Dispersal and population persistence of a threatened butterfly in the face of habitat fragmentation and environmental change

Alex Edward Blomfield

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Abstract

Butterflies are sensitive indicators, showing vulnerability to habitat and environmental change. The ecological requirements of Lepidoptera are relatively well documented, but in addition to the provision of adult and larval resources, species persistence in fragmented landscapes is influenced by the spatial configuration of habitat patches. This thesis aimed to assess population persistence and dispersal of the pearl-bordered fritillary, *Boloria euphrosyne*, a threatened butterfly in the UK. While the drivers of population decline in *B. euphrosyne* are likely multifactorial, site connectivity, climate and nitrogen deposition were shown to be important regulators of abundance and extinction risk. Notably, analysis of population abundance at sites across England suggested that the impacts of nitrogen deposition and elevated rainfall may be dependent on habitat type, with wooded sites likely to be most vulnerable. Population synchrony was used to investigate the implications of site connectivity on extinction risk and is suggested to be an effective indicator of functional connectivity between local populations. Population size, structure and mobility was also assessed directly, by mark-release-recapture, in the Morecambe Bay region, in north-west England. In addition, morphology was trialled as a proxy for mobility. Wing aspect ratio and wing loading were shown to be significantly related to the distance moved in individuals that were recaptured only once. Wing aspect ratio was positively correlated with the number of times an individual was recaptured, this may indicate sampling bias, but also suggests intraspecific variation in flight capacity. Comparisons between historical specimens and butterflies from current populations highlighted morphological changes including declines in thorax size and wing loading. Current populations in Morecambe Bay have become increasingly isolated and these morphological changes suggest altered flight capacity as a possible response. Overall, this thesis demonstrates that site isolation has consequences for population persistence and dispersal, including potential effects on extinction risk and flight capacity.

Contents

List of acronyms used

AIC - Akaike's Information Criterion

AONB - Area of Outstanding Natural Beauty

CI - Confidence interval

CWT - Cumbria Wildlife Trust

DF - Degrees of freedom

GAM - Generalized Additive Model

GLM - Generalised Linear Model

IQR - Interquartile Range

LNR - Local Nature Reserve

LWT - Lancashire Wildlife Trust

NNR - National Nature Reserve

NT - National Trust

OS - Ordinance Survey

RSPB - Royal Society for the Protection of Birds

SD - Standard Deviation

SE - Standard Error

SSSI - Site of Special Scientific Interest

TL - Thorax Length

TV - Thorax Volume

TW - Thorax Width

UK - United Kingdom

UK BAP - United Kingdom Biodiversity Action Plan

UKBMS - United Kingdom Butterfly Monitoring Scheme

WL - Wing Length

WW - Wing Width

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Declaration and funding statement

I declare that this thesis is my own work and has not been submitted in substantially the same form for the award of a higher degree elsewhere.

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Chapter 4 and 5 include the use of additional photographs taken by Hollie Blaydes for morphometric analysis (Blaydes, 2019).

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Chapter 1: Introduction and Literature Review

1.1 The landscape-scale approach to conservation

Rates of species loss are now considered to be on the scale of the 6th mass extinction, making global biodiversity loss one of the most pressing issues in conservation (Dirzo and Raven, 2003; Wake and Vredenburg, 2008; Barnosky et al. 2011). Biodiversity underpins ecosystem functioning, while providing a range of vital services including pollination and natural pest control (Tscharntke et al. 2012; Cohen et al. 2020). Humans have had a profound impact on the natural environment, to the extent that the 'Anthropocene' has been widely recognised as a new geological epoch (Crutzen, 2002). Currently, habitat destruction is the principal cause of global biodiversity decline (Dirzo and Raven, 2003). Habitat destruction can be broken-down into three components: habitat loss, fragmentation and deterioration in habitat quality. The negative impacts of climate change are predicted to accelerate over the coming decade and are likely to act synergistically with other factors including habitat loss and fragmentation (Millennium Ecosystem Assessment, 2005; Leadley et al. 2010).

Land conversion is a major cause of habitat destruction, both directly, via habitat loss, and indirectly, as remnant areas of suitable habitat become fragmented and isolated from other habitat patches (Cote et al. 2017). Factors including agricultural intensification have also contributed to habitat degradation, with localised impacts on farmland habitats themselves, in addition to widespread effects from agrochemical pollution (Vogels et al. 2020). Although agricultural conversion is a key driver of habitat loss, recent policy in the United Kingdom (UK) and other European countries, has been somewhat effective at restoring a component of biodiversity into farmland by increasing the heterogeneity of landscapes and introducing key resources such as overwintering refuges, nesting sites and food sources (Haaland et al. 2011; Cole et al. 2020; Graham et al. 2020). However, agri-environment schemes are likely to benefit only a limited subset of taxa and exclude the substantial component of biodiversity that is restricted to non-agricultural land (Wood et al. 2015). Whereas some generalist species may be able to persist in a variety of habitats, habitat specialists occupy a much narrower ecological niche and are often restricted to semi-natural habitat (Hill et al. 1999a; Clavel et al. 2011). Semi-natural habitat tends to be fragmented and exists within a wider agriculturally dominated landscape. Habitat generalists are typically less sensitive to the effects of fragmentation, yet agricultural mitigation strategies, such as improving biodiversity in field margins, are likely to benefit generalists while being of little value to specialists (Marvier et al. 2004). In the context of habitat fragmentation, species persistence is influenced not only by the abundance of required resources, but by the spatial configuration of habitat patches containing these resources (Turlure et al. 2010a). Knowledge of species' habitat requirements underpins habitat management, making knowledge acquisition an important first step to conserving species. But, in an increasingly fragmented landscape, conservation must take a broader approach and consider connectivity between sites and the dispersal ability of target species. Landscape-scale conservation requires a shift in focus from single sites to the coordinated management of whole site networks (Ellis et al. 2012).

Invertebrates are especially vulnerable to habitat loss and environmental change, making them sensitive environmental indicators (Fox et al. 2015). Butterflies are one of the best studied insect groups and are a commonly used model for understanding environmental change (Stevens et al. 2010; Warren et al. 2021). The United Kingdom Butterfly Monitoring Scheme (UKBMS) has reported that 76% of butterfly species in the United Kingdom have experienced declines in either range or abundance over the past four decades (Fox et al. 2015). Butterflies show significant interspecific variation in species traits including their degree of specialism and dispersal ability; unsurprisingly, sedentary, habitat specialist butterflies have experienced the most rapid population declines (Ellis et al. 2012). Dispersal ability is central for both species' persistence in fragmented landscapes and the ability of species to move to more suitable areas as the climate changes (Warren et al. 2001; Nowicki et al. 2014).

This thesis will address the factors regulating abundance, extinction risk and dispersal in one of Britain's most threatened butterflies, the pearl bordered fritillary, *Boloria euphrosyne.* In this chapter, the metapopulation approach is introduced as a framework for understanding the importance of dispersal for population persistence in patchy habitats (Section 1.2). Specific issues relating to the conservation of small populations are discussed with relevance to dispersal (Section 1.3). In addition to outlining methods used to quantify mobility (Section 1.4), factors affecting dispersal (Section 1.5) and the consequences of site isolation for long-term persistence (Section 1.6) will be highlighted. Key mechanisms of environmental change will also be discussed (Section 1.7). Background information will be provided on *B. euphrosyne,* with an outline of the distribution, status and ecology of the species (Section 1.8) in addition to details of its former English stronghold in Morecambe Bay (Section 1.9). Section 1.10 provides an overview of the thesis aims and outlines the focus of the subsequent chapters.

1.2 The metapopulation approach to understanding population persistence

Metapopulation theory explains habitat patch occupancy as a function of extinction-colonisation dynamics and provides a useful framework for understanding species persistence in fragmented landscapes (Levins, 1969; Hanski, 1999a). A metapopulation can be thought of as a ‘population of populations,’ being made up of local patches linked by dispersal (Levins, 1969). The proportion of occupied patches in a landscape at any given time is the result of extinction and colonisation rates. As such, dispersal between patches enables persistence at the metapopulation level, even where individual local populations are vulnerable to extinction (Hanski and Thomas, 1994). Site area and isolation are two fundamental parameters in metapopulation theory that influence the rates of extinction and subsequent recolonization (Schtickzelle and Baguette, 2003). Site area is assumed to correlate positively with population size, with larger sites predicted to support populations with higher abundance and to, therefore, have a lower extinction risk (Hanski and Thomas, 1994). Site isolation is a product of both a site’s distance from neighbouring habitat patches and their population sizes (Hanski, 1998). The probability of successful colonisation is higher where the distance between donor and recipient sites is short (Nowicki et al. 2014). As sites with high population abundance are more likely to produce net emigrants, population density also affects the likelihood of colonisation success (Bonsall et al. 2014). Habitat fragmentation, which results in areas of suitable habitat being reduced in size and becoming more isolated, can decrease the likelihood of successful movement between sub-populations and limit population persistence (Fourcade and Öckinger, 2017). For example, in the Glanville fritillary, *Melitaea cinxia*, the proportion of occupied patches was found to be reduced in a patch network comprised of small and isolated habitat patches (Hanski et al. 1995). Where the rate of local extinction exceeds that of colonisation, populations will be unable to persist in the long-term without intervention; this is one reason why population extinctions can occur despite the availability of suitable habitat (Amarasekare, 1998). Alternatively, habitat loss may reduce habitat availability such that species minimum area requirements are no longer met (Baguette and Stevens, 2013). The non-immediacy of extinctions following habitat loss is termed an extinction debt (Tilman et al. 1994). Recolonization following site extinction is also often non-immediate, and this is one reason why unoccupied patches of suitable habitat may be present in the landscape (Levins, 1969).

Extinction-colonisation dynamics interact, with well-connected populations less likely to go extinct. Indeed, the movement of individuals between sub-populations can facilitate the recolonization of formerly occupied sites, or prevent extinction of vulnerable populations, by the rescue effect (Brown and Kodric-Brown, 1977; Hanski, 1998). Colonisers act to rescue dwindling populations by mechanisms including improved genetic diversity and enhanced mate location and breeding success at higher population densities (Saccheri et al. 1998; Bonsall et al. 2014). Sub-populations may act as sources or sinks based on their population size and dispersal dynamics. Where the intrinsic rate of natural increase (r) is greater than zero, a population may be considered to be a source, whereas sink populations are characterised as having an r that is less than zero (Pulliam, 1988). As such, immigration is vital for the persistence of sink populations, which otherwise exist below an extinction threshold (Pulliam, 1988). By contrast, source populations are those that produce net emigrants, which disperse to other patches. Typically, populations occupying higher quality habitats are source populations, as these habitats have a greater carrying capacity and can support high growth rates. Conservation efforts are most effective when focused on source populations, however, population dynamics are not constant and source-sink dynamics may shift over time. Although source populations have unstable dynamics, characterised by frequent extinctions and recolonisations, they can be critical for persistence at the metapopulation level. While dispersal reduces extinction risk by promoting the recolonization of empty patches, dispersal is also a factor that can synchronise local population dynamics (Powney et al. 2011; Kahilainen et al. 2018). Where dynamics among patches are asynchronous, with populations reaching peak abundance in different years, a balance of source and sink populations is maintained through time, allowing dwindling sites to be rescued (Hanski, 1998). By contrast, if populations have highly synchronised dynamics, they are likely to be vulnerable to simultaneous extinction from environmental stochasticity (Hanski, 1998; Powney et al. 2010). Indeed, the loss of immigration across a network after the extinction of a single site, could synchronise the dynamics of remaining populations and is thought to promote further extinctions by this mechanism (Matter and Roland, 2010).

Metapopulation theory has practical applications for conservation in fragmented habitats and as there is good evidence that several species of butterfly form metapopulations, Lepidoptera are valuable model species (Hanski and Thomas, 1994; Davies et al 2005). Most notably, Glanville fritillary, *Melitaea cinxia* metapopulations have been studied extensively in Finland, but other butterfly species such as the silver studded blue, *Plebjus argus*, and the silver-spotted skipper, *Hesperia comma* are also reported to form metapopulations (Hanski et al. 1995; Thomas et al. 1998; Davies et al. 2005). It is worth noting that not all butterflies form metapopulations, as such, it has been suggested that the principles of metapopulation ecology may be relevant only to a small proportion of butterfly species (Ranius et al. 2011; Fronhofer et al. 2012). However, there is evidence that landscape characteristics influence population structure and metapopulation theory remains a useful framework for many species occupying fragmented landscapes. Although metapopulation models explain extinctions through environmental stochasticity, others have argued that species persistence is more often determined by their ability to track resources as habitats progress to later stages of ecological succession (Thomas et al. 2001). While habitat turnover may be a key process affecting the persistence of species occupying mid-successional habitats, the effects may be mitigated by the presence of factors that slow succession. For example, one explanation for the persistence of species such as the high brown fritillary, *Fabriciana adippe*, in Morecambe Bay, is the slow succession of well-drained limestone grassland. This slow regeneration is thought to have resulted in less rapid declines in the habitat quality in this area following abandonment, relative to other systems such as coppiced woodland, where succession is more rapid (Barnett and Warren, 1995). Patch connectivity plays a more important role where succession is slow because the relative threat of extinction from changing habitat is less immediate. Indeed, a key criticism of traditional metapopulation models is that they often do not account for factors such as habitat quality, this makes them potentially less applicable to heterogeneous and dynamic landscapes. The idea that patches are immediately available for recolonization following extinction overlooks the influence of factors such as habitat degradation in driving site extinctions. In dynamic landscapes where ecological succession is rapid, habitat turnover is an important driver of extinction and is likely to have a more immediate effect on population persistence than connectivity (Hodgson et al. 2009).

1.3 Population dynamics and extinction risk of small populations

Metapopulation theory provides a useful framework for understanding the demographic processes and elevated risk of extinction in small populations. As well as being more vulnerable to extinction from stochastic events, small populations often experience disproportionately low growth rates. This inverse density dependence is known as the Allee effect (Allee, 1931). Increased emigration from small populations is one mechanism that has been used to explain the rapid population declines in small populations of the high brown fritillary, *Fabriciana adippe,* in the UK (Bonsall et al. 2014). Although increased emigration at low densities may be adaptive where it reduces the risk of inbreeding, it can facilitate the extinction of small populations and may also result in mortalities during dispersal and lost mating opportunities if colonisation is not successful (Kuussaari et al. 1996). Other mechanisms that result in reduced growth rates at low population densities include limited mating opportunities and compromised fitness associated with a decline in genetic diversity in small populations (Kuussaari et al. 1998; Mattila et al. 2012). Many butterflies exhibit protandry, an adaptation that serves to maximise male mating success, as early eclosure in males allows them to set up territories prior to female emergence. However, asynchrony in emergence may also contribute to Allee effects by further reducing mating opportunities where mate location is already compromised by low population density (Kuussaari et al. 1998; Calabrese et al. 2008).

Population collapse can result in genetic bottlenecking, a process in which overall genetic diversity is reduced (Austin et al. 2011; Fountain et al. 2016). Inbreeding can also lead to an increase in the frequency of homozygous loci in a population (Saccheri et al. 1998; Mattila et al. 2012). Loss of fitness in inbred populations results from the expression of deleterious recessive alleles and inbreeding depression (Mattila et al. 2012). In the past it has been suggested that genetic factors are unlikely to play a key role in population extinctions as factors such as demographic and environmental stochastisity have a more immediate effect on populations (Caro and Laurenson, 1994). However, more recent studies have demonstrated the negative influence of low genetic diversity on fitness traits in butterflies including hatching rate and adult lifespan (Saccheri et al. 1998; Baguette et al. 2008; Mattila et al. 2012). Small populations are also at risk of losing genetic diversity by genetic drift. Loss of genetic variance and the loss of rare alleles from a population can limit species capacity to adapt to future environmental change (Anderson et al. 2008; Hanski, 2012). Indeed, a slow response to management may be attributable to population vulnerability following bottlenecks (Schmeller et al. 2011). The Allee effect explains why populations may experience an extinction threshold at low densities despite an apparent availability of suitable habitat (Amarasekare, 1998).

As discussed in the previous section, connectivity between populations can facilitate population rescue, as immigration has the potential to increase population size above the extinction threshold (Brown and Kodric-Brown, 1977; Hanski, 1998). For this reason, the dynamics of small populations can be strongly influenced by dispersal (Bonsall et al. 2014). Metapopulations close to the extinction threshold are more likely to persist if they have high levels of dispersal and one mechanism for this is increased genetic flow (Hanski et al. 2017). Accordingly, female dispersal can be of particular importance for metapopulation persistence, egg-laying in new patches both spreads the risk during oviposition and leads to gene flow (Lakovic et al. 2015)

1.4 Quantifying mobility

Dispersal can be regarded as the movement of individuals between sites, with the potential to result in gene flow (Ronce, 2007). Dispersal determines species survival in fragmented landscapes, influencing processes such as local adaptation, community dynamics and population genetic structure (Nowicki et al. 2014). As such, the dispersal ability of a target species is an important consideration in conservation. There are several approaches to quantifying dispersal in butterflies, including both direct and indirect means (Stevens, 2010). Mark-release-recapture (MRR) is a direct method of studying dispersal. Individuals are caught and marked with a unique code, allowing them to be identified. The location of capture and subsequent recapture points are recorded and Euclidean distance between successive capture points is commonly used to quantify distance travelled (Skórka et al. 2013). MRR is also a valuable tool in estimating population size and assessing connectivity between habitat patches. However, these studies are labour intensive and may not always identify long-distance dispersal events (Zimmermann et al. 2011). Behavioural studies, which record flight behaviour at habitat edges or point-release experiments can also be used to assess species dispersal propensity and willingness to enter matrix habitat (Leidner and Haddad, 2011).

Indirect methods for studying dispersal include genetic studies and the use of proxies such as population synchrony and wing morphology. Genetic differentiation between populations can give information on gene flow that may be used to infer effective dispersal. There is also good evidence that intraspecific variation in dispersal has a genetic basis (Fountain et al. 2017). For example, the gene encoding the glycolytic enzyme phosphoglucose isomerase (Pgi) is a well-studied candidate gene for dispersal. In *M. cinxia,* individuals that are heterozygous for the *Pgi* gene demonstrated increased dispersal and enhanced colonisation capacity (Watt, 1983). Further studies have also revealed differences in survivorship and fecundity between *Pgi* variants in this species (Orsini et al. 2009; Saastamoinen et al. 2009). Although similar links between *Pgi* and dispersal have been reported in the orange sulphur butterfly, *Colias eurytheme*, *Pgi* genotype was not found to influence dispersal in the marsh fritillary, *Euphydryas aurinia* (Smee, 2011). The importance of dispersal for metapopulation persistence is highlighted by significant differences in *M. cinxia* population growth rates based on *Pgi* genotype (Hanski and Saccheri, 2006). Allelic variation in the *Pgi* gene has been shown to account for as much as 30% of variation in metapopulation size (Hanski et al. 2017).

One mechanism for intraspecific variation in flight performance appears to be linked to flight metabolic rate (FMR), with heterozygotes having higher FMRs than the homozygotes. However, these differences in FMR interact with body size and ambient temperature, with heterozygotes able to fly under a broader range of environmental conditions (Niitepõld et al. 2009). Variation in the role of *Pgi* genotype is also dependent on sex, likely due to the differing roles of flight metabolic rate in breeding success in the two sexes. In the species *M. cinxia*, females with the *Pgi-f* genotype made more frequent inter-patch movements than *Pgi-non-f* females, whereas in males the genotypic effect was the opposite (Niitepõld et al. 2011). The finding that females from isolated, newly established populations are characterised by higher flight metabolic rates and had a higher frequency of *Pgi-f* genotypes relative to females from older populations supports the idea that individuals with high flight capacity are likely to be more successful colonisers (Haag et al. 2005).

Sex-biased dispersal is a major source of variation in dispersal behaviour within species. Many butterflies display sexual dimorphism in mobility, mate location strategy and morphology, due to the differing selection pressures acting on dispersal in males and females (Hill et al. 1999b; Ehl et al. 2017). Relative to makes, females are more likely to gain from dispersal, as colonising new habitat provides additional oviposition sites (Pellegroms et al. 2009). Movement between successive reproduction sites is adaptive, particularly in heterogeneous landscapes, as it represents a 'risk-spreading' oviposition strategy that allows species to track resources in dynamic systems (Lakovic et al. 2015). By contrast, as males gain fitness by maximising the frequency of lifetime matings, mate location is crucial. As such, males with a high flight capacity are likely to gain more by patrolling in search of females than by making long-distance movements (Breuker et al. 2007). Indeed, in males of the species *M. cinxia* there is a negative correlation between flight metabolic rate and dispersal, while the trend observed in females is the opposite (Niitepõld et al. 2011).

Previous studies have used a range of morphological traits (most commonly wingspan, reviewed in Sekar, 2012) as proxies for dispersal ability in Lepidoptera. For example, in the large blue, *Phengaris arion,* there was a positive relationship between wing length and within-patch mobility (Skórka et al. 2013). Morphology influences the energetic costs of flight, meaning that shifts in morphological traits can result in individuals being differentially adapted to optimise energy efficiency or flight speed. Wing loading is the ratio of body weight to wing area. Low wing loading can be an adaptation for sustained flight as it is associated with gliding, a form of flight that reduces energetic costs (Mattila et al. 2012). Wing aspect ratio, the ratio of wing length to width, gives a measure of the slenderness of the wing. Short, broad wings have a lower wing aspect ratio than narrow, elongated wings. Lower wing aspect ratios are associated with agile flight such as patrolling (Wickman, 1992; Cespedes et al. 2015). Indeed, interspecific comparisons of mate location strategies in butterflies have identified distinct morphological traits in males of perching and patrolling species. Perching species have larger thoraxes, higher wing loading and higher wing aspect ratios than males in patrolling species (Wickman, 1992). This is thought to reflect the need for perching males to perform short bouts of rapid flight rather than sustained movements. Studies on butterflies have also used the relative investment in the thorax, which contains the flight muscles, and the abdomen to quantify the trade-off between dispersal and reproduction (Gibbs and Van Dyck, 2010). Flight metabolic rate is important for dispersal and is thought to be strongly influenced by thorax mass. In the speckled wood, *Pararge aegeria*, colonising individuals were reported to have larger thoraxes relative to sedentary individuals (Hill et al. 1999b). Although the implications of morphological traits on flight performance are relatively well established, links between morphology and emigration propensity are somewhat unclear (Reim et al. 2018). While morphological traits influence the energetic costs of flight, dispersal propensity is influenced by several factors including population density, habitat quality and prevailing weather conditions, meaning that realised dispersal, does not necessarily relate to morphology (Kuussaari et al. 1998; Thomas, 2001; Kuussaari et al. 2016).

A further method of assessing dispersal behaviour is population synchrony, the degree of correlation in annual fluctuations of population densities between sites (Sutcliffe et al. 1996; Powney et al. 2011). Although synchrony between populations can result from factors such as shared environmental conditions (the Moran effect), dispersal between populations is also an important factor driving synchrony (Royama, 1992). By controlling for the synchronising effects of climate, residual population synchrony has been shown to be positively related to matrix permeability at both the inter- and intra-site level and has, hence been suggested to represent a proxy for dispersal (Powney et al. 2011; Powney et al. 2012). The relationship between population synchrony and functional connectivity has also been supported by empirical evidence from MRR data (Oliver et al. 2017). As such, population synchrony presents a promising method that may utilise pre-established monitoring data, such as that from the long-standing United Kingdom Butterfly Monitoring Scheme (UKBMS), to assess functional connectivity in populations (Powney et al. 2011).

1.5 Factors affecting dispersal in butterflies

Traditional metapopulation theory states that site connectivity is the key determinant of colonization probability (Hanski, 1998). However, a range of factors influence realised dispersal and colonization success in butterflies, resulting in considerable variation in dispersal propensity both within and between species (Stevens et al. 2010). Factors intrinsic to an individual, such as sex, genetics and morphology, influence dispersal and flight capacity as discussed above. In addition, extrinsic factors including population density, landscape structure and habitat quality play a role in influencing dispersal propensity.

Dispersal is a density dependent process and there is evidence of both positive and negative density dependent dispersal in butterflies (Nowicki and Vrabec, 2011; Konvicka et al. 2012).

Positive density dependent dispersal describes increased emigration at high population densities and is thought to be a mechanism to avoid inbreeding, kin competition, resource depletion and conspecific harassment (Baguette et al. 1998; Brunzel, 2002; Enfjäll and Leimar, 2005). However, higher population densities may also provide a cue to habitat quality, explaining why some species including *M. cinxia*, are observed to be less likely to leave a patch when conspecifics are present (Kuussaari et al. 1998). Factors such as limited mate availability may drive increased emigration at low densities (Kuussaari et al. 1998; Baguette et al. 2011). However, elevated emigration at low population densities can be detrimental to population persistence. For example, where habitat is scarcely distributed in a landscape, the likelihood of successful colonisation is low. In this way, increased emigration at low densities can drive Allee effects, such as those associated with population declines in the high brown fritillary, *Fabriciana adippe* (Bonsall et al. 2014).

Habitat quality is another important influence on dispersal, with evidence of increased emigration of butterflies from lower quality habitat patches, which are less likely to provide adequate resources (Thomas, 2001). Although host plant abundance is often used as the sole indicator of habitat quality for monophagous species, there are often several aspects of habitat quality (Nowicki et al. 2007). Host plant quality, the presence of symbiotic organisms and microclimate suitability may also be important components of habitat quality and can influence dispersal (Sivakoff et al. 2016). While climate change results in warming at the macro-scale, the converse can be true for microclimate conditions. The advanced onset of growing seasons in spring leads to the accumulation of green plant material, which relative to sparse vegetation, bare ground or leaf-litter, promotes cooler microclimatic conditions (Wallis De Vries and Van Swaay, 2006). Lepidoptera are vulnerable to such microclimate cooling and altered microclimate conditions are likely to be important drivers of species range shifts in response to climate change (Bennie et al. 2013). Management also has the potential to influence habitat suitability, via impacts on habitat structure and host plant quality. For example, biomass grown in full sun typically has a lower nutritional value, being characterised by a lower nitrogen and water content and higher levels of carbon (Sivakoff et al. 2016). In the Appalachian brown, *Satyrodes appalachia*, reduced host plant quality was associated with lower investment in fecundity and increased allocation of resources to dispersal as adults (Sivakoff et al. 2016). Similarly, experimental reduction of habitat quality, using grazing, resulted in increased emigration of the bog fritillary, *Boloria eunomia,* from low quality patches (Baguette et al. 2011). Larval habitat quality also influences adult morphology. For instance, speckled wood, *Pararge aegeria,* adults displayed lower wing loading where they had been subjected to food stress as larvae

(Pellegroms et al. 2009). As reduced wing loading is associated with long-distance dispersal, this example again suggests a tendency for increased emigration from low-quality habitat.

Dispersal behaviour is also likely to depend on the spatial distribution of resources in the landscape; where resources are common, organisms may evolve a restricted foraging range (Konvicka et al. 2012). For example, in a habitat with widespread nectar resources, butterflies displayed larger wing area and lower wing loading, traits that can be associated with limited movement (Turlure et al. 2010a). Traditional management techniques, which create ephemeral habitat patches on a rotational basis, allow butterflies to easily colonise adjacent habitat patches and may result in an evolutionary shift towards more sedentary behaviour (Thomas, 1994). Many specialists rely on early successional habitat, which only remains suitable for a limited period after management. For species with limited dispersal abilities, the maintenance of accessible, high quality habitat is crucial for their persistence. Accordingly, targeted coppice and ride management for the heath fritillary butterfly, *Melitaea athalia*, has allowed the butterfly to colonise new habitat and recover to pre-1980 levels at Blean Woods, in south-east England, following rapid population declines (Hodgson et al. 2009; Ellis et al. 2012).

Landscape factors, including patch size, isolation and matrix permeability influence the movement of individuals between populations. Willingness to emigrate has been shown to be mediated by patch area, with butterflies more likely to leave small patches (Schtickzelle and Baguette, 2003). As such, small populations are characterised by higher rates of population turnover than those occupying larger patches (Habel and Schmitt, 2009). However, this effect is not observed in more continuous landscapes, where emigration is often independent of patch area (Mennechez et al. 2003). Another example of condition dependent dispersal in fragmented landscapes relates to temperature. Emigration in the meadow brown, *Maniola jurtina,* is temperature dependent in fragmented landscapes, such that dispersal only occurs at optimum temperature conditions in these environments (Delattre et al. 2013). By contrast, emigration in this species is not influenced by temperature in continuous landscapes. Temperature dependent dispersal may be a means to offset the increased costs of dispersal in fragmented landscapes. Life history is also a key determinant of species' responses to fragmentation. Indeed, it has been demonstrated that dispersal propensity in *B. eunomia* is lower in highly fragmented habitat, while the converse is true for *M. cinxia*, which displays characteristics associated with elevated emigration in fragmented landscapes (Schtickzelle et al. 2006; Hanski, 2011). *M. cinxia* populations occupying more isolated sub-networks in the Åland Islands display higher frequency of the C allele in the single nucleotide polymorphism (SNP) *Pgi_111*, associated with increased dispersal rate (Hanski, 2011). The differing response of these butterflies to fragmentation is thought to be a result of differences in population dynamics between the two species, arising from their contrasting oviposition strategies (Hanski, 2011). In *B. eunomia,* population abundance is typically stable as their risk-spreading oviposition strategy, of laying several small clutches of eggs, results in low population turn-over rates (Choutt et al. 2011; Hanski, 2011). In stable populations, the benefits of dispersal are less apparent, therefore the increased costs of dispersal in a fragmented landscape promote sedentary behaviour in this species (Habel and Schmitt, 2009). By contrast, the high population turnover rates in *M. cinxia* populations, associated with laying large clutches of eggs, maintains selection on dispersal even in fragmented landscapes (Hanski, 2011). Dispersal is vital for recolonisation following site extinctions and for the maintenance of genetic diversity in unstable populations (Habel and Schmitt, 2009).

Functional connectivity, the measure of landscape permeability to individual dispersal, is an important consideration in managing populations (Tischendorf and Fahrig, 2000; Oliver et al. 2017). Landscape elements, including topography, vegetation structure and patch edges can act as barriers to dispersal. For example, conifer plantations limit movement in the highly sedentary butterfly, *M. athalia* (Ellis et al. 2012). Greater genetic differentiation between populations of the Rocky Mountain apollo, *Parnassius smintheus*, separated by forest, relative to meadow habitat, highlights the barriers that forest poses to dispersal in this species (Keyghobadi et al. 2005). In species that occupy different types of habitat, variation in mobility may be observed based on habitat structure. Conservation strategies, such as corridors and stepping-stones, can facilitate dispersal and mitigate the effects of fragmentation (Leidner and Haddad, 2011; Saura et al. 2014). Nevertheless, the presence of habitat corridors does not necessarily result in increased dispersal (Tischendorf and Fahrig, 2000) and some argue that their benefits as a conservation tool have been overstated (Simberloff et al. 1992). However, the relevance of such conservation strategies is likely to be dependent on population structure. Populations with high turnover rates exist in a balance of extinctions and colonisations. Promoting habitat connectivity is crucial for these, often small, populations that rely on dispersal and population exchange to maintain genetic diversity (Habel and Schmitt, 2009). However, stability in population abundance is important for maintaining the genetic diversity of populations with lower dispersal propensity and conservation strategies aimed at improving landscape connectivity are likely to be less successful than improving habitat quality in occupied patches (Habel and Schmitt, 2009).

1.6 Evolutionary consequences of habitat fragmentation

As discussed above, dispersal behaviour is influenced by multiple factors, both intrinsic and extrinsic to the disperser. In this section, the long-term consequences of landscape factors on species dispersal ability will be discussed. Habitat fragmentation increases the costs of dispersal and can select for reduced dispersal propensity in populations occupying fragmented habitats. For example, less dispersive phenotypes have been found in old, isolated populations of the Glanville fritillary, *Melitaea cinxia* (Hanski et al. 2004). Although habitat fragmentation renders the costs of dispersal prohibitively high for some species, for others it may still be adaptive (Bonte et al. 2012). For example, in *M. cinxia*, there is selection for enhanced colonization capacity in fragmented landscapes (Fountain et al. 2016). By contrast, the bog fritillary, *Boloria eunomia*, displays lower dispersal rates in fragmented landscapes (Baguette et al. 2003). This variation may be a result of differences in population turn-over rates, linked to oviposition strategy in these species (Hanski, 2011; Hanski 2012). In fragmented landscapes, emigrating individuals will be under strong selective pressure to have good colonisation capacities and to make long-distance movements (Nowicki et al. 2014). Accordingly, habitat fragmentation has been shown to influence butterfly flight behaviour; rapid and straighter flight in the matrix is associated with improved colonisation success and lower mortality rates during dispersal (Schtickzelle et al. 2006; Kuefler et al. 2010; Nowicki et al. 2014).

Altered dispersal strategies in fragmented habitats can be associated with changes in morphology. Given that the thorax contains the flight muscles, an increase in thorax size is likely to be associated with increased mobility (Norberg and Leimar, 2002). Abdomen size has been used as an indicator of investment in reproduction and there can be trade-offs between reproduction and investment in dispersal (Hill et al. 1999b). Indeed, allocation to the thorax, relative to the abdomen, was greater in the silver studded blue, *Plebjus argus,* when occupying fragmented habitat (Thomas, 1998). In the garden tiger moth, *Arctia caja*, a comparison of individuals from current and historical populations showed an increase in wing aspect ratio over time, also suggesting increased dispersal ability in fragmented habitats (Anderson et al. 2008). By contrast, a reduction in the ratio of thorax width to length, observed in swallowtail, *Papilio machaon*, specimens, was thought to have accompanied reduced mobility in an isolated population at Wicken Fen, in the South of England, prior to site extinction (Dempster et al. 1976).

Reduced connectivity between populations can lead to local adaptation but can also result in negative genetic effects (Mattila et al. 2012). For example, following habitat fragmentation in south west Finland, *M. cinxia* demonstrated allele frequency changes associated with enhanced colonization, this was, however, insufficient to prevent their extinction in the region (Fountain et al. 2016). Although isolated populations can maintain genetic diversity where populations are large and stable, smaller populations, with high turnover rates are more dependent on dispersal to maintain genetic diversity (Habel and Schmitt, 2009). Genetic deterioration and demographic processes contribute to extinction debt in fragmented landscapes (Fountain et al. 2016). As discussed above, small populations occupying fragmented habitats are likely to be particularly vulnerable to demographic stress.

1.7 Climate and environmental change

Although habitat loss is currently the main driver of species declines, the influence of climate change is expected to accelerate over the next decade (Millennium Ecosystem Assessment, 2005; Leadley et al. 2010). The impacts of climate change are particularly important to understand in species that have a limited dispersal ability, as these species are less likely to be able to adapt by colonising new areas (Warren et al. 2001). Furthermore, altered weather conditions and climate-induced changes in phenology have potential to impact species' dispersal trajectories (Bonte et al. 2012).

Temperature can influence populations through impacts on fecundity and species dynamics. For example, there is evidence for reduced larval survival at higher temperatures through mechanisms including reduced habitat quality and elevated susceptibility to fungal pathogens during milder winters (Turlure et al. 2010b; Radchuk et al. 2013). Temperature is also a key influence on the developmental rate of insects, leading to reduced development times and associated body size effects under warmer conditions (Stevens, 2004; Bowden et al. 2015). Accordingly, in the arctic fritillary, *Boloria chariclea,* and the northern clouded yellow, *Colias hecla,* wing length was shown to decrease in response to elevated temperature during larval development (Bowden et al. 2015). Individual size often has important implications for fecundity. For example, larger body size is associated with greater reproductive success in Glanville fritillary, *Melitaea cinxia,* females (Ahola et al. 2015). Body size also has implications for flight capacity, meaning that changes in morphology have potential to alter dispersal costs (Bonte et al. 2012). Although reduced duration of the larval period associated with elevated temperatures may generally decrease vulnerability to predators and parasites, altered timings of life stages can influence species interactions (Bonte et al. 2012). For example, spring temperature influences developmental rate in both the braconid wasp parasite, *Cotesia melitaearum* and its lepidopteran host, *M. cinxia,* and there is evidence that microclimate warming may increase the risk of *M. cinxia* parasitism by synchronising host-parasite dynamics (van Nouhuys and Lei, 2004). Altered species phenology may also be detrimental where it leads to asynchrony with resources such as larval hostplants (Rytteri et al. 2021). Furthermore, warming temperatures during the late summer or autumn can result in extended flight periods and partial second generations, as observed in species including the wall brown, *Lasiommata megera* (Van Dyck et al. 2015). Such 'suicide generations' have a detrimental effect on populations as individuals emerge too late in the year to breed successfully and are unable to survive the winter as adults. Climate change has also been shown to have a synchronising effect on metapopulation dynamics in species including *M. cinxia* (Kahilainen et al. 2018). Synchrony increases the risk of simultaneous regional extinction and reduces the likelihood of population rescue, meaning that climate change may reduce metapopulation viability.

In addition to climate effects on species interactions and survivorship, climate change is likely to impact populations via indirect effects on habitat. Sparse vegetation, where solar irradiation elevates the surface temperatures of dead plant material and leaf litter, provides warm microclimates crucial for larvae developing in the early spring (Barnett and Warren, 1995; Wallis De Vries and Van Swaay, 2006). Many Lepidoptera have a narrow thermal niche for larval development making them highly sensitive to microclimate cooling that can arise from climate-driven changes in vegetation structure such as elevated growth of green plant biomass during warmer winters (Wallis De Vries and Van Swaay, 2006; McDermott Long et al. 2017). Microclimate cooling is one such impact that is likely to interact with other mechanisms of environmental change, including nitrogen deposition, which also stimulates the growth of green plant biomass (Wallis De Vries and Van Swaay, 2006). Nitrogen enrichment represents an emerging research area in Lepidoptera conservation, in addition to microclimate cooling, there is evidence for a range of potential effects on habitat quality and species persistence through mechanisms including direct toxicity, changes in soil chemistry, altered foodplant quality and declines in botanical diversity (Wallis De Vries and Van Swaay, 2006; Habel et al. 2016; Zhu et al. 2016; Stevens et al. 2018). Indeed, there is some evidence that nitrogen deposition represents a more immediate threat to biodiversity than climate change (Plantlife, 2017). The low body tissue N:P ratio in Lepidoptera makes them especially vulnerable to changes in foodplant stoichiometry associated with nitrogen enrichment (Woods et al. 2004). As such, consumption of hostplants grown under elevated nitrogen conditions has been associated with reduced larval survival in several butterfly species (Fischer and Fiedler, 2000; Kurze et al. 2018). As host plant specialists are likely to occupy a narrower stoichiometric niche than generalists, they are likely to experience the most marked reduction in performance under elevated nitrogen conditions

(Vogels et al. 2020). Furthermore, the nutrient optimum of species' larval foodplants has also been related to their persistence. Ellenberg N indicator values describe the gradient in plant tolerance to soil fertility, values range from 1-9 with lower values reflecting nutrient sensitive species, which reach peak abundance in low-fertility soils (Hill et al. 1999c). Extinctions of Swedish grassland butterflies have been shown to be more common amongst species reliant on host plants with low nutrient tolerance (indicated by Ellenberg N values) (Öckinger et al. 2006).

1.8 Distribution, status and ecological requirements of *Boloria euphrosyne*

The pearl-bordered fritillary, *Boloria euphrosyne*, is a highly specialised, monophagous butterfly which, despite targeted management efforts in recent years, has continued to experience significant population declines in the UK. The combination of its narrow ecological niche and low abundance makes *B. euphrosyne* both a priority for conservation and a useful model species for studying the impacts of habitat fragmentation and environmental change.

In the UK, *B. euphrosyne* is univoltine, flying between late April and early June (Barnett and Warren, 1995). However, there are occasional reports of partial second broods in the late summer in southern England (Eeles, 2019). Males are reported to emerge several days earlier than females enabling them to establish territories prior to female eclosure (Al Dhaheri, 2009). Mate location behaviour is characterised by patrolling flight in males, while females are less active (Eeles, 2019). Eggs are laid singly, with areas of short sward and sparse grass cover selected for oviposition (Warren and Oates, 1994; Randle, 2009; Atkinson, 2016). Although eggs may be laid directly on the foodplant (*Viola sp.*), usually they are laid on leaf litter (Barnett and Warren, 1995; Atkinson, 2016). The first instar larvae emerge around two weeks after the eggs are laid (Eeles, 2019). Larval development is of long duration in *B. euphrosyne*, which overwinters in the larval stage, hibernating amongst leaf litter in its fourth instar (Eeles, 2019). Warm temperatures are required for larval development, and leaf litter is thought to play a critical role in providing a warm microclimate. Larvae feed during the day and bask on dead bracken fronds and leaf litter, which provide hotspots in the environment and allow them to raise their body temperature (Barnett and Warren, 1995; Eeles, 2019). Therefore, violets growing in sparse vegetation or through a thin (< 15cm) layer of leaf litter are favoured (Barnett and Warren, 1995). In the UK, *B. euphrosyne* larvae are reported to use common dog-violet, *Viola riviniana,* preferentially, although depending on habitat type and availability, heath dog-violet, *Viola canina*, and marsh violet, *Viola palustris*, are also used (Barnett and Warren, 1995). It has been suggested that *B. euphrosyne* shows a preference for violets of a small size class

(Thomas et al. 1991 in Greatorex-Davies et al. 1992; Randle, 2009). However, this may be a function of their preference for early successional habitat rather than violet size *per se*. The timing of emergence after pupation is temperature dependent, with emergence occurring at around nine days in warmer conditions and up to three weeks later at lower temperatures (Eeles, 2019).

