

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

H1: Ecological recovery is limited by the stream's ability to support the desired taxa.

H2: Ecological recovery is limited by our inability to detect organisms present at such low abundances as to be undetectable using current sampling methods.

H3: Ecological recovery is limited by a failure of fish and/or benthic macroinvertebrates to recolonize the stream. [Follows from failing to reject H1 and rejecting H2](#)

All three hypotheses use DNA sequencing methods

Microbes: Stream sediment microbial communities have successfully predicted stream condition

Fish and Benthos: 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 and sediments collected ~100m above the project and at the bottom of the restoration project

Single eDNA sample collected in spring

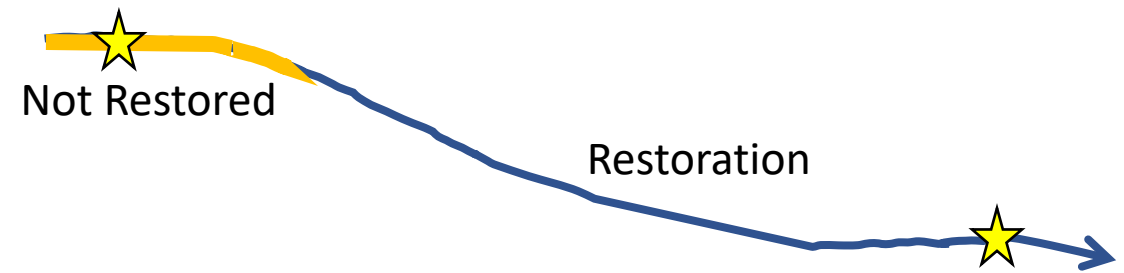
Across the urban gradient

RSC-ish and NCD-ish 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

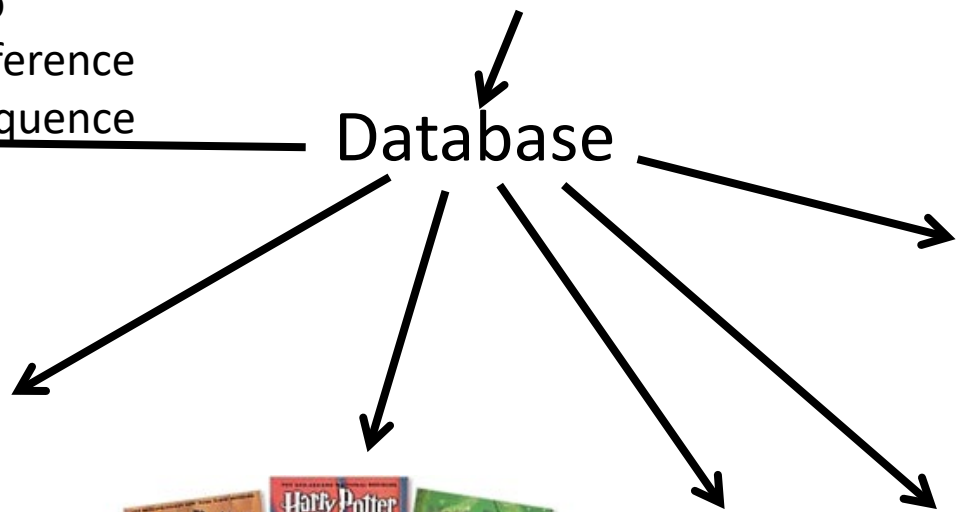
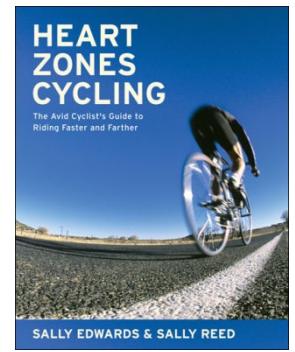
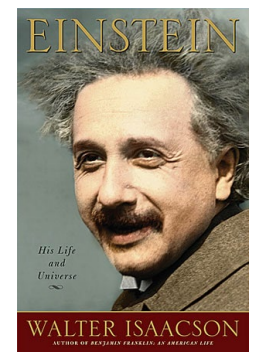
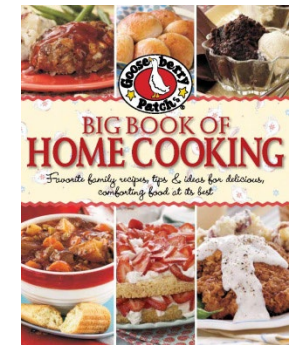
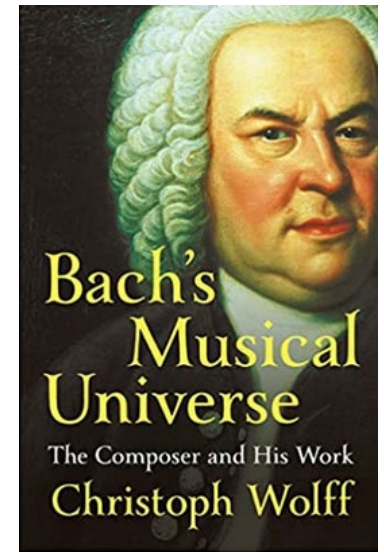


Imagine a library full of books. Each book is a different species. The specific letters on the page are the DNA base pairs of the genome



No reference sequence ?

Database



Results preview – evidence for restoration success ⁶

Microbial communities did not provide enough resolution for confidence in the predictions

Combined eDNA and physical sampling found **more fish and benthic macroinvertebrate taxa** in RESTORED sections compared to the upstream controls

Combined eDNA and physical sampling found **more sensitive fish and benthic macroinvertebrate taxa** in RESTORED sections compared to the upstream controls

eDNA identified **more fish and benthic macroinvertebrate taxa AND more sensitive fish and benthic macroinvertebrate taxa** than physical sampling of either fish or benthic macroinvertebrates

H1: Microbial communities do not provide much resolution on predicting stream condition: disappointing resolution



Site	Control	Restored	Site	Control	Restored
Bachelors Run	Fair	Fair	Mill Creek	Fair	Fair
Bacon Ridge Upper	Fair	Fair	Muncaster Mill	Fair	V Poor
Bramhope	Fair	Fair	Plumtree Creek	Fair	Fair
Cat Branch	Fair	V Poor	Red Hill Lower	Fair	Fair
Cowhide Branch	Fair	Fair	Red Hill Upper	Fair	Fair
Fosters Branch	Fair	Fair	Shepherd's Cove	Fair	V Poor
Greenbriar Branch	Fair	V Poor	UT Little Gunpowder	Fair	Fair
Howard Branch	Fair	Fair	Valley Mill Creek	Fair	Fair
Kelly Branch	Fair	Fair	Watkins	Fair	Fair
Little Catoctin	Fair	Fair	Minebank	Good	V Poor
Little Patuxent	Fair	Fair	Wheel Creek	Good	V Poor
Little Tuscarora	Fair	Fair	Bacon Ridge Lower	V Poor	Fair
Long Draught Branch	Fair	Good	Sandy Branch	V Poor	Fair
Maple Dell	Fair	Fair			

H2: Ecological recovery is limited by our inability to detect organisms present at such low abundances as to be undetectable using current sampling methods.

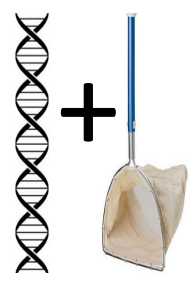
If yes: eDNA should identify additional taxa present, but not found in traditional monitoring AND

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

***Additional sensitive taxa

Key to comparisons on upcoming slides:

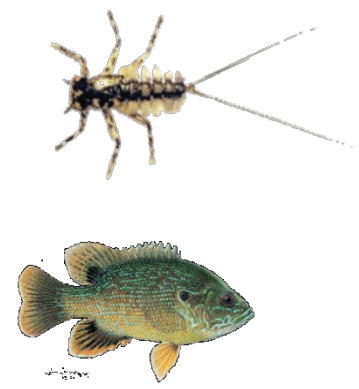
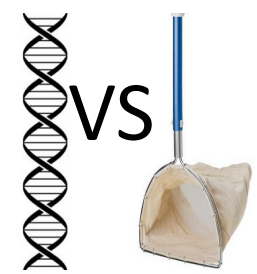
eDNA + Physical samples



