Earth Sciences
New Zealand

Environmental DNA indicators for supporting implementation of the National Objectives Framework (NOF) in the NPS-FM

On July 1, 2025 ***NIWA*** and ***GNS Science*** merged
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Incorporating eDNA into the FIBI

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Earth Sciences
New Zealand

Options to incorporate eDNA into estimates of FIBI

If we wish to use eDNA and fishing[1] data interchangeably to estimate FIBI, then we have two options:

1. **Simply input eDNA data into the current FIBI[2] without changes to the underlying FIBI algorithm.**
- The current FIBI assumes sampling method has no effect on the number of species detected.
- This option therefore assumes that the species richness's estimated with eDNA and fishing data are equivalent. If not, and eDNA detects more or less species than fishing, we would expect FIBI scores calculated from eDNA data to be inflated or deflated relative to scores calculated from fishing.

2. Develop a new eDNA-FIBI that accounts for the higher species richness's detected from eDNA
- If eDNA detected higher species richness than fishing, we may develop an eDNA-specific FIBI that accounts for the higher species richness.

[1]Data collected using protocols in Joy M, David B, and Lake M. 2013. New Zealand Freshwater Fish Sampling Protocols (Part 1): Wadeable rivers and streams
[2]Current FIBI refers to that used for the NPS-FM attribute calculated according to methods described by Joy M, Death R. 2004. Application of the Index of Biotic Integrity Methodology to New Zealand Freshwater Fish Communities. Environmental management 34:415-428.

1. Can we simply input eDNA data into the current FIBI?

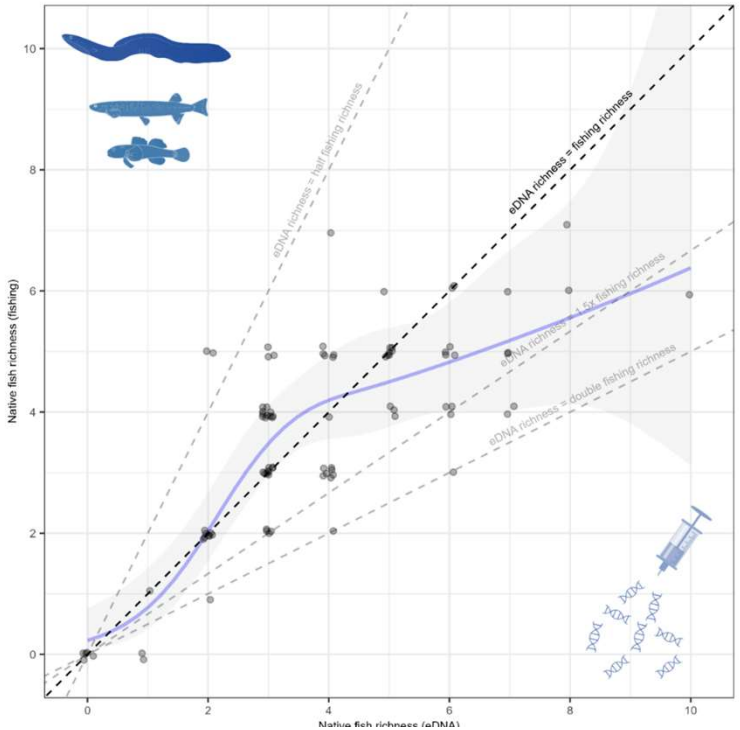
- If we are to simply input eDNA data into the current FIBI algorithm, then we assume species richness estimated with eDNA are equal to those estimated from fishing.

- To test this assumption we analysed the relationship between native fish species richness measured with eDNA and fishing.

- We used the paired eDNA-fishing data which consisted of:
  - 90 sites (72 within the Waikato region, 18 within the Te Hoiere/Pelorus catchment)
  - eDNA data collected using the 6-rep method, and data processed according to Melchior and Baker (2023)[1]
  - Fishing data collected using electric fishing and trapping protocols described by Joy et al. (2013)

[1]Melchior, M. & Baker, C. 2023. Environmental DNA guidelines and field protocols for lotic systems. NIWA Client Report No. 2023279HN

Is species richness from eDNA and fishing equivalent?



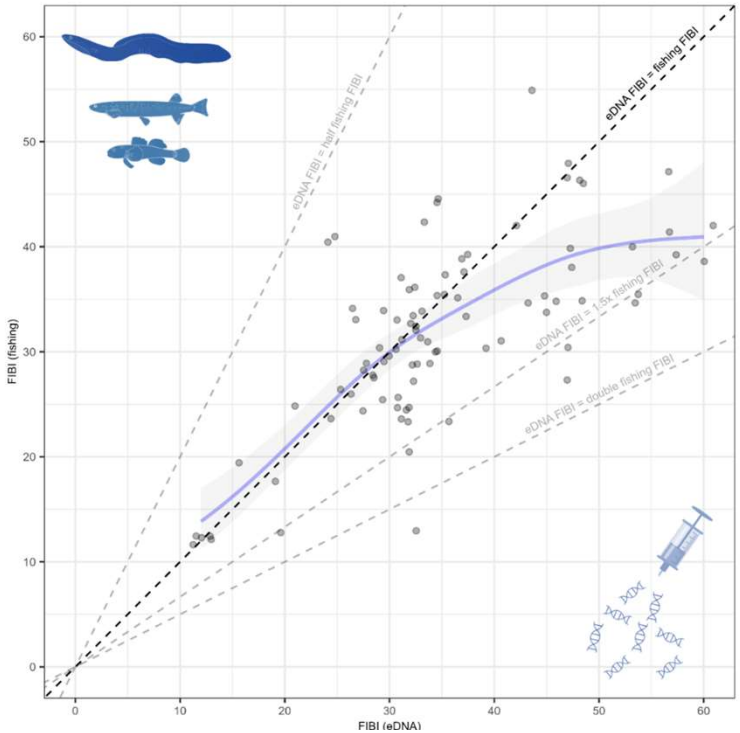
The relationship between native species richness estimated using eDNA and fishing. Dashed reference lines indicate where native species richness detected by eDNA is half (0.5x), equivalent to (1:1 line, black dashed), 1.5x, and double (2x) native species richness detected by fishing, within the same reach. Mean departure from the 1:1 line indicated by a smoother fitted using a Generalised Additive Model (mean, blue line; 95% confidence interval, shaded region). Locations where the blue line falls below or above the 1:1 line indicate where eDNA richness is greater, or less than fishing richness on average. Note that a +/- 0.1 random deviate has been added to points on both axes to aid interpretation.

- Native species richness detected using eDNA tended to be higher relative to fishing richness, particularly in species-rich communities.
- For all reaches with > five species, eDNA detected between 1-2x the number of species detected by fishing.
    - For example, in reaches where fishing detected three species, eDNA may detect an additional three species within the same reach at the same time/day – a doubling of species richness.
- **Conclusion**: The additional species richness detected by eDNA would likely lead to inflated FIBI scores using the current FIBI algorithm.

Relationship between FIBI estimated from eDNA and fishing

- If we make no changes to the current FIBI algorithm and input eDNA data, how will the additional species richness from eDNA affect FIBI scores compared to those estimated from fishing?
- We analysed the relationship between FIBI calculated using eDNA and fishing data.
- No changes to the FIBI algorithm were made, with FIBI being calculated as described by Joy & Death (2004).
- We used the same paired eDNA-fishing data as in the previous slide.

Relationship between FIBI estimated from eDNA and fishing



The relationship between FIBI calculated from eDNA and fishing data. Dashed reference lines indicate where eDNA FIBI is half (0.5x), equal to (1:1 line, black dashed), 1.5x, and double (2x) fishing FIBI, within the same reach. Mean departure from the 1:1 line indicated by a smoother fitted using a Generalised Additive Model (mean, blue line; 95% confidence interval, shaded region). Locations where the blue line falls below or above the 1:1 line indicate where eDNA FIBI is greater, or less than fishing FIBI on average. Note that a +/- 1 random deviate has been added to points on both axes to aid interpretation.

- FIBI estimated from eDNA tended to be higher than FIBI estimated from fishing when FIBI exceeded 30.
- The magnitude of the difference was such that FIBI estimated from eDNA was often more than 1.5x FIBI estimated from fishing.
    - For example, in reaches where fishing yielded a FIBI of 40, eDNA may yield a FIBI over 60 within the same reach.
- **Conclusion:** The magnitude of the eDNA bias would result in different FIBI attribute grades depending on whether eDNA or fishing data was used.

1. Can we simply input eDNA data into the current FIBI?

Overall conclusion: No

- eDNA detected a greater number of species than fishing, particularly in species-rich communities, inflating FIBI for those communities.

- The magnitude of this bias would result in different FIBI attribute grades depending on whether eDNA or fishing data was used.

- If we ignore these problems, and used eDNA and fishing data interchangeably with the current FIBI algorithm, spatial and temporal patterns in FIBI may be confounded by changes in sampling method[1].

