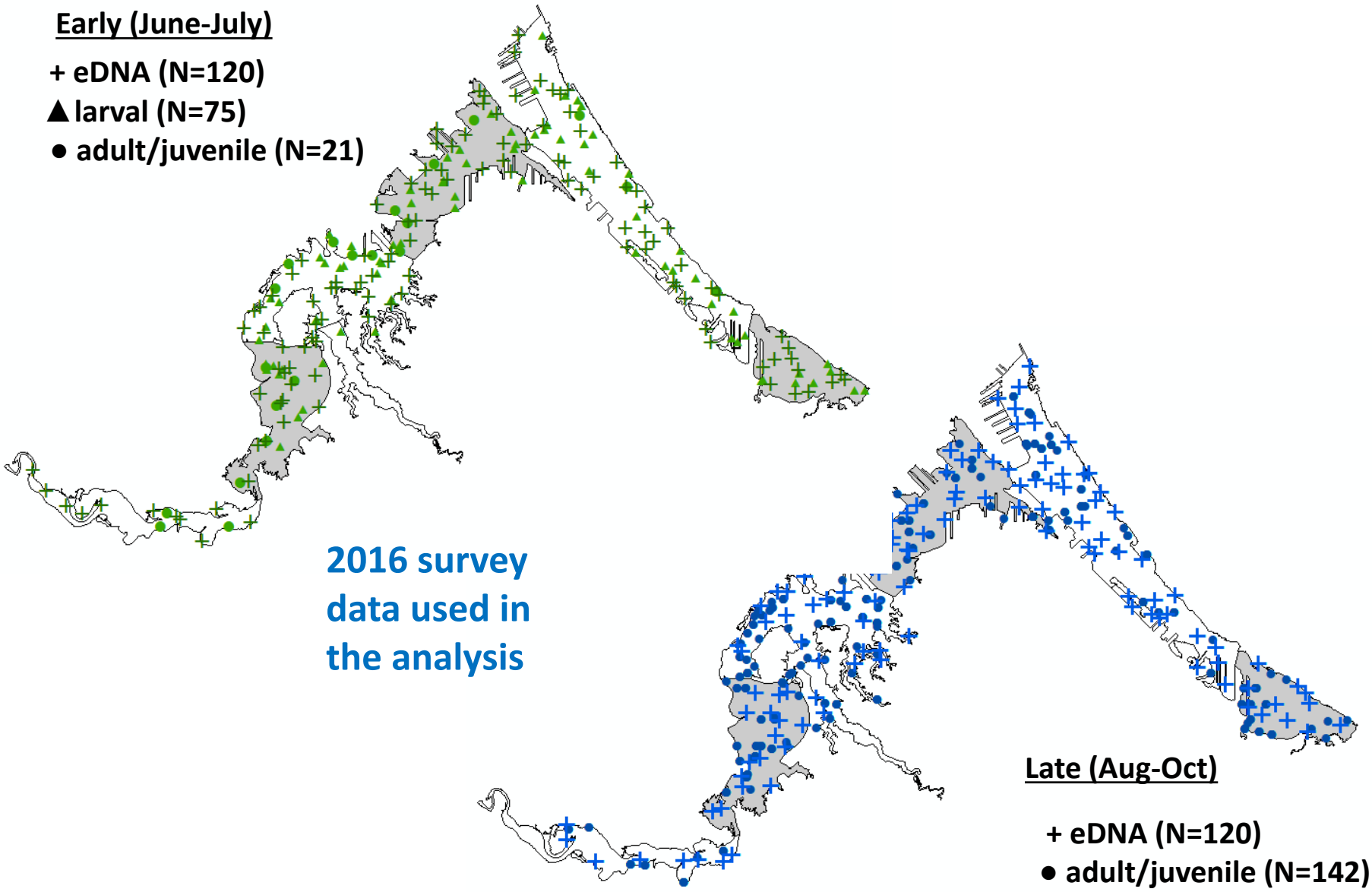
- Impetus: index sturgeon abundance
- 33 loc's, June, July, Sept
- Multi-panel gill nets

U.S. Fish & Wildlife Service:

- Impetus: AIS early detection
- 50 loc's, August
- Fyke nets, electrofish, bottom trawl

[MORE PHOTOS HERE]

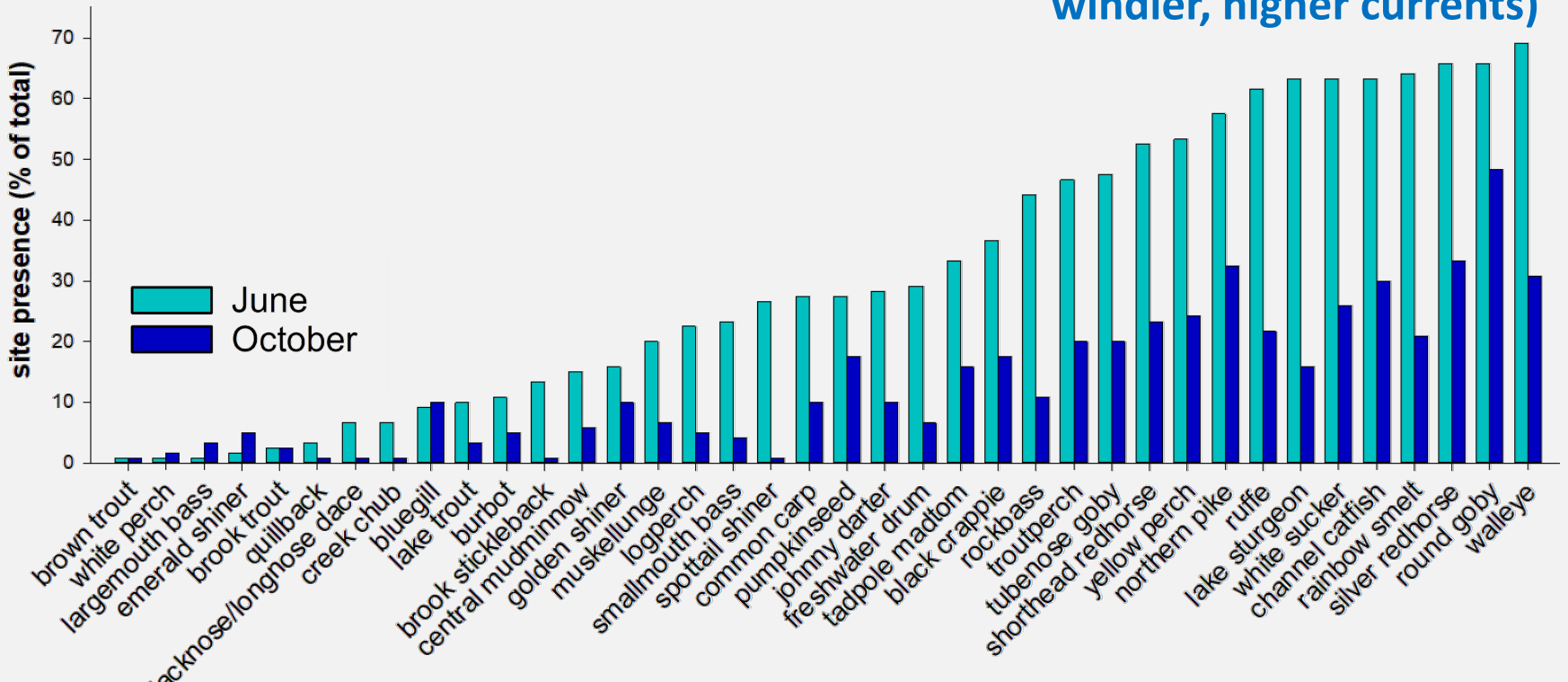
St Louis River estuary is a well sampled place



