Main goals

Since limited information had been available on reeds of running waters, this project was carried out to study thoroughly the establishment and genetic and phenetic differences of reed stands developing in various riverine habitats and also to reveal the effect of the diversity of those stands on other organism groups. Our main questions included the following:

1. How do reed stands establish in different riverine habitats, what is their genetic diversity and what kinds of colonization processes occur?
2. What is the phenotypic (morphological, anatomical and chemical) diversity in those stands?
3. Internal (genetic) determinacy or external constraints (and what sort of them) have stronger effects on reed structure and growth?
4. How the investigated stands affect their biotic and abiotic environments?
5. Can we reveal relationships between the differences of reed stands and the distribution and abundance of zooplankton assemblages?

Sampling

Study sites were chosen along the Danube River; reed stands in (i) an artificially controlled side arm with low water level fluctuation (Soroksári-Duna); (ii) an oxbow with significant water level fluctuations related to the flood events in the main river channel (Nyéki-Holt-Duna); and (iii) a separated oxbow without any connection with the main channel (which is supported by inland inundation, Riha-tó).

At each site, a 300-500 m long sector of reed stands was investigated along three transects parallel to the shore (Transect I was taken along the shore edge, Transect II situated in the middle of the stand and Transect III at the open water edge). Distances between sampling points along all transects ranged from 25 to 35 m, therefore, depending on the given site and reed stand, approximately 45 sampling points per site were selected. For anatomical and morphological investigations, three reed shoots were randomly collected at each sampling point.

Genetic investigations

Clonal composition and genetic diversity of reeds were investigated by microsatellite techniques. Genetic examinations were completed by the RAPD (random amplified polymorphic DNA) method to determine intraclonal genetic heterogeneity driven by somaclonal mutations.

Phenetic investigations

During the project, all morphological and anatomical measurements listed in the research plan were accomplished. The following characters and general measurements were considered: culm density; biomass, the length of leaf blades; the maximum width of leaf blades; the length of leaf sheaths; the length of internodes; the diameter of internodes measured at the middle; the total number of leaf sheaths and internodes in each ramet; the mean length of leaf sheaths and internodes in each ramet; and the total stem length; internode wall thickness; the radial thickness of epidermis and subepidermal tissue; the radial thickness of aerenchyma channels, cortical sclerenchyma and parenchyma; the cross-sectional areas of the aerenchyma channel and the innermost (largest) vascular bundle; the cross-sectional areas of the bundle sheath sclerenchyma, the phloem and the metaxylem vessels. Based on stem diameter data and measurements listed above, the cross-sectional area of the pith cavity, that of the internode wall and the total transversal area of aerenchyma channels were also calculated.

During the chemical analysis, pH, electric conductivity, oxygen content, redox potential, chlorophyll-a, temperature, $NH4^{+}$, PO_4^{3-}, NO_3^{-}, TC, TOC and TN of the water, and C, N, S and P content of the organs of the collected ramets were determined.

Compared to the submitted research plan, chemical analyses have been extended by ICP MS analyses of reed organs, water and sediment samples to determine the concentrations of further elements (Al, B, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Si, Sr, Zn).

Although basal internodes have been collected and stored for determination of amino acid content, we could not accomplish these investigations during the project.

Furthermore, our gas flow-meter broke down, preventing us to complete our *in situ* measurements on the convective flow rates of ramets.

Investigations on the diversity of some organism groups

In the last study year, the project was expanded to investigate thoroughly the diversity of communities living in the selected riverine habitats and to reveal potential relationships between the variability of reed stands and the abundance of different plant and animal species. Instead of using literature data, as it was undertaken in the research plan, we collected samples in the selected habitats for phytoplankton, zooplankton and prokaryote assemblages and we also assessed macrophyte communities.

Prokaryotic diversity was estimated both from the water and reed biofilm using the Denaturing Gradient Gelelectrophoresis (DGGE) method. The community DNA samples extracted for DGGE were involved in another project in cooperation with the Biological Research Centre, Szeged, and bacterial 16S sequences were analyzed using Illumina MiSeq platform. Thanks to that, we can collect metagenomic information on bacterial communities of the selected reed stands.

Main results

Our results demonstrate that water regime as external constraining factor may affect strongly the riverine reed stands in several ways.

