Determining the population connectivity of the endangered lycaenid butterfly *Lycaena helle* (Denis & Schiffermüller, 1775) in Luxembourg

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Abstract

The LIFE Éislek project aims to restore and maintain habitats for three target species: Violet Copper, Whinchat and Red-backed Shrike. This study focuses on the current distribution and population connectivity of the Violet Copper *Lycaena helle* butterfly, as well as its habitat use.

Of the total of 152 sites visited between mid May until the end of June, 102 sites were retained for further analysis. Of each occupied or potential but unoccupied site, the state was determined. For the habitat use of the butterfly, plant density cover was recorded at 2m radius where the butterfly has been recorded.

The condition and isolation of habitat patches were shown to have the greatest influence on the presence of *L. helle.* Depending on the state of the different sites, management plans of rotational light grazing and mowing efforts were adapted accordingly in order to guarantee maximum benefits for the target species. Great care was taken as to make sure that the proposed management regimes do not have any negative effects on *Proclossiana eunomia*, another glacial relict species occurring in this region.

The species has the potential to have a highly interconnected population in the northwest region of Luxembourg, if all potential sites are to be restored and maintained. However, if only areas within Habitat Directive areas are to be managed, the species is likely to be lost in the near future due to increased fragmentation and isolation of existing populations. The results of this study suggest that the Habitat Directive areas should be extended in order to include all suitable butterfly sites and that species-specific management should not be limited to these areas, but should also include Bird Directive sites as well as non-designated areas.

Based on the findings of this study, several adaptations to the current Action Plan for *L. helle* and propositions for future monitoring regimes have been suggested.

Introduction

Climate change has become an increasingly important topic in conservation biology (Walther *et al.,* 2002; Parmesan & Yohe, 2003; Sawchik *et al.,* 2005; Habel *et al.,* 2010 c). Changing global temperatures during glacial and interglacial periods have always influenced the distribution patterns of organisms, especially butterflies (Parmesan *et al.,* 1999; Turlure *et al.,* 2009; Habel & Assman, 2010; Habel *et al.,* 2010 c; Habel *et al.,* 2011;). These changes force species to adapt to new ecological conditions (Habel *et al.,* 2010 c) or have driven them into extinction whenever they were unable to adapt. The ongoing debate about the potential consequences of global climate change have made this an increasingly important research area to determine the future of certain species.

It has been estimated that the Earth's climate has increased by 0.6°C in temperature in the last 100 years (Walther *et al.,* 2002) and this is likely to continue (Parmesan *et al.,* 1999). The rate at which temperatures have increased during that period have been greater than at any other period during the last 1,000 years (Walther *et al.,* 2002). Additionally, agricultural intensification, industrialisation and human population growth have a major driver effect on environmental changes (Maes & Van Dyck, 2005; Sawchik *et al.,* 2005; Öckinger & Smith, 2006; Hudewenz *et al.,* 2012). This has major negative consequences for biodiversity, with almost every ecosystem on Earth going through strong anthropogenically-induced disturbances (Sawchik *et al.,* 2005), leaving mostly semi-natural habitats (Maes & Van Dyck, 2005; Öckinger & Smith, 2006).

Butterfly species are known to be extremely sensitive to changes (Maes & Van Dyck, 2001; Sawchik *et al.,* 2005). Consequently, due to habitat deterioration, habitat loss and the increasing isolation of suitable habitats, the distribution and abundance of many European butterflies have declined during the last 50 years (Maes & Van Dyck, 2001; Parmesan & Yohe, 2003; Öckinge & Smith, 2006; van Swaay *et al.,* 2012). Understanding the loss of plant diversity and of different types of habitat has been pointed out as being highly important for butterfly conservation (Hudewenz *et al.,* 2012).

It is therefore not surprising that many conservation studies nowadays are concerned with theoretical and experimental studies and modelling analysing the effects of fragmentation, habitat size and isolation, especially in previously continuous areas, with the primary interest in population ecology and genetics (Louy *et al.,* 2007; Vandergast *et al.*, 2009; Habel *et al.,* 2010 b & c; Habel *et al.,* 2011). The metapopulation theory is one such model and has become a paradigm in biodiversity conservation (Hanski & Gaggiotti, 2004), especially when it comes to trying to halt the ongoing decline of European butterflies that has been observed for several decades (Maes & Van Dyck, 2001; van Swaay *et al.,* 2012).

The metapopulation theory, exemplified by Levins' (1970) model or Hanski's (1994) incidence function model, explains the persistence of regional species with unstable populations and the concept of migration between local populations (Hanski, 1998; Nowicki *et al.,* 2007) focusing mainly on spatial presence-absence patterns (Nowicki *et al.,* 2007). Consequences of habitat size and isolation on migration, population extinction and (re-) colonisation are the main factors determining classic metapopulation dynamics (Hanski, 1998; Nowicki *et al.,* 2007; Bauerfeind *et al.,* 2009). According to the classic metapopulation concept, local populations maintain a balance between local extinctions and recolonisation.

For conservation efforts, however, network characteristics such as patch size, habitat quality and isolation are considered to be increasingly important issues to be considered when trying to re-establish a metapopulation and assure its long-term survival (Betzhold *et al.,* 2007; Bauerfeind *et al.,* 2009). Other vital factors, such as the dynamics and densities of individual populations are especially important for species with low turnover of local populations (Nowicki *et al.,* 2007) but are often not incorporated into metapopulation studies. Furthermore, as every species has different habitat requirements, different factors become more important for individual species, especially for specialist species. For example, for some metapopulations, habitat quality and isolation of the different patches is crucial for their survival, whereas for other populations habitat size in conjunction with isolation and quality is more important. Therefore both approaches (habitat quality and habitat network) now tend to be seen as two interconnecting factors to describe species distribution at a landscape scale (Thomas *et al.*, 2001; Betzhold *et al.,* 2007; Nowicki *et al.,* 2007).

Habitat quality for individual butterfly species is determined mostly by the presence, condition and organisation of different resources (Ellis, 2003; Betzhold *et al.,* 2007; Bauerfeind *et al.,* 2009; Turlure *et al.,* 2009). While each species of butterfly depends on different resources, the common need for any butterfly species can be characterised by a set of complementary resources, which are consumables (e.g. host-plants) and utilities (e.g. perch structures) (Bauerfeind *et al.,* 2009). Thus habitat quality and therefore the occurrence of any given butterfly species, is determined by the abundance and availability of the resources needed by the given species (Thomas *et al.*, 2001).

Habitat quality is often determined by the agricultural use of given sites. With increased specialisation towards either crop production or livestock husbandry, only the most productive and valuable agricultural sites are managed, while marginal sites are abandoned. This leads to some areas being managed very intensively, while invaluable sites often remain unmanaged (Sawchik *et al.,* 2005; Öckinger & Smith, 2006 and ref therein; Öckinger *et al.,* 2006).

These unmanaged sites become ecologically valuable for the first few years, but due to succession and afforestation, decrease rapidly in quality for many butterfly species. Intensively used sites, on the other hand, might remain open, but lose their quality for many species due to increasing nutrient and pesticide levels or intensive grazing pressures (Öckinger & Smith, 2006). Consequently, preserving habitats in good quality only works under the right management schemes or restorative efforts, which has become crucial for butterfly conservation (Öckinger *et al.,* 2006; Goffart *et al.,* 2010). The precise knowledge of the conditions and ecological resources requires by any given butterfly species, has become essential for conservation biology (Turlure *et al.,* 2009). The lack of information regarding specific requirements and limiting factors of different butterfly species (Bauerfeind *et al.,* 2009) often renders conservation efforts extremely difficult.

Another factor affecting habitat quality are climatic changes, which often have fundamental impacts on local ecosystems, and thus, can alter distribution patterns of certain species (Habel & Assman, 2010). Natural climate changes like glacial and interglacial periods have modified the distribution patterns of many organisms for thousands of years (Finger *et al.,* 2009).

The current climatic changes, however, have much more dramatic impacts on many biota forcing species to adapt much more rapidly to new ecological conditions (Parmesan & Yohe, 2003) in an increasingly fragmented landscape. As whole habitats can be altered by the rising temperatures rise, certain habitats can get an even more patchy distribution, which results in fragmented populations (Fisher *et al.,* 1999). Finger *et al.,* (2009) suggest that butterflies can survive in fragmented landscapes as metapopulations for long periods, as long as genetic exchange remains possible. Habitat fragmentations, however, will remain a threat to the survival of any butterfly species as patch occupancy is declining progressively due to decreasing habitat qualities (Hill *et al*., 1996), which in return decreases the potential for good dispersal abilities between sites (Fisher *et al.,* 1999).

But not just climatic changes cause habitat fragmentation. Many habitats are also lost due to urbanisation of the European countryside (Bauerfeind *et al.,* 2009; Stevens *et al.,* 2010). This causes an even more severe threat to the biodiversity as habitats are often not replaced by other types of suitable habitats, which could support new species of butterflies or other organisms (Vandergast *et al.,* 2007). This is particularly severe for species with narrow ecological niches, e.g. specialists (Finger *et al.,* 2009). These species often suffer extremely under habitat fragmentation due to disruption of habitat connectivity as a consequence of habitat loss. This often results in decreasing population sizes (Habel *et al.,* 2010 a).

In addition, small populations in a fragmented landscape suffer from increased isolation, which in turn reduces colonisation rates and so increases the risk of extinction (Hanski, 1999; Vandergast *et al.,* 2007). Again, this often leads to genetic depression, which negatively affects the species' long-term fitness and survival (Louy *et al.,* 2007 and ref therein; Habel *et al.,* 2010 a).

Thus, habitat fragmentation can have significant negative impacts on butterfly richness and genetic diversity (Wettstein & Schmid, 1999; Louy *et al.,* 2007; Vandergast *et al.,* 2007).

The dispersal ability between different sites is a crucial factor for a sustainable metapopulation (Bauerfeind *et al.,* 2009) and the evolutionary biology (Stevens *et al.,* 2010) of any given butterfly. To allow successful dispersal between sites, and thus, the ecological and evolutionary functioning of natural populations (Stevens *et al.,* 2010), butterfly species rely on the persistence of the different patches. If these patches are fragmented, they should be close enough to each other, hat they can serve as a network. According to Hanski (1998), populations in large and/or less isolated patches face less risk of extinction than populations in small and/or isolated patches. This is also supported by a study conducted by Hill *et al.* (1996) on the silver-spotted skipper.

Dispersal abilities are also dependent on the ecological demands of a given species. Landscapes, which may appear strongly fragmented for a specialist butterfly with low dispersal abilities, but may be continuous for a generalist butterfly with higher dispersal abilities. As for habitat quality, the dispersal ability is a highly variable factor for different butterfly species (Stevens *et al.,* 2010). Therefore, every species' dispersal ability has to be taken into consideration if conservation efforts are to be successful.

