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Proposal of a standardized EDGG surveying methodology for orthopteroid insects

Andreas Hilpold[1]*, Philipp Kirschner[2] & Jürgen Dengler[3,4,5]

[1]Institute for Alpine Environment, Eurac Research, Drususallee/Viale Druso 1, 39100 Bozen/Bolzano, Italy; andreas.hilpold@eurac.edu

[2]Department of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria; philipp.kirschner@gmail.com

[3]Vegetation Ecology, Institute of Natural Resource Sciences (IUNR), Zurich University of Applied Sciences (ZHAW), Grüentalstr. 14, 8820 Wädenswil, Switzerland; juergen.dengler@uni-bayreuth.de

[4]Plant Ecology, Bayreuth Center of Ecology and Environmental Research (BayCEER), Universitätsstr. 30, 95447 Bayreuth, Germany

[5]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

*) corresponding author

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Abstract: Orthopteroid insects (*Caelifera*, *Ensifera*, *Mantodea*) are characteristic of grassland ecosystems. They are often key species in grassland food webs, and many species have very specific requirements concerning habitat structure. Thus, orthopteroid insects are valued and widely used indicators in grassland ecology. Here we propose a standardised surveying methodology for orthopteroid insects in EDGG Biodiversity Plots. A variety of methods to survey orthopteroid insects have been used in the past, most of them based on sweep netting along a defined transect. The method proposed here is also based on this principle, but additionally utilises an exhaustive search to capture total species richness and estimate the frequency of the surveyed species. The method can be used in any grassland survey that is based on EDGG Biodiversity Plot and similar sampling designs. It was tested during the EDGG Field Workshop 2019 in Armenia, and has proven its applicability in a wide range of different grassland types.

Keywords: Biodiversity monitoring; bush cricket; EDGG Biodiversity Plot; grasshopper; grassland; mantid; *Mantodea*; *Orthoptera*; standardised sampling.

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Introduction

Grasshoppers (*Orthoptera*: *Caelifera*), bush crickets (*Orthoptera*: *Ensifera*) and mantids (*Mantodea*), together referred to as "orthopteroid insects" characterise grassland ecosystems like few other arthropod groups. In the Western Palaearctic, grasshoppers occur in all types of grasslands from the salt marshes of the Pannonian depression up to alpine meadows. In most of these ecosystems orthopteroid insects represent the major part of the herbivorous arthropod fauna (Curry 1994) and as such, they play a key role in many grassland food webs (Belovsky & Slade 2018). Grasshoppers, for example, have a large impact on litter decomposition and nutrient cycling in grasslands and thus can have an effect on plant abundance and plant species composition (Belovsky & Slade 2000). Most Palaearctic orthopteroid insect species are polyphagous (e.g. grasshoppers are all herbivores) or euryphagous (e.g. bush crickets are often canivorous or omnivorous). Whilst having a generalist diet, many grasshopper and bush cricket species are sensitive to habitat structure and microclimate (Ingrisch & Köhler 1998; Gardiner & Dover 2008), which renders them ideal ecological indicators for grassland ecology (Fartmann et al. 2012). Integrating abundance data and species composition has, for example, shown to be useful in assessing succession (e.g. Marini et al. 2009; Fartmann et al. 2012) or disturbance (Bhaskar et al. 2019).

In general, the assessment of orthopteroid insects in biodiversity studies is easy to carry out in comparison to other groups of invertebrates. Orthopteroid insect species can usually be identified in the field by morphological characters and/or species-specific vocalizations (e.g., Coray & Thorens 2001; Roesti & Rutschman 2020). Since they have relatively uniform annual cycles, one survey per year is in most cases sufficient to get representative data from a single site.

Here we suggest a survey methodology that can be applied in all kinds of grassland habitats, but may also be suited for

other kind of open habitats, such as grain fields or vineyards. It is already used in the Biodiversity Monitoring (BDM) South Tyrol, Italy (BMS; https://biodiversity.eurac.edu). It is based on previous suggestions of Dengler et al. (2016) and Kühnel et al. (2017). It is also particularly suitable in combination with the standardised EDGG multi-scale sampling methodology for vascular plant, bryophyte and lichen diversity (Dengler et al. 2016; 2020). It has been successfully applied in the EDGG Field Workshop in Armenia (Aleksanyan et al. 2020).