B. euphrosyne is reported to occupy three principal habitats in the UK: woodland, where it occupies rides and clearings, grass and bracken habitats with scattered scrub and finally, wood pasture (Barnett and Warren, 1995). Persistence in woodland habitats is dependent on management to produce a continuous supply of suitable habitat; population size is reported to peak two years following coppicing and declines as succession occurs (Warren and Thomas, 1992). While regular cutting or grazing contribute to maintaining an open habitat structure and promoting violet germination, where disturbance is too frequent, the development of an excessively grassy sward and loss of woodland flora may result (Clarke et al. 2011). Hence, rotational management that allows managed areas to regenerate before they are recut, is crucial. There is also concern that longer growing seasons and a changing climate may promote grass and herb growth (Ellis et al. 2019). An increase in grass cover is likely to be detrimental to *B. euphrosyne* as it results in a cooler microclimate, unsuitable for larval development. In coppiced woodland *B. euphrosyne* is sedentary, forming closed colonies, it is unable to colonise habitat patches more than a few hundred metres from an existing colony (Thomas, 1984). It is, therefore, important that rotational management provides new habitat patches that are readily accessible. *B. euphrosyne* has suffered from a decline in traditional woodland management practices and has become increasingly restricted to areas of rough grassland and bracken, where succession is less rapid (Greatorex-Davies et al. 1992). In more open habitats, it is thought that this species forms metapopulations, with movements of at least 4.5 km between adjacent colonies reported (Barnett and Warren, 1995). In these habitats, management may include bracken control and grazing, to prevent scrub encroachment. Bracken control will prevent leaf litter ('standing trash') building up to an extent that violet growth is excluded, but again, excessive management, on too short a rotation, may result in a grassy sward.

Historical declines in *B. euphrosyne* abundance and distribution, particularly in the north-east of England, were strongly linked to loss of suitable habitat, arising initially from the decline in traditional woodland management practices from the mid-nineteenth century (Barnett and Warren, 1995). In central and southern England, a large proportion of *B. euphrosyne* colonies were restricted to conifer plantations by the 1980s, as following World War II, considerable areas of ancient woodland were cleared for plantations (Warren, 1993). While *B. euphrosyne* was able to persist in conifer plantations initially, their maturation in subsequent decades resulted in colony losses as the habitat became unsuitable (Greatorex-Davies et al, 1992; Barnett and Warren, 1995). An increase in the number of site extinctions from the 1950s onwards was highlighted by a review of key sites in central southern England (Warren, 1993). Although southern sites were the most strongly impacted by post-war conifer plantations, the reported estimation that *B. euphrosyne* declines reached 39% per decade by the 1980s was thought to be similar across its range (Warren, 1993; Barnett and Warren, 1995). Despite revival of traditional woodland management practices in more recent years, populations of *B. euphrosyne* declined in abundance by 71% between 1976 and 2014, with one third of English colonies lost between 1997 and 2004 (Ellis et al. 2012; Fox et al. 2015). *B. euphrosyne* is also endangered in Ireland (Nash and Hardiman, 2013), while in Scotland the butterfly is faring slightly better, particularly in the highlands (Barnett and Warren, 1995). *B. euphrosyne* receives protection under Schedule 5 of the Wildlife and Countryside Act (1981) for sale only, it is also a United Kingdom Biodiversity Action Plan (UK BAP) priority species. National population declines in this species have resulted in the charity Butterfly Conservation categorising it as a Class A Priority Species, recognising that urgent action is required to conserve this species (Butterfly Conservation, 2016). Much conservation effort for this species has focused on habitat management and restoration to increase population abundance, although the importance of site connectivity is increasingly being recognised (Ellis et al. 2012).

Beyond the UK, the full range of *B. euphrosyne* includes western, central and eastern Palaearctic regions, with the butterfly found in northern Spain and across much of Europe and temperate Asia. Although *B. euphrosyne* is classified as ‘not threatened’ in Europe, it has gone extinct in the Netherlands where it was once widespread (Van Swaay, 1990; Barnett and Warren, 1995; Asher et al. 2001). Notably, there is some evidence that in Europe the butterfly has evolved a broadened dietary niche. In addition to *Viola sp*. the butterfly is reported to feed on Bog Bilberry, *Vaccinium uliginosum* as well as using members of the Rosaceae family as secondary host plants (Simonsen et al. 2010).

1.9 *Boloria euphrosyne* in Morecambe Bay

The Morecambe Bay Limestones, located in northern Lancashire in the north-west of England, are of national importance for biodiversity, particularly Lepidoptera (Skelcher, 2014). In 1996, a survey of bracken habitat in Morecambe Bay and the South Cumbria Low Fells identified fourteen pearl-bordered fritillary, *Boloria euphrosyne*, colonies (Stuart, 1996). A report from

2008 describes *B. euphrosyne* as occurring in eight landscape networks in the north-west of England (Ellis and Wainwright, 2008). At that time, 17 colonies over 37 sites were reported as occupied, with 68% of these sites monitored by butterfly transects under the UKBMS (Ellis and Wainwright, 2008). Recent data from UKBMS transects suggests that many of these colonies have now been lost (Botham et al. 2020a). Warton Crag, located within the Arnside and Silverdale Area of Outstanding Natural Beauty (AONB), is a particularly important site for fritillaries, hosting one of the largest remaining populations of *B. euphrosyne* in the area (Turner and Riden, 2016). In 2010, a mark-release-recapture study at Warton Crag showed that several *B. euphrosyne* colonies were present across the site, with some movement between them (Bennett, 2010). However, a subsequent study at Warton Crag in 2016 and 2017 showed that *B. euphrosyne* was occupying fewer habitat patches than in 2010 and that movement between these was limited (Davidson, 2017). *B. euphrosyne* is a key focus of targeted habitat management in the region (Turner and Riden, 2016). Nevertheless, continuing population declines and site extinctions in Morecambe Bay parallel the national trends for *B. euphrosyne*, despite the region's reputation as the national stronghold for this species (Ellis et al. 2012). Identifying the factors that have driven site extinctions in the past as well as identifying the factors limiting current populations is likely to be key for conserving this species.

Given the sedentary nature of *B. euphrosyne*, it is probable that long-distance movement, between landscape networks, is limited (Barnett and Warren, 1995). Movement between local sites, however, is likely and populations are suggested to exist as a metapopulation within networks (Ellis and Wainwright, 2008). Local sites are reportedly well connected, and, on this basis, site isolation has previously not been considered to be a significant factor influencing *B. euphrosyne* persistence. However, given the rate of site extinctions over the past decade, it is likely that landscape connectivity has declined. While site isolation may have been of less importance to this species' persistence in the past, it is suggested to be a key contributing factor to current declines in *B. euphrosyne*. Further insight into the dispersal behaviour and population structure of *B. euphrosyne* is likely to have important implications for its conservation.

1.10 Scope of the thesis

The objective of this study was to assess population persistence and dispersal behaviour of the pearl-bordered fritillary, *Boloria euphrosyne,* a rapidly declining specialist butterfly that is failing to respond to targeted management in north-west England. The principal aims are to:

- Investigate factors that have driven past extinctions and those that may govern or limit current populations.
- Assess population connectivity, factors influencing dispersal and adaptations to habitat fragmentation.
- Inform future conservation efforts and monitoring of *B. euphrosyne*, with focus on the Morecambe Bay region.

This thesis is structured in four primary data chapters each contributing to different aspects of the general aims.

Chapter two aims to highlight the factors that are important for species persistence. Environmental drivers of population change over the past 15 years were investigated at 46 sites in England monitored by UKBMS transects.

Chapter three concerns population connectivity. Population synchrony was used to investigate functional connectivity of *B. euphrosyne* populations in Morecambe Bay.

Chapter four details a mark-release-recapture study carried out at Whitbarrow National Nature Reserve (NNR), to assess the mobility, population size and structure of *B. euphrosyne* populations at this key site network in Morecambe Bay. Factors influencing dispersal are discussed, including the use of flight related morphological traits as an indicator of butterfly mobility.

Evidence for morphological change in populations is highlighted in chapter five. Morphological traits in museum specimens are compared to butterflies from current populations, with implications for dispersal capacity discussed.

A general discussion is presented in chapter six to highlight the implications of the research for conservation, providing advice for site managers and directions for future study.

Chapter 2: Modelling species persistence in a fragmented landscape

2.1 Abstract

Butterflies are sensitive to habitat fragmentation and changing climate. As a sedentary butterfly with a highly specialised ecological niche, the pearl-bordered fritillary, *Boloria euphrosyne*, is particularly vulnerable to perturbation. Despite targeted management efforts, *B. euphrosyne* population declines in the UK have been pronounced, particularly over the last decade in the Morecambe Bay region, a former UK stronghold for this species in north-west England. To better understand these population declines and to aid the management of remaining *B. euphrosyne* populations, factors driving population persistence and abundance in this species were investigated. The influence of variables relating to adult dispersal and larval niche in English populations were assessed over the 2002-2017 period, using data from the United Kingdom Butterfly Monitoring Scheme (UKBMS). Site connectivity was shown to be positively related with both species' abundance and persistence. Weather conditions during the flight period were also found to be important, with interacting effects between maximum May temperature and rainfall on abundance. In addition, factors relating to the larval niche, significantly influenced extinction risk and maximum abundance. Extinction risk (as measured by population status in 2017) was lower at sites with warmer annual maximum temperatures, while site aspect had a significant effect on maximum abundance. Maximum abundance was also influenced by atmospheric nitrogen deposition, the effects of which were dependent on rainfall and habitat type. It is suggested that the impacts of both nitrogen deposition and increased rainfall under climate change scenarios are likely to affect woodland sites most strongly. While these results reinforce the importance of habitat quality, the relevance of landscape-scale conservation is also highlighted. Conservation management often focuses on the provision and quality of habitat at the site-level, however, the results presented here show that even for habitat specialists, population connectivity is critical for long-term survival in fragmented landscapes, particularly in the face of climate and environmental change.

2.2 Introduction

Habitat quality plays a central role in driving intra-species variation in abundance but, in addition, the importance of landscape scale conservation is being increasingly recognised (Ellis et al. 2012; Curtis et al. 2015). Understanding the factors regulating species' persistence is crucial for managing populations and reversing their declines. However, difficulties in assessing species' responses to perturbation can arise where there are contrasting effects at different life stages (Radchuk et al. 2013). Impacts are dependent on their cumulative effects across each stage of the life cycle, as such, it is crucial to understand how habitat quality and availability interact with climate and other modes of environmental change, including habitat fragmentation and nitrogen deposition.

Shifting climatic conditions can have indirect impacts on species where climate mediated changes in vegetation and microclimate alter habitat suitability. Abiotic factors such as temperature and humidity are important influences on invertebrate oviposition site selection and larval survival (Eilers et al. 2013). Climate change is likely to have impacts on species' fecundity, particularly for specialist species that occupy a narrow ecological niche. The bog fritillary, *Boloria eunomia*, is vulnerable to an increased risk of disease and fungal pathogens during milder winters (Radchuk et al. 2013). Negative effects of increased temperature on larval survival have also been observed in other species, such as the cranberry fritillary, *Boloria aquilonaris*, in which rates of larval survival decline at higher temperatures in association with habitat condition. *Sphagnum* hummocks are key for thermal buffering of larvae and are reduced in degraded peat bogs (Turlure et al. 2010b). Other indirect climate effects include the prolonged grass and herb growth that occurs during milder winters; elevated green plant biomass facilitates microclimate cooling and can have a detrimental effect on species that require warm microsites for larval development (Fischer and Fiedler, 2000). Such climate driven cooling may be enhanced by nitrogen deposition, which has also been suggested to result in increased shading and microclimate cooling as it promotes green plant biomass (Wallis De Vries and Van Sway, 2006). Indeed, nitrogen associated impacts on microclimate, in combination with milder winters, have been linked to declines in species such as the wall brown, *Lasiommata megera* (Klop et al. 2015). Nitrogen deposition may also affect herbivorous invertebrates through changes in foodplant quality or availability (Habel et al. 2016; Zhu et al. 2016). The low body tissue N:P ratio of Lepidoptera make them particularly vulnerable to changes in foodplant stoichiometry (Woods et al. 2004; Vogels et al. 2020). Butterflies with limited dispersal capacity, and those which are host plant specialists, have been most strongly affected by nitrogen deposition, highlighting how life-history strategies and specialisms during different stages of the life cycle can interact (Hendriks et al. 2013; Roth et al. 2021).

Dispersal influences processes such as local adaptation, community dynamics and population genetic structure, making it vital for maintaining resilient populations in the face of environmental change (Nowicki et al. 2014). Dispersal is particularly critical for species' persistence in fragmented landscapes as it facilitates recolonisations, which may rescue sub-populations from extinction (Brown and Kodric-Brown, 1977). Site area and connectivity are two key parameters in metapopulation theory (Hanski, 1998). Habitat fragmentation results in patches becoming smaller and poorly connected, as suitable habitat is lost to conversion or by inappropriate management. Such landscape changes reduce the likelihood of rescue by dispersing individuals and species with limited mobility are particularly vulnerable to habitat fragmentation (Fourcade and Öckinger, 2017). Population persistence tends to be greater in well-connected patches as these aggregations of core habitat support metapopulations with more viable dynamics relative to those on the periphery (Thomas et al. 2008). Repeated site extinctions in a network lead to increased isolation of remaining populations, while smaller habitat patches are also less likely to provide necessary resources and support viable populations (Thomas et al. 2001). While the implications of fragmentation are dependent on species' mobility and life-history traits, other factors, including habitat structure and environmental gradients, also interact with site isolation to influence dispersal behaviour and species persistence (Schtickzelle et al. 2006; Hanski, 2012; Delattre et al. 2013). As such, there are often regional differences in the population trajectories of species occupying separate landscape networks (Thomas et al. 2008; Bell et al. 2021). As well as being vital for metapopulation persistence, landscape connectivity is important in facilitating species' range shifts in response to climate change. Altered climatic conditions have benefitted certain mobile, generalist species, such as the speckled wood, *Pararge aegeria*, resulting in notable range expansion in the UK (Hill et al. 1999b). However, other variables including habitat condition can have a more immediate impact than climate on population trajectories at species range margins (Bell et al. 2021). Furthermore, sedentary species that are less likely to be able to colonise new habitat patches, may struggle to adapt as climatic conditions shift and are likely to suffer population declines as a result (Warren et al. 2001).

Declines in butterfly populations have been well documented in the United Kingdom (Fox et al. 2015; Dennis et al. 2017) and beyond (Warren et al. 2021). As butterflies are sensitive to their environment and often display rapid, population-level responses to habitat and climatic change, this makes them a useful model species (Stevens et al. 2010; Ellis et al. 2012). Environmental pressures have been shown to have a differential effect on habitat specialists and the wider countryside butterflies (Warren et al. 2021). Habitat specialists, sedentary species and those that overwinter in the immature stages (as eggs or larvae) are overrepresented among declining species (Eskildsen et al. 2015). As a monophagous, habitat specialist butterfly that overwinters in the larval stage, the pearl-bordered fritillary, *Boloria euphrosyne*, possesses several factors associated with vulnerability to extinction (Eskildsen et al. 2015). *B. euphrosyne* is also relatively sedentary, although it is thought that dispersal behaviour in this species is habitat-dependant, with the butterfly showing greater mobility in habitats with an open structure, where it forms metapopulations (Barnett and Warren, 1995). Indeed, *B. euphrosyne* is a key species of conservation concern, with declines in abundance in England of 74% between 1978 and 2019 (Brereton et al. 2020). In terms of conservation management, much focus has been given to understanding species' ecological requirements and maintaining suitable breeding habitat (Ellis et al. 2012). Accordingly, the ecology and lifecycle of *B. euphrosyne* are thought to be relatively well understood, yet despite targeted management efforts, this butterfly continues to decline in the UK (Barnett and Warren, 1995; Botham et al. 2020a). Understanding how populations are affected by perturbations, based not only on intrinsic species traits, but on site-specific factors such as aspect, landscape configuration and habitat type, is crucial for managing declining species (Fourcade and Öckinger, 2017).

Here, the influence of variables affecting adult dispersal and larval niche were assessed at sites across England. The aim of this study was to investigate drivers of population abundance and highlight site attributes associated with extinction risk in *B. euphrosyne,* by disentangling the effects of factors acting at different times during the lifecycle. Following metapopulation theory, site area and connectivity were hypothesised to have a positive relationship with butterfly abundance and persistence. It was also predicted that there would be an interaction between the extent of woodland and site connectivity, reflecting the greater importance of dispersal in open habitats where the species is thought to form metapopulations (Barnett and Warren, 1995). It was hypothesised that open habitats provide more marginal conditions for breeding, therefore the extent of woodland was expected to be positively associated with abundance. Rainfall and maximum temperature were predicted to affect abundance and extinction risk, with weather conditions during the adult peak flight period in May likely to influence mate location and dispersal success. The long duration of the larval period and the specificity of the larval niche in this species, also mean that climate and site aspect may affect larval survival, with important potential population-level effects. In addition, nitrogen enrichment can lead to microclimate cooling, due to increased grass and herb growth. As such, nitrogen deposition was hypothesised to influence population abundance via impacts on larval performance.

2.3 Methods

2.3.1 Study species

B. euphrosyne is a univoltine, habitat specialist butterfly that flies between late April and June in England, with the peak flight period in May. *B. euphrosyne* is reliant on both a single larval food plant, *Viola sp*., and specific abiotic conditions for larval development. The species occupies early successional woodland habitats as well as limestone grassland with scattered scrub and bracken stands, where leaf litter and sparse vegetation provide a warm microclimate for developing larvae (Barnett and Warren, 1995). Eggs are laid singularly and hatch in late summer, meaning that this species has a long period of larval development and overwinters in the larval stage (Fig. 2.1).

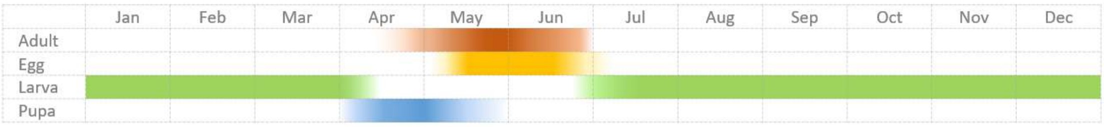


Figure 2.1. Timings of the lifecycle in *Boloria euphrosyne.* Approximate timings of the lifecycle showing the peak flight period and long duration of larval development. Figure based on that from Eeles (2019).

Adults from woodland colonies are characterised by sedentary behaviour, while in open habitats the butterfly is reported to be more dispersive and to form metapopulations (Thomas, 1984; Barnett and Warren, 1995). Despite targeted management efforts, *B. euphrosyne* population declines in the UK have been pronounced, particularly over the last decade in the Morecambe Bay region, a former stronghold for this species in England (Botham et al. 2020a).

2.3.2 Study sites and population metrics

The United Kingdom Butterfly Monitoring Scheme (UKBMS) is a long-standing programme of co-ordinated monitoring, which relies heavily on volunteers and is run in partnership between Butterfly Conservation, the UK Centre for Ecology and Hydrology, the British Trust for Ornithology, and the Joint Nature Conservation Committee. Under this scheme, butterfly species' abundance is recorded on fixed route transects at sites across the UK. Transects are walked on a weekly basis between April and September producing, ideally, 26 counts per year (Pollard and Yates, 1993). From these counts, annual species abundance indices are calculated at the site and national levels. However, missing data may be present due to incomplete sampling, for example, where minimum weather criteria are not met. Annual site indices are calculated using a general additive model and this can account for some missing data, but where the data are insufficient, due to limited recording during the peak flight period, indices are not able to be produced for that year (Botham et al. 2020a). UKBMS transects for which site location data were available (Botham et al. 2020b) were selected for study where *B. euphrosyne* was recorded on the transect in at least four years between 2002 and 2017 where recording effort during the peak flight period was sufficient to have allowed abundance indices to be produced. As there were few sites in Scotland and Wales which fulfilled these criteria, the analysis focused on English populations, with 46 transect sites across four regional networks in England selected for study (Fig. 2.2).

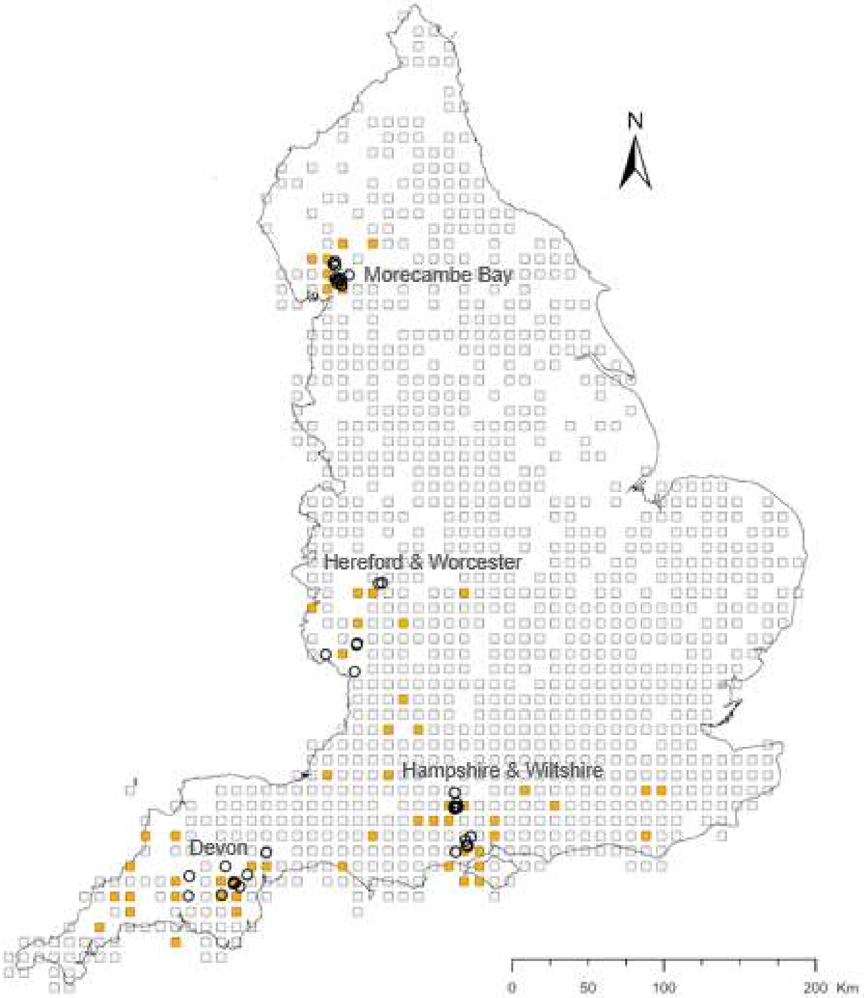


Figure 2.2. Study site locations. The locations of the 46 UKBMS sites selected for study (black circles show site centroids). Orange squares show *Boloria euphrosyne* distribution in England between 2002-2017 at the 10 km scale (NBN Atlas, 2021) Grey squares show 10 km grid squares monitored by UKBMS transects or the Wider Countryside Butterfly Survey during the same period (Botham et al. 2020b).

Sites were classified by their status in 2017, with site extinction defined as three consecutive years up to 2017 where *B. euphrosyne* was not recorded on the transect. Annual UKBMS indices were used to calculate mean abundance for each site over the 2002-2017 study period. To distinguish between true zero counts and false absences (due to low detection rate) averages only included counts from years where site indices were able to be calculated. Maximum abundance was also identified as the highest UKBMS index for each site within the study period.

2.3.3 Site attributes

The study sites were described using a range of spatial, habitat and climatic variables that related to *B. euphrosyne* adult or larval life stages (Supporting Information, Fig. S2.1). Transect route maps for each site were provided by the UKBMS. All spatial analysis was carried out in ArcGIS Pro 2.5.0 (Esri, 2020). Site boundaries were defined based on transect route maps, allowing site area to be calculated and attributes to be extracted. *Site connectivity* was calculated using the following equation (Hanski, 1999b):

$$S_i = \sum_{\mathrm{j \neq i}} exp\,(-\alpha d_{ij}) N_j.$$

Where d is the distance, in metres, between patches i and j. d was calculated as the direct geodesic distance between the start location of each transect route. α is a constant which describes the rate of decline in colonisation probability with distance, this was estimated using mark-release-recapture data and calculated as 1/average distance moved. In this case, an average distance moved by *B. euphrosyne* was calculated from mark release recaptures carried out in 2016, 2017 and 2019, to give an α value of 0.0035 (Davidson, 2017; Thesis Chapter 4). The α value used here is similar to values used in previous studies, using the same methodology (Fourcade and Öckinger, 2017). N_j is the population size of patch j (Hanski, 1999b). Site connectivity was calculated for each year, with UKBMS abundance indices used as N_j. The calculated values for each site were then averaged over the 2002-2017 period. When calculating connectivity, all abundance indices produced for *B. euphrosyne* at UKBMS transect sites within a 10 km radius of the focal site were used. Although this measure is referred to elsewhere as isolation, higher values indicate well-connected sites (Hanski, 1999b).

Rainfall and *maximum temperature* data from the HadUK-Grid 1 km Gridded Climate Observations were used to calculate averages for each site across the 2002-17 study period (Hollis et al. 2019). Both annual and daily values for May (the peak flight period) were extracted and averaged across the study period. *Woodland extent* was calculated using the 2015 Land Cover Map and describes the percentage of the total site area characterised by woodland habitat (this includes actively managed semi-natural woodland and recently felled areas and so represents broad habitat type rather than canopy cover) as opposed to grassland systems (Rowland et al. 2017). *Aspect*, referring to the average compass direction of a site, was calculated using OS Terrain 50 raster data (OS, 2015). Using nitrogen deposition data from the NERC Environmental Information Data Centre, average levels of *deposited nitrogen* were calculated for each site, over the study period (Tomlinson et al. 2020). The dataset used gives values of modelled annual nitrogen deposition in the UK at a 1 km × 1 km resolution, which are calculated from 1990 - 2017 nitrogen emissions data (Tomlinson et al. 2020). The values of deposited nitrogen calculated for each site in the current study represent the sum of wet and dry deposited nitrogen, including both reduced and oxidised forms.

2.3.4 Statistical analysis

The effects of site attributes and climatic variables on *B. euphrosyne* population status, average abundance and maximum abundance were investigated using generalized linear models (GLM). Due to collinearity between terms and the hypothesis that variables have distinct population-level effects associated with their impacts on *B. euphrosyne* at different life-stages, models were built for each population metric to assess potential factors affecting the butterfly during the adult and larval period separately. Interactions between factors were included in maximal models where biologically relevant. The explanatory variables for the adult period models were site area (m^2), connectivity, woodland extent (%), average maximum daily temperature during May (°C) and average daily May rainfall (mm). Climate data for May were used in the adult period models as May is the peak flight period. These models took the form:

y ~ area + connectivity + woodland + temperature + rainfall + area : connectivity + temperature : rainfall + woodland : temperature + woodland : rainfall

The explanatory variables for the larval period models were aspect (°), total deposited nitrogen (kg ha^{-1} $year^{-1}$), woodland extent (%), average daily maximum temperature for the whole year (°C) and average annual rainfall (mm). Given the long period of larval development in *B. euphrosyne* (Fig. 2.1) the larval period models used annual climate data averaged across the study period. These models took the form:

y ~ aspect + nitrogen + woodland + temperature + rainfall + aspect: temperature + aspect: rainfall + nitrogen : rainfall + nitrogen : woodland + temperature : rainfall + woodland : temperature + woodland : rainfall

In all maximal models, latitude was included as an additional explanatory variable and region (with sites grouped into four geographic areas as per Fig. 2.2) was included as a random effect. Model selection was carried out using a backwards stepwise procedure, using chi-square likelihood ratio tests (binomial and negative binomial errors) or F-tests (normal errors) to assess the significance of terms. Terms where $P < 0.05$ were considered significant and retained in the model (Crawley, 2007). Population status was assessed using a GLM with binomial errors and a logit link function. Average abundance was modelled using a GLM with normal errors, average abundance was log transformed to improve model fit. A negative binomial GLM with a log link function was used to assess the influence of site attributes and environmental variables on maximum abundance. Model fit was validated using residual plots and tests for over-dispersion using the R package DHARMa (Hartig, 2020). Tests for spatial autocorrelation were also carried out. Moran's I tests showed that spatial autocorrelation was not present in the model residuals (Supporting Information, Table S2.1). Building separate models ensured that terms in all models had variance inflation factors (VIF) of < 3 (Zuur et al. 2010). All statistical analysis was carried out in R Studio Version 4.0.3 (R Core Team, 2020).

2.4 Results

Ten (22%) of the 46 study sites were classified as extinct, with seven of these extinctions occurring in the Morecambe Bay area, in north-west England. Maximum abundance ranged from 6 to 441 (mean = 79.74 ± 13.56 SE). However, the majority of populations were small, with almost half of the sites (48%) with a maximum abundance of less than 45 individuals (Supporting Information, Fig. S2.2a). Average abundance over the study period ranged from 1 to 160 (mean = 29.8 ± 4.6 SE), with 37 sites (80%) having an average abundance of less than 50 and 18 sites (39%) having an average abundance of less than 10 individuals (Supporting Information, Fig. S2.2b).

2.4.1 Factors affecting populations during the flight period

Variables relating to the adult flight period significantly influenced all investigated population metrics. Site connectivity significantly affected population status and abundance of *B. euphrosyne* populations, with well-connected sites less likely to have become extinct (Table 2.1a; Fig. 2.3a).

Table 2.1. Model output: the effects of site attributes relating to the adult flight period on three population metrics in *Boloria euphrosyne.* Parameter estimates, standard errors (SE), degrees of freedom (d.f.), Chi-squared (χ^2) or F values and *P*-values from minimum adequate models assessing the effects of site attributes during the adult flight period on (a) population status, (b) average abundance and (c) maximum abundance at sites in England between 2002 and 2017. n = 46. Max May temp refers to the average maximum daily temperature in May (°C).

(a) Population Status

Independent Variables	Estimate	SE	d.f.	χ^2	*P*
Intercept	-28.930	11.632	1	-13.311	0.004
Connectivity	0.332	0.167	1	-6.881	0.009
Max May Temp	1.626	0.665	1	-8.841	0.004
May Rainfall	2.729	1.211	1	-8.298	0.003

(b) Log Average Abundance

Independent Variables	Estimate	SE	d.f.	F	*P*
Intercept	-36.181	10.748	4, 45	6.664	<0.001
Connectivity	0.208	0.045	1, 42	20.952	<0.001
Max May Temp	2.482	0.704	2, 43	6.330	0.002
May Rainfall	16.914	4.667	2, 43	7.806	<0.001
Max May Temp : May Rainfall	-1.095	0.31	1, 42	12.507	<0.001

(c) Maximum Abundance

Independent Variables	Estimate	SE	d.f.	χ^2	*P*
Intercept	-28.352	10.156	4	13.031	0.011
Connectivity	0.138	0.043	1	9.045	0.003
Max May Temp	2.107	0.665	2	10.978	0.004
May Rainfall	13.316	4.406	2	11.161	0.004
Max May Temp : May Rainfall	-0.867	0.292	1	10.377	<0.001

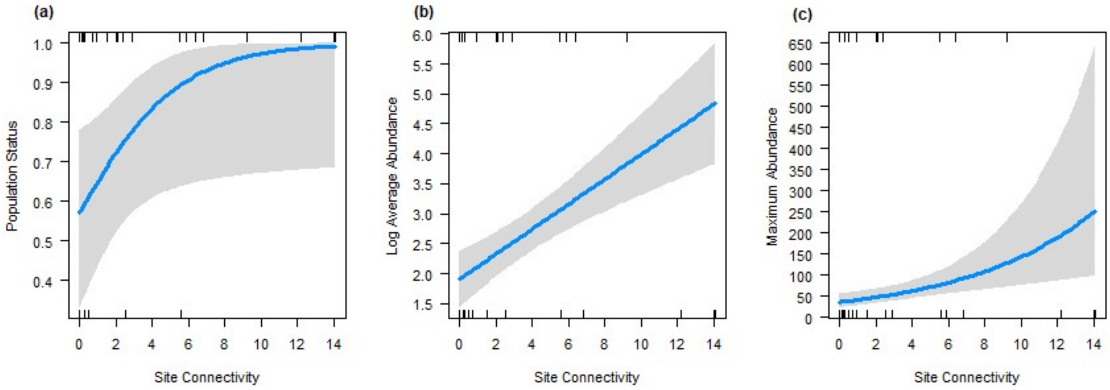


Figure 2.3. The model predicted relationships between site connectivity and three population metrics in *Boloria euphrosyne*. The relationship between site connectivity and (a) population status in 2017, (b) log average abundance, (c) maximum abundance of populations at 46 sites in England over the 2002-2017 period. Shaded areas show 95% confidence intervals and rug marks show partial residuals.

There was also a positive relationship between connectivity and both average abundance (Table 2.1b; Fig. 2.3b) over the study period and maximum abundance (Table 2.1c; Fig. 2.3c), demonstrating that populations occupying better connected sites are able to reach a larger size at their peak, relative to those at isolated sites.

Climatic variables during the adult peak flight period, also significantly affected populations (Table 2.1). Both rainfall and average maximum daily temperature in May were positively related with population status, showing that warmer, wetter sites were less likely to have gone extinct (Fig. 2.4).

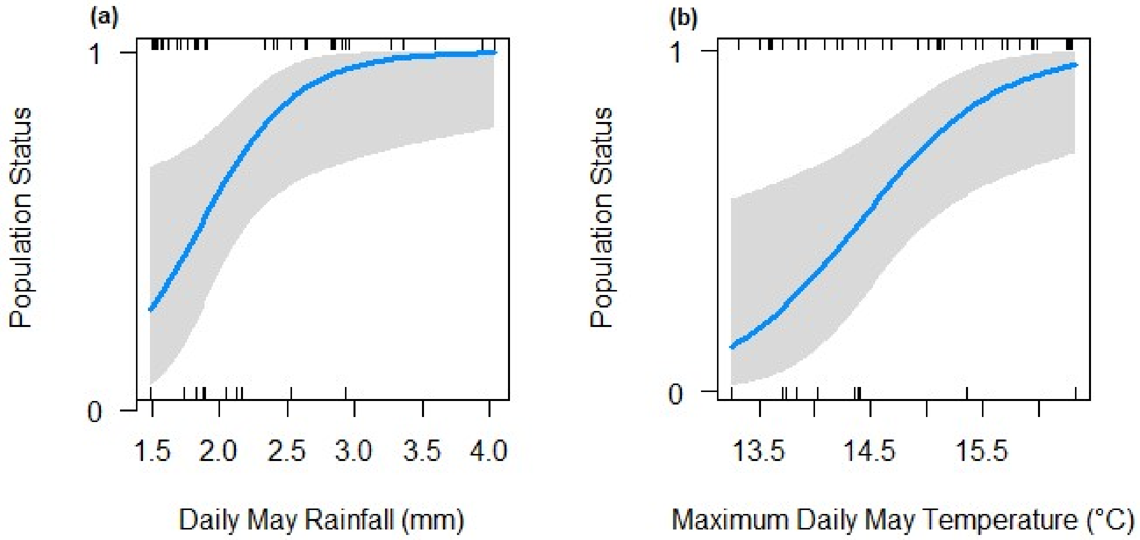


Figure 2.4. The model predicted relationships between two climatic variables and population status in *Boloria euphrosyne.* The relationship between population status of 46 English populations in 2017 and (a) Daily May rainfall, (b) Maximum daily May temperature. Shaded areas show 95% confidence intervals and rug marks show partial residuals.

For average and maximum abundance, however, there was an interaction between daily May rainfall and average maximum daily May temperature such that the relationship between abundance and temperature was only positive at lower levels of rainfall. Cross-sections were taken at the 10th, 50th, and 90th quantiles, showing that at levels of daily precipitation above approximately 2.6 mm^{-1} the relationship between abundance and maximum daily May temperature was negative (Fig. 2.5).

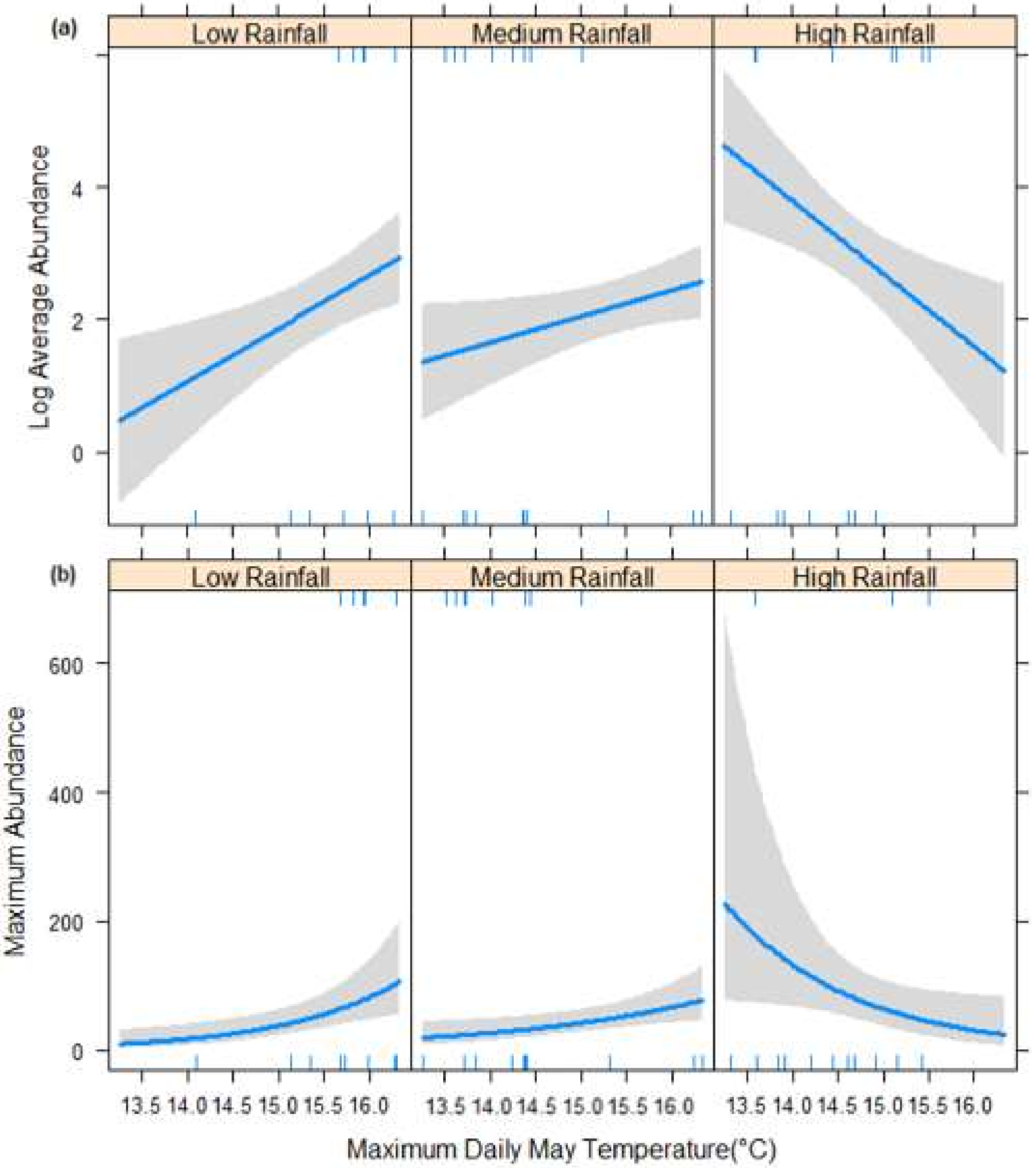


Figure 2.5. The model predicted relationships, showing the interacting effects of daily May rainfall and average maximum daily May temperature on two population metrics in *Boloria euphrosyne* (a) log average abundance and (b) maximum abundance of populations at 46 sites in England over the 2002-2017 period. Break points represent cross-sections taken at the 10th, 50th, and 90th quantiles, equivalent to 1.5 mm d^{-1}, 1.9 mm d^{-1} and 3.3 mm d^{-1} levels of rainfall. Shaded areas show 95% confidence intervals and rug marks show partial residuals.

2.4.2 *Factors affecting populations during the larval period*

Variables relating to the larval period were also found to have a significant impact on population status and maximum abundance (Table 2.2) although no variables were retained in the larval model built to explain average abundance. There was a positive relationship between population status and annual maximum temperature, with likelihood of extinction lower at warmer sites (Fig. 2.6).

Table 2.2. Model output: the effects of site attributes relating to the larval period on two population metrics in *Boloria euphrosyne*. Parameter estimates, standard errors (SE), degrees of freedom (d.f.), Chi-squared (χ^2) and P-values showing the effects of site attributes and environmental factors measured across the whole year on (a) population status and (b) maximum abundance at sites in England between 2002 and 2017. n = 46. Annual Max Temperature refers to average maximum daily temperature across the whole year (°C).

