EFFECT OF DNA EXTRACTION METHODS ON METABARCODING SUCCESS OF HOMOGENIZED FRESHWATER MACROINVERTEBRATE COMMUNITY SAMPLES

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Introduction
Metabarcoding based on homogenized bulk samples holds great potential for larger-than-ever scale monitoring of freshwaters. Several studies show that DNA extraction methods developed and optimized for the specific samples give the best results. Rather than being an exhaustive comparison, this study exemplifies that the choice of DNA extraction method may have a profound effect on the biodiversity analysis.

Conclusions
Based on our results, choosing the DNA extraction method for lotic environments is not as critical as it is for lentic environments - especially if the deep lentic samples contain only few target specimens in excess non-target material. The lotic monitoring sampling is based on kick-samples, and those samples contain mainly the target specimens while the lentic sampling is performed using grabs and the deep lake bottom samples contain mainly sediments. In our case, the PowerPlant kit with beads and inhibitor removal steps performed most consistently. However, none of the compared DNA extraction methods were able to recover the same community composition in the lake 15m subsamples (approximately 20 target specimens), indicating serious issues for repeatability of such samples.

Methods

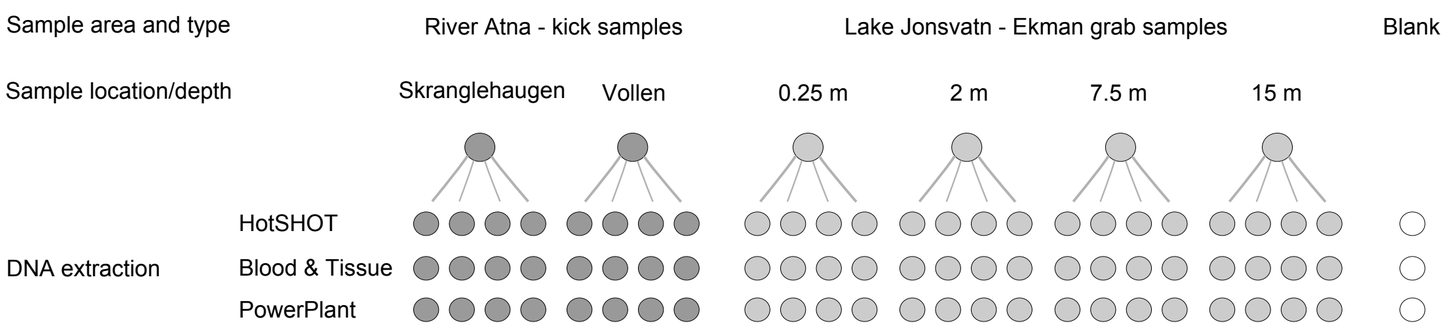

Fig. 1 Experimental set-up. Benthic macroinvertebrate samples were collected from two locations in River Atna and from a depth gradient in Lake Jonsvatn in Central Norway. Samples were homogenized using a blender. 16 subsamples were obtained from each samples and distributed to three groups subjected to DNA extraction, using (1) the HotSHOT extraction where the subsamples were lysed in an alkaline NaOH and neutralized with an acid Tris buffer, (2) the Qiagen DNeasy Blood and Tissue kit according to manufactuer's instructions, and (3) the MO BIO PowerPlant Pro DNA Isolation kit according to manufacturer's instructions. Three fragments of the mitochondrial COI gene were amplified and paired-end sequenced using Illumina MiSeq platform. The methods were compared, using DNA yield and OTUs matching with an invertebrate species in BOLD v.4 Species Level Barcode Records.

