



Demographic characteristics of populations of *Tofieldia calyculata* growing in three different habitat types – implications for species conservation

Tomáš Vlasta · Terezie Šimáková ·
Zuzana Münzbergová

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Abstract This study deals with *Tofieldia calyculata* (L.) Wahlenb., an endangered plant species associated with fen habitats. We compared the population dynamics of ten populations of the species in three different habitats (fens, wet slopes and alpine grasslands) in different mountain ranges and lowlands of Central Europe with the aim to propose appropriate management for declining lowland fen populations. We analysed the data using integral projection models. We also tested the best conditions for seedling recruitment by creating experimental sowing plots with four different management regimes. Most of the vital rates depended on the type of habitat. This strongly affected inflorescence damage and seedling establishment, with fen populations having more damaged inflorescences and lower seedling establishment, leading to their slow decline. Experimental disturbances and the removal of aboveground biomass and litter were necessary for seedling establishment

in fens. The management of the remaining lowland fen populations should focus not only on mowing, but also on litter removal and on the creation of disturbances to support seedling recruitment. Even a small proportion of seeds germinating in a disturbed plot can increase the population growth rate of fen populations to above 1. In contrast to fen populations, high seedling establishment and a low proportion of damaged inflorescences on wet slopes and alpine grasslands allows for high rates of generative reproduction, resulting in stable or growing populations. These populations currently do not need any specific management.

Keywords Fens · Habitat type · Integral projection models · Seedling establishment · *Tofieldiaceae*

Introduction

Calcareous fens and wet meadows are among the most endangered European habitats because they have experienced a sharp decline in the area and number of localities as well as their quality. A large part of these habitats has disappeared because of drainage and conversion into agricultural land. Other sites have changed into different habitat types through succession due to the abandonment of traditional management such as mowing (van Diggelen et al. 2006; Chytrý et al. 2020). Many plant species associated with these habitats are therefore currently highly

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T. Vlasta · T. Šimáková · Z. Münzbergová
Faculty of Science, Department of Botany, Charles
University, Benátská 2, Prague 12801, Czechia
e-mail: vlastat@natur.cuni.cz

T. Šimáková · Z. Münzbergová (✉)
Institute of Botany, Czech Academy of Sciences, Zámek 1,
Průhonice 25243, Czechia
e-mail: zuzana.munzbergova@ibot.cas.cz

endangered, for example *Gentiana pneumonanthe*, *Ligularia sibirica*, *Tofieldia calyculata*, *Liparis loeselii*, etc. (Oostermeijer et al. 1996; Gabrielová et al. 2011; Heinken-Šmídová and Münzbergová 2012; Urban et al. 2020). Another reason for the high vulnerability of species occurring in these habitats is that they are at the edges of their distribution in these habitats. Many of these species are considered (post-)glacial relicts (Pokorný et al. 2010; Hájek et al. 2011; Heinken-Šmídová and Münzbergová 2012). In the past, the survival of the species in these habitats was often supported by various traditional managements such as hay making, preparation of bedding for life stock or grazing (Middleton et al. 2006). As all these forms of management have recently been abandoned as unprofitable, so active management steps are needed to maintain the diversity of these habitats. Their proper planning should be based on detailed knowledge of the factors affecting the population dynamics of the species concerned.

Several previous studies have therefore focused on the population dynamics of various species of calcareous fens and wet meadows, including the role of environmental and habitat variability and different management regimes. These studies have shown that habitat productivity can serve as an important predictor of population performance, with better performance of populations in nutrient poor conditions due to less intense competition. This has been demonstrated for *Scorzonera humilis* by Colling and Matthies (2006) and for *Ligularia sibirica* by Heinken-Šmídová and Münzbergová (2012). Different management regimes have also been shown to explain the variability in the population dynamics in these habitats. For example, mowing has been shown to be beneficial for seedling recruitment in *Gladiolus imbricatus*, *Serratula tinctoria* and *Succisa pratensis* (Bühler and Schmid 2001; Bissels et al. 2004; Kose et al. 2019). Mowing has also been shown to support rapid population growth in *Dactylorhiza incarnata* (Schrautzer et al. 2011) and maintain optimal species composition of its habitats even in suboptimal conditions (Hájek et al. 2020). Grazing can also positively influence populations in fen and wet meadow habitats by litter removal and by increasing the availability of light, which leads to higher reproduction, as shown by Ågren et al. (2006) for *Primula farinosa*. The effect of grazing on individual growth and reproduction may, however, not always be positive (Bissels

et al. 2004; Brys et al. 2011; Evju et al. 2011; Kose et al. 2019). Removing the topsoil has been suggested as a very good management tool also for fen species because it removes nutrients and improves moisture and light conditions (Emsens et al. 2015; Klimkowska et al. 2015). Disturbances are often necessary for the successful seedling establishment of fen species such as *Parnassia palustris*, *Pinguicula vulgaris*, *Succisa pratensis*, *Tofieldia calyculata* (Stammel et al. 2006) and *Trollius altissimus* (Lemke et al. 2015). The absence of any management usually leads to succession of vegetation and to the decline of populations of the rare species restricted to these habitats.

Fen and meadow species are usually long-lived and their long lifespan enables them to survive in suboptimal conditions such as high competition in dense vegetation and high cover of litter preventing seedling recruitment for many decades forming so-called remnant populations. The deterioration of habitat conditions increases the importance of survival for the growth rate of these populations, as has been shown in *Gentiana pneumonanthe* (Oostermeijer et al. 1996), *Scorzonera humilis* (Colling and Matthies 2006) and *Ligularia sibirica* (Heinken-Šmídová and Münzbergová 2012). However, in the long term, without reproduction, these populations are doomed to extinction (Oostermeijer et al. 1996; Lemke et al. 2015).

Most studies exploring species of calcareous fens or wet meadows have focused on the population dynamics of species growing in these particular habitat types. However, many Central European fen species grow also in habitat of other types within their distribution range; for example, various mountain habitats (like alpine grasslands or wet slopes) where such species are usually more common, examples being *Parnassia palustris*, *Trollius altissimus* or *Gentiana verna* (Elkington 1963; Lemke et al. 2015). Some species which can grow not only in fen habitats, but also in mountain habitat types, have been studied, but only in lowland fens or meadows, for example *Arnica montana* (Vikane et al. 2019) or *Trollius altissimus* (Muncaciuc et al. 2010; Lemke et al. 2015). In an exceptional study, Maurice et al. (2012) studied populations of *Arnica montana* in mountain and lowland habitats of different types and found greater clonality and lower flowering in populations at lower elevations (Maurice et al. 2012). By comparing the dynamics of populations from different habitat types,

we can explore which vital rates are identical for each type of habitat and which are different, identifying the vital rates which may be targeted for supporting the growth of declining populations.

Here we describe the population dynamics of the long-lived perennial plant *Tofieldia calyculata* (L.) Wahlenb, a typical species having a distribution centre in the Alps with peripheral lowland fen populations. Lowland fen populations are currently strongly endangered in several European countries (Gabrielová et al. 2011). There is not much information about the population biology of the species in the literature. Several authors have described the habitat requirements of current lowland populations (Wisniewski 1992; Kolbek 2015; Sozinov and Kruchonok 2018) or the distribution of the species in the past and at present (Kaplan et al. 2015).

Stammel et al. (2006) investigated the effect of different types of management on the germination of peatland species, including *T. calyculata*. This research showed that the species is incapable of reproduction in abandoned areas. Of the species studied, the germination of the species was the most dependent on suitable microsites. The species germinated most on mown and trampled areas, but still very little compared to other peatland species. Other aspects of the population biology of the species have not been investigated so far.

We studied ten populations in three different habitat types (calcareous fens, wet slopes, alpine grasslands) in the mountains and lowlands of Central Europe. In all populations, we followed labelled plants in permanent transects for three or four years. We used these data to construct integral projection

models (IPMs) to describe the dynamics of the populations, to identify the extent of between-habitat variation in the population dynamics and to use this knowledge to identify the key parts of the life cycle differentiating presumably declining lowland populations from presumably stable mountain populations and suggesting optimal conservation strategies for the lowland populations of this species. Specifically, we ask the following questions: (1) What are the common features, differences and limiting factors in the population dynamics of *T. calyculata* in the three different habitat types? And (2) What conservation recommendations can be suggested based on the knowledge of the population dynamics in the three habitat types typical for the species?

