The ecology and conservation of *Leptidea reali* (Real's Wood White) in Northern Ireland

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Abstract

The 2001 discovery of *Leptidea reali* (Lepidoptera: Pieridae) represented the first new butterfly species to be found in the UK for over 100 years and is the only butterfly found in Northern Ireland that is not found anywhere else in the UK. However, little scientific research has been made into its ecology here. The present study used a combination of field observations and flight cage experiments to evaluate habitat usage and foodplant choice in populations of *L. reali* at two sites in County Armagh, throughout the 2008 flight period.

A total of 66 eggs were directly observed being laid or found in the field, with measurements made of the air temperature at the egg, the availability and percentage cover of all potential foodplants within a $1m^2$ quadrat, the height of the egg on foodplants, aspect and average sward height. The position of each egg location was mapped using GPS. A further 113 eggs were laid by 15 females during 20 flight cage experiments, with records made of where and on which foodplants all eggs were laid. At both sites a combined 151-nectaring events were recorded. A total of 6 specimens were confirmed as *L. reali* by dissection and examination of genitalia and a further 23 mid-leg samples were taken for future genetic analysis.

Results indicate that *L. reali* showed a preference towards *Lathyrus pratensis* for oviposition, both through field observed egg lays (78%) and in flight cages (80% of first eggs and 72% of all eggs). Eggs were also recorded in very small numbers on *Vicia sepium* and *Lotus penunculatus*. Nectaring was recorded on *V. sepium*, *L. pratensis, Veronica chamaedrys, L. corniculatus, Trifolium pratense, Vicia cracca* and *Lychnis flos-cuculi*. *L. reali* preferred *V. sepium* for nectaring on 57% of occasions and 81% of all nectaring events involved female butterflies. A series of further findings are described, including aspects of all stages of the *L. reali* life cycle, the occurrence of multiple egg laying events and courtship displays. A number of recommendations for general and site-specific management are made.

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1. INTRODUCTION

1.1 BACKGROUND

Butterflies provide vital ecological, economic and cultural services which are beneficial to human life on earth. These benefits range from inspiring poets, to pollination of commercial crops and indicators of climate change. They have also helped further scientific understanding of invertebrates, the constitution of species, and are fundamental to the study of mimicry rings.

However, these species face a number of threats, including habitat loss and poisoning of the countryside by increased use of pesticides. A combination of these and other factors can be used to explain the drastic decline in butterfly populations over the past 50 years across the UK. This has led conservation bodies to attempt to safeguard butterflies by a variety of methods, including habitat management and monitoring of populations.

Considering only 25 butterflies have been recorded in Northern Ireland (NI), it was a highly significant discovery in 2001, when the Real's Wood White butterfly *Leptidea reali* (Lepidoptera: Pieridae) was recorded there for the first time. The Northern Irish population not only represents 100% of the UK population but also a population at the western edge of its range.

However, little is known about the ecology of this important species in NI, including its habitat requirements, food plant preferences and oviposition. This review aims to gather what is known and what is not known about the status, conservation and ecology of *L. reali* in NI and how similar autecology studies on other butterfly species could be used to inspire future research. This is achieved through an appraisal of existing '*sinapis-reali* complex' literature and methodologies. Finally, an outline of recommended future research, including the aims of my own study, is presented.

1.2 'WOOD WHITE' BUTTERFLIES

1.2.1 THE *SINAPIS-REALI* COMPLEX

Much research had been made into the 'Wood White' butterfly in Europe before the description of *L. reali* in 1988 (Real, 1988). It was discovered in NI in 2001 (Nelson *et al.*, 2001). It is therefore crucial to point out that much of what has been written about *L. sinapis* prior to the discovery of *L. reali* could in fact be providing misleading information about the ecology of *L. sinapis*, or may actually be describing the ecology if *L. reali*, or indeed a combination of both. It is this confusion that prompted Benes *et al* (2003) to describe this dilemma as the "*sinapis-reali* complex". Thus, it is necessary to review the key literature on both "sister species" (Martin *et al*., 2003).

Discovery in Europe and Northern Ireland

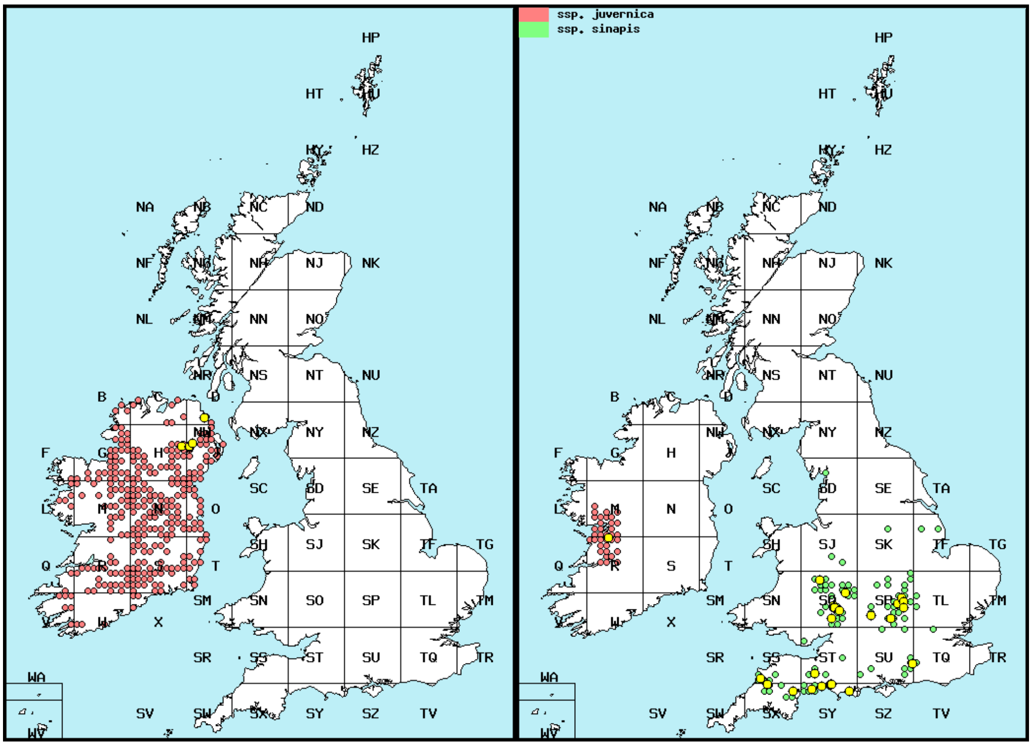
In 1988 a new species of *Leptidea* was described from specimens from the French Pyrenees by Real (1988), who noticed differences in the female ductus bursae between the well studied *L. sinapis* and this new species, which he called *L. lorkovicii*. The discovery was published in the little known journal '*Memoires de Comite*' and was not even mentioned in the title, a fact that meant it went almost unnoticed until a year later when Reisinger (1989) renamed the species *Leptidea reali*. Lorkovic (1993) showed that there were also consistent differences in the male aedeagus and saccus of both species and found that the female *L. reali* would not mate with male *L. sinapis*. This study also argues that minor differences in the form and colour of the pupae of the sibling species can be seen. Benes *et al*. (2003) describes the discovery as even more exciting since the new species was a sibling of one of the best-studied butterflies on the Continent.

It was not until 2001 that *L. reali* was confirmed in NI (Nelson *et al*., 2001). Examination of the genitalia of 34 specimens of *Leptidea* from across the UK and Ireland showed that *L. reali* was present in NI, while it also revealed that *L. reali* exists in at least 4 counties of the RoI. The 'sister species' *L. sinapis* was thought to be confined to 'The Burren' in County Clare, but recently its occurrence has been proven in the limestone pavement areas of

County Galway (Asher *et al*., 2006). The historic and current distribution of *Leptidea* in Britain and Ireland is shown in figure 1.

<u>Figure 1:</u> *The distribution of* L. reali *(A) and* L. sinapis *(B) in Britain and Ireland.*

(A) (B)



(Source: www.ukbutterflies.co.uk)

Separation from *L. sinapis*

L. reali is virtually impossible to recognise in the field on the basis of external morphology alone. Instead, a variety of techniques have been used to confirm its differentiation from *L. sinapis*.

Following Lorkovic's (1993) discovery that *L. reali* could be accurately identified by greatly prolonged genitalia alone, a number of other studies have used both the male and female genitalia to confirm *L. reali* in countries across Europe (Mazel & Leestmans, 1996; Karsholt, 1999; Freese & Fiedler, 2002). The main differences between the male and female genitalia of *Leptidea* can be seen in figure 2.

It has also been shown to be a new species through molecular evidence (Martin, 1997) and a pre-mating isolation mechanism (Freese, 1999). Furthermore, ecological studies have shown that the two species differ in foodplant preferences (Freese, 1999; Herman, 1999). *L. reali* prefers meadow vetchling *Lathyrus pratensis* while *L. sinapis* favours common bird's-foot-trefoil *Lotus corniculatus.* More recently the use of DNA-sequences (Martin *et al*., 2003) has supported the case of *L. reali* to be recognised as a species in its own right.

The separation of the 'cryptic species' by a range of techniques has been evaluated in publications by Freese and Fiedler (2002; 2004). They drew a number of conclusions that suggested *L. reali* and *L. sinapis* cannot be distinguished via the morphology of eggs, larvae or pupae or the colouration and patterns of their wings. All in, it can be stated that, with male as well as with females, the lengths of the genitalia are the most suitable characteristics for definition (Freese & Fielder, 2002). Freese & Fiedler (2002) aimed to investigate the possibility of speciation through sexual selection and female choice through controlled experiments, to add weight to the "anecdotal observational evidence" used to separate the species thus far. The study (Freese & Fiedler, 2002) found that, in mating experiments in flight cages, the females and males of both species discriminated among species during mate choice and only intraspecific mating was recorded, thus supporting the notion that *L. reali* and *L. sinapis* are good species with ethological reproductive isolation.

One area where it is possible to distinguish between the two species in the field is during courtship displays. Friberg *et al* (2008[1]) and Friberg & Wiklund (2007) both present parallel differences between the species. During courtship all *L. sinapis* males performed a couple of wing beats at irregular intervals during courtship, while all *L. reali* males were found to express quite the opposite, keeping their wings in a rested position throughout the courtship.

Key studies and their methods across Europe- pre and post discovery

Before the 1988 discovery, *L. sinapis* was a model for behavioural research (Wiklund, 1977[1,2] & 1984; Wiklund *et al*., 1979). In the UK it was one of the species for which the method of butterfly transects was developed (Pollard, 1982) and helped establish the active management of woodland for butterflies (Warren, 1985; Warren *et al*., 1986). Much detail was known about its biology and status in Britain (Warren, 1984) and it had also been studied by Heal (1965) in NI. The arrival of *L. reali* introduced confusion into the knowledge of this much-studied system (Benes *et al*., 2003).

The methods used to study *L. sinapis* pre 1988 were based mainly on observations from the field. These ranged from the purely "circumstantial" evidence of *L. sinapis* spreading via railway routes given by Heal (1965), to the more rigorous observations made by Warren (1984). The latter study summarises detailed ecological work carried out on *L. sinapis* over several years, mostly at two sites in England but also at "several localities" in Ireland in 1978. The studies aimed to detail the species' biology, behaviour and habitat use. The methods employed included observations on eggs and larvae by following their development on marked foodplants, with the position on plant and development stage of larvae recorded twice a week. Similarly, adult behaviour was studied by observing individual butterflies and recording time spent on various activities such as nectaring, courtship and oviposition. Attention was also given to the fact that all observations were carried out when weather conditions were ideal for adult activity; when the air temperature exceeded 17^{o}C, which harks back to the criteria of the Butterfly Monitoring Survey (BMS) proposed by Pollard (1977).

Since the discovery of *L. reali* in 1988, studies into the '*sinapis-reali'* complex have moved towards a more experimental perspective that allows the comparison of field and laboratory data. These range from the numerous techniques used to separate the species outlined above, to more experimental methodologies including the techniques of captive breeding, catch and release and flight cage experiments.

Benes *et al* (2003) pointed out the disparity that exists between studies on *Leptidea* that consider casual observational data compared to those based upon quantitative information that can be analysed statistically. They describe work up until 2003 as focused on fundamental questions of a rather descriptive nature. They also claimed that their study would be the first to quantitatively assess the habitat preferences of the two species. Freese & Fiedler (2002) are also critical of previous studies derived from evidence without controlling any confounding variables and without any statistical evaluation of the results. As a result, their study aims to use methods including mating experiments in flight cages, while ovipositioning preferences were gauged by putting live caught females into "glasses" and then offering them three different known foodplants and recording which they preferred. These studies represent a crucial change in the way *Leptidea* are studied.

