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

Bob Hilderbrand, Rodney Richardson, Regina Trott
UMCES Appalachian Lab, Frostburg, MD

Louis Plough UMCES Horn Point Lab, Cambridge, MD

Clay Raines
USGS Eastern Ecological Science Center, Leetown, WV

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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 possible, but 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 rejecting H1 and failing to reject H2

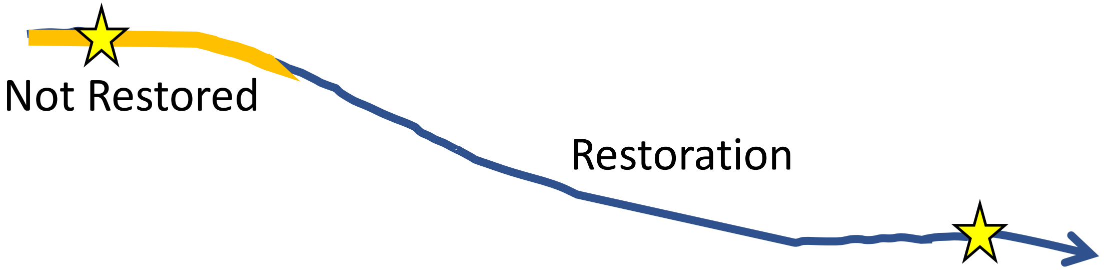
All three hypotheses use DNA sequencing methods

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.

Results preview – evidence for restoration success

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 sensitive benthic macroinvertebrate taxa** in RESTORED sections compared to the upstream controls

eDNA identified **more benthic macroinvertebrate taxa** AND **more sensitive benthic macroinvertebrate taxa** than physical sampling. Not the case for fish.

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

Microbial communities predicted the BIBI +/- 0.5 with 30% accuracy. Better than guessing (8 prediction categories), but not good enough

Increased to 60% accuracy at BIBI +/- 0.75

Low variability in predictions. Nearly every site was predicted to be BIBI=3.0.

No directional bias in over- or under-predicting the BIBI. It just didn't work well.

Precludes rigorous assessment of H1: We cannot independently assess if streams can harbor desired fish or benthics

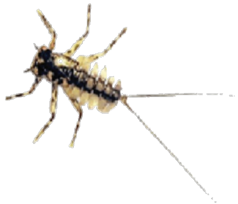
Ecological recovery is somewhat limited by our inability to detect organisms. eDNA improves this for benthics.

Restored sections had significantly more benthic macroinvertebrate taxa

AND

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

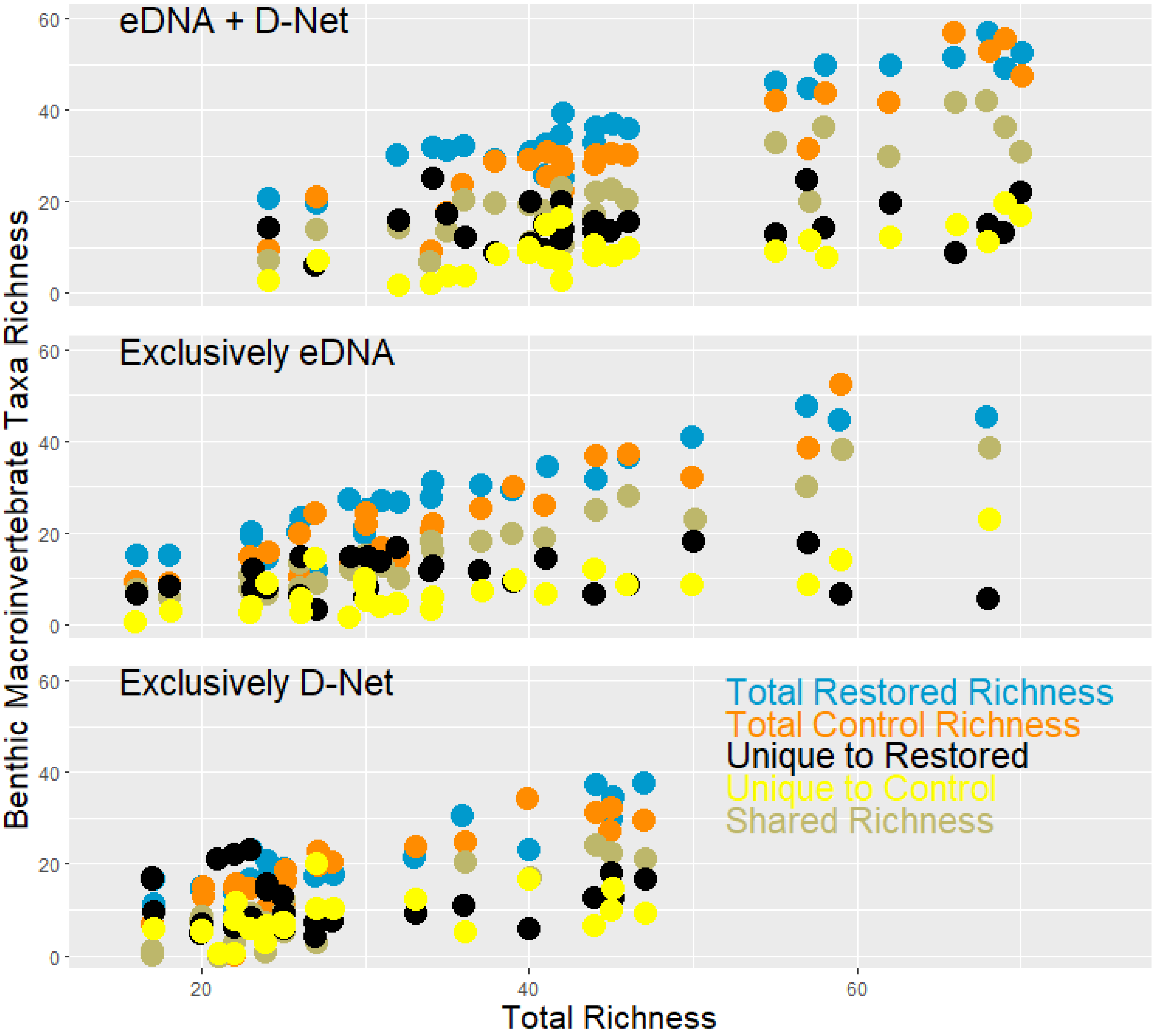
***This includes additional sensitive taxa eDNA identified additional taxa for benthic macroinvertebrates and provides additional sensitivity when combined with D-Net sampling