Results

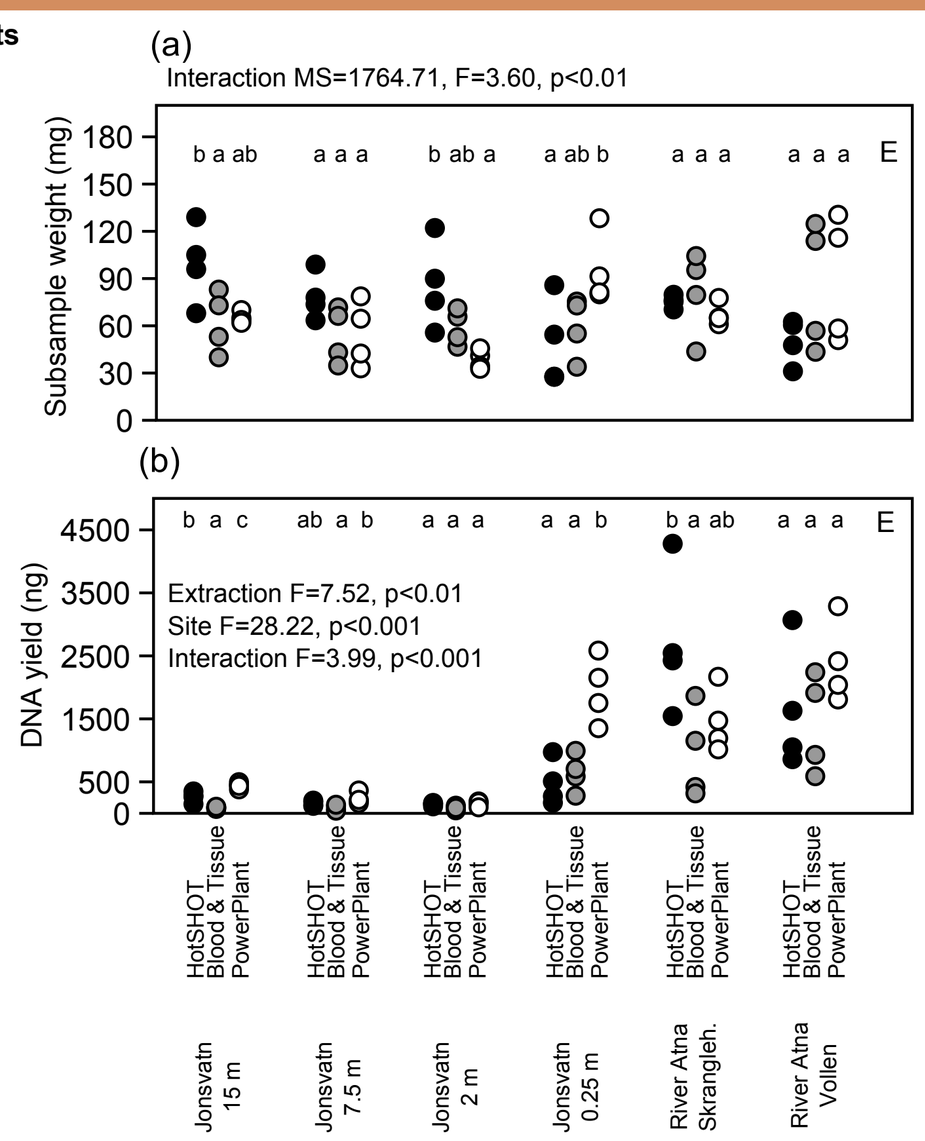

Fig. 2 Subsample size (a) and DNA yield in the subsamples (b). The weights of subsamples were similar overall despite the cross-over interaction (two-way ANOVA). The DNA yield correlated weakly with the subsample weights (Spearman's rho 0.31, p<0.01) due to strong correlation of PowerPlant kit subsample weight and DNA yield (rho 0.77, p<0.001). The highest DNA yield was achieved with the PowerPlant kit extraction (two-way ANOVA followed by Tukey's HSD). The F-statistic value (F) and significance (p) are given, and similar extraction methods (E) at a site are marked with a same letter.

