Using eDNA methods to extend biological sampling and identify candidate restorations for species reintroductions

Key Research Question: The effectiveness of biological community restoration at the project scale

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Key idea(s): Stream restorations are effective, but the biota cannot be detected / become established

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H1: Ecological recovery is limited by our inability to detect organisms present at such low abundances as to be undetectable using current sampling methods.

If yes: eDNA should identify additional taxa present, but not found in traditional monitoring AND

eDNA should identify additional taxa present in restored sections, but not found upstream of the restoration

Key idea(s): Stream restorations are effective, but the biota cannot be detected / become established

H1: Ecological recovery is limited by our inability to detect organisms present at such low abundances as to be undetectable using current sampling methods.

If no: We assess H2 and H3:

H2: Ecological recovery is limited by a failure of fish and/or benthic macroinvertebrates to recolonize the stream.

H3: Ecological recovery is limited by the stream's ability to support the desired taxa.

H2 and H3 are linked to microbial communities

H2: Ecological recovery is limited by a failure of fish and/or benthic macroinvertebrates to recolonize the stream. Indirect assessment.

Support for H2 will find no appreciable numbers of additional fish or benthic taxa, BUT the stream microbial community will indicate suitable conditions for taxa recovery (H3)

H3: The stream may be limited to support the desired taxa.

Stream sediment microbial communities may suggest suitable conditions for recovery of fish and benthos – possible candidate for reintroductions.

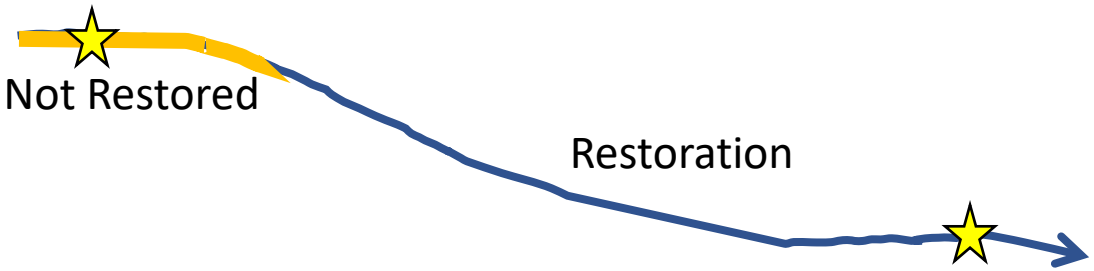
If microbes “say” NO, then conclude that the restoration has not provided suitable conditions for ecological uplift

All three hypotheses use DNA sequencing methods

eDNA metabarcoding is used for identifying the fish and benthic invertebrates in the stream. Data are geographically filtered to include only those taxa found in the 20+ years of MBSS sampling.

26 restorations examined using water samples collected ~100m above the project and at the bottom of the restoration project

- Single eDNA sample collected in spring
- Across the urban gradient
- RSC-ish and NCD restorations
- Various times since restored



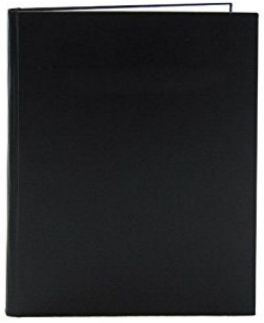
1. Compare taxa in restored vs above.
2. Compare taxa in eDNA vs physical collections

We should expect to see more taxa and more ‘desirable’ or sensitive taxa in restored sections.

How the bioinformatics works

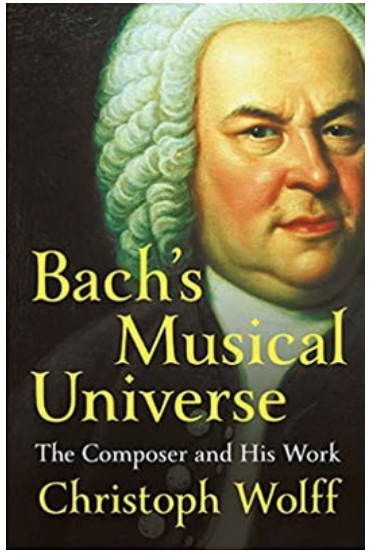
DNA sequences

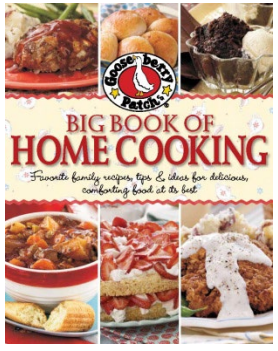
Imagine a library full of books. Each book is a different species. The specific letters on the page are the DNA base pairs of the genome

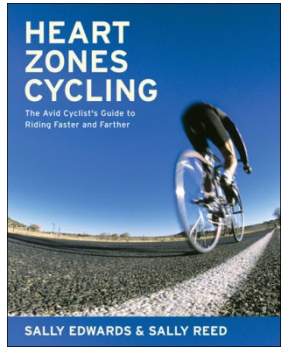


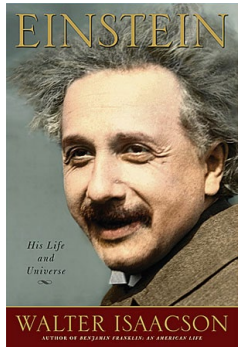
? No reference sequence

Database









H2: Ecological recovery is limited by a failure of fish and/or benthic macroinvertebrates to recolonize / establish in the stream.

