

# Managing calcareous grassland for the declining Duke of Burgundy *Hamearis lucina* butterfly: effects of grazing management on *Primula* host plants

Anne E. Goodenough<sup>1</sup> · Matthew H. Sharp<sup>1</sup>

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**Abstract** Many butterfly species are declining in range and abundance, sometimes to the point of becoming vulnerable to extinction. Several traits increase a species' vulnerability to population decline through stochastic processes, including high larval specificity and poor dispersal rate. The Duke of Burgundy *Hamearis lucina* relies on *Primula* as its sole larval host plant. This monophagus dependency, coupled with susceptibility to environmental stochasticity, low dispersal and poor recolonization potential, means it is vital that sites supporting this rapidly-declining species are managed optimally. Here, we use two calcareous grassland sites in the UK with different grazing systems to identify optimal grazing management for *Primula* abundance and, for the first time, *Primula* characteristics linked previously to Duke ovipositing preference and success: size, condition, succulence and surrounding sward height. We find that autumn and winter grazing intensity are both positively associated with *Primula* abundance, but there is a trade-off for winter grazing with negative effects on plant size, condition and succulence. Winter grazing also decreased the sward height below the optimum. Plants were bigger and better at the site managed using continuous (free-roaming) grazing versus the site managed using rotational (paddock-based) grazing. We recommend moderately high grazing intensity during autumn using a free-roaming system where possible to attain abundant *Primula*, with a reduction in grazing intensity or grazing removal during winter to ensure suitability of individual plants. This management would also benefit other host plants, such as bird's foot

trefoil *Lotus corniculatus*, which are vital for other declining butterflies that frequently co-occur with Dukes.

**Keywords** Butterfly conservation · Grazing regime · *Hamearis lucina* · Habitat management · Host plant choice · Ovipositing preference

## Introduction

Butterflies are an important taxonomic group in many ways. They can be excellent indicator species of ecosystem health, provide important services such as pollination and pest control, constitute important links in many food chains, act as hosts for other species, and are often charismatic flagship species for education and conservation. Many species, however, are declining in range and abundance, sometimes to the point of becoming vulnerable to extirpation or even extinction. Across Europe, 19% of the 482 species of butterfly are considered threatened or near threatened on the butterfly red list (van Swaay and Warren 1999), while a recent pan-European study on grassland butterfly species showed a 30% decline in a composite index of 17 species monitored in 22 different counties between 1990 and 2013 (van Swaay et al. 2015). In some countries, the situation is especially concerning: for example, in the UK, 41 of the 68 species have decreased in prevalence over the period 1976–2014 (Fox et al. 2015).

There are many reasons for decline including: habitat change and fragmentation, agricultural intensification, and climate change (Warren 1993a; Bubová et al. 2015). However, several traits make species especially vulnerable to the effects of stochastic processes that can lead to population decline and ultimately even extirpation or extinction. These include narrow niche breadth, high larval specificity,

✉ Anne E. Goodenough  
aegoodenough@glos.ac.uk

<sup>1</sup> University of Gloucestershire, Francis Close Hall, Swindon Road, Cheltenham, Gloucestershire GL50 4AZ, UK

and poor dispersal rate—especially if combined with short adult lifespan (Kotiaho et al. 2005). Taking the last two factors, some species are so specialized in their use of larval host plants that they are monophagous (i.e. only use one species or genus). This can be a risky strategy, especially in a changing environment, as monophagous species are inherently vulnerable to changes in host plant quantity or quality (Anthes et al. 2008). This can have a profound effect, especially if the species is also a poor disperser and thus has a limited ability to react to poor host plant conditions at a given site.

The Duke of Burgundy *Hamearis lucina* butterfly is both monophagous and very poor at dispersing. The larval host plant is *Primula*, primarily cowslip *P. veris* and primrose *P. vulgaris* but the hybrid of these two species (false oxlip *Primula vulgaris*  $\times$  *veris*) is used occasionally. Females live for an average of 5 days in the adult lifestage (Oates 2000), during which time they must mate and oviposit, as well as feed. Although individual females can sometimes move up to 250 m (Oates 2000), the species is generally extremely poor at dispersing. This is exemplified by new habitat patches, seemingly in prime condition, not being (re)colonized at distances of 75–450 m from extant populations (Anthes et al. 2008). The problems of chance extinctions driven by stochastic processes, and low recolonization potential, were highlighted as major extirpation drivers by Léon-Cortés et al. (2003).

Because of its monophagous dependency, susceptibility to environmental stochasticity, and low dispersal and recolonization potential, it is vital that optimal habitat management is undertaken at sites that still support Dukes. This is especially true given recent declines for this species across Europe: populations have decreased in at least 10 countries (Asher et al. 2001; Bourn and Warren 1998) and extirpation in two more (van Swaay and Warren 1999). In the UK, there have been substantial declines in both range (84% decrease between 1974 and 2014) and abundance (42% decrease over the same period) (Fox et al. 2015). Not only is the species declining in percentage terms; the actual number of sites, and the number of individuals per site, is very low. The species was found in 84 of the UK's 10  $\times$  10 km grid squares (compared to, for example, small tortoiseshell *Aglais urtica* in 2607 grid squares) and around 50% of sites have <10 individuals recorded annually (Fox et al. 2015; Ellis and Wainwright no date). Key reasons for decline are habitat change, especially out-competition of host plants by rank grasses and scrub encroachment, habitat fragmentation, and climatic change (Dennis and Shreeve 1991; Bourn and Warren 1998; Oates 2000; Léon-Cortés et al. 2003; Turner et al. 2009). The species is now listed as vulnerable (Fox et al. 2011) with one of the last UK strongholds being unimproved grassland within the Cotswolds region of central England (Bourn and Warren 1998).