A further Freese & Fielder study (2004) compared the usefulness of species separation techniques and introduced a number of 'new' techniques to the study of *Leptidea*. For instance, specimens of *L. sinapis* and *L. reali* were caught alive and allowed to lay eggs at a different location with the subsequent caterpillars being raised in climatised jars. These 'captive bred' specimens were then individually marked and used in mating experiments that took place in 'flight cages'.

A number of these new methodologies are amalgamated in the recent work of Friberg *et al*. (2008[1, 2]). This research combines observational field studies with laboratory and field experiments, to investigate fields such as the role of female choice in mating and niche separation in space, time and voltinism between *Leptidea* species. This latest research demonstrates the value of the techniques outlined in table 1.

<u>Table 1:</u> *Study techniques employed by Friberg* et al *(2008[1, 2]).*

- DNA sequencing techniques proposed by Martin *et al*. (2003) were used instead of genital examination to separate species.
- Used laboratory mating experiments of 20 "wild caught" *Leptidea* females. With females being offered two known foodplants for both species to study ovipositioning.
- Pupae were kept outdoors "on the balcony…of Stockholm University".
- Courtship displays were recorded by releasing a female into a flight cage and then introducing a male. Females of both species were presented to both conspecific and hetrospecific males. A further development was recording the displays on video.
- Used a field experiment where 28 *L. sinapis* and 34 *L. reali* females were individually released and followed until meeting a male. Any resulting courtship was again recorded by both stopwatch and video camera.
- Analysed over-wintering generations to assess whether the differences in phenology in the field were inherent or caused by differences in the habitats used by the two species.
- An example of using a controlled variable in an experiment is given through the analysis of the development rate of specimens at constant temperatures using varying daylengths and host plants. This was used to see if the difference in voltinism shown between the two species could be driven by habitat preference.

Some equally important points raised by the literature include the need to mark each individual specimen used in any experiment, in order to avoid pseudo-replication (Friberg &

Wiklund, 2007). It is also important to note that none of the post 1988 examples discussed are from NI or, for that matter, the UK.

Agreements, disagreements and omissions

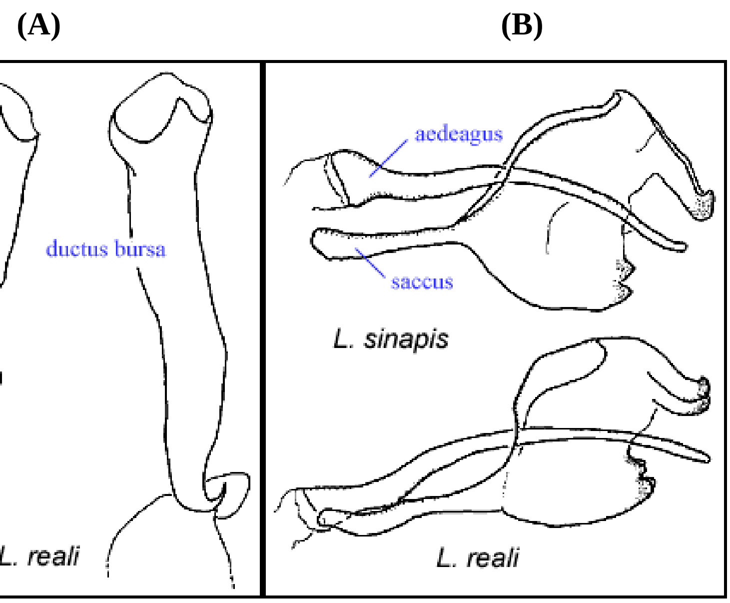
Other studies have shown *L. reali* to be widely distributed across Europe, often sympatrically with *L. sinapis*. For instance a study by Mazel & Leestmans (1996) shows this to be the case in France. This study also provides one of the many disagreements that appear in the literature. It is claimed that only *L. sinapis* is found in Corsica, yet the opposite is reported by Lorkovic (1993). Another disagreement comes in Lorkovic's description of differences in the morphology of pupae, since subsequent studies (e.g. Freese & Fiedler, 2004) could not find any secure features for separating the species using pupae, or indeed any other pre-imago phase.

Interestingly, in Britain populations of *L. sinapis* have been decreasing in its preferred habitat in woodland rides and glades (Warren & Bourn, 1998). In Ireland, where the species occurs in more open habitats, it has been increasing (Rippey, 1986). This spread is argued by Heal (1965) to have been facilitated by old railway lines in NI.

L. reali is the only species of butterfly which exists in Ireland that cannot be found in Britain. This seems extremely odd, considering that the conditions exist in Britain for it to colonise there and the vectors (e.g. humans or own flight) that brought *L. reali* to NI, must be assumed to have influenced Britain. Also, it is perplexing to think that *L. sinapis* does not exist in NI when it does in the Republic of Ireland. A number of interesting issues stem from the Nelson *et al* (2001) study, one of which is the use of only 34 specimens of *Leptidea* in a study to ascertain species existence at a number of sites across the UK and Ireland. Another is the decision not to include any specimens from the best known 'Wood White' sites in NI such as its strongholds at Craigavon Balancing Lakes, County Armagh or Montiaghs Moss NNR, County Antrim. Indeed 'Wood Whites' from only 8 different sites in NI were examined. A further source of confusion is over one specimen taken from the Ulster Museum (which beforehand was believed to contain specimens of solely English origin) that was found to belong to the *L. reali* species. Instead of representing the confirmation of the species in England, the specimen is described as "unknown" and "unlabelled". The authors do note, however, that this matter should be addressed. Assurance has also been given by the authors that several hundred specimens have now been examined and that details will be published sometime during winter 2008 (M. Hughes, pers. comm.).

Lorkovic (1993) states the belief that there is an increase in the size of genital apparatus from east to west Europe. Freese and Fiedler (2004) showed that the genitalia of male butterflies of both species raised under various laboratory conditions, showed considerable overlap in length of aedeagus between species. Is there not a case that perhaps the *Leptidea* in NI show their genitalia range due to climatic conditions, particularly temperature?

<u>Figure 2:</u> *A comparison of* Leptidea *genitalia, showing the female ductus bursae (A) and male aedeagus and saccus (B) of* L. reali *and* L. sinapis.



(Source: www.toyen.uio.no)

A grey area also exists in the literature when it comes to separating the two species using the length of genital apparatus (see figure 2). These lengths are given ranges in Freese & Fiedler (2004) of 1.64-1.70mm for the aedeagus and 0.64-0.72mm for length of saccus. Any specimen falling between these ranges is difficult to assign to species. The importance of this becomes clear when compared to the ranges given in Nelson *et al* (2001) used to separate the two species in several European countries. A number of countries show *L. reali* with aedeagus lengths within the grey areas given above. Perhaps these size differences can be explained by the overall smaller sizes of butterflies across Europe proposed by Lorkovic (1993), or this matter still needs to be resolved.

Moreover, Freese & Fiedler (2002) highlight the point that even though genitalia are known to exhibit phenotype plasticity and interspecific variation (Goulson, 1993; Monti *et al*., 2001), there is increasing evidence that in many cases genitalic differentiation is more related to sexual selection and cryptic female choice rather than maintaining reproductive isolation. Descimon & Mallet (2007) suggest that the techniques used to separate *Leptidea* may merely represent a 'genitalic polymorphism', similar to that found in *Melitaea athalia*, where two types of male genitalia were associated with two biogeographical entities and split into two species, only to later be found to represent a 'hybrid zone' between the two species. Similarly, Martin *et al*. (2003) found a 5% overlap between the *Leptidea* taxa, while Verovnik & Glogovcan (2007) found two specimens with *L. reali* genitalia measurements that genetically belonged to *L. sinapis* and showed species specific RAPD markers of both species, indicating a probable hybrid origin, thus challenging the species status of *L. reali*. It was perhaps, not before time then, that ways of discriminating one species from another grew from the preparation of genitalia to the variety of techniques available today.

Nevertheless, the discovery of the first new butterfly species in the UK since the Essex Skipper *Thymelicus lineola* in 1889 and the first to be found in Ireland since the Pearl-bordered Fritillary *Boloria euphrosyne* in 1922, has posed new challenges to butterfly conservation, while at the same time helping to solve others.

The discovery could explain why Wood Whites in Britain are mainly found in woodland habitats and are in decline, while the 'Wood Whites' of Ireland are expanding and live in more open habitats as observed by Rippey (1986). It has also focused conservation efforts in England towards saving the dwindling *L. sinapis* populations, while the challenges in NI are to discover why *L. reali* chose to colonise here and nowhere else in the British Isles (if this is confirmed to be the case). What, if any, adaptations have they made to life in NI (perhaps they prefer a different foodplant or produce less generations than their European conspecifics) and which conservation methods can be used to protect them?

1.2.2 *L. REALI* IN NI- WHAT WE KNOW AND WHAT WE DON'T KNOW

Put simply, we do not know a great deal about the ecology of *L. reali* in NI. Much of what is written actually refers to previous *L. sinapis* research (often from Britain) and records. Since the discovery of *L. reali* in NI (Nelson *et al*., 2001), little research has been carried out into this 'new' UK species. Certainly, none appears in the published literature. Therefore, much of what follows in this section is based upon the most up to date species accounts available via the Internet and a publication by Thompson & Nelson (2006). It is understood from these sources and through personal communication with BCNI, that some research into the species and its relationship with *L. sinapis* has been undertaken by Northern Irish experts.

Status

L. reali is a SOCC and a priority species in NI, making it an offence to collect for resale. It is not listed as a UK species requiring a BAP. It is not included in the Wildlife Act (NI) 1985 as one of the 7 butterfly species to gain legal protection. However, this species was only discovered in NI by Nelson *et al* (2001). The population in NI not only represents 100% of the species in the UK, but also a population at the outer range of its distribution. It has been listed as a priority species in NI due to a perceived decline in its population, although it is believed *L. reali* is increasing at some monitored sites (Nelson & Hughes, 2005).

The Environment and Heritage Service (EHS) states that a number of priority species, which do not have UK action plans, will require NI action plans. These are species which:

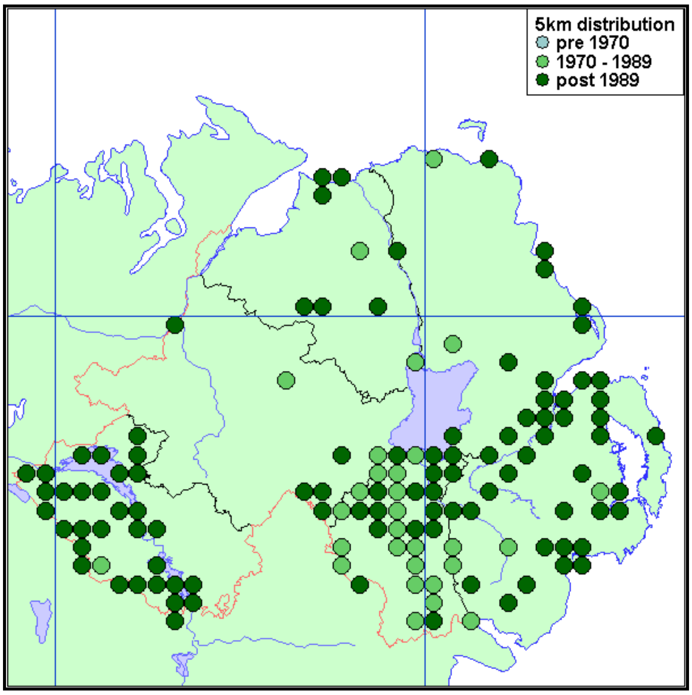
- Require urgent conservation action.
- Illustrate particular conservation problems.
- Are restricted to Northern Ireland in the UK and Ireland.
- Are declining flagship species which are viewed as important by the NI public and can be used to illustrate wider biodiversity issues.

It is thought that *L. reali* meets these criteria. However, little research as been made into its ecology in NI and, as yet, sites are not designated or managed for the species. This is where my study is intended to help. Records indicate that *L. reali* was exceptionally rare in NI and started spreading northwards in the 1950's (Heal, 1965) but now appears to be in retreat.

Distribution

It is widely assumed that past Northern Irish records of *L. sinapis* are, in fact, representative of *L. reali*. Therefore, the species can be found in all 6 counties of the jurisdiction. Figure 3 shows its distribution to be mostly southern, with strongholds around Craigavon in County Armagh and around the County Fermanagh lakes.

