Article S1. Environmental DNA extraction protocol

We used a chloroform-based DNA extraction method modified from (Turner et al. 2014) for all our eDNA samples. The extractions were done in a dedicated lab space with no other PCR-based work going on. The protocol is as follows:

1. Incubate the samples (i.e., 2 mL tubes with the filter paper) under 55 °C for 15 minutes.
2. This step should be done in a fume hood. Add equal volume of 24:1 chloroform: isoamyl ethanol (e.g., 500 µL CTAB, 480 µL chloroform and 20 µL isoamyl ethanol). Briefly mix the solution on a vortex mixer and shake it gently for 15 minutes on a reciprocal shaker. The filter paper should completely dissolve into chloroform and the solution should appear milky. Note that some insoluble matter (e.g., algae debris) might persist.
3. Centrifuge the tube with minimum 12,000 RPM for 30 minutes. At the end, the solution should appear clearly with two phases: top aqueous phase (supernatant) with dissolved DNA and lower chloroform phase which is heavier and hydrophobic. Sometimes there will be insoluble debris in between.
4. Carefully transfer the supernatant to a new tube. Avoid anything near the interface of the two phases.
5. Repeat step 2 to 4. The resulting solution should have a volume around 500 µL. Note: Sometimes the volume exceeds this because the wet filter paper introduced extra liquid at the outset.

6. Add 0.7 volume of cold isopropanol and gently invert the tube to mix. Incubate at 4 °C overnight[1].
7. Centrifuge at minimum 12,000 RPM for 15 minutes. The DNA pellet should have formed at the bottom of the tube. Might be visible as a small white spot. Discard the supernatant gently.
8. Add 200 µL of freshly made cold 70% ethanol and centrifuge at minimum 12,000 RPM for 5 minutes discard the supernatant gently. Repeat this step.
9. Remove the tube lid and let the remaining ethanol air evaporate. Under room temperature it should be done within 30 minutes. Provide protection on the tube top to avoid contamination via air.
10. Elute the DNA pellet with 100 µl pre-warmed TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at 55 °C.
11. Validate DNA content (e.g., electrophoresis and spectrophotometry) and store the sample at a maximum of -20 °C until subsequent analyses.

Reference

Turner CR, Barnes MA, Xu CCY, Jones SE, Jerde CL, and Lodge DM. 2014. Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution* 5:676-684. 10.1111/2041-210x.12206

[1] Standard DNA precipitation protocol requires salt added to high concentration (e.g., add 0.5 volume 5 M NaCl) during step. However, the 2% CTAB solution we used for filter preservation already contained 1.4 M NaCl that is more than enough for DNA to precipitate. Additional salt would not be helpful but in stead might cause trouble, as excessive salt could be carried over to DNA extractions and potentially inhibit subsequent PCR applications.