[1]Fish species richness data in the NZFFD used by Joy & Death (2004) to develop the FIBI were not collected using the standardised protocol of Joy et al. (2013) and often used a lower sample effort. The Joy et al. (2004) FIBI may therefore underestimate the species richness's expected from fishing data collected by the Joy et al. (2013) fishing protocol. In any case, the problem being addressed here is that the FIBI assumes sample method has no effect on species richness detected, and we break that assumption if we use eDNA and fishing data interchangeably, which confounds resulting FIBI scores. This problem would persist if the FIBI had been developed using data collected using the Joy et al. (2013) fishing protocol. The magnitude of this problem would likely be greater if we had compared FIBI calculated with eDNA and richness data from the NZFFD, because the differences in species richness's between these data types would be larger.

What are our options from here?

1. Apply a correction factor to eDNA richness such that it has the same range as fishing
   - Using models (e.g., previous figure) or
   - Calibrate the eDNA sampling protocol such that it yields equivalent fishing richness

2. Develop a new FIBI algorithm for eDNA data
   - Requires new maximum species richness lines (MSRLs) to account for the increased richness of eDNA

We don't recommend Option 1:

- Reduces full potential of eDNA – we lose information on species richness
- Requires quality national paired eDNA-fishing data across a broad spatial range and different elevations – which does not currently exist
- We already have national eDNA data for Option 2

In the next section we develop a preliminary eDNA-FIBI algorithm.

2. Developing a new eDNA-FIBI algorithm

- Development of a new eDNA-FIBI is eminently achievable, but would require us to address some of the deficiencies of the current FIBI algorithm.

- One major deficiency is the failure to estimate MSRLs using a defensible model.

- The current FIBI estimated MSRLs as a function of site distance from the coast (D) and elevation (E) by eye, which has several flaws:
  a) It falsely assumes the effects of distance and elevation on species richness are independent
  b) It is subjective, hence biased
  c) It is not repeatable, hence we need a new approach if we are to develop new MSRLs

- Flaw a) may lead to inflated estimates of maximum species richness for many catchments which, in turn, will result in FIBI scores in those catchments being reduced/penalised, purely as an artefact of the inappropriate statistics used.

2. Developing a new eDNA-FIBI algorithm

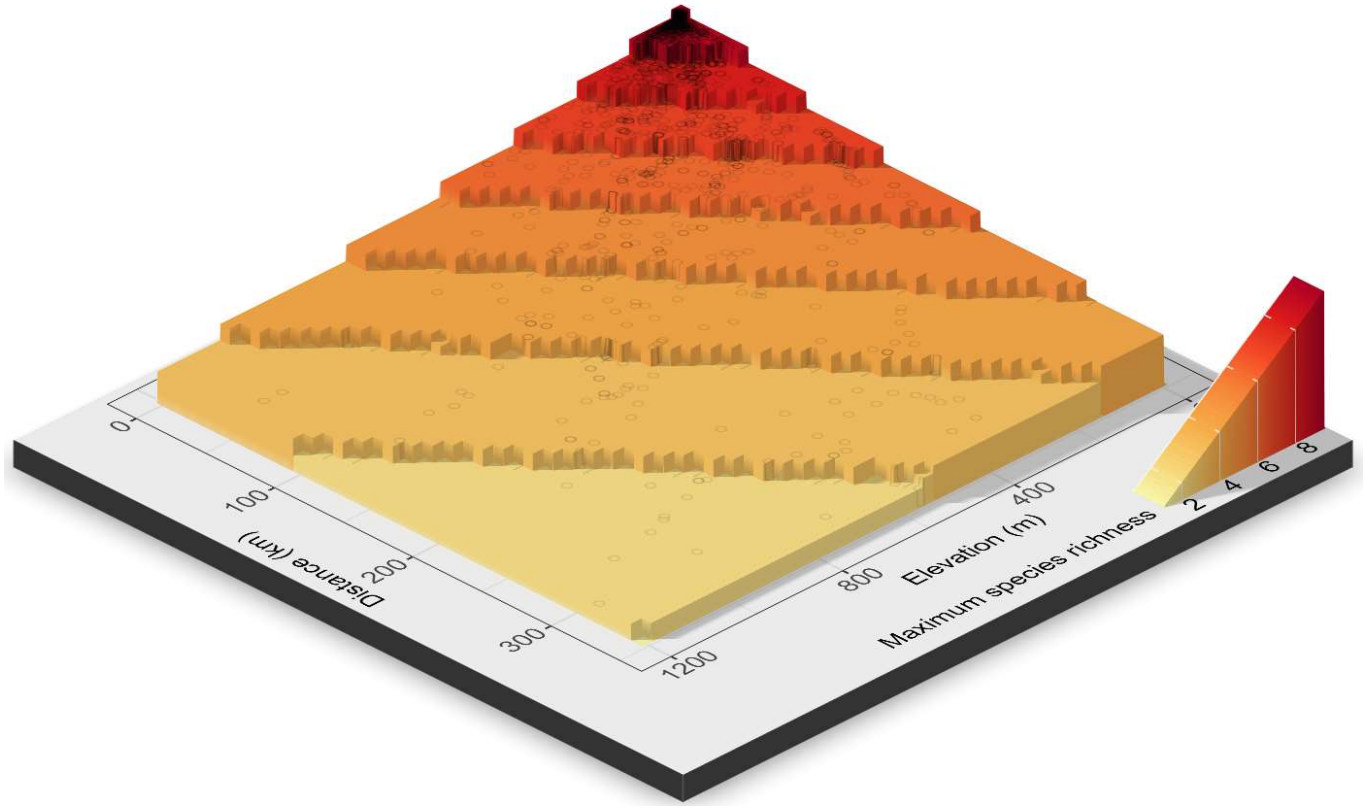
- In subsequent slides we present methods and results of a new approach to estimate MSRLs for all sub-metrics of the FIBI using eDNA data, and apply these MSRLs to calculate a new eDNA-FIBI.

- The MSRLs were estimated using Bayesian negative binomial Generalised Linear Models (GLMs), fitted using the national 6-rep eDNA dataset compiled from the Wilderlab database (1432 6-rep samples from 1356 stream reaches). Data was downloaded from Wilderlab on 6 March 2025.

- To illustrate the error introduced by assuming independent distance and elevation effects, we estimated two sets of MSRLs:
  1) Joint-effects MSRLs, whereby MSRLs were estimated for distance and elevation from a joint-effect GLM including both distance *and* elevation as covariates.
  2) Independent-effects MSRLs, whereby MSRLs for distance and elevation were estimated from separate independent-effect GLMs with distance *or* elevation as covariates.

- The two sets of MSRLs were then used to compute and compare a joint-effects eDNA-FIBI and an independent-effects eDNA-FIBI.

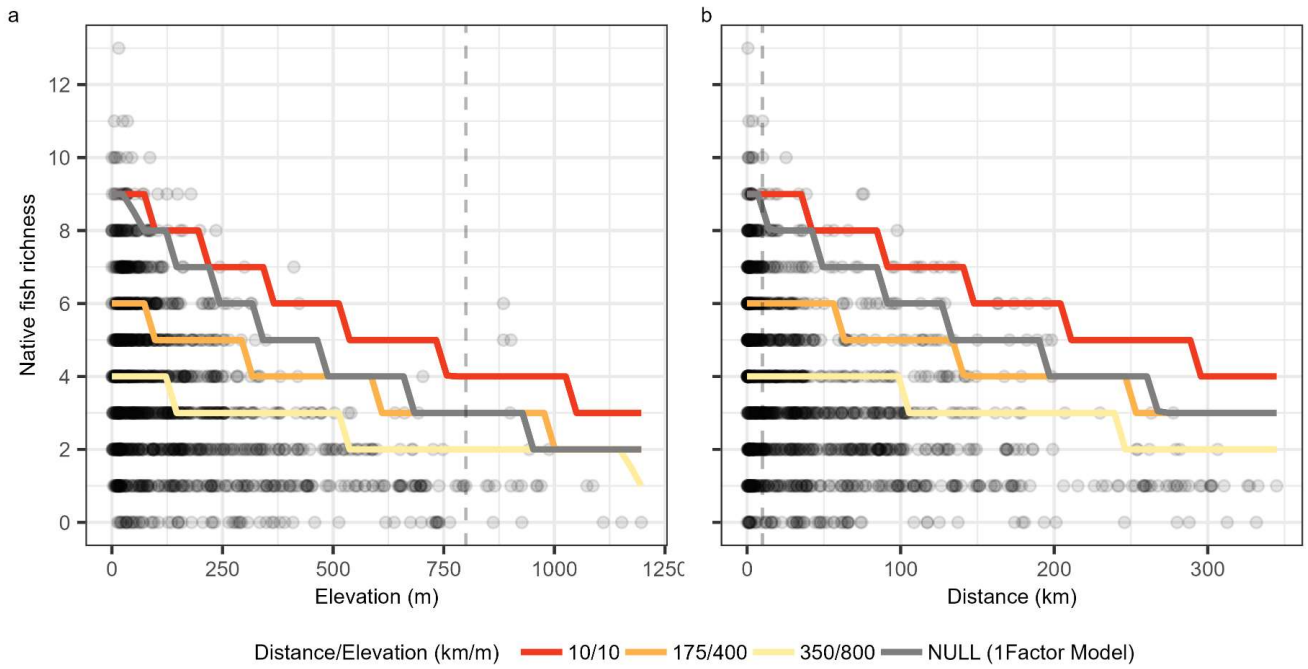
The joint effect of distance and elevation on native richness



Maximum native species richness arising from the joint effects of distance (km inland) and elevation (m above sea level; asl).
Maximum native species richness estimated from 95th quantile of the posterior predictive distribution of the joint-effects GLM. Observed points from the 6-rep national eDNA dataset positioned in 2D space, circles.