Water regime determines the ratio of vegetative and reproductive propagation which in turn affects reed propagation and colonization. In floodplains, where the frequency and duration of floods are high and long enough to impede germination and seedling development, the chance of generative reproduction is greatly reduced. At the Nyéki Holt Duna, for instance, low genetic diversity (i.e. few and large clones) was observed not only in the deepest water but also at the higher elevations; indicating the primary role of vegetative propagation in these habitats.

Contrariwise, when an oxbow has completely lost connection to the main river, reeds develop in the same way as in regular lakes without any sign of clonal pattern which would otherwise indicate riverine origin of the habitat. Due to the static water depth gradient (in Riha-tó, for instance), generative reproduction is possible only at the

landward edge of the stand where genetic diversity is thus the highest. In these habitat types, the deep water is occupied by large clones, i.e. the most successful ones with high competitive ability. However, the result of competition between the clones (for instance, the number and arrangement of the most successful clones in the deepest water) may depend on the distance taken by the genets.

The third type of reed development was observed in the Soroksári Duna. High clonal diversity (many small genets) was detected in all transects that run parallel to the riverbank, which can be maintained only by generative reproduction. Germination in the landward edge is reasonable, since here the water level does not rise above the soil surface (Soroksári Duna is an artificially controlled side arm). The reason for generative reproduction in the middle and at the open water edge of the stand is that reed forms floating mats thus providing suitable conditions for germination and seedling development in the entire stand. It means that reed can exclude the effect of water level fluctuation in this way.

These three types of reed stands demonstrate that the clonal composition of riverine reed stands can indicate the water regime of the habitat.

These findings were published in the Q1 journal Ecological Indicators and also presented as lectures at two conferences:

Engloner A., Szegő D., 2016. Genetic diversity of riverine reed stands indicating the water regime of the habitat. Ecological Indicators, 61: 846–849. dx.doi.org/10.1016/j.ecolind.2015.10.037. (IF 3.9)

Engloner A., Szegő D. 2015. A vízjárás hatása a nád szaporodására és klonális diverzitására. Előadás, 10. Magyar Ökológus Kongresszus, 2015. augusztus 12-14. Veszprém. Absztrakt kötet p:51.

Engloner A., Szegő D. 2015. Különböznek-e a tavi és folyóvízi nádasok? 1: Genetikai diverzitás. előadás, LVII. Hidrobiológus Napok „Genetikai és molekuláris-biológiai kutatások jelentősége a hidrobiológiában” Tihany, 2015. október 7-9. Összefoglalók: p:15-16.

The low clonal variability (i.e. few large clones occupying large areas with a wide range of water depths) found in the Nyéki-Holt-Duna allowed us to investigate the dependence of intraclonal genetic heterogeneity on geographical distance and water depth. By studying the same reed DNA samples by RAPD technique, we found that vegetative spreading increases the number of somaclonal mutations in riverine reeds. This, however, may depend on the direction of colonization: the extent of increase seemed to be lower if reed spreads parallel to the shore than perpendicularly. We assume that the reason for this phenomenon is that spreading towards deeper water may require higher adaptation than colonization in the same water depth.

Based on the above findings, an article is under preparation: Engloner A., Szegő D., Krizsik V. Podani J.: Dependene of intraclonal genetic heterogeneity of reeds on the geographical distance and water depth, which will be submitted to the Ecological Indicators.

Water regime may also affect remarkably the phenetic morphological variability of riverine reeds.

Of the investigated variables, the number of leaf blades and side shoots was outstandingly different in the Nyéki Holt Duna in comparison to the other two sites; the fewest leaf blades and the largest number of shoots were recorded in this habitat. On the basis of internodes, reed ramets from the Nyéki Holt Duna also showed clear separation: most of the internode characteristics attained their maximum value in this habitat. Since the Nyéki Holt Duna is regularly flooded and water level can easily rise several meters, reed plants can be damaged during these flood events by the running water (leaf blades may be broken and lost). Furthermore, the temporarily submerged plant parts can be covered by suspended materials transported by the water and left behind during its recession. Both phenomena decrease the photosynthetic capacity of reed plants possibly bringing about the formation of new (side) shoots. Of the three habitats, stems with the longest and thickest internodes were recorded also in this stand, indicating the highest mechanical resistance. The thickness of internode wall and most of the tissues constituting it was also the largest in these internodes.