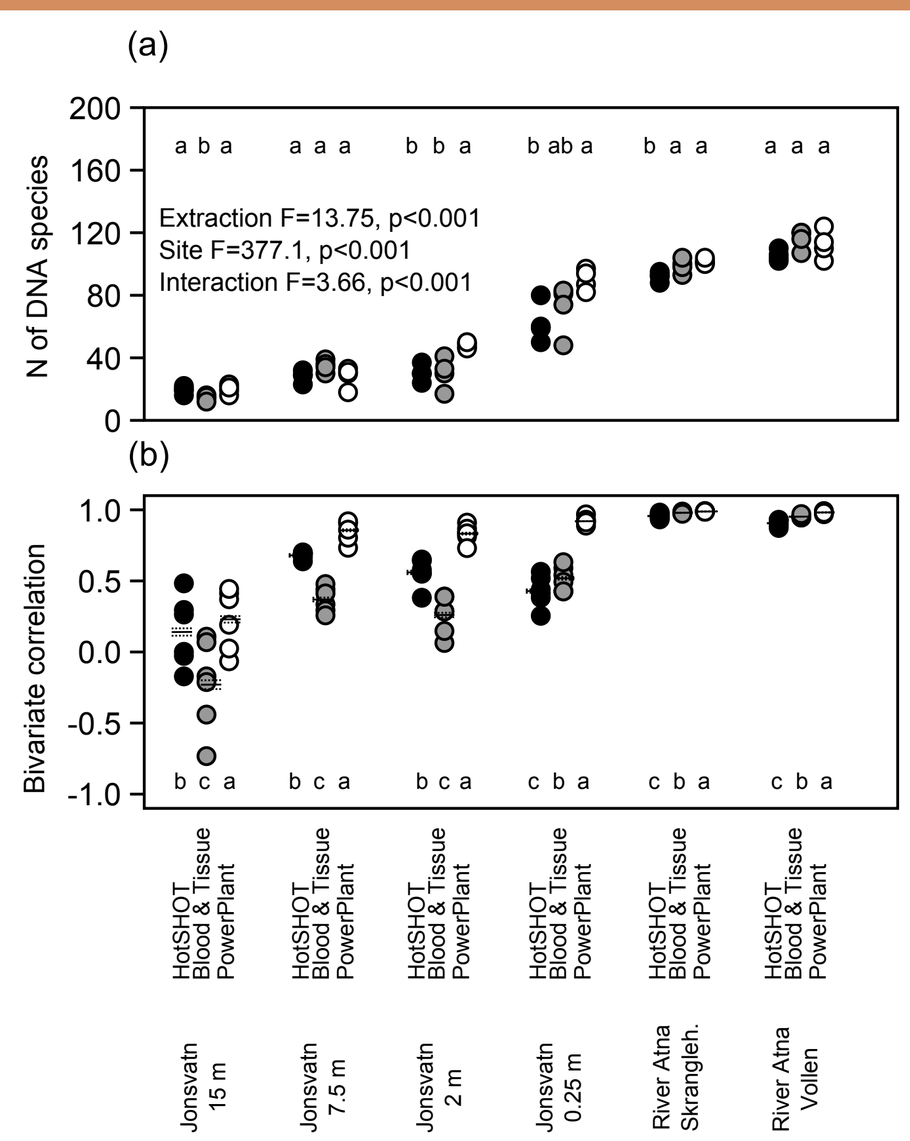

Fig. 3 Number of DNA species (a) and difference in the community composition using bivariate correlation values (b). In total, 443 OTUs were assigned to invertebrate species, and the highest number of species was achieved using the PowerPlant kit (two-way ANOVA and Tukey's HSD at each site). The bivariate Poisson lognormal correlation values were the highest at each site, using the PowerPlant kit (mean and 95% confidence intervals). The mean value was higher than 80 in all except the 15m subsamples using the PowerPlant kit, while using the Blood & Tissue kit and HotSHOT extraction it was higher than 80 only in the river samples. This indicates that the PowerPlant kit outperformed the other methods and the Blood & Tissue kit and HotSHOT extraction failed to recover the same community composition from the lake samples.

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