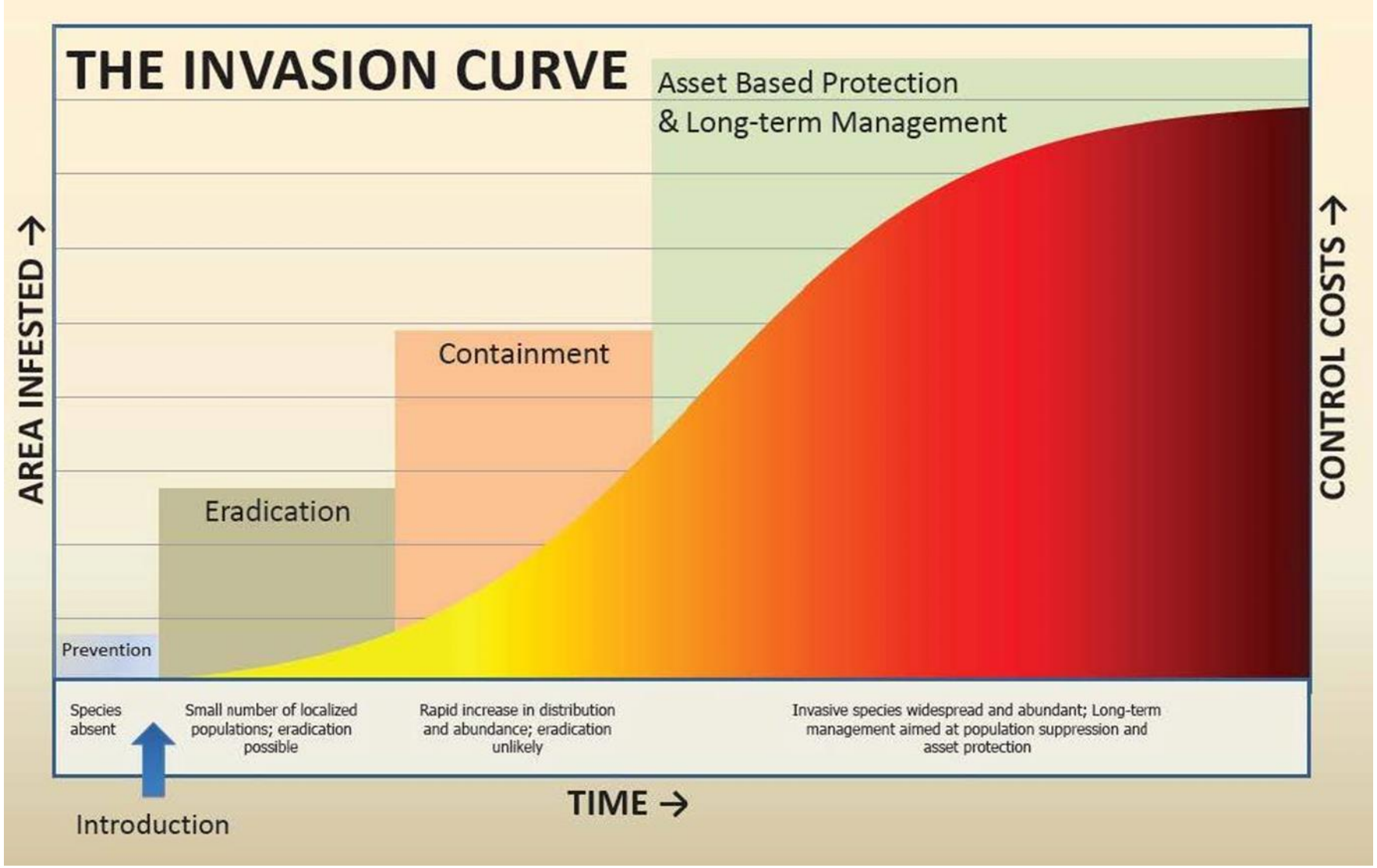
Comparing environmental DNA (eDNA) and traditional surveys of diversity and relative abundance: implications for invasive fishes

Nick Sard, **Seth Herbst,** Genelle Uhrig, Jeannette Kanefsky, and Kim Scribner

Fort Lauderdale, Florida

Oct 24, 2017

Importance of Early Detection



Rodgers, L., et al. "Status of nonindigenous species." *South florida environmental report* 1 (2012): 7-35.

Methods and Strategy for AIS Detection

- Standardized statewide Status and Trends sampling program to monitor fish populations
  - Also used for AIS detection, but how effective is this method?

- Traditional gear has been evaluated for maximizing early detection in Great Lakes harbors
  - Has led to targeted AIS sampling in high risk harbors in the Great Lakes (implemented by USFWS)
  - Little work has been done on inland lakes

Sampling Design for Early Detection of Aquatic Invasive Species in Great Lakes Ports

Joel C. Hoffman, Joshua Schloesser, Anett S. Trebitz, Greg S. Peterson, Michelle Gutsch, Henry Quinlan & John R. Kelly

eDNA Sampling for AIS

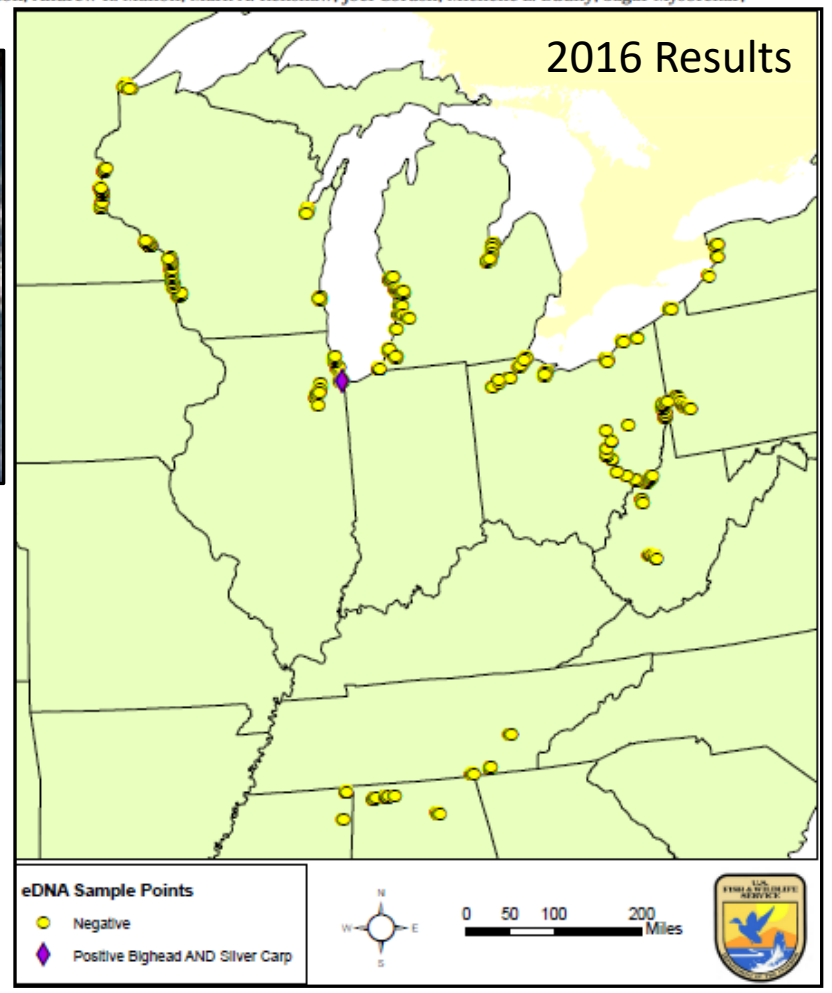
- Emerging genetic approaches (eDNA barcoding) used to detect species at rare or low densities
  - Primarily been used for Bighead and Silver Carps

NRC Research Press

RAPID COMMUNICATION

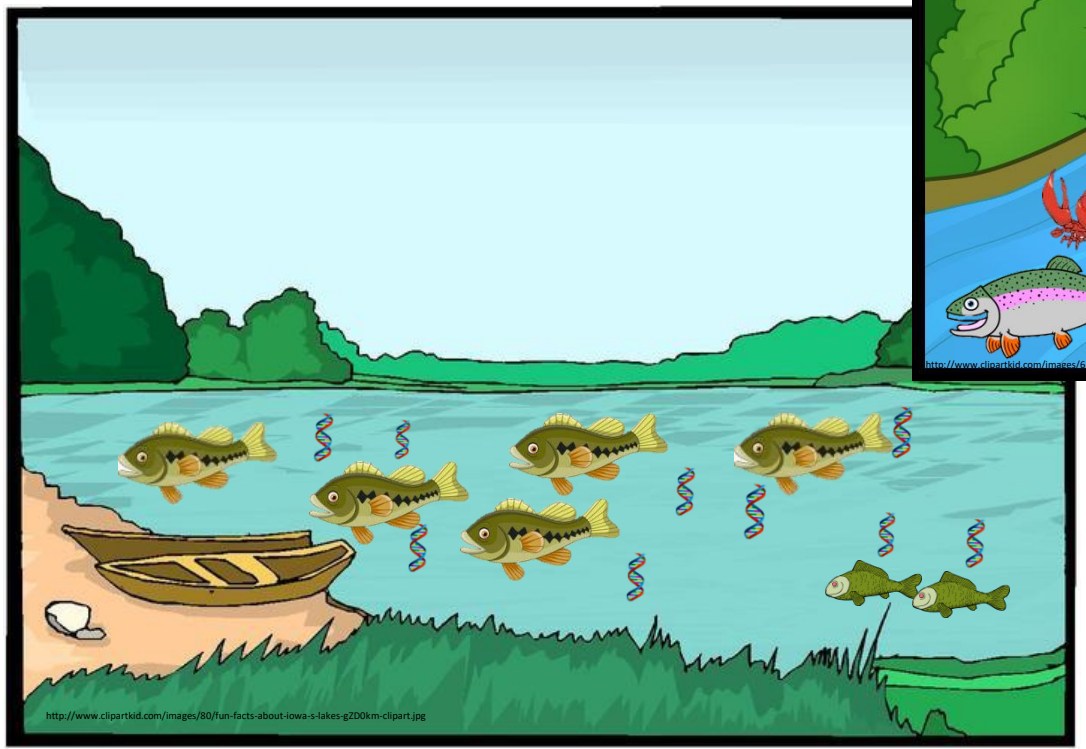
Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program

Christopher L. Jerde, W. Lindsay Chadderton, Andrew R. Mahon, Mark A. Renshaw, Joel Corush, Michelle L. Budny, Sagar Mysorekar, and David M. Lodge



What is DNA Barcoding?

- Plants and animals constantly extrude DNAs in environment (eDNA)
- Take water samples, extract eDNAs, and test for presence of a species
- Used to detect low abundance species (e.g. AIS and T & E)
- Single species focused



MOLECULAR ECOLOGY
Molecular Ecology (2016) 25, 3101–3119 doi: 10.1111/mec.13660

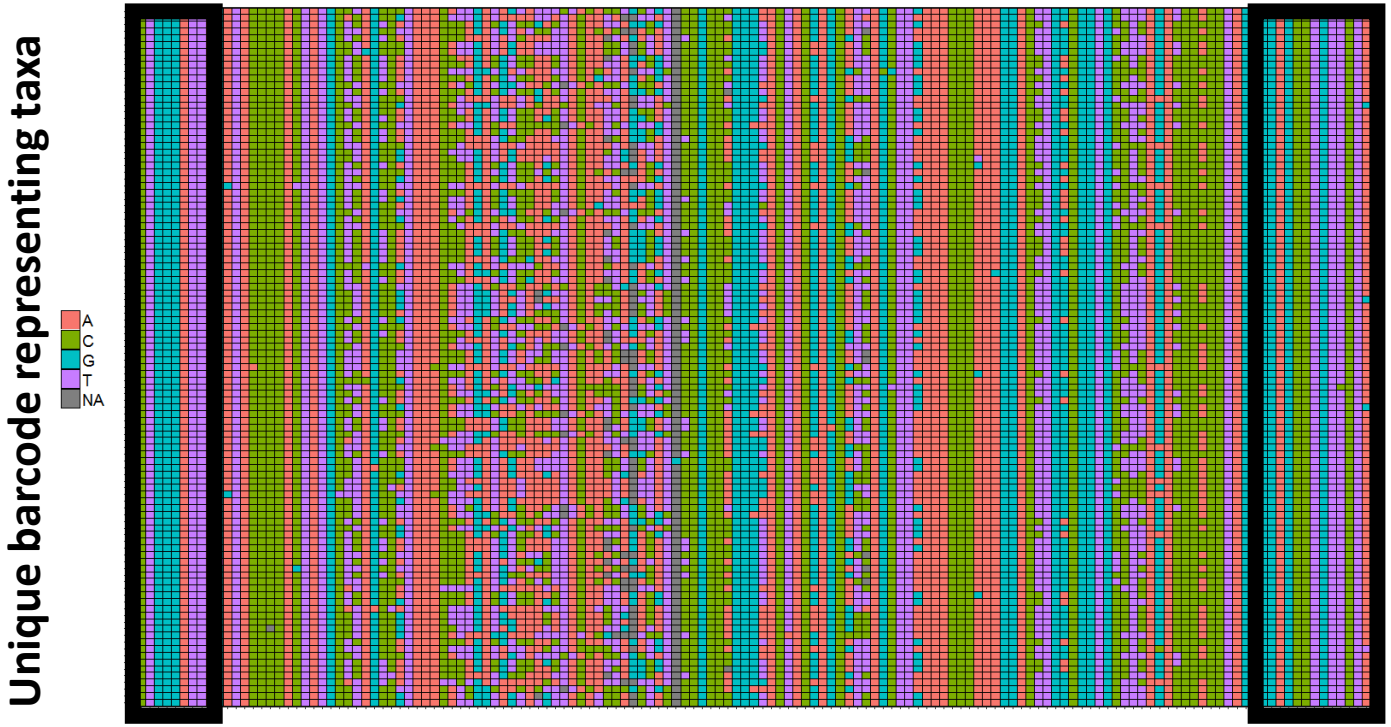
Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods

