The ecology and conservation of endangered saproxylic hoverflies (Diptera, Syrphidae) in Scotland

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Summary

Hoverflies are important for their roles in ecological and environmental services, and are also charismatic species of conservation interest in their own right. Almost half of all hoverflies are saprophages, which are organisms that feed on dead or decaying organic matter, and these include saproxylic species that depend on deadwood. Deadwood and its associated community are a rich source of forest biodiversity and are fundamental to forest function, but due to poor management, many saproxylics are threatened or endangered, and techniques for conserving saproxylic species are poorly developed. In this thesis I study the ecology and conservation management of an endangered UK saproxylic fly, the Pine hoverfly, *Blera fallax* (Linnaeus) (Diptera, Syrphidae) and the dispersal ability of the similarly endangered Aspen hoverfly, *Hammerschmidtia ferruginea* (Fallén) (Diptera, Syrphidae). My main goals were to clarify methods to support their recovery in active programmes of species conservation in Scotland, UK. For *B. fallax,* this included experimenting with habitat creation techniques, investigating the best conditions for larval growth and assessing competition effects. In addition, I evaluated the genetic variability of the remaining population in Scotland by comparing it with one in Europe to determine whether genetic constraints may limit recovery. For *H. ferruginea,* I determined dispersal ability with field experiments involving mark and recapture techniques.

By cutting holes at the surface of stumps of *Pinus sylvestris*, breeding habitat was created artificially for *B. fallax* at the remaining known locality for this species in the UK. Over 4 years, 81 % of holes were colonized by *B. fallax*, and by up to six

other saproxylic syrphid species. The most successful holes were those cut into the heartwood, seeded with pine chips and sawdust and partially covered, as indicated by a combination of field occupancy monitoring and lab growth experiments. Observations of larval morphology and behaviour within rot holes revealed specializations that largely segregate the species in both time and space, and may mitigate interspecific competition between *B. fallax* and three more common syrphid species. I further demonstrated that *B. fallax* has a life history that features facultative semivoltine development, which may be a bet-hedging strategy to cope with fluctuating levels of larval food. Fifty *B. fallax* larvae were successfully reared and bred in captivity and from these, 430 descendent laboratory reared larvae and adults were released across three relocation sites. After initial success at the first re-location site when a new generation of larvae appeared in holes in 2010, a population crash at all sites occurred in the following year, possibly caused by adverse weather conditions. This disappointing result highlights the vulnerability of small populations to stochastic events, and means that survival of *B. fallax* may now depend on those larvae that are semivoltine, supplemented by animals currently being reared in captivity. My genetic analyses revealed similarly troubling information that highlights the precarious existence of *B. fallax* in Scotland: compared with a population in Sweden, Scottish *B. fallax* had significant less neutral genetic variation, and showed signs of a recent and severe bottleneck that reduced the effective population size to just 12 (CI: 0 - 266) individuals at some point in the last 200 years. Mindful of these challenges, I exploit my new data on the ecology and life history of *B. fallax* and combine it with techniques for captive rearing and for monitoring the genetic health of *B. fallax* into specific protocols and general prescriptions for the on-going recovery and management of this species.

In order to assess the dispersal ability of *H. ferruginea* (and therefore its potential for recolonizing newly created habitat), in May to July over two years, adults were marked and released from a central point and subsequently monitored at the breeding site, decaying aspen wood *Populus tremula*, where adults tend to assemble for mating and oviposition. Adults were resighted visiting logs of decaying aspen set out at 1 km intervals along transects up to 7 km away. Up to 10 % of released individuals were resighted up to 5 km from the central release point. Most dispersing individuals (68 %) were resighted at 1 km, which I propose as the optimal distance for managing aspen for this species.

Both of these hoverflies are case studies of techniques for recovering endangered saproxylic flies. Overall, my findings greatly increase fundamental knowledge of the ecology and natural history of these flies, and clarify some of the practical approaches that will be required in their conservation.

Declaration

I hereby declare that this thesis has been composed by myself and that it embodies the results of my own research. Where appropriate, I have acknowledged the nature and extent of work carried out in collaboration with others.

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Ellen Louise Rotheray

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"But more than roles and research projects, is the fact that in taking a little time to become acquainted with hoverflies, is invariably to become smitten by them"

Rotheray and Gilbert 2011

Chapter 1

Introduction

1.1 Insect conservation

Possibly the most diverse group of organisms on Earth are the insects, comprising half of the world's described species (Primack 1998; Cardoso *et al*. 2011), so conserving insects is fundamental to maintaining biodiversity (Dunn 2005). Projections based on better-known taxa estimate that 40,000 insects have gone extinct in the last 600 years (Dunn 2005). Their specialisations and complex ecological associations make them particularly vulnerable to habitat fragmentation and land-use changes (Samways 1994). Their importance in ecosystem functioning is widely recognised and yet despite this, insects have been neglected in conservation studies worldwide, often due to a lack of basic knowledge of their biology and distribution (Dunn 2005; Balvanera *et al*. 2006; Samways 2006; Cardoso *et al*. 2011). Conservation efforts often rely on shortcuts for the maintenance of biodiversity and to monitor conservation problems such as umbrella species (species whose conservation confers protection to a large number of naturally co-occurring species), and indicator species (used to assess the condition of a particular habitat or ecosystem; Caro & O'Doherty 1999; Roberge & Angelstam 2004). While broad scale conservation management can be more efficient and economical, it can overlook critical small-scale habitats and special features. Due to scale, efficiency and tight budgets, there is an argument for broad conservation management using these surrogates (Simberloff 1998; Samways 2007). However, specialist and rare species are often over looked, and their specific needs are not provided for. In order to conserve these most threatened species, we need to focus investigation on their specific requirements and target habitat management accordingly.

While conservation objectives often favour more popular, charismatic species (Martín-López *et al*. 2009) this trend is gradually changing, with current on-going conservation management work focusing on several insect groups including butterflies (Thomas *et al*. 2009, 2011), and bumblebees (Goulson *et al*. 2011), and species-specific interventions such as relocation projects for the field cricket (*Gryllus campestris* (Orthoptera, Gryllidae)) (Hochkirch *et al*. 2007), the mottled grasshopper (*Myrmeleotettix maculatus* (Orthoptera, Acrididae)) (Gardiner 2010) and the southern damselfly (*Coenagrion mercuriale* (Odonata, Coenagrionidae)) (Purse 2002). Insects have been proposed as effective flagship species (taxa used to generate public support and funding for conservation goals) for particular habitats and for conservation in general (Caro & O'Doherty 1999; Guiney & Oberhauser 2009). These insect-focused conservation projects are still in the minority however, especially in terms of funding and resource allocation. Most of the current work relies heavily on charitable organisations such as Butterfly Conservation and the Bumblebee Conservation Trust raising the profile of these species groups. Very little conservation effort has been directed at hoverflies, a little known and often misunderstood group of insects. Why should we want or need to conserve hoverflies?

1.2 The importance of hoverflies

Diptera constitute the third most diverse order of insects, with an estimated 120,000 species, and hoverflies are one of the largest families of Diptera with more than 6,000 species worldwide (Gilbert *et al*. 1994; Rotheray & Gilbert 2011). There are 56 UK Red Data Book listed hoverflies, and seven have UK Biodiversity Action

Plans (Stubbs & Falk 2002; Ball *et al*. 2011). These plans highlight the need for focused investigation into unknown details of their natural history, especially concerning their life cycles, breeding sites and larval stages, behaviours and requirements (Drake & Baldock 2005).

Hoverflies provide essential ecological and environmental services such as the pollination of a variety of plants in both natural and agricultural systems, predation of aphids, control of invasive weeds, and recycling of wastes (Rotheray and Gilbert 2011). They are among the most frequent insect visitors to flowers, and visit a wide range of species (Gilbert 1980, 1981, 1985). They are known to pollinate up to 30 leading crops and cultivated plants (Ball *et al*. 2011; Rotheray & Gilbert 2011). In light of recent declines in bee species, hoverflies are becoming increasingly important pollinators (Biesmeijer *et al*. 2006; Jauker *et al*. 2009, 2011). They also form a vital resource for many kinds of predators, from invertebrates such as beetles, spiders, wasps and ants, to birds and mammals (Rotheray and Gilbert 2011). In contrast to the generally uniform feeding habits of adults, the larvae have a great diversity of form, feeding mode and place of development; from predation on aphids, ants, and bee and wasp larvae, to feeding on plants including invasive weeds, and organic decaying matter (Owen 1981; Gilbert 1986; Schönrogge *et al*. 2002).

Due to their diverse preferences and tolerances, species assemblages are characteristic of particular habitats and environmental conditions and therefore they can be used as indicators of site quality, and monitoring effects of environmental and landscape-level change (Sommaggio 1999; Burgio & Sommaggio 2007). Lists of hoverfly species have been drawn up as indicators of ancient woodlands, forming

groups that describe the condition of a woodland based on their abundance and species richness, aided by relatively easy identification and sampling (Stubbs 1982; Speight 1986; Maleque *et al*. 2009). Most hoverflies are specialists and many are geographically endemic and are therefore of greater biodiversity significance (Primack 1998; Rotheray & Gilbert 2011).

Almost half of all hoverflies are saprophages (Gilbert 1994). These are species that play pivotal roles in the decomposition and recycling of a vast range of materials from decaying vegetation in lakes, rivers and rot holes, to compost, dung and dead wood (Gilbert 1986). They break down waste from agricultural and industrial processes, which opens up possibilities for their commercial use. They can be found from coasts to mountaintops, in tropical and temperate forests, in grasslands and savannahs and even deserts (Rotheray & Gilbert 2011). Because of their often-striking colours, large size, stable taxonomy and recognisable hovering behaviour, hoverflies are a good flagship group for saproxylic species.

1.3 Saproxylics

Saproxylic organisms are a diverse group that includes invertebrates, fungi and micro-organisms that recycle minerals and nutrients and are part of a complex, and often specialised, community of decomposers (Grove 2002). Deadwood is a highly biodiverse, species rich resource (Carey 1989; Peterken *et al*. 1992; Humphrey 2005; Humphrey *et al*. 2005); in Scandinavia it is estimated that up to 7,000 species depend on deadwood (Marchetti 2005). In Britain, an estimated 6 % of the entire invertebrate fauna exclusively depend on saproxylic organisms or decaying wood

(Butler *et al*. 2002; Lassauce *et al*. 2011). Deadwood and its dependent community are recognised as fundamental to forest function through critical processes such as nutrient cycling (Butler *et al*. 2002; Grove 2002; Jonsson *et al*. 2005; Schmuki *et al*. 2006). Due to the specialised fauna and high number of associated threatened species, saproxylics can be used as bio-indicators of site quality, and thus indicators of forests of conservation importance (Speight 1989).

Less intensive forest management is better for saproxylics (Grove & Tucker 2000; Grove 2002). Recent research has highlighted the sensitivity of saproxylic species where in managed forests, fewer individuals and species are found compared to old growth or primary forests (Grove 2002; Humphrey 2005). Saproxylic organisms have suffered from unsympathetic forest management involving the removal of dead trees, branches and stumps that can obstruct forest operations, and are thought to be potential sources of outbreaks of pest species (Schiegg 2001). A large number of studies have highlighted the difficulties in prescribing management that meets the requirements of all saproxylic species (Jonsell *et al*. 2003; Jonsson *et al*. 2005; Davies *et al*. 2008; Lassauce *et al*. 2011). This is broadly due to a dearth of knowledge about their conservation needs, the dependence of species groups on different age and decay structures of decaying wood, and often complicated ecological relationships (Rotheray & MacGowan 2000; Yee *et al*. 2004; Smith *et al*. 2009; Weslien *et al*. 2011).

Understanding the dispersal abilities of species of conservation concern is critical, for this will determine the likelihood that they colonize any suitable habitat that is created for them. While many species have reportedly low powers of dispersal

relative to human-induced habitat fragmentation (Grove 2002; Ranius *et al*. 2011), little is known of the capability of saproxylic species to colonise new areas and compensate for local extinctions. A number of techniques have been used to determine the dispersal abilities of saproxylic beetles (Jonsson 2002; Ranius 2006; Svensson *et al*. 2011). These have mainly used mark recapture and telemetry methods, and genetic studies have complemented findings (Jonsson *et al*. 2003; Ranius 2006; Schmuki *et al*. 2006). However, few studies have measured the dispersal ability of saproxylic flies.

1.4 Conservation management and the case study of two hoverflies

Conservation management is the practical approach to preventing extinction of species, and if possible, reintegrating them into properly functioning ecosystems (Primack 1998). This is achieved through gathering information on population biology, ecology, and genetics and determining the best strategies for protecting rare species and designing the best recovery programmes.

This thesis empirically analysed several aspects of the conservation biology of two endangered saproxylic hoverflies in Scotland: the Pine hoverfly *Blera fallax* (Linnaeus) and the Aspen hoverfly *Hammerschmidtia ferruginea* (Fallén). These species have been the focus of survey and management work since the early 1990s (Rotheray & MacGowan 2000; Rotheray *et al*. 2001). They both meet criteria as indicator species as they are associated with a tree species characteristic of a particular woodland type (native boreal forests of the northern hemisphere), they are dependent on dead wood and are excessively localised (Speight 1989). They are

relatively large making them easy to find and recognise, and therefore are also appropriate as flagship species for their breeding habitat. Both species have recently been proposed for addition to Schedule 5 of the Wildlife and Countryside Act, 1981 (Ball *et al*. 2011).

The main motivation of this study was to fill the gaps in our knowledge of these species, in particular to identify factors affecting growth, survival, and recruitment, and potentially limiting the recovery of populations in Scotland. Successful techniques have been devised to investigate the requirements and habitat preferences of *H. ferruginea* and these have assisted conservation protocols (Rotheray *et al*. 2009). Similar techniques were required for *B. fallax*, in which the majority of efforts were focused.

1.5 The pine hoverfly Blera fallax

The Pine hoverfly *Blera fallax* is listed in the UK Red Data Book as category 1 (endangered) and it is a UK Biodiversity Action Plan priority species. This status was confirmed in 1999 after an extensive 12-year survey, which indicated a decline from 8 to 2 populations since the early 1900's mainly due to loss of habitat and changes in forestry management (Rotheray & MacGowan 2000). In 2007, *B. fallax* was included in the Species Action Framework (SAF), a Scottish Natural Heritage (SNH) initiative (Scottish Natural Heritage 2007), which focuses on funding projects to improve the status of species deemed significant to overall Scottish biodiversity. This species is arguably Britain's rarest resident insect.

Blera fallax biology

Blera fallax larvae filter-feed microbes in rot holes occurring in decaying roots and holes at the surface of stumps of Scots Pine, *Pinus sylvestris* L. In Scotland, *B. fallax* has not been found associated with any other tree species, however in Scandinavia it has been found in Norway Spruce *Picea abies* (Speight 2008). The larvae have an extended anal segment for respiration under water which gives them their common name of rat-tailed maggot or long-tailed larvae (Rotheray 1993). The complex mouthparts consist of a pump in the head skeleton and various filters that suck in fluid and filter out microbes, which are the source of food (Hartley 1963; Roberts 1970; Rotheray 1993). Larvae have a pair of spiracles at the front of the body, thought only to play a minor role in gas-exchange, and a coating of protective spicules across the front of the thorax, which may also assist in loosening microbes in the substrate (Rotheray & Gilbert 1999; Rotheray 1993). Electron micrographs of these filters and microbial cultures made from samples taken within the gut of closely related species indicate that larvae feed on bacteria and microbes of ~0.1μm (Mahmoud 1999). *Blera fallax* larvae are almost translucent at first but become opaque with accumulating white fat as they grow. Fat seems to be required for overwinter survival and pupation in the spring. *Blera fallax* adults are sexually dimorphic in colour pattern, and they have been observed feeding on wild raspberry (Stubbs & Falk 2002).

Blera fallax distribution and habitat decline

Blera fallax is found across the Palearctic as far east as Japan and south as the Pyrenees (Speight 2008). Based only on scant, intermittent records, it is considered locally rare or declining wherever it has been recorded (Speight 2008). Declines in Scotland are probably associated with changing forestry practices in Scotland. Since the mid 19th century, monoculture conifer plantations have expanded, reducing the size and age structure in woodlands to meet prime cultivation standards. Stumps are also often destroyed by forestry operations, removed for re-seeding, and sometimes used for biofuel (Walmsley & Godbold 2010).

Blera fallax as an umbrella species

Conserving *B. fallax* may confer protection to a large number of naturally co-occurring species. In Scotland, *B. fallax* shares its habitat with at least 30 endangered taxa from several groups including Diptera (Rotheray *et al*. 2001), parasitic Hymenoptera and Coleoptera (Alexander 1988; Butler *et al*. 2002). The pine stump habitat is not only important for rot hole dwelling species. The larvae of *Xylota jakutorum* (Diptera, Syrphidae) are known to develop in the borings of pine weevils (*Hylobius abietus*) in conifer stumps (Rotheray & Stuke 1998). Others include the hoverfly *Microdon mutabilis* (Diptera, Syrphidae) and their associated *Formica lemani* ant colonies, mason bees such as *Osmia unciniata*, the UK Biodiversity Action Plan species twinflower *Linnaea borealis*, and a range of lesser-known wood decaying fungi, lichen, mosses and bryophytes (Lonsdale *et al* 2008). By conserving *B. fallax*, other species in this rich community should also benefit.

1.6 Main objectives for B. fallax

Captive breeding and relocation

Due to the small number individuals that could only be found in one site at the beginning of this study, the decision was made to captive breed *B. fallax* in order to re-populate previously occupied sites. This involved meeting and discussing site creation possibilities with local foresters and land managers where habitat was deemed suitable enough for relocation i.e. pine trees were of a suitable size and age structure, and adult food plants were available. Three sites were selected based on these specifications, as well as a long-term commitment to monitoring and supplementation of breeding habitat by the owners. Relocations to each of these sites were planned in successive years, in accordance with Actions drawn up under the SNH SAF (Appendix 5.2).

Habitat creation

Experimental habitat creation for this species had not previously been attempted, but was clearly necessary at both extant sites and in sites where reintroductions were planned. Experimental techniques involved boring holes into stumps, either left after felling or newly felled, and then filling them with wood chips or sawdust.

Captive rearing larvae

At the same time as creating habitat for *B. fallax*, we needed to develop methods for rearing larvae ex-situ. Therefore, we formulated various larval growth experiments investigating the effects of substrate size, volume, and inter-specific competition, in addition to investigating whether *B. fallax* larvae can develop in other host tree species. The results from this work not only provide insight into the life history strategies of *B. fallax*, but may also identify sensitive stages in development that may require special or specific management.

Competition and co-existence

Understanding the ecology of species interactions and coexistence can clarify factors that may influence declining populations. Competitive exclusion may limit the success of relocations so understanding potential conflicts is critical. *Blera fallax* shares its rot hole habitat with eight other species of saprophagous fly. Occupancy is dominated by three more commonly found hoverflies including *Callicera rufa* (Schummel), *Myathropa florea* (Linnaeus) and *Sphegina clunipes* (Fallén) (Diptera, Syrphidae). These species differ in their morphology chiefly by the length of their posterior breathing tube, which may underpin resource partitioning by defining the depth that each species tends to occupy within a rot hole.

Myathropa florea may be the main competitor with *B. fallax*. It is widespread across the British Isles (Stubbs & Falk 2002) and can be found in a wide range of rot holes or water-filled crevices in both coniferous and deciduous trees (Rotheray 1993).

Callicera rufa is found in coniferous tree-holes, mainly spruce *Picea abies*, larch *Larix decidua* and pine (Stubbs & Falk 2002). Although *C. rufa* is fairly widespread, and has even recently been recorded in England (Shropshire and Nottinghamshire) (Nigel Jones pers. comm.), it is largely restricted to Caledonian pine woodland in the Scottish Highlands (MacGowan 1994; Rotheray *et al*. 2001). *Sphegina clunipes* is a common species, widespread across the British Isles as far north as Sutherland (Stubbs & Falk 2002). It is normally associated with deciduous woodlands, and most often found in decaying sap under bark (Rotheray 1993; Stubbs & Falk 2002), but has recently been found in numerous artificial pine rot holes.

In order to determine species-specific microhabitat use, laboratory observations on the four species in artificial rot holes were carried out to determine functional differences in relation to their characteristic morphological features.

Conservation genetics

Where captive breeding and translocation play a role in management protocols, inbreeding effects and effective population size become particularly relevant issues (Leberg 2005). Small populations are at risk of loss of genetic diversity through drift, inbreeding depression, and reduced adaptive potential (Frankham 1998). Reduced fitness caused by inbreeding has been demonstrated in numerous controlled experiments (Armbruster *et al*. 2000; Woodworth *et al*. 2002; Whitehorn *et al*. 2010) and in studies on wild populations (Brown & Brown 1998; Keller 1998; Saccheri *et al*. 1998). While the effects on different taxa and individual populations appear to vary (Elgar & Clode 2001), especially with respect to demographic and

environmental stochasticity, inbreeding depression is considered pervasive enough to have a generally detrimental effect on population persistence (Keller & Waller 2002).

We investigated effective population size and genetic diversity of the single remaining population in Scotland, to assess the signs of recent demographic changes such as a population bottleneck. We sought to place the genetic diversity of Scottish *B. fallax* in context by comparing it with Swedish samples, which were considered to be from a more genetically 'healthy' population. We were able to do this by using material from adults that died in captivity in the course of our captive breeding trials. The genetic data will also help to assess the feasibility of translocation and captive breeding from elsewhere in Europe if a genetic 'rescue' attempt is necessary. In addition, we investigated non-invasive techniques for extracting DNA in order to facilitate population monitoring for future conservation efforts without the risk of harming individuals.

1.7 The aspen hoverfly Hammerschmidtia ferruginea

The Aspen hoverfly *Hammerschmidtia ferruginea* is listed in the UK Red Data Book as a category 1 (endangered) species, and it is included in the UK Biodiversity Action Plan (UKBAP). Since 1999 the number of UK sites occupied by *H. ferruginea* decreased from 15 to 8 thought mainly due to loss or reduction of breeding habitat (Rotheray *et al*. 2009).

Hammerschmidtia ferruginea biology

Hammerschmidtia ferruginea is a specialist saprophage depending on a rare and temporary resource: decaying cambial layers under bark of dead aspen *Populus tremula* L. (Salicaceae). Previous attempts at utilising closely related Black poplar *Populus nigra* were unsuccessful, perhaps because of the depth or width of cambium rot (I. MacGowan pers. comm.). Over 200 larvae can occupy and emerge from a ~3-metre long *P. tremula* log in one year (Rotheray *et al*. 2009). Adults feed on rowan, bird cherry and hawthorn, and can locate breeding habitat up to 1 km from the emergence site (Rotheray *et al*. 2009). Males gather on logs and defend territories, and females visit logs to mate and oviposit eggs (Rotheray *et al*. 2009).

Hammerschmidtia ferruginea distribution and habitat decline

Hammerschmidtia ferruginea has a Holarctic distribution from Alaska to Japan, however it is locally rare (Speight 1989, 2008). In Continental Europe, *H. ferruginea* face different circumstances than in Britain as trees are taller, and thicker, and deadwood usually becomes available when they reach their natural age limit, rather than mainly due to intermittent stormy conditions in Scotland (Rotheray *et al*. 2009). From the time a tree falls or a branch breaks off, it can take up to two years for the cambial layers to become suitable for larval development and, depending on size and location, a piece of wood with cambial decay can last from just one to three years before the bark cracks and the decay dries out (Rotheray *et al*. 2009). Trees in Europe probably have a longer decay period due to their size. Aspen is threatened across Europe (Kouki *et al*. 2004), and in the UK there are few aspen woodlands

large enough (>100 trees) to maintain a constant input of dead wood. This means that intervention is required in Scotland to ensure a continuity of suitable deadwood to sustain remaining populations of *H. ferruginea*.

Hammerschmidtia ferruginea as an umbrella species

In Scotland, *H. ferruginea* is considered an umbrella species for a group of 13 other rare and similarly endangered Diptera dependent on aspen (Rotheray 2001). Aspen is an important element of the boreal habitat, which harbours a multitude of organisms that depend on different parts and stages of the aspen lifecycle, and therefore many species are threatened by its decline. The animals that depend on it are typically either herbivorous or saproxylics (Kouki 2008). These include aspen specialist fungi, lichens, moths, beetles and flies (Cosgrove *et al*. 2005). Seventeen moths predominantly depend on aspen in Scotland, including the UK BAP species the Dark Bordered Beauty *Epione vespertaria* (Leptidoptera, Geometridae). Aspen is capable of seeded growth, but it most often exhibits vegetative reproduction, where one parent plant has many suckers growing off roots. Aspen stands are diminishing, and those that remain are small and isolated. Grazing of palatable suckers, chiefly by deer, hampers expansion and regrowth efforts in the UK.

Main objectives for H. ferruginea

Dispersal

Nothing is known of the dispersal capability of *H. ferruginea*. The remaining localities for this species are centred in Strathspey, Scotland, where they are separated by a minimum of 5 km (Rotheray *et al*. 2009). It is unknown whether *H. ferruginea* are able to disperse such distances, and if not, management ought to consider providing linking pockets of breeding habitat. Therefore, two years of this study involved setting up mark and recapture experiments to determine how far *H. ferruginea* is capable of dispersing and detecting breeding habitat. In addition, observations were made of the behaviour of territorial males and ovipositing females on logs in order to investigate mate-seeking requirements and colonisation ability.

1.8 Summary of aims

The main aim of this study was to determine conservation and recovery requirements of two endangered saproxylic hoverflies in Scotland. This involved developing techniques to find the answers to the following main questions:

1. How does the rot hole substrate and competitive environment affect larval growth in *B. fallax*, and what are the consequences of unfavourable larval growth conditions for adult size (Chapter 2, 3 and 5)?
2. Is interspecific competition for resources in a rot hole likely to affect the recovery of *B. fallax* (Chapter 3)?
3. What is the genetic variation of the Scottish *B. fallax* population compared to Swedish flies, and might genetic constraints limit the recovery of this species (Chapter 4)?
4. What is the dispersive potential of *H. ferruginea* and how will this affect its conservation (Chapter 6)?

Chapter 2

Growth, development and life-history strategies in an unpredictable environment: case study of a rare hoverfly *Blera fallax* (Diptera, Syrphidae)

2.1 Abstract

The development of holometabolous insects varies in response to fluctuating environments. Adult characteristics are strongly influenced by larval conditions, and variation in phenotypic traits, such as size and development time, depends on available resources. The main aim of this study was to investigate the developmental requirements and phenotypic plasticity of the endangered pine hoverfly *Blera fallax* (Linnaeus) (Diptera, Syrphidae). *Blera fallax* is dependent on a scarce and ephemeral habitat in Scotland, Scots pine *Pinus sylvestris* L stump rot holes. Conservation management for this species involves creating habitat to expand existing populations, creating new areas for relocations, and captive breeding and rearing larvae to boost populations and provide material for relocations. In order to do this effectively, we need to determine what conditions best promote larval growth, identify the level of plasticity in larval and adult characteristics in potentially food-limited conditions, and establish ecological requirements of this species at the larval stage to guide research and management efforts. We manipulated the pine substrate volume, wood chip size and host species, and investigated intraspecific competition by increasing larval density in artificial larval microcosms. We assessed ontogeny by measuring growth rate, fat deposition, survival, time to maturation and male and female adult thorax and wing length. Individuals in low resource conditions not only took longer to develop but also had reduced thorax and wing lengths. Females were larger but had more variable size at maturity compared with males. Males typically did not emerge smaller in resource-limited conditions, presumably at the expense of developmental rate. In fact, males seemed more likely to become semivoltine, extending development time to achieve

similar sizes irrespective of environmental resource availability. The growth curve observed in the field was most similar to that in resource-limited conditions in the lab, suggesting that resources are limiting in nature. Between 2 and 20 % of larvae extended development over two years regardless of growth conditions, perhaps indicating a strategy to circumvent extinction during years with very low breeding success. We discuss the implications of our results for optimising captive breeding conditions for this species, and for creation of artificial rot hole habitat in the field.

2.2 Introduction

Unpredictability in food quantity and quality is a reality for many organisms and life-history characteristics can vary in response (Blanckenhorn 1998; Shertzer & Ellner 2002; Skalski *et al*. 2005; Hou *et al*. 2011). Organisms can regulate physiological processes over a wide range of environmental conditions (Sweeney & Vannote 1978; Taylor 1981; Schmidt-Nielsen 1997; Blanckenhorn 1999), and often demonstrate phenotypic plasticity, which consists of changes in an organism's phenotype in response to changes in the environment. Although some of the phenotypic variation induced by the environment can be non-adaptive, such as reduced overall size when food limitation impedes development, other aspects of development, including the resolution of trade-offs between growth and adult longevity, are probably adaptive, i.e. the phenotypic response is moulded by selection (Stillwell *et al*. 2010).

In holometabolous insects, most of the growth (which combines the rate of increase in mass and timing of maturation) occurs in larval form, a specialized life stage for accumulating resources (Abrams *et al*. 1996). Development time depends strongly on resource availability, and typically involves a trade-off between two important aspects of life history: spending more time in the feeding larval stage can increase adult size (which may in turn have beneficial consequences for mating success or fecundity; Roff 1992; Stearns 1992; Esperk & Tammaru 2004; Dmitriew 2011), but the extended developmental period will usually either restrict adult longevity (e.g., in seasonal environments) or increase generation time. Longer development may also increase exposure to predation or parasitism. Often size is more important than

the length of time it takes to develop, so many animals delay sexual maturation to increase in size despite the entailed costs (Policansky 1983; Reznick 1990). Although organisms vary in the mechanisms by which the trade-offs between growth and other life history traits are governed, in seasonal environments many invertebrates have a diapause stage in which growth is temporarily arrested to allow overwintering or aestivation (Smith & Smith 2006). As both diapause and developmental life history decisions are likely to be governed by environmental cues, we predict that diapause is one of the key life history traits that may show plasticity in response to growing conditions (Abrams *et al*. 1996).

In the context of studying adaptive plasticity across life history traits in response to food limitation, we investigated growth in the larval stage of the endangered pine hoverfly *Blera fallax* (Diptera, Syrphidae). Conservation management for this species involves creating breeding habitat at new locations and relocating individuals by captive breeding and rearing techniques (Chapter 5). In order to do this effectively, we need to know how to adjust conditions to enhance the fitness of individuals and populations. Therefore, we investigated the growth trajectories of larvae in response to several experimentally manipulated conditions (including characteristics of the larval substrate and the presence and intensity of intraspecific competition). Our findings may help identify aspects of the environment that are amenable to conservation management, and which have measurable effects on the fitness of developing hoverflies.

Natural history of B. fallax

Blera fallax is a specialist saproxylic insect; the larval stage develops on microbes in rot holes occurring in stumps and decaying roots of Scots Pine, *Pinus sylvestris* L. This microhabitat develops due to heart-rot fungi, chiefly *Phaeolus schweinitzi*, softening heartwood that is often exposed when the tree falls. A cavity then forms in the heartwood, which fills with rainwater and microbes. Rot holes also form in the exposed surface of stumps left behind after felling in pinewood plantations. After up to nine years, stumps decay and lose their capacity for water retention, and this occurs at a faster rate in smaller stumps (Graham E. Rotheray pers. comm.). As forestry practices have changed, stumps have become smaller, or have been removed completely for re-seeding, resulting in a reduction in new breeding habitat for *B. fallax*. This is probably the reason for the decline in Scottish populations from five to one in the last 20 years (Rotheray & MacGowan 2000; Chapter 5).

Due to the nature of their formation, rot holes are heterogeneous, varying in width and depth, host tree, location and content (Kitching 1971; Fisher *et al*. 1990; Sota *et al*. 1994; Yanoviak 1999; Paradise 2004; Bell *et al*. 2005). They contain a mass of decaying detritus, which is variable in nutrients, chemistry, fungi, bacteria and protozoa (Walker *et al*. 1991). The type and location of the substrate within rot holes can have significant effects on the fitness of organisms that feed in them (Fish & Carpenter 1982; Fisher *et al*. 1990; Walker *et al*. 1991; Srivastava & Lawton 1998; Kaufman *et al*. 2008). As part of developing techniques for artificial creation

of rot holes, it is important to investigate how rot hole conditions affect larval growth.

Rot holes often differ in quality according to the number of occupants. Through intermittent survey work over the past eight years, *B. fallax* larvae have been found at various developmental stages throughout the year, with from one to thirty individuals sharing a rot hole (Rotheray *et al*. 2001). In other Dipteran species inhabiting rot holes, the effects of competition are moderated because of Allee effects: larval activity may promote bacterial growth through grazing, which means that larvae with low densities of interspecific competitors outperform those living in isolation (Walker *et al*. 1991; van de Bund *et al*. 1994; Kaufman *et al*. 1999; Graca *et al*. 2000). However, most studies report competition for resources among filter feeding larvae (Livdahl 1982; Fisher *et al*. 1990; Broberg & Bradshaw 1995; Knight *et al*. 2004). This may place selection on ovipositing females, who must strategically spread their eggs among resource patches in a way that promotes development and avoids intraspecific competition for limited resources.

In the current study, our main questions concern how substrate conditions affect larval development, and the relative importance of intraspecific competition versus Allee effects on larval fitness. To answer these questions we altered the growing conditions for larvae and measured the response in larval growth, fat deposition, larval survival, development time, and pupal and adult size. Furthermore, because *B. fallax* depends on a fluctuating and heterogeneous resource, we may expect this species to adjust its life history strategy according to the available resources. We expect that larval growth will increase with increased substrate surface area to water

volume (which may promote microbe density). We further predict that intraspecific competition will affect larval growth in limited resource conditions, but will not be as important when microbes are less limiting, and if Allee effects are apparent. Finally, we expect larvae in poorer conditions to develop for longer but emerge at comparable sizes to those in high resources, especially in males, due to presumed advantages of achieving large size over early emergence (see Chapter 5).

2.3 Methods

We subjected larvae to several experimental conditions and monitored growth and survival at equal time intervals from the onset of each experiment until eclosion, and measured several adult traits upon emergence. The experiments were run over two years due to constraints on sample sizes and time imposed by the on-going captive breeding and conservation management efforts for this species. Maternal lineages were tracked in the second year.

All lab experiments were carried out using 250ml glass bottle microcosms with foam stoppers and pine bark ladders (9 x 3 x 1 cm^3), to allow larvae to adhere to and crawl closer to the surface to breathe. Moss plugs were provided towards the end of the developmental period. These were situated at the mouth of the microcosms for larvae to crawl into upon exiting the water to pupate. Microcosms were filled with pine chips or sawdust and bottled water (Highland Spring Ltd) and were left for 48 hours to allow the content to become saturated before introducing larvae. These were kept in climate-controlled facilities at temperatures and photoperiods corresponding with those that they would experience naturally in North Central

Scotland (Table 2.1). Environmental conditions were estimated using datalogger temperature readings and Met Office reports. Larvae were selected based on size (where possible < 7mm body length, which corresponds to the first or early second instar) and randomly assigned to treatments for each experiment. Larvae were starved for 24 hours before each experiment to minimise the effect of pre-experimental conditions.

To measure larval and pupal area and adult traits, we placed individuals on laminated lined paper for scale, captured a digital image, and estimated the two-dimensional area or length of adult trait using ImageJ software (Abràmoff *et al*. 2004), a public-domain software package for image processing. In experiment 2, after transferring larvae to filter paper to remove excess water, mass was also measured on a 0.001g resolution balance. Adult size traits included the length of the thorax from the point at which the neck meets the pronotum to the apex of the scutellum, the length between two wing veins (landmarks 1 and 3 in Milankov *et al*. 2010), and (for samples in year two) head length between the neck and the tip of the fronds (Stubbs & Falk 2002), and head width at the widest points of the eyes. Development time was assessed as the number of days from the start of the experiment until the day of eclosion.

2.3.1 Experiment 1.

Effect of pine woodchip size and Nipagen antifungal treatment

In addition to potential stress incurred due to substrate form and condition, rot hole inhabiting Syrphid larvae occasionally suffer from filamentous fungal growth on the integument, with likely negative consequences for their survival and health. Consequently we wished to simultaneously investigate the effects of particle size (wood chip/less surface area versus sawdust/greater surface area to water volume) and the application of Nipagen (a standard antifungal agent in experiments involving *Drosophila*, e.g., Tinsley *et al*. 2006). A replicate (x40) three-factor experiment included the following treatments: sawdust (S) (50 ml), chips (C) (50 cm^3), sawdust and chips (CS) (25 ml + 25 cm^3), and sawdust (50 ml) plus 0.5 ml (0.35%) Nipagen (NS), with one larva inhabiting each microcosm. We chose substrate volumes based on data from a previous study (see Chapter 3). Five millilitres of water was added to each microcosm every few months to replace losses due to evaporation. We took seventeen larval area measurements between 30th August 2009 and 14th July 2010. The measurement interval was increased to 30 days between November 2009 and January 2010, but otherwise measurements were taken at fifteen-day intervals.

Larval growth field comparison

We collected field growth data to study the 'natural' *B. fallax* larval growth trajectory over time. Between July and November 2009, twenty-seven artificially

bored pine rot holes containing *B. fallax* larvae were located in the field. These were visited five times in thirty-day intervals, and all larvae located in the stumps were photographed and measured for larval area. No manipulation was carried out in the field, and due to the large number of confounding variables, such as the size of bored hole, larval density and an inability to identify individual larvae, formal statistical comparisons between field data and in-situ experiments were not possible.

Fat deposition over time

Blera fallax larvae are almost transparent during growth and development, but they become opaque with white fat as they over winter or pupate. Fat deposition over time was also calculated (for C, CS, S and NS treatments) using the digital images by measuring the area of larval mass that did not contain fat and subtracting this from the total area.

2.3.2 Experiment 2.

Effect of intra-specific competition

A replicated (x20) 2x2x3 full factorial experiment was carried out in 2010 with a new generation of *B. fallax* larvae, which included two substrate levels (low = 40 and high = 80 ml pine sawdust), two water levels (low = 70 and high = 140 ml) and three larval densities (1, 2 and 3 larvae) (Table 2.2). We were unable to explore higher densities of competition because of the limited number of larvae available; furthermore we wanted to explore the possibility of Allee effects, which are most

likely to be evident in small groups. Larvae sharing a microcosm were from the same brood, i.e. were siblings through the maternal line, from one of thirteen dams. Four weight and area measurements were taken at fifteen-day intervals between 18th September and 10th November 2010.

Effect of tree species

Blera fallax appears to be a pine specialist in Scotland, but as they occur in other conifer hosts abroad, we wanted to know whether alternate species could support larvae, broadening options for future habitat creation. The effect of tree species on larval growth was investigated by a replicate (x20) experiment including 50 ml sawdust per microcosm of Pine *P. sylvestris*, Birch *Betula pubescens* and Spruce *Picea abies*. One larva was added to each microcosm and three area and weight measurements were taken at fifteen-day intervals from September until November 2010.

2.3.3 Statistical analysis

All statistical analyses were carried out using the statistical package R (version 2.13.1) (R Team 2011).

Fitting growth curves

We assessed pre-winter growth over the first four time intervals because the growth in this period appeared to be asymptotic (conforming to the most typical growth

functions). After these intervals, some individuals had entered winter diapause and started decreasing in area, which often prevented model convergence. We began by comparing the fits of several nonlinear functions, including asymptotic regressions, the Michaelis-Menton model, and von Bertalanffy (vB) growth functions. Likelihood ratio tests comparing these models revealed that the vB curve was at least as strongly supported as other approaches, and significantly better than two-parameter models. Furthermore, the parameters produced by this function (L0, size at hatching/birth; $L\infty$, mean asymptotic size, and K, a growth rate constant) are relatively easily interpreted in terms of the life history of the flies. Consequently, we focussed our model refinement on models using the vB function.

To determine whether individual treatments affected the parameter estimates, we grouped subjects by treatment and fit nonlinear functions conditional on the treatment level, as suggested by Crawley (2007). This method allows coefficients to vary by treatment group. When parameter estimates suggested different model fits between treatments, we subdivided the data by groups and created separate models for each treatment. This mixed nonlinear modelling (in which treatment is fitted as if it were a random effect) did not allow us to simultaneously estimate treatment effects and account for pseudoreplication. We verified that our parameter estimates were not biased by temporal autocorrelation (pseudoreplication because of repeated measurements on individuals) in two ways: by building linear mixed effects models with microcosm fitted as a random effect, and checking that these models produced similar parameter estimates to those of the fixed effects models; and by explicitly modelling the autocorrelation using a first order autoregressive, as suggested by Crawley (2007). Neither of these approaches suggested that the parameter estimates

were biased by pseudoreplication. In our results, we only report bootstrapped estimates and confidence regions from fixed effects models for simplicity. We conclude significant differences in parameter estimates whenever the bootstrapped mean for one treatment is not included in the 95% CI for another. Note that while we interpret each parameter in turn, the fitted parameter estimates are obviously not independent of one another. For example, the growth constant describes the average rate of growth over time until reaching the asymptotic size (i.e. average size achieved before winter diapause). In addition, the coefficients should not be interpreted as actual values. For example, time was recorded as 'day of experiment' rather than 'age of larva', therefore L0 will be an underestimate of actual size at hatching/birth.

This approach was used to compare larval growth across treatments in experiment 1 and 2. In addition, the data in each treatment were split by sex to assess sex differences in growth. We recorded both mass and larval area in the second experiment, but as the two variables were strongly collinear ($R = 0.97$), and the model outcome did not depend on the response used, we report only models based on larval mass below for simplicity.

Fat deposition over time

Fat deposition was a binomial response (the proportion of larval area that was opaque). We initially tried to fit generalized linear models with binomial error to model this response, but we were unable to reliably estimate the variance accounted for by individual microcosms. Consequently, we simply used non-parametric

Kruskal-Wallis tests to confirm between-treatment fat deposition differences two weeks before (day 49, 20th October) and after (day 230, 29th April) larvae became completely opaque with fat.

Survival analysis

Survival analysis was carried out up until day 300 in experiment 1, and 200 in experiment 2. We fitted survival curves using the non-parametric Cox's proportional hazards model, and compared the output with a parametric model, alternately assuming a constant hazard and a non-constant hazard with Weibull errors (Crawley 2007). A censoring vector was used because some individuals survived beyond the end of the experiment, and the time of death was unknown for others.

Pupation, emergence and adult size traits

Chi-square tests were used to assess sex ratios between treatments and over time, and to compare numbers eclosing between treatments. Linear regression was used to test the relationship between day of pupation and pupal period before eclosion.

We used multivariate analysis of variance (MANOVA) to determine the effect of treatment and sex on development time (1/day in experiment), pupal size (√pupal area; pupal area was square root transformed to convert it to the linear scale shared with size traits), and adult size traits. Model simplification was carried out using

likelihood ratio tests, and sequentially deleting terms (beginning with higher order interactions) that did not significantly decrease model deviance (Crawley 2007).

2.4 Results

2.4.1 Experiment 1. Effect of pine woodchip size and Nipagen

The nonlinear model using the vB function parameters would not converge when using the full dataset. Of 40 replicates, 3 each in treatments S (Sawdust) and NS (Nipagen and Sawdust), and 15 in C (Chips) did not appear to grow or did not pupate within the same year as the experiment (Table 2.2). No slow-growing individuals were found in treatment CS (Chips and Sawdust). Upon removing these replicates from the analysis, convergence was then achieved (Fig 2.1). The slow-growing individuals were not included in growth or fat deposition analysis, but were used to compare adult size traits between years. Below we will refer to the subset of individuals that grew slowly in the first year as semivoltine.

Larval growth

We predicted that larval growth would be adversely affected in the low surface area to water volume treatment i.e. treatment C; as predicted, individuals in treatment C reached a lower mean asymptotic ($L\infty$) size of 0.456 (0.426 – 0.523 confidence interval CI) than those in the S (0.60), NS (0.72) and CS (0.571) treatments (Fig 2.1, Table 2.3). The growth constant (K) was indistinguishable across treatments C

(0.089) and CS (0.095), but was greater in those treatments than in treatments NS (0.023) and S (0.041) (Fig 2.1, Table 2.3). Estimated size at hatching (L0) did not significantly differ across any of the treatments (Table 2.3). There was also no effect of sex on growth (data not shown).

The mean asymptotic parameter for growth data collected from the field was slightly greater than but comparatively similar to that for lab treatment C, and the growth constant (0.15, 0.098 – 0.325 CI) was greater than in all lab treatments (Fig 2.1, Table 2.3).

Fat deposition over time

Individuals in treatment C not only appeared to take longer to grow but also to build up fat reserves before winter, and appeared to lose a greater proportion of fat after winter while gaining in size (Fig 2.2). Two weeks before becoming completely opaque with fat (day 49, 20th October) a Kruskal-Wallis test indicated significant differences in the proportion of body area covered by fat between C and CS (χ^2 = 3.75, df = 1, P = 0.05), NS (χ^2 = 5.14, df = 1, P < 0.05) and S (χ^2 = 6.36, df = 1, P < 0.05). From November until March (days 79 to 164), fat deposition was 100 % in all treatments and feeding appeared to be suspended, indicated by a lack of dark colouration in the gut. Two weeks after winter (day 230, 29th April), similar significant differences were found, in which the proportion of fat was lower in individuals in treatment C compared with CS (χ^2 = 8.52, df = 1, P < 0.005), NS (χ^2 = 18.37, df = 1, P < 0.005) and S (χ^2 = 16.4, df = 1, P < 0.005) (Fig 2.2). Before pupation, fat deposition returned to 100 % and the gut area became clear.

Survival analysis

While the greatest mortality was in treatment CS (0.35, Table 2.2), we could not detect a statistically significant effect of treatment on survival ($Z = 1.2$, $P > 0.1$, Cox proportional hazard). The trend indicated that most deaths occurred early in the experiment, and few individuals died after the first 100 days.

Pupation, emergence and adult size

Seventeen large, opaque individuals (7 CS, 4 NS and 6 in S treatments) were found exiting the microcosms before winter, a response apparently indicating the completion of development, after which larvae begin searching for a place to pupate. These individuals were returned to microcosms, and moss plugs were provided within which larvae readily came to rest.

Individuals took between 13 and 36 days to develop and eclose depending on when in the year they pupated. The later the onset of pupation, the shorter the subsequent pupal period before eclosion ($r^2 = 0.79$, $F_{1,112} = 420.6$, $P < 0.001$). A significantly greater number of *B. fallax* eclosed in treatments CS (26), NS (25) and S (26) than in C (13, $\chi^2 = 14.9$, df = 3, $P < 0.005$, Table 2.2).

The sex ratio did not deviate significantly from 50:50 (M/F 45/44). In the first week of the emergence period, significantly more males emerged than females (M/F 38/22, $\chi^2 = 8.97$, df = 1, $P < 0.05$), and more females emerged in the final 23 days (M/F 7/22, $\chi^2 = 7.76$, df = 1, $P < 0.05$). Females overall took longer to develop to

eclosion (MS = 344.18, $F_{1,88}$ = 16.49, P = 0.0001). We assessed within treatment sex-differences in development time, which showed the same trend in CS, NS and S, but no significant difference between males and females in C (Table 2.4). Individuals in C took longer to develop (Table 2.4), and significantly fewer eclosed compared with each of the other treatments (χ^2 = 3.78, df = 1, p = 0.05, Table 2.2).

Individuals in treatment C had smaller pupal areas, thorax and wing lengths, and took longer to develop compared with individuals in treatments CS, NS and S (MANOVA, P < 0.005, Fig 2.3, Table 2.5). No difference was found between S, NS and CS (Fig 2.3). Female wing and thorax lengths were greater than those of males, and wing length in both males and females was significantly greater in treatments CS, NS and S than in C (MANOVA, P < 0.05, Fig 2.4, Table 2.4 and 2.5).

A significant treatment by sex interaction was found for thorax and wing lengths when semivoltine individuals were included in the model. These individuals were smaller compared with those that eclosed in the previous year, and males tended to have greater thorax lengths and longer wings than females, the opposite trend to the other treatments (MANOVA, P < 0.05, Fig 2.4, Table 2.4 and 2.5).

2.4.2 Experiment 2. Effect of intra-specific competition

As in experiment 1, between 2 and 20 % of larvae in each treatment did not appear to grow substantially in the first year. The nonlinear model using the vB function parameters would not converge with these replicates included in the model.

Therefore, 29 of 240 replicates (i.e. microcosms) were removed from the analysis (between 1 and 4 per treatment).

Larval growth

We predicted larval density would have an effect on growth, but as pine sawdust substrate and water volume increased, or in the presence of Allee effects, the impact of competition would be reduced. As there were no interactions between larval density, water and sawdust level (data not shown), we consider their effects on growth parameters sequentially, below.

The mean asymptotic size decreased significantly as the number of larvae increased from one larva per microcosm (0.100, 0.085 – 0.134 CI) to two (0.066, 0.06 – 0.083) and three (0.051, 0.048 – 0.058) (Fig 2.5, Table 2.3). The growth constants were lowest in single larva microcosms (0.035, 0.019 – 0.053 CI), and did not overlap with the confidence region for the growth constant of three larvae microcosms (0.068, 0.041 – 0.104 CI) (Fig 2.5, Table 2.3). In two larva microcosms the growth constant was intermediate to and indistinguishable from that for the other two treatments.

The mean asymptotic size was significantly lower in low water level (0.061, 0.056 – 0.069 CI) relative to high water treatments (0.084, 0.073 – 0.116 CI) (Fig 2.6, Table 2.3). The growth constant was greater in low water (0.056, 0.036 – 0.081 CI) compared with high water (0.036, 0.018 – 0.054) (Fig 2.6, Table 2.3). The mean asymptotic size was significantly lower in low sawdust (0.060, 0.055 – 0.068 CI)

relative to the high sawdust treatment (0.088, 0.076 – 0.127 CI) (Fig 2.6, Table 2.3). Growth constants were greater in low sawdust (0.064, 0.041 – 0.095 CI) compared with the high sawdust treatment (0.032, 0.016 – 0.048) (Fig 2.6, Table 2.3).

As for experiment 1, estimated size at hatching (L0) did not differ significantly across any of the treatments (Table 2.3). Male and female subsets also did not produce different parameter estimates (data not shown).

Effect of tree species

The mean asymptotic size was highest for pine (0.093, 0.073 – 0.238 CI) although this was statistically indistinguishable from the estimate for the birch sawdust treatment (0.079, 0.066 – 0.164 CI). Asymptotic size was significantly lower in spruce (0.06, 0.05 – 0.12 CI) (Fig 2.7, Table 2.3). Growth constants and size at hatching (L0) did not significantly differ across tree species (Table 2.3). As above, male and female subsets of data did not produce significantly different growth parameters (data not shown).