Material and methods

Study species

Tofieldia calyculata (L.) Wahlenb is a small polycarpic perennial plant from the *Tofieldiaceae* family (Fig. 1). A single individual usually consists of several rosettes of ensiform leaves (mean $\pm$ SD: 3.59 ± 4.9 rosettes per plant in our data) that are connected by a short rhizome (Štěpánková et al. 2010). The species usually flowers between June and September. The inflorescence is a raceme with a number of yellow small 6-petal flowers (Štěpánková et al. 2010, Fig. 1). Its fruit is a capsule containing many tiny seeds (mean $\pm$ SD: 40.4 ± 13.7 seeds per capsule – our unpublished data). The plants are self-compatible and viable seeds develop even in the absence of pollination



Fig. 1 Individuals of *Tofieldia calyculata* of different sizes **a** – one-year-old seedlings, **b** – juvenile plant (three years old), **c** – adult vegetative plant, **d** – adult flowering plant.

(our unpublished data). Individuals of *T. calyculata* are easy to differentiate because clonal reproduction of the phalanx type is limited to only a few centimetres (Klímešová et al. 2017); new rosettes can arise from rhizomes in close vicinity of the mother plant, but usually remain connected to it. Plants of this species are very long-lived. Based on our field observations of the growth of individual plants, they can be expected to take more than ten years before flowering for the first time.

Tofieldia calyculata grows in a wide range of calcareous habitats – fens and wet meadows, wet slopes, wet rocks, alpine grasslands, wet pine woods, etc. (Štěpánková et al. 2010). The centre of its distribution range is in the Alps and in the Western Carpathians. In the mountains, the species usually grows on wet slopes, wet rocks and in wet pine woods and alpine grasslands. Lowland populations are currently restricted mainly to calcareous fens and wet meadows (Štěpánková et al. 2010; Kolbek 2015) with a few remaining populations on wet slopes and in pine woods (Wisniowski 1992; Kolbek 2015; Sozinov and Kruchonok 2018). Lowland populations are highly endangered. Their endangerment is caused by a combination of their location at the edge of the species' distribution edge, the direct destruction of many localities and the suboptimal conditions of the remaining localities (drainage, absence of management leading to succession and overgrowth by competitively strong grasses, shrubs and trees). In the past, the species was more common in lowland areas but has experienced a sharp decline of the number of populations (Kaplan et al. 2015; Kolbek 2015). For example, there were more than 50 populations on the territory of Czechia in the last 150 years (Kaplan et al. 2015). In 2022, by contrast, there were only four. Furthermore, only one of them is large, consisting of approximately 400 individuals (unpublished data). The species is nowadays protected by law in some European countries, including Czechia and Poland (Gabrielová et al. 2011), where the species has been studied.

Collection of population data

Ten Central European populations were studied. These populations were chosen to cover all the main habitat types where the species commonly grows, both in lowland and mountain areas (fens, wet slopes below the timberline and alpine grasslands, Table 1).

We tried to select populations where the environmental conditions are as similar as possible within one habitat type. The studied mountain populations on wet slopes and alpine grasslands currently have no obvious management (they may occasionally be grazed by cattle and sheep – Gosausee, Dachstein). The fen populations are more variable, either mown (Jestřebské slatě, Cikánský dolík) or unmown (Belianské lúky, Sosnowiec-Bory). The populations were located in two mountain (Alps and Tatra Mts) and two lowland (northern Bohemia, Czechia, and southern Poland) regions – Table 1, Fig. 2. The two fen populations (Jestřebské slatě, Cikánský dolík) in Czechia were studied between 2017 and 2021, and the remaining eight populations were studied between 2018 and 2021. For more detailed information about the populations, see Table S1 in the electronic supplementary material.

In each population, at least 150 individuals covering all life-stages (seedlings, small juvenile plants, bigger vegetative and reproductive plants) of the species as evenly as possible, if available, were labelled with plastic tags. For better re-localization of the plants in the subsequent years, the labelled plants were located also using coordinates along permanent transects. This enabled us to re-localize the plant in case the labels were lost (in approx. 1% of individuals). One to three permanent transects were established in each population. The position of each transect was selected to ensure covering the variability of the vegetation in the locality (e.g. places with dense vegetation, with mosses, with less dense vegetation and with bare soil). We labelled all plants usually up to 100 cm distance from the transect, but the area and also the length of the transect (between 3 to 20 metres) varied between sites due to different densities of plants in different localities. When we labelled 50 individuals of a given life-stage (seedlings and juveniles, bigger vegetative and reproductive plants, Fig. 1), we skipped further individuals of this life-stage in the transect to ensure a balanced representation of all life-stages (Münzbergová and Ehrlén 2005). We used these life-stages only to ensure the sampling of a sufficient number of small and large, as well as reproductive and vegetative, individuals within each population. In the subsequent analyses, we did not work with these life-stages, but with plant size as a continuous variable (see below for details). We were unable to distinguish between seedling and juvenile

Table 1 List of populations studied and their characteristics

Population (id)	Habitat type	Years	N	Region	Elevation [m a.s.l.]	Estimated population size*	Latitude [° N]	Longitude [° E]	Description of vegetation		
									Dominants of the vegetation	Herb layer cover	Tree layer cover
Jestřebské slatě (Jba)	Fen	2017–2021	214–231 ⁺	northern Bohemia (CZ)	260	100	50.6028497	14.6222692	<i>Molinia caerulea</i> agg., <i>Carex panicea</i>	81.25%	Mown
									<i>Molinia caerulea</i> agg., <i>Carex flacca</i>	80%	Mown
									<i>hostiana</i> , <i>Molinia caerulea</i> agg., <i>Carex flacca</i>	75%	Currently unmown
Cíkánský dolík (Cd)		2017–2021	30–159	northern Bohemia (CZ)	371	25	50.2706556	13.8978928	<i>Molinia caerulea</i> agg., <i>Carex flacca</i>	80%	Mown
									<i>hostiana</i> , <i>Molinia caerulea</i> agg., <i>Carex flacca</i>	75%	Currently unmown
									<i>hostiana</i> , <i>Molinia caerulea</i> agg., <i>Carex flacca</i>	75%	Currently unmown
Belianské lúky (Bel)		2018–2021	156–205	Tatra Mts (SK)	677	1,500	49.214371	20.391761	<i>hostiana</i> , <i>Molinia caerulea</i> agg., <i>Carex flacca</i>	75%	Currently unmown
									<i>hostiana</i> , <i>Molinia caerulea</i> agg., <i>Carex flacca</i>	75%	Currently unmown
									<i>hostiana</i> , <i>Molinia caerulea</i> agg., <i>Carex flacca</i>	75%	Currently unmown
Sosnowiec-Bory (Sosn)		2018–2021	76–161	Southern Poland	260	75	50.2760711	19.2722917	<i>Molinia caerulea</i> agg., <i>Carex flacca</i>	47.5%	Unmown for a long time
									<i>Molinia caerulea</i> agg., <i>Carex flacca</i>	47.5%	Unmown for a long time
									<i>Molinia caerulea</i> agg., <i>Carex flacca</i>	47.5%	Unmown for a long time

Table 1 (continued)

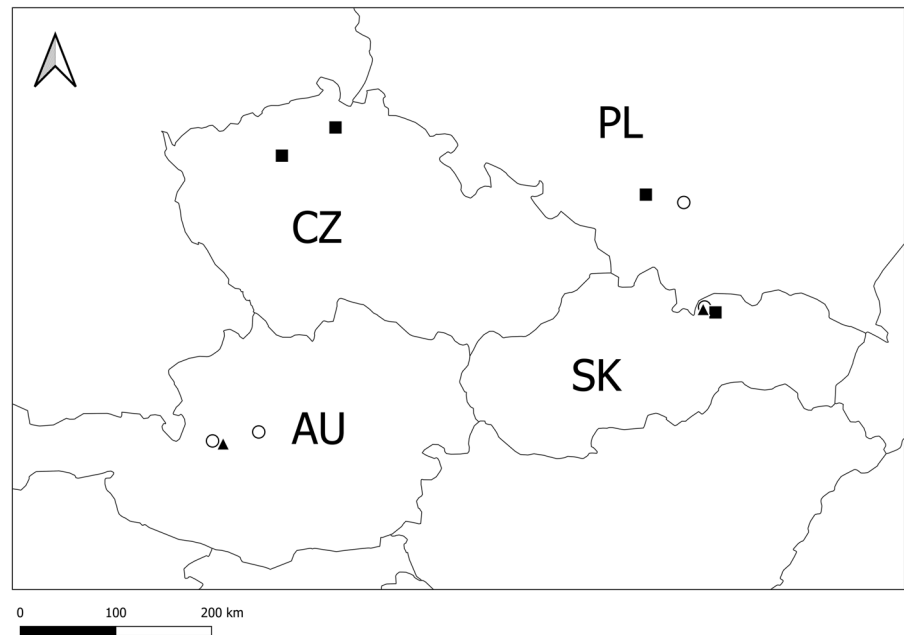
Population (id)	Habitat type	Years	N	Region	Elevation [m a.s.l.]	Estimated population size*	Latitude [° N]	Longitude [° E]	Description of vegetation		
									Dominants of the vegetation	Herb layer cover	Tree layer cover
Gosasee (Gos)	Wet slope	2018–2021	201–285	Alps (AU)	984	3,000	47.5243239	13.5105097	<i>Tofieldia calyculata</i> , <i>Picea abies</i> juv., <i>Fagus sylvatica</i> juv	41.25%	None, only occasionally grazed by cattle
									<i>Tofieldia calyculata</i> , <i>Calamagrostis varia</i> , <i>Sesleria caerulea</i>	61.67%	None
Weis-senbach (Wei)	Wet slope	2018–2021	175–251	Alps (AU)	750	350	47.6702111	14.1282278	<i>Erica carnea</i> , <i>Tofieldia calyculata</i> , <i>Calamagrostis varia</i> , <i>Sesleria caerulea</i>	61.67%	None
									<i>Calamagrostis varia</i>	28.25%	25%
Monkova dolina (Md)	Tatra Mts (SK)	2018–2021	179–244	Tatra Mts (SK)	1,000	150	49.25922	20.2309106	<i>Calamagrostis varia</i>	28.25%	None
Ojcow (Ojc)	Southern Poland	2018–2021	109–150	Southern Poland	365	1,500	50.2270342	19.8352011	<i>Tofieldia calyculata</i> , <i>Fragaria vesca</i> , <i>Leontodon hispidus</i> , <i>Prunella vulgaris</i>	42.5%	None
											24%