Given the vital importance of the larval host plant in this monophagous species, and the fact that the species is not generally considered to be nectar-limited (Garling 1984; Ottes 2000), conservation of Dukes needs to focus on ensuring the availability and suitability of *Primula* larval host plants. Several previous studies have been conducted on female ovipositing preference, host plant selection, and larval niche variation. For example, Fartmann (2005, 2006) and Anthes et al. (2008) found that butterflies occurring in calcareous grassland in Germany preferentially selected plants in more shaded conditions, with more vegetation coverage and a higher sward, probably to avoid host plant desiccation. The preference for plants in taller swards was also found in woodland clearings in the UK (Sparks et al. 1994) and scrub-pasture in Sweden (Persson 2006). Meanwhile Turner et al. (2009), studying a quarry-based population in the UK, showed that females preferentially chose plants with a larger spread and longer leaves, and those that occurred in dense patches. The above studies have provided a good understanding of the interactions of Dukes with *Primula*, but there has been virtually no work on how to manage grassland to facilitate growth of plants that are optimal for the butterfly's needs. A couple of studies have investigated the effect of grassland management—primarily grazing and mechanical cutting—on *Primula* (Brys et al. 2004; Brys and Jacquemyn 2009), but these have focused on the plant itself (population growth rate, flowering proportion, seed output) rather than those attributes known to be important for Dukes. Given that the mere presence of *Primula* is no guarantee that a site will support Dukes, even when abundant (Kruys 1998), lack of information on how to manage sites to promote “Duke-suitable” *Primula* is a vital missing link. Without such understanding, management at sites that hold the few remaining populations of Dukes in the UK (e.g. Ellis et al. 2011), and those at risk elsewhere in Europe, is not necessarily optimal.

In this study, we examine two calcareous grassland sites in the UK that have different grassland management to quantify the effects on *Primula* abundance and Duke-friendly characteristics as determined by previous studies: (1) rosette size; (2) leaf length; (3) number of other *Primula* plants within 50 cm; (4) mean vegetation sward height; and (5) percentage cover provided by surrounding vegetation. We also quantify plant condition and succulence because of the possible importance of desiccation. Both sites are within the UK Cotswolds stronghold for Dukes, have historically supported good numbers, and are managed specifically for Duke conservation. Because many sites that are managed for Dukes are also important for other butterfly species, including the two sites studied here, we also examine the effect of the different grassland management on other key larval host plant species.

## Methods

### Study area

This study was undertaken at two sites in the Cotswolds, Gloucestershire, UK; part of the main UK stronghold for the species (Bourn and Warren 1998). Primary fieldwork was undertaken between May and September 2013, supplemented by long-term grazing records supplied by Natural England (see below). The two sites, Cranham Common 51°48'54.87"N 2° 8'54.51"W and Edge Common 51°46'52.51"N 2° 13' 23.4624"W, are around 1.8 km apart and form part of the same National Nature Reserve (NNR) managed by Natural England. The sites support unimproved lowland calcareous grassland—a UK priority habitat—and are dominated by upright brome *Bromus erectus*, torgrass *Brachypodium pinnatum* and sheep's fescue *Festuca ovina*, with abundant quaking grass *Briza media* and a wide range of flowering herbs including bird's foot trefoil *Lotus corniculatus*. The sites are similar in terms of habitat and abiotic variables and occur on the same natural limestone escarpment. Both sites have historically supported the three *Primula* species/hybrids associated with Dukes, with abundant cowslip, some primrose, and very occasional false oxlips.

Grazing is an important aspect of management at both sites. At Cranham Common, grazing is controlled through a network of paddocks (13 across the whole 18.2 ha site), with a small herd of cows being rotated between small paddocks to create a grazing regime mosaic. At Edge Common, grazing is undertaken more extensively with cows roaming freely across the whole 20.5 ha site for about 7 months of the year, without using a paddock system, in a form of management called continuous grazing (Blanchet et al. 2003). Henceforth, these sites will be referred to as the rotationally-grazed site and the continuously-grazed site.

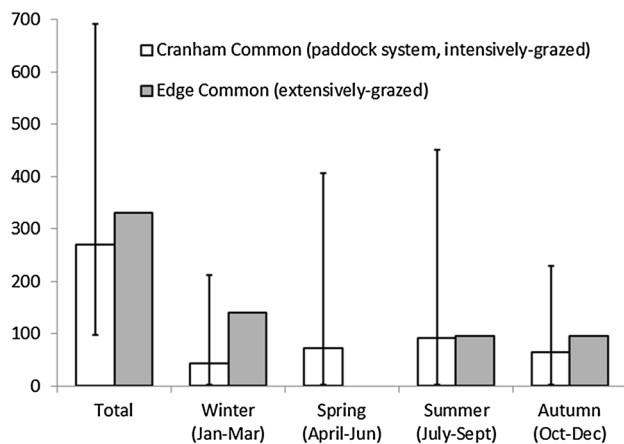
Both sites are designated as Sites of Special Scientific Interest (SSSIs), being notified for their butterfly fauna and, in the case of the continuously-grazed site, specifically because of the Duke of Burgundy (Nature Conservancy Council 1987). The sites are assessed through the Common Standards Monitoring (CSM) framework, invoked by Article 17 of the EU Habitats Directive for EU-designated sites and also used in the UK for nationally-protected SSSIs. At the most recent assessment of habitat condition (Natural England 2013), the rotationally-grazed site was found to be in favourable condition as it has been for >10 years, while the continuously-grazed site became favourable for the first time following a long period of unfavourable status; this was due to the commencement of regular grazing.