<u>Figure 3:</u> *The distribution of* L. reali *in Northern Ireland.*



(Source: www.habitas.org.uk)

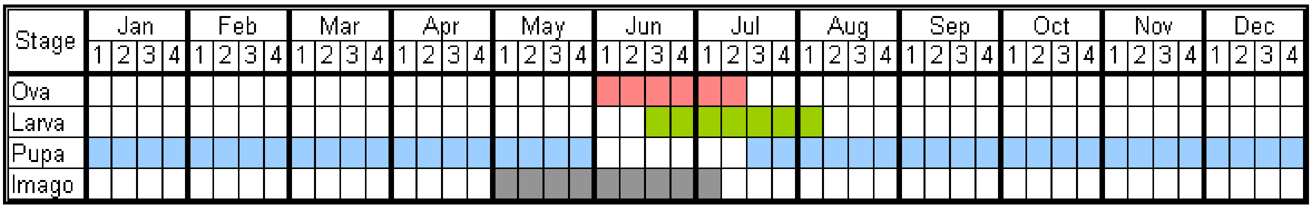
Habitat requirements

It breeds in mainly open areas with patches of scrub. Its preferred habitats include flower-rich grassland such as dunes, rough grassland, abandoned quarries, hedges, verges, limestone grassland and disused railway lines (Thompson & Nelson, 2006; www.habitas.org.uk; www.ukbutterflies.co.uk; www.butterfly-conservation.org).

Life cycle

The recorded flight period is 9th April to 21st September but the main flight period is in May and June, as shown in figure 4. Some late records in August and September suggest an occasional second brood. Eggs are laid singly on the upper parts of the larval foodplants meadow vetchling *Lathyris pratensis* and bird's-foot trefoil *Lotus corniculatus* and hatch after around ten days. The caterpillar has been found eating the younger, more nutritious growth at the top of the foodplant and, after about four weeks, moves off to hibernate in tall grasses or scrub where it overwinters as a pupa (Thompson & Nelson, 2006 & www.habitas.org.uk).

<u>Figure 4:</u> *The life cycle of* L. reali *in Northern Ireland.*



(Source: www.ukbutterflies.co.uk)

Food plant preferences

Work by Thompson & Nelson (2003) implied that further investigation was needed into the ecology of *L. reali* in NI and how it differs from the 'true' Wood White *L. sinapis*. At this time, the principal larval foodplant was thought to be meadow vetchling *Lathyrus pratensis*, but records suggested it also used bush vetch *Vicia sepium* and bird's-foot trefoil *Lotus corniculatus*. Furthermore, studies on the Continent suggested that *L. reali* only feeds on meadow vetchling but this, as yet, has not been confirmed in NI.

By 2006, Thompson & Nelson only state that larvae have been recorded on *L. pratensis* and *L. corniculatus*. The latest information from BC also agrees that foodplants are not well known, but now include the additional species of tufted vetch *Vicia cracca* and greater bird's-foot trefoil *Lotus pedunculatus* in NI. The available information suggests that *L. reali* caterpillars feed on various legumes in NI, but no research has yet been undertaken into the foodplants they actually prefer.

1.3 SUMMARY AND AIMS OF STUDY

"In conserving butterflies in the countryside it is necessary to know as much as you can about the role they play in the habitats in which you find them. This role in nature is called a niche" (Feltwell, 1995).

It is clear that even following 20 years of recognised existence of *L. reali* in Europe and over 7 in NI, the two *Leptidea* siblings still constitute an enigma (Benes *et al*., 2003). This review has highlighted the existence of a wide range of methods used to study *L. reali* across

Europe. It has also delivered the message that detailed ecological knowledge of the species is lacking in NI and that, without it, conservation attempts are not viable at the present time. This predicament has been realised by BCNI, who produced an outline of the research that is needed on *L. reali* in NI (www.irishmoths.fsnet.co.uk). The important elements are shown in table 2.

Table 2: *Planned future research into* Leptidea *in Northern Ireland.*

- **Morphology of larvae and pupae**- detailed descriptions needed. What differences, if any, can be seen between *Leptidea*?
- **Nomenclature/Taxonomy**- subspecies and species names to be reviewed.
- **DNA**- does genetic analysis split Irish specimens into two separate species?
- **Biology**- detailed ecological information is required about all stages of development.
- **Distribution and sympatry**- confirm speciation and distribution across NI and study populations in areas in RoI where both species live side by side.
- **Habitat preferences**- experimental research required to discover which habitat *L. reali* actually prefers out of the variety it inhabits.

(M. Hughes, pers. comm.)

My review of the existing literature on *L. reali* has revealed that a number of crucial issues are unknown surrounding its ecology and conservation in NI. The overall aim of this thesis is to provide ecological management information on *L. reali* that could be used to help produce a Northern Ireland Species Action Plan (NISAP). My study seeks to address some of the questions raised by this review, as shown in table 3.

Table 3: *Aims and questions of my own research.*

- Are all the butterflies I study *L. reali*?
- How many individuals are there per site studied?
- What does *L. reali* prefer as its foodplant in the field compared to choice in a flight cage experiment?
- Where on the foodplant does oviposition take place?
- How many eggs are laid per session and/or plant?
- Habitat requirements- gauged through a basic overall vegetation survey of sites and detailed botanical quadrats at each egg-laying location.

- The influence of vegetation cover, aspect and temperature on ovipositioning.
- How is the mating display initiated? How long does it last? What differences are there from *L. sinapis* displays?
- Sources of nectar?
- How long does it take an egg to become a caterpillar?
- How long does it take for an egg to become a pupa?

The 2001 discovery of *L. reali* in NI represented the first new butterfly species to be found in the UK for over 100 years. At first this finding aroused great excitement in the Lepidoptera world, but it soon became clear that very little was known about even the basics of its life cycle or distribution. Scientists began the process of monitoring, autecological and speciation studies, which so far have helped to confirm its trends, distribution and position as a true species. The species is nearing its northern and western range in NI and has colonised this location despite ecological isolation from Britain and the Continent. Yet, to this day, very little knowledge or understanding exists to explain why.

I believe that my detailed ecological study on the Northern Irish population of *L. reali* will aid conservation measures which need to be introduced to ensure the future of the species in Northern Ireland.

2. SITES AND METHODS

2.1 SITE LOCATIONS

The two sites used in the present study are both in County Armagh and were chosen as one (Craigavon Lakes) is believed to be the best site in Northern Ireland to see large numbers of *L. reali* in a confined area. The other (Oxford Island NNR) was chosen due to its proximity to Craigavon Lakes, its well-documented population of *L. reali* and the fact that it offers different habitats and vegetation. The use of two sites also benefits comparative analysis. The location of both sites is shown in figure 5.

Figure 5: *Map of study locations*

2.2 RELATIONSHIPS WITH PREVIOUS STUDIES

The techniques involved in my research to a large extent involved field observations and flight cages, not to mention the laboratory preparation of genitalia to determine speciation.

A study by Jeffcoate (2006) looked at the use of vegetation resources by *L. sinapis* in Britain and is particularly relevant to my research. Although Jeffcoate (2006) does not focus on *L. reali*, it does deal with most of the topics I cover in my thesis and presents a current guide to the techniques that are available. The following are just some of the methods I utilised:

- Focusing attention on one main site, with information backed up by several other sites. The grassland around Craigavon Balancing Lakes in County Armagh is believed to be the most populous *L. reali* site in NI. I carried out the bulk of my research at this site. Once a good grasp of techniques and an understanding of basic ecology had been gained, additional data were gathered from Oxford Island NNR, County Armagh.

- Meteorological notes were made (by Jeffcoate, 2006) of temperature, percentage of sun exposure and wind speed (again related to criteria for BMS proposed by Pollard, 1977). I recorded ambient shade temperature, temperature at eggs and the aspect of any slopes used for egg laying. Such measurements could allow, for example, the correlation between temperature and height above ground of egg laying to be estimated.

- The identification of live caught specimens only took place towards the end of the breeding period, when mating and egg laying were coming to an end. The identification of a number of specimens was confirmed by dissection of genitalia.

- The number of adults visible was recorded over the time period of each site visit, expressed as a timed count per hour. This information could be used (in conjunction with BMS data at some sites) to ascertain voltinism.

- All nectaring episodes were recorded with the plants used.

- The location chosen by butterflies to mate was also noted (Jeffcoate, (2006) as was the possibility of mating occurring at sites with “white objects”), as well as timing, observing and video recording courtship displays. Any multiple egg laying episodes were of particular interest.

- Egg laying- female ovipositioning, foodplant selection and use. Egg site details were noted including surrounding vegetation and height above ground. Foodplant preference were studied by introducing an egg laying female into a flight cage and offering it a number of known foodplants that are available in the field. This technique provided a way to discover which foodplant *L. reali* actually prefers when given a selection to choose from.

- The larvae and pupae- while not intended to be a major feature of my study, were followed and their development recorded and photographed, if the opportunity arose.

2.3 SITE DESCRIPTIONS

2.3.1 CRAIGAVON LAKES

Craigavon Balancing Lakes are situated to the south of Lough Neagh and to the north of Craigavon itself (grid reference J050580). The 'lakes' are actually one large body of water, joined by a culvert at its western end. The lakes were built as part of the original 1970's plan to create a new age city away from Northern Ireland's urbanized east coast. The purpose of the lakes was twofold, firstly to provide a recreational facility for the citizens of Craigavon but also to have a practical role. The south lake, which is slightly larger, acts as a balancing lake, releasing collected rainwater at a controlled rate, into Lough Neagh.

The lakes are shallow, with an average depth of 2 metres and were formed in a natural depression in boulder clay soil (Austin, 2004). The two bodies of water are separated by the Belfast to Dublin railway line, possibly a factor in *L. reali* emergence at this site (Heal, 1965). Anglers and water sports enthusiasts use the southern lake, while the north lake has been left to nature. Walkers and cyclists frequent the surrounding area.

The vegetation in the central area near the railway line is home to a wide range of plants, which support a diverse array of Lepidoptera. The vegetation of particular interest to the present study in this location, includes the potential larval foodplants of *Lathyrus pratensis, Vicia sepium, Lotus corniculatus* and *Vicia cracca.* I was able to draw upon previous vegetation studies at the site, to build up a mental and physical map (figure 6) of the most likely areas of importance for *L. reali*. This information came from a 2004 NVC habitat survey of 66 quadrats in the central section of the site (Austin, 2004). I was also able to pinpoint the areas to include in the present study from consulting previous butterfly Pollard data from the site (a weekly predetermined BMS transect through a site, counting butterfly numbers once a throughout the flight period).

In general the habitat on either side of the railway track, where a steep bank is present, is dominated by *Crataegus monogyna, Salix spp* and *Rubus fruticosus*. The northeast and southeast of the site have been planted with *Sorbus aucuparia*, *Sorbus aria*, *Prunus avium*, *Alnus glutinosa*, *Quercus robur* and *Fraxinus excelsior*.

Out of the 66 quadrats studied in 2004, a total of 41 quadrats (62%) contained *Lathyrus pratensis,* 22 quadrats (33%) contained *Vicia sepium*, 20 quadrats (30%) contained *Lotus corniculatus* and only 14 quadrats (21%) contained *Vicia cracca.* The vegetation for each quadrat sample was placed into a Vespan computer program, which matched the data with diagnoses for NVC habitats.

The results indicate that the main visual NVC habitat is MG 5. An MG 5 is a *Cynosurus cristatus – Centaurea nigra* grassland habitat which is represented by the following vegetation: *Festuca rubra, Cynosurus cristatus, Holcus lanatus, Dactylis glomerata, Agrostis capillaris, Anthoxanthum odoratum, Lolium perenne, Lotus corniculatus, Plantago lanceolata, Trifolium repens, Centaurea nigra, Trifolium pratense, Achillea millefolium, Prunella vulgaris, Leontodon autumnalis, Ranunculus acris* and *Rumex acetosa* (Francis and Dixie, 1996). This community is species-rich lowland meadow vegetation and its topography may contain some ridges (Rodwell, 1998).

The results also show that a MG 1-habitat type is present in the Craigavon Lakes area. A MG 1 habitat is *Arrhenatherum elatius* grassland and a characteristic species here is *Dactylis glomerata*. This habitat is ungrazed and is usually associated with road verges, railway embankments, churchyards and neglected agricultural and industrial habitats (Rodwell, 1998).