Survival analysis

Sawdust, water and larval density all had significant effects on survival (Sawdust, $Z = -2.5$, $P < 0.05$; Water, $Z = 5.8$, $P < 0.005$; Larvae, $Z = -4.3$, $P < 0.005$, Cox proportional hazard). Survival decreased significantly in the high water and low sawdust and larvae treatments (Fig 2.8). The greatest mortality was found in the high sawdust, high water and low larval density treatments (0.55 to 0.65, Table 2.2),

and the lowest was in the low water, high larval density treatments (0.03 to 0.15, Table 2.2).

Only two individuals died in the phase of the experiment on tree species during which we monitored growth (September to November 2010), therefore we did not conduct formal survival analysis for those samples. Mortality across tree species treatments was determined in June and July 2011, and in total, significantly more individuals died in the pine treatment (0.55), compared with birch (0.30) and spruce (0.10) ($\chi^2 = 32.10$, df = 2, $P < 0.005$, Table 2.2).

Pupation, emergence and adult size

Across treatments, the sex ratio was significantly female biased (M/F 64/102, $\chi^2 = 8.69$, df = 1, P = 0.003). A smaller proportion of *B. fallax* individuals eclosed within one year in low (0.33) compared with high sawdust treatments (0.47) and low (0.37) compared with high water treatments (0.44), and significantly fewer in high compared with low competition treatments (1 vs. 3 larvae, $\chi^2 = 9.14$, df = 1, $P < 0.005$, Table 2.2). There were significantly more males emerging (26) than females (18) in the first two weeks of emergence (M/F 0.41/0.18, $\chi^2 = 8.97$, df = 1, $P < 0.005$).

Larval density, water and sawdust level had significant effects on thorax and wing lengths, puparium areas, and times to eclosion, all of which were consistent with effects of these treatments on larval growth. Thorax and wing lengths and pupal areas decreased in increasing larval density and decreasing sawdust and water levels

(MANOVA, $P < 0.05$, Table 2.6). However, the effects of these treatments sometimes depended on sex. Compared with males, a greater negative effect is evident in female thorax and wing length and puparium area in increasing larval density treatments (sex by density interaction $P < 0.05$, Fig 2.9, Table 2.6). While male wing lengths did not differ according to sawdust levels, females had significantly longer wings in high sawdust treatments (sex by sawdust interaction $P < 0.05$, Fig 2.10, Table 2.6).

Females took longer to eclose (day in experiment, mean 254 ± 6.5 SD, standard deviation) than males (250 ± 5.2 SD), and females on average were larger than males (Table 2.4). However, development time was also significantly shorter for both males and females in high sawdust and water treatments (Table 2.4 and 2.6).

Effects of tree species

Chi-squared tests show no significant difference between the total number eclosing or the number of males and females in pine (M/F 2/6), birch (M/F 7/3) or spruce treatments (M/F 6/5). Tree species did have a significant effect on thorax length, wing length and puparium area (MANOVA, $P < 0.05$, Table 2.6): individuals in the spruce treatment had significantly smaller thorax and wing lengths and pupal areas (Table 2.6, Fig 2.11). We found no differences in development time between treatments.

2.5 Discussion

Rearing conditions had a strong effect on the growth, development time, and adult size traits of *B. fallax*. Individuals grew larger in treatments with smaller wood chip size, and in greater substrate and water volumes and lower larval densities. In conditions that do not sustain rapid growth, some individuals responded by extending development into an extra growing season, i.e. they became semivoltine. Surprisingly, individuals grew larger in birch (a species in which *B. fallax* are never found in nature) than spruce (a host species exploited by *B. fallax* in Europe). Males and females appeared to resolve the trade-off between development time and size at eclosion differently, with males prolonging development and females emerging smaller.

Growth and life history at the larval stage

Depending on available resources, larvae grew rapidly at the beginning of their development, but growth then slowed and individuals reached an asymptotic size before winter, a phase that may be governed by seasonal triggers such as photoperiod and temperature signals. Photoperiod is often the main environmental cue in insects (Nylin & Gotthard 1998). The initial growth trajectory probably reflects the period when all available resources are devoted to premature growth (Day & Taylor 1997). For larvae in good conditions, the asymptotic size defines the mass at eclosion, i.e. no more growth occurs before pupation, while for those in less favourable conditions, growth continues after winter.

Voltinism

Survey results from 2011 showed no new instars at any *B. fallax* site, the surviving populations consisting solely of larvae that were developing over two years (see Chapter 5). This suggests a complete failure of adult breeding at all sites, perhaps due to cold and wet weather during the adult breeding season. Therefore bet hedging by producing some slow growing, semivoltine offspring may improve the survival of *B. fallax* during unpredictable or unreliable periods. Theory suggests when the environment is unpredictable, females should increase the variation in their offspring as an adaptive strategy (Marshall *et al*. 2008; Crean & Marshall 2009; Monro *et al*. 2010). Such developmental plasticity has been reported in other insects such as stoneflies and damselflies (Cayrou & Céréghino 2005) but it has never been formally reported in hoverflies. In addition, this strategy may also reduce direct sibling competition within rot holes (Chapter 5).

Larval growth and the pine rot hole

Individuals grew faster and larger in treatments with sawdust than without. The ability of microbes to metabolize wood probably depends on what fraction of the wood is exposed to water. This increases dramatically in sawdust treatments relative to wood chips. Growth rates of filter feeding larvae have been found to increase with increased area of submerged surfaces (Juliano & Reminger 1992; Leonard & Juliano 1995; Eisenberg *et al*. 2000). Insect larvae have been observed grazing preferentially over the surface of the wood and leaf substrate in tree-holes, heavily affecting microbe abundance in these specific areas (Kaufman *et al*. 2001, 2002).

Different species of bacteria and other microbes adhere to and grow on the substrate surface, and increased abundance and species richness is found near the substrate surface as opposed to the water column (Harlan & Paradise 2006). In experiment 1, individuals in treatment CS (with both wood chips and sawdust) had a greater, though not significant, growth rate (Fig 2.1). The larger wood chips may provide a better surface area for larvae to move along, and dislodge and graze microbes from the surface, possibly assisted by their anterior spiracles. Providing this and the same volume of sawdust to increase the overall surface area will probably provide the best conditions for larval growth based on these results.

Our assessment of growth in the field revealed substantial variation in growth, but overall corresponded with the resource-limited conditions (C) in the lab suggesting that in nature, resources may often be limited. Therefore, ensuring rot hole surface area is maximised by, for example providing chips and sawdust, would benefit larval growth in the field.

Increasing larval abundance does not appear to promote microbial growth to the benefit of *B. fallax* larvae, as would be true if Allee effects were strong at low larval densities. Instead, the results correspond with studies that show density-dependent limitations on growth (see Bradshaw & Holzapfel 1992; Broberg & Bradshaw 1995). Presumably, larval grazing depletes the resources available; it may also select for bacterial populations that have a stronger ability to adhere to the substrate surface, or those that are resistant to digestion. Complex effects of filter feeding have been demonstrated in studies of mosquitoes, where the microbial community changes both directly due to removal of protozoa competitors for microbes and

indirectly due to changes in nutrient content (Kaufman *et al*. 2008; Addicott 1974; Paradise & Dunson 1997; Kaufman *et al*. 1999, 2008).

An important natural element that was missing in our study was rainwater or stemflow, and it may ultimately be this that limits larval growth (Kaufmen *et al* 2008). Stemflow has been found to neutralize density-dependent competition, suggesting a likely influence on nutrient dynamics and bacterial populations, and thus on larval Syrphid productivity (Walker *et al*. 1991). In addition, natural tree holes can also experience inputs of detritus such as leaves or pine needles, and these will provide more surface area and nutrients (Carpenter 1983; Daugherty *et al*. 2000; Daugherty & Juliano 2002, 2011). Rapid degradation in the quality of rot hole detritus has been found in recent studies (Maciá & Bradshaw 2000; Kaufman *et al*. 2008). The accumulation of waste products such as ammonia may be an additional time or density-dependent issue causing further depression in larval growth (Carpenter 1983). If this is true, another advantage of stemflow or rainfall may be diluting or flushing such waste metabolites. However, it is important to note that most studies assessing the content and dynamics in tree-holes involve buttress holes, which are lined with bark. Pine rot holes in stumps penetrate the hard wood where decomposition may contribute considerable differences in nutrient quality and therefore microbial abundance (Kitching 1971; Fish & Carpenter 1982).

Fat deposition

As winter approaches, energy seems to be re-directed from mass accumulation to pre-winter fat storage. Once fat storage is complete, signified by a once translucent

body becoming completely opaque with white fat, larvae appear to stop feeding and enter a slow-moving over-winter stasis, which is probably an energy conserving behavioural response (Hart & Bale 1997a). Fat storage is an important basic survival requirement for unproductive periods such as overwintering in many organisms, often involving trade offs in which energy has been directed from body and skeletal growth to fat reserves (Bull *et al*. 1996; Morgan & Metcalfe 2001; Shertzer & Ellner 2002). Fat deposition was slower in individuals in less favourable conditions, however by the over-winter period (November to March) these individuals had accumulated fat reserves that were indistinguishable observationally from those of rivals (they were 100 % opaque).

Individuals that had not completed development before winter (i.e. they continued to gain size after winter) became almost transparent again in spring, which suggests they probably utilised the fat for post-winter growth as well as gaining more energy from continued feeding. Similar findings have been reported in mosquitoes, which take advantage of the rise in temperature after winter to catch up with growth (Bradshaw 1973). In *B. fallax*, the post-winter growth rate did not seem to exceed that observed in the autumn, suggesting that growth may be limited by the environment rather than developmental constraints that are shed during compensatory growth phases.

When development is complete, individuals again appear to channel resources into fat storage before pupating. However some individuals continued to develop for another year. A small number of larvae appear to grow very little and take more than one year to develop independent of treatment.

Effect of tree species

Larvae grow as well in birch as they do in pine, and while growth is significantly inhibited in spruce compared to the other species, the same number of individuals eclosed in spruce as they did in birch, and more than pine within a year. Clearly the larval stage is capable of developing in a range of alternative resources. Ovipositing females with strong larval substrate preferences therefore may be limiting the success of the species by not utilising all the available alternative habitats. *Blera fallax* has not been found in any other tree species than pine in Scotland, and gravid females are disinclined to oviposit in rotting spruce or birch sawdust (Chapter 5). The specialisation on pine may occur because pine habitat was more available or productive than alternative tree rot holes at some past time, or it may be that our study did not include aspects of fitness that reveal costs of growth in alternative species of hosts, such as subsequent mating success or fecundity.

Wandering larvae and pupation

In lab conditions, *B. fallax* individuals that had completed development were observed 'wandering' before winter, the same behaviour observed in many holometabolous insects when they begin searching for a place to pupate (Huffaker & Gutierrez 1998). For *B. fallax*, this may leave individuals vulnerable to desiccation or predation, but may also protect them from freezing water in the rot hole, which they may no longer require for development. Clearly captive breeders will need to allow for wandering by developing larvae when rearing this species by applying moss plugs to microcosms as early as September.

Survival

Larval survival was significantly higher in low levels of water, high levels of sawdust, and high larval densities, and in spruce and birch treatments compared with pine. The treatment levels with lowest survival were also those in which growth rate was highest. Physiological costs associated with maximised growth may be the cause, whereby an organism's susceptibility to short term stresses are increased by higher metabolic demands necessary for faster growth (Blanckenhorn 1998), and many studies have evidence for such negative associations (Metcalfe & Monaghan 2001; Lavoie & Oberhauser 2004; Mangel & Munch 2005; Stoks *et al*. 2006; Dmitriew & Rowe 2007). However, the costs of growing slowly could also have been masked by the lab conditions, such as a lack of an unknown environmental stress (e.g., predation or pathogen pressure). We don't know how important water, sawdust or larval density is for survival in natural conditions, and measuring survival in the field is difficult, as larvae often crawl into tight crevices and often exit the rot hole completely before winter (E.L. Rotheray, pers. obs.).

Increasing larval density (from 1 to 3 larvae) and the application of the anti-fungal treatment Nipagen did not appear to affect survival. Fungal growth was not directly assessed in the Nipagen experiment, but while fungal growth was apparent in all treatments there were no noticeable differences in mortality across them. We conclude that while Nipagen does not appear to negatively affect larval growth or survival, its application similarly has no obvious beneficial impact on mortality.

Development, pupal and adult size

Individuals reared in low resource conditions had longer development times, lower single year eclosion rates, and smaller morphological traits. The sizes at eclosion appear to decrease with less productive conditions.

The duration of the pupal stage decreased significantly with the day in the year on which pupation took place, a phenomenon probably triggered by photoperiod (Gotthard *et al*. 1999; Gotthard 2008). While it is not known if there are any associated costs in *B. fallax*, other Dipteran species have demonstrated lower fecundity associated with decreased pupal development time (Telles-Romero *et al*. 2011).

Size may be important to females if it is associated with higher levels of fecundity. While it is likely to be an experimental artefact brought about by low sample size, female *B. fallax* in captivity have shown increased fecundity with decreasing wing length (Chapter 5). Most studies show a positive correlation of insect wing and thorax size with fecundity (Grimaldi & Jaenike 1984; Honěk 1993; Nylin & Gotthard 1998; Armbruster & Hutchinson 2002). Females probably allocate more energy than males to reproduction in low resource conditions thereby exhibiting a stronger response to lower resources. This in turn may explain the response of semivoltine individuals where resource levels may have deteriorated over time further reducing the threshold size of females below that of males in these conditions.

Females, which on average are larger than males, appear to vary more substantially in size at eclosion than males, who appear more likely to retain size at the expense of not achieving maturity and having to develop for another year. Eclosing at a smaller size may be compensated by avoiding mortality risks associated with increased development time, such as increased exposure to predation, bacterial and viral infection, and possibly increased over-wintering survival risks. For males, achieving a certain body size may be essential for mating success (see Chapter 5), which could explain why males are less likely to emerge smaller in restricted conditions. It often benefits males to emerge first, a feature known as protandry, in which males mature their reproductive organs and set up territories ahead of female emergence. This can lead to smaller size in males or changes in the levels of sexual size dimorphism throughout the season (Nylin & Gotthard 1998).

The contrasts across the sexes in the propensity to become semivoltine and the resolution of tradeoffs between development and adult size may reflect sex-specific adaptive differences in investment (Dmitriew *et al*. 2009). However, there are many unresolved questions about the nature of selection on the two sexes, including the sign and intensity of size associated sexual and fecundity selection (see Chapter 5) that will need to be resolved before we can state this with any confidence.

Conservation and animal husbandry for B. fallax

We found clear effects of several aspects of the rearing environment on development time, eclosion and adult size. Based on these results, the conditions that best enhance fitness for *B. fallax* reared ex-situ include a minimum sawdust

level of 40 ml and 140 ml water per larva, rearing in groups of no more than two per microcosm. Increasing the number to two per microcosm may improve survival. For semivoltine larvae, ex-situ conditions may require supplementation of new sawdust substrate and water in order to maximise growth in year two of their development.

Conservation management involves creating habitat for *B. fallax* by boring holes in pine stumps, and the nature of the hole determines the contents and the density (Chapter 5). The findings from this study suggest hole boring techniques such as the tools and methods used (see Chapter 5) will be important determinants of *B. fallax* larval growth, which is directly associated with individual fitness. Increasing the substrate and rot hole surface area or structural complexity will probably benefit larval growth by providing access to more resources. Wild densities can be much higher than the ones we manipulated which could potentially inhibit larval growth or be a source of mortality (Chapter 3) therefore it may benefit individual larval growth and survival if they were distributed across available habitat at *B. fallax* localities. Further study should investigate the effects of rainwater and natural detritus as well as efforts to increase the surface area in rot holes in the field. Monitoring larval growth and survival between July and no later than September allows for the initial growth trajectory to be measured, and reduces the chances of larvae exiting the rot hole before growth and survival data collection is complete. Because *B. fallax* can be semivoltine, monitoring should consider this time frame and management efforts should include a two-year plan whereby habitat is assured and secured all year round.

Table 2.1 Average monthly temperatures and mid-month light levels, as programmed in larval rearing climate-controlled facilities.

	Maximum temperature (°C)	Minimum temperature (°C)	Lights on	Lights off
January	7	2	08:30	16:25
February	4	1	07:45	17:15
March	8	4	06:45	18:40
April	13	5	05:30	20:50
May	17	7	05:00	21:15
June	18	9	04:15	22:15
July	19	10	05:10	21:35
August	18	8	05:30	21:15
September	15	8	06:30	20:00
October	10	5	07:50	18:10
November	7	4	07:45	16.15
December	7	1	08:45	15:30

Table 2.2 Year, treatments, total number of larvae included in each treatment, number of male and female adults emerged from each treatment, and percentage dead and pupated within one year. W, water; L, larvae.

Year	Substrate volume	Water volume	Treatment	Pre-winter larval area (mean ± standard error)		Total N at start	Female	Male	Dead (%)	Pupated (%)
				Initial area (mm^2)	Final area (mm^2)					
Experiment 1										
1	50	70	Chips	0.223 ± 0.102	0.274 ± 0.182	40	9	4	30	33
1	50	70	Chips + Sawdust	0.233 ± 0.113	0.374 ± 0.267	40	14	12	35	65
1	50	70	Sawdust + Nipagen	0.207 ± 0.168	0.359 ± 0.261	40	9	16	28	65
1	50	70	Sawdust	0.194 ± 0.112	0.381 ± 0.232	40	13	13	23	70
1	-	-	Field	0.182 ± 0.09	0.377 ± 0.162					
Experiment 2										
2	40	140	S40W140L1	0.207 ± 0.067	0.477 ± 0.064	20	6	2	55	40
2	40	140	S40W140L2	0.214 ± 0.080	0.404 ± 0.029	40	10	6	58	40
2	40	140	S40W140L3	0.217 ± 0.073	0.371 ± 0.034	60	11	4	25	27
2	80	140	S80W140L1	0.216 ± 0.075	0.444 ± 0.040	20	5	2	65	35
2	80	140	S80W140L2	0.202 ± 0.062	0.355 ± 0.034	40	13	15	15	73
2	80	140	S80W140L3	0.227 ± 0.058	0.313 ± 0.033	60	14	13	17	47
2	80	70	S80W70L1	0.210 ± 0.074	0.527 ± 0.066	20	6	5	25	55
2	80	70	S80W70L2	0.236 ± 0.065	0.470 ± 0.054	40	17	3	3	53
2	80	70	S80W70L3	0.235 ± 0.057	0.421 ± 0.052	60	7	3	7	18
2	40	70	S40W70L1	0.245 ± 0.039	0.485 ± 0.042	20	7	7	25	70
2	40	70	S40W70L2	0.240 ± 0.036	0.396 ± 0.029	40	4	3	10	18
2	40	70	S40W70L3	0.228 ± 0.040	0.347 ± 0.029	60	2	1	15	5
2	40	140	Birch	0.225 ± 0.091	0.373 ± 0.149	20	3	7	30	50
2	40	140	Spruce	0.226 ± 0.094	0.340 ± 0.138	20	5	6	10	55

Table 2.3 Bootstrapped von Bertalanffy parameter estimates and 95 % confidence intervals for models of larval mass increase across experimental treatments: chips, chips and sawdust, nipagen and sawdust and sawdust, and field larval growth (Experiment 1); and 1, 2 and 3 larval densities; low and high sawdust and water treatments; and birch, pine and spruce sawdust treatments (Experiment 2).

Treatment	Mean asymptotic size, L^{∞} (95% CI)	Growth constant, K (95% CI)	Mean size at hatching, L0* (95% CI)
Experiment 1			
Chips	0.456 (0.426 – 0.523)	0.089 (0.033 – 0.233)	-9.129 (-24.98 – -3.189)
Chip + Sawdust	0.571 (0.54 – 0.61)	0.095 (0.06 – 0.175)	-5.60 (-9.13 – -2.63)
Sawdust + Nipagen	0.72 (0.54 – 2.62)	0.023 (0.003 – 0.065)	-17.15 (-39.06 - -6.86)
Sawdust	0.60 (0.53 – 0.84)	0.041 (0.017 – 0.071)	-9.57 (-19.22 - -5.256)
Field	0.50 (0.46 – 0.53)	0.15 (0.098 – 0.325)	-2.026 (-3.84 - -0.42)
Experiment 2			
Low water level (70 ml)	0.061 (0.056 – 0.069)	0.056 (0.036 – 0.081)	-7.72 (-11.45 - -5.33)
High water level (140ml)	0.084 (0.073 – 0.116)	0.036 (0.018 – 0.054)	-7.63 (-12.07 - -5.02)
Low sawdust level (40ml)	0.060 (0.055 – 0.068)	0.064 (0.041 – 0.095)	-6.14 (-9.80 - -3.90)
High sawdust level (80ml)	0.088 (0.076 – 0.127)	0.032 (0.016 – 0.048)	-9.21 (-14.03 - -6.29)
1 larva/microcosm	0.100 (0.085 – 0.134)	0.035 (0.019 – 0.053)	-6.51 (-10.21 - -4.19)
2 larvae/microcosm	0.066 (0.060 – 0.083)	0.047 (0.025 – 0.070)	-8.20 (-13.13 - -5.31)
3 larvae/microcosm	0.051 (0.048 – 0.058)	0.068 (0.041 – 0.104)	-7.98 (-13.07 - -4.91)
Tree species			
Birch	0.079 (0.066 – 0.164)	0.067 (0.015 – 0.159)	-6.196 (-15.244 – -2.34)
Spruce	0.06 (0.05 – 0.21)	0.054 (0.006 – 0.19)	-9.72 (-26.68 – -2.99)
Pine	0.093 (0.073 – 0.238)	0.05 (0.01 – 0.097)	-4.99 (-10.55 – -2.29)

* Note that time was recorded as ‘day of experiment’ rather than ‘age of larva’, therefore L0 will be an underestimate of actual size at hatching/birth.

Table 2.4 Main *B. fallax* rearing conditions with trait measurements (mean ± standard deviation SD) in treatments; chips, chips and sawdust, nipagen and sawdust, and sawdust; 2010, semivoltine individuals (those that took two years to develop but from the same brood) (Experiment 1); and 1, 2 and 3 larval densities; low and high sawdust and water treatments; and birch, pine and spruce sawdust treatments (Experiment 2).

	Thorax length (mean ± SD)		Wing length (mean ± SD)		Pupal area (mean ± SD)		Day emerged (mean ± SD)	
Treatment	Female	Male	Female	Male	Female	Male	Female	Male
Experiment 1								
Chips	0.329 ± 0.03	0.322 ± 0.01	0.643 ± 0.05	0.610 ± 0.02	0.281 ± 0.04	0.269 ± 0.01	164 ± 10.2	158 ± 5.0
Chips + Sawdust	0.359 ± 0.01	0.347 ± 0.02	0.670 ± 0.03	0.645 ± 0.02	0.335 ± 0.03	0.352 ± 0.02	157 ± 3.1	154 ± 1.4
Sawdust + Nipagen	0.362 ± 0.02	0.350 ± 0.02	0.684 ± 0.02	0.654 ± 0.02	0.340 ± 0.02	0.340 ± 0.03	158 ± 3.7	154 ± 2.1
Sawdust	0.350 ± 0.02	0.346 ± 0.02	0.682 ± 0.04	0.659 ± 0.02	0.328 ± 0.03	0.327 ± 0.02	158 ± 3.2	154 ± 3.3
Semivoltine	0.261 ± 0.02	0.281 ± 0.03	0.546 ± 0.04	0.575 ± 0.04	0.202 ± 0.05	0.227 ± 0.03	540 ± 7.8	536 ± 7.4
Experiment 2								
1 larva	0.325 ± 0.02	0.316 ± 0.09	0.637 ± 0.04	0.606 ± 0.03	0.286 ± 0.03	0.265 ± 0.03	150 ± 6.5	146 ± 5.2
2 larvae	0.307 ± 0.02	0.304 ± 0.02	0.613 ± 0.02	0.603 ± 0.02	0.244 ± 0.03	0.258 ± 0.03	153 ± 6.2	149 ± 10.1
3 larvae	0.285 ± 0.03	0.291 ± 0.03	0.583 ± 0.03	0.584 ± 0.04	0.218 ± 0.02	0.241 ± 0.03	155 ± 10.5	150 ± 7.8
Water 70 (low)	0.308 ± 0.03	0.299 ± 0.02	0.608 ± 0.04	0.587 ± 0.03	0.247 ± 0.04	0.246 ± 0.03	154 ± 8.3	149 ± 7.3
Water 140 (high)	0.301 ± 0.03	0.305 ± 0.02	0.609 ± 0.04	0.603 ± 0.03	0.244 ± 0.04	0.259 ± 0.03	152 ± 8.0	149 ± 9.0
Sawdust 40 (low)	0.294 ± 0.03	0.301 ± 0.02	0.596 ± 0.04	0.598 ± 0.03	0.234 ± 0.04	0.246 ± 0.04	152 ± 7.9	148 ± 5.4
Sawdust 80 (high)	0.311 ± 0.02	0.304 ± 0.03	0.617 ± 0.03	0.597 ± 0.03	0.251 ± 0.04	0.259 ± 0.03	153 ± 8.4	149 ± 9.7
Birch	0.315 ± 0.01	0.306 ± 0.02	0.631 ± 0.03	0.619 ± 0.01	0.249 ± 0.02	0.277 ± 0.04	145 ± 9.1	140 ± 4.7
Pine	0.314 ± 0.02	0.342 ± 0.01	0.608 ± 0.05	0.619 ± 0.04	0.258 ± 0.03	0.277 ± 0.02	142 ± 6.0	144 ± 10.6
Spruce	0.294 ± 0.02	0.292 ± 0.02	0.596 ± 0.03	0.574 ± 0.03	0.217 ± 0.01	0.221 ± 0.02	144 ± 6.8	141 ± 2.9

Table 2.5 Multivariate (MANOVA) hypothesis test results (Experiment 1), between treatments Chips, Sawdust and Nipagen, Chips and Sawdust, and Sawdust and within treatment by sex differences. MS, mean sum squared value.

	Thorax length			Wing length			Pupal area			Development time		
Experiment 1	MS	F	Prob>F	MS	F	Prob>F	MS	F	Prob>F	MS (10^6)	F	Prob>F
Treatment	0.002	7.65	< 0.001	0.004	5.14	0.003	0.014	20.56	< 0.001	0.02	7.98	< 0.001
Sex	0.002	5.01	0.028	0.016	18.44	< 0.001	0.0002	0.31	0.582	0.04	14.37	0.0003
Treatment x Sex*	0.001	2.4	0.031	0.002	2.51	0.025	0.001	0.82	0.555			
Sex differences within treatment												
Chips + Sawdust	0.001	3.625	0.069	0.004	6.160	0.020	0.002	3.149	0.089	0.006	5.300	0.030
Chips	0.0002	0.322	0.582	0.003	1.400	0.262	0.0004	0.345	0.569	0.01	0.964	0.347
Nipagen + Sawdust	0.001	2.600	0.121	0.005	14.553	0.001	0.00002	0.005	0.945	0.01	9.560	0.005
Sawdust	0.0001	0.195	0.663	0.004	3.622	0.069	0.00001	0.012	0.915	0.01	7.204	0.013

* interaction results only for semivoltine adults included in model

Table 2.6 Multivariate (MANOVA) hypothesis test results investigating the effects of larval density (1/2/3 larvae), sawdust volume (high/low), water volume (high/low) and tree species (pine/birch/spruce) on adult traits (Experiment 2). MS, mean sum squared value.

	Thorax length			Wing length			Puparium area			Development time		
Experiment 2	MS	F	Prob>F	MS	F	Prob>F	MS	F	Prob>F	MS (10^5)	F	Prob>F
Larvae	0.014	34.1	< 0.001	0.021	30.8	< 0.001	0.031	49.9	< 0.001	0.05	4.4	0.014
Sawdust	0.010	24	< 0.001	0.011	15.7	< 0.001	0.021	34.9	< 0.001	0.001	0.1	0.736
Water	0.002	5.5	0.021	0.011	16.3	< 0.001	0.012	19.7	< 0.001	0.06	5.2	0.024
Sex	0.0003	0.8	0.369	0.008	10.8	0.001	0.002	3.1	0.080	0.003	8.9	0.003
Larvae x sex	0.0003	0.9	0.400	0.001	2.1	0.122	0.004	6	0.003	0.002	0.1	0.895
Sawdust x sex	0.002	5.6	0.019	0.006	8.8	0.003	0.001	1.7	0.194	0.002	0.1	0.725
Tree species												
Treatment	0.002	5.5	0.010	0.004	4.7	0.018	0.008	10.199	0.001	4.404	0.125	0.883

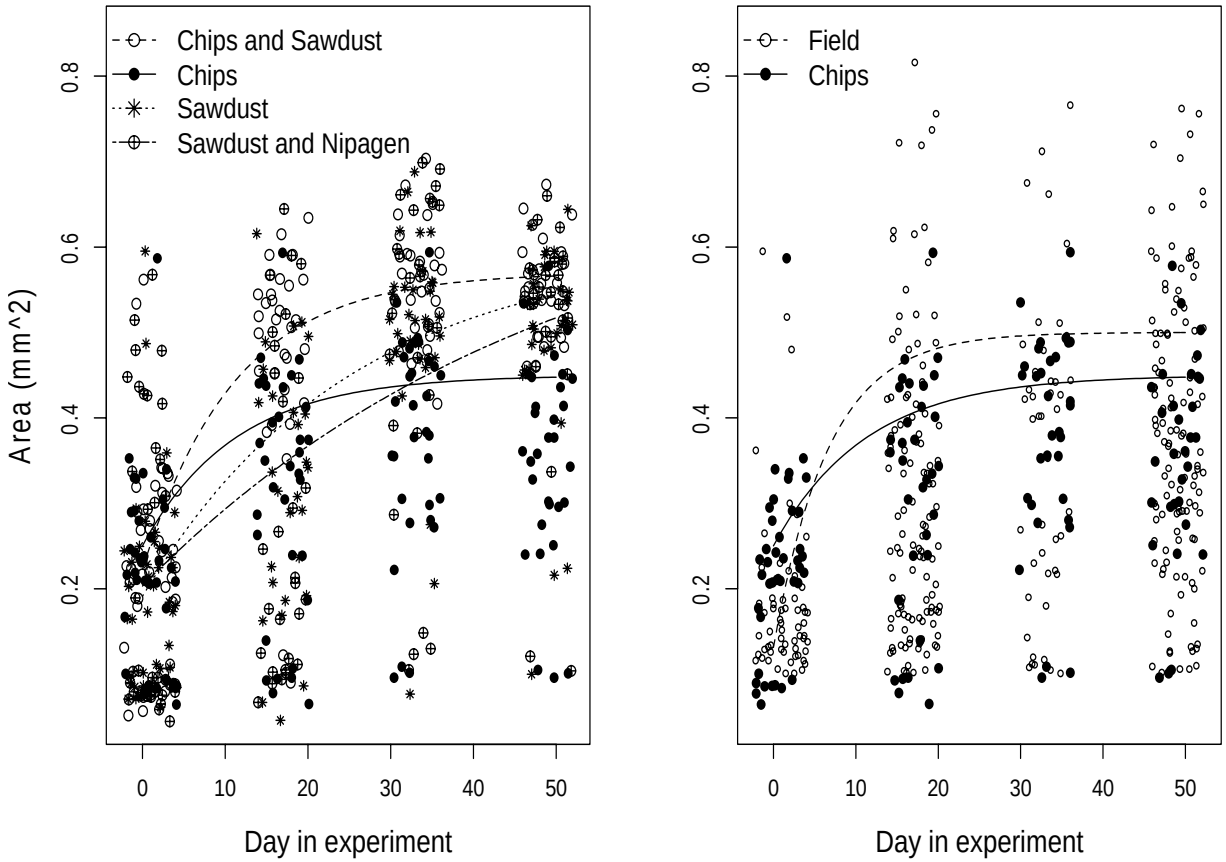


Figure 2.1 *B. fallax* larval growth curves implemented by the von Bertalanffy growth function fitted by nonlinear fixed effects models, and 'jittered' point data to illustrate the differences in pine wood treatments (Experiment 1): Chips and Sawdust, Chips, Sawdust, and Sawdust plus Nipagen fungal treatment (left) and comparing Chips and field growth data (right).

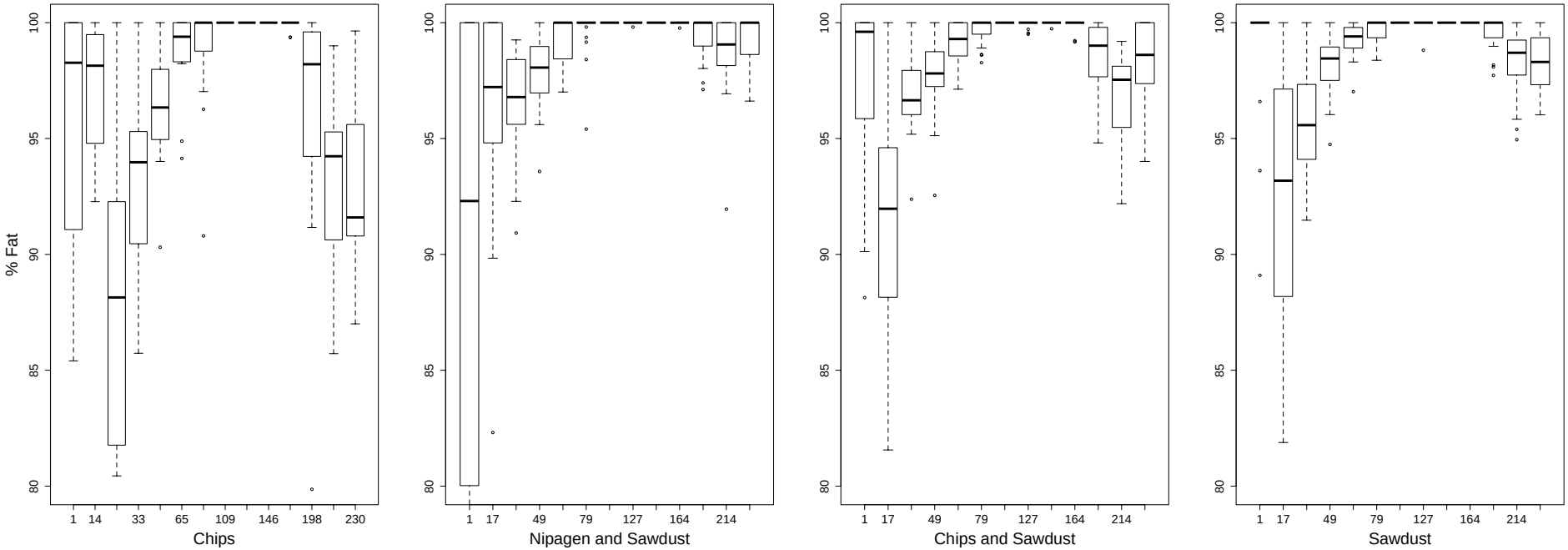


Figure 2.2 Percentage fat (y-axis) in each time interval representing August 2009 (day 1) until June 2010 (day 230) (x-axis), illustrated in separate graphs for each treatment; Chips, Sawdust and Nipagen, Chips and Sawdust, and Sawdust (Experiment 1).

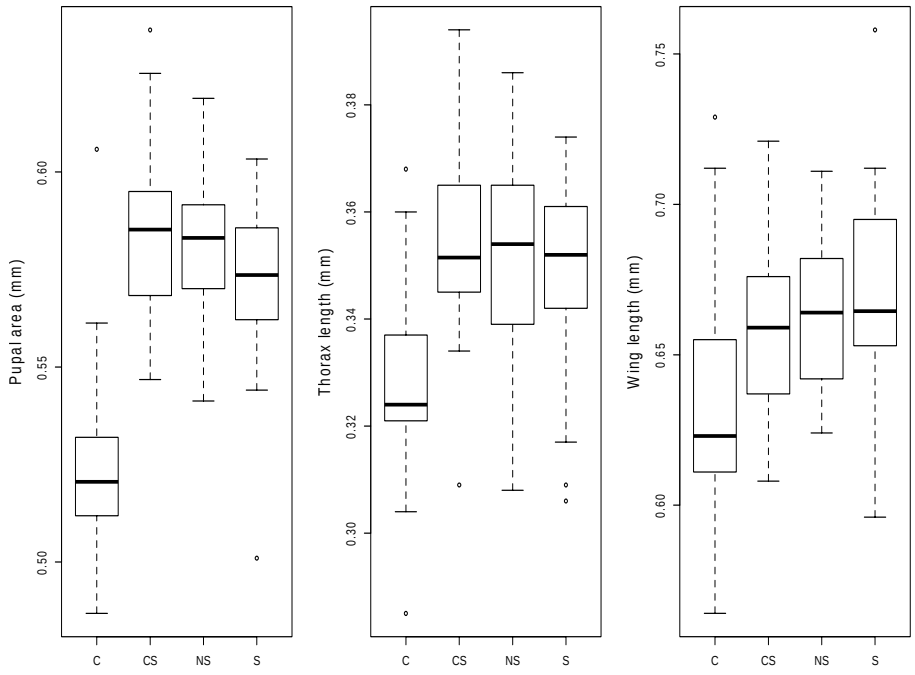


Figure 2.3 Boxplots illustrating the difference in √ pupal area (mm), and thorax and wing length between semivoltine individuals (those that took two years to develop) and in Chips C, Chips and Sawdust CS, Sawdust and Nipagen NS, and Sawdust S treatments (Experiment 1).

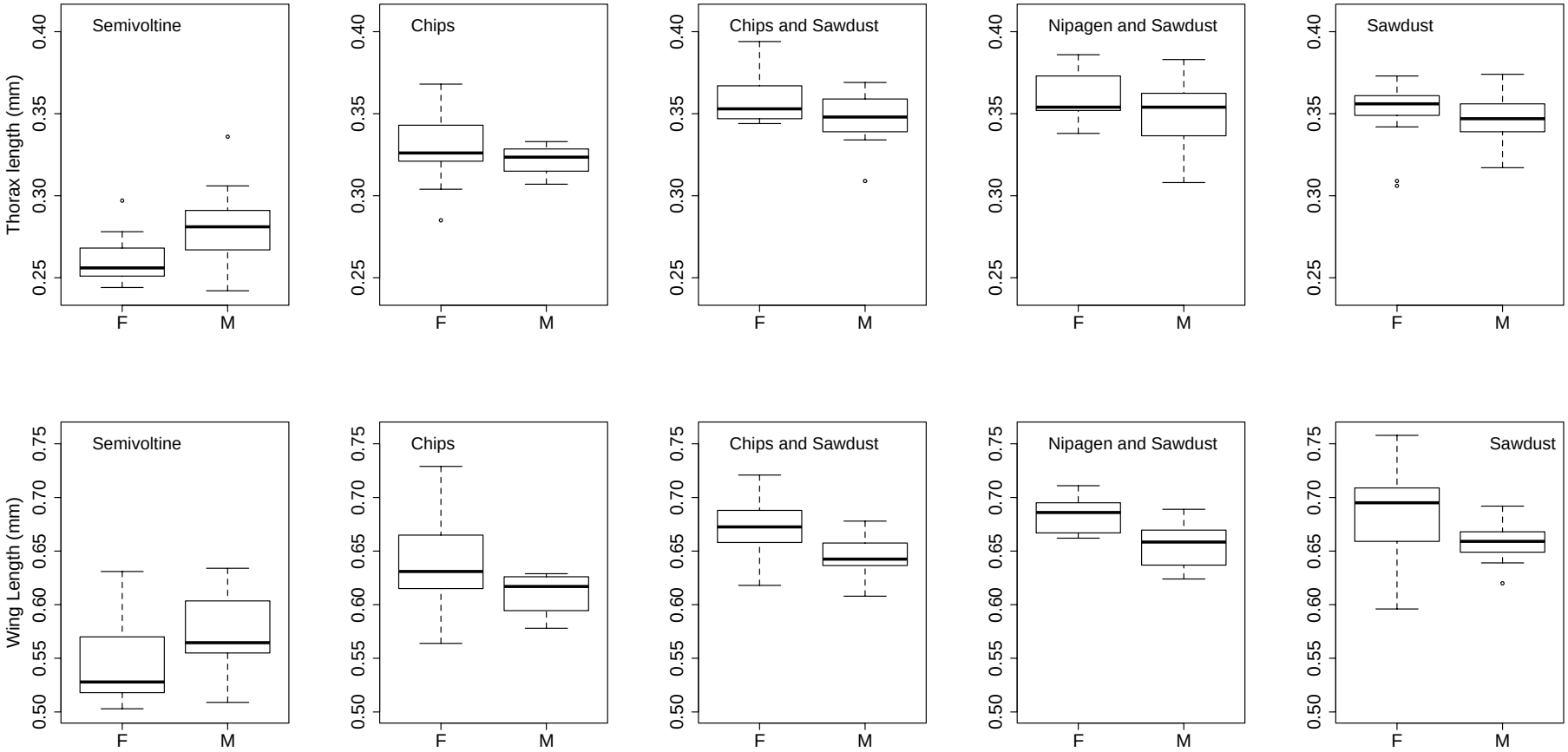


Figure 2.4 Boxplots illustrating the difference in thorax (top row) and wing length (bottom row) between males (M) and females (F) in semivoltine individuals (those that took two years to develop) and in Chips, Chips and Sawdust, Sawdust and Nipagen, and Sawdust treatments (Experiment 1).

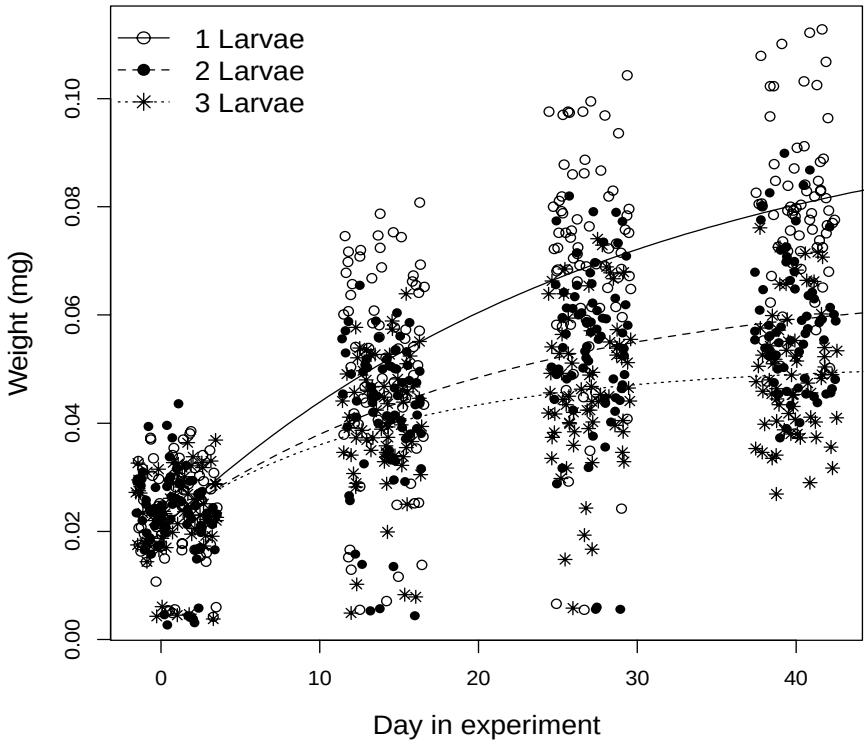


Figure 2.5 *B. fallax* larval growth curves implemented by the von Bertalanffy growth function fitted by nonlinear fixed effects models, and 'jittered' point data to illustrate the differences in 1, 2 and 3 larval density treatment groups (Experiment 2).

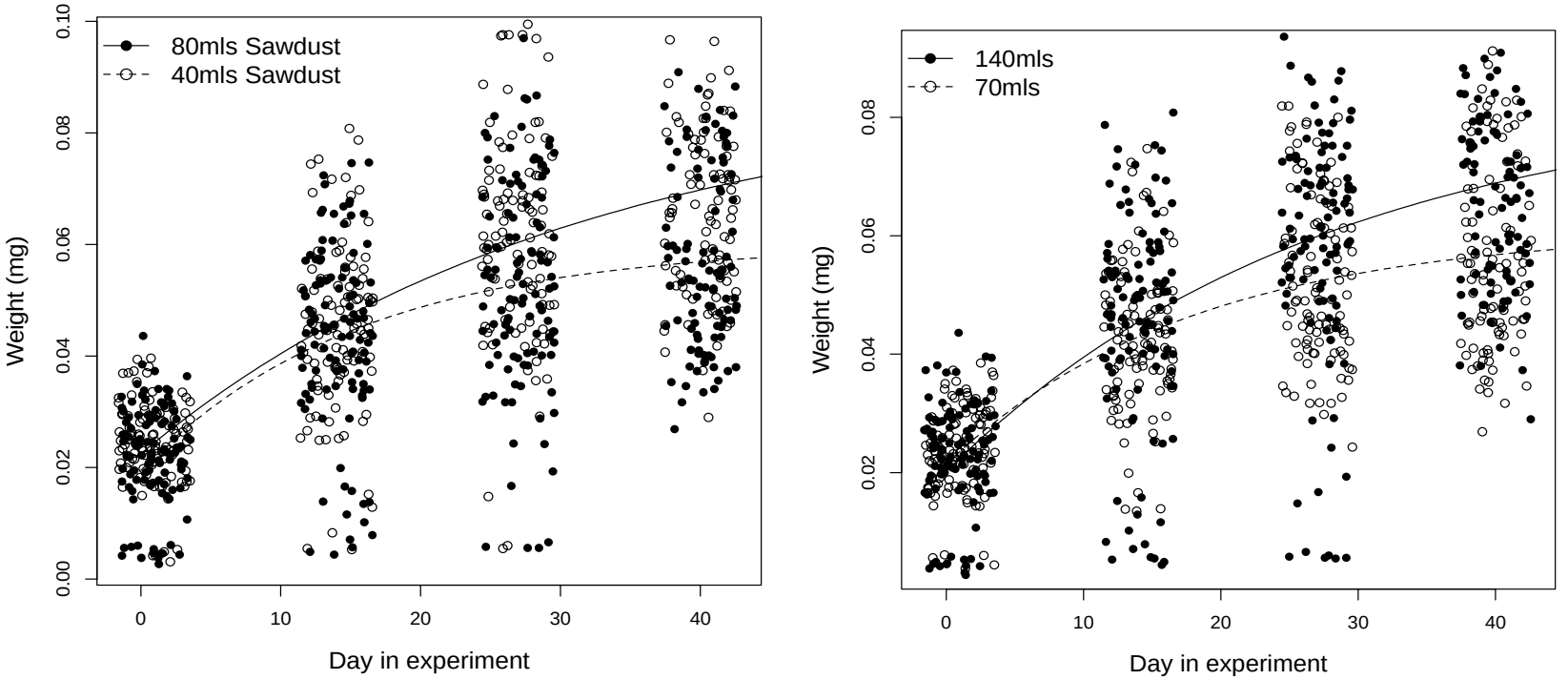


Figure 2.6 *B. fallax* larval growth curves implemented by the von Bertalanffy growth function fitted by nonlinear fixed effects models, and 'jittered' point data to illustrate the differences in grouped low (40 ml) and high (80 ml) pine sawdust treatments (left), and grouped low (70 ml) and high (140 ml) water treatments (Experiment 2).

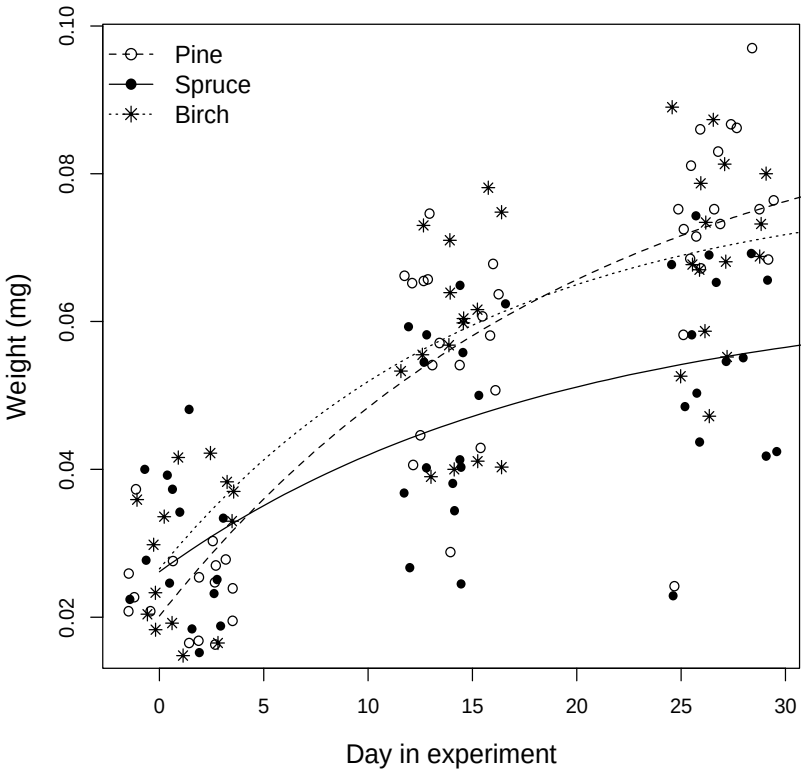


Figure 2.7 *B. fallax* larval growth curves implemented by the von Bertalanffy growth function fitted by nonlinear fixed effects models, and 'jittered' point data to illustrate the differences in pine, spruce and birch wood treatment groups (Experiment 2).