### Grazing data

Grazing data for both sites were obtained from Natural England. For the rotationally-grazed site, records contained data on when each paddock had been grazed, for how long, and by how many cows, for the period July 2011–June 2013 (i.e. 2 years prior to the start of the project). The grazing system used a fixed network of paddocks but the timing of grazing in each was haphazard rather than strategic, with paddocks being grazed according to local conditions and sward height rather than according to a set schedule. On average, each paddock was grazed twice per year, with each grazing period lasting 30 days on average (range 7–56 days). From these data, several grazing variables were calculated. To quantify grazing intensity, we quantified, on a per-paddock basis, the number of “cow days” (grazing duration in days multiplied by herd size at that time, which ranged between 5 and 9) that there had been over the preceding two year period for each season (winter=January–March; spring=April–June; summer=July–September; autumn=October–December). Grazing intensity was calculated by dividing number of cow days by the size of the paddock ascertained using GIS. It was important to account for season when quantifying grazing intensity since the interaction between grazing and abundance of seasonal plants, including *Primula*, could be affected by the time of year grazing occurred. We also quantified, for each paddock, the time interval (days) between the start of the project and last grazing. For the continuously-grazed site, where grazing was not paddock based, seasonal grazing intensity was calculated at site level rather than paddock level. The total amount of grazing (cow days per year per hectare) was fairly similar between the two sites: rotationally-grazed site=7.5; continuously-grazed=7.9. However, there were seasonal differences with more winter grazing at the continuously-grazed site but no spring grazing (Fig. 1).

### Field data

At the rotationally-grazed site, five parallel belt transects were marked out in each paddock; these were regularly spaced and ran the entire length of the paddock. Where terrain was not flat, transects were always positioned parallel to the slope. Transect length depended on the size of the paddock (range=25–210 m; mean=50 m), such that sampling effort was proportional to paddock size. Each transect was 2 m wide and was divided into 5 m long sections. The number of *Primula* was counted within each section to give *Primula* density per 10 m<sup>2</sup>. Slope angle was recorded using an Abney level (36,300, York Survey Supply, York, UK) and coded as follows: 0=flat (0–4°), 1=slight slope (5–9°), 2=moderate slope (10–14°), 3=steep slope



**Fig. 1** Grazing pressure at the rotationally-grazed and continuously-grazed site

(15°–19°), very steep slope ( $\geq 20^\circ$ ). Aspect was recorded. At the continuously-grazed site, 10 transects were laid out to span the entire site, again parallel to the slope. Because these transects covered the entire site, each was longer than those within individual paddocks at the rotationally-grazed site (range 150–400 m; mean=200 m). Slope angle and aspects were recorded. In total, data were collected from 75 transects (65 at the rotationally-grazed site and 10 at the continuously-grazed site), with a combined length of 6480 m and a combined survey area of 12,960 m<sup>2</sup>.

To complement the transect data at the rotationally-grazed site, and get a better insight into the potential impacts of paddock-based grazing, multiple quadrats were laid out to collect detailed data for *Primula* spp. and other butterfly larval foodplants (bird's foot trefoil *Lotus corniculatus*, marjoram *Origanum majorana*, wild thyme *Thymus polytrichus*, horseshoe vetch *Hippocrepis comosa*, kidney vetch *Anthyllis vulneraria*, meadow vetchling *Lathyrus pratensis*, and tufted vetch *Vicia cracca*). Each quadrat was 2×2 m arranged as a grid of 100 squares such that each square covered 4 cm<sup>2</sup>. The number of squares in which each of the target species was found was recorded as per Sutherland (2006). Again the sample effort was proportional to the size of the paddock: 167 quadrats studied overall (range=5–30 per paddock).

Finally, to collect data on plant biometrics, 150 *Primula* plants were studied in detail (rotationally-grazed site: 2 plants per transect \* 5 transects per paddock \* 13 paddocks=130; continuously-grazed site: 2 plants per transect \* 10 transects over the whole site=20). In all cases, the focal plants were selected systematically, the first being the nearest plant to one-third distance along the transect and the second being the nearest plant to two-thirds distance along the transect. Several measurements were taken: (1) maximum diameter across the plant rosette (mm); (2)

the length of the longest leaf from stem to tip (mm); (3) the number of other *Primula* plants within 50 cm; (4) mean vegetation sward height within 30 cm; (5) percentage cover provided by surrounding vegetation; (6) plant condition (ordinal scale=1 serious decay with plant being yellow/brown and damaged; 2=plant yellow/green with some damage; 3=light green with slight damage; 4=plant slightly wilting but no damage; 5=perfect condition). With the exception of plant condition, all variables had been recorded previously in studies of Duke of Burgandy ovipositing using the same or similar methods, (Fartmann 2006; Anthes et al. 2008; Turner et al. 2009) and found to be important. One (or occasionally two—see below) leaves were removed from each plant for laboratory analysis of succulence; this was done with the agreement of Natural England as the statutory regulator of the NNR/SSSI and after careful checking to ensure absence of Lepidoptera eggs/larvae.

The number of butterflies was recorded at each site by Natural England staff and submitted to the Gloucestershire Centre for Environmental Records. We also noted any Duke of Burgandy eggs on *Primula* when taking leaves for succulence work at both sites. Both datasets were utilized to put the *Primula* data, and management implications of this study, into a butterfly context.

### Laboratory data

Quantifying succulence—the amount of moisture within a leaf—is an important concept in plant-insect interactions, especially as a measure of plant suitability as a larval food source (high succulence=high suitability). There are several measures of leaf succulence, primarily: (1) quantifying maximum succulence in relation to leaf area (Mantovani 1999); and (2) comparing the amount of moisture actually in a leaf in field conditions to dry weight (Zotz and Winter 1994). To compare these methods, a pilot study was undertaken whereby pairs of leaves were removed from 10 separate plants. For each plant, succulence was measured using a different method on each leaf. To calculate maximum succulence, each leaf was placed in a sealed bag with moistened paper. After water imbibition had occurred to the point of saturation, Maximum Fresh Weight (MFW) was quantified and Leaf Area (LA) was measured. The leaf was then oven-dried (60°C for 48 h) and re-weighed to ascertain Dry Weight (DW). Succulence (S) was calculated using Mantovani's (1999) index of  $S = (MFW - DW) / LA$ . The second method involved quantifying the fresh:dry weight ratio, with leaves collected early in the morning before evapotranspiration commenced (Zotz and Winter 1994). The two succulence measures were compared on a per-plant basis using a Wilcoxon sign-rank test, which showed that there was no difference between the metrics

( $z = -0.051$ ,  $n=10$ ,  $p=0.959$ ). Accordingly the more common, and easier to standardise, maximum succulence method was adopted for this study.