The Vespan computer program compared all of the 66 quadrats and suggested that overall the Craigavon Lakes area can be classified as an NVC, MG 9 habitat. A MG 9 habitat is a *Holcus lanatus - Deschampsia cespitosa* grassland with both as widespread species. The vegetation is often mosaic and the habitat can be found on steep sloping pastures, woodland ridges and clearings, road verges, churchyards and fen margins (Austin, 2004).

The area included in the present study follows, to a large extent, the same area as the current butterfly Pollard transect. This Pollard was set up with the monitoring of *L.reali* in mind and covers the habitat where the largest numbers of the species are believed to occur. It is not surprising that this route also coincides with the areas where a large proportion of the ground is covered by potential larval foodplants and nectar sources. An important distinction to make between this study and the methodology of a BMS, is that I was not restricted to following a predetermined line through several transect sections. Allowances were made so that butterflies could be followed and a larger area was covered than would have been the case using the BMS methodology. Figure 6 shows the area studied throughout this project.

This area has recently been designated an LNR due to the large colony of *L. reali* it supports. The site is largely left unmanaged, apart from recent path improvement and gorse removal work on the southern side. Small areas are cut annually for hay in the late summer. I proposed to visit this site on 30 different occasions (3-7 visits per week) from mid April to mid July.

2.3.2 OXFORD ISLAND NATIONAL NATURE RESERVE

Oxford Island is situated at the southern end of Lough Neagh (grid reference J050610), the largest freshwater lake in the UK, covering an area of 383 km^2 (jncc.gov.uk). The landscape surrounding the lough is a mixture of pastures, wetlands, plantations and small settlements, many of which constitute high quality wildlife habitats (www.ehsni.gov.uk). The reserve is a popular location for families, school groups and wildlife enthusiasts. Several walking trails lead through the range of habitats and several bird hides are positioned along the banks of the lough.

The grasslands within Oxford Island NNR are managed in several different ways. Areas have been subject to annual cutting, grazing by livestock; or sites have not been managed and vegetation has been allowed to become more rank. Oxford Island NNR has records of butterfly surveys dating back to 1980, and therefore has a substantial long-term dataset that could be used to identify key *L. reali* sites.

The reserve underwent an NVC survey in 2006 (Davis) and from this and past BMS data, I was able to highlight areas suitable for study in this project and also to dismiss areas as unsuitable habitat for *L. reali*. The main area within Oxford Island NNR for *L. reali* is Kinnegoe Meadows. This area can generally be described as wet grassland pasture. It is grazed during the winter months to promote a short sward in the spring and summer to encourage a traditional wildflower meadow. The NVC survey (Davis, 2006) splits this area in two, Kinnegoe Meadows and Kinnegoe East. The former is classified as MG9 *Holcus lanatus-Deschampsia cespitosa* grasslands, while Kinnegoe East is classified as MG4 *Alopercurus pratensis- Sanguisorba officinalis* grassland; in other words a lowland grassland characteristic where traditional hay meadow treatment has been applied (Rodwell, 1998). This section of Kinnegoe Meadows is believed to be the most floristically rich on the reserve (M. Malley, pers. comm.) and would be expected to attract a large number of *L. reali* seeking nectar and foodplants.

Perhaps the most important difference between this site and Craigavon Lakes is that *Lotus corniculatus* is largely replaced by *Lotus penunculatus* (another potential foodplant) owing to the moist nature of the soil. Other areas deemed worthy of inclusion in the present study are the area described as the Western Shore and some other woodland clearings. These areas were chosen if they had received regular records of *L. reali* on past BMS transects, showed suitable habitat according to the 2006 NVC survey or were areas that looked floristically interesting during pre-study site visits.

During the 2006 NVC, Kinnegoe Meadows was classified through the study of 16 quadrats. Out of these, *Lotus pedunculatus* was present in 15 (94%), *Vicia cracca* was recorded in 12 transects (75%) and *Lathyrus pratensis* was present in 10 (63%).

Figure 7 shows the areas studied at this site. It was proposed to visit this site on 20 occasions (3-7 times per week) from mid April until mid July.

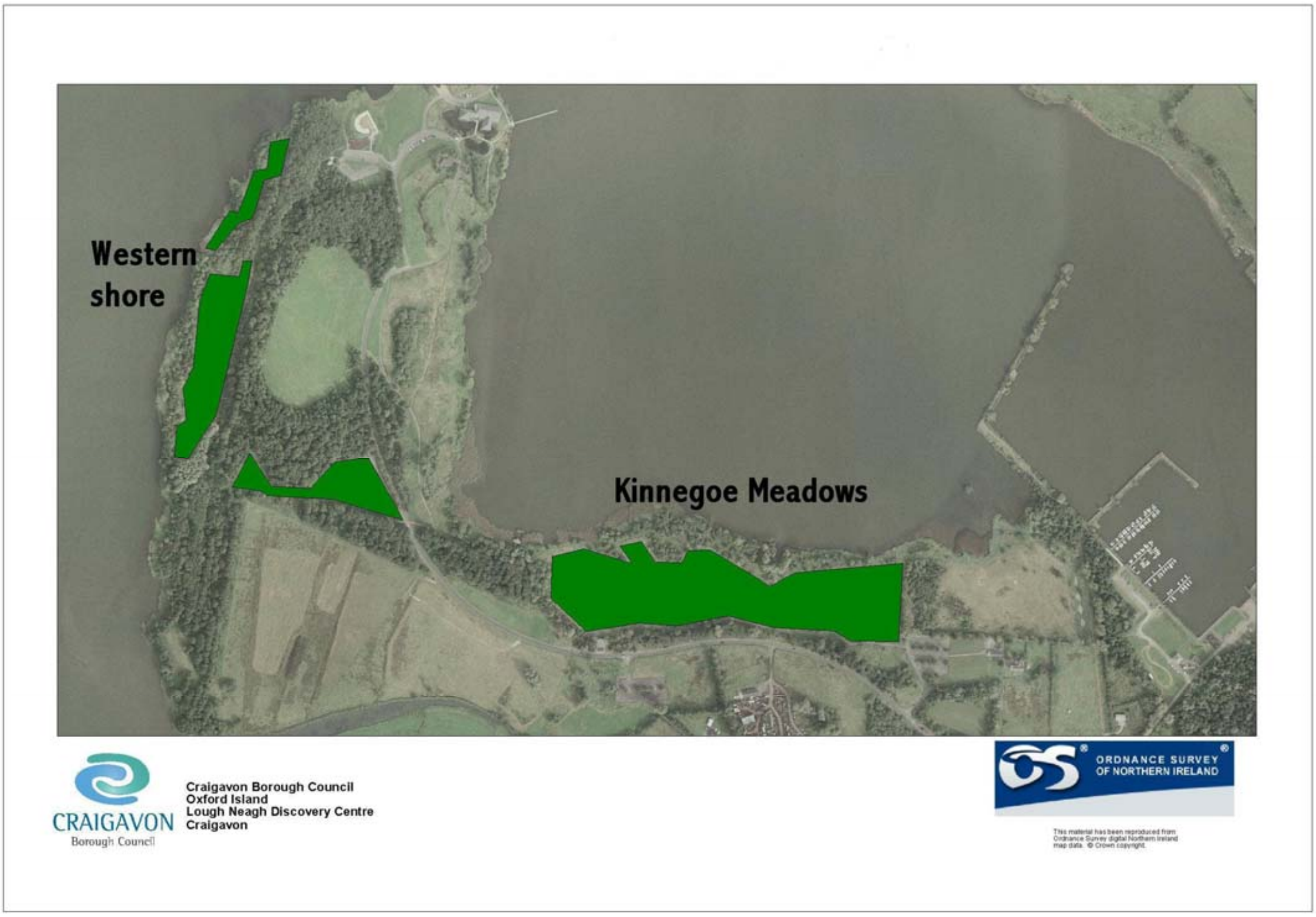
Figure 6: *Craigavon Lakes-area of study- highlighted green*

CRAIGAVON
Borough Council

Craigavon Borough Council
Oxford Island
Lough Neagh Discovery Centre
Craigavon

ORDNANCE SURVEY
OF NORTHERN IRELAND

Figure 7: *Oxford Island NNR- area of study-highlighted green*



2.4 NUMBERS

The number of visible adults was recorded over the period of each site visit. For each visit the total numbers seen will be expressed as a timed count per hour (achieved by following a transect through the main study areas). For each successive 7-day period (3-7 visits), the median number was calculated from the daily visits made to express the average adult count per hour for each week. This information was used to view the phenology of *L. reali* populations at each site over the flight period.

2.5 OVIPOSITION CHOICE-FLIGHT CAGE

Egg laying females (approx. 1 per week per site) were collected and stored in cardboard boxes under natural light and temperature, with a small amount of concentrated sucrose solution added for nourishment. Boxes were covered in gauze and lined with moist filter paper. Single females were placed into a flight cage (2m x1m x1m) offering three known foodplants available in NI (*L. pratensis, L. corniculatus and V. cracca*). The females were offered the same amount of each foodplant of a similar age and condition. The females and foodplants were left in the cage for 1 day (24hrs) then the number and position of eggs per plant were recorded. This process was repeated with several females using fresh food plants each time. Any specimens that died as a result of these experiments were then available for genitalia examination. All eggs were redistributed onto foodplants across the sites from which they came. Only the first egg laid by each female in the flight cage was used in statistical analysis, to ensure independence of data. Figure 8 shows an image of the flight cages used.

Figure 8: *Image of flight cages used*

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2.6 OVIPOSITION PREFERENCE-FIELD OBSERVATIONS

Females in egg laying mode were followed and their behavior recorded. Each ovipositioning act was timed and the site marked for measuring height of egg above ground, assessment of microhabitat (via 1-meter quadrat, recording vegetation cover, aspect, air temperature and availability of other potential food plants) and identification of the food plant. I also recorded and timed when a female laid several eggs, to assess short-term egg laying capacity. I marked and numbered each egg with GPS and placed a marker stick close to the plant and revisited daily and recorded its presence or absence, any change in colour and the addition of any new eggs and presence of early instar larvae. In addition any eggs found by accident, or searching adjacent plants, were recorded as above. When an egg that was observed being laid and dated and the presence of a first instar larva confirmed, this data were used to calculate the hatching time: the average time to be expressed as a median value. The total numbers of new eggs (laid plus found) were calculated per week (3-7 site visits) and their position was categorized by height- less than 10cm, less than 20 cm, less than 30cm and more than 30cm above ground level. All eggs were individually identified as described above, to avoid pseudo-replication.

2.7 LARVAE AND PUPAE

When possible eggs from the field or the flight cages were checked daily for presence or absence. This information was used to estimate how long it takes eggs to hatch. The first instar larva could then be followed and checked upon daily, to see how long it takes to become a fully-grown caterpillar and finally, a pupa. It was anticipated that most of this analysis would be made on eggs kept in captivity from the flight cages, as following caterpillars in the field could have proved very difficult.

2.8 NECTARING PREFERENCE

Each episode of nectaring was recorded, including the nectar plant, the sex of the butterfly and the number of events observed per week. This information was used to show if there was any change in nectar plant choice over the study period.

2.9 COURTSHIP

Each observed incident of courtship was recorded and the extent of the encounter timed. These events were video recorded when the opportunity arose.

2.10 SPECIES IDENTIFICATION

A number of mid-leg samples were collected from a range of butterflies; those observed in flight, those seen egg laying and those used in flight cages. These samples were to be used in future undergraduate work aimed at using DNA sequencing to separate *Leptidea* species. To avoid taking more than one mid-leg sample from the same butterfly, all specimens were marked on the abdomen with yellow paint using cocktail sticks and an insect marking cage, as shown in figure 9. The mid-leg samples were taken from the butterflies using the aid of an insect marking cage and a pair of tweezers. All samples were placed in a small container filled with ethanol and stored in a domestic fridge.

Figure 9: *A* L. reali *specimen about to be marked after collection of a mid-leg sample*

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The genitalia of several butterflies from both sites, including some previously used in the flight cages were examined (M. Hughes). This established if the male saccus and aedeagus and the female ductus bursae measurements coincided with the expected values for *L. reali*. The following methodology was used.

The abdomen was removed from the specimen and placed in a hot 10% solution of potassium hydroxide for 10 to 15 minutes. The abdomen (now soft) was transferred to a watch glass or petri dish containing water, with the addition of a few drops of alcohol to reduce the surface tension. Whilst viewing under a stereomicroscope, the genitalia were then removed from the abdomen, which was immersed in fluid (this prevented air bubbles entering the genitalia). The genitalia were then placed on a glass slide and measured through the microscope using a graticule. A reference slide was then prepared in the usual way (M. Hughes, pers. comm.).