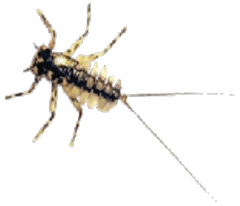
eDNA+DNet: RESTORED sections tend to have more benthic taxa

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

D-net only: No differences



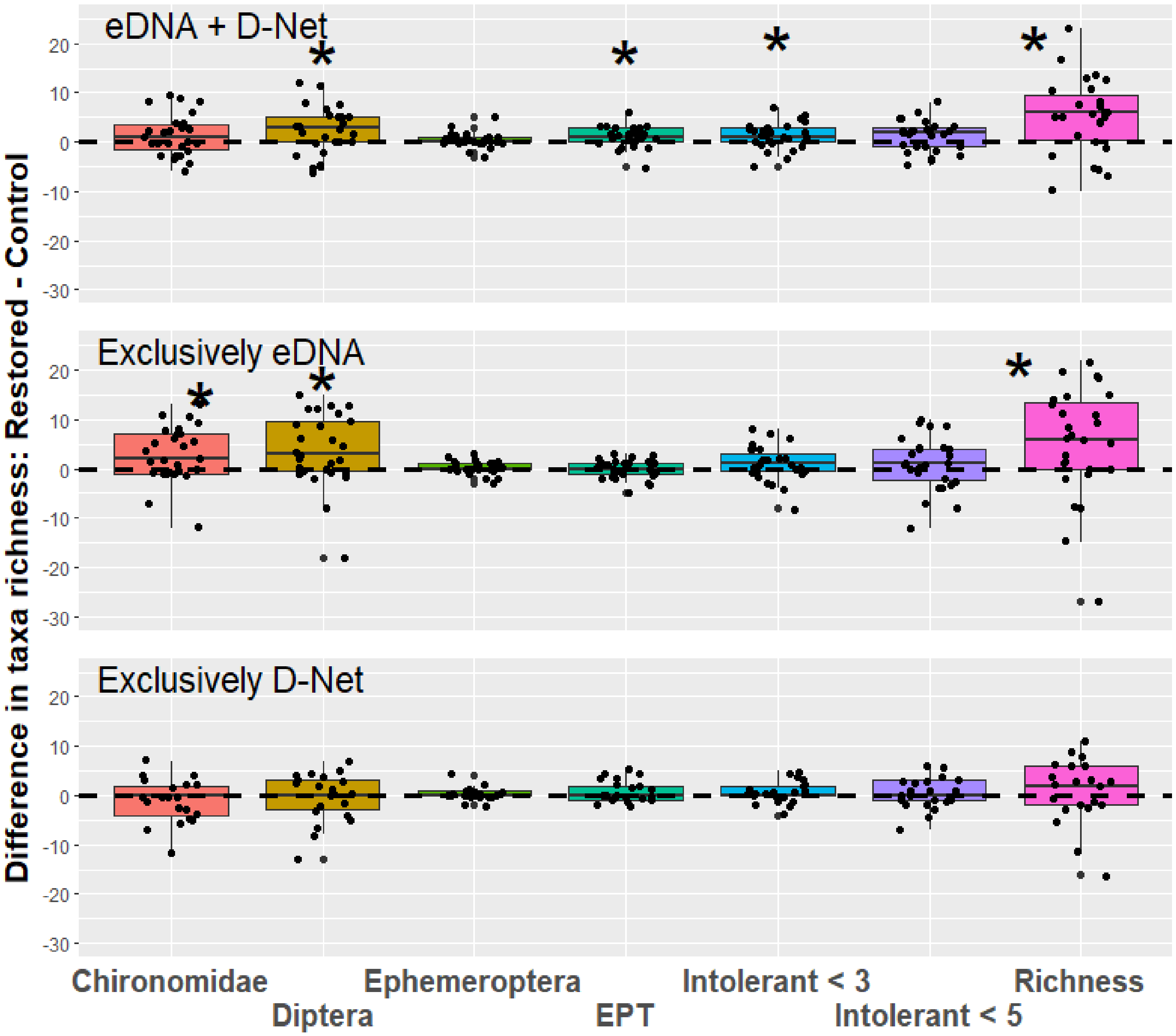
Benthics



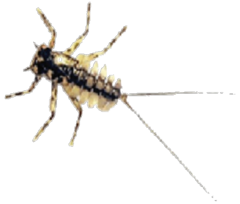
eDNA+DNet suggests RESTORED sections tend towards higher richness, more Dipterans, EPT, & sensitive taxa

