Supplementary information for

'Fishing in the water: effect of sampled water volume on environmental DNA-based detection of macroinvertebrates'

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Appendix I: PCR conditions

PCR mixture

Our PCR mixtures contained 1 X Buffer (Roche, Switzerland), 0.18 mM dNTP, 1 X BSA, 0.05 U/µL Taq (Roche, Switzerland), 2.0 mM $MgCl_2$ and 0.5 µM of each primer (see table below) and 2 µL of eDNA in a total reaction volume of 15 µL.

Primer

Polymerase chain reaction (PCR) primer pair sequences and annealing temperatures. Amplicon size includes primer pair lengths.

Species	Primer name	Primer sequence 5´–3´	Annealing temperature (°C)
Ancylus fluviatilis	Anf-F2	AATAGTTGAAGGAGGAGCAG	56
	Anf-R2	CAAATAAGGAAAGACGTTCC	
Baetis buceratus	Bab-F	GACTGCTACCCCCTTCATTG	51
	Bab-R	GCATGCGATCCAATGTTATC	
Gammarus pulex	Gap-F	TTTTGACTTTTACCTCCTTCYC	48.5
	Gap-R	GATATACCAGGTCTACGTATATTG	

PCR temperature regime

50 cycles

95° C	4 min
95° C	30 sec
Annealing temperature dependent on used primer, see table above	30 sec
72° C	1 min
72° C	5 min
10° C	forever

Figure S1: eDNA concentrations relative to used sampled volumes

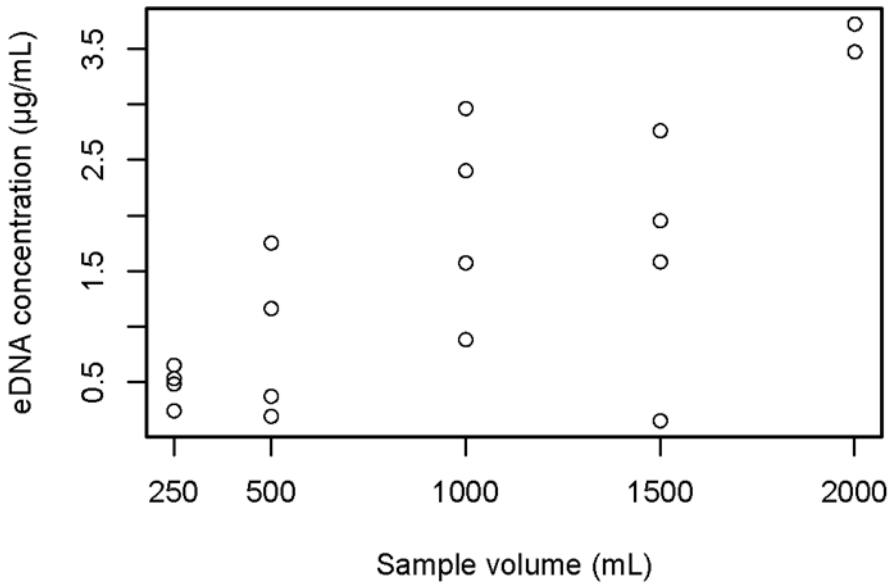

Fig. S1: eDNA concentration relative to the used sample volume. Two data points are missing for 2000 mL as we run out of sample to measure DNA concentration.