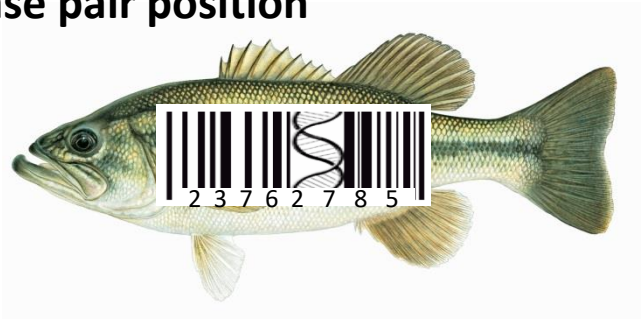
BERND HÄNFLING,*[1] LORI LAWSON HANDLEY,*[1] DANIEL S. READ,† CHRISTOPH HAHN,* JIANLONG LI,* PAUL NICHOLS,* ROSETTA C. BLACKMAN,* ANNA OLIVER† and IAN J. WINFIELD‡

eDNA Meta-barcoding Characterizes Entire Communities

- Standardized regions of DNA
  - Usually book-ended by highly conserved regions - universal primers
  - Contain areas with greater inter-specific differentiation or sequence divergence

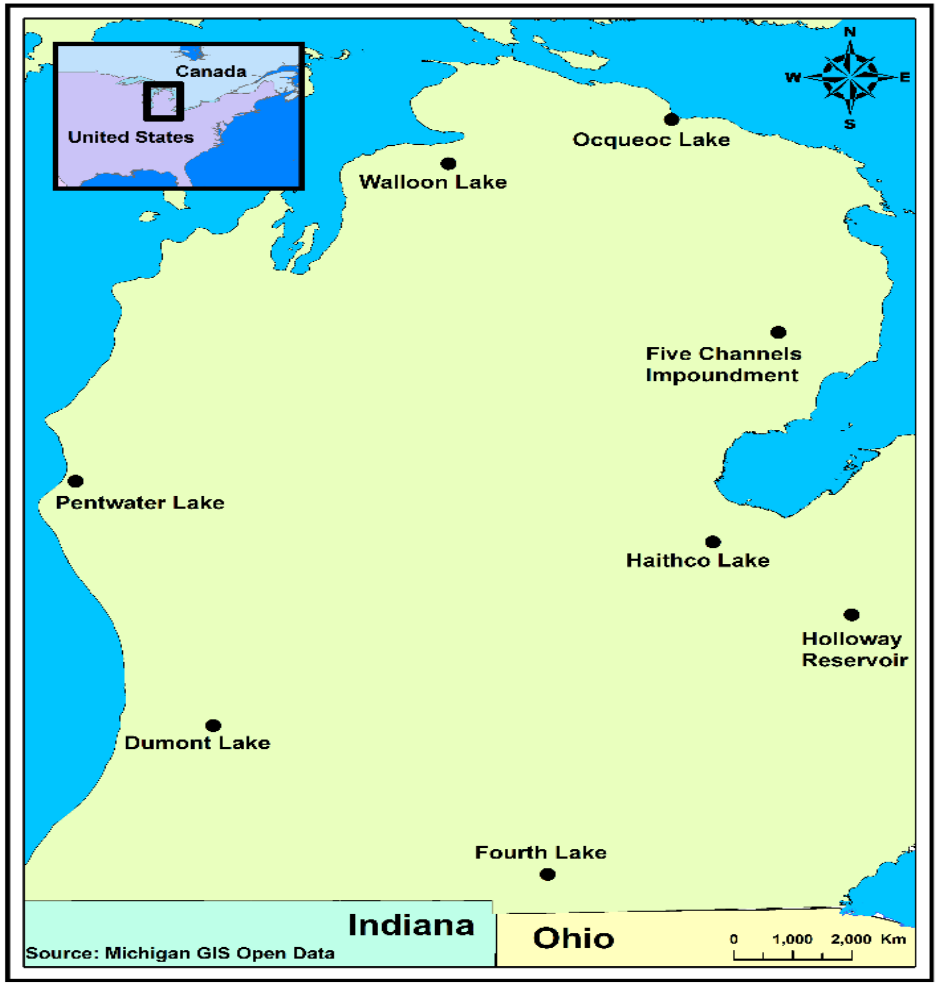
- Common regions used for barcoding
  - Cytochrome oxidase I (mtDNA)
  - **12S and 16S ribosomal unit (mtDNA)**
  - RuBisCo large subunit (plants)



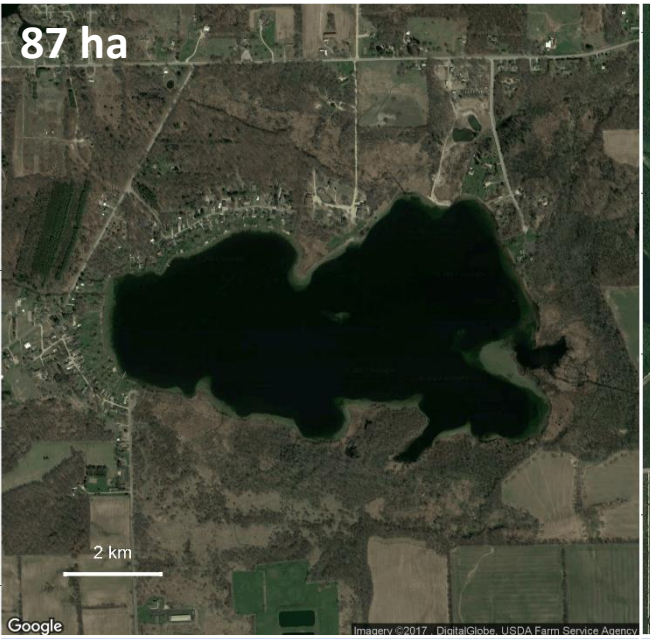


Goals: Evaluate effectiveness of Status and Trends monitoring program for AIS detection and the added value of incorporating eDNA sampling

- MDNR sampled eight lakes in 2016
  - Part of Status and Trends Survey
  - Multiple gear types used
- Sampled for eDNA one week following traditional sampling
- We collected 50 ± 8 (mean ± SD) eDNA water samples from each lake
  - 400 total samples collected