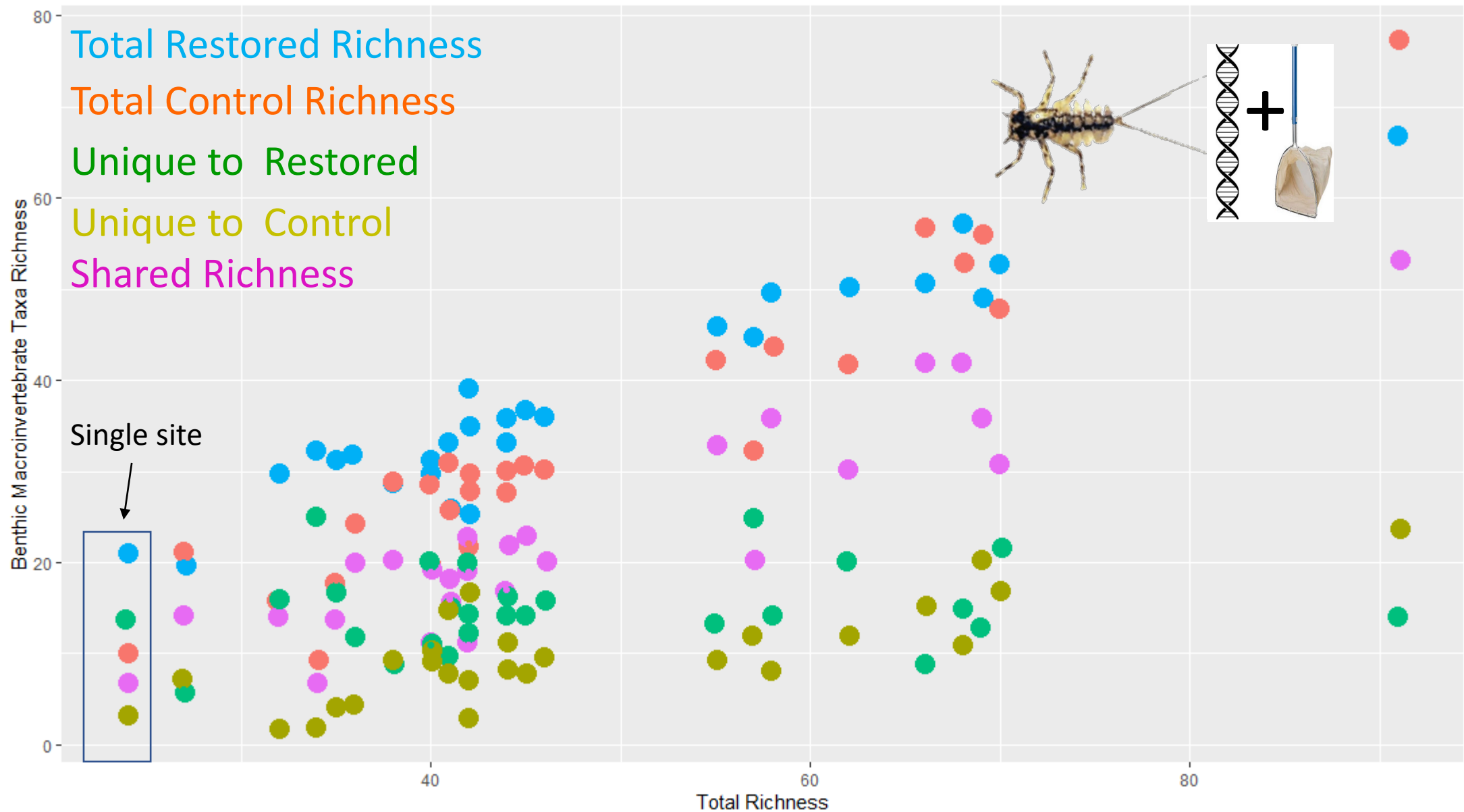
eDNA data only



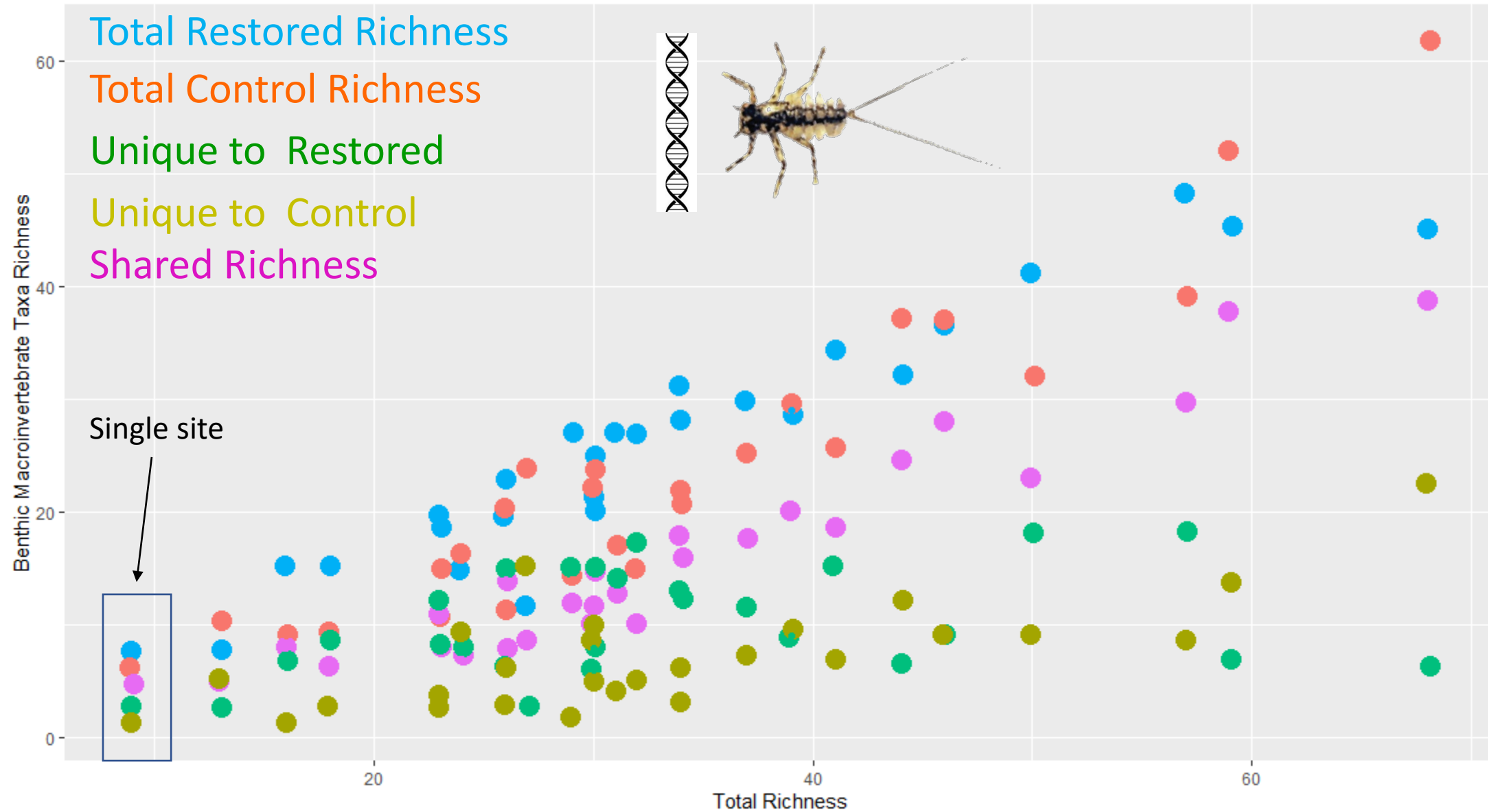
eDNA compared to Physical samples



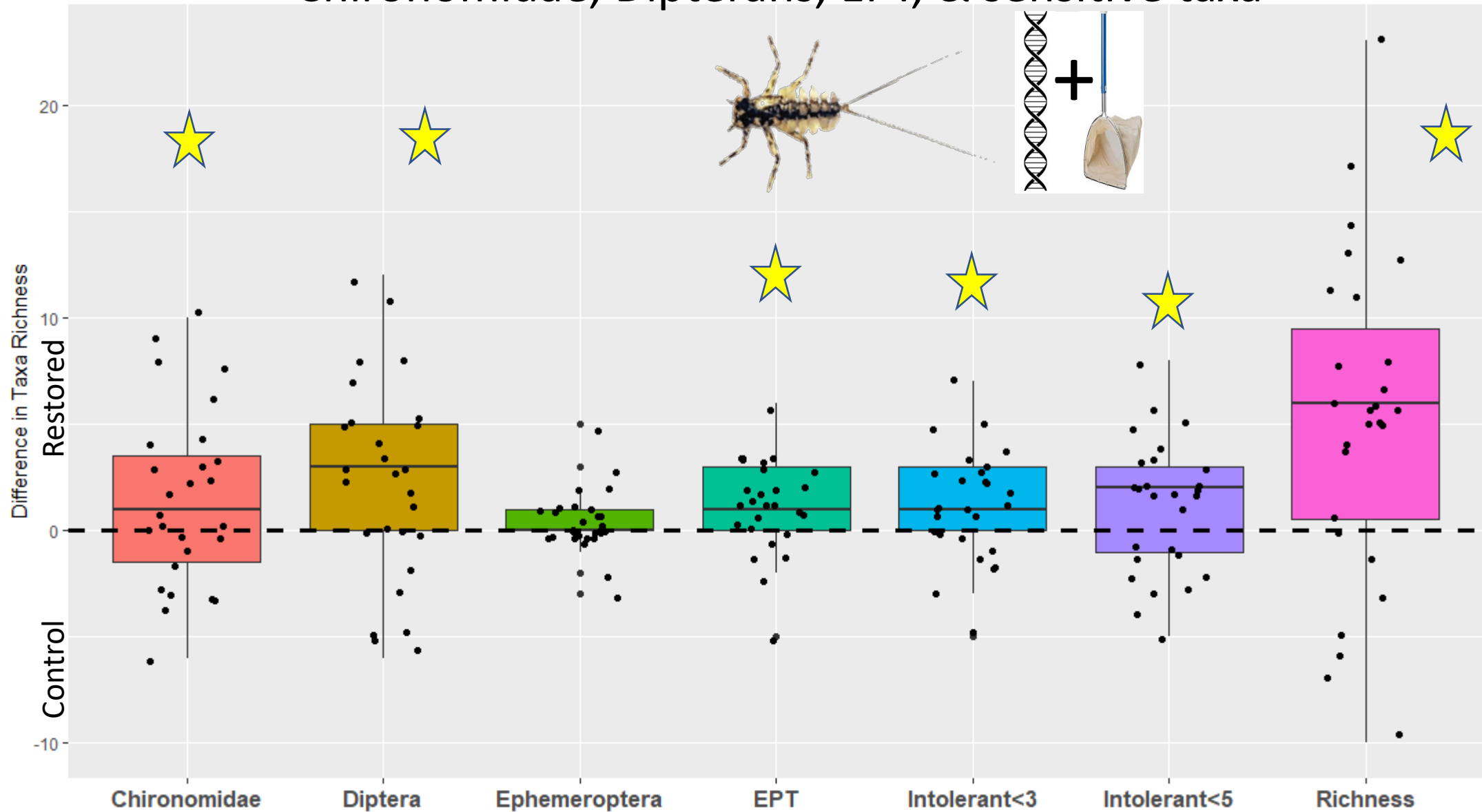
eDNA+DNet: RESTORED sections tend to have more benthic taxa



