Functioning of vertebrate metacommunities in dynamic riverine landscapes: an innovative approach using eDNA metabarcoding

A bilateral Austrian-Hungarian Joint Research Project (RIMECO)

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Summary report

In this project we examined which are the main determinants of metacommunity organization of vertebrate metacommunities in large rivers and their floodplains using traditional and eDNA metabarcoding based sampling methods. In WP1 we tested the applicability and representativeness of eDNA based samplings in river-floodplain systems regarding some key abiotic characteristics of the water body and transport of eDNA. In WP2 we characterized the structure of vertebrate (primarily fish and amphibian) metacommunities in an Austrian and a Hungarian floodplain and examined the effectiveness of traditional and eDNA based sampling methods in large floodplain rivers (Danube River). In WP3 we characterized fish and amphibian metacommunities at high spatiotemporal resolution and examined the temporal aspects of their dynamics in relation to environmental variability (especially hydrological connectivity and hydrological variability) of the waterbodies using eDNA. The main results are summarized below.

Generally, the project proceeded in a well-structured manner. However, the COVID-19 pandemic affected the capacity of the laboratory responsible for the eDNA analyses (sequencing), which caused significant delays in the delivery of the processed sample results. As a consequence, the analysis of spatiotemporal dynamics was also delayed, and we have only recently completed the preparation of several key manuscripts. These will be submitted to high-impact (D1 level) journals within the next three to five months. Nevertheless, we are able to provide a brief summary of the main findings (for further details, see WP3)

Overall, our high-quality publications provide clear and compelling evidence supporting the applicability of environmental DNA (eDNA) metabarcoding and quantitative PCR (qPCR) analyses for assessing the spatial and temporal dynamics of vertebrate communities in dynamic river-floodplain ecosystems. By integrating these molecular techniques, we demonstrate their effectiveness in capturing patterns of biodiversity and community composition across both space and time. These findings offer valuable insights into the underlying ecological processes that govern metacommunity organization in these complex and fluctuating environments. Furthermore, our results underscore the potential of eDNA-based approaches to inform environmental monitoring and assessment and provide actionable guidance for conservation and management strategies in riverine landscapes. Based on our multitaxa approach we also provide novel site specific and regional scale information on the diversity and organization of aquatic (fish), semiaquatic (amphibians) and both aquatic and terrestrial (birds) communities, which can be used in the sustainable management of Danubian floodplains by national parks and water authorities.

WP1

This WP focused on evaluating the uncertainties associated with the estimation of the absolute abundance of eDNA in the samples using both observational and experimental research.

We developed a method to infer the absolute abundance of fish species by combining metabarcoding with a PCR-based estimate of the total abundance of eDNA amplified with a universal marker. A 2850 km eDNA study in the Danube catchment, using a spatially integrated sampling protocol coupled with traditional electrofishing for estimating fish biomass and density, showed that total fish eDNA concentrations and total fish abundance were strongly correlated.

We also developed a model to predict the downstream transport distance of fish environmental DNA using the following conceptual framework. Environmental DNA (eDNA) is an effective tool for describing fish biodiversity in lotic environments, but the downstream transport of eDNA released by organisms makes it difficult to interpret species detection at the local scale. In addition to biophysical degradation and exchanges at the water–sediment interface, hydrological conditions control the transport distance. The new eDNA transport model considers downstream retention and degradation processes in combination with hydraulic conditions and assumes that the sedimentation rate of very fine particles is a correct estimate of the eDNA deposition rate. Based on meta-analyses of available studies, the particle size distribution of fish eDNA (PSD), the relationship between the sedimentation rate and the size of very fine particles in suspension, and the influence of temperature on the degradation rate of fish eDNA were successively modelled. After combining the results in a mechanistic- based model, the eDNA uptake distances (distance required to retain 63.21% of the eDNA particles in the riverbed) observed in a compilation of previous experimental studies were correctly simulated. eDNA degradation is negligible at low flow and temperature but has a comparable influence on background transfer when hydraulic conditions allow a long uptake distance. The wide prediction intervals associated with the simulations reflect the complexity of the processes acting on eDNA after shedding. This model can be useful for estimating eDNA detection distance downstream from a source point and discussing the possibility of false positive detection in eDNA samples.

A further paper is in preparation and will be submitted within the next six months. To better address the uncertainties associated with estimating species presence and relative abundance from environmental DNA water sample, an experiment was conducted in the Austrian Danube floodplain. Three sites with different connectivity to the main channel were sampled ten times using a similar integrative sampling protocol (replicates). Due to technical issues, the final concentration of total fish environmental DNA per litre (qPCR analysis) and the number of reads per species (metabarcoding analysis) were only available last year. The main objective of the performed data analysis and modelling is to show how the combined effect of environmental DNA concentration in the water and the effective sequencing depth after computational screening affect the number of species detected. The focus is on rare species whose specific read count is less than 1% of the total effective sequencing depth. The uncertainties association with the quantification of their relative abundance will be shown in this study.