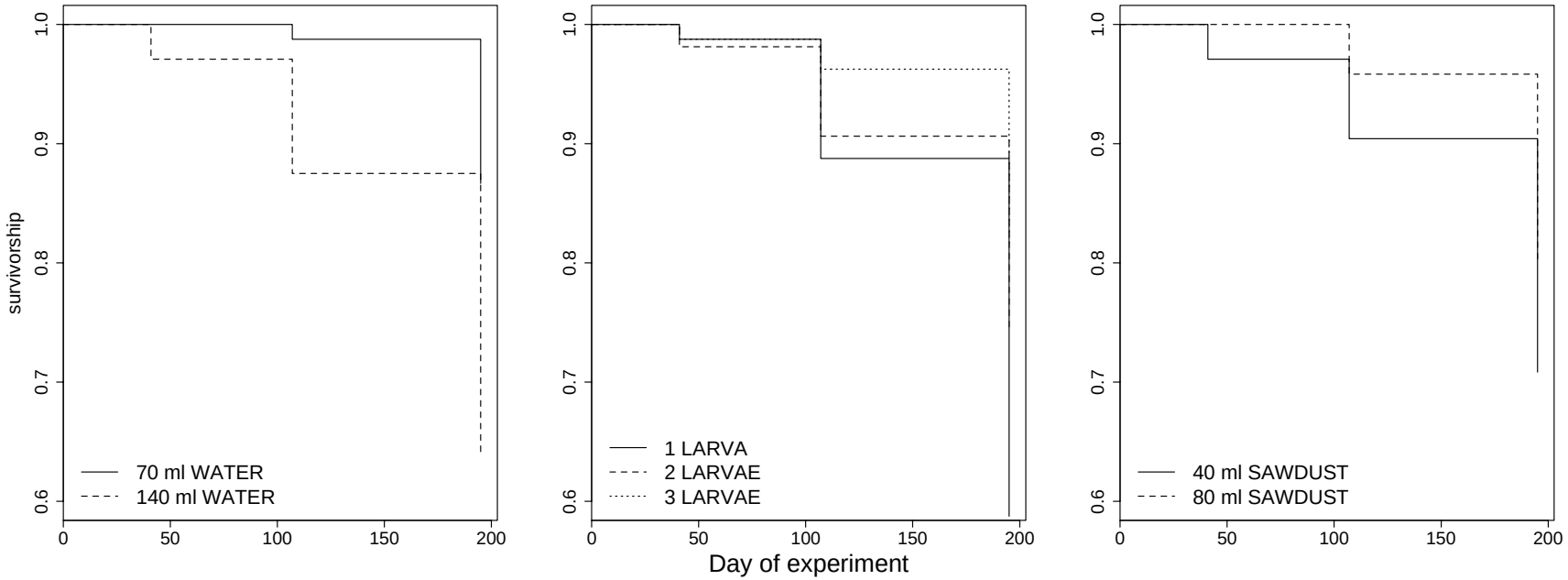


Figure 2.8 Cox proportional hazard survival curves drawn separately for water, larval density and sawdust treatments (Experiment 2).

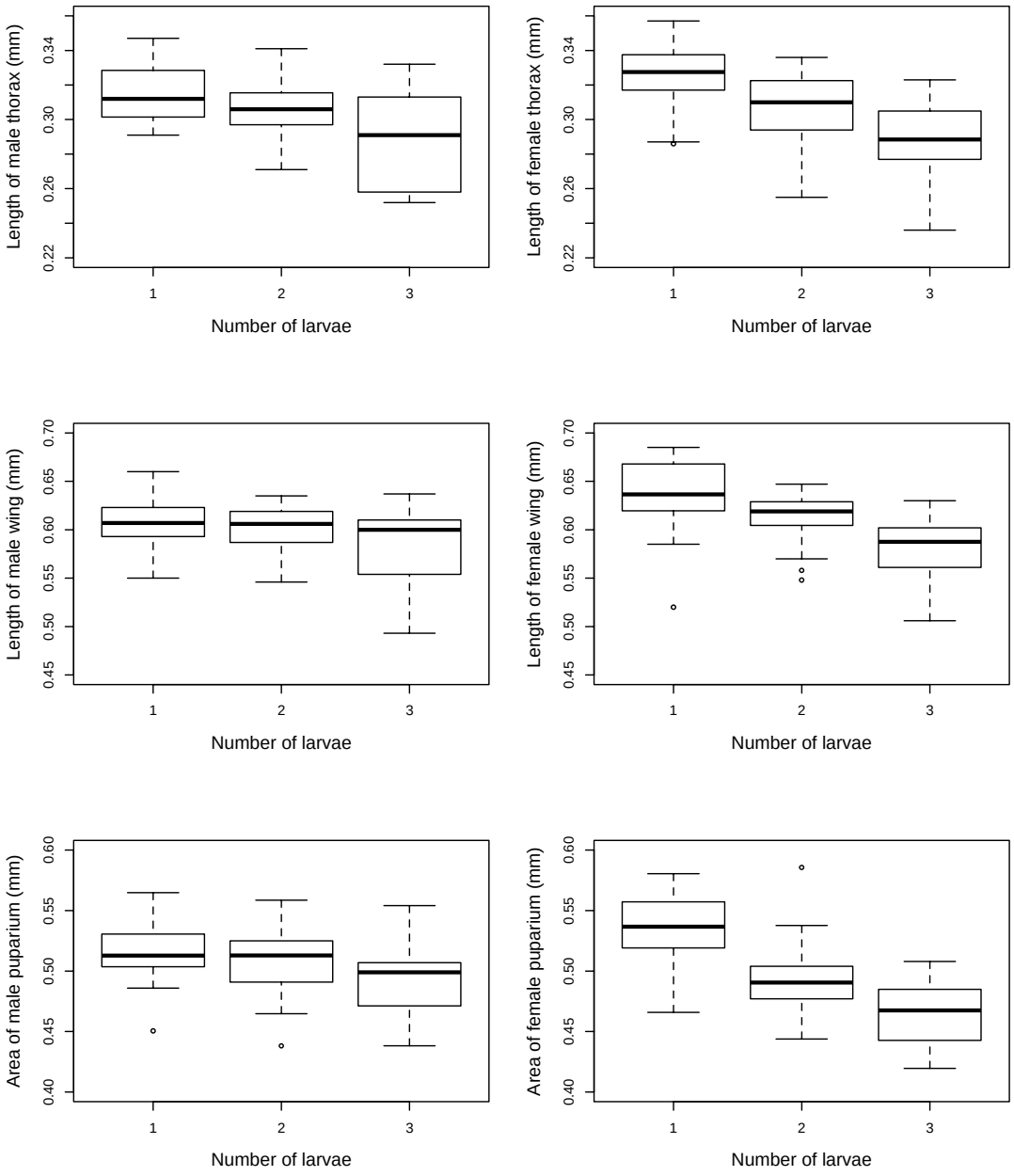


Figure 2.9 Boxplots showing response of male and female thorax (top row), wing length (middle row) and √ puparum area (bottom row) (mm) to larval density. The y-axis represents the mean value for all larvae in one microcosm.

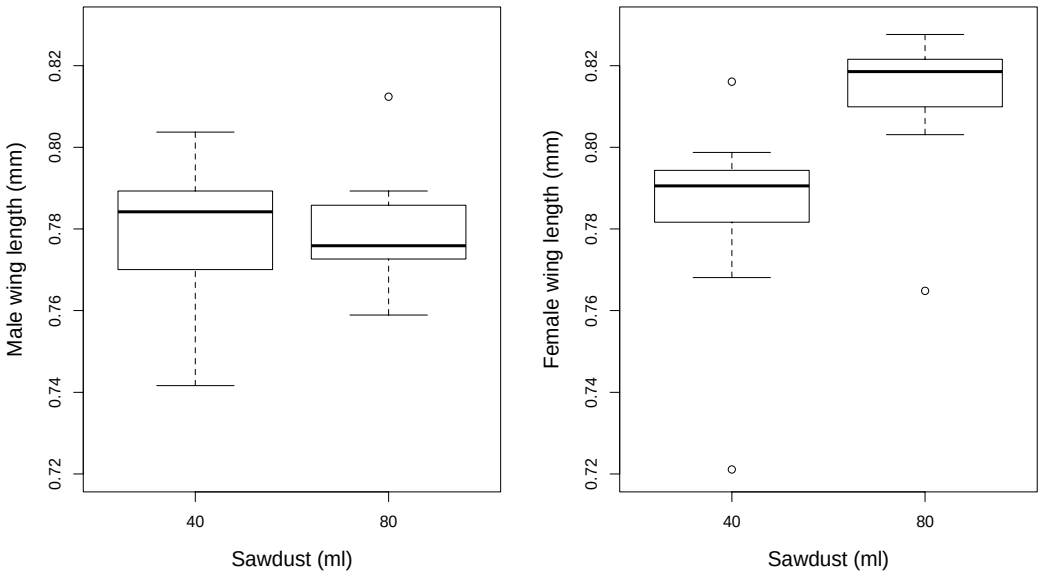


Figure 2.10 Boxplots showing different responses of male and female wing length (mm) to sawdust level (larval density = 1).

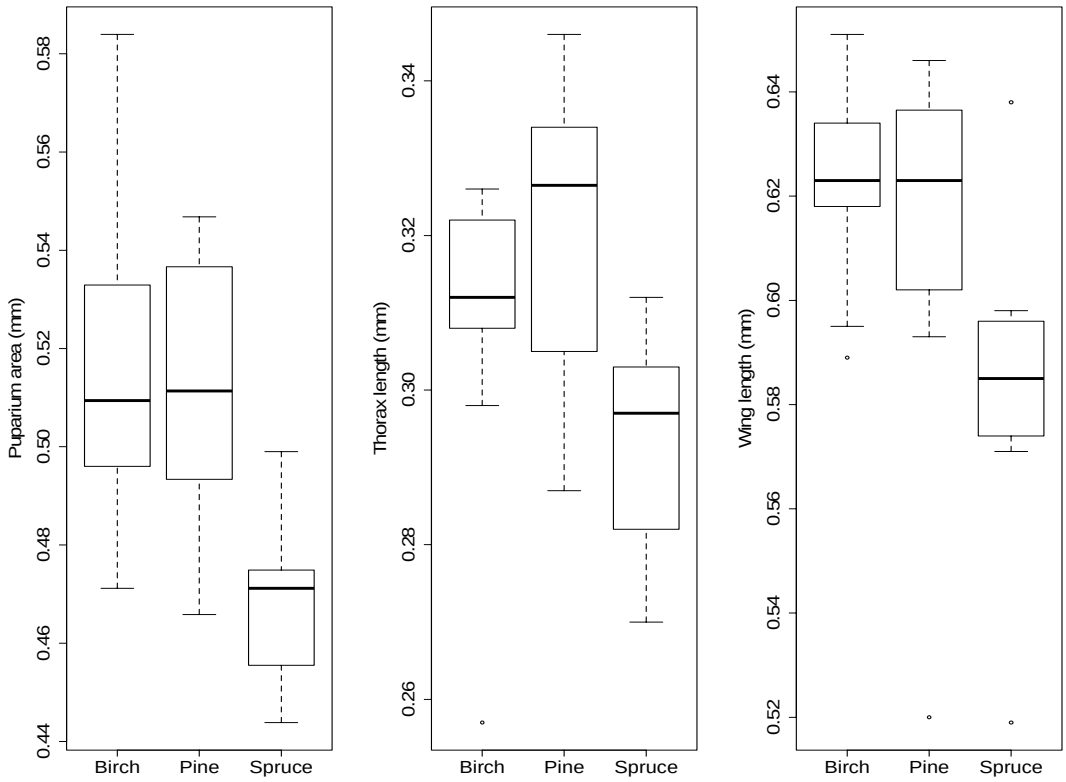


Figure 2.11 Boxplots showing different response variables: √ puparium area, and thorax and wing length (mm) to tree species birch, pine and spruce.

Chapter 3

Differences in ecomorphology and microhabitat use of four saproxylic larvae (Diptera, Syrphidae) in tree stump rot holes

3.1 Abstract

We explored co-existence and microhabitat partitioning in larvae of four species of hoverfly which occupy rot holes in Scots Pine *Pinus sylvestris* L. in Scotland, UK: the endangered pine hoverfly *Blera fallax* (Linnaeus), and three more common species, *Callicera rufa* (Schummel), *Myathropa florea* (Linnaeus) and *Sphegina clunipes* (Fallén) (Diptera, Syrphidae). Our primary aim was to investigate competitive exclusion risks to *B. fallax*, a species that now survives at only one site in the UK. We examined morphological differences between species and compared these to microhabitat use in an artificial rot hole. In addition, we measured larval growth for three of the species in different volumes of pinewood substrate to investigate differences in development in response to varying substrate levels. Field surveys confirmed that *B. fallax*, *C. rufa* and *M. florea* co-occur in pine rot holes. *Sphegina clunipes* was not abundant and only occurred with *B. fallax* on two occasions. Species differed in their growth rates and responses to variation in substrate level. *Blera fallax* developed quickly before winter, and decreasing substrate volume significantly inhibited growth, while *C. rufa* and *M. florea* took 6 months longer to achieve critical size for eclosion. Each species inhabited a distinct depth in the rot hole and exhibited correspondingly different behaviours associated with respiration and the length of their posterior breathing tubes. *Blera fallax* has an extendable breathing tube, which extended up to four times the body length (up to 46 mm) but is shorter than the body when fully retracted. *Blera fallax* occupied all areas of the rot hole. *Myathropa florea* has a long, extendable breathing tube (up to 63 mm) that is longer than the body when fully retracted, and was observed most regularly at the deepest points in rot holes. *Callicera rufa* has a short, non-

extendable breathing tube (mean length 0.96 mm), was highly sensitive to disturbance, and was most regularly found at an intermediate depth. *Sphegina clunipes* is the smallest species with a non-extendable, body length-long breathing tube (mean length 2.95 mm) and was most often found near the surface. The microhabitat partitioning observed in this study may facilitate the coexistence of these four species, and suggests that competitive exclusion will not hamper conservation management efforts for *B. fallax*.

3.2 Introduction

Closely related species that have similar niches may compete where their ranges overlap, but under the competitive exclusion principle they should be living in ways that minimise competition (Armstrong & McGehee 1980; Hardin 1960). Such adaptations can arise through coevolved niche shifts, or if already present upon first encounter may maintain co-existence (Lotka 1932; MacArthur 1972). Rare or specialised species less tolerant to competition may be vulnerable to even small competitive differences resulting in extinction (Hardin 1960). Coexistence mechanisms may buffer against extinction by providing advantages to rare or endemic species (Levins & Culver 1971; Lankau 2011). Fluctuations in environmental quality may affect competition and the consequent diversity in a habitat (Gotthard 2008; Pal *et al*. 2009; Chakraborty & Li 2010). Determining whether natural populations are food limited, and how different species respond to fluctuating levels in resources, is important in the study of species interactions in communities (Lenski 1984; Juliano 1986; Olson & Olson 1989). Rot hole communities provide excellent opportunities to study inter and intra-specific competition as they are discrete, spatially limited and capable of being experimentally manipulated (Kitching 1971; Srivastava & Lawton 1998; Srivastava 2005).

Saproxylic or dead wood syrphid larvae (Diptera, Syrphidae) have undergone considerable ecological radiation and occupy diverse microhabitats in woodlands, especially tree-holes, where they are well adapted for developing on decaying matter while being immersed (Gilbert *et al*. 1994). Modifications in Syrphid

saprophages in relation to other lower Cyclorrhapha (a higher taxonomic rank under the order Diptera), include greater size and enlarged mouthparts with a mechanism for filtering microbes suspended in fluids (Gilbert *et al*. 1994; Rotheray & Gilbert 1999, 2011). Being semi-aquatic and unable to swim, they need to reach the water surface in order to respire, where they may be particularly vulnerable to natural predators. To move underwater they have prolegs with crochets to grip substrates and an extended posterior breathing tube, which allows respiration while grazing on microbes in an aquatic environment, and limits exposure to terrestrial predators (Gilbert *et al*. 1994; Rotheray & Gilbert 1999). The degree of modifications varies across rot hole species, which may give rise to resource partitioning between otherwise potentially competing species (MacArthur 1972; Pfennig *et al*. 2007).

Four saprophagous syrphid species co-occur in pine, *Pinus sylvestris* L. tree rot holes in Scotland, UK, including the endangered pine hoverfly *Blera fallax* (Linnaeus), and three more common species, *Callicera rufa* (Schummel), *Myathropa florea* (Linnaeus) and *Sphegina clunipes* (Fallén) (Diptera, Syrphidae) (Fig 3.1). These species are chiefly univoltine, with overlapping flight periods between June and August for *B. fallax* and *C. rufa*, while *M. florea* is on the wing between May and October and *S. clunipes*, between May until September (Stubbs & Falk 2002). They also vary in the length of their breathing tubes and in the range of habitats they are associated with i.e. the condition and the species of their host tree. Unlike the other species, *B. fallax* is confined to *P. sylvestris*, and probably due to habitat loss currently exists at only one site in Scotland (Rotheray & MacGowan 2000; Rotheray 2010). *Myathropa florea* is widespread across the British Isles (Stubbs & Falk 2002) and can be found in a wide range of rot holes or water-filled

crevices in both coniferous and deciduous trees (Rotheray 1993). *Blera fallax* and *M. florea* have long, extendable posterior breathing tubes, and are thought to be the most derived of the four saprophages (Gilbert *et al*. 1994). *Callicera rufa* is found in coniferous tree-holes, mainly spruce *Picea abies*, larch *Larix decidua* and *P. sylvestris* (Stubbs & Falk 2002). Although *C. rufa* is fairly widespread, and has recently been recorded in England (Shropshire and Nottinghamshire) (Nigel Jones pers. comm.), it is largely restricted to Caledonian pine woodland in the Scottish Highlands (MacGowan 1994; Rotheray *et al*. 2001). *Sphegina clunipes* is a common species, widespread across the British Isles as far north as Sutherland (Stubbs & Falk 2002). It is normally associated with deciduous woodlands, and most often found in decaying sap under bark (Rotheray 1993; Stubbs & Falk 2002), but has recently been found in numerous artificial *P. sylvestris* rot holes (see Chapter 5). *Sphegina clunipes* and *C. rufa* have shorter, non-extendable posterior breathing tubes, and *C. rufa* has two sets of three large anterior hooks, the function of which is unclear although they may assist in excavation of rot hole substrates (Rotheray 1993). It is unknown if these differences partition the microhabitat of larvae sufficiently to eliminate or reduce competition for resources, and how such restrictions within the rot hole may affect their potential vulnerability to natural enemies or varying environmental conditions. It is particularly important that we determine how the pine rot hole community interacts in order to support conservation management for *B. fallax*. Effort is currently focused on retaining and expanding populations by relocating larvae into artificially created habitat at sites in the historical range for this species (Rotheray 2010; see Chapter 5). We need to confirm *B. fallax* will not be competitively excluded, or less able to cope in the

conditions created for its recovery, in which case an alternative management approach would be required.

There have not been any previous studies of saproxylic fauna in pine rot holes or involving the four fly species investigated here. Rotheray & MacGowan (2000) suggested that the quantity of wet decay found in rot holes is sufficient not to cause competition for resources between these four species. While some studies looking at increasing densities of rot hole filter-feeding communities detect no adverse effect on survival or biomass (Walker *et al*. 1991; van de Bund *et al*. 1994; Kaufman *et al*. 1999; Graca *et al*. 2000), most studies suggest competition in rot holes have negative effects (Livdahl 1982; Fisher *et al*. 1990; Broberg & Bradshaw 1995; Knight *et al*. 2004). Microbial diversity and abundance in a rot hole varies depending on the size of hole, tree species, and location, and environmental variables such as light availability (Kitching 1971; Sota 1998; Strand *et al*. 1999; Maciá & Bradshaw 2000; Paradise 2004; Bell *et al*. 2005). This in turn affects insect productivity which is directly related to the type and volume of detritus substrate (Fish & Carpenter 1982; Walker *et al*. 1991; Srivastava & Lawton 1998; Paradise 2004; Kaufman *et al*. 2008). Syrphid abundance in particular correlates with detritus volume (Srivastava & Lawton 1998).

Resource partitioning may reduce levels of competition, as has been found in a number of other insect communities inhabiting similar discrete water-filled environments (Levot *et al*. 1979; Seifert & Seifert 1979; Bradshaw & Holzapfel 1992; Fincke 1992; Sota *et al*. 1994). Insect larvae have been recorded preferentially grazing on the surface area of rot holes and leaf detritus, and on

particular bacterial taxa, decreasing abundance and microbial community structure associated with these specific areas (Kaufman *et al*. 2001, 2002, 2008). If larvae from different species segregate spatially, and these areas vary in productivity, we might predict different tolerance across taxa to changes in substrate levels. We wanted to test whether differences in larval behaviour covaried with differences in the response of the larvae to experimentally manipulated conditions in artificial rot holes. We predicted that, as a pine specialist, *B. fallax* growth response to resource-depleted conditions would equal or better co-occurring species.

Pine rot hole syrphids appear to vary in their characteristic responses to physical disturbance and freezing conditions (E.L. Rotheray, pers. obs.), which may be important for escaping from predators and surviving overwinter. While there are no known predators within the rot holes, a species of parasitoid, *Rhembobius perscrutator* Thunberg (Hymenoptera, Ichneumonidae) attack puparia of *B. fallax*, *C. rufa* and *M. florea*, all of which exit the rot hole in order to pupate. Beetles, birds and mammals are probably opportunistic predators of both larvae and pupae. Movement within rot holes may have been shaped by predation pressure if the different parts of the hole are subject to contrasting predation risk (Sih 1986; Relyea 2001; Schulte *et al*. 2004). Over-wintering larvae often move out of water upon freezing to avoid conditions, which can affect fly larva survival (Teets *et al*. 2011). This may make them more vulnerable to predation during these periods, and hence is likely to be important when considering the shape and depth of artificial rot holes created as part of conservation management protocols. Therefore, in addition to investigating microhabitat use and resource accessibility, we investigated inter-specific differences in response to disturbance and freezing conditions in the four

species. We predicted that species with longer breathing tubes would inhabit deeper areas in the rot hole, where they are less vulnerable to predation, but exit a rot hole less upon freezing in winter months.

3.3 Methods

3.3.1 Field data collection

To assess co-occurrence among the four species in this study, nine larval surveys took place at Curr Wood in Strathspey, Scotland, UK (57°18' N, 3°39' W) between November 2007 and February 2011. For each survey, the detritus content of ~100 pine stump rot holes was searched through, and a plastic pipette was used to carefully probe cracks and crevices deeper in the hole. Larval characters, such as the breathing tube and anterior spiracles, were used to identify each species (Rotheray 1993; see Appendix 5.3). The abundance of each species and degree of co-occurrence of *B. fallax*, *C. rufa*, *M. florea* and *S. clunipes* was recorded.

3.3.2 Morphological measurements

Ten larvae from each species (*B. fallax*, *C. rufa*, *M. florea* and *S. clunipes*) were collected from Curr Wood on 11th October 2008 and reared in captivity. In May and June 2009, final instar body length (from the tip of the anterior to the base of the posterior breathing tube), and posterior breathing tube length (from base of the

posterior breathing tube to the tip of the tube) was measured using a digital calliper. Maximum observed breathing tube lengths of *B. fallax* were also measured. This was made possible due to the tendency of *B. fallax* individuals to move along excavated tunnels in the sawdust adjacent to the transparent wall of glass microcosms, while the tip of their breathing tubes remained at the surface. The length was estimated using digital callipers and/or measuring tape. This was not possible for *M. florea* as larvae were never fully visible in this way, however in some cases the breathing tubes were not retracted upon removal allowing them to be measured. After transferring larvae to filter paper to remove excess water, weight was measured on a 0.001g resolution balance. The number of each species surviving to eclosion was also recorded.

3.3.3 Location and microhabitat use

Ten artificial pine 'rot hole' microcosms were created using 1000 ml glass jars filled with 200 ml pine sawdust, ~200cm^3 pine wood chips and 200ml bottled spring water. Microcosms were kept in climate-controlled facilities on 12-hour temperature and photoperiod cycles corresponding with those that they would experience naturally in North Scotland. These were estimated using data logger temperature readings and Met Office reports (although they were generally kept above 1°C to avoid mortality due to freezing) (Table 2.1). Microcosms were left for 48 hours to allow the substrate to become saturated. One larva of each species was placed into each microcosm. After 24 hours acclimation, instantaneous sampling techniques were used to record the location and activity of each individual (Altmann 1974). Three variables were recorded: 1) Position. The microcosm content (9 cm in height)

was divided into equal parts designated as surface (6 to 9 cm), middle (3 to 6 cm), or bottom (0 to 3 cm). 2) Respiration. Two respiratory organs are used for air exchange in syrphid larvae; the posterior breathing tube or the anterior spiracles, which are thought only to play a minor role (Gilbert *et al* 1994). Utilisation was determined by observing if either was in contact with the water surface. 3) Movement. Individuals were recorded as either moving or stationary. Spot observations were repeated twenty-one times for a total of 40 larvae between 7th and 24th November 2008.

Vibrations were an inevitable consequence of tracking activity and location of each species in the microcosms. *Callicera rufa* was highly sensitive to vibrations of any kind, causing individuals to quickly retreat if near the surface. Therefore, in order to obtain a more accurate description of natural behaviour, an additional observation was carried out focusing on single *C. rufa* activity throughout 60-minute periods. A stop clock was used to record the time each individual spent completely submerged or breathing (i.e. posterior breathing tube breaking the water surface). This was then repeated once for each individual ensuring a minimum 24-hour interval.

3.3.4 Response to freezing

During surveys carried out in the winter months when rot holes were frozen, small numbers of *C. rufa* and *B. fallax* were found either resting on the frozen water surface or in crevices in the stump or around the hole. After noting differences among species in their response to freezing in the field, an experiment investigating behavioural response to falling temperature was carried out in December 2008. Ten

microcosms already described above containing one of each of the four species were supplemented with an additional six microcosms each containing 3 *B. fallax* larvae, and temporarily moved into a climate-controlled cabinet programmed to the same daily cycles but with a lower minimum temperature of -2^{o}C. Once the water surface became frozen, the location of each individual was recorded (i.e. submerged or above the surface), and the temperature was returned to cycles reaching minimum 1°C (see Table 2.1) to allow larvae access to the surface to breathe. A Chi-squared test was used to assess differences between species by numbers at the surface or submerged.

3.3.5 Disturbance response

Two experiments were carried out to investigate response to disturbance. Both experiments required that individuals had their posterior breathing tubes in contact with the water surface. The first experiment was designed to mimic disturbance that a foraging predator may cause at the water surface. A plastic pipette was used to briefly touch the water, at least 4cm from where the breathing tube was breaking the surface, and the immediate response of the individual was recorded i.e. if the tube or larva retracted or withdrew from the surface. These tests were repeated three times with 48-hour intervals for a total of 40 individuals (10 of each species).

The second experiment was designed to determine how larvae cope if they are dislodged from a substrate. Individual larvae were gently detached from their anchorage, either from in the sawdust or on the bark ladder, using the end of a plastic pipette. Individuals were then observed for ten minutes or until they sunk or

found substrate, during which their behaviour was monitored and the response (positive or negative buoyancy) was recorded.

All statistical analyses were carried out using the statistical package R (version 2.13.1) (R Team 2011). Linear mixed effects (LME) models in the 'nlme' package (Bates & Maechler 2008) were used to account for repeat measures. This method uses restricted maximum likelihood to produce unbiased estimates of model parameters to test hypotheses. LME provide estimates of the influence of fixed effects on the mean as well as influence of random effects on variance, allowing correlation between within-group errors and unequal variances. We used frequency of microhabitat-use or behaviours (respiration and movement) to predict location in the rot hole and behaviour by species with individual larva identity fitted as a random effect in both tests. We report significance tests for analysis of variance (ANOVA) with restricted maximum likelihood (REML) used to compare models with and without species as an explanatory variable.

We used generalised linear mixed effects models with Binomial error distribution to model species differences in response to disturbance (no withdrawal/withdrawal) and dislodging (float/sink), with individual larva fitted as random effects. Due to the unbalanced design, we report significance tests for type-II sums-of-squares implemented in the 'car' package (Weisberg & Fox 2010).

3.3.6 Growth experiments

Growth experiments to assess differences among species in resource acquisition were carried out using manipulated quantities of *P. sylvestris* sawdust collected from a pine wood mill in Abernethy Forest, Scotland in the same volume of water. Fourteen 250ml glass microcosms with foam stoppers were filled with 10 - 70 ml (in 10 ml increments) pine sawdust, 70mls water, and bark ladders (9 x 3 x 1 cm^3) to allow larvae to crawl closer to the surface to breath. Microcosms were left for 48 hours to allow the content to become saturated.

On 29th August 2009, fourteen captive bred ~1st instar *B. fallax* larvae were selected based on size (< 7mm body length). Larvae were starved for 24 hours to minimise the effect of pre-experimental conditions, before transferring one randomly designated individual into each microcosm. Initial larval measurements were taken on 29th August and a further two on 2nd and 20th October 2009. Individual larvae were measured following methods outlined in Chapter 2. Individuals were removed from the microcosm using a plastic pipette and transferred to laminated lined paper for scale. Digital images of each larva were taken, and its two-dimensional area was calculated using ImageJ software (Abràmoff *et al*. 2004). To avoid excessive *B. fallax* mortalities, after the final measurement in October, surviving individuals in low sawdust volumes (< 50 ml) were provided with additional substrate. No further larval measurements were made, although the number of individuals surviving to eclosion was recorded.

In 2010, these methods were repeated using twenty-one larvae each of *C. rufa* and *M. florea* collected from the Curr Wood. Due to logistical reasons, *B. fallax* was not included in this experiment. The experiment was started slightly later in the year as no *C. rufa* larvae were found in breeding sites until late August. Each volume (10 to 70ml) was duplicated three times for each species. Initial larval area measurements were taken and a further two at fourteen-day intervals between 23rd September and 21st October 2010, plus one additional measurement on 16th May 2011. Weight was also measured at the initial and first two time intervals, and in July 2011 (for logistical reasons, area was not measured in July). Larvae were transferred to filter paper to remove excess water, and weight was measured on a 0.001g resolution balance. Individuals surviving to eclosion were recorded.

Linear models were used to investigate the effect of sawdust volume on growth for each species. Larval area (mm^2) was used for time intervals 1 (August/September) to 4 (May), and weight (for *C. rufa* and *M. florea*) for time intervals 1 to 5 (July). Although the differences in size measures across species prohibit direct comparisons, weight and area are strongly correlated and should reflect similar patterns overall; corresponding results were found using either measurement (see Chapter 2). Total area change (and weight change for *C. rufa* and *M. florea*) between the first two time intervals (pre-winter growth) was modelled for each species as a function of sawdust volume, and separate models were used to measure change in area between the initial time interval and May (time interval 4), and weight between the initial time interval and July (time interval 5) for *C. rufa* and *M. florea*. Chi-squared tests were used to measure differences in the number surviving

between species, and in low volumes (< 40 ml) compared with high volumes (> 30 ml).

In order to visualize the effect of volume on growth across species, nonparameteric thin-plate splines of body area and mass as a function of sawdust volume and time were generated, implemented using the 'fields' package for R (Furrer *et al*. 2009). The smoothing parameter (lambda) for the response surface was determined using generalised cross-validation (GCV).

3.4 Results

3.4.1 Field data collection

Between 2007 until 2011, larvae of the four species were found in sixty-two rot hole breeding sites. In 35% of holes larvae co-occurred: 9% with three species mainly *M. florea*, *B. fallax* and *C. rufa*; and 27% with two species, mainly *B. fallax* and *M. florea* (18%). Rot holes containing one species (65%) were mainly *B. fallax* (32%). *Sphegina clunipes* was mainly found alone, co-occurring with *B. fallax* in one rot hole, and with *B. fallax* and *M. florea* in one other.

The abundance of each species varied throughout the year. The most abundant was *M. florea* and the least abundant was *S. clunipes* (Table 3.1). All four species were present as 1st instar larvae in rot holes between July and August, but *C. rufa* tended to appear later than the other species (Table 3.1). The abundance of *M. florea* and *B.*

fallax appeared to decrease dramatically after winters 2007/08 and 2010/11, decreasing by 84 and 62% in *M. florea*, and 94 and 79% in *B. fallax*, respectively (Table 3.1). *Callicera rufa* also dropped in abundance in 2010/11 by 59% (Table 3.1). Larval density per rot hole varied from one to eight (mean 4.1 ± 2.2 SD) for *B. fallax*, three to nine (mean 5.4 ± 2.76 SD) for *C. rufa*, two to ten (mean 5.74 ± 2.76 SD) for *M. florea* and five to eleven (mean 6.04 ± 3.88 SD) for *S. clunipes* (Table 3.1).

3.4.2 Morphology

Based on body weight, on average *C. rufa* and *M. florea* were the largest of all four species at 0.177 ± 0.05 and 0.167 ± 0.04 milligrams (mg) respectively, and *S. clunipes* was the smallest at 0.011 ± 0.002 mg (mean ± Standard Deviation SD) (Table 3.2). *Myathropa florea* had the longest breathing tube, which measured roughly double the length of the body (24.6 ± 14.6 mm, Table 3.2) and extended up to four times body length reaching 62.8 mm in one case. The *B. fallax* breathing tube length was an average half the length of the body (7.2 ± 1.98, Table 3.2), but could also extend up to four times body length reaching up to 46.2 mm.

A significant difference was found between the numbers of individuals surviving between each species ($\chi^2 = 58.5$, df = 3, $P < 0.005$). The number of captive reared larvae surviving to eclosion in 2008 to 2009 was least in *S. clunipes* (40%) and greatest in *C. rufa* (100%), with intermediate survival in *M. florea* (70%) and *B. fallax* (66%) (Table 3.2). The larvae were not monitored periodically so exact time of death is not known, preventing further survival analysis.

3.4.3 Location and microhabitat use

The generalised linear models compared using REML suggested a strong difference between species in their rot hole location ($L = 145.9$, $p < 0.0001$), and between species in their behaviour (respiration and movement) ($L = 59.6$, $p < 0.0001$) in a rot hole. *Myathropa florea* was most often observed at the bottom of the microcosm, either completely submerged or with the breathing tube at the water surface (Fig 3.2 and 3.3). In contrast *S. clunipes* was most often found near the surface, but again it tended to be either completely submerged or with the breathing tube at the water surface (Fig 3.2 and 3.3). *Callicera rufa* was almost always found in the middle of the microcosm and completely submerged (Fig 3.2 and 3.3). *Blera fallax* appeared to inhabit all areas equally (Fig 3.2), was the most mobile of the four species, and was regularly observed with the head end at the surface (Fig 3.3).

During a 60-minute period, *C. rufa* (n = 10) moved to the water surface to respire a minimum of three and maximum of seven times (mean 4.7 ± 1.2 SD). Upon submergence, individuals moved to the central part of the microcosm. Per surface/submergence interval, the maximum-recorded length of time spent at the surface was 11:05 min (1:7 ± 1:7 min) and submerged was 28:02 min (10:2 ± 6.38 min) (Fig 3.4).

3.4.4 Response to freezing

Twenty-five (86%) *B. fallax* and eight (80%) *C. rufa* were found on the frozen water surface in the incubators, while all *M. florea* and *S. clunipes* remained submerged ($\chi^2 = 58.5$, df = 3, $P < 0.005$).

3.4.5 Disturbance response

The generalised linear mixed-effect model showed a strong effect of species on buoyancy (i.e. floating upon dislodging) ($\chi^2 = 15.65$, $p = 0.001$), but no significant response for disturbance (i.e. withdrawing upon disturbance) ($\chi^2 = 6.36$, $p = 0.09$) (Table 3.3). However, the binomial model did not fit well because all *C. rufa* and *S. clunipes* individuals behaved identically. Comparing the remaining two taxa showed that *B. fallax* was more likely to respond to disturbance than *M. florea* ($\chi^2 = 5.86$, $p = 0.016$) and was more likely to float upon dislodging ($\chi^2 = 11.68$, $p = 0.001$) (Table 3.3). *Callicera rufa* showed a consistent withdrawal response to disturbance, and was positively buoyant where upon losing anchorage they would arch and twist at the water surface until finding substrate to grip. *Sphegina clunipes* did not retract upon disturbance and was consistently negatively buoyant, and on reaching the bottom of the microcosm remained motionless. Most *B. fallax* withdrew upon disturbance (73%), and 83% were positively buoyant upon dislodging (Table 3.2). *Blera fallax* response to losing anchorage was similar to *C. rufa* although twisting movements were less pronounced. *Myathropa florea* was 40% positively buoyant and responded to stimulus 33% of the time (Table 3.2). *Myathropa florea* was

consistently more slow-moving than *B. fallax* and *C. rufa* whether at the surface or submerged.

3.4.6 Growth experiments

Blera fallax growth was significantly affected by sawdust volume (Parameter estimate 0.005 ± 0.001 SE, t = 7.17, P < 0.001) (Table 3.4). Larvae grew more slowly in lower volumes of sawdust, and *B. fallax* accumulated most of their mass before the winter, while the other taxa experienced growth before and after overwintering (Table 3.4, Fig 3.5). In November all remaining *B. fallax* individuals in volumes greater than 50 ml had exited the rot hole, presumably to pupate (see Chapter 2; Rotheray & MacGowan 2000). Mortality was lower in higher volumes of sawdust (14%) compared with those in 30ml sawdust and less (29%) ($\chi^2 = 5.23$, df = 1, P < 0.05).

Pre-winter growth was not significantly affected by sawdust volume in *C. rufa* (Parameter estimate -0.0002 ± 0.001 SE, t = -0.36, P = 0.724) or *M. florea* (Parameter estimate 0.001 ± 0.0004 SE, t = 1.32, P = 0.203). In early spring, volume had a significant effect on growth in *M. florea* (Parameter estimate 0.002 ± 0.001 SE, t = 2.08, P = 0.05), but not in *C. rufa* (Parameter estimate 0.001 ± 0.001 SE, t = 1.13, P = 0.274). Using total growth change from time interval 1 to 5 (September to July), a significant effect of sawdust volume on growth was evident in both *C. rufa* and *M. florea* (Table 3.4, Fig 3.5).

All *C. rufa* and *M. florea* individuals in treatments above 20 ml eclosed in July, and overall mortality was lower than *B. fallax* (43%) at 14% in *M. florea*, and 10% in *C. rufa* ($\chi^2 = 29.04$, df = 2, $P < 0.005$).

3.5 Discussion

The co-occurring species assessed in this study exhibited variation in development time, feeding strategies and microhabitat preferences, which may be evidence for resource partitioning reducing effects of competition in pine rot holes.

Co-occurrence in the field was confirmed in at least three of the four species irrespective of time of year, and larval density was similar across co-occurring species. The exception was *S. clunipes*, which was not as abundant, was smaller than the other species, and was only twice observed co-occurring with *B. fallax* and *M. florea*. This suggests competition for resources could potentially occur between *B. fallax*, *C. rufa* and *M. florea*.

3.5.1 Growth experiments

It is important to address the restrictions of this experiment, which limit interpretation. Primarily, the experiment assessing *B. fallax* growth was carried out in a previous year and at an earlier time in the year therefore, while it is a true representation of the timing of the lifecycle of these taxa, it limits direct comparisons between *B. fallax* and the other taxa. Furthermore, varying sawdust

volume alone creates different conditions in a microcosm. The response of larval growth may not be caused by volume as much as the conditions created by increasing the sawdust to water ratio (see Chapter 2). In order to derive a more direct assessment of how larvae are affected by volume of pine wood substrate, water volume would need to be manipulated also.

Blera fallax and *M. florea* both achieved considerable growth before winter, whereas *C. rufa* appeared to grow gradually throughout the developmental period. During the winter, like many overwintering larvae, feeding is probably suspended as a means to survive freezing conditions (Hart & Bale 1997a, 1997b; Bale 2002; Chapter 2). Unlike *B. fallax* in good quality conditions, *C. rufa* and *M. florea* may be more dependent on the following spring and early summer to achieve critical size for eclosion.

While growth in both *C. rufa* and *M. florea* is inhibited in low volumes of pine sawdust, *B. fallax* appears to be worst affected. Both *C. rufa* and *M. florea* accessed enough food for growth in even the lowest sawdust volume, but took longer to develop to a sufficient size for eclosion. While *C. rufa* and *M. florea* may be better able to develop in resource limited habitats, it is unknown if *B. fallax* would be subjected to similarly low detritus conditions in natural circumstances. However, larvae developing in lab conditions exhibited similar growth to *B. fallax* in the field (Chapter 2), which suggests that resources may also be limiting in natural circumstances. *Blera fallax* can continue to develop for another year if conditions are poor (Chapter 2). Controlled comparative field studies using similarly sized

stump rot holes with the same content as microcosms, would be required to assess this further.

Blera fallax was able to achieve more growth in a shorter period of time, which may be part of a strategy for avoiding competition with larger species that arrive later and take longer to develop. As indicated by the 'exiting' behaviour observed in *B. fallax* upon completing development, individuals in high sawdust volumes had probably achieved enough growth for pupation before winter and therefore entered into a long period of diapause before pupating in the spring (Chapter 2). By growing fast, *B. fallax* may also reduce negative effects of competition due to their greater size compared with new larval instars of their competitor species, a finding reported in mosquitoes where larger size leads to competitive advantages in tree-holes (Livdahl 1982). A similar strategy is reported in *Mecistogaster* damselflies (Odonata) colonising tree-holes earlier in the wet season than other larger guild members thereby avoiding competition and gaining a head start in growth critical to its survival (Fincke 1992). Size may in fact partition resources further as reported by Srivastava and Lawton (1998) in *M. florea* which, as the larger larval filter feeder, appears to have very little effect on the abundance of smaller species inhabiting the same rot hole. The size of a filter feeder can shape the size and abundance of the microbe community and can even lead to phenotypic plasticity within certain bacterial taxa where individuals in a population change in size to decrease grazing vulnerability (Gerritsen 1984; Hahn & Höfle 2001; Matz & Kjelleberg 2005; Salcher *et al*. 2005). If this occurs in pine rot holes, different larval instars may be exploiting different bacterial species or microbial populations thus reducing competition further.

As well as size, activity and ability to move through dense substrate may affect larval growth. *Myathropa florea* showed signs of being growth-inhibited in the higher volumes, which may be related to the more compacted substrate as the sawdust to water ratio increases. Indeed a previous study has found that *M. florea* prefer low detritus tree holes (Schmidl *et al*. 2008). *Myathropa florea* was also particularly slow-moving, which in less densely packed habitat may otherwise improve feeding efficiency by using less energy to move around (Reinhold 1999). The anterior hooks of *C. rufa* may assist passage through the substrate and thus provide access to more microbes. This may serve as an extra advantage in such conditions and possibly explains the constant gradual growth and lack of inhibited growth in higher volumes. *Blera fallax* was not inhibited in higher sawdust volumes, however inhibited growth in higher volumes has been evident in another study (Chapter 2). Rot hole content in the field becomes compact during dry periods, particularly during late summer (E.L. Rotheray pers. obs.), so having the ability to cope with such conditions is likely to be important.

3.5.2 Location and microhabitat use

As predicted, *M. florea* was most often observed in the deepest areas of the microcosm where they tended to remain, anchored in the sawdust. In contrast, *B. fallax* was more active, and apparently capable of occupying all areas in the microcosm. This may be an advantage of the readily extendable breathing tube of *B. fallax*. In contrast with *M. florea*, *B. fallax* could retract their breathing tube to less than the length of their body, which may allow for less restricted movement if

breathing tubes can become tangled or trapped. Rot in pine stumps can travel deep into the root system (Rotheray & MacGowan 2001) thus being able to search for food in such small cracks and crevices may be advantageous.

Callicera rufa appeared to be restricted to the centre of the microcosm probably due to the necessity for it to access the surface to breathe at least three times every hour. This requirement may make them more vulnerable to predation, and consequently select for more antipredatory behaviour. The anterior hooks of *C. rufa* may provide a further advantage in assisting anchorage, which may be particularly important for this species in light of its buoyancy. Thrashing at the surface may increase the risk of predation, as found in tree-hole inhabiting mosquitoes (Juliano & Reminger 1992). *Callicera rufa* has no extendable breathing tube, therefore moving quickly to safety may be more important and advantageous than sinking to the bottom where they run the risk of drowning. The ability to sink has previously been recorded in *M. florea* where they reportedly hold or expel air from their breathing tube to retain or lose buoyancy (Buckton 1895; Greig 1989). *Myathropa florea* was also less sensitive to disturbance, probably due to reduced vulnerability being deep in the rot hole for most of the time. *Sphegina clunipes* was not mobile, sensitive to disturbance, or buoyant, and showed no physical reaction upon sinking. The greatest proportion of mortality was also found for *S. clunipes*. Pine rot holes may be a sub-optimal habitat for this species, possibly utilised when their preferred habitat (decaying sap under bark) is rare or unavailable.

3.5.3 Response to freezing

Blera fallax and *C. rufa* were observed resting on the frozen surface of microcosms upon freezing while *M. florea* and *S. clunipes* remained below the surface. A survival tactic of final instar larvae i.e. those that have finished feeding, might be to quit rot holes during freezing if survivorship is greater outside than inside a freezing tree hole. Artificial habitat created for *B. fallax* may be more vulnerable to freezing completely for longer periods than natural rot holes, due to the shallow cavity compared with natural holes that can extend deep into the roots of a tree (Rotheray & MacGowan 2000). Larvae can withstand being frozen having been chipped from solid ice and found alive and moving (G.E. Rotheray pers. comm.), but they may be vulnerable to constriction and physical rupture if trapped in the solid ice for extended periods. In the field, a number of *C. rufa* larvae have been observed trapped exiting rot holes in winter, and *M. florea*, whenever observed during these periods, has been trapped under the frozen surface (E.L. Rotheray pers. obs.). Subsequent surveys suggested these individuals from both species had died. *Callicera rufa* and *B. fallax* move to the surface of the rot hole when it freezes, and *B. fallax* moves out of the hole completely if fully grown (see Chapter 2) possibly giving them a strategic advantage over *M. florea* and *S. clunipes*. A more detailed study is required into the specific dynamics within a rot hole over the winter period, and how larvae survive these conditions.

This study provides insight into the mechanisms that may facilitate resource partitioning and permit coexistence of these syrphids in pine tree rot holes. It also demonstrates variable avoidance tactics and survival strategies used by different

species to exist in these seasonal, heterogeneous habitats. Intra-specific competition for resources was not directly assessed, but the inter-specific response to altered resource levels suggests that further experiments would be worthwhile. Differences in time of feeding and feeding strategies probably minimises the overlap among species with similar diets. *Blera fallax* develops earlier than the other taxa and may have a further advantage in being able to inhabit all depths of a rot hole, which may allow it more flexibility in adjusting feeding depth to maximize food intake. These findings suggest competitive effects are likely not to impact on conservation strategies for this species.

Table 3.1 Total abundance of *B. fallax*, *C. rufa*, *M. florea* and *S. clunipes* present in rot holes, the number of rot holes occupied (No. RH), and the number of new and previously occupied rot holes in each year at Curr Wood recorded between 2007 until 2011.

										Rot holes		
		B. fallax	No. RH	*C. rufa*	No. RH	*M. florea*	No. RH	*S. clunipes*	No. RH	Total occupied	Previously occupied	Newly occupied
2007	November	35	10	16	6	118	15	5	1	20	-	20
2008	April	2	1	21	5	19	6	0	0			
2008	July	5	5	8	2	4	2	17	2			
2008	August	109	16	19	3	182	23	19	3			
2008	October	100	13	27	5	113	23	23	2	32	15	17
2009	July	142	24	0	0	162	16	0	0			
2009	September	59	13	33	4	91	16	18	2	33	23	10
2010	August	111	22	61	7	101	14	7	1	33	25	8
2011	February	37	11	25	4	39	14	7	1	20	20	0

- initial survey

Table 3.2 Larval body and posterior breathing tube measurements (mean ± SD), final instar weight and mortality of *B. fallax*, *C. rufa*, *M. florea* and *S. clunipes* from May and June in year one, 2008.

Species	Body length (mm)	Tube length (mm)	% tube to body length	Final instar weight (mg)	% Mortality*	% Buoyant	% Disturbance responsive
B. fallax	13.5 ± 2.11	7.2 ± 1.98	54 ± 11	0.072 ± 0.03	34	83	73
C. rufa	17.7 ± 3.2	0.96 ± 0.3	6 ± 1	0.177 ± 0.05	0	100	100
M. florea	13.4 ± 3.3	24.6 ± 14.6	187± 88	0.167 ± 0.04	30	40	33
S. clunipes	6.2 ± 0.9	2.95 ± 0.8	49 ± 18	0.011 ± 0.002	60	0	0

* n = 10 except *B. fallax* where n = 50

Table 3.3 Parameter estimates for generalised linear mixed-effects models intra-specific response to disturbance (withdrawal upon disturbance) and dislodgement (positive or negative buoyancy).

	Parameter estimate ± SE	z-value	p-value
Disturbance			
B. fallax	4.765 ± 2.15	1.895	0.058*
C. rufa	1.86 ± 2.165e+04	0.001	0.999
M. florea	-8.214 ± 3.282	-2.503	0.012*
S. clunipes	-1.02 ± 1.225e+07	0.000	1.000
Buoyancy			
B. fallax	2.65 ± 0. 96	2.758	0.005*
C. rufa	18.95 ± 8.996e+04	0.007	0.9944
M. florea	-4.527 ± 1.32	-3.063	0.002*
S. clunipes	-22.18 ± 3.166e+0.4	-0.008	0.993

* <0.005 significance

Table 3.4 Parameter estimates for general linear model of *B. fallax* larval area change over 50 days (August to October) and *C. rufa* and *M. florea* weight (mg) over 250 days (September to July), in 20 to 70ml sawdust treatments.

	Parameter estimate ± SE	t-value	p-value
Growth response to volume			
B. fallax			
Intercept	-0.025 ± 0.034	-0.737	0.438
Sawdust volume	0.005 ± 0.001	7.174	<0.001*
C. rufa			
Intercept	-0.002 ± 0.012	-0.154	0.88
Sawdust volume	0.002 ± 0.0003	8.111	<0.001*
M. florea			
Intercept	0.029 ± 0.012	2.479	0.024
Sawdust volume	0.001 ± 0.0003	3.29	0.004*

* <0.005 significance

Figure 3.1 Four final instar saprophagous syrphid species co-occurring in *Pinus sylvestris* tree rot holes. From left *Myathropa florea, Blera fallax, Sphegina clunipes* and *Callicera rufa* (Diptera, Syrphidae)

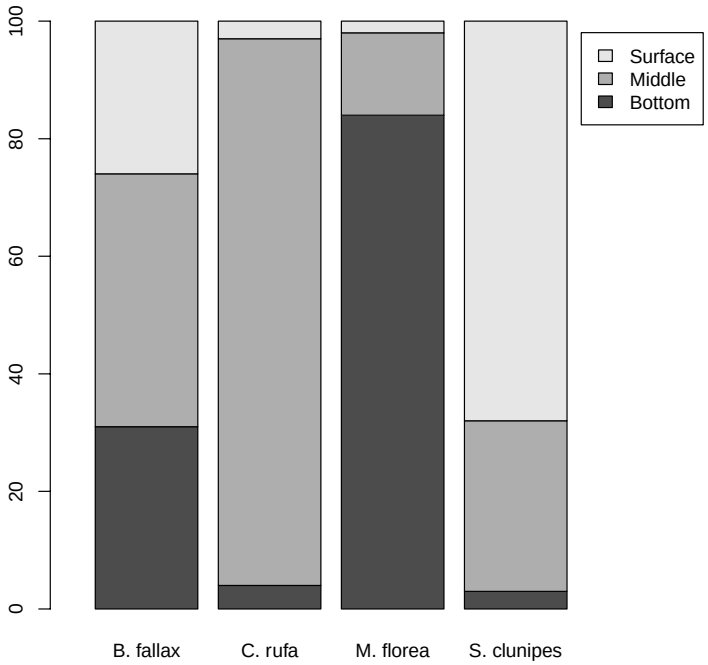


Figure 3.2 Stacked column plot illustrating number of times each species, *B. fallax*, *C. rufa*, *M. florea* and *S. clunipes*, was recorded at three locations, surface (6 to 9 cm), middle (3 to 6 cm) and bottom (0 to 3 cm) in 1000ml glass artificial rot holes (21 instantaneous observation sampling were made over 17 days).