Table 1 (continued)

Population (id)	Habitat type	Years	N	Region	Elevation [m a.s.l.]	Estimated population size*	Latitude [° N]	Longitude [° E]	Description of vegetation		
									Dominants of the vegetation ^o	Herb layer cover	Tree layer cover
Dachstein (Dac)	Alpine grassland	2018–2021	171–310	Alps (AU)	1,788	1,500	47.5134189	13.6616417	<i>Dryas octopetala</i> , <i>Rhododendron hirsutum</i> , <i>Homogyne alpina</i>	69%	Occasionally grazed by sheep
									<i>Carex sempervirens</i> , <i>Dryas octopetala</i> , <i>Vaccinium gaultherioides</i> , <i>Pulsatilla alpina</i> , <i>Vaccinium myrtillus</i> , <i>Sesleria caerulea</i> , <i>Salix reticulata</i>	30%	None
Široké Sedlo (Ss)		2018–2021	180–214	Tatra Mts (SK)	1,825	350	49.2371867	20.213386			0%

N minimal and maximal number of labelled plants, CZ Czechia, SK Slovakia, PL Poland, AU Austria* estimated number of flowering individuals in the first year of the experiment, + individuals from sowing plots are not included, ° species with at least 5% of cover at least in one relevé; for more information about the populations studied, see the electronic supplementary material, ° species with at least 5% of cover at least in one relevé

Fig. 2 Map of the populations studied. Squares – calcareous fens, circles – wet slopes, triangle – alpine grassland, CZ – Czechia, SK – Slovakia, PL – Poland, AU – Austria



individuals; however, given that only seedlings newly found in the second year of the study were used to calculate generative reproduction, this does not affect our models (see below). The variability within flowering and non-flowering plants was quite large, from smaller plants (but not seedlings) to large old individuals with many rosettes, which allowed us to capture all possible sizes needed to parametrize our models (see below).

Since the second year, we searched for new seedlings and labelled them, too. The small plants that were found for the first time in the parts of permanent transects, where all plants had been labelled during the previous year, were regarded as new seedlings. We usually searched for new seedlings up to 100 cm from the transect, but the area varied between sites due to different seedling densities. The aim was to locate enough seedlings in an area which has been completely explored and still keep the task feasible. However, the searched area was selected to ensure covering the variability of the vegetation at the locality (e.g. places with dense vegetation, with mosses, with less dense vegetation and with bare soil).

We visited each locality every year. We tried to visit each locality approximately in the same period each year to be sure that the population was always in the same stage. We tried to visit the populations after flowering, during the fruiting period. For

mountain populations, this meant visiting them in the second half of July and the first half of August. We visited lowland populations, which flower continuously between June and September, in the second half of August and the beginning of September. Usually, almost all individuals were fruiting at the time of measurement with the exception of some individuals in lowlands and in alpine grasslands, where the individuals usually started flowering later than in mountain populations on wet slopes. Because of the high number of populations and four geographic regions, it was not possible to visit all ten populations at the same time.

Every year, we measured the length and width of the longest leaf (as an estimation of the leaf area) of each labelled plant and counted the number of vegetative and reproductive rosettes. For reproductive rosettes, we recorded the fate of inflorescence (flowering, damaged, fruiting). The cause of damage was probably herbivory (snails and possibly also ungulates) or drought. Unfortunately, we were not able to identify the specific causes of the damage for each plant, so we did not distinguish between the causes further.

To calculate the seed production, we collected five to ten fruiting inflorescences every year from each population if possible. And counted the seeds produced by these inflorescences. We calculated the

average number of seeds per capsule and inflorescence for each population and year.

Seed bank experiment

We tested if the species has a permanent seed bank at the Czech fen locality Jestřebské slatě. We did not test survival in the seed bank at the remaining localities because we visited the localities in Austria, Slovakia and Poland only once a year because they were hard to access for us. At the second Czech locality (Cikánský dolík), there are very few fruiting plants and removing too many seeds for the experiment could endanger the population. We tested if the species has a permanent seed bank by burying seeds in nylon bags into the soil at the locality (see Münzbergová 2004; Dostálek and Münzbergová 2013). The nylon used is a material with little holes, approx. $266 \times 162 \mu\text{m}$. By utilizing nylon for the bags, we are ensuring that we can easily find the seeds after several years, that the moisture levels inside them are similar to the surrounding soil and that the soil microbiota can enter the bags. Although the bags do not allow larger organisms to enter, still this is the best compromise between realism and relocation of these very tiny seeds that we are aware of, and it has been used in a range of previous studies (e.g. Dostálek and Münzbergová 2013; Rokaya et al. 2017; Moravcová et al. 2022).

We buried bags with 100 local seeds each into soil (approximately 10 cm below ground) in September 2017 at the Jestřebské slatě locality. Altogether, we buried 15 bags at three different locations within the locality (approximately 10 m apart). Every year in August, we dug out three bags, one from each of the three locations, and tested their germination in a growth chamber (the same conditions as described above). Only ten seeds germinated in 2018, that is, after one year in the soil. After two years in the soil, in 2019, the seed coats were already degraded and the seeds were almost decomposed. This indicates that the species does not have a permanent seed bank. We therefore do not consider the seed bank in any of the following analyses.

Analysis of population data

Analysis of single vital rates

Prior to analysis, we log-transformed plant size (= length of the longest leaf $\times$ width of the leaf $\times$

number of rosettes). Then we used it as a predictor of plant performance for modelling vital rates in subsequent analyses. We used the product of these three variables because it described the observed variation among the individuals. Based on our observation of the plants over time, we identified that the number of rosettes per individual increases over time. Especially within individuals having fewer rosettes, there is also large variation in leaf width and length, with younger individuals having narrower and shorter leaves. By contrast, the number of leaves within a rosette is quite constant and number of leaves was therefore not considered. Excluding the number of leaves also made recording plant size in the field much faster, especially in individuals with many rosettes. The data from the first season (2017) were not used in the model, because they were available only for two fen populations.

We modelled the effect of the type of habitat, year, plant size at time t and their two-way interactions on vital rates: the probability of plant survival at time $t + 1$, growth (size at time $t + 1$), the probability of flowering and of fruiting at time t and the number of flowering and fruiting rosettes at time t . We did not evaluate triple interactions, as their estimates were not reliable. We used generalized linear mixed-effect models with binomial distribution for the probability of survival, flowering and fruiting, with Poisson distribution for the number of fruiting rosettes, and a linear mixed-effect model for growth. The number of fruiting rosettes was only tested in plants that fruited whereas all the other effects were tested using the whole dataset. The population was set as a random factor in all analyses. The analyses were performed in R version 3.6.3 (R development team 2022) using packages 'lme4' (Bates et al. 2015) and 'lmerTest' (Kuznetsova et al. 2017).