Regarding the habitats separately, internodes were the thickest and longest in Transect I and Transects I-II in the Nyéki Holt Duna and the Riha-tó, respectively. The weakest separation of transects was revealed in the Soroksári Duna; Transect III was between the other two transects with remarkable overlap. Most of the anatomical features reached the largest extension in the thickest internodes, i.e., for instance, in Transect I, in the case of the Nyéki Holt Duna.

In habitats where river bed morphology provides either static or, due the water level fluctuations, changing water depth gradient (i.e. Riha-tó and Nyéki Holt Duna), larger internodes developed at lower elevations (in Transects I-II at Riha-tó and Transect I at Nyéki Holt Duna where water depth was 50-170 and 45-85 cm, respectively, at the time of sampling) than in transects with water depth $\leq$ 0 cm. At the deepest transects the longest internodes of ramets occurred close to the bottom. These tendencies, however, were not detected in Soroksári Duna where reed forms floating mats, that is, the effects of water depth gradient or water level fluctuation are excluded.

These results were presented at the 33rd Congress of the International Society of Limnology and the 58th Conference of the Hungarian Hydrobiologists:

Engloner A.I., Szakály Á. 2016. Morphological and anatomical diversity of reed stands developed in different water bodies of the Danube River. 33rd Congress of the International Society of Limnology, Torino 31 July - 5 August. Book of abstracts p. 355.

Szakály Á., Engloner A., 2016. Különböznek-e a tavi és folyóvízi nádasok? 2: Morfológiai és anatómiai diverzitás. Előadás, LVIII. Hidrobiológus Napok „Hosszútávú hidrobiológiai kutatások a Kárpát-medencében" Tihany, 2016. október 5-7. Összefoglalók: p. 16.,

and an article will be submitted to Aquatic Botany: Engloner A.I., Szakály Á. Morphological and anatomical diversity of reed stands developed in different water bodies of the Danube River.

Chemical investigations confirmed that the nutrient content of reed may depend on the morphology of river banks (i.e. on water depth at which reed develops). In deep water, the uptake of some minerals can be impeded by anoxia, which is common in lakes. However, anoxia may occur not only in habitats that completely lost connection to the main river and became a lake-like habitat (the oxbow Riha-tó) but also, for instance, in the Nyéki-Holt-Duna where water level fluctuates regularly.

Furthermore, the variance of the elemental concentrations in the sediment samples was the lowest at Nyéki Holt Duna, which may indicate that floods, i.e. the suspended materials left behind during the recession of water, diminish the effect of river bank morphology on sediment chemistry.

The lowest element content in the sediment was found in the Riha-tó, because there is no chance for allochtonous element (nutrient, contaminant) input transported by the river.

These results were given as an oral presentation in the following conference:

Szakály Á., Óvári M., Engloner A., Záray Gy. 2015. Folyami élőhelyen nőtt közönséges nád (Phragmites australis) elemanalitikai vizsgálata. In: Adányiné Kisbocskói N., Demeter Á., Farkas E., Kardos Zs., Nyitrai L., Simonné Sarkadi L., Wölfking J. (szerk.) Magyar Kémikusok Egyesülete 2. Nemzeti Konferencia, Hajdúszoboszló, 2015.08.31-09.02., ISBN 978-963-9970-57-1, p.102.

Preliminary chemical results were presented in two MSc theses:

Gere Dóra (2016) A közönséges nád (Phragmites australis) folyóvízi állományainak elemösszetétel vizsgálata. (Examination of the element content of riverine reed stands (Phragmites australis). Szakdolgozat (MSc), Vegyész Mesterszak, ELTE TTK Kémiai Intézet Analitikai Kémiai Tanszék. Témavezető: Engloner Attila, Belső konzulens: Óvári Mihály. Pp. 48.

Stefán Dávid (2016) Folyami nádasok üledékének elemanalízise. (Elemental characterisation of the sediment of riverine reed stands.) Szakdolgozat (MSc), Vegyész Mesterszak, ELTE TTK Kémiai Intézet Analitikai Kémiai Tanszék. Témavezető: Engloner Attila, Belső konzulens: Óvári Mihály. Pp. 50.

The manuscript by Engloner A., Stefán D., Gere D., Dobosy P., Németh K., Szakály Á., Záray Gy., Óvári M. entitled “Chemical differences between reed stands developing in riverine habitats with different water regimes“ is being prepared and will be submitted to Microchemical Journal.