Results – eDNA by month

5 eDNA samples yielded no fish at all

38 shared taxa; generally higher presence in June (spawning season but also windier, higher currents)



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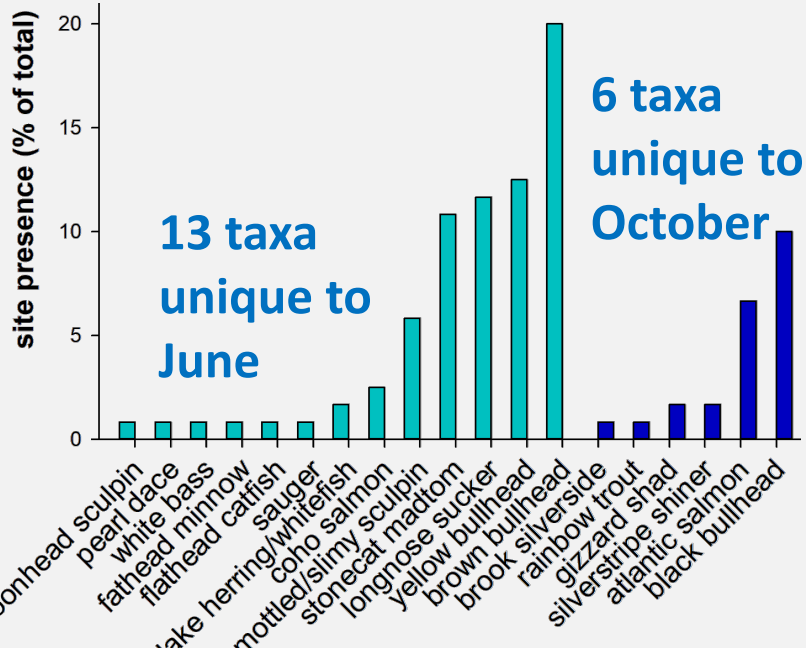