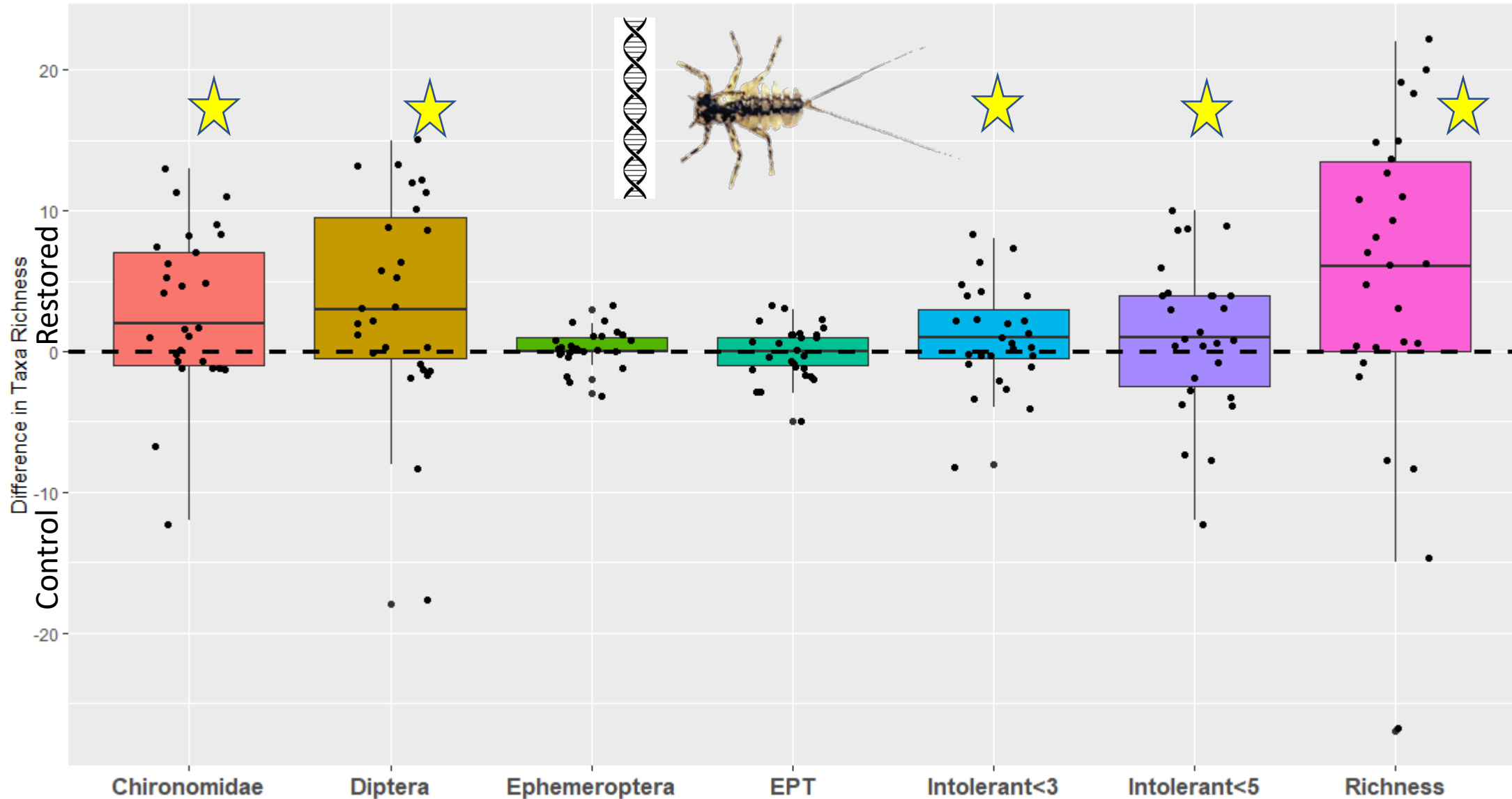
eDNA-only: RESTORED sections tend to have more benthic taxa



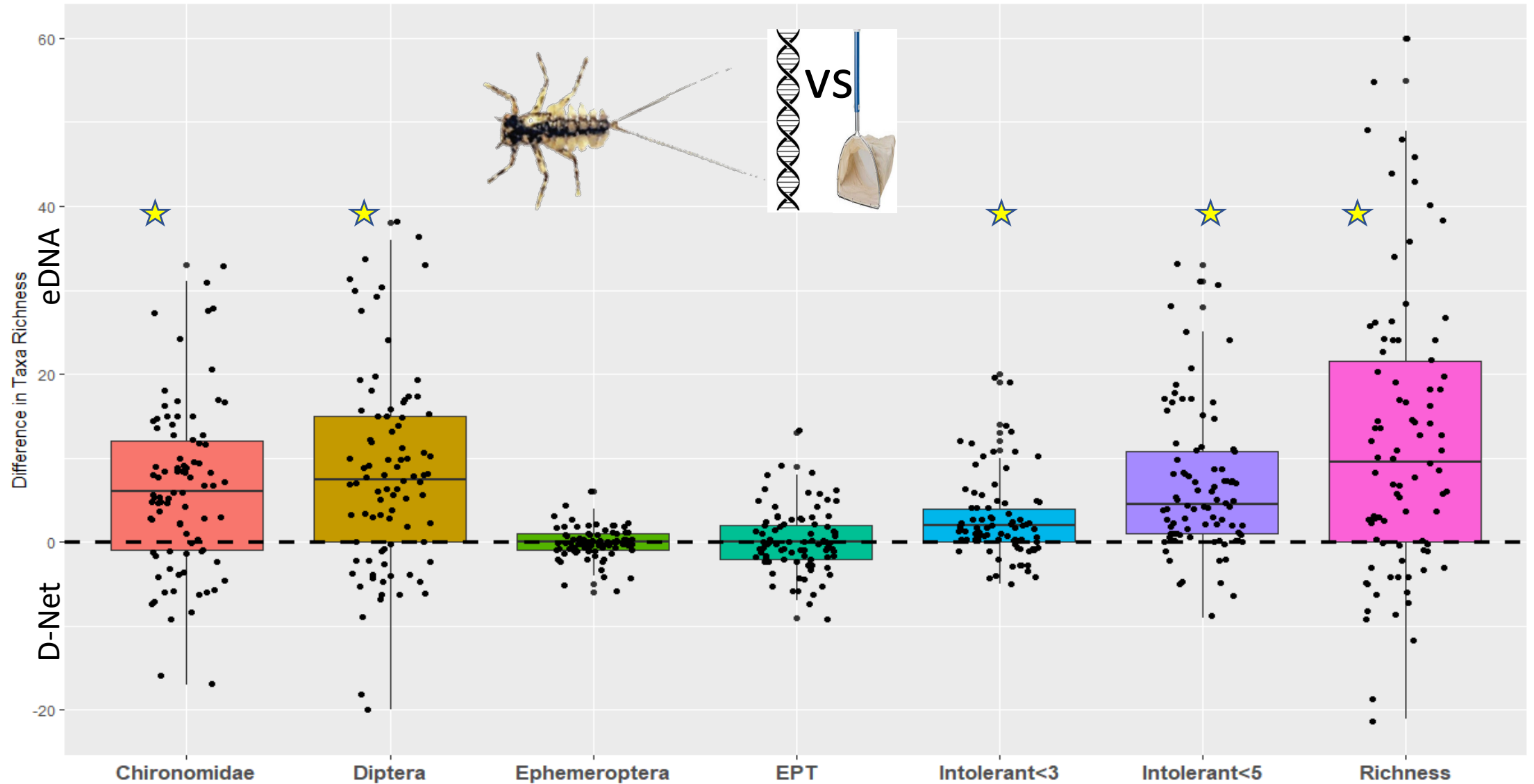
eDNA+DNet suggests RESTORED sections tend towards higher richness, more Chironomidae, Dipterans, EPT, & sensitive taxa



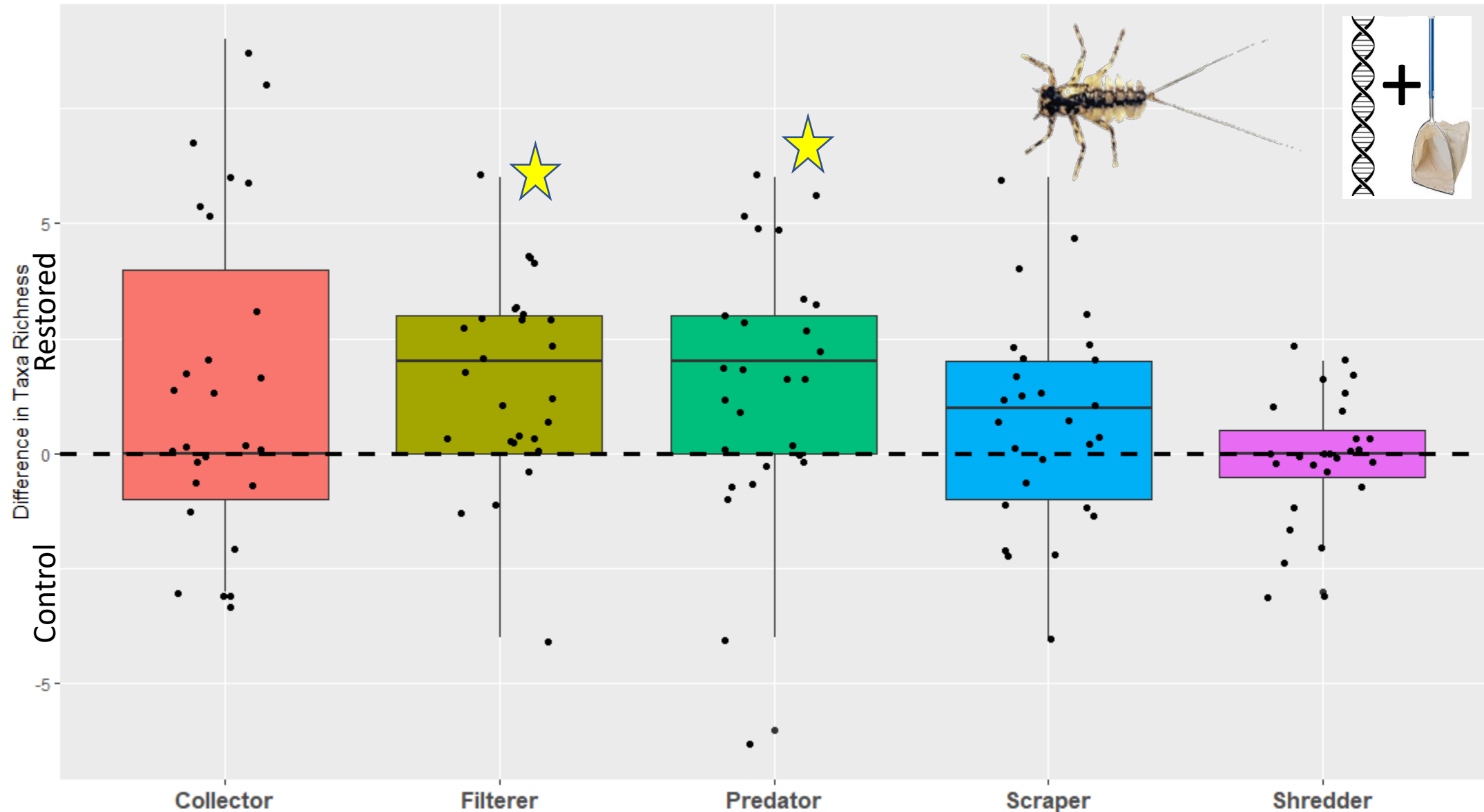
eDNA-ONLY: suggests RESTORED sections tend towards higher richness, more Chironomidae, Dipterans & sensitive taxa