Dumont Lake

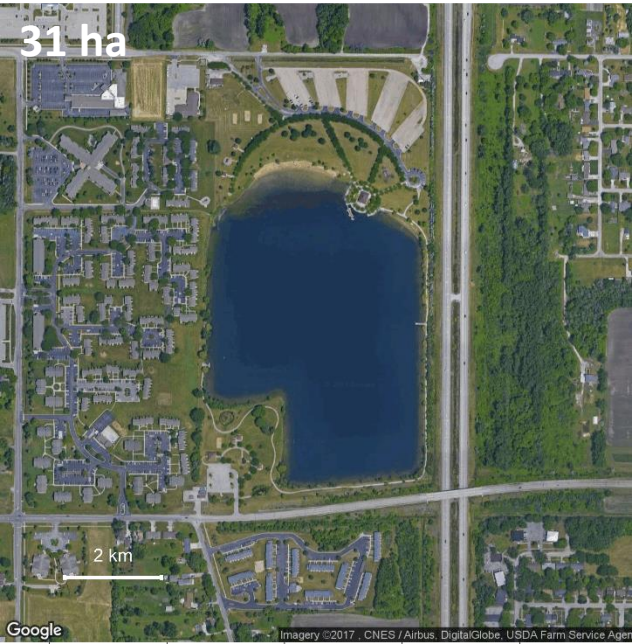


Five Channels Impoundment

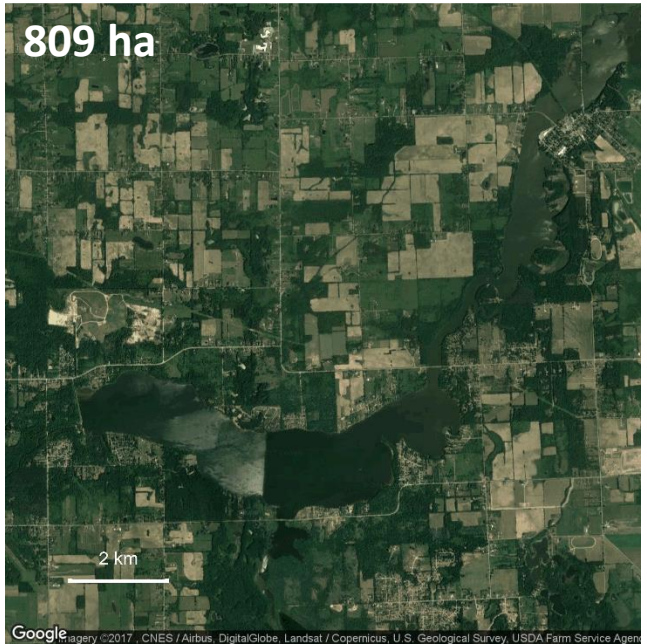
Fourth Lake

Ocqueoc Lake

Haithco Lake



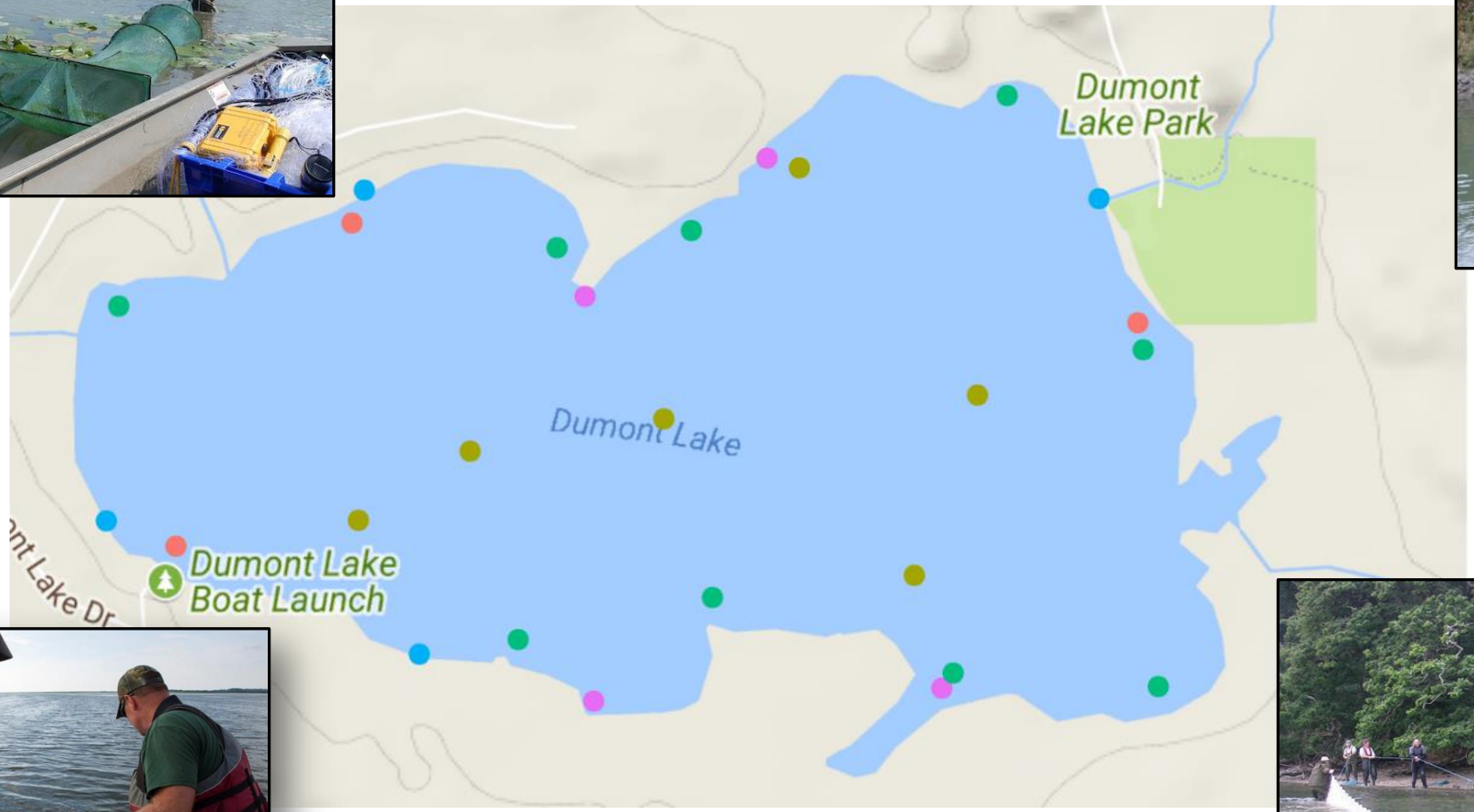
Holloway Reservoir



Pentwater Lake

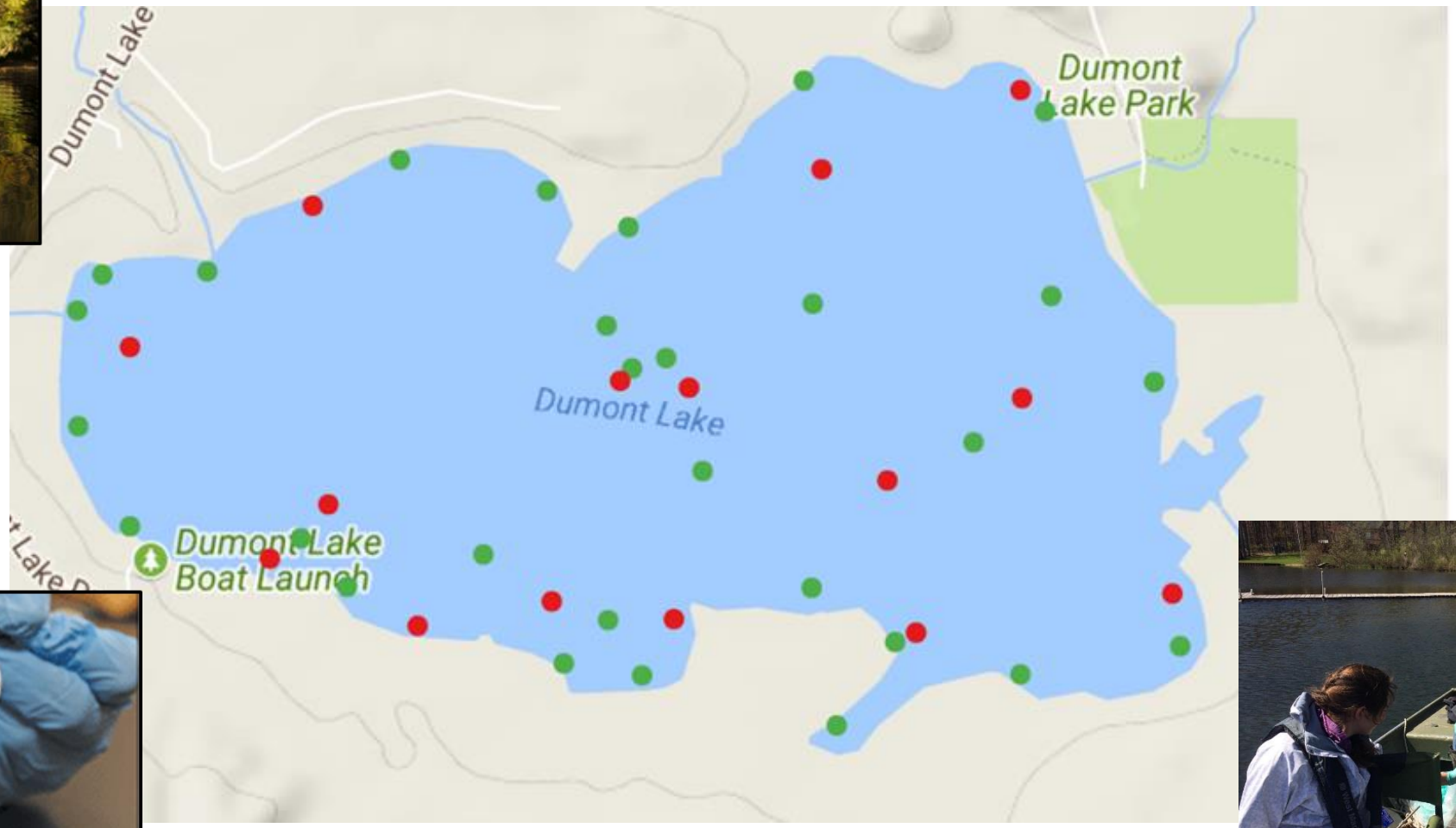
Walloon Lake

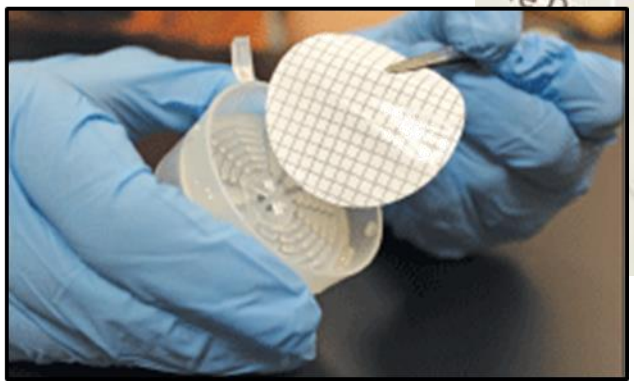
Fisheries Division Status and Trends Sampling Sites



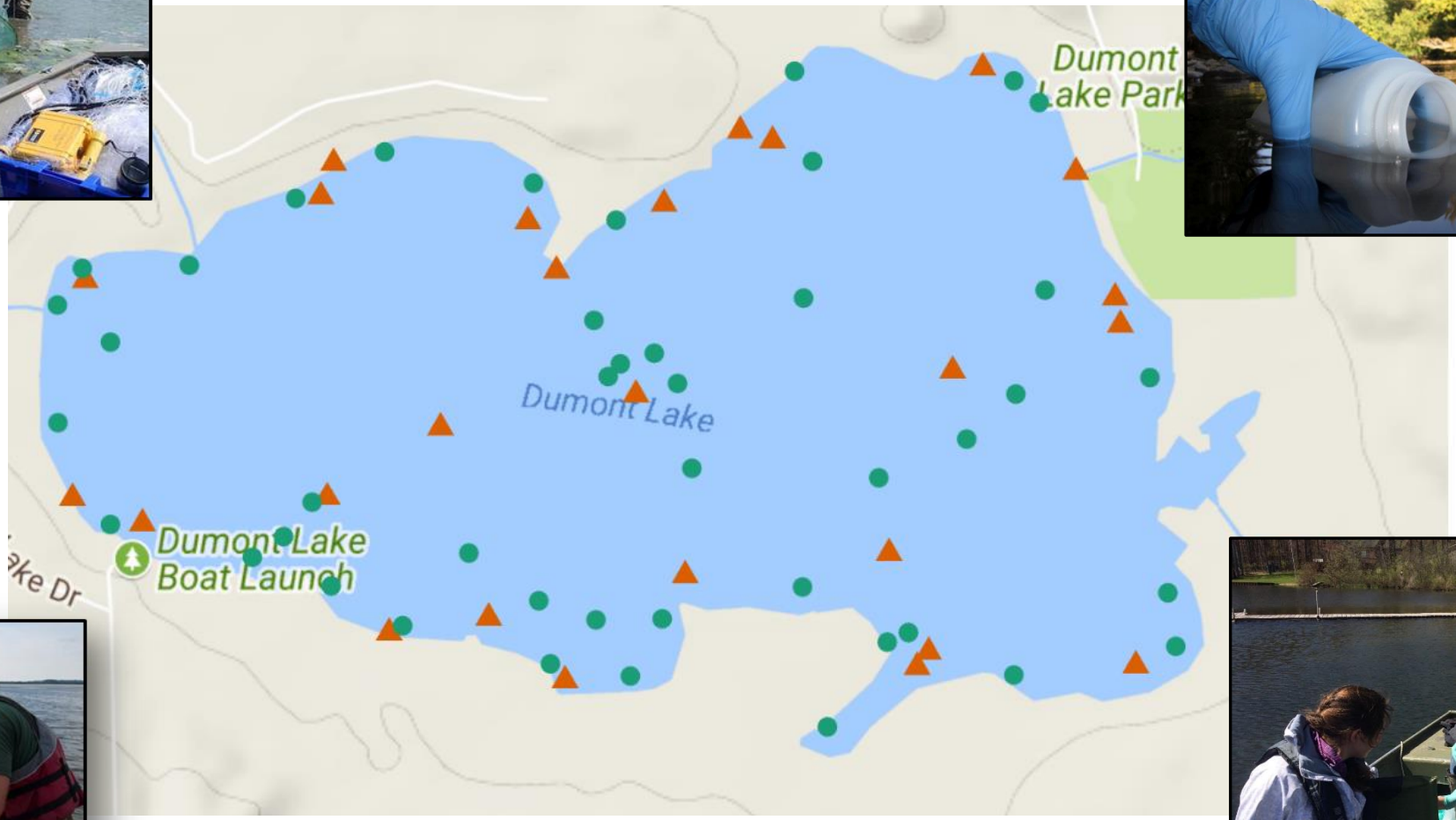
Gear type
- Boomshocker
- Experimental gillnet
- Large mesh fyke net
- Seine
- Small mesh fyke net

MSU eDNA Sampling Sites





Comprehensive Sampling Strategy

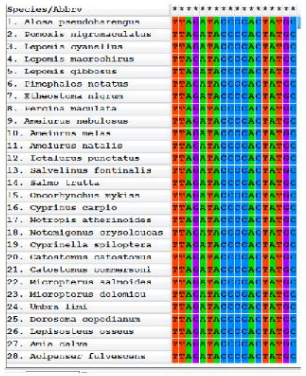


eDNA Metabarcoding Process to Determine Community Composition

Water sampling & eDNA isolation

DNA sequence collection & comparison

Design of "Universal" Primers



Testing for ability of selected gene region(s) to amplify & discriminate <u>all</u> target species

Simultaneous PCR amplification of target amplicon(s) in each eDNA sample

Amplicon library prep & combined HTS for each eDNA sample

Data indexing, cleaning, filtering & bioinformatic analysis

Comparison of eDNA sample sequence data to reference sequence database

= Black Crappie

= Bluegill

= Round Goby

= Yellow Perch

ID of target species' DNA sequences in each sample

Status of eDNA project

Water sampling & eDNA isolation

DNA sequence collection & comparison

Design of "Universal" Primers

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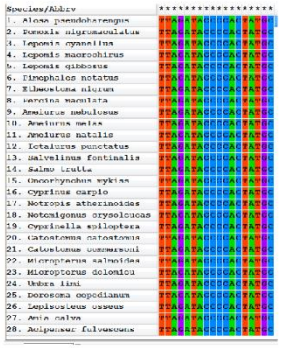

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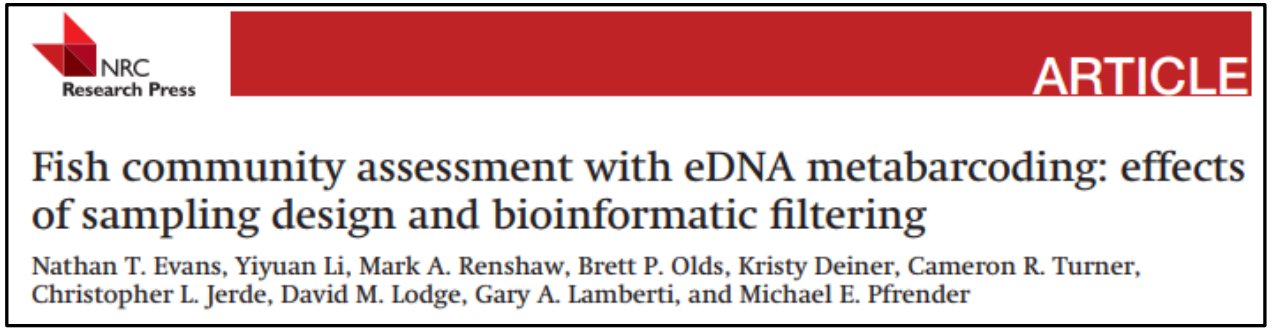
ID of target species' DNA sequences in each sample

Bioinformatics Stringency Criteria for Species Detection

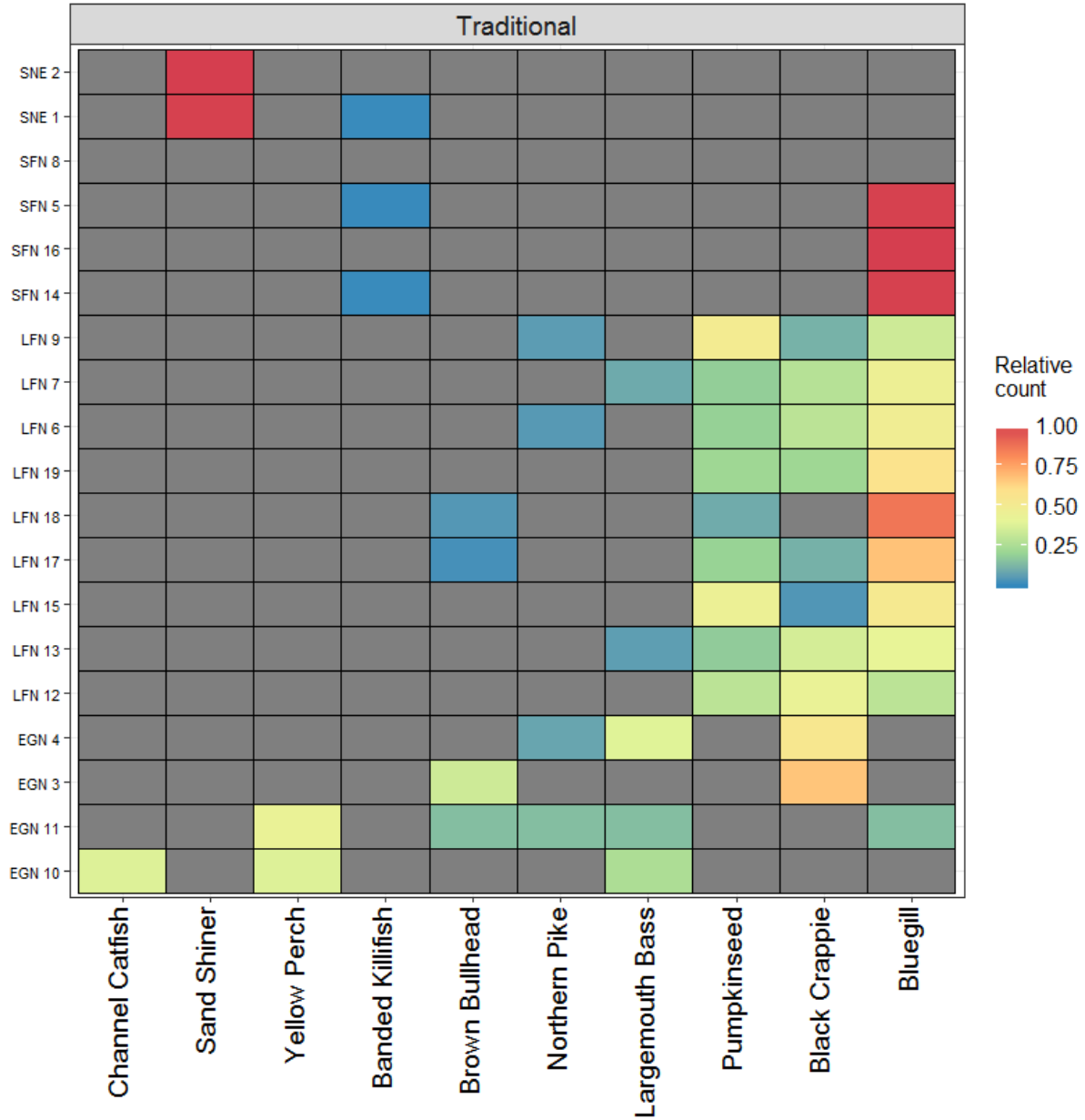
1. Liberal criteria for AIS observation
   - Before any filtering based on negative (no DNA) controls
   - For each lake:
     - Were any AIS sequences observed?
     - In how many samples?
     - Mean number of reads per sample?
     - May warrant additional sampling
2. Conservative criteria for community diversity estimates
   - Ask the same questions above
   - Account for negative controls, and remove unclassified columns
     (*can bias species detection low*)
   - Compare measures of diversity with traditional methods