- Under the joint-effects GLM, the highest potential native species richness occurs only when *both* distance and elevation are low.
- Conversely, species richness is lowest when *both* distance and elevation are high
- In contrast, the independent-effects GLMs assume that only distance *or* elevation must be low to achieve the highest potential native species richness

Native richness error introduced by ignoring joint effects



Maximum species richness lines (MSRLs) for native species for (a) elevation and (b) distance.
Grey solid lines are MSRLs estimated independently for distance (D) and elevation (E) (from independent-effects GLMs). Coloured lines are MSRLs estimated from the joint effect of D and E (joint-effects GLM), with MSRLs shown for (a) E at fixed values of D (10, 175 and 350 km) and for (b) D at fixed values of E (10, 400 and 800 m). The MSRLs are estimated from 95th quantile of the posterior predictive distribution of the models. Points are the observed national 6-rep eDNA data used to fit the models. Grey dashed lines provide a reference for a hypothetical river with D=10 km and E= 800 m.

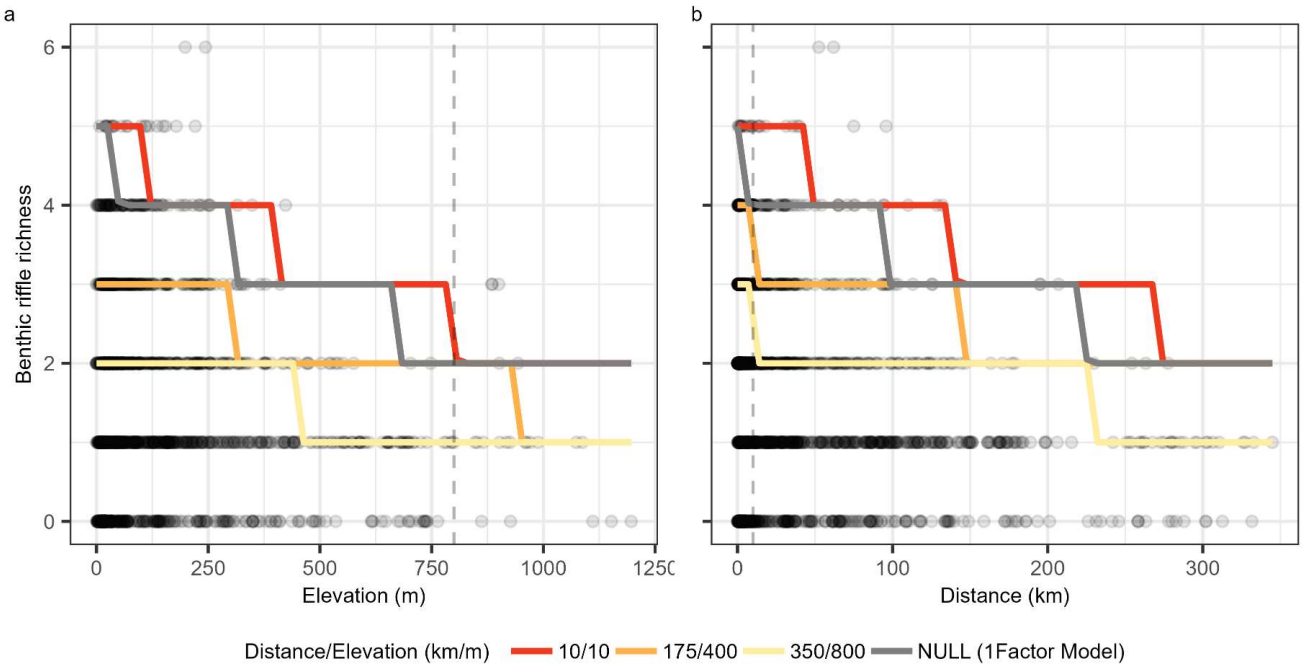
- Independent-effects models (grey-solid lines) overestimate maximum native species richness by more than double in some rivers.
- Consider, for example, the effect of D in rivers near the coast (e.g., D=10 km, grey dashed line, panel b), but high in elevation (e.g., E=800 m, yellow line, panel b)
- The joint-effects MSRL (yellow line) estimates the maximum number of native species at this river = four
- In contrast, the independent-effects MSRL (grey-solid line) wrongly estimates double the number of species should be present.

2. Developing a new eDNA-FIBI algorithm

- By ignoring the joint effects of distance and elevation on species richness, the MSRLs of the original FIBI likely overestimate the number of native species expected in some rivers.
- In other rivers, the original FIBI likely under-estimates the number of native species expected
- These biases likely lead to many rivers falling in FIBI bands that are unduly harsh or lenient.
- In subsequent slides, we present joint-effects and independent-effects MSRLs for the remaining sub-metrics of the FIBI (benthic-riffle, benthic-pool, pelagic-pool and intolerant species richness).
- We then computed a joint-effects eDNA-FIBI and independent-effects eDNA-FIBI, and from these computed the error in FIBI scores and the National Policy Statement for Freshwater Management (2020)[1] (NPSFM 2020) National Objective Framework (NOF) attribute bands introduced by ignoring the joint effects of distance and elevation.

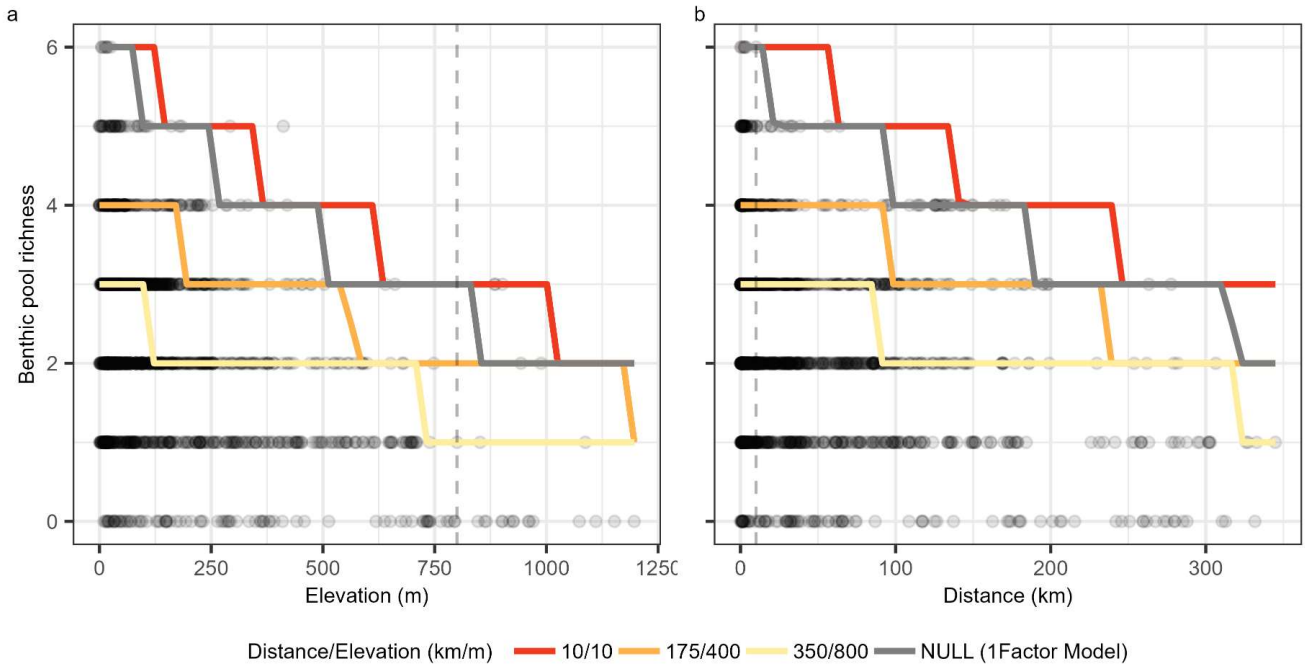
[1]Ministry for the Environment (2020). National Policy Statement for Freshwater Management 2020. Ministry for the Environment, Wellington, New Zealand. 70 p.

