

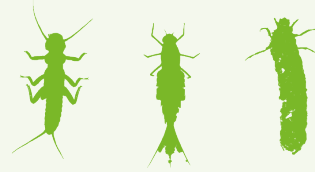


Aquatic Macroinvertebrates

Objective: Increase abundance and diversity of macroinvertebrates in restored river sections

Carpathian Mountains

status: no change observed



Problem: Unsustainable land use practices including logging in riparian areas have led to degraded riparian ecosystems. Beavers, a key ecosystem engineer, have also been absent from the landscape for two centuries. This has led to a reduction in aquatic biodiversity and a decline in the [condition of aquatic ecosystems](#) and [riparian habitats](#).

Action: Foundation Conservation Carpathia have reintroduced 62 beavers to the area. Beaver activity, including dam-building, is expected to improve habitat condition by retaining water in the landscape for longer and creating a greater range of heterogeneous aquatic and riparian habitats.

Indicator: A shift in the composition of aquatic macroinvertebrate communities, and an increase in macroinvertebrate biodiversity, indicate recovery of river ecosystems. Macroinvertebrates are commonly used to indicate river condition since they are sensitive to changes in water quality and other environmental conditions.



A beaver being released. Photo: Călin Șerban.

Methods

Within the area planned for beaver reintroduction, five river sections were selected for macroinvertebrate sampling: Valea Vladului, Valea lui Coman, Valea Chilie, Valea Bătrâna, and Valea Bălțatu. On each

selected river, 20 sites were sampled along a 200m long stretch. Site selection aimed to cover all microhabitats present in the river section, based on substrate and flow regime. The same sections were used for [monitoring physicochemical water quality parameters](#).

Samples were collected in June and November each year between November 2019 and June 2024, to capture the full diversity of macroinvertebrate species present. Sampling at one of the five river sections, Valea Bălțatu, started only in November 2021, while sampling at Bătrâna was paused between June 2021 and June 2023 due to the effects of flooding at the site. The baseline period, prior to beaver reintroduction, was between 2019 and 2021. Beaver reintroduction started in November 2021 and continued until April 2024, by which point 62 beavers had been successfully released: 19 individuals were released on the upper Dâmbovița Valley around Valea Vladului and Valea Bălțatu sampling sites; 21 individuals were released on the lower Dâmbovița Valley around Valea lui Coman and Valea Chilie sampling sites; 22 individuals were released on the upper Raul Targului Valley around Valea Bătrâna sampling site.

Reintroduced beavers showed very exploratory behaviour after release, making it difficult to predict where they would establish. This meant that sites selected for baseline data collection were not always the same sites where beavers subsequently established. Beaver activity was high around Valea Bătrâna and Valea Bălțatu, low around Valea Chilie and Valea lui Coman, and absent around Valea Vladului (Fig. 1). However, monitoring at Valea Vladului was still informative as a comparative control for the sites where beavers did establish.