### Statistical analysis

To establish whether there were differences between the two study sites, and, for the rotationally-grazed site, a difference between the different paddocks, a separate mixed-effect nested ANOVA was undertaken for each of the six dependent variables (*Primula* abundance, rosette diameter, leaf length, *Primula* plants within 50 cm of the focal plant, plant condition, and succulence). In all cases, site ID was entered as the first fixed factor. Paddock ID was entered as a second fixed factor nested within site ID since this applied to the rotationally-grazed site only. Transect ID entered as a random factor. The rationale for paddock ID being a fixed factor, rather than a random factor, was that each paddock was a fixed spatial entity that was consistent over time. In addition, all land at the rotationally-grazed site was assigned to a paddock. In other words, paddocks were not sampling units; the sum of the land in the 13 paddocks equaled the total land area at that site. More importantly, there was a biological reason for examining paddocks specifically rather than simply allowing for variability (i.e. using a fixed factor framework not a random factor framework): grazing was managed using the paddock system, such that each individual paddock was grazed differently. For all models, the marginal  $R^2$  ( $R^2_m$ ) was calculated to describe the proportion of variance explained by the fixed factors in the model (site and paddock), while the conditional  $R^2$  ( $R^2_c$ ) was calculated to describe the proportion of variance explained by the random factor (transect) as per Nakagawa and Schielzeth, H. (2013). To complement the transect analysis, a further ANOVA was run on *Primula* coverage using the more detailed quadrat data (paddock ID once again entered as a fixed factor and transect ID entered as a random factor). Quadrat data were only collected at the rotationally-grazed site so site ID was not entered as a factor in these analyses; a nested design was thus unnecessary.

To establish potential effects of abiotic and management factors on *Primula* abundance and characteristics, hierarchical models were created. Separate Multiple Linear Regression analyses were run for the two measures of *Primula* abundance (transect count data and quadrat coverage data), and for the six plant biometrics listed above. In all cases, the abiotic factors of slope angle and aspect were entered into the regression models first via forced entry (stage 1). This ensured that the topographic variability within and between the sites was allowed for before variables describing the grazing management, and resultant habitat parameters, were entered. Following the forced entry

of the abiotic factors, five grazing factors (grazing intensity in each of the four seasons, plus time since last grazing in days) and two vegetation parameters (sward height; percentage cover) were made available. Finally, to assess any remaining effects of site (and thus potentially grazing regime: rotational or continuous), site ID was included as a candidate variable in a third and final model-creation stage for all models except *Primula* coverage (quadrat data were only collected at the rotationally-grazed site). In stages 2 and 3, a stepwise entry approach was adopted with the entry criterion set at  $\alpha=0.05$  (Field 2000). The same approach was used to analyse the effect of grazing on other butterfly larval food plants. The field data met, and indeed exceeded, the minimum case:variable ratio of 3:1 as recommended by Tabachnick and Fidell (1989), such that analysing a relatively large number of independent variables in one analysis was valid. The assumptions of normality and homoscedasticity were assessed by examining residual plots (Berry and Feldman 1985; Fox 1991) and were met. The assumption of orthogonality was tested using the variance inflation factor (VIF) and tolerance collinearity statistics according to the suggested criteria: (1) VIF of all variables  $< 10$  (Myers 1990); and (2) tolerance  $> 0.2$  (Menard 1995) and was met.

## Results

### Between- and within-site comparisons

Of the three species/hybrids of *Primula* known to have occurred on the site in the past, only cowslips were found during this study. It should be noted that results here are based on correlative analyses rather than a standardized manipulative experiment. Moreover, although grazing data from 2 years were used, the effects on grazing on plants were studied in one season only. Despite these limitations, analyzing the number of *Primula* using transect data from both sites showed that there was a significant difference between them (rotationally-grazed site:  $8.562 \pm 0.369$  SEM *Primula* per 10 m<sup>2</sup>; continuously-grazed site:  $3.437 \pm 0.380$  SEM *Primula* per 10 m<sup>2</sup>; Table 1; Fig. 2a). While the largest amount of variance in the data was explained by site, there was also a difference in *Primula* abundance between paddocks at the rotationally-grazed site. This suggests that grazing regime might affect *Primula* abundance in more subtle ways, probably due to the timing of grazing within the year. The overall marginal  $R^2$  value ( $R^2_m$ ) summarizing the amount of variance that could be explained by the fixed factors of site and paddock was 0.399, while the conditional  $R^2$  value ( $R^2_c$ ) summarizing the amount of variance that could be explained by the random factor of transect was much

**Table 1** ANOVA results comparing *Primula* abundance (number of plants from transect data and coverage from quadrat data) in relation to site (number of *Primula* only), grazing paddock, and transect replicate

	Number of <i>Primula</i>			<i>Primula</i> coverage		
	F	d.f	P	F	d.f	P
Site ID: whether continuously- or rotationally-grazed (fixed)	10.798	1	<b>0.001</b>			
Paddock ID (fixed; nested in site ID)	11.688	13	<b>&lt;0.001</b>	1.990	11	<b>0.035</b>
Transect ID (random)	1.570	9	0.119	0.849	4	0.497
	R <sup>2</sup> =0.410 (R <sup>2</sup> <sub>m</sub> =0.399; R <sup>2</sup> <sub>c</sub> =0.011)			R <sup>2</sup> =0.173 (R <sup>2</sup> <sub>m</sub> =0.173; R <sup>2</sup> <sub>c</sub> =0.000)		

The marginal R<sup>2</sup> value (R<sup>2</sup><sub>m</sub>) describes the proportion of variance explained by the fixed factors in the model (site and paddock), while the conditional R<sup>2</sup> value (R<sup>2</sup><sub>c</sub>) describes the proportion of variance explained by the random factor in the model (transect). Significant results are shown in bold

lower at just 0.011. This, taken together with transect being non-significant, suggests *Primula* was distributed fairly evenly within each paddock at the rotationally-grazed site or across the whole area at the continuously-grazed site. A similar pattern was also shown when *Primula* coverage data from quadrat sampling at the rotationally-grazed site alone, when paddock was a significant fixed factor (R<sup>2</sup><sub>m</sub>=0.173) but transect was a non-significant random factor (R<sup>2</sup><sub>c</sub>=0) (Table 1).

