Final report on the NKFI-FK 127939 Grant

Colonization-naturalization ability of alien aquatic plants and trait based-assembly of their communities

RESEARCH RESULTS

In our project, we undertook an exploration of hypotheses that have received limited attention in the context of aquatic alien plant communities. Our research looked at different facets of functional differences between alien and native aquatic plant species. We sought to identify the specific traits that provide alien species with competitive advantages over their native counterparts, and to elucidate the resulting implications for species assembly within these ecosystems. Our primary focus was to investigate two key stages of biological invasion within aquatic plant communities. We investigated the following questions related to the naturalization and introduction phases of invasion:

1.1 What is the extent of phenotypic plasticity against the environmental variables among the most abundant alien macrophyte species in the Pannonian Ecoregion?

We set up a microcosm experiment lab at the Department of Biology at the University of Nyíregyháza. This facility enabled us to manipulate the temperature, light and nutrient availability of the species, therefore we could set up different climate-change scenarios. We investigated climate change induced phenotypic plasticity of native and alien aquatic plants. We paired native-alien species that are congeneric or belongs to the same growth-form. The species list is: *Salvinia molesta, S. natans, Limnobium laevigatum* vs. *Hydrocharis morsus-ranae, Myriophyllum aquaticum M. spicatum, Hygrophyla polysperma, Ludwigia palustris, Potamogeton lucens, Elodea nuttallii, Limnophila sessiflora* vs. *Gymnocoronis spilanthoides, Pistia stratoites, Rotala rotundifolia.*

In all our experiments, we placed apical shoots into 2-liter aquaria, setting two distinct nutrient concentration levels: eutrophic (0.5 mg N L^{-1}, 0.05 mg P L^{-1}) and hypertrophic (2 mg N L^{-1}, 0.2 mg P L^{-1}). These experiments were conducted under varying light intensities, spanning from heavily shaded to well-illuminated conditions, with four specific levels: 25, 67, 230, and 295 µmol m^{-2} s^{-1} PAR photon flux density. To maintain controlled conditions, the plants were cultivated in moderately cold and warm water (21.5 and 27.5 ± 0.5 °C) using a temperature-regulated water bath.

Our research has unveiled significant differences in the phenotypic plasticity of these species. Notably, select findings from these species pair comparisons have already been published (**Szabó et al. 2019** in Plant Biology, **Szabó et al. 2020** in Frontiers in Plant Science, **Koleszár et al. 2022** in Ecology and Evolution). Currently, we are diligently working on a comprehensive analysis that encompasses all the data from these species, although this study is currently in progress and experiencing some delays. The overarching objective of this study is to address the question of how freshwater plant communities may be impacted by projected climate change.

It is worth noting that all the raw trait data collected in our research has been made publicly available in a trait database paper by **Sonkoly et al. (2023)** in Scientific Data. Additionally, these data have been used in our revision of plant life-form classifications, including aquatic plants, see **Botta-Dukát et al. (2023)** in Acta Botanica Hungarica.

1.2 How alien species affect community assembly and alter ecosystem functioning (i.e. biomass production)? How alien and native communities differ in its functional diversity, redundancy, phylogenetic diversity and species diversity?

Functional diversity indices that summarize trait variation between organisms within communities are widely used tools in community ecology. Although intraspecific trait variation is often an important component of the total variation, traditional functional diversity indices neglect this component. Unfortunately, a recent publication by Carmona et al. does not contain any guidance on pooling data before fitting TPDs. A common challange is that reliable fitting of TPDs needs larger sample size than usually used. This larger sample size can be reached pooling of data comes from different localities. If the TPD is the same in these localities, pooling results in a more reliable estimation. Moreover, if pooled data represent different TPDs, the fitted distribution is an artefact. In our study (**Botta-Dukát & Lukács 2021** in Global Ecology and Biogeography) we suggested an algorithm for the automated selection of optimal pooling. It is based on fitting Gaussian mixture models and model selection using Bayes information criterion (BIC). The new algorithm was able to select the optimal pooling of data, which is illustrated using artificial data. Analysis of field data examples showed that optimal pooling is often not obvious: both merging all data and analysing measurements from each locality separately might result in unreliable estimates. To test its applicablity we preperad an other study where we investigated the effects of intraspecific trait variability on functional diversity indices.