Dispersal ability is not just an important factor in functioning metapopulations, but also crucial for species' survival in the face of climatic changes and altering local microclimates (Parmesan *et al.,* 1999). Non-migratory species in particular are affected by climatic changes often resulting in extinction along the southern tip of their range due as their habitat becomes increasingly unsuitable. If suitable habitats become available along the northern edge of their distribution, this may result in the colonisation of new areas and a northward shift of the entire population (Parmesan *et al.,* 1999; Finger *et al.,* 2009; Habel & Assman, 2010). Finger *et al.* (2009), for example separated the different species reactions into different ecological groups. Mediterranean species still maintain the ability to enlarge their distribution range by expanding northwards and uphill (e.g. Parmesan *et al.,* 1999). According to Parmesan *et al.* (1999), 63% of 35 non-migratory European butterfly species showed a northward shift during this century, but only 3% showed a southward shift. Alpine species, on the other hand, having adapted to moist and cool environments, can only retreat into higher altitudes or latitudes (Finger *et al.,* 2009). Such shifts in distribution remain possible in high mountain systems for example. Many mountain regions in Europe, however, rarely exceed 1000 m and therefore do not offer new suitable escape areas if overall temperatures rise any further. This will eventually lead to the local extinction of these species (Parmesan & Yohe, 2003).

<u>Relict species</u>

Cyclic changes of warm and cold periods of the Pleistocene have resulted in anthropogenic responses with two different groups: during warm periods, warm adapted species expanded their distribution range, whereas cold adapted species decreased their range, and vice versa during cooler periods (Varga & Schmitt, 2008; Habel & Assman, 2010; Habel *et al.,* 2010 c; Habel *et al.,* 2011). Species, which have survived and formed fragmented, isolated populations throughout their distributions, are called relict species (Fisher *et al.,* 1999; Finger *et al.,* 2009; Habel & Assman, 2010). The populations of these species are typically small and often severely restricted in their geographical ranges (Habel & Assman, 2010). Postglacial warming has caused species to undergo severe range shifts into higher altitudes and latitudes (Turlure *et al.,* 2009; Habel *et al.,* 2010 b & c; Habel *et al.,* 2011). As these populations are often very isolated from each other, regional extirpations through demographic and genetic stochasticity can be induced. This in return can severely reduce individuals' fitness and finally result in the complete collapse of entire metapopulations (Frankham, 2005). If climate was to cool down again, these relict species are likely to increase their ranges and become widely distributed again (Habel & Assman, 2010).

With continuously rising temperatures, Arctic and alpine species will have to shift their ranges further into higher altitudes and/or latitudes (Habel *et al.,* 2010 b & c). Boreomontane species, on the other hand, which represent relict species with mixed characteristics of continental, Arctic and alpine species (Habel *et al.,* 2010 b & c) and with remnant populations in low altitudinal mountainous regions have often reached their limits of their current distribution (Turlure *et al.,* 2009; Habel *et al.,* 2010 b). Climate Envelop Models for *L. helle,* for example, predict a loss of major parts of suitable habitats resulting in extinction of most populations in the west if climate will continue to warm (Habel & Assman, 2010; Habel *et al.,* 2010 b; Habel *et al.,* 2011). *L. helle* of the alpine regions will be less affected as it can still respond with altitudinal shifts, an option which the remaining populations in lower altitudes do not have, as the maximum altitude has been reached (Habel *et al.,* 2010 b).

The wet meadows of the Ardennes high plateaus in Belgium and Luxembourg represent such a region of lower altitudes. Still offering appropriate ecological conditions, which closely resemble those of Northern Europe, these areas allow the persistence of some relict butterfly species, such as *Proclossiana eunomia* or *Lycaena helle* (Goffart *et al.,* 2010). As with many populations of relict species, the Ardennes' populations are spatially trapped, as there is no possibility for a distribution shift to the North, where suitable habitats are lacking (Parmesan *et al.,* 1999; Turlure *et al.,* 2009). Equally, there is no possibility for an uphill shift, as the upper altitudinal limits are reached for the region (Turlure *et al.,* 2009; Goffart *et al.,* 2010; Habel *et al.,* 2010 b). Some of these relict species, however, have become genetically unique (e.g. *L. helle* – Meyer, 1981 a, b; Habel *et al.,* 2010 b & c) and therefore deserve special conservation efforts.

Other lists with species under conservation concern have been set up by the Habitat Directives of the European Union in 1994 (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora): Annex II – lists species whose conservation requires designation of Special Areas of Conservation; Annex IV

- lists species of community interest in need of strict protection. The Habitat Directives include a series of other Annexes, most of them concerning 'habitats' and species of European Community concern, and have become an increasingly important tool for implementing nature conservation efforts in the European Union. Every member state is required to designate Natura 2000 sites for the species listed in Annex II. These habitat patches are usually defined through vegetation types or biotopes using a given set of diagnostic plant species. The overall aims are to protect around 220 habitat types and approximately 1,000 species within the EU. Around 29 butterfly species (and 2 sub-species) are listed in the Annex II and IV, of which 12 are classified as 'Threatened', 5 as 'Near Threatened' (IUCN). Given the fact that many of these species listed under the Habitat Directive are also valuable indicator species for certain important habitats, all conservation efforts targeting these species will also bring great benefits for other species and biodiversity as a whole (van Swaay *et al.,* 2010).

To support nature conservation and environmental projects in Habitat (and Bird) Directive sites, the EU has created a financial instrument called 'LIFE' in 1992 and has since co-financed over 3,104 projects worth approximately €2.2 billion (van Swaay *et al.,* 2010).

<u>LIFE 'Éislek'</u>

The LIFE 'Éislek' project aims to restore wetlands in 11 Natura 2000 sites of the Ösling region in the north of Luxembourg, in order to improve habitat quality for three specific target species: Red-backed Shrike (*Lanius collurio*), Whinchat (*Saxicola rubetra*) and Violet Copper (*L. helle*). These aims will be achieved by protecting suitable habitats (through agri-environment schemes, land purchase, etc.), restoring degraded sites, and adapting management options to be more beneficial for the three target species. Monitoring and raising public awareness will be other important components of the 'Life Éislek' project, which will run for a total of 5 years.

The project will be run by natur&ëmwelt 'Fondation Hëlef fir d'Natur' and the Centrale ornithologique Luxembourg', but will be carried out in close collaboration with the 'Ministère du Dévelopement Durable et des Infrastructures du Luxembourg', the 'Comité National pour la Défense Sociale' and the 'Chambre d'agriculture'.

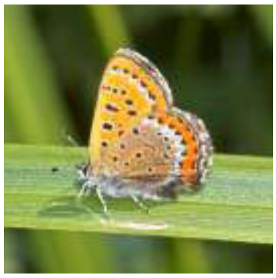
Aim

The aim of this project is to determine how the butterfly uses its habitats in order to propose appropriate management options. Whether or not the management Habitat Directive areas only will be sufficient to secure a viable population in the north-western region of Luxembourg will be a second aspect of this paper.

Methods

Study species

The violet copper *Lycaena helle* (Denis & Shiffermüller, 1775) is a boreomontane species ranging from Central Europe to Scandinavia over to Northern Asia (Meyer, 1981 a & b; Habel *et al.,* 2008; Finger *et al.,* 2009). It is a typical postglacial relict species. Despite several locally stable populations (Bauerfeind *et al.,* 2009; van Swaay *et al.*, 2012), it is considered as one of the rarest butterfly species in Central Europe and has shown a continued decline throughout its range (Fisher *et al.,* 1999; Wipking *et al.,* 2007; Finger *et al.,* 2009; van Swaay *et al.*, 2012). With its low dispersal ability (Meyer, 1981 b; Fisher *et al.,* 1999; Chuluunbaatar *et al.,* 2009) it has been assumed that its distribution has always been restricted to relatively few localities in the past (Meyer, 1981 b; Fisher *et al.,* 1999; Bauerfeind *et al.,* 2009). Genetic evidence, however, suggests that there has been a homogenous distribution over the majority of the western Palearctic during the early postglacial (Habel *et al.,* 2008; Habel *et al.,* 2010 b). The current distribution in Central Europe is patchily scattered over different regions, and limited to altitudes above 400 m (e.g. Ardennes, Eifel, Westerwald, the Massif Central and Vosges) (Meyer, 1981 b; Wipking *et al.,* 2007; Habel *et al.,* 2008).



Lycaena helle

Bogs or moorlands represent the main habitat in which *L. helle* occurs. Due to the lack/scarcity of these natural habitats, however, most populations tend to be found on abandoned wet meadows along streams, springs or lakes (Fisher *et al.,* 1999; Wipking *et al.,* 2007; Bauerfeind *et al.,* 2009; Chuluunbaatar *et al.,* 2009; Finger *et al.,* 2009; van Swaay *et al.*, 2012). Males are highly territorial. Their territories tend to be in close proximity to shrubs or trees, which provide protection from the wind. Females will only enter the males' territories to mate, afterwards returning to more open sites, where they lay their eggs. Both male and female adults roost in tall trees (Goffart *et al.*, 2001, 2010). The univoltine populations of Central Europe fly from mid

May until the end June (Meyer, 1981 c; Fisher *et al.,* 1999; Wipking *et al.,* 2007; Finger *et al.,* 2009). Adults have an average flight period of 10 days (Meyer, 1981 c; Fisher *et al.,* 1999), but extremes of 34 days have been recorded (Fisher *et al.,* 1999). While adults feed on as many as 30 different plant species (Wipking *et al.,* 2007), the larva is restricted to a single host-plant, the Common bistort *Polygonum bistorta* (Wipking *et al.,* 2007; Finger *et al.,* 2009; van Swaay *et al*., 2012). In its northern range, other host-plants like *Rumex sp.* and *Bistorta vivipara* are also accepted (Wipking *et al.,* 2007). The butterfly overwinters as a pupa in the leaf litter (Fisher *et al.,* 1999; Wipking *et al.,* 2007; van Swaay *et al*., 2012).

The main threats are habitat loss due to intensification of agricultural practices (land drainage and deterioration of wetlands), afforestation, increased fragmentation as well as ever increasing isolation of the different populations (Fisher *et al.,* 1999; Wipking *et al.,* 2007; Bauerfeind *et al.,* 2009; Chuluunbaatar *et al.,* 2009; van Swaay *et al*., 2012). Throughout Europe nine different subspecies have been recognised (Meyer, 1981 a, Habel *et al.,* 2008; Habel *et al.,* 2010 c; Habel *et al.,* 2011).

The species is classified as endangered under the IUCN Red List for European Butterflies (Van Swaay *et al.,* 2010) and is listed on the Annex II and IV of the Habitat Directive (EEC 92/43/EWG) of the European Union.

<u>Study Area</u>

After the loss of the only other known population in the southwest of Luxembourg due to construction (Meyer, 1981 b), *L. helle* is now limited to the "Ösling region" (Meyer, 1981 b; Wipking *et al.,* 2007; Habel *et al.,* 2008). The study area stretches over an area of 20 km^2 (see Map 1) and contains eight Habitat Directive and three Bird Directive sites (see Table 1 & Map 1).

Table 1: Habitat and Bird Directive Sites situated in the study area. For the exact location of the different sites see Map 2.