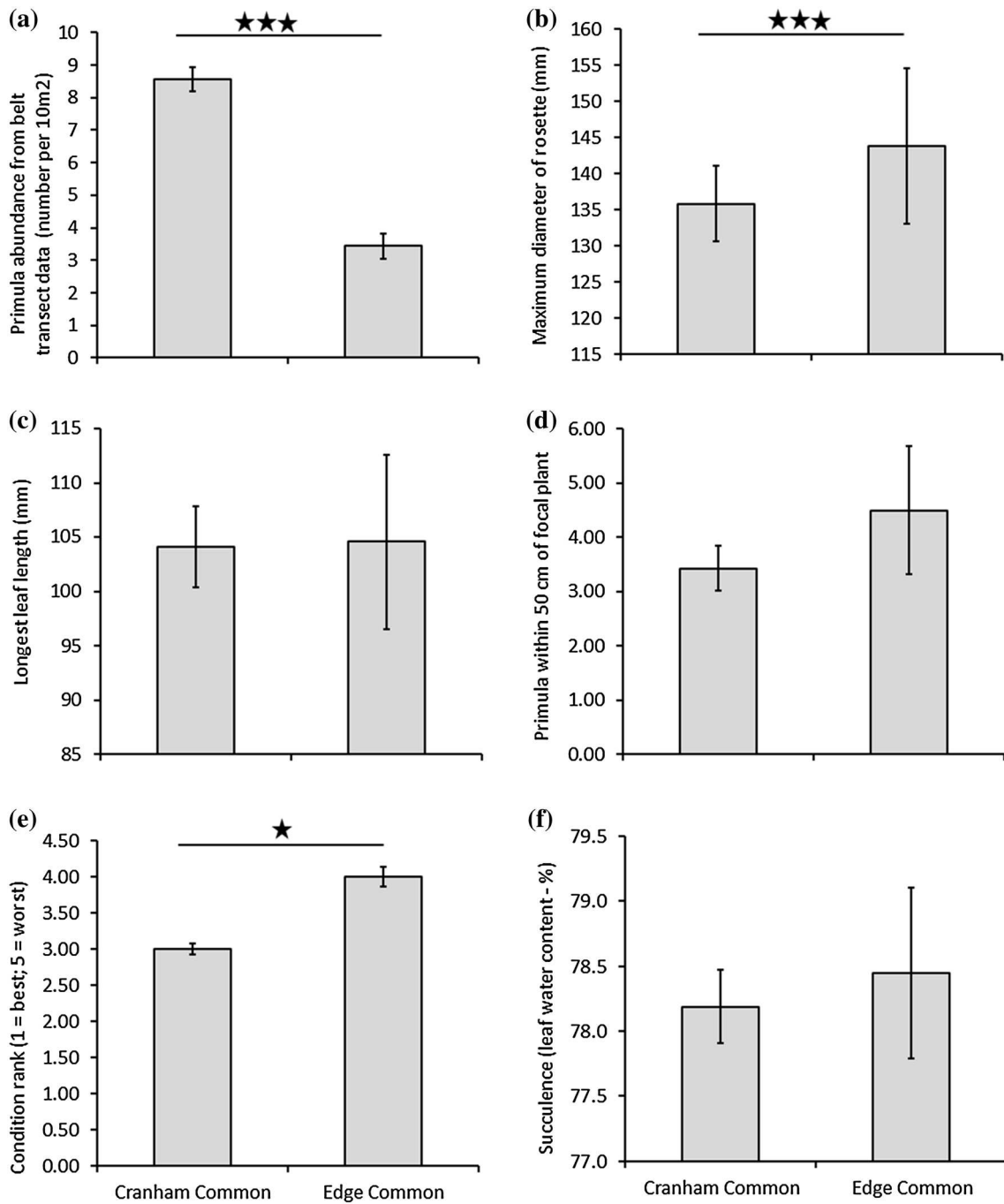
There were also differences in plant biometrics (Table 2). Although the continuously-grazed site had fewer plants (see above), the plants were larger at this site compared to those at the rotationally-grazed site (rosette spread 143.8 mm ± 10.768 SEM versus 135.6 mm ± 4.236 SEM; Fig. 2b). This difference was significant (Table 2). However, there was no site-level difference in the length of the longest leaf (Fig. 2c), suggesting site did not affect growth of individual leaves despite differences in overall rosette size. Both size parameters differed significantly between paddocks at the rotationally-grazed site. There was no site-level difference between the number of other *Primula* plants within 50 cm of each focal plant (Fig. 2d), implying that plant clustering did not differ between sites. This lack of clustering was also suggested by the fact that transect ID was non-significant and unimportant in the ANOVA analyses of *Primula* abundance and coverage; see above and Table 1. There was, however, a site-level difference in condition, with plants at the continuously-grazed site being in better condition than those at the rotationally-grazed site (median score on an ordinal scale from 1 (worst) to 5 (best): rotationally-grazed site=3; continuously-grazed site=4; Fig. 2e). Surprisingly, given the difference in plant condition between the sites, there was no measurable site-specific difference in succulence, possibly because of the high variability in this measure (Fig. 2f). In all analyses, the amount of variance explained by transect as a random factor was negligible, especially when compared to the amount explained by site and/or paddock as fixed factors (R<sup>2</sup><sub>m</sub> >> R<sup>2</sup><sub>c</sub>; Table 2).