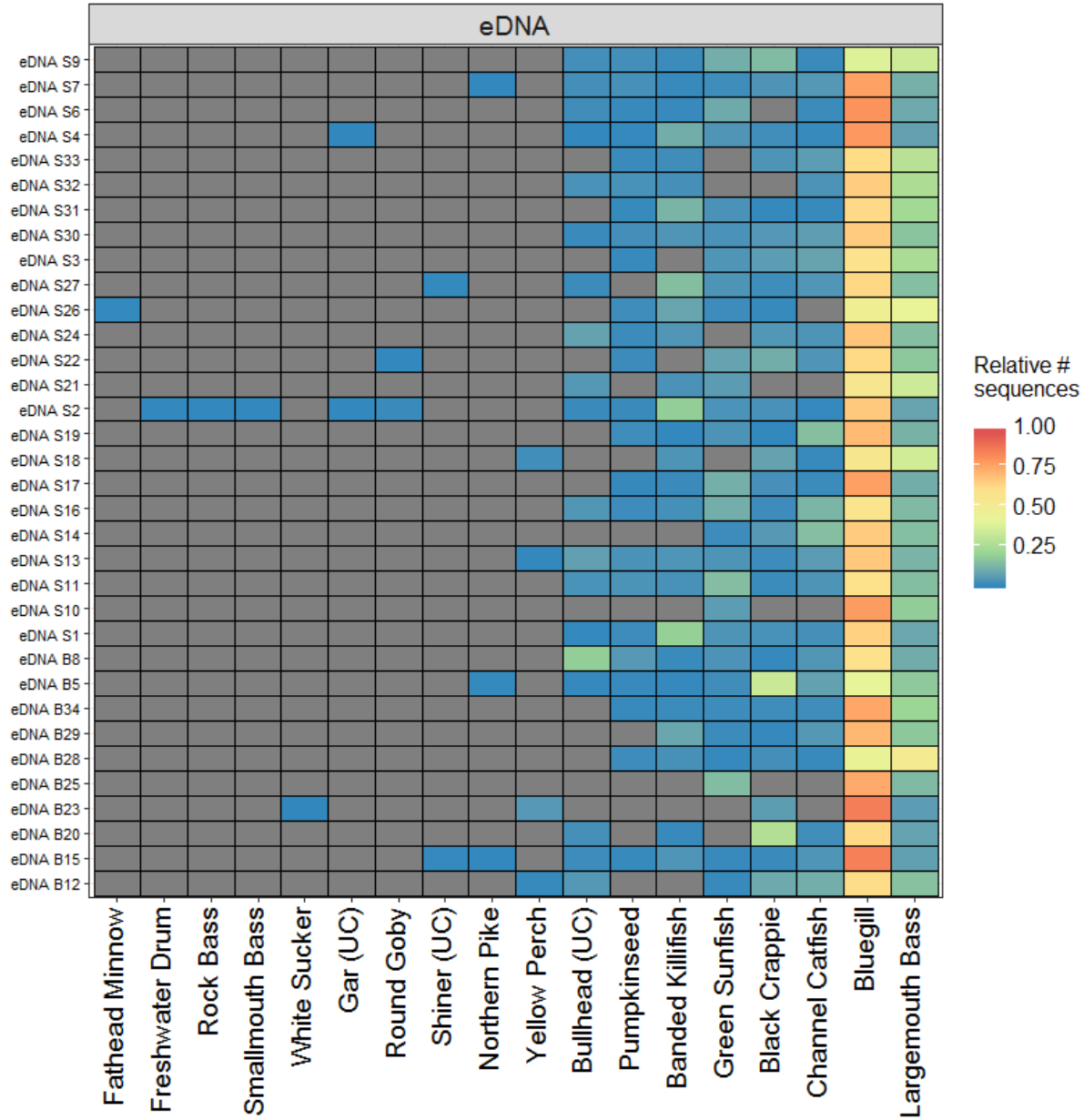


eDNA sample				…
1	11,100	691	0	…
2	3,334	52	0	…
…	…	…	…	…
400	2	3,076	5	…

52 Unique classifications
(48 to species; 4 only to genus)

Haithco Lake: eDNA and Traditional Community Matrices





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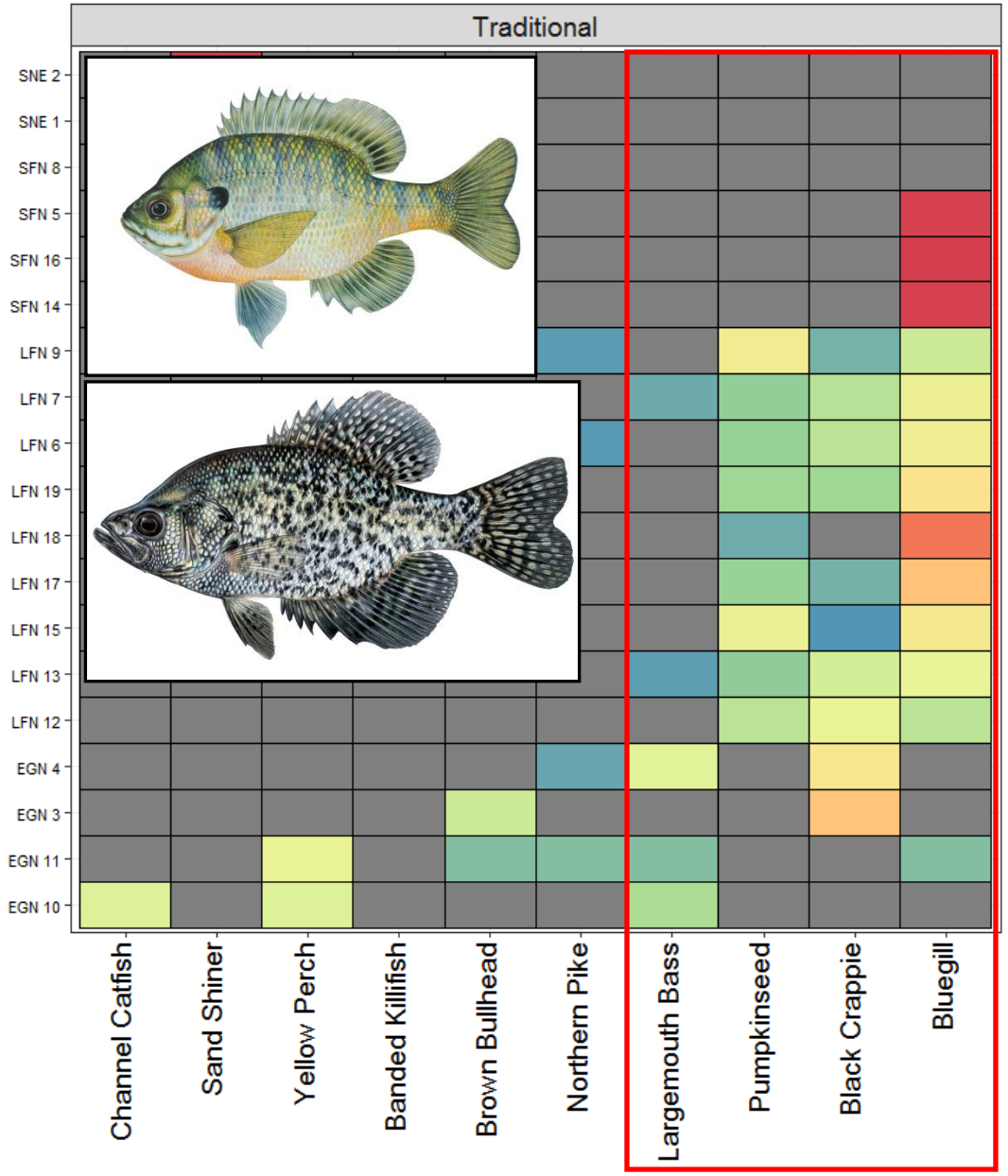

