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Conference Abstract

Tracing the almost extinct mayfly Prosopistoma pennigerum (Müller, 1785) – an eDNA approach

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Abstract

The rare mayfly *Prosopistoma pennigerum* was once widely distributed across Europe and occurred virtually in every large river. Today, it holds fast against the ever-growing destruction of its habitat with a few relic populations remaining. Preliminary data and information on its congeners suggest that free-flowing rivers with near-natural hydrodynamics are its primary habitat. Rivers where the species currently occurs should be primary targets for large-scale landscape conservation and protection. According to the Water Framework Directive, achieving longitudinal and lateral connectivity is a priority target, and occurrence and viability of *Prosopistoma pennigerum* populations could be an indicator of restoration success.

We developed and validated a targeted qPCR protocol to detect this mayfly and applied it to standardized water samples filtered for eDNA analysis from the Vjosë (Albania) and Volga (Russia), two rivers with extant populations of *P . pennigerum* . In the Vjosë river, 45 sampling sites were sampled three times in 2018 and 2019. In the Volga river, we focused on a site with >15 years of continuous records of *P . pennigerum* at Rzhev and two downstream locations, where eDNA samples were collected in 2017.

At each sampling site in the Vjosë, eDNA samples were collected by filtering 0.5 L of stream water through each of two 0.45 µm Sterivex filters. In the Volga, 2 L of stream water were filtered through a total of eight 0.7 µm glass fibre filters. Filters were stored and shipped at -20°C until further processing. Environmental DNA extraction was performed using the DNeasy® PowerWater® Sterivex™ Kit following the Experienced User protocol for Vjosë samples and via a Phenol-Chloroform Isoamyl extraction for the Volga samples.
A Taqman qPCR assay was developed using a newly designed primer and probe set. Standard curves obtained from an amplicon dilution series yielded a reaction efficiency of 88% and an R 2 -value of 0.995, with a calculated limit of quantification of 1801 copies/µL and a limit of detection of 59 copies/µL. Each eDNA sampling replicate was tested using five qPCR replicates, yielding ten qPCR reactions per sampling site in the Vjosë river and 40 qPCR reactions in the Volga river. With each batch of eDNA samples, four negative and two positive controls were analysed.

At each site, we also collected benthic MHS samples with a 25x25 cm 500 µm net where 20 samples were taken to reflect microhabitat distribution and dominance. Benthos samples were subsampled in the lab and all zoobenthos hand-picked. *Prosopistoma pennigerum* occurrence was assessed as areal density (number of specimens per m 2).

While *P . pennigerum* occured in high frequencies and abundances in benthic samples along the Vjosë river main stem in all sampling seasons, qPCR of eDNA samples suggested slightly different occurrence patterns. Conversely, *P . pennigerum* was detected with high consistency at two sites in the Volga river (at Rzhev and a site 99 km downstream), where its frequency is much lower than in the Vjosë river. The success of detecting a benthic species in eDNA samples depends on a variety of factors that may have affected DNA quality and prevented better detection of *P . pennigerum* in the Vjosë river eDNA samples.

We demonstrate the principal applicability of molecular methods to search for rare species in hot-spots of biodiversity in Central Europe. The remnant populations of *P . pennigerum* in the hydrodynamically minimally impaired Vjosë and the Volga highlight the conservation and protection needs in Eastern and Southern Europe. At the European scale, restoration efforts should be geared towards creating viable habitat conditions for large-river species such as *P . pennigerum* . Here, our qPCR assay can deliver crucial data for better management of Europe’s large rivers.

Keywords

eDNA, BQE zoobenthos catchment scale, large rivers, Vjosë, Volga

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