(a) Population Status

Independent Variables	Estimate	SE	d.f.	χ^2	*P*
Intercept	-10.951	6.358	1	-4.090	0.043
Annual Max Temperature	0.882	0.463	1	-4.090	0.043

(b) Maximum Abundance

Independent Variables	Estimate	SE	d.f.	χ^2	*P*
Intercept	-31.190	11.890	7	19.858	0.006
Aspect	0.006	0.003	1	4.683	0.030
Nitrogen Deposition	1.760	0.697	3	7.669	0.053
Woodland	0.243	0.091	3	11.753	0.008
Annual Rainfall	0.017	0.006	3	9.859	0.020
Nitrogen Deposition : Annual Rainfall	-0.001	0.000	1	6.725	0.010
Woodland : Annual Rainfall	-4.38E-05	0.000	1	5.350	0.021
Woodland : Nitrogen Deposition	-0.011	0.005	1	4.533	0.033

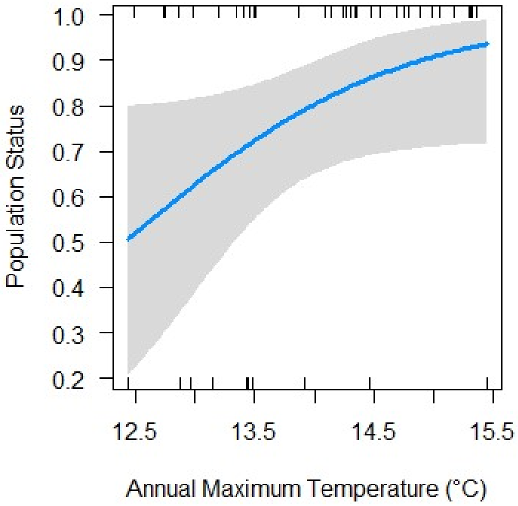


Figure 2.6. The model predicted relationship between annual maximum temperature and population status in *Boloria euphrosyne.* The relationship between population status of 46 English populations in 2017 and annual maximum temperature over the 2002-2017 period. Shaded areas show 95% confidence intervals and rug marks show partial residuals.

Site aspect, nitrogen deposition, percentage woodland and annual rainfall were found to significantly influence maximum abundance (Table 2.2b). Site aspect refers to the majority compass direction that sites sloped; study sites varied in aspect from 40.9 to 253.1 degrees, reflecting a range of aspect from north-north-easterly to westerly. Site aspect was positively related with maximum abundance (Fig. 2.7) reflecting higher maximum abundance at sites with a south to south-westerly aspect.

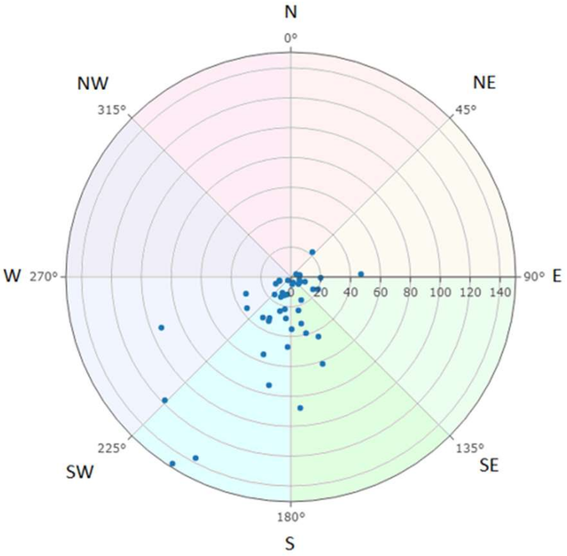


Figure 2.7. Site aspect and abundance of *Boloria euphrosyne* populations. The relationship between site aspect and maximum abundance of populations at 46 sites in England. Aspect is shown in degrees and majority compass direction.

Maximum abundance was also significantly influenced by annual rainfall, although the direction of effect was dependent on both levels of nitrogen deposition and habitat type. There were significant interactions between annual rainfall and nitrogen deposition (Fig. 2.8).

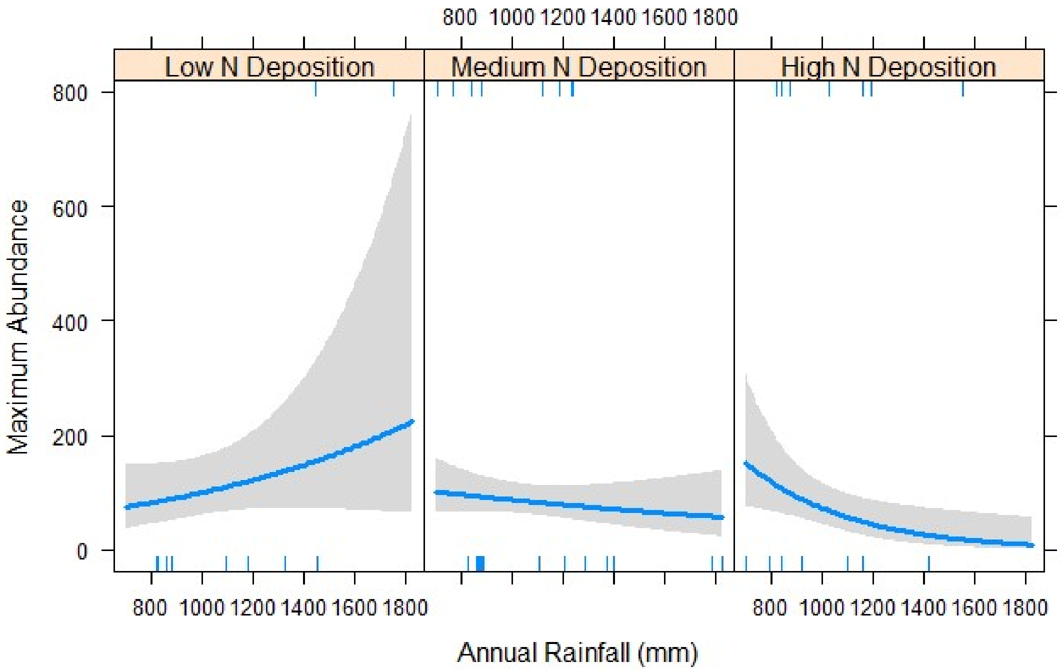


Figure 2.8. The model predicted relationship showing the interacting effects of annual rainfall and nitrogen deposition on *Boloria euphrosyne* abundance. The relationship between maximum abundance populations at 46 sites in England showing the interaction between annual rainfall and levels of deposited nitrogen. Break points represent cross-sections taken at the 10th, 50th, and 90th quantiles, equivalent to 14.1 kg ha^{-1} year^{-1}, 15.9 kg ha^{-1} year^{-1} and 18.4 kg ha^{-1} year^{-1} levels of nitrogen deposition. Shaded areas show 95% confidence intervals and rug marks show partial residuals.

Site values for nitrogen deposition ranged from 13.3-20.6 kg ha^{-1} year^{-1}. At sites with low levels of nitrogen deposition (below approximately 15 kg ha^{-1} year^{-1}) there was a slight positive relationship between annual rainfall and maximum abundance (Fig. 2.8). However, the relationship levelled off with increased nitrogen deposition and sites with high levels of nitrogen deposition (above approximately 17.2 kg ha^{-1} year^{-1}) were negatively affected by increased annual rainfall (Fig. 2.8).

The effect of average rainfall on maximum abundance also varied with habitat type, being positive at sites with up to 50% woodland extent, while the modelled relationship for sites with a higher coverage of woodland showed that high levels of annual rainfall were associated with lower maximum abundances (Fig. 2.9).

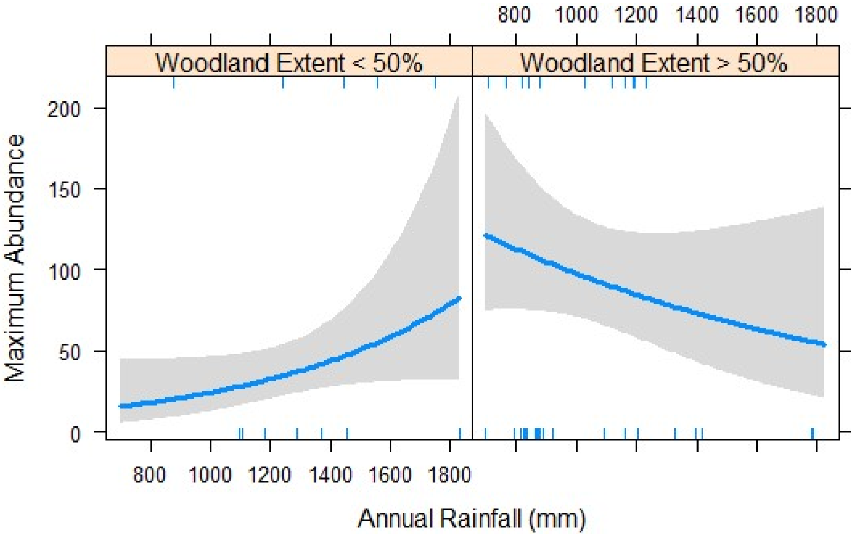


Figure 2.9. The model predicted relationship showing the interacting effects of annual rainfall and woodland extent on *Boloria euphrosyne* abundance. The relationship between maximum abundance of populations at 46 sites in England showing the interaction between annual rainfall and the extent of woodland cover. Break points in woodland extent represent sites with <50% and >50% cover of woodland habitat. Shaded areas show 95% confidence intervals and rug marks show partial residuals.

There was also a significant interaction between woodland extent and nitrogen deposition. Nitrogen deposition had a positive effect on maximum abundance at sites with up to 50% woodland extent, but a negative effect on abundance at woodland dominated sites (Fig. 2.10).

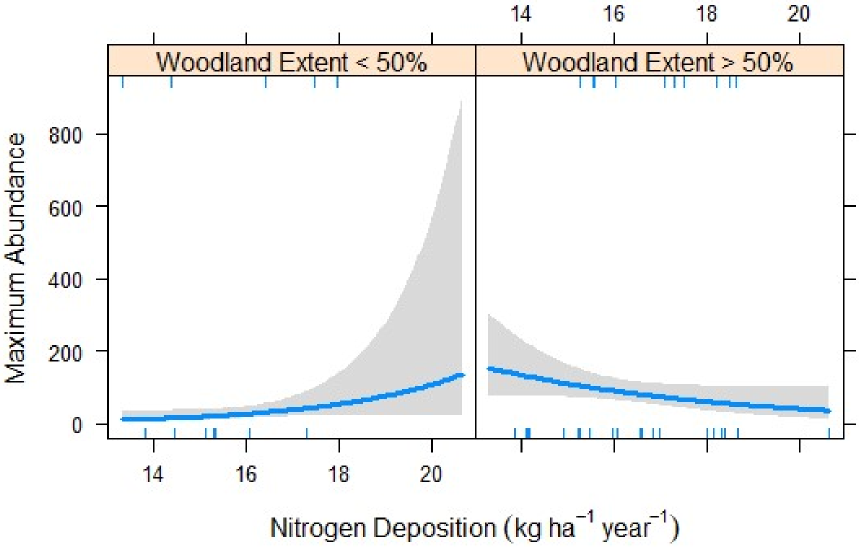


Figure 2.10. The model predicted relationship showing the interacting effects of nitrogen deposition and woodland extent on *Boloria euphrosyne* abundance. The relationship between maximum abundance of populations at 46 sites in England showing the interaction between the extent of woodland and nitrogen deposition. Break points in woodland extent represent sites with <50% and >50% cover of woodland habitat. Shaded areas show 95% confidence intervals and rug marks show partial residuals.

2.5 Discussion

The influence of variables affecting *B. euphrosyne* during the adult flight period and larval stages were assessed in English populations over the 2002-2017 period. Population metrics were shown to be significantly affected by factors relating to adult dispersal, in addition to variables likely to influence larval survival. It was demonstrated that site connectivity and weather conditions during the peak flight period in May are important for the maintenance of large, viable populations, as they were shown to influence abundance and extinction risk. Abundance responses to rainfall and temperature during the flight period showed an interaction, with abundance positively related with maximum daily May temperature, but only at sites with limited rainfall. Extinction risk was also lower at sites with warmer temperatures both in May and across the whole year. Maximum abundance was significantly influenced by site aspect, average annual rainfall, nitrogen deposition and the extent of woodland. Interacting effects suggest that the impacts of climate and other environmental changes on breeding success may have differential effects across sites with different habitat characteristics.

The results provided here highlight that many *B. euphrosyne* populations in England are concerningly small and that site connectivity plays an important role in influencing both population size and extinction risk. Better connected sites supported populations with higher abundance and were less likely to have faced extinction during the 2002-2017 period. These results are consistent with previous studies on butterfly metapopulations that have demonstrated higher levels of population survival in core areas of habitat, relative to those on the periphery. For example, *E. aurinia* survival has been shown to be greatest in 10 km grid squares surrounded by other occupied 10 km grid squares, as these aggregations of habitat are better able to support colonies with viable metapopulation dynamics (Thomas et al. 2008). As a sedentary butterfly, *B. euphrosyne* is likely to be particularly vulnerable to habitat fragmentation and isolation. The dynamics of small, fragmented populations are also strongly influenced by dispersal and immigration between sub-populations plays an important role in sustaining populations that exist close to the extinction threshold (Bonsall et al. 2014). Such 'rescue effects' may provide one explanation for the relationship between connectivity and the population metrics assessed in the current study (Brown and Kodric-Brown, 1977; Hanski, 1998). In this way, populations with sink-type dynamics and those occupying sub-optimal habitats can be maintained by dispersal. Conversely, where dwindling populations with low abundance are isolated, they are unlikely to be rescued from extinction. Where sites are isolated, emigration can also be detrimental to population persistence, as successful colonisation of another patch is unlikely (Schtickzelle et al. 2006). For example, in the high brown fritillary, *Fabriciana adippe,* elevated emigration from isolated populations at low densities has been linked to their population decline in the UK (Bonsall et al. 2014). This study suggests that site isolation may play a greater role in regulating *B. euphrosyne* populations than previously thought, with small populations at isolated sites likely to be highly vulnerable to extinction. Nevertheless, metapopulation modelling has suggested that, due to 'extinction debt,' current UK distributions of species such as *E. aurinia* overestimate the likelihood of future species' persistence and the concept of extinction debt may also be applicable for other species inhabiting fragmented landscapes, including *B. euphrosyne* (Bulman et al. 2007).

It is worth considering that the index of connectivity in the current study used Euclidean distance between UKBMS sites as an indicator of the likelihood of colonisation. In reality, landscape permeability is more complex, with habitat features and prevailing weather conditions having the potential to facilitate or impede dispersal (Powney et al. 2012, Vindstad et al. 2019). As not all *B. euphrosyne* populations are monitored by butterfly transects, the index of connectivity used here may exaggerate the extent to which the study sites are isolated. However, the proportion of *B. euphrosyne* populations monitored by UKBMS transects is likely to be high. In the Morecambe Bay region, for instance, a reported 68 % of *B. euphrosyne* sites are monitored by transects (Ellis and Wainwright, 2008). Population metrics were calculated using data from the 2002-2017 period, however not all sites were fully surveyed every year. The number of years used to calculate population metrics for each site ranged from 4-16. As such, the population metrics in the current study may be sensitive to the years in which sampling was conducted.

Population status and abundance were also shown to be significantly influenced by weather conditions, particularly during the peak flight period in May. Sites with higher maximum temperatures both in May and across the whole year were less likely to have gone extinct. One explanation for this is that population rescue is more likely to occur at sites that experience favourable weather conditions during the flight period, as dispersal is often temperature dependent (Delattre et al. 2013; Kuussaari et al. 2016). In addition, warm temperatures during the flight period may also promote population persistence through successful breeding and enhanced mate location. Butterflies patrol more actively during warm weather and patrolling behaviour is necessary in *B. euphrosyne* males for mate location and territory defence (Ide, 2010; Barnett and Warren, 1995). Furthermore, larval development in *B. euphrosyne* occurs in the early spring and is temperature dependent, with warm microclimate conditions required (Barnett and Warren, 1995). Extinction was also shown to be less likely at sites with higher levels of daily precipitation in May. The positive role of rainfall during the peak flight period seems somewhat paradoxical given the species' habitat requirements and oviposition preference for sites with a warm microclimate, associated with dry leaf litter (Atkinson, 2016). Furthermore, high levels of rainfall have been shown to limit dispersal in other species (Kuussaari et al. 2016). On the other hand, drought events can be detrimental, affecting both nectar availability and oviposition site suitability, with potential population-level effects (Sutcliffe et al. 1997; Phillips et al. 2018; Salgado et al. 2020). It is likely, therefore, that there is an optimal level of rainfall for *B. euphrosyne* persistence. The interacting effects of rainfall and maximum daily May temperature on average and maximum abundance, highlight these potential trade-offs. At sites with higher levels of daily May rainfall there was a negative relationship between abundance and temperature (Fig. 2.5). One explanation is that warmer, wetter conditions could have negative population-level effects due to influences on habitat. Given that maximum May temperatures are correlated with maximum temperatures across the whole year, these results may reflect impacts across life-stages. Elevated temperatures and rainfall may promote grass and herb growth and increase the rate of regeneration in early-successional habitats, leading to a microclimate cooling and a decline in oviposition site suitability (Ellis et al. 2019). Such temperature-driven vegetation growth may be limited at sites with low rainfall.

Maximum abundance at sites over the study period was also shown to be strongly influenced by factors relating to larval niche. Emergence in the early spring makes *B. euphrosyne* particularly sensitive to spring weather and individuals are reported to select the warmest, driest areas for oviposition (Barnett and Warren, 1995). Accordingly, site aspect was shown to have significant influence on maximum abundance, with higher abundance found at sites with south to south-westerly aspects. Sites with southerly aspects tend to be more sheltered and warmer, making these habitats well-suited for larval development. Nevertheless, overwintering as larvae may bring elevated risk from desiccation as seasons become warmer, microclimate heterogeneity arising from variation in topography may help to buffer populations against the negative effects of climate change in the future (Breed, 2013; Suggitt et al. 2018). While site aspect was shown to influence *B. euphrosyne*, latitude was not retained in the models as it did not have a significant influence on the population metrics assessed. Previous studies have highlighted regional differences in population trajectories and variation in the stages of decline of populations persisting in separate landscape networks. For example, population declines in the grizzled skipper, *Pyrgus malvae*, have been most pronounced in the north and west of their range in the UK, relative to sites in the south and east (Bell et al. 2021). Declines in *E. aurinia* across Europe have also highlighted higher rates of survival in southern and eastern countries (Thomas et al. 2008). Such variation may be driven by large-scale environmental gradients, but variables including habitat condition can often have a more immediate impact on population trajectories (Bell et al. 2021). Previous studies have suggested that the present distribution of *B. euphrosyne* is only explained by climatic variables to a limited extent and in the current study, there was no evidence to suggest regional variation in the drivers of population change in *B. euphrosyne* in England (Settele et al. 2008).

Annual average rainfall was also shown to have a significant effect on maximum abundance, however, the effects of rainfall interacted with both woodland cover and nitrogen deposition. There was a positive relationship between maximum abundance and annual rainfall at sites with up to 50% woodland cover and lower levels (equivalent to 15 kg ha^{-1} $year^{-1}$) of nitrogen deposition, while beyond these levels, abundance declined with elevated rainfall. Therefore, the results suggest that wooded sites and those with high levels of nitrogen deposition are likely to be most vulnerable to increased annual rainfall under climate change scenarios. Previous studies have highlighted the negative effects of extreme precipitation events during the pupal and larval life-stages of univoltine butterfly species (McDermott Long et al. 2017). In woodland, increased shading and moisture retention means that elevated levels of rainfall are more likely to have a detrimental effect on habitat quality. High levels of rainfall can compromise larval survival in the early spring, while milder, wetter winters may be associated with increased pathogen damage to overwintering larvae. By contrast, rainfall in habitats with a more open structure may be important for preventing desiccation of larvae and foodplants. In woodland, habitat suitability for *B. euphrosyne* has been reported to peak after two years following coppicing and to decline thereafter as succession occurs (Warren and Thomas, 1992). However, more recent studies have suggested that rapid succession, due to changing climatic conditions, is resulting in a deterioration in habitat quality, and woodland habitats are likely to degenerate more rapidly than open grassland habitats, where succession is less rapid (Barnett and Warren, 1995; Ellis et al. 2019).

Increased rainfall, in combination with warming temperatures may facilitate grass and herb growth, resulting in cooling of the microclimate and negative effects on larval survival (Wallis De Vries and Van Swaay, 2006). These microclimate effects are likely to be exacerbated in areas of high nitrogen deposition, where nitrogen enrichment and rainfall combine to stimulate green plant biomass. The interaction between nitrogen deposition and rainfall in this study, supports this hypothesis, showing that maximum *B. euphrosyne* abundance is negatively affected by elevated rainfall at sites with high levels of nitrogen deposition. The hostplant, *Viola sp.,* is sensitive to nutrient enrichment, highlighting the potential for nitrogen deposition to impact *B. euphrosyne* via changes in foodplant availability or quality (Hill et al. 1999c).

In addition to the interaction between nitrogen deposition and rainfall in this study, the effects of nitrogen deposition on maximum *B. euphrosyne* abundance were dependent on habitat type. Nitrogen deposition was shown to have strong negative effects on maximum abundance at sites with higher levels of woodland cover, while at sites where woodland cover was less than 65 % the relationship was positive. Previously, vulnerability to nitrogen enrichment has been shown to vary between habitats, with species occupying nutrient limited, oligotrophic habitats, showing stronger declines in distribution, relative to those occupying mesotrophic and eutrophic habitats (Maes and Van Dyck, 2001). The differential vulnerability of different systems to nitrogen deposition is highlighted by variation in the critical load (the level of pollutant below which ‘significant harmful environmental effects’ are not known to occur) between habitats (Hall et al. 2015). For instance, semi-dry calcareous grassland is characterised by a critical load of 15-25 kg N ha^{-1} $year^{-1}$, while broadleaved woodland has a lower critical load of 10-20 kg ha^{-1} $year^{-1}$ (Hall et al. 2015). *B. euphrosyne* occupies both open grassland habitats and actively managed areas of semi-natural broadleaved woodland (Barnett and Warren, 1995). The differential effects of nitrogen deposition on maximum abundance at sites with different habitat types, observed in the current study, may represent this variation in critical load between habitats. The strong negative effects of nitrogen deposition on maximum *B. euphrosyne* abundance in broadleaved woodland could be explained by the lower critical load of woodland relative to grassland, as woodland habitats are likely to be more strongly impacted by the same levels of nitrogen deposition than systems with a higher critical load. Values of total nitrogen for sites in the current study ranged from 13.3-20.6 kg ha^{-1} year^{-1} showing that all sites exceeded the critical load relating to broadleaved woodland, while the average of 16.2 kg ha^{-1} year^{-1} suggests that many sites may fall below the critical load for calcareous grassland. In woodland habitats, species' persistence is dependent on management to produce a continuous supply of suitable early successional habitat. Therefore, in woodland systems the negative impacts of nitrogen deposition may be more immediate as nitrogen enrichment may increase the rate of succession resulting in even shorter periods of habitat suitability. Historical decline in traditional woodland management practices in the UK have reputedly led to *B. euphrosyne* becoming largely restricted to areas of rough grassland and bracken, as woodland colonies have gone extinct (Greatorex-Davies et al. 1992). As succession is reported to be less rapid in the more open, grassland, habitats occupied by *B. euphrosyne,* these habitats are likely to be less vulnerable than woodland to changes in habitat regeneration associated with elevated levels of nitrogen (Barnett and Warren, 1995). The differential effects of nitrogen deposition at wooded sites and those with a more open structure highlighted in this study, may provide an alternative explanation for *B. euphrosyne* declines, particularly in recent years at wooded sites, such as Gait Barrows in the north-west of England, which is actively managed for this species (Ellis and Wainwright, 2008; Botham et al 2020a). The observed impacts of nitrogen deposition on *B. euphrosyne* population metrics are consistent with previously reported impacts on Lepidoptera, nevertheless when interpreting these results it may be worth considering the potential impacts of correlated large-scale environmental gradients and factors associated with the mechanisms of deposition that may be driving population declines. For example, levels of dry deposition of nitrogen oxides are higher in urban areas and negative trends could be driven by environmental gradients in the intensity of urbanisation or agriculture. Furthermore, the interaction between nitrogen deposition and rainfall may be driven, in part, by the process of wet deposition. The loading of nitrogen in wet deposition is dependent on levels of precipitation, with higher precipitation levels resulting in lower concentrations of deposited nitrogen. There is evidence that the form of nitrogen deposition may affect environmental outcomes, but this is complex and not fully understood (Stevens et al. 2018).

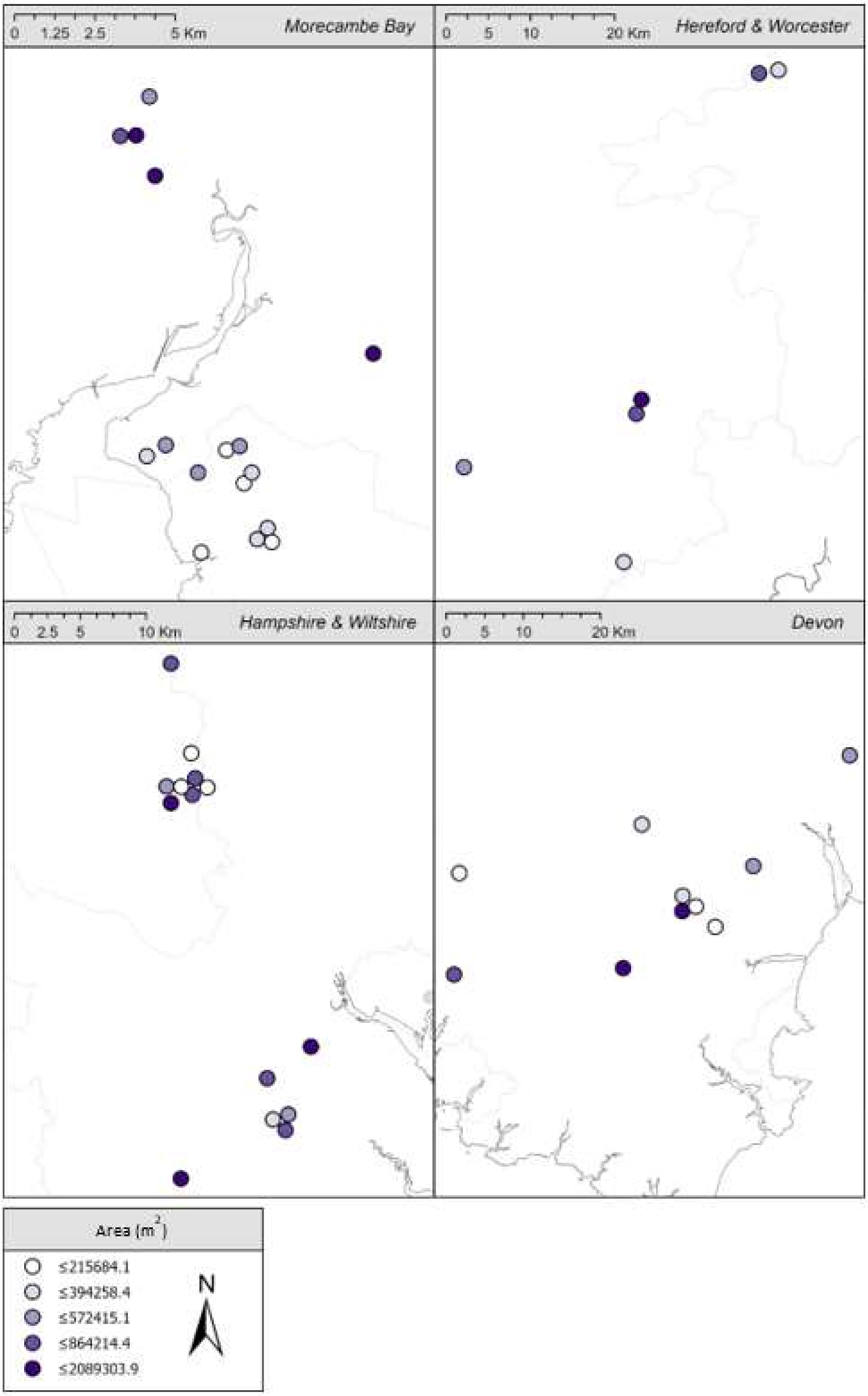
Effective conservation management should consider multiple spatial scales and impacts across life-stages. This study demonstrates that while habitat quality is likely to have important population-level effects, factors affecting adult life-stages, including site connectivity are also important for both population abundance and extinction risk. This analysis raises the possibility that nitrogen deposition is an important factor influencing carrying capacity, but that sites may vary in their susceptibility to nutrient enrichment depending on habitat type and levels of precipitation. In addition to a habitat-based approach, which targets existing populations and aims to increase abundance by supplying high quality habitat, considering conservation on a landscape-scale is crucial. Management regimes for early successional specialist species should also consider the progression of vegetation regeneration and manage small areas on a rotation so as to provide a continuous supply of readily colonisable habitat. Creation of landscape heterogeneity may also help to buffer populations against environmental change. Although the habitat requirements of many species of Lepidoptera are relatively well understood, in the context of environmental change and an increasingly fragmented landscape, the need to review and adapt management recommendations is pertinent.

2.6 Supporting Information

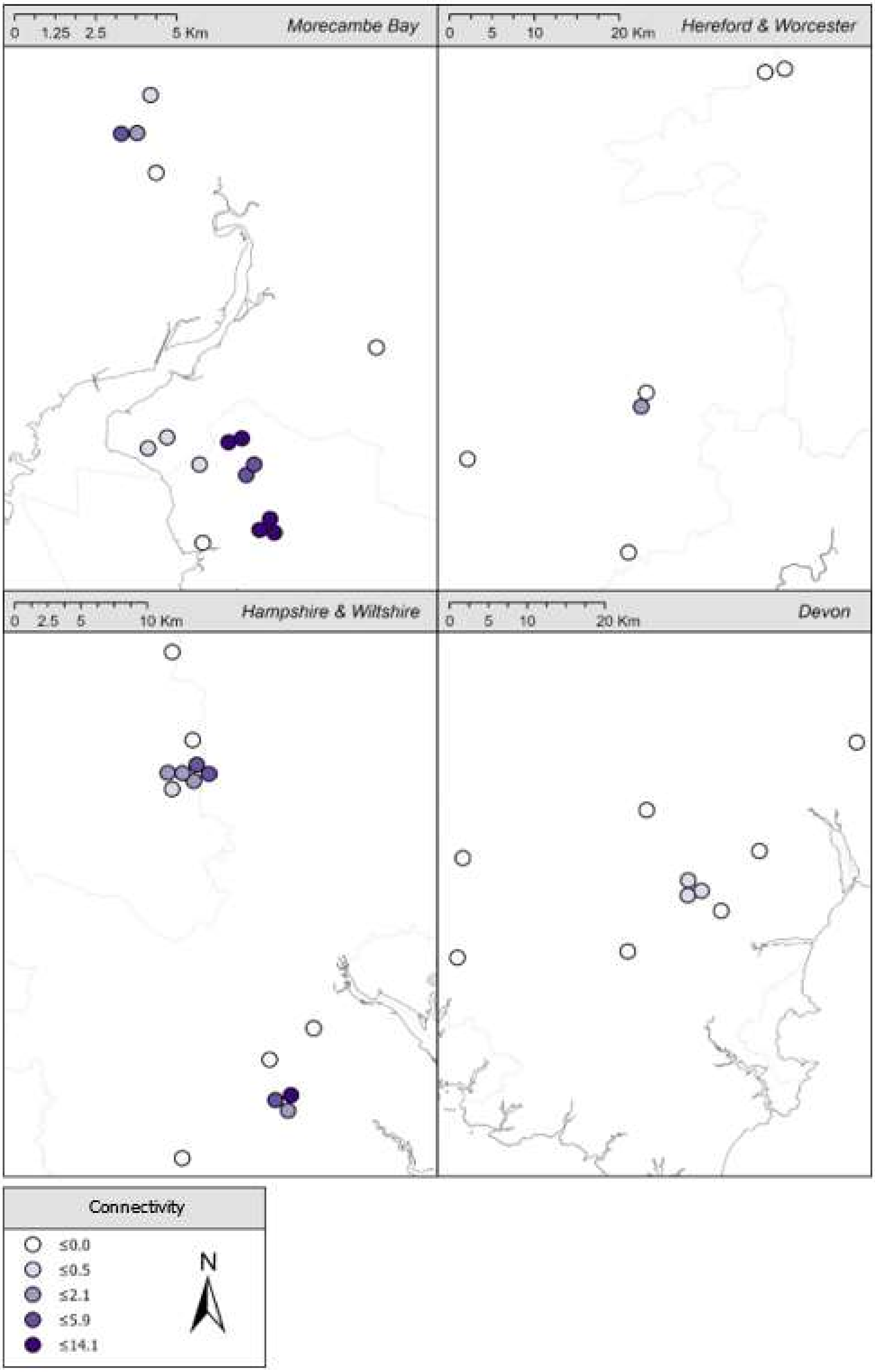
Table S2.1. Moran's I test results for model residuals.

Population Metric	Model	Observed	Expected	SD	P
Status	Adult	-0.015	-0.022	0.082	0.929
	Larvae	-0.015	-0.022	0.082	0.203
Average Abundance	Adult	0.028	-0.022	0.082	0.539
	Larvae	0.060	-0.022	0.082	0.319
Maximum Abundance	Adult	-0.034	-0.022	0.082	0.881
	Larvae	-0.042	-0.022	0.082	0.809

(a)

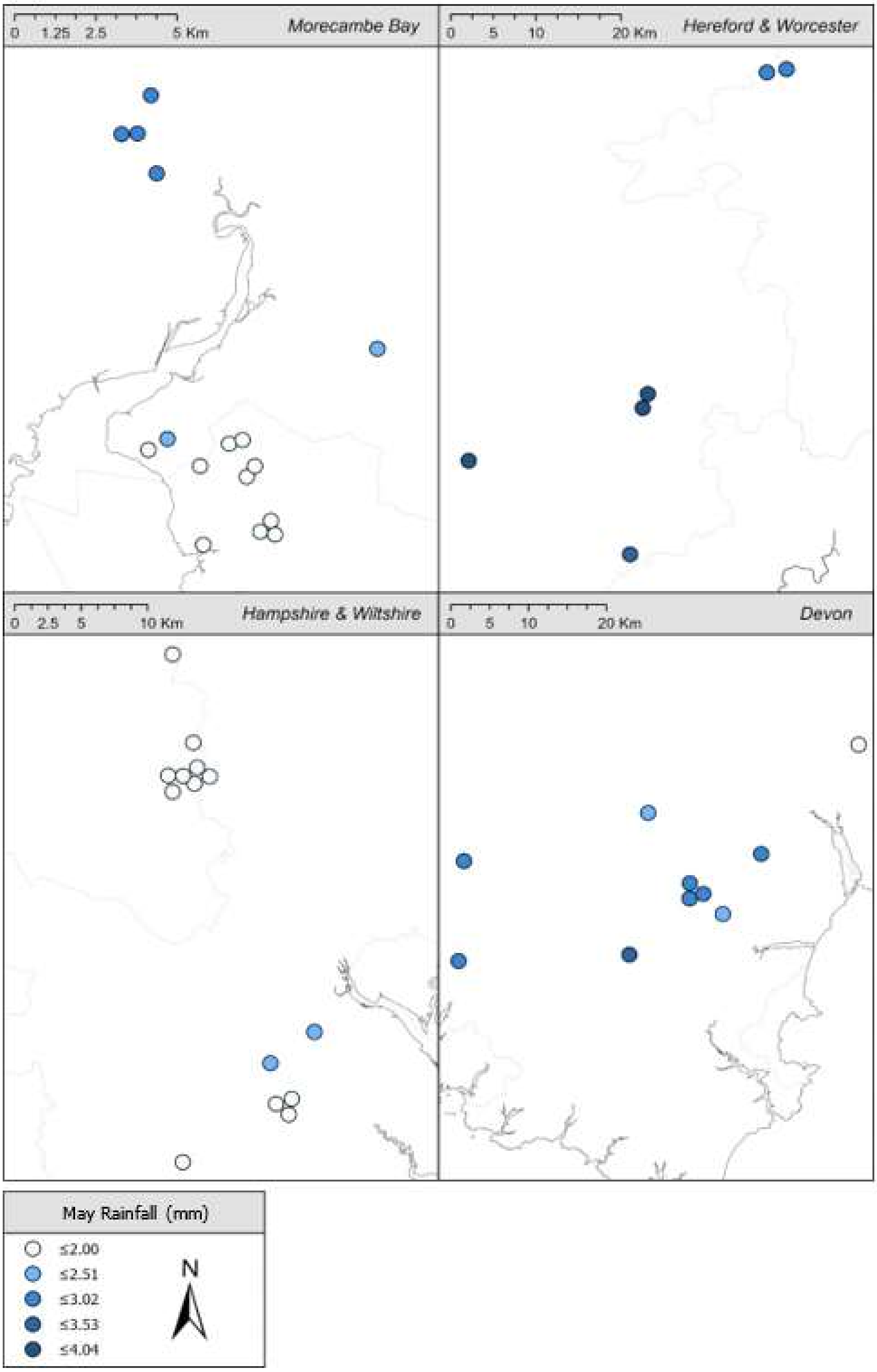

0 1.25 2.5 5 Km
Morecambe Bay
0 5 10 20 Km
Hereford & Worcester
0 2.5 5 10 Km
Hampshire & Wiltshire
0 5 10 20 Km
Devon
Area (m^2)
≤215684.1
≤394258.4
≤572415.1
≤864214.4
≤2089303.9
N

(b)

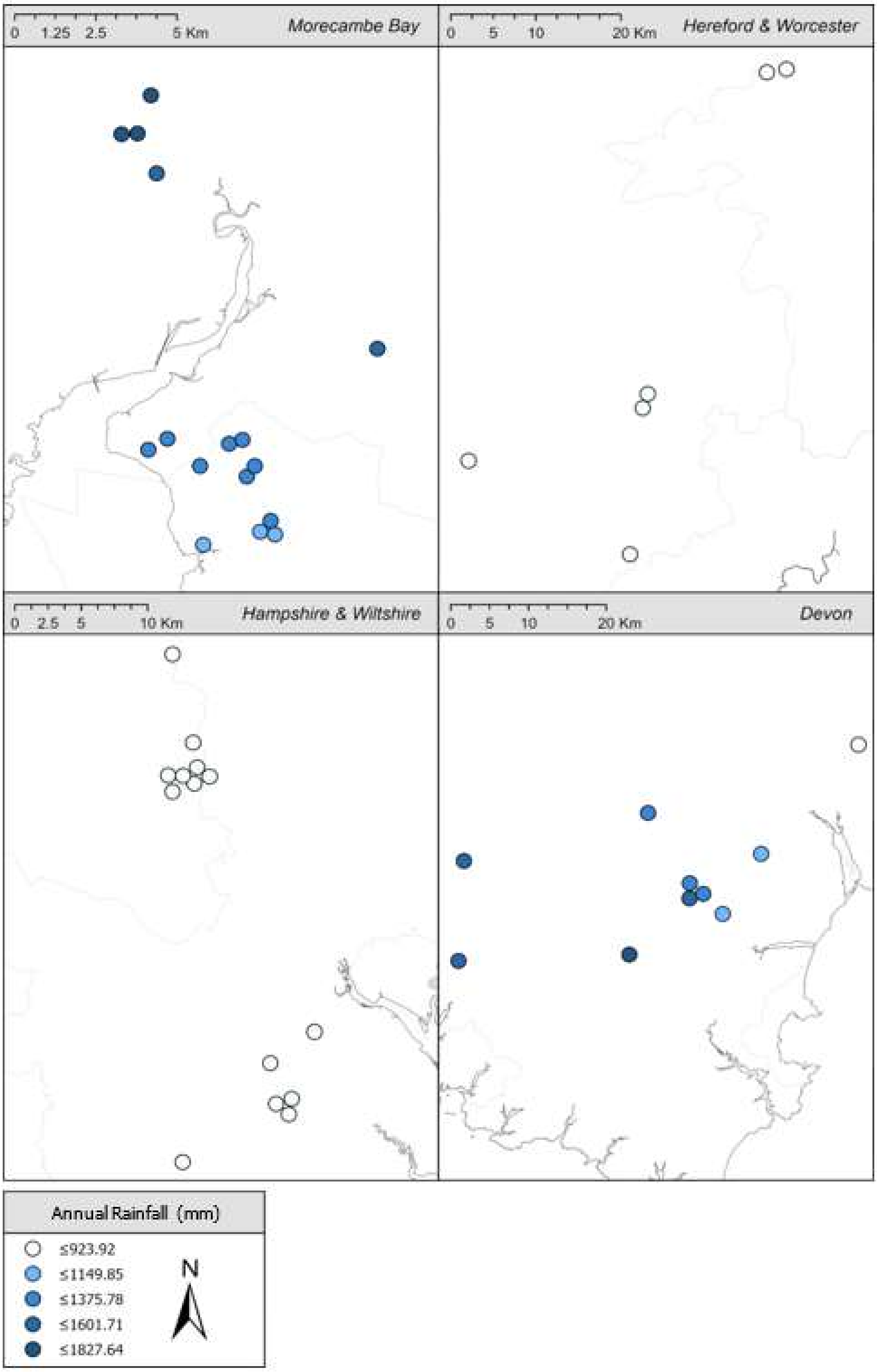


0 1.25 2.5 5 Km
Morecambe Bay
0 5 10 20 Km
Hereford & Worcester
0 2.5 5 10 Km
Hampshire & Wiltshire
0 5 10 20 Km
Devon
Connectivity
≤0.0
≤0.5
≤2.1
≤5.9
≤14.1
N