WP2

By the joint consideration of environmental and dispersal processes, metacommunity theory provides a useful framework to understand patterns in nature, including spatial and temporal variability in biological diversity. However, information is largely lacking on how these processes interact with inherent biological features of the organisms, such as their habitat affinity and dispersal ability, which subsequently influence patterns in alpha and beta diversity. Therefore, we examined the relative role of habitat specialist vs generalist species, and the spatial configuration, connectivity, and different environmental characteristics of river-floodplain habitats to get a more mechanistic understanding of patterns in the diversity of fish metacommunities. We used eDNA metabarcoding to characterize species (taxa) richness and composition in two separate floodplains of the Danube River (Austria and Hungary) during two different hydrological conditions. Results showed that the number of specialist and generalist species can largely determine the responses of alpha and beta diversity, and the richness difference (nestedness) and replacement (turnover) components of beta to environmental gradients. The variance in these diversity metrics could be effectively predicted by a bunch of environmental and spatial variables, despite high environmental variability, which characterises floodplain-river ecosystems. However, the relative proportion of the pure vs the shared fractions of environmental and spatial variables varied rather inconsistently, and did not depend on species level resolution, spatial scale or sampling occasion. Consideration of context dependency in both local and regional scale processes are thus important in the preservation of alpha and beta diversity, which should be considered by environmental management.

We also assessed how different faces of functional structure changes along the lateral hydrological connectivity gradient (LHC), the most important environmental structuring force in river-floodplain ecosystems. We characterized how attributes of fish community traits, specifically composition, divergence, and temporal variability respond to LHC in the Austrian-Hungarian floodplains of the Danube River using environmental DNA (eDNA) metabarcoding. Trait composition was quantified by community-level weighted means (CWM) as the degree of changes in trait responses along the LHC gradient from isolated oxbows to the main river. Divergence was measured using Rao's quadratic functional diversity index and a null model approach to calculate standardized effect sizes (SES), with larger SES values indicating greater divergence and smaller values indicating convergence. Temporal variability, representing the degree of instability in community traits over time, was calculated using a functional beta diversity measure for multiple communities. Our findings revealed apparent compositional changes for many trait variables, highlighting the significance of LHC in shaping community functional diversity. Divergence patterns indicated that isolated habitats foster trait convergence presumably due to habitat filtering, whereas more connected areas promote trait divergence due to higher species richness and habitat availability. Temporal variability of traits associated with flow preference exhibited a hump-shaped relationship with LHC, suggesting intermediate connectivity zones are hotspots of ecological dynamism. The study suggests that examining composition, divergence, and temporal variability together provides a more complete understanding of trait responses to connectivity, stressing the importance of maintaining diverse connectivity levels in river-floodplain systems for effective conservation and ecosystem management.

Our eDNA survey of fish assemblages proved to be also highly useful to obtain more detailed information on the occurrence and distribution of non-native and invasive fishes. For example, the Amur sleeper is one of the most rapidly spreading invasive fish in Eurasia. The species was detected in one of the 15 water bodies sampled with temporally intensive samplings. At this site all samples were tested positive for the species and the detection was additionally confirmed by electrofishing. This was the first detection of the species in this Danubian floodplain. Since the area maintains a high level of biodiversity with numerous protected and rare aquatic invertebrates and vertebrates, we highlighted the need of urgent management actions to prevent the further spread of Amur sleeper within and outside of the floodplain. Additionally, we provided a further example of the practical application of eDNA methods in the early detection of invasive species. As a forthcoming project we analyse the habitat use of invasive species in the studied Austrian and Hungarian floodplains to get a more detailed information on their ecology and to aid the work of environmental management. A manuscript is under preparation of this work.

Our study examined the ecological factors shaping riparian and aquatic bird communities in a relatively intact floodplain of the Danube River, Hungary. We assessed the influence of local habitat structure, land cover, and spatial configuration on taxonomic and functional community composition. Land cover and spatial factors played a greater role than local habitat characteristics in structuring both aquatic and terrestrial bird communities. The importance of land cover varied by scale and bird community type. Our findings highlight the need for conservation strategies that integrate both local and landscape-scale habitat features to maintain habitat connectivity, support species dispersal, and enhance floodplain biodiversity. As a continuation of the work, we examine how the complex interaction of waterbody characteristics, land use structure (with special regard to riparian forest structure) influence keystone vertebrate species (woodpeckers and beaver) and their interaction in the riparian zone of the waterbodies of the Gemenc floodplain. We have already finished the field surveys and started data entry and preliminary evaluations.

