Final scientific report

Effect of connectivity loss on biodiversity in experimental metacommunities (CLOSER)
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Project summary:
Habitat loss due to anthropogenic activities is one of the main causes of global biodiversity decline. There is, however, a major disagreement regarding the relative role of the total loss of habitat in the landscape compared to the role connectivity loss the remaining habitats undergo. This dispute is largely due to a lack of suitable data that would enable disentangling the two mechanisms in action. A better understanding is increasingly important under the current climate change scenarios, where these mechanisms interact with intensifying environmental stress. In this project, we investigated how connectivity and its loss directly influences biodiversity in habitat networks, and whether this changes when connectivity loss is coupled with a climatic stressor. In two large-scale aquatic ecology experiments, we investigated the direct effect of connectivity loss on local and regional biodiversity loss in metacommunities and its interactions with the effects of recurrent heatwaves, an increasingly common form of climate change related stressors. We have explicitly shown that connectivity loss is a direct driver of microbial biodiversity loss even if habitat amount and quality remain unchanged. We have also provided direct evidence that connectivity between habitats can mitigate the adverse effects of climate change on biodiversity. These results provide important proof as to the effects of connectivity loss on biodiversity under an increasingly extreme climate.

Description of the results achieved during the project:
The project had two main work packages (WPs). WP1 aimed to reveal the direct role of loss in explaining local and regional biodiversity loss in metacommunities, while WP2 combined this with the interaction of climate change.

In WP1, we compared biodiversity patterns (alpha, beta, gamma) in connected vs. fragmented habitat networks to explore the role of connectivity loss as the main driver of biodiversity loss under habitat fragmentation. With the same amount of habitat patches (five mesocosms representing regional networks, i.e., metacommunities, with homogenous environment) which stayed constant during the experiment, we followed changes in local and regional biodiversity in a 4-month long experiment. We used plankton as model organisms, including bacterio-, phyto- and zooplankton. All experimental metacommunities were treated the same way during the first month of the experiment, where connectivity was realised as a 1% weekly water exchange between the five mesocosm of each network. After the first month, connectivity loss was applied by stopping weekly dispersal events among the members of the fragmented habitat networks. We monitored all the 30 mesocosms for another three months to track changes in local and regional biodiversity in the metacommunities and link these to direct and indirect treatment effects.

Our findings were particularly striking for unicellular microeukaryotes. Connectivity loss led to significant declines in their diversity at both local and regional levels, highlighting that fragmentation can directly drive biodiversity loss, even under controlled circumstances (i.e., with habitat amount kept constant, and without any treatment-specific variation in abiotic

environment). Both rare and abundant species were impacted, suggesting that fragmentation represents a widespread and severe threat to microbial biodiversity. In contrast, prokaryotes appeared more resilient, though we observed signs of a potential extinction debt, where biodiversity loss may emerge over longer timescales.

Beyond biodiversity, the study also highlighted how connectivity loss disrupts trophic interactions. Zooplankton grazers, which interact closely with microbial communities, experienced reduced biomass in fragmented habitats, further exacerbating the decline in diversity and community evenness of microeukaryotes. These findings highlight the interdependence of organism groups within ecosystems and the cascading impacts that habitat fragmentation can have on biodiversity and ecosystem functioning.

Our results clearly show that connectivity plays a crucial role in maintaining local and regional biodiversity, and its effect is independent of the amount of remaining habitats. The findings were published in Global Change Ecology, one of the flagship high-impact D1 journals of the field (IF2023: 10.8), which I would like to highlight as the most important output of the project (among several other publications mostly in D1 journals listed below). Besides, our publication has already made it to the top 5% of all similar research outputs scored by Altmetric.