Benthic riffle species joint- and independent-effects MSRLs



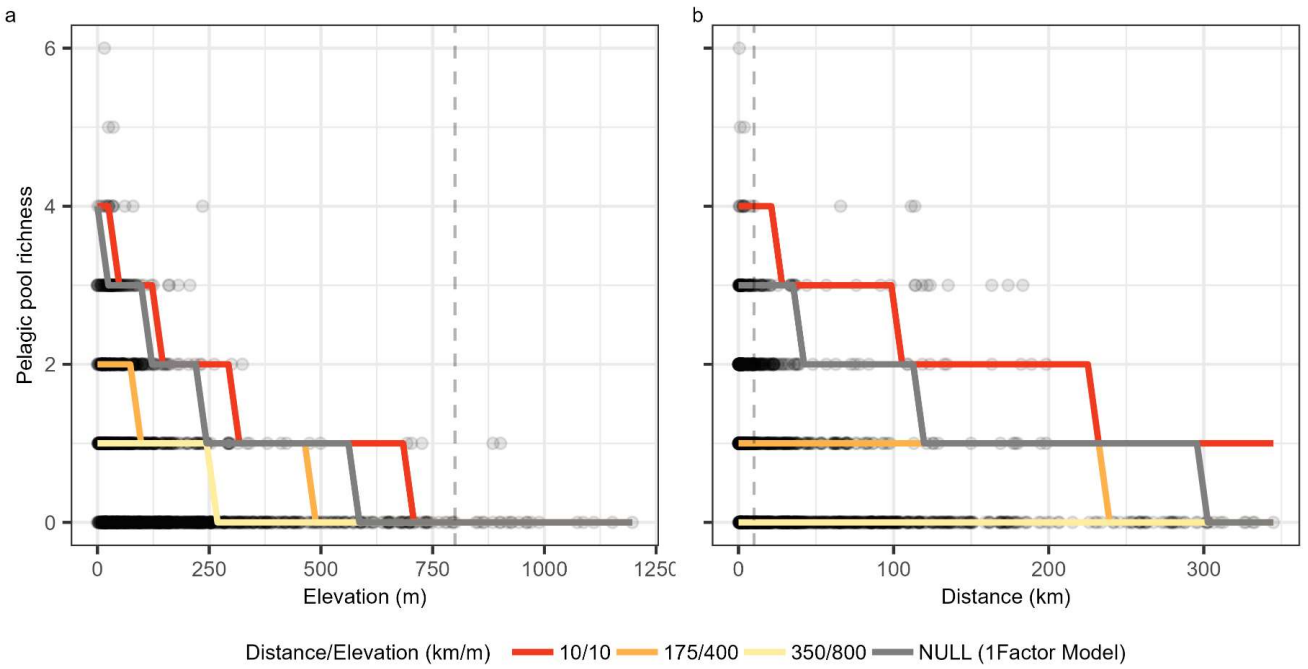
Maximum species richness lines (MSRLs) for benthic riffle species for (a) elevation and (b) distance.
Grey solid lines are MSRLs estimated independently for distance (D) and elevation (E) (from independent-effects GLMs). Coloured lines are MSRLs estimated from the joint effect of D and E (joint-effects GLM), with MSRLs shown for (a) E at fixed values of D (10, 175 and 350 km) and for (b) D at fixed values of E (10, 400 and 800 m). The MSRLs are estimated from 95th quantile of the posterior predictive distribution of the models. Points are the observed national 6-rep eDNA data used to fit the models. Grey dashed lines provide a reference for a hypothetical river with D=10 km and E= 800 m.

Benthic pool species joint- and independent-effects MSRLs



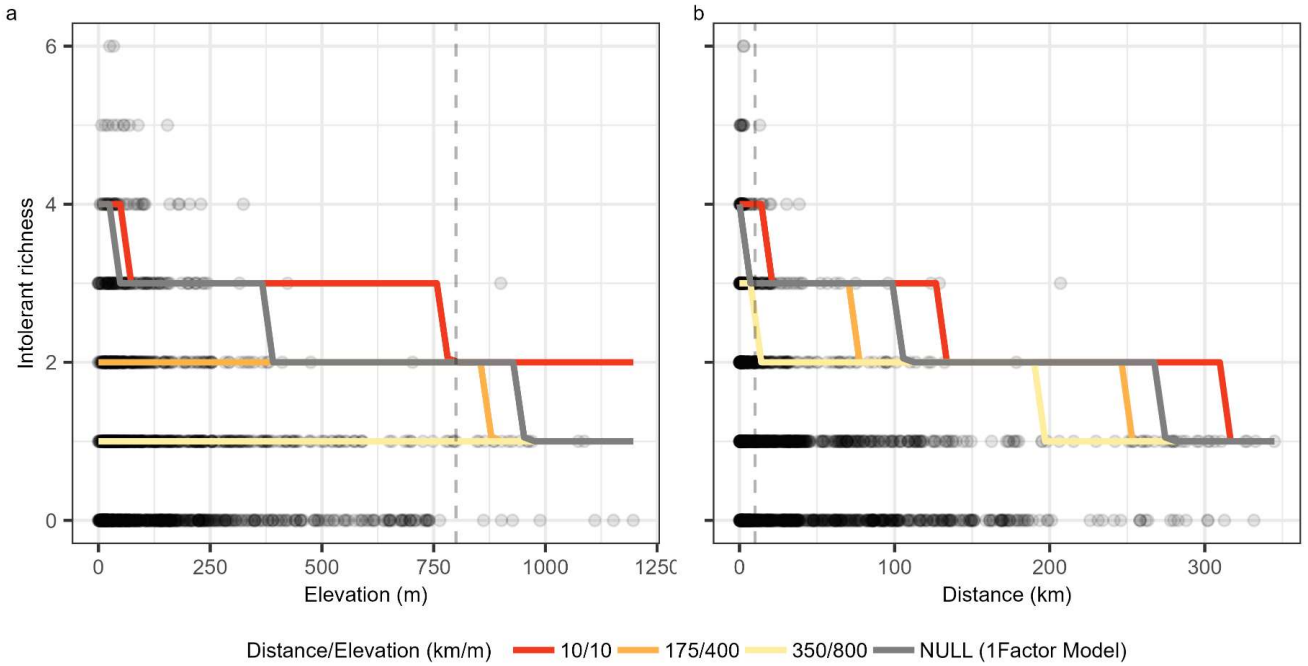
Maximum species richness lines (MSRLs) for benthic pool species for (a) elevation and (b) distance.
Grey solid lines are MSRLs estimated independently for distance (D) and elevation (E) (from independent-effects GLMs). Coloured lines are MSRLs estimated from the joint effect of D and E (joint-effects GLM), with MSRLs shown for (a) E at fixed values of D (10, 175 and 350 km) and for (b) D at fixed values of E (10, 400 and 800 m). The MSRLs are estimated from 95th quantile of the posterior predictive distribution of the models. Points are the observed national 6-rep eDNA data used to fit the models. Grey dashed lines provide a reference for a hypothetical river with D=10 km and E= 800 m.

Pelagic pool species joint- and independent-effects MSRLs



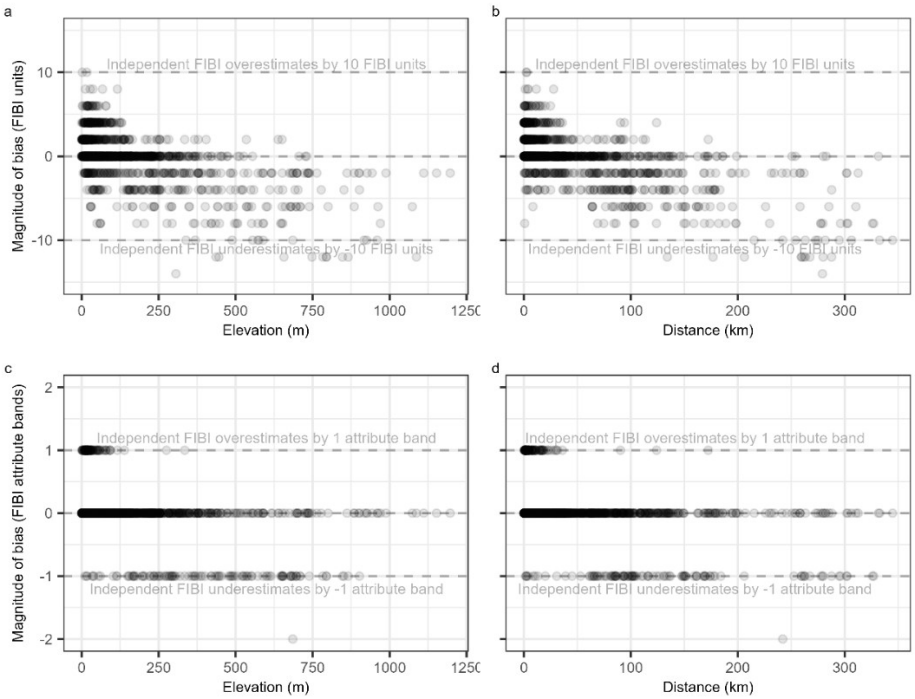
Maximum species richness lines (MSRLs) for pelagic pool species for (a) elevation and (b) distance.
Grey solid lines are MSRLs estimated independently for distance (D) and elevation (E) (from independent-effects GLMs). Coloured lines are MSRLs estimated from the joint effect of D and E (joint-effects GLM), with MSRLs shown for (a) E at fixed values of D (10, 175 and 350 km) and for (b) D at fixed values of E (10, 400 and 800 m). The MSRLs are estimated from 95th quantile of the posterior predictive distribution of the models. Points are the observed national 6-rep eDNA data used to fit the models. Grey dashed lines provide a reference for a hypothetical river with D=10 km and E= 800 m.

Intolerant species joint- and independent-effects MSRLs



Maximum species richness lines (MSRLs) for intolerant species for (a) elevation and (b) distance.
Grey solid lines are MSRLs estimated independently for distance (D) and elevation (E) (from independent-effects GLMs). Coloured lines are MSRLs estimated from the joint effect of D and E (joint-effects GLM), with MSRLs shown for (a) E at fixed values of D (10, 175 and 350 km) and for (b) D at fixed values of E (10, 400 and 800 m). The MSRLs are estimated from 95th quantile of the posterior predictive distribution of the models. Points are the observed national 6-rep eDNA data used to fit the models. Grey dashed lines provide a reference for a hypothetical river with D=10 km and E= 800 m.