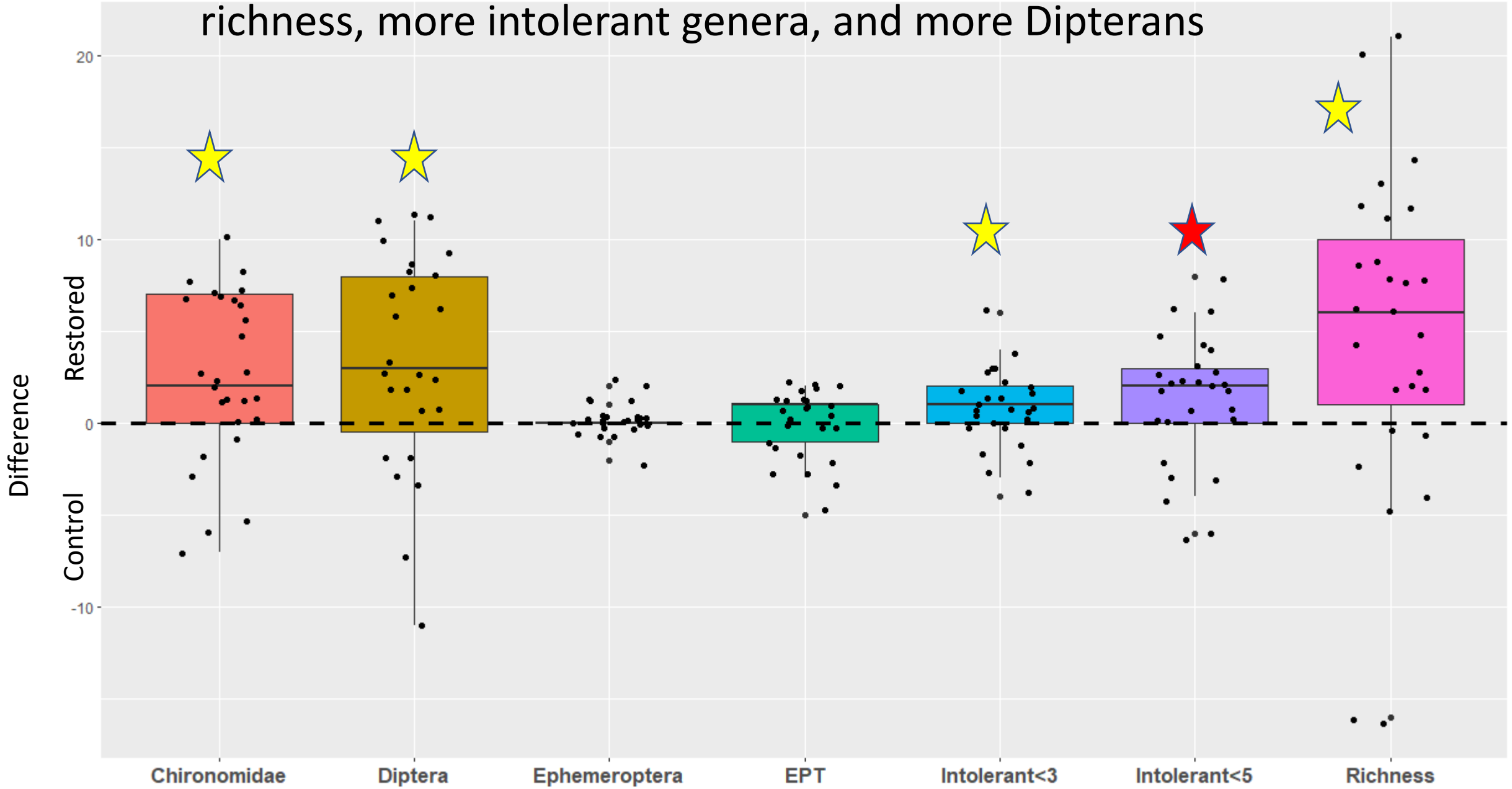
We use the same samples and eDNA techniques as in H1

H2 is supported by a lack of difference between upstream and restored AND evidence from the stream microbial communities