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

D-Net ONLY: No differences found between Restored and CONTROL sections

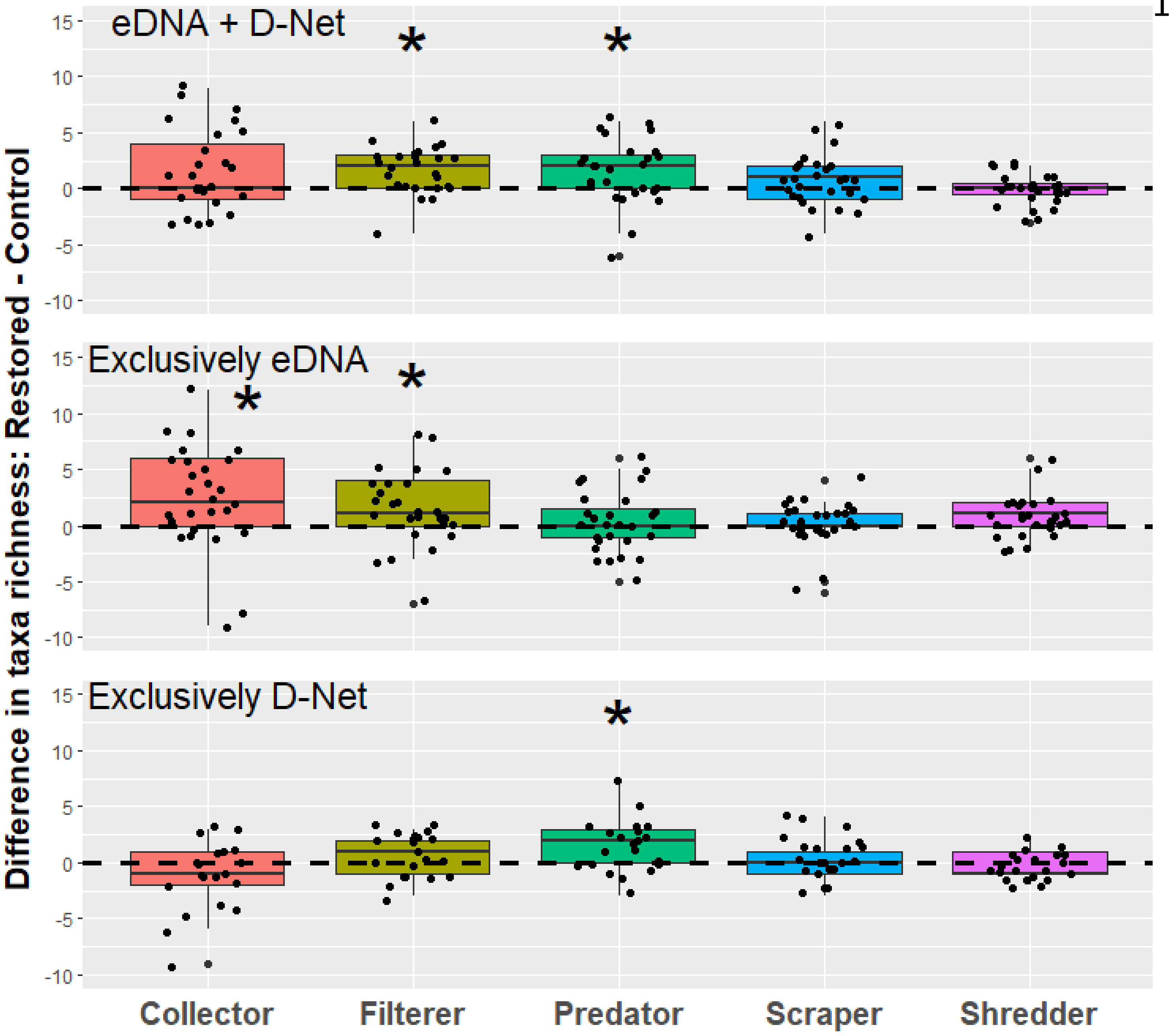


Benthics



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