Bias in FIBI introduced by ignoring joint effects of distance (D) and elevation (E)



- Compared to the joint-effects eDNA-FIBI, the independent-effects eDNA-FIBI tended to overestimate FIBI in streams with both low elevation and distance by almost 10 units (panels a, b).
- In contrast, as distance and elevation increase (panels a, b), the independent-effects eDNA-FIBI tended to underestimate FIBI by -10 units.
- These biases result in the independent-effects eDNA-FIBI falling in attribute bands that are too high in streams with low distance and elevation, and too low in streams with high distance and elevation (panels c, d).
- By extension, these biases are also expected to occur in the original FIBI that also assumes independent effects of distance and elevation.

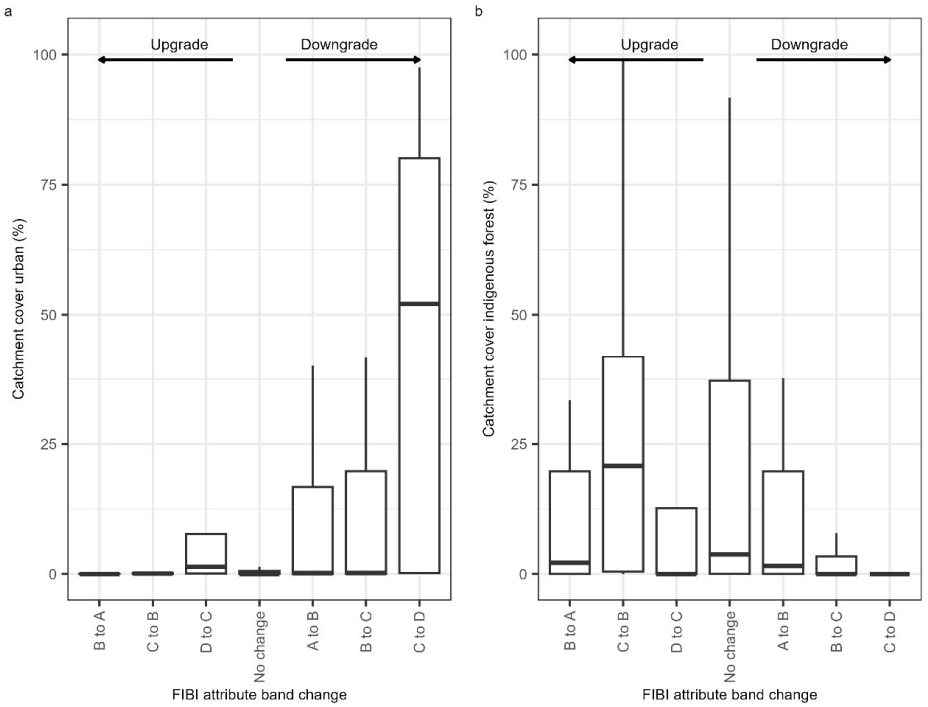
Magnitude of bias in the independent-effects eDNA-FIBI in both FIBI units as a function of a) elevation and b) distance and in attribute band units as a function of c) elevation and d) distance.
Magnitude of bias in panels a) and b) was calculated by subtracting the joint-effects eDNA-FIBI from the independent-effects eDNA-FIBI, for each sample in the national 6-rep eDNA dataset. Thus, points > 0 show where the independent-effects eDNA-FIBI scores were greater than the joint-effects eDNA-FIBI, hence are an overestimate of FIBI (vice versa for points < 0). Panels c) and d) show this same bias, but represented as the difference in FIBI attribute bands. Hence points = 1 imply the independent-effects eDNA-FIBI falls in attribute bands that are 1 band higher than the joint-effects eDNA-FIBI, hence are an overestimate (vice versa for points = -1).

Bias in FIBI introduced by ignoring joint effects of distance (D) and elevation (E)

- The independent-effects eDNA-FIBI results in FIBI attribute bands that are unduly-lenient in lowland/coastal streams, but unduly-harsh in high elevation/inland streams.
- The effects of landuse change typically propagate downstream towards the coast. So we may expect the joint-effects eDNA-FIBI will result in harsher FIBI attribute bands in streams more affected by landuse, and more lenient bands in streams less affected by landuse.
- To test this hypothesis, we computed the change in FIBI attribute bands resulting from switching from the independent-effects eDNA-FIBI, to the joint-effects eDNA-FIBI, for each sample in the national 6-rep eDNA data set.
- We then explored how these attribute band changes were distributed among catchments affected by varying amounts of urban and indigenous forest cover.
- Upstream urban and indigenous forest cover was computed for all river segments sampled in the national 6-rep eDNA data set using the Landcover Database V5[1].

[1]Fraser, C & Snelder, T. 2021. Update to REC Land Cover categories and review of category membership rules. LWP Client Report, 2021-18.

NOF attribute band changes based on the joint-effects FIBI



Distribution of a) urban land cover and b) indigenous forest cover among river reaches whose FIBI attribute bands changed after switching to the joint-effects eDNA FIBI from the independent-effects FIBI. X-axis labels describe the change in FIBI attribute bands resulting from switching from the independent-effects FIBI to the joint-effects FIBI. For example, "C to D" implies the FIBI attribute band changed from C, when scored using the independent-effects eDNA FIBI, to D, when scored using the joint-effects eDNA FIBI, which is a downgrade. Such downgrades occurred more frequently in catchments with high urban cover a), while upgrades were more frequent in catchments with high indigenous forest cover b).

- The joint-effects eDNA-FIBI results in more attribute band downgrades in urban catchments, but more attribute band upgrades in forested catchments.
- Consequently, the joint-effects eDNA-FIBI could be more effective at directing remediation resources to where it is most needed.

Conclusions and recommendations for using eDNA data to estimate FIBI

1. **We can't simply input eDNA richness data into the current FIBI algorithm**

- In species-rich communities, eDNA detects more species than fishing leading to higher FIBI scores using the current algorithm. Compared to fishing data, the higher estimates of eDNA-based FIBI scores fall into higher NPSFM (2020) NOF attribute bands.
- If we ignore these problems and used eDNA and fishing data interchangeably with the current FIBI algorithm, we risk confounding spatial and temporal patterns in FIBI with choice of sampling method.

2. A joint-effects FIBI should be developed and used for both eDNA and fishing data

- The current FIBI, which assumes independent effects of distance and elevation on species richness, results in attribute bands that are too lenient in streams more affected by land-use, and too harsh for streams less affected by land-use.
- The joint-effects eDNA-FIBI, developed here, corrects this issue for eDNA data, and better directs remediation resources to where they are likely needed most.
- However, the new eDNA-FIBI is not compatible with the original FIBI developed for fishing data.
- If we wish to use eDNA and fishing data interchangeably for estimating fish community states, we must also develop a new joint-effects FIBI for fishing data as well.

- Continued on next slide...

Recommendations for using eDNA data to estimate FIBI

3. Further development of the joint-effects eDNA-FIBI is required

- Because the joint-effects eDNA-FIBI estimates maximum species richness from the joint effects of distance and elevation, it is no longer necessary to estimate sub-metrics for distance and elevation separately - as undertaken with the current FIBI. Removing this step would result in an index with approximately half the range of the current FIBI, without loss of information.
- Reconsider which sub-metrics are necessary. The maximum number of species expected for some sub-metrics is so low, that it becomes very difficult for models to distinguish rivers into different bands of impairment. For example, the estimated maximum number of pelagic-pool species is 0 for many rivers, making it impossible to detect impairment in pelagic-pool species richness for those rivers. Such sub-metrics may offer little value in distinguishing sites by impairment.
- The rationale for species membership in some sub-metrics is also unclear. For example, why might some species be considered for membership in the “Intolerant” sub-metric and not others? How is “intolerant” defined and is this ecologically relevant? Inaccurate allocation of species to different sub-metrics could confound the FIBI, and make the index harder to interpret.
- Selection of appropriate sub-metrics, and membership of species to those sub-metrics, should be based on a clear understanding of what the index is designed to indicate.

- Continued on next slide...

Recommendations for using eDNA data to estimate FIBI

4. Redefine attribute bands for the new joint-effects eDNA FIBI

- Following previous recommendations will result in the new joint-effects eDNA-FIBI having a different range to the current FIBI. Consequently, previously used NPSFM (2020) NOF attribute bands will be incompatible with the new index and will need to be redefined.

5. Documentation

- The methods used to derive models underlying the new joint-effects eDNA-FIBI, and calculation of the resulting index, needs to be transparently documented so that the index can be recalculated by others. This may involve development of software that can be used to calculate the index with new data sets.
- Model evaluation/performance needs to be comprehensively documented

6. Continue collecting eDNA data using established protocols

- As we have demonstrated here, development of an eDNA-FIBI is eminently achievable using available data.
- Provided eDNA data are collected using established 6-rep protocols, these data can be used to back-calculate any finalised eDNA FIBI that is developed. Thus, the pending development of a new eDNA-FIBI should not prevent eDNA being used to monitor fish communities using existing protocols.