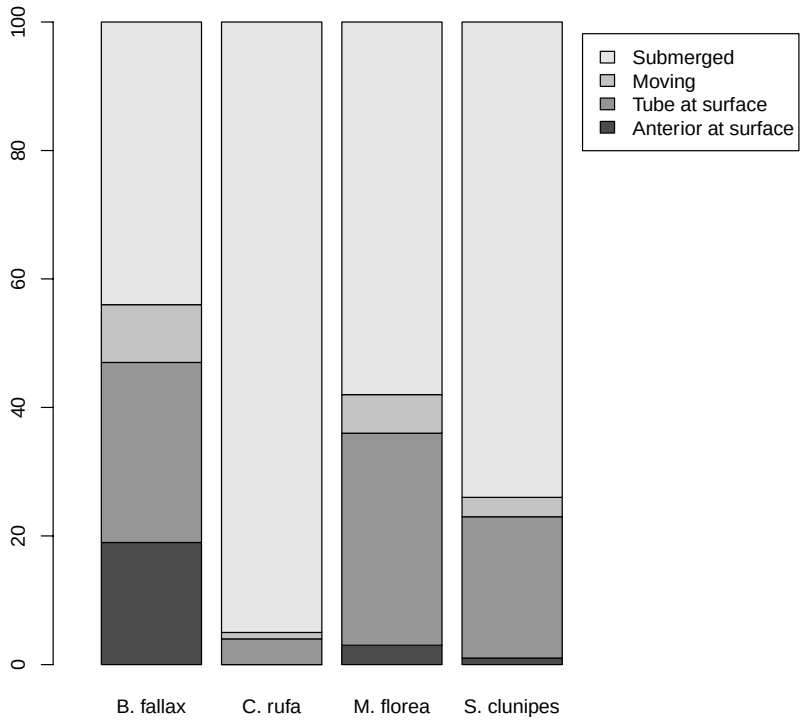


Figure 3.3 Stacked column plot illustrating number of times each species, *B. fallax*, *C. rufa*, *M. florea* and *S. clunipes*, was recorded as completely submerged, moving, or anterior/posterior breaking the water surface (21 instantaneous sampling observations were made over 17 days).

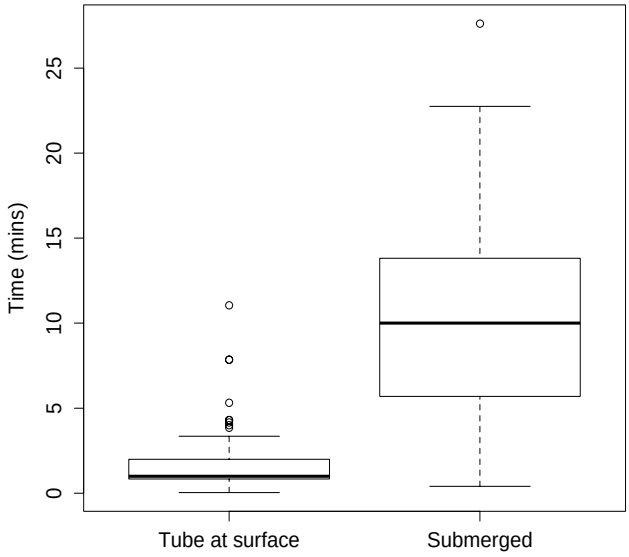
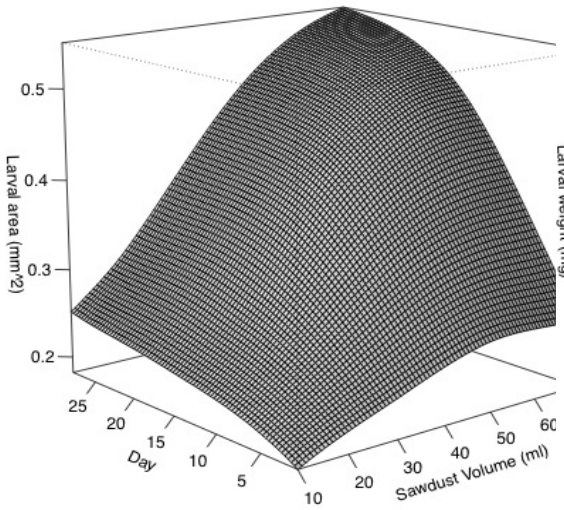
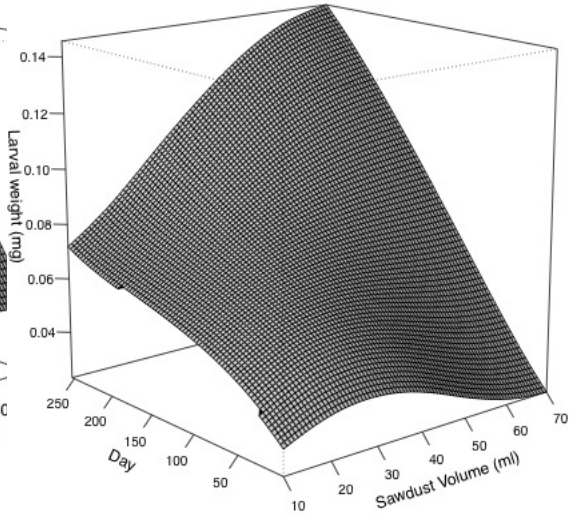


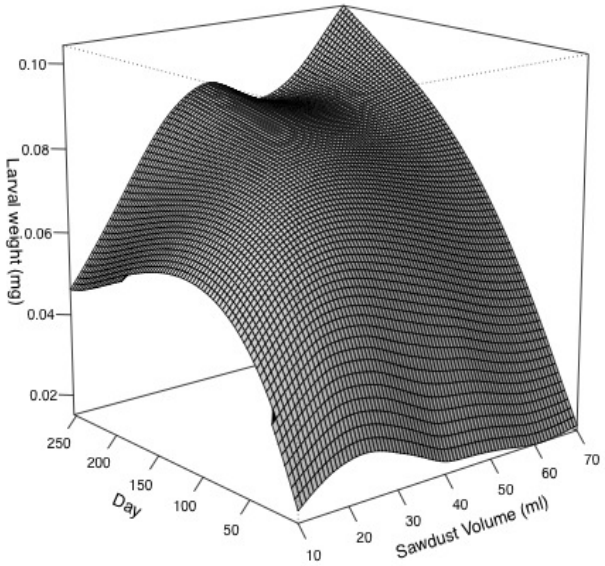
Figure 3.4 Boxplot illustrating the time *C. rufa* (n = 10) spent with the posterior breathing tube breaking the water surface or completely submerged over 60-minute periods.



B. fallax



C. rufa



M. florea

Figure 3.5 Plotted thin plate spline surface for larval body weight over time (day in experiment) in sawdust volumes 10 to 70 ml for *B. fallax*, *C. rufa*, *M. florea*. Lambda was estimated by generalized cross-validation (GCV). Note difference in time, which reaches a maximum 28 days for *B. fallax* and 250 for *C. rufa* and *M. florea.*

Chapter 4

Genetic variation and population decline of an endangered hoverfly *Blera fallax* (Diptera: Syrphidae)

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4.1 Abstract

Genetic diversity is one of several factors affecting extinction risk in vulnerable populations. In addition to informing conservation management strategies, data on genetic variability can also shed light on the recency and magnitude of historic bottlenecks. The pine hoverfly *Blera fallax* is one of the rarest invertebrates in the UK, known from just two populations in Scotland. It belongs to an often overlooked, species-rich community that is fundamental to forest function, the saproxylics (that depend on dead wood). To assist current conservation management for *B. fallax*, including captive breeding and translocations, it is important to know whether genetic factors will limit the success of recovery. Using 12 microsatellite loci, we compared the genetic variation in Scottish with Swedish specimens (Swedish populations are thought to represent a more outbred *B. fallax* population). As expected, the Scottish population showed significantly lower levels of polymorphism, expected heterozygosity and allelic richness than the Swedish population. Furthermore, significant genetic differentiation was found between the two *B. fallax* populations (F_{ST}=0.134). We then used an allele frequency-based approach and a Bayesian coalescent-based method to assess genealogical history and detect recent changes in population size. Unexpectedly, data from not only the Scottish but also the Swedish population indicated a strong and relatively recent decline that was more pronounced in Scotland. We discuss the implications of our findings for future conservation management planning, the first undertaking of its kind for saproxylic species in Britain.

4.2 Introduction

The conservation management of endangered species involves ensuring the survival of viable populations and increasing their abundance and distribution (Primack 1998). This requires knowledge of the behaviour and ecology of a species in order to identify the causes of decline and manage accordingly, but it also involves assessing genetic diversity in the frequently small, isolated and threatened populations, which can limit the adaptive potential of the species (Lande 1988). Populations with limited genetic diversity are more susceptible to environmental change and thus at greater risk of extinction (Frankham 1995, 1998, 2005). Where captive breeding and translocation play a role in management protocols, inbreeding effects and effective population size become particularly relevant issues (Leberg 2005). Reduced fitness caused by inbreeding has been demonstrated in numerous controlled experiments (Armbruster *et al*. 2000; Woodworth *et al*. 2002; Whitehorn *et al*. 2010) and in studies on wild populations (Brown & Brown 1998; Keller 1998; Saccheri *et al*. 1998), and while the effects on different taxa and individual populations appear to vary (Elgar & Clode 2001), especially with respect to demographic and environmental stochasticity, inbreeding depression is considered pervasive enough to have a generally detrimental effect on population persistence (Keller and Waller 2002). Where populations are considered highly vulnerable, conservation management often involves introducing individuals from a genetically and demographically healthy population to improve fitness (Dowling *et al*. 2005; Edmands 2007; Biebach & Keller 2010). While this has shown to be highly effective across a number of studies (Tallmon *et al*. 2004) such intentional hybridization can also lead to a subsequent reduction in fitness known as outbreeding depression (Templeton 1986; Lynch 1991; Edmands 2007), an effect that often becomes apparent in later generations (e.g., Armbruster *et al*. 2000;

Aspi 2000). While evidence for outbreeding depression is scarce, it is important to consider this risk and assess genetic and adaptive similarity between populations intended for translocation and hybridization (Edmands 2007).

Our main objective was to investigate the genetic diversity of the UK endangered hoverfly *Blera fallax* (Diptera, Syrphidae) by comparing a Scottish population with one in Continental Europe which, based on the distribution and condition of its pine wood habitat (Willis *et al*. 1998), is assumed to be less isolated and more panmictic (G. Rotheray pers. comm.). Our data will facilitate population monitoring and the design of conservation strategies for *B. fallax* in Britain as well as help to assess the feasibility of translocation and captive breeding of *B. fallax* from elsewhere in Europe if a genetic 'rescue' attempt is necessary.

Historically, *B. fallax* was probably an early coloniser of the Caledonian pine wood habitat; the larva filter-feeds on microbes in rot holes occurring in decaying roots and holes in the surface of stumps of Scots Pine, *Pinus sylvestris* L. (Rotheray & Stuke 1998; Rotheray *et al*. 2000). This microhabitat develops due to heart-rot fungi softening heartwood that is often exposed when a tree falls or is felled. Based on survey results from five consecutive years, *B. fallax* over-winters at the larval stage and primarily has a univoltine life cycle (Rotheray *et al*., unpub. data). As a saproxylic species, *B. fallax* is an important bio-indicator of habitat quality and is part of a very diverse, species-rich group of organisms that play a vital functional role in forest ecosystems, and include a high proportion of threatened species (Speight 1989; Grove 2002; Jonsson *et al*. 2005; Lassauce *et al*. 2011). *Blera fallax* is found across the Palearctic as far as Japan and South as far as the Pyrenees and, based only on scant, intermittent records collected over

the past 250 years, it is considered locally rare or declining wherever it has been recorded (Speight 2008). No detailed information on the distribution or health of these Palearctic populations exists. In Scotland, *B. fallax* shares its habitat with at least 30 endangered taxa from several groups including Diptera (Rotheray *et al*. 2001), parasitic Hymenoptera, Coleoptera, fungi and lichens (Alexander 1988; Butler *et al*. 2002). *Blera fallax* is listed in the UK Red Data Book as category 1 (endangered). It is a Biodiversity Action Plan priority species and is one of 32 species listed in the Species Action Framework, a Scottish Natural Heritage initiative that focuses on improving the status of species deemed significant to overall Scottish biodiversity (Scottish Natural Heritage 2007).

Based on historical pine pollen records and indications derived from fossils of colonizing arthropods during the Holocene epoch (Birks 1970; Bennett 1984; Whitehouse 2006), *B. fallax* has probably been isolated in Scotland since the last glaciation 7000 to 10000 years ago. Its geographic range underwent a severe decline from eight to two sites between 1950 and 2000 due to loss of habitat and changes in forestry management (Rotheray & MacGowan 2000). Larval counts and extensive habitat surveys indicate that just a few hundred individuals remain across both sites (I. MacGowan pers. comm.). Furthermore, survey work during the past four years has failed to locate signs of *B. fallax* at one of these sites. The remaining population may be highly isolated, inbred and have limited dispersal ability. Therefore current conservation practices involve captive breeding and translocation to historically inhabited sites in Scotland where new habitat has been created. To assist this effort, we urgently need data on the effective population size and genetic diversity of the remaining population. In this context we sought to estimate the genetic diversity of Scottish *B. fallax*, to compare it with Swedish samples, and to assess the signs of recent demographic changes in both populations.

4.3 Methods

Sampling and DNA extraction

In October 2008, after extensive searches identified just one remaining locality for this species, fifty *B. fallax* larvae were collected from forty pine rot holes at Curr Wood in Strathspey, Scotland, UK (57°18' N, 3°39' W). No more than two larvae were collected from one rot hole. These were reared to eclosion, and bred in captivity as part of a captive breeding program (Rotheray 2010). Seventeen individuals that had endured substantial wing damage while in captivity were frozen upon death, while the rest were released at the collection site in Curr Wood in an attempt to minimize the impact of our sampling on the source population. Between June and November 2009, twenty-two larvae and one adult *B. fallax* were collected by Hans Bartsch from a pine woodland site in Järfälla, Sweden (59°24' N, 17°52' E) (Fig. 4.1). We selected this site because of its similar latitude and its proximity to a colleague who could collect and identify specimens. No other extant sites were readily available for sampling at this stage. Although no detailed surveys of the current health of this population (or any other Palearctic population) exists, we expected based on the availability of habitat that the Swedish population should be larger. All Swedish larvae were frozen before being transferred to 90% ethanol, and the adult female was pinned dry.

Whole larvae were used to extract genomic DNA. The hind legs were removed from adults and stored frozen as a reference collection in case of contamination or loss of samples, while the rest of the body was used for DNA extraction (Rotheray *et al*. 2011). Twelve species-specific microsatellite markers (HF_8RB, HF_S56, HF_WMK, HF_JRW, HF_C4A, HF_OH2, HF_0IY, HF_AN4, HF_5VB, HF_AMQ, HF_FCT, HF_RKX)

developed from Scottish *B. fallax* were used for population genetic analysis (Rotheray *et al*. 2011). Polymerase chain reaction (PCR) and genotyping was carried out as in Rotheray *et al*. (2011).

Statistical analysis

The statistical analyses to determine allelic richness, heterozygosity and population differentiation were performed with Fstat 2.9.3 (Goudet 1995), GenALEx 6.1 (Peakall & Smouse 2006) and Arlequin 3.11 (Excoffier *et al*. 2005). Population structure was inferred using a Bayesian clustering method implemented in STRUCTURE 2.3.3 (Pritchard *et al*. 2000), which allocates individual genotypes into groups (K number of populations) by an estimated membership coefficient Q for each individual based on allele frequencies at unlinked loci. An admixture model was used with default parameter settings. One to five possible populations (K) were tested; each run had a burn in period of 50,000 steps, followed by 100,000 steps of Markov chain Monte Carlo (MCMC) sampling, and was iterated five times for both populations together and for each separately. Convergence was checked by comparing the output between runs.

In order to detect population decline and find evidence for historical bottlenecks we used two methods. The first analysed allele frequencies in BOTTLENECK 1.2 (Piry *et al*. 1999). Here we used two models of mutation: the more conservative stepwise mutation model (SMM) and the less conservative infinite allele model (IAM) (Luikart & Cornuet 1998; Maudet *et al*. 2002). The second employed likelihood-based Bayesian methods that inferred population demographic history by coalescence from the full allelic distribution, implemented in MSVAR version 1.3 (Beaumont 1999). This assumes an ancestral effective population size N_1 that gradually changed to a recent effective population size

N_0 at time T_a generations. We assumed a single generation per year (based on results from field surveys and larval growth studies, Rotheray *et al*., unpub. data), an exponential population size change and wide, log-normal distribution prior for the mean value of the demographic parameters across loci: mean 4 and variance 3 for N_1 and N_0; mean 3 and variance 2 for time since population size change T_a. The prior of the mutation parameter μ was set to a relatively low level of mean -4 and variance 0.5, based on the mixture of di, tri and tetra-nucleotidic repeat motif in the microsatellites used (Rotheray *et al*. 2011). A wide prior with a low mean is necessary due to the lack of empirical and independent data on *B. fallax* microsatellite mutation rate. All demographic and mutational parameters were allowed to vary among loci using the hierarchical model implemented in MSVAR (Beaumont 1999) and setting a mean of 0 and a variance of 0.5 for the variance parameters. Each Markov chain was run for 5 x 10^9 steps recording the parameter values every 50,000 steps for a total of 100,000 output lines. We ran five independent chains using different starting values to assess convergence using the Gelman-Rubin diagnostic (Gelman & Rubin 1992) implemented in the R package *coda* (Plummer *et al*. 2006), after cutting off the first 10% of each chain as a burn in period. The chains were then combined to estimate the mode of the posterior density of the model parameters and their 90% High Probability Density (HPD) using the R package *boa* (Smith 2007).

4.4 Results

Genetic variation

The percentage of polymorphic loci was 91.67% in the Swedish population and 66.67% in the Scottish population. The inbreeding coefficient (F_{IS}) across all loci in both

populations showed no significant deviation from zero (Table 4.1), indicating a lack of evidence for non-random mating and further population substructure. After Bonferroni corrections, locus HF_8RB showed significant deviation from Hardy-Weinberg in the Swedish population and was excluded from further analyses.

The maximum number of alleles per locus varied from four (mean 2.08 ± 0.29 SE) to six (mean 3.12 ± 0.46 SE) in the Scottish and Swedish populations respectively. One private allele was found in the Scottish population while twelve were found in the Swedish (Table 4.1). Overall, expected heterozygosity (H_E) and allelic richness was significantly lower in the Scottish population (H_E 0.30 ± 0.08 SE; allelic richness 2.0 ± 0.26 SE) than in the Swedish population (H_E 0.49 ± 0.06 SE; allelic richness 3.3 ± 0.5 SE) (one-tailed Mann-Whitney; H_E and allelic richness P=0.05) (Table 4.1).

Population genetic structure

Genetic differentiation between the two populations was significant (F_{ST}=0.134, P<0.001, Table 4.1). The STRUCTURE analysis showed the highest likelihood of the data with two populations (K = 2, results not shown) and clear segregation was found from the assignment test; 93% of Scottish individuals were assigned to one population and 83% of the Swedish individuals to the other (Fig. 4.2). No subdivision was found within either population; all individuals were assigned to each cluster (K = 2 to 5) with equal probability. This demonstrates an absence of immigration from other populations i.e. a lack of population substructure.

Inferences of population demographic history

Under IAM, both the Scottish and Swedish populations showed significant signs of recent bottlenecks (Wilcoxon test, one-tailed for heterozygote excess, Scottish p = 0.039 and Swedish p = 0.009) while under the more conservative SMM there was a marginally non-significant trend in the Scottish population (Wilcoxon test, one-tailed for heterozygote excess, P = 0.055), and no evidence for the Swedish population (Wilcoxon test, one-tailed for heterozygote excess, P = 0.313).

Using Bayesian methods (Beaumont 1999), we found a clear, strong signal for a population decline in both populations (Table 4.2). The five independent analyses of the two populations yielded convergent chains according to the Gelman-Rubin diagnostic (Gelman & Rubin 1992). The genetic data contained useful information to estimate the demographic parameters as shown by the posterior distributions that differ substantially from the prior distributions (Figure 4.3. A, B and C). As expected, there was no information relative to the mutation rate, as the posterior and the prior distributions for the mutation rate parameter are similar (Figure 4.3. D). This implies that the demographic parameter estimates depend on the mutation rate prior, which was confirmed by repeated analysis using different mutation rate priors (data not shown). Contemporary effective population size, N_0, is relatively smaller in Scotland (12 [0-266] individuals, mode and 90% HPD) than in Sweden (80 [1-1654]) (Table 4.2). These low estimates are more than two orders of magnitude smaller than the large ancestral effective population sizes for the two populations: 47567 [5456-446937] and 31169 [4224-227798] individuals for the Scottish and Swedish populations, respectively (Table 4.2). The time since population size change is slightly more recent for the Scottish population, 167 [3-3159] years, compared to 344 [4-6578] for the Swedish population. The high magnitude of population

size change, computed as $\log_{10}(N_0/N_1)$, of -3.49 [-5.46 - -2.27] and -2.39 [-4.62 - -1.43] for the Scottish and Swedish population respectively, means that it is very unlikely that such high estimates result from a confounding effect such as population substructure or sampling bias alone (Chikhi *et al*. 2010). This likelihood is verified by the lack of substructure found by the STRUCTURE analysis within each population. These results clearly indicate a very strong and relatively recent decline in effective population size in both populations, which is more pronounced and probably more recent for the Scottish one.

4.5 Discussion

As expected, the geographically isolated population of *B. fallax* in Scotland has less genetic diversity than the population in Sweden. Moreover, the two populations are clearly genetically distinct. More unexpected was the finding that both the Scottish and Swedish population appear to have gone through a fairly recent decline, which has direct consequences for the conservation of the species and suggests that *B. fallax* may be especially vulnerable to fragmentation.

Genetic evidence for population isolation in Scotland is apparent through the allele frequency-based bottleneck analysis. The less conservative IAM model suggests that both Scottish and Swedish populations have gone through a recent bottleneck, whereas under the SMM model, (which is considered more suitable for microsatellites; Luikart & Cornuet 1998), the test for the Scottish population was marginally non-significant (Wilcoxon Test: PSMM=0.055), and there was no evidence for a bottleneck in the Swedish population. A conservative interpretation of these results would suggest the

Scottish population has gone through a recent bottleneck (Luikart & Cornuet 1998). This initially confirmed our presumption that the population from Sweden would show less evidence for a decline due to greater habitat continuity. Swedish populations are less isolated and likely to experience immigration from surrounding localities, i.e. experience greater gene flow, and the habitat is considered to be less fragmented which facilitates gene flow. However, the Bayesian modeling method suggested that both populations have undergone a severe decline, which occurred approximately 200 years earlier in the Swedish population. This estimate accords reasonably well with human changes in land use in the 1700's; forest fires were repressed and woodlands were felled for construction and timber (Zackrisson 1977). It may have been forest fires, which happened on an approximately eighty-year cycle (Zackrisson 1977) that caused an initial bottleneck in the population, or proceeding deforestation since fires were repressed. We would need more data about the past and current population and habitat structure in Sweden, and to sample more Swedish *B. fallax* populations in order to comment further. The discrepancy between the bottleneck analysis and the Bayesian inference is probably due to inefficient use of the genetic information for the former approach (Felsenstein 1992). Similar situations where the SMM failed to detect a bottleneck have been found in other studies (Olivieri *et al*. 2008; Craul *et al*. 2009). If the Bayesian analysis is correct, it indicates that *B. fallax* may be particularly vulnerable to habitat fragmentation and, as suggested by observations of the Scottish population, have limited dispersal ability even in pine woodlands that appear to be fairly well connected (Willis *et al*. 1998).

The Scottish population shows a more severe decline, estimated to have occurred more recently approximately 167 years ago. This estimate is supported by the history of woodland management in Strathspey recorded for this period. Individuals sampled in

Scotland are from a population that, since the 1800's, may have been restricted to a 200-hectare pine plantation, Curr Wood. This woodland was planted with native *P. sylvestris* in 1796 and was established woodland by 1858 (Dunlop 1993). Between 1750 and 1850, and during World War 1 and 2, substantial clear felling was carried out in Strathspey (Worrell & Dunlop 2003). While this may have provided a lot of *B. fallax* habitat at the time, i.e., numerous pine stumps left to decay, after a period of natural regeneration these areas were extensively re-planted and re-seeded which involved ploughing and up-rooting stumps (Dunlop 1994). Accidental fires associated with the felling process were frequent, and destroyed vast areas of woodland; a six day long fire in 1948 destroyed the woodland near to and surrounding Curr Wood, which has never recovered (Dunlop 1994). During this time Curr Wood survived and, while thinning continued periodically, it was left to regenerate naturally (Worrell & Dunlop 2003) which is probably how *B. fallax* became isolated but persisted there.

The more recent population decline and isolation in Scotland may explain the reduced genetic diversity when compared to Sweden, however occurring on the edge of a species' range can also have consequences for the genetic diversity of populations (Hewitt 1996, 2000; Ibrahim *et al*. 1996). Such genetically impoverished populations have been found in many studies across taxa from European pool frogs *Rana lessonae* (Zeisset & Beebee 2001), and European hedgehogs *Erinaceus europaeus* and *E. concolor* (Seddon *et al*. 2001) to the grasshopper *Chorthippus parallelus* (Cooper *et al*. 1995) and the butterfly *Polyommatus coridon* (Krauss *et al*. 2004). The Scottish *B. fallax* population is on the edge of its West European range while the Swedish population is in the centre of its North Westerly range. Investigation into the genetic diversity of *B. fallax* across its European range will be necessary in order to explore this further.

Conservation and future research

The species geographic range and the recent bottleneck may explain the reduced genetic diversity observed in the Scottish population, but we do not yet know the fitness consequences of this reduction. While evidence has shown reduced genetic diversity has a deleterious effect on fitness (Reed & Frankham 2003), the effect of inbreeding on fitness in wild populations is not only difficult to measure, it also varies a great deal depending on different evolutionary and environmental factors, such as past founding events, genetic purging or complex ecological associations (Hedrick & Kalinowski 2000). Ongoing monitoring of the Scottish population will be necessary in order to detect such detrimental effects. Since 2007, in an effort to recover *B. fallax* in Scotland, active conservation management has included captive breeding from the remaining Scottish population and relocation to historic native pine woodlands where rot holes have been artificially created. While preliminary results for the translocation look promising (Rotheray 2010), the long and short-term effects this will have on genetic diversity, especially with regard to the number of individuals bred and subsequently translocated, are unclear. Due to lack of records and experts outside of Britain, sampling was only possible from one other population in Europe to compare with what appears to be the sole remaining UK population. Future work should assess and compare the diversity of populations across the Palearctic in order to better evaluate the condition of the Scottish population, as well as gain a better sense of *B. fallax* population genetic structure, effective population size and evolutionary potential. This may be achieved through nonlethal methods utilising empty puparia, which can be located within and around the rot hole habitat (see Appendix 4.2). Sampling historic genetic data using museum specimens has been done successfully for butterflies and bumblebees (Harper *et al*. 2006; Strange *et al*. 2009; Lye *et al*. 2011), and we consider this to be another potential source

of informative genetic data. Results from such studies may also clarify the probable viability of future relocations on newly founded Scottish sites. Maudet *et al*. (2002) showed through simulations that only a small number of immigrants from a well-differentiated, variable population are required to improve and sustain heterozygosity. However, if such translocations are to be carried out as part of ongoing conservation efforts, care should be taken with regard to possible adaptive differentiation across populations. The clear genetic distinction between the Scottish and Swedish populations is not unexpected, as they may have been separated for up to 10,000 years. It is possible that due to this separation and resulting divergence, the two populations have become locally adapted, in which case hybridization of these stocks could have negative genetic repercussions (Templeton 1986; Lynch 1991; Edmands 2007). Translocation efforts using individuals from Sweden could fail due to them being maladapted, or could cause outbreeding depression. Evidence of outbreeding depression has been demonstrated for hybridizing populations of mosquito fish *Gambusia holbrook* just 100 meters apart (Templeton 1986) and bark beetles *Xylosandrus germanus* 6km apart (Peer & Taborsky 2005), and second generation fitness problems arose after crossing *Drosophila* from geographically isolated populations (Aspi 2000). Even though the evidence for such local adaptation causing outbreeding depression is relatively scarce compared to that for inbreeding, translocation of individuals for hybridization with those in Scotland should only be carried out if the population is clearly suffering from inbreeding depression.

Saproxylic organisms form a complex and specialized community of decomposers, fundamental to forest function (Speight 1989; Grove 2002; Schmuki *et al*. 2006). Modern forestry practices continue to overlook the importance of retaining dead wood, often opting for an over-managed, ‘tidy’ woodland system (Butler *et al*. 2002; Humphrey

2005; Humphrey *et al*. 2005; Lonsdale *et al*. 2008). Due to the limited and temporally unpredictable availability of dead wood and the dependency of many species on specific stages of its decay, many saproxylic populations are characteristically small and isolated, but often exhibit limited dispersal abilities (Speight 1989; Butler *et al*. 2002; Ranius 2006). This causes particular susceptibility to adverse effects including woodland fragmentation (Jonsson 2003; Schmuki *et al*. 2006) and, especially where they depend more on plantations rather than natural mixed-age woodland, demographic fluctuations such as boom and bust cycles (Rotheray *et al*. 2009). For *B. fallax*, the microsatellite markers used here to assess genetic diversity can be further utilized by investigating fine-scale population genetic structure to better understand mating systems and dispersal ability, thus inform efficient conservation management strategies.

Table 4.1 Population statistics comparing Scottish and Swedish *B. fallax* populations per locus [FST, differentiation coefficient; P FST, p-value of observed FST under the assumption of panmixia; NA, number of alleles and private alleles; AR, allelic richness; HE, expected heterozygosity; FIS, inbreeding coefficient; P FIS, p-value of observed FIS under the assumption of panmixia]

			Scotland					Sweden				
Locus	F_{ST}	P F_{ST}	NA/private	AR	H_E	F_{IS}	P F_{IS}	NA/private	AR	H_E	F_{IS}	P F_{IS}
HF_8RB	-0.001	0.375	4/0	2	0.747	-0.271	0.972	4/0	4.00	0.758	-0.269	0.989
HF_C4A	0.148	0.010	2/0	3	0.337	0.130	0.537	2/0	2.00	0.511	-0.109	0.823
HF_JRW	-0.021	0.538	3/1	3	0.508	0.076	0.440	2/0	2.43	0.449	-0.066	0.786
HF_S56	0.104	0.000	3/0	2	0.683	-0.304	0.941	3/0	3.98	0.590	0.268	0.068
HF_WMK	0.154	0.005	2/0	3	0.059	0.000	-	3/0	2.98	0.503	0.139	0.333
HF_0IY	0.151	0.000	3/0	1	0.570	-0.032	0.556	6/3	5.34	0.763	-0.143	0.742
HF_5VB	0.078	0.063	1/0	2	0.000	*	*	3/2	2.37	0.237	-0.105	1.000
HF_AN4	0.155	0.000	2/0	1	0.487	-0.344	0.979	4/2	3.43	0.675	-0.229	0.950
HF_OH2	0.338	0.000	1/0	1	0.000	*	*	6/5	4.75	0.706	0.078	0.271
HF_AMQ	*	*	1/0	2	0.000	*	*	1/0	1.00	0.000	*	*
HF_FCT	0.034	0.248	1/0	2	0.000	*	*	2/0	1.82	0.125	-0.048	1.000
HF_RKX	-0.023	1.000	2/0	2	0.337	0.130	0.537	2/0	2.00	0.348	-0.257	1.000
Over all loci	0.134	0.000	2	2	0.298	-0.0884	0.537	3	3.16	0.491	-0.037	1.000

*monomorphic, – no p-value due to calculated F_{IS} value of zero

Table 4.2 Posterior distribution estimates: the mode and the 90% High Probability Density interval in brackets estimated from the posterior density kernel of the log scale parameter (as showed in Figure 5.3) back transformed in natural scale. N_0 and N_1 are expressed in number of individuals, T_a in years assuming a generation time of one year and μ in 10^{-4} mutations per generation per haploid genome.

Population	Contemporary effective population size (N_0)	Ancestral effective population size (N_1)	Time since population size change (T_a)	Mean mutation rate (μ)
Scotland	12 [0-266]	47567 [5456-446937]	167 [3-3159]	0.73 [0.12-4.46]
Sweden	80 [1-1654]	31169 [4224-227798]	344 [4-6578]	0.76 [0.13-4.81]

Figure 4.1 Map showing locations of the Scottish (left circle) and Swedish (right circle) *B. fallax* populations used in the study.

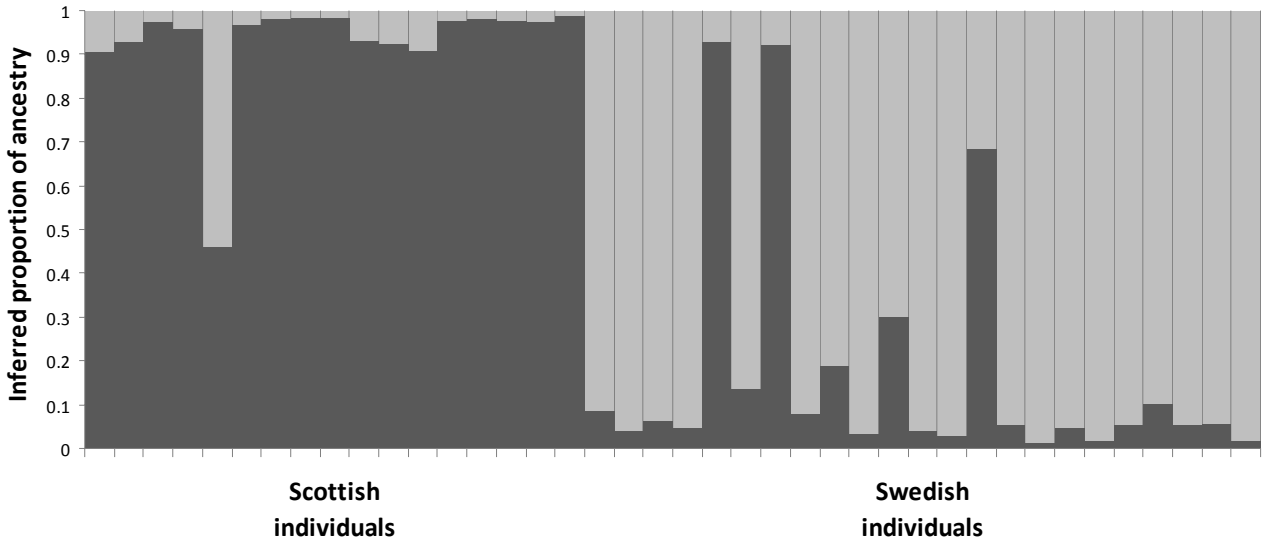

Figure 4.2 Bar plot showing inferred population ancestry (membership coefficient Q) for two assumed clusters (K=2) indicated by dark and light grey bars (first 17 bars are individuals sampled from the Scottish population, and the remaining 23 are Swedish).

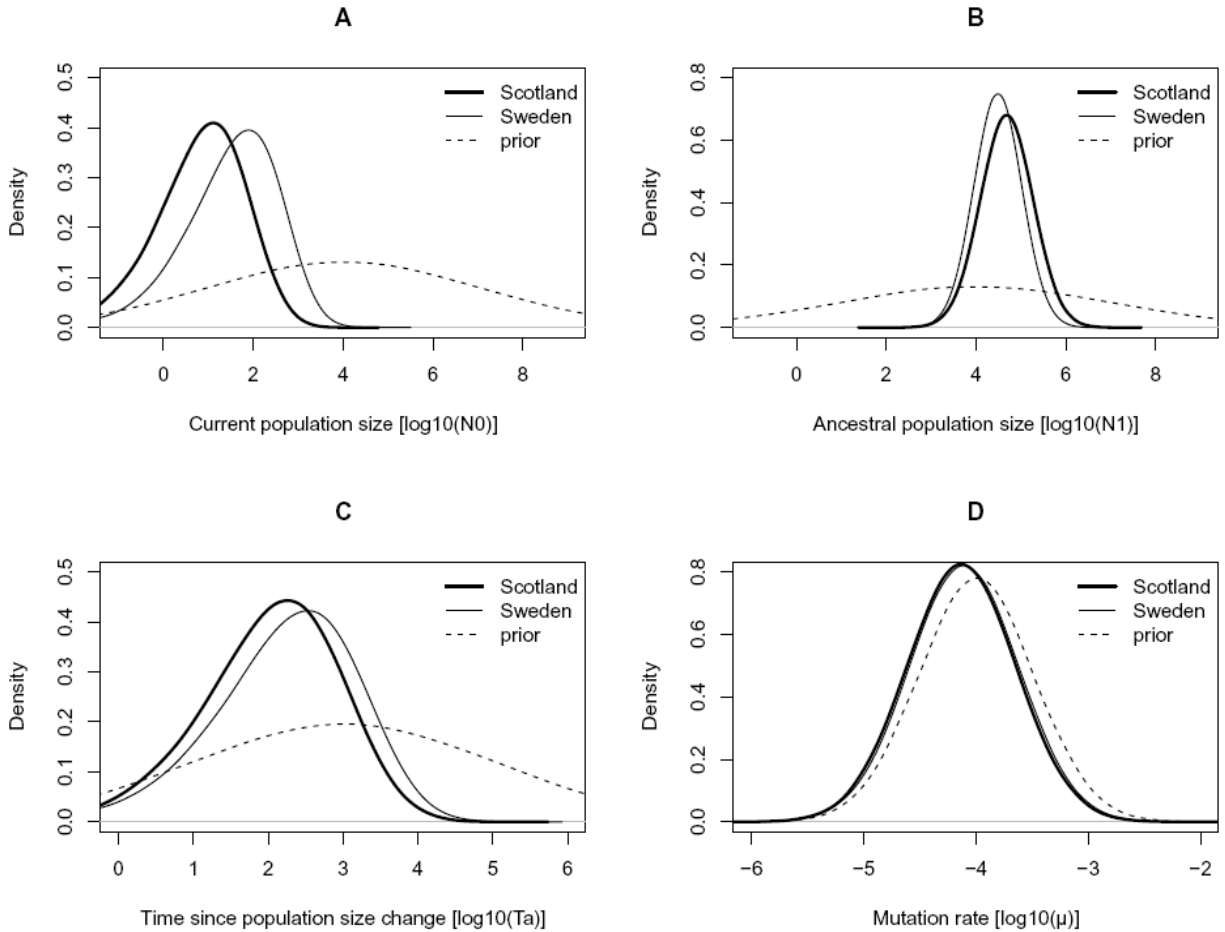

Figure 4.3 Posterior density distributions for (A) current and (B) ancestral effective population size, (C) time since the population decline and (D) microsatellite mutation rate for Scottish and Swedish populations of *B. fallax*. The dashed lines represent the prior distributions of the parameters, the thick, dark and thin, light grey lines represent the Scottish and Swedish populations respectfully.

Appendix 4.1

Polymorphic microsatellite loci for the endangered pine hoverfly *Blera fallax* (Diptera: Syrphidae)

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Abstract

We describe eleven polymorphic microsatellite loci developed for *Blera fallax* using 'next generation' 454 whole genome shotgun sequencing, along with conditions for three multiplex PCR reactions. We tested allelic variation on forty *B. fallax* individuals from Scotland and Sweden. Allelic richness and expected heterozygosity were 3.03 ± 0.274 (mean ± Standard Deviation) and 0.391 ± 0.057 respectively. The number of alleles per locus ranged from 2 to 6. We anticipate that these loci will assist conservation management by allowing the monitoring of translocated populations, estimating effective population size, and assessing population structure and dispersal in Scotland and across Europe.

Introduction

The pine hoverfly *Blera fallax* has declined in Scotland, UK and is now confined to just two sites (Rotheray & MacGowan 2000). It is listed in the Species Action Framework (Scottish Natural Heritage 2007), a Scottish Natural Heritage initiative for biodiversity significant species. Actions stipulate expanding the range of *B. fallax* from two to five localities by 2012. The small source population has probably gone through a recent genetic bottleneck, so we urgently need data on how translocation might affect genetic variability. Microsatellite markers have been developed for just one syrphid species with limited success (Schönrogge *et al*. 2006). Here we describe the development of polymorphic microsatellites using 454 pyrosequencing (Santana *et al*. 2009) to investigate the genetic diversity within *B. fallax*.

We collected 50 larvae from Dulnain Bridge, Scotland, and reared them to eclosion for captive breeding (Rotheray 2010). We froze seventeen adults after death for sequencing and genotyping. We obtained 22 further larvae and one adult from a pine woodland in Järfälla, Sweden. These larvae were frozen before storage in 90% alcohol, and the adult was pinned.

Method

We extracted genomic DNA (gDNA) using the DNeasy Blood & Tissue Kit (Qiagen), including optional RNase treatment. DNA yield was quantified using a ND-1000 spectrophotometer (NanoDrop). Pooled gDNA of two male and two female Scottish flies was used for shotgun sequencing of 1/8 plate on a 454 Genome

Sequencer FLX with Titanium chemistry (454 Life Sciences, Roche). We obtained 71,804 reads (25,184,020 total bases), of which 74% were longer than 300 base pairs (average read length = 351 bp). We *de novo* assembled the reads using Newbler 2.3 (454 Life Sciences), which resulted in 1150 contigs with an average size of 714bp (21.58% of the reads assembled). Both unassembled reads and contigs were used to identify microsatellite loci using the programs iQDD 0.9 (Meglecz *et al*. 2007) and Msatcommander 0.8.2 (Faircloth 2008). In total, we obtained 330 uninterrupted microsatellite loci with repeat motifs between two and six bp and at least five repeats for tetra and penta motifs, six for trinucleotide and eight for dinucleotide motifs. For 75 microsatellite loci we could design PCR primers with the PRIMER 3 package (Rozen & Skaletsky 2000) implemented in iQDD and Msatcommander (T_M > 60°C, otherwise default settings).

Of these, we selected 28 loci to test for PCR amplification using gDNA of one male and female from the Scottish population. The PCR reactions contained 10 ng template DNA, 1x Phire Hot Start Reaction Buffer including 1.5 mM $MgCl_2$ (Finnzymes), 0.25 µM forward and reverse primer, 0.2 mM dNTPs, 0.2 µl Phire Hot Start DNA Polymerase (Finnzymes) and ddH_2O to 10 µl total volume. PCR amplifications were performed in a Veriti^{TM} Thermal Cycler (Applied Biosystems) with two different cycling profiles. First, we tested a standard profile with three different annealing temperatures (PCR1, Table 1). In cases where this profile did not result in a specific PCR product, we applied a touchdown profile (PCR2, Table 1). Of the twenty-eight primer pairs tested, sixteen amplified a specific product of the expected size, as verified on 1.5% agarose gels. These sixteen loci were tested for polymorphisms on eight Scottish and six Swedish *B. fallax* using 2.5% agarose

gels. Of these, thirteen primers pairs were selected based on band patterns within and between populations. Forward primers were fluorescently labeled and combined into three multiplex PCR reactions, named Fallacplex 1, 2 and 3. PCR reactions contained 10 ng template DNA, 1x Multiplex PCR Master Mix (Qiagen), between 0.08 and 0.5 µM of each primer (Table 2) and ddH_2O to 10 µl. Fallacplex 1, 2 and 3 PCR conditions are described in Table 1. We then genotyped 17 Scottish (7 adult males, 10 adult females) and 23 Swedish (1 adult female, 22 larvae) *B. fallax* individuals. Fragment length analysis was conducted using a 3730 DNA Analyzer and GENEMAPPER 4.0 software (both Applied Biosystems). The statistical analyses were performed with Fstat 2.9.3 (Goudet 1995), GenALEx 6.1 (Peakall & Smouse 2006) and GENEPOP 4.0 (Raymond & Rousset 1995).

Results and discussion

We achieved consistent PCR amplification for all forty individuals. Of thirteen microsatellite loci, one locus failed to amplify in the multiplex reaction, and one was monomorphic for both populations. No significant linkage disequilibrium could be found at any pair of loci in either population, and no evidence for null alleles was detected. The number of alleles per locus ranged from 2 to 6 with a total allelic richness of 3.03 (±0.274). Expected and observed heterozygosities ranged from 0.059 to 0.763 (mean 0.391 ±0.057) and from 0.059 to 0.957 (mean 0.426 ±0.067), respectively (Table 2). One locus (HF_S56) showed significant deviation from Hardy-Weinberg equilibrium after sequential Bonferroni correction in one of the two populations (Table 2) (Rice 1989).

The novel microsatellite markers presented here not only assist conservation for this species in Scotland, but have wider applications for describing the distribution and dispersion of *B. fallax* across Europe. Our study confirms the effectiveness of long read, high-throughput sequencing for developing polymorphic microsatellites in non-model invertebrate species.

Table 1 PCR cycling conditions for the initial testing (PCR1 and PCR2), and the three final multiplexes (Fallac 1, 2 and 3).

	PCR1	PCR2	Fallacplex 1 & 2	Fallacplex 3
Initial denaturation	98 °C, 30 sec	98 °C, 30 sec	95 °C, 15 min	95 °C, 15 min
TD denaturation		98 °C, 5 sec		94 °C, 30 sec
TD annealing		64 -0.5 °C/cycle, 10 sec		64 -0.5 °C/cycle, 1 min 30 sec
TD extension		72 °C, 20 sec		72 °C, 1 min
		15 cycles		15 cycles
Denaturation	98 °C, 5 sec	98 °C, 5 sec	94 °C, 30 sec	94 °C, 30 sec
Annealing	58/61/64 °C, 10 sec	56 °C, 10 sec	64 °C, 1 min 30 sec	56 °C, 1 min 30 sec
Extension	72 °C, 20 sec	72 °C, 20 sec	72 °C, 1 min	72 °C, 1 min
	35 cycles	25 cycles	35 cycles	25 cycles
Final extension	72 °C, 1 min	72 °C, 1 min	60 °C, 30 min	60 °C, 30 min

TD, touchdown PCR profile

Table 2 Characteristics of 12 microsatellite loci in *Blera fallax*

Locus GenBank no.	Repeat Motif		Primer sequence	Conc. (μM)	Allele size range (bp)	Fallac -plex	Ta (°C)	Pop	Na	H_E	H_o	P
HF_8RB	$(CT)_{10}$	F	*6FAM*-TCGCCCATCTACGTTCACC	0.1	169-188	1	64	1	4	0.747	0.941	0.273
JN206627		R	GTTTCCACCGAAAGCAGTACACG	0.15				2	4	0.758	0.957	0.003*
HF_S56	$(AC)_{11}$	F	*NED*-CTCTCGCGCAAACTTTAAATCC	0.15	263-279	1	64	1	3	0.683	0.882	0.017
JN206628		R	GTTTATCGCGTGATGTTGCGAAG	0.2				2	3	0.590	0.435	0.312
HF_WMK	$(ACAT)_7$	F	*VIC*-AGGACAGTGCAGAGGTTGC	0.1	187-199	1	64	1	2	0.059	0.059	-
JN206629		R	TAGGCCGTTCACTATCCGC	0.15				2	3	0.503	0.435	0.170
HF_JRW	$(ACC)_9$	F	*6FAM*-GCATTCAGCAACACAAAAACAGATAAA	0.3	310-322	1	64	1	3	0.508	0.471	0.567
JN206630		R	AGGGGTGCACGACGACTACAG	0.4				2	2	0.449	0.478	1.000
HF_C4A	$(AATG)_6$	F	*PET*-TGATGCAACAGATGCTGGG	0.1	255-267	1	64	1	2	0.337	0.294	0.537
JN206631		R	GTCCTCGGCGGTGAAATAC	0.15				2	2	0.511	0.565	0.689
HF_OH2	$(AC)_{11}$	F	*PET*-ATTAAACTATGAGCGATGTCTGG	0.15	189-205	2	64	1	1	0.000	0.000	-
JN206632		R	GTTTGAATGCACTGCGTCACTCC	0.2				2	6	0.706	0.652	0.980
HF_0IY	$(AGC)_{10}$	F	*NED*-CGATCGGCAACTCATGTGG	0.15	242-257	2	64	1	3	0.570	0.588	0.536
JN206633		R	TACACAGGGTAAGCTCGGC	0.2				2	6	0.763	0.870	0.379
HF_AN4	$(ACG)_{12}$	F	*VIC*-AGGCACTGAGAACGAAAAGAATG	0.1	170-185	2	64	1	2	0.487	0.647	0.303
JN206634		R	GCAGCGAGGCAGACGATAG	0.15				2	4	0.675	0.826	0.253
HF_5VB	$(ACAT)_6$	F	*6FAM*-AGGGCCCAGTATTTGGTTG	0.15	291-303	2	64	1	1	0.000	0.000	-
JN206635		R	GAATTTGGGCCGGTAACGAG	0.2				2	3	0.237	0.261	1.000
HF_AMQ	$(GT)_{10}$	F	*NED*-GGCAGTCGGGATTTCTTCC	0.4	188	3	64-56 TD	1	1	0.000	0.000	-
JN206636		R	GTTTCTCTCCCGCCAGGATACTC	0.5				2	1	0.000	0.000	-
HF_FCT	$(AC)_{10}$	F	*PET*-ACCCCTTTTGTCGTTCGTTTAGT	0.08	277-283	3	64-56 TD	1	1	0.000	0.000	-
JN206637		R	GTTTCATTCAGGTGAGATTCGCTTTTG	0.12				2	2	0.125	0.130	1.000

HF_RKX	$(ACAT)_7$	F	*6FAM*-CAGGAAGAAAGAATCGGCAA	0.08	211-215	3	64-56 TD	1	2	0.337	0.294	0.537
JN206638		R	GAGTAGTTCCCTGTTGGGCA	0.12				2	2	0.348	0.435	0.538

F, forward primer sequence; R, reverse primer sequence; Conc., primer concentration in the PCR; Ta, annealing temperature; TD, touchdown PCR; Pop, population (1: Scotland, 2: Sweden); Na, number of alleles; H_E, expected heterozygosity; H_o, observed heterozygosity; P, P-value of probability test for deviation from Hardy-Weinberg equilibrium (HWE). Fluorescent dye labels are shown in italics at the 5' end of each forward primer. PIG-tail bases (Brownstein *et al*. 1996) are presented underscored. *significant deviation from HWE after sequential Bonferroni correction (Rice 1989), (-) monomorphic loci

Appendix 4.2

Nonlethal DNA sampling methods for the endangered Scottish hoverfly *Blera fallax* (Diptera, Syrphidae)

Abstract

We present non-lethal methods of extracting hoverfly DNA sufficient for microsatellite analysis from small pieces of adult tissue such as single terminal tarsi and wing tips, and sections of year old empty puparia. Verified by gel electrophoresis, the DNA extracted was sufficient for amplifying microsatellite primers developed to assist conservation management of the endangered hoverfly *Blera fallax* (Diptera, Syrphidae) in Scotland. This work will not only assist captive breeding and on-going monitoring of populations in Scotland, but it also demonstrates how molecular markers could be used to monitor populations of endangered insects without using potentially harmful sampling procedures.