Habitat Directive Sites	
National Code	**Natura2000 Name**
LU0001003	Vallée de la Tretterbaach
LU0001004	Weicherange – Breichen
LU1001005	Vallée supérieure de la Wiltz
LU0001007	Vallée supérieure de la Sûre/Lac du barrage
LU0001033	Conzefenn
LU0001038	Troisvierges – Cornelysmillen
LU0001042	Hoffelt – Kaleborn
LU0001043	Hoffelt-Sporbaach
Bird Directive Sites	
National Code	**Natura2000 Name**
LU0002001	Vallée de la Woltz et affluents de la source à Troisvierges
LU0002002	Vallée de la Tretterbaach et affluents de la frontière à Asselborn
LU0002004	Vallée supérieure de la Sûre et affluents de la frontière belge à Esch-sur-Sûre

Site Selection

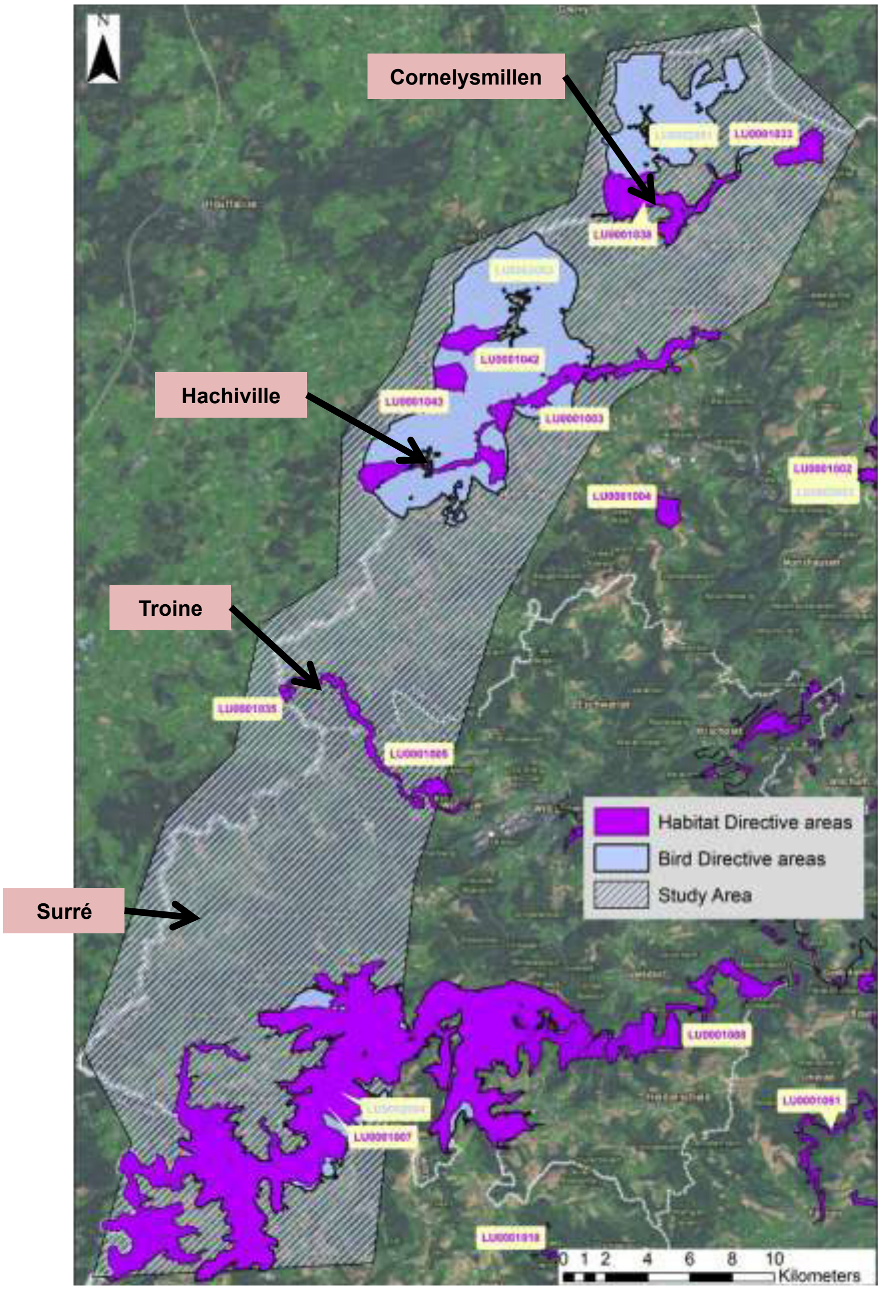
The whole study area was divided into four different zones. For each one of these zones, known and potential sites were identified to better organise monitoring. Potential sites were chosen using high-resolution areal images via the Luxemburgish geoportal (map.geoportal.lu). For each map there were between 30 and 60 sites of various sizes to be monitored. A total of 152 sites were visited of which 88 were kept for further analysis.

Monitoring

Monitoring was carried out from mid May (starting the 18th May 2013) till end of June (ending on 30th June 2013). As monitoring was limited to a single species, a zig-zag transect was used to count the different individuals. Due to a prolonged period of bad weather in May, the initial aim of establishing a population estimate was changed to a simple presence/absence study and an analysis of habitat use. Each site was visited for a minimum duration of 30 minutes (if no butterflies were found) or until no new individuals were seen.

For each individual the sex, its proximity to the closest hedge (shrub above 1.5 metre) and the plant cover (in % - using different categories: bistort, meadsweet, nettles & other plants) were recorded. For each observation, the exact coordinates were taken using a handheld satellite GPS.

Sites from which the butterfly was absent were classified into two different categories: Potential – Low Management (site needs continued management efforts, but is already a suitable habitat for the butterfly to colonise) and Potential – High Management (site has potential, but needs high management efforts before butterfly can colonise).



Map 1 – Study area, including all Habitat & Bird Directive Sites (name of each site shown as label). For orientation, the location of villages mentioned throughout the text is shown.

Additionally, each site was then further divided into one of the following “overall states”, as suggested by CRNFB (2006). An additional state was introduced to account for those sites that are currently unsuitable (Table 2): 1 – good; 2 – satisfactory; 3 – unfavourable; 4 – unsuitable at the moment.

Table 2: Criteria by which different states were assigned to individual sites/areas, depending on the overall habitat quality, as suggested by CRNFB (2006). State 'unsuitable' was added as an additional state for habitats where no host plants were present but could become potentially important sites due to their geographic location or as a result of ongoing current restoration efforts.

Indicator	State 'good'	State 'satisfactory'	State 'unfavourable'	State 'unsuitable at the moment'
Minimal surface	> 0.75 acre	0.50 – 0.75 acre	< 0.50 acre	N/A
Density of host-plant	> 50%	25 – 50 %	< 25 %	0 %
Density of flowering plants	> 5 %	1 – 5 %	< 1 %	N/A

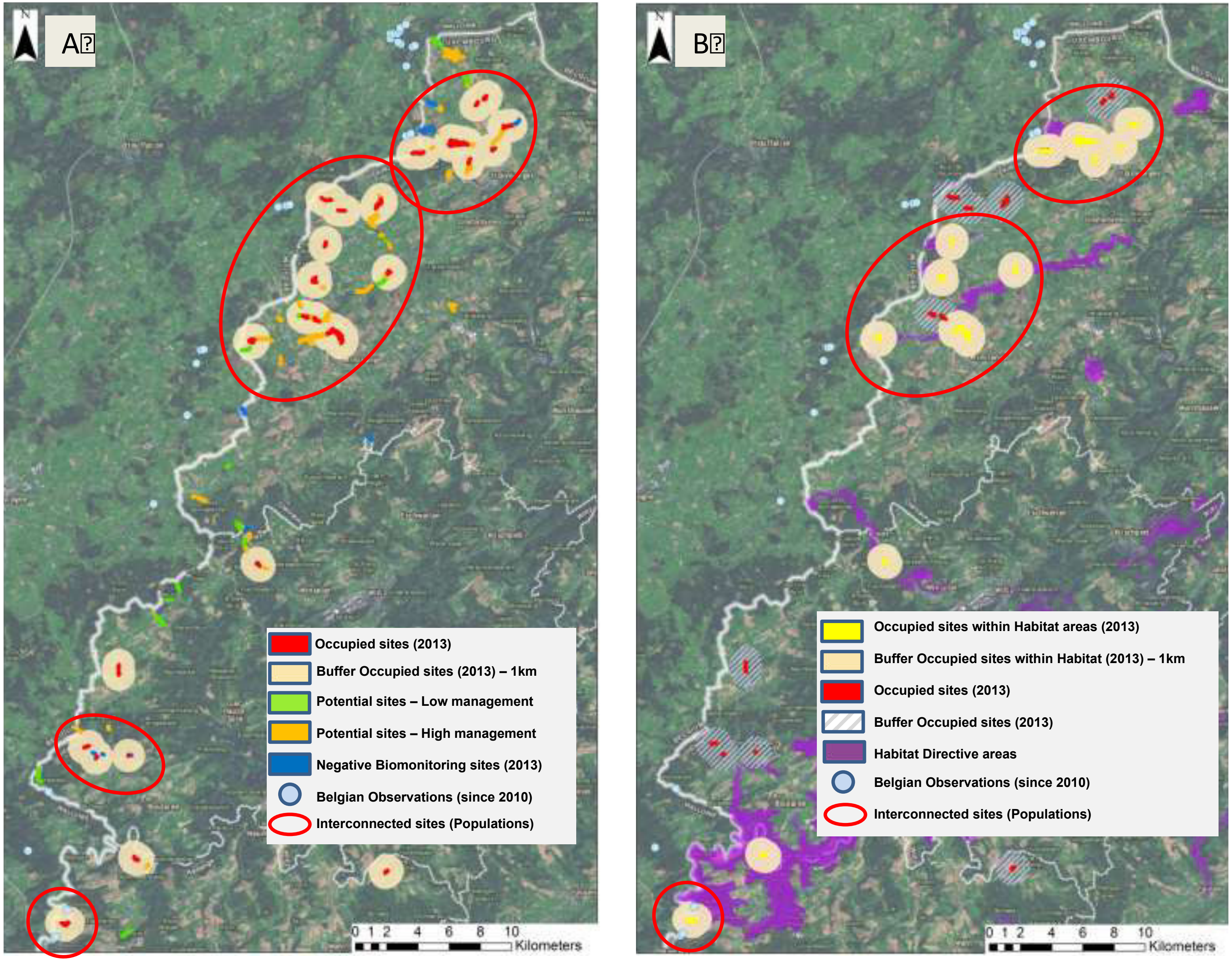
<u>Analysis</u>

Maps of the different situations (showing the 2013 population, the 2010 to 2013 population and potential future distributions in the whole study area or just within Habitat sites) were produced using ArcGIS. Polygons of each site were drawn using the boundaries of suitable habitat. The area (in m^2) of each site was calculated using the Geometry calculator. Distances (in m) between patches were determined using 'Near' tool for between occupied sites (limiting the distance to 2 km as the favourable maximum distance between sites for *L. helle* (CRNFB, 2006)) and from negative to occupied sites (no limitation in distances). To better visualise the connectivity of the different sites, a buffer zone of 1 km was used (half the favourable maximum distance between sites – CRNFB, 2006).