### Abiotic and management drivers of differences in *Primula* abundance and biometrics

The above ANOVA analyses essentially quantified differences between- and within-sites rather than deepening understanding of the abiotic and management factors potentially driving such differences. Hierarchical regression models undertaken to consider the data for the two sites in more detail showed that topography affected *Primula* abundance and many of the biometrics (Table 3). Generally, steeper slopes had higher *Primula* abundance and plants were in better condition compared to flat or gentle slopes, while slopes facing SW, W or NW tended to have more plants and bigger plants than slopes with a more easterly aspect. Once topography had been accounted for, the timing and intensity of grazing had a substantial additional effect on *Primula* ecology. There was a general trend for abundance of *Primula* to increase with increasing grazing intensity, with autumn grazing (October–December) being particularly valuable in this regard (Table 3). This possibly reflects the importance of grazing to reduce the abundance of dominant grass species, thereby decreasing competition for light early in the growing season (i.e. from February onwards). After all of these effects had been accounted for, there was still a difference between the sites (greater *Primula* abundance at the rotationally-grazed site).

There was no spring grazing at the continuously-grazed site and spring grazing was not included in any models. To establish whether this was due to there being little functional significance of spring grazing or whether the lack of data for the continuously-grazed site was skewing the model, an additional univariate analysis was undertaken using the quadrat data collected from the rotationally-grazed site only. This showed that there was no relationship between *Primula* abundance (coverage) and spring grazing (regression analysis: F<sub>1,165</sub> = 0.378; P = 0.540; R<sup>2</sup> = 0.002).

While higher grazing intensity was largely advantageous for *Primula*, the timing of this was vital for plant morphology and physiology. Winter grazing correlated negatively



**Fig. 2** Differences in *Primula* abundance and parameters between sites. Error bars shown SEM. Significant differences highlighted using convention criteria (\* = 0.05; \*\*\* <0.001)

with plant size (rosette diameter and longest leaf size). The two other main influences on plant size were sward height (positive) and percentage coverage (negative), suggesting that the plants needed some protection from surrounding vegetation but also needed high light levels. More importantly, winter (and autumn) grazing were also negatively associated with *Primula* condition and succulence, with

plants being in poorer condition when winter grazing intensity was high. Both succulence and condition were positively correlated with percentage cover, suggesting that while sunlight is important for plant growth, increased coverage is important for longevity, likely due to decreased desiccation. Once all of these factors had been accounted for, there was still an effect of site on succulence and

**Table 2** ANOVA results of *Primula* plant biometrics in relation to site, paddock, and transect

	Rosette spread			Longest leaf length			Primula plants within 50 cm			Condition			Succulence		
	F	d.f	P	F	d.f	P	F	d.f	P	F	d.f	P	F	d.f	P
Site ID (whether continuously- or rotationally-grazed; Fixed)	3.986	1	<b>0.048</b>	0.164	1	0.687	0.982	1	0.324	15.984	1	<b>&lt;0.001</b>	0.527	1	0.470
Paddock ID (nested in Site ID; Fixed)	3.009	11	<b>0.001</b>	2.403	11	<b>0.049</b>	1.279	11	0.244	2.686	11	<b>0.006</b>	<b>3.472</b>	7	<b>0.003</b>
Transect ID (random)	0.583	4	0.679	1.156	4	0.334	1.824	4	0.128	1.624	4	0.177	0.881	4	0.479
	$R^2=0.476$			$R^2=0.452$			$R^2=0.092$			$R^2=0.464$			$R^2=0.248$		
	$R^2_m=0.476$			$R^2_m=0.397$			$R^2_m=0.064$			$R^2_m=0.644$			$R^2_m=0.248$		
	$R^2_c=0.000$			$R^2_c=0.055$			$R^2_c=0.028$			$R^2_c=0.013$			$R^2_c=0.000$		

The marginal  $R^2$  value ( $R^2_m$ ) describes the proportion of variance explained by the fixed factors in the model (site and paddock), while the conditional  $R^2$  value ( $R^2_c$ ) describes the proportion of variance explained by the random factor in the model (transect). Significant results are shown in bold type

condition (higher at the continuously-grazed site). Interestingly, for succulence, this difference did not show up in the ANOVA analysis (Table 2), probably because of the high within-site variability (Fig. 2f); when this was accounted for in the hierarchical models, the effect of site became evident. In all other cases, the presence or absence of an additional effect of site after allowing for all other site-based factors matched the initial ANOVA analyses.

Taken together, the results suggest that moderate to high grazing intensity, especially during the autumn, is important for *Primula* abundance, but that grazing should be reduced, or even removed during the winter to ensure that the *Primula* plants are in good condition and have high succulence. Although winter grazing is associated with further increases in *Primula* abundance, the negative impacts on condition and succulence are substantial. As there was only one rotationally-grazed site and one continually-grazed site it is difficult to disentangle the degree to which any site-level differences are due to the difference in grazing regime rather than other site-specific factors.

### Duke of Burgundy data

In 2013, the year in which the study was conducted, 13 adult Duke of Burgundy butterflies were recorded at the continuously-grazed site, while no adults were recorded at the rotationally-grazed site. Duke eggs were recorded on three *Primula* plants at the continuously-grazed site versus none at the rotationally-grazed site.

### Factors affecting abundance of other butterfly larval food plants.

Of the nine other butterfly larval food plants surveyed (at the rotationally-grazed site only; see methods), only three were found—birds foot trefoil, kidney vetch and meadow vetchling. Of these, birds foot trefoil was positively associated with grazing during spring and summer (Table 4); indeed there were positive, albeit non-significant, relationships to grazing intensity during autumn and winter too. Meadow vetchling showed an opposite pattern, with an inverse relationship with autumn grazing (Table 4). Kidney vetch did not correlate significantly with any topographical or grazing variables.