The loss of plant functional diversity associated with biological invasion is a main subject of invasion biology, but still understudied in case of aquatic plants. In a publication (see **Koleszár et al. 2023** in Hydrobiologia) we delt with this issue, calculated functional richness, evenness, divergence and community-weighted mean trait values of aquatic plant communities for 20 plots, half invaded and half non-invaded by alien species, in a thermal effluent of West Hungary. Three leaf traits were considered to explain how alien species alter ecosystem function. We differentiated interspecific and intraspecific trait variation and investigate its effect on the community-level functional diversity. We found that alien species invasion causes significant changes in the functional composition of aquatic plants whereby alien species become able to absorb light more efficiently, which will directly enhance their increased biomass production. Our study highlights the importance of local adaptation, showing that calculating functional diversity with global pooling of trait measurements causes significant over- or underestimation of functional diversity indices. Consequently, this can potentially result in erroneous conclusions regarding the impact of invasion.

1.3. Are there any differences in long distance dispersal capability between alien and native congeneric macrophytes?

The expansion of alien plant species is of global concern, yet our understanding of their dispersal mechanisms is limited. In a study (**Lovas-Kiss et al 2023** in Freshwater Biology) we address the potential of alien plant seeds to disperse via ingestion, transport and egestion in waterfowl (endozoochory). Based on their general rapid expansions, we expected alien plant species to have several advantages for endozoochory compared to native plant species. We hypothesised that seeds of alien species would have higher passage rate, longer gut retention times, higher germinability after gut passage and shorter time-to-germination after egestion by waterfowl. In order to test our hypotheses, we compared the endozoochorous dispersal ability of six pairs of congeneric alien and native wetland plant species in a feeding experiment with mallards (*Anas platyrhynchos*). We focused on differences in seed survival, gut retention time, germinability and time-to-germination. In the analyses we corrected for seed shape and volume as these seed traits are known to have important effects. With gut passage, alien species had higher passage rates and germinated slower, whereas native species had shorter retention times and greater germinability. Controlling for seed traits did not alter these conclusions, but seed traits affected all aspects of the endozoochory process. This suggests that alien species may have particular traits correlated with a higher endozoochory potential. Among control seeds, alien seeds germinated faster and their germinability was higher than natives. Seed traits explained differences in germinability and time-to-germination in control seeds. Seeds of alien plant species have traits that correlate with successful endozoochory. This may provide alien species with a competitive advantage over native plant species by ensuring higher endozoochory rates in new environments, potentially enabling their rapid expansions. Our study underlines the important role of seed traits in the endozoochory potential of alien and native plant species, notably through their influence on retention time and germination.

The feeding experiment mentioned above presented us with a valuable opportunity to explore the potential for alien species to spread more effectively in different scenarios. Leveraging the same birds, we conducted a subsequent feeding experiment to assess the dispersal capacity of eggs belonging to invasive (Prussian carp) and native fish (Common carp) species. Fish have somehow colonized isolated water bodies all over the world without human assistance. It has long been speculated that these colonization events are assisted by waterbirds, transporting fish eggs attached to their feet and feathers, yet empirical support for this is lacking. Recently, it was suggested that endozoochory (i.e., internal transport within the gut) might play a more important role. Our study (**Lovas-Kiss et al. 2020** in PNAS) identified an overlooked dispersal mechanism in fish, providing evidence for bird-mediated dispersal ability of soft-membraned eggs undergoing active development; morover implies that invasive fishes have an advantage on long-distance dispersal. While we acknowledge a significant departure from the initial project aims on the organism groups, it's important to emphasize that the question we are now addressing remains closely interconnected with our overall research objectives.