In WP2, our key research question was the interplay between the disruption of metacommunity processes (via connectivity loss) and relevant stressors under climate change, which were applied in the form of repeated heatwaves to our experimental metacommunities. We carried out a large-scale mesocosm experiment in 60 mesocosms at our new mesocosm facility in Vácrátót (Tízhold experimental site, https://mesocosm.org/mesocosm/cer-mesocosms/). We had 20 experimental metacommunities, each consisting of three mesocosms (experimental ponds) of 200 litres. Connectivity was realised as a 1% weekly water exchange between the connected mesocosms while we applied no water exchange among the fragmented metacommunities during the duration of the experiment, which was two months in total. Two recurring heatwaves were applied, each with a +6°C increase in temperature compared to the ambient mesocosms, lasting 7 days.

We compared biodiversity patterns (alpha, beta, gamma), community biomass and composition in connected vs. fragmented networks crossed with the disturbance effect of repeated heatwaves. We used plankton as our model organisms, including bacterio-, phyto- and zooplankton, and added artificial substrates (tiles) for studying benthic biofilms. We found strong effects on both zooplankton composition and phytoplankton biomass after the first heatwave. Heatwaves exerted a strong negative impact on zooplankton abundance, leading to weaker top-down control on phytoplankton, resulting in higher chlorophyll concentrations. Connected metacommunities exhibited a faster recovery in terms of zooplankton abundance and community composition than fragmented metacommunities.

Our results clearly pointed at the fundamental role of spatial insurance in connected habitat networks and that these connections might counteract the negative effects of climatic stressors under the increasingly challenging climatic conditions. These results have important implications for the long-term conservation of habitat networks and provide important direct evidence for the role connectivity can play in safeguarding biodiversity under the intensifying local stressors linked to climate change. Conservation efforts need to focus on habitat (pond) networks rather than individual habitats to maintain biodiversity and ecosystem functioning in the era of environmental change.

I would also like to acknowledge that the project provided core funding to starting my research group when I moved to Hungary in 2019. It has therefore significantly contributed to my scientific career as an early-career scientist and new group leader, providing support to establishing my own group and research avenues.

Delays due to the COVID pandemic and related time plan adjustments:
The work suffered delays due to the pandemic. There were repeated periods where we had to limit the physical number of personnel per laboratory, which slowed the practical molecular work and microscopic identification. There was also a minor delay during the first project year due to COVID restrictions due to which we started the experiment a few months later than originally planned, but the milestones promised in the work plan were finally achieved by the end of the year.

Acknowledging force majeure difficulties related to the pandemic, a 12-month extension was allocated to the project by the NKFI office, which shifted the original time plan of the second part of the project. This way, WP2 took place in project years 4-5 instead of 3-4. The project extension also ensured that all the proposed practical tasks could be successfully finished in WP1.

At the same time, we could also use the affected time period to work on related biodiversity and connectivity analyses with existing metacommunity data, which was possible even during the pandemic. This resulted in the publication of a paper on connectivity provided by waterbirds (Szabó et al 2022 Journal of Biogeography), a publication on the role of connectivity for metapopulation and metacommunity structure (Horváth et al 2024 Oikos),), a publication on phylogenetic patterns linked to past dispersal events (Lukić et al. 2021), a publication on regional biodiversity patterns analysed in the same metacommunity (Márton et al 2023 Scientific Reports), a publication on spatio-temporal analyses of metacommunity patterns (Guzman et al 2022 Ecology), and a publication on the role of network position as an important measure of connectivity (Barta et al 2024 Ecography). The empirical data used in these works are all from the two sets of habitats serving as the inoculum of the experiment in WP1 (soda pans and ponds). Overall, results of these natural pond networks align well with the results derived from the experiments, contributing to an overall stronger and more general message on the importance of connectivity for the conservation of biodiversity.

Smaller adjustments to methodology:
The experimental designs were slightly modified building on comments from one of the original reviewers of the project and our collaborators in WP1, and experience from another experiment comparing the effects of constant warming to heatwaves (Huynh Ngoc et al. 2024 Ecology and Evolution) in WP2. This did not affect the main research questions but provided more robustness to the final experimental setups in both WPs.