2.11 OTHER MEASUREMENTS

Ambient shade temperature was recorded at the mid point of each visit (around 13.30 h). A standard air/soil digital thermometer was used, accurate to 0.1 degree centigrade. Photographs were taken of each stage of the life cycle.

3. RESULTS

Site visits were made from the 22nd April until the 25th June, with 28 separate visits made to Craigavon Lakes and 22 to Oxford Island NNR. The most site visits made per week was 5 and both sites were visited on at least 3 occasions per week throughout the flight period. Fieldwork was blessed with fine weather conditions for most of the study period and inclement weather conditions were only encountered on a few occasions towards the end of June. Normally, fieldwork was carried out between 10.30am and 16:30pm on any day when weather conditions were suitable (i.e. those recommended for BMS). During each site visit, the same route was taken throughout.

3.1 NUMBERS

Craigavon Lakes

The first adult *L. reali* was observed on the 5th May 2008 and the last on the 23rd June 2008, giving a flight period of exactly 8 weeks. The peak count for any single site visit reached a maximum of 79 individuals on the 30th May. Figure 10 shows that the phenology at this location appears to be univoltine, with only a few discrepancies away from an ideal curve. When the peak counts per site visit are re-calculated as average numbers of adults per hour, a strikingly similar pattern emerges, with a peak count of 32 butterflies per hour again occurring on the 30th May.

Oxford Island NNR

The first adult emergence was also recorded at this site on the 5th May, with adults on the wing until the 20th June. This gives a flight period at this site of 7 weeks and 4 days. The peak count for any single site visit reached a maximum of 46 on the 25th May. Figure 11 shows that this site also has a univoltine phenology, with an almost ideal curve in the latter part of the flight period. The average numbers per hour are too closely matched with peak counts to merit inclusion, other than to say that the maximum of the median number per hour (29) was encountered on the 2nd June.

Figure 10: *Peak adult counts and median adult numbers per hour, Craigavon Lakes*

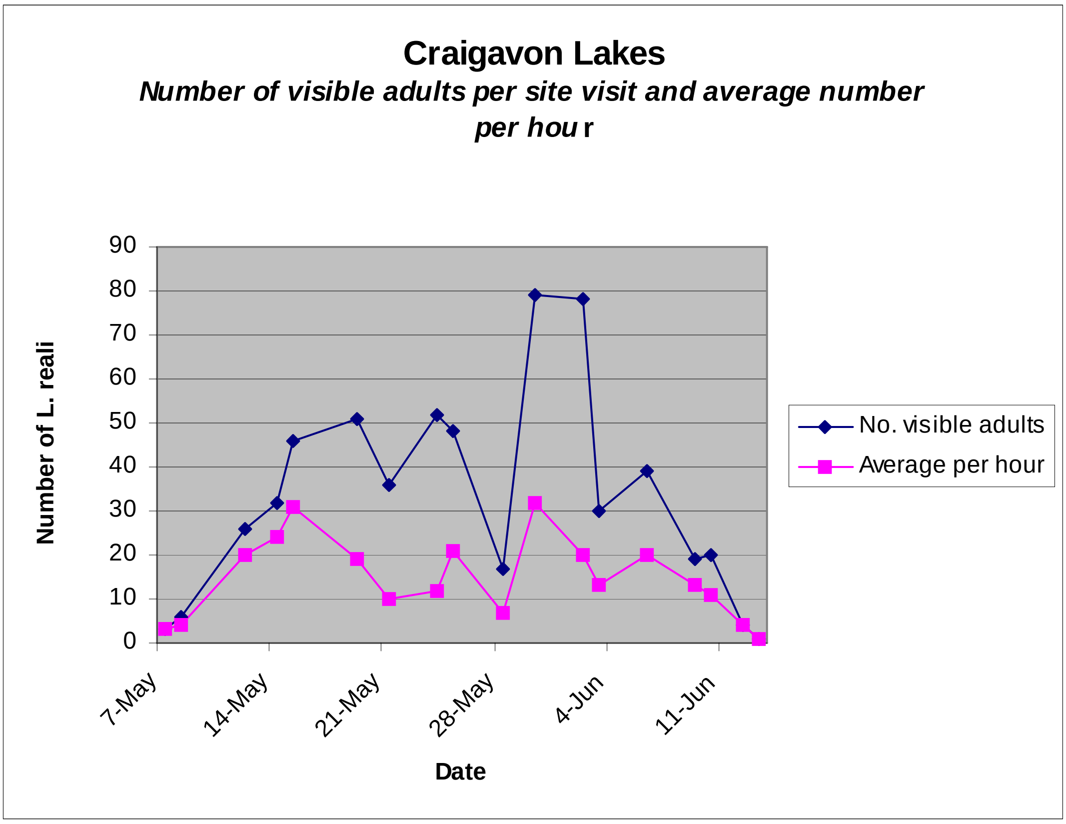
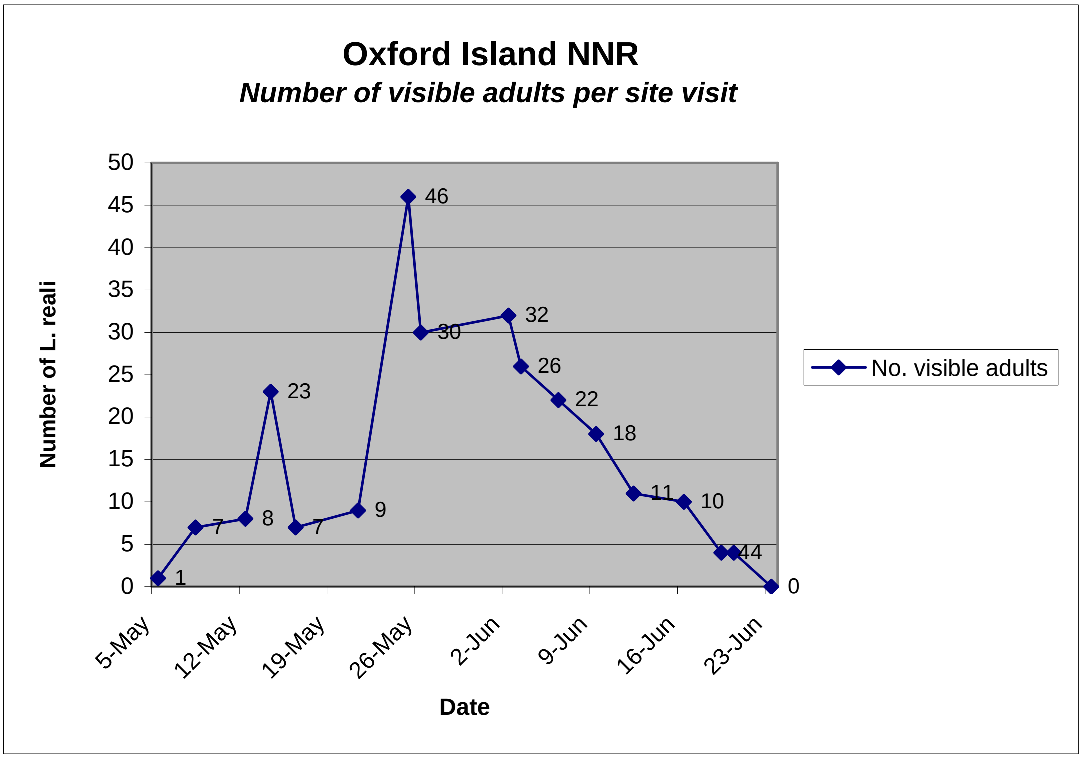


Figure 11: *Peak adult counts per site visit, Oxford Island NNR*



3.2 OVIPOSITIONING CHOICE- FLIGHT CAGE EXPERIMENT

A total of 20 flight cage experiments were carried out between 26th May and 19th June that resulted in 113 eggs being laid by 15 females. Females were placed into flight cages for a period of 24 hours and were offered three potential foodplants- *Lathyrus pratensis, Vicia sepium* and *Lotus corniculatus*, which were planted in equal sized pots and all plants were of equal health, age and size. Each potted plant was used only once and then discarded. Results indicate that female *L. reali* show a marked choice for *Lathyrus pratensis* for ovipositioning, with 12 out of 15 (80%) egg laying females choosing this plant to lay their first egg. Indeed 81 out of the 113 (72%) total eggs laid were positioned on *Lathyrus pratensis.*

However, some individuals preferred *Lotus corniculatus* as their first choice food plant, with 3 out of 15 (20%) egg laying females choosing this plant. Thirty eggs out of the total of 113 (26%) were laid on *Lotus corniculatus.* On no occasion was the first egg laid of any female observed on *Vicia sepium*, with only 2 eggs of the total laid (2%) being found on this species. Even then, one of these was on the stem of the plant.

Five of the females used in the experiment failed to lay any eggs and the most eggs laid by a single female was 20. On average, the female *L. reali* used in the cages laid 6 eggs each (n=20).

On average, eggs were laid on the second frond of the foodplants at a height of 18cm above the ground. Nine of the 15 egg laying females only laid eggs on a single plant species, while 6 females opted to share their eggs between 2 or more plant species. Of the 9 females to use only one plant species for ovipositioning, 8 used *Lathyrus pratensis* and 1 used *Lotus corniculatus* exclusively. The raw data from the flight cage experiments can be found in appendix 6. Only 4 of the 20 females used in this part of the study were from Oxford Island, thus making comparison between sites weak, although all 14 eggs were laid on *L. pratensis*.

3.3 OVIPOSITIONING PREFERENCE- FIELD OBSERVATIONS

Females that appeared to be in 'egg laying mode' (see section 3.11) were followed for up to 10 minutes to see if any egg laying took place. If ten minutes elapsed and the female showed no sign of egg laying, a new female was observed. A combined 66 eggs were observed in the field, with 57 of these directly observed being laid and a further 9 being found by searching suitable locations nearby and through searches of suitable foodplants during inclement weather conditions. Forty-nine of these ovipositioning events were observed at Craigavon Lakes and 17 at Oxford Island NNR. Egg laying was observed between 19th May and 19th June 2008.

Craigavon Lakes

Seventy six percent of all eggs (37/49) either observed or found, were laid on *Lathyrus pratensis.* Of the total number of eggs 22% (11/49) were positioned on *Lotus corniculatus.* Only 1 egg (2%) was found on *Vicia sepium.* Seventy four percent of eggs were located on the top two fronds of the foodplant. Two eggs were laid on prominent plants. Seventy eight percent of eggs were found in quadrats with other potential foodplants present, 22% had only 1 foodplant present.

Table 4: *Vital oviposition statistics from Craigavon Lakes*

Property	Average	Standard Deviation	*n*
1. Average height on foodplant	**17.4 cm**	6.07	49
2. Average height of sward	**33.1 cm**	7.94	49
3. Average ratio between 1 and 2	**1.91**	0.59	49
4. Average % ground cover MV	**11**	8.03	49
5. Average % ground cover BV	**1**	2.59	49
6. Average % ground cover BFT	**7.8**	11.08	49
7. Average % ground cover TV	**0.6**	2.22	49
8. Average air temperature at egg	**24 ^{o}C**	4.67	40
9. Average distance to larger vegetation	**3.5 m**	3.33	49

The information in table 4 demonstrates the typical habitat and temperature conditions found at a preferred egg location (*n*=49). Females tend to prefer a reasonably short sward and place their eggs on the top two fronds of a plant approximately half the height of the surrounding sward. In most cases a small ground covering of *Lotus corniculatus* (BFT) and *Lathyrus pratensis* (MV) was recorded. Egg sites were on average 3.5 metres from the nearest large vegetation (willow and gorse species in this instance) and the average air temperature (*n*=40) around an egg was 24 ^{o}C.

The height of an egg on a plant varied between 5.5 and 38cm and the average sward height ranged between 20 and 60cm. The maximum ground cover percentages found in the quadrats for each potential foodplant were 35, 10, 40 and 15% for *Lathyrus pratensis, Vicia sepium, Lotus corniculatus* and *Vicia cracca* respectively. The minimum air temperature at an egg location was 19.1 ^{o}C with a maximum of 35.3 ^{o}C. The furthest down a plant that an egg was found was on the 5th frond and the maximum distance an egg was laid from large vegetation was 15 metres.

