Supplementary Information

Watered-down biodiversity? A comparison of metabarcoding results from DNA extracted from matched water and bulk tissue biomonitoring samples

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Table S1. Summary of reads and ESVs in all taxa

	AD		BE		
	Benthos	**Water**	**Benthos**	**Water**	**Total**
Raw	N/A		N/A		48,799,721 x 2
Paired	N/A		N/A		42,317,963
Primer Trimmed	4,377,830	345,014	4,001,465	1,561,360	8,724,309
ESVs	2,581	1,099	4,831	9,571	16,841
Reads in ESVs	2,614,558	238,697	1,774,386	780,079	5,407,720
Proportion of raw reads in ESVs (%)	5.4	0.5	3.6	1.6	11.1

Table S2. Summary of reads and ESVs assigned to the Arthropoda

	AD		BE		
	Benthos	**Water**	**Benthos**	**Water**	**Total**
ESVs	1,735	280	2,398	491	4,459
Reads in ESVs	2,541,062	174,605	1,554,853	129,429	4,399,949
Proportion of raw reads in ESVs (%)	5.2	0.4	3.2	0.3	9.0
Proportion of all ESVs that are Arthropoda	67.2	25.5	49.6	5.1	26.5
Proportion of all reads in ESVs that are Arthropoda	97.2	73.1	87.6	16.6	81.4

Table S3. EPTO ESVs can be used to separate rivers using either benthos or water samples. Sample replicates were pooled. No significant beta dispersion was detected within groups (collection method, river). No significant interaction between groups was detected (collection method, river). Summary of PERMANOVA results based on a Sorensen dissimilarity matrix of EPTO ESVs. Significant p-values are in bold. Based on normalized data.

Source of variation	Df	MS	F	R^2	P
A) Interaction between groups					
Collection method	1	0.80	2.09	0.13	**0.002**
River	1	0.66	1.73	0.11	**0.021**
Collection method : River	1	0.44	1.16	0.07	0.282
Residuals	11	0.38		0.69	
Total	14			1.00	
B) Variation due to collection method					
Collection method	1	0.80	1.95	0.13	**0.011**
Residuals	13	0.41		0.87	
Total	14			1.00	
C) Within each collection method, variation due to river					
River, Stratum = Collection Method	1	0.65	1.56	0.11	**0.031**
Residuals	13	0.41			
Total	14				

Df = Degrees of freedom; MS = MeanSqs; F = F.Model; P = P-value

Figure S1. Rarefaction curves are saturated. Benthos samples from each site are shown in green and water samples are shown in blue. The vertical line shows the number of reads that would be included after normalizing library size down to the 15th percentile (reads = 2,099).