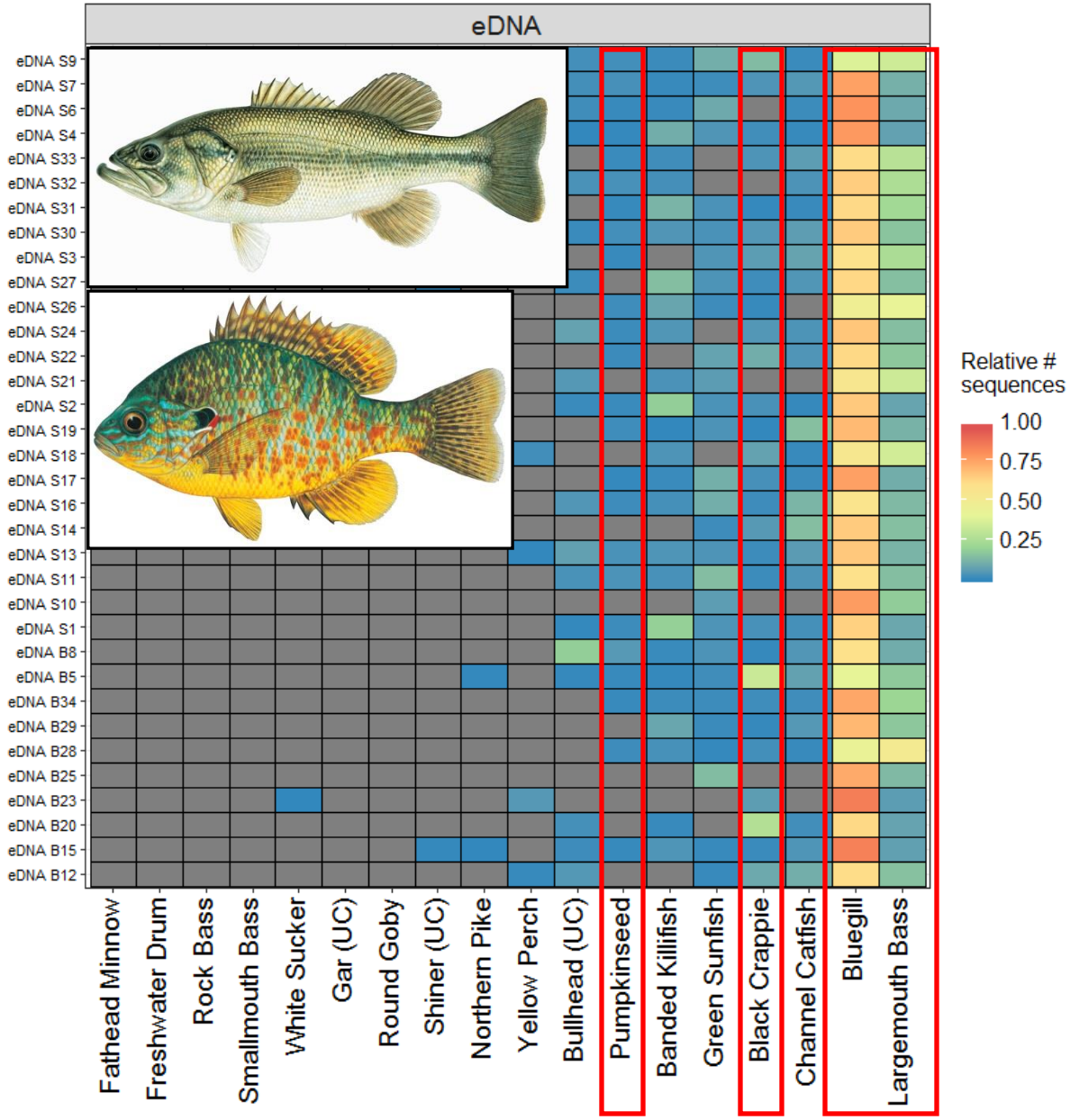

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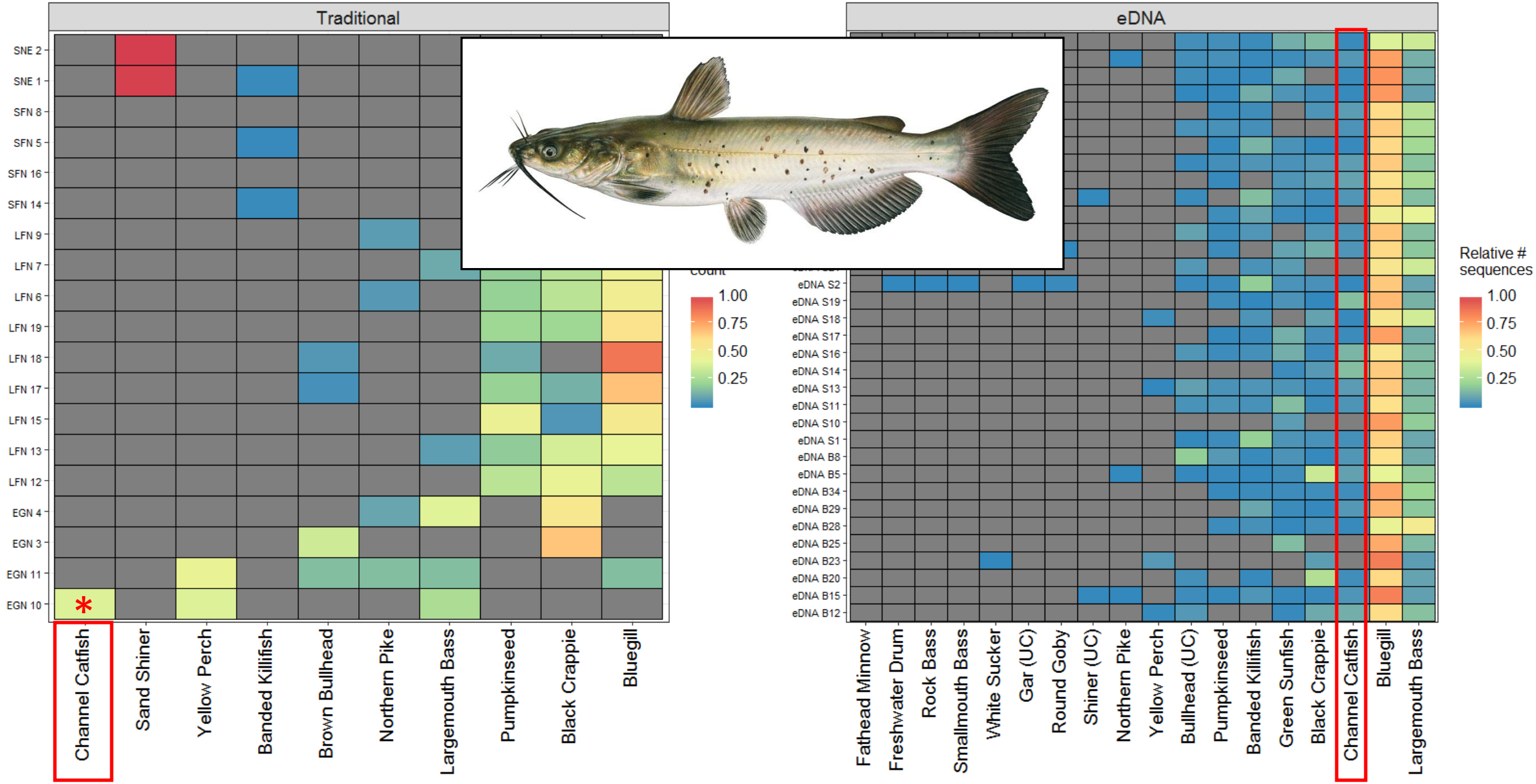
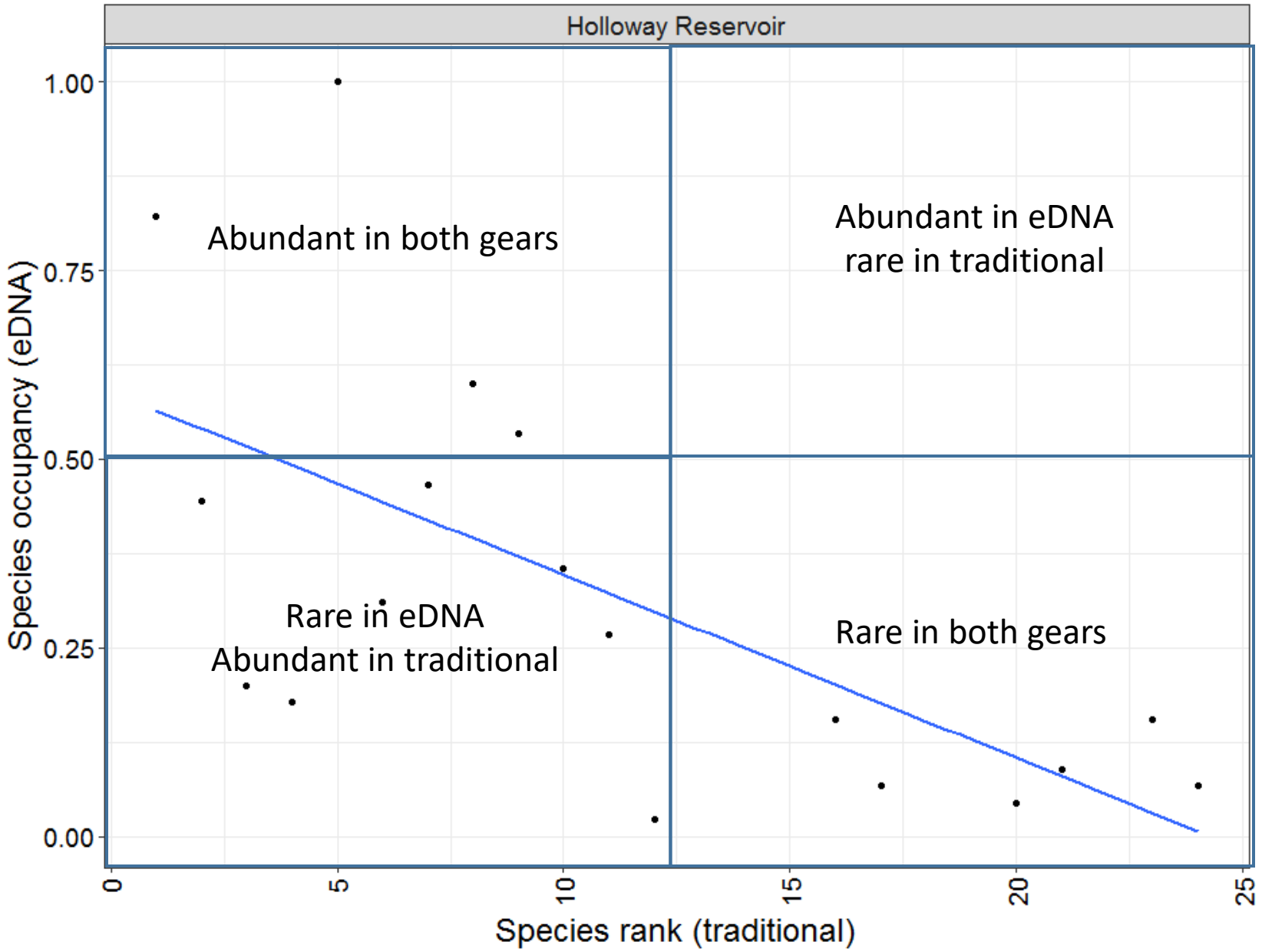


Correlation of Species Occupancy (eDNA) and Species Rank (traditional)



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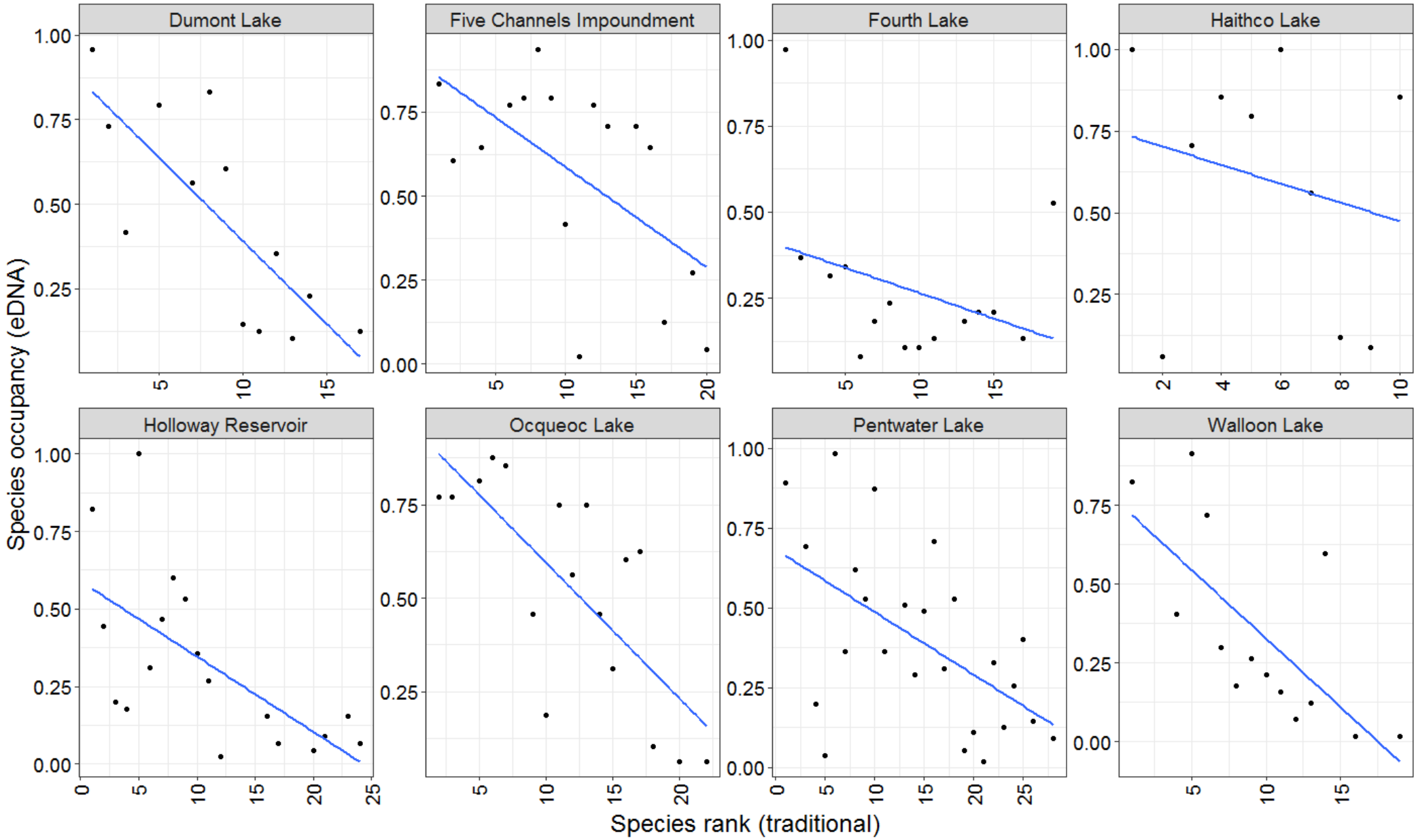
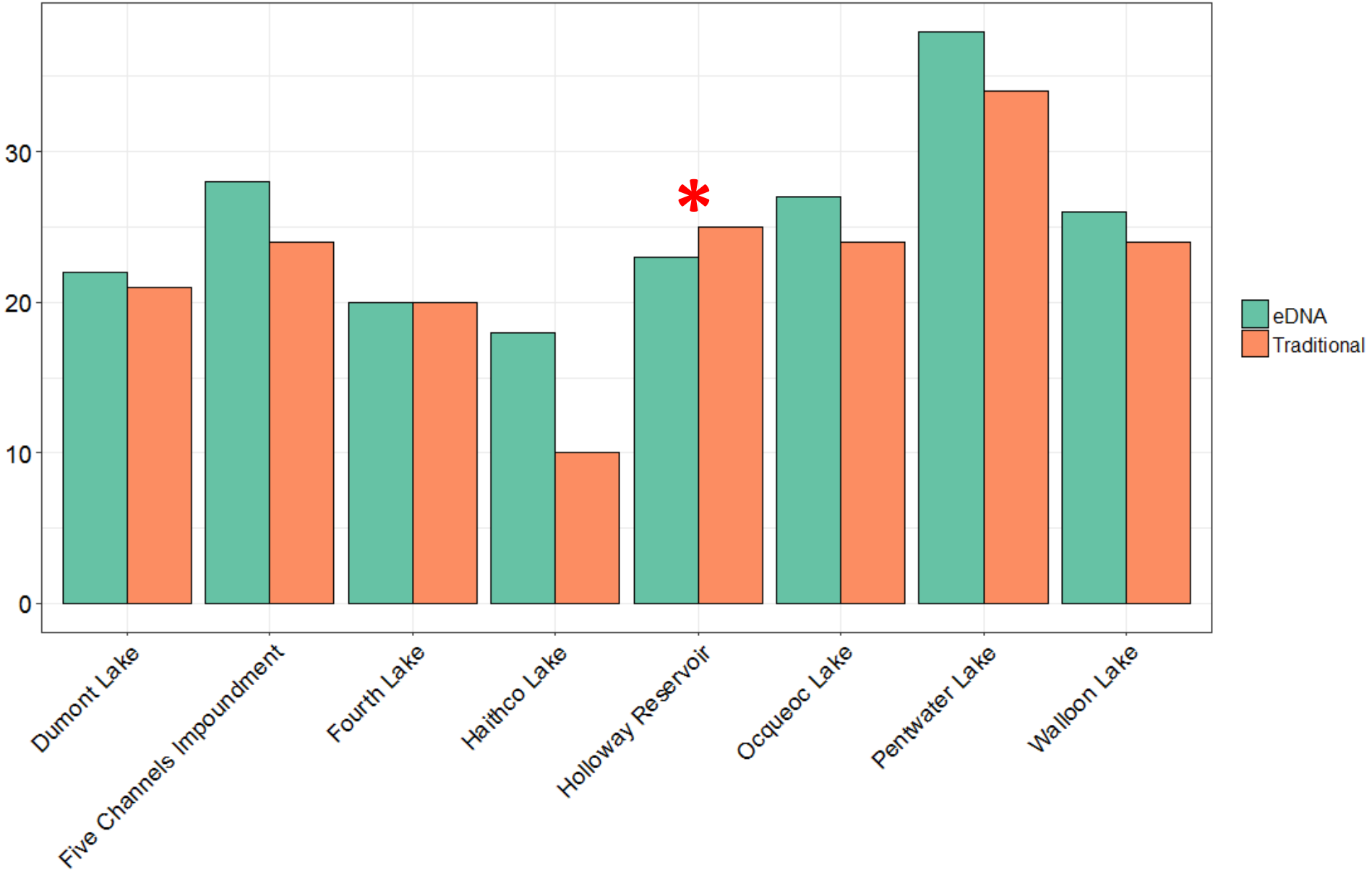


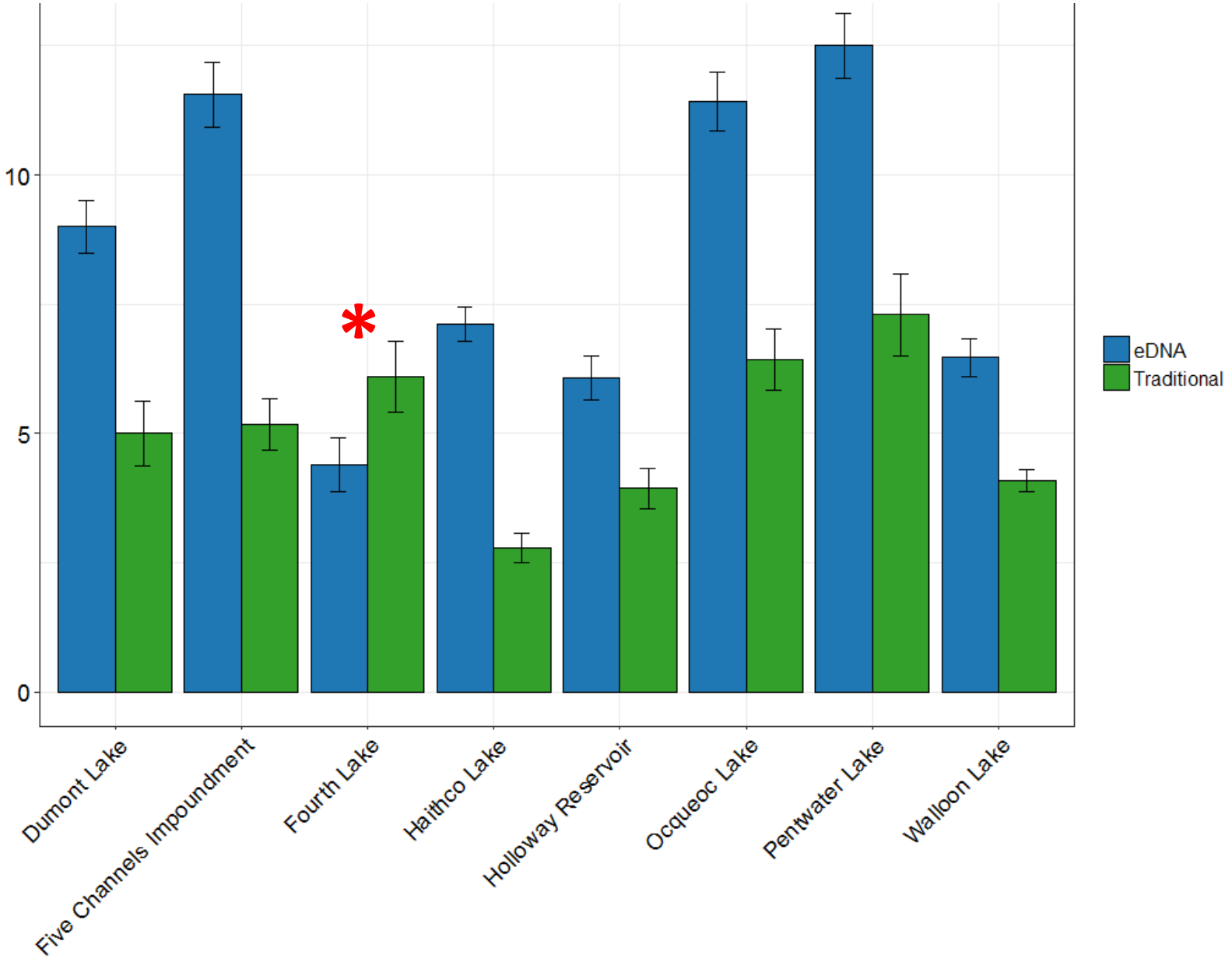
Total Species Detection: eDNA vs. Traditional Methods