Another important factor in female egg location preference is thought to be aspect, with some species preferring the warmth afforded by a south-facing slope. Table 5 shows that 55% of eggs were laid on flat ground, suggesting that microclimatic factors and shelter may be more important in oviposition preference at this location. Females showed no preference for north or south facing aspects, but avoided any locations with an east or westerly aspect. Interestingly, the egg found in the hollow (a depression surrounded by tall, thick grass on all sides) was also the egg that was recorded lowest to the ground (5.5cm) and one of the few to appear on a prominent plant.

Table 5: *Aspect preference at egg locations, Craigavon Lakes*

Aspect	Number of eggs	%
Flat	27	55
North	11	22
South	8	16
South west	1	2
North east	1	2
Hollow	1	2
Total	**49**	**100**

Another recorded aspect of female oviposition preference was the occurrence of multiple egg laying events. Table 6 shows that a total of 8 of these events took place between 21st May and 12th June. The average time taken to lay a single egg was 4 minutes 6 seconds, with a maximum of 4 eggs laid by any one female. The raw data that relate to this section of the results can be found in appendix 1. The location of all eggs can be seen in figure 12, which shows their distribution to be focused along the northern railway section.

Table 6: *Occurrences of multiple egg laying events at Craigavon Lakes*

Date	Multiple egg laying event Number	No. Eggs	Total time	Average time per egg (in seconds)
21-May	1	4	1318	330
24-May	2	3	1080	360
30-May	3	3	600	200
02-Jun	4	3	1140	380
03-Jun	5	2	420	210
06-Jun	6	2	405	203
06-Jun	7	4	187	62
12-Jun	8	2	452	226
			Average	**246**
				4 mins 6 secs

Oxford Island NNR

All 17 eggs at this site were recorded as direct observations. Eighty eight percent (15/17) of eggs were observed being laid on *Lathyrus pratensis,* with single eggs (6%) recorded being laid on *Lotus corniculatus* and *Lotus pedunculatus.* No eggs were recorded on either *Vicia* species. Seventy one percent of all eggs were laid on either the first or second frond of the foodplant. Only a single egg was positioned on a prominent plant. Eighty two percent of the quadrats studied surrounding the foodplant had at least 1 other potential foodplant present, with 18% recording a single foodplant species.

Table 7: *Vital oviposition statistics from Oxford Island NNR*

Property	Average	Standard Deviation	*n*
1. Average height on foodplant	**23.4cm**	13.42	17
2. Average height of sward	**43.1cm**	11.91	17
3. Average ratio between 1 and 2	**2.3**	1.12	17
4. Average % ground cover MV	**7.5**	5.32	17
5. Average % ground cover BV	**0.6**	1.37	17
6. Average % ground cover BFT	**0.6**	1.66	17
7. Average % ground cover TV	**3.1**	5.16	17
8. Average % ground cover GBFT	**1.2**	3.63	17
9. Average air temperature at egg	**28 ^{o}C**	4.22	17
10. Average distance to larger vegetation	**16m**	11.17	17

Table 7 shows many similarities to the results found at Craigavon Lakes, but also highlights some key differences. The average sward height used by females here is slightly taller than at Craigavon (confirmed statistically using a two-sample *t*-test, $t= -2.33$ and $p= 0.030$ at the $p<0.05$ significance level), but the eggs remained positioned at a ratio of close to 2 between the height of oviposition on the foodplant and the height of the sward. The influence of different habitat characteristics at this site can also be seen. *Lotus corniculatus* is largely replaced by *Lotus pedunculatus* in the quadrats, but females still seem to prefer a sparse ground covering of *Lathyrus pratensis.* The most striking difference between the two sites is the distance of egg locations from the nearest large vegetation, with eggs at Oxford Island an average of 16 metres away. This is representative of how the main habitat here is more of an open, managed hay meadow compared to the scrubby, largely unmanaged embankments of Craigavon. This great difference in distance to larger vegetation may have implications for the distances larvae have to travel to pupate and ultimately may affect their survival.

The vital statistics related to oviposition at Oxford Island (n=17) in table 7 are consistent with those for Craigavon, with a few exceptions. The maximum percentage groundcovers at egg locations for each potential foodplant are 20, 5, 5, 15 and 20% for *Lathyrus pratensis, Vicia sepium, Lotus corniculatus, Vicia cracca* and *Lotus pedunculatus* respectively. The main difference comes in the distances to larger vegetation measurements, with records of up to 30 metres at this site. There is statistical evidence to suggest that the mean distance from larger vegetation varies greatly between the two sites (using a two-sample *t*-test, $t= -4.46$ and the p-value was 0.000 at the $p<0.05$ significance level).

Table 8: *Aspect preference at egg locations, Oxford Island NNR*

Aspect	**Number of eggs**	**%**
Flat	5	29
South	4	24
West	4	24
Hollow	2	12
South west	2	12
Total	**17**	**100**

More obvious differences can be seen through the aspects chosen for oviposition by females at this site. Again, flat areas are the most popular, but table 8 also shows that females prefer aspects between the south and west. A further 2 eggs were found in hollows.

Only 3 observations of multiple egg laying were recorded at Oxford Island and these are shown in table 9. These events were recorded between 26th May and 19th June and the most eggs laid by one female was again found to be 4. Interestingly, the average time taken to lay an egg was 4 minutes 6 seconds, the same as for Craigavon Lakes. The raw data that relate to this section of the results can be found in appendix 2. Figure 13 shows the distribution of egg locations to be concentrated in Kinnegoe Meadows, with a single observation at the Western shore.

Table 9: *Occurrences of multiple egg laying events at Oxford Island NNR*

Date	Multiple egg laying event Number	Number of eggs	Total time	Average time per egg (in seconds)
26-May	1	4	1594	399
02-Jun	2	3	414	138
19-Jun	3	3	600	200
			Average	**246**
				4 mins 6 secs

3.4 LARVAE AND PUPAE

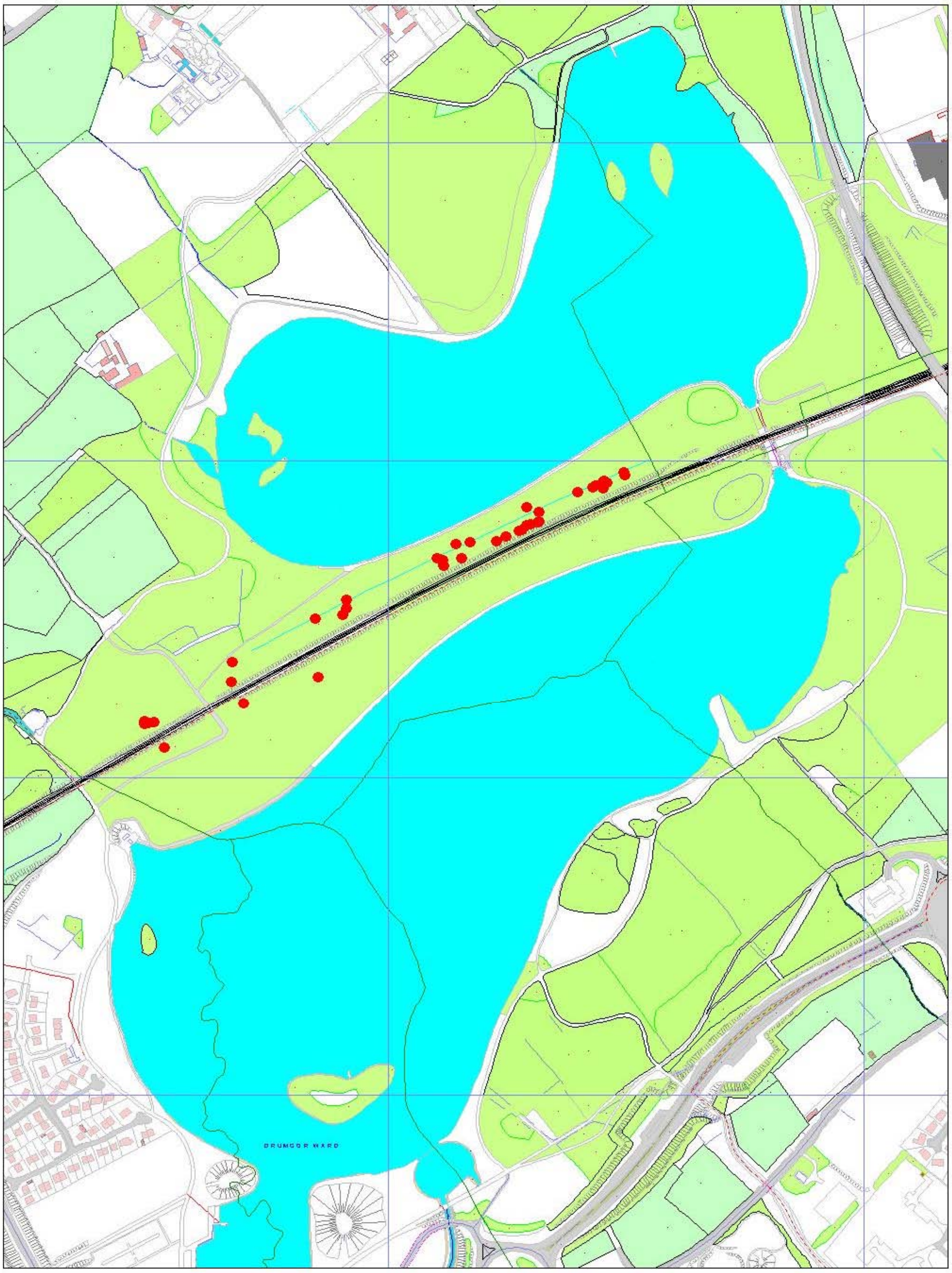
The data gathered for this section come from a combination of regular checks of field laid eggs and daily checks of a number of eggs kept in captivity, that were laid in the flight cages. This approach allowed me to observe 14 eggs being laid through to hatching into 1st instar larvae. The time taken for each of these eggs to hatch is shown in table 10. These values combine to give an average hatch time of 13 days.

Table 10: *Times taken for eggs to hatch into 1st instar larvae*

Egg hatch number	Time (days)
1	18
2	14
3	14
4	14
5	14
6	11
7	11
8	12
9	13
10	13
11	13
12	14
13	9
14	9