### Discussion

In a previous study, Brys et al. (2004) showed that removal of grass in the autumn through grazing or mowing was the most favourable management scenario for



**Table 3** Hierarchical Multiple Linear Regression models of *Primula* abundance/biometrics in relation to topography and grazing

Parameter	Correlation direction	Individual P value	Parameter	Correlation direction	Individual P value
Primula abundance (number of plants; transect data)			Primula abundance (coverage; quadrat data) <sup>b</sup>		
Slope <sup>a</sup>	+	<b>0.003</b>	Slope <sup>a</sup>		0.570
Aspect <sup>a</sup>	SW/W/NW better	<b>&lt;0.001</b>	Aspect <sup>a</sup>		0.305
Total grazing intensity	+	<b>0.002</b>	Total grazing intensity	+	<b>0.022</b>
Autumn grazing intensity	+	<b>0.011</b>	Autumn grazing intensity	+	<b>&lt;0.001</b>
Site ID	More at rotational	<b>&lt;0.001</b>			
Overall model: $F_{5,1085} = 9.490$ ; $R^2 = 0.205$ ; $P < 0.001$			Overall model: $F_{4,162} = 4.512$ ; $R^2 = 0.205$ ; $P = 0.002$		
Rosette spread			Longest leaf length		
Slope <sup>a</sup>		0.904	Slope <sup>a</sup>		0.507
Aspect <sup>a</sup>	SW/W/NW better	<b>0.030</b>	Aspect <sup>a</sup>	SW/W/NW better	<b>0.004</b>
Winter grazing intensity	+	<b>0.021</b>	Winter grazing intensity	+	<b>0.025</b>
Sward height	+	<b>&lt;0.001</b>	%Cover	–	<b>0.003</b>
%Cover	–	<b>&lt;0.001</b>	Sward height	+	<b>&lt;0.001</b>
Site ID	Larger at continuous	<b>&lt;0.001</b>			
Overall model: $F_{5,133} = 17.430$ ; $R^2 = 0.396$ ; $P < 0.001$			Overall model: $F_{5,133} = 19.035$ ; $R^2 = 0.417$ ; $P < 0.001$		
Succulence			Condition		
Slope <sup>a</sup>		0.658	Slope <sup>a</sup>	+	<b>0.038</b>
Aspect <sup>a</sup>		0.056	Aspect <sup>a</sup>		0.675
Winter grazing intensity	–	<b>0.001</b>	Winter grazing intensity	–	<b>0.025</b>
%Cover	+	<b>0.048</b>	%Cover	+	<b>0.023</b>
Site ID	Greater at continuous	<b>0.002</b>	Site ID	Greater at continuous	<b>0.002</b>
Overall model: $F_{6,92} = 4.803$ ; $R^2 = 0.239$ ; $P < 0.001$			Overall model: $F_{4,134} = 2.900$ ; $R^2 = 0.080$ ; $P = 0.024$		
Primula plants within 50 cm					
Slope <sup>a</sup>		0.046			
Aspect <sup>a</sup>		0.797			
No grazing variables entered					
Overall model: $F_{2,136} = 2.918$ ; $R^2 = 0.071$ ; $P = 0.370$					

Topographic variables (slope; aspect) entered via forced entry; grazing variables then available for stepwise entry: (1) time since last grazing; (2) grazing intensity over previous 2 years; (3–6) grazing intensity for winter, spring, summer, and autumn over previous 2 years. For analyses of *Primula* biometrics, sward height and percentage cover were also available for stepwise entry. Finally grazing regime (rotationally-grazed using small paddock or continuously-grazed) was available as a candidate variable. The optimal (final) model is shown in all cases. Bold values show significance ( $P < 0.05$ ); correlation direction is given for significant variables only

<sup>a</sup>Forced entry

<sup>b</sup>Grazing regime not a candidate variable (quadrat data only collected at intensively-grazed site)

*Primula* in terms of proportion of flowering individuals and seed output. Here, our analyses suggest that autumnal and winter grazing is beneficial not only for producing good numbers of plants but also for producing plants that have some of the characteristics that make them optimal larval host plants for Duke of Burgundy butterflies, namely large rosette spread and increased leaf length (Turner et al. 2009). However, winter grazing was also negatively associated with other characteristics that make *Primula* optimal larval host plants, in particular condition and succulence, which link to desiccation risk (Fartmann 2006; Anthes et al. 2008). Winter grazing also decreased

the height of the surrounding sward—ideally the sward should be fairly high around *Primula* plants for optimal oviposition (Sparks et al. 1994; Fartmann 2005, 2006; Persson 2006; Anthes et al. 2008).

Even after topography and the intensity and seasonality of grazing had been accounted for, site ID was still an important and significant factor in several models, including *Primula* abundance, size, succulence and condition. There were more plants at the rotationally-grazed site, but the plants were bigger, more succulent, and in better condition, at the continuously-grazed site. As noted in the results, because there was only one rotationally-grazed

**Table 4** Hierarchical Multiple Linear Regression models of abundance of butterfly larval plants in relation to topography and grazing

Parameter	Correlation direction	Individual P value
Birds foot trefoil abundance		
Slope*	+	<b>0.002</b>
Aspect*	SW/NW better	<b>0.018</b>
Spring grazing intensity	+	<b>0.001</b>
Autumn grazing intensity	+	<b>0.024</b>
Overall model: $F_{4,162} = 9.490$ ; $R^2 = 0.160$ ; $P < 0.001$		
Kidney vetch abundance		
Slope*		0.317
Aspect*		0.783
Overall model: $F_{2,164} = 0.558$ ; $R^2 = 0.007$ ; $P = 0.020$		
Meadow vetchling		
Slope*	-	<b>0.001</b>
Aspect*		0.132
Autumn grazing intensity	-	<b>0.003</b>
Overall model: $F_{3,163} = 17.430$ ; $R^2 = 0.106$ ; $P < 0.001$		