Incorporating eDNA into macroinvertebrate metrics

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Using eDNA for macroinvertebrate metrics

1. Are NPSFM (2020) NOF attributes the Macroinvertebrate Community Index (MCI), the Quantitative Macroinvertebrate Community Index (QMCI), and the Macroinvertebrate Average Score Per Metric (ASPM), when calculated using eDNA data equivalent to metrics calculated from physical samples?
    - If eDNA detects more or less species than physical sampling, particularly species with high or low tolerance values, we would expect macroinvertebrate metric scores calculated from eDNA data to differ from scores calculated from physical samples.

    - Does the taxonomic resolution of eDNA metrics affect the correlation?
        - MCI tolerance values are assigned at genus level or higher, whereas eDNA provides species-level identification.
        - *Note: species-level tolerance values for eMCI are currently under investigation as part of an Envirolink project led by University of Waikato.*

    - Does the physical sampling collection method affect the correlation?
        - Some councils use habitat-proportional sampling as recommended by the National Environmental Monitoring Standard (NEMS), others are still using Stark C3/C4 protocols (run/riffle habitats in hard-bottomed streams, macrophyte beds in soft-bottomed streams).

Using eDNA for macroinvertebrate metrics

- Analyses used 174 spatially and temporally paired eDNA-physical samples
    - Bay of Plenty – 92 pairs (60 sites over 2 years)
        - Physical samples collected from five locations, in proportion to the distribution of habitat types within the reach (NEMS protocol).
    - Hawke's Bay – 82 pairs (68 sites over 2 years)
        - Physical samples collected from run/riffle habitats in hard-bottomed streams and macrophytes or woody debris in soft-bottomed streams.

eDNA processing

eDNA data was collected using the 6-rep method, with data from all six replicates combined into a single 'composite' sample. Processed of data was according to Melchior and Baker (2023), and filtered as follows:

1. Groups represented by the MCI: insects, worms, molluscs, crustaceans, springtails, cnidarians, mites, ribbon worms, roundworms, flatworms, spiders.

2. Remove non-aquatic/freshwater taxa*
   - Coarser taxonomic resolutions (e.g., Order or Class) used for the MCI can encompass both freshwater and non-freshwater taxa (e.g., Oligochaeta). Aggregating eDNA data at these levels without filtering can lead to artificially elevated estimates of species richness and 'abundance' (read counts) relative to physical sampling, which predominantly captures aquatic taxa.

3. Remove any taxa outside their well-known distribution*
   - Incomplete or outdated libraries can result in incorrect IDs; for example, we removed an *Aucklandobius* stonefly detection from the Hawke's Bay dataset, as *Aucklandobius* are endemic to the sub-Antarctic islands.

*Require judgement calls that could introduce bias/variation between analyses

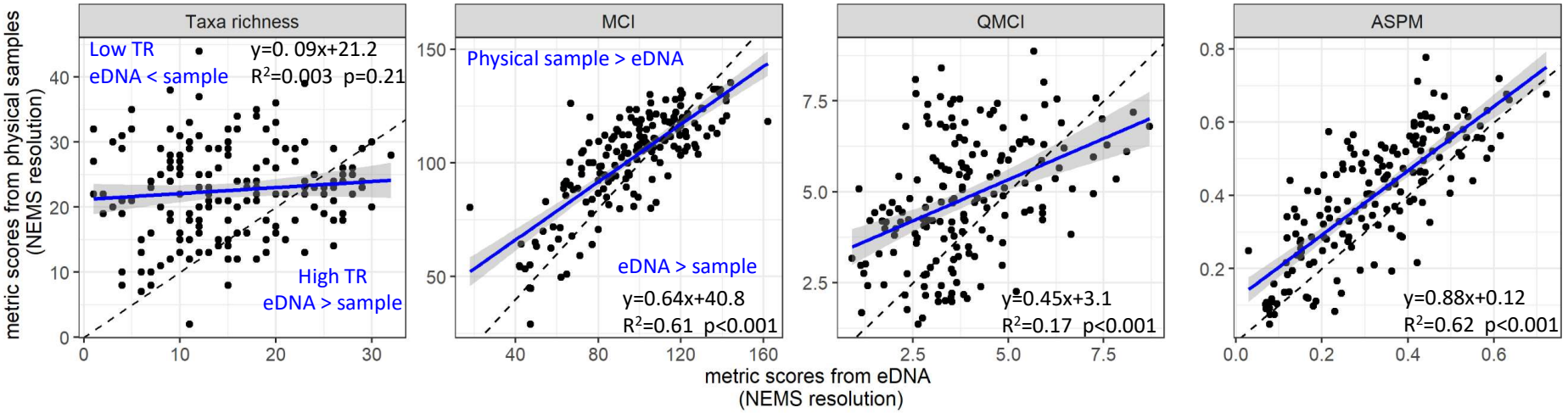
Metric calculation

1) Using NEMS-level resolution
    - which was also used for metric calculation from physical samples
    - genus for most insects, higher level (family, order, class) for other taxa
    - e.g. multiple species of Oligochaetes grouped – 1 score of 1

2) Using genus-level resolution
    - eDNA data identified to species, aggregated to genus because MCI tolerance values assigned at genus level
    - e.g. 3 genus of Oligochaetes – each score 1
    - taxa currently aggregated at higher taxa resolution tend to be more tolerant/lower scoring[1], therefore we would expect genus-level resolution to result in lower MCI scores from eDNA data

[1]Suren, A., Burdon, F.J., Wilkinson, S.P. 2024. eDNA Is a Useful Environmental Monitoring Tool for Assessing Stream Ecological Health. Environmental DNA, 2024; 6:e596.

Are metrics calculated using eDNA equivalent to metrics calculated from physical samples?



The relationships between macroinvertebrate metrics calculated using eDNA data versus physical sampling. The dashed 1:1 line indicates where the metrics are equivalent. The blue line is the fitted linear regression between the two metric scores with shaded 95% confidence interval. Locations where the blue line is above or below the 1:1 line indicate where eDNA metric scores were lower or greater, respectively, than metric scores calculated using physical sampling data.

No correlation between taxa richness from eDNA and physical sampling

Physical sampling detected more species (points above dashed 1:1 line), particularly when taxa richness was low

MCI scores from eDNA samples and physical samples are strongly correlated

Points generally above 1:1 line

Physical samples had higher MCI scores except when scores were very high (>130 indicative of pristine freshwater environments)

QMCI scores from eDNA and physical samples are weakly correlated

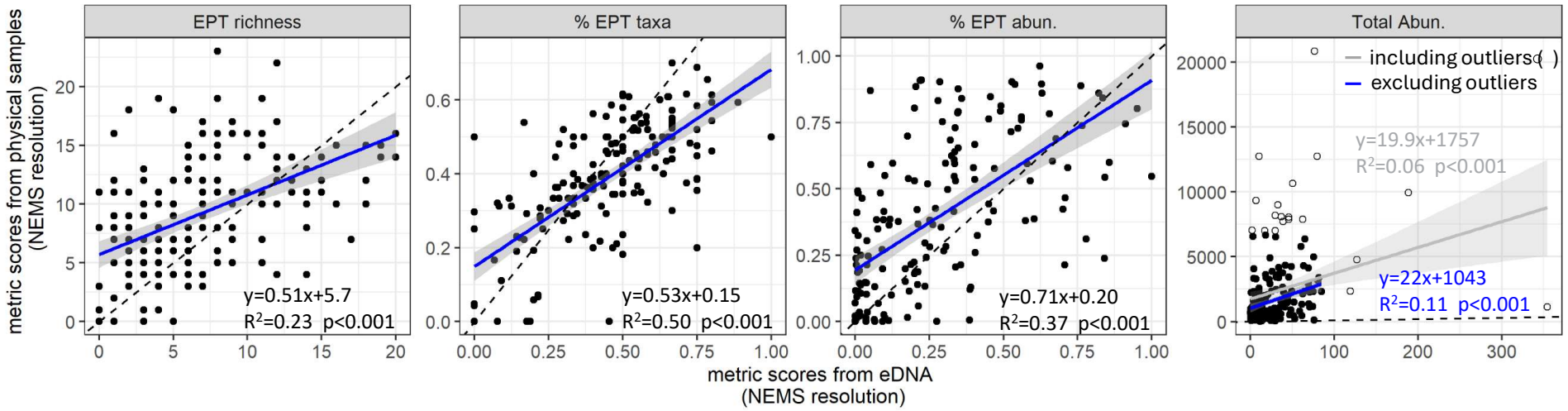
More points above 1:1 line

Note: this uses read counts as a proxy for abundance; read counts will also vary with environmental conditions, DNA shedding rates between organisms, etc

ASPM scores from eDNA samples and physical samples are strongly correlated

Majority of points above 1:1 line

Are metrics calculated using eDNA equivalent to metrics calculated from physical samples?



The relationships between macroinvertebrate metrics calculated using eDNA data versus physical sampling. The dashed 1:1 line indicates where the metrics are equivalent. The blue line is the fitted linear regression between the two metric scores with shaded 95% confidence interval. Locations where the blue line is above or below the 1:1 line indicate where eDNA metric scores were lower or greater, respectively, than metric scores calculated using physical sampling data.