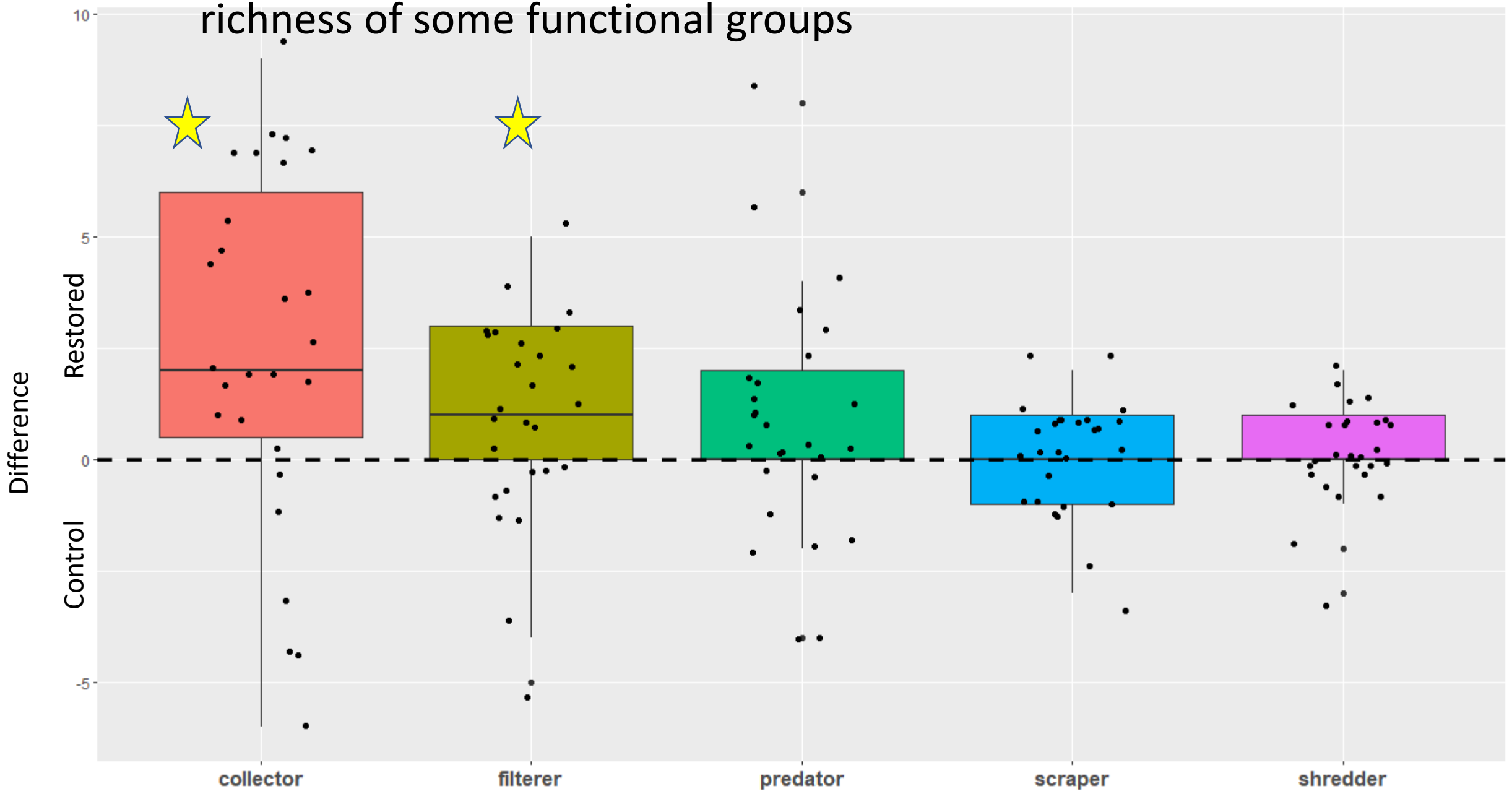
We're not yet at the point to evaluate the microbial communities. We have the data, but have not yet run the models…..I figured you would be more interested in the eDNA results for fish and benthos for H1.

ALL RESULTS ARE PRELIMINARY AND ARE VERY LIKELY TO CHANGE - ESPECIALLY FOR THE FISH!!!!!!!!!!!!!!!!!!!!!!!!!

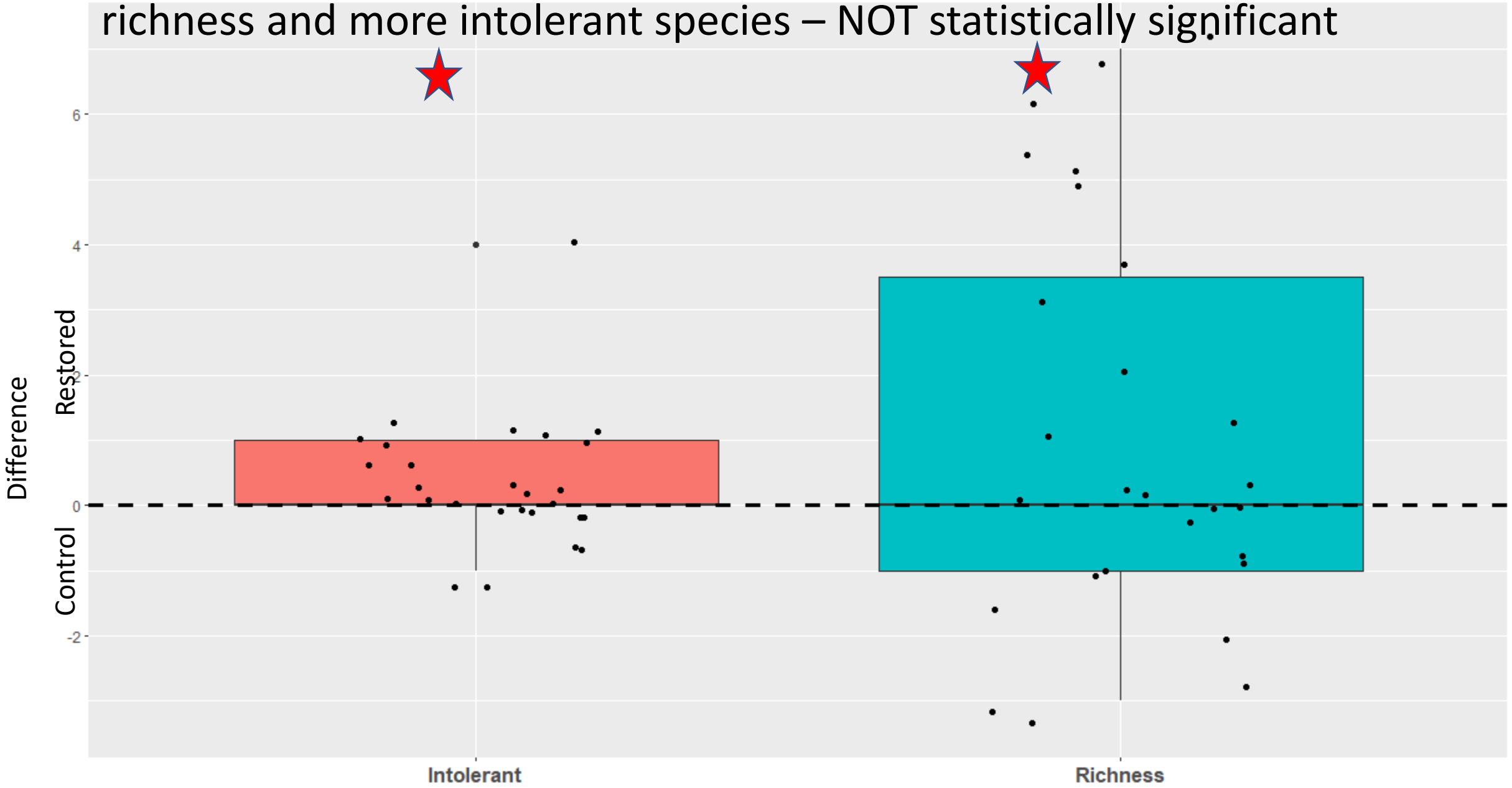
Benthos: eDNA suggests restored sections tend towards higher richness, more intolerant genera, and more Dipterans