Endozoochory by waterfowl is generally important for a broad range of angiosperms, most of which lack a fleshy fruit. This dispersal function contributes to the formation and maintenance of plant communities and may allow range shifts for plant species under global change. However, our current understanding of what seed or plant traits are important for this dispersal mechanism, and how they relate to variation in waterbird traits, is extremely limited. In a publication (see **Almeida et al. 2022** in Frontiers in Plant Science) we addressed this question using a unique dataset identifying the plant species (aquatic, wetland and terrestrial) whose seeds are ingested by 31 different waterfowl species in Europe. We used RLQ and fourth-corner analyses to explore relationships between (1) bird morphological and foraging strategy traits, and (2) plant traits related to seed morphology, environmental preferences, and growth form. We then used Generalized Additive Models to identify relationships between plant/seed traits and the number of waterfowl species that disperse them. Of course, this study not investigate specifically the differences between native and alien species, but have an importance on how seed and vegetative traits affect the chance of bird mediated dispersal. We found that the number of waterfowl vector species (up to 13 per plant species) increases for plants with greater soil moisture requirements and salinity tolerance, reflecting the inclination of most waterfowl species to feed in coastal wetlands. Our findings underline the importance of waterfowl dispersal for plants that are not strictly aquatic, as well as for plants associated with high salinities. Furthermore, our results reveal a soil moisture gradient that drives seedbird interactions, in line with differences between waterfowl groups in their microhabitat preferences along the land-water continuum. This study provides an important advance in our understanding of the interactions that define plant dispersal in wetlands and their surroundings, and of what plants might be affected by ongoing changes in the distributions of waterfowl species.

DEVIATION FROM THE ORIGINAL PLAN

Initially, our project set out to address more questions pertaining to the introduction and naturalization phases of biological invasion. However, we have encountered certain challenges and constraints that have led us to reconsider the scope of our project. Some of these questions have proven to be overly ambitious and cannot be fully answered due to technical (not enough quality data gained, the closure of Herbarias during COVID) or personal limitations (lack of lab time need for genetical analyses). This notably includes inquiries related to the phylogenetic relatedness, climatic analyses and the floristic review of helophyte alien species in Hungary; all of which have become unattainable within the research timeframe. However, our focus shifted towards providing a more comprehensive examination and elucidation of the other pertinent questions. The modification in the project's focus has not had any impact on the budget table.

CONTINUATION OF THE PROJECT

The project has produced a wealth of data that will require processing in the coming months. We are currently in the process of conducting a thorough evaluation of climate change-induced plasticity in both native and alien aquatic plant species, with the aim of drafting a manuscript in the near future. Throughout the evaluation of the project, we've set up many new research questions. These questions are closely tied to our mesocosm experiment and primarily revolve around the role of initial species diversity and heatwaves in shaping interspecific competition. These questions, and the mesocosm approach will be in the focal point of our future research.

PUBLICATIONS

Papers published in peer-reviewed scientific journals strictly related to the study aims and support of NKFI-FK 127939 Grant. Asterisk (*) indicates the researchers involved, and hashmark (#) indicates the students involved in the research project.