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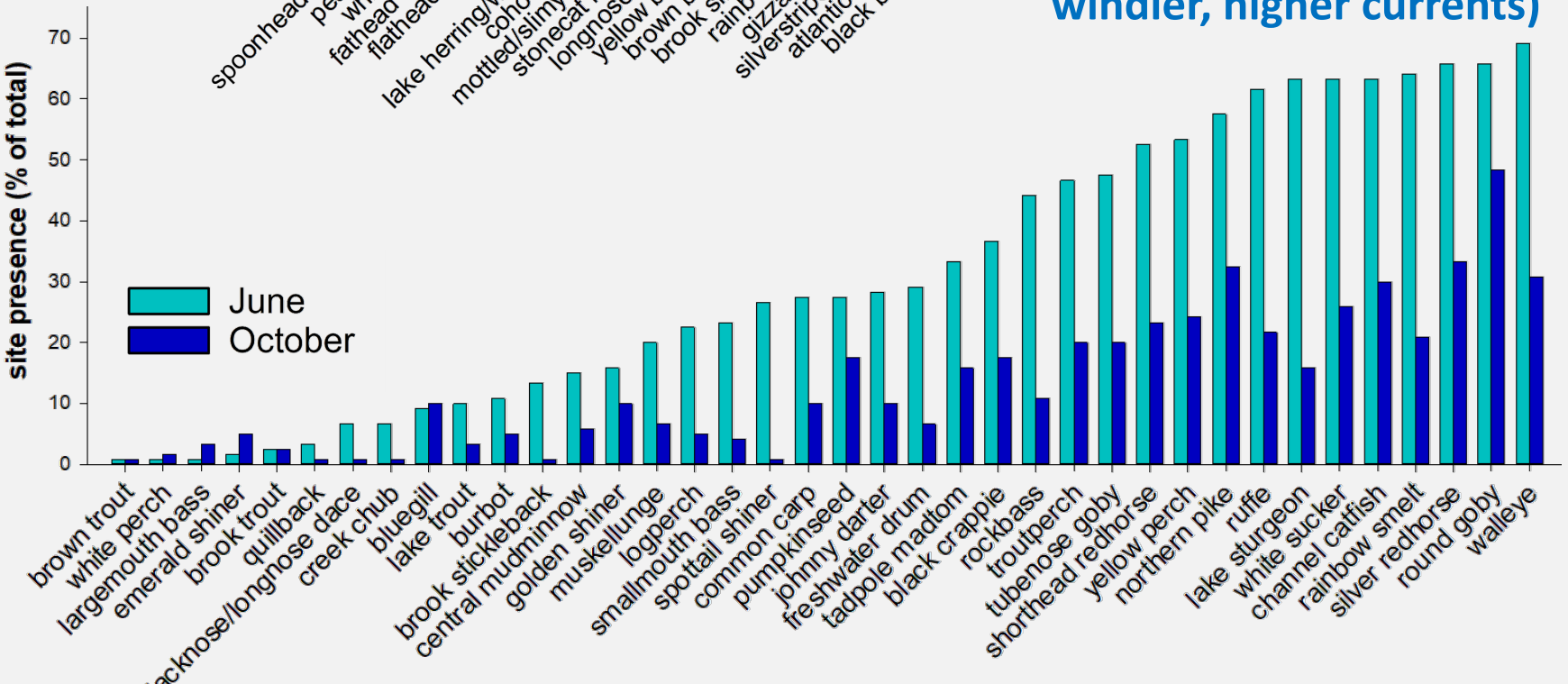
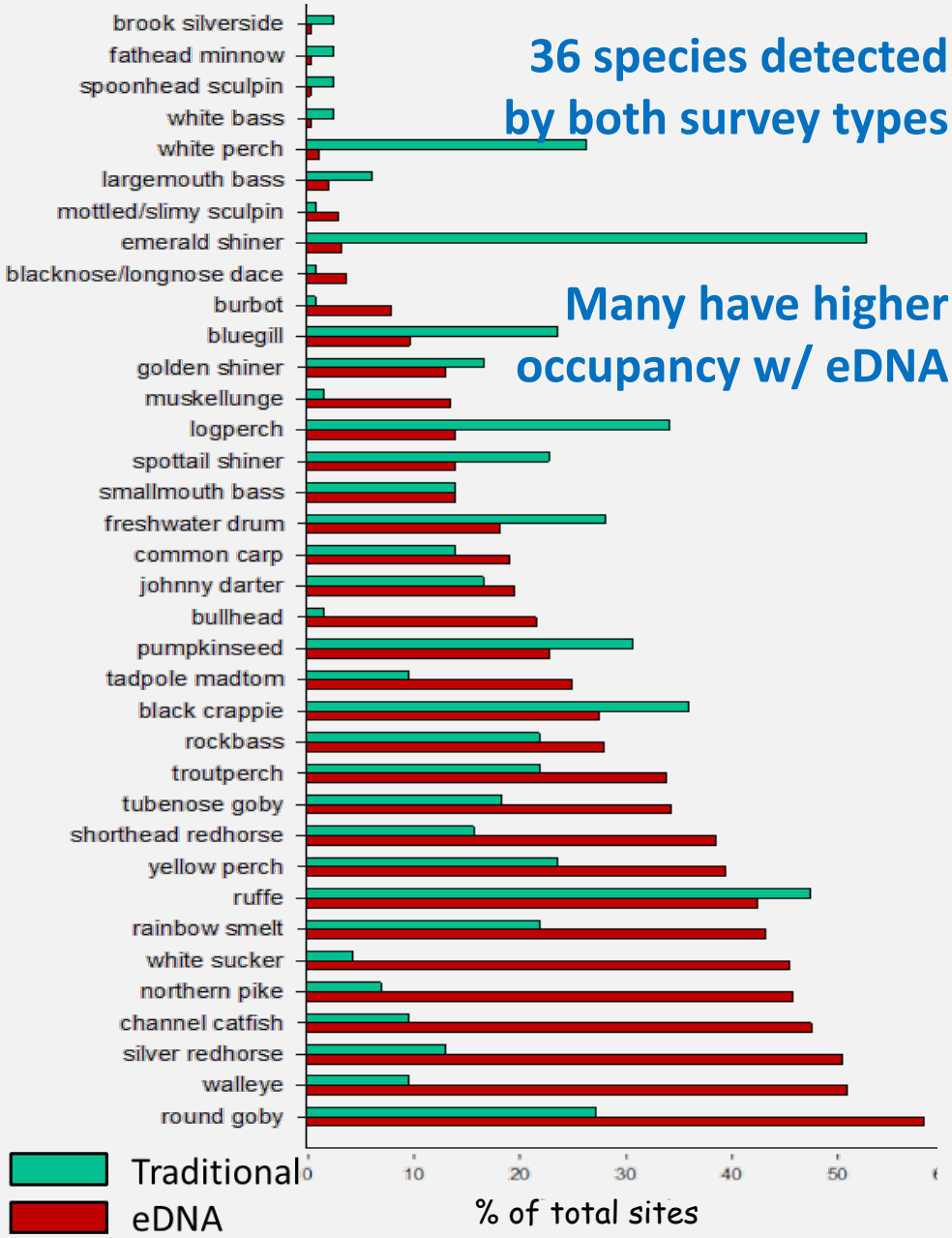