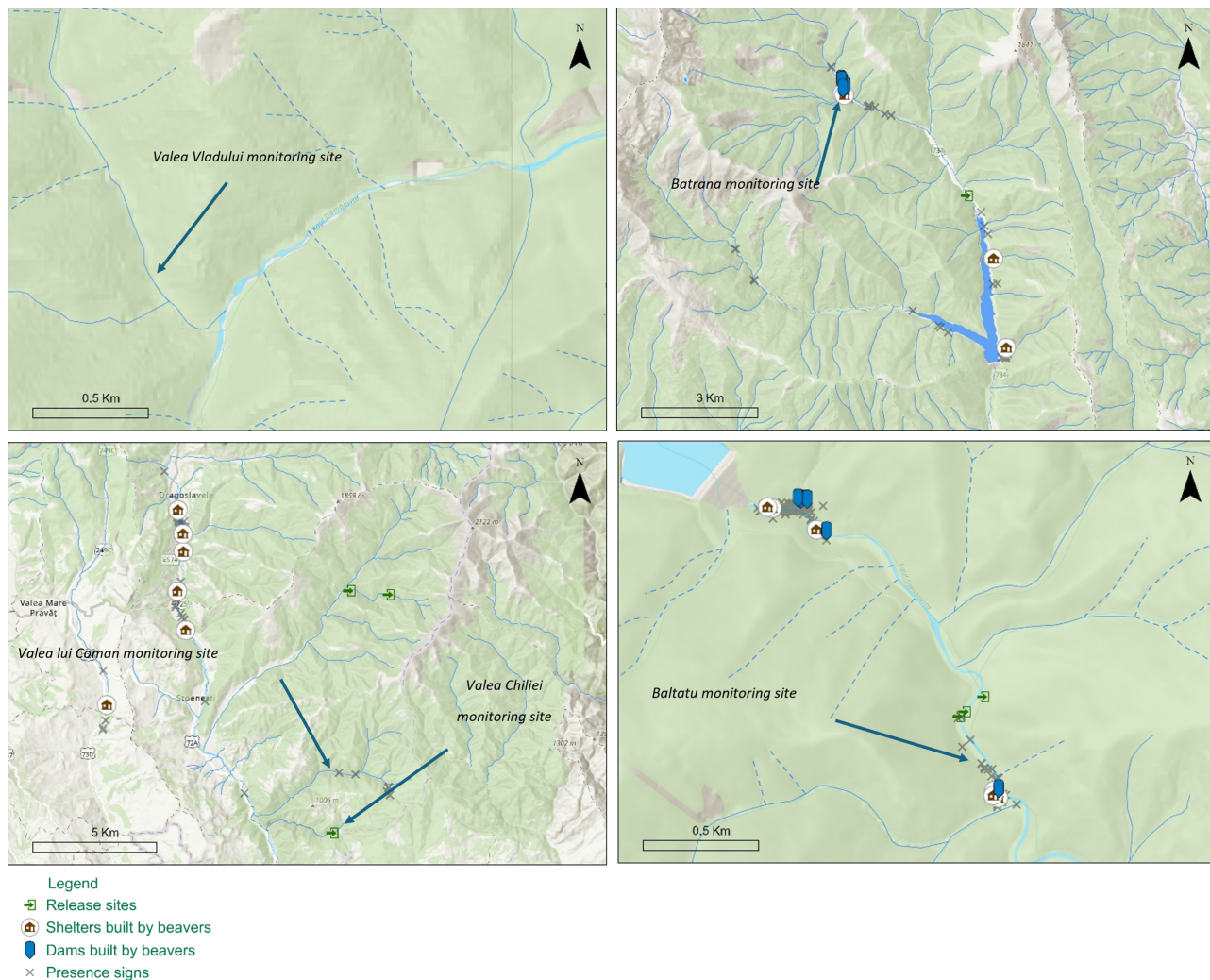


Figure 1: Location of monitoring sites relative to sites where beaver activity was recorded.

Macroinvertebrate samples were collected using a 30x30cm Surber sampler. The material collected was stored in plastic bags with 96% ethanol for preserving purposes. Additionally, in November 2021, eDNA sampling was also carried out in order to compare results from this method with results from traditional sampling. For eDNA sampling, water samples were collected at 8 randomly-selected sites within the selected river sections. 2200 ml water was collected in sterile plastic bags at each site and filtered in situ using NatureMetrics eDNA disc filters.



Sampling for aquatic macroinvertebrates using a Surber sampler. Photo: Foundation Conservation



eDNA sampling for aquatic macroinvertebrates. Photo: Foundation Conservation Carpathia.

Sampled macroinvertebrates were identified to order level based on their morphological characteristics, and assigned to functional groups based on their feeding and locomotion types ([Pastorino et al., 2020](#)). eDNA samples were analysed by NatureMetrics. Macroinvertebrate counts were then used to calculate EPT score for each site. This is a calculation of the percentage of macroinvertebrate community samples which are composed of the orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies). These taxa are particularly sensitive to pollutants and high nutrient levels, so are informative indicators. They are also sensitive to the muddy substrates that result from beaver activities.



Invertebrate taxa used to calculate the EPT index of sensitive groups: Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies). Photo: Foundation Conservation Carpathia.

Results

In baseline surveys between 2019 and 2021, macroinvertebrate abundance and EPT scores were similar across rivers and seasons, although abundance and EPT scores were higher in the southern rivers of Valea lui Coman and Valea Chilieii, probably due to their differing environmental conditions, with lower altitude and more open vegetation.