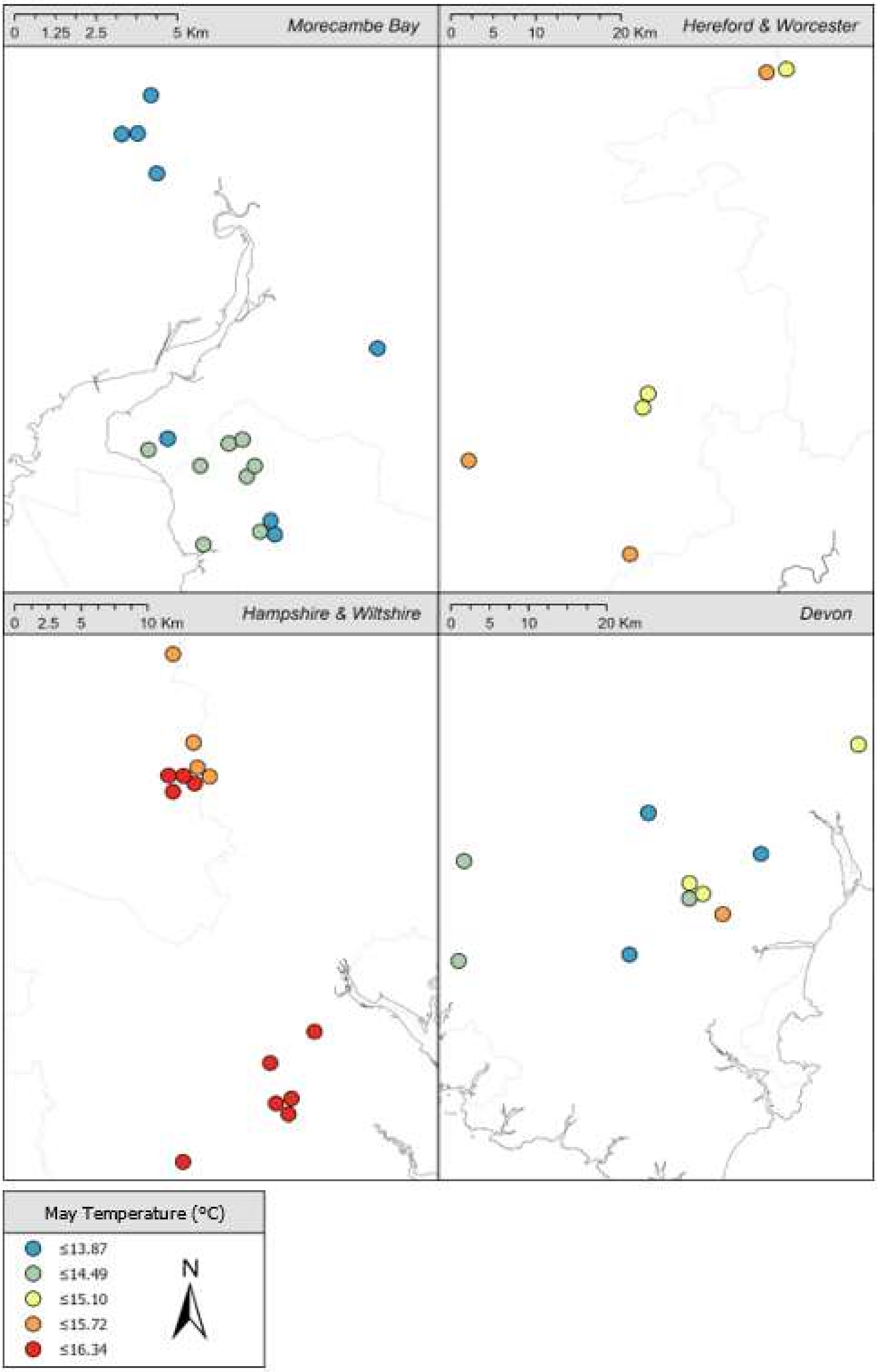
(c)


0 1.25 2.5 5 Km
Morecambe Bay
0 5 10 20 Km
Hereford & Worcester
0 2.5 5 10 Km
Hampshire & Wiltshire
0 5 10 20 Km
Devon
May Rainfall (mm)
○ ≤2.00
● ≤2.51
● ≤3.02
● ≤3.53
● ≤4.04
N

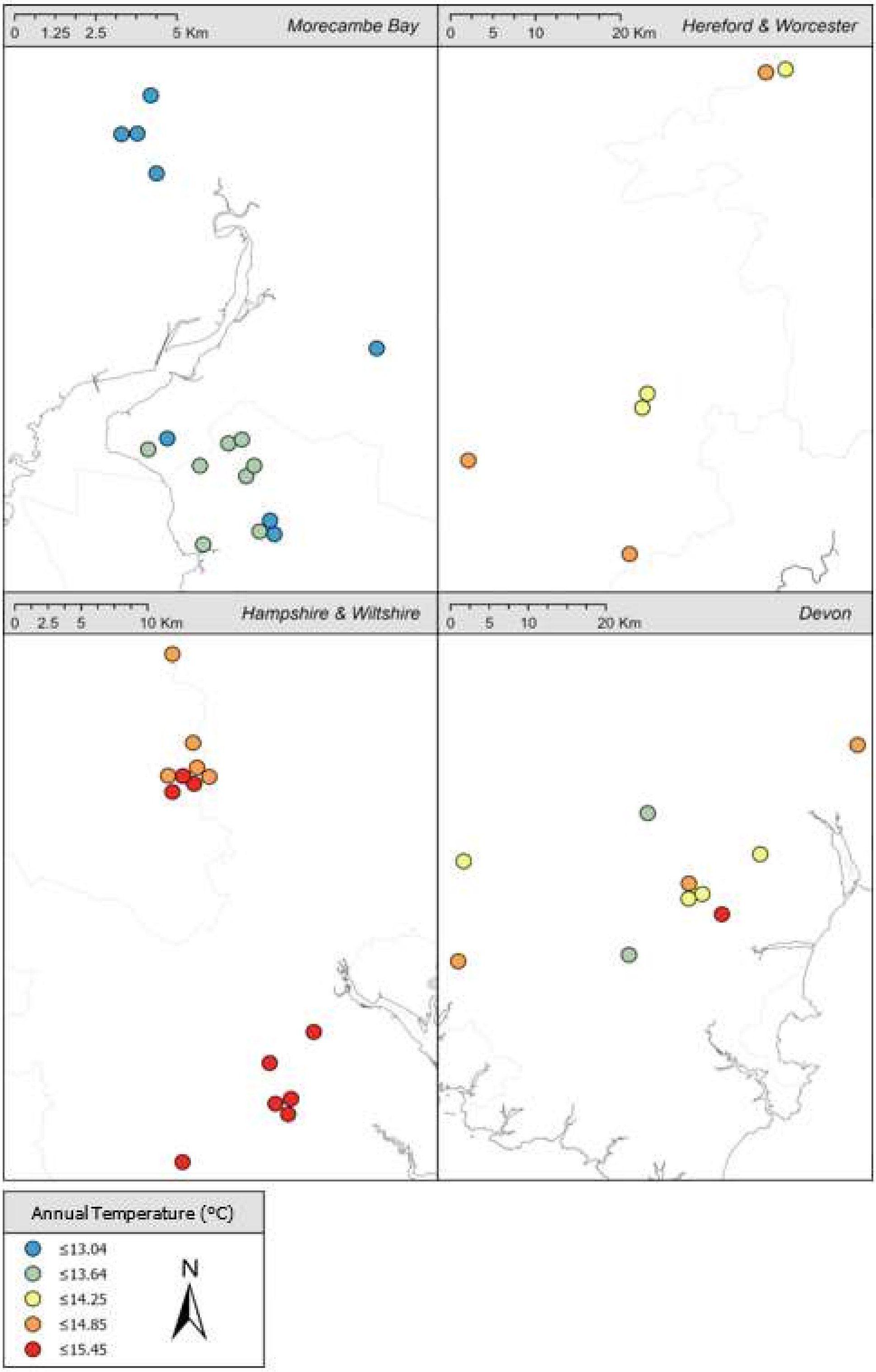
(d)


0 1.25 2.5 5 Km
Morecambe Bay
0 5 10 20 Km
Hereford & Worcester
0 2.5 5 10 Km
Hampshire & Wiltshire
0 5 10 20 Km
Devon
Annual Rainfall (mm)
○ ≤923.92
● ≤1149.85
● ≤1375.78
● ≤1601.71
● ≤1827.64
N

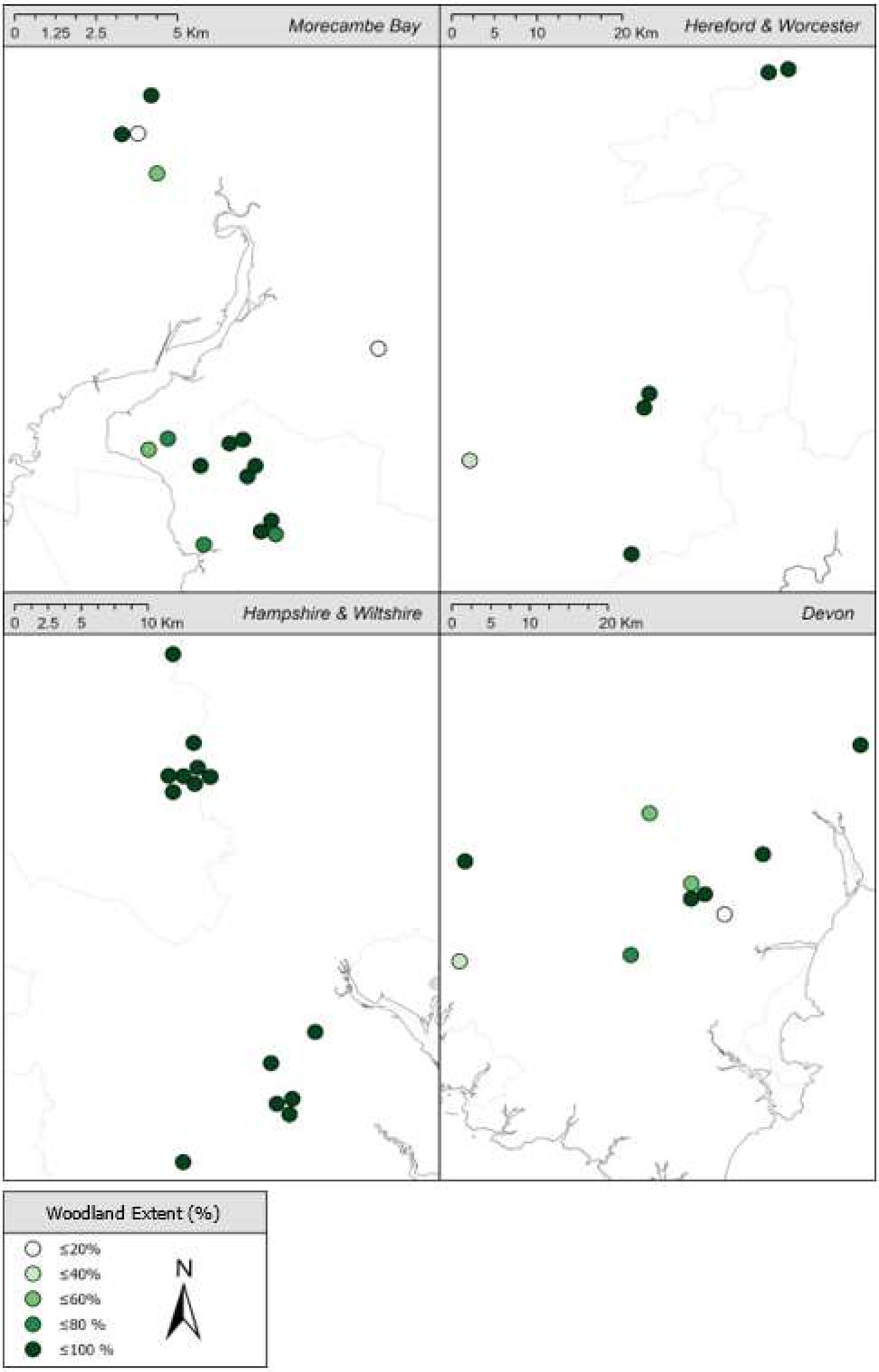
(e)


0 1.25 2.5 5 Km
Morecambe Bay
0 5 10 20 Km
Hereford & Worcester
0 2.5 5 10 Km
Hampshire & Wiltshire
0 5 10 20 Km
Devon
May Temperature (°C)
≤13.87
≤14.49
≤15.10
≤15.72
≤16.34
N

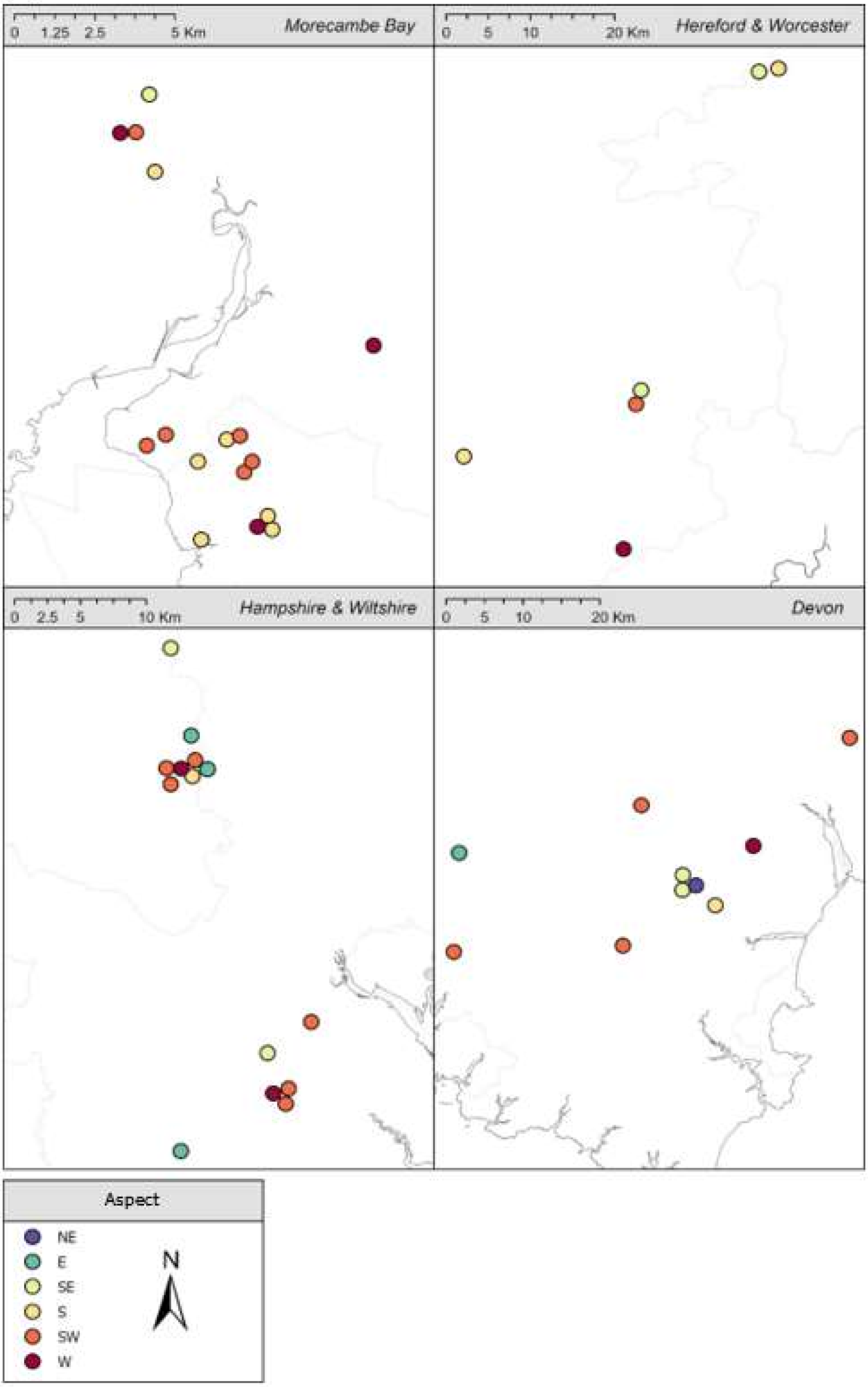
(f)


0 1.25 2.5 5 Km
Morecambe Bay
0 5 10 20 Km
Hereford & Worcester
0 2.5 5 10 Km
Hampshire & Wiltshire
0 5 10 20 Km
Devon
Annual Temperature (°C)
≤13.04
≤13.64
≤14.25
≤14.85
≤15.45
N

(g)


0 1.25 2.5 5 Km
Morecambe Bay
0 5 10 20 Km
Hereford & Worcester
0 2.5 5 10 Km
Hampshire & Wiltshire
0 5 10 20 Km
Devon
Woodland Extent (%)
≤20%
≤40%
≤60%
≤80 %
≤100 %
N

(h)


0 1.25 2.5 5 Km
Morecambe Bay
0 5 10 20 Km
Hereford & Worcester
0 2.5 5 10 Km
Hampshire & Wiltshire
0 5 10 20 Km
Devon
Aspect
NE
E
SE
S
SW
W
N

(i)

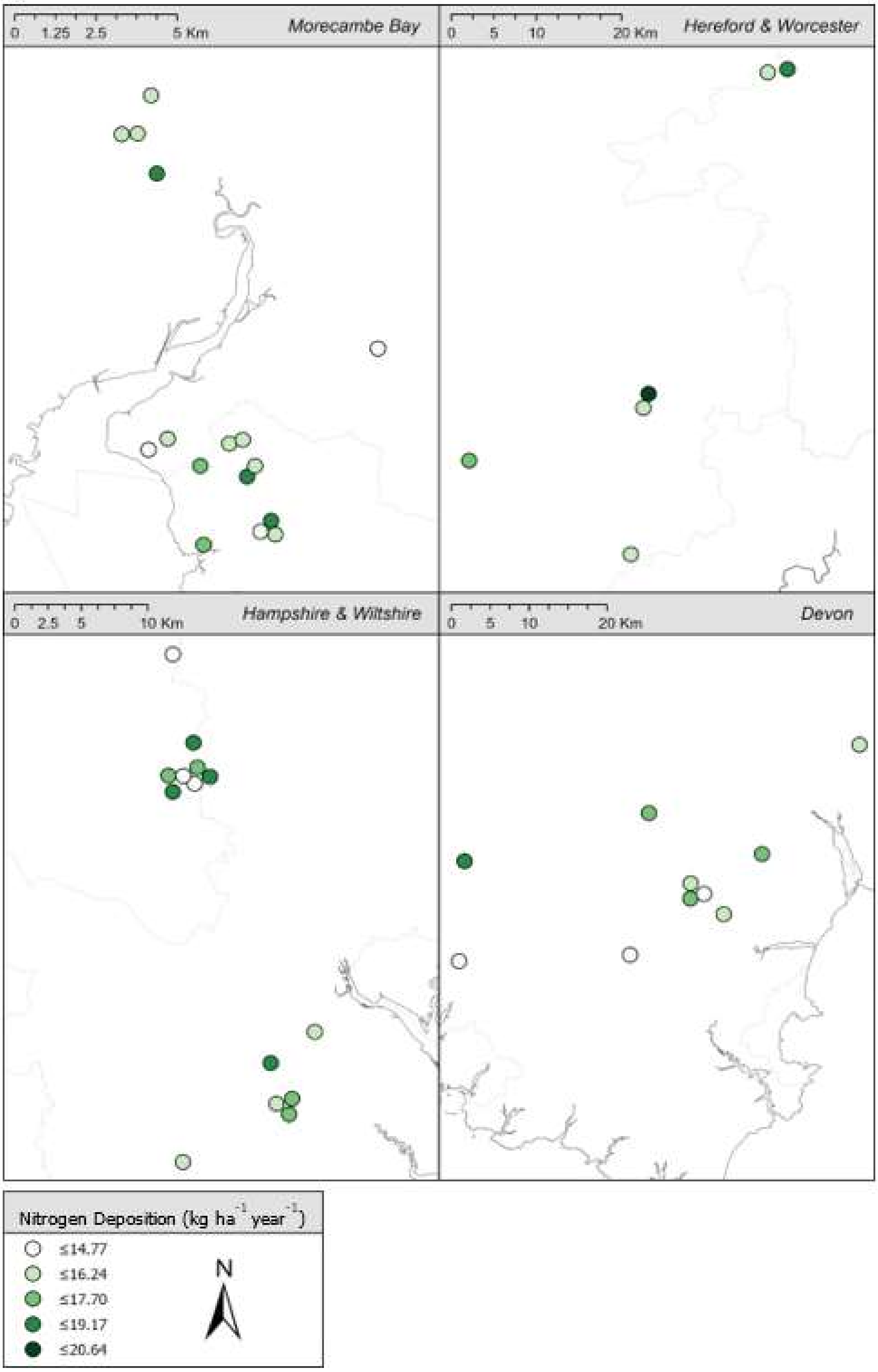


Figure S2.1. Site attributes summarised by geographic region. (a) Site area, (b) connectivity, (c) May rainfall, (d) annual rainfall, (e) May temperature, (f) annual temperature (g) woodland extent, (h) site aspect and (i) deposited Nitrogen. Points show centroids of the 46 study sites in England within four geographic regions and are shaded by attribute.

Table S2.2. Site attributes summarised by geographic region. Sample size, number of extinctions the occurred between 2002-2017 and average site attributes by region.

Region	Sites (n)	Extinctions (n)	Aspect (°)	Nitrogen Deposition (kg ha^{-1} year^{-1})	Woodland (%)	Area (m^2)	Connectivity
Devon	10	1	137.30	15.73	68.20	565738.86	0.08
Hampshire and Wiltshire	14	2	63.36	16.30	97.93	653086.61	1.96
Hereford and Worcester	6	0	121.45	17.20	88.83	572835.63	0.15
Morecambe Bay	16	7	87.83	16.00	74.44	560552.98	4.31

Region	May Rainfall (mm)	Annual Rainfall (mm)	May Temperature (°C)	Annual Temperature (°C)
Devon	2.64	1297.43	14.30	14.13
Hampshire and Wiltshire	1.66	853.21	15.91	14.98
Hereford and Worcester	3.42	782.28	15.20	14.31
Morecambe Bay	2.11	1332.54	13.95	13.07

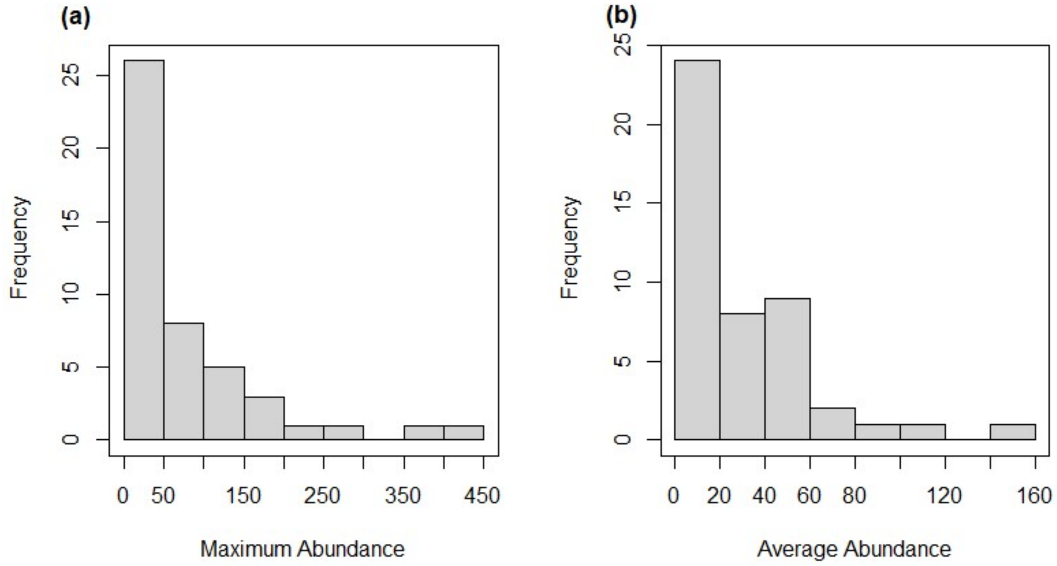


Figure S2.2. Frequency distribution of *Boloria euphrosyne* site abundance. (a) maximum abundance and (b) average abundance of *B. euphrosyne* at 46 sites in England over the 2002-2017 period.

Chapter 3: Population synchrony as an indicator of functional connectivity

3.1 Abstract

Functional connectivity describes the extent to which habitat structure facilitates or impedes movement and is a key influence on species' persistence, particularly in the context of habitat fragmentation and environmental change (Tischendorf and Fahrig, 2000). However, conventional methods for assessing dispersal, such as mark-release-recapture studies, are time consuming and labour intensive. Previously, residual population synchrony has been demonstrated to be an effective proxy for dispersal in mobile butterflies (Powney et al. 2012). Here, the utility and limitations of population synchrony as an indicator of functional connectivity is highlighted, in a specialist, sedentary butterfly, at a range of spatial scales. While at the local scale, population synchrony is likely indicative of dispersal in the pearl-bordered fritillary, *Boloria euphrosyne,* over larger scales, habitat is likely to influence population dynamics. Although declines in local-scale synchrony conformed with typical movement in this species, synchrony showed no significant trend with distance when studied at larger (between-site) scales. By focusing on specific site comparisons, heterogeneity in habitat successional stage is concluded to drive asynchrony between sites at larger distances and is, therefore, likely to be a more important driver of population dynamics over large distances than dispersal. Within-site assessments of synchrony highlight differences in dispersal based on habitat type, with movement shown to be most inhibited between transect sections with contrasting habitat permeability. While synchrony has implications for metapopulation stability and extinction risk, no significant difference was found in average site synchrony between sites where *B. euphrosyne* had become extinct during the study period and those remaining occupied. Population synchrony is demonstrated to be a useful tool to assess local-scale movement between sedentary populations, as well as to understand barriers to dispersal and guide conservation management.

3.2 Introduction

Dispersal is critical for population persistence in fragmented habitats (Hanski, 1999a). Understanding how landscape connectivity facilitates dispersal is crucial for predicting how species will respond to habitat fragmentation and environmental change, as well as for informing the design and management of reserves (Fourcade and Öckinger, 2017). In the face of climate change, landscape connectivity can promote persistence by facilitating range expansions, while local-scale movement between heterogeneous habitat patches allows species to select microclimates that can buffer against extreme events (Saura et al. 2014; Suggitt et al. 2018). Movement of individuals between sub-populations is also key for metapopulation stability as it enables the rescue of dwindling populations and recolonisation of empty habitat patches (Hanski, 1998).

Given the significance of dispersal, it is important to be able to assess functional connectivity (the extent to which habitat structure facilitates or impedes movement of a species through the landscape) (Tischendorf and Fahrig, 2000; Hanski et al. 2004). While landscape structure may be used to infer dispersal, it describes habitat connectivity irrespective of species' use (Powney et al. 2012). Indeed, physical landscape features, such as corridors do not necessarily reflect realised dispersal (Tischendorf and Fahrig, 2000). Given the limitations of structural connectivity for making predictions about realised dispersal, it is often necessary to study species' movements in order to understand functional connectivity. Butterflies provide good model organisms for studying dispersal and environmental change (Stevens et al. 2010). However, conventional methods for assessing dispersal, such as mark-release-recapture studies, are time consuming and labour intensive, meaning that their spatial coverage is often limited. Such studies may also overlook long-distance dispersal events, which, though rare, can have a profound impact on population persistence and genetic structure (Zimmermann et al*.* 2011).

An alternative method to describe functional connectivity is population synchrony, the correlation in annual variation in abundance between separate populations. As population dynamics are more closely synchronised in populations connected by frequent movement of individuals, synchrony is an indicator of dispersal (Oliver et al. 2017). Indeed, population synchrony has been shown to correlate with factors affecting dispersal such as landscape permeability or prevailing weather conditions and to decline with distance between sites (Thomas, 1991; Powney et al. 2012; Vindstad et al. 2019). For example, in the silver-studded blue, *Plebejus argus*, synchrony between local patches declined with distance and broke down beyond 600 m (Thomas, 1991). However, in addition to dispersal, several other factors can synchronise population dynamics. Synchrony has been shown to be higher in populations at the edges of species range margins, as well as being driven by correlation in environmental conditions at geographically close sites (the Moran effect) and trophic interactions (Royama, 1992; Powney et al. 2010; Kahilainen et al. 2018). Natural enemies can have a profound impact on host abundance, for example, the parasitic wasp, *Apanteles bignellii,* is thought to drive strong inter-annual population fluctuations in the marsh fritillary, *Euphydryas aurinia* (Porter, 1983).

The role of microclimate similarities and trophic interactions in driving synchrony is difficult to account for. However, by controlling for the synchronising effects of large-scale, shared climatic conditions on populations, residual synchrony has been used as an effective proxy for butterfly dispersal at a range of spatial scales (Powney et al. 2011; Powney et al. 2012). Furthermore, by comparing population synchrony in different landscape contexts, landscape features that promote or pose barriers to dispersal can be determined. For example, distance along forest edge was shown to be a better predictor of population synchrony in ringlet, *Aphantopus hyperantus,* populations than direct distance between patches, with populations separated by open habitat displaying greater synchrony than populations separated by woodland (Powney et al. 2012). In the speckled wood, *Pararge aegeria,* residual synchrony was also shown to be greater between patches where the intervening matrix was more permeable to dispersal, however, matrix suitability was of greatest importance for dispersal between sites over 20 km apart (Powney et al*.* 2011). Unlike geographically close sites, which are likely to be readily colonisable by this mobile butterfly, regardless of matrix characteristics, further apart sites are likely to be more reliant on matrix features to facilitate successful dispersal.

It has previously been suggested that given the limited dispersal propensity of many butterfly species, dispersal will be insufficient to synchronise their dynamics at large spatial scales, although there is evidence that habitat stepping-stones can synchronise population dynamics over long distances (Pollard and Yates, 1993; Powney et al. 2011). For example, in mobile species such as *P. aegeria,* dispersal is thought to drive synchrony between populations up to 160 km apart (Powney et al. 2011). However, synchrony has been shown to decline more rapidly with distance in sedentary populations than in those that are more mobile (Sutcliffe et al*.* 1996). Nevertheless, population synchrony is likely to be indicative of dispersal operating at smaller scales, such as within sites and this has been demonstrated empirically with mark-release-recapture data (Powney et al. 2012; Oliver et al*.* 2017). For example, in the bog fritillary, *Boloria eunomia*, synchrony was correlated with the frequency of inter-patch movements (Oliver et al*.* 2017). The presence of distance-decay in synchrony at these small spatial scales suggests that population synchrony may be relevant for understanding dispersal in sedentary butterflies, in addition to more mobile species. While previous studies linking residual synchrony to dispersal have often focused on mobile, generalist butterflies, a better understanding of within-site movement would aid conservation management for sedentary butterflies, which are overrepresented in declining species (Powney et al. 2012; Eskildsen et al. 2015; Warren et al. 2021). In addition, population synchrony may be used to highlight the impacts of environmental change on populations. Previously, encroaching forest has been associated with the decoupling of population dynamics, due to reduced dispersal between populations of the Rocky Mountain Apollo butterfly, *Parnassius smintheus* (Roland and Matter, 2007).

Population synchrony also has important implications for extinction risk. Local extinction events can drive synchrony, resulting in positive feedback in extinction risk. This was demonstrated by the experimental removal of *P. smintheus* from two sub-populations, which resulted in a marked increase in synchrony between remaining populations in the network (Matter and Roland 2010). Local extinction results in a decline in immigration across the network. Where this leads to a simultaneous reduction in abundance in remaining populations, it can synchronise their dynamics (Matter and Roland, 2010). Asynchrony at the metapopulation level promotes persistence as it can buffer species against regional extinction in suboptimal years and surviving populations can act as sources for recolonisation (Hanski, 1998; Kahilainen et al*.* 2018). Even though dispersal promotes local-scale persistence, it also contributes to the synchronising of population dynamics. In this way, population synchrony is understood to pose something of a ‘double edged sword’ to extinction risk (Hudson and Cattadori, 1999). As there is likely to be an optimum level of synchrony for long-term metapopulation persistence, a greater understanding of the links between population synchrony and extinction risk could help to predict population vulnerability to extinction, allowing management to be targeted accordingly (Powney et al. 2010).

Here, the use of population synchrony is investigated as an indicator of functional connectivity in a sedentary, specialist butterfly, the pearl-bordered fritillary, *Boloria euphrosyne.* Residual synchrony (synchrony after controlling for climatic factors) was assessed in the Morecambe Bay region, in north-west England, at two spatial scales, within- and between-sites. Within the maximum dispersal distance of the study species (< 4.5 km), it was predicted that population synchrony would be indicative of dispersal and therefore would decline with distance. At larger scales (> 4.5 km), the frequency of dispersal was hypothesised to be insufficient to synchronise population dynamics and instead, habitat similarity was predicted to synchronise dynamics. While *B. euphrosyne* is described as a sedentary butterfly, landscape structure is reported to influence mobility, with open habitat likely to facilitate dispersal (Barnett and Warren, 1995). To investigate this, population synchrony was used to assess the influence of landscape permeability on dispersal in this species. It was predicted that synchrony would be dependent on habitat structure, as such, a stronger distance-decay effect was predicted in habitats with a closed structure relative to those with an open habitat structure. Additionally, the role of site-specific factors in driving population dynamics was investigated. It was hypothesised that extrinsic factors such as vegetation successional stage may drive asynchrony between management units. Finally, the implications of synchrony for extinction risk were investigated. As inter-patch movements are likely to be infrequent, yet important for population persistence, it was hypothesised that populations from sites with higher average synchrony would have a lower risk of extinction.

3.3 Methods

3.3.1 Study Site Selection and the UKBMS

Butterfly abundance data were obtained from the United Kingdom Butterfly Monitoring Scheme (UKBMS), which is a long-standing programme of coordinated monitoring under which butterflies are recorded along fixed route transects (Pollard and Yates, 1993). Residual population synchrony, over the 1978-2016 period was assessed in *B. euphrosyne* populations at 15 UKBMS transect sites in the Morecambe Bay region (Fig. 1; Supporting Information, Table S3.1).

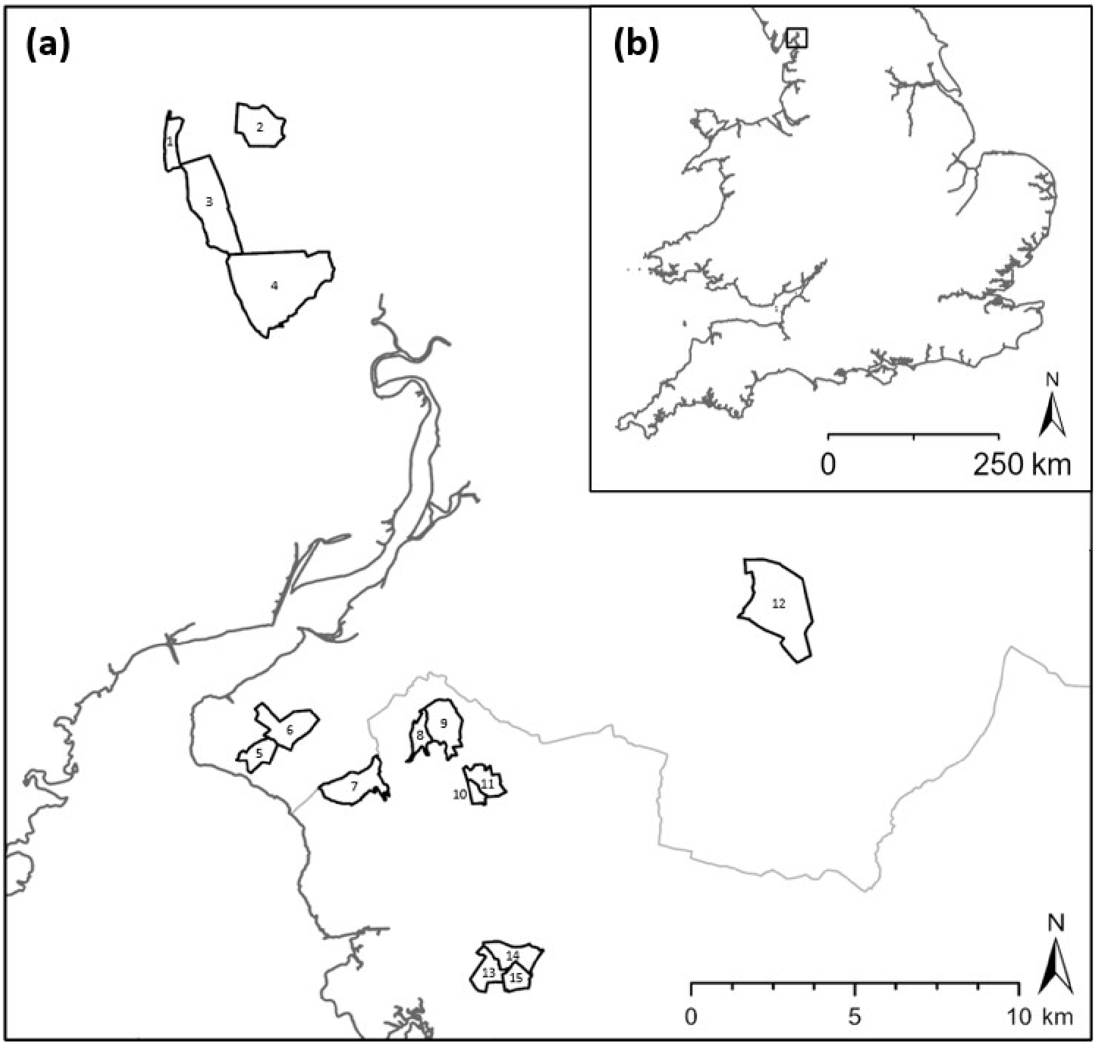


Figure 3.1. Study site locations. The location of the 15 UKBMS sites selected for study, showing (a) local and (b) national context. County boundaries (Lancashire and Cumbria) are shown in grey. See Supporting Information, Table S3.1. for transect site details.

Transect route maps for each site were provided by Butterfly Conservation. Butterfly transects are split into sections usually based on broad habitat type or landscape features (for the study sites these ranged from 7-15 sections per transect, with an average length of approximately 300 m). Analysis was performed at both the site and transect section level. Only site or section pairs that had at least 7 years of common data were included in analysis (Powney et al. 2011). As a chain of zero counts over the time series can inflate synchrony values, years with zero counts that were not bounded by a positive value were also excluded (Powney et al. 2011). Site-level analysis included pairwise comparisons across 15 sites. Section-level synchrony was assessed for 106 transect sections from 13 sites (sites 1 and 6 were excluded from section-level analysis due to transect sections at these sites having fewer than 7 years of data where zero counts were bounded by a positive value).

3.3.3 Population Synchrony and Site Attributes

Butterfly transects are recorded weekly from April 1st until September, but where minimum weather criteria are not met, missing data may be present. UKBMS site indices are calculated using a general additive model, to account for missing data (Botham et al. 2020a). To account for differential recording effort between transects when analysing synchrony at the section-level, section abundance indices, calculated proportionally from the UKBMS index for the whole site were used. Weekly counts from each transect section were summed to calculate the proportional contribution of each section to the site's annual abundance. Where multiple records for a site were submitted in a single week, counts were averaged. UKBMS site indices were multiplied by the section proportional contribution, to produce section-level indices. Where recording effort was insufficient for the UKBMS index to have been calculated, those comparisons were excluded.

Correlation of long-term population trends in a region could inflate synchrony (Paradis et al. 1999). To control for such trends, residuals from a linear model of index and year were extracted and used in both site- and section-level analysis (Powney et al. 2011). The data were also 'Pre-whitened' in order to account for the role of climate in synchronising population dynamics (Powney et al. 2012). This approach involved multiplying the average abundance count over the study period by the national population index for each given year and subtracting this value from the raw annual abundance count for the site or transect section, as summarised in the following equation:

$$d_{it} = c_{it} - m_i I_t$$

d_{it} is the pre-whitened count for site or transect section i in year t. c_{it} is the raw abundance count for site or transect section i in year t. m_i is the mean abundance for site or transect section i over the study period, and I_t is the national population index in year t. The national indices used were the English UKBMS national log collated indices for *B. euphrosyne* (Botham et al. 2019). These national indices were standardised using the following equation:

$$I_t = (n_t - m)+1$$

Where m, the overall mean of national index values for the 1978-2016 period, was subtracted from each annual value, n. Population synchrony was then calculated using the pre-whitened, standardised abundance data by carrying out a Spearman's correlation for all transect site and section pairs. The resulting Spearman's correlation coefficient value gives a measure of the extent to which population dynamics are synchronised.