Survey design

Most surveys of orthopteroid insects are done via sweep netting along a transect (Gardiner et al. 2005). Box quadrat sampling, a method in which enclosures are placed onto the patch to be surveyed, has also been used. However, in most instances, these methods produce results that are statistically indiscernible (Gardiner & Hill 2006). The survey method proposed here is designed to match the size of the EDGG Biodiversity Plots (Dengler et al. 2016). This is to account for habitat heterogeneity, and to enable comparability between both survey methods. We suggest to use the diagonal of EDGG Biodiversity Plots as survey transect (= 100 m² plots = 10 m × 10 m = 14.14 m diagonal). By doing so, all parameters assessed in the standard EDGG vegetation survey, such as vegetation height, cover of different layers, topographic and soil variables as well as plant community composition (for details, see Dengler et al. 2016) can directly be used as covariates when analysing the animal data. This will enable the detection of orthopteroid insect species that have very specific habitat preferences, such as those that occur on locally confined, small patches of open soil (e.g. *Oedipoda caerulescens*), or those which are mainly found in shrubs or tall tussocks of herbs such as some bush cricket species (e.g. *Polysarcus denticauda*).

Dengler et al. (2016), based on the first implementation of *Orthoptera* sampling in EDGG Biodiversity Plots (see Kühnel et al. 2017), suggested sampling grasshoppers and bush crickets by sweep netting along a transect in the 14.14 m diagonal of the Biodiversity Plot before the botanical survey. However, according to our experience, such short transects are not sufficient to representatively capture the species richness in the surveyed plots (cf. Kühnel et al. 2017). Whilst such short transects can be sufficient to assess the species richness in dense, mesic grassland types with high grasshopper abundances, they fail to do so in grassland types with low vegetation cover. In such cases, we observed that grasshoppers on bare soil easily escape sweep netting. This has

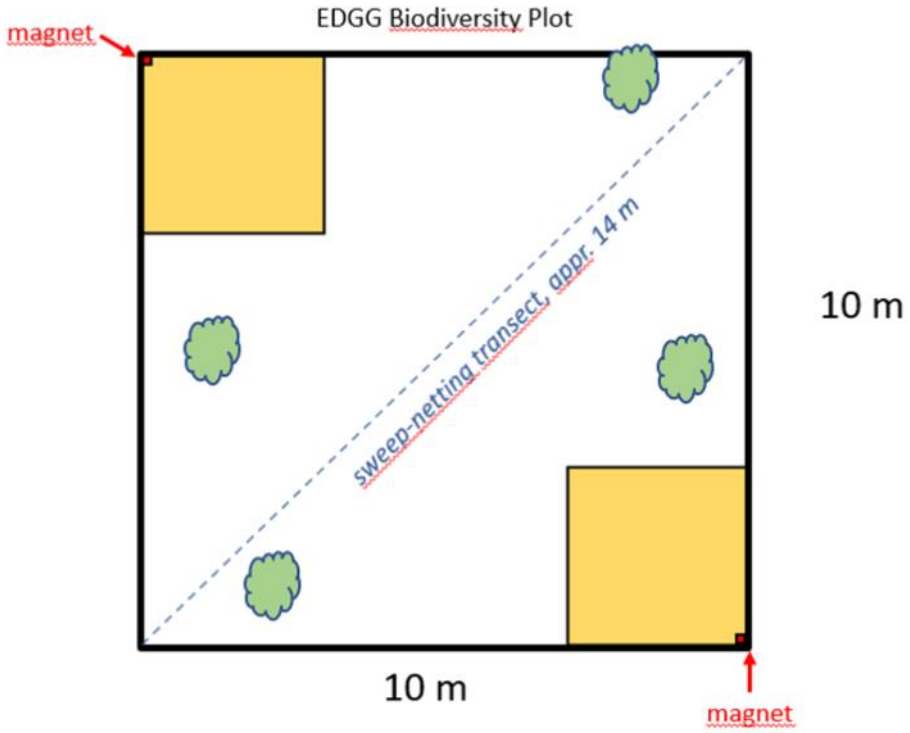

Fig. 1. Scheme for survey of orthopteroid insects. The survey is started by sweep-netting the diagonal transect, follwed by an exhaustive search in the 100 m² plot. It is important to check shrubs and grass tussocks for *Ensifera* species and sites with bare ground for certain *Caelifera* species (e.g. *Tetrix* spp., *Oedipoda* spp.). The yellow squares show the 10-m² subplots within the EDGG Biodiversity Plots in which the vegetation surveys are conducted.