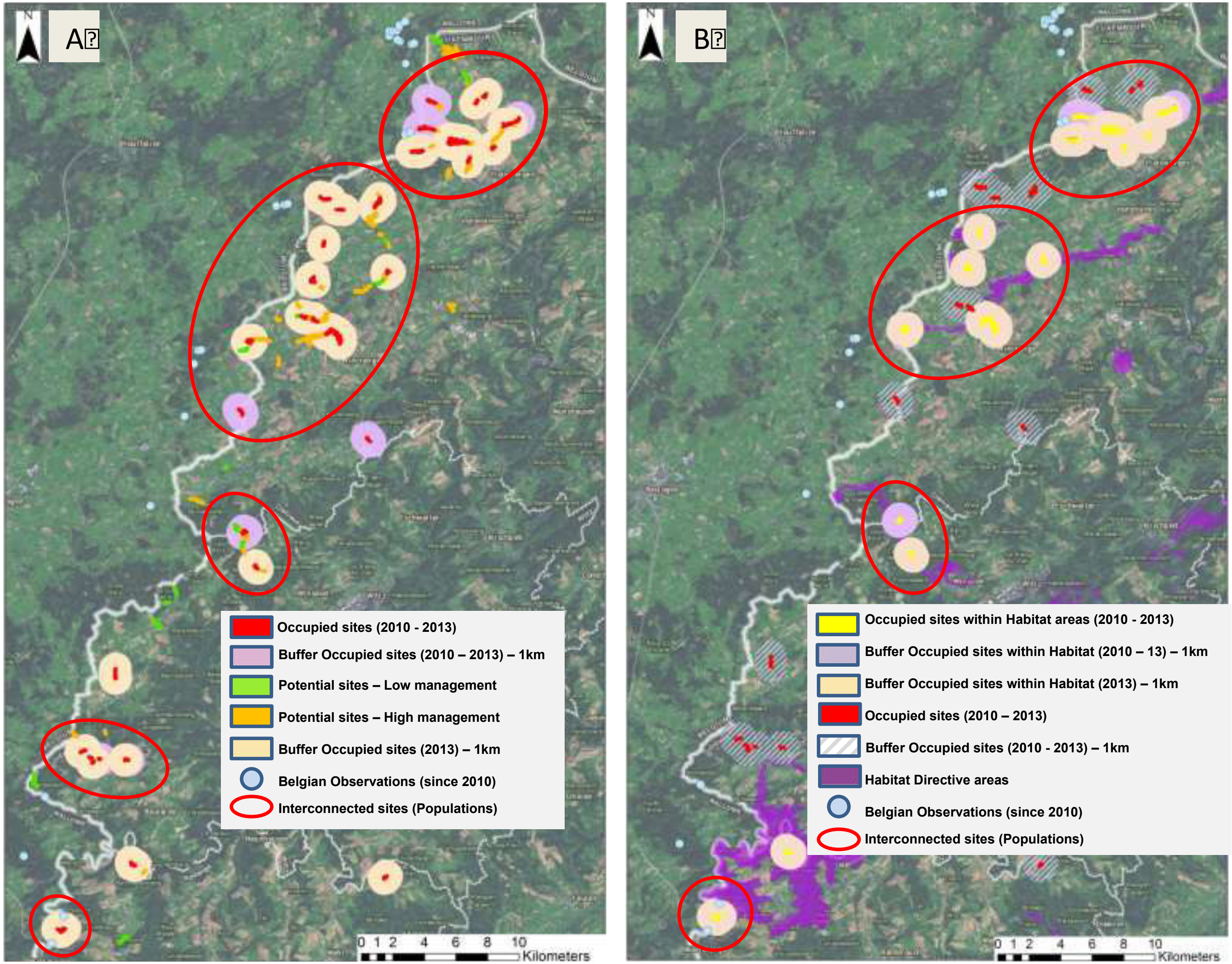
Data was exported from ArcGIS to be analysed in SPSS. As data was not normally distributed, a Mann Whitney-U test was conducted in order to determine whether or not males and females have different habitat requirements (i.e. distance to the nearest hedge, plant percentage cover for each individual plant or group of plants).

A logistic regression was conducted to determine the importance of various factors, such as area, isolation and state of habitats.

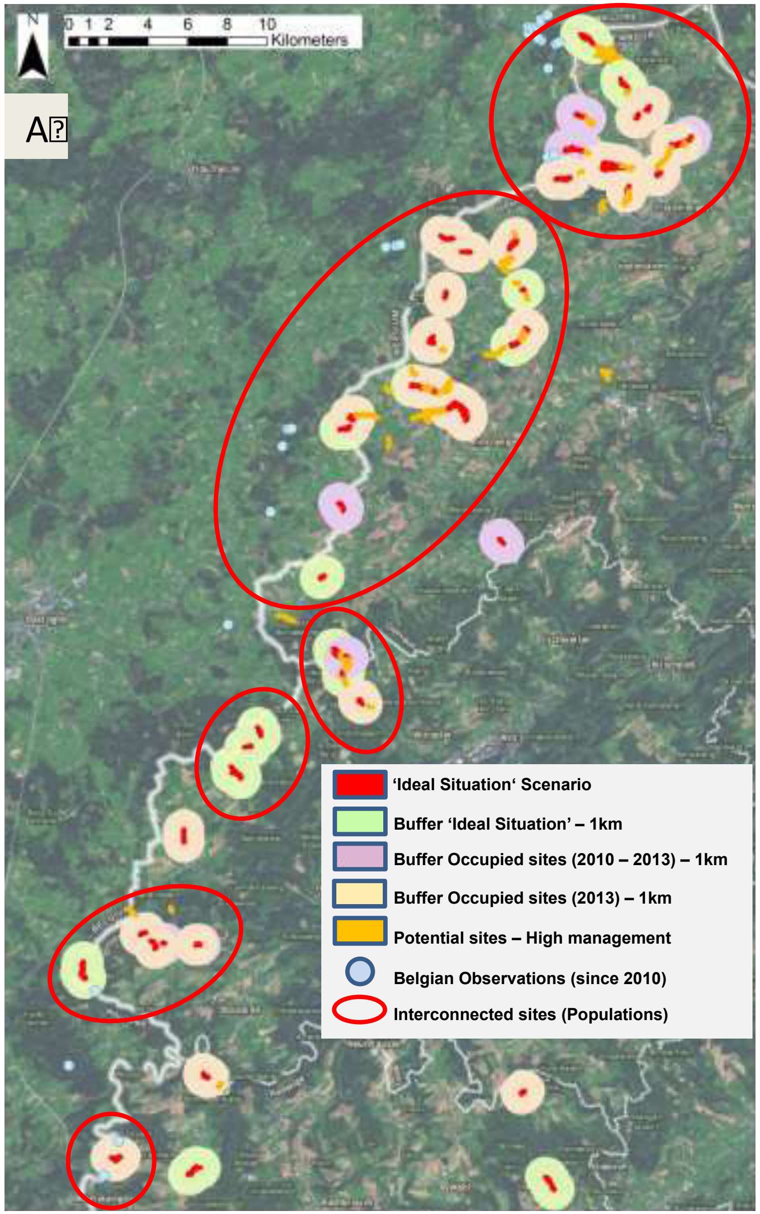
Results

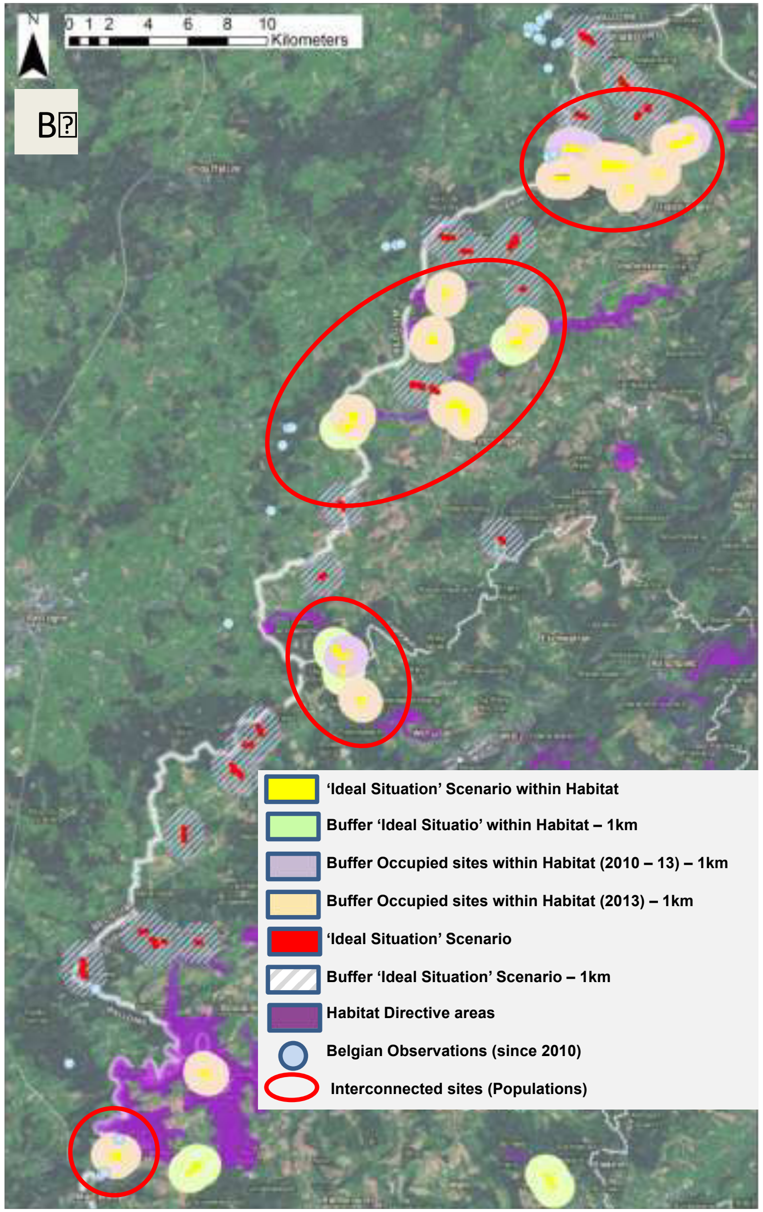


Map 1 – The distribution pattern of *L. helle* in 2013. A) shows occupied sites in the complete study area whereas B) only shows occupied sites within Habitat Directive areas. A buffer zone of 1 km around occupied sites is also shown. The red circles indicate individual populations of interconnecting sites. As these were not tested using mark-recapture experiments, these should only be seen as assumptions.