Establishment rate was calculated as the number of newly established seedlings per number of fruiting rosettes in the parts of transects where all plants were labelled the previous year. This was calculated for each population and year. Then we tested the effect of habitat type on establishment rate and on the percentage of damaged inflorescences using the Kruskal–Wallis test. We selected the non-parametric Kruskal–Wallis test because the data were largely overdispersed and did not fit any standard distribution (Zar 1996). At the same time, this test is suitable for comparing multiple independent samples, in

our case represented by habitat type. Then we used the pairwise Wilcoxon test for a multiple comparison between habitat types with a BH correction for multiple testing (Benjamini and Hochberg 1995). Pairwise Wilcoxon test is a pairwise modification of the Kruskal–Wallis test that is suitable for pair-wise comparisons (Zar 1996). This test was performed in the base version of R version 3.6.3 (R development team 2022).

Integral projection models

To analyse the dynamics of the populations, we used integral projection models – IPMs. We used data from all individuals; only seedlings emerged in sowing plots were not included in this analysis. All analyses were performed in R version 3.6.3 (R development team 2022), using the methodology described by Merow et al. (2014) to create separate IPM kernels for each transition interval and population. IPMs use continuous plant size instead of discrete stages of the life cycle in classical matrix models. The IPM kernel is analogous to matrix in matrix models consisting of a survival/growth kernel and a reproduction kernel (Merow et al. 2014). Each kernel (survival, growth and reproduction kernel) represents a single generalized linear model predicting the given vital rate (i.e. survival). The overall IPM kernel is a model combining all the partial models into one complex model.

We modelled the relationship between log-transformed plant size at time t and vital rates: the probability of survival at time $t + 1$, growth (size at time $t + 1$), the probability of fruiting at time t and the number of fruiting rosettes at time t . The number of fruiting rosettes was only tested in plants which fruited. Because of the occurrence of inflorescence damage, only fruiting rosettes were used for all analyses within IPMs instead of flowering rosettes. An exception was made in the cases of two populations in alpine grasslands, where the plants had not fruited yet at the time of the visits. Because we did not see any signs of damage in these populations, we decided to regard them as fruiting.

In our models, the term ‘reproduction’ only includes generative reproduction whereas clonal ‘reproduction’ in our models is included under growth, because clonal reproduction is strongly limited and new rosettes remain part of the mother

plant. If new rosettes emerge, they remain part of the mother plant, resulting in an increase in plant size.

We used generalized linear models (GLM) with binomial distribution for survival and fruiting, GLM with Poisson distribution for the number of fruiting rosettes and the linear model for growth. We initially compared models including three different types of predictors – size only, size plus its second power, and size plus its second and third powers. Then we compared the models by AIC and BIC. As all the predictors fitted the data relatively well in the size only model and some of the higher-level models produced skewed predictions due to the low sample size in some parts of the range, we decided to only use size in all subsequent models.

In some cases, our data did not allow us to devise reliable functions for each year in each population separately. This happened when mortality was too low (< 10 individuals per year) or when the number of fruiting rosettes was too low (< 10 per year). In these cases, the respective functions were created using all the data from the given population (combining all the years), and these functions were then incorporated in all the year-specific models.

We set the maximum size (as the maximum size of the plants in each modelled population) of the plant in the model to avoid eviction (Williams et al. 2012). The mean and standard deviation of the size of new seedlings was calculated from new seedlings found at each locality across all years (because of their low numbers in some years in some populations). These parameters were used, together with the parameters from models describing vital rates and with establishment rate (described above), to create the survival probability function, growth function, fruiting probability function and reproduction function. These functions were used to make the kernel of survival and growth, kernel of reproduction and the full kernel combining both kernels. Finally, we calculated the population growth rate (λ), elasticity (= the proportional change of population growth rate for proportional change in some vital rate; Benton and Grant 1999) and stable size distribution (a size distribution in which the relative number of individuals in each size is constant; Hasting 2011) for each full kernel. We also calculated the stochastic growth rate of each population combining λ from all transitions. Calculation of a stochastic growth rate is a standard approach allowing us to combine models from

different transitions intervals together, specifically, in our case to predict the population growth rate of each population given the observed between-year variation (Münzbergová 2007). We performed this calculation using IPMPack version 2.1 (Metcalf et al. 2013).

Seed germination and seedling establishment

Seed collection and germination in growth chambers

The seeds for the experiments were collected several weeks before we performed the sowing and seed bank experiments. We did not distinguish between healthy and unhealthy seeds when sowing them, because the seeds are very tiny and it would be very hard and time-consuming. We tested the germination rate of the seeds from each locality and year in a growth chamber on filter paper in Petri dishes. First, we stratified the seeds by leaving them in wet dark conditions for six weeks (at 5 °C). Under these conditions, the most seeds germinated in the experimental study, and therefore we used these conditions for stratification (Maas 1989). The seeds were then germinated on filter paper in growth chambers. The length of the day and night was 12 and 12 hours, respectively. The temperatures were set to 20 °C during the day and to 10 °C during the night. The seeds were checked every week and germinated seeds were removed. The mean germination rate in growth chambers was 69.6%; for details see Table S2 in the electronic supplementary material.

Seedling establishment in field conditions – sowing plots

At the two fen localities with dense vegetation where almost no seedlings were found (Jestřebské slatě, Cikánský dolík), we also established sowing plots in the field to test what are the best microsite conditions for seedling establishment. We established 5–7 pairs of plots of 50 × 50 cm at each locality. The two plots in the pair were always established next to each other: One plot was sown whereas the second plot in the pair was a control and was unsown (see below). The different pairs of plots were approximately 5 m apart. At each locality, sowing plots were established in two different years (Jestřebské slatě – 2017 and 2019, Cikánský dolík – 2018, 2019) to cover the variability in

environmental conditions in different years. Each plot was divided into four subplots of 25 × 25 cm, simulating different management regimes/microsite conditions: (1) removal of all aboveground biomass and litter and manual disturbance of the soil surface using a spade (digging – dg); (2) removal of all aboveground biomass and litter to the bare soil but without severe disturbance of the soil surface (biomass – bs); (3) removal of aboveground biomass and litter but without disturbance of the soil surface and moss layer (moss) – establishment in the moss layer; and (4) mown plot (standard management of both localities – mown).

In sown plots, each subplot was sown in August and September with unstratified seeds collected from the same locality (see above). At the Jestřebské slatě locality, each subplot was sown with 200 seeds. At the locality Cikánský dolík, each subplot was sown with only 100 seeds because of the small number of fruiting plants at the locality. The control plots were left unsown to distinguish the germination of sown seeds from natural seed rain.

Data analysis

We analysed data from both localities (Jestřebské slatě, Cikánský dolík) and years. The number of seedlings in the control plots was very low (see the electronic supplementary material), so we decided to neglect it and not to subtract seedlings which emerged on unsown control plots from the number of seedlings on sown plots. Then we used a generalized linear model (GLM) to test the effect of locality, management regime and their interaction on the number of seedlings. We used a quasi-binomial distribution due to the high ratio of residual deviance to degrees of freedom. Similarly, we tested the effect of locality, management regime and their interaction on seedling survival (survival of seedlings to the second year) on sowing plots, using the generalized linear model with binomial distribution.

We tested the effect of year, alone and in interaction with management, on the germination and survival of seedlings to the second year for each locality separately, because data from the two localities did not come from the same years. We used the same models with the same settings as models for both populations described above.

Modelling population dynamics with presence of disturbances in fen populations

In the fen populations Jestřebské slatě and Cikánský dolík, where almost no seedlings were recruited because of dense vegetation, we also incorporated data from the sowing plots into the IPMs, modelling a situation with the presence of plots with a disturbed soil surface. For these models, we used the data from sowing plots with the bare soil regime (bs regime), which represented disturbances with the highest seedling establishment and survival. In these models, we modelled the establishment probability in the situation when 5–100% of seeds can germinate in bs plots. In IPMs, the mean and standard deviation of the size of new seedlings reflected sizes found on these plots. To estimate establishment probability, we first calculated the median number of seedlings established on disturbed plots (bs) per seed sown. Subsequently, we calculated the mean number of seeds per fruiting rosette and the expressed number of seedlings per fruiting rosette. We decided to use the median instead

of the mean for seedling numbers because the mean was strongly affected by a few outliers. For the 100% scenario, we assumed a 100% rate of germination in disturbed plots. For the 5% of disturbed plots scenario, we calculated the probability of establishment as if 95% of seeds fell onto non-disturbed vegetation and 5% onto disturbed plots. We constructed the IPM kernel and calculated the population growth rate (λ) for every situation.

Results

Analysis of vital rates

All vital rates increased with plant size (Fig. 3, Table 2) and were significantly affected by year. The survival rate was very high in all populations, years and habitat types (Fig. 3). Specifically, 84.77% of plants survived to the next year, in adult plants as many as 95.52% survived, and 76.01% of labelled plants survived after the three years of the

Fig. 3 Effect of size at time t on the probability of survival at time $t + 1$, on size at time $t + 1$ (growth), probability of fruiting at time t and number of fruiting rosettes in fruiting individuals at time t . The effect of size was significant for all vital rates (see Table 4 for details).