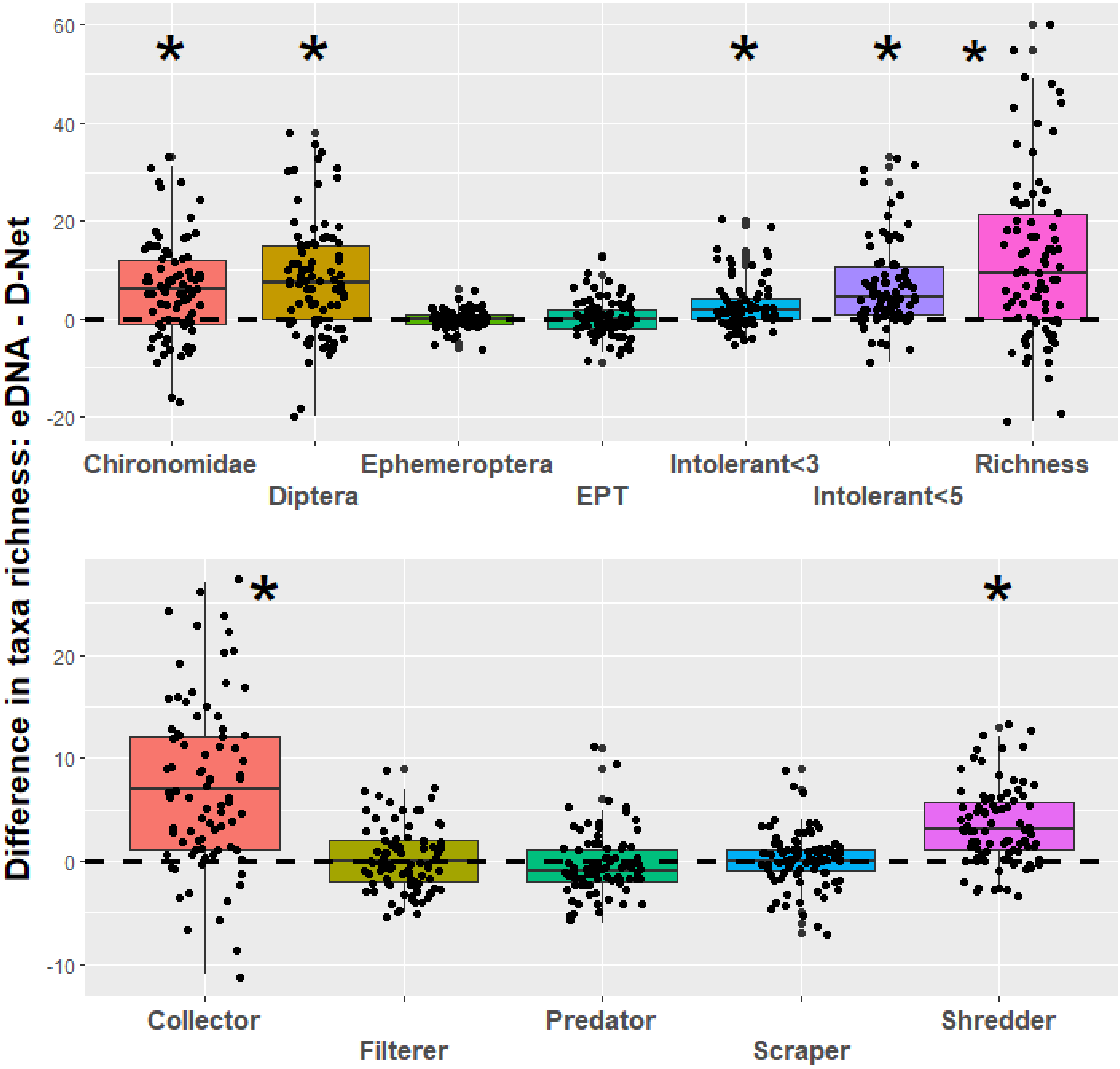


eDNA vs D-Net sampling

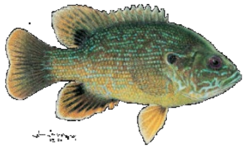
VS

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

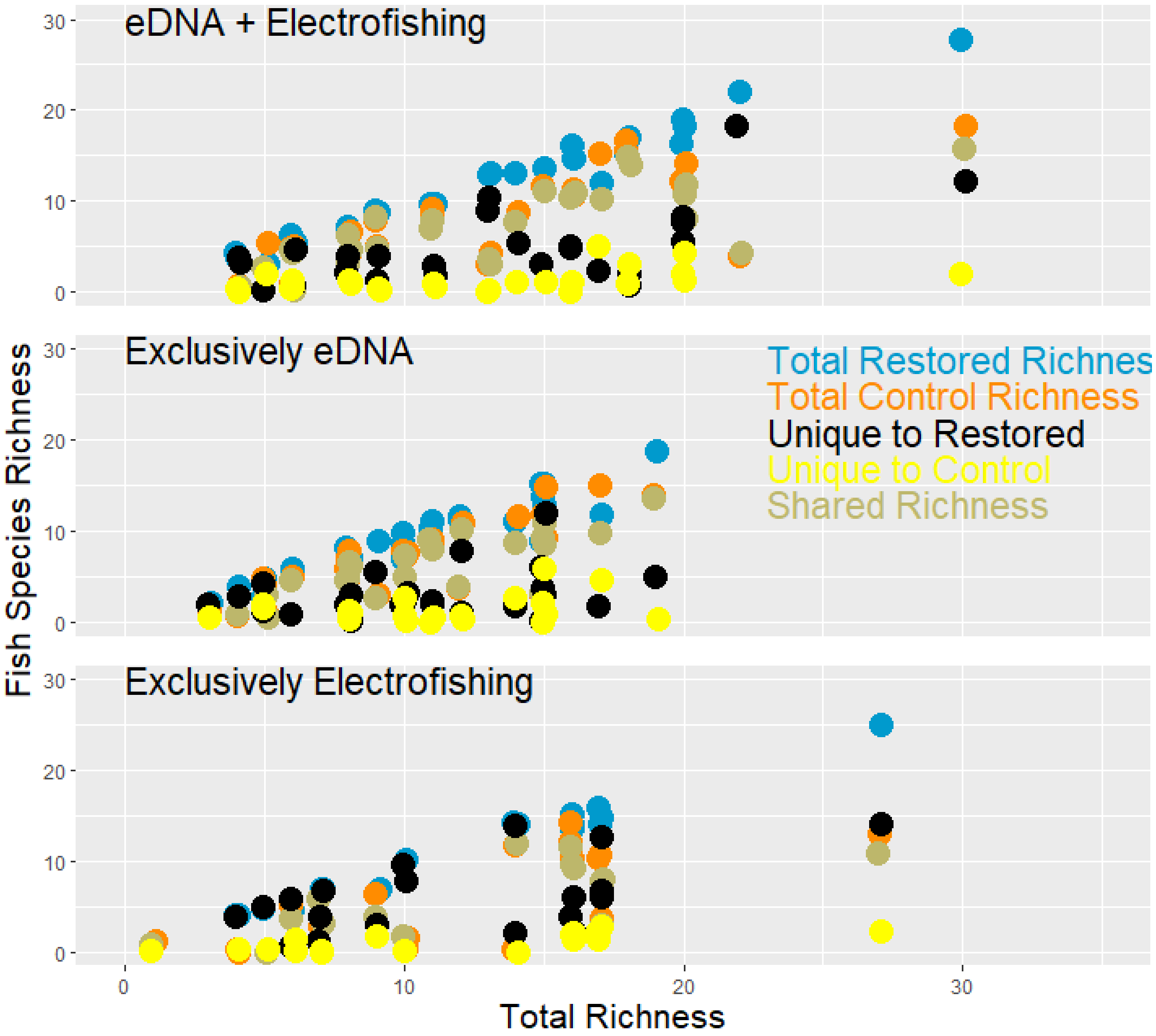


Fish

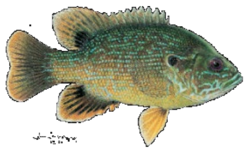