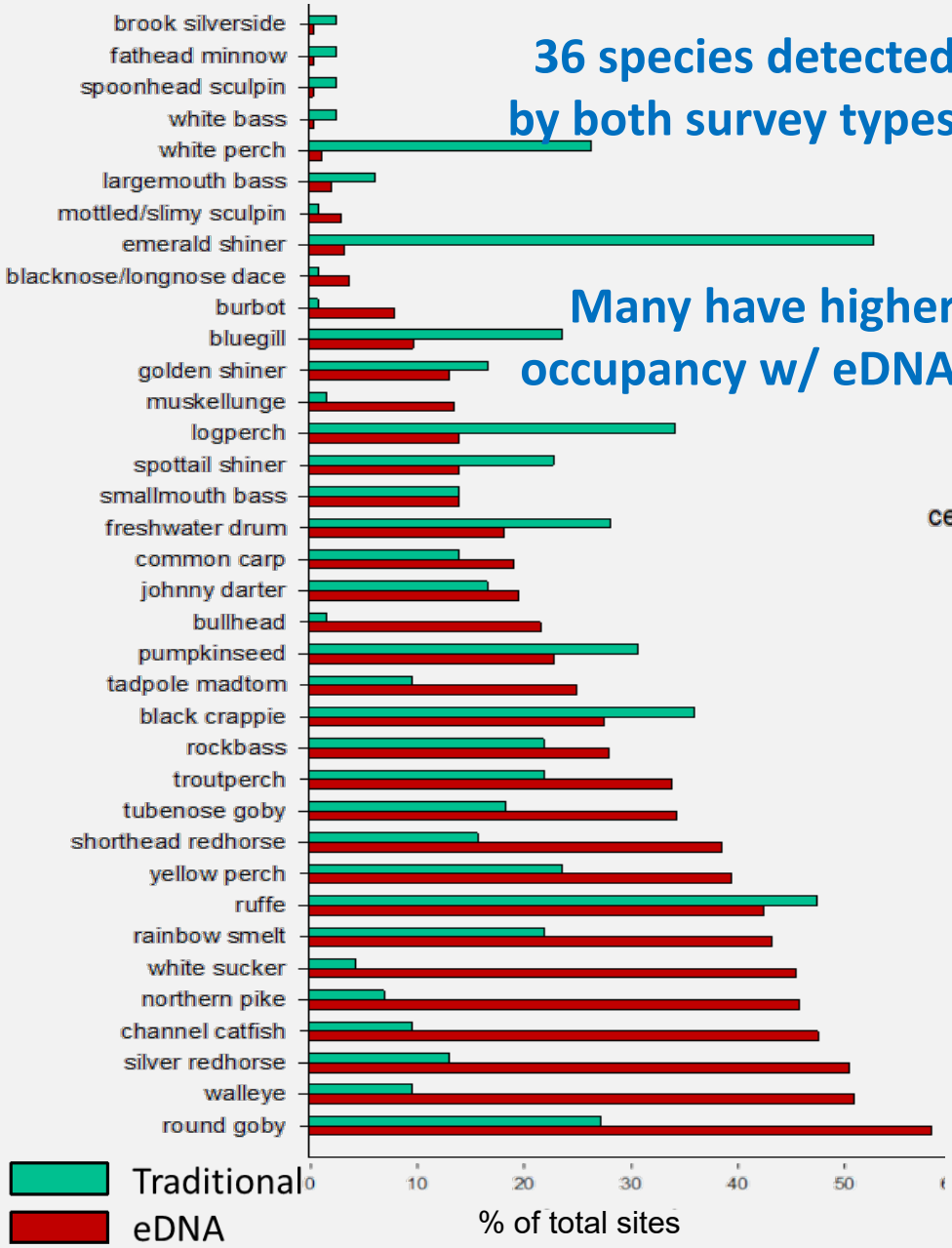
Results – eDNA vs. physical survey



36 species detected by both survey types

Many have higher occupancy w/ eDNA

Results – eDNA vs. physical survey



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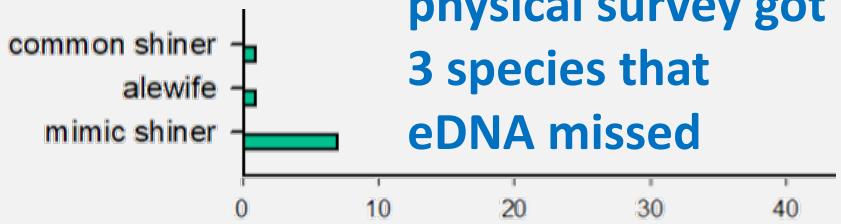
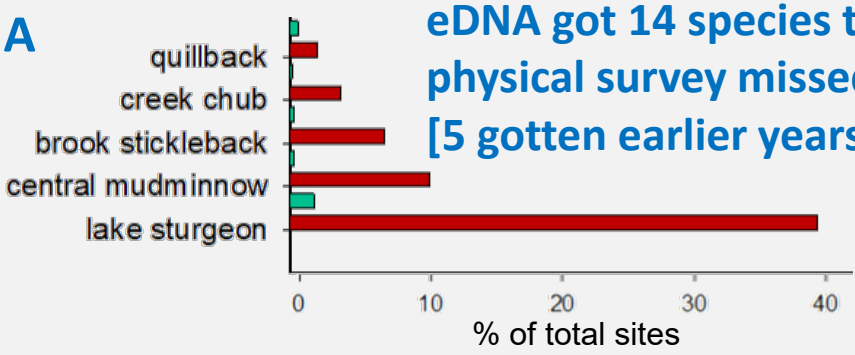


physical survey got 3 species that eDNA missed



eDNA got 14 species that physical survey missed [5 gotten earlier years]

- **Upstream or Lake residents – *eDNA present due to flow or mixing?***
- **Benthic/sedentary/cryptic species - *not captured with traditional gear? mis-ID with traditional methods?***
- **Low abundance - *rare species or newly established AIS***

eDNA detected 3 unexpected species

Gizzard Shad
(*Dorosoma cepedianum*)

12s, 2 sites, 77019 reads
- physical specimens collected in 2017 (one year later)

Flathead Catfish
(*Pylodictus olivarius*

12s, 1 site, 29873 reads
- look a lot like bullheads, easy to miss in trad survey
- Not yet detected in traditional survey
- Source from ballast water?
- Informed monitoring agencies to look more closely at bullheads

Silverstripe Shiner
(*Notropis stilbius*)

16s, 4 sites, 4522 reads
- DNA error? Most likely Emerald Shiner
- Or DNA from ballast water

What do we do about such cases?

- **Rare signals are important for AIS early detection**
- **Need to determine if such species are false positives or valid signals**
- **eDNA alone can't address this – physical survey follow-up needed**

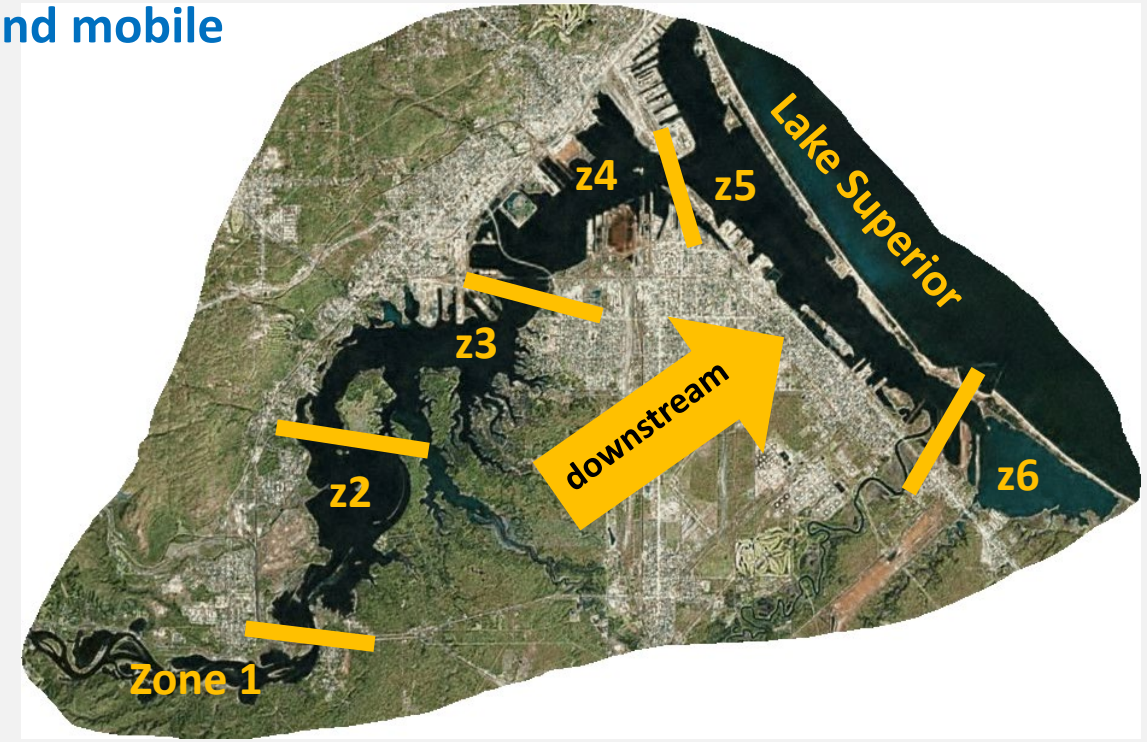
Data compilation – spatial pattern analysis