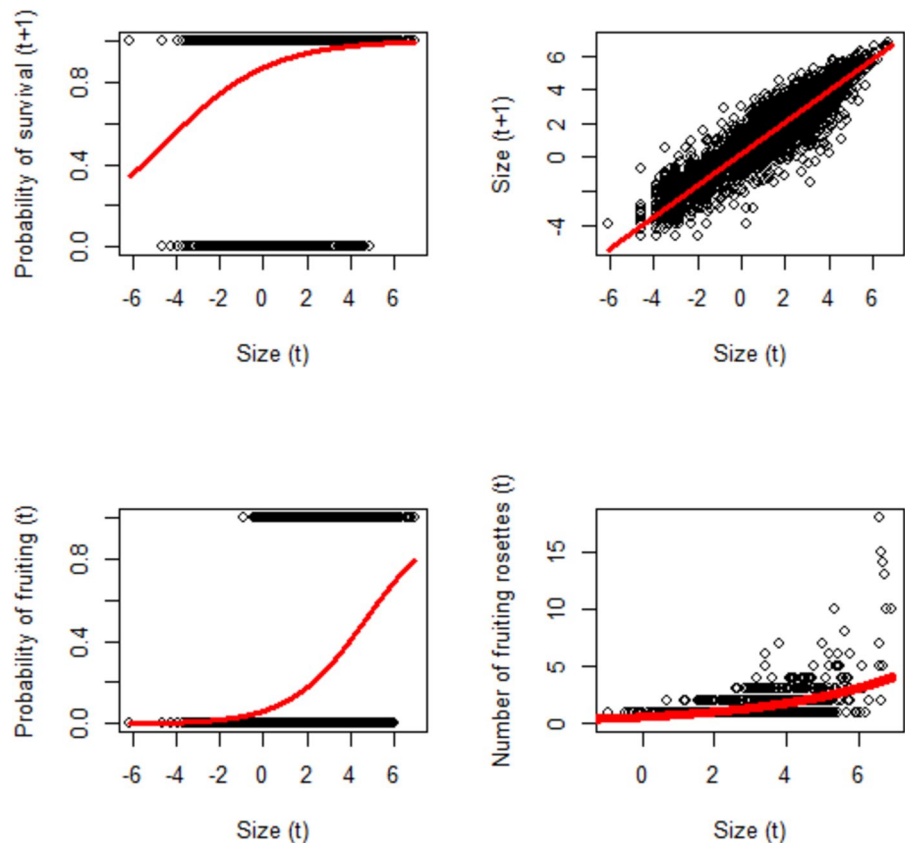


Table 2 Effect of habitat type, individual size, year and their two-way interactions on the vital rates of individuals of *T. calyculata*

		Probability of survival			Growth			Probability of flowering			Probability of fruiting			No. of flowering rosettes in flowering individuals			No. of fruiting rosettes in fruiting individuals		
		Deviance	P		F	P		Deviance	P		Deviance	P		Deviance	P		Deviance	P	
Habitat type	2	4.89	0.087		0.73	0.518		9.07	0.011		5.71	0.058		6.09	0.048		6.49	0.039	
Year	2	7.19	0.027		10.04	< 0.001		62.39	< 0.001		55.41	< 0.001		15.38	< 0.001		6.91	0.031	
Size	1	809.94	< 0.001		30030.36	< 0.001		2259.88	< 0.001		1586.30	< 0.001		412.21	< 0.001		180.50	< 0.001	
Habitat type : size	2	24.66	< 0.001		26.79	< 0.001		0.81	0.667		0.19	0.909		14.80	< 0.001		21.34	< 0.001	
Habitat type : year	4	56.66	< 0.001		4.38	0.002		15.54	0.004		6.60	0.159		4.69	0.32		2.07	0.72	
Size : year	2	62.39	< 0.001		21.73	< 0.001		10.02	0.007		0.17	0.921		12.63	0.002		2.15	0.341	

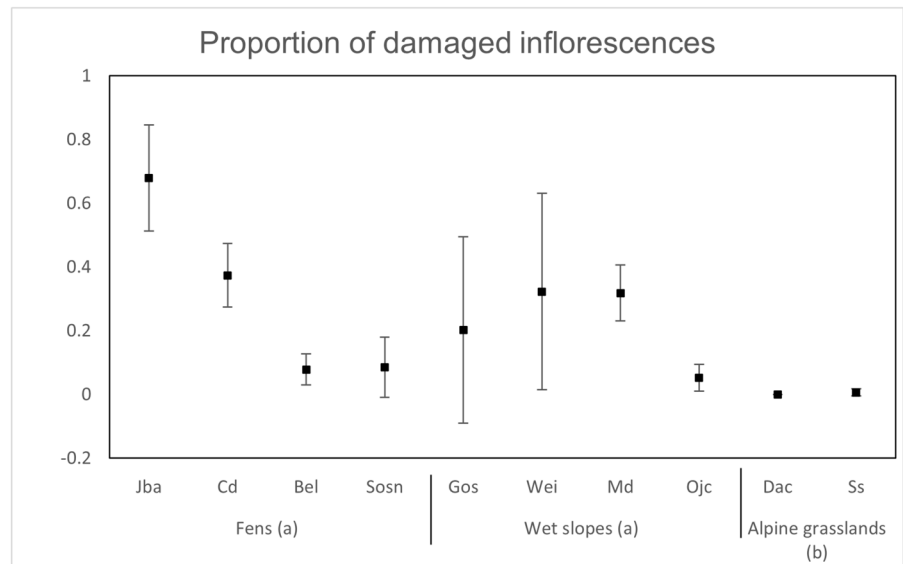
Data combining all years and populations were analysed using generalized mixed effect models with population as a random factor (see the Material and methods section for the specific distribution of each different dependent variable). Bold values are significant at $P \leq 0.05$.

experiment. The sole exception was the population at Sosnowiec-Bory where 39.7–51.5% of plants died each year. In most individuals, it was not possible to identify the cause of death. The observed causes of death included disturbances by animals and humans, dry conditions or flooding for seedlings and also competition from *Pinus mugo* and *Dryas octopetala* in alpine grassland populations. The effect of habitat type was marginally significant for survival (Table 2). The highest survival was observed in alpine grasslands – 92.2%, the lowest in fens – 82.5%.

The effect of habitat type was also significant for the probability of flowering, number of flowering rosettes, and number of fruiting rosettes, and marginally significant for the probability of fruiting (Table 2). Individuals from wet slopes and fens produced more flowering rosettes and on wet slopes also a greater number of fruiting rosettes than individuals from alpine grasslands. The type of habitat also had a significant effect on damaged inflorescences (Kruskal–Wallis test, $\chi^2 = 16.62$, $P < 0.001$, Fig. 4). The pairwise Wilcoxon test showed a significant difference between fens and alpine grasslands ($P = 0.001$) and between wet slopes and alpine grasslands ($P < 0.001$). There was no significant difference between fens and wet slopes ($P = 0.288$), although the highest percentages of damaged inflorescences were observed in fens (especially in the Jestřebské slatě population – 69.3% of all inflorescences in all years, maximum 90.4% in 2018, and in the Cikánský dolík population – 41.6%). There were almost no damaged inflorescences in alpine grasslands.

For the survival, growth and probability of flowering, the effect of habitat type was significant in interaction with year. The effect of habitat type interacted also with size for the probability of survival, growth and number of flowering and fruiting rosettes. As for survival, small individuals in alpine grasslands had higher survival compared to wet slopes and fens. Smaller individuals from alpine grasslands had more flowering and fruiting rosettes than individuals of the same size from wet slopes and fens. The effect of size also interacted with year for the survival, growth and number of flowering rosettes (Table 2). Higher survival of small individuals was observed in the 2019/2020 season in all habitat types; in the remaining seasons it was quite low in fens and wet slopes and high in alpine grasslands. In the 2018/2019

Fig. 4 Proportion of damaged inflorescences in each population and habitat type summarized across years (mean $\pm$ SD of year-specific data). Different letters indicate statistically significant differences between habitat types.



season, the plants flowered and fruited at smaller sizes than in the two remaining seasons in all habitat types.

The number of newly established seedlings per fruiting rosette was significantly different between habitat types (Kruskal–Wallis test, $P < 0.001$, $\chi^2 = 18.28$) with wet slopes and alpine grasslands having more established seedlings per fruiting rosette (wet slopes – mean = 1.88 seedlings per fruiting rosette; median = 1.61, maximum = 6.77, alpine grasslands – mean = 6.64, median = 6.61, maximum = 12.67) than fens (mean = 0.63, median = 0, maximum = 8.17). The difference was significant between all habitat types (pairwise Wilcoxon test, $P < 0.05$).