Of our investigations concerning on the diversity of some organism groups living in the riverine habitats, results on macrophytes have been processed and published.

During the project, a new method to estimate plant abundance data was developed. In aquatic macrophyte ecology, species abundance is usually estimated by cover values expressed on the ordinal scale, however, there has been increasing demand for three-dimensional estimates of plant abundance. To extend ordinal cover data into three dimensions, the new formula considers the vertical developmental types of plants. In this formula, a parameter k is used with three different values reflecting three groups of macrophytes, namely the "free floating leaved"; "rooted, floating leaved" and "submersed leaved" species. By using the new method, inappropriate conversion and evaluation of ordinal abundance data occurring frequently in the literature may also be avoided.

The new method was presented at a conference and in a published article:

Engloner A. 2014. Új módszer vízi makrofitonok relatív abundanciájának becslésére. LVI. Hidrobiológus Napok: A hidrobiológiai kutatások eredményei és gyakorlati hasznuk, Tihany, október 1-3. Szerk: Bíró P., Reskóné Nagy M., Kiss Keve T. p12.

Engloner A.I. 2015. Proposal for estimating volume based relative abundance of aquatic macrophytes. Community Ecology 16:33-38.

The OTKA Research Grant made possible to investigate macrophytes not only in the selected study sites, but also in the main Danube channel and we revealed correlations between macrophyte occurrence and environmental characteristics. According to our results, the mostly perennial, rooting species prefer the gravelly habitats, while the non-rooting, free-floating macrophytes occur mainly in the sandy stretches. Based on current

velocity and Secchi transparency, these stretches seemed to provide “more lotic” and “rather lentic” habitats. It was also revealed that the closer are the river stretches to a water course discharging upstream the more free-floating aquatic plants occur in the main Danube channel.

These results were published in:

Engloner, A.I., Szalma, E., Sipos, K., Dinka, M. 2013. Occurrence and habitat preference of aquatic macrophytes in a large river channel. Community Ecology, 14:243–248. (IF 1.6)

During the project, all samplings for additional phytoplankton, zooplankton and prokaryote investigations were accomplished, but the microscopic identification of phytoplankton, zooplankton assemblages and data analysis are still in progress.

In riverine habitats, bacterial diversity is increased by reed stands: DGGE investigations revealed higher bacterial diversity in reed biofilm than in water samples in each reed stand. (The lowest variety of planktonic bacterial communities was found in Soroksári-Duna, where the water flows and can be mixed for most of the year.) On the basis of biofilm bacteria, Nyéki Duna and Riha-tó were similar to each other, while these two habitats separated well from Soroksári-Duna.

Metagenomic sequences of the samples were investigated by using two different approaches. At first, community composition was investigated by similarity based binning using Megan. For this purpose, the sequences were aligned to Silva database using MALT aligner, resulting in a total of 6189680 alignments, or 65860 alignments in each sample on the average. As an alternative approach, for the network analysis OTU picking was carried out using Qiime software suite. Altogether 257106 OTUs (as read sets having at least 97% sequence identity) were found across the whole set of samples and these were annotated using the GreenGenes database. The metagenomic data are being processed.

Summary

Our project provides the very first data on the colonization and the genetic and phenetic differences of reed stands developing in various riverine habitats. It was demonstrated that water regime as external constraining factor may affect riverine reed stands strongly and several ways. It determines the ratio of vegetative and reproductive propagation, therefore colonization processes and genetic diversity in riverine reed stands can differ from those in lakes. However, when an oxbow has completely lost connection to the main river, reeds develop in the same way as in regular lakes without any sign of clonal pattern that would indicate riverine origin of the habitat. Furthermore, we found that vegetative spreading increases intraclonal genetic heterogeneity which, however, may depend on the direction of colonization.

It was also found that water regime may affect remarkably the phenetic (morphological, anatomical and chemical) variability of riverine reeds. Compared to the submitted research plan, our examinations were expanded by some further investigations.

The diversity of some organism groups living in the selected riverine habitats was also investigated in order to reveal the potential relationships between the variability of reed stands and the abundance of different plant and animal species. During the project all additional sampling and lab works were accomplished, but data evaluation is still in progress. A new method was developed to estimate plant abundance: the new proposed formula considers the vertical developmental types of plants.

In addition to published work, further papers are under preparation which will be submitted to Q1 journals.