also been shown for other species that tend to dwell e.g. in grass tussocks (Gardiner & Hill 2006). Whilst selecting a longer transect might be a way to address this problem, such an approach might at the same time render incongruence with the surveyed parameters of the EDGG vegetation plots. Depending on the type of surveyed grassland, increasing the transect length might still be an option. For example, in plots that have a vegetation cover below 50%, a second diagonal could be sampled. Such an approach showed to be efficient in assessing changes in grasshopper community composition in disturbed subtropical grasslands (Bhaskar et al. 2019). Being aware of the advantages and caveats of transect methods, we suggest using a combination of transect sweep netting and exhaustive search as the standardized grasshopper sampling method for EDGG Biodiversity Plots (Fig. 1).

In such an exhaustive search, the grasshopper surveyors should be able to assess the species richness comprehensively within the EDGG biodiversity plot. This should be done via additional sweep net beats and a detailed search by eye within the 100-m² EDGG biodiversity plot. In an exhaustive search, survey time needs to be standardised, and should, based on our experience, not be longer than 30 minutes. However, it might be shorter, if the surveyor concludes that the grasshopper community of the site is exhaustively assessed (e.g. in sparsely vegetated grassland types). Foremost, this exhaustive search should assure that the full heterogeneity of a plot is assessed, and no species are missed. If there is more than one surveyor, the maximum survey time should be divided by the number of surveyors. Once the species list is complete, a frequency class is assigned to every species (Table 1). The frequency class is based on the number of single animals encountered in the plot. An accurate counting is, especially for abundant species, only possible during the transect survey. An exact counting of individuals within the exhaustive search is prone to double counting and therefore not meaningful.

Phenological issues

In contrast to many other animal groups, sampling orthopteroid insects is relatively easy. In most cases a single survey in mid-summer, when all species are represented by adult individuals, will be sufficient to assess the full species spectrum. However, the presence of adults is crucial, as many taxa cannot be determined to species level in their larval stages. Thus, the period in which to survey orthopteroid insects needs to be adapted correspondingly.

In grasslands of the planar to colline belts, the presence of at least some adults can be expected as early as late June or July for most species. In steppe-like grasslands of the montane belt, adults may occur later, i.e. by the end of July (e.g. *Chorthippus mollis*) (Zuna-Kratky et al. 2017). In subalpine and alpine grasslands, occurrence of adult specimens cannot be expected before the beginning of August. However, a few genera deviate from this pattern: some crickets (e.g. *Gryllus campestris*) overwinter as subadults and in high summer adults tend to be rare. The same holds true for many groundhoppers (*Tetrix* spp.), with the ideal sampling period is probably being mid to late July until September. It should be noted that *Gryllus* and *Tetrix* can often be identified as larvae, which means that a survey in mid or late summer should cover all grasshopper species.

As emphasized, a single survey between July and September might be sufficient to assess species richness and abundance of orthopteroid insects. However, EDGG grassland plots are frequently surveyed earlier (e.g. June or early July for steppe-like grasslands, May to Mid-July for hay meadows). If a postponement of the vegetation survey to a later period of the year is not possible (due to phenological reasons), we recommend splitting the surveys according to an appropriate time. In such a case, an exact localization of the plots in course of the vegetation survey is crucial. Thus, we recommend locating the plot with GPS coordinates and additionally, if compatible with the management of the habitat, also with additional signals (e.g. colour signs or sticks) or magnets buried in the soil. In the BDM South Tyrol, the survey of vascular plants and grasshoppers are mostly conducted separately; vegetation survey is conducted from April to August, grasshopper survey from the end of July until the beginning of September.

For expeditions, where a splitting of survey periods is not feasible, we suggest organising the vegetation surveys as late as possible. For alpine habitats for example, a vegetation survey in August is still possible. In Mediterranean habitats, grasshopper phenology is even quicker, and a vegeta-

Table 1: Frequency classes used in the sampling.