1. Lovas-Kiss Á., Navarro M.J., Vincze O., Löki V*., Urgyán R., Pallér-Kapusi F., van Leeuwen C., Green A.J. & **Lukács B.A*.** (2023). Alien advantage in endozoochorous seed dispersal of aquatic plants. – FRESHWATER BIOLOGY 68: 1703–1715. https://doi.org/10.1111/fwb.14154.
2. Koleszár G*., Szabó S*., Kékedi L.#, Löki V*., Botta-Dukát Z*. & **Lukács B.A*.** (2023) Intraspecific trait variability is relevant in assessing differences in functional composition between native and alien aquatic plant communities. – HYDROBIOLOGIA (Accepted) https://doi.org/10.1007/s10750-023-05313-4.
3. Sonkoly J., Tóth E., Balogh N., Balogh L., Bartha D., Bata K., Bátori Z., Békefi N., Botta-Dukát Z*., Bölöni J., Csecserits A., Csiky J., Csontos P., Dancza I., Deák B., Dobolyi K.Z., E-Vojtkó A., Gyulai F., Hábenczyus A.A., Henn T., Horváth F., Höhn M., Jakab G., Kelemen A., Király G., Kis Sz., Kovacsics-Vári G., Kun A., Lehoczky É., Lengyel A., Lhotsky B., Löki V.*, **Lukács B.A.*,** Matus G., McIntosh-Buday A., Mesterházy A., Miglécz T., Molnár V.A.*, Molnár Zs., Morschhauser T., Papp L., Pósa P., Rédei T., Schmidt D., Szmorad F., Takács A., Tamás J., Tiborcz V., Tölgyesi C., Tóth K., Tóthmérész B., Valkó O., Virók V., Wirth T. & Török P. (2023). PADAPT 1.0 – The Pannonian database of plant traits. – SCIENTIFIC DATA (Accepted).
4. Botta-Dukát Z.*, Bartha D., **Lukács B.A.*** & Pinke Gy. (2023). Adaptation of life-form categorization of Ellenberg and Müller-Dombois to the Hungarian flora. – ACTA BOTANICA HUNGARICA 65: 1–34. https://doi.org/10.1556/034.65.2023.1-2.1.
5. Koleszár G.*, **Lukács B.A.***, Nagy P.T. & Szabó S*. (2022). Shade tolerance as a key trait in invasion success of submerged macrophytes. – ECOLOGY & EVOLUTION 12: e9306 https://doi.org/10.1002/ece3.9306. *First two authors contributed equally.*
6. Almeida B.A., **Lukács B.A.*,** Lovas-Kiss Á*., Reynolds C. & Green AJ. (2022). Functional traits drive dispersal interactions between European waterfowl and seeds. – FRONTIERS IN PLANT SCIENCE 12: 795288. https://doi.org/10.3389/fpls.2021.795288
7. Botta-Dukát Z.* & **Lukács B.A.*** (2021). Optimal pooling of data for the reliable estimation of trait probability distributions (TPDs) – GLOBAL ECOLOGY AND BIOGEOGRAPHY 30 (6): 1344-1352.

8. Lovas-Kiss Á., Vincze O., Löki V*., Pallér-Kapusi F., Halasi-Kovács B., Kovács G., Green A.J. & **Lukács B.A*.** (2020). Experimental evidence of dispersal of invasive cyprinid eggs inside migratory waterfowl. – PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA (PNAS) 117: 15397–15399.
9. Szabó S.*, Peeters ETHM, Borics G.*, Veres Sz., Nagy P.T. & **Lukács B.A.*** (2020): The ecophysiological response of two invasive submerged plants to light and nitrogen. – FRONTIERS IN PLANT SCIENCE 10: 1747.
10. Szabó S.*, Peeters E., Várbíró G., Borics G. & **Lukács B.A.*** (2019). Phenotypic plasticity as a clue for the invasion success of the submerged aquatic plant *Elodea nuttallii*. – PLANT BIOLOGY 21: 54–63.

Papers published in peer-reviewed scientific journals partly related to the study aims and support of NKFI-FK 127939 Grant. These studies are not detailed in the final report.

1. Lukácsné Urgyán R**., Lukács B.A**., Fekete R., Molnár V.A.*, Nagy A., Vincze O., Andy J.G. & Lovas-Kiss Á. (2022). Plants dispersed by a non-frugivorous migrant change throughout the annual cycle. – GLOBAL ECOLOGY AND BIOGEOGRAPHY https://doi.org/10.1111/geb.13608
2. **Lukács B.A,** Molnár V.A., Mászáros A., Lovas-Kiss Á., Vincze O., Süveges K., Fekete R. &. Mesterházy A. (2020): The decline and recovery of populations of *Potamogeton coloratus* in Hungary. – PRESLIA 92: 73–86.
3. García-Girón J., Heino J., Baastrup-Spohr L., Clayton J., de Winton M., Feldmann T., Fernández-Aláez C., Ecke F., Grillas P., Hoyer M.V., Kolada A., Kosten S., **Lukács B.A**., Mjelde M., Mormile R.P., Rhazi L., Rhazi M., Sass L., Xu J. & Alahuhta J. (2023). Compositional breakpoints of freshwater plant communities across continents. – LIMNETICA 42: 291–301. DOI: 10.23818/limn.42.21

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