Across rivers, macroinvertebrate abundance and EPT scores decreased after 2021 (Fig. 2).

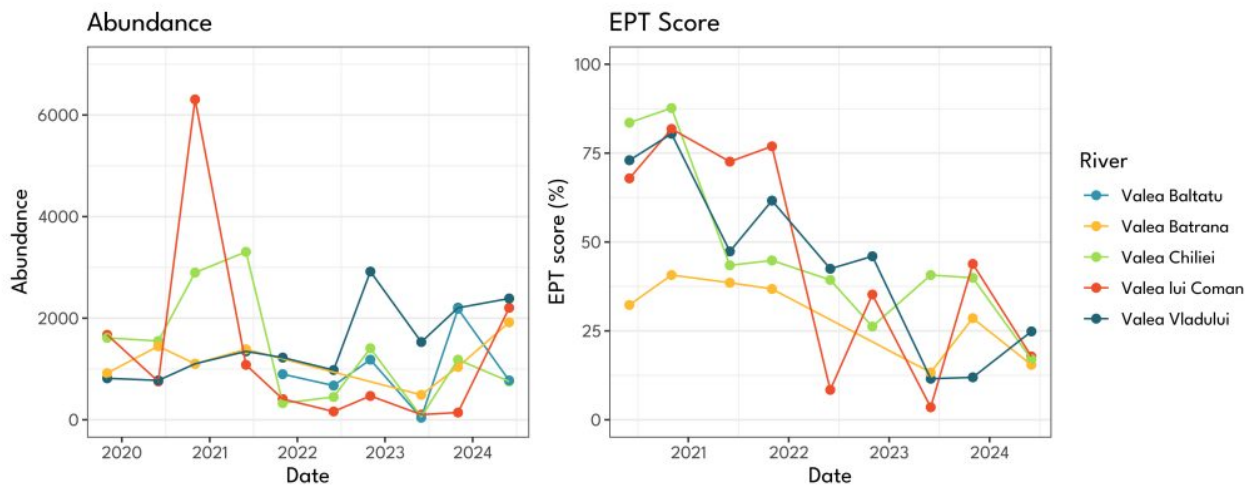


Figure 2: Change over time in macroinvertebrate abundance and EPT score in the five sampled river sections.

There were some taxon-level changes following flooding and beaver reintroduction. However, these relationships could not be statistically verified. At Valea Bătrâna, Gammaridae decreased in abundance following flooding in 2021. In Valea lui Coman and Valea Chilie there was a drop in EPT taxa after the 2021 flooding. At Valea Bălțatu, Gammaridae increased in abundance after beaver reintroduction. At Valea Chilie, Tricladida and Planaria were no longer detected after beaver reintroduction, while Simuliidae appeared. However, abundance of these taxa was too low for this to be a robust result, and it may be due to random sampling effects.

eDNA sampling identified much higher species diversity than manual sampling, and included the detection of elusive and low-density species (Fig. 3). In addition to macroinvertebrates, it also detected 62 species from other taxa – birds, amphibians, fish and mammals. However, similar to manual sampling the orders with highest relative abundance (which may not be an accurate reflection of true abundance) were Diptera (flies), Ephemeroptera (mayflies) and Plecoptera (stoneflies).