Class	Criteria
I	1 individual. In addition there should be no sign of more individuals of the same species outside of the plot.
II	2–5 individuals in the plot; if you have only one individual in the plot, but you have observed more of them close to the plot give also II
III	6–10 individuals
IV	11–20 individuals
V	21–50 individuals
VI	More than 50 individuals

tion survey in June, when both grasshoppers and plants are in a stage that allows identification, will be possible without missing a significant number of species. This was confirmed by our experiences during the EDGG Field Workshop in Armenia 2019 (Aleksanyan et al. 2020): In the end of June and in the beginning of July, most species were present with at least some adult individuals that allowed an identification to species level. Of course, in some cases a determination to the species level within every genus was not possible, e.g. *Platycleis*. However, the option for a delayed joint vegetation survey in favour of a more complete orthopteroid sampling also depends on the type of vegetation surveyed. In annual-dominated grasslands, for example, a late vegetation survey would likely miss a significant number of plant species.

Additional data

Data from pitfall traps or casual findings can be used to complement species lists. However, we emphasize that the suggested survey methodology is superior to other methods in representatively capturing the species richness of orthopteroid insects. In course of the BDM South Tyrol, findings from sweep-net surveying and from pitfall traps were compared. In most cases, pitfall traps only captured a fraction of the species richness observed via direct surveys. Inversely,in a few cases pitfall traps captured species that were missing in the direct survey data (pers. observation A. Hilpold)

The proposed method at a glance

General considerations

- The grasshopper survey includes grasshoppers and locusts (*Caelifera*), bush crickets and related groups (*Ensifera*) and mantids (*Mantodea*).
- The weather conditions are important for a successful survey of orthopteroid insects since most orthoptera species avoid singing at low temperatures. Preferably, surveys should be conducted between 9 a.m. and 5 p.m. However, this is highly dependent on the local climatic conditions and the surveyor has to decide from case to case if a survey is meaningful or not.
- The survey starts with sweep-netting along a transect that is marked by the diagonal of the Biodiversity plot. If the botanical survey is being conducted at the same time, the diagonal that does not include the two 10-m^2 vegetation plots that should be surveyed. If this is not the case, directionality is not relevant. The transect must be walked at a slow pace (approximately 2 km/h). Sweeping height depends on the height of the vegetation: where vegetation is low sweeping must be done directly at the ground surface; where vegetation is high (e.g. in mesic hay meadows) sweeping is done in the upper part of the vegetation layer.
- If only small 10 m² vegetation plots are surveyed, orthopteroid sampling might still be conducted. In such a case, the orthopteroid survey should be conducted using the extent of a biodiversity plot, using one corner of the small plot as a starting point for the sweep-net transect. In this case it is important to document transect directionality to ensure reproducibility.
- Ideally, the grasshopper survey should be conducted before the vegetation survey of the 10-m^2-plots or the 100 m^2-plots to prevent some species being chased away. In our experience, this effect was negligible for small and less mobile grasshoppers, but problematic for larger species with good flying ability. Alternatively, the grasshopper survey may be postponed until after the vegetation survey is completed.
- Juveniles caught in the sweep net should also be checked as those of many genera (e.g. *Mantis*, *Gryllus, Oecanthus* or *Pyrgomorpha*) can be identified already at an early stage.

Step 1: Transect

- Count every individual caught in the net. By opening the net a small cleft you can catch adult individuals first. Once the adult individuals are assessed they can be released or, if identification is doubtful, should be collected and preserved for later identification.
- Count juvenile individuals. If juveniles are very abundant all caught individuals could be transferred to some sort of transparent container that makes counting easier (after having removed the adults).
- Always note the most accurate taxonomic level that can be distinguished. Many groups can be identified in the larval phase, e.g. *Calliptamus* spp. If you cannot recognise the genus, note the family or sub-family. This could enable species assignment at a later stage after additional specimens have been caught or after examining material collected from the respective site.

Step 2: Exhaustive search

- Start with an acoustic assessment. Some species groups are much easier to distinguish by their songs than by their morphology (e.g. *Chorthippus biguttulus* group). The acoustic survey helps to find elusive taxa. Some bush-dwelling *Ensifera* are cryptic and very mobile and it is hardly possible to detect them with methods other than by song. If you are not familiar with the songs of each species (e.g. in expeditions), a mobile sound device (cell phone) is very helpful and helps to memorise the song (e.g., Roesti & Rutschmann 2020, for Central European *Orthoptera*).
- Carefully check patches with bare ground as certain species prefer this microhabitat type, e.g. *Oedipoda* spp., *Tetrix* spp.
- Check single shrubs or large herbs and grass tussocks for *Ensifera* and mantids.