The study sites were classified by population status and dominant habitat type. Population status was classed as extinct if *B. euphrosyne* was absent from the transect for three consecutive years, up to and including 2018. Habitat type was defined at the site and transect section level as the most frequently occurring habitat type, using the 2015 Land Cover Map (Rowland et al. 2017; Supporting Information Table S3.1). For the analysis, habitat similarity was classified using these land cover categories, with “1” referring to paired transect sites or sections sharing the same broad habitat type and “0” to those with different habitat classifications. Also based on the classifications of broad habitat type, transect sections were classed as having either an “open” or “closed” habitat structure. Sections with a dominant habitat type of grassland or arable, have a high permeability to dispersal and were classed as “open,” while woodland habitats were classed as “closed”. As the analysis involved pairwise comparisons between all transect sections, this classification resulted in three categories of habitat structure, with “open” or “closed” describing pairwise comparisons between sections that shared the same habitat structure, while “mixed” described transect section pairs with contrasting habitat structures (“open” vs “closed”). Distance between transect pairs was calculated in ArcGIS Pro 2.5.0 as the Euclidean distance between site and transect section midpoints (Esri, 2020).

3.3.3 Statistical Analysis

For the site-level analysis, linear models were used to examine the relationship between population synchrony and distance. Dispersal has been reported to synchronise population dynamics over large spatial scales (Powney et al. 2011). However, *B. euphrosyne* is a sedentary species and although it has been reported to move up to 4.5 km between sites, such long-distance dispersal events are rare (Barnett and Warren, 1995). For this reason, separate models were built to assess synchrony between all possible pair-wise site comparisons and for those less than 4.5 km apart. To avoid pseudoreplication, synchrony values relating to only one of each site pair were used in analysis. Habitat similarity was included as an explanatory variable. Average synchrony was calculated at the site level and related to population status (occupied or extinct) using a generalised linear model with binomial errors and a logit link function. Population synchrony was also investigated at the transect section level. A generalized additive model (GAM) was fitted in order to investigate threshold effects in the relationship between synchrony and distance between transect sections less than 4.5 km apart.

In order to investigate drivers of asynchrony between sites at longer distances, synchrony between transect sections was also examined at key sites (sites 2, 8, 9, 14 and 15; Supporting Information Table S3.1). Key sites were those which had over 350 data points. Here, linear mixed effects models were built to incorporate all pairwise comparisons for the selected site. The site of each corresponding transect section was included as a random effect. Linear mixed effects models were also used to assess the influence of habitat and distance on local synchrony, by assessing synchrony within-sites, at the transect section level. Transect site was included as a random effect. In addition to building a model for all within-site pairwise comparisons (n = 515), the effect of habitat structure was investigated. The data were split into section pairs where both sections were "open" (n = 50), both were "closed" (n = 388) or "mixed" (n = 77), where one section was closed and the other open, with separate models built for each category. All analysis was carried out in R Studio Version 4.0.3 (R Core Team, 2020).

3.4 Results

3.4.1 Population Synchrony

The relationship between population synchrony and distance was non-significant for pairwise comparisons at the site level. However, habitat similarity was shown to have a significant effect on population synchrony over these large spatial scales, with greater synchrony between sites sharing the same habitat type (Table 3.1a; Fig. 3.2).

Table 3.1. Model output: the effects of distance and habitat similarity on between-site population synchrony. Parameter estimates, standard errors (SE), t and P-values showing the effects of distance and habitat on between-site population synchrony for (a) all site pairs (n = 77, (b) site pairs less than 4.5 km apart (n = 34). Habitat similarity is classed as "1" for site pairs which share the same category of broad habitat type and as "0" for sites of different habitat types. Significant relationships are highlighted in bold.

Predictor	Coefficient	SE	t	*P*
a) All Sites				
Distance (km)	0.006	0.007	0.859	0.393
Habitat Similarity		0.078	3.110	**0.003**
0	-0.071			
1	0.172			
b) < 4.5 km Apart				
Distance (km)	-0.089	0.046	-1.920	0.064
Habitat Similarity		0.238	0.560	0.579
0	0.248			
1	0.382			

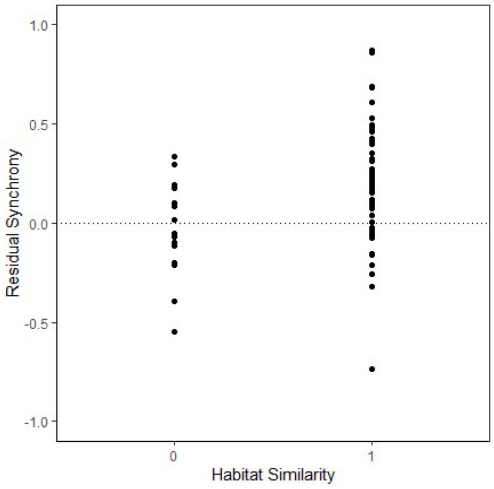


Figure 3.2. The relationship between population synchrony and habitat similarity. Residual population synchrony and habitat similarity for all transect site pairs. Habitat similarity is classed as "1" for site pairs that share the same category of broad habitat type and as "0" for sites of different habitat types. Above the dashed line population dynamics are synchronised, below the line dynamics are asynchronous.

As the reported maximum dispersal distance for *B. euphrosyne* is 4.5 km, population synchrony was also assessed for transect sites within this distance. There was some evidence that synchrony of populations located less than 4.5 km apart may decline with distance, though this was only marginally significant (Table 3.1b; Supporting Information, Fig. S3.2). Site-level synchrony was also not significantly influenced by habitat similarity at this scale (Table 3.1b). Population synchrony was not related to extinction risk, with no significant difference found in average synchrony between populations classed as "extinct" or "occupied" (estimate = 1.71; SE = 3.31; z = 0.52; p-value = 0.61; d.f. = 13).

Butterfly transects are sub-divided into sections, so population synchrony was also assessed at this level. Synchrony between transect sections less than 4.5 km apart, showed a significant trend with distance (GAM: e.d.f. = 8.662; Ref.d.f. = 8.967; F = 26.85; P > 0.001). The trend was generally negative, though some fluctuations in synchrony were present (Fig. 3.3).

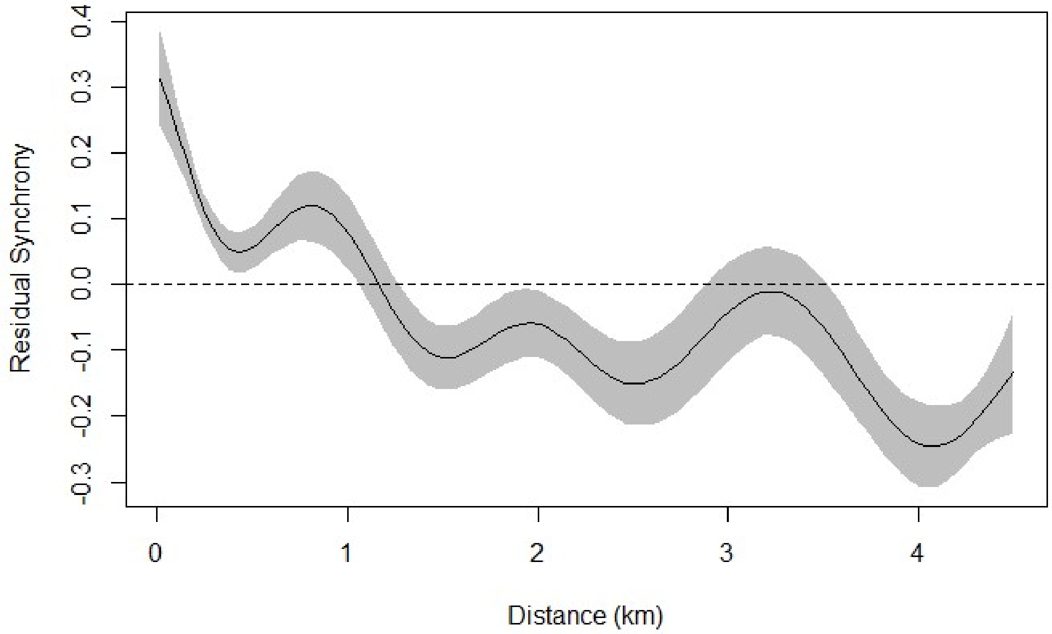


Figure 3.3. The model predicted relationship between residual synchrony and distance between UKBMS transect sections. GAM showing residual population synchrony and distance between transect section pairs less than 4.5 km apart. Above the dashed line population dynamics are synchronised, below the line dynamics are asynchronous. The shaded area shows the 95% CI.

Population synchrony showed a rapid decline with distance between 0 - 425 m, with synchrony falling close to 0 at around 425 m. There was an increase in synchrony between approximately 425 – 900 m, before a further decline. Populations showed fluctuating, asynchronous dynamics at distances above 1200 m, which could be due to site specific factors. To investigate this further, models were built for key sites (those which had over 350 data points). Although comparisons between transect sections were only statistically significant for two of the five key sites analysed (Table 3.2), there was a general decline in synchrony with distance, with synchrony dropping to near 0 (representing uncorrelated dynamics) at around 2 km (Fig. 3.4).

Table 3.2. Model output: the effects of distance on population synchrony at five sites in north-west England. Parameter estimates, standard errors (SE), t values, degrees of freedom (d.f.) and P-values showing the effects of distance on population synchrony (between transect section pairs) with separate models built for each selected site to incorporate all pairwise comparisons between transect sections less than 4.5 km apart (both within the selected site and with sections from other sites). Significant relationships are highlighted in bold.

Predictor	Coefficient	SE	n	t	d.f.	*P*
a) Site 2						
Distance (km)	-0.083	0.044	289	-1.880	163.324	0.062
b) Site 8						
Distance (km)	-0.102	0.037	381	-2.763	13.409	**0.016**
c) Site 9						
Distance (km)	-0.100	0.033	487	-3.057	16.127	**0.007**
d) Site 14						
Distance (km)	-0.072	0.044	336	-1.640	4.583	0.167
e) Site 15						
Distance (km)	-0.035	0.026	308	-1.352	7.508	0.216

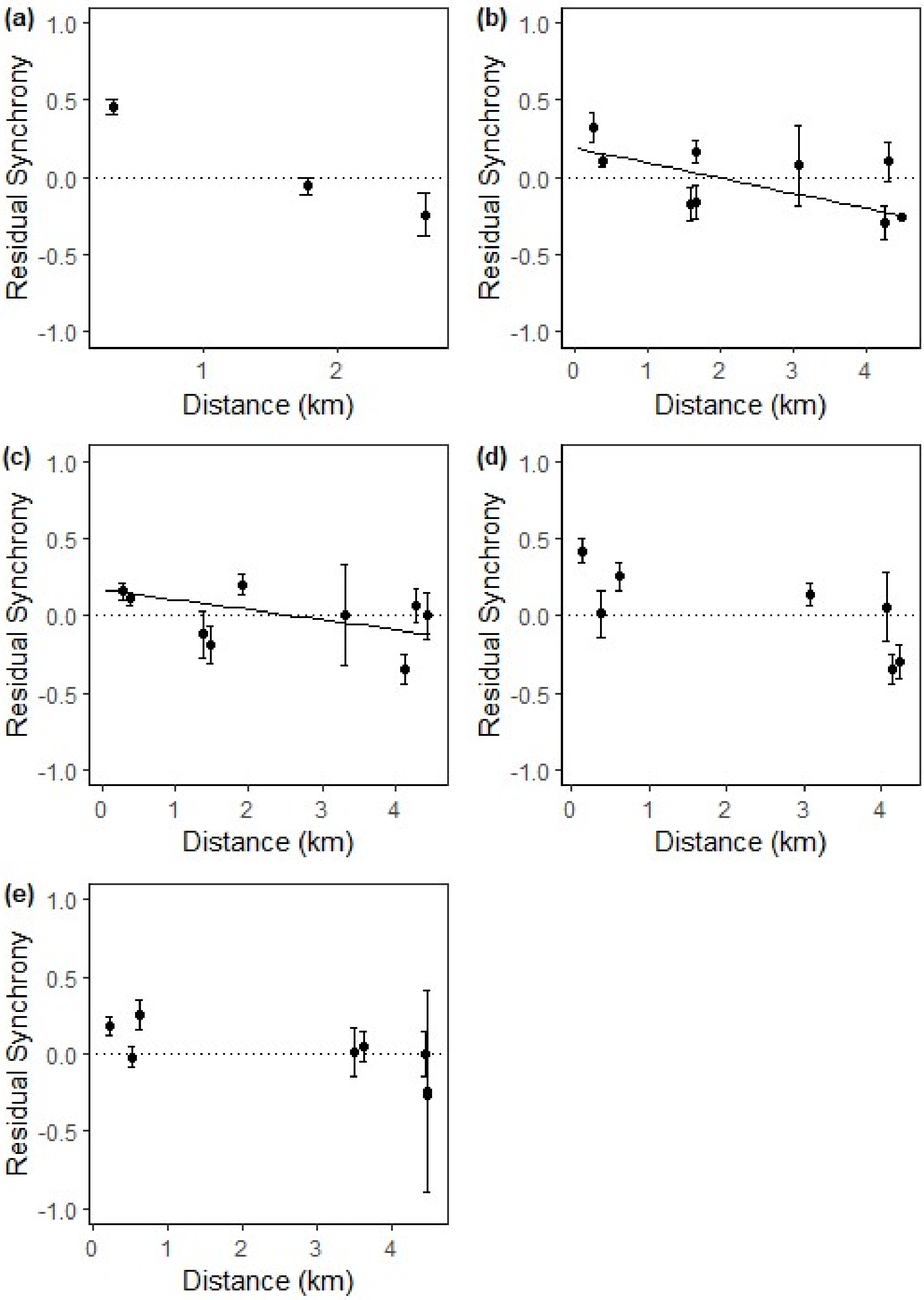


Figure 3.4. Relationships between residual synchrony and distance at five sites in north-west England. The relationship between residual population synchrony and distance for site specific comparisons between transect sections at (a) Site 2, (b) Site 8, (c) Site 9, (d) Site 14, (e) Site 15 and corresponding transect sections up to 4.5 km apart (both within the site and with sections from other sites). Points show average synchrony for each site pair. Error bars show the 95% CI. Above the dashed line population dynamics are synchronised, below the line dynamics are asynchronous. Site numbers as per Fig.3.1

There was also some variation in the degree of synchrony between sites at similar distances, with population dynamics at some sites becoming asynchronous (negatively correlated) while others remained synchronised (Fig. 4).

3.4.2 Local-Scale (within site) Synchrony

In order to investigate local-scale movement, population synchrony was assessed within sites (pairwise comparisons of sections within the same transect). To investigate the effect of habitat structure on population synchrony, the data were split based on the habitat of the pair of transect sections compared (open habitat, closed habitat or mixed habitat) and analysed separately. For all transects, synchrony declined with distance between transect section pairs (Table 3.3a; Supporting Information, Fig. S3.3). However, the relationship between distance and synchrony was dependent on the habitat structure of the pair of sections compared. Synchrony declined significantly with distance for sections with a mixed habitat structure but not for section pairs with similar habitat structure (Table 3.3; Fig. 3.5).

Table 3.3. Model output: the effects of habitat similarity and distance on local-scale population synchrony. Parameter estimates, standard errors (SE), t values, degrees of freedom (d.f.) and P-values showing the significance of habitat similarity and distance on local-scale population synchrony (between pairs of sections within the same transect site) for (a) all section pairs within a transect site (b) section pairs with a closed habitat structure, (c) section pairs with an open habitat structure and (d) section pairs that had different habitat structures. Significant relationships are highlighted in bold.

Predictor	Coefficient	SE	n	t	d.f.	*P*
a) All sections						
Distance (km)	-0.117	0.059	515	-1.956	352.314	**0.047**
b) Closed habitat sections						
Distance (km)	-0.152	0.092	388	-1.662	352.648	0.097
c) Open habitat sections						
Distance (km)	0.019	0.111	50	-0.171	37.701	0.865
d) Mixed habitat sections						
Distance (km)	-0.449	0.144	77	-3.116	70.441	**0.003**

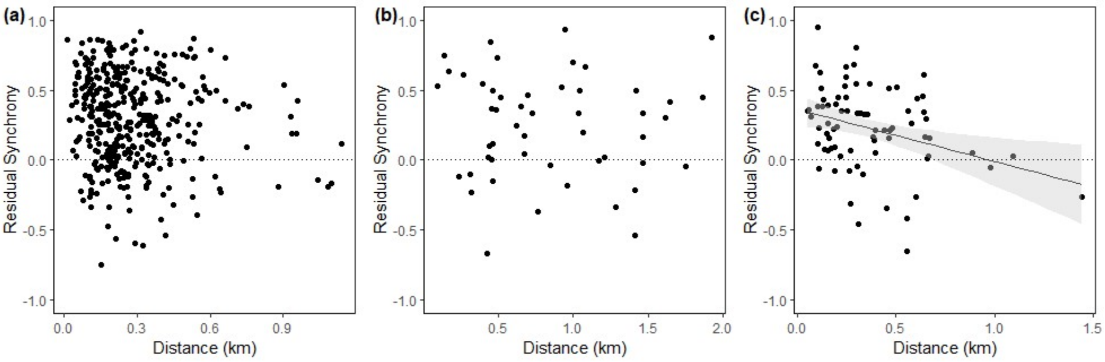


Figure 3.5. Relationships between residual synchrony and distance within-sites, showing habitat differences. The relationship between residual population synchrony and distance for within-site transect section pairs with (a) closed habitat structure (b) open and (c) mixed habitat structure (closed vs open). The shaded area shows 95% CI. Above the dashed line population dynamics are synchronised, below the line dynamics are asynchronous.

3.5 Discussion

Site-level analysis demonstrated that at large spatial scales (between sites) synchrony was not related to distance, while habitat similarity synchronised population dynamics, with sites sharing the same broad habitat type, showing greater synchrony. By contrast, in populations located less than 4.5 km apart, synchrony marginally declined with distance but there was no effect of habitat similarity. When studied at the transect-section level, trends in population synchrony related well with typical dispersal distance in the study species; significant declines in synchrony were present, with the most rapid declines within 1 km. At larger scales, comparisons of key sites revealed that synchrony over longer distances is site specific. Within-site assessments of synchrony highlighted differences in dispersal based on habitat structure, with declines in population synchrony only significant when comparing transect sections that had different habitat structures (closed vs open). Contrary to predictions, population synchrony was not significantly related to extinction risk.

Dispersal is suggested to synchronise *B. euphrosyne* population dynamics at small scales (between transect sections), but not over larger distances (between sites). In the current study, the average distance between sites was 6.6 km. Although dispersal has been reported to synchronise dynamics over large spatial scales in mobile butterfly species, given that *B. euphrosyne* has a maximum dispersal distance of 4.5 km, it is perhaps not surprising that synchrony was not significantly related to distance between sites (Barnett and Warren, 1995;

Powney et al. 2011). Previous studies have also found synchrony to be dependent on spatial scale, with a greater rate of decline in synchrony at the local scale than between sites (Sutcliffe et al. 1996). While there is potential for habitat-driven asynchrony to be averaged out when studied at larger spatial scales, such as between transect sites rather than sections, mobility is also a key determinant on the rate of distance decay in synchrony. As the decline in synchrony with distance, at the transect section-level, relates well with reported movement in *B. euphrosyne,* it is likely attributable to a decline in movement frequency with distance, which would facilitate the decoupling of population dynamics (Barnett and Warren, 1995). The rapid decline in synchrony with distance up to 425 m conforms well with the average movement distance of 315 m observed in a mark-release-recapture study of *B. euphrosyne* and suggests that dispersal is most common over short distances, such as occurs within-sites (Davidson, 2017). Indeed, the average distance between transect sections within the same site in our study system is around 347 m, which supports this interpretation. These results provide good evidence that residual population synchrony is an effective indicator of local-scale movement in sedentary species. However, an increase in synchrony between approximately 425 - 750 m was also observed; at these distances most pairwise comparisons (64%) were of transect sections between- rather than within-site. Between sites, habitat management is likely to be independent, whereas, rotational management within-sites can drive asynchrony if for example, adjacent transect sections are at different stages of ecological succession following management. The slight increase in synchrony at distances where the transition is made from within- to between-site comparisons, could be explained by habitat effects, with habitat-driven asynchrony being a less strong driver of population dynamics between sites than within.

The GAM also highlighted fluctuating, asynchronous dynamics above distances of approximately 1200 m. As dispersal between populations over these larger distances is likely to be very limited, these fluctuations in synchrony are best explained by variation in habitat type and management regime, rather than dispersal. The site-specific models supported this interpretation, with variation in the degree of synchrony between sites at similar distances. Although this species is reported to move as far as 4.5 km, these movements are likely rare, and habitat effects may offset any synchronising effects that dispersal would have on population dynamics at this scale. As a specialist of early successional habitat, *B. euphrosyne* populations are strongly influenced by vegetation successional stage. Abundance in woodland colonies is reported to peak around two years following coppice management, as succession progresses, breeding success declines as the habitat becomes unsuitable (Thomas et al. 1991; Barnett and Warren, 1995). Therefore, where sites are managed over different timescales (for example, an uncoordinated program of scrub clearance or coppicing) variation in successional stage may drive asynchrony between populations occupying different management units. This has been observed in other early successional butterfly species such as the heath fritillary, *Melitaea athalia*, as well as *P. argus* where neighbouring colonies occupying patches of heathland at different successional stages, were shown to fluctuate asynchronously (Warren, 1987; Thomas et al. 1991). Indeed, given the role of habitat in driving variation in population dynamics, it is worth noting that the boundaries of UKBMS transect sections are generally defined according to habitat type. As such, even where transect sections share the same broad habitat type (e.g. open versus closed), within-site variation may mean that sections within transects are more different than sections compared between transects.

Habitat similarity was shown to have a synchronising effect on *B. euphrosyne* populations over large spatial scales, this result is consistent with previous research in other butterfly species (Powney et al. 2010). In the study region, in addition to breeding in early successional woodland and bracken habitats, such as in the south of England, *B. euphrosyne* also occupies well-drained grassland, scree and scrub habitats (Barnett and Warren, 1995). Limestone grasslands in this region are reported to regenerate slowly and thus may provide suitable breeding habitat for an extended period (Barnett and Warren, 1995). Slow regeneration of habitat may be associated with greater stability in population abundance, which is likely to promote synchrony in population dynamics (Sutcliffe et al. 1996). Habitat-dependent carrying capacities or responses to environmental perturbations are also potential mechanisms that could drive stronger synchrony in populations occupying sites with the same habitat type (Powney et al. 2010; Powney et al. 2011). Although similarity in broad habitat type between transect sites was demonstrated to have a synchronising effect on population dynamics at large spatial scales, no significant effect of habitat similarity was found when comparing sites less than 4.5 km apart. Again, at these smaller distances, differences in timing of habitat management, which are likely to generate heterogeneity in the successional stage of vegetation, may have decoupled population dynamics between sites sharing the same broad habitat type. Conversely, when synchrony is examined at larger scales, the timing of management is likely to be independent and therefore management driven heterogeneity may be averaged out (Sutcliffe et al. 1996).

Population synchrony was also used to investigate variation in *B. euphrosyne* dispersal, based on habitat structure. Habitat structure was predicted to influence synchrony, with stronger distance decay predicted in closed habitats (e.g. woodland), than in those with a more open structure (e.g. grassland). This prediction was based on previous research on other butterfly species, showing that populations occupying grassland-dominated transects were characterised by more synchronised dynamics than those in woodland transects (Powney et al. 2012). This prediction was also based on the current understanding of the mobility of the species. *B. euphrosyne* is described as highly sedentary in woodland habitats, where it forms discrete colonies with limited dispersal (Barnett and Warren, 1995). By contrast, in open habitats, where there are fewer barriers to dispersal, the butterfly is thought to be more mobile and to form metapopulations. Indeed, the results here show population synchrony in open habitats to be independent of distance, while synchrony showed a tendency to decline with distance in closed habitats. One explanation is that in open habitats individuals are able to move freely between transect sections over the distances studied, resulting in population dynamics being synchronised equally with distance, whereas woodland habitats pose greater barriers to dispersal. The rapid decline in synchrony with distance, observed between transect section pairs with contrasting habitat structures (mixed), may indicate that individuals are unwilling to move between habitats. Alternatively, asynchrony between transect section pairs with a mixed habitat structure could be climate mediated. Indeed, there is good evidence that population responses to interannual climatic variation are dependent on habitat structure (Sutcliffe et al. 1997; Weiss et al. 1988; Suggitt et al. 2018). Studying functional measures of distance and the permeability of matrix habitat, in addition to habitat patch characteristics and Euclidean distance, may allow for better understanding of the landscape features that promote or inhibit dispersal. However, these results do suggest that *B. euphrosyne* populations occupying different habitats are likely to be effectively isolated and variation in habitat structure, even over relatively short distances, may pose a barrier to dispersal.

No relationship between average site synchrony and population status was found. Although it was predicted that population persistence would be associated with higher average site synchrony, asynchrony also is important for persistence at the metapopulation level, as it reduces the likelihood of simultaneous extinction and can facilitate re-colonisation (Hanski, 1998). Many of the *B. euphrosyne* populations studied are small (over the study period 60% of annual site indices were ≤ 50). Asynchrony between populations may be particularly important for species with low abundance, as these are likely to be more vulnerable to extinctions associated with local stochasticity than those with higher abundance. Furthermore, landscape connectivity may facilitate the dispersal of parasites and natural enemies between populations. Potentially, any beneficial effects of dispersal on population extinction risk could be offset by these factors. Pre-whitening of the data allowed the synchronising effects of shared climate to be controlled for, but the potential role of biotic interactions, such as the movement of natural enemies between populations, are more difficult to account for (Oliver et al. 2017). Although synchrony was not found to relate to extinction risk here, given the role of habitat in synchronising population dynamics, landscape changes such as biotic homogenisation may increase population synchrony and therefore future vulnerability to extinction (Powney et al. 2010; Pandit et al. 2016). Studying temporal change in population synchrony, particularly following habitat fragmentation or the extinction of one or more patches in a network, could further our understanding of the impacts of environmental change on dispersal and population persistence.

The sensitivity of population synchrony to local-scale and likely, infrequent, dispersal in the current study shows that synchrony has utility for assessing functional connectivity in sedentary species. While population synchrony is interpreted as the result of local dispersal in *B. euphrosyne*, beyond management units, population dynamics are likely to be influenced by vegetation successional stage, with asynchrony between units driven by timing of the management regime. Although this study shows no clear implications of synchrony on population persistence at the site level, these results have important implications for landscape design and management. Asynchrony is key for regional metapopulation persistence and habitat management may be used as a tool to maintain asynchrony between populations, decreasing the risk of simultaneous extinction. The strong distance decay in synchrony between transect sections with contrasting habitat structures also observed suggests that, even over short distances, populations occupying patches with different habitat types can be functionally isolated. These results highlight the need to consider habitat structure during management planning so as to ensure that rotational management produces habitat that is readily colonisable.

3.6 Supporting Information

Table S3.1. Study site details. Site details of the 15 UKBMS butterfly transects used in synchrony analyses. Classifications of habitat type were made based on the dominant and secondary habitats present at the site level using the 2015 Land Cover Map (Rowland et al. 2017). Site 10 (Leighton Moss) is predominantly reed bed and as *B. euphrosyne* was only regularly recorded on two of the transect sections, habitat type refers to these sections only.

Site Number	Transect Name	Grid Reference	Number of Sections	Transect Length (m)	Dominant Habitat Type	Secondary Habitat Type
1	Whitbarrow - Howe Ridding NNR	SD434881	15	1410	Broadleaved Woodland	NA
2	Whitbarrow North	SD44X88X	15	2870	Broadleaved Woodland	Acid Grassland
3	Whitbarrow NNR - Hervey CWT	SD441869	11	4000	Acid Grassland	Broadleaved Woodland
4	Whitbarrow NNR - Farrer's Allotment	SD452855	15	4100	Acid Grassland	Broadleaved Woodland
5	Heathwaite NT	SD448768	7	3500	Broadleaved Woodland	Calcareous Grassland
6	Arnside Knott NT	SD453773	12	4700	Broadleaved Woodland	NA
7	Eaves Wood NT	SD466762	11	4500	Broadleaved Woodland	NA
8	Gait Barrows	SD477771	9	1400	Broadleaved Woodland	NA
9	Gait Barrows NNR (Warden's)	SD481773	15	1900	Broadleaved Woodland	Arable
10	Leighton Moss	SD487760	15	5922	Broadleaved Woodland	Improved Grassland
11	Yealand Hall Allotment	SD489762	14	4000	Broadleaved Woodland	NA
12	Holme Park Fell	SD542794	10	3315	Calcareous Grassland	Calcareous Grassland
13	Warton Crag RSPB	SD489727	7	2800	Broadleaved Woodland	NA
14	Warton Crag LWT	SD493730	15	5400	Broadleaved Woodland	NA
15	Warton Crag LNR	SD494726	15	3181	Broadleaved Woodland	Calcareous Grassland

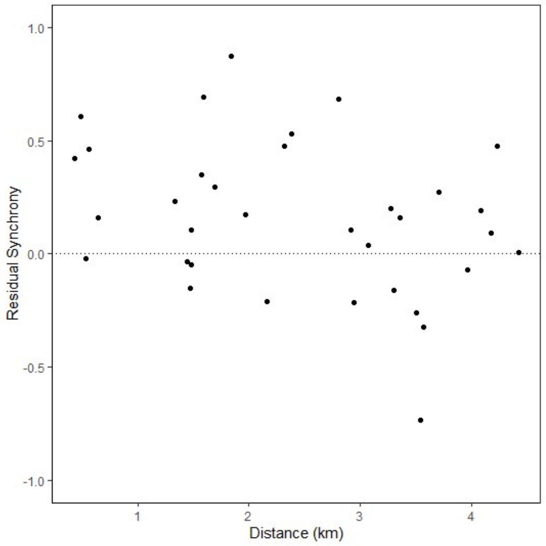


Figure S3.1. Between-site residual population synchrony and distance. Residual population synchrony and distance. Site-level pairwise comparisons were made between 15 UKBMS transects, for site pairs less than 4.5 km apart. The shaded area shows the 95% CI. Above the dashed line population dynamics are synchronised, below the line dynamics are asynchronous.

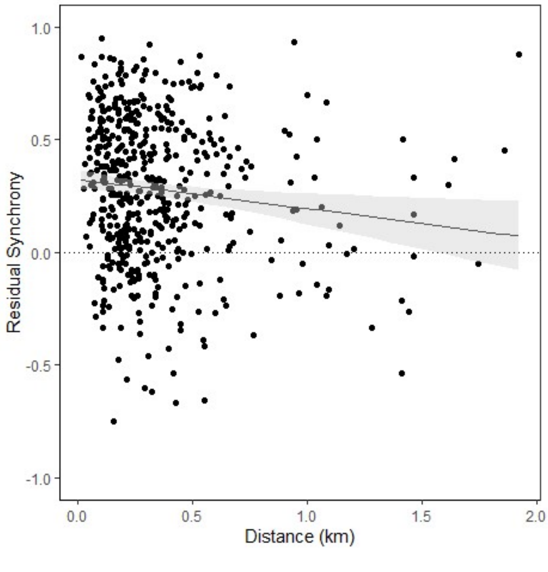


Figure S3.2. The relationship between within-site residual population synchrony and distance. The relationship between residual population synchrony and distance for all within-site comparisons. Above the dashed line population dynamics are synchronised, below the line dynamics are asynchronous. The shaded area shows 95% CI.

Chapter 4: Factors influencing dispersal propensity in *Boloria euphrosyne*

4.1 Abstract

As dispersal plays a key role in population persistence, an understanding of species mobility is important for conservation. However, dispersal is difficult to quantify and is often condition dependent, being influenced by traits intrinsic to an individual, such as sex or morphology, in addition to external factors including landscape structure. To assess the population structure and factors influencing mobility in a threatened, habitat-specialist butterfly, *Boloria euphrosyne,* a mark-release-recapture (MRR) was carried out at Whitbarrow, a key site for the species in north-west England. Butterfly morphology is linked to flight capacity, but the relationship with dispersal propensity is often more complex. Here, the effects of flight related morphological traits on individual dispersal propensity and displacement distance were investigated in *B. euphrosyne*. Wing aspect ratio and wing loading were found to be significantly related with displacement distance, but only for those individuals that were recaptured once. Variation in morphological traits, based on the number of times individuals were recaptured, may indicate sampling bias, but also suggests intraspecific variation in flight capacity and behaviour in this species. There were, however, no observable differences in morphology between individuals making inter-patch movements and those recaptured in the same patch. The MRR showed that most movements in *B. euphrosyne* were less than 100 m, although some individuals moved considerably further. The study sites appear to support healthy populations, with a widespread distribution. The observed transfer of one individual between adjacent study areas suggests that between-site movement is infrequent, but it is likely important for genetic flow and population persistence in the network.

4.2 Introduction

Dispersal is a key factor underpinning persistence in spatially structured populations (Hanski, 1998; Fourcade and Öckinger, 2017). Dispersal facilitates genetic flow and the colonisation of new habitat patches, in addition to preventing extinction by 'population rescue' (Brown and Kodric-Brown, 1977; Saccheri et al. 1998; Nowicki et al. 2014). Drivers of environmental change including habitat fragmentation and climate change are likely to alter selection pressures on dispersal capacity (Hill et al. 1999d), and an understanding of species' mobility can be essential for successful conservation action. However, there is considerable variation in spatial population structure, flight capacity and individual dispersal propensity in butterflies (Stevens et al. 2010). Dispersal propensity is often context dependent, being influenced by a range of factors including population density, landscape structure, habitat quality and weather conditions (Nowicki and Vrabec, 2011; Sivakoff et al. 2016; Kuussaari et al. 2016). In addition, dispersal can be influenced by intrinsic factors including sex, individual condition and morphology (Breuker et al. 2007).

In insects, a range of morphological traits have been used as proxies for dispersal ability (Sekar, 2012). The energetic costs of flight are influenced by morphology, this explains why variation in morphological traits can be indicative of individual differences in flight ability (Vincze et al. 2019). As the thorax contains the flight muscles, thorax volume is often positively correlated with flight speed and displacement distance in butterflies (Dudley and Srygley, 1994; Turlure et al. 2010a). Investment in energy efficient flight and flight speed have been highlighted as contrasting flight optimisation strategies (Lindström and Alerstam, 1992; Vincze et al. 2019). While there is evidence that flight speed and manoeuvrability are important for individuals moving through the matrix to colonise new patches, slower, energy efficient flight can be necessary for long distance dispersal (DeVries et al. 2010). High wing loading (the ratio of body volume to wing area) is associated with larger thoraxes and high acceleration capacity. Conversely, low wing loading (reflecting a small body size and large wing area) is associated with limited acceleration but energy efficient and sustained flight (Davis and Holden, 2015; Sivakoff et al. 2016). A further factor influencing flight energetics and manoeuvrability is the shape of the forewing (described by the wing aspect ratio). Narrow, elongated wings have a higher wing aspect ratio than short, broad wings and are associated with energy efficient, gliding-type flight, such as observed in long-distance migration (Johnson et al. 2014; Cespedes et al. 2015). By contrast, butterflies with low wing aspect ratios are suited for rapid, powered flight, which may be adaptive for individuals crossing inhospitable matrix habitat in order to colonise new habitat patches (DeVries et al. 2010). Indeed, populations occupying recently established colonies tend to show a high frequency of morphological traits associated with colonisation capacity (Hill et al. 1999b). For example, individuals from recently colonised populations have been shown to have lower wing aspect ratios than butterflies from long-standing speckled wood, *Pararge aegeria*, colonies (Hill et al. 1999b).

A primary source of intraspecific variation in dispersal behaviour relates to sexual dimorphism. There are demonstratable trade-offs between flight and fecundity, which lead to sex-bias in flight behaviour and dispersal propensity (Hill et al. 1999b). Often males are more mobile than females, as males play an active role in mate location (Breuker et al. 2007). Distinct morphological traits based on mate location strategies have been identified in butterflies. For example, *P. aegeria,* shows intraspecific variation in mate location strategy, with males characterised by either 'perching' or 'patrolling' behaviours (Shreeve, 1987). Males that show perching behaviours tend to have larger thoraxes, higher wing loading and higher wing aspect ratios relative to males that actively search for females by patrolling (Wickman, 1992). These differing morphological traits are thought to reflect the need for perching males to perform short bouts of flight and to facilitate rapid take-offs in pursuit of females, in contrast to sustained flight involved in patrolling.

While there are well established links between morphology and flight performance, the relationship between morphology and dispersal propensity is often more complex, likely due to the range of factors, both intrinsic and extrinsic to an individual, that influence realised dispersal (Reim et al. 2018). Functional connectivity describes the extent to which landscape structure facilitates or impedes dispersal (Tischendorf and Fahrig, 2000). In addition, patch characteristics, such as resource availability, influence dispersal propensity in butterflies. Given the condition dependent nature of dispersal, altered selection pressure on dispersal is likely in changing environments. Indeed, previous studies have highlighted interspecific variation in dispersal behaviour, both with reduced dispersal (Merckx et al. 2003; Schtickzelle et al. 2006) and elevated dispersal (Hanski, 2012) in fragmented landscapes. One reason for variation in species' responses is that different life history strategies influence the relative costs and benefits of dispersal in a fragmented landscape (Hanski, 2012). There is good evidence that in small populations with high turnover rates, fragmentation provides strong selection pressure for increased flight capacity and dispersal (Duplouy et al. 2013). Habitat quality and population density are also likely to be important influences on dispersal. There is evidence particularly for elevated emigration of females at high densities in order to avoid conspecific harassment. Female dispersal is also adaptive as colonisation of new patches reduces kin competition and spreads the risk when ovipositing (Pellegroms et al. 2009; Niitepõld et al. 2011; Lakovic et al. 2015). Conversely, low population densities can also trigger increased emigration, due to limited mate location or because low densities indicate poor habitat quality (Kuussaari et al. 1998). Butterflies disperse more readily from patches with reduced habitat quality, while displacement distance is also lower in habitats where resources are abundant (Turlure et al. 2010a; Sivakoff et al. 2016). The ability to track resources in the environment is particularly important for habitat specialists, which are often reliant on transient, early successional habitats.

This study aimed to assess the influence of morphology on dispersal in the pearl-bordered fritillary, *Boloria euphrosyne,* a threatened, habitat specialist butterfly in the UK. The relationship between flight-related morphological traits (wing loading and wing aspect ratio) and the distance moved by individual butterflies was assessed in a mark-release-recapture field study. Individuals showing greater displacement distances and making inter-patch movements were predicted to have higher wing aspect ratios (associated with long distance dispersal and energy efficient flight; Cespedes et al. 2015) and lower wing loading (associated with limited acceleration, but sustained flight; Davis and Holden, 2015). Due to the differential selection pressures on males and females, morphology and dispersal behaviour were also expected to differ by sex. Males were predicted to have higher wing aspect ratios relative to females, as is generally the case in species where males use patrolling flight in mate-location (Cespedes et al. 2015). Adaptations for rapid flight were hypothesised to be more important in females, as colonising new habitat patches can be important in a risk-spreading oviposition strategy (Lakovic et al. 2015). Additionally, population size and structure at the study site was assessed to provide an indication of the current population status and highlight potential issues with the long-term persistence of this threatened species.

4.3 Methods

4.3.1 Study system

Boloria euphrosyne is a sedentary, habitat-specialist butterfly. In the UK, the species is highly threatened; with historical national declines in abundance and distribution strongly linked to loss of suitable habitat, arising from the abandonment of traditional woodland management practices, such as coppicing, from the mid-nineteenth century onwards (Greatorex-Davies et al. 1992). As a result, *B. euphrosyne* has become increasingly restricted to open areas of well-drained grassland and bracken dominated habitats (Barnett and Warren, 1995). The species is reported to show intraspecific variation in dispersal, with adults in woodland colonies characterised by sedentary behaviour, while in open habitats the butterfly is thought to be more dispersive and to form metapopulations (Thomas, 1984; Barnett and Warren, 1995). The

Morecambe Bay Limestones in north-west England are notable, both as a hotspot for lepidopteran diversity generally, and as a stronghold for *B. euphrosyne* in England (Ellis et al. 2012). Nevertheless, despite targeted management efforts, *B. euphrosyne* population declines over the last decade in the Morecambe Bay Region have continued (Botham et al. 2020a).

The study was carried out at Whitbarrow Scar, in south Cumbria, a north-south ridge of Carboniferous Limestone, with an elevation of 215 m (Natural England, 1990). Whitbarrow is an extensive (1156.8 ha) and diverse area of nationally important semi-natural habitat, which comprises a network of separately owned and managed sections and is designated as both a National Nature Reserve (NNR) and a Site of Special Scientific Interest (SSSI) (Fig. 4.1).

In addition to supporting 49 nationally scarce species of invertebrate, Whitbarrow is a core site network for violet feeding fritillary butterflies in the Morecambe Bay area (Natural England, 1990; Ellis and Wainwright, 2008). The site comprises mosaics of scrub and well-drained limestone grassland on thin soils, dominated by blue moor grass, *Sesleria caerulea,* with limestone outcrops and scree, in addition to sections of predominantly broadleaved woodland at the lower elevations and to the east of the site (Ellis and Wainwright, 2008; Fig. 4.2).

