UNIVERSITY OF SZEGED
Faculty of Science and Informatics
Department of Ecology
Doctoral School of Environmental Sciences

TRUE BUGS (HETEROPTERA) AS A CONSERVATION TOOL IN HABITAT ASSESSMENT AND MONITORING: CASE STUDIES ON THE PANNONIAN SALINE GRASSLANDS

PhD dissertation

JELENA ŠEAT

Supervisor

DR ATTILA TORMA

Szeged, 2022

1. INTRODUCTION

Europe has a long history of various extensive farming traditions, resulting in the development of mosaic-like landscapes consisting of semi-natural habitat patches and agrarian lands. European farmlands have been attractive to many species and have been successfully supporting local biological diversity for centuries, and today the existence of some plants and animals is completely dependent on the farmlands. In the European Union, farmlands of conservation importance make up 40% of the total area included in Natura 2000. Rich biodiversity here is usually related to low productive agricultural systems like extensively used semi-natural grasslands, among which are saline pastures.

Saline or halophytic habitats of the Pannonian Region encompass the whole spectrum of different types of habitats, from grasslands and wetlands to alkaline (soda) lakes. These saline habitats form the most continuous inland salt-affected landscape in Europe covering more than 210,000 ha. All saline habitats of the Pannonian Region are listed in Annex I of the Habitats Directive by its code name 1530 *Pannonic salt steppes and salt marshes (Council Directive 92/43/EEC). These habitats are marked as areas of high conservation importance due to their restricted geographic distribution and unique taxa which cannot be found anywhere else in Europe. Unfortunately, human activities in Europe caused a huge loss of saline habitats and nowadays remaining 20% of these habitats is of ancient origin. The uniqueness of saline grasslands is the fine-scale zonation of vegetation with around 50 distinct plant communities. At the same time, plant diversity in those communities is relatively species-poor consists mostly of several

characteristic halophytes and salt-tolerant grasses. Compositional mosaicism of vegetation in saline grasslands is driven by microtopography, seasonal changes in astatic groundwater, and salt content in the upper layer of the soil. Although wide areas of Pannonian saline grasslands in Hungary are comparatively well taken care of, small grassland fragments at the edge of the ecoregion experience greater pressure and accelerated change in their original biodiversity (Rabitsch, 2012).

In the last two decades, terrestrial true bugs have been increasingly used as model organisms and bioindicators in ecological studies. Most of these experiments have been studying the effects of grassland management, landscape heterogeneity and habitat quality on true bugs as surrogates of local biodiversity. Duelli & Obrist (1998) ranked true bugs as one of the most suitable indicator groups for the evaluation of habitat quality and approximation of biodiversity in European farmlands.

Most of the terrestrial true bugs are phytophagous species with remarkable species richness in grasslands and other open grassy habitats. The herbivory of true bugs (with different degrees of specialization) makes them sensitive to any change in vegetation composition, structure or quality. Taxonomy and systematic relationships of most of the European true bugs are stable. They also have a very diverse and broad range of bionomic features (e.g. body size and shape, wing and leg length). These insects can be sampled easily by standardized methods, plus, the cost of collecting and storing them is low. Achtziger *et al.* (2007) considered true bugs as favourable indicators in assessments related to nature conservation because they have a high indicator value and comparatively low processing effort.

2. AIMS

True bugs have a good potential to become a staple group in all kinds of biodiversity and habitat assessments of the European mainland where the true bug fauna is well-known. The recognition of rare, endemic, and endangered true bugs can raise the value of protected sites or assign conservation significance to unprotected ones. Their numerous species have vast ecological functions and their response to environmental changes can help to create fine-tuned and specific management according to local needs. The following aims of this thesis were addressed by using true bugs as bioindicators:

(i) Assessing the conservation status of saline grasslands at the edge of the Pannonian Region and identifying the key elements of true bug community structure in saline grasslands (Study 1);

(ii) Assessing the effects of grazing as the most applied management type on biodiversity of saline grasslands (Study 2);

(iii) Assessing the importance of drainage canals as secondary habitats in biodiversity conservation of saline grasslands (Study 3).