Weak correlation between EPT taxa richness from eDNA and physical sampling

Physical sampling detected more species (points above dashed 1:1 line), particularly when taxa richness was low

The percentage of EPT taxa detected from eDNA samples and physical samples was strongly correlated

Points generally below 1:1 line

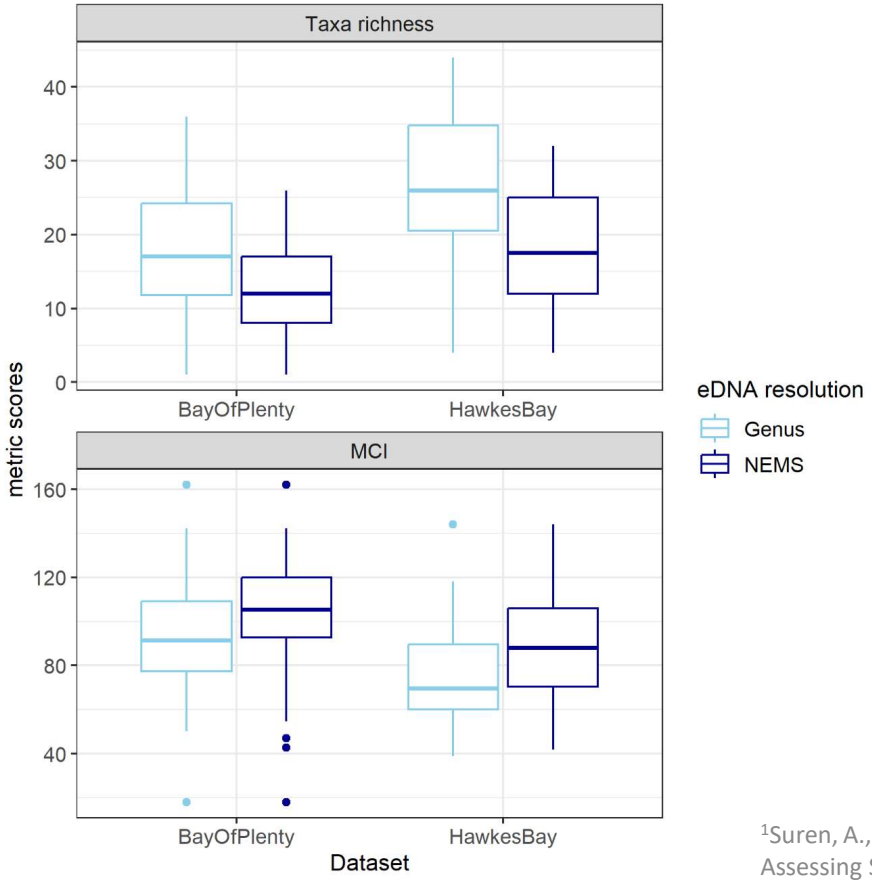
eDNA detected more EPT taxa when the percentage of EPT was higher

The percentage of total EPT abundance from eDNA read counts was weakly correlated with the percentage in physical samples

More points above 1:1 line

Total macroinvertebrate read counts from eDNA was not correlated with total abundance of organisms in physical samples

Does taxonomic resolution influence metric scores?



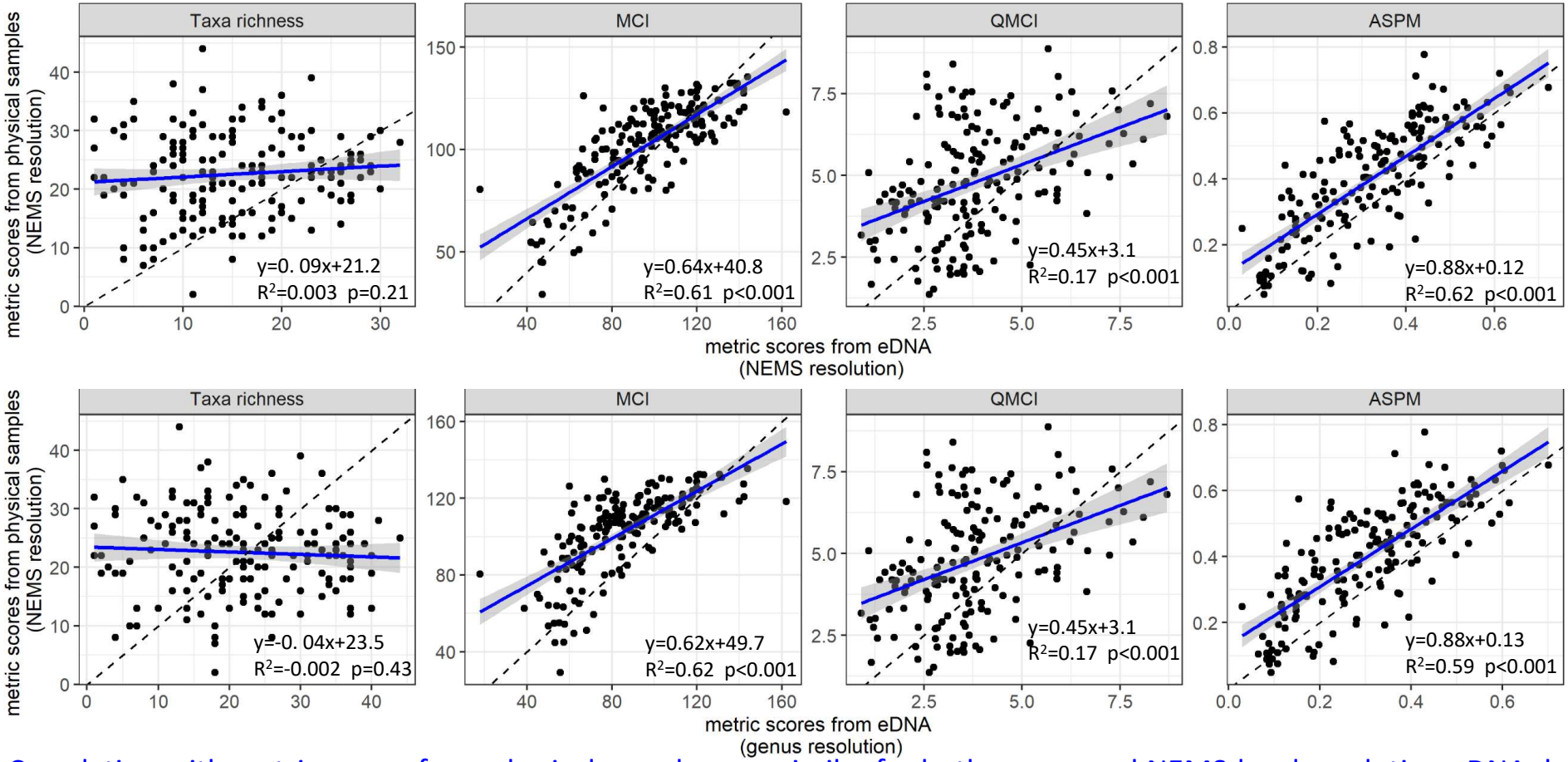
Results were similar to those found by Suren et al. (2024)[1]:

- Greater taxa richness using genus-level eDNA
- MCI scores calculated using genus level eDNA data were lower than metrics at genus resolution due to high diversity of taxa typically aggregated into a higher level groups with low MCI tolerance values (e.g. Oligochaetes, Hydra, Ostracods, Nematoda, Platyhelminthes).

Note: Suren et al. also used Bay of Plenty SoE data, but didn't have temporally paired physical samples and eDNA samples.

[1]Suren, A., Burdon, F.J., Wilkinson, S.P. 2024. eDNA Is a Useful Environmental Monitoring Tool for Assessing Stream Ecological Health. Environmental DNA, 2024; 6:e596.