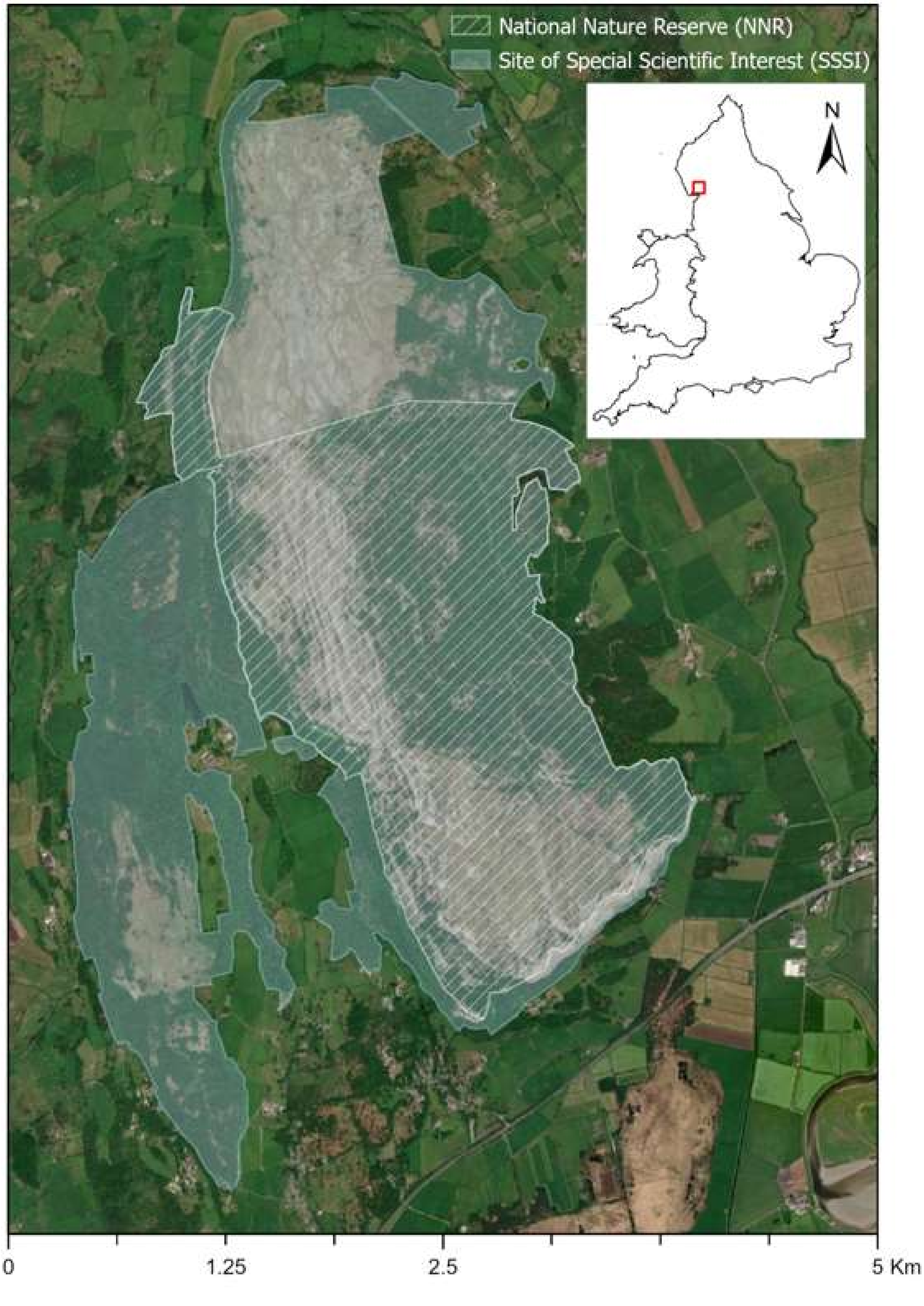


Figure 4.1. Map of the Whitbarrow site network. The insert shows site location in the UK and the Aerial photograph shows National Nature Reserve (NNR) and Site of Special Scientific Interest (SSSI) boundaries. *Arial imagery source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN and the GIS User Community.*

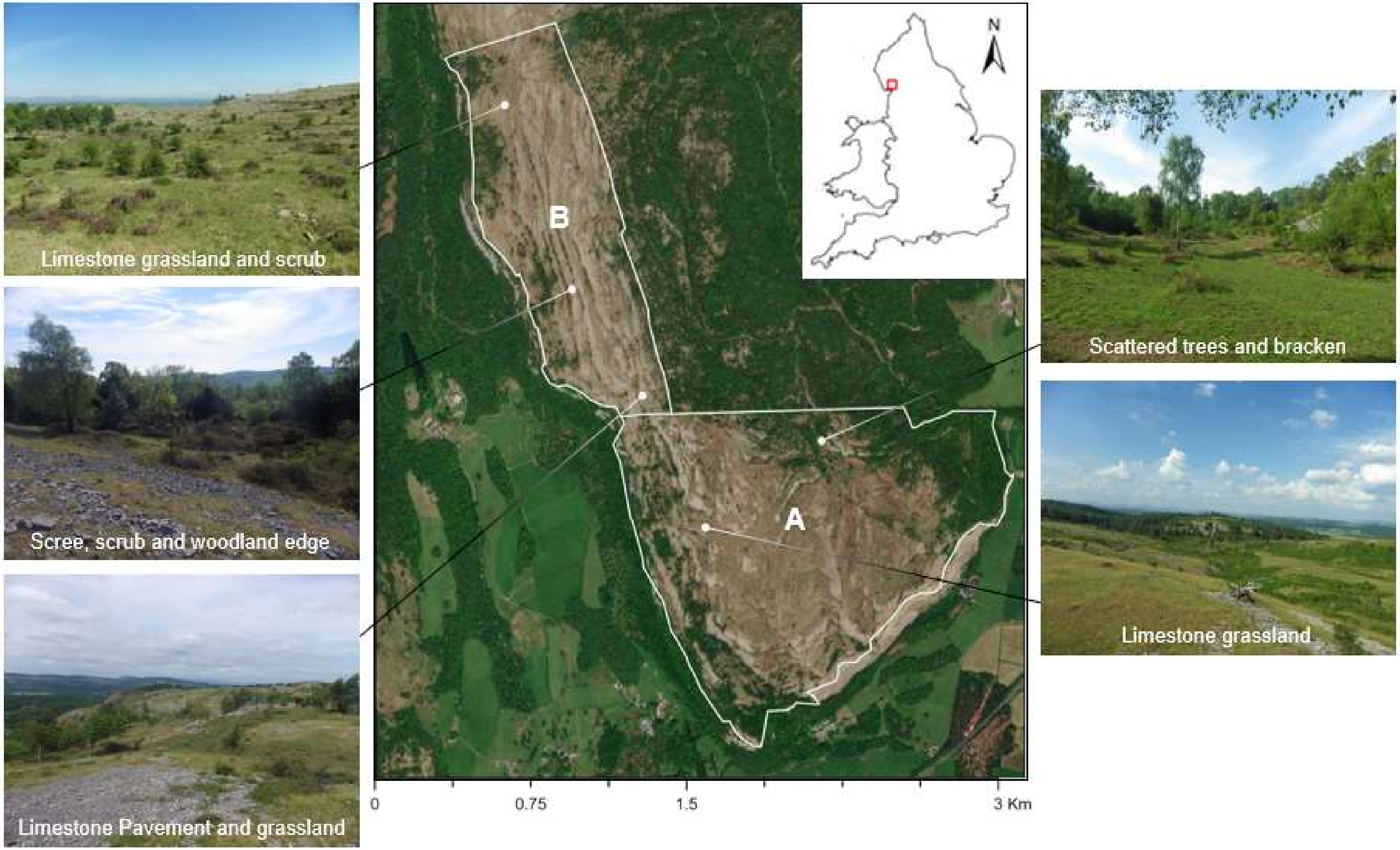


Figure 4.2. Boundaries and habitat characteristics of study areas within the Whitbarrow network. (A) Farrer's Allotment, (B) Hervey. *Arial imagery source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN and the GIS User Community.*

4.3.2 Mark-release-recapture

A mark-release-recapture (MRR) was carried out in 2019, across two areas of the Whitbarrow network: Farrer's Allotment and Hervey (Fig. 4.2). Study sites were visited over 20 days between 30th April and 28th May, when weather conditions were suitable. The mark-release-recapture was carried out with assistance from two volunteers who were involved with marking the butterflies and others who submitted occasional sightings of marked individuals. Recording effort was kept consistent across the flight period. The route, based on selected sections of UKBMS transects and additional areas of suitable habitat, was walked at a steady pace, with the aim of covering the whole site in a day. Where this was not possible, due to poor weather, areas that had not been covered the previous day were given priority the subsequent day. The route was walked starting at different locations each day to ensure equal coverage and variation in the time of day that patches were visited. On initial capture, butterflies were sexed by examining the abdomen and then marked on one wing with a permanent marker giving each individual a unique ID number (Fig. 4.3).

Figure 4.3. Marking code used in the mark-release-recapture. The location of marked points corresponds to the numbers shown (a) these are summed to give each individual a unique number. (b) An example of a marked butterfly, the individual marked is number 19.

A total of 195 individuals were marked between 30th April and 16th May. Due to the permit restriction issued by Natural England, after this number was reached, no further individuals were marked. When unmarked individuals were encountered, their location was recorded (Fig. S4.1) and they were immediately released unmarked. The recapture location of all marked individuals was recorded but repeat recaptures of an individual on the same day were excluded from analysis. The date and location of captures and recaptures were recorded using a GPS to obtain a 12-figure British grid reference (accurate to within 3-4 m).

4.3.3 Morphological measurements

On recapture, individuals were photographed for subsequent morphometric analysis (n = 66). Butterflies were caught using a net and placed into a cool box for a minute to calm them to allow photography and reduce handling time. Individuals were immobilised with the wings open in polythene envelopes and photographed from the dorsal side, using a Pentax X-5 digital camera (Fig.4.4).

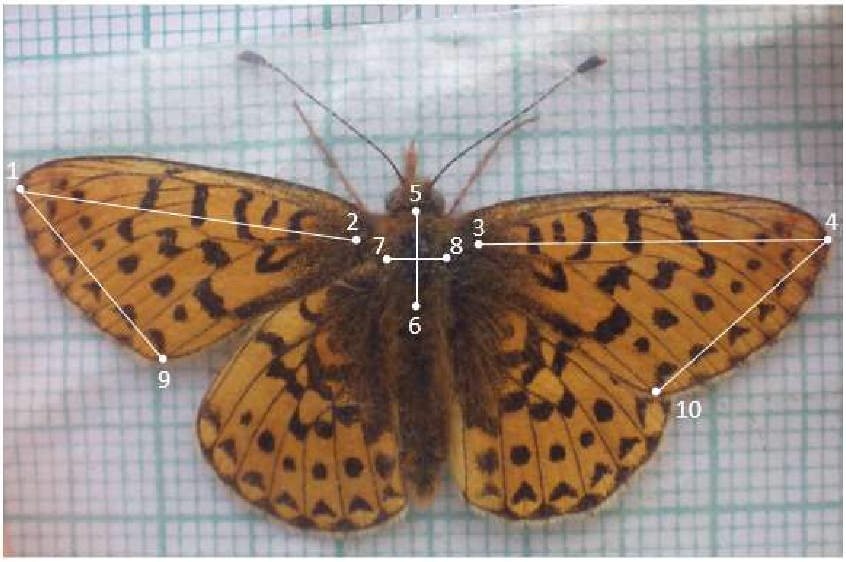


Figure 4.4. Photography for morphometric analysis of *Boloria euphrosyne*. An individual photographed using the polythene envelope method, with locations of landmarks used in morphometric analysis

A small number of photographs were discarded where the quality was not sufficient for reliable measurements to be taken. In addition to photographs taken at Farrer's Allotment (n = 26) and Hervey (n = 35), photographs taken by a MSc student a nearby section of Whitbarrow during the same flight period, using the same methodology were used (n = 5; Blaydes, 2019; Fig. S4.2). Flight-related morphological traits were measured from the photographs using landmarks digitised in tpsDig232 (Rohlf, 2017; Fig. 4.4). Thorax length (TL) was measured at the midpoint, starting below the eyes for consistency (Fig. 4.4, landmarks 5-6). Thorax width (TW) was measured at the widest point (Fig. 4.4, landmarks 7-8). Wing length (WL) was measured from the base to the end of the R4 vein (Turlure et al. 2016; Fig. 4.4, landmarks 2-1 and 3-4). Wing width (WW) was measured from the end of the R4 vein to the end of the 1A + 2A vein (Turlure et al. 2016; Fig. 4.4, landmarks 1-9 and 4-10). Measurements of the length and width of the thorax and the left and right forewings were calculated using the R package '*geomorph'* (Adams et al. 2021). Where possible, the average forewing width and length was calculated for each individual and used in analysis. Where wing condition precluded accurate measurements being taken from both wings (n = 10) measurements from a single wing were used. The thorax volume (TV), wing loading and wing aspect ratio were calculated using the following equations based on Johnson et al. (2014) and Turlure et al. (2016).

$$\mathrm{TV} = \frac{4}{3}\pi \times \left(\frac{\mathrm{TW}}{2}\right)^2 \times \frac{\mathrm{TL}}{2}$$

$$\text{Wing loading} = \frac{\mathrm{TV}}{\mathrm{WL}}$$

$$\text{Wing aspect ratio} = \frac{\mathrm{WL}}{\mathrm{WW}}$$

4.3.4 Statistical analysis

Population estimates for each site were calculated using the Rcapture package in R (Rivest and Baillargeon, 2019). Loglinear models for open populations were used to estimate population size, based on the frequency of capture of individuals marked during the MRR. Separate models were built with the probability of recapture set to either 'equal' or 'unconstrained' probability, these were compared and the model with the lowest Akaike's Information Criterion (AIC) was selected for each site. In addition, an alternative estimate of population size was calculated using the following equation (Blaydes, 2019):

$$\text{Population size} = \frac{\text{Total marked x Total captured}}{\text{Number of marked recaptures}}$$

Here, total captured refers to both marked and unmarked captures in addition to all recapture events. The number of marked recaptures relates to the total number of recapture events, rather than the number of individuals recaptured. While this method of population estimate is likely to be less robust than the Loglinear model, which accounts for dispersal and mortality, it does consider the large number of individuals (n = 428) encountered that were not marked due to permit limits. As such, this alternative measure of population size should give an assessment based on abundance across the whole flight period, while the estimate from the Loglinear model will be of population size during the period of marking only.

Boloria euphrosyne distribution, based on capture and recapture points of marked individuals, was mapped in ArcGIS Pro 2.5.0 (Esri, 2020). An aggregation of individuals with at least three captures and one recapture, separated from other recapture points by at least 100 m, were defined as a patch. This delimitation was made based on those used in previous studies (Davidson, 2017) and considering the most frequent recorded movements of < 100 m observed in this study. Butterflies were classed as 'dispersers' or 'non-dispersers.' Non-dispersers were defined as individuals recaptured in the original patch in which they were marked, while dispersers were those recaptured elsewhere, either in a different patch, or in the matrix. Euclidean distance between capture and recapture locations for each recaptured individual were used to calculate the average distance moved between capture points (Fig. 4.5a) and the maximum displacement distance (longest single distance between capture points; Fig. 4.5b). For individuals that were recaptured only once, displacement distance was simply the Euclidean distance between the point of initial capture and subsequent recapture. Multiple recaptures made on the same day were excluded from analysis. Additionally, to control for the frequency of recapture, a further distance metric was used, referring to the distance between initial capture point and the location of the first recapture (Fig. 4.5, C to R1).

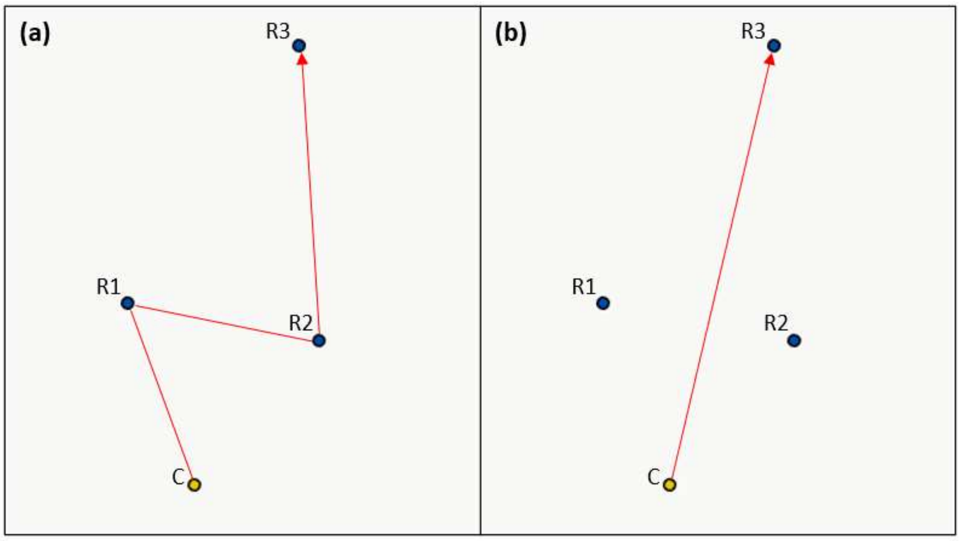


Figure 4.5. Methods used to calculate distance metrics used in the current study. (a) the distance between capture and all subsequent recapture locations was averaged to calculate the mean distance moved (b) maximum displacement distance was measured as the longest single distance travelled by an individual. The initial capture point (C) is shown in yellow, and recaptures (R) are shown in blue, with numbers corresponding to the order of subsequent recaptures.

As morphological measurements were found to be highly correlated, only wing aspect ratio and wing loading were used to assess the relationship between morphology and mobility (Supporting Information, Fig. S4.3). Forewing morphology is an important component of butterfly flight performance. Wing aspect ratio describes the shape of the forewing and is an important influence on flight energetics. Wing loading refers to the size of the thorax relative to forewing area; thoracic mass is also important for flight speed. As both traits are ratios derived from direct measurements, they are independent of overall body size, eliminating bias due to individual size differences. The influence of morphology on distance metrics were modelled using a linear model with normal errors. Distance measurements were log transformed to improve model fit. Differences in morphological traits between dispersers and non-dispersers were assessed using a generalized linear model with binomial errors and a logit link function. Quasi-Poisson models were used to assess morphological variation based on the number of times an individual was recaptured during the mark-release-recapture study as well as to assess the influence of recapture frequency on displacement distance between the initial capture point and first recapture for all individuals. The quasi-Poisson model type was used to account for under-dispersion in the data. These models highlighted both morphological variation and variation in displacement distance in individuals relating to the frequency of capture. To account for this, an additional model was built to assess the influence of individual morphology on displacement distance in individuals that were marked and subsequently recaptured only once during a mark-release-recapture study. Sex was also included in all models to assess sexual dimorphism. Interactions between morphological traits and sex were also assessed. Model selection was carried out using a backwards stepwise procedure, using chi-square likelihood ratio tests (binomial errors) or F-tests (normal errors) to assess the significance of terms. Terms where $P < 0.05$ were considered significant and retained in the model (Crawley, 2007). All statistical analysis was carried out in R Studio Version 4.0.3 (R Core Team, 2020).

4.4 Results

4.4.1 Population size, structure, and mobility at Whitbarrow

A total of 112 (n = 34 female; 78 male) individuals were initially marked at Hervey and 83 (n = 20 female; 63 male) at Farrer’s Allotment (Fig. 4.6).

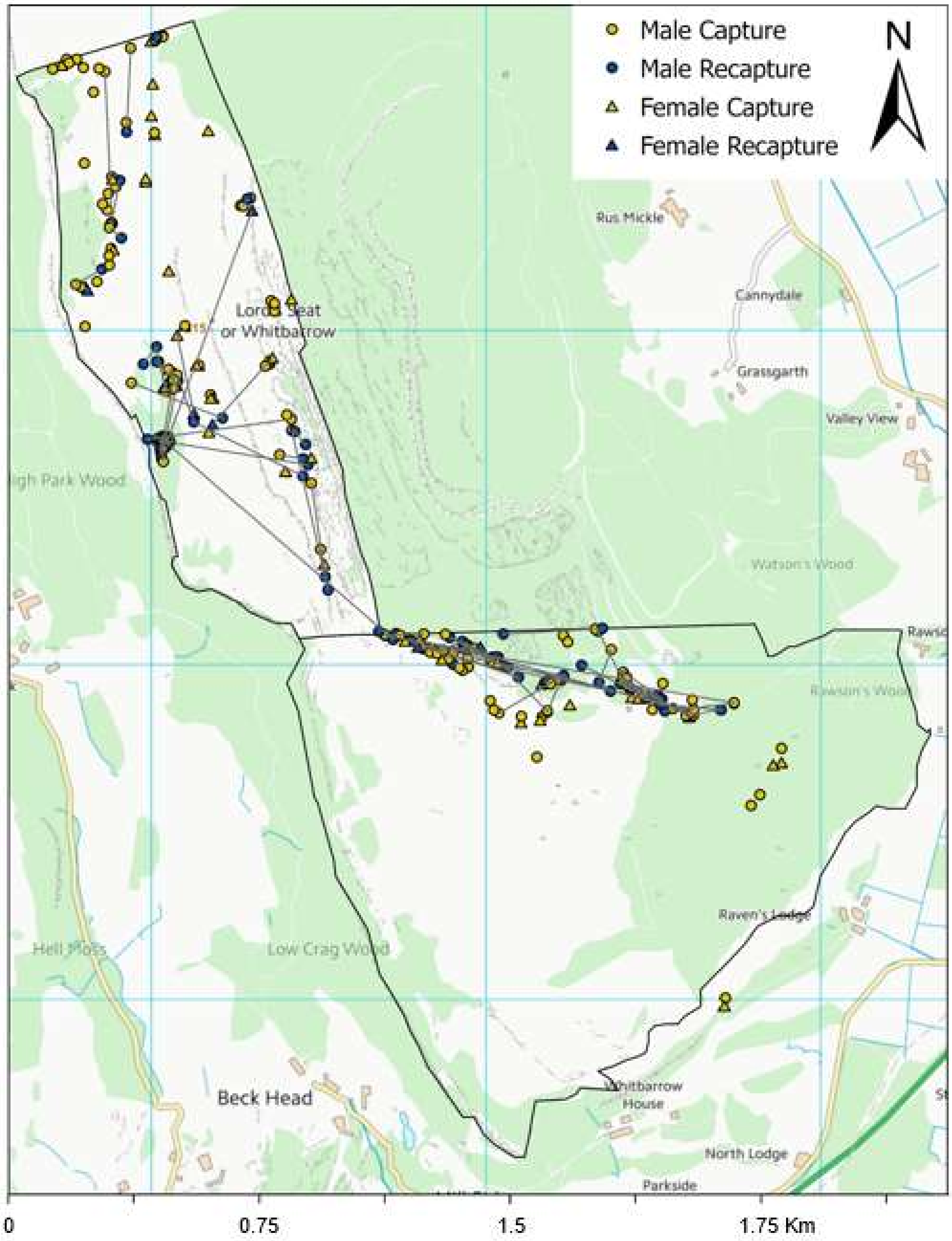


Figure 4.6. ***Boloria euphrosyne*** **distribution at Whitbarrow.** Capture/recapture locations (initial capture points shown in yellow, recaptures shown in blue) and sex (males shown as points, females as triangles) of individuals marked at Whitbarrow NNR during a mark-release-recapture study in 2019. Black polygons show site boundaries for Hervey (top left) and Farrer's Allotment (bottom *right).* Grey lines show Euclidian distance between successive capture points. *Contains OS data: OS Open Raster © Copyright and database right 2020.*

In total, 103 recaptures (53 at Hervey; 50 at Farrer's Allotment) were made (excluding same-day recaptures), with 70 individuals recaptured at least once (Fig. 4.6). The overall recapture rate (proportion of marked individuals that were recaptured at least once) was 35.9% with similar recapture rates between Hervey and Farrer's Allotment (35.7% and 36.1% respectively). However, recapture rate was higher in males than in females (40.4% of marked males were recaptured relative to only 24.1% of females). Female recapture rate was also lower at Farrer's Allotment at 17.6% relative to Hervey where the rate was 35.0%.

Distance moved by *B. euphrosyne* individuals in the MRR ranged from 9 m to 949 m. Although across the sample, the mean average distance moved across all successive captures was 168.6 m ± 24.2 SE and the mean maximum displacement distance was 187.1 m ± 24.9 SE, most recorded movements were less than 100 m (Fig. 4.7).

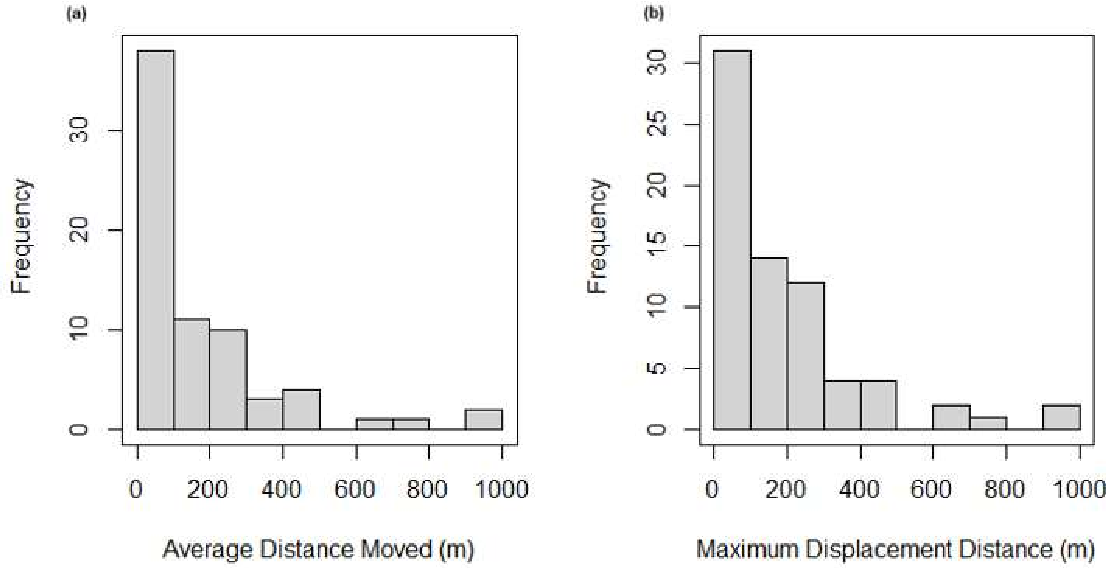


Figure 4.7. Frequency distribution of distance moved by *Boloria euphrosyne*. (a) the average distance across all successive captures and (b) maximum distance moved by individuals at Whitbarrow NNR, during a mark-release-recapture study in 2019.

One individual was recorded moving between the adjacent areas of the reserve, being marked in Hervey and moving a total of 917 m to be recaptured in Farrer's Allotment (Fig. 4.6). However, the largest maximum displacement distance moved by an individual was within Farrer's Allotment (949 m). Although average distance moved was higher in females (266.8 m ± 85.3 SE) relative to males (146.2 m ± 21.9 SE) this difference was not statistically significant (Welch t-test: t = 1.69; d.f. = 13.22; *P*-value = 0.11).

Aggregations with at least three captures and one recapture, separated from other points by at least 100 m apart, were defined as a patch. Seven patches were identified at Hervey, while only one was identified at Farrer's Allotment (Fig. 4.8).

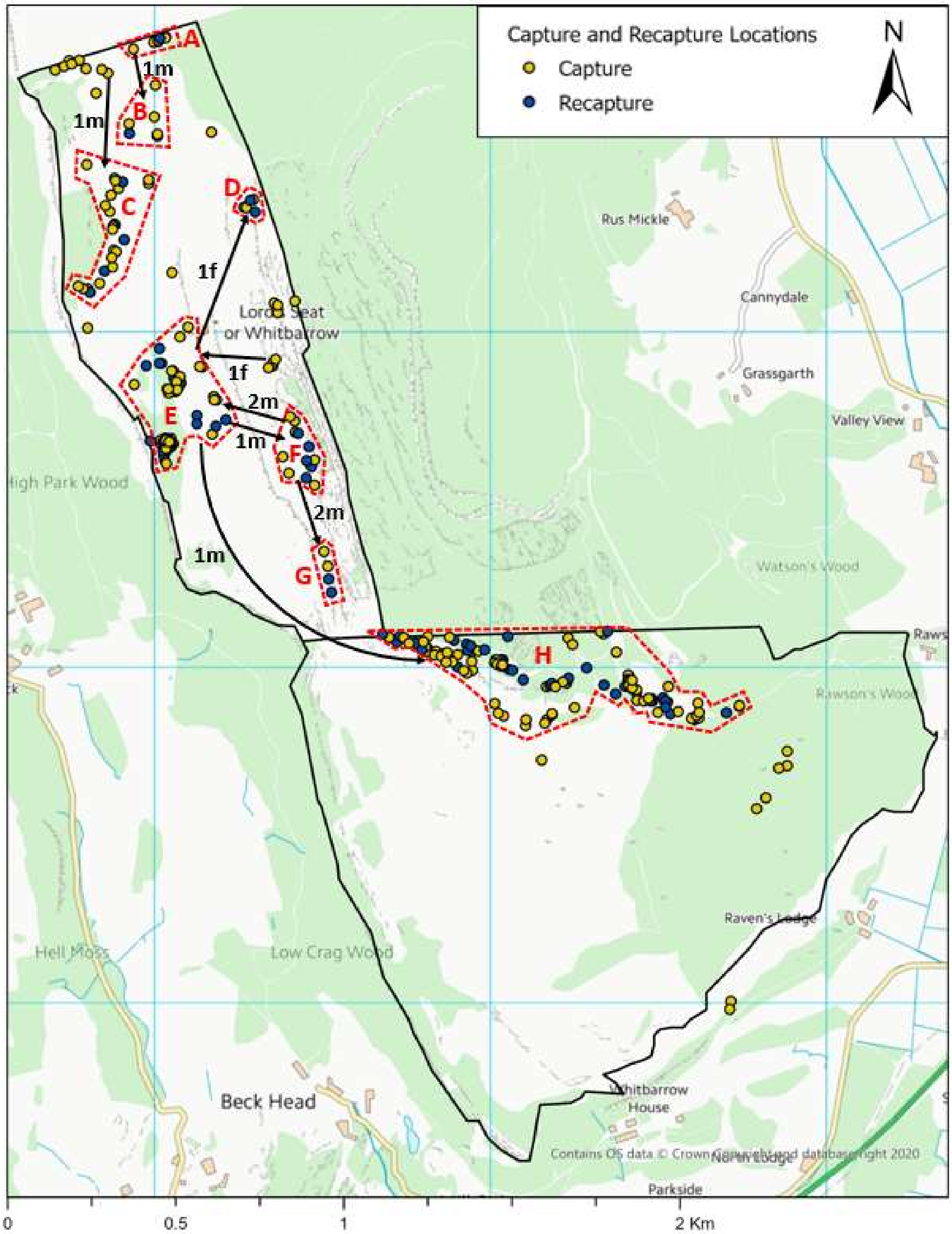


Figure 4.8. Patch boundaries and inter-patch movements of *Boloria euphrosyne* at Whitbarrow. The red dashed lines show the boundary definitions of patches A-H, based on capture and recapture locations (initial capture points shown in yellow, recaptures shown in blue) of individuals marked at Whitbarrow NNR during a mark-release-recapture study in 2019. Black arrows show direction and number of inter-patch movements of males (m) and females (f). Black polygons show site boundaries for Hervey and Farrer's Allotment. *Contains OS data: OS Open Raster © Copyright and database right 2020.*

Eight individuals (six males and two females) were observed making inter-patch movements. These individuals were recaptured outside of the initial patch where they were marked, either in a different patch or in the matrix (Fig. 4.8).

The estimated population size using Loglinear models was 499 (± 178 SE) individuals for Hervey and 112 (± 18 SE) for Farrer's Allotment. The less robust measure of population size, which also considered unmarked individuals gave estimates of 968 individuals at Hervey and 460 at Farrer's Allotment.

4.4.2 Morphology and mobility

Morphology and movement data were collected for 66 individuals (55 male; n = 11 female). Summaries of the morphological measurements for males and females are given in Table 4.1.

Table 4.1. Summary of morphological traits in *Boloria euphrosyne* at Whitbarrow. Mean and standard errors (SE) of morphological measurements from males (n = 55) and females (n = 11) caught at Whitbarrow NNR during a mark-release-recapture study in 2019.

Morphological Trait	Male	SE	Female	SE
Wing aspect ratio	1.59	0.01	1.58	0.01
Wing Loading	1.16	0.03	1.14	0.07
Forewing Length (mm)	19.37	0.12	20.44	0.27
Forewing Width (mm)	12.22	0.08	12.98	0.15
Thorax Volume (mm^3)	22.47	0.60	23.28	1.52

Neither morphology nor sex had significant main or interacting effects on the distance metrics investigated. There was no significant effect of sex or morphology on displacement distance (Table 4.2a, 4.2b and 4.2c). In addition, individuals making inter-patch movements did not differ morphologically from those that were recaptured in the same patch as initial capture (Table 4.2d).

Table 4.2. Model output: the effects of *Boloria euphrosyne* morphology on four distance metrics. Parameter estimates, standard errors (SE), degrees of freedom (d.f.), Chi-squared (χ^2) or F values and *P*-values from separate models to assess the impact of morphology on (a) average distance moved, (b) maximum displacement distance (c) distance between the point of initial capture and first recapture and (d) inter-patch movement in *Boloria euphrosyne*, while accounting for sex. n = 66.

(a) Average Distance Moved

Variable	Estimate	SE	d.f.	F	*P*
(Intercept)	-2.938	4.352	1, 65	1.910	0.137
Sex	0.445	0.390	1, 63	1.305	0.258
Wing Aspect Ratio	3.817	2.743	1, 63	1.936	0.169
Wing Loading	1.005	0.646	1, 63	2.416	0.125

(b) Maximum Displacement Distance

Variable	Estimate	SE	d.f.	F	*P*
(Intercept)	-4.949	4.443	1, 65	2.251	0.091
Sex	0.346	0.398	1, 63	0.757	0.388
Wing Aspect Ratio	5.180	2.800	1, 63	3.424	0.069
Wing Loading	1.009	0.660	1, 63	2.337	0.131

(c) Initial Capture to First Recapture

Variable	Estimate	SE	d.f.	F	*P*
(Intercept)	-0.942	4.498	1, 65	1.994	0.124
Sex	0.608	0.403	1, 63	2.276	0.137
Wing Aspect Ratio	2.296	2.834	1, 63	0.656	0.421
Wing Loading	1.180	0.668	1, 63	3.120	0.082

(d) Inter-patch Movement

Variable	Estimate	SE	d.f.	χ^2	*P*
(Intercept)	-21.733	12.554	3	7.297	0.063
Sex	1.675	0.915	1	3.208	0.073
Wing Aspect Ratio	9.949	7.754	1	1.713	0.191
Wing Loading	2.877	1.815	1	2.718	0.099

However, both morphology and displacement distance were shown to influence the frequency of individual capture during the mark-release-recapture field study. Recaptured individuals were captured a total of two, three or four times during the flight period, depending on how frequently they were encountered and successfully caught. The number of times an individual was recaptured was positively related with wing aspect ratio (Table 4.3; Fig. 4.9a) and negatively related with distance travelled between initial capture and the first recapture (estimate = -0.0004; SE = 0.0002; d.f. = 1,65; F = 4.495; p-value = 0.038; Fig. 4.9b).

Table 4.3. Model output: the effects of *Boloria euphrosyne* morphology on recapture frequency. Parameter estimates, standard errors (SE), degrees of freedom (d.f.), F values and *P*-values from a model to assess the impact of morphology on the number of times an individual was recaptured during a mark-release-recapture study at Whitbarrow NNR in 2019. Significant results are highlighted in bold. n = 66.

Variable	Estimate	SE	d.f.	F	P
(Intercept)	-2.329	0.905	1, 65	6.819	**0.002**
Wing Aspect Ratio	1.995	0.573	1, 65	12.015	**<0.001**
Wing Loading	0.164	0.136	1, 64	1.453	0.233

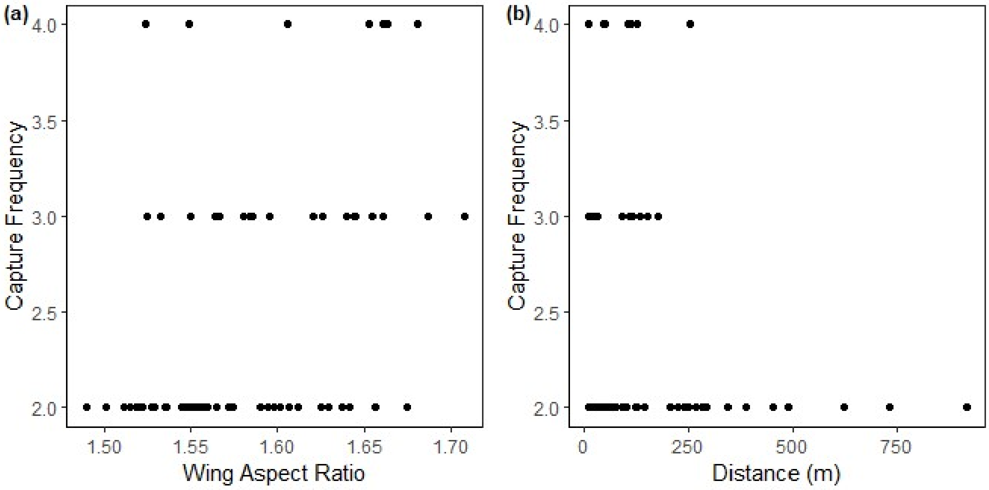


Figure 4.9. Individual differences in wing aspect ratio and displacement distance relating to recapture frequency. (a) Frequency of capture and wing aspect ratio and (b) frequency of capture and distance moved by *Boloria euphrosyne* individuals during a mark-release-recapture study at Whitbarrow NNR in 2019.

To account for the observed relationship between recapture frequency and both wing aspect ratio and displacement distance, individuals that were recaptured once (n = 40) were analysed separately. For those individuals that were recaptured once, there was a significant effect of morphology on displacement distance, with positive relationships between both wing aspect ratio and wing loading and distance between points of capture (Table 4.4). As such, individuals with narrower, elongated wings (wing aspect ratio) and a larger thorax size in relation to wing area (wing loading) were found to disperse further (Fig. 4.10).

Table 4.4. Model output: the effects of *Boloria euphrosyne* morphology on distance travelled in individuals recaptured once during the mark-release-recapture study. Parameter estimates, standard errors (SE), degrees of freedom (d.f.), F values and *P*-values from a model to assess the impact of morphology on displacement distance, while accounting for sex, in individuals marked and subsequently recaptured only once during a mark-release-recapture study at Whitbarrow NNR in 2019. Significant results are highlighted in bold. n = 40.

	Estimate	**SE**	**d.f.**	**F**	***P***
(Intercept)	-12.964	6.319	3, 39	4.340	**0.010**
Sex	0.556	0.442	1, 37	1.584	0.216
Wing Aspect Ratio	9.249	4.030	1, 37	5.267	**0.028**
Wing Loading	1.982	0.827	1, 37	5.748	**0.022**

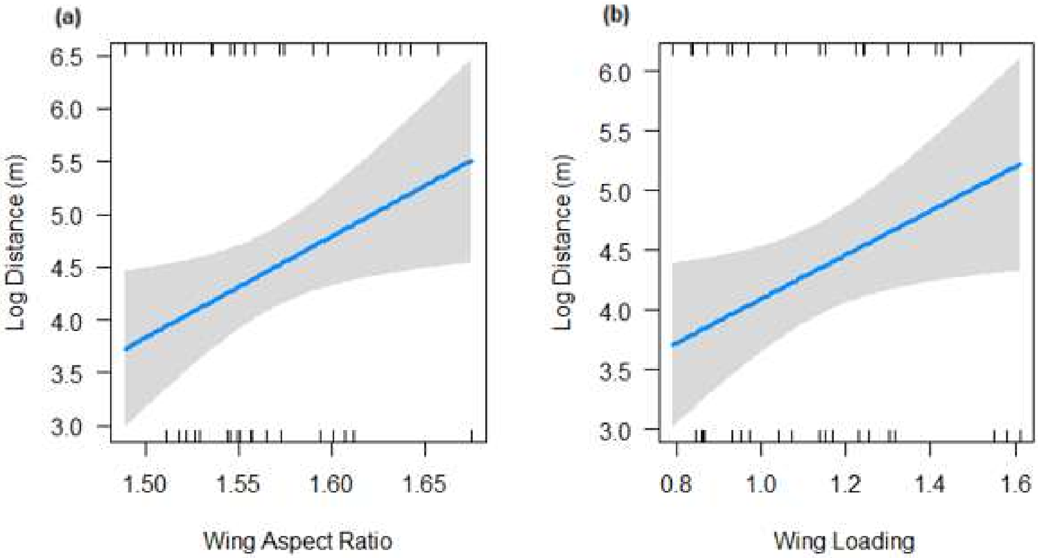


Figure 4.10. Model predicted relationships between morphology and displacement distance in *Boloria euphrosyne* individuals recaptured only once. (a) wing aspect ratio and (b) wing loading on displacement distance in individuals marked and subsequently recaptured only once during a mark-release-recapture study at Whitbarrow NNR in 2019. Shaded areas show 95% confidence intervals and rug marks show partial residuals.