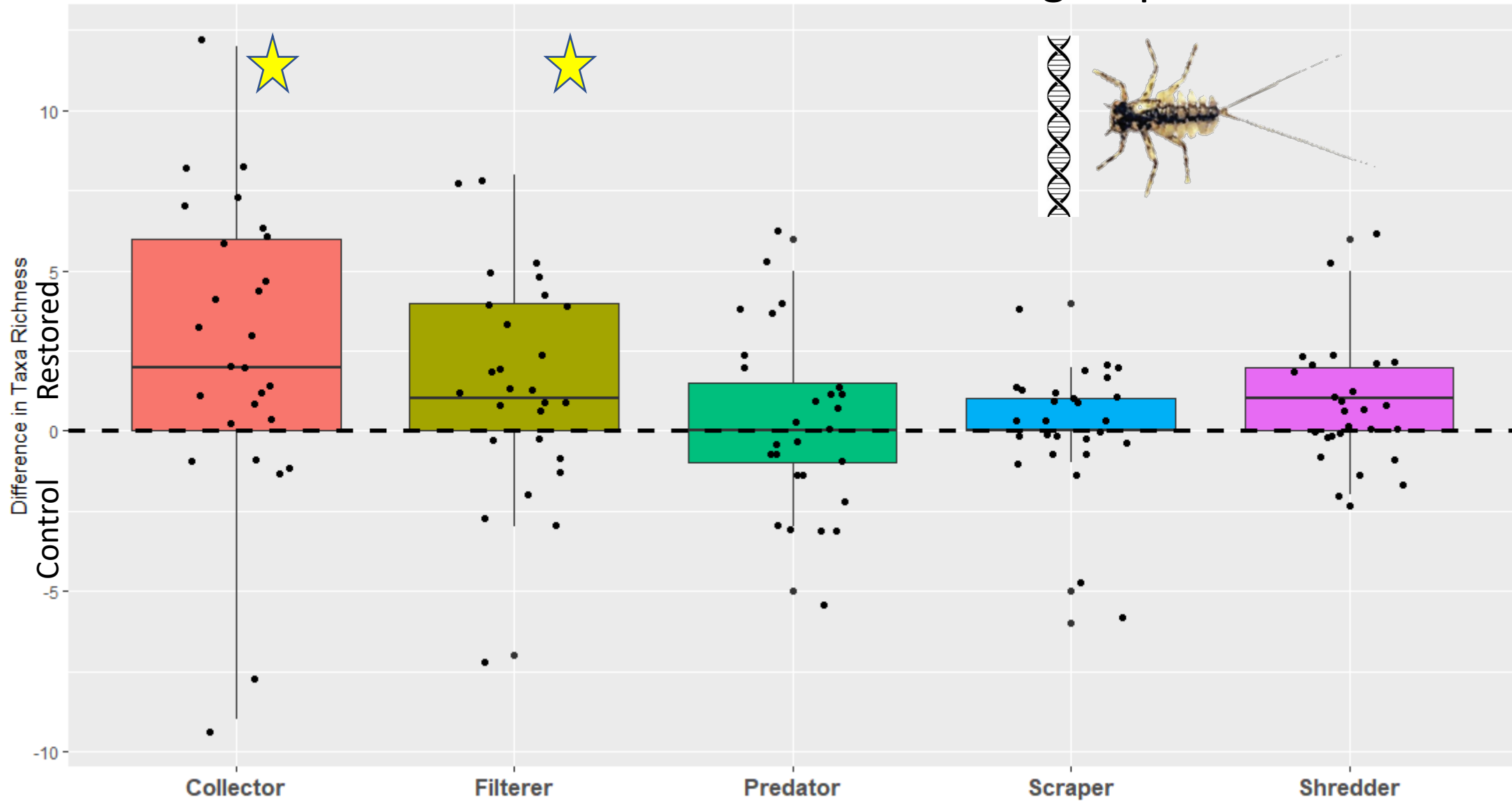
eDNA tends to better sample intolerant taxa, Dipterans, and overall biodiversity than D-Net sampling



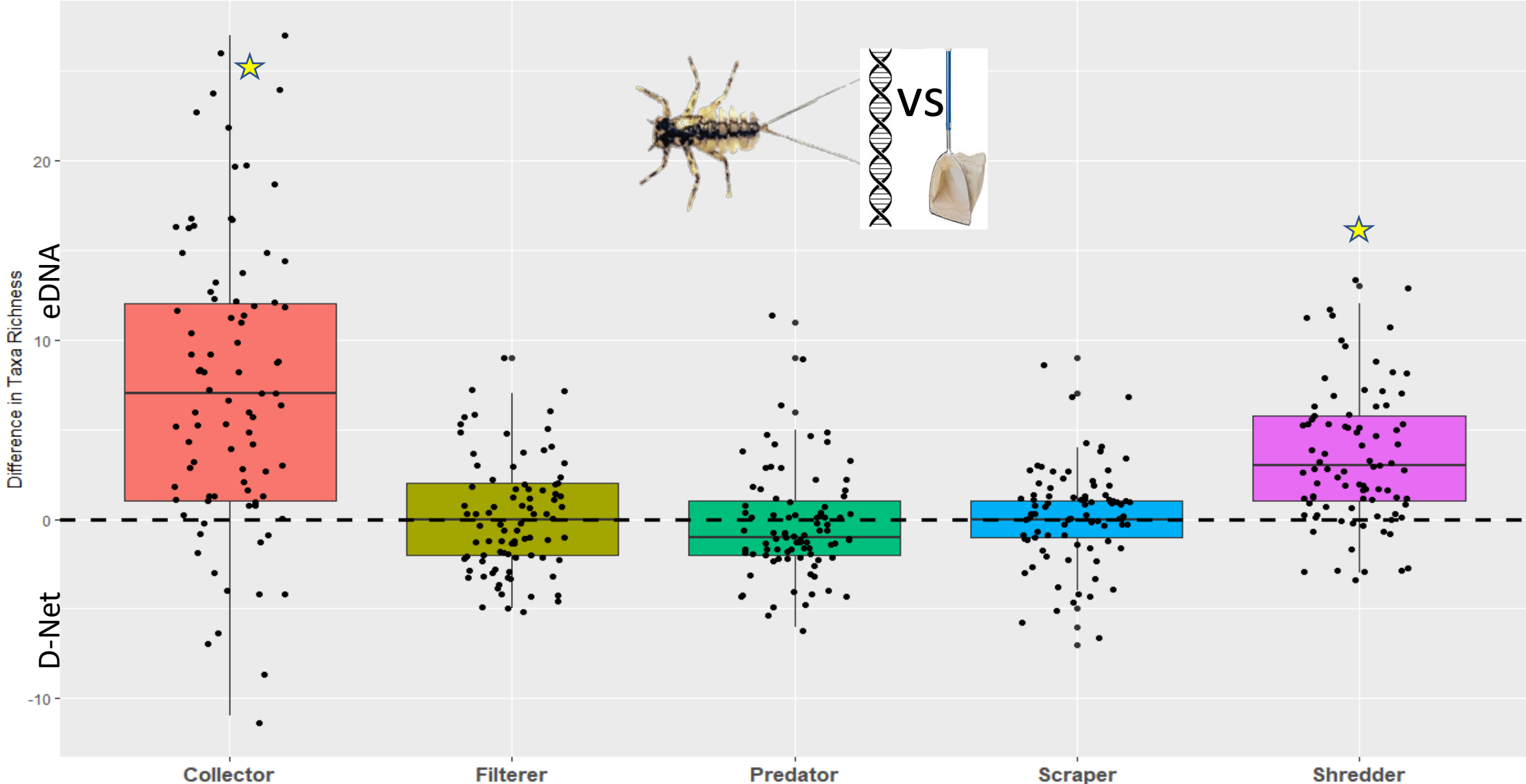
eDNA+DNet suggests restored sections tend towards higher richness of Filterer and Predator functional groups



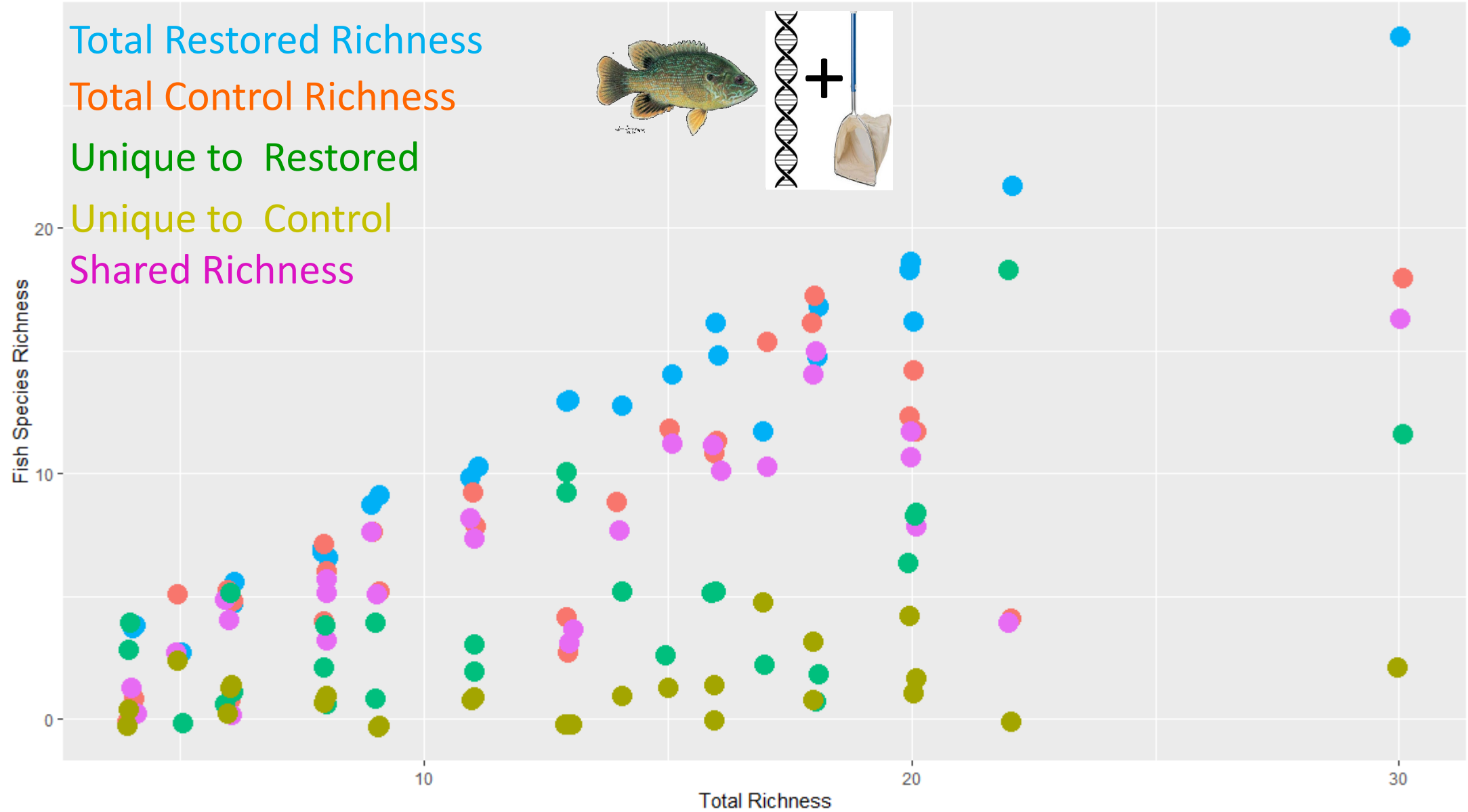
eDNA-ONLY suggests restored sections tend towards higher richness of Collector and Filterer functional groups