More fish species were found in RESTORED sections compared to the CONTROL sections for all collection methods analyzed.

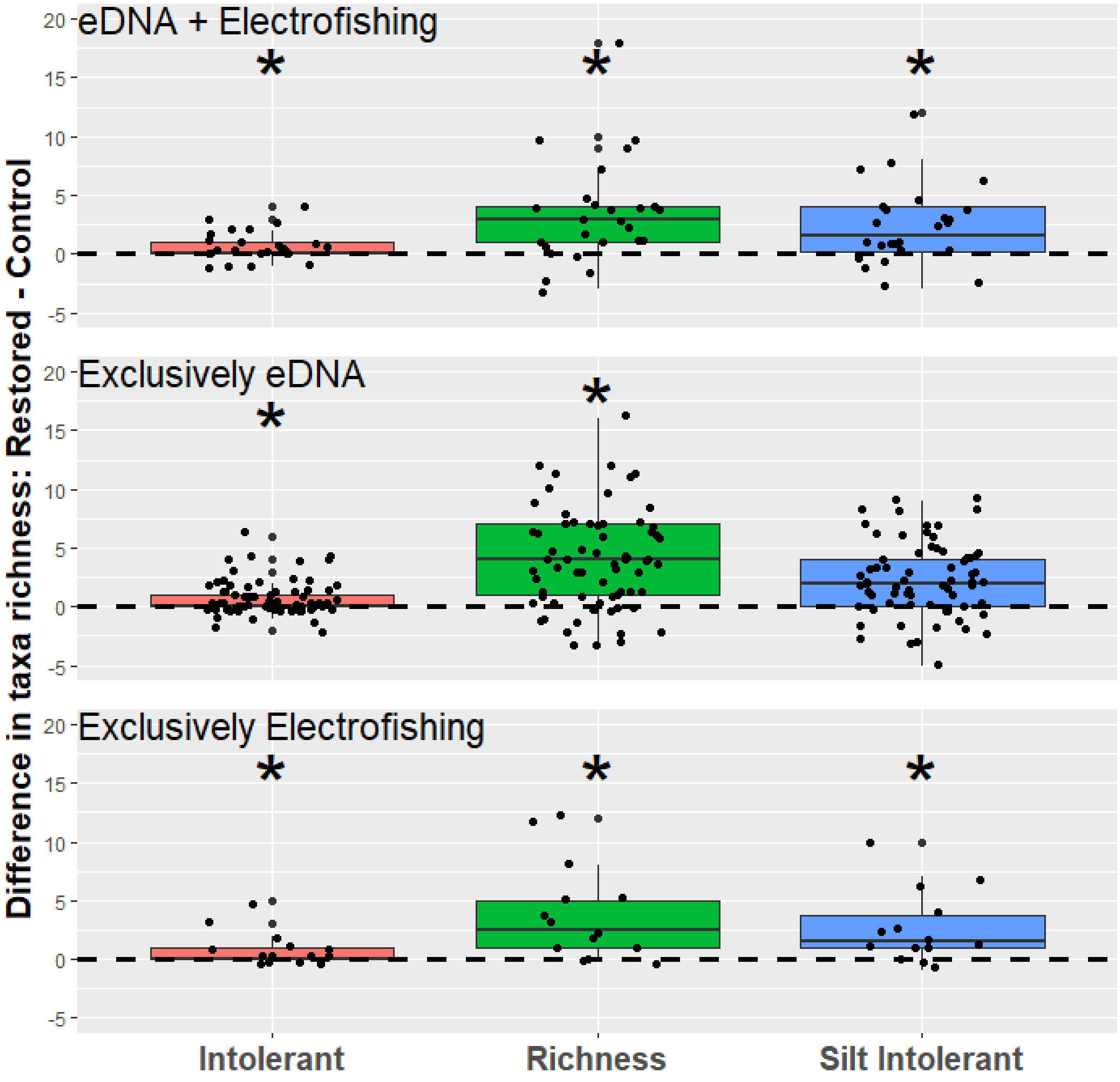
Including eDNA with the electrofishing data tended to improve the number of species found at a site.