Introduction

The main aim was to investigate non-lethal means of extracting DNA sufficient for monitoring the genetic variation and population structure of the pine hoverfly *Blera fallax* (Diptera, Syrphidae) in Scotland. Current conservation efforts include attempts to characterize genetic diversity within and among populations throughout Europe. However, this species is extremely rare in Scotland, with only one small surviving population, so destructive sampling for genetic studies is not possible. Hence here we develop protocols for non-destructively extracting DNA from larvae and puparial cases.

Survey work mainly involves searches for the larval stage in the rot holes in stumps of *P. sylvestris* where they develop, although this can be difficult as the deep cracks and crevices within the holes are often inaccessible. *Blera fallax* larvae exit the rot hole habitat in order to pupate. Whole or parts of empty puparia can often be found outside the rot hole, and in some cases this is the only evidence we have for *B. fallax* presence and successful eclosion. Adult *B. fallax* are elusive and rarely seen.

A captive breeding programme for this species is underway. In addition to monitoring genetic diversity in wild populations, analysing DNA from captive individuals may be valuable in revealing signs of inbreeding, and in matching adults for breeding purposes.

A number of techniques for non-lethal extraction of DNA from insects have been investigated. For example, no negative survival impacts were found in *Bombus terrestris* when removing the terminal portion of the tarsus of the mid leg or hind

leg for DNA extraction, nor were there any found for rare butterflies' *Vanessa cardui* and *Satyrodes eyrydice* upon removal of small parts of their hind wings (Holehouse *et al*. 2003; Hamm *et al*. 2010). Wing tips have also often been used as a source of non-lethal extraction in honeybees *Apis mellifera* and butterflies such as *Parnassius apollo* (Lushai *et al*. 2000; Châline *et al*. 2004). However, to our knowledge attempts to extract DNA from empty puparia have not been previously explored. Such methods could be generally applicable across a broad range of invertebrate taxa.

Method and Results

Several possible sources of DNA were investigated: adult wing tips and terminal single tarsal segments; empty year-old puparia stored at room temperature and fresh < 1-month-old puparia stored at -4°C; and water collected from a *B. fallax* larval rot hole. Sterile blades and fine tweezers were used to remove wing clippings of no more than 2 mm from the tip of the wing from adults that died in captivity. The same sterilised tools were used to remove single terminal tarsi, and for dividing puparia. Whole empty puparia as well as the posterior and anterior halves were used in separate extractions. Water was taken directly from artificial rot hole microcosms inhabited by *B. fallax* larvae, as well as from 2 ml spring water from a sterile petri dish where a larva was left overnight. No more than 0.5 ml of water was transferred to a 2 ml eppendorf tube, centrifuged for 3 minutes, and using a pipette, the bottom 0.1 ml was removed for DNA extraction. Each DNA source was sampled three times.

We extracted DNA using the HotShot protocol (Holehouse *et al*. 2003) and the

DNeasy Blood and Tissue Kit (Qiagen). DNA yield was quantified using a ND-1000 spectrophotometer (NanoDrop). Two species-specific microsatellite markers (HF_WMK and HF_OIY) developed from Scottish *B. fallax* were used (Rotheray *et al*. 2011). Amplification was carried out in a 10 μL final volume using QIAGEN Multiplex PCR kits. Each reaction contained 1 μL Q-solution, 2 μL H_2O, 5 μL PCR Master Mix, 1 μM of primer and 1 μL (undiluted) DNA product. A *B. fallax* positive control (previously sequenced DNA product) was used, as well as a negative control (lacking DNA product) to account for possible contamination. Polymerase chain reaction (PCR) was carried out as in Rotheray *et al* (2011) and amplification of the product of the expected size was verified on 1.5% agarose gel.

We achieved amplification for wing tip, terminal tarsi, whole and anterior portions of fresh puparia and year-old puparia. No amplification was achieved for rot hole water (69.3 ± 15.32 ng/μl). The DNA yield estimated by the spectrophotometer does not correlate with band strength on gels, which increased from the wing tip (5.9 ± 2.12 ng/μl), whole puparia (119.06 ± 106.78 ng/μl) and anterior section of puparia (4.2 ± 4.14 ng/μl), year-old puparia (28.63 ± 47.26 ng/μl) to the terminal tarsi (2.42 ± 0.79 ng/μl). Excess RNA, and microbial DNA from the rot hole water, may have caused the variation found in DNA yield concentrations. While our results require verification and sizing by DNA sequencing, based on the size of product and control band amplification used, our results suggest that sufficient DNA can be extracted from not only fresh, whole puparia, but also smaller pieces of puparium up to one year old. Our findings will not only assist genetic monitoring of *B. fallax* but widens the possibilities for non-destructive genetic studies of all holometabolous insects.

Chapter 5

Habitat restoration and captive breeding techniques for the endangered Pine hoverfly *Blera fallax* (Diptera, Syrphidae) in Scotland

Abridged version of chapter published as (Appendix 5.1):

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5.1 Abstract

Conservation management aims to prevent extinctions and restore endangered species in an effort to preserve biodiversity and a healthy functioning ecosystem. This involves determining causes of population decline and investigating autecology in order to develop effective strategies to restore species or habitats. The current study investigates the ecological requirements of a highly endangered saproxylic insect, the Pine hoverfly *Blera fallax* (Diptera, Syrphidae), while at the same time evaluating the success of practical conservation management efforts for this species. This involved developing techniques for creating and expanding effective breeding habitat (*Pinus sylvestris* stump rot holes), finding the optimum growing conditions for larvae, developing procedures for captive breeding, and monitoring relocated and source populations. In addition, an objective of this study was to assess the factors affecting successful monitoring, management and translocation. Larvae were successfully reared in captivity in 40 ml *P. sylvestris* substrate immersed in 70 ml water, and took between 270 and 415 days to eclose. In captivity, adults were on the wing between 11th May and 24th August, with males and females having an average lifespan of 38 and 34 days, respectively. Adults fed on a range of flowering plants but preferred Rosaceae, particularly *Sorbus aucuparia*. Oviposition was apparently triggered by water-soaked *P. sylvestris* sawdust, and captive females produced up to 188 eggs each. In the first year of relocation, 179 captive-bred individuals (84 larvae and 95 adults) were released, and 43 1st instar larvae were subsequently found in artificial breeding habitat at the release site, and 1 km away. These findings on the biology, life strategies and

techniques for monitoring the genetic health of *B. fallax*, are combined to develop protocols for on-going conservation management.

5.2 Introduction

Conservation management decisions are often necessary before data is obtained on species threatened with extinction (Primack 1998). But in order to improve the effectiveness of such decisions, we need to understand the ecological requirements and reasons for decline.

Blera fallax (Diptera, Syrphidae) is listed in the UK Red Data Book as category 1 (endangered) and it is a UK Biodiversity Action Plan (BAP) priority species. This status was confirmed in 1999 after an extensive 12-year survey, which indicated a population decline from 8 to 2 known populations since the early 1900's (Rotheray & MacGowan 2000). *Blera fallax* was included in the Species Action Framework (SAF), a Scottish Natural Heritage (SNH) initiative, which focuses on funding projects to improve the status of species deemed significant to overall Scottish biodiversity. Priority is given to those that are likely to benefit from determined conservation management plans based on known species requirements (Scottish Natural Heritage 2007).

Having been first discovered in the late 19th century (Verrall 1901) very little was known about *B. fallax* until 1998 when the larval stage was first described (Rotheray & Stuke 1998). *Blera fallax* is a specialist saproxylic insect, the larval stage developing on microbes in rot holes occurring in stumps and decaying roots of Scots Pine, *Pinus sylvestris* L. In Scotland, *B. fallax* has not been found associated with any other tree species, however in Scandinavia it does develop in Norway Spruce *Picea abies* L. (Speight 2008). As a saproxylic species it is an important bio-

indicator of habitat quality and is part of a very diverse, species-rich and specialised group of organisms that play a vital functional role in forest ecosystems (Grove 2002; Jonsson *et al*. 2005; Speight 2008; Lassauce *et al*. 2011). *Blera fallax* is found across the Palearctic, but has been scantily recorded (Speight 2008). In Scotland *B. fallax* shares its larval habitat with at least 30 endangered insect taxa from diverse groups (Rotheray *et al*. 2001). It is part of a unique community of invertebrates that make up the Caledonian Forest ecosystem (Shaw & Thompson 2006).

Action 3 of the SNH SAF prescribes that the number of sites inhabited by *B. fallax* be increased from 2 to 5 localities by 2012 (Appendix 5.2). Breeding habitat loss over the last 100 years is thought to be the main factor that caused the decline in *B. fallax* populations, and availability of habitat is thought to limit population expansion today, therefore restoring suitable breeding habitat is the highest priority for conservation management (Rotheray & MacGowan 2000). This requires characterising the habitat at extant *B. fallax* sites, developing techniques for habitat creation, and preparing sites for relocation. In order to source a sufficient number of individuals for relocation and reduce the impact on the remaining population, captive breeding and rearing are required.

Captive rearing is an increasingly used interim strategy particularly for butterflies while long-term recovery techniques are developed, although it has had varied success (Crone *et al*. 2007; Schultz *et al*. 2008). As a consequence of captive breeding, genetic deterioration or diseases may lead to lower fitness, reducing population growth (Frankham 1998; Woodworth *et al*. 2002; Leberg & Firmin 2008). Reductions in mating success and selection for smaller body and wing size

have been reported from other captive breeding attempts (Joron *et al*. 2003; Schultz *et al*. 2009). Nevertheless, captive breeding is sometimes the only option for maintaining severely at-risk populations in the short-term (Crone at al. 2007).

Below, we describe several progressive stages of research on this species and its conservation management. These are the first attempts to develop captive breeding protocols for saproxylic hoverfly conservation.

5.3 Habitat creation techniques

Following successful techniques of habitat creation for the pine rot hole inhabiting *Callicera rufa* (Diptera, Syrphidae) (MacGowan 1994), we created rot holes by boring holes into the centre of stumps left after felling, using a chainsaw or drill, thereby mimicking the rotting process (see Appendix 5.3 and figures therein). Using a chainsaw, holes were created by making two parallel 15cm deep cuts straight down into the surface of the stump. These were positioned either side of the heartwood centre roughly 15 to 20 cm apart. Two further cuts were made perpendicular to and connecting the initial cuts to complete a square on the surface. These were made at 45º angles into the centre of the stump to join at a ~15cm deep point thus cutting out a triangle-shaped wedge. The hole was filled with either sawdust (untreated from a local sawmill) or wood chips, or sawdust and chips, and the triangle wedge was used to partially cover the hole to protect the content from evaporating while allowing rainwater to fill the cavity.

The second method was carried out using a petrol-powered drill and 25mm auger bit. Roughly 10cm diameter circular holes were created by boring repeatedly into the centre of the stump resulting in a 15 cm deep cavity occupying the heartwood. Sawdust created by the drilling process was used to fill the hole, and thick bark was used to partially cover the cavity.

Often suitable stumps are not available so plastic pots were also tested as an alternative breeding habitat. Pots (18 x 18 wide x 24 cm deep) were sunk into the earth and filled completely with pine wood chips. Six small holes were pierced into the sides of the pots near the top to allow excess water to escape, and bark was loosely placed over the top for protection, and to prevent passing invertebrates from falling in.

All rot holes were tagged with plastic laminated identity codes, and GPS coordinates were taken in order to monitor occupation and water retention over time.

5.4 Extant B. fallax field sites and habitat creation

Curr Wood

This site is a 200-hectare plantation (57°18' N, 3°39' W) (Figure 5.1). It is privately owned and has no conservation notifications. It consists mainly of *P. sylvestris* but also has deciduous trees such as *Sorbus aucuparia* (Rosaceae) and *Betula pubescens* (Betulaceae). Curr Wood was planted with native *P. sylvestris* in the late 18th century, thinned periodically, and left to regenerate naturally (Worrell & Dunlop

2003). Consequently, several hundred large *P. sylvestris* stumps exist at the site. *Blera fallax* was first recorded near Curr Wood in the early 20th century, and empty puparia were found on-site in 1997 (Rotheray & MacGowan 2000).

Artificial breeding habitat was previously created within a 10-hectare (Ha) area in 2003. Forty-two rot holes were bored using a chainsaw and fifteen plastic pots were set out. Three pots were positioned in an exposed, clear felled area and the remaining twelve were positioned within the woodland under canopy cover. Through 2008 until 2010, an additional 134 chainsaw-bored holes were created within the same area, and in June 2011, these were supplemented with 50 drill-bored holes (Table 5.1).

Anagach Wood

Anagach is a long-established pinewood plantation surrounding Grantown-on-Spey, 6 km North East of Curr Wood (57°19' N, 3°36' W) (Figure 5.1). It is a community owned woodland of about 400 Ha. After the original ancient pinewood was lost due to timber extraction, the site was re-planted with *P. sylvestris* 30 years before Curr Wood in 1766 (Worrell & Dunlop 2003). *Blera fallax* was first recorded at Anagach in 1949, and in 1997 over 10 empty puparia were found, however, no evidence of *B. fallax* has been confirmed since then (Rotheray & MacGowan 2000).

In 2007 through 2008, ten plastic pots were placed at one metre points along a straight transect within the woodland, and 25 holes were cut in stumps using a

chainsaw and filled with pine wood chips (Table 5.1). These were within a 10 Ha area and were created near locations where *B. fallax* had been recorded previously.

5.5 Breeding habitat surveys

Fourteen rot hole surveys were carried out at Curr and Anagach Woods between November 2007 and February 2011. We searched the detritus content of chainsaw-bored rot holes, plastic pots, and stumps with natural heart-rot for *B. fallax* presence. A plastic pipette and pastry brush were used to carefully probe cracks and crevices deeper in the hole.

Curr Wood

Each year through 2007 to 2011 we searched the detritus content of 59 chainsaw-bored rot holes, 15 plastic pots, and two stumps with natural heart-rot (in total 76) for *B. fallax* presence. Of these, 7 had chips alone, 22 sawdust, 47 chips and sawdust, and 46 were exposed (> 5 metres from canopy cover). All other naturally rotted holes (28) were too difficult to access due to the depth and small opening to the hole, and the remaining bored stumps (47 %) did not retain water. Half of these bored stumps were lacking the wedge or bark cover used to partially protect the hole from drying out which may have been the cause of water loss.

In addition to surveys, nine variables were measured for each rot hole in Curr Wood. These included the date the hole was bored, height and circumference of stump, hole circumference, hole depth, exposure (> 5 metres from woodland edge), content

type (sawdust, chips, or chips and sawdust), cover type (bark, wood chunks or wedge), and pH using a pHep5 Meter (Hanna HI-98128).

We used generalized linear models (GLMs) with Binomial error distributions to assess the effect of these nine stump variables on the presence or absence of species in a stump (Crawley 2007). Our maximal models included main effects and all second-order interactions, and were simplified using stepwise comparisons of model AIC implemented with the 'stepAIC' function from the 'MASS' (Venables & Ripley 2002).

Five temperature data loggers were used to determine the thermal conditions in the artificial rot holes, and whether there were any differences between exposed stumps, those within the woodland, and in plastic pots. Four aquatic dataloggers (Gemini Tinytag Aquatic TG-4100) were positioned at the deepest point within artificial rot holes (three in Curr Wood and one at the first proposed relocation site, Rothiemurchus Estate). The dataloggers were set to take a temperature measurement every hour. The first was placed in a 14cm deep hole in an exposed stump 30 metres from the woodland edge, and collected measurements from December 2007 until July 2010. The second was positioned in one of the exposed 24 cm deep plastic pots, which were sunk into the earth, two metres from the exposed stump. It took measurements for 18 months from April 2008 until October 2009. The third was positioned in a 13cm deep hole in a stump within the woodland, 40 m from the other dataloggers but on the same elevation (240 m), and it took measurements for 15 months from April 2009 until July 2010. The fourth was positioned in a 14 cm deep rot hole created in a *P. sylvestris* stump in

Rothiemurchus Estate, from May 2009 until July 2010. The fifth was an Air datalogger (Tinytag Transit TG-4080) and was positioned in Curr Wood at the base of a tree inside a sealed wooden bird box from April 2008 until July 2010. Differences in the range of temperatures between types of rot hole environment were assessed using one-way analysis of variance (ANOVA).

Blera fallax larvae were found in 48 (81%) chainsaw-bored holes (5.3 ± 5.5 larvae per hole) (mean ± Standard Deviation SD), and both of the stumps with natural heart rot (13 ± 10.4). No *B. fallax* larvae were found in plastic pots. *Blera fallax* were repeatedly found in the same artificially bored rot holes over the four years: eight were occupied every year, an additional eight were occupied for three years, and nineteen were occupied for two years. Total larval density over four years in one rot hole varied from 1 to 78 (14.13 ± 19.64). Of stumps containing *B. fallax* larvae, 13 (± 18.99) were exposed and 16 (± 20.76) were unexposed. More larvae were found in rot holes with both chips and sawdust (16.8 ± 19.2) than sawdust (11.3 ± 22.1) or chips alone (5 ± 9.4). The number of *B. fallax* found in stumps created in 2003 (12.6 ± 19.5), 2007 (16.6 ± 20.87) and 2009 (15.7 ± 19.6) did not differ significantly ($X^2 = 0.57$, $P = 0.75$). No measured habitat trait was found to predict *B. fallax* presence (all GLM, $P > 0.1$, see Table 5.3 for a comparison of means for occupied and unoccupied stumps).

Between April 2008 and July 2010, air temperature reached a maximum of 33.56 °C and a minimum of -14.39 °C. Average monthly water temperature fluctuations in rot holes fell within the range of the air temperature (Fig 5.2). The temperature reached a lower minimum in exposed pine stump rot holes (-9.19 °C) compared

with plastic pots sunk into the earth (-4.36 °C) and the unexposed stump (-1.56 °C). Maximum temperatures were near equal in all breeding habitats, reaching one degree higher in the exposed stumps (24.10 °C). No significant difference was found between monthly maximum, minimum or mean temperature readings from stump or plastic pot rot holes (ANOVA, $P > 0.1$).

Anagach Wood

Of ~200 *P. sylvestris* stumps located in Anagach Wood, approximately 50 older stumps (at a more advanced stage of decay) were exhaustively searched for suitable water-retaining cavities, or wet rot. Of these, 10 stumps had naturally rotted holes that retained water. No *B. fallax* larvae or puparia were found in any breeding habitat artificially created or natural in Anagach Wood. While *C. rufa* and *M. florea* were found (~ 4 per stump or pot), *S. clunipes* was the most numerous species, occurring in similar quantities (~10 per breeding habitat) in artificially bored rot holes and plastic pots.

5.6 Adult ecology

During 2007 and 2008, we investigated several aspects of adult ecology, including the nature of food plants, mating behaviour and dispersal ability (following methods as in Rotheray *et al* 2009). We positioned 104 emergence traps over felled stumps with natural heart rot (37), as well as all artificially bored rot holes (67) at Curr Wood before May 14, 2008. The emergence traps were made from 3 x 3 metre white cotton netting and malleable fence wire. The traps were secured to the ground

using pegs fashioned from the fence wire and heavy branches. In total, six *B. fallax* (3 males, 3 females) were caught in four emergence traps over one naturally rotted and three artificially bored *P. sylvestris* stumps. Other insects caught in the traps included several true flies, *M. florea* (5), *C. rufa* (6), *Sericomyia lappona* (6), *Rhingia campestris* (27), *Helophilus pendulus* (5), *Speghina clunipes* (15), *Microdon* sp., *Xylota segnis* (1), and a single species, *Rhembobius* sp., of Ichneumonid parasitoid of flies (5) (Table 5.4). By mid June, all traps were removed to allow ovipositing females access to the rot holes.

For a mark-recapture experiment, we surveyed a 3 km transect that covered the area where stumps and traps were located and where flowering trees and shrubs were found, including large patches of *S. aucuparia*, *Cytisus scoparius* (Fabaceae), and *Rubus idaeus* (Rosaceae). Each trap was checked at least daily and the transect surveyed twice a day (10am until 1pm, and 2pm until 5pm, weather permitting) from 14th May until 31st July 2008. Two unmarked females were observed ovipositing in a naturally rotted stump and one that was artificially bored, 400 metres apart. Both females were marked and released, but never re-sighted. There were no additional sightings of *B. fallax* during transect surveys.

5.7 Captive breeding for relocation

In October 2008, a survey identified 100 *B. fallax* larvae in the artificially created rot holes at Curr Wood. Because this was probably a small fraction of the actual population size based on the amount of available naturally rotted habitat, we removed 50 larvae for captive breeding in the laboratory. From this initial sample,

more than 1,200 larvae have been bred in captivity to date, of which 430 have been released at relocation sites and the source site (Table 5.2).

Rearing larvae to eclosion

Larvae were transferred individually to 1000 ml glass bottle microcosms, which were designed to simulate rot holes. A mix of ~200 ml each of *P. sylvestris* sawdust, chips and spring water were allotted to each bottle based on observation of rot holes in the field. Larvae were kept in climate-controlled facilities with thermal conditions that mimicked those experienced in the field in Strathspey, estimated using on-site dataloggers and Met Office reports. However, to avoid mortality due to the water in the microcosms freezing, temperatures were kept above 1°C (see Table 2.1).

During the captive breeding period, we conducted experiments to study how larvae responded to substrate conditions and intra-specific competition effects (see Chapter 2). We also conducted behavioural observations to inform rearing and habitat creation techniques, as well as to investigate microhabitat use and life history (see Chapter 3). These experiments suggest that optimal growth requires a minimum of 40 ml *P. sylvestris* sawdust (or chips and sawdust) and 70 ml of spring water per larva (Chapter 2). We provided bark pieces to allow larvae to crawl out of the water, or approach the surface to breathe, as well as moss plugs at the top of the microcosm, which fully developed larvae require for overwintering or pupation (Chapter 2). Sponge stoppers were also used to prevent larvae from wandering out of the microcosm completely. Mortality was proportionally greater in 2009 (46 individuals, 29 %) than 2010 (114 individuals, 22 %). In 2009, 85 % (39

individuals) of the individuals that died did so in the first 15 days (early instars) of development (Table 5.5).

Upon pupation, each individual was weighed on a 0.001g resolution balance, and carefully transferred to individually labelled plastic cups filled with tissue paper and moss to prevent pupae from incurring damage while in transit. A piece of cotton netting was secured over the top of the cup with an elastic band. Newly emerged adults tend to sit on this netting while their cuticle hardens and wings expand, which conveniently makes them visible for collection and transferral to breeding cages. Upon ensuring that the adult's wings had completely extended, each one was transferred to laminated lined paper, and a photograph was taken for measuring adult traits, including wing and thorax length (and leg length in 2009). All individuals eclosed and were kept in captivity in 2009 (19/19 Males/Females), and in subsequent years a random selection of 7 to 10 individuals from each larval growth treatment (see Chapter 2) were kept for captive rearing (15/24 M/F in 2010, 30/30 M/F in 2011) while the rest were released at the relocation site. Individuals that were not kept for captive breeding were transferred, on the day of their emergence, to 9 x 2 cm tubes for transportation to their release site. Those kept for captive breeding were individually marked using non-toxic enamel paint and moved into breeding cages.

Adult flight cages

Two types of cage were tested: one large outdoor cage positioned on-site at the release location where an observer could enter to record adult behaviour and time

budgets in a close-to natural situation; and four small indoor cages that provided better control over light and temperature and were used primarily to observe mating and oviposition behaviour. The four indoor cages (45 x 45 x 60 cm) were constructed using white cotton netting, malleable wire and ten linked strip lights (90cm, 21 Watt fluorescent bulbs) (Fig 5.3). The outdoor cage was constructed using white cotton netting (for the roof and door) and polyethylene mesh (for the walls) over a polypropylene frame (roughly 195cm height, 375cm length, 90cm width) (Fig 5.3). Cotton was more appropriate for the roof as plastic tended to collect water droplets that could damage or drown the insects, which tended to gather on the roof of the cage. The cage was constructed by mid-May in a partially shaded position at the relocation site. Large pine branches were fixed along the walls and roof of the cage to provide extra shade and roosting habitat, and two 3-litre plastic freezer bags sunk into the ground and filled with wet *P. sylvestris* sawdust and chips as artificial rot holes.

A range of food-plants were presented to adults in the indoor and outdoor cages based on what was available in and around Curr Wood. They were collected from the field and positioned in bottles filled with water, plugged with netting to prevent individuals from falling in. Feeding was recorded when adults visited flower heads and moved mouthparts over the stamens and at the base of the perianth. Food plants were removed if after two days no adults were observed feeding on them, and all flowers that were used were replaced on a two-day cycle. An additional nectar source was provided by soaking cotton wool in dilute honey solution (1ml honey/10ml water), wrapping it in cotton mesh and securing it to the roof or walls

using a crocodile clip. In the outdoor cage, wood ants became attracted to the honey solution making it necessary to alter its location each day.

Daily, 2 to 4 hour-long observations were made in the outdoor cage (between the hours of 8am and 8pm, weather permitting) to ascertain behaviours, mating frequencies, and estimate time budgets. The main behavioural categories were feeding, resting, ground searching and mate seeking. Ground searching was defined as adults moving over the ground vegetation. Sexual activity was defined as males chasing females or each other. For each category the time, individual and length of time per individual was recorded.

Fifty-one hours were spent observing adults in the outdoor cage. Adults were on the wing in captivity from 11th May until 24th August, and males and females had a mean lifespan of 38 (± 17.59) and 34 days (± 16.4) respectively (Table 5.5). In total, 44 and 43 % of the day (between 8am and 8pm in good weather conditions) was spent feeding and resting respectively. Individuals were most often observed resting on the walls and roof of the netting. Adults fed on *Sorbus aucuparia* (Rosaceae) (66%), *Stellaria holostea* (Caryophyllaceae) (27%), *Umbelliferae spp.* (Apiaceae) (23%), Bedstraw (*Galium spp*.) (Rubiaceae) (2%), *Rosa canina* (Rosaceae) (<1%), and Buttercups *Ranunculus spp*. (Ranunculaceae) (< 1%). Adults did not feed on *Cytisus scoparius* (Fabaceae), *Ajuga reptans* (Lamiaceae), *Senecio jacobaea* (Asteraceae), *P. sylvestris*, *Prunus padus* (Rosaceae), or *Rubus idaeus* (Rosaceae).

Adults were observed behaving territorially 6 %, and searching on the ground 7 % of the time. Ground searching was recorded a total of 36 times, of which the mean

time spent was 8.4 minutes. During this time, adults would move their mouthparts over damp patches of earth and in the bags of wet sawdust. Upon subsequent visits at the beginning and end of the observation period, the ground and the netting were liberally sprayed with water, and often adults were observed drinking immediately.

In indoor cages, adults spent most of their time on the roof of the cage at the closest point to the light source. Flowers had to be positioned near this area in order for adults to land on them and feed. Water was imbibed only when the netting near the top of the cage was sprayed, after which adults would often drink immediately.

Mating requirements

As well as general observations of individuals in both outdoor and indoor environments, in order to gather information on sexual behaviour, we studied how the density of adults affected the number of mating attempts. Using the indoor cages as replicates, we varied the number of males and females from 2 to 10 (always with a unity sex ratio). The number of copulation events per individual, and copulation duration was recorded. Copulation was defined by physical contact between male and female genitalia. All copulations were assumed to be of equal value.

We assessed directional sexual selection on phenotypic traits using standardized linear selection gradients (ß), which are partial coefficients from regressions of all relevant phenotypic traits on relative fitness (Arnold & Wade 1984a, 1984b). Our index of male mating success was the number of matings acquired. For comparison, we also measured partial coefficients between female traits and mating frequency,

although for females we have no a priori reason to expect that mating success covaries with fitness. For each analyses, we first included all mated and unmated individuals in a generalised linear model with binomial error distribution. We then eliminated the zero fitness class (i.e., individuals who never mated, or females who laid no eggs), because membership in the zero class could arise as an artefact of the artificial rearing regime and consequently obscure more interesting patterns of variation among mating or fecund individuals. All coefficients and standard errors come from Gaussian linear models featuring standardized thorax width, wing length and pupal mass. P-values were obtained from generalized linear models with Poisson error distribution. Our low sample size prevented us from analysing patterns of nonlinear selection.

We assessed the effect of the number of previous matings on female copulation duration using linear mixed effects (LME) models implemented in the 'nlme' package (Bates & Maechler 2008). We modelled copula duration as a function of the mating number (first, second or third mating), including the random effect of female identity to account for repeated measures on individuals.

Outdoor cage

Adult males aged 15 days or older could be observed chasing passing male and female individuals and repeatedly returning to within inches of the same location, from here on termed 'temporary territories'. Males would keep these temporary territories for up to 13 minutes (3.77 ± 3.37) (mean ± SD) often in a sun-lit area on the ground or in vegetation. This behaviour was only recorded between 12:30 and

16:35, at temperatures between 15 and 24°C, on 26 separate occasions. Upon landing on a female, the male's hind legs were secured around the female's abdomen and copulation was apparently initiated by stroking the underside of the female abdomen with the tip of the male abdomen.

Indoor cages

Males aged >11 days and females aged >15 days were observed mating in the indoor cages (Table 5.5). In the five days following the first recorded mating event, mating occurred slightly more regularly in higher density cages (0, 1, 3 and 3 matings in densities 1:1, 2:2, 3:3 and 5:5, respectively), although the small sample precludes drawing strong conclusions from this. Moving individuals in preparation for the density experiment led to a cessation of mating attempts for several days. Therefore, to encourage as much mating as possible the remaining individuals were divided into two cages and no further manipulations were made.

In total, eleven males and thirteen females were recorded mating in the first year of experimentation (of total 19/19 M/F). Females copulated up to 9 times (3.5 ± 2.11), and males copulated up to 8 times (4.75 ± 2.67). Copulations lasted between 1.5 and 70 minutes (26.7 ± 15.8). Among the 12 females that mated more than once, we found a significant effect of mating number on copulation duration (1^{st}, 2^{nd} and 3^{rd}) (Parameter estimate 8.35 ± 3.21 SE, $t = 2.59$, $P < 0.05$). Duration increased by an average of 10.9 ± 14.46 (SD) minutes between first and second copulation.

Analyses that included mated and unmated individuals detected no significant results (data not shown). However, in spite of our small sample, analyses that eliminated the zero fitness class detected marginally nonsignificant (P = 0.089) directional sexual selection for longer thoraces in males (whole model $R^2 = 0.700$, $F_{3,7} = 5.438$, P = 0.030; Fig 5.4, Table 5.6). Wing length and pupal mass had substantially smaller parameters, and did not significantly predict mate number. By contrast, none of the morphological traits predicted female mating success (whole model $R^2 = 0.145$, $F_{3,8} = 0.452$, P = 0.723; Fig 5.4, Table 5.6).

Oviposition requirements

A number of different techniques were attempted in the small cages to induce oviposition and determine preferences. These included providing 0.5 and 3 litre freezer bags, and 250 and 1000 ml jars, half-filled or completely filled with *P. sylvestris*, *P. abies* or *B. pubescens* sawdust and water. These were introduced to each cage 24 hours after a mating event was observed, and left for either one hour or the duration of the observation period (up to 3 hours).

We assessed fecundity selection on morphological traits in a similar way to our analysis of sexual selection, above, estimating standardized linear selection gradients (ß) as the partial coefficients of linear multiple regressions of thorax length, wing length and pupal mass on the number of eggs laid. As above, we eliminated the zero fecundity class to prevent these individuals from skewing patterns of covariance among fecund females. Unlike for mate number models, P-values for this analysis came from the same Gaussian error models that provided the

coefficients (i.e., we assumed that fecundity had normal error, even though the small sample prevented validation of this assumption).

Females were not observed ovipositing in artificial rot holes positioned either in the outdoor or indoor cage environment. Oviposition was triggered only upon sealing gravid females into 0.5 to 3 litre freezer bags, with 0.25 to 1.5 litres of wet *P. sylvestris* sawdust. *P. abies* and *B. pubescens* did not trigger oviposition. Females would begin ovipositing eggs after 1 to 20 minutes of being sealed into the bag.

Females were gravid no sooner than 14 days after first mating. The oviposition period in captivity was between 24th June and 24th August. Each female produced 10 to 50 eggs per clutch, and up to 188 per female (Table 5.5). In 2009, five females survived to oviposit resulting in ~463 larvae, and in each of 2010 and 2011, fourteen females produced ~800 larvae.

Although there is usually positive directional selection for fecundity in female insects, we detected significant negative directional selection for shorter wings, i.e. greater fecundity was recorded in females with shorter wing length (whole model $R^2 = 0.738$, $F_{3,6} = 5.644$, $P = 0.035$) (Fig 5.4, Table 5.6). In fact the coefficients for both wing length (ß = -1.022) and pupal mass (ß = -0.261) were negative, although the latter was indistinguishable from zero.

Rearing captive-bred larvae

Freezer bags of water-soaked *P. sylvestris* sawdust and *B. fallax* eggs were sprayed with spring water daily but otherwise remained un-manipulated for several weeks to avoid harming the eggs and first instars. If larvae had grown sufficiently, i.e. were clearly visible (body length > 0.5 mm long), the content of the bags was carefully searched, and larvae were counted and transferred into glass microcosms prepared with substrate as described above (Table 5.5). Between September and November, a random selection of 2nd and 3rd captive bred instars (body length > 1 cm long) were transferred to breeding habitat created at each relocation site (84 in 2009, 51 in 2010, 40 in 2011, Table 5.2).

5.8 Relocation sites and habitat preparation

Three relocation sites were selected based on four main criteria:

1. They were within the species historic range and surveys over a number of years had confirmed its absence at the time of relocation.
2. Sites were of sufficient distance (> 5 km) from existing populations that natural colonisation is unlikely.
3. The site had sufficient suitable habitat and potential for long-term habitat supplementation.
4. The site will receive a long-term (50 years +) commitment to protecting, monitoring and supplementing habitat.

A standard approach at each site was used to determine the presence of *B. fallax* and the condition of the habitat. Thorough surveys of the area were carried out by means of exhaustive searches, emergence traps over rotting pine stumps, and artificial rot holes created at least a year in advance, to confirm as far as possible the absence of *B. fallax* within the area for relocation. Thereafter, habitat creation techniques varied across sites depending on what habitat was available, as detailed below.

Rothiemurchus Estate

This site is 20 km south from Curr Wood on the privately owned, 3,000 Ha Rothiemurchus Estate (57°10' N, 3°48' W) (Figure 5.1). *Blera fallax* was first recorded in nearby Aviemore in the late 19th century, and was last recorded in 1942 (Rotheray & MacGowan 2000). This site was considered suitable for the first relocation attempt due to the large number of *P. sylvestris* stumps available for easy habitat creation, and the keen interest of foresters to develop the site for this species as part of their biodiversity action remit.

In 2003, holes were cut in 18 stumps within a 500 m^2 area using a chainsaw as described above. These were filled with pine chips and sawdust, and the hole was partially covered with the wedge created in the cutting process. In 2007, *C. rufa* and *M. florea* were found in 12 of 18 rot holes in Rothiemurchus Estate. Based on empty and live puparia found on site, at least 7 *C. rufa* and 19 *M. florea* eclosed in August and September the same year.

In July 2008, two additional groups of holes in stumps were created, spaced one km from each other and the original site forming three points of an equilateral triangle. While the design was based on groups of available stumps within the area, this enabled a first estimate of dispersal ability. The two groups consisted of 46 and 30 stumps, giving a total of 94 chainsaw-cut rot holes. Fifteen Norway spruce *P. abies* stumps (8 and 7 at each site) were used in addition to *P. sylvestris* to investigate the option of utilising another species of tree to create habitat for *B. fallax*, the stumps of which are large and abundant at the site. These were created in the same way using a chainsaw, and filled with pine chips and sawdust.

In 2008, just 4 weeks after boring, *C. rufa* and *M. florea* were found in 15 newly bored *P. sylvestris* stumps, as well as in three *P. abies* stumps. *Speghina* spp., *Chalcosyrphus nemorum*, *Clusoides geomyzinus* and *Myetophilidae* spp. were also found in *P. sylvestris* rot holes (Table 5.4).

In September 2009, 84 captively reared 2nd and 3rd instar *B. fallax* larvae were transferred in groups of 3 into 28 bored *P. sylvestris* stumps at the most northern of the three sites created at Rothiemurchus. This site was chosen because it was the greatest distance from the road, and had the greatest number of bored stumps (46). In May and June 2010, 95 (M/F 50/45) captively reared adults were released at the same site (Table 5.2). In September, 43 first instar *B. fallax* larvae were found in 12 stumps, four of which were found in the southwest group 1 km away from the release site, demonstrating that mating and oviposition had successfully occurred.

In June 2011, thirty additional drill-bored holes were created in *P. sylvestris* stumps at the most northern site (Table 5.1). Between May and June, 48 (M/F 24/24) adults were released at the same site in Rothiemurchus Estate, and in September, three large larvae were found that were considered to be semivoltine i.e. larvae from the previous year developing for two years (Table 5.2; Chapter 2). Surveys indicated a total of 37 larvae found at Curr Wood were also semivoltine (Table 5.2).

Abernethy Forest and Dell Woods

The second relocation site was Abernethy Forest National Nature Reserve (57°15' N, 3°40' W), 8 km south of Curr Wood, owned and managed by the Royal Society for the Protection of Birds (RSPB). It extends over 2,800 Ha, two thirds of which is native Caledonian forest (Summers *et al*. 1997) (Figure 5.1). *Blera fallax* was first recorded at Loch Garten in Abernethy Forest in 1934, and was last seen in the same area in 1982 (Rotheray & MacGowan 2000). Since then habitat creation in the form of plastic pots and bored holes have been tried and tested but no *B. fallax* larvae have been found. Stump habitat in Abernethy Forest is limited, so we had to fell trees in order to create enough stumps for hole boring and relocation.

The site selected for relocation was a 10 Ha plantation where *P. sylvestris* range from ~20 to 50 cm diameter (24.67 ± 10.55) (mean ± SD). The site was planted in 1958, and is close to the last observation site for *B. fallax* at Loch Garten. In August 2010, 100 trees of 28 to 40 cm diameter (32.76 ± 2.55), 95 to 131 cm circumference (113.61 ± 8.25) were felled. These were distributed evenly across a 10-hectare area. They were cut at minimum 28 cm height (63.78 ± 13.02) from the ground in order

to insure enough depth for bored holes. The holes were drilled using a generator-powered drill and 25 mm auger bit. Holes were partially covered using large chain-sawed slabs from the felled tree, and monitored for water retention over several months after creation. Due to a lack of rain and concern over whether the stumps would retain water, each hole was filled or topped up using bottled spring water or water from Loch Garten one month after boring. We studied the relationship between several stump variables (including diameter, circumference, height, and hole diameter) and water retention, which was measured as height of water in bored hole. In addition to holes in stumps, 10 holes were bored in the side of felled trees to attempt an alternative form of habitat creation.

In 2008, only *Speghina* spp. was found inhabiting the artificial breeding sites created at Abernethy Forest. In October and November 2010, 87 % of bored holes were retaining water two months after felling and hole boring. No relationship was evident between any stump variable measured and water holding capacity. None of the holes (n = 10) bored in the side of felled trees appeared to retain water. In September 2010, 51 *B. fallax* larvae were transferred in groups of 3 into 17 holes. In April 2011, 10 empty *B. fallax* puparia and 11 live pupated *B. fallax* were found around the holes. In May and June 2011, 78 adults (M/F 30/48) were released at Abernethy Forest (Table 5.2). In September 2011, *Speghina* spp. larvae (~30) and three *B. fallax* larvae, considered to be semivoltine, were found in bored holes (Table 5.2).

SNH-owned Dell Woods is 375 hectares of native pinewood and is part of Abernethy Forest, 5 km from Curr Wood (57°15’ N, 3°39’ W) (Figure 5.1). Due to

a lack of suitably sized stumps or available *P. sylvestris* trees for felling, breeding habitat was created by sinking 16 plastic pots into the earth and filling them with *P. sylvestris* chips. They were situated every two metres along a transect extending from inside the woodland to an exposed area. Surveying here began in 2007 and continued annually until 2010.

Four species have been identified inhabiting plastic pots at Dell Woods. These include *C. rufa*, *M. florea*, *Speghina* spp and *Xylota* spp (Table 5.4). No *B. fallax* larvae or evidence of occupation has been found.

Inshriach Forest

The third relocation site was Inshriach Forest, which comprises 3,000 Ha of forest, is 8 km south of Rothiemurchus Estate, and is owned and managed by Forestry Commission (57°07' N, 3°53' W) (Figure 5.1). Like Abernethy Forest, Inshriach lacked a sufficient number of large stumps and thus felling was required in order to create habitat. A 5 Ha *P. sylvestris* plantation site was identified where enough suitable trees were available for felling and long-term breeding habitat supplementation. This site was planted between 1949 and 1950. As part of investigating habitat creation techniques, it is important to know if suitable habitat can be created as part of normal harvesting rotations. In order to test this, 160 trees were felled included two size ranges (20cm and 30 cm diameter), and two height ranges (60 cm and 'normal felling height' roughly 20 cm or as close to the ground as machines allow), duplicated forty times. Both a petrol-powered drill and chainsaw (due to logistical and mechanical issues) were used to create holes. Each

stump was filled with sawdust created from the boring process and partially covered with the bored wedge, or large pieces left from creating the cavity. Felling and hole-boring were complete by November 2011. Due to a lack of rain, bottled water was used to confirm water retention in 10 bored holes, and 40 2nd and 3rd instar larvae in groups of 4 were transferred into these holes in November 2011 (Table 5.2).

5.9 Conservation genetics and population supplementation

Supplementation of breeding habitat (minimum 10 new bored rot holes per year), and of captively reared larvae and adults will continue at each site as necessary, until monitoring shows signs that a self-sustaining population exists. To further inform management of these small populations, DNA extracted from *B. fallax* individuals that died in captivity was used to develop microsatellite markers (Appendix 4.1). Genetic variation was measured by comparing the Scottish population with Swedish *B. fallax* specimens thought to represent a more outbred population (Chapter 4). While the genetic variation was lower in the Scottish population than the Swedish, the fitness consequences of this difference are unknown. Therefore, on-going monitoring is necessary in order to detect detrimental effects such as those from inbreeding (Frankham 1995, 1998, 2005). To assist these plans, a preliminary investigation into non-invasive techniques for extracting DNA was carried out (Appendix 4.2). Sufficient DNA for microsatellite amplification was extracted not only from small pieces of adult tissue such as single terminal tarsal segment and wing tips, but also from sections of year-old empty puparia (Appendix 4.2).

5.10 Discussion

Restoration ecologists must understand the ecology and biology of a species in order to create the conditions required for its successful re-establishment. Through experiments and observations over the past four years on both the larval and adult stages of *B. fallax*, we have developed important methods to help restore this species. Using techniques developed for captive breeding and animal husbandry at every life history stage, we have relocated individuals to previously occupied sites, and now have data to show successful utilisation of habitat created at two previously occupied locations in Scotland. Furthermore, we were able to identify adult food plants as mainly coming from the family Rosaceae, and demonstrated a minimum dispersal distance of 1 km. As this is the first attempt at conservation management of this kind for saproxylic hoverflies, there is little against which to compare progress. However, we have met targets set by the SNH SAF and UK BAP steering group (Appendix 5.2), and have protocols in place for taking *B. fallax* conservation management forward.

Habitat assessment

Ensuring that suitable breeding habitat is available over space and time is critical to the success of restoration and relocation. Genetic and historic evidence suggests the population at Curr Wood became recently isolated, probably due to forestry practices such as uprooting stumps, re-seeding and burning habitat in the area (Worrell & Dunlop 2003). Where *B. fallax* occurred in the past, intensive or unsympathetic management has resulted in large patches of old growth forest being

replaced by young plantation trees lacking structural diversity. This probably isolated and fragmented *B. fallax* populations, which eventually died out. Therefore, creating suitable breeding habitat was the highest priority. This required studying the habitat at the extant *B. fallax* site, Curr Wood, selecting a relocation site based on similar characters, and re-creating enough breeding habitat to re-establish a population.

The distribution of larvae in rot holes depends on female oviposition preferences, and probably a range of factors affecting detection. Larvae were repeatedly found occupying the same superficially similar stump rot holes over up to four years, while never found in others. While habitat preferences were not tested directly, the time the rot hole was created, and the dimensions of the stump or hole did not appear to explain this preference. It may be due to a number of untested factors such as resin acids, tree host-related chemistry or rot hole content, or a female's ability to detect some other trait such as differences in surface area within a rot hole or other factors that promote microbe abundance (Jonsell *et al*. 2001; Kainulainen & Holopainen 2002). *Myathropa florea* has been found in more detritus-rich sites, suggesting that they are able to select more productive breeding habitat (Srivastava & Lawton 1998). Further study through controlled experimental manipulations or measures of microbial diversity and abundance would be required to gain a better understanding of female oviposition preference.

Blera fallax larvae were never found in plastic pots filled with pine chips and sawdust in Curr Wood. Once captive females were sealed in bags containing chips and sawdust, oviposition was eventually triggered, so perhaps the volatile chemicals

that might attract females to oviposit were too dilute in plastic pots in-situ. Oviposition could not be triggered by any other species of tree, including *P. abies* which is known to be inhabited by *B. fallax* abroad. *Blera fallax* was not found in rot holes created in *P. abies*, even when filled with *P. sylvestris* wood chips. *Picea abies* is not native to the UK, which could explain why Scottish *B. fallax* might not be attracted to it. *Picea abies* also rots far more quickly than *P. sylvestris* (Vollbrecht *et al*. 1995; Marchetti & metsäinstituutti 2005; Mäkinen *et al*. 2007). Stumps that decay slowly may retain water for longer, which may explain the preference for *P. sylvestris*. In addition, while larvae find enough resources for growth in different tree species including *P. abies,* they do grow faster in *P. sylvestris,* and adults are significantly larger (Chapter 2). Perhaps this is a feature of the decaying process and associated microbes in *P. sylvestris* wood.

Water retention is a central concern when creating artificial rot holes. A large percentage of stumps did not retain water in Curr Wood. These stumps were fairly old, having remained for 10 years since the last clear fell rotation. As well as becoming dry and rotten, it was likely that they had a high number of beetle-excavated tunnels and holes. At Abernethy Forest, we tested a different form of habitat creation by using a drill, which created a smaller hole with less surface area and exposure to the elements, but of the same depth (see Appendix 5.3). While these holes appear to retain water in the short-term, their long-term suitability is unknown. Another species of rot hole dwelling insect (*Sphegina* spp.) was attracted to these holes, and we have evidence from captive release experiments that *B. fallax* larvae develop and overwinter successfully in this breeding habitat, but it remains to be seen if this type of hole will attract gravid females.

The rot hole environment is prone to temperature fluctuations, reaching -9°C in winter. The more exposed rot holes tended to drop to a lower minimum temperature. Whether this has any effect on larval survival is not known. Larval occupancy did not differ between more exposed rot holes and those within canopy cover, or in the vicinity of the woodland edge. Temperatures were milder, though not significantly so, in plastic pots sunk into the earth compared to an exposed stump rot hole, but whether this trend explains the lack of colonisation of plastic pots is unclear.

Adult emergence traps caught few flies. This may be explained by the tendency of *B. fallax* larvae to develop quickly and move out of and away from the stump before winter (Chapter 2 and 3). Dipteran larvae can move more than 30 metres in search of a suitable place to pupate (Hagstrum & Subramanyam 2010). This may be part of their natural 'wandering' phase triggered by a critical size threshold. At this time, they conserve energy over the winter by entering into a stasis or diapause phase in the surrounding vegetation. There may be additional survival advantages associated with this behaviour such as avoiding detection by parasitoids (Hagstrum & Subramanyam 2010; Chapter 3); *B. fallax* has one known predator, the parasitic *Rhembobius perscrutator* Thunberg (Hymenoptera, Ichneumonidae) which is a generalist parasitoid of syrphid pupae (Rotheray & MacGowan 2000).

Captive breeding

By 2007, it was apparent that only one population of *B. fallax* survived in the wild in the UK. Therefore, the decision was taken to attempt captive rearing to boost numbers and provide material for relocations.

One problem encountered when keeping adults in cages was the provision of water. In the outdoor cages, both males and females spent time in the ground vegetation, probably in search of water. Rain and damp earth alone may not be enough to provide drinking water for hoverflies restricted to cages during warmer, drier periods. Adults were only observed drinking water in the indoor cages once it was sprayed in areas they were likely to visit due to the heat and light. Developing better techniques of providing water, pollen and nectar for adult hoverflies would probably improve their survival in captivity. Because indoor cages allow better control over thermal and humidity conditions, greater capacity for monitoring survival and condition of adults, and greater ability to re-capture females for oviposition, this was our most effective way to captive breed *B. fallax*.