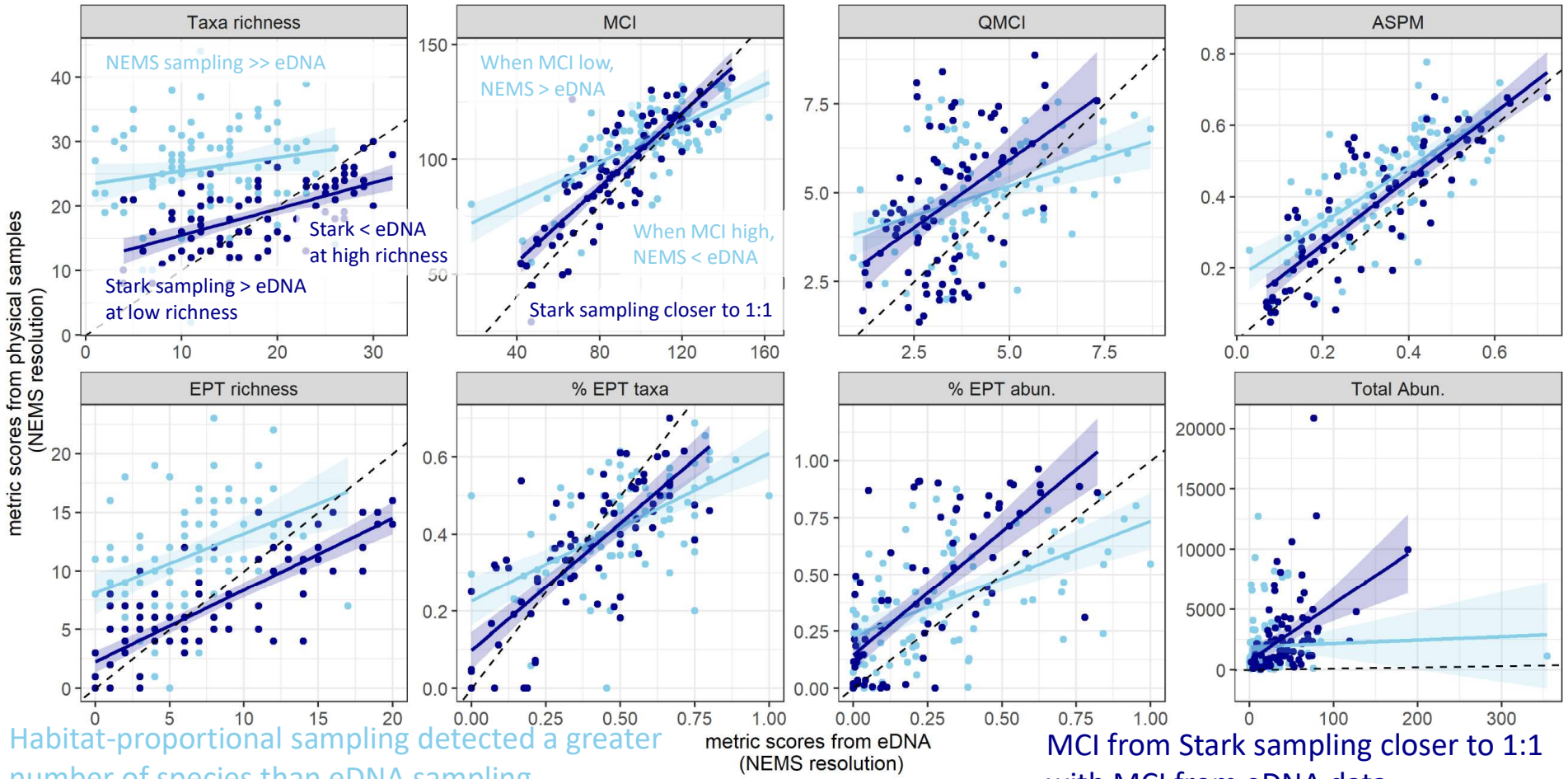
Does taxonomic resolution influence correlations?



Correlation with metric scores from physical samples was similar for both genus- and NEMS-level resolution eDNA data

Does physical sampling method matter?

- Bay of Plenty – habitat proportional (NEMS)
- Hawke's Bay – Stark C3/C4 (riffles – hard-bottomed, macrophyte beds – soft-bottomed)



Habitat-proportional sampling detected a greater number of species than eDNA sampling

MCI from Stark sampling closer to 1:1 with MCI from eDNA data

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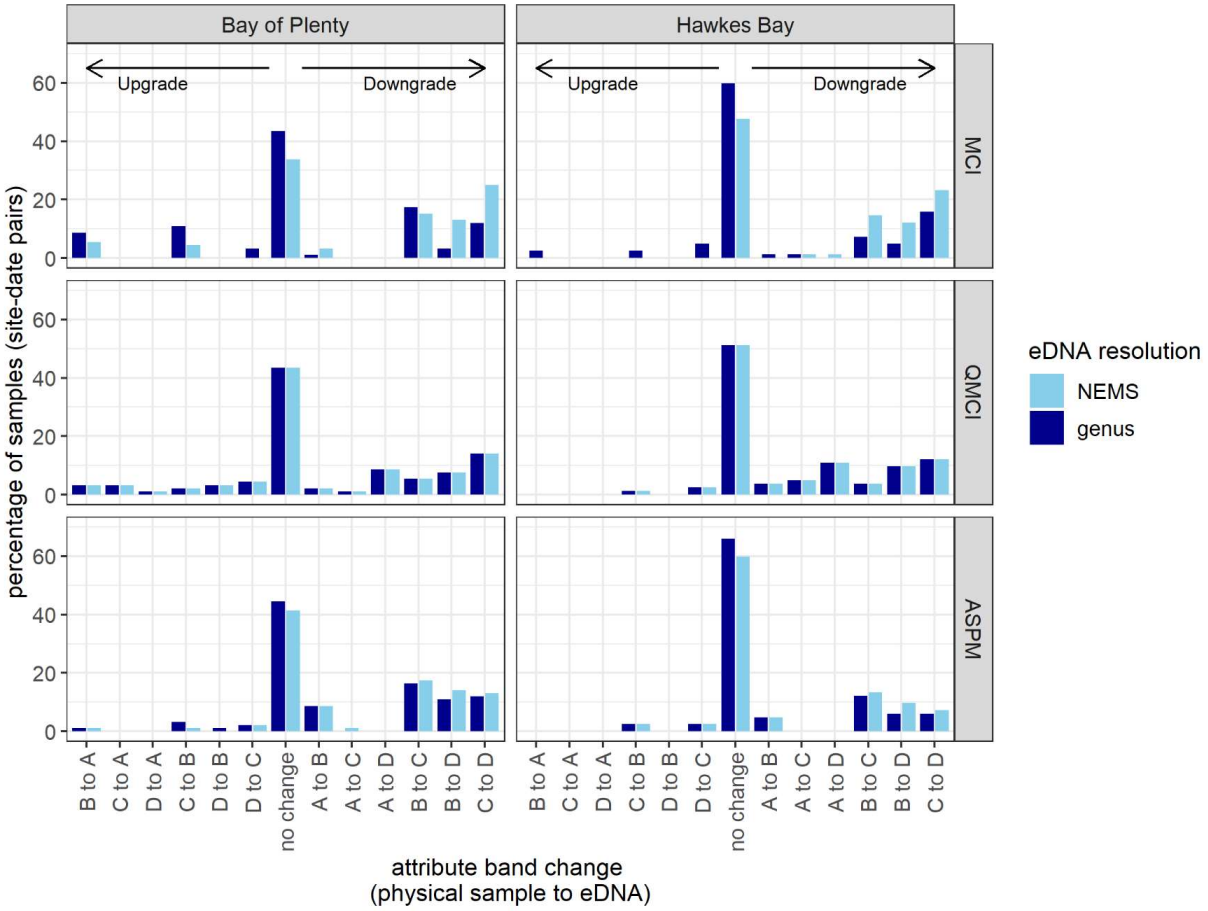
What are the implications for the NOF attribute bands?



- Compared to physical sampling, metrics calculated using eDNA resulted in different NOF attribute bands* in 40-60% of samples for all metrics.

  *single point rather than 5-year median

- Overall more sites fell in lower attribute bands using eDNA-based metrics compared to physical sample-based metrics.

Conclusions

1. The eDNA-based metrics and physical sampling metrics – MCI, QMCI, ASPM– are correlated but not equivalent (1:1). There was no correlation between taxa richness from eDNA and physical sampling.

2. Linear regression indicates it may be possible to derive a conversion factor between eDNA-based metrics and physical sampling metrics. However, regional- or physical sampling method-based conversions may be required to maintain continuity in macroinvertebrate time series if councils were to switch to only eDNA sampling, because: Correlation varied between physical sampling methods
    - The NEMS habitat-proportional sampling method detects more species than eDNA
        - A comparison of paired physical samples using NEMS vs. Stark methods also found greater taxa richness from NEMS sampling, but no consistent difference in MCI scores (Greenwood et al. 2023[1])
    - Metrics for physical samples collected using the Stark method are closer to 1:1 with metrics calculated from eDNA, particularly MCI
- Using genus-level or NEMS-level resolution for eDNA metrics did not impact the correlation with physical sample metrics. However, genus-level metric scores were generally lower than NEMS resolution metric scores
    - Likely due to greater numbers of low-scoring taxa at finer resolution

3. Switching from physical sampling to eDNA may result in a large proportion of sites (~50%) falling in a different NOF attribute bands, with a greater number of sites falling in lower attribute bands.

[1]Greenwood, M.J. et al. 2023. Implications for regional council SOE monitoring from adopting the NEMS macroinvertebrates protocols: analysis of the macroinvertebrate metric scores from paired sampling. Prepared for Northland Regional Council. Envirolink Report. NIWA Client Report 2023309CH. 68 p.

Recommendations to improve the use of eDNA for NOF attributes

1. Improved libraries
   - Currently some detections must be excluded due to insufficient taxonomic resolution (e.g. “Hydrobiosidae”) for calculating MCI.
   - Note: Varying levels of resolution is also a larger issue with MCI – example of Chironomids that if identified to genus score much higher than if only IDed to “Chironomidae” (e.g. *Parochlus*).

2. National standardisation (i.e., NEMS for eDNA) to eliminate the need for judgement calls which could introduce bias/noise
   - Create national and regional taxa lists
     - Freshwater vs terrestrial
       e.g. Diptera, Oligochaetes, at different levels of taxonomic resolution
     - Regional sense-checks – is this taxa likely found there based on known distributions
   - Data processing
     - Treatment of six replicates – as one composite sample (approach used here) vs. independent samples (i.e. MCI calculated for each replicate and averaged)

3. Stressor-based macroinvertebrate indices with eDNA-enabled taxonomic resolution
   - MCI known to respond similarly to multiple stressors (organic enrichment, sediment, upland-lowland gradient)

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