Integral projection models

The population growth rate was greater than 1 in almost all populations and transitions for wet slope and alpine grassland populations (Table 3) whereas it was smaller than 1 in all fen populations and years (Table 3).

We found high elasticities of survival (especially of medium and big individuals) in all populations and transitions in all habitats. In alpine grassland populations, survival of small individuals had high elasticity, too. In populations with high seedling establishment (mainly on wet slopes and alpine grasslands), we also found relatively high elasticity values of reproduction (Fig. 5).

The comparison of stable size distribution showed that fen populations had the highest proportion of large individuals whereas populations of wet slopes and alpine grasslands had a high proportion of small individuals such as seedlings and juvenile plants (Fig. 5).

Seedling establishment in sowing plots

Only five seedlings emerged on control plots, and the rest of seedlings emerged on sown plots (386 seedlings, electronic supplementary material). The mean percentage of established seedlings across all management regimes was 3.88% of sown seeds. The number of established seedlings was significantly influenced by the management regime and by the interaction of locality and management regime, but not by locality alone (Table 4, Fig. 6). The highest establishment was found in plots with bare soil (bs regime, Fig. 6), fewer seedlings were found on dug plots (dg regime) and in the moss regime (with the exception of the Cikánský dolík locality in 2019 with the highest rate of establishment in the moss regime). Almost no seedlings established in the mown regime at Jestřebské slatě (Fig. 6), but some seedlings were present in mown plots at the locality Cikánský dolík, where the vegetation is not so dense and enables seedling establishment even without disturbances. In a separate analysis of both populations, the management regime was highly significant in the population

Table 3 Population growth rates of the populations under study. The populations are divided by habitat type

Population (id)	Habitat type	Population growth rate (λ)				
		2017/2018	2018/2019	2019/2020	2020/2021	Stochastic growth rate
Jestřebské slatě (Jba)	Fen	0.947	0.976	0.878	0.937	0.963
Cikánský dolík (Cd)		0.848	0.951	0.980	0.978	0.928
Belianské lúky (Bel)		–	0.973	0.975	0.952	0.959
Sosnowiec-Bory (Sosn)		–	0.510	0.524	0.621	0.555
Gosauee (Gos)		–	0.993	1.080	0.953	1.004
Weissenbach (Wei)	Wet slope	–	1.161	1.011	1.015	1.114
Monkova dolina (Md)		–	1.083	1.039	1.136	1.101
Ojcow (Ojc)		–	0.973	1.011	1.065	1.042
Dachstein (Dac)	Alpine grassland	–	1.092	1.255	0.960	1.107
Široké Sedlo (Ss)		–	1.191	0.940	1.110	1.079

For each population, we present the population growth rate for each transition interval (pair of years, e.g. 2017/2018) separately, as well as for all the years combined into a single value expressed as a stochastic growth rate. Values smaller than one indicate declining populations and values greater than one indicate growing populations.

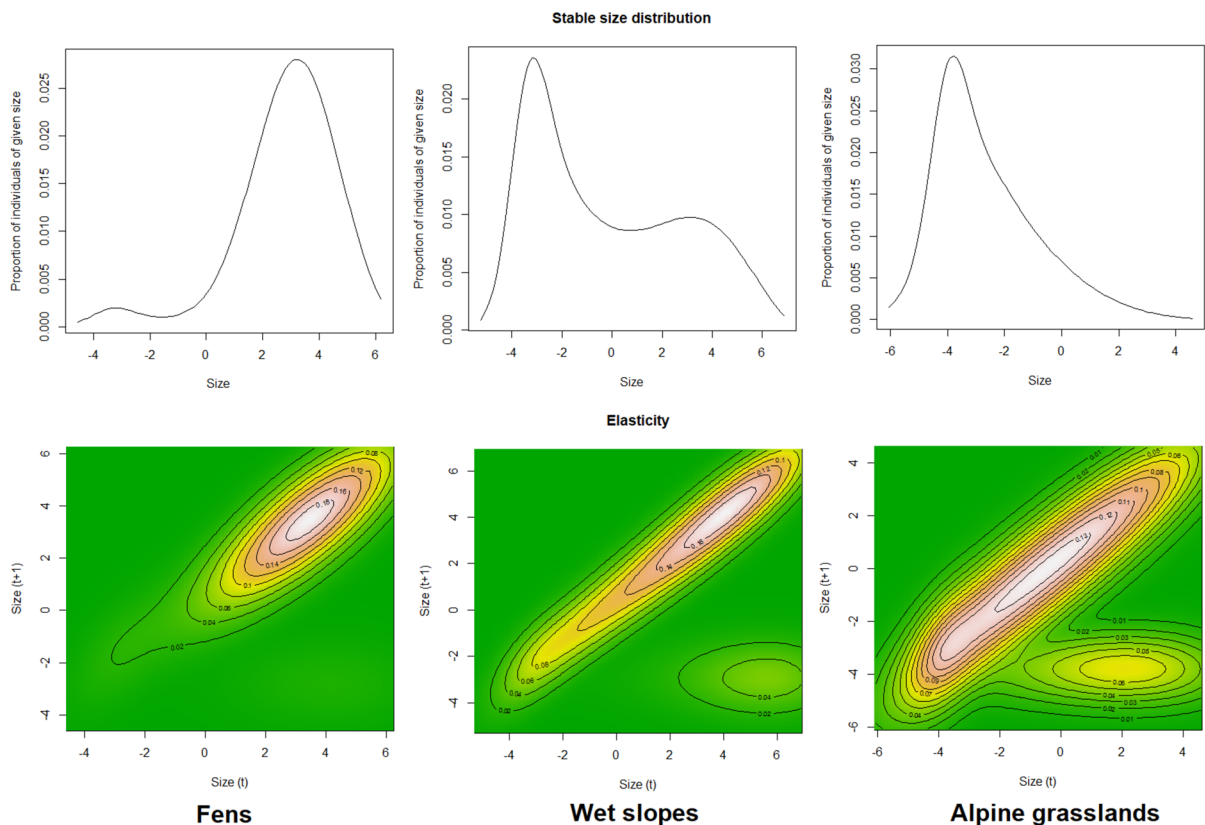


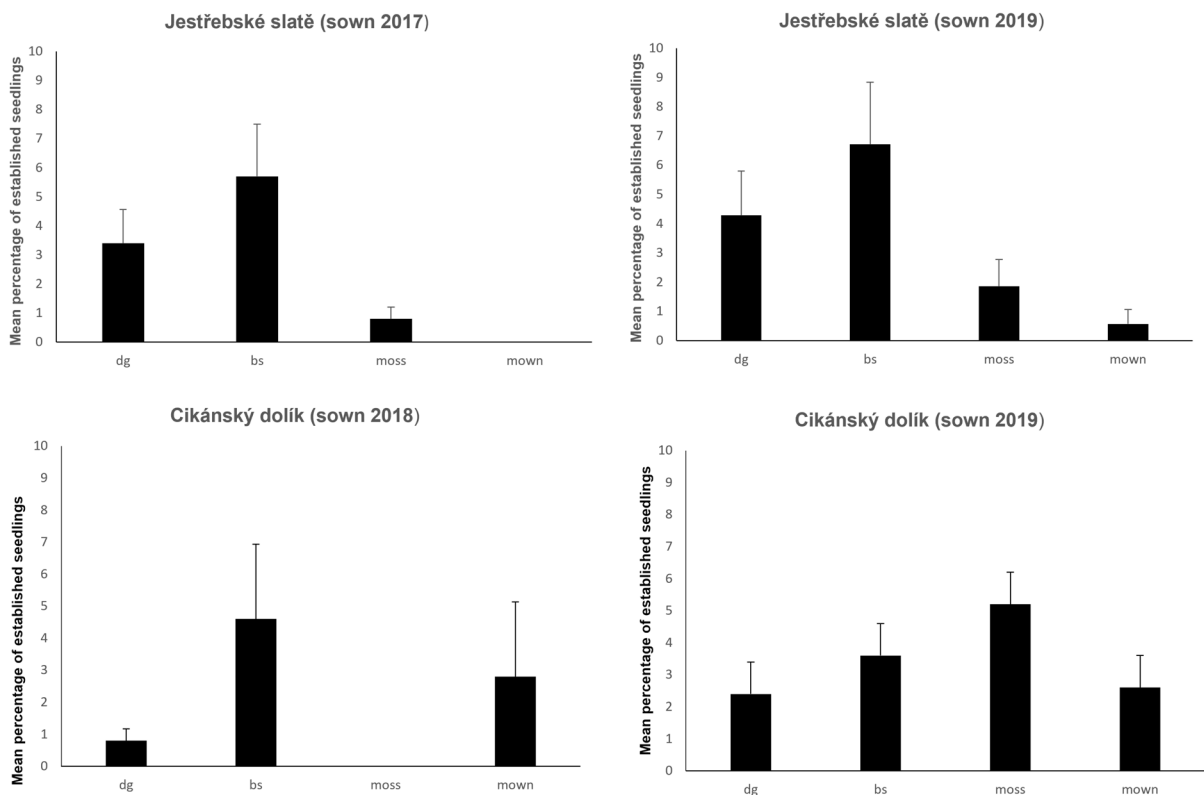
Fig. 5 Stable size distributions and elasticities habitats of the three types studied. Data from all transition intervals and populations of the given habitat type were used to construct each figure. Lines in elasticity figures indicate probabilities

of a given transition between size at time t to size at time $t + 1$. Reproduction (number of newly established individuals) is visualized in the bottom right corner.