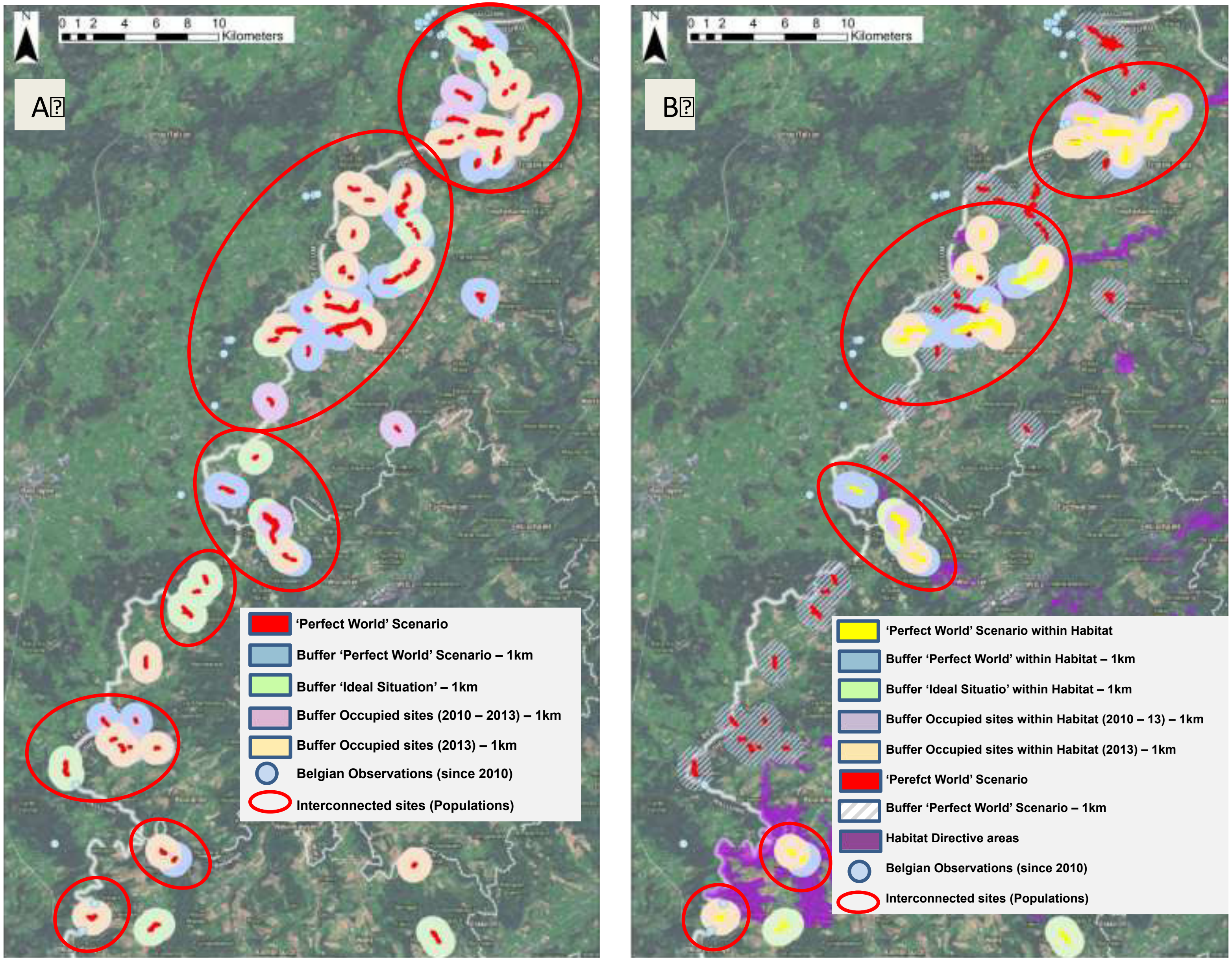


Map 2 – The distribution pattern of *L. helle* between 2010 and 2013. A) shows all occupied sites in the complete study area and B) only within Habitat Directive areas since 2010. A buffer zone of 1 km around occupied sites is also shown. The red circles indicate individual populations of interconnecting sites. As these were not tested using mark-recapture experiments, these should only be seen as assumptions. Buffer zones of sites occupied only in 2013 are also shown.

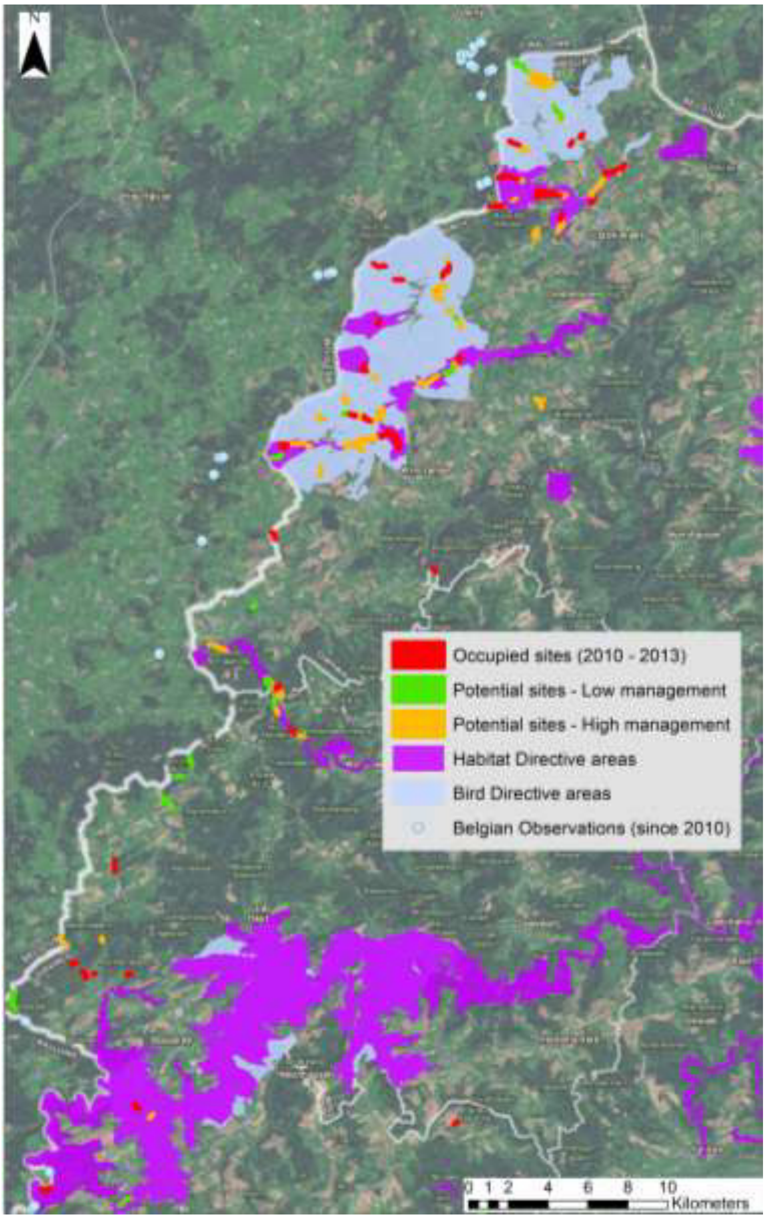




Map 3 – Ideal Situation: the expected distribution pattern of *L. helle* if all sites classified as 'Potential – Low management' were occupied. A) shows potentially occupied sites in the complete study area, B) only within Habitat Directive areas. A buffer zone of 1 km around occupied sites is also shown. The red circles indicate individual populations of interconnecting sites. As these were not tested using mark-recapture experiments, these should only be seen as assumptions. Buffer zones of site occupied only in 2013 and in 2010 - 2013 are also shown.



Map 4 – Perfect World: the expected distribution pattern of *L. helle* if all sites classified as 'Potential – Low management' & 'Potential – High management' were occupied. A) shows potentially occupied sites in the complete study area, B) only within Habitat Directive areas. A buffer zone of 1 km around occupied sites is also shown. The red circles indicate individual populations of interconnecting sites. As these were not tested using mark-recapture experiments, these should only be seen as assumptions. Buffer zones of site occupied only in 2013, in 2010 - 2013 and of the 'Ideal Situation' scenario are also shown.



Map 5 – Showing the different sites (occupied and potential sites), the Habitat and Bird Directive areas. Considering managing sites within Bird Directive areas would connect two major populations in the top North of Luxembourg. This would highly increase survival chances of these populations.

Given the current distribution (maps 1.A and 2.A) and the poor dispersal abilities of *L. helle* it is unlikely that the remaining populations across the losing region represent a self-sustaining metapopulation. Many populations remain highly isolated from each other. Only the top north-western region might represent two viable populations, with good connections to neighbouring populations in Belgium. If management and restoration efforts succeed in restoring “low & high management” sites, the resulting metapopulation could become large enough to be self-sustaining (Map 4.A).

Table 3: Sites at which the species was present in 2013 and from 2010 till 2013 or could be present in the ‘Ideal Situation’ scenario (see Map 3) and ‘Perfect World’ scenario (see Map 4). Population, site counts and area (in acre) are shown for the complete study area or only within Habitat Directive areas. ‘Populations, sites and area lost’ indicates the amount of populations, sites and area (in acre) that will be lost if sites would only be managed within Habitat Directive areas.

		Total study area	Within Habitat Directive areas	Populations, sites and area lost
Occupied sites (2013)	Current populations	4	3	1
	Occupied sites (and area in acre) within the given populations	29 (126 acres)	15 (93 acres)	14 (33 acres)
	Total of occupied sites (and area in acre)	31 (132 acres)	17 (112 acres)	14 (20 acres)
Occupied sites (2010 – 2013)	Current populations	5	4	1
	Occupied sites (and area in acre) within the given populations	36 (175 acres)	17 (120 acres)	19 (55 acres)
	Total of occupied sites	40 (195 acres)	19 (138 acres)	21 (57 acres)
“Ideal Situation” Scenario	Current populations	6	4	0
	Occupied sites (and area in acre) within the given populations	54 (494 acres)	27 (307 acres)	27 (187 acres)
	Total of occupied sites (and area in acre)	60 (533 acres)	30 (327 acres)	30 (206 acres)
“Perfect World” Scenario	Current populations	7	5	2
	Occupied sites (and area in acre) within the given populations	94 (1035 acres)	56 (612 acres)	44 (423 acres)
	Total of occupied sites (and area in acre)	103 (1111 acres)	61 (635 acres)	42 (476 acres)

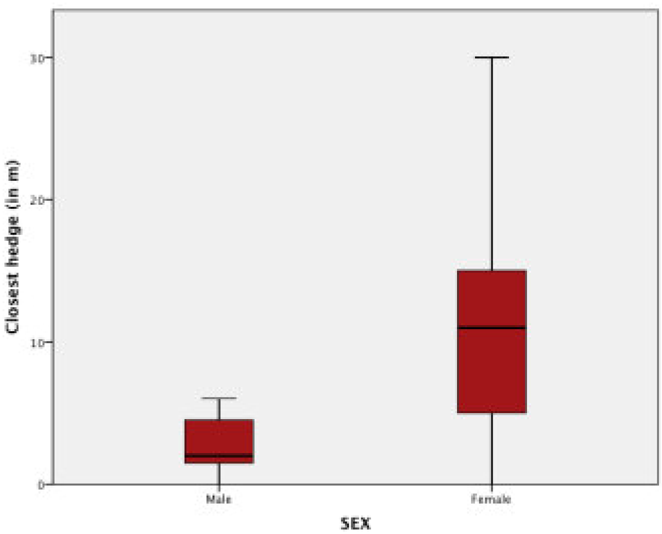
f conservation efforts are limited to sites within the Habitat Directive (as is required under the current LIFE Éislek project) the size of the populations and the number of sites that are available for management would be significantly decreased (Map 1.B & Map 2.B; Table 3) and will almost certainly not be sufficient to maintain the populations of *L. helle* in the long term. Even if all potential sites inside Habitat Directive areas that only require low management efforts were to be colonised eventually, satisfactory big populations would probably only occur in the top north-western part of the Ösling (Map 3.B). Equally, if all potential sites (low and high management) within Habitat Directive areas were to be colonised by *L. helle* ("Perfect World" scenario – Map 4), the populations still show no suitable interconnectivity for a sustainable metapopulation that would span the entire Ösling region (Map 4.B).

Habitat use

Table 4: Showing the average of the different data collected for habitat use. The distance is shown in m, whereas the different plant covers are shown in % for both sexes. The significance values of the Mann Whitney-U tests are also given.