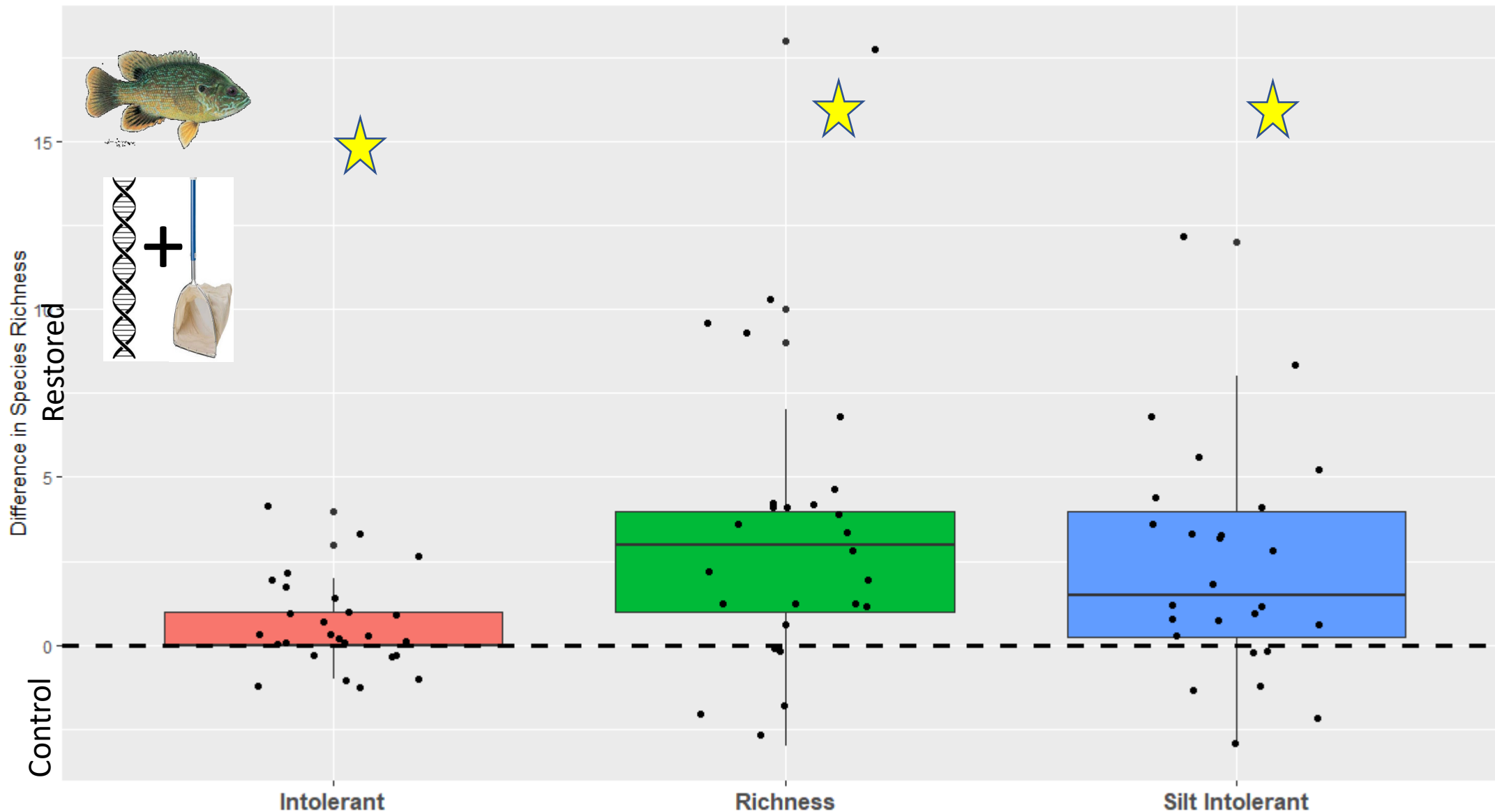
eDNA samples Collectors and Shredders better than DNet



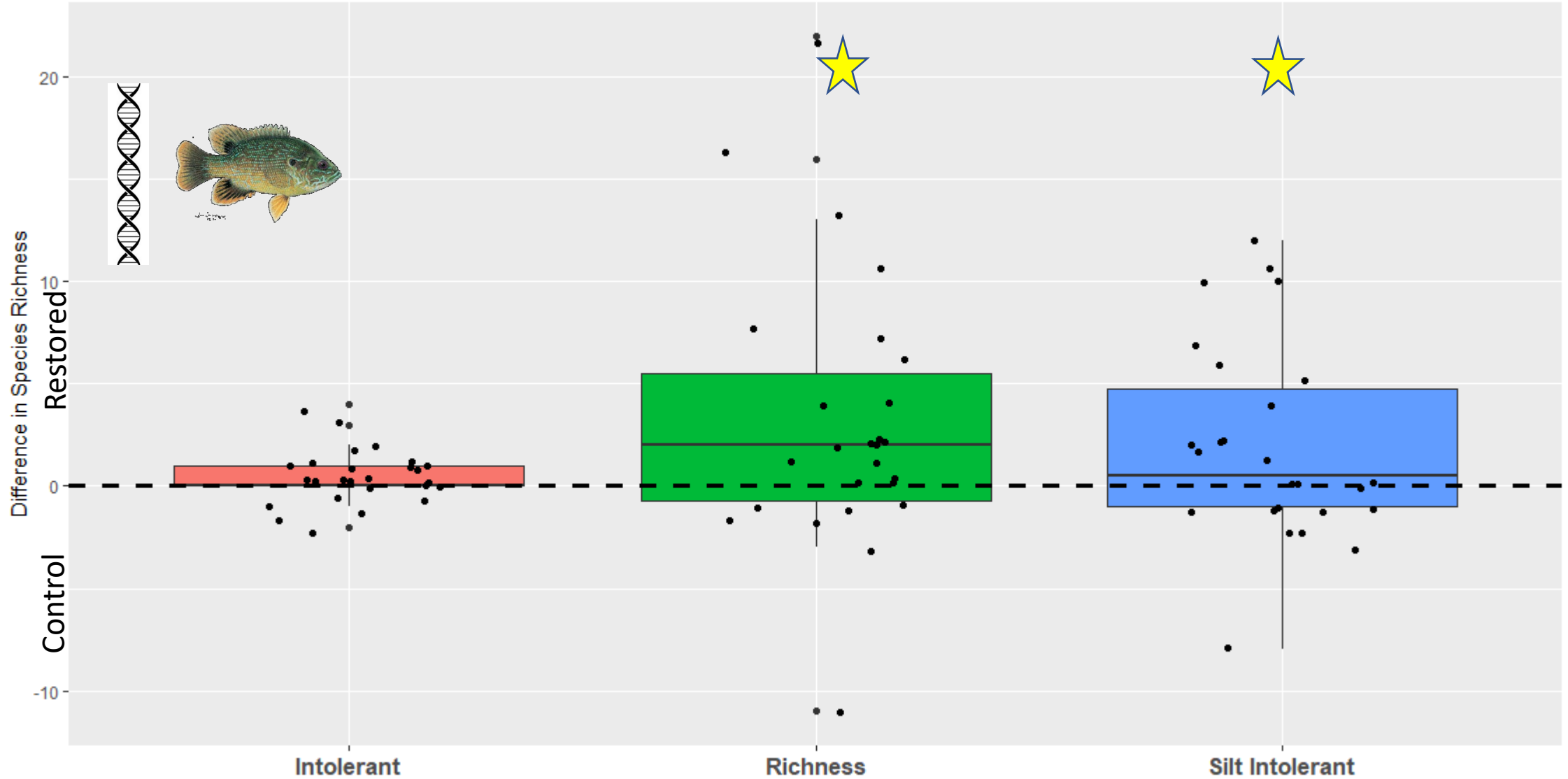
RESTORED sections tend to have more fish species