In WP1, we decided for a slight change in the practical implementation of the project regarding taxonomical identification. We opted for analysing eukaryotes (including phytoplankton) also by eDNA (similar to what was originally planned for bacteria), which resulted in higher resolution data for phytoplankton diversity, and also provided information on other unicellular organisms e.g., ciliates. The experimental design was also slightly modified from the one presented in the proposal, based on the reviewers' suggestions: instead of three large networks, we created six smaller ones, and three of them were not subjected to fragmentation, to have a three-fold replicate control at the network level, as suggested by the third reviewer.

In WP2, a more intense pulse disturbance of repeated 6 ºC heatwaves was applied to our experimental metacommunities instead of the originally planned warming. We opted for this considering that the frequency, duration, and intensity of heatwaves are increasing globally, and heatwaves generally have a more immediate effect on biodiversity than the more moderate increase in annual average temperatures (Huynh Ngoc et al. 2024 Ecology and Evolution).

Peer-reviewed publications in Q1 and D1 journals supported by the project:
Our results were published in 7 D1 and one Q1 publications detailed below, with a cumulative impact factor of 39.

1. *Szabó, B., *Váczy-Földi, M., Vad, C.F., Pálffy, K., Huynh, T.-H., Dobosy, P., Fierpasz, Á., Márton, Z., Felföldi, T. & Horváth, Z. 2024. Connectivity loss in experimental pond networks leads to biodiversity loss in microbial metacommunities. **GLOBAL CHANGE BIOLOGY** 30(12), e70001. [IF_{2023}: 10.8; $SCimago_{2023}$: D1] *equal contribution
2. Horváth Z., Haileselasie T.H., Vad C.F., Ptacnik R. & De Meester L. 2024. Parallels and divergences in landscape genetic and metacommunity patterns in zooplankton inhabiting soda pans. **OIKOS,** e10887, 10.1111/oik.10887 [IF_{2023}: 3.1; $SCimago_{2023}$: D1]
3. Barta B., Szabó A., Szabó B., Ptacnik R. & *Vad C.F., *Horváth Z. 2024. How pondscapes function: Connectivity matters for biodiversity even across small spatial scales in aquatic metacommunities. **ECOGRAPHY** 2024(2), e06960 [IF_{2023}: 5.4; $SCimago_{2023}$: D1] *equal contribution
4. Huỳnh Ngọc T.H., Horváth Z., Pálffy K., Kardos V., Szabó B., Dobosy P. & Vad C.F. Heatwave-induced functional shifts in zooplankton communities result in weaker top-down control on phytoplankton. **ECOLOGY AND EVOLUTION** 14(8): e70096 [IF_{2023}: 2.3; $SCimago_{2023}$: Q1]
5. Márton Z., Szabó B., Vad C.F., Pálffy K. & Horváth Z. 2023. Environmental changes associated with drying climate are expected to affect functional groups of pro- and microeukaryotes differently in temporary saline waters. **SCIENTIFIC REPORTS** 13, 3243. [IF_{2023}: 3.8; $SCimago_{2023}$: D1]
6. Szabó B., Szabó A., Vad C.F., Boros E., Lukić D., Ptacnik R., Márton Z. & Horváth Z. 2022. Microbial stowaways–waterbirds as dispersal vectors of aquatic pro-and microeukaryotic communities. **JOURNAL OF BIOGEOGRAPHY** 49: 1286–1298 [IF_{2022}: 3.9; $SCimago_{2022}$: D1]
7. Guzman L.M., Thompson P.L., Viana D.S., Vanschoenwinkel B.J., Horváth Z., Ptacnik R., Jeliazkov A., Gascón S., Lemmens P., Anton-Pardo M.T., Langenheder S., De Meester L. & Chase J. M. 2022. Accounting for temporal change in multiple biodiversity patterns improves the inference of metacommunity processes. **ECOLOGY** 103: e3683 [IF_{2022}: 4.8; $SCimago_{2022}$: D1]
8. Lukić D., Pinceel T., Marrone F., Mioduchowska M., Vad C.F., Brendonck L., Ptacnik R. & Horváth Z. 2021. Pleistocene allopatric differentiation followed by recent range expansion explains the distribution and molecular diversity of two congeneric crustacean species in the Palaearctic. **SCIENTIFIC REPORTS** 11, 22866. [IF_{2021}: 4.996; $SCimago_{2021}$: D1]

Talks and presentations
Our results were presented by the core project team and our supervised students as part of two invited plenary talks at international conferences, seven invited seminars at universities and research institutions, and in the form of 10 regular oral presentations at international meetings and one at a Hungarian meeting.