Total number of species observed

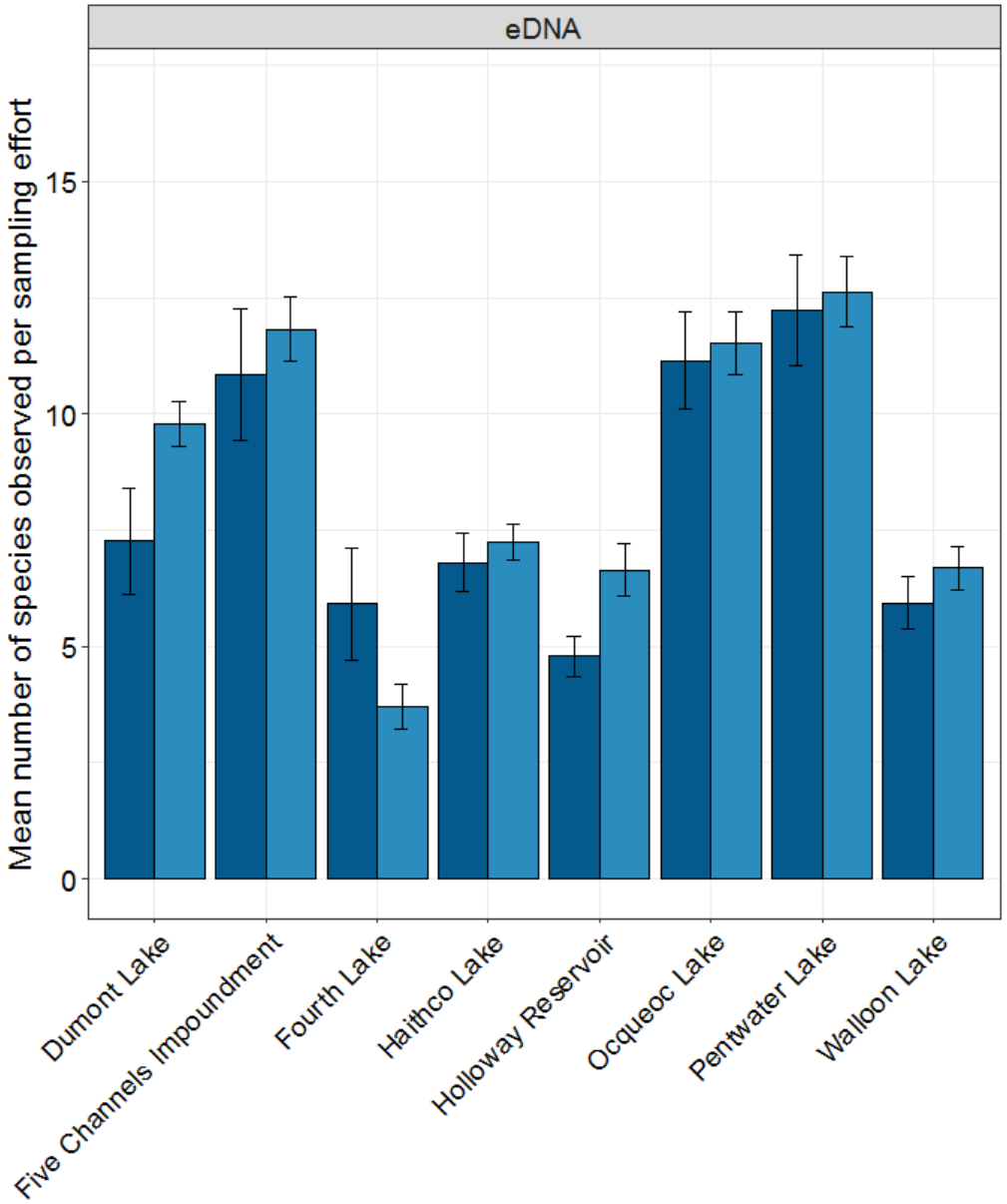


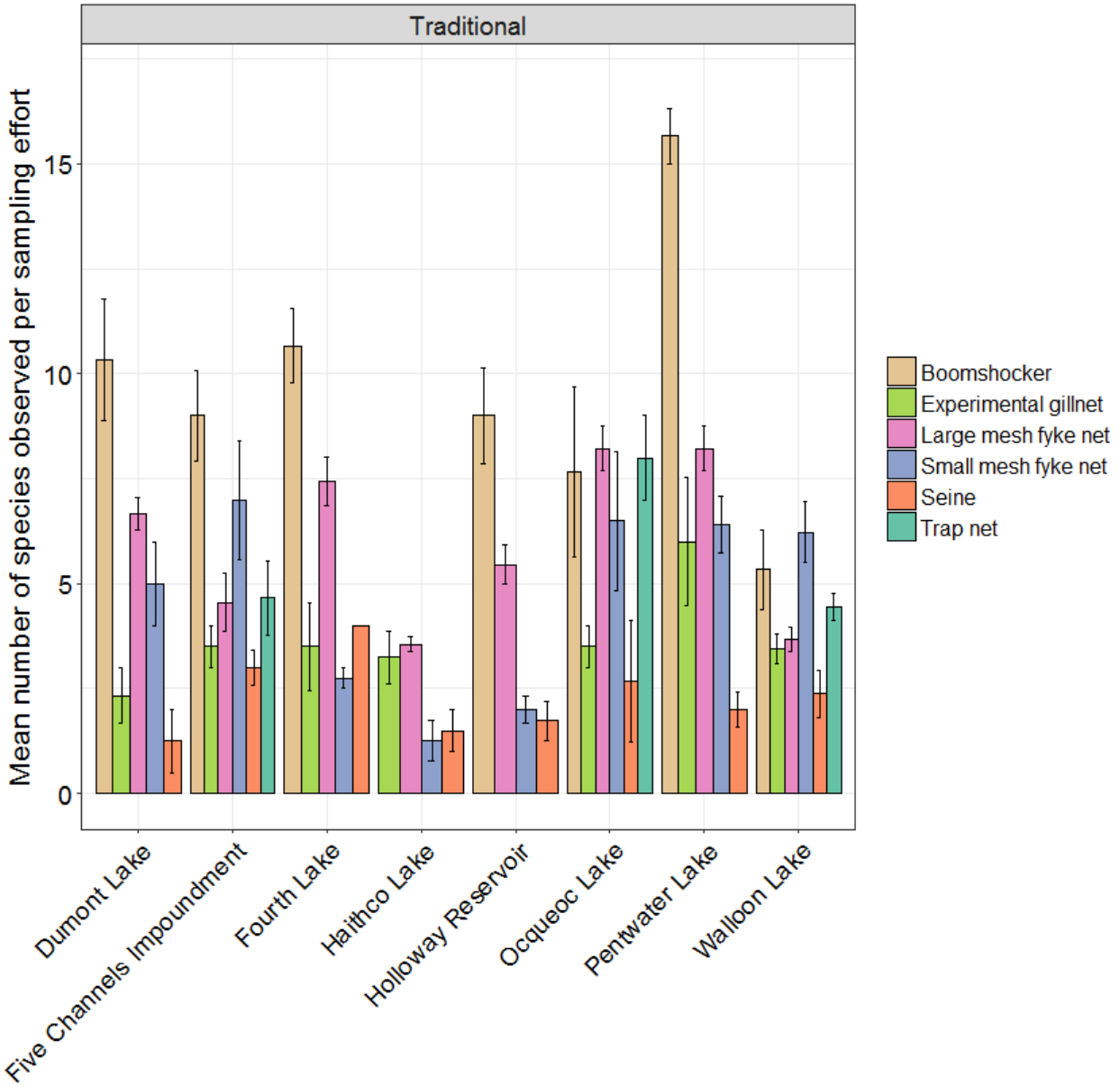
Mean Species Detection: eDNA vs. Traditional Methods