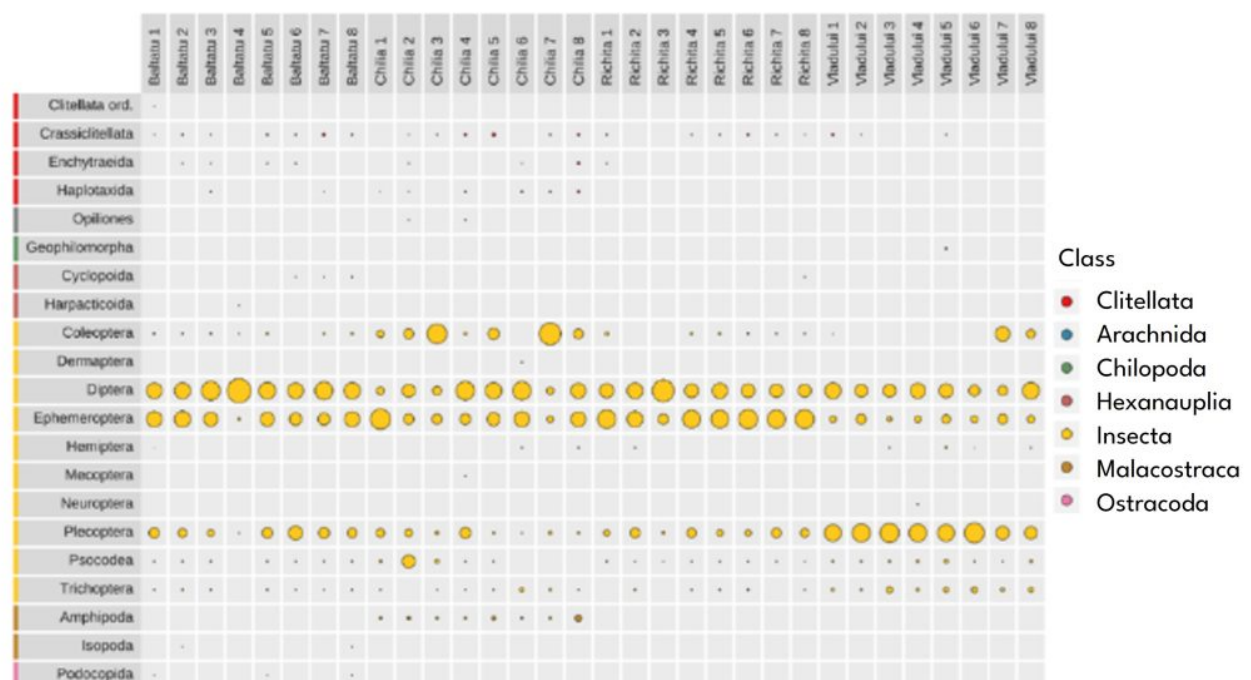


Figure 3: Results of eDNA sampling. The figure represents the proportion of the sequencing output allocated to the

different orders (rows) within each sample (columns). The size of each bubble represents the relative abundance of sequence reads for that order, relative to the total number of sequence reads in the entire sample.

Interpretation

Baseline data showed healthy macroinvertebrate communities, with high EPT scores indicating good water quality, especially in lowland rivers like Valea Chilei and Valea lui Coman.

In July 2021 extreme floods affected the entire area, after 3 consecutive days with record-breaking rain. All sampled rivers were severely affected by this event. The floods washed all sediments down to the bedrock, reshaping the entire river morphology. The new habitat conditions significantly impacted macroinvertebrate communities, with large decreases in relative abundance and EPT scores after June 2021. In particular, the fact that beaver reintroduction started shortly after the flooding occurred means it is impossible to separate the effects of beavers from the effects of flooding on macroinvertebrate communities, so changes over the period cannot be attributed to beavers. The observed decline in macroinvertebrate abundance and EPT scores was probably caused by the flooding rather than beaver reintroduction, particularly since it occurred even at control sites where no beaver activity was recorded. Changes in particular taxa may have been at least partly due to beaver activity, such as the increase in Gammaridae at Bălțatu, which might have been caused by the impact of beavers on riverbed substrates. However, a longer period of data collection will be needed to detect longer-term impacts of beavers on macroinvertebrate communities.



River during the extreme flood events of July 2021. Photo: Foundation Conservation Carpathia.

Lessons learned

Although the monitoring period reported here was too short to detect long-term ecological changes, this dataset will provide a valuable baseline to compare future survey data against. In addition, the invertebrate monitoring data provided a useful picture of short-term changes in water quality and habitat condition, allowing Foundation Conservation Carpathia to determine whether other interventions were needed.

The fact that the taxa with highest relative abundance were consistent between the eDNA and manual sampling suggests that eDNA could be a sensitive and representative method for sampling for long-term monitoring.

Citations

Pastorino, P., Zaccaroni, A., Doretto, A., Falasco, E., Silvi, M., Dondo, A., Elia, A. C., Prearo, M., & Bona, F. (2020). Functional Feeding Groups of Aquatic Insects Influence Trace Element Accumulation: Findings for Filterers, Scrapers and Predators from the Po Basin. [*Biology*, 9\(9\), 288.](#)

Partners