3. MATERIAL AND METHODS

Study 1: <u>True bugs of saline grasslands at the edge of the Pannonian Region</u>

For a pioneer work on saline true bug fauna in the northern Serbian province of Vojvodina, 138 locations were visited and samples were collected from 2015 to 2020. Numerous sampling sites covered a variety of habitats and

vegetation types (i.e. primary and secondary saline grasslands, meadows and steppes, saline marshlands), but *Artemisia* salt steppes were the main focus of the study. Terrestrial true bugs were sampled by sweep-netting, but in 2015, night sampling was also carried out.

In addition, 17 saline pastures were selected as sampling sites to study nested patterns of true bugs. Following criteria were considered during the site selection: (i) only grasslands with typical *Artemisia* steppe vegetation were sampled, (ii) exclusively grazed parts of grasslands were selected, and (iii) parts of grasslands with homogenous plant cover were selected (vegetation approx. 5–20 cm high without shrubs and trees). True bugs were sampled by sweep-netting every 3–4 weeks from April to October.

Three different sampling procedures were applied for this part of the study, varying in sampling effort and methodology: (i) sweep-netting along a single 20 m long transect per site in 2015, (ii) sweep-netting along with three parallel 30 m long transects per site in 2017, (iii) lastly, in 2015, the total inventory of true bugs was applied. In the last sampling procedure, consecutive samplings along random paths inside a buffer zone of approx. 100 m around central points of the sampling sites were performed. All three sampling procedures were repeated seven times, i.e. seven sampling occasions for every procedure.

For the study on nestedness, vegetation was also sampled in a 5 m × 5 m plot per sampling site in May–June and July–September periods. The standard methodology of the Braun-Blanquet cover-abundance scale was used for the vegetation survey. Landscape features of sampling sites surrounding were derived from satellite images with the help of QGIS tools;

five land cover classes were distinguished in the landscape: grassland, arable land, shrubs and trees, orchard and 'uninhabited area' for terrestrial true bugs.

Collected specimens of adult true bugs were identified up to species level. The conservation status of species in the countries of the Pannonian Region mostly follows the IUCN Red List categorization.

The dataset on true bugs of the selected seventeen pastures was analyzed for nestedness in the form of both binary and weighted matrices (except total inventory data). To analyze binary matrices, NODF (Nestedness metric based on Overlap and Decreasing Fill) and spectral radius metrics were used to measure the degree of nestedness and on weighted ones, WNODF (Weighted NODF) and spectral radius were applied. To test the statistical significance of nestedness in the binary matrices, Swappable–Swappable and Cored–Cored null models were used and for weighted matrices, Binary Shuffle and Row Column Totals Average null models were used. Requirements for choosing null models were that they should conserve the size (number of rows and columns) and fill (percentage of filled cells in a matrix) of an original matrix. Spearman's rank correlation method was applied to test possible correlations between the site ordering and assessed true bug and vegetation data, as well as, assessed landscape features of sampling sites.

Study 2: <u>True bugs in the assessment of saline grassland management</u>

The study was conducted in the Körös-Maros National Park in southern Hungary and one of its Natura 2000 and nationally protected areas, Csanádi puszták. The dominant open semi-natural habitat type in the region is a loess steppe, but saline vegetation developed in depressions of solonetz soils. In

the past an extensive area of loess steppes was ploughed and transformed into arable land, however, some localities like Csanádi puszták still preserve the original vegetation of saline grasslands. Wet (alkali meadows) and dry (*Artemisia* salt steppes) saline grassland patches cover 22.1% and 26.5% of the protected area, respectively.

A sampling site was defined as a 50 m × 100 m fenced area (hereafter experimental plot) with an inner ungrazed area and extensively grazed grassland outside the fence. In total, 12 sampling sites were surveyed, 6 in each saline grassland type (wet and dry grasslands). Each experimental plot was divided into two equal sub-plots (50 m × 50 m) to implement two different collecting methods for true bugs (sweep-netting and pitfall-trapping) without them being interfering with each other. The sampling of true bugs was conducted in four different regimes: (i) in the centre of an experimental plot (ungrazed centre), (ii) on the inner sides of a plot (ungrazed edge), (iii) on the outer sides of a plot (grazed edge) and (iv) at a distance of 25 m from a plot (grazed centre). True bugs were firstly sampled in Montág puszta in mid-May 2018. The second sampling was done in Királyhegyesi puszta in mid-June 2018.