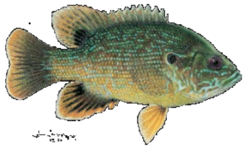
Fish



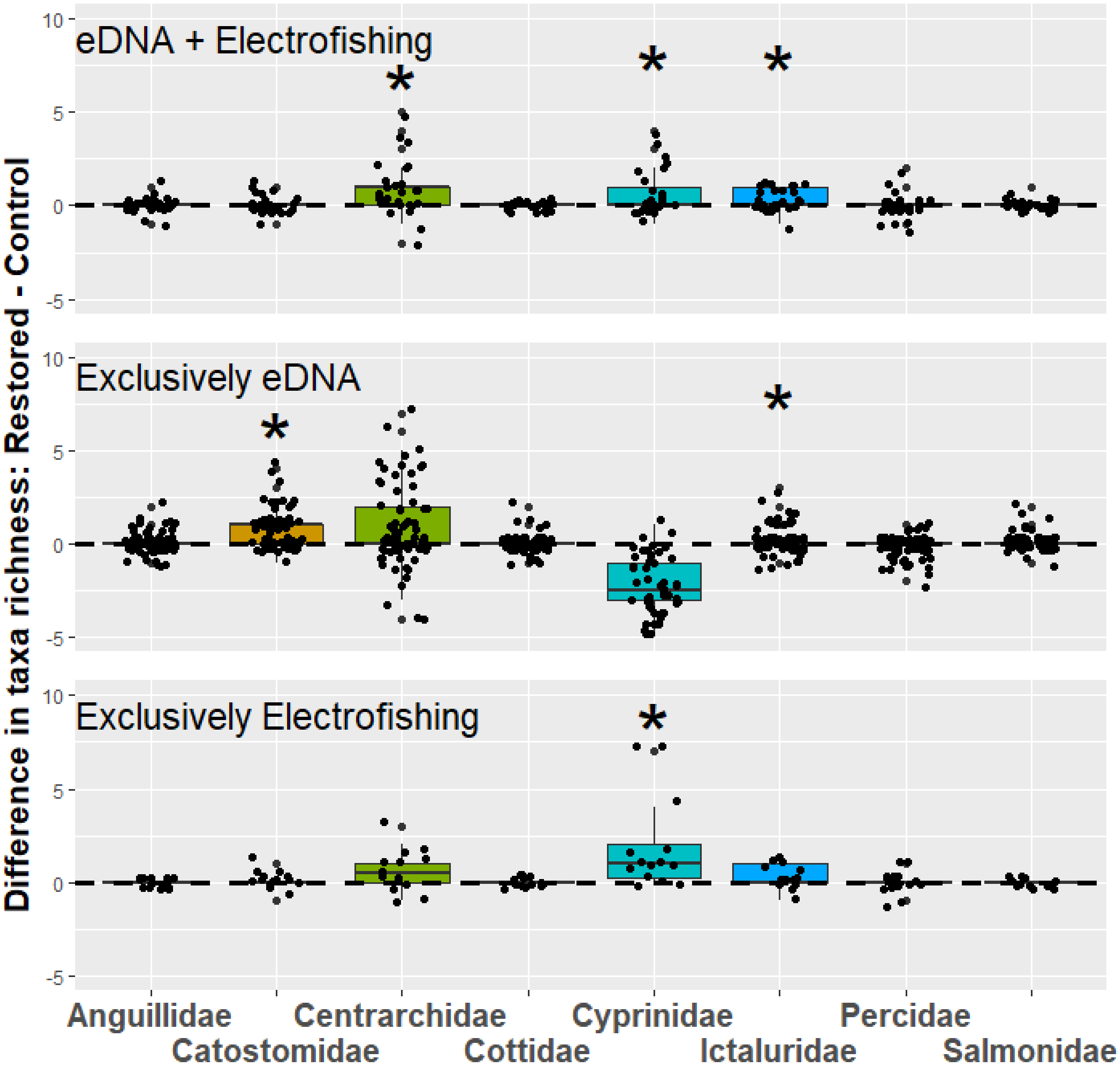
Across all aspects shown, RESTORED sections had significantly more fish species than CONTROL sections.