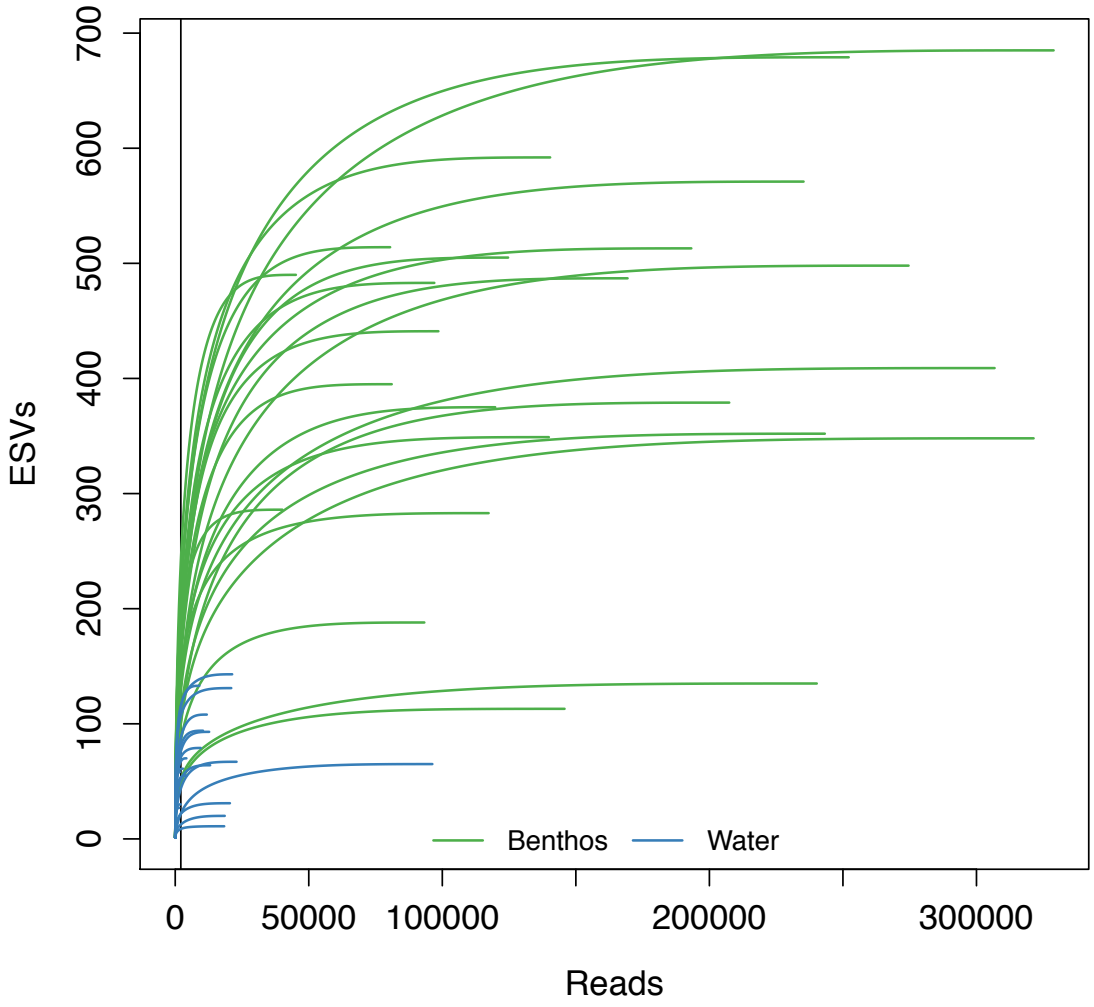

Figure S2. The recovery of reads and ESVs from benthos is much greater than that from water. Results summarized before normalization.