Through captive breeding, we were able to observe *B. fallax* behaviour and reproductive biology, none of which has been reported previously. Females mated up to nine times, and copulation duration tended to increase with the number of copulations. This is not unusual in insects when competition causes males to attempt displacement of sperm stored from previous copulations (Price *et al*. 1999; Snook & Hosken 2004). Males with greater thorax lengths were also more successful in copulating with females, which corresponds to sexual differences in

development as shown in Chapter 2 where experiments on larval growth suggest that for males, thorax length is more important than risks involved in taking longer to develop. Male size may be related to or important for mate-seeking behaviour; males were observed holding temporary territories in outdoor cages, but we did not observe overt aggression between males that might favour larger individuals in contests. Where mating takes place in the field is unknown. While other saproxylic flies (e.g., Neriids and aspen hoverflies, Preston-Mafham 2001; Rotheray *et al*. 2009) gather at the breeding site, we never observed male *B. fallax* defending or attending pine stumps in the field.

Data from the small number of females induced to oviposit eggs shows that shorter wings correlate with fecundity. Most studies show insect wing and thorax size positively correlating with fecundity (Grimaldi & Jaenike 1984; Honěk 1993; Nylin & Gotthard 1998; Armbruster & Hutchinson 2002). Our finding could be an experimental artefact brought about by difficulties in providing the appropriate conditions for oviposition and low sample size. Inducing oviposition took two weeks after the first mating, and was not achieved by any other means than sealing females in bags full of wet pine sawdust. This suggests the conditions provided are not ideal, which may also explain low female survival to oviposition (26% in the first year, 47 % in the second and third year). It would be useful to study vitellogenesis, egg retention and oviposition in future years, should the population become healthy enough, to determine whether the negative covariance between wing length and fecundity reflects genuine selection or not.

The status of the relocation and conservation management

Relocations appeared to be initially successful, particularly the first release at Rothiemurchus in 2009. Over 40 new larvae were found in artificially created rot holes up to one km from the release site demonstrating successful survival, mating, local dispersal and oviposition. Unfortunately, 2011 appears to have been a very poor year for *B. fallax* at all sites, including the sole natural wild population at Curr Wood. No new larvae were found at any *B. fallax* site in 2011, the surviving populations consisting solely of larvae that were developing over two years. This suggests a complete failure of adult breeding at all sites, perhaps due to cold and wet weather during the adult breeding season. Large population fluctuations due to stochastic events are not uncommon in insects. *Blera fallax* may have a bet-hedging strategy in order to cope during these adverse periods involving a number of larvae developing over two years regardless of growth conditions (Chapter 2). However, with the current precariously low population size, bust periods could readily drive the species to extinction.

The success of re-establishment depends on on-going management at relocation sites and at Curr Wood, where expansion and supplementation of the breeding habitat is imperative. This requires long-term cooperation of landowners and managers implementing informed, conservation based practices. Management for *B. fallax* is inexpensive, and can probably fall in line with normal harvesting rotations. This is currently being investigated at Inshriach Forest. While harvesting procedures often already involve leaving a number of tall stumps to create diversity of habitat structure and habitat for nesting birds (Summers 2007) stumps are still being

unnecessarily treated with fungicides. Recommendations for habitat creation will include refraining from this practice as well as providing tall stumps for hole-boring (Appendix 5.3).

The overlying feature of Rothiemurchus Estate, Abernethy Forest and Inshriach Forest is that these areas are cultural landscapes or owned or under management actions that aim at conserving and increasing biodiversity using a level of intensive management that would be uneconomical for commercial forestry. Commercial thinning continues in Curr Wood, the most recent round of which aimed to remove up to 30% of the trees in January 2012. Although the future of the site is uncertain, short-term viability of *B. fallax* habitat has been assured (A. Elliot, pers. comm.). Management plans for the next few years involve expanding breeding habitat in the 5-mile radius around Curr Wood, which covers 1000 Ha of woodland managed by Scottish Woodlands. The intention is also to link up pockets of woodland such as the community-owned Ellan Wood in Carrbridge where habitat creation has already begun.

Monitoring

Having an effective means to measure abundance of any endangered species is essential to monitor population trajectories, the effectiveness of management actions, and to measure the impact of environmental change. There are many advantages to concentrating on the early, more abundant larval stages rather than the elusive adults when monitoring species that are rare and difficult to find (Rotheray *et al*, 2001). Based on our findings on *B. fallax* larval phenology, optimal detection time

is late summer (August to September). Often empty puparia are the only evidence found for *B. fallax* occupancy, especially because natural habitat, which often extends deep into the roots of pine stumps, is difficult to search (Rotheray & MacGowan 2000). We may also be able to monitor the genetic health of populations by extracting DNA from empty puparia (Appendix 4.2). Acquiring DNA in this way will also facilitate monitoring of captive bred populations, where non-invasive sampling methods are required to prevent the inadvertent mortality of rare captive specimens.

Conservation significance

Blera fallax is one of many saproxylic organisms, a group which also includes diverse invertebrates, fungi and micro-organisms that recycle minerals and nutrients and are part of a complex, and often specialised, community of decomposers. This resource and its community are now recognised as fundamental to forest function through critical processes such as nutrient cycling (Grove 2002; Schmuki *et al*. 2006). Saproxylics can be used as bio-indicators of site quality, and thus indicators of forests of conservation importance (Speight 1989). *Blera fallax* meets the criteria as an indicator species as it is associated with a tree species that is characteristic of a particular woodland type (native boreal forests of the northern hemisphere), it is dependent on dead wood and is excessively localised (Speight 1989). In addition, unlike the adults, the larvae are relatively easy to find and recognise; hence *B. fallax* is suitable to play a role as a flagship species. *Blera fallax* is also found in association with a range of other saproxylic Diptera that will benefit from management promoting mature timber or old growth forests.

Saproxylic organisms have suffered from unsympathetic forest management involving the removal of dead trees because fallen branches, trees and stumps obstruct forest management and are thought to be potential sources of outbreaks of pest species (Schiegg 2001). Stumps are not only destroyed by forestry operations in plantation woodlands, but are being removed for re-seeding, and even used for biofuel (Walmsley & Godbold 2010). The pine stump habitat is not only important for rot hole dwelling species, such as most of those found in association with *B. fallax* in this study. The larvae of *Xylota jakutorum* (Dipetera, Syrphidae) are known to develop in the borings of the pine weevil *Hylobius abietus* (Coleoptera, Curculionoidea) in conifer stumps (Rotheray & Stuke 1998). Others include *Microdon mutabilis* (Diptera, Syrphidae) and their associated *Formica lemani* (Hymenoptera, Formicidae) ant colonies, mason bees such as *Osmia unciniata* (Hymenoptera, Megachilidae), the UK Biodiversity Action Plan species twinflower *Linnaea borealis*, and a range of lesser-known wood decaying fungi, lichen, mosses and bryophytes (Lonsdale *et al* 2008). Forestry in the UK now includes retaining deadwood, including stumps, as part of their guidance and good practice, however widespread understanding and appreciation of the biodiversity importance of deadwood is needed (Forestry Commission 2002).

Conclusion

Well-managed breeding programmes as properly integrated components of wider efforts have good conservation potential. This study is a significant example of the benefits, as well as tribulations, of captive breeding in invertebrate conservation.

The main difficulty is often that there are few analogous studies and very little background information, unlike the case for most vertebrates. But this study is an example of what can be achieved in four years of focused research, in cooperation with foresters, landowners and managers, to re-establish an endangered insect.

Table 5.1 Artificial habitat created at six sites over eight years.

	Number of artificial rot holes created						
Sites	2003	2007	2008	2009	2010	2011	Total
Curr Wood	52	17	15	60		50	194
Anagach Wood	10			25			35
Rothiemurchus Estate	18		43	30		30	121
Abernethy RSPB					100	10	110
Dell wood SNH		16					16
Inshriach FC						160	160

Table 5.2 Maximum larval abundance per year at each site (N) and number of adults (released between May and July) or larvae (L) (released between September and October) (R) at each site.

		2007	2008	2009		2010		2011	
Site	Owner/Manager	N	N	N	Released	Released	N	Released	N
Curr Wood	Private	35	109	142	0	10 (5/5)*	111	24 (8/16)*	37
Anagach Wood	Community owned	0	0	0	0	0	0	0	0
Rothiemurchus Estate	Private	0	0	0	84 (L)	95 (50/45)*	43	48 (24/33)*	3
Abernethy Forest	RSPB	-	0	0	0	51 (L)	-	78 (30/48) *	3
Inshriach Forest	Forestry Commission	-	-	-	-	0	0	40 (L)	-

- not surveyed * Males/Females

Table 5.3 Pine stump and rot hole dimensions for those occupied versus those never occupied by *B. fallax* through 2007 until 2011 at Curr Wood.

Pine stump and rot hole dimensions	Occupied by *B. fallax*	Not occupied by *B. fallax*
Circumference (cm)	153.84 ± 17.86	128.42 ± 35.3
Height (cm)	34.96 ± 12.88	29.84 ± 14.4
Hole depth (cm)	13.32 ± 6.22	13.04 ± 4.9
Hole opening area (cm^2)	256.02 ± 67.35	204.54 ± 110.3
pH	5.06 ± 0.27	5.13 ± 0.28

Table 5.4 List of species or groups of species, description (and any listed status), locality and biology, found in association with habitat created for *B. fallax*.

Species	Description	Locality	Biology
Callicera rufa	Diptera, Syrphidae	Rot hole	Scottish coniferous woodland; saproxylic larvae; rot holes and water-filled cavities in conifers
Chalcosyrphus nemorum	Diptera, Syrphidae	Rot hole	Deciduous woodland; saproxylic larvae; decaying sap under fallen wood
Microdon spp.	Diptera, Syrphidae	Emergence trap	Coniferous woodland; larvae predatory in ant nests in *P. sylvestris* stumps
Myathropa florea	Diptera, Syrphidae	Rot hole	Deciduous and coniferous woodland; saproxylic larvae; holes and water-filled cavities
Sphegina clunipes	Diptera, Syrphidae	Rot hole	Deciduous and coniferous woodland; saproxylic larvae; decaying sap under bark and water-filled cavities
Sphegina sibirica	Diptera, Syrphidae	Rot hole	Pine woodland; larvae probably saproxylic under bark of decaying wood
Clusiodes geomyzinus	Diptera, Clusiidae (BAP species)	Rot hole	Coniferous woodland; saproxylic larvae; decaying fallen branches and stumps water-filled cavities
Mycetophilidae spp.	Diptera, Mycetophilidae	Rot hole	Deciduous and coniferous woodland; saproxylic larvae; fruiting fungi and water-filled cavities
Rhembobius perscrutator	Hymenoptera, Ichneumonidae	Emergence trap	Parasitoid of large Cyclorrhaphan Diptera puparia
Sericomyia lappona	Diptera, Syrphidae	Emergence trap	Wet woodlands and moorland; saproxylic larvae; peaty pools and boggy streams
Rhingia campestris	Diptera, Syrphidae	Emergence trap	Saproxylic larvae; animal dung, silage and decaying organic matter
Helophilus pendulus	Diptera, Syrphidae	Emergence trap	Wet habitats; saproxylic larvae; pools and stream sides
Xylota segnis	Diptera, Syrphidae	Rot hole	Saproxylic larvae; wet decaying organic matter including water-filled cavities and compost heaps

Table 5.5 Life history, fecundity, and oviposition, *B. fallax* larval and adult rearing requirements.

Life history		Days	Range
Time from oviposition to 1st visible instar	24th June to 15th July	21	21 - 45
Time from visible instar to final instar	24th June to 16th June	326	28 – 326*
Pupation period	15th April to 16th June	62	13 - 36
Time from first instar to adult	16th July to 11th May	415	270 - 357*
Emergence	11th May to 30th June	41	
Flight period	11th May to 24th August	105	7 - 105
Time from emergence to mating	11th May until 27th June	47	11 - 30
Time from emergence to oviposition	11th May to 10th July	60	14 - 30
Oviposition	24th June to 24th August	61	5 - 61
Maximum recorded adult age	31st May to 24th August	86	7 - 86
Biology			
Fecundity	188 maximum eggs per female		5 -188
Oviposition stimuli	Water soaked *P. sylvestris* sawdust (0.5 L)		
Early instar mortality	24 %*[1]		
Late instar mortality	4 %*[1]		
Rearing/dietary requirements			
Larval	Minimum 40 ml *P. sylvestris* sawdust + 70 ml water per larva		
Adult	Pollen + nectar (particularly Rosacea) + dilute honey solution and water		

* Estimated (due to unknown individual 1st instars), and based on univoltine life cycle (see Chapter 2)
*[1] Based on mortality in 2009 larval growth conditions

Table 5.6 Standardized linear selection gradients (ß) for male and female mating success, and female fecundity, for a small number of caged *Blera fallax*. All coefficients and standard errors come from Gaussian linear models featuring standardized thorax width, wing length and pupal mass. P-values were obtained from generalized linear models with Poisson error distribution.

	Male mating success				Female mating success				Female fecundity			
Trait	ß	SE	z	P	ß	SE	z	P	ß	SE	t	P
Thorax length	0.593	0.259	1.699	0.089	-0.144	0.697	-0.282	0.778	0.222	0.283	0.786	0.462
Wing length	-0.165	0.253	-0.512	0.609	0.195	0.658	0.389	0.697	-1.022	0.269	-3.794	0.009
Pupal mass	0.192	0.192	1.056	0.291	0.237	0.251	1.158	0.247	-0.261	0.259	-1.007	0.353

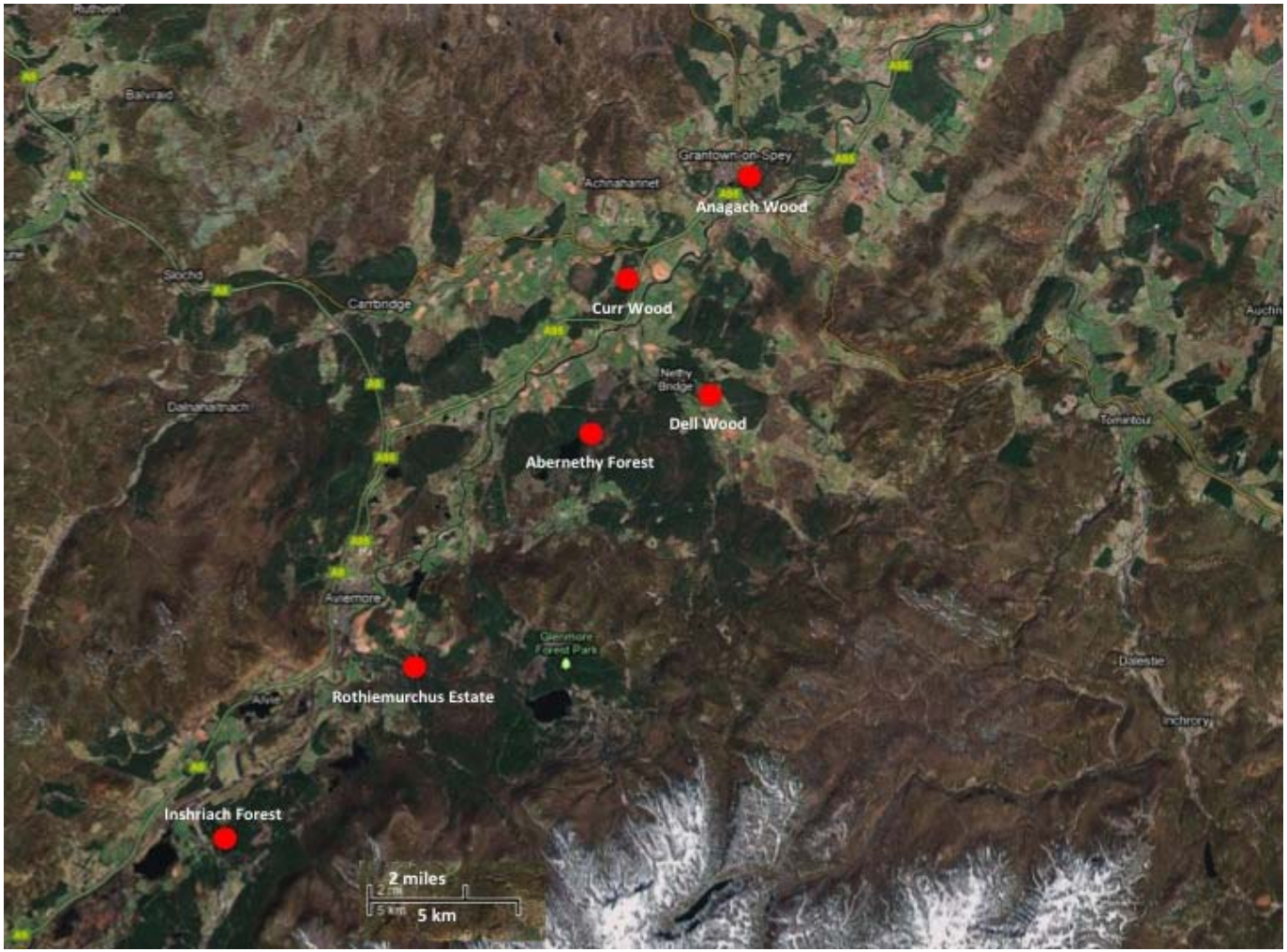


Figure 5.1 Map illustrating extant *B. fallax* sites Curr Wood and Anagach Wood, and relocations sites Rothiemurchus Estate, Abernethy Forest (and Dell Wood), and Inshriach Forest

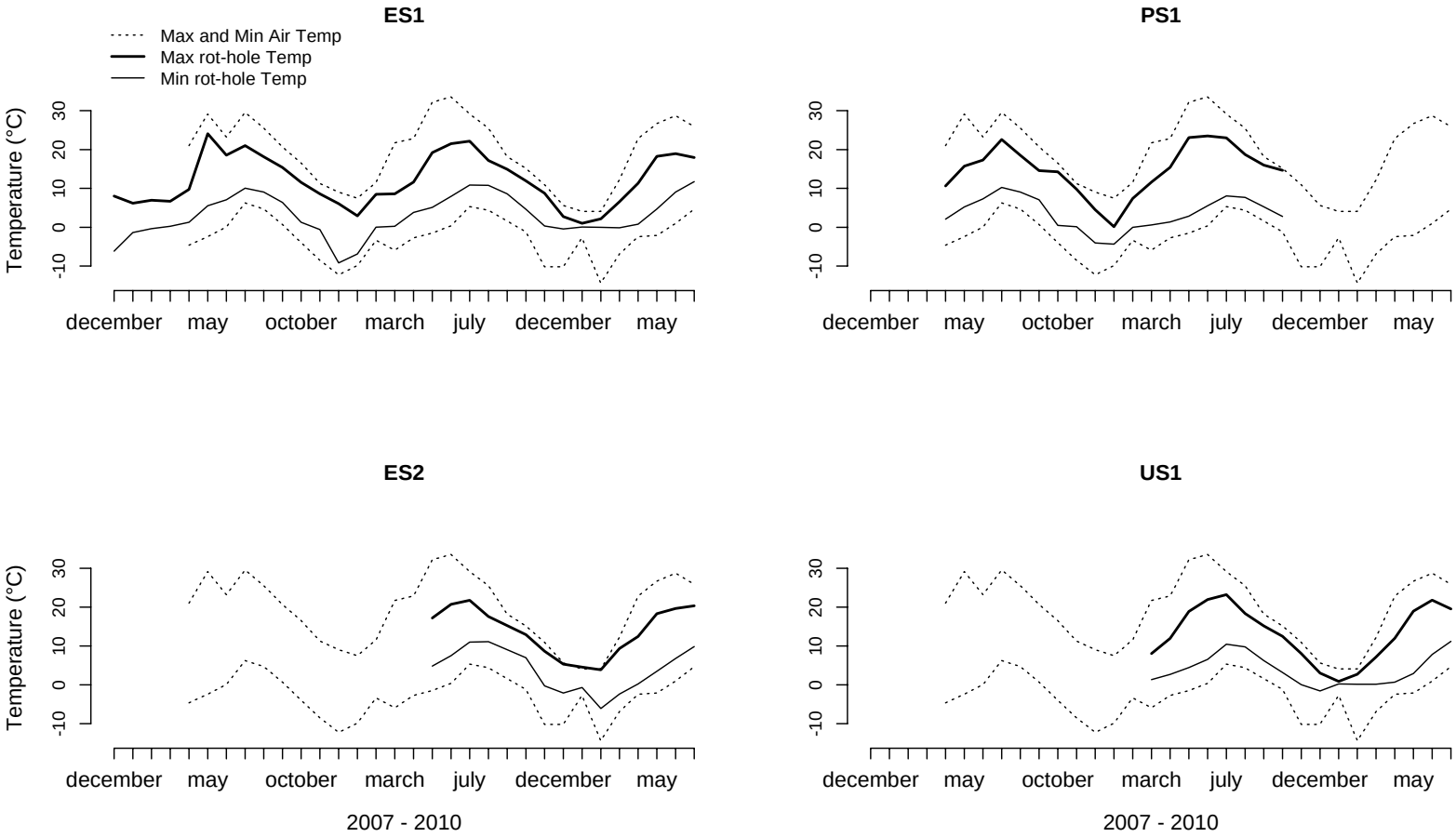


Figure 5.2 Average monthly maximum and minimum air temperature over time at site 1 (Curr Wood) and separate graphs for maximum and minimum temperatures in each rot hole type; exposed site 1, ES1; plastic pot site 1, PS1; exposed site 2 (Rothiemurchus Estate), ES2; and Unexposed site 1, US1.

Figure 5.3 Outdoor cage for captive breeding and observing behaviour of *B. fallax* constructed using white cotton netting (for the roof and door) and polyethylene mesh (for the walls) over a polypropylene frame (roughly 195cm height, 375cm length, 90cm width) (left) and indoor cages (45 x 45 x 60 cm) constructed using white cotton netting, malleable wire and ten linked strip lights (90cm, 21 Watt fluorescent bulbs) (right).

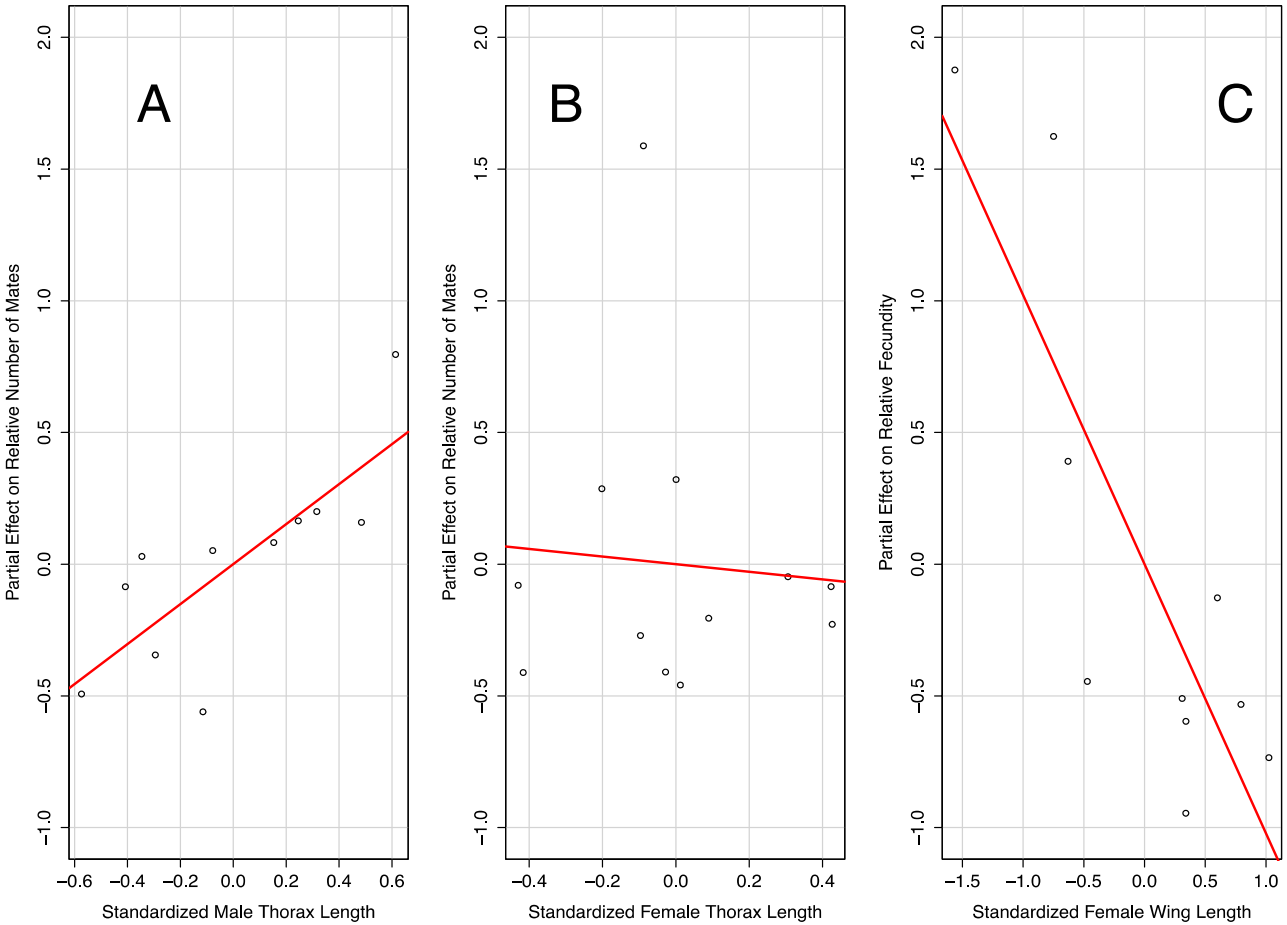


Figure 5.4 Added variable plots illustrating the partial effects of A) male thorax length on relative male mating success; B) female thorax length on relative mate number; and C) female wing length on relative fecundity.

Appendix 5.1

Restoring the endangered Pine hoverfly, UK

From the: Global Re-introduction Perspectives: 2010
IUCN/SSC Re-introduction Specialist Group (RSG). Edited by Pritpal S. Soorae
Published as:

Rotheray E.L. (2010) 'Restoring the endangered pine hoverfly in the UK' In: Global Re-introduction Perspective: 2010. IUCN/SSC Re-introduction Specialist Group & Environmental Agency (ed. Soorae, P.S.), 21-24

Introduction

The endangered status of the Pine hoverfly (*Blera fallax*) (Diptera, Syrphidae) was confirmed in 1999 after a 12-year investigation (Rotheray & MacGowan, 2000). The study concluded that the species had probably existed in the British Isles for several millennia, but that in the last hundred years it had declined in distribution from eight to just two known sites, both confined to the central highlands of Scotland. In 1999, the pine hoverfly was listed in the UK Red Data Book as category 1 (endangered), it is also a UK Biodiversity Action Plan priority species, and is one of 32 species listed in the Species Action Framework (2007), a Scottish Natural Heritage (SNH) initiative which focuses on improving the status of species deemed significant to overall Scottish biodiversity. Very little is known about the ecology of the pine hoverfly. In particular the elusive adults are very difficult to find; during the 12-year study no adults were observed (Rotheray & MacGowan, 2000). However breeding sites were identified where larval stages could be found and intervention is essential if we wish to safeguard UK populations of this species. In 2008 the first attempts were made to re-locate the pine hoverfly to its historic sites in Scotland.

Goals

Goal 1: Identify at least two potential re-location sites within the species' historic range.
Goal 2: Increase breeding resources at re-location sites.
Goal 3: Establish populations of pine hoverflies at two re-location sites.
Goal 4: Carry out annual monitoring to record progress and prepare additional sites to link populations.

Success Indicators

Indicator 1: Self‐sustaining populations established at re-location sites.
Indicator 2: Distribution of the pine hoverfly extended in Scotland.

Project Summary

Feasibility: In Scotland the Pine hoverfly's preferred habitat is Scots pine (*Pinus sylvestris*). It is a specialist saprophage: it develops in rotting pine stumps. Heart rot fungus (*Phaeolus schweinitzi*) attacks the centre of the tree causing it to weaken, fall and snap at the base revealing a hole that fills with rain water, and it is in this cavity that the larvae filter feed. Currently in Scotland this micro-habitat is rarely found in native pine woodlands due to a lack of veteran and senescent trees. The remaining populations survive in non-native plantations where rot holes are formed in pine stumps left vulnerable to decay after felling. It is possible to create breeding sites by boring holes in stumps, filling them with pine chips or sawdust and allowing the rain to fill the cavity. Habitat creation in this way began in the 90's and proved successful for a closely related species, *Callicera rufa* (MacGowan, 1994). In 2003, the same methods were used at pine hoverfly sites and by the following year it was confirmed to have been similarly effective (Rotheray, 2006). Due to these simple, swift and inexpensive methods of management, re-locating this species to historic sites in Scotland is a practical option, which appeals to site owners and managers alike. The pinewood sites proposed for re-location are historic sites for the pine hoverfly with a characteristic ground flora and associated shrubs. These plants, particularly rowan (*Sorbus aucuparia*) provide food for adults in the form of pollen and nectar. At these sites, the pine wood habitat has improved since the last records of the pine hoverfly due to the positive management actions under the influence of the SSSI (Sites of Special Scientific Interest) and SAC (Special Areas for Conservation) designations, which cover the sites. Both sites have included provision of artificially created rot holes as part of their agreed long term forest planning.

Implementation

The number of individuals to be released at re‐location sites is under investigation and the implementation process is being developed and agreed between the BAP coordination group and the Species Action Framework management group. Rather

than directly transferring individuals from one site to another, in June 2009 an attempt to captive breed the pine hoverfly was made. This species had never previously been bred in captivity and this type of re-location of a saproxylic insect has never been attempted anywhere in the world. In November 2008, fifty larvae were removed from the wild and reared in captivity in jars filled with water and pine wood chips. In June 2009, thirty-eight of them emerged as adults and were split between one large on-site cage (designed to observe adult behaviour in a more natural setting) and four small indoor cages. Over a period of 2 months the captive adults were successfully fed on pine woodland associated flora, mated in on-site and indoor cages, and several females oviposited a total of about 460 eggs, of which roughly 300 larvae have survived to date. Although both cage methods were successful, the smaller indoor cages are considered more advantageous due to the greater amount of control, protection and ease of assembly. In October 2009, 85 of the captive bred larvae that had reached the final stage in development were transferred to 28 bored stumps at one of the new sites where three groups of 30 bored stumps had been created within a kilometre of each other. In June 2010, 95 adults were released at the same site and the remainder entered into a second generation of captive breeding. To avoid inbreeding and 2nd generation habituation (adaptation to captive conditions) individuals from the original site will be included in captive breeding efforts during 2010. Although recent surveys show that the removal of 50 larvae from the original population has not had a measurable negative affect on the population, it is proposed that of the captive bred stock 50 adults will be released at the original site in 2011 to supplement the population.

Post-release monitoring

The relocation site is being monitored monthly and each larva that is located is photographed to follow development. Sixty percent of the released larvae were found in the cut stump holes four weeks after release. Eight weeks after release, a total of 15% were located in the holes. It is known that during winter, fully developed larvae of the pine hoverfly tend to move out of the water and into leaf litter on the ground or into deep cracks in the stumps where they are very hard to locate, while smaller larvae remain in the holes and complete their development in

spring. This may explain the low numbers of larvae remaining in holes. In August 2010, 43 new *B. fallax* larvae were found in 12 stump holes we created, four of which were 1 km away from the site they were released.

Major difficulties faced

Because of the lack of scientific research on the ecology of this species, in particular the adult requirements for feeding and breeding, much of the project involved trial and error.
Lack of large pine stumps for habitat creation (holes cut in small stumps tend to only temporarily hold water).

Major lessons learned

New understanding of insect husbandry, in particular the ability to rear adult flies in small indoor cages while utilising large outdoor cages to investigate pine hoverfly behaviour.

Success of project
Reason(s) for success/failure

Having started in November 2008, the re-location of the pine hoverfly is in its early stages. As yet we do not know if the population at the re-location site will establish itself, however having found a new generation of larvae there, this has been taken as an indicator of success at this preliminary stage.

References

Rotheray, G.E. and MacGowan, I. 2000. Status and breeding sites of three presumed endangered Scottish saproxylic syrphids (Diptera, Syrphidae). *Journal of Insect Conservation*, 4, 215-223

Rotheray, G.E. 2006. Restoring the BAP Hoverflies: *Blera fallax* and *Hammerschmidtia ferruginea* (Diptera, Syrphidae). Unpublished report of research,

survey and recovery attempts 2002-2005 for Scottish Natural Heritage and Royal Society for the Protection of Birds.

MacGowan, I. 1994. Creating breeding sites for *Callicera rufa* Schummel (Diptera, Syrphidae) and a further host tree. *Dipterists Digest*, 1, 6-8.

Appendix 5.2

Actions and performance targets for the Species Action Framework (SAF) plan to manage the Pine hoverfly

Actions and performance targets for the SAF plan to manage the Pine hoverfly

Action 1. Ensure the maintenance of viable populations.
Performance measure: minimum number of breeding locations maintained at 2007 levels, to end of project.

Action 2. Increase the amount of breeding habitat
Performance measure: double the number of active breeding locations by 2009.

Action 3. Increase the range from 2 to 5 localities by 2012.
Performance measure: three new localities stocked with larvae by 2011.

Action 4. Develop techniques for using artificial breeding sites.
Performance measure: demonstrate increased productivity of individual breeding sites to 2011.

Action 5. Monitoring effectiveness of actions and reporting outcomes.
Performance measure: populations monitored to end of project.

Action 6. Investigate autecology particularly larval survival and adult dispersal.
Performance measure: detailed understanding fed into more effective management by 2010.

Action 7. Develop partnerships with site owners and others.
Performance measure: three new localities stocked with larvae by 2011.

Action 8. Prepare guidance material on habitat management.
Performance measure: guidance material produced by March 2010.

Action 9. Manage the project effectively.
Performance measure: project managed effectively with reports, meeting and reviews, to end of project

Appendix 5.3

Forest Operations and Guidance for conserving the Pine Hoverfly *Blera fallax*

Forest Operations and Guidance for conserving the Pine Hoverfly *Blera fallax*

Summary:

The Pine hoverfly *Blera fallax* is a saproxylic insect dependent on Pine *Pinus Sylvestris*. It is endangered in the British Isles, and active conservation is managed under the Scottish Natural Heritage Species Action Framework. The larval stage of this insect develops in water-filled rot holes which occur naturally in broken or wind blown trees and also in mature pine tree stumps. However due to the removal of of stumps and felling policies in the past, *B. fallax* breeding habitat has become extremely rare.

The Pine hoverfly *Blera fallax*

In the British Isles, the few remaining population of the Pine hoverfly occur Strathspey, Scotland. Captive breeding and re-location projects are underway to expand the species distribution to previously occupied sites: Rothiemurchus Estate, Abernethy Forest, Glenmore Forest and Ishriach Forest.

Aim: Incorporate Pine hoverfly habitat creation into standard forestry management and its promotion in forestry best practice guidelines.

Habitat creation: Refrain from using chemical treatment on stumps. Create rot holes by boring holes into pine stumps using either a chainsaw or drill. Retention of water is vital and this is optimised by boring into the heartwood centre only (normally no more than ~10 cm diameter wide in the centre of a stump).

	Stump and hole specifications (cm)
Stump Height	30 to 60
Stump Width	> 25
Hole Width	10 x 10
Hole Depth	15

Drill-boring rot holes: Using a petrol powered drill and 25mm auger bit, make ~10cm diameter circular holes by boring repeatedly into the stump resulting in a 15 cm deep cavity occupying the heartwood centre. Fill the cavity with sawdust created in the boring process.

Chainsaw-boring rot holes: Make two parallel, 15cm deep cuts into the surface of the stump positioned either side of the heartwood centre roughly 15 to 20 cm apart. Make two further cuts, perpendicular to and connecting the initial cuts to complete a square on the surface. Make these at 45º angles into the centre of the stump to join at a ~15cm deep point boring out a triangle-shaped wedge. Fill the cavity with sawdust created in the boring process, and place the triangle wedge partially over the hole to protect the content from evaporating while allowing rainwater to fill the cavity.

Bored rot holes created using a chainsaw (left) and drill into the heartwood (right)

Distribution and supplementation: Habitat creation must be done annually (10 to 20 holes bored) to account for holes that don't retain water, and attempts must be made to link up and create continuous areas of habitat within 1km distances.

Identification and biology: Pine hoverfly larvae have long breathing tubes, which they telescopically extend to breathe while feeding deep in the rot hole. They can be found in rot hole between July and May however the best time to survey is September. Most often they develop within a year, and are on the wing between May and July.

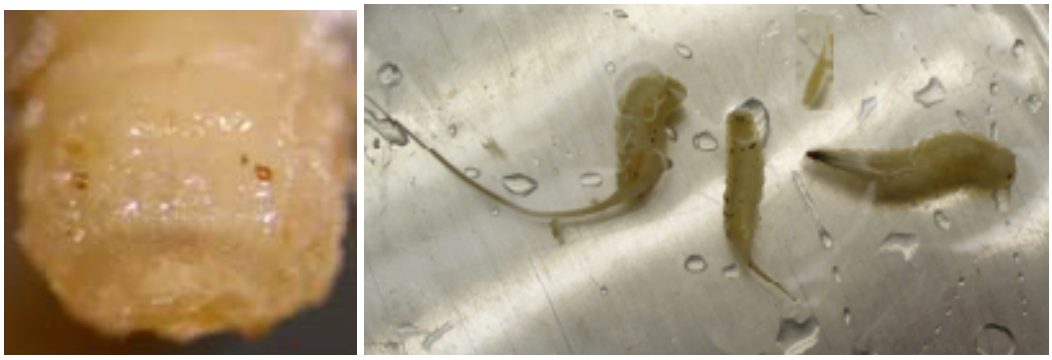
Table showing dates and time of four different stages of life history

Life history	Date	Number of days (range)
Larval development in rot holes	24th June to 16th June	28 to 326+
Pupation period	15th April to 16th June	13 to 36
Adult emergence period	11th May to 30th June	50
Adult flight period	11th May to 24th August*	7 to 105

* based on adults in captivity

Pine hoverfly larva (left) and developing puparia in moss (right)

Larvae: Larval body length (not including breathing tube) varies from 2 mm (1st instar) to 16 mm long (final instar). They can be identified based on two main characters. 1. A completely white, extendable breathing tube with brown tip. 2. An arch of moustache-like anterior spicules.



Pine hoverfly larva showing arching moustache-like spicules (left) and four most common species found in a pine rot holes: from left *Myathropa florea*, the Pine hoverfly *Blera fallax*, *Speghina clunipes* and *Callicera rufa* (right).

Adults: From the head to wing tip adults measure 1.2 cm. Adults mainly feed on Rowan *Sorbus aucuparia* but will feed on a variety of pine wood associated flora such as Greater Stitchwort *Stellaria holostea*, Umbellifers (Apiaceae), Bedstraw (Rubiaceae), Dog-Rose *Rosa canina* and Buttercup (Ranunculaceae).

References and sources of information

Rotheray E.L. (2010) 'Restoring the endangered pine hoverfly in the UK' In: Global Re-introduction Perspective: 2010. IUCN/SSC Re-introduction Specialist Group & Environmental Agency (ed. Soorae, P.S.), 21-24.

Malloch Society Website

www.mallochsociety.org.uk/blera-2006-status

Chapter 6

Mark recapture estimates of dispersal ability and observations on the territorial behaviour of the rare hoverfly, *Hammerschmidtia ferruginea* (Diptera, Syrphidae)

6.1 Abstract

In order to effectively manage habitat for fragmented populations, we need to know the capacity of species to colonise unoccupied habitat patches. Dispersal is vital in maintaining viable populations in increasingly fragmented environments by allowing re-colonisation of areas in which populations have gone extinct. The endangered aspen hoverfly *Hammerschmidtia ferruginea* (Fallén, 1817) (Diptera, Syrphidae) depends on a limited and transient breeding habitat: decaying aspen wood *Populus tremula* L. (Saliaceae). Conservation management for *H. ferruginea* involves encouraging aspen expansion across Scotland, and ensuring retention, maintenance and continuity of dead wood where *H. ferruginea* has been recorded and in areas that may link populations. In order to do this effectively we need to know how far *H. ferruginea* can disperse. By taking advantage of the tendency of adults to group on decaying aspen logs, we estimated dispersal ability through mark and recapture techniques. In the first year, 1,066 flies were marked as they emerged from aspen logs and 78 were re-sighted at artificially-placed decaying aspen logs, one of which was 4 km from the release site along a transect including "stepping stones" of breeding habitat separated by 1 km. In the second year, of 1,157 individually marked flies, 112 were re-sighted and one was observed 5 km from the release site with no intermediately spaced stepping-stones of breeding habitat. Aspen logs are probably important mate-seeking sites, which should be left undisturbed during the flight period between early May and late July. Territorial behaviour was recorded at all (19) decaying aspen log locations. In total, 72 males were recorded defending territories, which overlapped with 68% of female oviposition sites. Male dispersers had longer wings, and males recorded in territorial

disputes on aspen logs had longer thoraces than the average for re-sighted and total emerged males. While these results show *H. ferruginea* is capable of locating decaying logs up to 5 km away, most dispersing individuals (68%) were recorded at 1 km, which should be taken into account in developing management protocols. If enough dead wood is available it should be distributed within a radius of 1 to 2 km, and where possible, as stepping-stones linking up aspen woodlands.

6.2 Introduction

In the UK, the aspen hoverfly *Hammerschmidtia ferruginea* is listed in the UK Red Data Book as a category 1 (endangered) species, and it is included in the UK Biodiversity Action Plan (UKBAP). It is listed as an indicator of internationally important forests, and is rare throughout its Holarctic distribution (Speight 1989, 2008). *Hammerschmidtia ferruginea* is considered a flagship species for a group of 13 other rare and similarly endangered Diptera in Scotland that all depend on aspen, *Populus tremula* L. (Salicaceae) (Rotheray 2001).

Since 1999 the number of UK sites occupied by *H. ferruginea* has decreased from 15 to 8 (Rotheray *et al*. 2009). The main reason for this decline is probably that it is a specialist saprophage depending on a rare and temporary resource: decaying cambial layers under bark of dead aspen wood. From the time a tree falls or a branch breaks off, it can take up to two years for the cambial layers to become suitable for larval development and, depending on its size and location, a piece of wood with cambial decay can last from just one to three years before drying out (Rotheray *et al*. 2009). During periods when habitat is in short supply, *H. ferruginea* can also develop in small pockets of decaying sap that exude from damage on living trees (Rotheray 1991). In the UK there are few aspen woodlands large enough (>100 trees) to maintain a constant input of dead wood, and in Scotland where some large groups of aspen stands exist, unpredictable winds and storms are the chief cause of fluctuations in the amount of dead wood present.

As part of the UKBAP process, strategies must be designed for the restoration, protection and monitoring of *H. ferruginea*. Conservation management involves encouraging the expansion of aspen and insuring continuity of deadwood (Rotheray *et al*. 2009). This includes detecting the quantity and state of decay of dead aspen wood at all *H. ferruginea* sites and supplementing breeding habitat as necessary by severing branches or whole trees (Rotheray *et al*. 2009). In order to plan this effectively we need to know how far individuals can disperse. In 2006, a mark and recapture experiment estimated the dispersal ability of *H. ferruginea* at no less than 1 km, but the hoverfly was considered to be capable of moving further than this based on known distances of up to 4 km between populated sites (Rotheray *et al*. 2009). Therefore, we carried out a two-year project to specifically investigate the dispersal ability of *H. ferruginea*. In addition to this, breeding sites were evaluated for their utilisation as mate-seeking sites, a gap in the known ecology of this endangered hoverfly.

6.3 Methods

Field site

The study involved surveying *H. ferruginea* localities in Strathspey as described by Rotheray *et al*. (2009). Dispersal experiments took place at Insh Marshes National Nature Reserve (NNR), Inverness-shire, Scotland (57°05’ N, 3°58’ W), which is owned and managed by the Royal Society for the Protection of Birds (RSPB). The reserve is primarily a wetland floodplain with wet woodland fringed by birch *Betula pubescens* and aspen at higher elevations.

6.3.1 Distribution and habitat quality

All aspen woodland sites were located using aerial photographs of the area, which identify aspen due to its tendency to flush later in the year than more common species such as *Betula* spp. (Kouki 2008). These were surveyed for decaying aspen wood and early larval stages of *H. ferruginea* in order to avoid any bias within the experimental areas, and build a basic distribution map of Strathspey populations. Survey methods for larvae and puparia were those described previously by Rotheray *et al*. (2009).

Dispersal experiments

Two experiments were carried out between May and August 2009 and 2010. The first sought to investigate dispersal ability using decaying aspen logs set out from a central point at 1 km intervals to a maximum of 4 km. The second experiment was designed to estimate maximum dispersal ability from 1 to 7 km, and to assess morphological differences between dispersers (those observed at 1 km +) and non-dispersers (those only observed at the release location), and males defending territories on the logs. Data on emergence, size differences and sex ratio over time were also assessed in both years.

6.3.2 Experiment 1: stepping-stone dispersal ability

In 2008, nine aspen trees were felled on private land local to Insh Marshes, and donated to the reserve to provide habitat for *H. ferruginea*. These were cut into twenty logs of similar length (mean 113 ± 26.7 SD cm) and width (32 ± 7.4 cm). In 2009, two 3 km and one 4 km long transects were set up using decaying aspen logs extending out from a central location at ~120° angles from each other, pointing south, northwest and northeast (Fig 6.1). Two logs were positioned at the central meeting point of the transects, and at 1, 2 and 3 km "stepping stone" points along each transect. One additional log was positioned at 4 km on the NE transect. We conducted extensive searches to confirm no other decaying aspen branches or logs were within 1 km of the experimental area.

From the 14th May until 24th June, emergence traps were constructed over six decaying fallen or severed aspen on and around Insh marshes. The traps were simple constructions using pesticide-free mosquito netting and malleable fence wire. Each trap was checked for emergence every morning and afternoon, between 7am and 8pm. All *H. ferruginea* caught in traps were removed individually using a 3 x 10 cm collection tube, and marked on the thorax using a dry grass stem and non-toxic enamel paint using a different colour or combination of colours for each day of emergence. Marking was carried out in the tube, or within a marking cage made from a plastic open-ended 3 x 10 cm tube with a cork plunger and flexible netting over one end where the insect could be gently immobilised (Bonduriansky & Brooks 1997). Occasionally, marking required moving adults into cool bags for several minutes to reduce activity. Each adult was photographed on laminated lined

paper for scale, and the length of the thorax, from where the neck meets the pronotum to the apex of the scutellum, and the length between two wing veins (landmarks 1 and 3 in Milankov *et al*. 2010) were measured using ImageJ software (Abràmoff *et al*. 2004). Adults were released the same day at the central meeting point of the transects.

From 16th May until 5th July, 60-minute observation sessions were spent at each group of decaying logs in succession throughout the day, with the starting location rotated each day (from 9/10am to 7/8pm depending on weather). We noted the local wind direction, and recorded the number of marked and unmarked *H. ferruginea* individuals throughout each session. Digital images of marked individuals were taken when possible, and were used to identify individuals by comparing mark shape and location on the thorax; otherwise sex and colour combination was recorded.

6.3.3 Experiment 2: estimated maximum dispersal ability

In 2010, similar techniques were used as in experiment 1, however this time two decaying logs were placed at eight points from 0 to 7 km from one central release location with no intermediately placed stepping stones (Fig 6.2). Logs were sourced from a local golf course where three aspen trees had fallen naturally and snapped at the base. These were cut into similar lengths (128 ± 26.7 cm) and were of similar width (28 ± 6.8 cm).

From 17th May until 22nd June, we constructed emergence traps over seven decaying logs or trees in and around RSPB Insh Marshes, and one west of Newtonmore (57°04' N, 4°07' W). We used the same methods as described above for experiment 1 to collect and mark *H. ferruginea* caught in the traps, however each individual was this time given a unique mark using a combination of colours and locations on the thorax.

From 4th June until 14th July, each log was observed as described in experiment 1, except that adult activity, location on the aspen log, territorial behaviour and oviposition were also recorded. Behaving territorially was defined as males chasing passing insects and repeatedly returning to a similar location on the aspen log. 'Contests' were defined as events involving two males in physical contact. A male was considered to have 'won' a contest if it returned to the original location and continued territorial behaviour, while a 'losing' male would either leave the site or take up a territory elsewhere. Oviposition was defined as when the female ovipositor could be observed probing cracks in the bark. Territory and female oviposition locations were recorded on basic illustrative representations of the logs. Territories were described as overlapping oviposition locations if they were within 20 cm on the log.

Males and females appear to darken in colour as they age (Rotheray *et al*. 2009). To test the accuracy of thorax colour or shade as a prediction of age, we compared each marked individual to a strip of paper with seven progressively darker shades labelled 1 to 7, and assigned each individual to the most similar colour category.

This category was later compared with known adult ages based on dates of emergence.

Statistical analysis

Chi-squared tests were used to assess deviations from the unity sex ratio over the emergence period. We used general linear models to assess differences in adult thorax and wing length between sexes, associations between thorax and wing length and day of emergence, and associations between the direction of dispersal and local wind direction. We used generalised linear models (GLMs) with Binomial error distribution to model the influence of wing and thorax length on dispersal (a binomial response, with individuals recaptured at 1 or more km away from their capture site as dispersers) and the outcome of territorial contests between males. Finally, we used a linear model to predict thorax shade (with category number treated as a continuous variable) as a function of adult age. All statistical analyses were carried out using the statistical package R (version 2.13.1) (R Team 2011).

6.4 Results

6.4.1 Distribution and habitat quality

In June 2011, decaying aspen wood and newly fallen trees and branches were found at all *H. ferruginea* sites in Strathspey (see Rotheray *et al*. 2009). Empty puparia or signs of *H. ferruginea* were found in one fallen aspen tree at Creagan Bruegach

(57°05’ N, 3°58’ W), two at Kinveachy (57°05’ N, 3°58’ W) and one at Newtonmore (57°05’ N, 3°58’ W). Each site, including *H. ferruginea* localities pre and post 2006 (Rotheray *et al*. 2009), was mapped to indicate potentially overlapping areas, and areas where breeding habitat supplementation should be focussed (Fig 6.3).