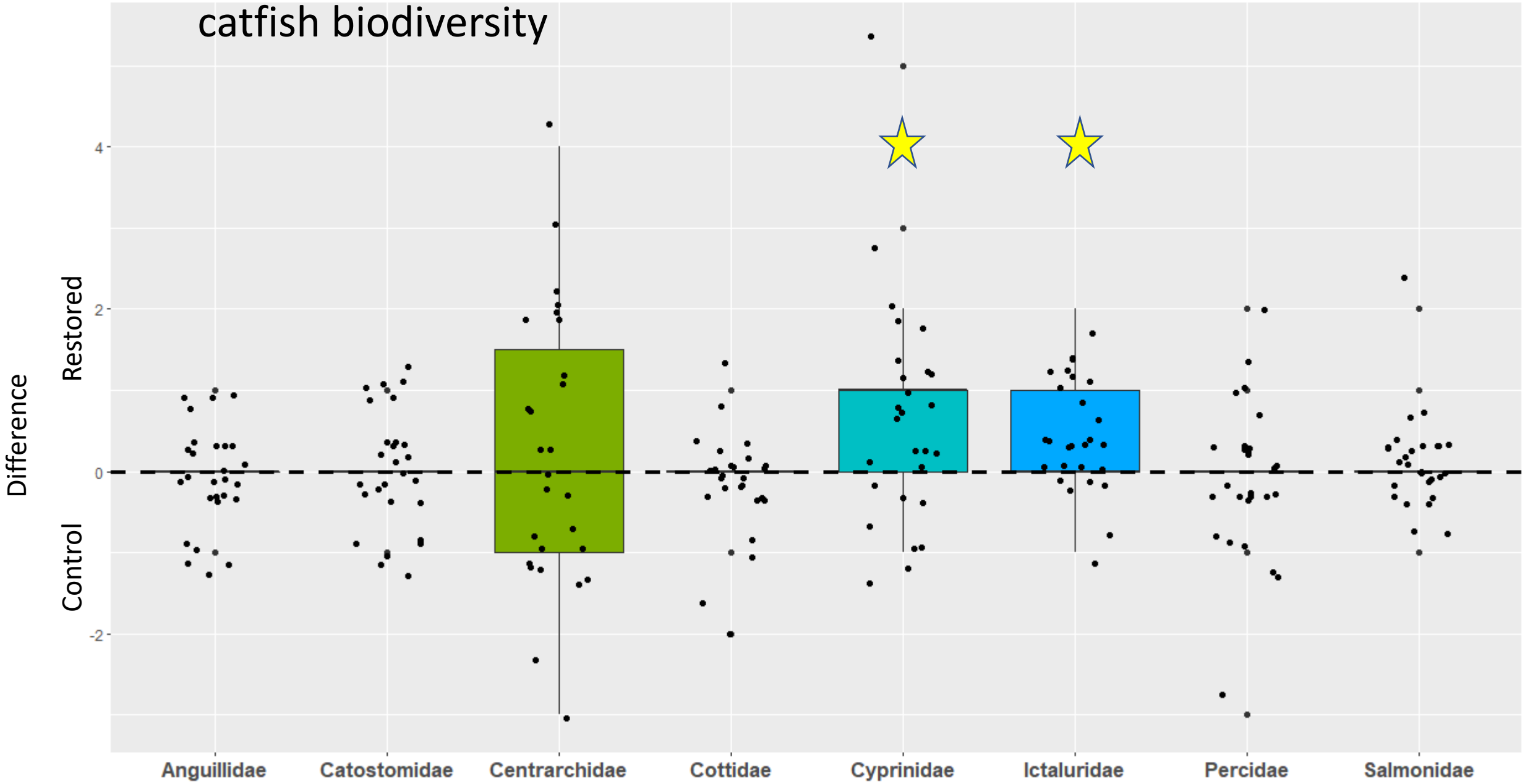
Benthos: eDNA suggests restored sections tend towards higher richness of some functional groups