Figure 12: *Map showing the distribution of egg locations at Craigavon Lakes.*

Craigavon Lakes

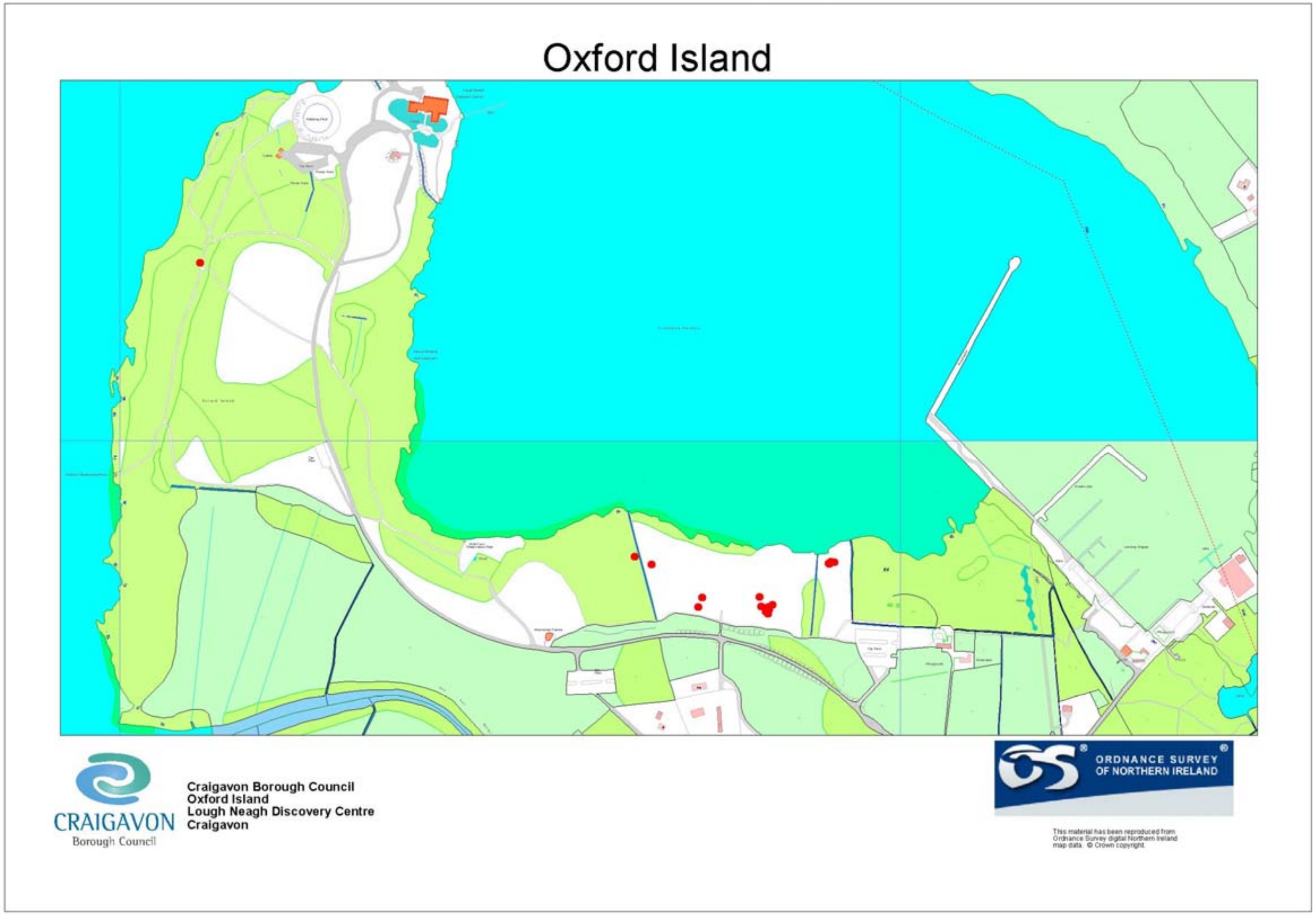


CRAIGAVON
Borough Council

Craigavon Borough Council
Oxford Island
Lough Neagh Discovery Centre
Craigavon

ORDNANCE SURVEY OF NORTHERN IRELAND

<u>Figure 13:</u> *Map showing the distribution of eggs at Oxford Island NNR*



Furthermore, a small number of eggs (3) laid in the flight cages were kept in captivity (a small jar kept indoors, with new foodplants offered daily) to develop through to pupae. The large majority (110) of eggs from the flight cages were returned to suitable locations at the sites from where their parents came. The information in table 11 relates to these 3 eggs and shows that, upon hatching as 1st instar larvae, they all measured 1.5mm and took approximately 13 days to develop into caterpillar form (see morphological differences in figure 18). Their size steadily increased until they were fully grown by day 27, measuring 20mm. By day 33 all caterpillars had begun to pupate, becoming fully enclosed pupae by day 34. These pupae will be kept until hatching as adults next spring and will then be released at Craigavon Lakes.

Table 11: *Duration of development from 1st instar to pupa*

Number of days hatched	Length (mm)	State
1	1.5	1st instar
8	5	1st instar
13	9	Caterpillar
19	13	Caterpillar
24	17	Caterpillar
27	20	Caterpillar
34	21	Pupa

These three eggs also gave information on the development rate of a *L. reali* butterfly from being laid until pupation. The three eggs were laid on the 30th May and hatched on the 12th June (14 days); from hatching they took until 25th June to resemble caterpillars (27 days) and until 10th July to become fully grown (41 days). They reached pupae form on the 17th July (48 days). So from the day they were laid until they reached pupation took approximately 7 weeks. This should have implications for site management in terms of cutting and grazing timings and will be dealt with in more detail in the discussion.

3.5 NECTARING PREFERENCE

A total of 151 nectaring events were recorded, with 110 from Craigavon Lakes and 41 from Oxford Island NNR. Both sites showed that the majority of nectaring events involved female butterflies, 82% at Craigavon Lakes (90/110) and 80% at Oxford Island (33/41). Nectaring took place between 8th May and the 23rd June.

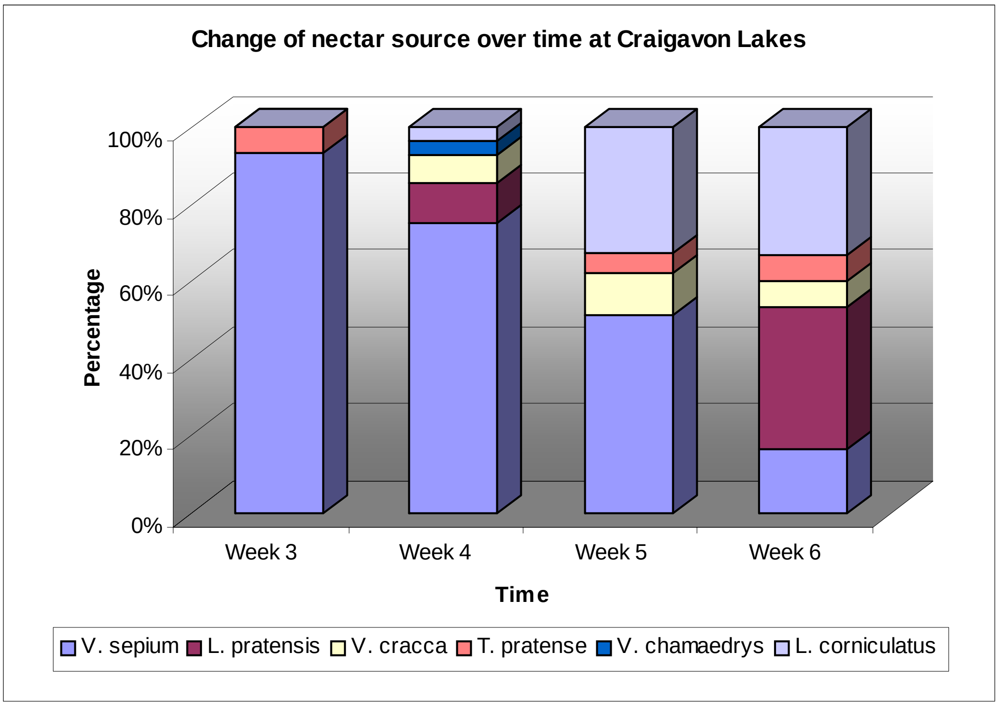
Craigavon Lakes

Nectaring was observed on 6 different plant species. These are shown in table 12, including the percentage each was used out of all nectaring events:

Table 12: *Nectar sources and their usage at Craigavon Lakes*

Plant species	Percentage use
Vicia sepium	53%
Lathyrus pratensis	14%
Veronica chamaedrys	1%
Lotus corniculatus	21%
Trifolium pratense	5%
Vicia cracca	7%

Figure 14: *Percentage change over time in nectar sources at Craigavon Lakes*



In the first week of adult activity (nectaring not recorded until week 3), 93% of nectaring events took place on *Vicia sepium*. However, through the course of the flight period the amount of nectaring on this species began to decrease and other nectar sources became more important. This trend is illustrated in figure 14, which shows how the percentage of total nectaring events changed over time. *Vicia sepium* shows a gradual decrease in usage and, by week 6, played only a minor role as a nectar source (17%). As butterflies used this species less and less, an increase in the use of *Lotus corniculatus* is shown to take its place, rising from 0% in week 3 to 33% in week 6. By week 6 *Lathyrus pratensis* had become the most widely used nectar source, with 37% of all events occurring on this species. *Vicia cracca* played a supporting role throughout the latter stages of the flight period and *L. reali* were also observed nectaring on *Veronica chamaedrys* and *Trifolium pratensis*, but these events were very infrequent.

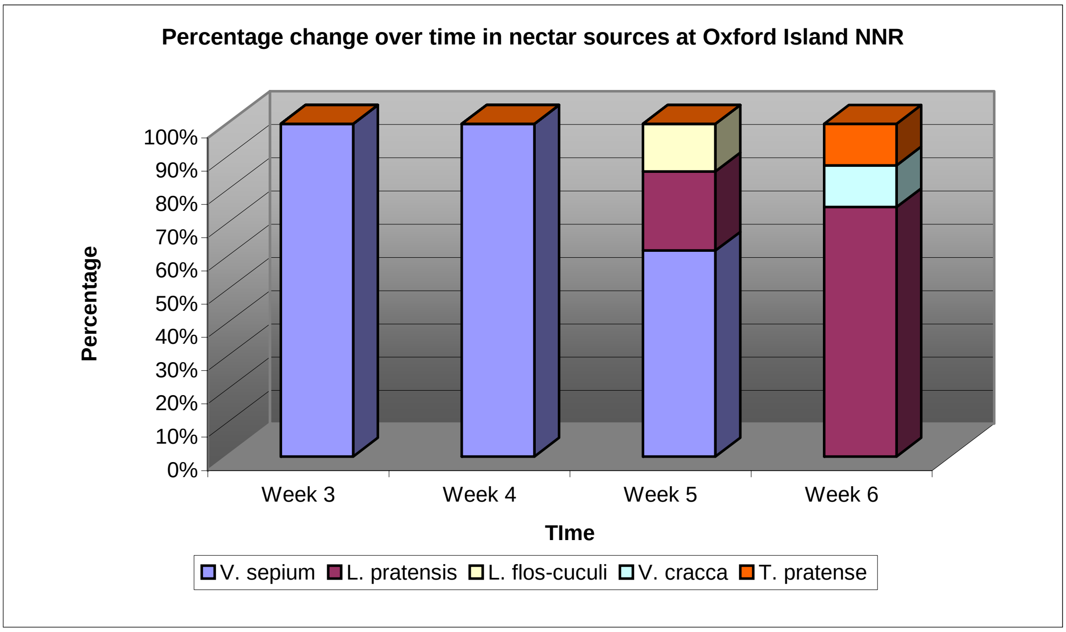
Oxford Island NNR

Nectaring was observed at this site on 5 different plant species. These are shown in table 13, including the percentage each was used out of all nectaring events:

Table 13: *Nectar sources and their usage at Oxford Island NNR*

Plant species	Percentage use
Vicia sepium	61%
Lathyrus pratensis	27%
Lychnis flos-cuculi	7%
Trifolium pratense	2%
Vicia cracca	2%

Figure 15: *Percentage change over time in nectar sources at Oxford Island NNR*



Once again the most popular choice of nectar plant at this location was *Vicia sepium* (61%), followed by *Lathyrus pratensis* (27%). But as figure 15 illustrates, the percentage of total usage of these plants shows great variability through time. In fact, during the first two weeks of the flight period (weeks 3 and 4) 100% of nectaring events were recorded on *Vicia sepium.* But by week 6 this species recorded no nectaring events whatsoever and was replaced by *Lathyrus pratensis* as the nectar source of choice, with 75% of all events occurring on this species. After weeks 3 and 4 some of the less abundant nectar sources became more important such as *Lychnis flos-cuculi, Trifolium pratense* and *Vicia cracca.*

Of particular note is the fact that the *L. reali* at this site apparently avoided *Lotus sp.* as nectar sources, in contrast to Craigavon Lakes, where *Lotus corniculatus* was the second most widely used nectar source. It should also be pointed out at this stage that *L. reali* at both sites avoided other widely available nectar sources such as *Leucanthemum vulgare* and *Ranunculus acris.*

3.6 COURTSHIP

Only 11 courtship events were observed at either site during the course of the study. These ranged in duration from as little as 9 seconds to as long as 7 minutes 51 seconds. Only one of these events was recorded at Oxford Island and this occurred on the last day any adults were recorded at the site. These events occurred from the 19th May until 20th June and are shown in table 14. The average duration of a courtship display was 2 minutes 51 seconds.

Table 14: *Courtship event times from both sites*

Date	Courtship display No.	Duration (secs)	Site (C/O)
19-May	1	15	C
19-May	2	37	C
19-May	3	327	C
21-May	4	60	C
24-May	5	9	C
24-May	6	392	C
02-Jun	7	123	C
06-Jun	8	301	C
06-Jun	9	471	C
06-Jun	10	100	C
20-Jun	11	45	O
	Average	171	**2 minutes 51 seconds**

C/O; C=Craigavon Lakes, O= Oxford Island

Three courtship display events were recorded using a digital camera. On no occasion did courtship lead to an attempt at mating. Mating *L. reali* were not observed throughout the present study.

3.7 SPECIES IDENTIFICATION

From around the middle of the flight period, a number of butterfly specimens were collected from both sites to have their genitalia examined by Maurice Hughes. A total of six adult butterflies were collected from the field or after being used in flight cage experiments. From this analysis, the species and sex of each butterfly were confirmed. Table 15 shows how the measurements of genital apparatus in the present study compare to accepted figures used to confirm separation from *L. sinapis*. Full details of this analysis are given in appendix 7.