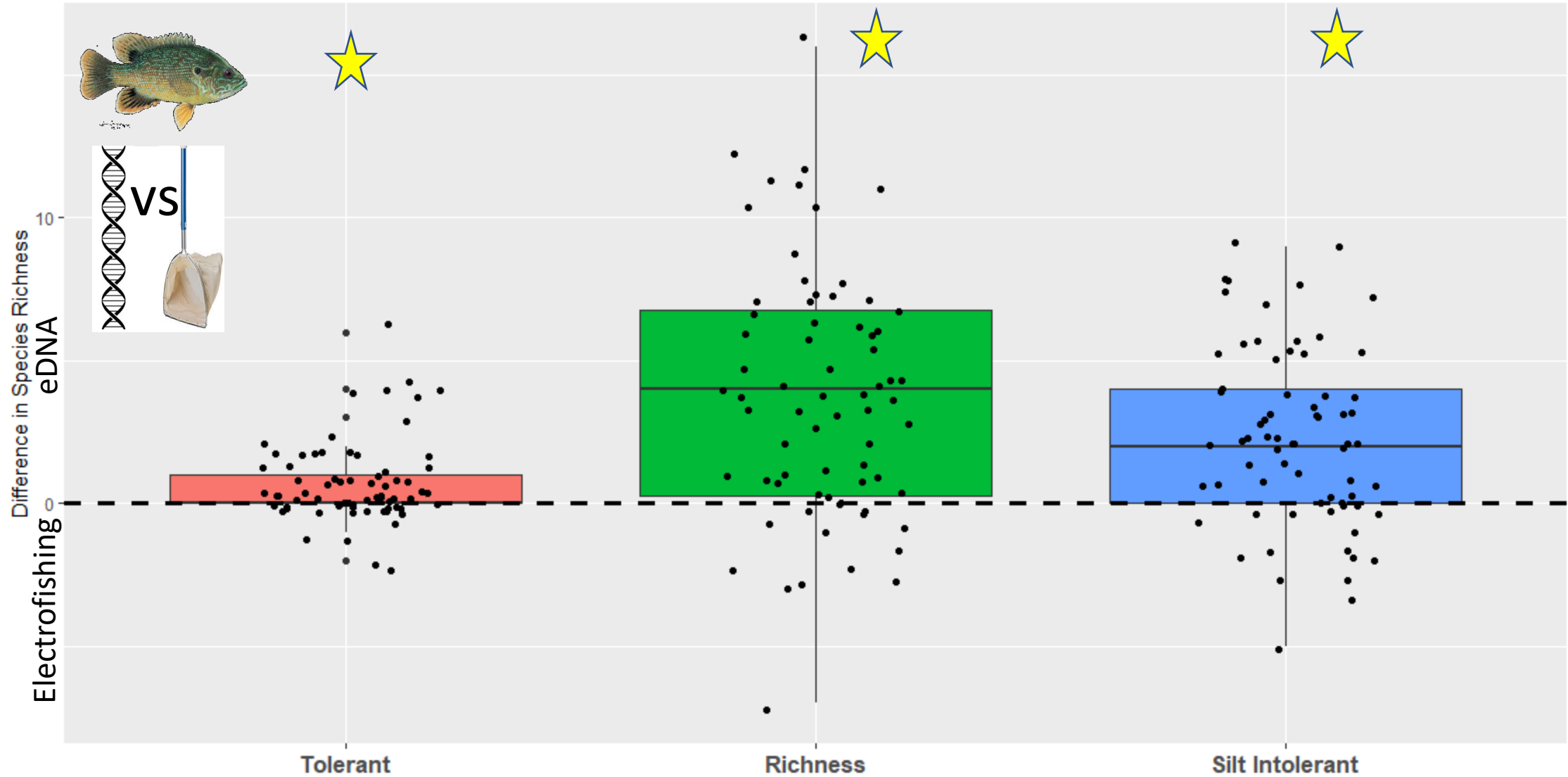
eDNA+Electrofishing strongly suggests RESTORED sections have higher fish species richness, more intolerant, and more silt intolerant species



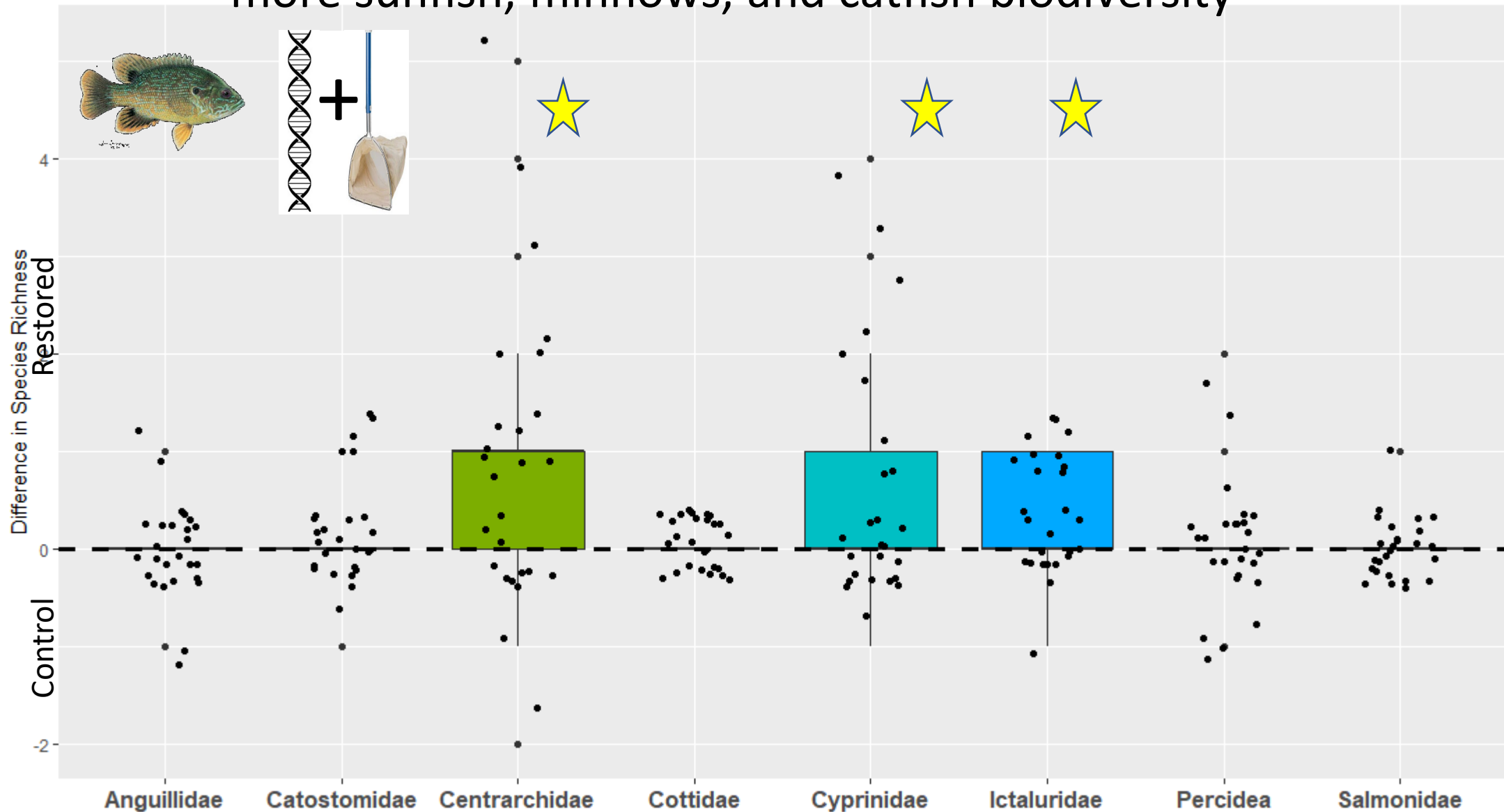
eDNA-ONLY suggests RESTORED sections have higher fish species richness and more silt intolerant species



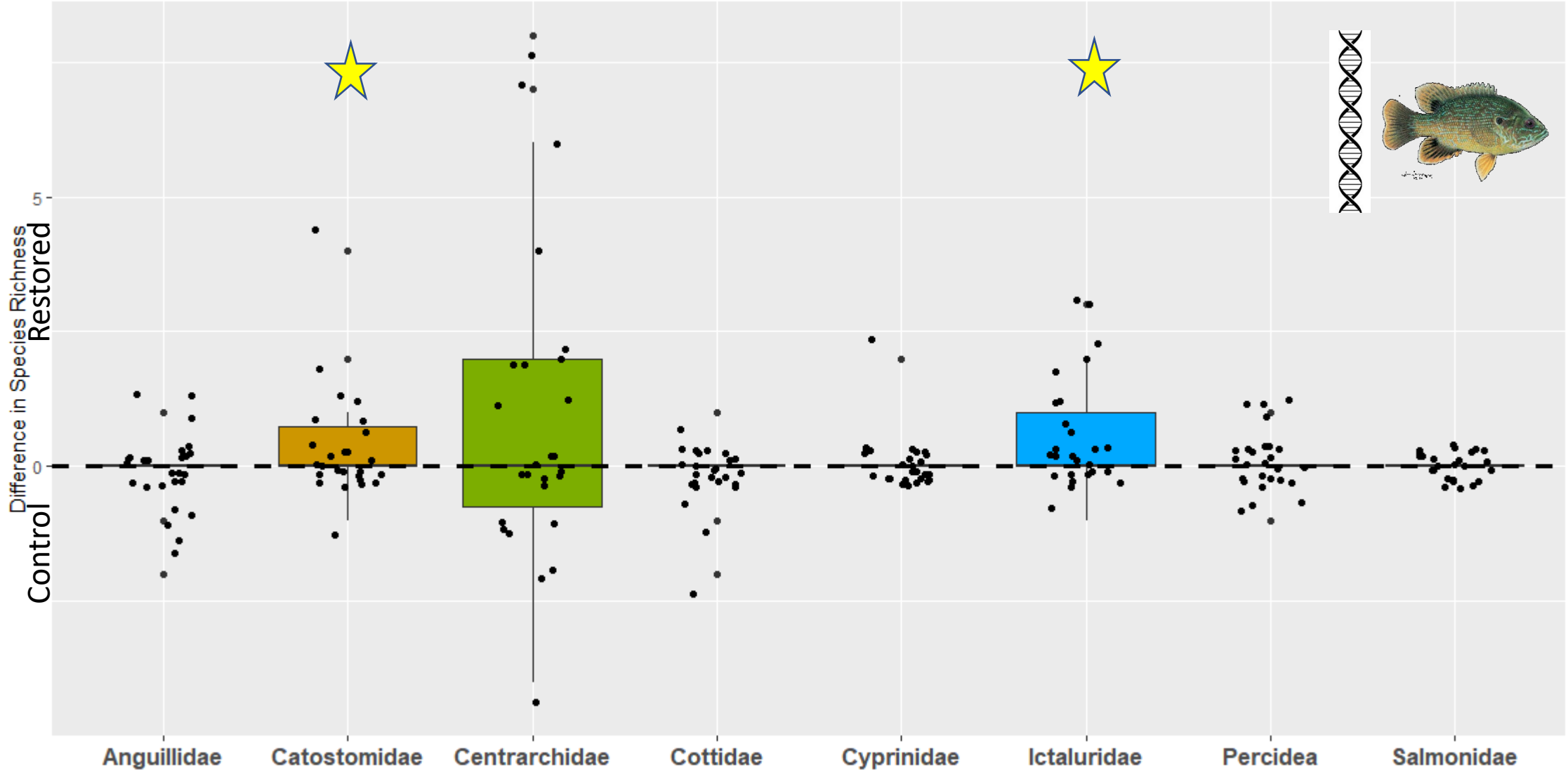
eDNA identifies more fish species, more tolerant, and more silt intolerant species than electrofishing