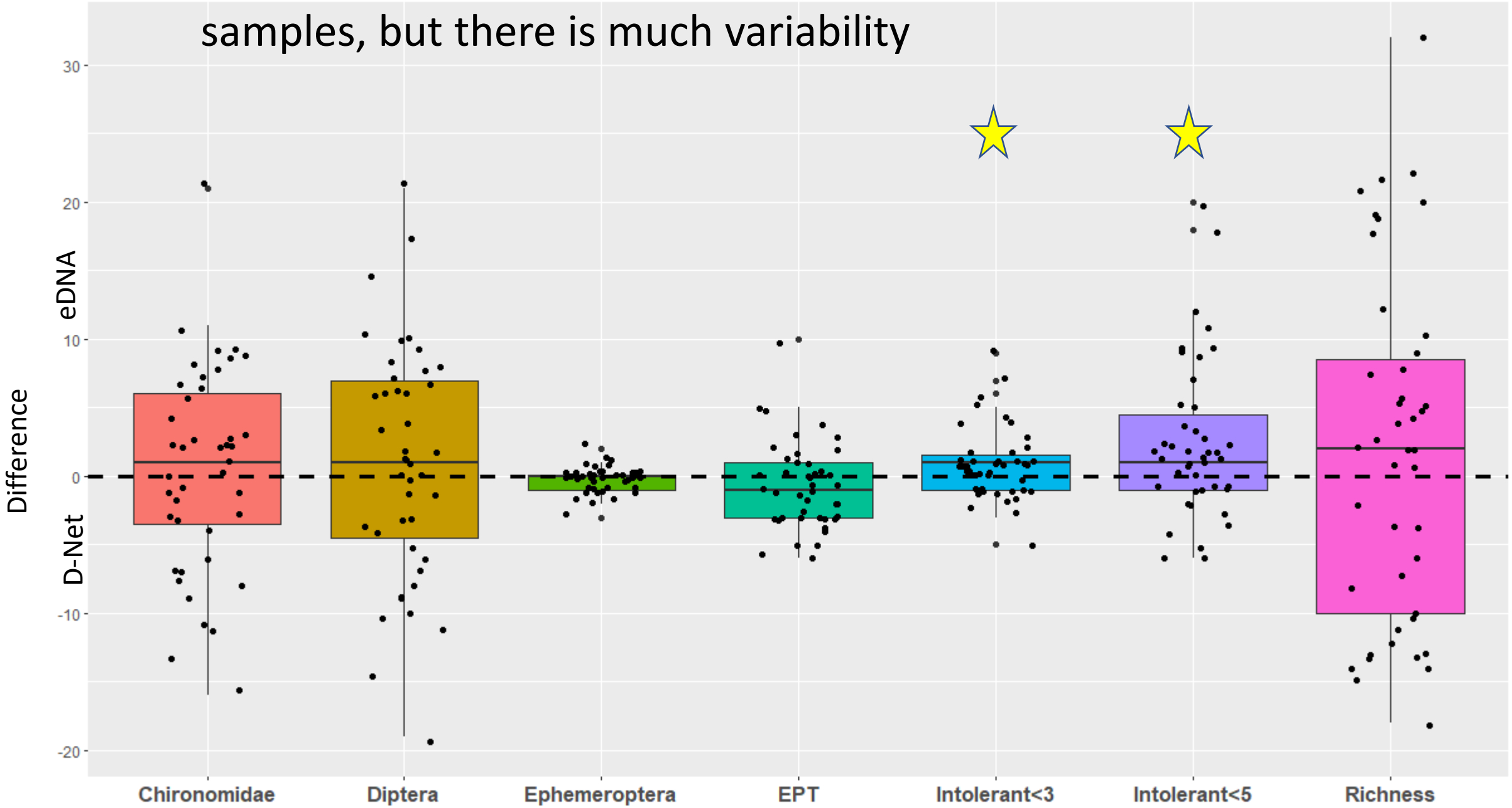
eDNA suggests restored sections tend towards higher fish species richness and more intolerant species – NOT statistically significant