4.5 Discussion

This study shows that morphology may be an indicator of mobility in *B. euphrosyne*, as forewing aspect ratio and wing loading were found to be positively related with distance moved, at least for those individuals that were recaptured only once during the mark-release-recapture field study. However, morphology was shown to be a poor predictor of the investigated distance metrics across the whole sample of individuals and there was no morphological variation between individuals making inter-patch movements and those recaptured in the same patch. The interpretation of the role of morphology on movement in this study is complicated by an effect of wing aspect ratio on the frequency of individual recapture. For this reason, analysis excluding butterflies that were recaptured more than once is also presented. The effect of wing aspect ratio on capture frequency may indicate sampling bias, but could also suggest intraspecific variation in flight capacity in *B. euphrosyne*. Indeed, the distance moved between the initial capture point and first recapture point was negatively related with recapture frequency, suggesting that individuals that were encountered multiple times displayed more sedentary behaviour. The MRR highlighted that *B. euphrosyne* is well distributed across Hervey and that both Hervey and Farrer's Allotment are likely to support viable populations. While most observed movements were less than 100 m, some individuals do move considerably further, suggesting that populations at Whitbarrow are likely to exist as part of a larger metapopulation, linked by local dispersal. Although the transfer of individuals between Farrer's Allotment and Hervey is suggested to be infrequent, it is likely sufficient for gene flow.

The positive relationship between wing aspect ratio and displacement distance, shown in individuals that were recaptured only once during the MRR, could support the idea that high wing aspect ratios (narrow, elongated wings) are an adaptation that promote energy efficient flight and therefore enable individuals to disperse further (Cespedes et al. 2015; Ehl et al. 2017). This being said, high wing aspect ratios are usually observed in individuals making long-distance, migratory movements, while the positive trend observed in the current study, between wing aspect ratio and displacement distance was over a relatively small spatial scale (Johnson et al. 2014). Individuals with low wing aspect ratios are characterised by a high flight capacity and adaptations for flapping, powered flight, with good manoeuvrability, as such, patrolling individuals are characterised by low wing aspect ratios (Wickman, 1992; DeVries et al. 2010). Patrolling is an important behaviour in *B. euphrosyne* males for mate location; males with a high flight capacity are likely to maximise their fitness by patrolling, rather than by dispersing long-distances (Wickman, 1992; Barnett and Warren, 1995). Patrolling individuals are also likely to make repetitive, routine movements and as a result to have low displacement distances

(Breuker et al. 2007). Therefore, patrolling behaviour and associated low total displacement and low wing aspect ratios could explain the positive trend between wing aspect ratio and displacement distance observed in the current study.

A positive relationship between wing loading and displacement distance was also observed in individuals that were recaptured only once during the MRR. Previously, wing loading has been shown to have implications for flight speed and energetics; low wing loading facilitates limited acceleration capacity, but energy efficient, sustained flight, and has been described as a potential adaptation for long-distance dispersal (Davis and Holden, 2015, Sivakoff et al. 2016). In the current study, however, wing loading was shown to be positively related with displacement distance. This relationship could be explained by the relatively short displacement distances observed in the current study and the role of flight speed in shorter distance movements. Due to the link between thorax size and flight speed, high wing loading is associated with rapid flight and flight speed could explain the positive relationship observed here. Indeed, previous studies have shown that flight speed positively affects displacement distance (Dudley and Srygley, 1994; Turlure et al. 2010a).

Although wing aspect ratio and wing loading were significantly related with displacement distance in individuals that were recaptured only once, when the whole sample of individuals was assessed the distance metrics investigated did not differ with morphology. Therefore, this study suggests that in addition to morphology, other factors influencing mobility are likely to be important. In the current study, individuals that were encountered more frequently were shown to have higher wing aspect ratios. This variation suggests sampling bias, potentially arising where morphology facilitates greater ease of capture of certain individuals. Individuals with low wing aspect ratios are reported to have agile flight and may thus be better able to evade capture, this could explain why individuals that were recaptured only once had lower wing aspect ratios relative to individuals caught multiple times (DeVries et al. 2010). In addition, the frequency of individual capture was negatively related with displacement distance. As such, sampling bias could arise as sedentary, perching individuals are more likely to be encountered multiple times and to be recaptured near to the location of initial capture. By contrast, individuals characterised by a dispersive phenotype may be less likely to be recaptured repeatedly if they have dispersed out of the study area. Indeed, previous studies have identified intraspecific variation in butterfly mobility, including morphological differences between perching and patrolling individuals (Wickman, 1992). Higher wing aspect ratios have been found in individuals characterised by perching rather than patrolling behaviours. Differing flight strategies could also explain why morphology was related with dispersal distance only in individuals that were recaptured once.

Morphological traits relating to flight energetics are likely to be of most relevance to mobile individuals, while displacement distance in sedentary individuals is more likely to be independent of morphology. This study suggests that intraspecific variation in dispersal propensity is likely to be present in *B. euphrosyne* populations. It also raises questions about the extent to which MRR methodology provides a representative assessment of dispersal ability in a population.

There was no significant morphological variation between individuals making inter-patch movements and those recaptured in the same patch. However, limited numbers of individuals making inter-patch movements were observed in the current study, so this test has limited statistical power. Patches were defined as an aggregation of individuals with at least three captures and one recapture, separated from other recapture points by at least 100 m. This definition is consistent with those used in previous studies (Davidson, 2017) and is justified by the fact that most recorded movements observed in this study were < 100 m. However, it is worth considering that these results may be influenced by the patch definitions used in the current study and that these distances may represent too small a spatial scale for morphological variation in dispersal to be apparent. Although seven patches were identified at Hervey (Fig. 4.8, Patches A-G) this site is likely to represent a continuous area of broadly suitable habitat, with few barriers to dispersal between these patches. Previous criticisms of MRR studies include the fact that they may frequently overlook long-distance dispersal events, as such, it is plausible that over larger spatial scales, morphological differences between sedentary individuals and those making long-distance movements, may be apparent (Zimmermann et al. 2011).

Morphology may be a poor predictor of realised dispersal where factors such as landscape structure and habitat quality exert an important influence on mobility (Reim et al. 2018). Although previous studies have demonstrated links between butterfly mobility and morphology through traits such as flight behaviour or acceleration capacity, the plastic and context dependent nature of emigration propensity has also been highlighted (Skórka et al. 2013; Cespedes et al. 2015; Reim et al. 2018). Population density and the distribution of host-plant resources in the landscape is, for example, known to influence mobility (Konvicka et al. 2012). Landscape structure is also an important influence on realised dispersal, with patch area, permeability of matrix habitat and distance between patches impacting both the willingness of individuals to cross patch boundaries and subsequent colonisation success (Schtickzelle and Baguette, 2003; Nowicki et al. 2014). Given the specialised habitat requirements of *B. euphrosyne,* including its reliance on a single host plant species, the influence of habitat quality on dispersal behaviour should not be discounted. A better understanding of the influences of habitat quality may therefore be required for a full understanding of mobility in this species.

Recapture rates are influenced by patch demographics, with low recapture rates potentially attributable to high immigration and high mortality (Polic et al. 2021). Although the current study suggests some variation in overall distance moved between males and females, with average distance travelled higher in females (266.8 m ± 85.3 SE) relative to males (146.2 m ± 21.9 SE) there were no significant sex differences in dispersal. Accordingly, the differing recapture rate between males and females observed here is most likely a result of sex-bias in detection due to variation in male and female flight behaviour, rather than a result of differences in immigration rate. Males are more likely to be readily detected as they spend more time actively patrolling, while typical female behaviour in *B. euphrosyne* is cryptic, with individuals characterised by flying low to the ground and resting amongst vegetation (Bennett, 2010). As females making longer distance movements may be more readily observable, low detection rates of non-dispersing females is one explanation for the greater displacement distances and higher standard errors observed in females.

The current study highlighted a widespread distribution of *B. euphrosyne* at sites in the Whitbarrow network. These results also support the idea that in open habitats *B. euphrosyne* forms metapopulations and may be more mobile than in woodland (Barnett and Warren, 1995). The population estimate for Farrer's Allotment (112 ± 18 SE) was lower than for Hervey (499 ± 178 SE) and only one patch was identified at Farrer's Allotment (Fig. 4.8; Patch H). However, patch H was large and the MRR demonstrated frequent intra-patch movement across the site. Recording effort at Farrer's Allotment focused mostly to the north of this site during the MRR, although it is likely that the core breeding areas were surveyed, occasional sightings are reported across the more exposed areas of the site to the south (Cumbria Branch Butterfly Conservation Sightings, 2017). The butterfly was also found to be well distributed across Hervey, with seven patches (Fig. 4.8, Patches A-G) identified and some transfer of individuals between them.

It is worth noting that the population estimates presented here are based on recapture rates following the marking of only 195 individuals. As the data collected only refers to population abundance during the early stages of the flight period, it is likely that population size has been underestimated. After limits on the number of individuals marked were reached, an additional 428 unmarked individuals were caught and released unmarked (Fig. S4.1). The alternative measure of population size, which also considered unmarked individuals, gave estimates of 968 individuals at Hervey and 460 at Farrer's Allotment. Although this second estimate of population size gives a measure of abundance across the whole flight period, rather than just during the period in which individuals were marked, this is likely to give an overestimate of population size as recapture of unmarked individuals multiple times was not accounted for. A further consideration when comparing the population estimates between sites is that phenology appears to have been later at Farrer's Allotment, this is likely to have resulted in a smaller proportion of the population being marked than at Hervey. To calculate robust population estimates, ideally a larger proportion of the population would have been marked. As this was not possible, the population estimates here should be interpreted with caution. It is also worth considering these population size estimates in the context of weather conditions. Dry, sunny weather during the flight period made 2019 an uncommonly good year, with abundance indices on UKBMS transects up by 141% and 267% on the previous year at Hervey and Farrer's Allotment respectively (Botham et al. 2020a). Due to the condition-dependent nature of dispersal, it is also possible that these weather conditions may have promoted elevated mobility (Delattre et al. 2013; Kuussaari et al. 2016). In addition to evidence of increased flight activity of butterflies during warm weather, expansion into marginal habitat during good years can result in the widespread distribution of individuals across a site, when conditions are favourable (Ide, 2010). Therefore, it is possible that the patches identified in the MRR represent marginal populations that may be transient, in addition to core colonies. However, the patches are broadly consistent with observed distribution during fieldwork at the site in 2018 (pers. obs.).

The Whitbarrow network comprises an extensive area of continuous, semi-natural habitat and a core site network for *B. euphrosyne* in north-west England. Although *B. euphrosyne* is monitored by seven transects across the Whitbarrow network, the large extent of the site means that complete surveying is difficult (Ellis and Wainwright, 2008). It is suggested that the likely distribution of the butterfly is widespread at Whitbarrow, beyond the extent of areas monitored by UKBMS transects. Dispersal of individuals across the network is likely to be crucial for long term persistence in this region. While transect records at Whitbarrow have shown abundance declines in recent years, the population estimates suggest that Hervey and Farrer's Allotment still support well sized and viable populations (Botham et al. 2020a). Nevertheless, rapid population declines in other areas in Morecambe Bay, including the Arnside and Silverdale network, highlight the fragile nature of population persistence in the region and reinforce the need for continued population monitoring.

4.6 Supporting Information

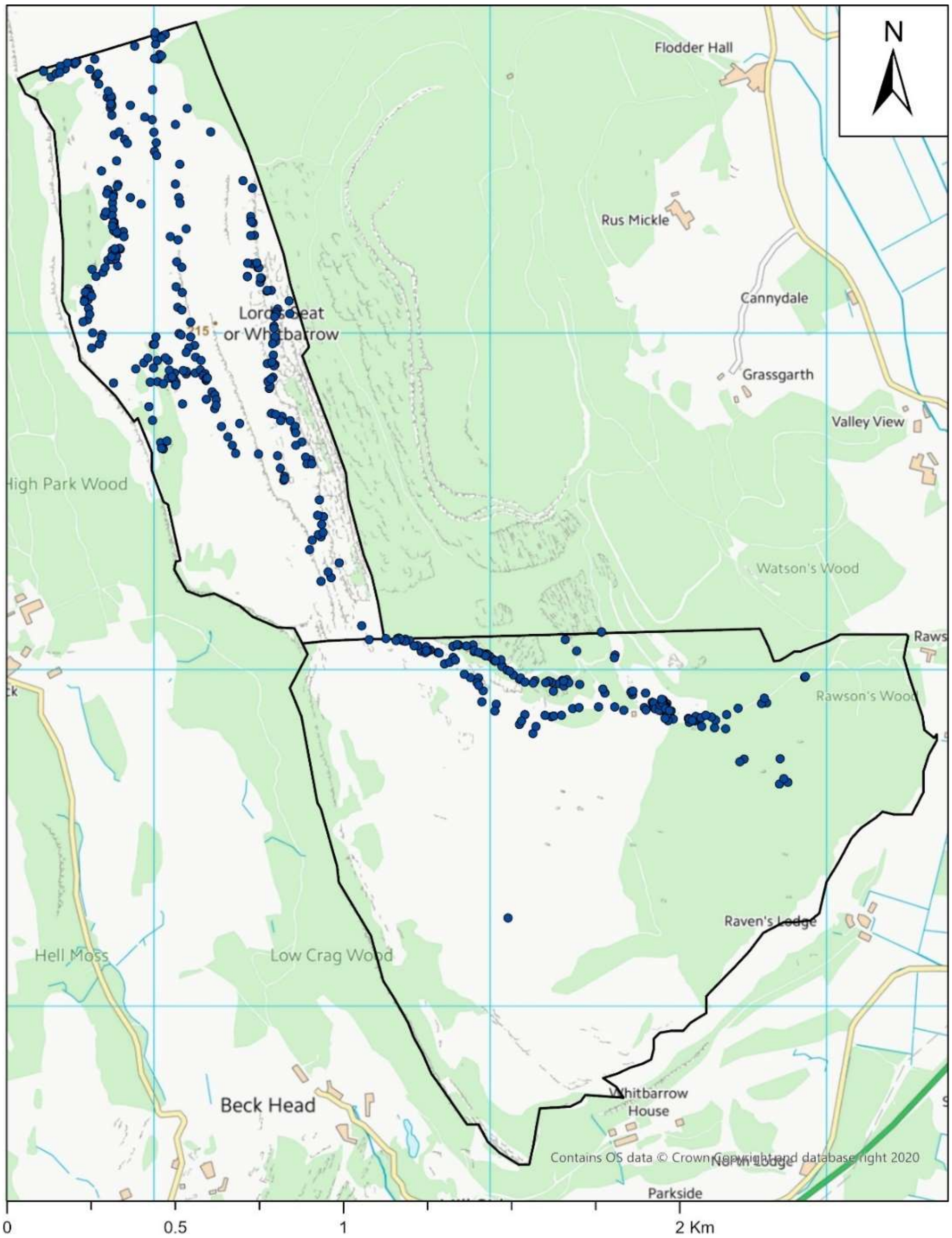


Figure S4.1. Distribution of unmarked individuals at Whitbarrow. Capture point locations of unmarked *Boloria euphrosyne* individuals observed during a mark-release-recapture study at Whitbarrow NNR in 2019 after the permit limit for marking had been reached. Black polygons show site boundaries for Hervey and Farrer's Allotment. *Contains OS data: OS Open Raster © Copyright and database right 2020.*

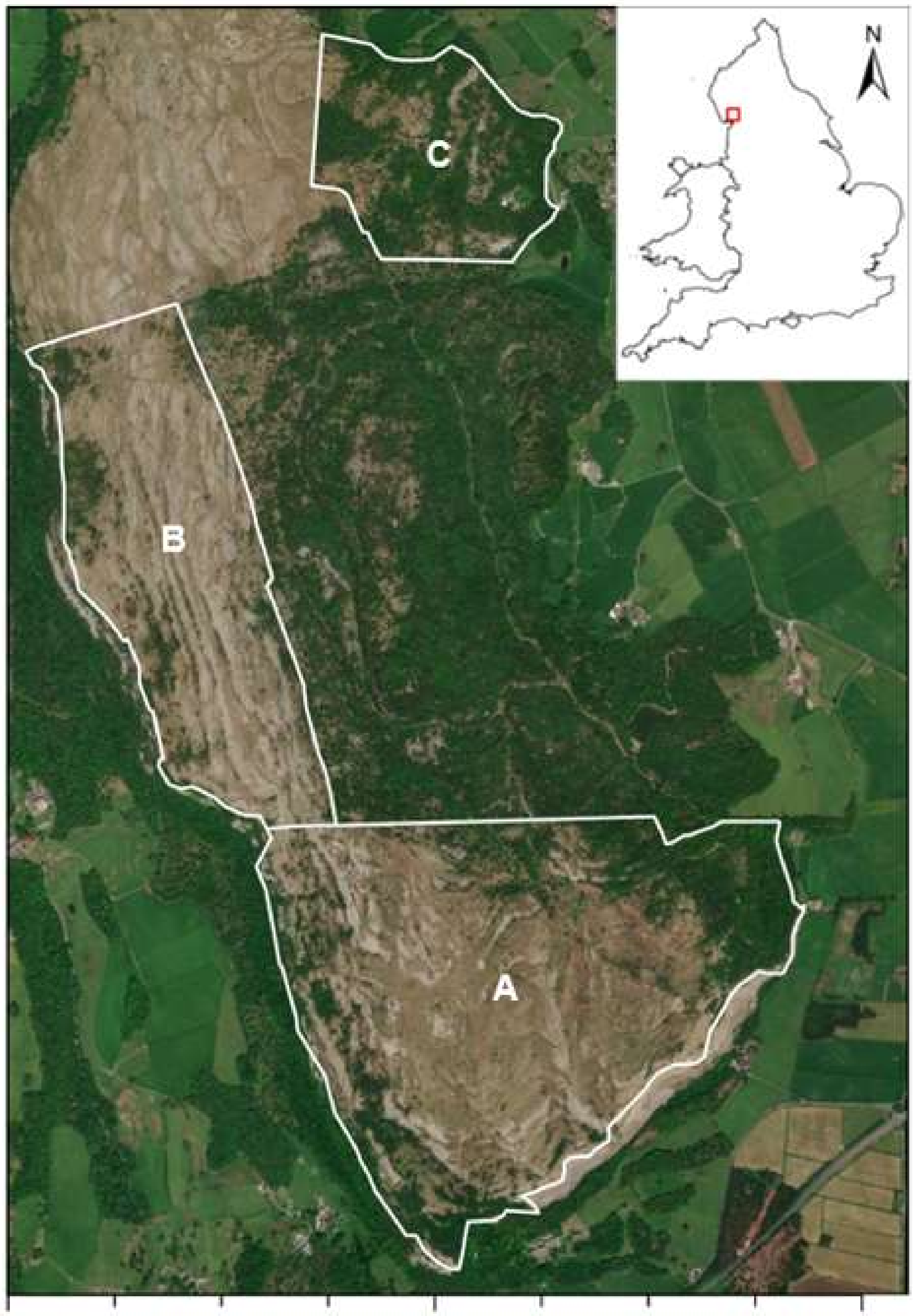


Figure S4.2. Location of 2019 study areas within the Whitbarrow network. (A) Farrer's Allotment, (B) Hervey and (C) Site C. Arial imagery source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN and the GIS User Community.

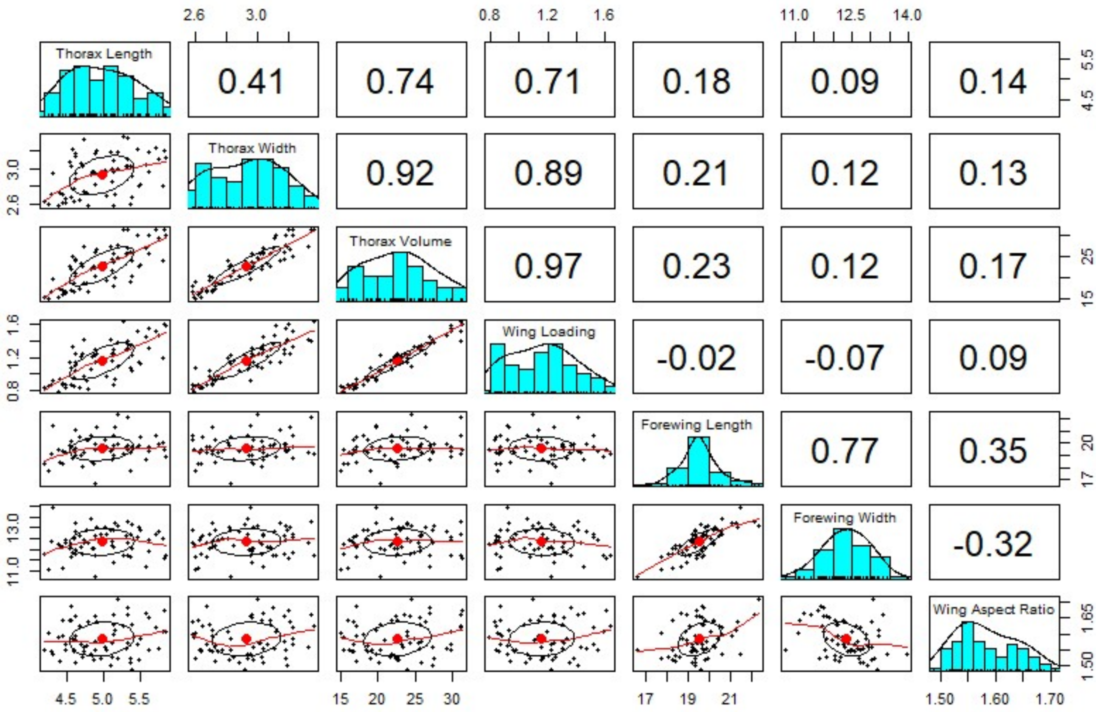


Figure S4.3. Correlation matrix for *Boloria euphrosyne* morphological traits.

Chapter 5: Morphological change suggests altered flight capacity in a fragmented landscape

5.1 Abstract

Species' dispersal capacity and responses to site isolation are important considerations in management strategies, particularly in the face of habitat fragmentation and environmental change. In butterflies, morphology is often indicative of dispersal ability, hence, flight-related morphological traits can be used to understand selection on dispersal in response to factors such as habitat structure and isolation. Here, non-lethal methods of collecting morphological data under field conditions were used to investigate spatial and temporal variation in flight-related morphological traits in a threatened butterfly in the UK. Morphology was compared between extant populations of the pearl-bordered fritillary, *Boloria euphrosyne,* in north-west England and museum specimens collected from the area historically. There were no significant morphological differences between sites in the current populations. However, morphometric analysis revealed that strong population declines in the region from the 1970s onwards have been accompanied by phenotypic changes. There was significant variation between the museum specimens and current populations, with individuals in extant populations showing increased forewing size, smaller thoraxes and lower wing loading. The observed changes in morphology, which are indicative of gliding rather than rapid, powered flight, are associated with improved energy efficiency and are therefore consistent with the hypothesis that current populations are better suited to make sustained, long-distance movements. Although such long-distance movements could be adaptive in fragmented habitats, at low population densities, where colonisation success may be limited, elevated emigration could have detrimental effects for population persistence.

5.2 Introduction

Species' capacity to respond to perturbations is key to their survival in a changing landscape (Nowicki et al. 2014). Insects respond rapidly to their environments and butterflies are common model organisms used to investigate the effects of climate and habitat change (Stevens et al. 2010). Habitat fragmentation is a widespread and pressing issue, particularly for sedentary, habitat specialist butterflies (Cote et al. 2017; Warren et al. 2021). Dispersal is critical in fragmented landscapes, where butterflies in small habitat patches may only persist as part of a metapopulation (Hanski, 1999a; Fourcade and Öckinger, 2017). In addition to physical fragmentation, through habitat loss or degradation of habitat quality, population decline and extinctions lead to site isolation. Over the long term, isolation may affect species through altered selection on dispersal (Nowicki et al. 2014). Selection on dispersal may influence both emigration propensity and flight capacity, while isolation increases the costs of dispersal, and this may lead to a reduction in dispersal events, it may also provide selection pressure to promote dispersal success (Baguette et al. 2003; Kuefler et al. 2010).

Species' responses to isolation are likely to depend on intrinsic factors including life history strategies as well as extrinsic factors such as the degree of isolation and timescale over which it occurred (Schtickzelle et al. 2006; Hanski, 2012). In addition, willingness to disperse is mediated by patch characteristics and environmental conditions, with, for example, butterflies more likely to emigrate from smaller habitat patches and those of lower quality, which are less likely to provide adequate resources (Thomas 2001; Schtickzelle and Baguette, 2003). However, emigration from small populations may be detrimental to survival where dispersal mortality is high and colonisation success limited. As such, increased emigration at low densities has been linked to population declines in the high brown fritillary, *Fabriciana adippe*, in the UK (Bonsall et al. 2014).

For dispersing individuals, energy efficiency and time efficiency (mediated by flight speed) have been highlighted as contrasting flight optimisation strategies (Lindström and Alerstam, 1992; Vincze et al. 2019). Long-distance dispersal, such as in a highly fragmented habitat, requires adaptations for energy efficiency. By contrast, intermediate levels of fragmentation may promote adaptations for maximising the time efficiency of movements, as rapid flight will minimise the time spent moving through a potentially resource poor or inhospitable matrix (Kuefler et al. 2010). There are links between dispersal behaviour and several aspects of butterfly morphology; in particular, wing morphology and thorax size are indicative of flight performance (Reim et al. 2018). As morphology influences the energetic costs of flight, shifts in morphological traits can result in individuals being differentially adapted to optimise energy efficiency or flight speed (Vincze et al. 2019). Hence, flight-related morphological traits may be used to infer species' dispersal capacities and responses to habitat fragmentation. Although wing length is a commonly used proxy for mobility in butterflies and is often reported to be positively associated with dispersal distance, other morphological traits are also likely to be important (Sekar, 2012; Skórka et al. 2013). For example, wing aspect ratio, which describes the shape of the wings, influences flight energetics and manoeuvrability. Narrow, elongated wings have a higher wing aspect ratio than short, broad wings, resulting in less energetically demanding, gliding-type flight, associated with sustained movements, such as long-distance migration (Johnson et al. 2014; Cespedes et al. 2015). By contrast, low aspect ratios are associated with rapid, powered flight and greater manoeuvrability, traits that can be important in ensuring colonisation success (DeVries et al. 2010). Indeed, colonising individuals from recently established speckled wood, *Pararge aegeria*, populations have been shown to have lower wing aspect ratios than butterflies in well-established colonies (Hill et al. 1999b). Wing loading is the ratio of body volume to wing area. Low wing loading reflects a small body size and large wing area and is associated with limited acceleration capacity but energy efficient, sustained flight (Davis and Holden, 2015, Sivakoff et al. 2016). Morphological comparisons between Glanville fritillary, *Melitaea cinxia*, populations showed that individuals occupying an isolated, island population had lower wing loading relative to those in a well-connected metapopulation (Mattila et al. 2012). Low wing loading may potentially be an adaptation for long-distance dispersal. The thorax contains the flight muscles and is indicative of butterfly dispersal ability, with thorax volume shown to be positively correlated with flight speed, as well as displacement distance in mark-release-recapture studies (Dudley and Srygley, 1994; Turlure et al. 2010a).

Natural history collections are a valuable resource and have been used to make inferences about species' responses to climate change and habitat fragmentation (Fenberg et al. 2016; Kharouba et al. 2019; Wilson et al. 2019). For example, swallowtail, *Papilio machaon,* specimens, occupying an isolated site at Wicken Fen, showed a reduction in the ratio of thorax width to length over time, suggesting evolution towards reduced mobility resulting from site isolation (Dempster et al. 1976). Comparisons using museum specimens and individuals from current populations are a particularly useful indicator of the impacts of environmental change. However, most previous studies investigating butterfly morphology have involved lethal techniques and these methods are unsuitable for the study of rare species (e.g. Turlure et al. 2010a; Taylor-Cox et al. 2020; but see Kalarus et al. 2013). Of the few studies involving photography of live butterflies for morphometric analysis, specimens have been hand-reared in the lab enabling photography under standardised conditions (Breuker et al. 2007). Here, two non-lethal methods of collecting data on flight-related morphological traits under field conditions were used.

The current study used flight-related morphological traits in the pearl-bordered fritillary, *Boloria euphrosyne,* to investigate population responses to site isolation. As a sedentary species, *B. euphrosyne* is likely to be strongly impacted by site isolation, yet the implications of site connectivity are frequently overlooked where management for this species focuses primarily on the provision and quality of habitat at the site-level. It was predicted that there would be morphological variation between museum specimens collected from the Morecambe Bay region historically and butterflies from current populations. Increased site isolation was hypothesised to have resulted in altered selection on dispersal, promoting morphological traits associated with enhanced long-distance movement abilities. Although some species demonstrate reduced dispersal propensity in fragmented landscapes, as a specialist of early-successional habitat, ecological succession drives population turn-over in *B. euphrosyne* and is likely to maintain selection on dispersal in this species (Barnett and Warren, 1995). Thorax size was predicted to be smaller in current populations, while wing size was predicted to be greater. An increase in wing aspect ratio and reduced wing loading was also predicted, reflecting shifts to energy efficient, gliding flight and long-distance movements, as populations have become more isolated.

5.3 Methods

5.3.1 Study area and species

Boloria euphrosyne is a sedentary, specialist butterfly of early-successional habitat, which has faced significant population declines, making it one of the UK's most threatened butterflies (Barnett and Warren, 1995; Fox et al. 2015). Historically, national declines in *B. euphrosyne* have been strongly linked to loss of suitable habitat, arising from the abandonment of traditional woodland management practices from the mid-nineteenth century onwards (Barnett and Warren, 1995). The Morecambe Bay Limestones in the north-west of England, often referred to as a national stronghold for this species, are one region where *B. euphrosyne* populations have persisted (Ellis et al. 2012). In Morecambe Bay, the slow regeneration of vegetation, in the well-drained limestone grassland, scree and scrub habitats characteristic of the area, is likely to have maintained the suitability of breeding habitat for an extended period following historical abandonment (Barnett and Warren, 1995). Nevertheless, several site extinctions have occurred over the past decade and with many *B. euphrosyne* populations in this region now characterised by low abundance, remaining populations are increasingly isolated (Botham et al. 2020a).

Study sites were selected using transect records from the United Kingdom Butterfly Monitoring Scheme (UKBMS) to identify sites in the Morecambe Bay area supporting *B. euphrosyne* populations (Botham et al. 2020a; Botham et al. 2020b). Five transects were walked in 2018, this was narrowed to three transects, in the Whitbarrow network, in the 2019 field season, during which data were also collected on butterfly mobility (Supporting Information, Table S5.1; Thesis Chapter 4). Museum specimens collected from north Lancashire and south Cumbria were also identified for use in the study. All individuals were classed by the location of capture as either north or south of the Kent estuary. Locations of sampling sites are shown in Fig. 5.1.

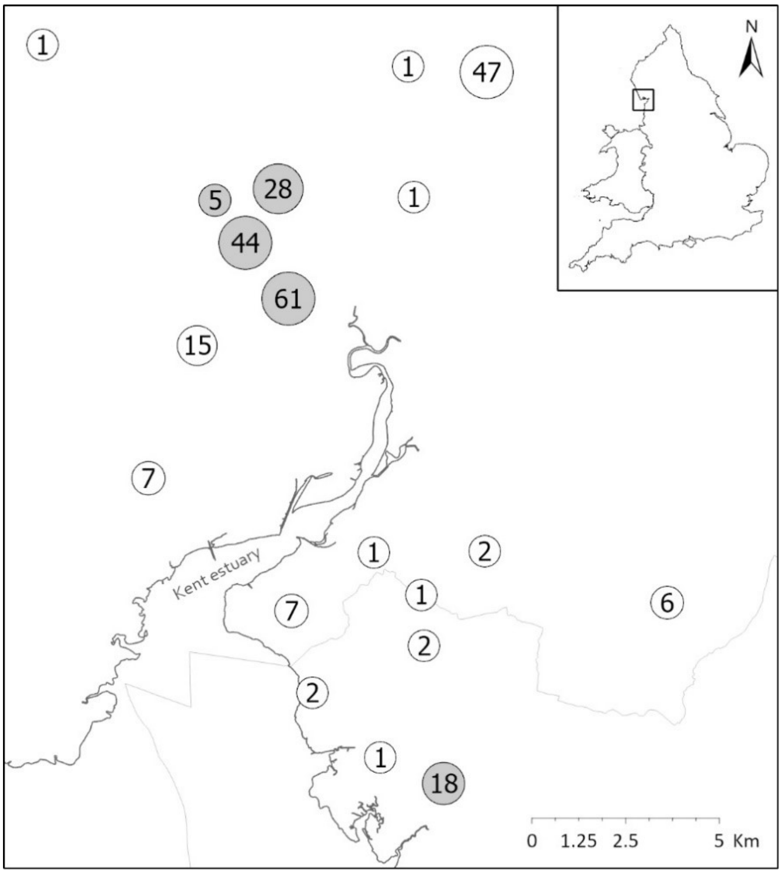


Figure 5.1. Location of sampling sites. The distribution of sampling sites for morphometric analysis in north-west England, relative to the Kent estuary. County boundaries (Lancashire and Cumbria) are shown in grey. Proportional symbols show the location and number of samples from each locality. Current samples are shown by the filled symbols, historical samples by the clear symbols. Historical sample locations generally correspond to 'nearest named place' rather than true sampling locality.

5.3.2 *Current populations*

Morphological data from current populations were collected over two flight seasons (May - June 2018 and 2019). In total 156 butterflies were caught and photographed. These included 90 (71 male and 19 female) during the 2018 flight period and 66 (55 male, 11 female) in 2019. During the flight period, study sites were visited daily, where weather conditions were suitable, with a fixed route, based on the UKBMS transect route and adjacent areas of suitable habitat, walked.

Butterflies were caught using a net and placed into a cool box for a minute to calm them to allow photography. Individuals were photographed from the dorsal side with the wings open, using a Pentax X-5 digital camera. Individuals were held in containers with a graph paper insert at the base and photographed while basking with the wings open (year 1; Fig. 2a) or immobilised with the wings open in polythene envelopes (year 2; Fig. 1b).

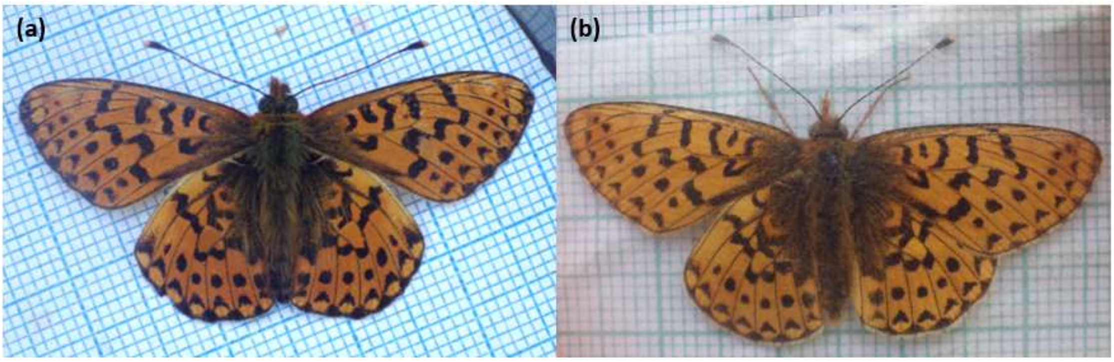


Figure 5.2. Photography for morphometric analysis of *Boloria euphrosyne* (a) Individuals photographed in year 1 using the dorsal basking method and (b) in year 2 using the polythene envelope method.

The alternative method used in year 2 was developed to reduce handling time of the butterflies and with the aim of holding them flatter. The sex of each butterfly was determined by examining the abdomen. Individuals were marked with permanent pen prior to release to prevent repeat photography of the same individual.

There was no significant variation in morphological traits between sites in the current populations (Supporting Information, Table S5.2). Measurements of morphological traits were consistent across the current sample, with the exception of wing aspect ratio, which differed between the two photography methods (Supporting Information, Table S5.2). Individuals photographed in 2018 using the dorsal basking method showed slightly higher wing aspect ratios than those photographed in 2019 (1.60 ± 0.01 vs 1.58 ± 0.01). Therefore, in addition to the main analysis, the effect of collection period was assessed for each photography method separately.

Collection period only had a significant effect on wing aspect ratio for current individuals photographed in 2018 using the dorsal basking method (Supporting Information, Table S5.3).

5.3.3 Museum specimens

In total 94 (60 male, 34 female) museum specimens collected from north Lancashire and south Cumbria were used in morphometric analysis (Fig. 5.1). The specimens were collected over the 75-year period between 1895-1970, pre-dating major population declines in the region, with the majority (n = 55) collected before 1930. Photographs of pinned specimens were obtained from the British Natural History Museum's online database (n = 25), along with details of the locality and year of capture (Natural History Museum, 2014). Specimens from Tullie House (n = 9) and Kendal Museum collections (n = 60) were directly photographed, using a Pentax X-5 digital camera. A grid-scale, fixed at the height of the wings, was included in each photograph. For consistency, only specimens pinned and photographed from the dorsal side were included in analysis.

5.3.4 Morphological measurements

Flight-related morphological traits of both the museum specimens and butterflies photographed in the field were measured using landmarks digitised in tpsDig232 (Rohlf, 2017; Fig. 5.3).

Figure 5.3. Locations of landmarks used in morphometric analysis

Desiccation and damage to pinned specimens prevented accurate abdomen measurements from being taken. Thorax length (TL) was measured at the midpoint, starting below the eyes for consistency (Fig. 5.3, landmarks 5-6). Thorax width (TW) was measured at the widest point (Fig. 5.3, landmarks 7-8). Wing length (WL) was measured from the base to the end of the R4 vein (Turlure et al. 2016; Fig. 5.3, landmarks 2-1 and 3-4). Wing width (WW) was measured from the end of the R4 vein to the end of the 1A + 2A vein (Turlure et al. 2016; Fig. 5.3 landmarks 1-9 and 4-10). Head width was measured as the distance between the eyes (Fig. 5.3, landmarks 11-12). Measurements of the length and width of the thorax and the left and right forewings were calculated using the R package '*geomorph'* (Adams et al, 2021). Where possible, the average forewing width and length was calculated for each individual and used in analysis. Where wing condition precluded accurate measurements being taken from both wings ($n = 21$) measurements from a single wing were used. The thorax volume (TV), wing loading and wing aspect ratio were calculated using the following equations based on Johnson et al. (2014) and Turlure et al. (2016).

$$\mathrm{TV} = \frac{4}{3}\pi \times \left(\frac{\mathrm{TW}}{2}\right)^2 \times \frac{\mathrm{TL}}{2}$$

$$\text{Wing loading} = \frac{\mathrm{TV}}{\mathrm{WL}}$$

$$\text{Wing aspect ratio} = \frac{\mathrm{WL}}{\mathrm{WW}}$$

5.3.5 *Statistical analysis*

Variation in morphological traits between historical and current populations were assessed using generalised linear models with normal errors. The significance of interaction terms and the location of capture (grouped as specimens collected from sites north or south of the Kent estuary; Fig. 1) was assessed using a backwards selection procedure, with terms retained in the model where F-tests indicated $P < 0.05$. Sex was included as a covariate in all analyses. To standardise morphological measurements between individuals and to account for biases in the samples arising from potential collectors' preference for larger individuals, head width was also included as a covariate in the analysis of thorax measurements and wing width. As aspect ratio and wing loading are calculated as proportions and are therefore dimensionless, head width was not used as a covariate in these models. All analysis was carried out in R Studio Version 4.0.3 (R Core Team, 2020).

5.4 Results

Summaries of the morphological measurements for individuals from both historical and current populations are given in Table 5.1.

Table 5.1. Summary of morphological traits in *Boloria euphrosyne* specimens. Mean and standard error (SE) of morphological measurements of individuals from current (n = 126 male; 30 female) and historical (n = 60 male; 34 female) populations in north-west England.

	Historical				Current			
	Male		Female		Male		Female	
Morphological Trait	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Head Width (mm)	1.23	0.03	1.22	0.03	1.17	0.02	1.18	0.06
Thorax Width (mm)	3.04	0.03	3.06	0.04	2.94	0.02	3.00	0.04
Thorax Length (mm)	5.26	0.05	5.12	0.06	4.95	0.04	5.14	0.07
Thorax Volume (mm^3)	25.79	0.63	25.28	0.80	22.57	0.38	24.41	0.81
Forewing Length (mm)	18.97	0.12	19.27	0.15	19.56	0.09	20.34	0.20
Forewing Width (mm)	12.08	0.08	12.14	0.08	12.29	0.06	12.82	0.13
Wing Aspect Ratio	1.57	0.01	1.59	0.01	1.59	0.01	1.59	0.01
Wing Loading	1.36	0.03	1.31	0.04	1.15	0.02	1.21	0.04

Individuals in current populations were shown to have smaller thoraxes relative to the historical sample. Indeed, significant differences were observed in measurements of thorax width and volume between the collections while accounting for sex and head width, as a surrogate for body size (Table 2; Fig. 5.4).

Table 5.2. Model output: morphological comparisons between historical and current *Boloria euphrosyne* specimens. Parameter estimates, standard errors (SE), degrees of freedom (d.f.), F values and *P*-values from morphological comparisons between current populations and museum specimens (collection period) accounting for sex differences, body size (using head width) and the location of capture. Significant results are highlighted in bold.