To detect possible edge effects and to analyze the response of true bugs to grazing in different grassland types, generalized linear mixed models were applied. Species richness, abundance and community weighted mean trait values of true bugs were used as response variables. Vegetation type (wet *vs*. dry grassland), management treatment (grazed *vs*. ungrazed) and location of a sample (edge *vs*. centre) were used as explanatory variables, whereas the experimental plot was considered as a random effect. Interactions of the main effects were also incorporated into models. For the

visualization of true bug communities in dry and wet saline grasslands, non-metric multidimensional scaling was applied as the ordination method. To detect species associated with a certain vegetation type or with grazed/ungrazed parts of grasslands, indicator species approach was applied.

Study 3: The role of drainage canals in preserving saline grassland true bugs

The study area covers part of the Danube-Tisza interfluve of central Hungary and overlaps with the protected area of Kiskunság National Park. This region is known for its well-preserved sandy habitats and landscapes like forest-steppe and sand dunes, however, the substrate here is diverse, thus certain zonality of the soils can be recognized. The coarse sand prevails in the central zone of the region, saline loam along the bordering rivers (Danube and Tisza), and peaty loam (fen substrate) between the sandy and saline zones. According to dominant soil types, the main open habitats present here are fens, sandy grasslands, saline grasslands, and marshlands.

The first type of sampling site was a 200 m long section of an agricultural (bordering cropland) or a grassland (bordering grassland) drainage canal. In total 60 canals were surveyed, 30 of each surrounding landscape matrix type. In consistence with soil distinctions, three habitat types were recognized in canals: fen, saline habitat and sandy habitat (20 of each type). The second type of sampling site was a 5 m × 200 m transect in the grassland area (hereafter reference transect) parallel to every grassland canal. Plant species were recorded in 8 × 1 m^2 plots per sampling site. Each canal bank was assessed for reeds and woody vegetation abundance on both sides of the canals' transects with expressed maximum coverage of 400 m (2 × 200 m). The sampling of true bugs was done alongside a 200 m canal

section or reference transect. One sample contained specimens swept from four evenly spaced 25 m long subtransects in a row (i.e. 4 × 25 sweeps). To record seasonal changes in true bug communities, three sampling periods were designated in May, July and September of year 2018.

Standardization of the species richness scores of canals to habitat-specific average reference species richness scores was performed. The result of standardization was ‘species excess‘ (expressed in %), a value that represented proportional excess or deficit of species richness compared to habitat-specific reference averages. Linear mixed-effects models were applied to evaluate how environmental factors determine the true bug species richness (i.e. species excesses as response variable). Explanatory variables were landscape matrix (grassland *vs*. agrarian), habitat type (fen *vs*. sandy *vs*. saline), size (large *vs*. small) and season as categorical ones, and abundance of woody vegetation and reeds as continuous. Canal identity was a random effect in the models. The mean score of species excesses in each level of the categorical and explanatory variables was also checked to see whether it differed from the reference level, i.e. from the score 0. For the analysis, reduced models were used including only one explanatory variable at a time and the random term of canal identity if multiple data were available for each canal.

For further analyses, data from different seasons were pooled to create joined species composition matrix of true bugs. Reference transects were treated as the third level of the surrounding landscape matrix. True bugs data from different habitats (defined by substrate and/or landscape matrix) were analyzed for dissimilarity in their community composition by permutational multivariate analysis of variance. RLQ and fourth-corner

analyses were applied to investigate how environmental factors shape true bug community composition and what habitat attributes are associated with certain traits of true bugs. To separate functional groups (i.e. trait syndromes) of true bugs, hierarchical cluster analysis was applied. ANOVA and Tukey's test were performed to validate the distinction of traits values among species clusters. Commonness index was calculated for all true bugs aiming to detect the rarest and the most common species in the dataset.