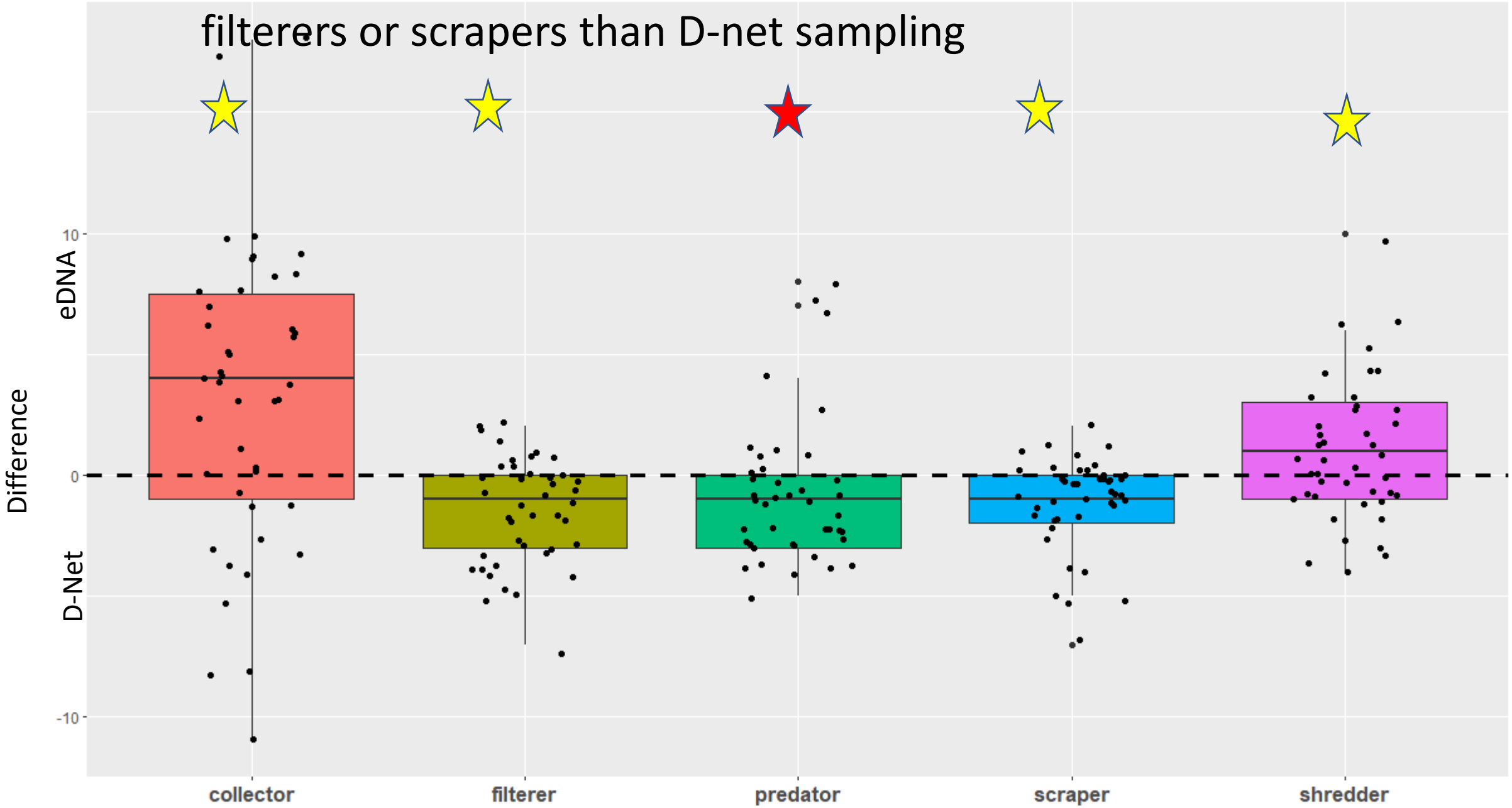
eDNA suggests restored sections may have more minnow, and catfish biodiversity



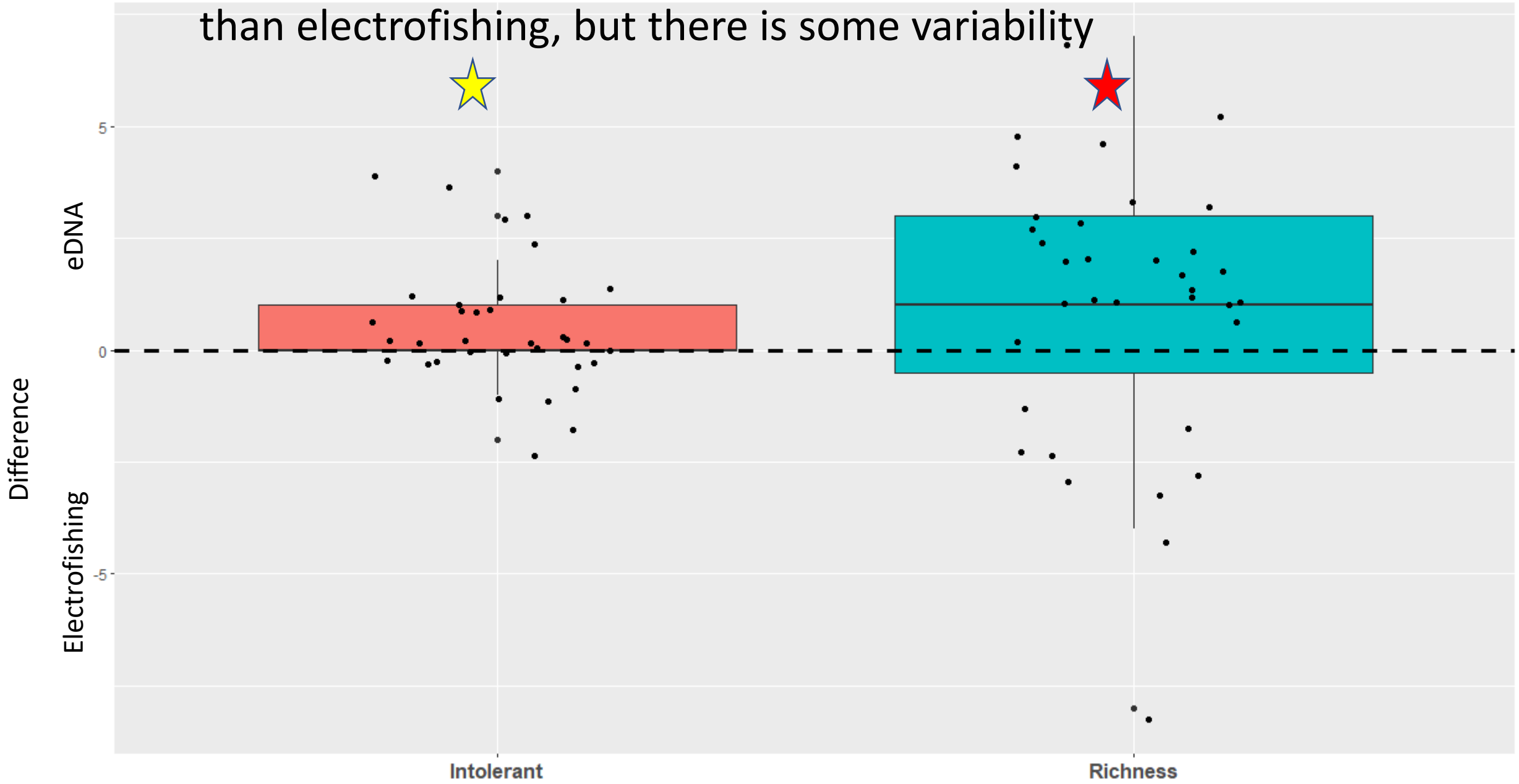
Benthos: eDNA picks up more intolerant genera than D-net samples, but there is much variability