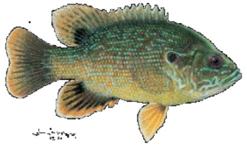
Fish



Including eDNA with electrofishing data tended to increase the sensitivity of the findings.



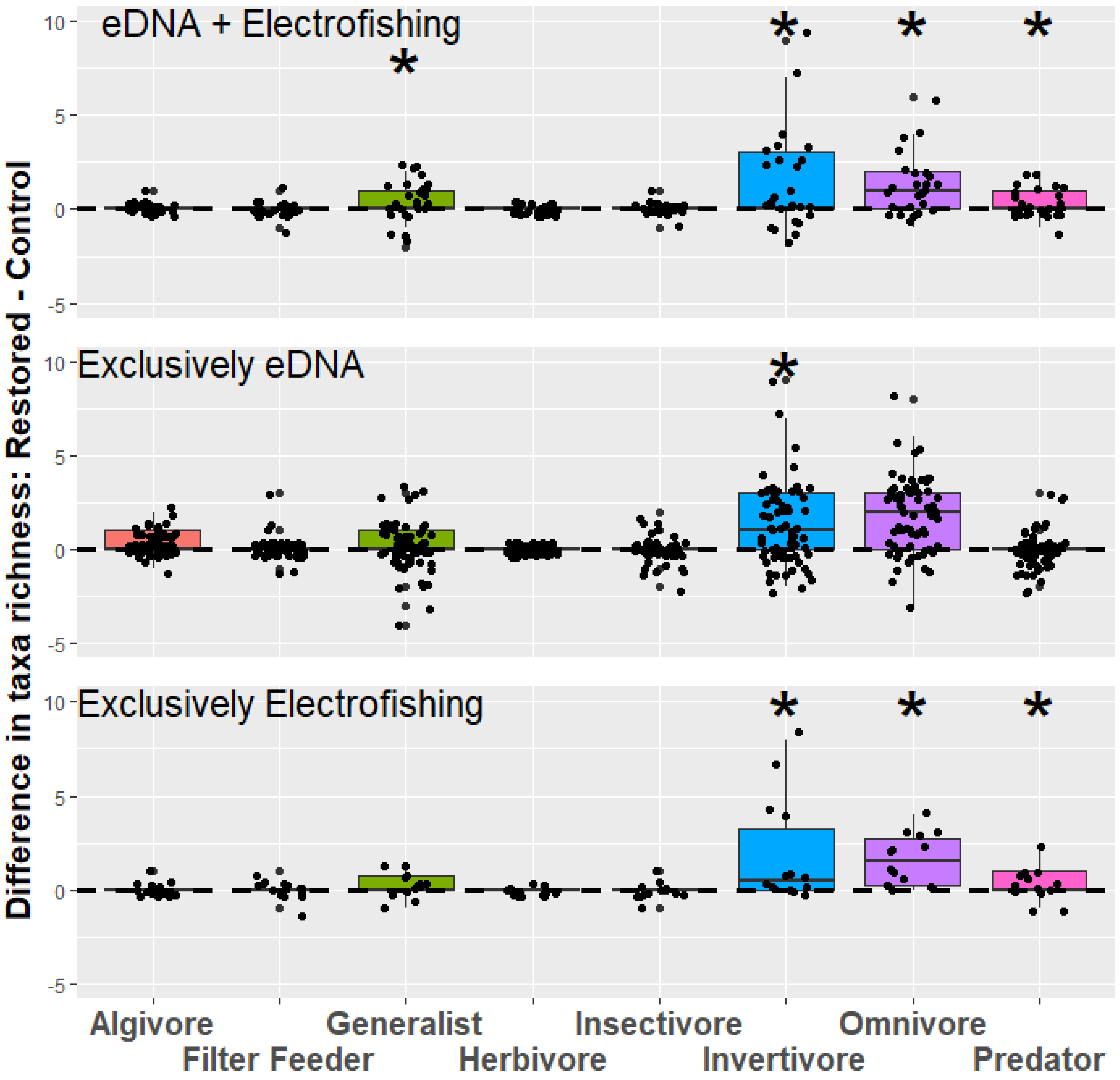
Fish



RESTORED sections had significantly more species in several trophic groups compared to CONTROL sections

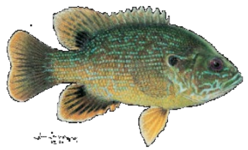
Including eDNA with electrofishing data tended to increase the sensitivity of the findings.

eDNA alone was not as good as electrofishing in identifying differences between RESTORED and CONTROL sections