Invited plenary talks:

1. Horváth Zs. XVIII Brazilian Congress of Limnology, online conference, 21 Nov 2022
2. Horváth Zs. 10th International Shallow Lakes Conference, 21-26 June 2020; Natal, Brazil – postponed and held online between 1-5th March 2021

Invited seminars:

1. Horváth Zs. Research Department for Limnology, University of Innsbruck, Mondsee, Austria, 4 Dec 2024
2. Horváth Zs. WasserCluster Lunz, Austria, 11 Sept 2024

3. Horváth Zs. University of Florida, Gainesville, Florida, USA, 5 March 2024
4. Szabó B. Leibniz-Institut für Gewässerökologie und Binnenfischerei (IGB), Neuglobsow, Germany, 16 November 2023
5. Horváth Zs. KU Leuven, Leuven, Belgium, 6 January 2023
6. Horváth Zs. ELTE BTDK Spring school, Budapest, Hungary (10 April 2022)
7. Horváth Zs. Invited seminar at 'BioMove seminars', University of Potsdam, Germany, 26 Nov 2021

Oral presentations at international meetings:

1. Huynh Ngoc T.H., Szabó B., Vad C.F., Lukić D., Márton Z., Pálffy K., Barrios M., Changtong S., Stamenković O., Aydin G., Kratina P., Smeti E., Quiroz M., Redón S., Laskai Cs., Bene K., Dobosy P, Biró A., Siniakova T., Baranya E., Knyesko A. & Horváth Z.: Multiple stressors in the Anthropocene: effects of habitat fragmentation and heatwaves on planktonic pond metacommunities. 3rd International Symposium on Aquatic Mesocosm-Based Research, 7-10 November 2023, Antalya, Türkiye
2. Horváth Zs., Váczy-Földi M., Vad Cs.F., Dobosy P., Felföldi T., Fierpasz Á., Huynh Ngoc T.H., Márton Zs., Pálffy K., Szabó A., Szabó B. Connecitivity loss as a driver of biodiversity loss in pond networks. SIL Austria, 28-30 Sept 2022, Illmitz, Austria
3. Szabó B., Váczy-Földi M., Vad C.F., Pálffy K., Huynh Ngoc T.H., Dobosy P., Fierpasz Á., Márton Z., Felföldi T., Szabó A. & Horváth Z.: Experimental evidence for the effects of connectivity loss on the biodiversity of aquatic microorganisms in pond networks. 3rd International Symposium on Aquatic Mesocosm-Based Research, 7-10 November 2023, Antalya, Türkiye
4. Szabó B., Váczy-Földi M., Vad C.F., Fierpasz Á., Pálffy K., Dobosy P., Huynh Ngoc T.H., Márton Z., Szabó A., Felföldi T., Horváth Z.: Effects of connectivity loss on microbial communities in experimental pond networks. ASLO Aquatic Sciences Meeting 2023, 4-9 June 2023, Palma de Mallorca, Spain
5. Márton Zs., Szabó B., Vad Cs.F., Pálffy K., Horváth Zs. Environmental drivers of microbial communities in the Seewinkel soda pans. SIL Austria, 28-30 Sept 2022, Illmitz, Austria (accepted talk with valid registration canceled due to illness)
6. Szabó B., Szabó A., Vad Cs.F., Boros E., Lukić D., Ptacnik R., Márton Zs., Horváth Zs. Waterbirds as dispersal vectors of aquatic pro- and microeukaryotes. SIL Austria, 28-30 Sept 2022, Illmitz, Austria
7. Horváth Zs. Connectivity loss as a driver of biodiversity loss in pondscapes. World Biodiversity Forum, Davos, Switzerland, 26 June – 1 July 2022, hybrid meeting.
8. Barta B., Horváth Zs., Szabó A. & Vad Cs. Biodiversity in a pondscape - relative roles of environmental filtering and network position. Symposium for European Freshwater Sciences, 25-30 July 2021
9. Szabó B., Szabó A., Vad Cs.F., Boros E., Lukić D., Ptacnik R., Márton Zs. & Horváth Zs. Microbial stowaways: can waterbirds facilitate the dispersal of aquatic microorganisms? 10th International Shallow Lakes Conference, 1-5th March 2021
10. Huỳnh Ngọc T.H., Horváth Zs., Dobosy P., Kardos V., Pálffy K., Szabó B. & Vad Cs.F. Means and extremes - effects of climate warming scenarios on plankton communities. 10th International Shallow Lakes Conference, 1-5th March 2021