4. RESULTS AND DISCUSSION

Study 1: True bugs of saline grasslands at the edge of the Pannonian Region

In saline grasslands of northern Serbia, 210 true bug species were identified. The most important are records of new species for Serbian fauna: *Agramma ruficorne*, *Chlamydatus saltitans*, *Solenoxyphus fuscovenosus*, *Lygaeosoma anatolicum*, *Emblethis brachynotus*, Geotomus *punctulatus*, and *Phimodera flori*. Among many dwellers of saline habitats in Serbia, species strongly connected to salt-affected areas are of special interest in conservation and can be divided into three groups: (i) trophic specialists (*Solenoxyphus fuscovenosus*, *Antheminia varicornis*) always appear in combination with a host plant specialized to a salty soil (halophytes), (ii) habitat specialists (*Conostethus hungaricus*, *Henestaris halophilus*) do not have a preference for a single host plant or the host is not known and typically are present in *Artemisia* steppe vegetation, (iii) the last group consists of species that are highly associated with saline grasslands in the Pannonian Region (*Lygaeosoma anatolicum*, *Peritrechus meridionalis*, *Crypsinus angustatus*).

Regardless of their exclusivity and prioritization in conservation, saline specialists can be highly frequent and even dominant at a certain part of a season (e.g. *Conostethus hungaricus*) or in a certain vegetation type (e.g. *Solenoxyphus fuscovenosus*, *Antheminia varicornis*). On the other hand, 10 species recorded in saline grasslands in Serbia had in total three records altogether with historical and new data. Rare species deserve a special place in red lists and conservation planning; species in the data deficient category should be treated as threatened, following the precautionary principle.

All three sampling procedures (varying in sampling intensity) confirmed that true bug communities in saline grasslands are highly nested, so even the minimal sampling effort—collection of true bugs along single 20 m long transect—resulted in significant nestedness. Grass-feeders are the most abundant and the most represented in saline grasslands, they are the core of a true bug community and to some extent, their exclusion from the analyses could be considered as a reduced sampling effort. However, true bug communities without grass-feeders continued to be nested, which means grass-feeders were not responsible for this structural pattern, but less numerous polyphagous generalists were. This study also confirmed that a higher number of species (matrix size) and more frequent species occurrences in different sites (matrix fill) provided higher values of nestedness and a more steady pattern. The results of this study showed no correlation between grassland patch size and site ranking in nested matrices, which confirms the results obtained by other authors that nestedness is not necessarily affected by the habitat area. In the case of invertebrates who use space at a fine scale, nestedness is most probably caused by habitat nestedness as a result of differences in habitat quality or diversity of microhabitats.

Study 2: True bugs in the assessment of saline grassland management

The season has the strongest effect on true bug communities in different saline grasslands, and greatly influenced the effects of grazing as well, but the location of a sample (edge *vs.* centre) showed a minor effect on true bugs. Grazing was the main factor that determined the functional attributes of true bug communities in May, but in June, it affected their species richness and abundance. Removal of grass biomass by spring grazing was the most influential for the plant-dwellers in more productive wet saline grasslands, causing a shift in community composition to more drought-tolerant and smaller size species. Later in the season, the effects of vegetation type dominated in shaping true bug communities. In summer, dry grasslands compared to wet ones became much drier, warmer and with specific food sources for specialist herbivores. Plant-dwelling true bug communities of dry saline grasslands were on average represented by bigger and more polyphagous species. Specialized grass-feeders from dry grasslands in foraging fresh food moved to wet ones. All aforementioned habitat changes in saline grasslands greatly influenced communities of plant-dwellers, whereas ground-dwellers showed minor sensitivity to changes in vegetation cover caused by management or seasonal dynamics. Communities of plant-dwelling nymphs more or less mirrored patterns of plant-dwelling adults. Indicator species analysis showed the dominance of grass-feeders in both dry and wet saline grasslands. Recorded grass-feeding species appeared to have preferences for a certain grassland type and many of them were indicators of ungrazed parts as well.