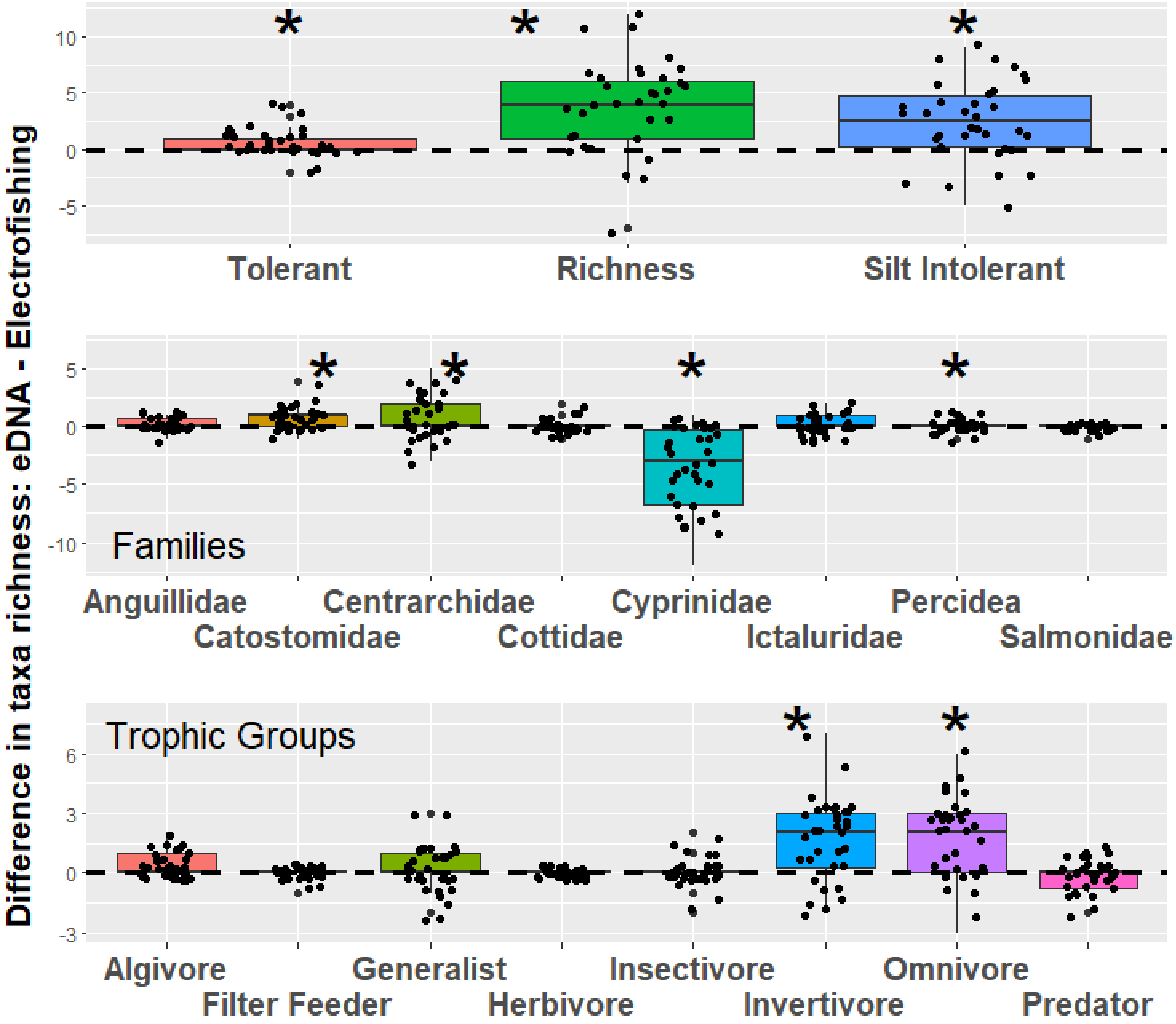
eDNA vs Electrofishing

VS



Compared to electrofishing, eDNA identified significantly more species, more sensitive species, and across different taxonomic and trophic groups.

The minnows (Cyprinidae) were the only group where electrofishing was superior to eDNA in finding more species.



Summary - eDNA is worth the effort

Increased sensitivity of eDNA showed differences between RESTORED and CONTROL sections that traditional D-Net sampling did not find for the benthics

eDNA consistently identified more taxa for both fish and benthics, and more sensitive taxa than traditional monitoring

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

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

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

Final Thoughts

Restorations are still missing most of the indicator taxa & adding eDNA data did NOT change the IBI scores. There are still limitations in terms of the restorations themselves and in how we evaluate them.

My white whale – An eDNA IBI not part of this project. A story and a caution with AI

Results were better than guessing a stream's FIBI or BIBI, but not by much and is unreliable based on my attempts – Random Forest, Ordinal Logistic Regression

eDNA is not a replacement for physical sampling, but it substantially adds to the knowledge at each site, even for the fish

Microbial communities are not reliable predictors of condition using my methods

Translation Slides

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

Translation Slides by

Jay Killian, MD DNR, MBSS

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 <u>no changes</u> observed in EPT and other important indicators that would change an IBI

What does this mean for me?

- 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 long is eDNA detectable before degrading into something not useful in monitoring?
    - Can eDNA be developed into its own new assessment tool?