Table 4 Effect of locality, management regime, year and their interactions on the number of established seedlings and seedling survival to the second year within sowing plots with different types of management

Population	Predictor	No. of established seedlings			Survival of established seedlings		
		<i>D.f.</i>	<i>F</i>	<i>P</i>	<i>D.f.</i>	Deviance	<i>P</i>
Both populations	Locality	1	0.040	0.842	1	3.69	0.055
	Management regime	3	7.680	< 0.001	3	60.57	< 0.001
	Locality × management regime	3	3.300	0.025	3	41.89	< 0.001
Jestřebské slatě population	Management regime	3	0.995	< 0.001	3	92.925	< 0.001
	Year	1	1.011	0.321	1	11.918	< 0.001
	Management regime × year	3	0.579	0.632	2	0.656	0.720
Cikánský dolík population	Management regime	3	0.750	0.531	3	9.534	0.023
	Year	1	1.553	0.222	1	0.433	0.511
	Management regime × year	3	2.675	0.064	2	2.796	0.247

Bold values are significant at $P \leq 0.05$. The table shows a model for both localities combined as well as for each locality separately. The effect of year was not tested in the combined model, because we did not have data from the same years in both localities. Data were analysed using generalized linear models (GLM)

**Fig. 6** Percentage of established seedlings in sowing experiments under different management regimes (dg, bs, moss, co) at two localities (Jestřebské slatě and Cikánský dolík). The column of each treatment shows the mean percentage ($\pm$ SE)

of established seedlings in the first year. For the significance of the differences between localities and management regimes, see Table 4. For more detailed data, see Table S4 and Table S5 in the electronic supplementary material.

at Jestřebské slatě (Table 4), while the effect of the management was not significant in the population at Cikánský dolík. This confirms that seedlings can emerge in all types of plots at this locality. In neither of the localities did we detect a significant effect of the sowing year (Table 4).

The survival of seedlings to the second year was also significantly affected by the management regime (Table 4). The survival of new seedlings was the highest on bare soil (44.6%) followed by moss (35.5%) and mown plots (29.03%). The lowest survival rate was observed on dug plots (10.4%). The effect of the locality was only marginally significant (Table 4), but it significantly interacted with the management regime (Table 4). In a separate analysis of both populations, the effect of the management regime on the survival of seedlings was significant in both populations. The effect of year was significant only in the Jestřebské slatě population, with lower survival in the dry year 2018 (Table 4, Fig. 6).

Modelling population dynamics with the presence of disturbances in fen populations

At the Jestřebské slatě fen locality, 5% of seeds falling into plots with bare soil could lead to a population growth rate greater than 1 in the 2017–2018

and 2019–2020 periods. By contrast, 10–20% would be needed in the other years (periods) (2018/2019, 2020/21) because of the high mortality of adults and extensive damage to inflorescences (Fig. 7). In the Cikánský dolík population, more seeds germinating on disturbed plots will be necessary, at least 30% in years with higher lambda values. In periods with lower lambda (as in 2017/2018, when lambda was the lowest), not even a 100% success rate of seed germination in disturbed plots would increase the population growth rate to above 1 (Fig. 7).

Discussion

Differences in populations dynamics between habitat types

In this study, we found a significant effect of habitat type or its interactions with size and year on most of the vital rates studied (survival of plants of all sizes, probability of flowering, proportion of damaged inflorescences, establishment rate). In general, survival was very high across habitat types and it was marginally affected by the habitat type, with the highest survival rates on alpine grasslands and the lowest but still very high in calcareous fens,

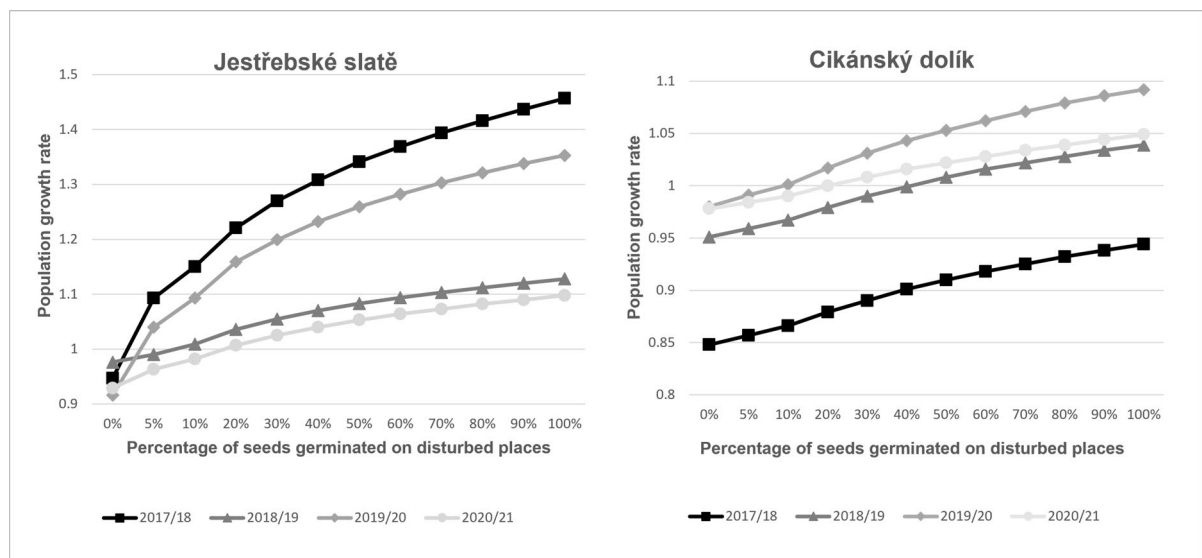


Fig. 7 Effect of percentage of disturbed places (bare soil regime) available for seedling establishment on the rate of population growth in the fen populations Jestřebské slatě and

Cikánský dolík. 0% means no disturbed places (standard management – mowing); 100% means that all seeds can germinate in disturbed plots (bs regime).

confirming that even in harsh alpine conditions, the survival of small individuals can be very high (Forbis 2003). A very high mortality was observed in fen population Sosnowiec-Bory. In this population, there is a high cover of young shrubs and trees due to succession (high competition, low light availability). The soil surface was also highly disturbed in 2019. We think that the high mortality in this population was caused mainly by these two factors.

We are not aware of any studies comparing similar habitats or species as in our study. Only comparisons of forest and grassland populations or different types of forests are available. In these study systems survival was affected by habitat type (Hesse et al. 2008; Jacquemyn et al. 2010; Mayberry and Elle 2010; Černá and Münzbergová 2013; Andrieu et al. 2017).

We observed different patterns in flowering and fecundity in habitats of different types. For example, wet slopes and calcareous fens had a significantly higher probability of flowering and a higher number of flowering rosettes. Wet slopes also had a higher number of fruiting rosettes than the other habitats. Differences in flowering and fecundity patterns between habitats have also been observed in other systems, for example, comparing forests vs grasslands (Wesselingh et al. 1997; Hesse et al. 2008; Bermingham 2010; Černá and Münzbergová 2013) or forests with different light conditions (Jacquemyn et al. 2010). Higher fecundity in later successional stages of one habitat type (abandoned meadows, shrubs) is also common (Wallace and Prather 2013; van der Meer et al. 2014; Lemke and Salguero-Gómez 2016).

Populations of *T. calyculata* from various habitat types also significantly differed in seedling establishment. We discovered significantly lower seedling establishment in fens than in other habitats, caused by the lack of places with bare soil without litter. We showed that in these habitats, even a small number of seeds, which can germinate in bare soil, can raise the population growth rate above 1. This is consistent with information for many other grassland species, which also need bare soil for seedling recruitment (Bissels et al. 2004; Stammel et al. 2006; Lemke et al. 2015; Janišová et al. 2016; Kladviová and Münzbergová 2016), because dense vegetation and litter serve as seed traps (Ruprecht and Szabó 2012). The lack of seedlings of grassland species in later successional stages with dense

vegetation and a high litter layer is very common (Oostermeijer et al. 1996; van der Meer et al. 2014; Lemke and Salguero-Gómez 2016).