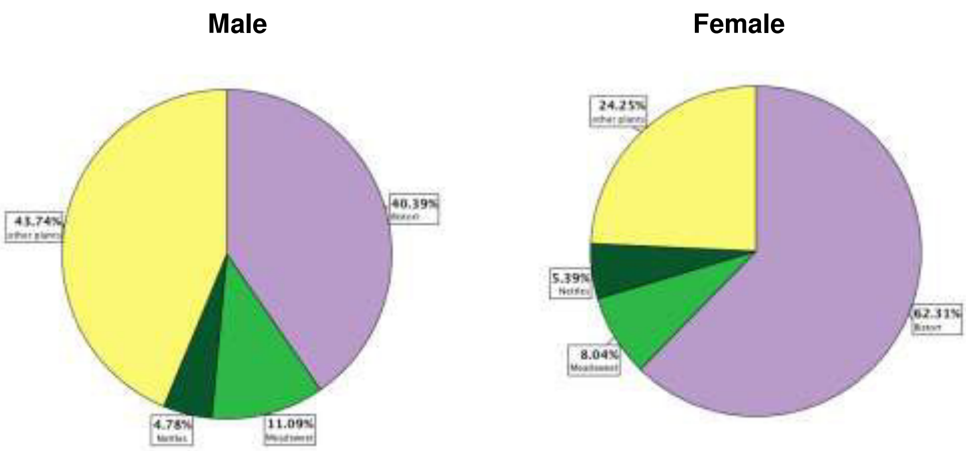
	Average Distance (in m)	% cover			
		Bistort	Meadsweet	Nettles	Other plants
Male	2.7	39.9	7.4	0.9	42.8
Female	11.4	63.3	5	2.6	24
Significance	>0.0001	0.041	0.931	0.612	0.145

As indicated by the results of the Mann Whitney-U test, males and females differed significantly in their habitat requirements, both in terms of shrub prevalence and percentage cover of the larval food plant (bistort) but not for any other plants. The median distance to the nearest hedge differed significantly between sexes ($Z = -4.83$; $p > 0.001$), with females being located further away from the nearest hedge (11 ± 1.3 m). Males on the other hand were always found in close proximity to a hedge (2 ± 0.43 m).



Graph 1: Boxplot showing the difference in median distance from closest hedge for both sexes.

The Mann Whitney-U tests showed a clear difference in plant percentage covers (in %) between sexes for bistort only ($Z = -2.048$; $p = 0.041$). All other plant percentage covers did not differ significantly between sexes (Meadsweet: $Z = 0.087$; $p = 0.931$; Nettles: $Z = -0.507$; $p = 0.612$; Other plants: $Z = -1.459$; $p = 0.145$). Both sexes seem to avoid high densities of both nettles and meadsweet (Graph 2). Occurrence of other plant species, other than nettles and meadsweet, does not seem to affect the presence of neither male nor female (Graph 2).



Graph 2: Average density cover of bistort, meadsweet, nettles and other plants in a 2m radius of occurrence for both sexes of *L. helle.*

<u>Occurrence pattern</u>

The minimum adequate model (MAM) for the logistic regression shows that there is a significant increase in patch occupancy with Isolation ($x^2 = 27.266$, d.f. = 1, $p < 0.0001$). Equally, there is a significant increase in patch occupancy with the State of habitat ($x^2=53.9$, d.f. = 1, $p < 0.0001$). Neither area nor any of the interactions showed any effect on the occurrence pattern for *L. helle*.

Discussion

Habitat use

The findings of this study suggest that the area of any given habitat has no influence on the presence or absence of the butterfly. While this does not concur with the suggestions of Hanski (1998), who postulated that populations on larger sites should be less likely to be threatened by extinction. As stated by the CRNFB (2006), sites should be no less than 0.50 acres (see Table 2). With all sites of this study (both occupied and unoccupied) being well over this threshold - the smallest one being approximately 2800 m^2 (~ 0.70 acre) – it is not surprising that the overall habitat size was not significant. Given the poor quality of many of the larger sites, however, it becomes obvious that a site's quality is much more important than its size per se. Many of the smaller sites with favourable conditions were therefore equally likely to be occupied. This also agrees with findings by Bauerfeind *et al.*, (2009) whose study found that patch size is the least important factor in determining the presence of *L. helle*.

Isolation and quality (state) of habitat remain the most important factors considered here for the presence of *L. helle* on any given site, which also agrees with the results of Bauerfeind *et al.* (2009). Given the low dispersal abilities of the species (Fisher *et al.,* 1999; Chuluunbaatar *et al.,* 2009), with an average flight distance of 181m for females and 44m for males (Chuluunbaatar *et al.,* 2009), it is little surprising that patch isolation is a crucial factor for *L. helle* when it comes to effectively colonising new sites.

For Luxembourg, many sites are in close proximity to each other, especially in the north-western region of the Ösling, where many sites remain unoccupied (see Map 1 A) & 2 A)). As the analyses show, the state of the habitat also plays a crucial role. A potential site can be in close proximity to a colonised site, even be immediately adjacent to it, but if the quality of the site is not sufficiently high, the butterfly is unable colonise it.

The significant differences in habitat use that were found between males and females of *L. helle* clearly show that females are dependent on a high density of bistort, the host plant. The percentage coverage of bistort is therefore also one of the most important factors for a site to be suitable for the butterfly and be considered as being of 'good quality' (Wipking *et al.,* 2007; Turlure *et al.,* 2009; Goffart *et al.,* 2010). Males, on the other hand, do not rely on an equally high density of bistort but require the presence of sufficient structures that provide protection from the wind and from where they can perform their territorial display flights and/or mate with females.

This this suggests that males are able to colonise low quality sites in close proximity to high quality sites. Given their weak dispersal ability and the females' reliance on high densities of common bistort, it would be unfavourable for males to set up their territories too far away from potential egg-laying sites. So males are indirectly dependent on the quality of the site to increase the likelihood of females being present.

Good quality habitat of *L. helle*

The same is true for females, who are indirectly dependent on the presence of some structures, such as trees and shrubs, for wind protection and to increase the likelihood of males being present. Females will try and find a male to mate with, only then to return to egg-laying areas. So, both sexes are dependent on a good quality habitat, which consists of a mosaic of different structures, some trees to roost in, and a high-density coverage of bistort. The combinations of these factors are also always given as minimum adequate necessities for the survival of the butterfly on any patch of land (Wipking *et al.,* 2007; Turlure *et al.,* 2009; Goffart *et al.,* 2010).

There are, however, sites that are in unfavourable condition where the butterfly still occurs (personal observation). These sites were often highly overgrown by either nettles or meadsweet and so contained a low density of bistort. These could either be dispersing individuals moving between sites or remnant individuals of a time when these patches were still in favourable condition. Lack of management or wrong management has turned these sites into lower quality sites for the butterfly. Some of these sites, for example, are grazed by high stock densities (of sheep) every year, between June and August (Claude Schiltz, personal communication). As sheep usually avoid nettles and meadsweet, these two plant species greatly benefit from this management regime and slowly overtake the entire site, gradually outcompeting bistort, which is eventually lost from the site or only present in very low densities.

Other sites lie downstream or in close proximity of intensively used lands. Fertiliser run-off increases the nutrient level in potential butterfly habitats (Öckinger & Smith, 2006; Wipking *et al.,* 2007). This, in turn promotes the growth of nettles. Unless more appropriate management regimes are applied and surrounding sites are obliged to adhere to certain restrictions (see proposed management options), these remnant populations are likely to be lost in the near future.

Wipking *et al.* (2007), in their study of the exact same populations in 2005 and 2006, came to the same conclusions. In addition, the authors strongly criticised the use of high-density sheep grazing as a management option on potential and occupied sites, and concluded that the butterfly had probably been lost or populations severely depleted as a result.

The persistence of any butterfly species is dependent on the maintenance of a network of local populations with potential exchange between them (Bauerfeind *et al.,* 2009; van Swaay *et al.*, 2012). Not every patch of potential habitat has to be suitable at any one time. A core of good sites should, however, remain (Goffart *et al.,* 2010; van Swaay *et al.*, 2012). Some studies have shown that management techniques like mowing, grazing and tree cutting can have positive effects on biodiversity as they reduce the dominance of highly competitive plants (Goffart *et al.,* 2010 and ref therein). Inappropriate management efforts, on the other hand, can have detrimental effects for the survival of many butterfly species. Ellis (2003), for example, showed that the Brown Argus *Aricia agestis* adults were much less abundant in grazed compared to un-grazed areas. Öckinger *et al.* (2006), managed to show that sheep have a negative effect on species richness and that grazing should preferably be done by cattle or horses. Goffart *et al.* (2010) showed that mowing at the wrong time reduced the population of certain butterflies (here *P. eunomia* & *L. helle*) by 50% or more. Lack of management, on the other hand, can be equally detrimental, as was shown by Öckinger et al. (2006), who have shown that species richness of grassland specialists (both butterflies and plants) decreased when semi-natural pastures were abandoned.

Given that *L. helle* predominantly exists on semi-natural lands in the Ardennes region (Wipking *et al.,* 2007; Goffart *et al.,* 2010; PAE, 2013), it is important to apply the appropriate management options in order to guarantee the long-term survival of the species. As disturbances, such as fires or storms, which naturally rejuvenate biotopes no longer play a vital role, semi-natural sites need to be managed to remain in a suitable condition (Meyer & Helminger, 1994; Turlure *et al.,* 2009; Goffart *et al.,* 2010; PAE, 2013).

This is especially important for *L. helle*, since it already shows highly fragmented populations over its entire distribution range (Turlure *et al.,* 2009; Goffart *et al.,* 2010). It is however also crucial to acknowledge the needs of other threatened butterfly species, such as *Proclossiana eunomia,* that might co-exist with *L. helle* in the same area.

P. eunomia is also a glacial relict species with both adults and larvae being dependent on the same host plant as *L. helle.* Consequently one might assume these two species would compete with one another, however, due to significant differences in ecological needs (Tolman & Lewington, 2008; Turlure *et al.,* 2009; Goffart *et al.,* 2010), both species can co-exist.

P. eunomia is currently listed on neither Annex II nor IV of the Habitat Directives or on the IUCN Red List. It is however listed as Endangered on the Red List of Luxembourg (Meyer, 2000). Its status across Europe should not jeopardise its survival and so sites should be managed to benefit both butterfly species in all their different stages at the same time.

<u>Current management schemes</u>

Management of *L. helle* habitats currently takes one of two forms: sites are either grazed by high densities of sheep or left aside (personal observation). This is contrary to the recommendations of most studies, which suggest that grazing should be done by cows or horses (ideally Fjord Ponies), while sheep are least favourable (Wipking *et al.,* 2007; Turlure *et al.,* 2009; Goffart *et al.,* 2010; van Swaay *et al.*, 2012), as they have strong negative effects on the host-plant due to trampling of the rhizomes.

In addition, grazing is often conducted much too early, at the beginning June. Sheep preferably feed on bistort, largely ignoring all other plants that occur in wet meadows. This not only reduces the availability of food plants for the butterfly but also removes all larvae and pupae that might occur on the plant. Both direct and indirect effects sheep grazing can be highly detrimental for the butterfly population (Turlure *et al.,* 2009; Goffart *et al.,* 2010; van Swaay *et al.*, 2012).

Site being grazed by cows

Site after being grazed by sheep

Unmanaged sites, on the other hand, are slowly invaded and overgrown by shrubs and trees, eventually replacing all wet meadows with woodlands. Host-plants are also often outcompeted by nettles or meadsweet, which benefit from fertiliser runoff from intensively used agricultural fields in the direct vicinity.

Natural habitats, like moorlands, can be preserved by occasional shrub clearing to prevent afforestation. Semi-natural wetlands can only be preserved by both mowing and extensive grazing. While both management options will result in some negative effects for both butterfly species, these can be minimised if used appropriately. Under no conditions should sites be left unmanaged, as this would eventually lead to afforestation. Management should never be too intensive. If in unfavourable condition, stronger management options can be considered for a period of time in order to restore the site into a higher quality habitat. Once this status has been reached, management options need to be adapted so butterflies can (re-) colonise.

