ARPHA Conference Abstracts 4: e65022
doi: 10.3897/aca.4.e65022

Conference Abstract

Wet grinding of invertebrate bulk samples – a scalable and cost-efficient protocol for metabarcoding and metagenomics

Dominik Buchner[‡], Peter Haase[§], Florian Leese[‡]

‡ University of Duisburg-Essen, Aquatic Ecosystem Research, Essen, Germany
§ Senckenberg Reseach Institute and Natural Histroy Museum Frankfurt, Gelnhausen, Germany

Corresponding author: Dominik Buchner (dominik.buchner524@googlemail.com)

Received: 25 Feb 2021 | Published: 04 Mar 2021

Citation: Buchner D, Haase P, Leese F (2021) Wet grinding of invertebrate bulk samples – a scalable and cost-efficient protocol for metabarcoding and metagenomics. ARPHA Conference Abstracts 4: e65022. https://doi.org/10.3897/aca.4.e65022

Abstract

DNA metabarcoding is an efficient tool to characterize invertebrate species composition in environmental samples. Most metabarcoding protocols for invertebrate bulk samples start with sample homogenization, followed by DNA extraction, amplification of a specific marker region and sequencing on a high-throughput sequencer.

Many of the above-mentioned laboratory steps have been verified thoroughly and best practice strategies exist. Giving the amount of research done to validate almost all laboratory steps of metabarcoding workflows it is surprising that there is no clear recommendation for the basis of almost all metabarcoding studies: the homogenization of samples itself.

For homogenization, different devices are used that can be divided into two major categories: bead mills and blenders. While bead-mills accelerate small, hard particles in a closed container or tube to break down specimens into small tissue fragments, blenders work with a rapidly rotating blades that reliably slice specimens as well as other substrate of the sample. Both methods are currently used in metabarcoding studies and have downsides to consider.

Bead-mills rely on single-use plastics and therefore produce a lot of waste and are expensive. In addition to that, processing times can go up to 30 minutes making them unsuitable for large scale studies. Blenders on the other hand offer the opportunity to be cleaned and can handle larger sample volumes in shorter time, with an increased risk of cross-contamination.

We here aimed to develop a fast, robust, cheap and reliable sample homogenization protocol that overcomes the above-mentioned limitations of both methods, i.e. does not produce difficult to discard waste and avoid single-use plastics while reducing overall costs. We tested the performance of the new protocol using six sorted Malaise trap insect samples and six unsorted stream macroinvertebrate kick-net samples. We used 14 technical replicates of each sample and many negative controls per sample (Fig. 1) to quantify impacts of i) insufficient homogenization and ii) possible sources of cross-contamination.

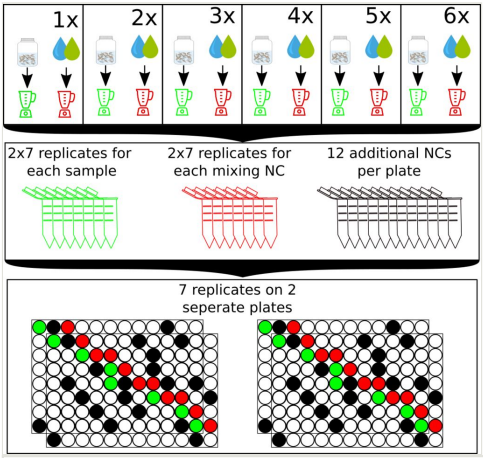

Figure 1. doi

Overview of the study design. Between each sample the blender was rinsed 1-6 times with ddH20 or sterilisation solution. After each washing step a negative control of 100% EtOH was taken. Each of the samples as well as the negative controls was technically replicated 14 times, equally distributed over 2 plates, that were never opened at the same time. In addition to that, 12 negativ controls that never had contact with the blender were distributed across each of the 4 plates to control for background noise.

Our results show that homogenization is sufficient after 3 minutes of homogenization. Rinsing the blender with water is sufficient in most cases but leads to low read-numbers in some of the negative controls. These could be further reduced by rinsing the blender with self-made drain-safe sterilization solution based on bleach but far less corrosive than pure bleach. Our results suggest that rinsing 1-2 times for 20 seconds is sufficient to avoid any cross-contamination.

The improvements of the protocol in terms of speed, ease of handling, overall reduction of costs as well as the documented reliability and robustness make it an important candidate

for sample homogenization after sampling in particular for large-scale and regulatory metabarcoding biodiversity assessments and monitoring programs.

Keywords

metabarcoding, homogenization, wet grinding

Presenting author

Dominik Buchner

Presented at

1st DNAQUA International Conference (March 9-11, 2021)