Removing biomass to expose the soil without disturbing it proved to be the most efficient regime with the highest germination and high survival rates. By contrast, plots with a more disturbed soil (dug) regime showed lower establishment and very low survival. The same was observed also in *Trollius altissimus*, with low seedling survival in the regime with the most severe disturbance (Lemke et al. 2015). Heinken-Šmídová and Münzbergová (2012) observed that germination of *Ligularia sibirica* is the highest in the moss layer in comparable fen habitats. This may indicate that severe disturbances are not as effective for seedling establishment as less severe disturbances. On the other hand, even larger and more severe disturbances, such as peat or topsoil removal, were shown highly beneficial for rare vascular plants and mosses of calcareous fens habitats (Emsens et al. 2015; Klimkowska et al. 2015; Singh et al. 2021). Wet slopes and alpine grasslands had many suitable places for seedling establishment. In these two habitats, seedlings occur in bare soil as well as in low mosses. No seedlings were present in places with dense vegetation and in high mosses (pers. observ.). It is possible that places with bare soil are a product of landslides, which may sometimes occur on wet slopes.

Another difference between habitat types was inflorescence damage with very high damage in fen populations, lower on wet slopes and almost none in alpine grasslands. We are not aware of any studies comparing similar habitats or species as in our study. However, studies from other systems showed various effects of herbivory on populations growing in different habitats (Mayberry and Elle 2010; Dostálek et al. 2018). It is clear that herbivore pressure (Bermingham 2010; Brys et al. 2011; Katz 2016; Dostálek et al. 2018; Song et al. 2020) and especially florivory (Flaherty et al. 2018) and seed herbivory (Münzbergová 2005; Katz 2016; Kurkjian et al. 2017) can affect the population dynamics of a species by reducing its population growth rate. In our study system, the severe inflorescence damage was also probably the cause of the significant interaction of size and habitat type in the case of the number of fruiting rosettes, because while the numbers of flowering rosettes were very similar in

wet slopes and calcareous fens in all sizes, larger individuals from calcareous fens had fewer fruiting rosettes than individuals from wet slopes.

Inflorescences may be damaged by multiple factors (pers. observ.). In summer 2018, an extremely dry season, we observed some inflorescences damaged by drought in the calcareous fen population at Jestřebské slatě. However, it is more probable that most of the inflorescences were damaged by herbivores (molluscs, ungulates), because many inflorescences were also damaged in wetter years in the Jestřebské slatě population as well as at other localities of calcareous fens and on wet slopes with a more stable water regime. In plants cultivated in a garden in our side study, we also observed that the inflorescences were sometimes damaged for no obvious reason (pers. observ.). The reasons for damage are thus unclear in most cases.

Differences in seedling establishment and inflorescence damage in fen populations led to differences in population growth rates, elasticities and stable size distribution with a low population growth rate, a high elasticity of survival of large individuals, and a high proportion of large individuals in the stable size distribution. The same pattern was observed in different successional stages in similar habitat types in several previous studies – the low number of young plants, vigorous flowering but low reproduction and greater importance of adult plants in late successional habitats for the fen meadows species *Gentiana pneumonanthe*, *Succisa pratensis* and *Trollius europaeus* (Oostermeijer et al. 1996; van der Meer et al. 2014; Lemke and Salguero-Gómez 2016). Due to the high elasticity of the survival of adult plants, populations in fen habitats can be relatively stable for many years without any generative reproduction. Moreover, from our demographic data showing slow growth and high survival, it seems that *T. calyculata* is a long-lived species and that even many years without generative reproduction may not be problematic for this type of species. However, from the long-term point of view, reproduction in at least some years is crucial for the long-term survival of the species' populations. Successful generative reproduction in these habitats depends on the availability of suitable microsites and on a reduced level of inflorescence damage. On the contrary, the population growth rate of populations on wet slopes and alpine grasslands was above one and these populations also had high elasticities for

seedling establishment. Moreover, small individuals prevailed in the stable size distribution.

It is possible that the described differences between habitat types were mainly caused by the current suboptimal conditions at fen localities (absence of mowing, drainage, high cover of litter) rather than by the fen habitat type itself. Research based on data from more fen localities with different successional stages or different management could show more specific results. In our study, we have explored the effects of different small-scale interventions on seedling establishment in fens. However, information of the effect of large-scale management regimes, such as mowing, grazing or topsoil removal, on the whole life cycle of the species is still lacking. Also, it would be important to identify to what extent we can improve the growth of fen populations by interventions in the water regime of the habitats.

Temporal variation in population dynamics

Plant populations are commonly affected by temporal variability in climatic conditions (Svensson et al. 1993; Evju et al. 2011; Nicolè et al. 2011). This can be especially true in cases of extreme climatic conditions such as drought (Toräng et al. 2010). Our study was performed mainly in dry years, but some years were extremely dry (especially 2018) in Central Europe (historical data of the Czech Hydrometeorological Institute). For example, in the case of the Jestřebské slate population, the annual precipitation sum was only around 400 mm instead of the usual 634 mm (average data from the three nearest climatological stations of the Czech Hydrometeorological Institute). Data from wet seasons and well-preserved fen localities are necessary for a better understanding of the population dynamics of *T. calyculata* because fen vegetation can change in connection to dry or moist site conditions (Fojt 1994; Hájek et al. 2020) which could also lead to changes in the population dynamics. In our study, drought led to the withering of inflorescences (pers. observ.) and to a very high mortality of seedlings. On the other hand, we also observed a high mortality of seedlings if the soil was flooded (pers. observ., e.g. at Sosnowiec-Bory in 2020 and at Belianské lúky and Cikánský dolík in 2021). This suggests that conditions which are either too dry or too wet limit successful seedling establishment. Similar relationships were also observed in populations

of *Trollius europaeus* in Romania, with less juvenile plants in wetter sites (Muncaciu et al. 2010). The different effect of moisture was also observed in populations of *Liparis loeselii*, in which density of the plants was negatively associated with soil moisture (Oostermeijer and Hartman 2014), but the effect on flowering was positive (Urban et al. 2020).

Conservation recommendations

The two main factors limiting populations of *T. calyculata* in calcareous fens are poor seedling establishment caused by dense vegetation and litter and extensive damage to inflorescences caused by drought and herbivory. To reverse the unfavourable status of these populations, it is necessary to remove biomass and litter as part of conservation management. Currently applied management, such as mowing and the removal of shrubs and trees, is very important to maintain the species composition of fen vegetation even in the current dry conditions (Middleton et al. 2006; Hájek et al. 2020), but it may not be sufficient for the long-term persistence of populations of *T. calyculata*. Therefore, optimal management should, for instance, include yearly mowing in late summer, creation of disturbances in some years, and, as support, the sowing of locally collected seeds to ensure that they land on disturbed plots. We tested the effect of small disturbances, but it has been shown that even larger disturbances can be beneficial for many rare mosses and vascular plants of fen habitats with a relatively quick restoration of the target habitat after topsoil removal (Emsens et al. 2015; Klimkowska et al. 2015; Singh et al. 2021). In the case of *T. calyculata*, we recommend to test larger disturbances first, for example only in one part of the locality, because we have only tested the effect of small disturbances. We recommend to locate disturbances to those parts of localities where there are few or no adult individuals. This type of management should ensure good conditions not only for seedling establishment, but also for the high survival of adult plants. This is because disturbances were often the cause of death of adult plants and adult survival plays a key role in the growth of fen populations. An approach ensuring good conditions for seedling establishment as well as for the survival of adults will be beneficial for populations of *T. calyculata*. The same approach has been proposed as the best for other grassland species with

similar ecology and population biology (Oostermeijer et al. 1996; Heinken-Šmídová and Münzbergová 2012; van der Meer et al. 2014).

Populations on wet slopes and alpine grasslands do not need any specific management actions. Better performance of these populations can be supported by shrub cutting and biomass removal, although it seems that there are enough places for seedling establishment. Despite high lambdas in alpine grasslands, we observed death of some plants that were overgrown by *Pinus mugo* and *Dryas octopetala*. Alpine populations can be potentially threatened by climate change-induced tree line shift caused by climate change, as competitively stronger herbs, shrubs and trees can outcompete species such as *T. calyculata*.

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Data Availability Data are available at the authors upon request.

Declarations

Conflicts of interests The authors have no financial or proprietary conflicts of interests. The experiments comply with the current laws of the countries in which they were performed.

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