Morphological Trait	**Estimate**	**SE**	**n**	**d.f.**	**F**	***P***
Thorax Width	2.905	0.091	250	3, 249	4.518	**0.004**
Sex	0.041	0.034		1, 247	1.448	0.230
Head Width	0.101	0.071		1, 247	2.036	0.155
Collection Period	-0.084	0.031		1, 247	7.313	**0.007**
Thorax Length	5.244	0.161	249	4, 248	6.381	**<0.001**
Sex	-0.135	0.087		2, 246	4.039	**0.018**
Head Width	0.007	0.124		1, 245	0.003	0.957
Collection Period	-0.307	0.064		2, 246	8.392	**<0.001**
Sex:Collection Period	0.330	0.119		1, 245	7.653	**0.006**
Thorax Volume	23.029	1.757	249	3, 248	7.563	**<0.001**
Sex	0.802	0.660		1, 246	1.474	0.226
Head Width	1.790	1.371		1, 246	1.704	0.193
Collection Period	-2.360	0.599		1, 246	15.527	**<0.001**
Forewing Length	18.307	0.427	244	4, 243	11.862	**<0.001**
Sex	0.600	0.144		1, 240	17.323	**<0.001**
Head Width	0.157	0.302		1, 240	0.269	0.604
Location of Capture	0.476	0.171		1, 240	7.758	**0.006**
Collection Period	0.682	0.132		1, 240	26.653	**<0.001**
Forewing Width	11.716	0.280	243	5, 242	6.997	**<0.001**
Sex	0.077	0.135		2, 239	9.691	**<0.001**
Head Width	0.135	0.197		1, 238	0.471	0.493
Location of Capture	0.248	0.112		1, 238	4.881	**0.028**
Collection Period	0.189	0.102		2, 239	10.928	**<0.001**
Sex:Collection Period	0.496	0.188		1, 238	6.952	**0.009**
Wing Aspect Ratio	1.574	0.006	242	2, 241	2.938	0.055
Sex	0.005	0.008		1, 240	0.369	0.544
Collection Period	0.018	0.007		1, 240	5.856	**0.016**
Wing Loading	1.331	0.026	243	2, 242	17.323	**<0.001**
Sex	0.013	0.033		1, 241	0.161	0.689
Collection Period	-0.171	0.030		1, 2	32.251	**<0.001**

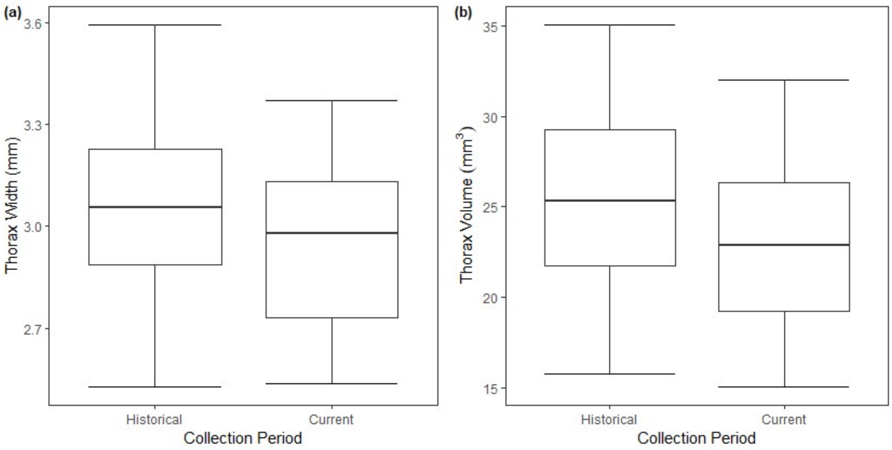


Figure 5.4. Variation in thorax size between historical and current specimens of *Boloria euphrosyne*. (a) Thorax width and (b) thorax volume in museum specimens (Historical) and individuals from current populations in north-west England. Horizontal lines within the boxes represent the median, box edges show the interquartile range (IQR). Whiskers show the extent of the data, which are at most 1.5 times the IQR from the box edges.

Thorax length was shorter in the individuals from current populations (Table 5.2). There was also a significant interaction between sex and collection period, with thorax length only significantly shorter in males from current populations relative to the historical specimens (Fig. 5.5a, Table 5.2).

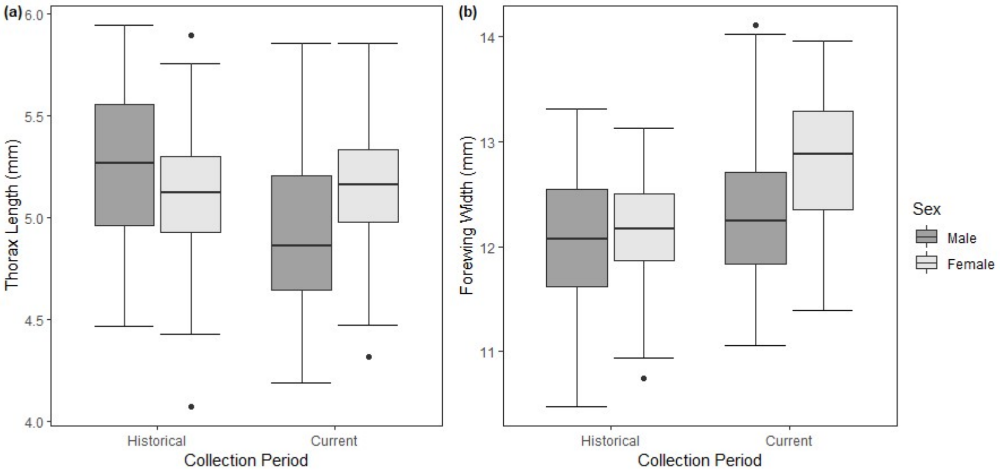


Figure 5.5. Variation in morphology between historical and current specimens of *Boloria euphrosyne*, showing differences based on sex (a) Thorax length and (b) forewing width in male and female museum specimens (historical) and individuals from current populations in north-west England. Horizontal lines within the boxes represent the median, box edges show the interquartile range (IQR). Whiskers show the extent of the data, which are at most 1.5 times the IQR from the box edges, outliers (shown as points) are data values falling beyond this.

There was evidence of sexual dimorphism in wing size, as both forewing length and width was greater in females relative to males (Table 5.2). However, for forewing width there was a significant interaction between sex and collection period, with increases in wing width over time most pronounced in females (Fig. 5.5b, Table 5.2). Additionally, there was variation in wing size based on specimen locality; specimens collected from sites north of the Kent Estuary had longer, wider forewings (Table 5.2, Fig. 5.6). In addition to the influence of locality on wing size, forewing measurements were shown to have increased over time, with current populations supporting individuals with wider, longer forewings (Table 5.2, Fig. 5.5b, Fig. 5.6).

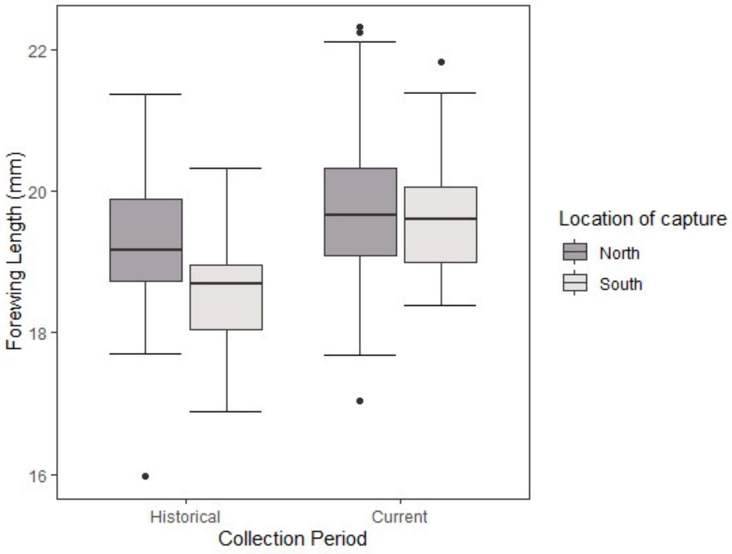


Figure 5.6. Variation in forewing length between historical and current specimens of *Boloria euphrosyne*, showing differences based on location of capture. Forewing length in museum specimens (historical) and individuals from current populations showing differences by location of capture (grouped as specimens collected from sites north or south of the Kent estuary). Horizontal lines within the boxes represent the median, box edges show the interquartile range (IQR). Whiskers show the extent of the data, which are at most 1.5 times the IQR from the box edges, outliers (shown as points) are data values falling beyond this.

Higher wing aspect ratios were also observed in the current sample (Fig. 5.7a), showing that wings in the current populations are relatively longer and more slender, although the effect size was small (Table 5.1). When the data were split by photography method, the difference in wing aspect ratios was only significant for individuals measured in 2018 (Supporting Information, Table S5.3). Wing loading was also significantly lower in current populations, highlighting the decline in body size relative to wing area in current populations (Fig. 5.7b, Table 5.1).

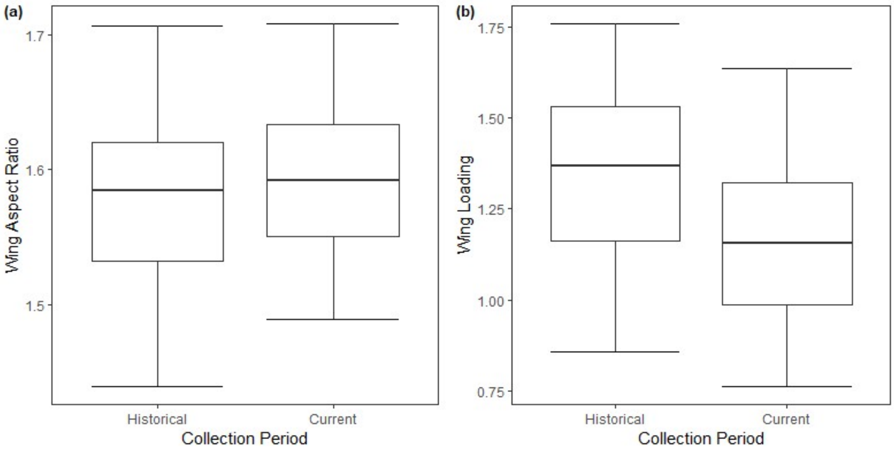


Figure 5.7. Variation in wing aspect ratio and wing loading between historical and current specimens of *Boloria euphrosyne*. (a) Wing aspect ratio and (b) wing loading in museum specimens (historical) and individuals from current populations in north-west England. Horizontal lines within the boxes represent the median, box edges show the interquartile range (IQR). Whiskers show the extent of the data, which are at most 1.5 times the IQR from the box edges.

5.5 Discussion

The current study highlights population responses to site isolation in *B. euphrosyne* using flight-related morphological traits. Morphometric analysis revealed that population declines from the 1970s onwards have been accompanied by phenotypic changes in populations in the Morecambe Bay region, with significant variation in flight-related morphological traits between museum specimens collected from the area between 1895 and 1970 and individuals from current populations. Reductions in thorax size and wing loading were observed, in addition to increases in forewing measurements. Although there was no significant variation in morphological characteristics between sites in current populations, locality of collection did have an effect, with specimens collected from sites north of the Kent Estuary generally showing longer, wider forewings. The analysis also highlighted sexual dimorphism in morphology, with females having larger forewings than males, a difference that was most pronounced in the individuals from current populations. Indeed, there was a significant interaction between sex and collection period with increases in forewing width over time greater in females. There was also an interaction between sex and collection period for thorax length, with the decline between the historical and current specimens only present in males. Given the links between butterfly morphology and flight performance, it is likely that the observed morphological changes will have implications for dispersal.

Thorax size is positively correlated with flight speed and dispersal distance, as such, investment in the thorax can act to maximise the time efficiency of flight (Dudley and Srygley, 1994; Turlure et al. 2010a). This study highlighted a reduction in thorax size over time, suggesting that current populations have adapted to maximise energy efficiency, over flight speed. Individuals with large thoraxes are reported to be good colonisers, likely because of the positive influence of thorax size on flight performance and the importance of rapid flight for successful dispersal through resource-poor or inhospitable matrix habitat (Hill et al. 1999b; Fountain et al. 2016). However, the strategy of maximising flight speed conflicts with energy efficiency, making rapid flight unsustainable over long distances (Vincze et al. 2019). Reductions in wing loading were also observed in individuals from current populations, reflecting the decrease in thorax size relative to wing area. High wing loading is associated with powered flight and increased acceleration capacity, but as these types of movements are energetically costly, individuals with higher wing loading may be reluctant to disperse long-distances (Berwaerts et al. 2002; Sivakoff et al. 2016). Given these trade-offs, the low wing loading and reduced thorax size present in current populations of *B. euphrosyne* implies evolution towards sustained flight but declines in flight speed, consistent with an energy-saving flight strategy and long-distance dispersal (Cespedes et al. 2015; Vincze et al. 2019).

Individuals from current populations displayed larger forewings and slightly higher wing aspect ratios relative to museum specimens. The increase in wing aspect ratio demonstrates a greater increase in forewing length, relative to width, in the current populations, resulting in narrow, slender wings, while historical populations had relatively shorter, broader wings. In butterflies, movement distance is often positively correlated with wing length (Skórka et al. 2013; Flockheart et al. 2017). High wing aspect ratios are also associated with long-distance dispersal and are present in migratory species such as the monarch, *Danaus plexippus* (Davis et al. 2005). Wing aspect ratio is negatively related with flight speed, again highlighting the trade-off between long-distance movements and rapid flight (Dudley and Srygley, 1994). It is suggested that the potential changes in forewing size and shape observed in current populations could reflect an adaptation for making long-distance movements. However, in the current study the effect size was small and differences in wing aspect ratio were not significant when comparing historical populations with only specimens photographed in 2019, using the polythene envelope method. Observed differences in wing aspect ratio between individuals photographed in 2018 and 2019 suggest that photography method may have influenced the measurements taken. The method used in 2019 is more likely to give accurate results as individuals were held and photographed ‘flatter’ than when using the dorsal basking method. Therefore, these results should be interpreted with caution. Nevertheless, other morphological traits and their relationships with collection period were consistent across the two methods used.

Photography of live butterflies presents an opportunity for morphological studies on rare species, for which lethal sample methods are inappropriate. However, there may be issues with the comparison of pinned specimens and live butterflies, particularly as desiccation may exaggerate size differences between the two samples. Although the water content of butterfly's wings is usually very small, meaning that drying should not affect wing measurements, dehydration of museum specimens is likely to result in shrinkage of butterfly thoraxes (Van Hook et al. 2012). Interestingly, in the current study, thorax measurements were greater in the pinned specimens relative to the live sample, contrary to what would be predicted if desiccation was driving variation between the samples. Given this, perhaps the observed differences in thorax size are even greater than suggested here. To further validate this method, it could be useful to calibrate measurements by using captive reared butterflies and comparing measurements made on live individuals with follow-up measurements on the same individual after preservation.

Previous studies have demonstrated sex bias in flight behaviour in butterflies; while males are often more active and display traits associated with increased flight ability, females show a greater tendency to disperse (Berwaerts et al. 2002; Reim et al. 2018). Due to differing selection pressures on males and females, sex-specific flight behaviours are likely to contribute to sexual dimorphism in morphology and may influence responses to site isolation (Cespedes et al. 2015). The results presented here highlight variation in the responses of males and females to site isolation, with declines in thorax length only present in males, and increases in forewing width most pronounced in females. Males of this species spend considerable time patrolling habitat and searching for mates (Barnett and Warren, 1995). Gliding flight, typical of male territorial behaviour, is often associated with smaller thorax measurements (Turlure et al. 2010a). Differences in flight behaviour could explain why declines in thorax length were only present in males. Mobility in females is important in finding suitable breeding habitat; female behaviour in *B. euphrosyne* is characterised by flying low to the ground, searching for oviposition sites and resting on vegetation (Barnett and Warren, 1995). Female dispersal also has key implications for metapopulation persistence. Metapopulations with limited female dispersal are highly vulnerable to extinction as dispersal plays an important role in the rescue effect and in the colonisation of new habitat patches (Örvössy et al. 2013). Due to the energetic costs of carrying eggs, greater thorax size may be necessary to support female flight (Ehl et al. 2017). Therefore, thorax width is likely to represent a more fixed trait in females, this could explain the smaller changes in thorax length in females relative to males. There was also sexual dimorphism observed in forewing measurements, with females displaying larger wings relative to males, which could suggest that females have greater mobility. However, while previous mark-release-recapture studies on *B. euphrosyne* in the region have suggested that females may have higher emigration propensity in comparison to males, females are generally thought to move short distances (Bennett, 2010; Davidson, 2017).

Optimal wing morphology will be dependent on the type of flight, although the focus of this study has been on dispersal, selection acting on routine or station keeping movements may confound the effects of site isolation on flight-related morphological traits (Van Dyck and Baguette, 2005). To account for sexual dimorphism, sex was included as a covariate in all analyses. Mobility is vital where resources are rare or patchily distributed in the landscape, hence, factors such as resource availability and habitat quality may act as alternative explanations for the observed changes in morphology. Indeed, 'resource grain' has been shown to influence mobility, with the spatial dimension of nectar resources negatively correlated with distance moved in a mark-release-recapture study (Turlure et al. 2010a). Additionally, butterflies emerging from plots experimentally manipulated to have reduced habitat quality were found to disperse more readily than those occupying high quality habitat (Sivakoff et al. 2016). Furthermore, dispersing individuals were observed to have lower wing loading. Particularly as a monophagous, habitat specialist, the dispersal behaviour of *B. euphrosyne* may conceivably be influenced by the spatial distribution of the larval foodplant, *Viola sp*. However, if foodplant availability was the driving force behind the morphological changes observed in the current study perhaps significant differences between sites would be expected in current populations, given the variation in habitat quality and violet abundance (Thesis Chapter 4).

Temperature is an additional factor impacting on insect morphology. Therefore, climatic changes occurring in the intervening years between the historical and current sample could provide an alternative explanation for the observed morphological changes. Increased temperatures can result in longer growing seasons, which can increase resource access for developing arthropods and could lead to traits such as the larger forewing size observed here (Faldyn et al. 2018). Conversely, food plant quality can be negatively affected by warming temperatures and as insects have elevated metabolic rates at warmer temperatures, body size will be smaller where these greater energy demands cannot be offset (Bowden et al. 2015). To control for allometry and these potential climate effects on body size, head width was used as a covariate in analysis.

Boloria euphrosyne is reportedly a relatively sedentary butterfly, however, the current study highlights morphological changes that are suggestive of adaptations for making longer distance movements. Overall, the observed variation in morphology between the pinned and live specimens indicates that current populations are likely to be better adapted for gliding flight. Gliding flight is associated with reduced energetic costs and may promote sustained, long-distance movements. However, these morphological changes also imply a decline in flight speed, which may compromise the dispersal success and colonisation capacity of butterflies in current populations. Similar differences in morphology as observed in *B. euphrosyne* in the current study, including reduced wing loading and thorax size, were identified in an isolated, island population of *M. cinxia*, relative to individuals in a well-connected metapopulation (Mattila et al. 2012). The isolated population was also characterised by compromised fitness and a high genetic load, highlighting that morphological changes do not in themselves necessarily indicate adaptive change or population fitness. Given both the influence of abiotic factors on dispersal success and the trade-offs between dispersal capacity and fecundity, flight-related traits may not provide an accurate measure of effective dispersal (Turlure et al. 2010a; Kuussaari et al. 2016; Lancaster and Downes, 2017). It is also worth noting that while morphological traits have some predictable implications for flight capacity in butterflies, this does not necessarily relate to emigration propensity (the willingness of an individual to leave a patch) (Reim et al. 2018). This being said, given the habitat preferences of the study species, ecological succession is likely to drive population turn-over in *B. euphrosyne* and promote dispersal in fragmented habitats, as colonising new habitat patches at an appropriate successional stage is essential for population persistence. Population density is also an important influence on dispersal behaviour (Kuussaari et al. 1996; Sivakoff et al. 2016). High rates of population decline, and site extinctions have occurred in the Morecambe Bay region, meaning that remaining populations are both highly isolated and likely to be persisting at low densities. The negative implications of elevated emigration at low densities, where successful dispersal is limited, have been highlighted in *F. adippe* where emigration from small populations has been linked to population densities falling below an 'Allee threshold', resulting in increased extinction risk (Bonsall et al. 2014). Indeed, in addition to adaptations for efficiency of flight, the morphological changes in current populations suggest limited acceleration capacity, meaning that flight speed may compromise colonisation success. Therefore, while the adaptations to reduce the energetic costs of flight and make longer distance movements observed in the current study may be adaptive, their implications for population persistence are not clear, as elevated emigration at low population densities could be actively increasing extinction risk.

5.6 Supporting Information

Table S5.1. UKBMS transect site details. All transect sites were walked in 2018, only those marked with an asterisk (*) were walked in 2019.

Transect Name	Grid Reference	Number of Sections	Transect Length (m)
Warton Crag LNR	SD494726	15	3181
Whitbarrow - Howe Ridding NNR	SD434881	15	1410
Whitbarrow NNR - Farrer's Allotment *	SD452855	15	4100
Whitbarrow NNR - Hervey CWT *	SD441869	11	4000
Whitbarrow North *	SD44X88X	15	2870

Table S5.2. Model output: morphological comparisons between *Boloria euphrosyne* individuals at currently occupied sites. Parameter estimates, standard errors (SE), degrees of freedom (d.f.), F values and *P*-values for morphological comparisons of current specimens by site, accounting for sex differences, body size (using head width) and photography method. Significant results are highlighted in bold.

Morphological Trait	Estimate	SE	n	d.f.	F	*P*
Thorax Width	2.825	0.113	156	3, 155	1.084	0.358
Sex	0.059	0.046		1, 153	1.672	0.198
Head Width	0.101	0.086		1, 153	1.375	0.243
Site	-0.003	0.012		1, 153	0.043	0.836
Thorax Length	4.676	0.208	155	3, 154	3.495	**0.017**
Sex	0.200	0.083		1, 152	5.757	**0.018**
Head Width	0.280	0.158		1, 152	3.149	0.078
Site	-0.022	0.022		1, 152	0.979	0.324
Thorax Volume	19.729	2.166	155	3, 154	2.867	**0.039**
Sex	1.875	0.868		1, 152	4.666	**0.032**
Head Width	2.807	1.646		1, 152	2.908	0.090
Site	-0.171	0.233		1, 152	0.539	0.464
Forewing Length	19.871	0.505	152	3, 151	5.046	**0.002**
Sex	0.797	0.207		1, 149	14.880	**<0.001**
Head Width	-0.161	0.388		1, 149	0.173	0.678
Site	-0.047	0.055		1, 149	0.729	0.395
Forewing Width	12.300	0.332	150	3, 149	5.239	**0.002**
Sex	0.529	0.136		1, 147	15.134	**<0.001**
Head Width	-0.031	0.256		1, 147	0.015	0.903
Site	0.011	0.036		1, 147	0.085	0.772
Wing Aspect Ratio	1.638	0.014	150	3, 149	3.803	**0.012**
Sex	-0.006	0.010		1, 147	0.385	0.536
Method	-0.025	0.009		1, 147	8.383	**0.004**
Site	-0.004	0.003		1, 147	1.833	0.178
Wing Loading	1.189	0.035	151	2, 150	1.740	0.179
Sex	0.066	0.043		1, 149	2.285	0.133
Site	-0.014	0.011		1, 149	1.572	0.212

Table S5.3. Model output: variation in wing aspect ratio between historical populations and current *Boloria euphrosyne* specimens sampled in 2018 or 2019. Parameter estimates, standard errors (SE), degrees of freedom (d.f.), F values and *P*-values for morphological comparisons of wing aspect ratio, based on collection period and photography method in current samples. (a) comparisons between museum specimens and current *Boloria euphrosyne* populations sampled in 2018 and photographed using the dorsal basking method (n = 90). (b) comparisons between museum specimens and current populations sampled in 2019 and photographed using the polythene envelope method (n = 66). Significant results are highlighted in bold.

(a)

Morphological Trait	**Estimate**	**SE**	**d.f.**	**F**	***P***
Wing Aspect Ratio	1.572	0.007	2, 175	5.446	**0.005**
Sex	0.012	0.009	1, 174	1.658	0.200
Collection Period	0.026	0.008	1, 174	10.327	**0.002**

(b)

Morphological Trait	**Estimate**	**SE**	**d.f.**	**F**	***P***
Wing Aspect Ratio	1.574	0.007	2, 157	0.450	0.638
Sex	0.006	0.010	1, 156	0.323	0.570
Collection Period	0.008	0.009	1, 156	0.751	0.387

Chapter 6: General Discussion

6.1 Context of study and research aims

The pearl-bordered fritillary, *Boloria euphrosyne*, a specialist butterfly of early successional habitat, has faced significant declines in both abundance and distribution in the UK (Ellis et al. 2012; Fox et al. 2015). The Morecambe Bay Limestones, located in the north-west of England, are of national importance for biodiversity, and have a reputation as a national stronghold for this species (Ellis et al. 2012; Skelcher, 2014). Nevertheless, populations in this region are in decline, with several colonies being lost over the last decade (Ellis and Wainwright, 2008; Botham et al. 2020a).

The thesis aims were to investigate population declines in *B. euphrosyne* and to assess the implications of site isolation for population persistence. By studying both historical abundance data from populations across England and assessing the functional connectivity of colonies in the Morecambe Bay region, this study aimed to identify factors driving site extinctions and those that are limiting current populations. Focus was given to understanding the influence of site isolation on species persistence in addition to the longer-term implications of fragmentation on dispersal capacities and population structure. A greater understanding of the factors associated with population declines in this species may be used to assess the extinction risk of current populations and highlight priorities for management. This research will also be considered in a broader context, with the implications for conservation of species inhabiting fragmented landscapes discussed more generally.

6.2 Synopsis of key findings

The factors driving *B. euphrosyne* population persistence and abundance in England were investigated (Chapter 2). This chapter highlights the critical role of population connectivity for long-term survival of populations across fragmented landscapes, particularly in the face of environmental change. All population metrics were positively related with site connectivity, demonstrating the likely importance of dispersal between local sites in maintaining viable populations. Weather conditions, both during the adult flight period and throughout larval development, also have potential to drive population-level effects. Variation in vulnerability to perturbation was highlighted between populations occupying woodland dominated systems and those occupying habitats with a more open structure, including grassland and bracken dominated sites. Nitrogen deposition and increased rainfall under climate change scenarios are likely to impact vegetation structure and lead to declines in breeding habitat suitability via microclimate cooling, which are suggested to affect woodland sites most strongly.

Chapter 2 highlights the importance of site connectivity for population persistence, and to investigate these effects further, population synchrony was trialled as a method to assess effective dispersal in *B. euphrosyne* (Chapter 3). Population synchrony is suggested to be an effective indicator of dispersal at local scales, while at larger scales, landscape heterogeneity is likely to influence population dynamics. Although average site synchrony is not related to population status, with no significant difference between levels of synchrony observed in extinct or currently occupied sites, population synchrony has much potential as a tool to investigate changes in site connectivity over time using pre-existing monitoring data. In addition, the assessment of within-site synchrony highlights potential barriers to dispersal and suggests that *B. euphrosyne* movement is likely to be less frequent between transect sections with contrasting habitat types.

Chapter 4 builds upon the previous chapters to investigate dispersal behaviour in *B. euphrosyne* using direct methods. The effects of flight related morphological traits and sex on individual dispersal propensity were assessed in a mark-release-recapture (MRR) field study. Wing aspect ratio and wing loading is positively related with displacement distance in *B. euphrosyne,* although this was only the case for individuals that were recaptured only once. Indeed, frequency of individual capture was shown to be positively related to wing aspect ratio and negatively related with displacement distance, which may confound general patterns between movement and wing morphology. While this variation may indicate sampling bias, it also suggests that there is intraspecific variation in flight capacity. Additionally, the MRR highlights the widespread distribution of *B. euphrosyne* across the study areas and likely metapopulation structure at this core site network in the Morecambe Bay region.

The influence of morphology on movement behaviour demonstrated in Chapter 4 suggests that if adaptive changes in dispersal behaviour, associated with population isolation, have occurred, they may have been accompanied by changes in flight-related morphological traits. Chapter 5 investigated this hypothesis, using morphological comparisons to assess changes in flight capacity following historical population declines. Morphometric comparisons between current populations and pinned museum specimens highlight potential adaptations associated with energy efficiency of flight in current populations, including declines in thorax size, longer forewings and lower wing loading.

6.3 Drivers of decline in *B. euphrosyne*

6.3.1 Habitat quality and environmental change

Habitats differ in their carrying capacities and vulnerability to environmental change. Species distribution modelling has previously highlighted that woodland cover is an important factor determining *B. euphrosyne* distribution in the UK (Ball, 2012). Woodland colonies are, however, particularly vulnerable to deterioration in habitat quality associated with ecological succession, and this thesis has highlighted additional pressures from elevated rainfall and nitrogen deposition that have potential to disproportionately affect species occupying woodland habitats (Ellis and Wainwright, 2008). Woodland systems have lower critical loads for nitrogen deposition, with nitrogen deposition levels in woodland systems higher than in open habitats, due to greater deposition velocities of nitrous gases and particulates onto tall vegetation (Rowe et al. 2019). The habitat-dependent nature of butterfly population declines have been noted previously (Ellis and Wainwright, 2008). In woodland habitats, lack of suitable management, including the abandonment of coppicing, has been reported as a leading cause of population decline as it leads to rides and open clearings becoming overgrown and unsuitable for breeding. Succession can also pose physical barriers to dispersal and may act to isolate remaining woodland colonies further. Indeed, prior studies have linked abundance trends in violet feeding fritillary butterflies in the Morecambe Bay region to woodland management activity, with stable population trends observed at actively managed sites in contrast to abundance declines of 74% at sites without active management (Ellis et al. 2012). Population declines of *B. euphrosyne* at grassland sites are generally associated with declining habitat quality, linked to changing management patterns (Ellis and Wainwright, 2008). For example, agricultural abandonment has seen a decline in active grazing of sites . Grazing is important both for limiting succession and for creating variation in sward structure, while trampling by cattle can help to break up dense bracken stands and reduce excessive build-up of standing trash.

While detailed assessments of habitat quality were beyond the remit of this thesis, habitat quality is undoubtably an important factor governing *B. euphrosyne* and other habitat specialist species. The ecology of the high brown fritillary, *Fabriciana adippe* is broadly similar to *B. euphrosyne;* both are violet feeding fritillaries, which have associations with bracken, *Pteridium aquilinum* (Ellis et al. 2019; Eeles, 2019). *Fabriciana adippe* has also faced strong population declines in the Morecambe Bay region, where declines of 92% between 1990 and 2017 have occurred, relative to declines of 85% across the rest of the UK (Ellis et al. 2019). One explanation for *F. adippe* declines in the Morecambe Bay region is deterioration in habitat condition. There is evidence for declines in *Viola sp.* cover, in addition to broad scale changes in habitat structure, with reductions in bracken litter and increased grass cover reported at several sites (Ellis et al. 2019). The reasons for *Viola sp.* decline are not clear, nitrogen deposition has been shown to affect insect herbivores via a range of mechanisms including impacts on host plant abundance and quality (Wallis De Vries and Van Sway, 2006; Habel et al. 2016; Zhu et al. 2016). This thesis suggests a potential influence of nitrogen deposition on *B. euphrosyne* populations in England and impacts on host plant abundance provide one plausible explanation. Indeed, the sensitivity of *Viola sp.* to nutrient enrichment lend weight to this hypothesis. *Boloria euphrosyne* larvae are reported to use common dog-violet, *Viola riviniana,* preferentially, although depending on habitat type and availability, heath dog-violet, *Viola canina*, and marsh violet, *Viola palustris*, are also used (Barnett and Warren, 1995). The Ellenberg N score (indicative of plant tolerance to soil fertility) for *V. riviniana* falls towards the mid-range at 4, while *V. canina* and *V. palustris* have scores of 2, demonstrating their sensitivity to nutrient enrichment (Hill et al. 1999c). Microclimate impacts provide a further possible mechanism for the detrimental effects of nitrogen deposition on *B. euphrosyne.* In particular, synergy between nitrogen deposition and changing climate has potential to impact populations via microclimate cooling, associated with increased grass cover and shading (Wallis De Vries and Van Sway, 2006; Roth et al. 2021). There is evidence that nitrogen enrichment alters sward structure and plant communities, and by stimulating an increase in overall biomass, acts to cool microclimates. Increased rainfall and longer growing seasons are also predicted to have similar effects on plant communities. As well as resulting in cooling of the microclimate, excessive grass growth is likely to suppress violet abundance.

This thesis highlighted the potential for habitat differences in population responses to nitrogen deposition and changing climate. In the future, the intensity of management may need to be altered where changing environmental conditions and longer winter growing seasons promote grass growth, for example, *P. aquilinum* management that is too frequent promotes increased grass cover (Ellis et al. 2019). Management approaches may include creating areas of suitable habitat at sites where variation in topography has potential to buffer populations against future environmental change. Butterflies use microclimate selection for thermoregulation, this behaviour highlights the importance of habitat heterogeneity for persistence (Kleckova et al. 2014; Bladon et al. 2020). In the face of climate and other environmental changes, traditional management techniques may need to be re-evaluated, as the focus of management must be on outcome. Novel approaches including the application of wood chips to sites to improve microclimate suitability for fritillaries have also been trialled in the Morecambe Bay area with some success (Firth, 2012). The importance of management outcome is also demonstrated by a study comparing butterfly species abundance between Morecambe Bay transect sites (Taylor et al. 2013). This study found that there was no significant difference in the abundance of UK Biodiversity Action Plan (UK BAP) fritillary species between sites where coppice management was used for conservation relative to commercially managed sites. As such, this study highlights the importance of woodland management for violet feeding fritillaries, even where sites are managed commercially rather than for conservation, and suggests that sustainable harvesting of wood-fuel has great potential to deliver conservation benefits.

6.3.2 Site connectivity and implications of isolation

In addition to the provision of high-quality breeding habitat, site connectivity is a key factor influencing species occupying fragmented landscapes. This thesis builds on previous research that has shown that following regional declines, core areas of habitat promote survival by support viable metapopulations (Thomas et al. 2008). Although specialist species tend to be considered to be less dispersive than generalists, long-distance dispersal can be essential in allowing species with a narrow ecological niche to access suitable habitat (Warren et al. 2001; Polic et al. 2021). By demonstrating that, even as a sedentary butterfly, site connectivity is likely to have important implications for *B. euphrosyne* persistence and dispersal, this thesis highlights the vulnerability of populations to isolation resulting from population declines across a landscape network. Site isolation can provide selection favouring dispersal, which may mitigate the negative effects of habitat loss (Hanski et al. 2017). However, fragmentation can also select for sedentary behaviour in stable populations or where the costs of dispersal are prohibitively high (Hanski, 2012). This thesis highlights variation in *B. euphrosyne* dispersal propensity and displacement distance based on morphology, supporting the conclusion that observed changes in morphology between butterflies from current populations and museum specimens, reflect altered dispersal capacity in a fragmented landscape. Assessments of local scale movement within sites, using both population synchrony and direct mark-release-recapture methods demonstrated that movement frequency declines with distance and that dispersal is unlikely between patches with contrasting habitat structure. The relationship between population synchrony and distance conforms well with displacement distances observed in the mark-release-recapture, demonstrating that movement frequency declines with distance and that dispersal is unlikely between patches with contrasting habitat structure. Population synchrony represents a promising method that could utilise existing monitoring data to aid the understanding of dispersal in fragmented landscapes. Understanding dispersal has particular relevance in the context of climate change, where ability to move between sites could allow range expansions and buffering against climate extremes (Hill et al. 1999d; Warren et al. 2001).

6.4 Implications for conservation

An 'extinction debt,' exists where perturbation causes extinctions which are non-immediate. As such, where extinction debts occur, current species distributions overestimate the likelihood of future persistence (Bulman et al. 2007). Management of small populations should focus on maintaining population abundance at levels above the extinction threshold, in addition to promoting emigration success (Bonsall et al. 2014). It is possible that in some landscapes *B. euphrosyne* may be in stages of an extinction debt. Dispersal-limited, specialist species are most likely to experience delayed extinctions, with the probability of an extinction debt occurring also tending to be highest in landscapes where large habitat patches and high connectivity buffer populations against immediate extinction in the event of perturbation (Kuussaari et al. 2009). However, the population response of *B. euphrosyne* at Whitbarrow in 2019 highlights the species resilience at Whitbarrow and the ability of these larger colonies to 'bounce back' in good years provides an optimistic outlook for the species in this region. Re-colonisations tend to occur in 'good' years where favourable weather conditions promote dispersal. The ability of populations to 'bounce back' is dependent on the carrying capacity of the landscape and population densities; populations persisting at low densities are less likely to be able to take advantage of favourable conditions and recover (Schmeller et al. 2011). In addition, management outcomes can be unpredictable, particularly in the context of environmental change, and even where management results are positive, population responses to management can be slow (Ellis et al. 2019).

A further explanation for the continued declines of *B. euphrosyne* in the UK, is an elevated extinction risk at low population densities, generated by Allee effects (Allee, 1931). In the Glanville fritillary, *Melitaea cinxia*, Allee effects have been shown to occur through increased emigration rate and reduced mating at low densities (Kuussaari et al. 1998). In *F. adippe,* 75% of UKBMS sites assessed between 1976 - 2010 were shown to be influenced by Allee effects, with 40% of these sites characterised as being isolated and displaying sink-type population dynamics (Bonsall et al. 2014). Moreover, *F. adippe* populations where abundance was lower than 500 individuals were suggested to be strongly affected by immigration and Allee effects (Bonsall et al. 2014). For *B. euphrosyne,* it appears that many of the remaining populations in England are concerningly small. Indeed, a mark-release-recapture study at Warton Crag in 2021 gave a peak population estimate of just 71 individuals, a value significantly below the suggested Allee threshold in *F. adippe* (Bonsall et al. 2014; Gage, 2021).

6.5 Future work

An alternative explanation for the reported failure of populations to respond to targeted management, is loss of genetic diversity and the reduced capacity of populations to adapt to a changing landscape following a bottleneck event (Botham et al. 2020a). The majority of the remaining populations of *B. euphrosyne* in Morecambe Bay have undergone bottlenecks at some stage. Historical specimens provide a valuable resource for studying past populations and genetic analysis to compare historical specimens with current populations would be an interesting avenue for future research, although this was sadly beyond the remit of this project.

Nitrogen deposition and the range of impacts on insect herbivores is an emerging research area, and one that is of particular relevance to Lepidoptera and Diptera, due to their low body tissue N:P ratio relative to other groups (Woods et al. 2004; Vogels et al. 2020). Given that the average levels of nitrogen deposition shown to impact *B. euphrosyne* in the current study were relatively low compared with levels reported in previous studies, it is likely that increased levels of nitrogen deposition will have negative effects on a range of species (Roth et al. 2021). Further research into likely mechanisms and potential mitigation strategies would be of great value.

Given the implications of a changing climate for habitat quality and the suggestion that management techniques may need to be adapted in the future, studies to trial alternative management approaches would also be of value.

6.6 Concluding remarks

Boloria euphrosyne represents a highly specialised butterfly with a narrow ecological niche. Several traits including limited dispersal capacity and univoltinism make this species highly vulnerable to habitat fragmentation and other modes of environmental change (Middleton-Welling et al. 2020). Although the butterfly has shown some positive responses to targeted management in England, in some areas, most notably the Morecambe Bay region, declines have continued (Ellis et al. 2012; Botham et al. 2020a). Previous studies have highlighted the crucial role that woodland management plays on population persistence in early successional species and this thesis highlights that woodland habitats are likely to face additional pressures from environmental change (Ellis et al 2012; Taylor et al. 2013). Site connectivity and maintaining population abundance above an Allee threshold is crucial to ensure the long-term viability of small populations and those occupying fragmented landscapes (Bonsall et al. 2014).

6.7 Supporting Information

Table S6.1. Morecambe Bay key site details. Site details of 15 key UKBMS butterfly transects recently occupied by Boloria euphrosyne in the Arnside and Silverdale Network and Whitbarrow Network. Site Numbers correspond to Figure 6.1. Classifications of habitat type were made based on the dominant and secondary habitats present at the site level using the 2015 Land Cover Map (Rowland et al. 2017).

Site Number	Transect Name	Grid Reference	Number of Sections	Transect Length (m)	Dominant Habitat Type	Secondary Habitat Type
1	Whitbarrow - Howe Ridding NNR	SD434881	15	1410	Broadleaved Woodland	NA
2	Whitbarrow North (Site C)	SD44X88X	15	2870	Broadleaved Woodland	Acid Grassland
3	Whitbarrow NNR - Hervey CWT	SD441869	11	4000	Acid Grassland	Broadleaved Woodland
4	Whitbarrow - Wakebarow	SD450870	15	3505	Broadleaved Woodland	NA
5	Whitbarrow NNR - Farrer's Allotment	SD452855	15	4100	Acid Grassland	Broadleaved Woodland
6	Heathwaite NT	SD448768	7	3500	Broadleaved Woodland	Calcareous Grassland
7	Arnside Knott NT	SD453773	12	4700	Broadleaved Woodland	NA
8	Eaves Wood NT	SD466762	11	4500	Broadleaved Woodland	NA
9	Gait Barrows	SD477771	9	1400	Broadleaved Woodland	NA
10	Gait Barrows NNR (Warden's)	SD481773	15	1900	Broadleaved Woodland	Arable
11	Leighton Moss	SD487760	15	5922	Broadleaved Woodland	Improved Grassland
12	Yealand Hall Allotment	SD489762	14	4000	Broadleaved Woodland	NA
13	Warton Crag RSPB	SD489727	7	2800	Broadleaved Woodland	NA
14	Warton Crag LWT	SD493730	15	5400	Broadleaved Woodland	NA
15	Warton Crag LNR	SD494726	15	3181	Broadleaved Woodland	Calcareous Grassland

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