Omitted species not found in both survey types

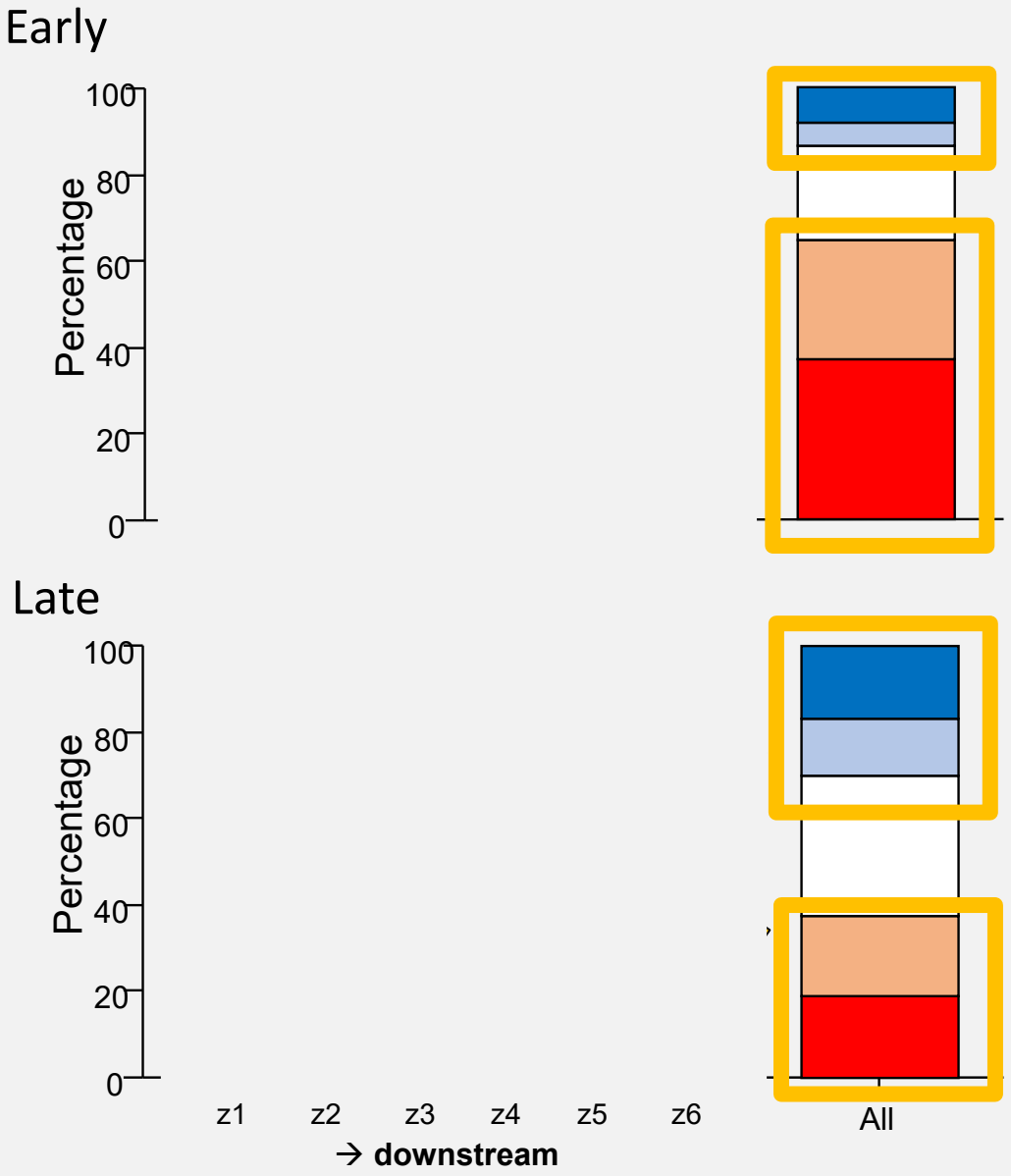
Constructed Guilds:

- benthic -- sedentary and somewhat cryptic but widely distributed
- littoral invertivores -- small home ranges primarily in veg
- pelagic invertivores -- primarily in deeper or more lentic regions
- territorial piscivores –mobile but small home ranges
- rovers -- widely dispersed and mobile

Examined estuary by spatial zone and by physical features



Results – Comparison of shared species



Time matters

← Early:

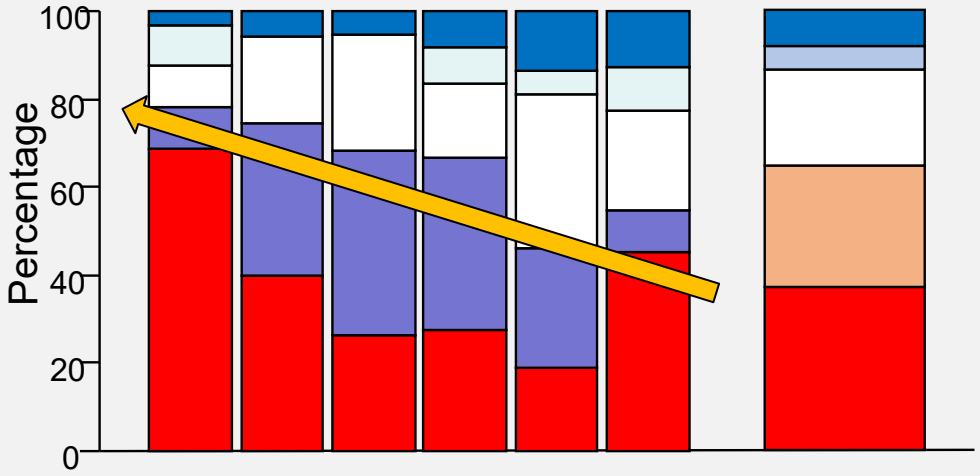
- Much higher occurrence with eDNA than physical surveys

← Late:

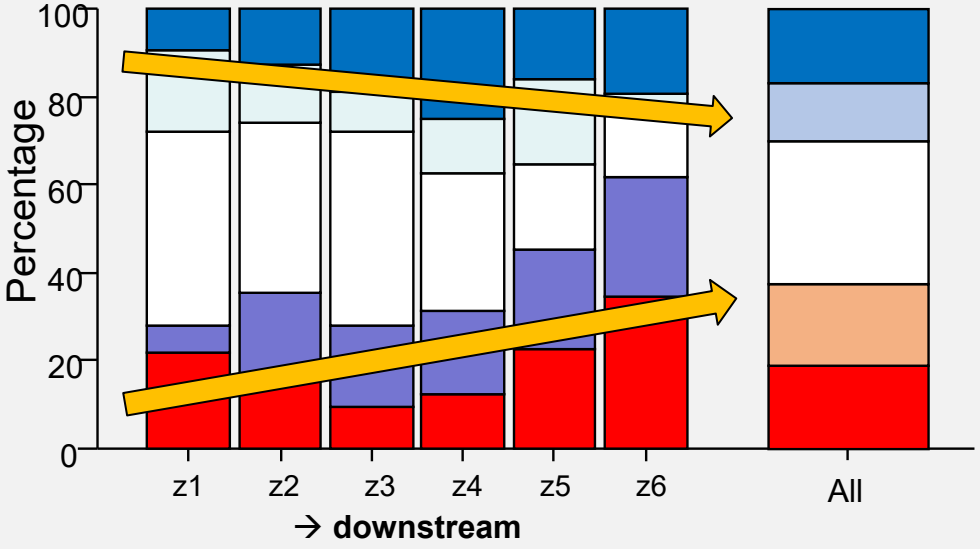
- Two survey types had similar performance overall

Results – Comparison of shared species

Early



Late



Time matters, and place

← Early:

- Much higher occurrence with eDNA than physical surveys
- eDNA outperformance increased in upstream direction

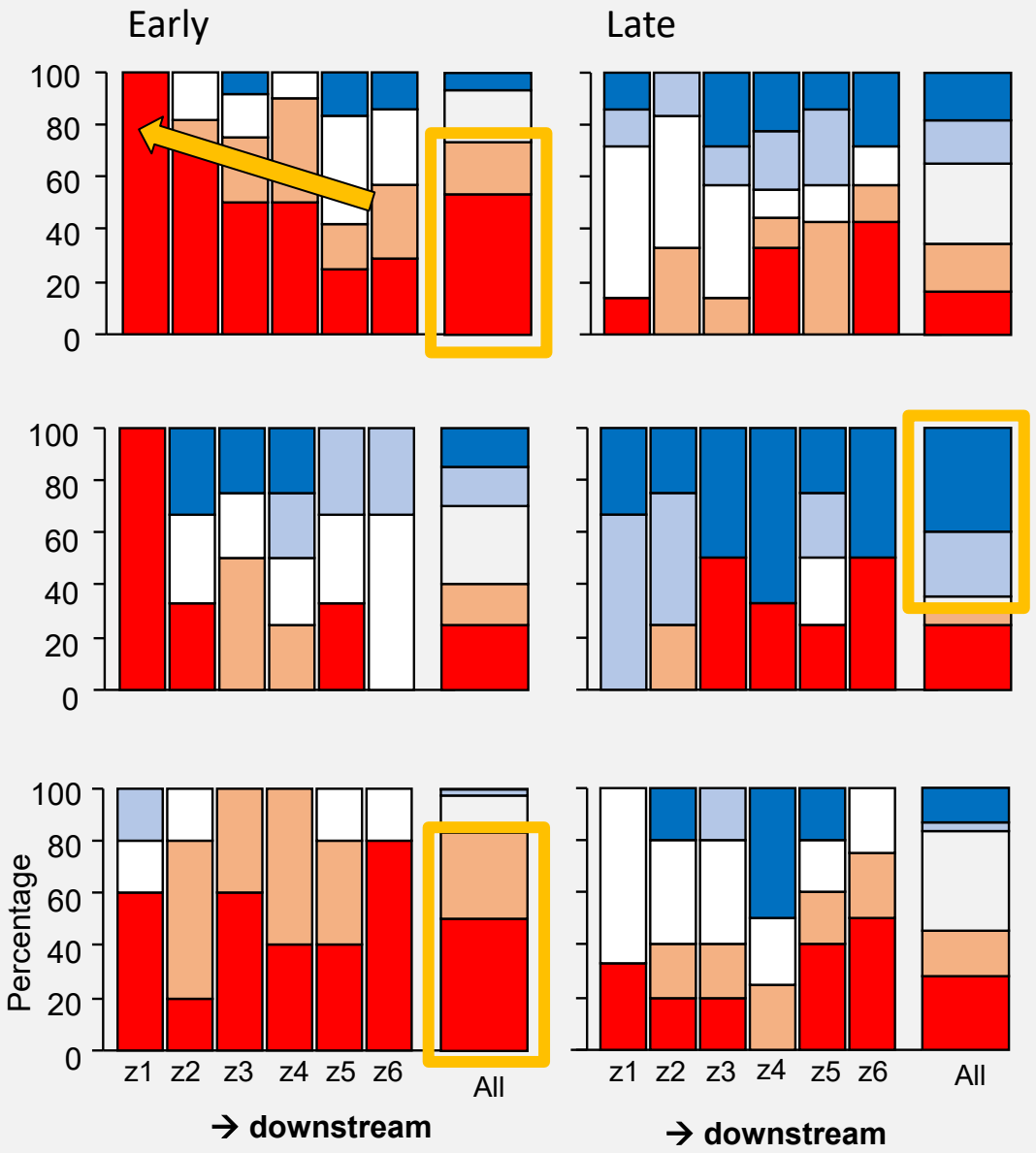
← Late:

- Two survey types had similar performance overall
- performance inequalities increased in downstream direction

phys only
phys > eDNA
eDNA = phys
eDNA > phys
eDNA only

Based on pres/abs categories:
0 = absent,
1 = up to 33,
2 = up to 66%,
3 = up to 100% occurrence

Results – Comparison of shared species by guild



Guild matters

← Sedentary benthic:

- eDNA outperforms phys survey early but not late
- upstream gradient early vs little gradient late

← Pelagic invertivore:

- surveys equivalent early, physical outperforms late
- no clear spatial pattern

← Territorial piscivore:

- eDNA outperforms early vs. survey equivalence late
- no clear spatial pattern

phys only
phys > eDNA
eDNA = phys
eDNA > phys
eDNA only

Results – Comparison of AIS detection

		Z1	Z2	Z3	Z4	Z5	Z6
Round goby 	eDNA	45	47	77	83	82	67
	phys	0	14	5	19	22	56
Tubenose goby 	eDNA	45	53	50	57	36	50
	phys	0	14	32	19	19	67
rainbow smelt 	eDNA	45	47	54	74	88	67
	phys	0	0	5	24	74	78
common carp 	eDNA	73	40	42	39	21	8
	phys	0	0	36	38	4	0
ruffe 	eDNA	36	73	69	74	73	75
	phys	67	71	27	52	70	67
brook silverside 	eDNA	0	0	0	0	0	0
	phys	0	0	5	5	0	0
white perch 	eDNA	0	0	4	0	0	0
	phys	67	57	18	52	56	44
white bass	eDNA	0	0	4	0	0	0
	phys	0	0	5	5	15	11

Species matters

← Higher detect rate with eDNA – round goby, tubenose goby, rainbow smelt, common carp

← Similar detect rates both surveys – eurasian ruffe, brook silverside

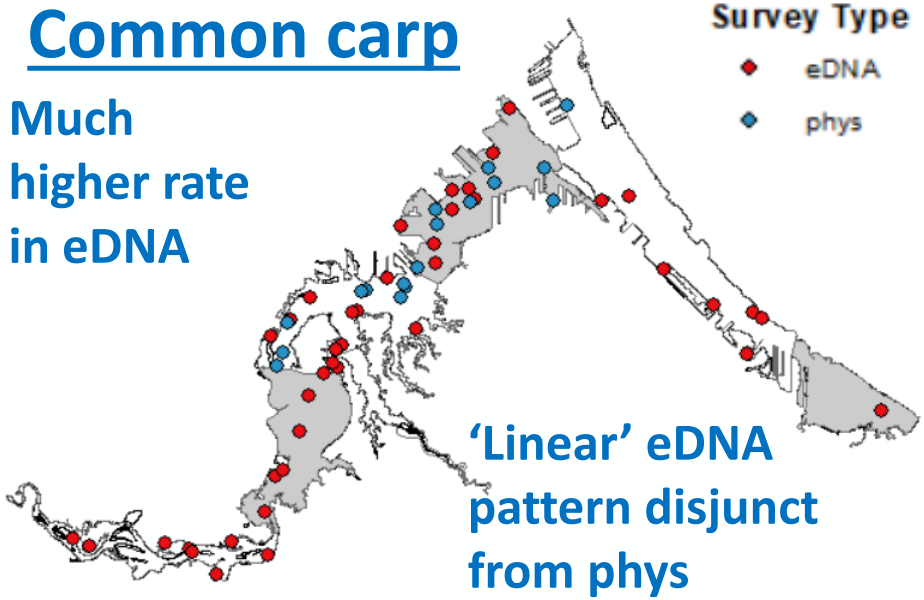
← Higher detect rate in physical survey – white perch, white bass

(Showing 'early' but similar pattern 'late')

Results – Widely varying spatial distribution

Common carp

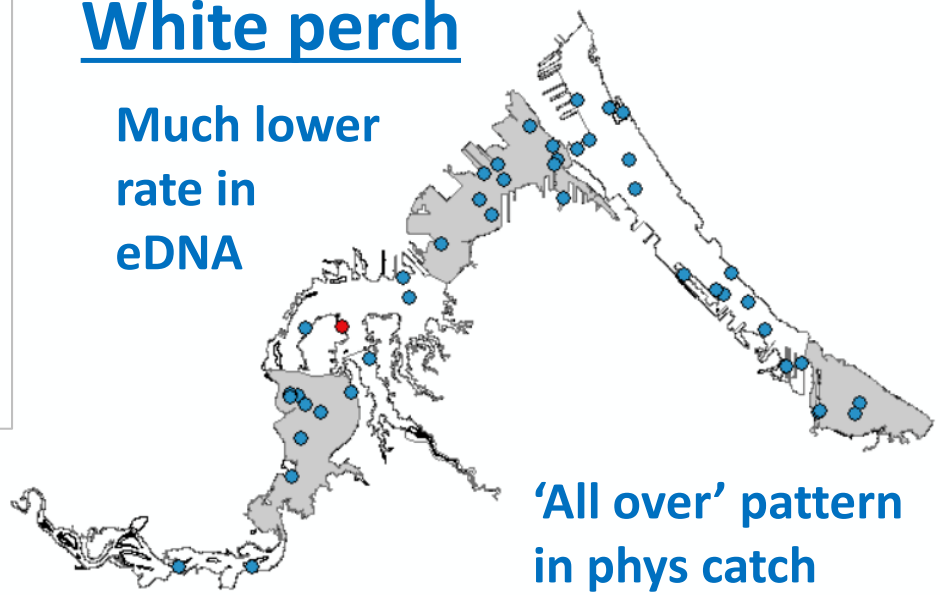
Much higher rate in eDNA



'Linear' eDNA pattern disjunct from phys

White perch

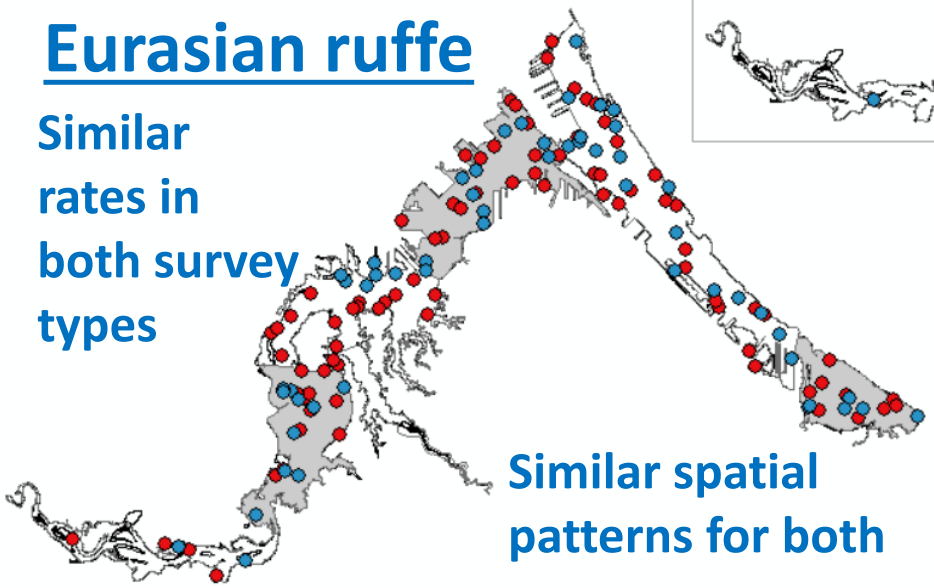
Much lower rate in eDNA



'All over' pattern in phys catch

Eurasian ruffe

Similar rates in both survey types



Similar spatial patterns for both

Early eDNA vs. physical survey patterns for 3 AIS

Discussion – what to make of spatial patterns?