Based on the findings of this study and a thorough literature research, site-specific management plans for each type and/or state of habitat (e.g. occupied sites, unoccupied sites with high potential, etc.) have been devised and can be found in the appendix. The new proposed plans are set up to benefit both *L. helle* and *P. eunomia* and ideally should replace all previous management plans.

Proposed changes to the Action Plan for *L. helle*

A management plan for *L. helle* in Luxembourg has been set up by natur&ëmwelt – Fondation Hëlef fir d'Natur in 2013. These plans propose grazing of the complete site as sole management option and consider mowing as an inappropriate option. As proposed above, however, grazing should never be considered as the only option for either occupied or unoccupied (potential) sites, especially if sheep are the only livestock to be used for the grazing. It is highly recommended to respect the alternation of grazing and mowing, always leaving an area unused as has been suggested by many different authors (Meyer & Helminger, 1994; Wipking *et al.,* 2007; Turlure *et al.,* 2009; Goffart *et al.,* 2010; van Swaay *et al.*, 2012).

Occurrence pattern in the study area

The management or restoration of sites alone, however, is not enough to ensure the survival of *L. helle* in Luxembourg. Habitat patches also need to be close enough to each other to ensure the connectivity of the different populations. Looking at the whole area of the north-western parts of the Ösling region, the recent data (2010-13) suggests that populations of *L. helle* might be relatively stable but not self-sustaining in the long term. Many populations still remain highly isolated from each other and are likely to become extinct over time, leaving only the two bigger populations in the top north-western corner. However, if all potential sites found during this study, will be adequately managed and colonised, there could be an interconnected metapopulation in Luxembourg with a good connectivity to populations in Belgium.

However, as can be seen from the various maps produced for this study, only 18 occupied sites of a total of 37 sites lie within Habitat areas (see Map 1 B) & 2 B); Table 3), which means over half the sites are not available for management under the LIFE Éislek Project. Restricting management to those sites within Habitat Directive areas dramatically decreases the availability of both good and potential habitats. This is unlikely to be sufficient to guarantee the long-term survival of *L. helle* in Luxembourg, as the progressive loss of suitable habitat can lead to losses of local populations, which in turn may eventually result in regional extinctions (van Swaay *et al.*, 2012). The current extent of the Habitat Directive areas in Luxembourg, is not sufficient to maintain a viable population in Luxembourg. The loss of the Luxemburgish population could have severe consequences for the nearby Belgian populations.

Including sites occurring within Bird Directive areas, however, will add another 8 sites that could be potentially managed under the LIFE Éislek Project (see Map 5). Although this still leaves 11 sites potentially unmanaged, this will significantly increase the two major populations in the top north-western region of the Ösling. While European regulations dictate that – within Life projects - Bird Directive sites may only be managed for birds (but not for other fauna), some of the management options for the Red-backed Shrike may be beneficial for *L. helle*. Thus, the management of these sites could be adapted to benefit both the bird and butterfly species. Management for the Red-backed Shrike usually involves light grazing while keeping other areas as fallow land. A rotational grazing regime which is limited to half of each site, while keeping the other half unused may thus be beneficial for both species. Additional mowing of every 3 to 5 years would ensure that the site will not be encroached by dominating plant species, which also have negative effects on the Red-backed Shrike.

Other populations further south, however, will be lost in the future if they are ignored now. As fragmentation negatively influences species richness (Hill *et al.,* 1996; Fisher *et al.,* 1999; Wettstein & Schmid, 1999; Louy *et al.,* 2007; Bauerfeind *et al.,* 2009), reconsideration of the delineation of some of the Habitat Directive areas is highly advisable. Especially since some boundaries/areas have proven to occur on the wrong place (e.g. LU0001010 – Grosbous 'Néibrouch') or simply follow roads instead of vegetation types. The area around Surré, Troine and Hachiville should be highly considered to be included into the Natura 2000 Habitat network. Additionally, not only sites occupied by *L. helle* should be under protection, but also adjacent potential sites. These could act as corridors between sites and, thus, enhance the population connectivity.

<u>The current state of *L. helle* in Luxembourg and proposed monitoring plan</u>

The lack of data that was available at the time may well be one of the reasons why the boundaries of the Habitat Directive areas were rather crude and in some cases even inappropriate. Exemplifying for this situation, this is reflected by the knowledge of the distribution of *L. helle* in Luxembourg over the past 40 years. In 1981, Meyer produced a document on the ecology and distribution of *L. helle* for the whole of Europe (Meyer, 1981 a, b, c). At that point only 5 sites were known in the Ösling region. Before this, the only known population occurred near Steinfort in the southwest of Luxembourg. In 1990/92 and 2005/06 more studies on the distribution of *L. helle* were conducted by the Natural History Museum of Luxembourg (MNHN) (Meyer & Helminger, 1994, Wipking *et al.,* 2007), reporting it from 1 and 23 sites respectively. Only with the start of the butterfly biomonitoring in 2010 (in collaboration of the MNHN, CRPGL* and MDDI**) data started to be collected in a systematic way and provided a much more thorough estimate of the actual distribution and population status of this species. Many new sites have since been found. As the biomonitoring is limited to randomly chosen 5x5 km squares, additional sites were found during the monitoring for this and the LIFE Éislek projects.

*CRPGL – Centre de Recherche Public Gabriel Lippmann

**MDDI – Ministère de Dévelopement Durables et des Infractructures

The number of new, previously unknown sites is particularly surprising, given the fact that this year's adverse weather conditions made field work very difficult and meant for much lower butterfly abundances present at any one site.

This suggests that there may be additional occupied sites that are still unknown. For the protection of this species, however, it is crucial to get the best possible idea about its distribution in Luxembourg. As the butterfly occurs in a highly fragmented population, restricted to only a few suitable habitats, it is crucial to find all sites where the butterfly still occurs, so that these can be protected and properly managed.

The huge lack on information of the distribution pattern of *L. helle* in Luxembourg has been largely due to lack of financial support from the government. Only when the butterfly Biomonitoring started in 2010, was financial support made available for butterfly monitoring. This Biomonitoring, however, does not concentrate on one species only, but tries to estimate the current population state of all the butterflies occurring in Luxembourg. Therefore, it is even more crucial that during the LIFE Éislek, monitoring should play a vital role during the project.

As the monitoring, which is carried out as part of the LIFE Éislek project, is limited to sites within Habitat Directive Areas, it should be possible to visit all potential sites over the next 4 years.

To efficiently monitor the whole area and every suitable habitat, it is crucial to prioritise monitoring to those areas that are considered to have high potential but for which the butterfly has not been found yet rather than focusing on sites that are already known to be occupied. Potential sites should be visited at least twice during the season.

Occupied sites, which are under management, should be visited once during the peak flight period (first week of June) to make sure that management efforts have no negative or detrimental effects on the population. If the population shows any kind of decline, management efforts should be re-evaluated.

Whenever possible, Bird Directives areas should also be monitored for *L. helle*. These can be combined with the monitoring for both bird species (and vice versa) that is being carried out as part of the Life project. This will ensure that no suitable habitat is managed solely for the benefit of a single species, bird or butterfly.

Ideally, potential sites directly outside Habitat Directive areas should be monitored, too. If occupied sites are found, this data could be used as evidence to re-evaluate the boundaries of certain Habitat Directive areas or for the designation of new ones.

<u>The future of *L. helle* in Luxembourg: Why save a glacial relict species?</u>

Apart from its fragmented distribution in Luxembourg, there are other factors, which make the survival of *L. helle* in the Ardennes region very difficult in the long term. Rising temperatures and changes in agricultural practices have caused a severe reduction of wet meadows (Finger *et al.,* 2009; Habel *et al.,* 2010 c). While agricultural practices could potentially be controlled, climate change cannot. As a result of the increasing isolation and habitat fragmentation, many populations are being threatened by their reduced genetic diversity (Finger *et al.,* 2009; Habel & Assman, 2010; Habel *et al.,* 2010 c). The Ardennes' population is genetically different from both the next closest populations in the Eifel and Westerwald (Finger *et al.,* 2009; Habel & Assman, 2010; Habel *et al.,* 2010 a, c). Furthermore, Finger *et al.* (2009) showed high genetic differentiation within the Ardennes region, indicating highly disrupted population connectivity. As isolation and small population size can lead to inbreeding, and thus extinction (Frankham, 2005; Habel *et al.,* 2010 b), the most imminent goal should be to substantially increase the population sizes and to enhance gene flow.

Climate Envelope Models, however, have predicted that *L. helle* will probably go extinct in regions with lower altitudes if climate will continue to warm (Habel & Assman, 2010; Habel *et al.,* 2010 b; Habel *et al.,* 2011). So why invest money into saving a species that is doomed to go extinct? Habel *et al.* (Habel & Assman, 2010; Habel *et al.,* 2010 b & c; Habel *et al.,* 2011), whose many studies predict the extinction of *L. helle* in the Ardennes region, suggested many times that the genetic uniqueness for this region alone deserves protection. Furthermore, the studies indicate that the perspective doom of *L. helle* might not be as dramatic as shown by these models. It is, therefore, crucial to obtain the largest possibly populations within areas most threatened by climate change to give the butterfly the best possible genetic advantages to potentially adapt to new upcoming situations. Thus, the highly fragmented habitats of the Ardennes region must be connected again to establish a good gene flow (Habel *et al.,* 2011).

Containing unique alleles should, however, not be the only reason why *L. helle* should be protected. The Ösling region is one of the most intensively used agricultural areas of Luxembourg. As a result, natural habitats only remain in small isolated pockets, as does the wildlife that is associated with these habitats. Many species have become dependent on the maintenance and restoration of these few remnants of natural habitats, which can have a beneficial impact on many different species. The restoration efforts of moorland-like habitats around the Cornelysmillen for example, have improved the habitat to the point that now a total of 258 plant species, 86 bird species, 107 lepidopterans (including *L helle*) and 57 coleopterans have been detected on these sites (natur&ëmwelt, unpublished data). So investing money into the protection of *L. helle* does not just save one species, but will support a much wider biodiversity.

<u>*L. helle* as a flagship species of wet meadows</u>

Many studies have shown that improving an area for one target species can increase overall species richness of such habitats (Maes & Van Dyck, 2004; Sawchik *et al.,* 2005). Being such a great survivor by clinging on to isolated fragmented populations all over Europe and being a good indicator species for wet meadows, make *L. helle* a great potential flagship species of wet alpine grasslands. Increasing public awareness to the threat this species is facing might result in greater public understanding and thus, support to protect this species. Additionally, focal species can become a valuable tool for the proposition and evaluation of management practices for a wider range of biodiversity conservation (Sawchik *et al.,* 2005).

Conclusion

With the current rate of climate change, all conservation efforts invested now into saving the population of *L. helle* in Luxembourg might prove fruitless in the future. It is nevertheless worth protecting a species, if the management efforts that are carried out to help its survival will also benefit many other species. Habitat requirements of *L. helle* are sufficiently low, for the species to persist in the small remnants of suitable habitats that are still left in the Ösling region of Luxembourg. Current efforts to only manage habitats within Habitat Directives area, however, are unlikely to guarantee the species' survival. The overall goal of the species' long-term survival can only be achieved if all stakeholders (nature conservation, government officials, land owners and farmers) manage to work together, both inside and outside of Habitat and/or Bird Directive Areas. Higher financial support from the government to restore and/or maintain all occupied sites outside Habitat areas should be given to both private landowners and nature conservation organisations (e.g. “natur&ëmwelt – Fondation Hëlef fir d’Natur” for Luxembourg).