- Carefully check small patches of short vegetation in the plot, as additional species might be restricted to such patches (e.g. *Omocestus haemorrhoidalis, O. petraeus*).
- Take no more than 30 minutes and divide this time by the surveyors present (e.g. two surveyors have 15 minutes each).

Step 3: Assigning frequency classes

- The frequency classes in Table 1 refer to adult and subadult individuals. If specimens are observed in an early larval stage that cannot be identified, it is recommended to assess their frequency class anyway, and on an as accurate taxonomic level as possible (e.g. *Acrididae* juvenil = IV). Such information could be informative in later analyses, if, for example, total orthopteroid frequencies between plots are compared.

Conclusions and outlook

With our proposal, we refine a previous proposal for standardized sampling of orthoptera species in EDGG Biodiversity Plots (Dengler et al. 2016; Kühnel et al. 2017). The method has proven to be suited for a wide range of grassland types, is fast and efficient and typically can be conducted during an EDGG Field Workshop or similar sampling campaigns on the same day as the vegetation sampling, thus making it logistically more feasible in remote areas. When the vegetation sampling is phenologically too early, plots need to be marked (nowadays EDGG Biodiversity Plots are marked with magnets by default) to allow for a later survey of orthopteroid species. Since EDGG Biodiversity Plots and EDGG Field Workshops are specifically aimed at standardised multi-taxon sampling for analyses of cross-taxon patterns and drivers of biodiversity, adding orthopteroid insects as fourth group next to vascular plants, bryophytes and lichens (Dengler et al. 2016) would be particularly valuable. This adds to previous attempts to integrate spider (Polchaninova et al. 2018) and leafhopper sampling (Filibeck et al. 2018) in a standardised way to the EDGG Biodiversity Plots, which allowed many comparative studies of biodiversity patterns and drivers between vascular plants, bryophytes and lichens (e.g. Kuzemko et al. 2016; Dengler et al. 2020). The joint analysis of multiple taxonomic groups from various trophic levels can be particularly insightful (Zulka et al. 2014).

However, the proposed method is not restricted to be used in conjunction with the EDGG sampling methodology for plant diversity in grassland vegetation, but it can be viewed as an efficient method that is universally applicable in standardised surveys and monitoring of biodiversty. In South Tyrol, the methodology is used for all assessed terrestrial habitats except for urban habitats and lake shores, where transect surveys are applied. In the case of forest sites the plot size is enlarged by a factor of 10. Results of the grasshopper survey of the first BMS field season in 2019 are in favour of the method's efficacy in capturing species diversity: 58% of known grasshopper diversity of South Tyrol could be captured after surveying 56 Biodiversity Plots that are distributed evenly over the region and represent most of the region's habitats (Hilpold et al. 2020).

Author contributions

J.D. had the idea of conducting standardised *Orthoptera* sampling on EDGG Biodiversity Plots and tested it with a student class. A.H. and P.K. implemented and refined the method during the EDGG Field Workshop in Armenia and in the South Tyrolean biodiversity monitoring. A.H. and P.K. led the writing of the article, while J.D. made smaller contributions.

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Appendix 1: EDGG Protocol for grasshopper survey (*Caelifera*, *Ensifera*, *Mantodea*)

Leg.:_____
Date: _____ Plot ID EDGG: _____ Alternative plot number _____
Weather conditions _____ Temperature : _____°C

	TRANSECT 14.14 m				
Nr.[1]	**Species/genus/family**	**# adult**	**# subad.**	**# juv.**	**Freq. class**[2]
	EXHAUSTIVE SEARCH (only additional species, not found in transect)				
	Species/genus/family	**Adult y/n**	**Subad. y/n**	**Juv. y/n**	**Freq. class**[2]

[1] Species richness in the plot. Every species should be counted only once. For example, if you have a subadult *Platycleis* sp. and an adult *Platycleis albopunctata*, count it only once, unless it is likely that they are two different species.
[2] Frequency classes: I = 1 individual, II = 2-5 ind.; III = 6-10 ind.; IV = 11-20 ind.; V = 21-50 ind.; VI > 50 ind.