As part of the project's practical application, we also assessed the diversity of habitats and ecological communities in the Hungarian floodplain to support future conservation and restoration planning efforts. Our approach involved using our data on the multitaxa diversity of vertebrate communities, reviewing existing literature, and analyzing historical and remote sensing data of the landscape. Additionally, we engaged stakeholders to help identify and prioritize the most valuable aquatic habitats for conservation purposes. The study underscored that despite substantial landscape alterations resulting from past river regulation projects, many waterbodies and areas of the landscape still retain significant potential for maintaining both habitat diversity and biodiversity. Furthermore, our analyses demonstrate how data on habitat changes and diversity can inform sustainable management planning.

WP3 This project addresses the evaluation of the temporal dynamics of fish and amphibian metacommunities, and their interactions. We have successfully collected all the eDNA samples over the past three years, totalling 11 occasions throughout the three-year period from both floodplains. Unfortunately, there was a delay in the laboratory work, which prevented us from obtaining the data and halted our progress in the analyses. Nevertheless, we have already analysed the data and have started submitting materials into SCI journals. In the main body of work we developed a novel metacommunity model to analyse the organization of fish metacommunities in dynamic landscapes. The theoretical background and the obtained results are summarised in a manuscript which is under an exceptionally long review process presently (at Ecological Monographs). We have also just prepared a manuscript which introduces a novel framework for understanding the internal structure of metacommunities, focusing on the role of species- and site-level attributes in shaping community dynamics. By applying this framework to fish community data from the Danube River floodplains using eDNA metabarcoding, we uncover site level heterogeneities in the different ecological processes that provides insights for context-sensitive conservation strategies—advancing both metacommunity theory and its practical application in dynamic landscapes.

Our study also evaluated the direct and indirect effects of lateral hydrological connectivity (LHC) derived habitat changes on the two most abundant Amphibian species in the studied riverine floodplains. Our results showed that LHC did not directly influence Pelophylax spp. and B. bufo abundance but affected them indirectly and species-specifically through environmental drivers. Hydrology negatively and indirectly impacted Pelophylax spp. through vegetation cover and the physical and chemical properties of waterbodies. B. bufo was affected in a similar way but primarily through habitat structure. Our findings highlight the importance of understanding species-specific responses to lateral hydrological connectivity in dynamic environments, as river regulation altering LHC can differently impact amphibian species in riverine floodplains.

Additionally, we analyse the fish and amphibian metacommunity as complex networks. Using an approach based on Bayesian networks we analyse for species co-occurrence patterns accounting for spatial, temporal, and dynamic autocorrelation as well as environmental conditions. In the second step, we applied a graph-theoretic approach to depict and analyse the relationships between species and define discrete communities. We identified different communities in the system, varying in their migration intensity, interaction with other communities and dependency on environmental conditions. Also, species interactions such as predator-prey interactions within fish as well as between fish and amphibians, are well delineated in the network. A paper is in preparation and will be submitted within the next three months.

Finally, we also assess how eDNA metabarcoding can be applied to address typological questions regarding Danubian fish communities, by comparing these novel results with our previous work based on traditional fishing methods. These findings contribute to evaluating the applicability of eDNA-based sampling methods for the bioassessment of large rivers such as the Danube. A manuscript is currently in preparation and is expected to be submitted within the next five months.

Scientific papers in different stages of publication, still not accepted at journals

- Feng, K., Czeglédi, I., Funk, A., Hein, T., Pont, D., Meulenbroek, P., Valentini, A., Erős, T. A unified framework for unlocking metacommunity processes in dynamic landscapes (R1 version submitted to Ecological Monographs, has been sitting in the journal overall for more than eight months)
- Czeglédi, I., Funk, A., Pont, D., Hein, T., Meulenbroek, P., Preiszner, B., Valentini, A., Erős, T. Local and regional processes affect the distribution and population dynamics of alien fishes in river-floodplains (target journal Freshwater Biology)
- Feng, K., Czeglédi, I., Funk, A., Hein, T., Pont, D., Meulenbroek, P., Valentini, A., Erős, T. (in prep.) Unveiling the internal structure of metacommunities: integrating site and species attributes to ecological processes (target journal Ecography)
- Funk, A., Czeglédi, I., Erős, T., Lukas, L., Meulenbroek, P., Pont, D., Valentini, A., Hein, T. Analysis of species interaction networks in a fish and amphibian floodplain metacommunity using eDNA metabarcoding. (target journal Oikos)
- Meulenbroek P., et al. (in prep.) Longitudinal typology of the Danube using fish assemblage data from eDNA metabarcoding. (target journal Ecological Indicators)
- Mészáros, B., Funk, A., Hein, T., Lukas, L., Meulenbroek, P., Pont, D., Valentini, A., Schmera, D., Czeglédi, I., Erős, T. Effects of lateral hydrological connectivity on the relative abundance of water frogs (Pelophylax spp.) and common toads (Bufo bufo) using eDNA surveys (under review at Hydrobiologia)