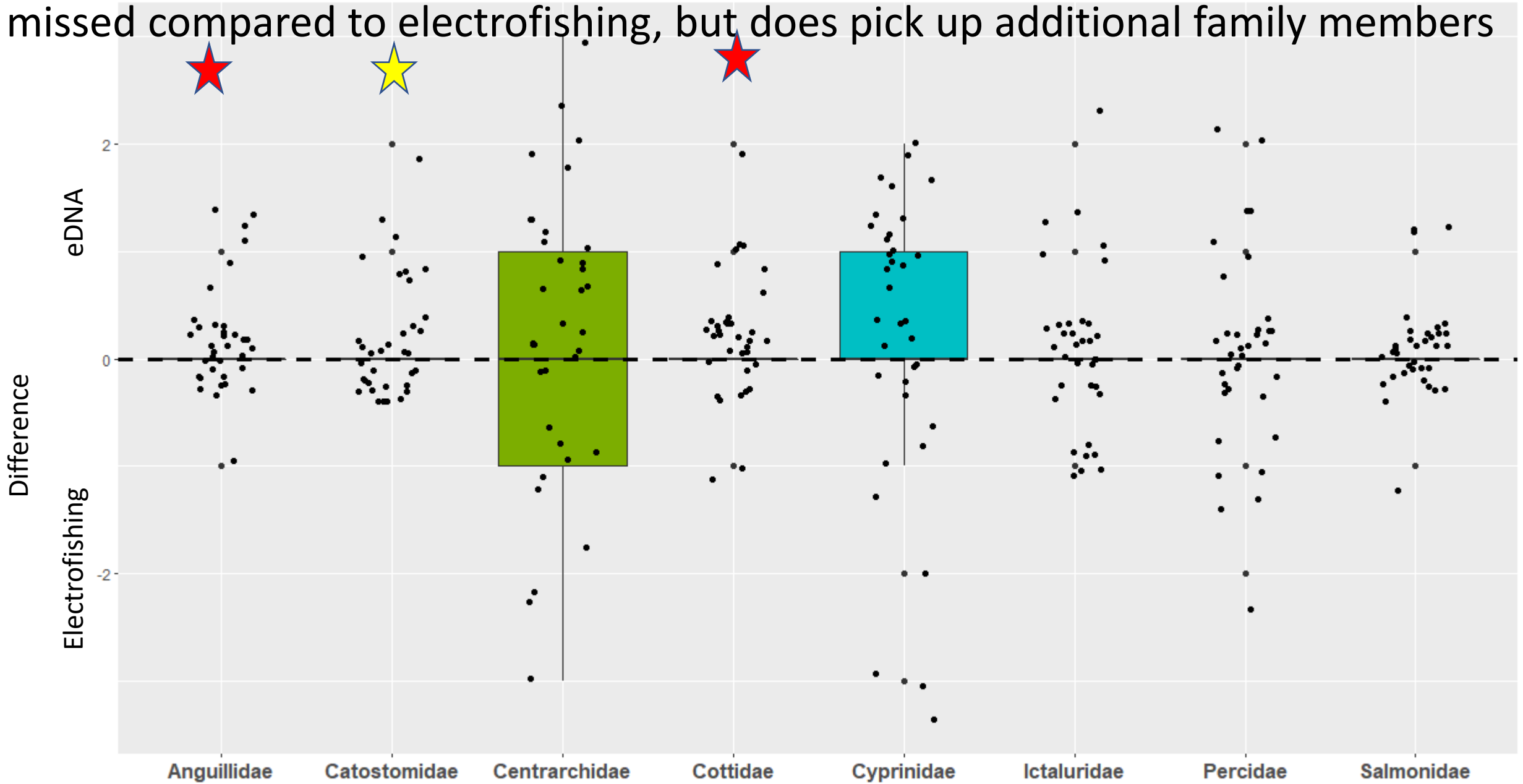
Benthos: eDNA picks up more collectors and shredders, but fewer filterers or scrapers than D-net sampling



Fish: eDNA picks up more species and more intolerant species than electrofishing, but there is some variability