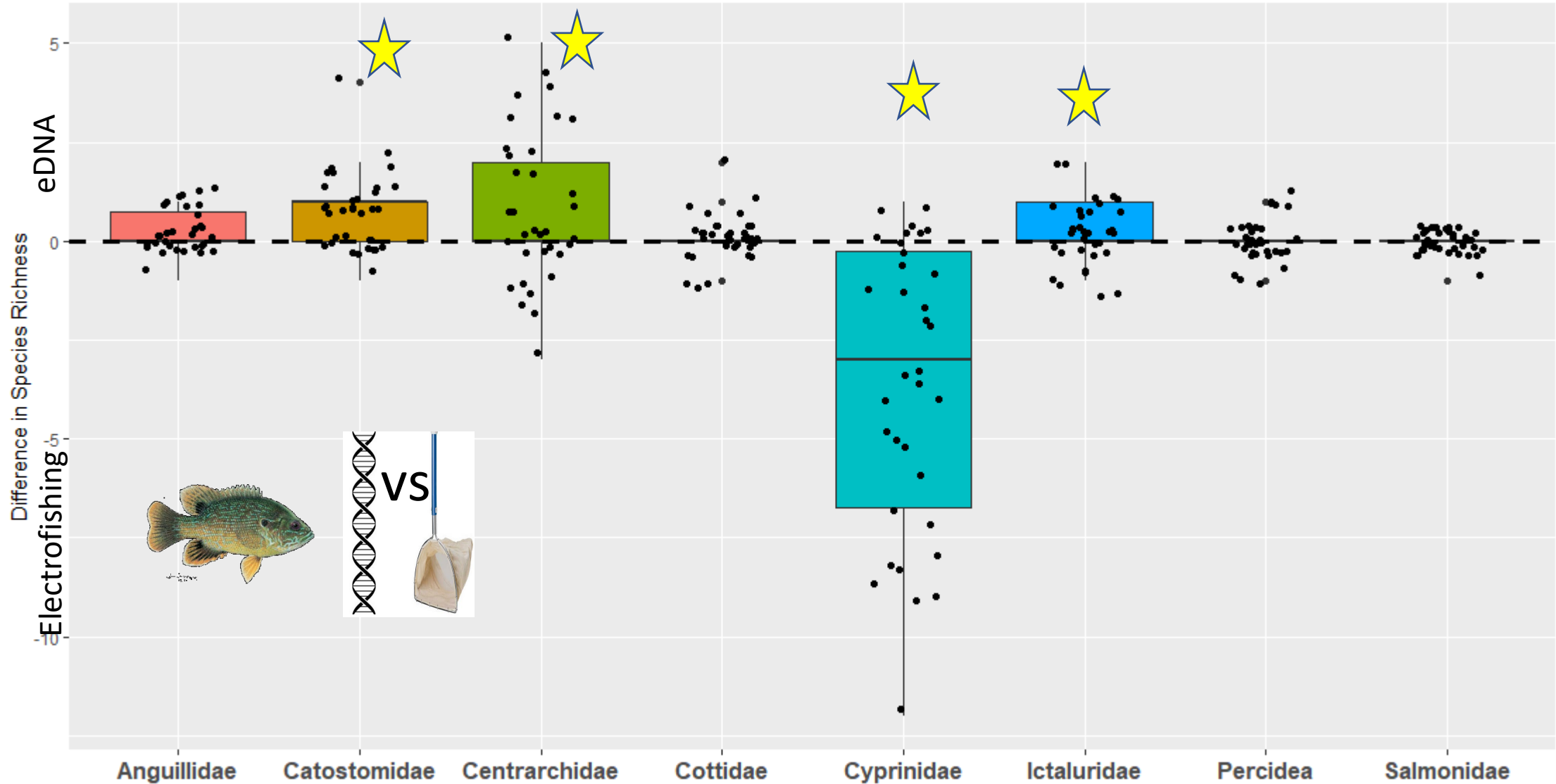
eDNA+Electrofishing suggests RESTORED sections may have more sunfish, minnows, and catfish biodiversity



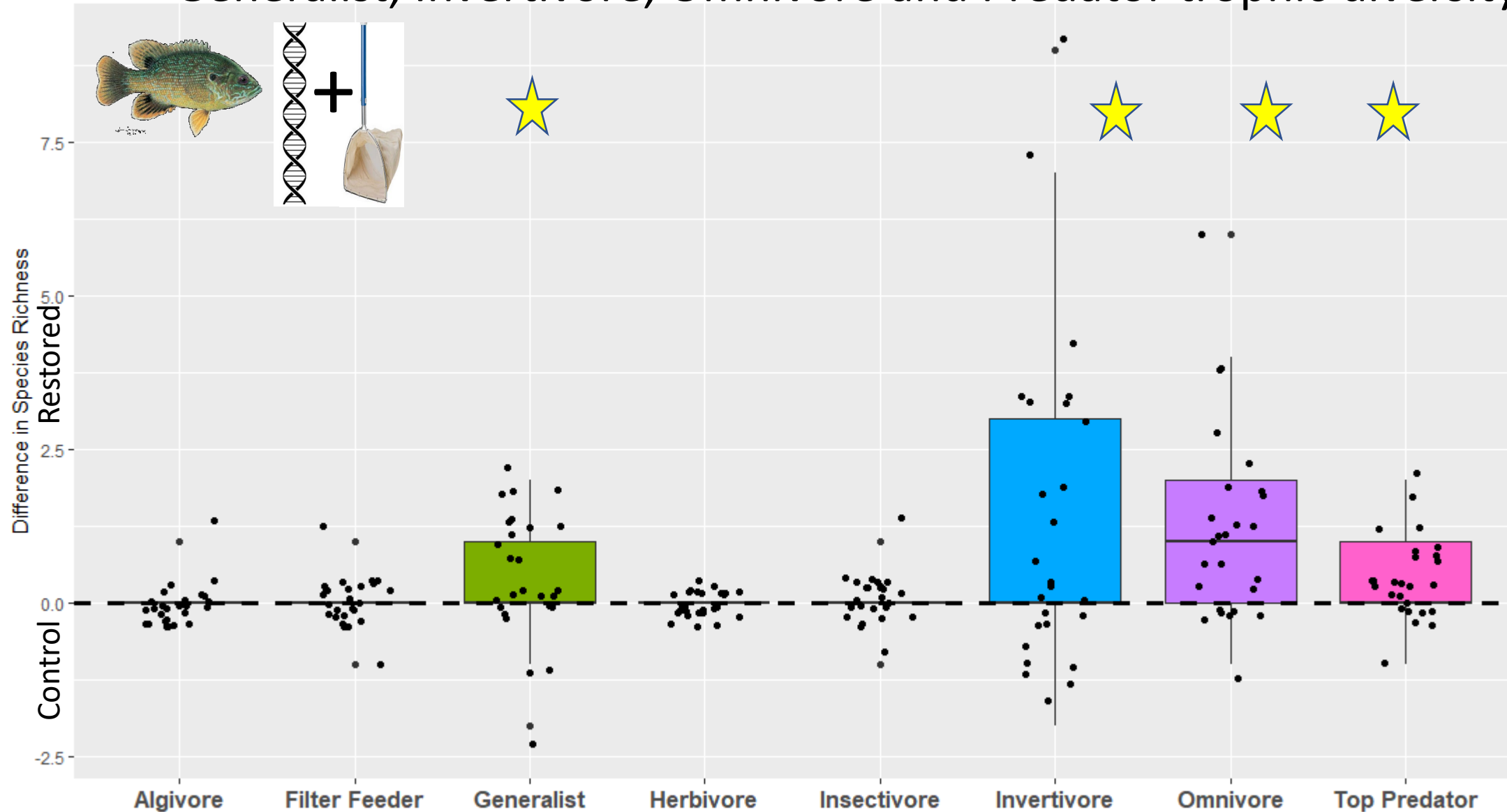
eDNA-ONLY suggests RESTORED sections may have more sucker and catfish biodiversity



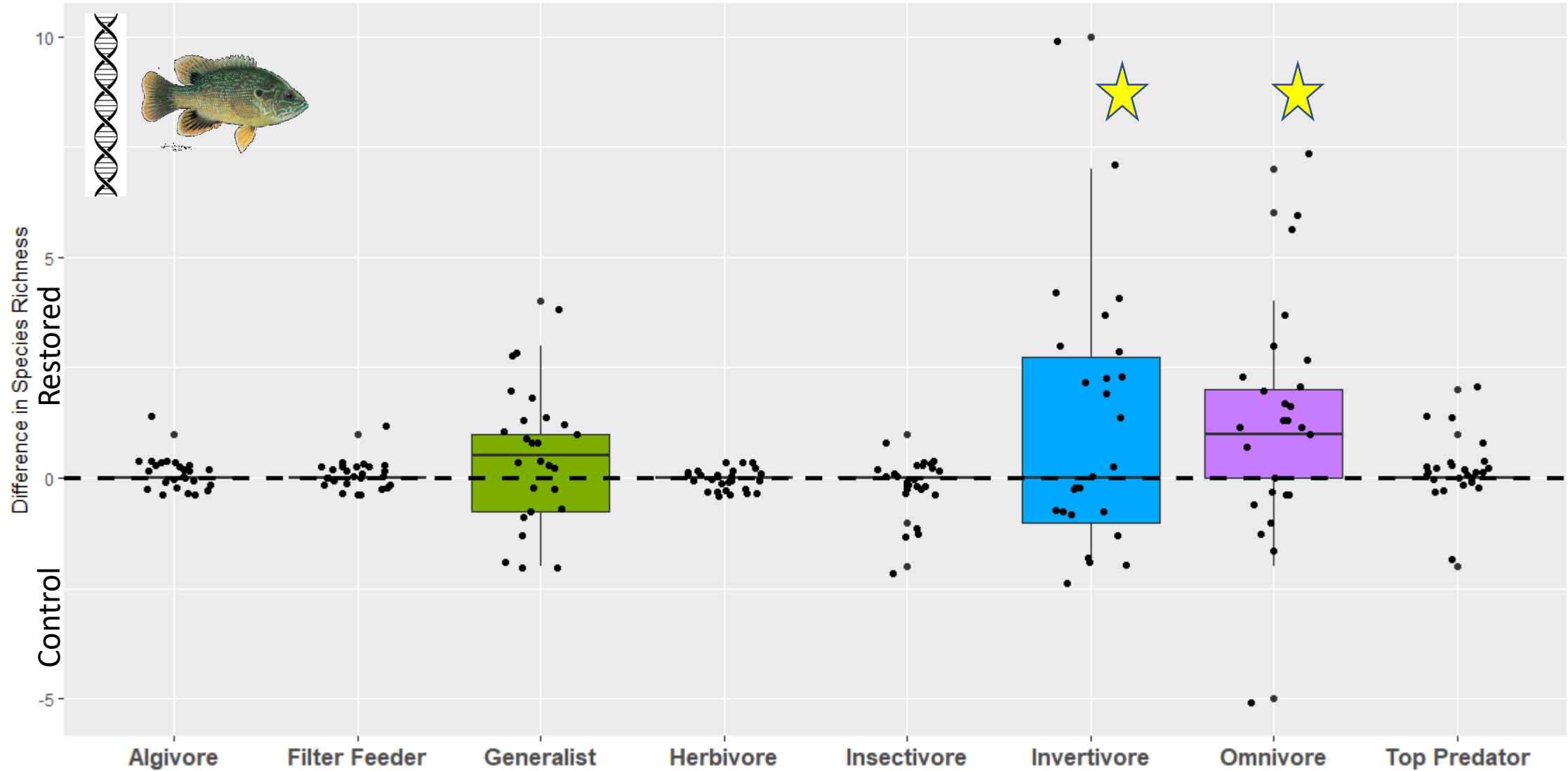
eDNA identifies more suckers, sunfish, and catfish than Electrofishing, but far fewer minnow species*