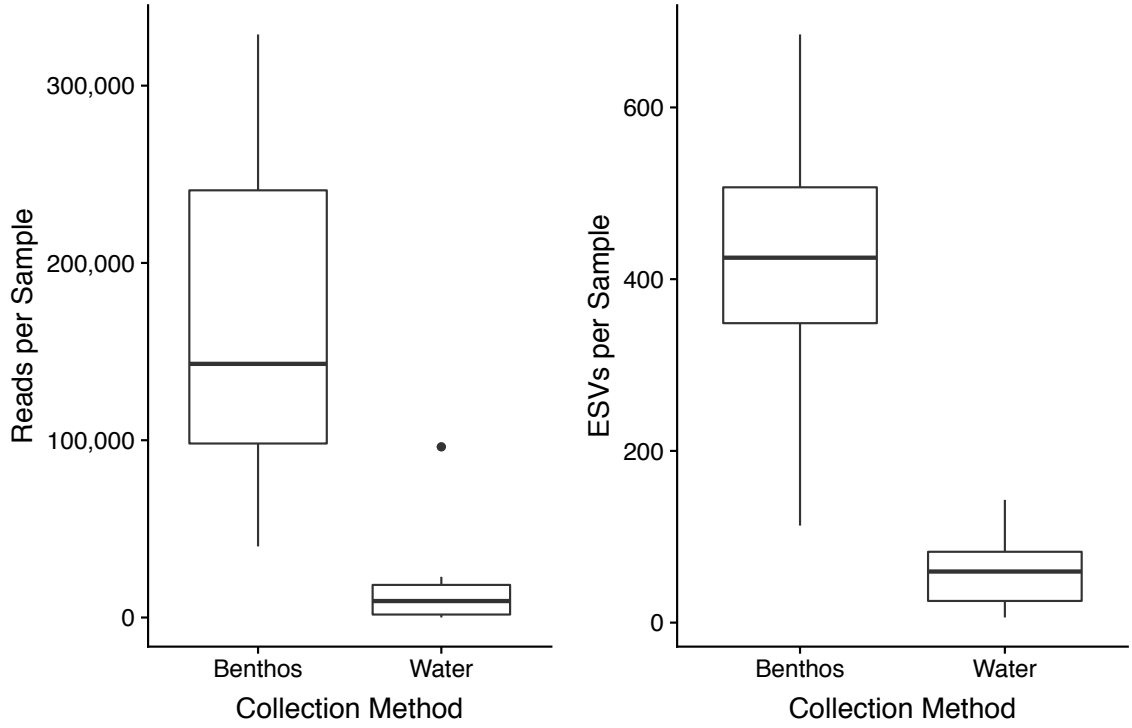

Figure S3. Only some Arthropod ESVs could be assigned with high confidence. Results summarized before normalization.

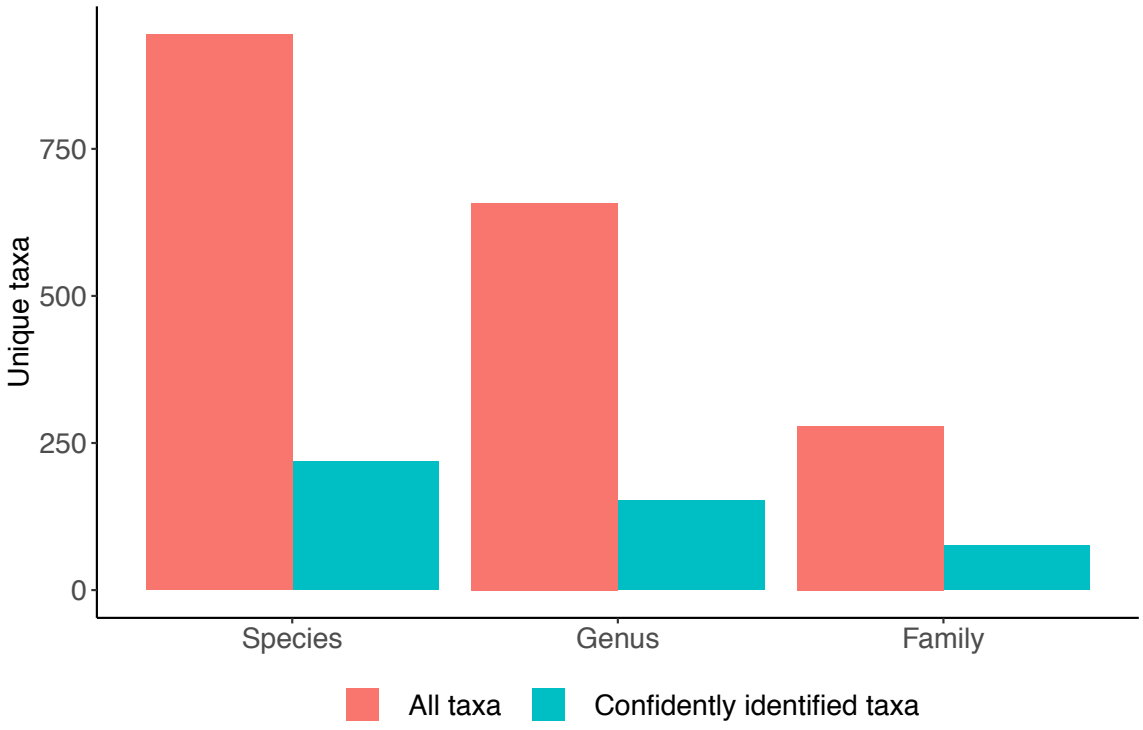

Figure S4. River sites are separated whether Arthropod ESVs are detected from benthos or water samples. NMDS ordination distances well-represent observed Sorensen dissimilarities (Benthos stress = 0.08, R^2 = 0.95; Water stress = 0.09, R^2 = 0.95). PERMANOVA shows that river groupings are significant and explain 14-19% of the variation in beta diversity (Benthos R^2 = 0.19, p-value = 0.001; Water R^2 = 0.14, p-value = 0.001). Results based on normalized data.