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Appendix – Management Plans for *L. helle*

All management options are based on recommendations by Goffart *et al*. (2010), Turlure *et al*. (2008), Meyer & Helminger (1994), as well as van Swaay (2012).

Mowing

Mowing at any time of the year will always result in detrimental effects on *P. eunomia, L. helle* being only sensitive to too early mowing. Late mowing (after August) has negative effects on *P. eunomia* as it will remove grass tussocks on which caterpillars are dependent. It has, however, no negative effects on *L. helle*. Early mowing (between May and July) has detrimental effects on both butterfly species, as caterpillars and pupae will be killed or removed from the site.

Mowing itself can have beneficial effects, as it increases the abundance of flowering plants. As a result, mowed sites increasingly attract adults of *L. helle* both for feeding and for laying eggs *. A*dults of *P. eunomia,* however, strongly avoid these patches the following year due to a lack of grass tussocks.

All cut material should be removed from site to prevent increases in soil nutrients.

Grazing

Overall, the impact of grazing on butterfly abundance tends to be lower than for mowing. However, inappropriate or intensive grazing regimes that are conducted either too early (before end of July) or using high stock densities can have strong negative effects on both species. *P. eunomia* seems to benefit more from late grazing and/or alternately grazed plots. Late grazing (from August) prevents trampling of both host plants and early stages of development of both species and the removal of nectar sources during flight period of *L. helle*. The main threat from grazing results from trampling of the subterranean rhizomes of the host plant (Mireille Molitore, personnel communication). Inappropriately high livestock densities would therefore be likely to exacerbate this effect.

Best results for both species were achieved by alternating grazing regimes.

Grazing is best conducted by cattle or horses (ideally Fjord Pony). If this is not possible, grazing by sheep is an option. Under no circumstances, however, should this exceed the maximum stocking rate of 0.2 Livestock Unit (LU)/ha. Ideally, sheep flocks should be accompanied by a shepherd to guarantee equal grazing of all areas.

If sites are too small (<1 ha), grazing as a management option should be re-evaluated or only conducted over a short period of time (maximum 1 week).

Neither management option (grazing or mowing) should be considered as the only management tool on favourable sites. Under no circumstances should early mowing or grazing be applied to the whole site or to the same site for several consecutive years, as it might not only wipe out the entire population of larvae and/or pupa of both butterfly species, but also remove all grass tussocks for subsequent years.

Unmanaged areas

On sites where the butterfly already occurs or could occur, leaving unmanaged areas each year is highly recommended. These areas provide important refuge areas for both butterfly species throughout all life stages.

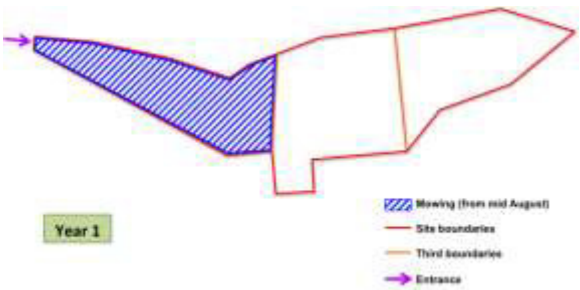
Planting

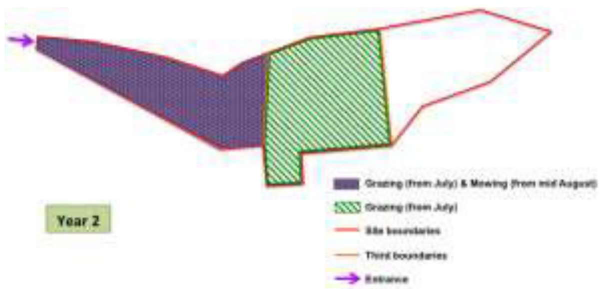
If it is necessary to plant trees and/or shrubs, all plants should be protected against grazing for the first few years. Planting of shrubs should occur randomly (e.g. not in line) and should be completely exposed. The species of shrubs and trees to be planted should be selected according to their natural occurrence on wet meadows or in the region (e.g. shrubs: *Salix* sp.; trees: *Betula alba*).

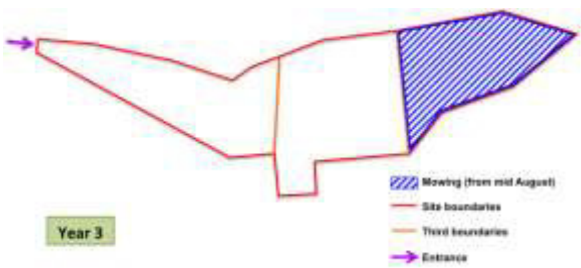
Sites with favourable conditions (occupied or unoccupied)

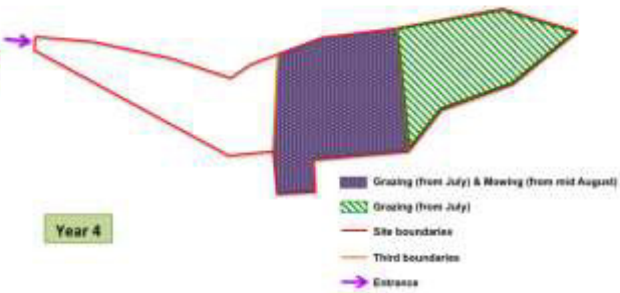
- Sites should be divided into thirds (Every third should include: high density of food plants, shrubs and be of approximately equal size. None of the thirds should be more than 500 m from the closest tree line or group away to maintain roosting opportunities.)
- Extensive grazing on 2/3 with <0.2 LU/ha/year every other year after July
- Rotational mowing of 1/3 every year after September (if too high abundance of nettles or meadsweet – mid June to mid July).
- Leave 1/3 management every year

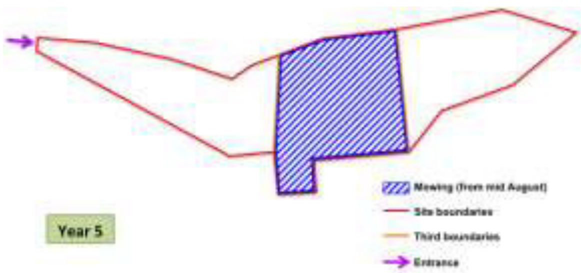
Site 1: Site ‘Brouch’ near Bigonville – Occupied site in favourable condition.









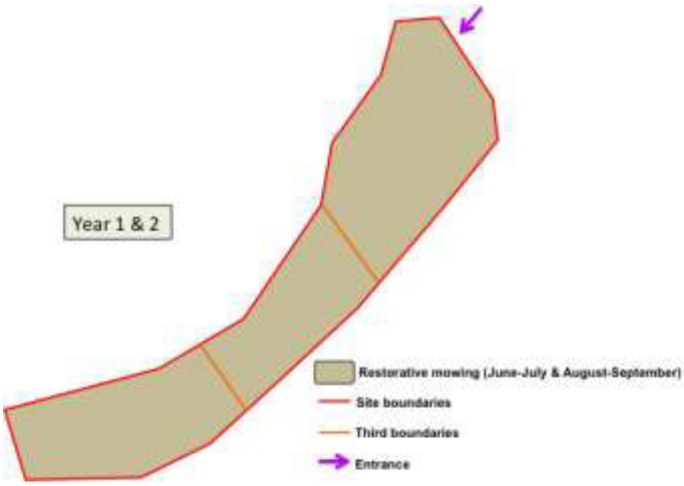


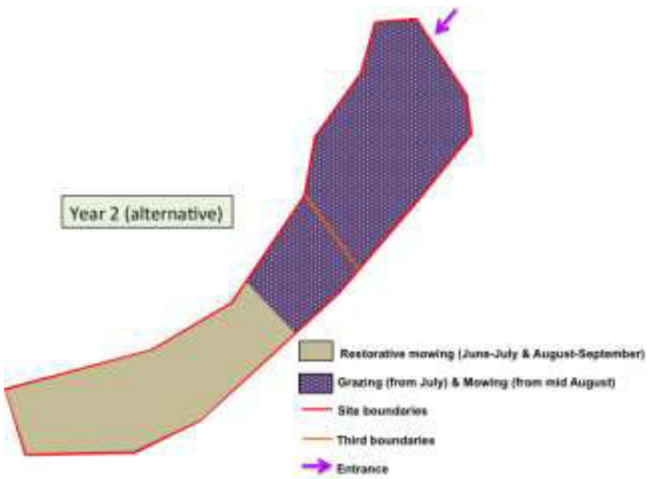
Sites under unfavourable conditions (low host plant density present)

- Restorative mowing for first two years (at least twice during the season). Grazing can be used on later dates, but never as sole option.

  For very large areas: divide site into two – each ½ is alternatively mowed or grazed every year.
- After two years reassessment of site
  - Still unfavourable condition: continue restorative mowing
  - Condition favourable: adapt management option for favourable conditions.
- Planting of shrubs and/or trees should be considered if none occur on site.

Site 2: Site 'Géidermillen' at Goedange-Moulin – site in unfavourable conditions





Year 3 (and following) – Reassess site, adapt management plan accordingly

Restoration of potential but currently unsuitable sites

On clear-felled sites:

- for the first two years after clear-felling: remove dead left-over material (plant debris), then mulch the site
- Restorative mowing for 3 to 4 years, with sowing or planting of bistort if colonisation unlikely. Grazing can also be used as an additional option.
- Reassessment of site after 5 years to adapt management options

Previously intensive agricultural land:

- Removal of excess nutrients/chemicals by mowing and subsequent removal of all plant material to prevent eutrophication
- Remove any drainage systems to restore natural hydrology
- Planting of shrubs and/or trees should be considered if none occur on site
- Reassessment of site after 4 year to adapt management options

Proposed restrictions to sites immediately adjacent or directly upstream from occupied habitats

- Buffer zone of 25 meters around occupied and potential habitats, as well as any water system occurring upstream from sites, for which the use of fertilisers, herbicides and pesticides should be banned
- Limited access of cows to streams or rivers, with the majority of the water body being fenced off (buffer zone of 5 m).

Table 1: Timeline of the different management options for the different types of habitat. The thirds of favourable habitats are divided into part A, B and C.

	Favourable Habitats	Unfavourable Habitats	Restoration
Year 1	Mow part A (after mid August) Leave part B & C unused	Restorative mowing (once between mid June and July; second mow between August & September) (Other option: graze on half, mow the other)	First year after clear-cut: mulch habitats (when ground frozen in Winter or dry in Summer)
Year 2	Mow part B (after mid August) Graze part B & C together (after July) Leave part A unused	Restorative mowing (Other option: graze on half, mow the other by rotating from last year)	Mulch habitat if not done so already
Year 3	Mow part C (after mid August) Leave part A & B unused	Reassess habitat in May 1) switch to "Favourable Habitat" management 2) continue restorative mowing	Start restorative mowing (or option with grazing)
Year 4	Mow part A (after mid August) Graze part A & B together (after July) Leave part C unused	If 1) start from Year 1 If 2) reassess habitat	
Year 5	Mow part B (after mid August) Leave part A & C unused	Continue chosen management option	
Year 6	Mow part C (after mid August) Graze part C & A together (after July) Leave part B unused	Continue chosen management option	
	Repeat Year 1 to 6	Continue chosen management option	

If occupied or favourable habitats do not belong to "natur&ëmwelt" and cannot be purchased, adequate agro-environmental schemes should be set up with owners to secure the quality of these sites.