6.4.2 Experiment 1: stepping-stone dispersal ability

Emergence

Between 15th May and 14th June 2009, 1,066 individuals were caught in six emergence traps, most of which came from one severed tree (664). Overall the sex ratio was female biased (M/F = 465/573, $\chi^2 = 11.24$, df = 1, $P < 0.001$). The sex ratio was significantly male biased in the first fifteen days (15th until 29th May) of the emergence period ($\chi^2 = 6.55$, df = 1, $P < 0.05$) and significantly female biased over the final fifteen days ($\chi^2 = 28.32$, df = 1, $P < 0.001$). The average daily emergence was 34 flies (± 30.37 SD) with a peak of 107 on May 30th.

Dispersal

In total, 105 hours were spent observing aspen logs (~10 hours at each) during which 78 (7.3%) marked *H. ferruginea* were re-sighted. Most individuals were re-sighted at the release location (62 %). Of those that dispersed (28), 39% were observed at 1 km, 43% at 3 km, and one individual was re-sighted at 4km (Table 6.1). More individuals were observed at logs extending northeast from the release

point (18) than the southwest (3) and south (7) ($\chi^2 = 12.9$, df = 2, $P < 0.005$), and this didn't appear to relate to wind direction ($P > 0.05$). Male and female maximum longevity (estimated as the latest recapture date) was 41 (11 ± 14.67) and 28 (11 ± 8.64) (mean ± SD) days respectively, and there was no difference between the number of males (20) and females (23) dispersing ($\chi^2 = 0.21$, df = 1, $P = 0.65$).

Adult size

Males had significantly longer wings (MS = 0.69, $F_{1,995} = 196.64$, $P < 0.001$) and thoraces (MS = 0.303, $F_{1,995} = 316.36$, $P < 0.001$) than females (Table 6.2). Of 78 re-sighted individuals, only three females and one male could be individually identified from photographs, therefore we were unable to conduct analyses of morphological correlates of dispersal in this experiment.

6.4.3 Experiment 2: estimated maximum dispersal ability

Emergence

On the 17th May until 20th June 2010, eight emergence traps constructed over decaying aspen logs caught 1157 *H. ferruginea* adults, 94% of which emerged from one fallen tree located near Newtonmore. Overall the sex ratio was male biased (M/F = 592/519, $\chi^2 = 4.79$, DF = 1, $P < 0.05$). The sex ratio was significantly male biased in the first fifteen days of the emergence period (M/F = 431/254, $\chi^2 = 45.7$, DF = 1, $P < 0.001$) and significantly female biased in the final nineteen days (M/F =

161/265, $\chi^2 = 25.39$, DF = 1, $P < 0.001$). There was an average daily emergence of 37 (± 56.2 SD) with a peak of 247 individuals on 23rd May.

Dispersal

In total, 249 hours were spent observing aspen logs (~31 hours each) during which 115 hoverflies (10%) were re-sighted. The release site at 0 km had the greatest number of individuals recorded (87 marked and 76 unmarked) (Table 6.1, Fig. 6.2). Of those that dispersed (28), 68 % were observed at 1 km, and one male was observed at 5km (Table 6.1). Males were found to repeatedly visit logs, as part of their territorial behaviour, whereas females were recorded visiting infrequently (M/F = 252/131, $\chi^2 = 38.2$, df = 1, $P < 0.005$). There was no significant difference between the number of males and females dispersing (M = 58, F = 49, $\chi^2 = 0.76$, df = 1, $P > 0.05$). No significant linear relationship was found between local wind direction and the direction of dispersal ($P > 0.05$). Maximum male and female longevity was 45 (30.7 ± 7.2) and 45 (25.7 ± 8.2) days respectively.

Mate seeking

Between 4th June and 13th July, we recorded 72 males defending territories and 46 females ovipositing on the aspen logs.

Females observed ovipositing were between 10 and 42 days old (25.46 ± 8.05) (mean ± SD). Of visiting females observed, 44 % were observed ovipositing (Table 6.1). Of all male territorial locations on aspen logs, 68% were on or near (< 20cm

distance) female oviposition locations. Males were observed hovering over females briefly, landing on them and immediately carrying them away from the oviposition resource and out of sight. Females often oviposited within male territories during male territorial behaviour. The maximum number of individuals at a log during one hour was nine at 0 km (Table 6.1).

Adult size

Males had significantly longer wings (MS = 0.48, $F_{2,1208} = 187.27$, $P < 0.001$) and thorax lengths (MS = 0.16, $F_{2,1034} = 147.04$, $P < 0.001$) than females (Table 6.2). No linear relationship was found between size traits and time of emergence ($P > 0.05$).

On average, males and females from 2009 had significantly smaller thoraces (male: MS = 0.007, $F_{1,1089} = 6.2$, $P < 0.001$, female: MS = 0.02, $F_{1,1110} = 24.51$, $P < 0.001$) and wing lengths (male: MS = 0.27, $F_{1,1001} = 89.32$, $P < 0.001$, female: MS = 0.16, $F_{1,1027} = 53.63$, $P < 0.001$) than those in 2010 (see Table 6.2 for means).

The generalised linear model showed a strong effect of male wing and thorax length on dispersal (GLM, $P < 0.005$, Table 6.3). Wing length was greater in dispersing males (0.784 ± 0.052, mean ± SD (mm)) than re-sighted (at 0 km only) males (0.747 ± 0.053) and total emerged males (0.75 ± 0.05). Thorax length was smaller in dispersing males (0.365 ± 0.028) than total emerged males (0.379 ± 0.04) but not re-sighted males (0.370 ± 0.032). No effect was found of female wing or thorax length on dispersal (see Fig 6.4 and Table 6.2 for means).

In total, 21 contests were recorded involving 23 uniquely identified males. Twelve of these contests involving 14 males took place at 0 km. The generalised linear model showed a strong effect of male wing length on the outcome of territorial contests ($Z = 2.247$, $P < 0.05$) but no effect of thorax length ($Z = -0.844$, $P = 0.4$, Table 6.3). Males in territorial contests had longer wings (0.783 ± 0.04) than non-dispersing, re-sighted males (0.747 ± 0.053) and total emerged males (0.750 ± 0.05). No effect of thorax or wing length was found on 'winning' males in contests, which had on average greater thorax (0.379 ± 0.041) and wing length (0.789 ± 0.043) than contest 'losers' (thorax: 0.360 ± 0.023, wing: 0.776 ± 0.039) (See Fig 6.5 for means). No linear relationship was found between age and 'winning' or 'losing' males ($P > 0.05$).

Adult age positively affected thorax shade ($r^2 = 0.38$, $F_{1,99} = 62$, $P > 0.001$) (Fig 6.6).

6.5 Discussion

In Scotland, six of eight remaining *Hammerschmidtia ferruginea* sites are located in Strathspey (Rotheray *et al*. 2009). Each known site is separated by up to 5 km along a 40 km length of Strathspey, from the most southern at Creagan Bruegach to the most northern at Grantown-on-Spey (Rotheray *et al*. 2009). The findings in this study imply that *H. ferruginea* is capable of dispersing at least 5 km, suggesting the network of sites in Strathspey probably form a metapopulation, i.e. a group of unstable, local populations occupying discrete habitat patches linked by dispersal (Hanski 1998). Although large aspen stands (>5 Ha) are rare in Scotland, aspen is widely distributed, and recent surveys using aerial photographs have identified

several small pockets of aspen woodland outside previously mapped areas in Strathspey (Worrell 1993; MacGowan 1997; Kouki 2008). *Hammerschmidtia ferruginea* can probably detect and locate decaying aspen in these more isolated areas, using them as stepping-stones between larger woodlands, and as habitat availability fluctuates through space and time. This may explain how populations have survived in Strathspey while elsewhere they have declined.

A lack of deadwood can cause abrupt local extinctions, possibly isolating populations across the landscape. Current conservation management efforts aim to link up fragmented habitat for this hoverfly (*H. ferruginea* UKBAP Steering Group pers. comm.), and the findings from this study broaden the management options by enabling exploitation of more isolated aspen woodlands. However, it is important to note that fewer individuals will locate breeding habitat at greater distances. While as many individuals were observed at 3 km as there were at 1 km in 2009, in 2010 most dispersing individuals (68 %) were observed only at 1 km from the release location. Moreover, despite more than double the observation effort, fewer decaying logs to observe, and ~100 more marked individuals in 2010, the same number (28) were observed dispersing in both years. It is likely that stepping-stones augment *H. ferruginea* dispersal, but this result could also be due to several other factors. For example, differences in quality between the decaying logs within and between the two experiments, may alter detection and attraction, or the location and direction of transects may have an effect. Indeed in 2009, the number of re-sighted individuals observed on the transect extending northeast was greater than those found to the south and southwest, which did not appear to correspond with the prevailing wind direction. Dispersal propensity is probably strongly affected by the landscape.

Transects in 2009 extended south toward valleys where there are no aspen woodland. In 2010, more activity was observed at logs closer to and around Insh Marshes reserve, 4 and 6 km southwest from the release site. Breeding habitat has been continuous at the reserve for at least the last 20 years (Rotheray & MacGowan 2000; Rotheray *et al*. 2001, 2009) and probably attracts individuals from further north by way of volatiles evaporating from the decay.

For *H. ferruginea*, wings are essential for tracking changing resources. Dispersing males had significantly longer wings than those that were re-sighted at the release location, and compared to the average population wing length. Dispersal ability often positively correlates with indices of body size and wing length (Denno 1994; Hoffmann *et al*. 2007; Sekar 2011; Stevens *et al*. 2012). While no significant difference was found, thorax length was greater in males that won contests compared with losers, and males that dispersed. There may be a trade off with dispersal and thorax length, related to winning contests. Variation among individuals in insect wing development and associated muscle tissue are often regulated by trade-offs between flight capability and reproduction (Zera & Denno 1997). The ability to disperse can also have direct consequences on mating success. For example, the planthopper *Prokelisia dolus* (Hemiptera: Delphacidae) has a flightless morph which outcompetes with those that are capable of flight in mating success (Langellotto *et al*. 2000).

Wing length, as well as thorax length may be important to males in winning territorial contests. Winning males also had longer wings, and those involved in contests had significantly longer wings than the average for the population.

Territorial behaviour in flies commonly involves male defence of oviposition sites to monopolize incoming females (Maier & Waldbauer 1979; Preston-Mafham 2001). Gravid females are attracted to the breeding site, and presumably to the particular areas that offer the best conditions for larval development. The hypothesised resource-dependant polygyny in this system was supported by the number of territories that overlapped with oviposition sites on the logs, and by males repeatedly defending these areas. While we could not directly study paternity success in the current experiment, we expect that males who patrolled and won contests for high quality territories achieve better insemination success with gravid females visiting the site.

If sexual selection based on resource defence polygyny is strong in this species, it may have implications for the conservation genetics of this species. For example, if just a few males secure most of the copulations, this could dramatically reduce the effective population size. Current habitat management for *H. ferruginea* often involves cutting up aspen trees in an attempt to extend the time one fallen aspen provides breeding habitat (Rotheray *et al*. 2009), but this results in reducing the area available for males to set up territories, potentially increasing the intensity of sexual selection and the resulting skew in mating and paternity success. This may have knock-on effects on genetic variation in *H. ferruginea* populations, a question deserving more scrutiny in future work.

All individuals were significantly smaller in 2009 than those in 2010. The primary source tree in 2010 was fairly isolated at 8 km from known localities for *H. ferruginea*, and the tree had fallen and snapped at the base, whereas the main source

for individuals in 2009 was a severed tree in the middle of a known locality for this species. Both sources were large whole trees that had been decaying and therefore available as breeding habitat for three years, and were not suitable as breeding habitat in subsequent years after the experiments as the rotting layer had dried out. The difference in fly size between years may be an indicator of resource condition due to severing which appears to dry the rot faster. Testing this hypothesis would require experiments that assess the effects of severing trees on the rot layer.

Like many insects that are relatively large, agile flyers and are dependent on a local and restricted resource, *H. ferruginea* appears to be capable of substantial dispersal. However, deadwood insects are often associated with low dispersal rates due to the fluctuations in breeding habitat availability. Relatively small species and intermediate dispersers are thought to be more vulnerable to fragmentation, thus better subjects for detecting the effects of habitat fragmentation (Bailey 2007; Watanabe *et al*. 2010). Habitat isolated by a few hundred metres has an effect on specialist insect beetles associated with aspen (Ranius *et al*. 2011). Diptera that depend on the same transient habitat as *H. ferruginea* may exhibit similar requirements, however further investigation into the dispersal abilities of these species is necessary to confirm *H. ferruginea* as a suitable umbrella species.

For a detailed study of individual populations, it is advantageous to be able to estimate the age of individuals. Our work has confirmed that thorax shade provides a reasonable estimate of adult age in *H. ferruginea*, with our categorical assignations of thoracic shade capturing 38% of the variation in adult age. This technique could therefore be used to help monitor local populations, by indicating

when, where and how adults are active, mating and ovipositing, and in which part of their flight period they may be. This is important to ensure that the resources required are protected when these activities take place. In our study, this critical period was between early May to late July. Such data are important for managing populations because at these times in particular, fallen timber should not be disturbed.

Conservation management

Current *H. ferruginea* population declines have probably resulted from a lack of periodic storms that boost deadwood input (Rotheray *et al*. 2009). Due to the small size of aspen woodlands, enough dead wood is frequently not available for saproxylic insects that specialize on aspen. Functional connectivity among populations will depend on the natural tendency of flies to disperse which may in turn depend on population density. While *H. ferruginea* is rare, due to its dependence on a characteristically varying level of breeding resource, it may have evolved to cope with fluctuating levels of suitable fallen wood. When breeding habitat is scarce, *H. ferruginea* have been found in sap runs, which occur on live, damaged aspen trees (Rotheray & MacGowan 2000). However, patch abundance and distribution may alter such that population dynamics break down, and continuity of breeding habitat within colonisation distance and through time is crucial (Grove, 2002). Studies of how breeding habitat quality, landscape elements and composition affect *H. ferruginea* dispersal may clarify factors limiting colonisation. Understanding movement dynamics at the landscape level could be determined through non-invasive molecular techniques (Jonsson *et al*. 2003;

Vinatier *et al*. 2011; Appendix 4.2). Current conservation management should focus on maintaining breeding habitat continuity by supplementing gaps in dead wood in addition to creating new habitat within colonisation distance of about 5 km. It is also important to preserve existing aspen connected networks where natural dynamics can be relied on to produce new suitable breeding habitat.

Table 6.1 Total number of marked *H. ferruginea* individuals re-sighted at each distance from the release site in 2009 and 2010, and for each distance: total marked and unmarked individuals, maximum number observed over a 1 hour session, % of total males and females observed, % of visiting females observed ovipositing per site.

	Number of re-sighted individuals				2010 total observed *H. ferruginea* at each location				
Km	2009	2010	Male	Female	Total	Maximum no. per hour	% males of total	% females of total	% visiting females ovipositing per site
0	45	87	51	36	163	9	40	44	39
1	11	19	10	9	60	6	14	17	64
2	4	4	1	5	16	5	1	11	29
3	12	2	0	2	18	3	6	2	100
4	1	2	1	1	46	6	13	5	43
5	-	1	1	0	21	3	6	5	50
6	-	0	0	0	58	7	14	16	43
7	-	0	0	0	18	4	5	2	50

Table 6.2 Male and female thorax and wing measurements in 2009 and 2010, and separately for the subsets of dispersing individuals (1 km +) in 2010 (Dispersers), and males that 'won' and 'lost' in territorial contests.

	Total	Thorax length (cm) (mean ± SD)		Wing length (cm) (mean ± SD)	
Total in Year		M	F	M	F
2009	1066	0.373 ± 0.034	0.337 ± 0.029	0.72 ± 0.06	0.67 ± 0.059
2010	1157	0.379 ± 0.04	0.35 ± 0.028	0.75 ± 0.05	0.69 ± 0.05
Dispersal 2010					
Dispersers	28	0.365 ± 0.028	0.337 ± 0.034	0.784 ± 0.052	0.693 ± 0.066
Re-sighted	50	0.370 ± 0.032	0.344 ± 0.025	0.747 ± 0.053	0.691 ± 0.048
Contests 2010					
Winners	24	0.379 ± 0.041	-	0.789 ± 0.043	-
Losers	19	0.360 ± 0.023		0.776 ± 0.039	

Table 6.3 Parameter estimates for generalised linear models with Binomial error distribution modelling the influence of wing and thorax length on dispersal in 2010 in separate models for dispersing males (1 km +) and total emerged males in 2010; dispersing (1 km +) and re-sighted males (at 0 km only) in 2010; and males that were re-sighted (at 0 km only) and in territorial contests.

	Parameter estimate ± SE	z-value	p-value
Disperse v emerge 2010			
Intercept	-8.62 ± 4.58	-1.882	0.059
Thorax length	-17.05 ± 8.29	-2.057	0.039*
Wing length	14.86 ± 5.528	2.688	0.007*
Disperse v re-sight			
Intercept	-9.07 ± 5.685	-1.596	0.111
Thorax length	-9.06 ± 10.759	-0.842	0.399
Wing length	14.45 ± 6.426	2.248	0.025*
Contest v non-disperse			
Intercept	- 9.06 ± 5.686	-1.592	0.111
Thorax length	-9.09 ± 10.766	-0.844	0.398
Wing length	14.44 ± 6.427	2.247	0.025*

* <0.05 significance

Figure 6.1 Map of experiment 1 illustrating the location of each group of decaying logs (red circles) at 1 km points from the central release location (red circle with central 'R') (maps: http://maps.google.co.uk/maps).

Figure 6.2 Map of experiment 2 illustrating the location of each group of decaying logs (red circles) from 1 to 7 km from the central release location (red circle with central 'R') (maps: http://maps.google.co.uk/maps).

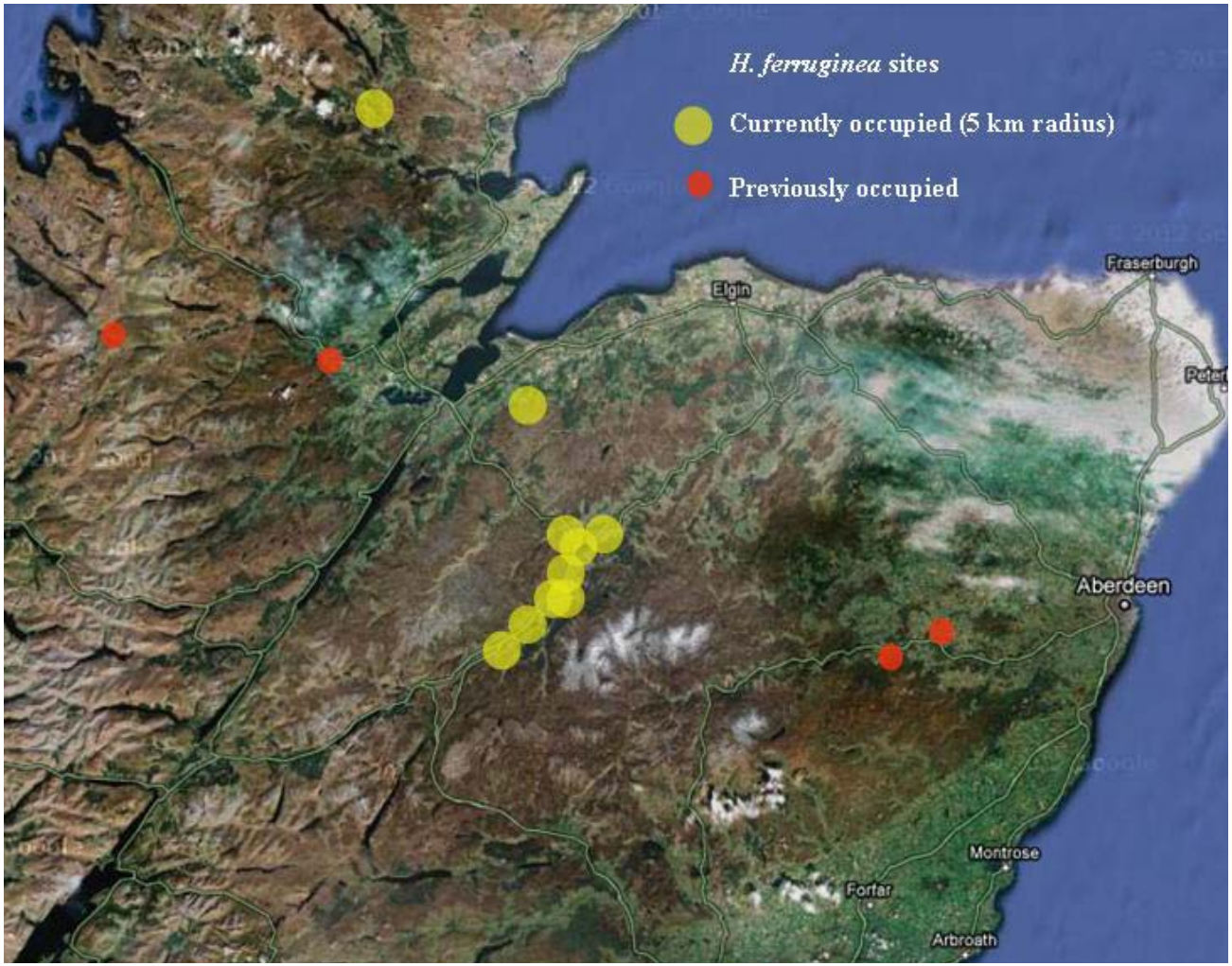


Figure 6.3 Map of North Scotland indicating *H. ferruginea* localities with 5km dispersal rings where it was last recorded 2006 - 2011 (yellow circles) and previously occupied pre-2006 *H. ferruginea* sites (red circles) (maps: http://maps.google.co.uk/maps).

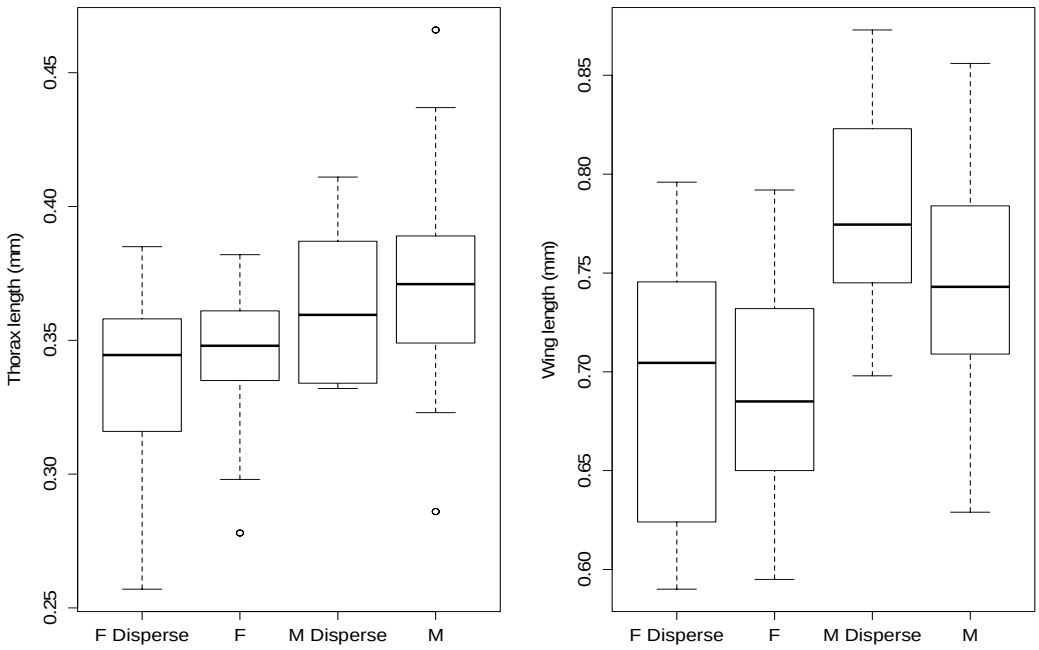


Figure 6.4 Boxplot to illustrate thorax and wing length of males (M) and females (F) re-sighted at the release location, and re-sighted at 1 to 5 km (Dispersers, Experiment 2).

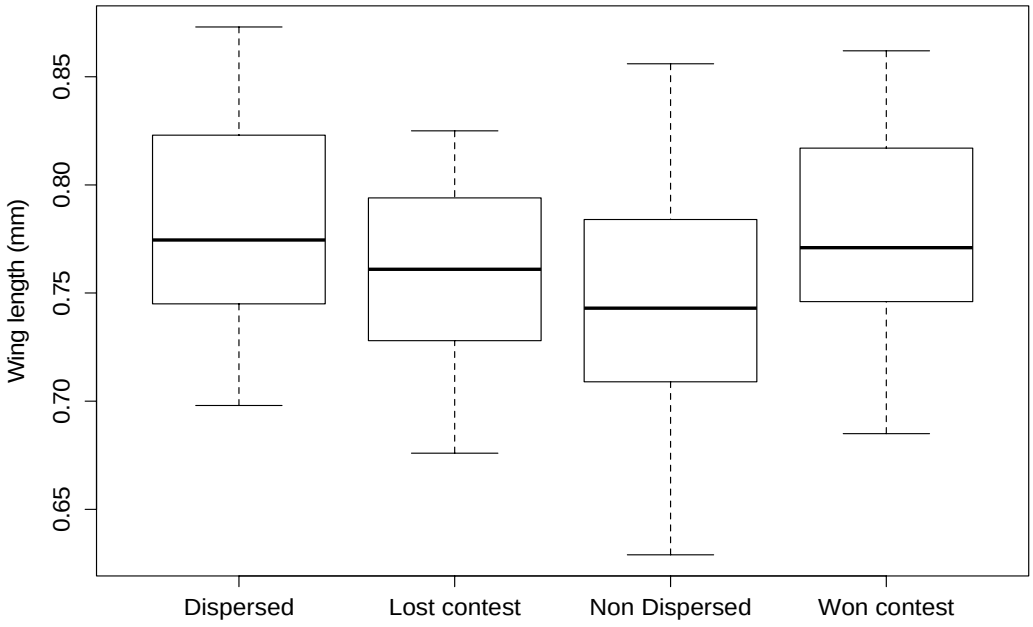


Figure 6.5 Boxplots to illustrate wing length of males re-sighted at 1 to 5 km (Dispersers), males re-sighted at the release site (Non Dispersed) those in territorial contests that 'lost' (Lost contest) and 'won' (Won contest) (Experiment 2).

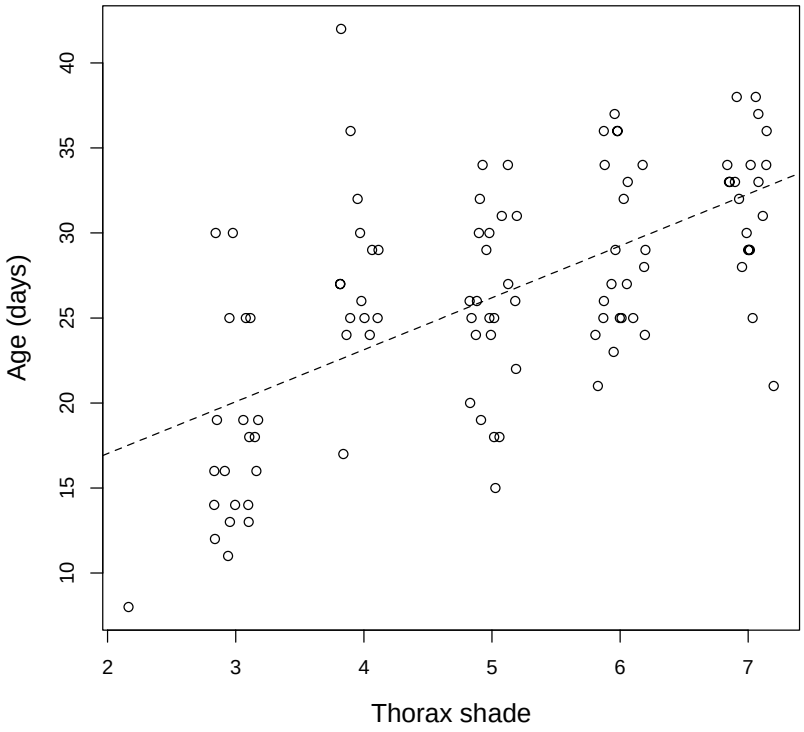


Figure 6.6 Linear regression of individual 'jittered' thorax shade assigned by observers (treated as a continuous variable) and age of *H. ferruginea* individuals (Experiment 2).

Chapter 7

Discussion

7.1 Summary of aims

This thesis investigated the adult and larval requirements of a saproxylic hoverfly *Blera fallax* (Diptera, Syrphidae) in conjunction with practical conservation management. Captive breeding and rearing enabled a detailed examination of larval life history traits and microhabitat use, as well as identification of adult food plants. It also demonstrated the potential of these hoverflies to be useful models for studying life history strategies and how they enable populations to cope with characteristic, fluctuating resources.

In addition, using mark and recapture methods, it assessed the dispersal ability of another endangered saproxylic hoverfly, *Hammerschmidtia ferruginea*. These are the first attempts of their kind to identify the requirements of endangered saproxylic hoverflies and apply the findings to encourage and conserve populations.

Main findings:

1. The rot hole substrate and competitive environment negatively affects larval growth in *B. fallax*, which has consequences on adult size (Chapter 2, 3 and 5).
2. Interspecific competition for resources in a rot hole is not likely to affect the recovery of *B. fallax* (Chapter 3).
3. Lower levels of genetic variation were detected in the Scottish *B. fallax* population compared to Swedish flies; genetic constraints that may limit the recovery of this species (Chapter 4).

4. *Hammerschmidtia ferruginea* was recorded dispersing up to 5km from a release site, informing conservation management protocols for this species, and providing a first estimate for saproxylic fly dispersal (Chapter 6).

7.2 Captive breeding and relocation of B. fallax

The aim of relocation projects is to reduce the risk of extinction for an endangered species by creating more self-sustaining populations. Intervention of this kind is usually undertaken in situations where so much habitat has been lost that the rate of local extinctions threatens to completely eliminate a species. Previous insect captive breeding and/or relocation attempts have mainly involved butterflies, but have also included crickets and grasshoppers, dragonflies and damselflies, stick insects and burying beetles (Amaral *et al*. 1997; Witkowski *et al*. 1997; Sherley 1998; Berggren 2005; Hannon & Hafernik 2007; Hochkirch *et al*. 2007; Honan 2008; Gardiner 2010). Results have been highly variable, and there does not appear to be a formula for success as every species, population, location and circumstance is different. Careful selection of the source population appears to be important. The source populations for most successful relocation attempts were large, viable and from nearby locations. Risks associated with translocation include a loss of genetic variability due to small population size, which may lead to lower fitness and reduce population growth (Frankham 1998; Woodworth *et al*. 2002; Leberg & Firmin 2008). However, sustainable populations can be established despite some loss of genetic variability (Brookes *et al*. 1997; Witzenberger & Hochkirch 2008). Relocation of just 50 individuals has reportedly established a viable and genetically diverse population of the mountain endemic butterfly *Erebia epiphron silesiana*

(Schmitt *et al* 2005). There was only one source population of *B. fallax* available for captive breeding and relocation in this study, it was not large, and seems to have limited genetically variability (Chapter 4).

Extinction risk is elevated by inbreeding depression, and low genetic variability appears to reduce the fitness of a species before it goes extinct (Saccheri *et al*. 1998; Spielman *et al*. 2004), so inbreeding depression effects must be closely monitored both in wild and lab-reared stocks. In the case of Scottish *B. fallax*, augmentation through reintroduction from populations elsewhere in Europe is an option. However, these populations have probably been separated for up to 10,000 years and we have no detailed understanding of the degree of local adaptation there may be in the Scottish population. Such adaptation is known to occur over hundreds as opposed to thousands of years, and so is likely to be present (Stockwell *et al*. 2003). Before considering introducing individuals from abroad, we would need to sample more populations of *B. fallax* in Europe in order to determine what the genetic variation of populations is, and culture and cross populations in the lab to investigate compatibility and outbreeding effects. If the effective population size of *B. fallax* in Scotland is as low as our analysis suggests ($N_e = 12$, albeit with a fairly large confidence interval, see Chapter 4), this may have consequences for the adaptive evolution regardless of the quality of any newly introduced alleles (Charlesworth 2009). This is because in small populations the effects of genetic drift can swamp even strong selection. Scottish *B. fallax* may require significant augmentation in order to have any affect on the genetic variability and adaptive potential of the population.

7.3 Determining habitat requirements of B. fallax

Providing the correct habitat is probably the most important factor that determines the success of relocation attempts. Success can only be evaluated by close monitoring of the relocated population. A study which relocated over 1,600 tiger beetles *Cicindela dorsalis dorsalis* (Coleoptera: Cicindelidae) had promising initial results, only to experience a massive decline in the proceeding years, the cause of which was unidentified due to lack of post-release monitoring (Knisley *et al*. 2005). Damselflies relocated in California experienced a similar failure due to not accounting for ecological requirements at the occupied sites (Hannon & Hafernik 2007). Specialised ecological relationships, involving highly interdependent pairs or groups of species such as the Large Blue butterflies *Maculinea spp.* and their *Myrmica* ant hosts (Thomas *et al*. 2009), highlight the importance of understanding the ecology and cause of decline in species before attempting relocations. Baseline information on all developmental stages is required for adequate conservation management.

In the course of this thesis, we encountered difficulties studying *B. fallax* adult requirements in the field. It is not surprising that our attempts were unsuccessful; other studies of rare hoverflies have had similarly limited results when concentrating on the elusive adult stage (Drake & Baldock 2005). Location of adult hoverflies is often only possible if they have a mate-seeking strategy that concentrates adults at an identifiable resource in the field, such as in the territorial behaviour of *H. ferruginea* on fallen aspen wood (Chapter 6). By focusing on observing breeding habitat, *B. fallax* adults were eventually observed. However, the

very low catch per unit effort (two female sightings over 6 weeks of surveying time, Chapter 5) suggests that limited conservation resources should be spent elsewhere. Only by surveying for larvae were we able to deduce that adults can locate habitat up to 1 km from a release or emergence site (Chapter 5).

Some useful information on adult biology was acquired through captive breeding. The most useful finding in terms of the relocation was that adults fed on a variety of food plants (Chapter 5), which suggests that like most adult hoverflies, *B. fallax* is a generalist and is not limited by availability of food plants. This fact broadens the potential benefits of conserving *B. fallax* by confirming its role as a pollinator in pine woodlands. Through captive breeding, we were also able to document new details of *B. fallax* behaviour, reproductive biology and longevity, including that males appear to hold temporary territories, that females mate up to 9 times, and that both can live beyond 50 days in captivity. These observations have implications for captive breeding purposes, for example, where more successful males may dominate paternity in cages. A greater number of cages with fewer males per cage may be beneficial for sustaining the limited genetic diversity of Scottish flies in future captive breeding efforts.

Stimulation of oviposition was only possible by enclosing females with wet pine sawdust (Chapter 5), which probably means there are unknown elements that females require to trigger oviposition. We have yet to establish the pine stump or rot hole traits that determine female selection preferences for oviposition. It may be an olfactory response to heart-rot fungus, which causes the initial decay. Investigations into beetles in spruce stumps found that the height of stumps was not as important

as the presence of two species of fungus which appeared to have an association with beetle presence (Jonsell & Weslien 2003). Much more work is needed on the oviposition preferences of *B. fallax*, and other saproxylic hoverflies. We know that we can create habitat that is suitable in pine stumps, indicated by *B. fallax* larval occupancy every year for 4 years in artificially created holes (Chapter 5), and we know we should only utilise pine stumps for habitat creation (Chapter 2 and 5). We have been able to show that viable holes can be created in smaller trees between 25 and 30 cm width, which makes use of plantation-sized trees being felled as part of normal pinewood harvesting rotations (Chapter 5). The suitability of these holes has been suggested by their ability to retain water and their occupation by other species of Diptera (Chapter 5). How deep the cavity can be before losing water retention, or the optimal depth and area we can bore into stumps at normal harvesting height for long-term water retention, is yet to be determined. Continued monitoring and experimental habitat creation should clarify this.

It is important to identify the reason for decline or any factors that may limit the success of relocation to a new site. Intraspecific larval growth experiments demonstrated that competition for resources can occur in artificial rot holes and this can limit adult size (Chapter 2). Therefore, future relocations should ensure that the smallest possible number of *B. fallax* larvae is introduced into each rot hole. While interspecific competition in a rot hole may not limit *B. fallax* based on microhabitat use observations (Chapter 3), further experimentation may identify whether there are competition effects between species for resources that may inhibit growth and larval survival in *B. fallax*. Observations on microhabitat use identified *B. fallax* as the only species of four to inhabit all areas of the rot hole (Chapter 3). This

strengthens the argument that *B. fallax* may be an effective umbrella species for the pine rot hole habitat.

It is cost-effective to focus survey work on the larval stage of *B. fallax* as they occupy a discrete, easily monitored habitat, sampling is not invasive, and we now know enough about the life cycle in order to design suitable protocols. *Blera fallax* appears to have a flexible life history strategy where, depending on levels of larval food, semivoltism may occur. Similar semivoltine life histories have been reported in dragonflies, damselflies and stoneflies (Purse & Thompson 2002; Schultheis *et al*. 2002; Cayrou & Céréghino 2005; Watts *et al*. 2005; Raebel *et al*. 2010) but no evidence has been reported for hoverflies. Facultative semivoltinism in *B. fallax* may reflect a bet-hedging strategy that enables a proportion of the population to survive years when conditions are unsuitable for adult activity (Schultheis 2002). There are probably costs associated with this strategy, such as increased risk of mortality over time, but it may allow some individuals to survive catastrophic events. For example, in 2011, breeding appears to have been at a very low level or to have failed altogether at all sites, including the one natural population, probably due to cold and wet weather during the adult flight period. Only semivoltine larvae appear to have survived this event. Weather is a major factor known to influence the success of insect translocation projects (Pearce-Kelly *et al*. 1998; Hochkirch *et al*. 2007). The translocation of the field cricket *Gryllus campestris* has shown a consistent population and range expansion that was probably assisted by suitable weather in each year after an initial release of 200 individuals (Hochkirch *et al*. 2007). The recent population crash in *B. fallax* highlights the vulnerability of such small populations to natural stochastic events. It is not yet known whether the

population will recover, and if so whether there may be an additional loss of genetic variability through bottlenecking.

It is difficult to learn from failed relocations due to the lack of published data on those that are unsuccessful (Fischer 2000). Studies from even very similar species from the same family can have considerably different results or require alternative techniques (Pearce-Kelly *et al*. 1998) so comparing attempts, especially those in different family groups, may not improve the success rate.

7.4 Conservation of saproxylic hoverflies

In Europe, the importance and diversity of saproxlic hoverflies is slowly being recognised, and through focused survey work the composition of communities are being established in order to focus conservation priorities (Reemer 2005; Ricarte *et al*. 2007, 2009). Good forestry practice in the UK now includes retaining deadwood (Forestry Commission 2002), however a more widespread understanding and appreciation of the importance of deadwood is needed. Leaving habitat entirely unmanaged is rarely optimal and broad or best-fit management strategies for saproxlic species do not yet exist (Davies *et al*. 2008). Saproxylic communities change over time and differ in their requirements and needs (Ranius *et al*. 2011; Weslien *et al*. 2011). The principal lesson of species-level conservation studies is that the requirements of individual species vary, so to ensure survival, tailored actions are required. It is important to determine requirements and provide for every stage of an insect life cycle. This is evident from my thesis, where two species depend on a relatively short-lived habitat window. Deadwood can also be an

important mate-seeking site, and size, location and volume may be important for the species in this study, especially if it has an effect on decay time. For species like *H. ferruginea*, intervention is required to ensure constant deadwood input, but manipulating deadwood is a short-term solution. Even though experimentally controlled and replicated evidence is lacking, currently the general prescriptions for saproxylic species are to increase forest area, the number of large trees, the age structure, and to allow dead or moribund trees to decay naturally (Davies *et al*. 2008). In addition, encouraging adult food plants through diversity of structure is also necessary (Fayt *et al*. 2006; Gittings *et al*. 2006).

We probably need appropriate representative umbrella species or species groups to represent microhabitats for conservation planning in woodlands. Sub groups of saproxylic hoverflies can act as indicators for each other (Smith *et al*. 2009). Whether *B. fallax* or *H. ferruginea* are good umbrella species is an important question for future study. Compared with other saproxylic species, the colonisation ability of *H. ferruginea* appears to be good (dispersing up to 5 km distance, see Chapter 6), but many hoverflies have limited dispersal abilities (Wratten *et al*. 2003). Like the saproxylic beetle *Bolitophagus reticulates* (Jonsell *et al*. 2003), *H. ferruginea* appears to more frequently disperse short distances, which suggests that new deadwood breeding habitat should be linked to existing populations by islands of habitat spaced no more than 1 km apart (Chapter 6). Using population genetic techniques (by sampling DNA from populations that move between overlapping habitat patches) has proven useful in understanding dispersal patterns and abilities in other taxa (Jonsson *et al*. 2003; Watts *et al*. 2004; Ranius 2006; Schmuki *et al*. 2006), and this could be aided by our ability to nonlethally sample from the

discarded puparia of emerged flies (Appendix 4.2). Using molecular markers, we will be able to detect much more precisely rates of migration between adjacent habitat patches, which could substantially improve practices for managing the landscape for deadwood insects. For example, this information may help in prioritising investment of scarce resources into populations that need it most.

The approach to habitat management for species such as *H. ferruginea* requires not just focusing on the localities where they occur, but it also requires landscape level conservation i.e. of fragments of habitat adjacent to the main areas that provide additional support for populations. This will assist long-term persistence and survival by forming a metapopulation landscape (Dover & Settele 2009). Such habitat corridors or stepping-stones may need to be managed on different scales for different species groups depending on dispersal and colonisation abilities. These should be created with short-term and long-term movement in mind, therefore conserving individual species and biodiversity. This requires maintaining heterogeneity in land quality while reducing the contrast between habitat patches, connecting patches and reducing isolation.

In order to conserve saproxylic hoverflies i.e. create sustainable populations, we need to confirm their status and distribution, and identify threats and gaps in the knowledge about adults and larval requirements. To ensure that conservation action will ensue and for funding, time and labour to be made available, some level of legislation or national or regional plan needs to be in place under which conservation action can be authorised. The support, agreement and enthusiasm of landowners and managers at localities holding populations or habitat in the area is

crucial to successful short and long-term implementation (Hochkirch *et al*. 2007; Daniels 2009). Deadwood conservation considered in this study is both cost effective and simple. It involves monitoring the deadwood habitat, ensuring suitability and overlapping resources, protecting veteran trees, and encouraging regeneration. In addition, by implementing actions over the short-term, greater long-term benefits are gradually accrued, due to the habitats conserved for the aspen and pine hoverflies, which assist other invertebrates, vertebrates, plants, fungi and lichens and beyond.

Blera fallax and *H. ferruginea* are relics of our ancient, boreal, Caledonian pine wood forest, existing now only in Scotland in the British Isles, making them an important part of our natural heritage. Our appreciation of this importance is exemplified by the enthusiastic participation of numerous organisations, private landowners and managers throughout the course of my thesis work.

Some difficult triage decisions are likely to be necessary as increasing numbers of insect species reach endangered status and where conservation is hampered by limited resources such as money, space, time and materials. Saproxylic hoverflies such as the ones in this study stand as an example showing that such problems and difficulties can be overcome. They also stand proxy for large, species rich groups that include organisms other than insects sharing or relying on similar habitats and where, as we have shown, techniques for their conservation are simple, inexpensive and can be immediately rewarding in terms of maintaining biodiversity. Overall, my findings greatly increased fundamental knowledge of the ecology and natural history of these flies, and clarified some of the practical approaches that will be

required in their conservation. This work demonstrates what can be achieved for saproxylic species conservation, and insect conservation as a whole.

References

Abràmoff, M.D., Magalhães, P.J. & Ram, S.J. (2004). Image processing with ImageJ. *Biophotonics international*, 11, 36–42.

Abrams, P.A., Leimar, O., Nylin, S. & Wiklund, C. (1996). The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. *American Naturalist*, 147, 381–395.

Addicott, J.F. (1974). Predation and prey community structure: an experimental study of the effect of mosquito larvae on the protozoan communities of pitcher plants. *Ecology*, 55, 475–492.

Alexander, K.N.A. (1988). The development of an index of ecological continuity for deadwood associated beetles. *Antenna*, 12, 69–70.

Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour,* 3, 227–266.

Amaral, M., Kozol, A. & French, T. (1997). Conservation status and reintroduction of the endangered American burying beetle. *Northeastern Naturalist*, 4, 121–132.

Armbruster, P. & Hutchinson, R.A. (2002). Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). *Journal of medical entomology*, 39, 699–704.

Armbruster, P., Hutchinson, R.A. & Linvell, T. (2000). Equivalent inbreeding depression under laboratory and field conditions in a tree-hole-breeding mosquito. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267, 1939–1945.

Armstrong, R.A. & McGehee, R. (1980). Competitive exclusion. *American Naturalist*, 115, 151–170.

Arnold, S.J. & Wade, M.J. (1984a). On the measurement of natural and sexual selection: applications. *Evolution*, 38, 720–734.

Arnold, S.J. & Wade, M.J. (1984b). On the measurement of natural and sexual selection: theory. *Evolution*, 38, 709–719.

Aspi, J. (2000). Inbreeding and outbreeding depression in male courtship song characters in Drosophila. *Heredity*, 84, 273–282.

Bailey, S. (2007). Increasing connectivity in fragmented landscapes: An investigation of evidence for biodiversity gain in woodlands. *Forest Ecology and Management*, 238, 7–23.

Bale, J. (2002). Insects and low temperatures: from molecular biology to distributions and abundance. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 357, 849–862.

Ball, S. Morris, R.K.., Rotheray, G.E. & Watt, K.R. (2011). Atlas of the Hoverflies of Great Britain (Diptera, Syrphidae). *Centre for Ecology and Hydrology*.

Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.S., Nakashizuka, T. & Raffaelli, D. (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters*, 9, 1146–1156.

Bates, D. & Maechler, M. (2008). Dai B. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-28; 2008, URL http//CRAN. R-proyect. org

Beaumont, M.A. (1999). Detecting population expansion and decline using microsatellites. *Genetics*, 153, 2013–2029.

Bell, T., Ager, D., Song, J.I., Newman, J.A., Thompson, I.P., Lilley & A.K. (2005). Larger islands house more bacterial taxa. *Science*, 308, 1884.

Bennett, K.D. (1984). The post-glacial history of *Pinus sylvestris* in the British Isles. *Quaternary Science Reviews*, 3, 133–155.

Berggren, A. (2005). Effect of Propagule Size and Landscape Structure on Morphological Differentiation and Asymmetry in Experimentally Introduced Roesel's Bush Crickets. *Conservation Biology*, 19, 1095–1102.

Biebach, I. & Keller, L. (2010). Inbreeding in reintroduced populations: the effects of early reintroduction history and contemporary processes. *Conservation Genetics*, 11, 527–538.

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M. & Peeters, T. (2006). Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. *Science*, 313, 351–354.

Birks, H.H. (1970). Studies in the Vegetational History of Scotland: I. A Pollen Diagram from Abernethy Forest, Inverness-Shire. *The Journal of Ecology*, 58, 827–846.

Blanckenhorn, W.U. (1998). Adaptive phenotypic plasticity in growth, development, and body size in the yellow dung fly. *Evolution*, 52, 1394–1407.

Blanckenhorn, W.U. (1999). Different growth responses to temperature and resource limitation in three fly species with similar life histories. *Evolutionary Ecology*, 13, 395–409.

Bonduriansky, R. & Brooks, R.J. (1997). A technique for measuring and marking live flies. *The Canadian Entomologist*, 129, 827–830.

Bradshaw, W.E. (1973). Homeostasis and polymorphism in vernal development of *Chaoborus americanus*. *Ecology*, 54, 1247–1259.

Bradshaw, W.E. & Holzapfel, C.M. (1992). Resource Limitation, Habitat Segregation, and Species Interactions of British Tree-Hole Mosquitoes in Nature. *Oecologia*, 90, 227–237.

Broberg, L. & Bradshaw, W.E. (1995). Density-dependent development in *Wyeomyia smithii* (Diptera: Culicidae): intraspecific competition is not the result of interference. *Annals of the Entomological Society of America*, 88, 465–470.

Brookes, M.I., Graneau, Y.A., King, P., Rose, O.C., Thomas, C.D. & Mallet, J.L.B. (1997). Genetic Analysis of Founder Bottlenecks in the Rare British Butterfly *Plebejus argus*. *Conservation Biology*, 11, 648–661.

Brown, J.L. & Brown, E.R. (1998). Are inbred offspring less fit? Survival in a natural population of Mexican jays. *Behavioral Ecology*, 9, 60–63.

Brownstein, M.J., Carpten, D. & Smith, J.R. (1996). Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. *Biotechniques*, 20, 1004–1010.

Buckton, G.B. (1895). The natural history of *Eristalis tenax* or the drone-fly. *Macmillan, London*.

Bull, C.D., Metcalfe, N.B. & Mangel, M. (1996). Seasonal matching of foraging to anticipated energy requirements in anorexic juvenile salmon. *Proceedings of the Royal Society: Biological Sciences*, 263, 13–18.

Burgio, G. & Sommaggio, D. (2007). Syrphids as landscape bioindicators in Italian agroecosystems. *Agriculture, ecosystems & environment*, 120, 416–422.

Butler, J., Alexander, K. & Green, T. (2002). Decaying wood: an overview of its status and ecology in the United Kingdom and Continental Europe. In:

Proceedings of the Symposium on the Ecology and Management of Dead Wood in Western Forests. pp. 11–19.

Cardoso, P., Erwin, T.L., Borges, P.A.V. & New, T.R. (2011). The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation*, 144, 2647–2655.

Carey, A.B. (1989). Wildlife associated with old-growth forests in the Pacific Northwest. *Natural Areas Journal*, 9, 151–162.

Caro, T.M. & O'Doherty, G. (1999). On the use of surrogate species in conservation biology. *Conservation Biology*, 13, 805–814.

Carpenter, S.R. (1983). Resource limitation of larval treehole mosquitoes subsisting on beech detritus. *Ecology*, 64, 219–223.

Cayrou, J. & Céréghino, R. (2005). Life-cycle phenology of some aquatic insects: implications for pond conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 15, 559–571.

Chakraborty, A. & Li, B.L. (2010). The role of fluctuating resource supply in a habitat maintained by the competition-colonization trade-off. *Annals of the New York Academy of Sciences*, 1195, E27–E39.