Oral presentation at a Hungarian meeting

1. Horváth Zs. "Az élőhelyvesztés és fragmentáció hatása a szikes tavi közösségek diverzitására". Balmazújváros, Hungary, 28 Feb 2022

Dissemination activities
We regularly disseminated project results to the public in the forms of various social media posts, videos, and interviews on the radio, television and online news platforms.

1. Media outputs:
    - International press release on EurekAlert about the results of WP1 published as Szabó et al. 2024 Global Change Biology
    - Interview and online article about the WP2 experiment in Zöld Hang with Zsófia Horváth (18 Sept 2023)
    - Live radio interview: Tudás és Hatalom with Zsófia Horváth (Tilos Rádió, 21 June 2022)
    - Radio interview: A Hely ("The Place") with Zsófia Horváth, Csaba Vad, Károly Pálffy (Kossuth Rádió, 3 Nov 2020)
    - Radio interview: Felfedező a tudományról ("Discovering science") with Beáta Szabó (Kossuth Rádió, 29 Oct 2020)
2. Regular posts (pictures and videos) on the research team's website and social media pages (Facebook, Instagram, Twitter, Bluesky)
3. Participation in Researchers' Night: In 2020, we filmed a short video presenting our work for the Researchers' Night 2020 event (digital program due to the pandemic). In 2021 and 2022, we organised the event of our host institute in person with hands-on activities.

Education
The project team hosted several students and carried out multiple teaching activities to facilitate the use of project results in higher education and contribute to knowledge transfer to early career scientists.

Engaging young people in research:

- 12 summer interns: eight summer interns (Budapest University of Technology and Economics, Central European University, University of Veterinary Medicine, Eötvös Loránd University, Hungary; University of Belgrade, Serbia) participated in the summer experiment in WP1, and four in WP2 (Eötvös Loránd University, University of Debrecen, Hungary; METU Middle East Technical University, Türkiye)
- One TDK and OTDK (student competition) thesis: Váczy-Földi M. A fragmentáció planktonikus pro- és eukarióta egysejtű közösségekre gyakorolt hatásának kísérletes vizsgálata. 1st place at the ELTE BTDK Student competition, 2nd place at the national final (OTDK).
- Four MSc theses (two from each work package) at Eötvös Loránd University, Budapest
- One student has successfully applied for a Stipendium Hungaricum PhD scholarship to carry out her work as part of the project and is already in her third year (ELTE)

Teaching:

- Spring school of AQUACOSM-plus: Talk on habitat fragmentation and connectivity loss by Zsófia Horváth, and mesocosm demo by Csaba Vad. 25-29 April 2022 Budapest, Hungary
- Fall school of AQUACOSM-plus: Talk on habitat fragmentation and connectivity loss by Zsófia Horváth. 24-28 Oct 2022 Cluj-Napoca, Romania
- Course of Zsófia Horváth at ELTE Eötvös Loránd University for MSc and PhD students: "Spatial Ecology: from Islands to Metacommunities". Course material includes topics covered by the project in both WPs and the presentation of the experiment in WP1. The course was held during the autumn semesters during the project duration.