- **eDNA generally finds more species and higher occurrence rates** but spatiotemporal details are quite variable

- **Physical surveys miss species that are present**

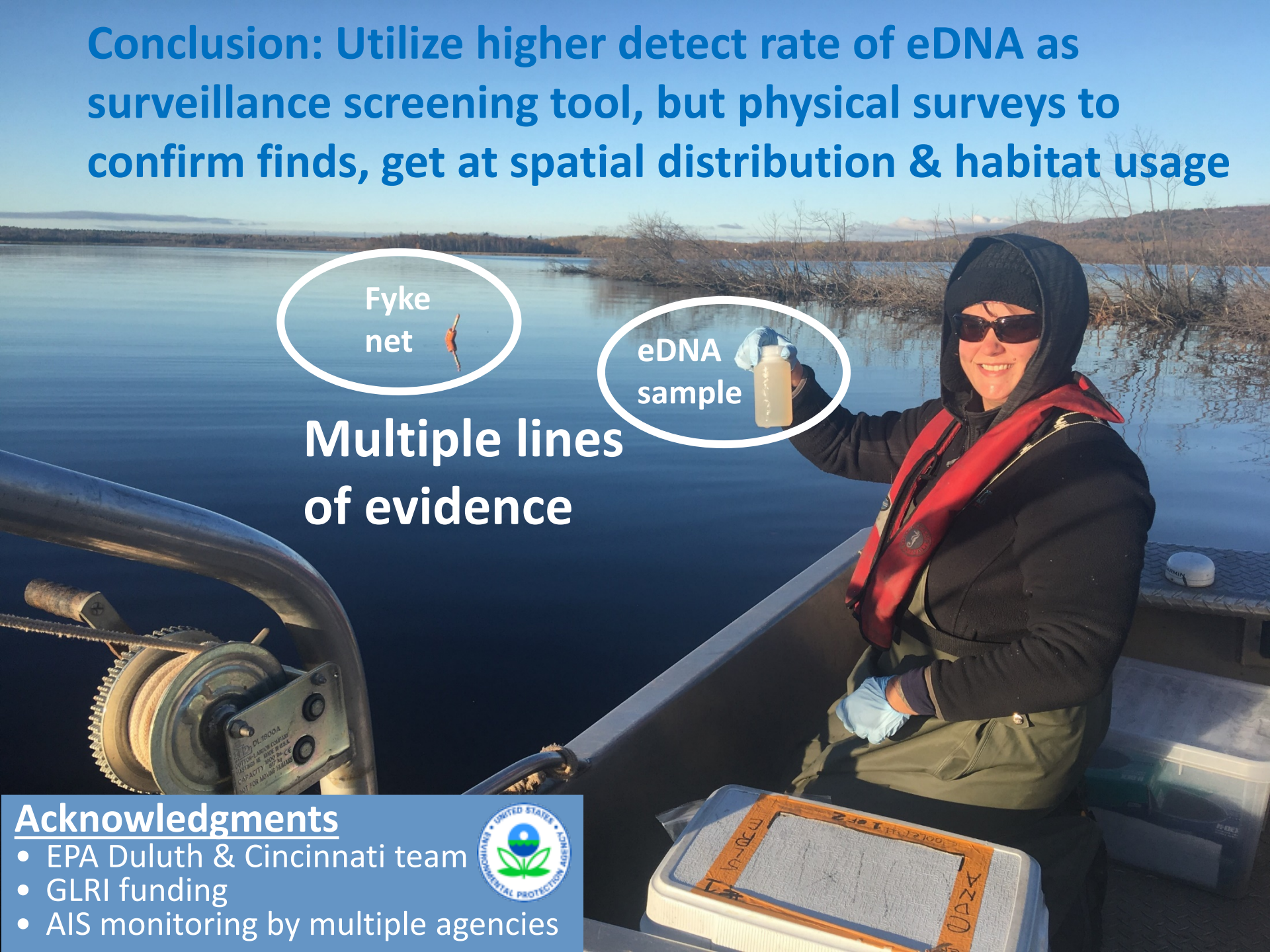
Example: unlikely that all 7 salmonidae species that physical surveys missed was just eDNA pushed in from lake or upstream.

- **eDNA places species contrary to habitat associations**

Example: round goby not usually in shallow veg, rainbow smelt not usually in upper system. Spread by current, wind, boats?

- **Unclear if new AIS are more vulnerable to eDNA or physical survey.** Both examples occurred in this dataset.

Conclusion: Utilize higher detect rate of eDNA as surveillance screening tool, but physical surveys to confirm finds, get at spatial distribution & habitat usage



Acknowledgments

- EPA Duluth & Cincinnati team
- GLRI funding
- AIS monitoring by multiple agencies

ABSTRACT: Analysis of environmental DNA (eDNA) offers a means of detecting target species and characterizing biological communities without having to collect the organisms themselves. The potential for eDNA to disperse widely from the organisms that generated it is a major reason for its appeal as a sampling target, but also raises important questions concerning what can be expected of spatial patterns arising from eDNA data relative to physical catch data. We explore these questions for fish communities in the St. Louis River Estuary -- a hydrologically open and spatially complex freshwater estuary of Lake Superior -- via the comparison of eDNA to physical survey data (~ 240 samples each) for 41 shared fish species. Comparisons among 6 broad spatial zones showed eDNA generally outperforming physical surveys in the early but not the late season, with details including a spatial gradient across zones and differences among the fish guilds involved. Four non-indigenous species were better detected with eDNA surveys, but two others were better detected with physical surveys. NMDS ordinations showed more spatial differentiation in fish structure in the late than early season for both survey types, but with relationships to fetch and vegetation more pronounced for physical surveys. GIS-based 'hot-spot' analyses showed much more pronounced spatial clumping of many fish species with physical surveys than with eDNA data. eDNA surveys provides a sensitive tool for establishing species presence at the system scale but tends to obscure spatial distribution information that is relevant to location-specific restoration and management actions.