Mean number of species observed per sample

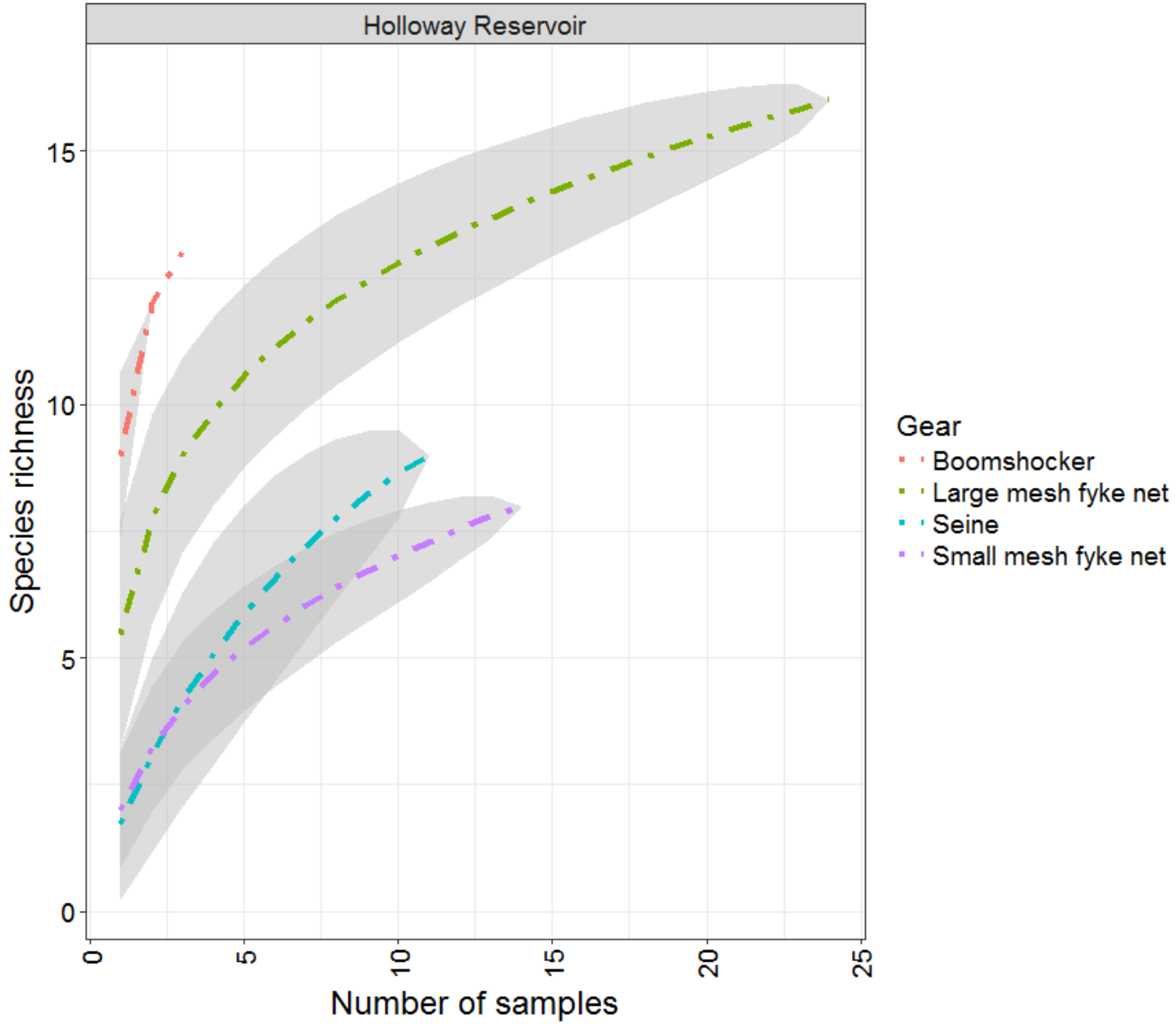


Variation among eDNA and Traditional Sampling Approaches

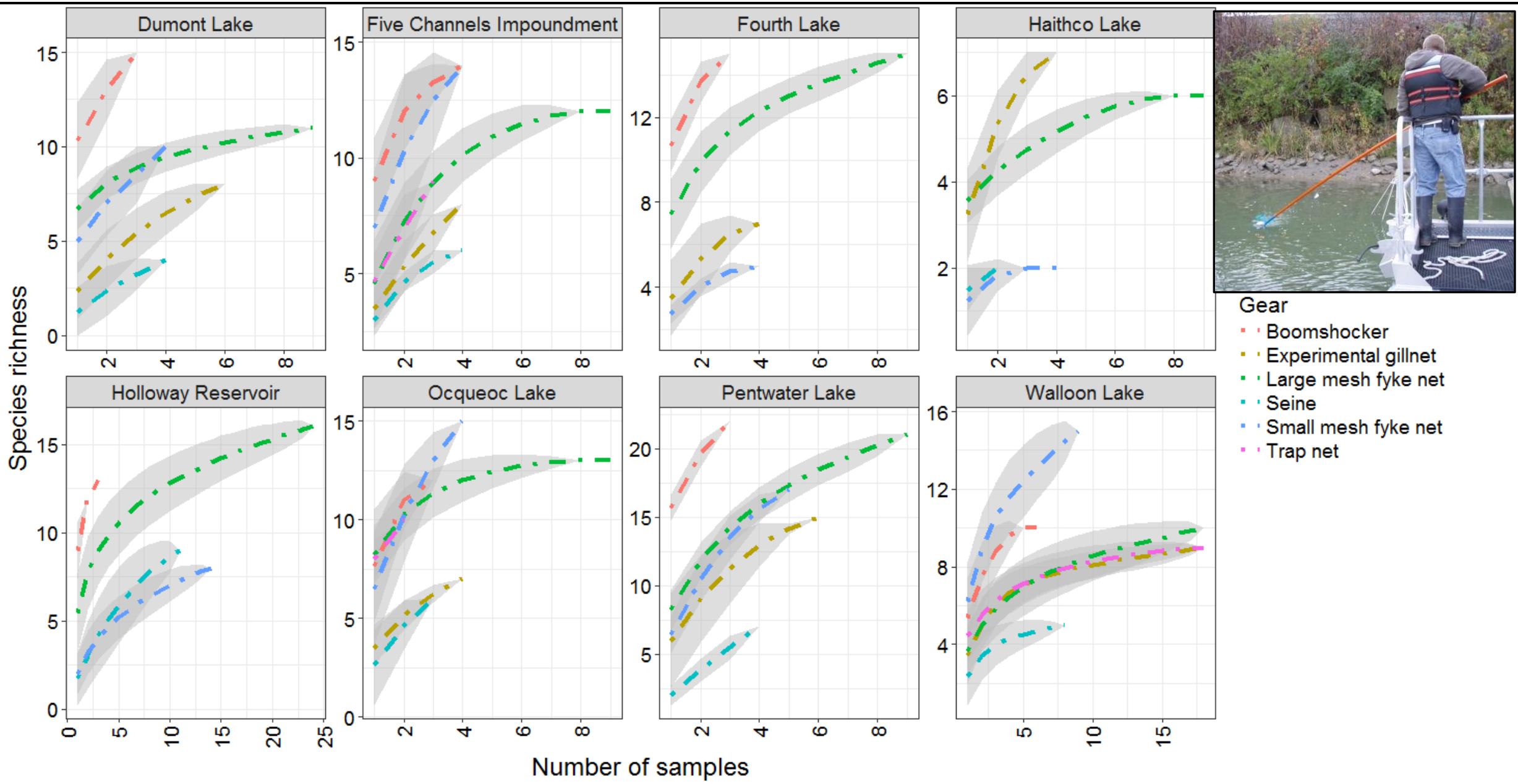




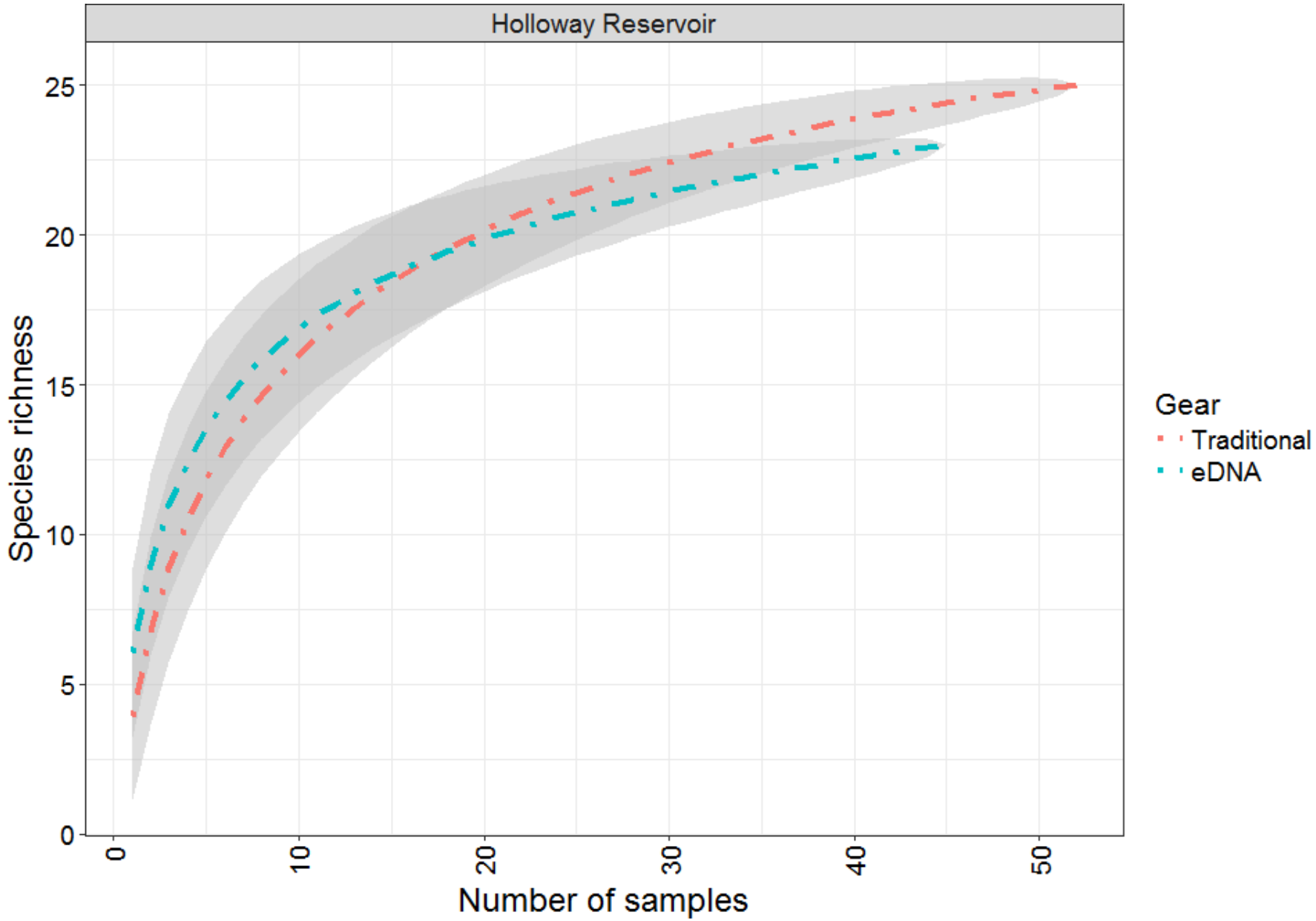
Holloway Reservoir: Species Accumulation Curve with Traditional Gear



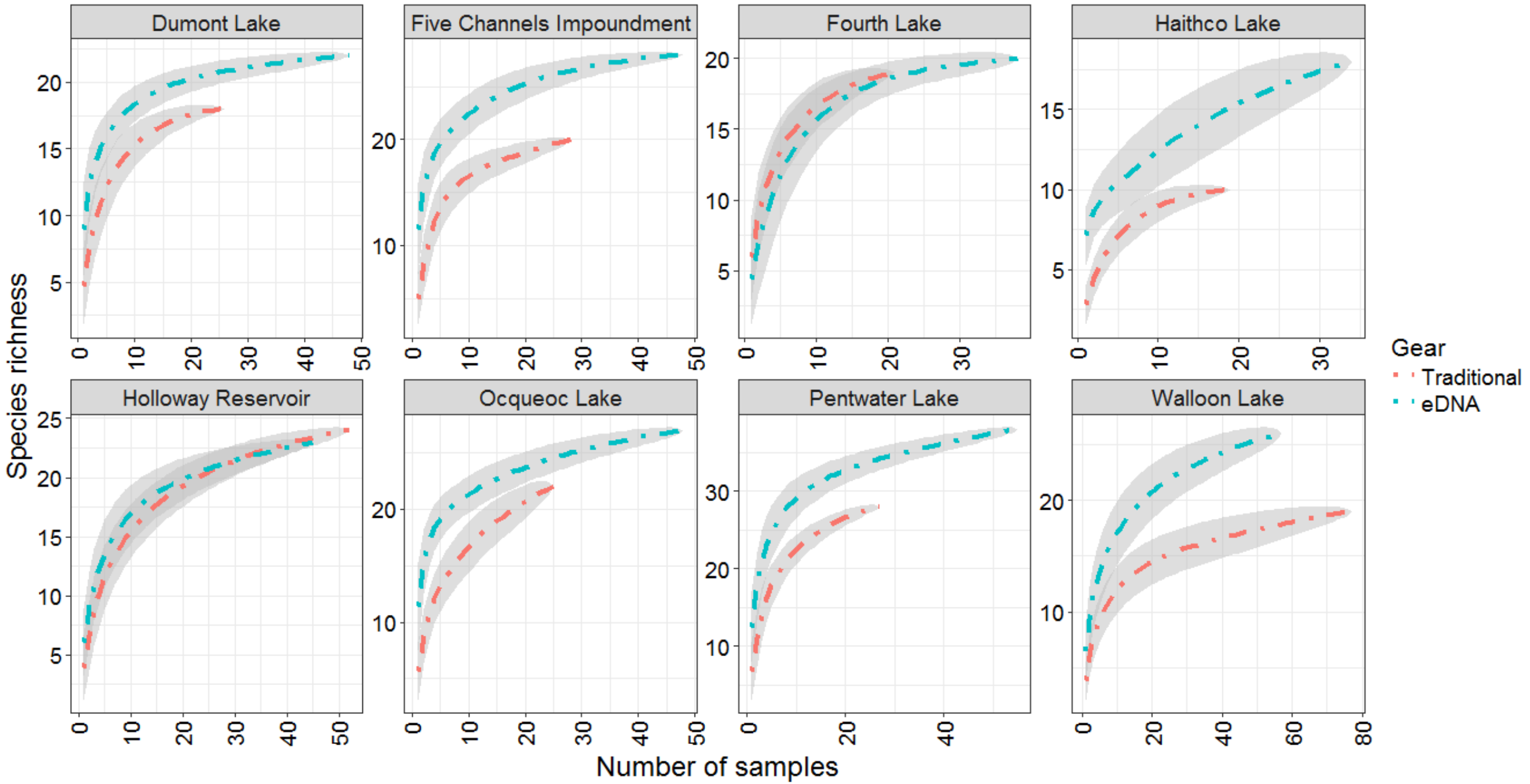
Species Accumulation Curves: Individual Traditional Methods



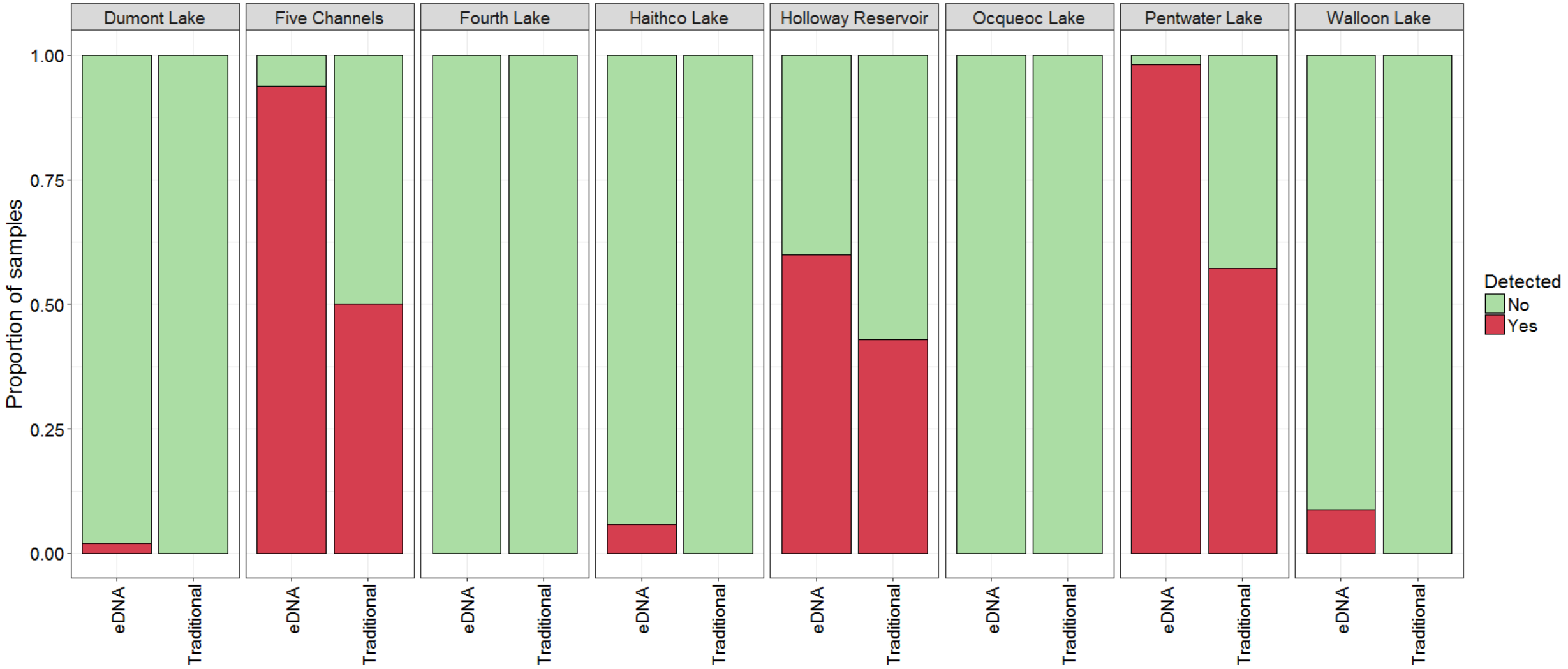
Holloway Reservoir: Traditional Gear Species Accumulation Curve