Fish: eDNA does not appear to have a taxonomic bias in which species are missed compared to electrofishing, but does pick up additional family members



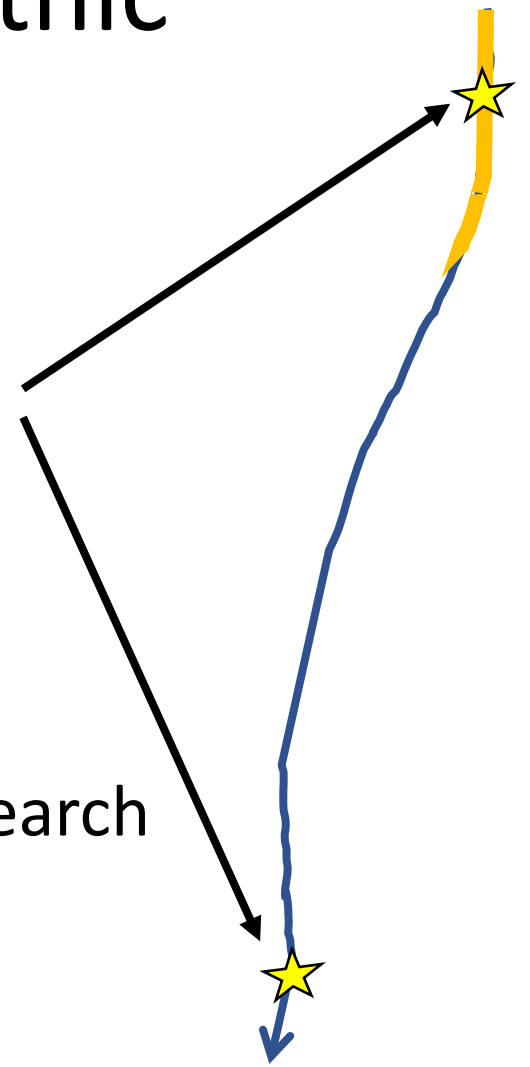
eDNA suggests restorations have benthic taxa that sections upstream do not*

Higher overall biodiversity in restored sections

- Could be sampling effect: 2x more sampling for restored
- More analysis required

Greater numbers of sensitive taxa in restored sections

- Mostly from Diptera and NOT from EPT
- Could explain lack of IBI score differences in previous research



eDNA suggests restorations may have positive fish response*

VERY PRELIMINARY and NOT STATISTICALLY SIGNIFICANT!!!!!!!

Trend towards more fish species and more sensitive species in restored sections

- Could also be a sampling effect – 2x more sample in restored

Higher biodiversity of minnows and catfishes

- Keep in mind this is only 1-2 more species

eDNA results are mixed compared to D-net sampling for benthos

eDNA picks up more intolerant benthic genera than D-net sampling

Several functional group differences

- eDNA finds more collectors and shredders
- D-net finds more filterers and scrapers and possibly predators

eDNA seems to pick up more fish species than electrofishing

Trend for more fish species with eDNA, but not statistically significant

Greater numbers of sensitive species with eDNA

eDNA will probably show more improvements once we clean up the taxa lists

- e.g., genome variation caused several eels and sculpins to be assigned to species not in the MBSS database, but were almost certainly American eel or Blue Ridge sculpin

Final Thoughts

There have been some improvements in restorations that are not found in the upstream areas – Good News!

- This should NOT be viewed as “Mission Accomplished”

Restorations are still missing most of the indicator taxa. There are still limitations.

- Habitat (in)stability and intolerant dipterans/chironomids
  - Substrates for reproduction by EPT?
- External gills of EPT indicators
  - Chemical sensitivity? Abrasion sensitivity?
- Might not be fixing the actual problems

Microbes still need to be evaluated to determine extent of potential uplift and reintroductions of benthos and fish

Translation Slides

What are the take home points? What does this mean for me?

Translation Slides by

Jay Kilian, MD Dept. of Natural Resources, Resource Assessment Service

Take-home messages from this research:

- eDNA detects higher richness in benthic and fish communities not detected using traditional methods (e.g., D-net, electrofishing). This is likely due to:
  1) eDNA samples "all" habitats (e.g., not just 20 ft^2 of best available habitat)
  2) traditional rapid assessment methods do not provide a <u>complete</u> census of all taxa living in a stream.

- eDNA detected subtle biological changes (e.g., addition of taxa) associated with restoration
  - "New" intolerant taxa found downstream, but no changes observed in EPT and other important indicators

Take-home messages from this research:

- eDNA used in tandem with traditional methods may provide a more complete picture of the biological changes resulting from restoration

- eDNA is a promising technique for stream bioassessments, however much research is still needed to:
  - Reliably compare results from eDNA and traditional sampling methods
  - Correlate abundance of eDNA with the abundance of actual taxa
  - Determine the best time of year to sample using eDNA
  - Evaluate eDNA performance over habitat types (e.g., blackwater), land use gradients, and biodiversity gradients.

How it works - metabarcoding

Water sample is collected and DNA extracted

PCR for specific primers targeting fish or benthos

Index barcodes added so multiple samples can be sequenced together

DNA sequencing on Illumina MiSeq (or other platform; 20 million reads)

Bioinformatics: Trim indexes, QA/QC, compare against reference db, cluster for similarity, assign taxonomic identity

Typical workflow for eDNA

Collect water samples

Filter on-site or in the lab