Châline, N., Ratnieks, F.L., Raine, N.E., Badcock, N.S. & Burke, T. (2004). Non lethal sampling of honeybee, *Apis mellifera*, DNA using wing tips. *Apidologie*, 35, 311–318.

Charlesworth, B. (2009). Effective population size and patterns of molecular evolution and variation. *Nature Reviews Genetics*, 10, 195–205.

Chikhi, L., Sousa, V.C., Luisi, P., Goossens, B. & Beaumont, M.A. (2010). The confounding effects of population structure, genetic diversity and the

sampling scheme on the detection and quantification of population size changes. *Genetics*, 186, 983–995.

Cooper, S., Ibrahim, K. & Hewitt, G. (1995). Postglacial expansion and genome subdivision in the European grasshopper *Chorthippus parallelus*. *Molecular Ecology*, 4, 49–60.

Cosgrove, P., Amphlett, A., Elliot, A., Ellis, C., Emmett, E. & Prescott, T. (2005). Aspen: Britain's missing link with the boreal forest. *British Wildlife*, 17, 107–115.

Craul, M., Chikhi, L., Sousa, V., Olivieri, G.L., Rabesandratana, A. & Zimmermann, E. (2009). Influence of forest fragmentation on an endangered large-bodied lemur in northwestern Madagascar. *Biological Conservation*, 142, 2862–2871.

Crawley, M.J. (2007). The R book. *Wiley*.

Crean, A.J. & Marshall, D.J. (2009). Coping with environmental uncertainty: dynamic bet hedging as a maternal effect. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364, 1087–1096.

Crone, E.E., Pickering, D. & Schultz, C.B. (2007). Can captive rearing promote recovery of endangered butterflies? An assessment in the face of uncertainty. *Biological Conservation*, 139, 103–112.

Daniels, J.C. (2009). Cooperative conservation efforts to help recover an endangered south Florida butterfly. *Insect Conservation and Diversity*, 2, 62–64.

Daugherty, M.P., Alto, B.W. & Juliano, S.A. (2000). Invertebrate carcasses as a resource for competing *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of medical entomology*, 37, 364–372.

Daugherty, M.P. & Juliano, S.A. (2002). Testing for context-dependence in a processing chain interaction among detritus-feeding aquatic insects. *Ecological entomology*, 27, 541–553.

Daugherty, M.P. & Juliano, S.A. (2011). Factors affecting the abundance of scirtid beetles in container habitats. *Journal of the North American Benthological Society*, 20, 109–117.

Davies, Z.G., Tyler, C., Stewart, G.B. & Pullin, A.S. (2008). Are current management recommendations for saproxylic invertebrates effective? A systematic review. *Biodiversity and Conservation*, 17, 209–234.

Day, T. & Taylor, P.D. (1997). Von Bertalanffy's growth equation should not be used to model age and size at maturity. *The American Naturalist*, 149, 381–393.

Denno, R.F. (1994). The evolution of dispersal polymorphisms in insects: the influence of habitats, host plants and mates. *Researches on Population Ecology*, 36, 127–135.

Dmitriew, C. & Rowe, L. (2007). Effects of early resource limitation and compensatory growth on lifetime fitness in the ladybird beetle (*Harmonia axyridis*). *Journal of Evolutionary Biology*, 20, 1298–1310.

Dmitriew, C., Carroll, J. & Rowe, L. (2009). Effects of early growth conditions on body composition, allometry, and survival in the ladybird beetle *Harmonia axyridis*. *Canadian Journal of Zoology*, 87, 175–182.

Dmitriew, C.M. (2011). The evolution of growth trajectories: what limits growth rate? *Biological Reviews*, 86, 97–116.

Dover, J. & Settele, J. (2009). The influences of landscape structure on butterfly distribution and movement: a review. *Journal of Insect Conservation*, 13, 3–27.

Dowling, T., Marsh, P. & Kelsen, A. (2005). Genetic monitoring of wild and repatriated populations of endangered razorback sucker (*Xyrauchen texanus*, Catostomidae, Teleostei) in Lake Mohave, Arizona-Nevada. *Molecular Ecology*, 14, 123–135.

Drake, M. & Baldock, N. (2005). The Bog Hoverfly on Dartmoor. *British Wildlife*, 17, 102–109.

Dunlop, B. (1993). The origin and history of Curr Wood, Strathspey. Unpublished paper available at Scottish Natural Heritage, Achantoul, Aviemore.

Dunlop, B. (1994). The native woodlands of Strathspey. Unpublished paper available at Scottish Natural Heritage, Achantoul, Aviemore.

Dunn, R.R. (2005). Modern Insect Extinctions, the Neglected Majority. *Conservation Biology*, 19, 1030–1036.

Edmands, S. (2007). Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology*, 16, 463–475.

Eisenberg, J.N., Washburn, J.O. & Schreiber, S.J. (2000). Generalist feeding behaviors of *Aedes sierrensis* larvae and their effects on protozoan populations. *Ecology*, 81, 921–935.

Elgar, M.A. & Clode, D. (2001). Inbreeding and extinction in island populations: a cautionary note. *Conservation Biology*, 15, 284–286.

Esperk, T. & Tammaru, T. (2004). Does the 'investment principle'model explain moulting strategies in lepidopteran larvae? *Physiological Entomology*, 29, 56–66.

Excoffier, L., Laval, G. & Schneider, S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol Bioinform Online*, 1, 47–50.

Faircloth, B.C. (2008). msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources*, 8, 92–94.

Fayt, P., Dufrêne, M., Branquart, E., Hastir, P., Pontégnie, C., Henin & J.M. (2006). Contrasting responses of saproxylic insects to focal habitat resources: the example of longhorn beetles and hoverflies in Belgian deciduous forests. *Journal of Insect Conservation*, 10, 129–150.

Felsenstein, J. (1992). Estimating effective population size from samples of sequences: inefficiency of pairwise and segregating sites as compared to phylogenetic estimates. *Genetic Research*, 59, 139–147.

Fincke, O.M. (1992). Interspecific competition for tree holes: consequences for mating systems and coexistence in neotropical damselflies. *American Naturalist*, 139, 80–101.

Fischer, J. & Lindenmayer, D.B. (2000). An assessment of the published results of animal relocations. *Biological conservation*, 96, 1–11.

Fish, D. & Carpenter, S.R. (1982). Leaf litter and larval mosquito dynamics in tree-hole ecosystems. *Ecology*, 63, 283–288.

Fisher, I.J., Bradshaw, W.E. & Kammeyer, C. (1990). Fitness and its correlates assessed by intra-and interspecific interactions among tree-hole mosquitoes. *Journal of animal ecology*, 59, 819–829.

Forestry Commission. (2002). A guide to managing deadwood in Forestry Commission forests.

Frankham, R. (1995). Conservation genetics. *Annual Review of Genetics*, 29, 305–327.

Frankham, R. (1998). Inbreeding and extinction: island populations. *Conservation Biology*, 12, 665–675.

Frankham, R. (2005). Genetics and extinction. *Biological Conservation*, 126, 131–140.

Furrer, R., Nychka, D. & Sain, S. (2009). fields: Tools for spatial data. Rpackage version, 6, URL http://cran.r-project.org/web/packages/fields/index.html

Gardiner, T. (2010). Successful translocation of the locally rare mottled grasshopper *Myrmeleotettix maculatus* to Jaywick flood defences in Essex, England. *Conservation Evidence*, 7, 106–110.

Gelman, A. & Rubin, D.B. (1992). Inference from iterative simulation using multiple sequences. *Statistical science*, 7, 457–472.

Gerritsen, J. (1984). Size Efficiency Reconsidered: A General Foraging Model for Free-Swimming Aquatic Animals. *The American Naturalist*, 123, 450–467.

Gilbert, F. (1980). Flower visiting by hoverflies (Syrphidae). *Journal of Biological Education*, 14, 70–74.

Gilbert, F. (1986). Hoverflies. Naturalists' Handbook 5. *Cambridge University Press, Cambridge, UK*.

Gilbert, F., Rotheray, G., Emerson, P. & Zafar, R. (1994). The evolution of feeding strategies. *Phylogenetics and Ecology*, 323–343.

Gilbert, F.S. (1981). Foraging ecology of hoverflies: morphology of the mouthparts in relation to feeding on nectar and pollen in some common urban species. *Ecological Entomology*, 6, 245–262.

Gilbert, F.S. (1985). Diurnal activity patterns in hoverflies (Diptera, Syrphidae). *Ecological Entomology*, 10, 385–392.

Gittings, T., O'Halloran, J., Kelly, T. & Giller, P.S. (2006). The contribution of open spaces to the maintenance of hoverfly (Diptera, Syrphidae) biodiversity in Irish plantation forests. *Forest ecology and management*, 237, 290–300.

Gotthard, K. (2008). Adaptive growth decisions in butterflies. *Bioscience*, 58, 222–230.

Gotthard, K., Nylin, S. & Wiklund, C. (1999). Seasonal plasticity in two satyrine butterflies: state-dependent decision making in relation to daylength. *Oikos*, 84, 453–462.

Goudet, J. (1995). FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of heredity*, 86, 485–486.

Goulson, D., Rayner, P., Dawson, B. & Darvill, B. (2011). Translating research into action; bumblebee conservation as a case study. *Journal of Applied Ecology*, 48, 3–8.

Graca, M., Newell, S. & Kneib, R. (2000). Grazing rates of organic matter and living fungal biomass of decaying *Spartina alterniflora* by three species of salt-marsh invertebrates. *Marine Biology*, 136, 281–289.

Greig, C. (1989). Overwintering Behaviour of the larva *Myiatropa florea* (L.) (Diptera: Syrphidae). *Dipterist Digest*, 2, 11–16.

Grimaldi, D. & Jaenike, J. (1984). Competition in natural populations of mycophagous Drosophila. *Ecology*, 65, 1113–1120.

Grove, S. & Tucker, N. (2000). Importance of mature timber habitat in forest management and restoration: what can insects tell us. *Ecological Management and Restoration*, 1, 68–69.

Grove, S.J. (2002). Saproxylic insect ecology and the sustainable management of forests. *Annual Review of Ecology and Systematics*, 33, 1–23.

Guiney, M.S. & Oberhauser, K.S. (2009). Insects as flagship conservation species. *Terrestrial Arthropod Reviews*, 1, 111–123.

Hagstrum, D.W. & Subramanyam, B. (2010). Immature Insects: Ecological Roles of Mobility. *American Entomologist*, 56, 230–241.

Hahn, M.W. & Höfle, M.G. (2001). Grazing of protozoa and its effect on populations of aquatic bacteria. *FEMS Microbiology Ecology*, 35, 113–121.

Hamm, C.A., Aggarwal, D. & Landis, D.A. (2010). Evaluating the impact of non-lethal DNA sampling on two butterflies, *Vanessa cardui* and *Satyrodes eurydice*. *Journal of Insect Conservation*, 14, 11–18.

Hannon, E.R. & Hafernik, J.E. (2007). Reintroduction of the rare damselfly *Ischnura gemina* (Odonata: Coenagrionidae) into an urban California park. *Journal of insect conservation*, 11, 141–149.

Hanski, I. (1998). Metapopulation dynamics. *Nature*, 396, 41–49.

Hardin, G. (1960). The competitive exclusion principle. *Science*, 131, 1292–1297.

Harlan, N.P. & Paradise, C.J. (2006). Do habitat size and shape modify abiotic factors and communities in artificial treeholes? *Community Ecology*, 7, 211–222.

Harper, G.L., Maclean, N. & Goulson, D. (2006). Analysis of museum specimens suggests extreme genetic drift in the adonis blue butterfly (*Polyommatus bellargus*). *Biological Journal of the Linnean Society*, 88, 447–452.

Hart, J. & Bale, S. (1997a). Factors affecting the freeze tolerance of the hoverfly *Syrphus ribesii* (Diptera: syrphidae). *Journal of Insect Physiology*, 44, 21–29.

Hart, J. & Bale, S. (1997b). Evidence for the first strongly freeze-tolerant insect found in the U.K. *Ecological Entomology*, 22, 242–245.

Hartley, J. (1963). The cephalopharyngeal apparatus of syrphid larvae and its relationship to other Diptera. In: *Proceedings of the Zoological Society of London*, 261–280.

Hedrick, P.W. & Kalinowski, S.T. (2000). Inbreeding Depression in Conservation Biology. *Annual Review of Ecology and Systematics*, 31, 139–162.

Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, 405, 907–913.

Hewitt, G.M. (1996). Some genetic consequences of ice ages, and their role, in divergence and speciation. *Biological Journal of the Linnean Society*, 58, 247–276.

Hochkirch, A., Witzenberger, K.A., Teerling, A. & Niemeyer, F. (2007). Translocation of an endangered insect species, the field cricket (*Gryllus campestris* Linnaeus, 1758) in northern Germany. *Biodiversity and Conservation*, 16, 3597–3607.

Hoffmann, A., Ratna, E., Sgro, C., Barton, M., Blacket, M. & Hallas, R. (2007). Antagonistic selection between adult thorax and wing size in field released *Drosophila melanogaster* independent of thermal conditions. *Journal of evolutionary biology*, 20, 2219–2227.

Holehouse, K.A., Hammond, R.L. & Bourke, A.F.G. (2003). Non-lethal sampling of DNA from bumble bees for conservation genetics. *Insectes Sociaux*, 50, 277–285.

Honan, P. (2008). Notes on the biology, captive management and conservation status of the Lord Howe Island Stick Insect (*Dryococelus australis*) (Phasmatodea). *Journal of Insect Conservation*, 12, 399–413.

Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, 66, 483–492.

Hou, C., Bolt, K.M. & Bergman, A. (2011). A general model for ontogenetic growth under food restriction. *Proceedings of the Royal Society B: Biological Sciences*, 278, 2881–2890.

Huffaker, C.B. & Gutierrez, A.P. (1998). Ecological Entomology. 2nd edition. John Wiley and sons, Blackwell publishing.

Humphrey, J.W. (2005). Benefits to biodiversity from developing old-growth conditions in British upland spruce plantations: a review and recommendations. *Forestry*, 78, 33–53.

Humphrey, J.W., Sippola, A.L., Lempérière, G., Dodelin, B., Alexander, K.N.A. & Butler, J.E. (2005). Deadwood as an indicator of biodiversity in European forests: from theory to operational guidance. In: Marchetti, M (Ed.), Monitoring and Indicators of Forest Biodiversity in Europe–From Ideas to Operationality. *EFI Proceedings*, 51, 193–206.

Ibrahim, K.M., Nichols, R.A. & Hewitt, G.M. (1996). Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity*, 77, 282–291.

Jauker, F., Bondarenko, B., Becker, H.C. & Steffan-Dewenter, I. (2011). Pollination efficiency of wild bees and hoverflies provided to oilseed rape. *Agricultural and Forest Entomology*, 14, 81–87.

Jauker, F., Diekötter, T., Schwarzbach, F. & Wolters, V. (2009). Pollinator dispersal in an agricultural matrix: opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat. *Landscape Ecology*, 24, 547–555.

Jonsell, M., Nordlander, G. & Ehnström, B. (2001). Substrate associations of insects breeding in fruiting bodies of wood-decaying fungi. *Ecological Bulletins*, 49, 173–194.

Jonsell, M., Schroeder, M. & Larsson, T. (2003). The saproxylic beetle *Bolitophagus reticulatus*: its frequency in managed forests, attraction to volatiles and flight period. *Ecography*, 26, 421–428.

Jonsell, M. & Weslien, J. (2003). Felled or standing retained wood–it makes a difference for saproxylic beetles. *Forest Ecology and Management*, 175, 425–435.

Jonsson, B.G., Kruys, N. & Ranius, T. (2005). Ecology of species living on dead wood–lessons for dead wood management. *Silva Fennica*, 39, 289–309.

Jonsson, M. (2002). Dispersal ecology of insects inhabiting wood-decaying fungi. Unpublished PhD thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Jonsson, M. (2003). Colonisation ability of the threatened tenebrionid beetle *Oplocephala haemorrhoidalis* and its common relative *Bolitophagus reticulatus*. *Ecological Entomology*, 28, 159–167.

Jonsson, M., Johannesen, J. & Seitz, A. (2003). Comparative genetic structure of the threatened tenebrionid beetle *Oplocephala haemorrhoidalis* and its common relative *Bolitophagus reticulatus*. *Journal of Insect Conservation*, 7, 111–124.

Joron, M., Brakefield, P.M. & others. (2003). Captivity masks inbreeding effects on male mating success in butterflies. *Nature*, 424, 191–194.

Juliano, S.A. (1986). Food limitation of reproduction and survival for populations of *Brachinus* (Coleoptera: Carabidae). *Ecology*, 67, 1036–1045.

Juliano, S.A. & Reminger, L. (1992). The relationship between vulnerability to predation and behavior of larval treehole mosquitoes: geographic and ontogenetic differences. *Oikos*, 63, 465–476.

Kainulainen, P. & Holopainen, J. (2002). Concentrations of secondary compounds in Scots pine needles at different stages of decomposition. *Soil Biology and Biochemistry*, 34, 37–42.

Kaufman, M.G., Bland, S.N., Worthen, M.E., Walker, E.D. & Klug, M.J. (2001). Bacterial and fungal biomass responses to feeding by larval *Aedes triseriatus* (Diptera: Culicidae). *Journal of medical entomology*, 38, 711–719.

Kaufman, M.G., Chen, S. & Walker, E.D. (2008). Leaf-associated bacterial and fungal taxa shifts in response to larvae of the tree hole mosquito, *Ochlerotatus triseriatus*. *Microbial ecology*, 55, 673–684.

Kaufman, M.G., Goodfriend, W., Kohler-Garrigan, A., Walker, E.D. & Klug, M.J. (2002). Soluble nutrient effects on microbial communities and mosquito production in *Ochlerotatus triseriatus* habitats. *Aquatic microbial ecology*, 29, 73–88.

Kaufman, M.G., Walker, E.D., Smith, T.W., Merritt, R.W. & Klug, M.J. (1999). Effects of larval mosquitoes (*Aedes triseriatus*) and stemflow on microbial community dynamics in container habitats. *Applied and environmental microbiology*, 65, 2661–2673.

Keller, L.F. (1998). Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution*, 52, 240–250.

Kitching, R.L. (1971). An Ecological Study of Water-Filled Tree-Holes and their Position in the Woodland Ecosystem. *Journal of Animal Ecology*, 40, 281–302.

Knight, T.M., Chase, J.M., Goss, C.W. & Knight, J.J. (2004). Effects of interspecific competition, predation, and their interaction on survival and development time of immature *Anopheles quadrimaculatus*. *Journal of Vector Ecology*, 29, 277–284.

Knisley, C.B., Hill, J.M. & Scherer, A.M. (2005). Translocation of Threatened Tiger Beetle *Cicindela dorsalis dorsalis* (Coleoptera: Cicindelidae) to Sandy Hook, New Jersey. *Annals of the Entomological Society of America*, 98, 552–557.

Kouki, J. (2008). Aspen and forest biodiversity in North European boreal forests. In: *Proceedings from 2008 HAG Aspen Conference*, 1 – 6.

Kouki, J., Arnold, K. & Martikainen, P. (2004). Long-term persistence of aspen - a key host for many threatened species - is endangered in old-growth conservation areas in Finland. *Journal for Nature Conservation*, 12, 41–52.

Krauss, J., Schmitt, T., Seitz, A., Steffan-Dewenter, I. & Tscharntke, T. (2004). Effects of habitat fragmentation on the genetic structure of the monophagous

butterfly *Polyommatus coridon* along its northern range margin. *Molecular Ecology*, 13, 311–320.

Lande, R. (1988). Genetics and demography in biological conservation. *Science*, 241, 1455–1460.

Langellotto, G., Denno, R. & Ott, J. (2000). A trade-off between flight capability and reproduction in males of a wing-dimorphic insect. *Ecology*, 81, 865–875.

Lankau, R. (2011). Rapid Evolutionary Change and the Coexistence of Species. *Annual Review of Ecology, Evolution, and Systematics*, 42, 335–354.

Lassauce, A., Paillet, Y., Jactel, H. & Bouget, C. (2011). Deadwood as a surrogate for forest biodiversity: Meta-analysis of correlations between deadwood volume and species richness of saproxylic organisms. *Ecological Indicators*, 11, 1027–1039.

Lavoie, B. & Oberhauser, K.S. (2004). Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. *Environmental entomology*, 33, 1062–1069.

Leberg, P. (2005). Genetic approaches for estimating the effective size of populations. *Journal of Wildlife Management*, 69, 1385–1399.

Leberg, P.L. & Firmin, B.D. (2008). Role of inbreeding depression and purging in captive breeding and restoration programmes. *Molecular Ecology*, 17, 334–343.

Lenski, R.E. (1984). Food limitation and competition: a field experiment with two Carabus species. *The Journal of Animal Ecology*, 53, 203–216.

Leonard, P.M. & Juliano, S.A. (1995). Effect of leaf litter and density on fitness and population performance of the hole mosquito *Aedes triseriatus*. *Ecological Entomology*, 20, 125–136.

Levins, R. & Culver, D. (1971). Regional coexistence of species and competition between rare species. *Proceedings of the National Academy of Sciences*, 68, 1246–1248.

Levot, G.W., Brown, K.R. & Shipp, E. (1979). Larval growth of some calliphorid and sarcophagid Diptera. *Bulletin of Entomological Research*, 69, 469–475.

Livdahl, T.P. (1982). Competition Within and Between Hatching Chorots of a Treehole Mosquito. *Ecology*, 63, 1751–1760.

Lonsdale, D., Pautasso, M. & Holdenrieder, O. (2008). Wood-decaying fungi in the forest: conservation needs and management options. *European Journal of Forest Research*, 127, 1–22.

Lotka, A.J. (1932). The growth of mixed populations: two species competing for a common food supply. *Journal of the Washington Academy of Sciences*, 22, 461–469.

Luikart, G. & Cornuet, J.M. (1998). Empirical Evaluation of a Test for Identifying Recently Bottlenecked Populations from Allele Frequency Data. *Conservation Biology*, 12, 228–237.

Lushai, G., Fjellsted, W., Marcovitch, O., Aagaard, K., Sherratt, T.N. & Allen, J.A. (2000). Application of molecular techniques to non-lethal tissue samples of endangered butterfly populations (*Parnassius apollo* L.) in Norway for conservation management. *Biological conservation*, 94, 43–50.

Lye, G.C., Lepais, O. & Goulson, D. (2011). Reconstructing demographic events from population genetic data: the introduction of bumblebees to New Zealand. *Molecular Ecology*, 20, 2888–2900.

Lynch, M. (1991). The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution*, 45, 622–629.

MacArthur, R.H. (1972). Geographic ecology. Patterns in the Distribution of Species. Princeton Univ. Press, Princeton.

MacGowan, I. (1994). Creating breeding sites for *Callicera rufa* Schummel (Diptera, Syrphidae) and a further host tree. *Dipterists Digest*, 1, 6–8.

MacGowan, I. (1997). The entomological value of aspen in the Scottish Highlands. Unpublished Malloch Society Research Report, URL http://www.mallochsociety.org.uk

Maciá, A. & Bradshaw, W.E. (2000). Seasonal availability of resources and habitat degradation for the western tree-hole mosquito, *Aedes sierrensis*. *Oecologia*, 125, 55–65.

Mahmoud, m. (1999). Eristaline larvae and the role of bacteria in their feeding. *J.Union Arab Biol*, 11A, 417–433.

Maier, C.T. & Waldbauer, G. (1979). Diurnal activity patterns of flower flies (Diptera: Syrphidae) in an Illinois sand area. *Annals of the Entomological Society of America*, 72, 237–245.

Mäkinen, H.M.H., Hallaksela, A.M.H.A. & Isomäki, A.I.A. (2007). Increment and decay in Norway spruce and Scots pine after artificial logging damage. *Canadian Journal of Forest Research*, 37, 2130–2141.

Maleque, M.A., Maeto, K. & Ishii, H.T. (2009). Arthropods as bioindicators of sustainable forest management, with a focus on plantation forests. *Applied entomology and zoology*, 44, 1–11.

Mangel, M. & Munch, S.B. (2005). A life-history perspective on short-and long-term consequences of compensatory growth. *American Naturalist*, 166, E155–E176.

Marchetti, M. & metsäinstituutti, E. (2005). *Monitoring and indicators of forest biodiversity in Europe: from ideas to operationality*. European Forest Institute.

Marshall, D.J., Bonduriansky, R. & Bussière, L.F. (2008). Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. *Ecology*, 89, 2506–2517.

Martín-López, B., Montes, C., Ramírez, L. & Benayas, J. (2009). What drives policy decision-making related to species conservation? *Biological Conservation*, 142, 1370–1380.

Matz, C. & Kjelleberg, S. (2005). Off the hook-how bacteria survive protozoan grazing. *Trends in microbiology*, 13, 302–307.

Maudet, C., Miller, C., Bassano, B., Breitenmoser-Würsten, C., Gauthier, D. & Obexer-Ruff, G. (2002). Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in Alpine ibex (*Capra ibex* (ibex)). *Molecular Ecology*, 11, 421–436.

Meglecz, E., Anderson, S.J., Bourguet, D., Butcher, R., Caldas, A. & Cassel-Lundhagen, A. (2007). Microsatellite flanking region similarities among different loci within insect species. *Insect Molecular Biology*, 16, 175–185.

Metcalfe, N.B. & Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution*, 16, 254–260.

Milankov, V., Francuski, L., Ludoški, J., Stahls, G. & Vujic, A. (2010). Genetic structure and phenotypic diversity of two northern populations of *Cheilosia aff. longula* (Diptera: Syrphidae) has implications for evolution and conservation. *Eur. J. Entomol*, 107, 305–315.

Monro, K., Sinclair-Taylor, T. & Marshall, D.J. (2010). Selection on offspring size among environments: the roles of environmental quality and variability. *Functional Ecology*, 24, 676–684.

Morgan, I.J. & Metcalfe, N.B. (2001). Deferred costs of compensatory growth after autumnal food shortage in juvenile salmon. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268, 295–301.

Nylin, S. & Gotthard, K. (1998). Plasticity in life-history traits. *Annual Review of Entomology*, 43, 63–83.

Olivieri, G.L., Sousa, V., Chikhi, L. & Radespiel, U. (2008). From genetic diversity and structure to conservation: genetic signature of recent population declines in three mouse lemur species (*Microcebus* spp.). *Biological Conservation*, 141, 1257–1271.

Olson, R.R. & Olson, M.H. (1989). Food limitation of planktotrophic marine invertebrate larvae: does it control recruitment success? *Annual Review of Ecology and Systematics*, 20, 225–247.

Owen, J. (1981). Trophic variety and abundance of hoverflies (Diptera, Syrphidae) in an English suburban garden. *Ecography*, 4, 221–228.

Pal, S., Chatterjee, S., Chattopadhyay, J. & others. (2009). Role of competition in phytoplankton population for the occurrence and control of plankton bloom in the presence of environmental fluctuations. *Ecological Modelling*, 220, 96–110.

Paradise, C.J. (2004). Relationship of water and leaf litter variability to insects inhabiting treeholes. *Journal of the North American Benthological Society*, 23, 793–805.

Paradise, C.J. & Dunson, W.A. (1997). Insect species interactions and resource effects in treeholes: are helodid beetles bottom-up facilitators of midge populations? *Oecologia*, 109, 303–312.

Peakall, R.O.D. & Smouse, P.E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6, 288–295.

Pearce-Kelly, P., Jones, R., Clarke, D., Walker, C., Atkin, P. & Cunningham, A. (1998). The captive rearing of threatened Orthoptera: a comparison of the conservation potential and practical considerations of two species' breeding programmes at the Zoological Society of London. *Journal of Insect Conservation*, 2, 201–210.

Peer, K. & Taborsky, M. (2005). Outbreeding depression, but no inbreeding depression in haplodiploid ambrosia beetles with regular sibling mating. *Evolution*, 59, 317–323.

Peterken, G.F., Ausherman, D., Buchenau, M. & Forman, R.T.T. (1992). Old-growth conservation within British upland conifer plantations. *Forestry*, 65, 127–144.

Pfennig, D.W., Rice, A.M. & Martin, R.A. (2007). Field and experimental evidence for competition's role in phenotypic divergence. *Evolution*, 61, 257–271.

Piry, S., Luikart, G. & Cornuet, J.M. (1999). Computer note. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. *Journal of Heredity*, 90, 502–503.

Plummer, M., Best, N., Cowles, K. & Vines, K. (2006). CODA: Convergence diagnosis and output analysis for MCMC. *R News*, 6, 7–11.

Policansky, D. (1983). Size, age and demography of metamorphosis and sexual maturation in fishes. *American Zoologist*, 23, 57–63.

Preston-Mafham, K. (2001). Resource defence mating system in two flies from Sulawesi: *Gymnonerius fuscus* Wiedemann and *Telostylinus sp. near duplicatus* Wiedemann (Diptera: Neriidae). *Journal of Natural History*, 35, 149–156.

Price, C.S.C., Dyer, K.A. & Coyne, J.A. (1999). Sperm competition between Drosophila males involves both displacement and incapacitation. *Nature*, 400, 449–452.

Primack, R.B. (1998). Essentials of Conservation Biology, Second Edition. Sinauer Associates, Inc.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.

Purse, B. (2002). The Ecology and Conservation of the Southern Damselfly (*Coenagrion mercuriale–Charpentier*) in Britain. Unpublished PhD Thesis, University of Liverpool, Liverpool.

Purse, B.V. & Thompson, D.J. (2002). Voltinism and larval growth pattern in *Coenagrion mercuriale* (Odonata: Coenagrionidae) at its northern range margin. *European Journal of Entomology*, 99, 11–18.

R Team. (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Raebel, E.M., Merckx, T., Riordan, P., Macdonald, D.W. & Thompson, D.J. (2010). The dragonfly delusion: why it is essential to sample exuviae to avoid biased surveys. *Journal of Insect Conservation*, 14, 523–533.

Ranius, T. (2006). Measuring the dispersal of saproxylic insects: a key characteristic for their conservation. *Population ecology*, 48, 177–188.

Ranius, T., Martikainen, P. & Kouki, J. (2011). Colonisation of ephemeral forest habitats by specialised species: beetles and bugs associated with recently dead aspen wood. *Biodiversity and Conservation*, 20, 2903–2915.

Raymond, M. & Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of heredity*, 86, 248–249.

Reed, D.H. & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conservation biology*, 17, 230–237.

Reemer, M. (2005). Saproxylic hoverflies benefit by modern forest management (Diptera: Syrphidae). *Journal of insect conservation*, 9, 49–59.

Reinhold, K. (1999). Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Functional Ecology*, 13, 217–224.

Relyea, R.A. (2001). The relationship between predation risk and antipredator responses in larval anurans. *Ecology*, 82, 541–554.

Reznick, D.N. (1990). Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): an experimental evaluation of alternative models of development. *Journal of evolutionary Biology*, 3, 185–203.

Ricarte, A., Jover, T., Marcos-García, M., Micó, E. & Brustel, H. (2009). Saproxylic beetles (Coleoptera) and hoverflies (Diptera: Syrphidae) from a Mediterranean forest: towards a better understanding of their biology for species conservation. *Journal of Natural History*, 43, 583–607.

Ricarte, A., Marcos-García, M.A.A., Pérez-Bañón, C. & Rotheray, G.E. (2007). The early stages and breeding sites of four rare saproxylic hoverflies (Diptera: Syrphidae) from Spain. *Journal of Natural History*, 41, 1717–1730.

Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution*, 43, 223–225.

Roberge, J.M. & Angelstam, P. (2004). Usefulness of the umbrella species concept as a conservation tool. *Conservation Biology*, 18, 76–85.

Roberts, M.J. (1970). The structure of the mouthparts of syrphid larvae (Diptera) in relation to feeding habits. *Acta zoologica*, 51, 43–65.

Roff, D.A. (1992). The evolution of life histories: theory and analysis. NY: Chapman and Hall, New York.

Rotheray, E.L. (2010). Restoring the endangered pine hoverfly in the UK. In: Global Re-introduction Perspectives: 2010 Additional case-studies from around the globe (ed. Soorae, P.S.). IUCN/SSC Re-introduction Specialist Group, 21–24.

Rotheray, E.L., Greminger, M.P., Nater, A., Krützen, M., Goulson, D. & Bussière, L.F. (2011). Polymorphic microsatellite loci for the endangered pine hoverfly *Blera fallax* (Diptera: Syrphidae). *Conservation Genetic Resources,* 4, 117–120.

Rotheray, E.L., MacGowan, I., Rotheray, G.E., Sears, J. & Elliott, A. (2009). The conservation requirements of an endangered hoverfly, *Hammerschmidtia ferruginea* (Diptera, Syrphidae) in the British Isles. *Journal of Insect Conservation*, 13, 569–574.

Rotheray, G. (2001). Aspen, a vital resource for saproxylic flies. In: *The Biodiversity and Management of Aspen Woodlands*, 29 – 31.

Rotheray, G. & Gilbert, F. (1999). Phylogeny of Palaearctic Syrphidae (Diptera): evidence from larval stages. *Zoological Journal of the Linnean Society*, 127, 1–112.

Rotheray, G.E. (1991). Larval stages of 17 rare and poorly known British hoverflies (Diptera: Syrphidae). *Journal of natural history*, 25, 945–969.

Rotheray, G.E. (1993). Colour guide to hoverfly larvae (Diptera: Syrphidae). *Dipterist Digest No. 9*. Derek Whitely Sheffield, United Kingdom.

Rotheray, G.E. & Gilbert, F.S. (2011). The Natural History of Hoverflies. Forrest Text.

Rotheray, G.E., Hancock, G., Hewitt, S., Horsfield, D., MacGowan, I. & Robertson, D. (2001). The Biodiversity and Conservation of Saproxylic Diptera In Scotland. *Journal of Insect Conservation*, 5, 77–85.

Rotheray, G.E. & MacGowan, I. (2000). Status and Breeding Sites of Three Presumed Endangered Scottish Saproxylic Syrphids (Diptera, Syrphidae). *Journal of Insect Conservation*, 4, 215–223.

Rotheray, G.E. & Stuke, J. (1998). Third stage larvae of four species of saproxylic Syrphidae (Diptera), with a key to the larvae of British Criorhina species. *Entomologists Gazette*, 49, 209–217.

Rozen, S. & Skaletsky, H. (2000). Primer3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology*, 132, 365–386.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. (1998). Inbreeding and extinction in a butterfly metapopulation. *Nature*, 392, 491–494.

Salcher, M.M., Pernthaler, J., Psenner, R. & Posch, T. (2005). Succession of bacterial grazing defense mechanisms against protistan predators in an experimental microbial community. *Aquatic microbial ecology*, 38, 215–229.

Samways, M.J. (1994). Insect Conservation Biology. Chapman & Hall, London.

Samways, M.J. (2006). Insect extinctions and insect survival. *Conservation biology*, 20, 245–246.

Samways, M.J. (2007). Insect conservation: a synthetic management approach. *Annual Review of Entomology*, 52, 465–487.

Santana, Q.C., Coetzee, M.P.., Steenkamp, E.T., Mlonyeni, O.X., Hammond, G.N.. & Wingfield, M.J. (2009). Microsatellite discovery by deep sequencing of enriched genomic libraries. *BioTechniques*, 46, 217–223.

Schiegg, K. (2001). Saproxylic insect diversity of beech: limbs are richer than trunks. *Forest Ecology and Management*, 149, 295–304.

Schmidl, J., Sulzer, P. & Kitching, R. (2008). The insect assemblage in water filled tree-holes in a European temperate deciduous forest: community composition reflects structural, trophic and physicochemical factors. *Hydrobiologia*, 598, 285–303.

Schmidt-Nielsen, K. (1997). Animal physiology: adaptation and environment. Cambridge University Press.

Schmitt, T., Cizek, O. & Konvicka, M. (2005). Genetics of a butterfly relocation: large, small and introduced populations of the mountain endemic *Erebia epiphron silesiana*. *Biological Conservation*, 123, 11–18.

Schmuki, C., Vorburger, C., Runciman, D., Maceachern, S. & Sunnucks, P. (2006). When log-dwellers meet loggers: impacts of forest fragmentation on two endemic log-dwelling beetles in southeastern Australia. *Molecular Ecology*, 15, 1481–1492.

Schönrogge, K., Barr, B., Wardlaw, J.C., Napper, E., Gardner, M.G. & Breen, J. (2002). When rare species become endangered: cryptic speciation in

myrmecophilous hoverflies. *Biological Journal of the linnean Society*, 75, 291–300.

Schönrogge, K., Gardner, M.G., Elmes, G.W., Napper, E.K.V., Simcox, D.J. & Wardlaw, J.C. (2006). Host propagation permits extreme local adaptation in a social parasite of ants. *Ecology Letters*, 9, 1032–1040.

Schulte, J., Losos, J., Cruz, F. & Nunez, H. (2004). The relationship between morphology, escape behaviour and microhabitat occupation in the lizard clade *Liolaemus* (Iguanidae: Tropidurinae: Liolaemini). *Journal of Evolutionary Biology*, 17, 408–420.

Schultheis, A.S., Hendricks, A.C. & Weigt, L.A. (2002). Genetic evidence forleaky'cohorts in the semivoltine stonefly *Peltoperla tarteri* (Plecoptera: Peltoperlidae). *Freshwater Biology*, 47, 367–376.

Schultz, C.B., Dzurisin, J.D. & Russell, C. (2009). Captive rearing of Puget blue butterflies (*Icaricia icarioides blackmorei*) and implications for conservation. *Journal of Insect Conservation*, 13, 309–315.

Schultz, C.B., Russell, C. & Wynn, L. (2008). Restoration, reintroduction, and captive propagation for at-risk butterflies: a review of British and American conservation efforts. *Israel Journal of Ecology and Evolution*, 54, 41–61.

Scottish Natural Heritage. (2007). A Five Year Species Action Framework: Making a difference for Scotland's Species. Scottish Natural Heritage Publications: Battleby.

Seddon, J., Santucci, F., Reeve, N. & Hewitt, G. (2001). DNA footprints of European hedgehogs, *Erinaceus europaeus* and *E. concolor*: Pleistocene refugia, postglacial expansion and colonization routes. *Molecular Ecology*, 10, 2187–2198.

Seifert, R.P. & Seifert, F.H. (1979). A Heliconia insect community in a Venezuelan cloud forest. *Ecology*, 60, 462–467.

Sekar, S. (2011). A meta-analysis of the traits affecting dispersal ability in butterflies: can wingspan be used as a proxy? *Journal of Animal Ecology*, 81, 174–184.

Shaw, P. & Thompson, D.B.A. (2006). The Nature of the Cairngorms: Diversity in a changing environment. The Stationery Office.

Sherley, G.H. (1998). Translocating a threatened New Zealand giant orthopteran, *Deinacrida* sp.(Stenopelmatidae): some lessons. *Journal of Insect Conservation*, 2, 195–199.

Shertzer, K.W. & Ellner, S.P. (2002). State-dependent energy allocation in variable environments: life history evolution of a rotifer. *Ecology*, 83, 2181–2193.

Sih, A. (1986). Antipredator responses and the perception of danger by mosquito larvae. *Ecology*, 67, 434–441.

Simberloff, D. (1998). Flagships, umbrellas, and keystones: is single-species management passé in the landscape era? *Biological conservation*, 83, 247–257.

Skalski, G.T., Picha, M.E., Gilliam, J.F. & Borski, R.J. (2005). Variable intake, compensatory growth, and increased growth efficiency in fish: models and mechanisms. *Ecology*, 86, 1452–1462.

Smith, B.J. (2007). boa: An R package for MCMC output convergence assessment and posterior inference. *Journal of Statistical Software*, 21, 1–37.

Smith, G.F., Gittings, T., Wilson, M., French, L., Oxbrough, A. & O'donoghue, S. (2009). Identifying practical indicators of biodiversity for stand-level

management of plantation forests. *Plantation Forests and Biodiversity: Oxymoron or Opportunity?*, 9, 67–91.

Smith, R.L. & Smith, T.M. (2006). Elements of ecology. Benjamin Cummings.

Snook, R.R. & Hosken, D.J. (2004). Sperm death and dumping in Drosophila. *Nature*, 428, 939–941.

Sommaggio, D. (1999). Syrphidae: can they be used as environmental bioindicators? *Agriculture, ecosystems & environment*, 74, 343–356.

Sota, T. (1998). Microhabitat size distribution affects local difference in community structure: metazoan communities in treeholes. *Researches on population ecology*, 40, 249–255.

Sota, T., Mogi, M. & Hayamizu, E. (1994). Habitat stability and the larval mosquito community in treeholes and other containers on a temperate island. *Researches on population ecology*, 36, 93–104.

Speight, M. (1986). Criteria for the selection of insects to be used as bio-indicators in nature conservation research. *Proceedings of the 3rd European Congress of Entomology in Amsterdam*, 3, 485–488.

Speight, M.C.. (1989). Saproxylic invertebrates and their conservation. Council of Europe.

Speight, M.C.D. (2008). Species accounts of European Syrphidae (Diptera) 2008. *Syrph the Net, the database of European Syrphidae*, 55, 262.

Spielman, D., Brook, B.W. & Frankham, R. (2004). Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of the United States of America*, 101, 15261–15264.

Srivastava, D.S. (2005). Do local processes scale to global patterns? The role of drought and the species pool in determining treehole insect diversity. *Oecologia*, 145, 204–214.

Srivastava, D.S. & Lawton, J.H. (1998). Why more productive sites have more species: an experimental test of theory using tree-hole communities. *American Naturalist*, 152, 510–529.

Stearns, S.C. (1992). The Evolution of Life Histories. Oxford University Press, USA.

Stevens, V.M., Trochet, A., Van Dyck, H., Clobert, J. & Baguette, M. (2012). How is dispersal integrated in life histories: a quantitative analysis using butterflies. *Ecology Letters*, 15, 74–86.

Stillwell, R.C., Blanckenhorn, W.U., Teder, T., Davidowitz, G. & Fox, C.W. (2010). Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. *Annual review of entomology*, 55, 227–245.

Stockwell, C.A., Hendry, A.P. & Kinnison, M.T. (2003). Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution*, 18, 94–101.

Stoks, R., Block, M.D. & McPeek, M.A. (2006). Physiological costs of compensatory growth in a damselfly. *Ecology*, 87, 1566–1574.

Strand, M., Herms, D.A., Ayres, M.P., Kubiske, M.E., Kaufman, M.G. & Walker, E.D. (1999). Effects of Atmospheric CO2, Light Availability and Tree Species on the Quality of Leaf Detritus as a Resource for Treehole Mosquitoes. *Oikos*, 84, 277–283.

Stubbs, A. (1982). Hoverflies as primary woodland indicators with reference to Wharncliffe Woods. *Sorby Record*, 20, 62–67.

Stubbs, A.E. & Falk, S.J. (2002). British Hoverflies: an illustrated identification guide. British Entomological and Natural History Society.

Strange, J.P., Knoblett, J. & Griswold, T. (2009). DNA amplification from pin-mounted bumblebees (*Bombus*) in a museum collection: effects of fragment size and specimen age on successful PCR. *Apidologie*, 40, 134–139.

Summers, R. (2007). Stand selection by birds in Scots pinewoods in Scotland: the need for more old-growth pinewood. *Ibis*, 149, 175–182.

Summers, R.W., Proctor, R., Raistrick, P. & Taylor, S. (1997). The structure of Abernethy Forest, Strathspey, Scotland. *Botanical Journal of Scotland,* 49, 39–55.

Svensson, G.P., Sahlin, U., Brage, B. & Larsson, M.C. (2011). Should I stay or should I go? Modelling dispersal strategies in saproxylic insects based on pheromone capture and radio telemetry: a case study on the threatened hermit beetle *Osmoderma eremita*. *Biodiversity and Conservation*, 20, 2883–2902.

Sweeney, B.W. & Vannote, R.L. (1978). Size variation and the distribution of hemimetabolous aquatic insects: two thermal equilibrium hypotheses. *Science*, 200, 444–446.

Tallmon, D.A., Luikart, G. & Waples, R.S. (2004). The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology & Evolution*, 19, 489–496.

Taylor, F. (1981). Ecology and evolution of physiological time in insects. *American Naturalist*, 117, 1–23.

Teets, N.M., Kawarasaki, Y., Lee, R.E. & Denlinger, D.L. (2011). Survival and energetic costs of repeated cold exposure in the Antarctic midge, *Belgica*

antarctica: a comparison between frozen and supercooled larvae. *Journal of Experimental Biology*, 214, 806–814.

Telles-Romero, R., Toledo, J., Hernández, E., Quintero-Fong, J. & Cruz-López, L. (2011). Effect of temperature on pupa development and sexual maturity of laboratory *Anastrepha obliqua* adults. *Bulletin of entomological research*, 101, 565–571.

Templeton, A.R. (1986). Coadaptation and outbreeding depression. In: Conservation Biology, Sunderland, (ed. Soule ME), Mass: Sinauer, 105–116.

Thomas, J.A., Simcox, D. & Clarke, R.T. (2009). Successful conservation of a threatened *Maculinea* butterfly. *Science*, 325, 80–83.

Thomas, J.A., Simcox, D.J. & Bourn, N.A.D. (2011). The restoration of the large blue butterfly to the UK. Global Re-introduction Perspectives: 2011 (ed. Soorae, P.S.). IUCN/SSC Re-introduction Specialist Group, 10–14.

Tinsley, M., Blanford, S. & Jiggins, F. (2006). Genetic variation in *Drosophila melanogaster* pathogen susceptibility. *Parasitology*, 132, 767–773.

van de Bund, W.J., Goedkoop, W. & Johnson, R.K. (1994). Effects of deposit-feeder activity on bacterial production and abundance in profundal lake sediment. *Journal of the North American Benthological Society*, 13, 532–539.

Venables, W.N. & Ripley, B.D. (2002). Modern applied statistics with S. Springer.

Verrall, G.H. (1901). British Flies: Platypezidae, Pipunculidae, and Syrphidae of Great Britain. Gurney & Jackson, London.

Vinatier, F., Tixier, P., Duyck, P.F. & Lescourret, F. (2011). Factors and mechanisms explaining spatial heterogeneity: a review of methods for insect populations. *Methods in Ecology and Evolution*, 2, 11–22.

Vollbrecht, G., Johansson, U., Eriksson, H. & Stenlid, J. (1995). Butt rot incidence, yield and growth pattern in a tree species experiment in southwestern Sweden. *Forest Ecology and Management*, 76, 87–93.

Walker, E.D., Lawson, D.L., Merritt, R.W., Morgan, W.T. & Klug, M.J. (1991). Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. *Ecology*, 72, 1529–1546.

Walmsley, J. & Godbold, D. (2010). Stump harvesting for bioenergy–a review of the environmental impacts. *Forestry*, 83, 17–38.

Watanabe, K., Monaghan, M.T., Takemon, Y. & Omura, T. (2010). Dispersal ability determines the genetic effects of habitat fragmentation in three species of aquatic insect. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 20, 574–579.

Watts, P.C., Kemp, S.J., Saccheri, I.J. & Thompson, D.J. (2005). Conservation implications of genetic variation between spatially and temporally distinct colonies of the endangered damselfly *Coenagrion mercuriale*. *Ecological Entomology*, 30, 541–547.

Watts, P.C., Rouquette, J.R., Saccheri, I.J., Kemp, S.J. & Thompson, D.J. (2004). Molecular and ecological evidence for small-scale isolation by distance in an endangered damselfly, *Coenagrion mercuriale*. *Molecular Ecology*, 13, 2931–2945.

Weisberg, S. & Fox, J. (2010). An R companion to applied regression. Sage Publications, Inc.

Weslien, J., Djupström, L.B., Schroeder, M. & Widenfalk, O. (2011). Long-term priority effects among insects and fungi colonizing decaying wood. *Journal of Animal Ecology*, 80, 1155–1162.

Whitehorn, P.R., Tinsley, M.C., Brown, M.J.F., Darvill, B. & Goulson, D. (2010). Genetic diversity, parasite prevalence and immunity in wild bumblebees. *Proceedings of the Royal Society B: Biological Sciences*, 278, 1195–1202.

Whitehouse, N.J. (2006). The Holocene British and Irish ancient forest fossil beetle fauna: implications for forest history, biodiversity and faunal colonisation. *Quaternary Science Reviews*, 25, 1755–1789.

Willis, K.J., Bennett, K.D. & Birks, H.J.B. (1998). The late Quaternary dynamics of pines in Europe. In: Ecology and biogeography of Pinus, (ed. Richardson, D.M.). Cambridge University Press, 107–121.

Witkowski, Z., Adamski, P., Kosior, A. & Plonka, P. (1997). Extinction and reintroduction of *Parnassius apollo* in the Pieniny National Park (Polish Carpathians). *Biologia*, 52, 199–208.

Witzenberger, K. & Hochkirch, A. (2008). Genetic consequences of animal translocations: A case study using the field cricket, *Gryllus campestris* L. *Biological Conservation*, 141, 3059–3068.

Woodworth, L.M., Montgomery, M.E., Briscoe, D.A. & Frankham, R. (2002). Rapid genetic deterioration in captive populations: causes and conservation implications. *Conservation Genetics*, 3, 277–288.

Worrell, R. (1993). The location of aspen clones in Scotland. Unpublished report available from Scottish Natural Heritage Research and Advisory Directorate, Edinburgh.

Worrell, R. & Dunlop, B. (2003). The Influence of Past Management of Pinewoods on the Occurrence of Twinflower. Unpublished report available from Plantlife, URL http://www.plantlife.org.uk

Wratten, S.D., Bowie, M.H., Hickman, J.M., Evans, A.M., Sedcole, J.R. & Tylianakis, J.M. (2003). Field boundaries as barriers to movement of hover flies (Diptera: Syrphidae) in cultivated land. *Oecologia*, 134, 605–611.

Yanoviak, S.P. (1999). Community structure in water-filled tree holes of Panama: effects of hole height and size. *Selbyana*, 20, 106–115.

Yee, M., Grove, S.J., Richardson, A. & Mohammed, C.L. (2004). Brown rot in inner heartwood: why large logs support characteristic saproxylic beetle assemblages of conservation concern. In: Insect biodiversity and dead wood. Proceedings of a symposium at the International Congress of Entomology, Brisbane, Australia, August, 42–56.

Zackrisson, O. (1977). Influence of forest fires on the North Swedish boreal forest. *Oikos*, 29, 22–32.

Zeisset, I. & Beebee, T.J.C. (2001). Determination of biogeographical range: an application of molecular phylogeography to the European pool frog *Rana lessonae*. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268, 933–938.

Zera, A.J. & Denno, R.F. (1997). Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology*, 42, 207–230.