Study 3: The role of drainage canals in preserving saline grassland true bugs

Landscape matrix, season and habitat (i.e. substrate) end up being the most influential factors in shaping species richness and community composition of true bugs in drainage canals. Other attributes of canals (i.e. canal size, species richness of plants and invasive plants, the abundance of reed, and woody vegetation) had a negligible effect. Agrarian canals had the highest number of species among saline habitats with many singletons. Grassland canals were more similar in species richness of true bugs to corresponding reference saline grasslands, but their community composition was distinctive. Remnants of salt marsh vegetation were more frequent in these canals than in agrarian ones and provided better support for marshland true bug fauna and saline specialists. Based on functional and life-history traits of true bugs, three trait syndromes were recognized which separated species from saline habitats into three species clusters. Species of A- and B-clusters were associated with open grassland-like habitats. A-cluster contained species of low dispersive abilities and with preferences for humid grasslands and saline marshlands, whereas B-cluster comprised highly mobile species of dry saline grasslands. C-cluster species were associated with agricultural canals and mostly consisted of bigger size habitat generalists with good dispersal abilities. Rare species in the study were mostly associated with reference grasslands and most saline habitat specialists were associated with the same habitats as well. In the end, grassland canals are better secondary habitats for saline habitat specialists, but agricultural canals accommodated more species and diversified local true bug fauna.

5. LIST OF PUBLICATIONS

MTMT Author ID: 30796299

Publications related to the PhD thesis:

Tölgyesi, C., Torma, A., Bátori, Z., Šeat, J., Popović, M., Gallé, R., Gallé-Szpisjak, N., Erdős, L., Vinkó, T., Kelemen, A.; Török, P. (2022): Turning old foes into new allies—Harnessing drainage canals for biodiversity conservation in a desiccated European lowland region. *Journal of Applied Ecology* 59(1): 89–102. DOI: 10.1111/1365-2664.14030 [**IF: 6.53**, **Scimago: D1**, number of independent citations: (MTMT): 0]

Šeat, J., Nadaždin B., Milić N., Ćuk M., Torma A. (2021): How steady is the nested pattern in saline grassland true bug communities? Effects of sampling effort and data completeness on nestedness. *Acta Oecologica* 110: 103670. DOI: 10.1016/j.actao.2020.103670 [**IF 1.674**, **Scimago: Q2**, number of independent citations: (MTMT): 0]

Šeat, J., Nadaždin B. (2021): True bugs (Heteroptera) of the Pannonic salt steppes and salt marshes in Serbia and their conservation status in the Pannonian countries. *Annales de la Société entomologique de France (N.S.)* 57(2). DOI: 10.1080/00379271.2021.1888155 [**IF: 1.111**, **Scimago: Q2**, number of independent citations: (MTMT): 0]

Other publications:

Kaur, H., Torma, A., Gallé-Szpisjak, N., Šeat, J., Lőrinczi, G., Módra, G., Gallé, R. (2019): Road verges are important secondary habitats for grassland arthropods. *Journal of Insect Conservation* 23: 899-907. DOI: 10.1007/s10841-019-00171-9 [**IF: 1.553, Scimago: Q1**, number of independent citations: (MTMT): 2]

Popović, M., Šašić, M., Medenica, I., Šeat, J., Đurđević, A., Crnobrnja-Isailović, J. (2017): Living on the edge: population ecology of Phengaris teleius in Serbia. *Journal of Insect Conservation* 21: 401-409. DOI: 10.1007/s10841-016-9922-6 [**IF: 1.562, Scimago: Q1**, number of independent citations: (MTMT): 0]

Cumulative impact factor (IF): 12.430

Co-author statement in connection with submission of PhD thesis

With reference to the Regulations of the Environmental Science Doctoral School of University of Szeged, statement from the Author in charge about Jelena Šeat's contribution in the shared work, which is already published and included in the PhD thesis of the applicant (Tölgyesi et al.: Turning old foes into new allies—Harnessing drainage canals for biodiversity conservation in a desiccated European lowland region. Journal of Applied Ecology 59 (1), 89-102, 2022), must be presented to the PhD Committee.

The Author in charge states that the published work, or the indicated part of the work, has not been and will not be used in another Ph.D. thesis.

20.06.2022. Dr. Tölgyesi Csaba