Topographic variables (slope; aspect) entered via forced entry; grazing variables then available for stepwise entry: (1) time since last grazing; (2) grazing intensity over previous 2 years; (3–6) grazing intensity for winter, spring, summer, and autumn over previous 2 years. The optimal (final) model is shown in all cases. Order of entry for grazing variables reflects their importance. Bold values show significance ( $P < 0.05$ ); correlation direction is given for significant variables only

site and one continually-grazed site it is difficult to disentangle the degree to which site-level differences are due to grazing regime rather than other site-specific factors. However, the sites occur in very close proximity (~1.8 km), are very similar in habitat, and share the same geology and elevation on the same limestone escarpment, and are part of the same National Nature Reserve. Moreover, the site factor was significant even after allowing for the effects of topography and grazing management in terms of intensity and grazing. It can thus be tentatively suggested that the *method* of grazing might be important with a continuous-grazing regime being better than a rotational one. This is the first time that this has been studied and suggests that the way that grazing stock are managed as regards their movement across a site is important over-and-above management of stocking density and grazing seasonality. Plants being larger and in better condition under continuous grazing would make sense given that when animals roam freely across an entire site, grazing pressure is *de facto* lower at any one given location than when animals are contained within one of a network of small paddocks and rotated around these, even if the overall stocking density is the same when considered across the whole site for a whole year. Having low, consistent, grazing pressure is thus more likely to result in larger plants and, because sward height

is generally higher, better condition and succulence as desiccation will be lower. Conversely, as *Primula* colonization and germination is higher when there is some disturbance (Brys and Jacquemyn 2009), having periodic but more intense grazing as per a rotational system would benefit plant abundance.

Fartmann (2005) found that W/SW slopes were preferred for female oviposting in calcareous grassland in the Diemeltal region of Germany. He attributed this to potential host *Primula* being too desiccated on Southern and Eastern slopes. Host plant desiccation is a frequent problem in the Diemeltal region, as well as in the UK (Anthes et al. 2008), so this explanation is certainly plausible and indeed had previously been postulated by Warren (1993b). Here, however, we have shown that *Primula* abundance is highest on slopes facing Southwest, West or Northwest while succulence is unaffected by aspect. Accordingly, it is possible that the host selection preference for SW-NW slopes is driven, at least in part, by greater *Primula* abundance decreasing host plant search-cost (i.e. the time and energy used in finding a suitable resource). Search-cost has been previously found to be important in butterflies, especially for monophagous species that experience high plant selectivity (Janz and Nylin 1997), especially if their egg load is comparatively low (Odendaal and Rausher 1990). As the search-cost involved in finding a ‘better’ plant

increases as the number of plants decreases, concentrating effort on areas where the number of *Primula* is high would make sense, especially given the risk of losing the current plant in search of something better. This is especially true for species that have a very short adult life stage such as Dukes, where mean adult lifespan is 5 days (Oates 2000). Moreover, selecting a host plant in an area of high *Primula* density also means that larvae can move from the primary plant to secondary plants to avoid starvation or intense competition if necessary (Oates 2000; Anthes et al. 2008). Regardless of the biological mechanisms involved, we suggest that preference be given to preserving and managing steep slopes facing SW-NW if any conservation prioritization decisions are needed, since these are associated with more, and larger, *Primula*. There might also be some benefits for succulence in water-restricted years.

The Duke of Burgundy is one of the few endangered butterfly species that tend to occur at sites with high richness of butterflies in general (Franzén and Ranius 2004). This means that managing habitat optimally for this species is likely to have cascade effects for the Lepidoptera more generally. Here, we have shown that steep SW-NW slopes that are grazed in the Autumn are not only suitable for the growth of *Primula*, but also bird's foot trefoil, which is the primary host plant of numerous other species that are also declining and that often co-occur with Dukes: dingy skipper *Erynnis tages*, green hairstreak *Callophrys rubi*, chalkhill blue *Polyommatus coridon*, common blue *Polyommatus icarus*, and silver-studded blue *Plebejus argus* (Dennis 2010). All these species except chalkhill blue are found at both sites used in this study. Bird's foot trefoil is also used as a host plant by the wood white *Leptidea* species complex, as is meadow vetchling. However, in the case of meadow vetchling, abundance is decreased by autumn grazing. As wood whites tend to use areas of meadows at the edges of woodland, it might be sensible to leave a buffer strip free from autumn grazing where woodland borders grassland if these species are present.

While grazing has long been understood as a primary management intervention for managing calcareous grassland for Duke of Burgundy, we have shown here that the intensity and timing of grazing, as well as potentially the grazing management system (rotational or continuous), is also important. Grazing not only affects the number of *Primula*, but, more specifically, for growth of plants that are suitable for use as larval host plants. Although it should be noted that our findings are based on one year of correlative analyses of just two sites, such that caution is needed when speculating on casual mechanisms especially as regards grazing regime, our study has provided numerous recommendations that should help existing conservation schemes—such as the pioneering work in north England outlined by Ellis et al. (2011)—become even more

effective. We recommend that sites with Dukes are managed with moderately high grazing intensity during the autumn to attain abundant *Primula*, but that grazing should be reduced, or even removed, during the winter to ensure that the *Primula* plants are in good condition, have high succulence, and are surrounded by sward of an appropriate height. This management would also benefit other host plants, such as bird's foot trefoil, which are vital for other declining butterflies that frequently co-occur with Dukes. It should be noted that although spring grazing was not entered into any model for *Primula* abundance or biometrics, and was not significantly related to *Primula* coverage based on analysis of just the rotationally-grazed site (the only site where spring grazing occurred), care might need to be taken to avoid too much spring grazing in areas where Duke-suitable *Primula* grow. This is because grazing at this time might remove valuable host plants immediately before ovipositing or, worse, destroy eggs when ovipositing has already occurred. Finally, we recommend that having a continuous, free-roaming, grazing regime *might* be better than a rotational one. More studies are needed, covering more sites and over a longer time period, to confirm the generality of this finding. This recommended as a priority, especially given that the rotationally-grazed site in our study did not support Duke of Burgundy during our study, possibly because the larval host plants were simply not Duke-suitable despite occurring in high numbers. This suggests that plant quality might need to take precedence over plant quantity (at least above a certain threshold) if management for Duke of Burgundy is to be as successful as possible.

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