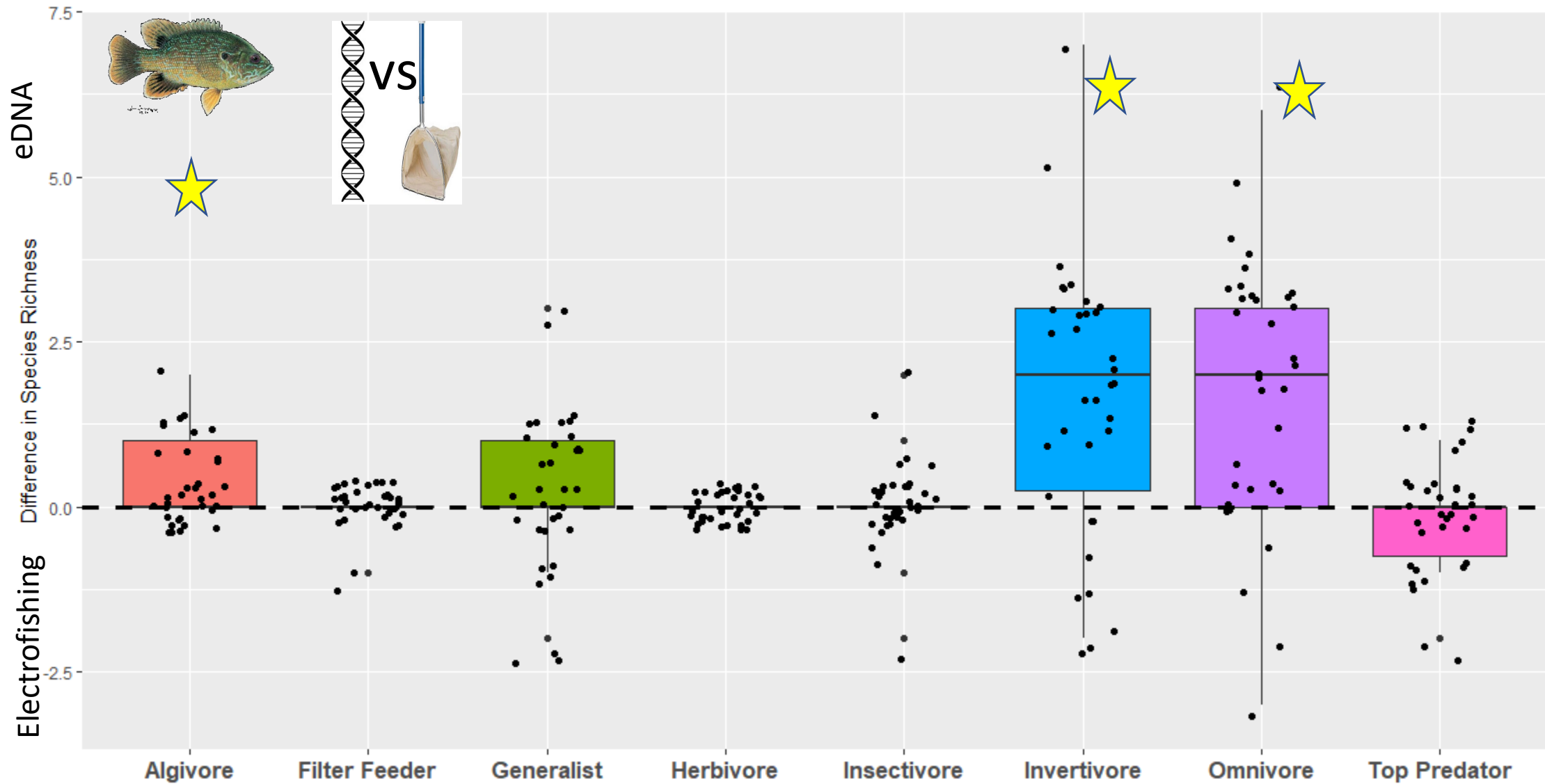
eDNA+Electrofishing suggests RESTORED sections may have more Generalist, Invertivore, Omnivore and Predator trophic diversity



eDNA-ONLY suggests RESTORED sections may have more Invertivore and Omnivore trophic diversity



eDNA identifies more Algivore, Invertivore, and Omnivore trophic diversity than Electrofishing



Summary – evidence for restoration success

Microbial communities did not provide enough resolution to provide confidence in the predictions

Combined eDNA and physical sampling found **more fish and benthic macroinvertebrate taxa** in RESTORED sections compared to the upstream controls

Combined eDNA and physical sampling found **more sensitive fish and benthic macroinvertebrate taxa** in RESTORED sections compared to the upstream controls

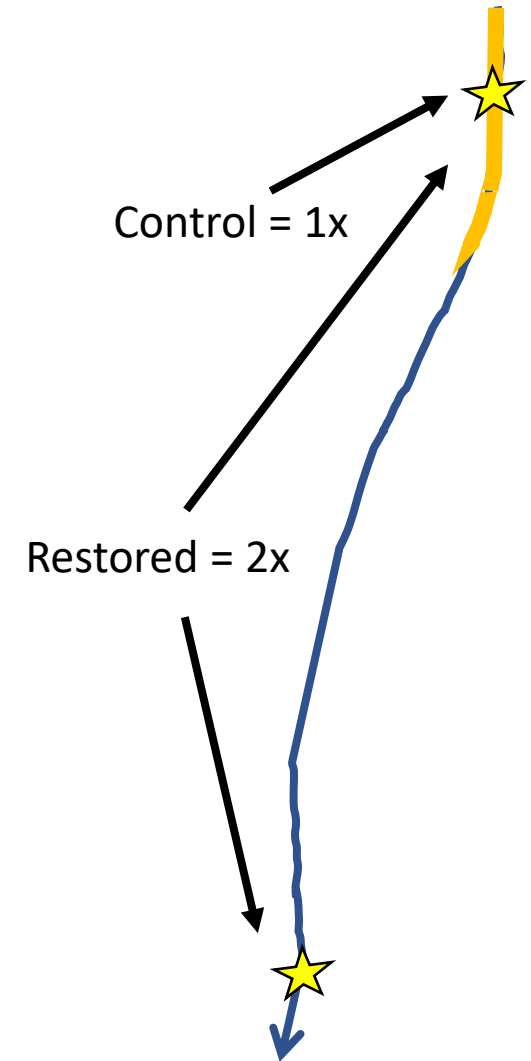
eDNA identified **more fish and benthic macroinvertebrate taxa AND more sensitive fish and benthic macroinvertebrate taxa** than physical sampling of either fish or benthic macroinvertebrates

*Adding eDNA information did not change IBI scores for fish or benthics

Caveat – Restored sections may have 2x sampling effort

eDNA from the control sections could also be in the restored sections

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



Final Thoughts – mostly the same as last year

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 & adding eDNA data did NOT appreciably change the IBI scores. There are still limitations.

Habitat (in)stability and intolerant dipterans/chironomids

More generations for chironomids

Substrates for reproduction by EPT?

External gills of EPT indicators

Chemical sensitivity? Abrasion sensitivity?



eDNA is not a replacement for physical sampling, but substantially adds to knowledge

Microbes seem to indicate possible reintroductions of benthos and fish for most streams - CAUTION: Results seem a bit weird

Translation Slides

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

Translation Slides by
Kara Kemmerer, MDE
Chris Victoria, Anne Arundel County

Kara: Take-home messages from this research

- Restored stream reaches contain higher richness in both benthic and fish communities, supported by physical sampling and eDNA metabarcoding, together.
- eDNA methods detect higher species richness and sensitive species richness for benthics and fish, above physical sampling.
- More work needs to be done to get actual abundances since eDNA alone does not capture magnitude of species.

Kara: What this means to me?

- Regulatory - Better informs permitting protocol, monitoring initiatives, and decision making to protect environmental resources.
- Monitoring - Represents an opportunity for MDE to expand its monitoring guidance and data. It is possible that eDNA sampling methods can be used to contextualize data obtained from traditional physical sampling.
- Guiding Implementation - This work provides a foundation for targeting implementation. It can be used to define success of restoration practices as well as pinpoint areas where biological uplift is lagging, allowing for jurisdictions to do more adaptive management in the future.

Chris: Take-home messages from this research

- Environmental DNA can provide important insights into restoration site recovery
- eDNA detects species not observed with traditional sampling, but seems to provide a more complete characterization of stream communities when used together
- eDNA seems to indicate that Mayflies and other EPT species do not return to restored reaches preferentially over upstream controls

Chris: What this means to me?

- Less expensive and less labor intensive in the field than traditional sampling = potentially more site assessment work possible
- eDNA technology is easily within grasp of local government practitioners and their consultants
- Further work is needed to better match eDNA outcomes to those of traditional sampling, upon which regulatory drivers are based
- Work is still needed before eDNA could begin to replace traditional sampling:
 - Ecological information lacking about microbial community interactions hampers interpretation of these results
 - Abundance of fish and benthics cannot be determined using this method, so it cannot substitute directly for traditional stream sampling (i.e.- BIBI and FIBI)
 - Current IBIs cannot be directly calculated with the data produced with eDNA sampling
 - At least one fish group (minnows) is not well sampled using eDNA
 - Sampling protocols for eDNA still vary considerably—no “20 jab” method specificity for eDNA work; QA/QC approaches need further development
 - Interpretation of the actual raw sequence data (Bioinformatics) is a major bottleneck in getting to an outcome with this approach
 - Statistical issues with sample independence for this work (IMO)—How far upstream should the control site be?