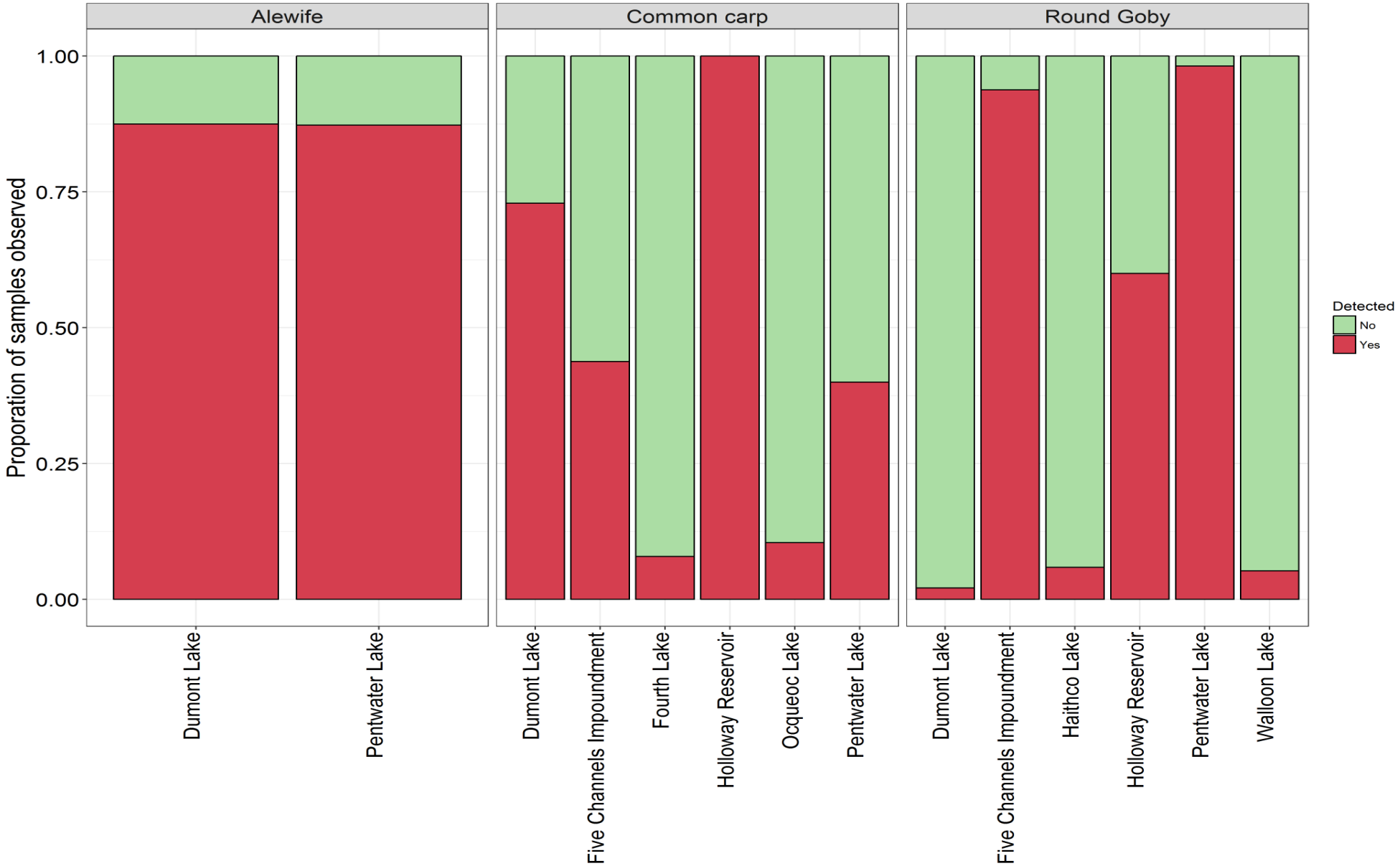
Species Accumulation Curves: eDNA vs. Combined Traditional Methods



eDNA Approach Effectively Detected Round Goby

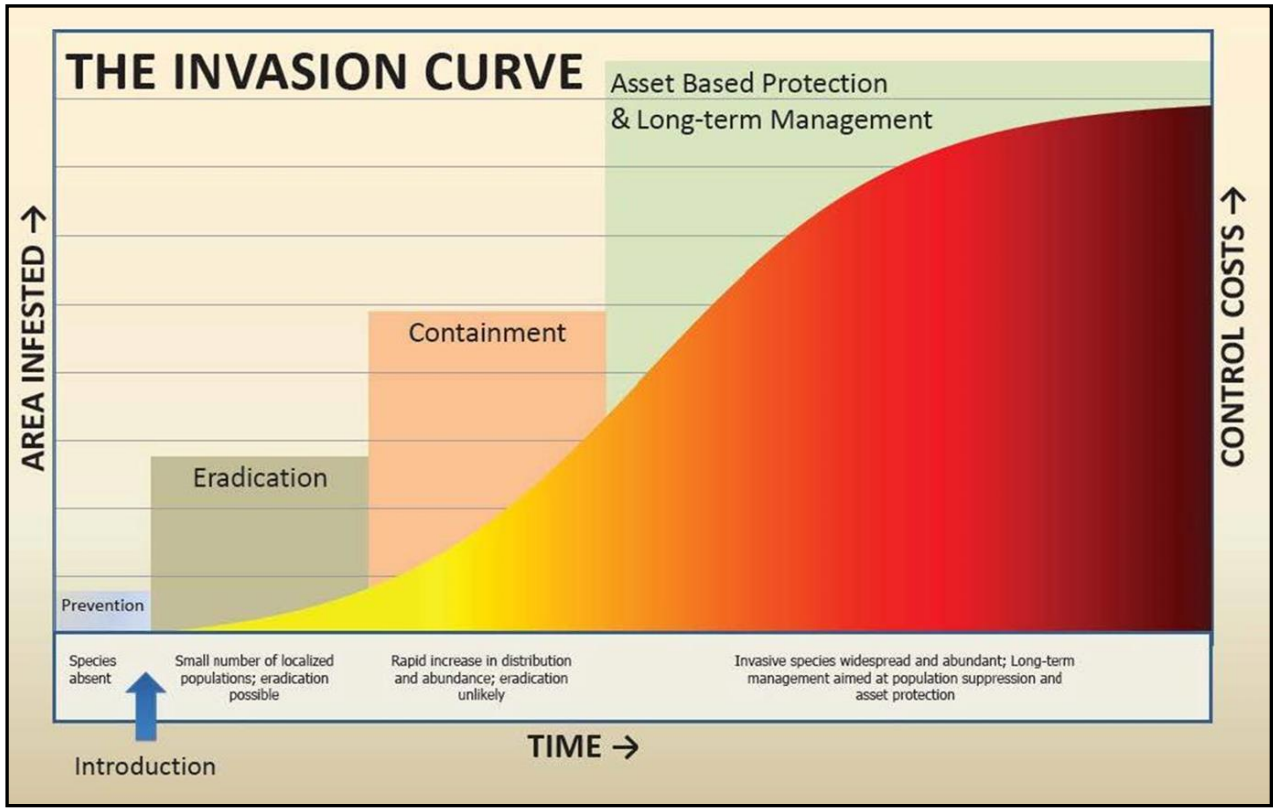


eDNA Approach Effectively Detected Non-native Species



Summary

- More species are detected in a single eDNA sample
- eDNA samples are effective at detecting AIS
- Comprehensive approach for species detection
  - Agency response will be dependent on risk and level of uncertainty
  - Consider contamination sources
- Future work with multiple loci
  - Finish 12S processing
  - Process 16S data
- Approach could be use to sample for
  - T&E
  - Non-fish species
- Cost needs to be considered for implementation



Acknowledgements

Funding

Great Lakes Restoration Initiative

Michigan DNR

Field Assistance

Michigan DNR

Michigan DEQ

Michigan State University

Smith-Root eDNA Backpack (ANDe)

Fish Illustrations within provided by Joseph R. Tomelleri ©

Mothur pipeline specifics

9.5 M paired-end (PE) 150 base pair (bp) sequences (reads)

Merged PE to reads (accounting for quality scores)

Removed poorly merged reads based on size (>160 bp) and ambiguous nucleotide calls

7.5 M reads (merged)

Aligned reads to reference database

Removed poorly aligned reads based on size (>152 bp or < 139 bp) and homo-polymer size (>5 nucleotides)

5 M reads (250 K unique reads)

Assembled Operational Taxonomic Units (OTUs) at 99% identity

62 K OTUs

For each OTU, classified based on taxonomic database

For each OTU, quantified how many reads were observed in each sample

Sample	OTU1	OTU2	…	OTU62000
ID1	1000	56	…	1
ID2	334	1	…	0
…	…	…	..	…
ID413	2	386	..	2

Removed non-fish OTUs

42 K OTUs (3.3 M reads)

BLASTed OTUs not classified to species

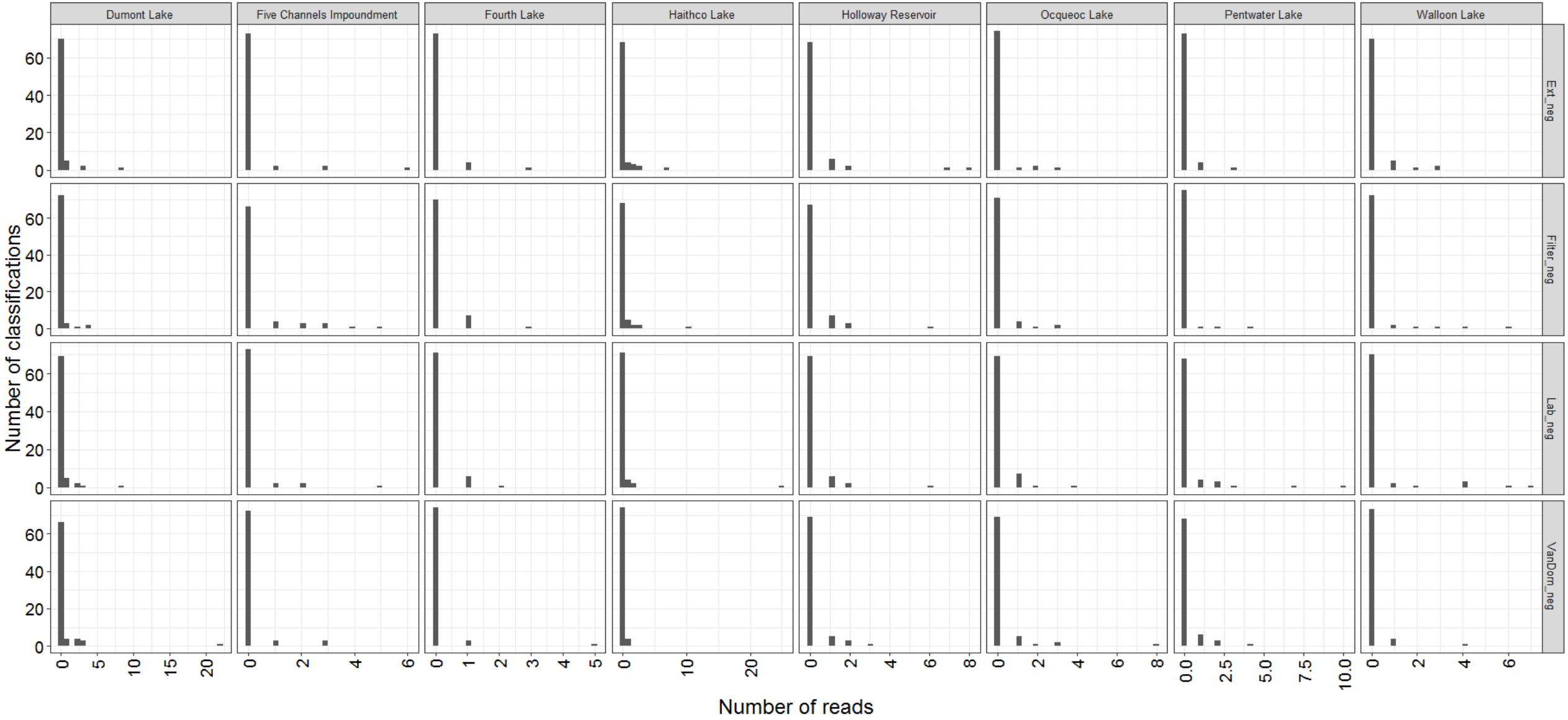
Condensed matrix to unique classifications - multiple OTUs represent the same classification

*Modified some classifications due to lack of resolution in database to genus (Bullhead, gar, redhorse, shiners)

52 Unique classifications (48 to species)

Sample	Perch	Bluegill	…	Redhorse (UC)
ID1	11100	690	…	1232
ID2	3334	45	…	0
…	…	…	..	…
ID413	2	3076	..	2

Little to no fish DNA contamination in samples



Example of classifications observed in negative samples

Lake_name	spp	Ext_neg	Filter_neg	Lab_neg	VanDorn_neg	total_surface	total_benthic	rel_surface	rel_benthic
Haithco Lake	Lepomis_macrochirus	7	10	25	1	42	43	0.52	0.51
Haithco Lake	Micropterus_salmoides	3	3	1	0	7	7	0.09	0.08
Haithco Lake	Neogobius_melanostomus	2	3	2	0	7	7	0.09	0.08
Haithco Lake	Ameiurus_unclassified	2	1	2	1	5	6	0.06	0.07
Haithco Lake	Ictalurus_punctatus	2	1	1	0	4	4	0.05	0.05
Haithco Lake	Lepomis_unclassified	3	0	0	0	3	3	0.04	0.04
Haithco Lake	Ambloplites_rupestris	0	2	0	1	2	3	0.02	0.04
Haithco Lake	Lepomis_gibbosus	0	2	0	0	2	2	0.02	0.02
Haithco Lake	Pomoxis_nigromaculatus	1	1	0	0	2	2	0.02	0.02
Haithco Lake	Actinopterygii_unclassified	1	0	0	0	1	1	0.01	0.01
Haithco Lake	Cyprinus_carpio	0	0	1	0	1	1	0.01	0.01
Haithco Lake	Esox_lucius	1	0	0	0	1	1	0.01	0.01
Haithco Lake	Fundulus_diaphanus	0	1	0	0	1	1	0.01	0.01
Haithco Lake	Micropterus_dolomieu	1	0	0	0	1	1	0.01	0.01
Haithco Lake	Perca.flavescens	0	1	0	0	1	1	0.01	0.01
Haithco Lake	Sander_vitreus	0	0	1	0	1	1	0.01	0.01
Haithco Lake	Etheostoma_nigrum	0	0	0	1	0	1	0.00	0.01
Haithco Lake	Alosa_pseudoharengus	0	0	0	0	0	0	0.00	0.00
Haithco Lake	Amia_calva	0	0	0	0	0	0	0.00	0.00
Haithco Lake	Aplodinotus_grunniens	0	0	0	0	0	0	0.00	0.00
Haithco Lake	…	…	…	…	…	…	…	…	…
Haithco Lake	Xyrauchen_texanus	0	0	0	0	0	0	0.00	0.00

**78 classifications before accounting for negative controls*