Table 15: *Sex and speciation separation by genital apparatus*

Specimen number	*Sex*	*Length of ductus bursae*	*Length of saccus*	*Length of aedeagus*
1	Female	0.953mm	na	na
2	Female	0.953mm	na	na
3	Female	0.953mm	na	na
4	Female	1.089mm	na	na
5	Female	*	na	na
6	Male	na	0.772mm	1.907mm

N.B * this sample was confirmed as *L. reali* before it was accidentally lost (M. Hughes, pers.comm.). The threshold measurements given in Freese and Fiedler (2004) that confirm separation from *L. sinapis* are >0.75mm for the female Ductus bursae, >0.77mm for the male Saccus and >1.76mm for the male Aedeagus.

These measurements confirm that all 6 specimens were of the species *L. reali*. However, it is important to state that this analysis by no means confirms that all butterflies used throughout this study were of this species, but rather it suggests that this is likely to be the case. Much greater knowledge of speciation will be gained when the morphological species trait details given here are compared to future genetic analysis of mid-leg samples. Specimens 1 to 6 all had mid-leg samples taken, for future study.

Between 8th May and 19th June a total of 23 mid-leg samples were collected from *L. reali* butterflies from both sites and from females used in flight cage experiments. It is proposed that the genetic analysis of these samples will form part of an undergraduate research project in 2008/09 at Queen's University Belfast, under the supervision of Dr. Robert Paxton with guidance from the present author. All information relevant to the mid-leg samples, including how many eggs each corresponding female laid in the flight cage and on which plant species, can be found in appendix 6. Figure 16 shows digital images, taken under the microscope by Maurice Hughes, of the typical genital apparatus of both sexes of *L. reali* used in the speciation work outlined earlier.

<u>Figure 16:</u> *Example images of a female ductus bursae (A) and a male saccus and aedeagus (B) of two of the specimens used in speciation designation by genitalia examination, including reference codes and apparatus measurements.*

(A) Female

(B) Male

3.8 OTHER MEASUREMENTS

Ambient shade temperature

Ambient shade temperature was recorded during the mid point of each site visit. All readings at Craigavon Lakes were taken under the western railway underpass and all readings at Oxford Island were recorded in the woodland outside the entrance to Kinnegoe bird hide. Sites were visited in a random order each day to avoid a temperature bias that may have occurred by always visiting the same site first.

Figure 17: *Ambient shade temperatures (AST) per week at Craigavon Lakes and Oxford Island NNR*

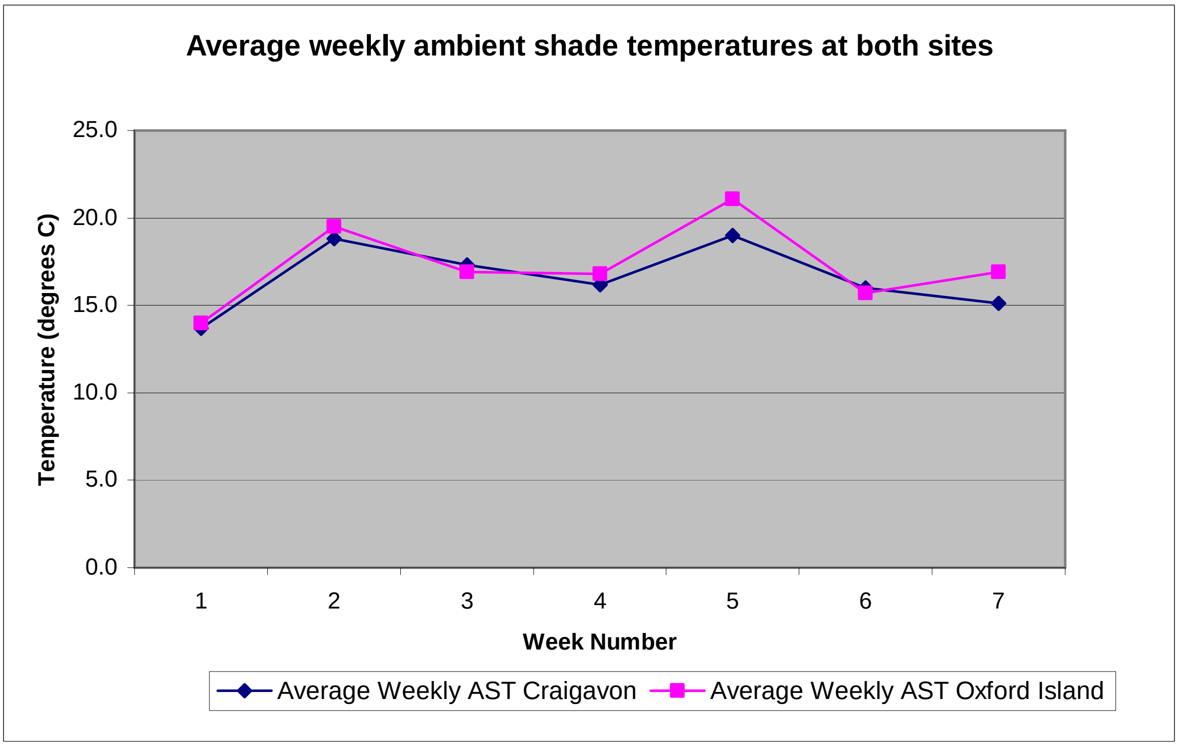


Figure 17 shows that over the flight period, the average temperature at Oxford Island was slightly higher than at Craigavon Lakes. Week 5 recorded the highest average weekly temperatures, 19 ^{o}C for Craigavon and 21.1 ^{o}C for Oxford Island.

This pattern of higher temperatures at Oxford Island is also shown through the overall average temperature and the maximum and minimum records. The overall average temperature at Craigavon was 16.7 ^{o}C. The minimum temperature recorded at Craigavon was 13.2 and the maximum was 21.8 ^{o}C. This compares to 17.4 ^{o}C, 13.7 ^{o}C and 23.4 ^{o}C at Oxford Island.

Photographing each life stage

Figure 18, on the next page, shows images of all stages of the *L. reali* life cycle taken throughout the course of the present study and the corresponding development times found for each stage.

<u>Figure 18</u>

Leptidea reali-

Life cycle and development times

Courtship and Mating

Eggs laid

1st Instar larva- eggs hatch after 13 days

Fully grown caterpillar- 41 days

Pupa- 48 days

Adult

3.9 STATISTICAL ANALYSIS

It is hotter closer to the ground, therefore it was expected that females would lay eggs higher up the vegetation on warm days. The results for air temperature at egg locations were correlated with height above ground. It seems reasonable to expect that with increasing temperature towards the ground, females would chose to oviposit towards the top of the vegetation. However correlation coefficients of –0.095 (*n*=40, p=0.558) for Craigavon Lakes and –0.050 (*n*=17, p=0.849) for Oxford Island suggest that these two variables are very much independent.

To compare the number of *L. reali* adults observed using the methodology of the current study with those gained through a BMS transect, a paired *t*-test of the two datasets (median weekly counts vs. BMS counts) was carried out in Minitab. This test was selected as data were collected from two related methodologies from the same sites, the differences were normally distributed and the observations were independent of each other. Results show no significant difference between the ranges of data gained by the different methods (p=0.128, p, 0.05) at Craigavon Lakes, or at Oxford Island (p=0.063, p<0.05). This suggests that the numbers of adults counted using both methodologies are consistent. This is especially encouraging for the BMS transect method, as it appears that the figures recorded may represent an accurate account of butterfly numbers at each site per week.

To compare oviposition records for each site, a Chi-squared test was used. This entailed comparing the relative abundance of each potential foodplant at a given site (estimated using NVC and my own quadrat data) with the observed use of each foodplant for oviposition. This should give some indication of foodplant preference. The null hypothesis is: *L. reali* lays eggs evenly over all potential foodplants when the abundance of each is equal. The result for Craigavon Lakes gives a X^2 value of 15.096 (*n*=49, degrees of freedom=3, p<0.01). This suggests that the null hypothesis should be rejected and points to a preference for one foodplant above all others *(Lathyrus pratensis)* at this site.

The result for Oxford Island gives a X^2 value of 8 (*n*=17, degrees of freedom=4, p<0.1). This means that it is not possible to reject the null hypothesis. This result indicates that *L. reali* at this site may not be preferential towards one particular foodplant, but it is also important to note that this site offers the additional choice of *Lotus penunculatus* compared to Craigavon Lakes.

Oviposition observations per plant, made during the flight cage experiments, were compared with the relative abundance of each foodplant offered in the cages, by means of a further Chi-squared test. The expected values are proportional to the frequency of the foodplants (in this case equal amounts i.e. a third of each). Only the first egg laid by the same butterfly was included in this analysis, as any additional eggs laid would not be statistically independent. The result gives a X^2 value of 15.6 (n=15, degrees of freedom=2, p<0.01), This means the null hypothesis that *L. reali* show no foodplant preference can be rejected. With an observed frequency of 12 against an expected frequency of 5, *Lathyrus pratensis* again appears to be the foodplant of choice. *L. reali* were observed on only 3 occasions egg laying on *Lotus corniculatus* and rejected *Vicia sepium* in all flight cages as their first choice foodplant.

3.10 UNUSUAL RESULTS

One obvious result that merits further discussion comes from the genitalia analysis of a female used in one of the flight cages. This relates to specimen code C16FC19, meaning that this female was the 16th mid-leg sample taken from Craigavon and took part in flight cage experiment 19 on the 19th June. The dissection of this specimen's genitalia revealed ductus bursae of 1.089mm, compared to measurements of 0.953mm for all other females. The fact that this specimen showed enlarged genitalia becomes even more intriguing when cross-referenced with the results from the flight cage, which show this to be the only female in the present study to exclusively use *Lotus corniculatus* for oviposition of eggs. It will be of great interest to see in which species future genetic analysis places this specimen.

Another important point to mention is some of the more unusual places *L. reali* females chose to oviposit. I have already outlined the characteristics of a typical egg location, but a small number of eggs were found in unusual locations, which suggests *L. reali* are able to oviposition in less than 'typical' places. This may point to the importance of other factors, not measured in this study, such as how the condition and/or age of foodplants influences oviposition behaviour. For instance, females were perfectly willing to lay eggs in areas with a maximum sward height of 1.1 metres and in areas of thick vegetation.

An example of this includes the egg laid on the 6th June at Oxford Island NNR. This egg was observed being laid outside the fence line at Kinnegoe Meadows, at a location shown in picture A of figure19. The height the egg was laid on the foodplant was 55cm, in an area with an average sward height of 66cm, a maximum sward height of just over a metre and dominated by tall grasses and *Filipendula ulmaria*, all of which were not characteristic of the typical egg locations found thus far.

Another example of an unusual egg location is shown in picture B of figure19. This egg was laid on the 25th May at Oxford Island. The fact that this was the only observed egg laid during study at the Western shore was unusual in itself, but with the egg-recorded 51cm up the foodplant and an average sward height of 59cm, this result becomes even more outstanding. As can be seen in the image of the egg location, the area is dominated by grasses, nettles and brambles and is very close to a thick hedge. A possible explanation for the choice of this location for oviposition is that it is south facing and would act as a suntrap.

At the other extreme, an egg laid on the 12th June at Craigavon Lakes was only 5.5cm off the ground on a plant measuring only 12cm high. The average sward height here was only 20cm. However this egg was afforded the protection and shelter by being right beside a gorse bush and only 1m from a large willow tree.

<u>Figure 19:</u> *Examples of unusual oviposition locations (marked in blue).*

(A) O13E 6th June, Oxford Island NNR

(B) O1E 25th May, Oxford Island NNR

3.11 FIELD OBSERVATIONS

Separating species, sex and 'egg laying mode' females

It proved relatively straightforward to separate *L. reali* from other white butterfly species by their small size and dainty flight, low to the ground. However, it has been suggested that one of the easiest ways to separate male and female *L. reali* in the field is through the more pronounced black tips of the male forewing (www.butterfly-conservation.org). The present study required the quick identification of potential egg laying females and it was found that this characteristic was of little use in separating the sexes. This was due to the rapid wing beats of *L. reali* in flight and the closed position of the wings when at rest.

Instead, it became apparent that sex could first of all be distinguished by flight characteristics and behaviour, then in many cases confirmed when a butterfly was at rest, by its size and the angle and position it held its wings. To separate on wing tip colour alone would require capturing and inspecting every butterfly.

Males were found to have a faster, more direct flight pattern covering large distances and did not stop to inspect nectar sources or potential foodplants. Females were more likely to be found at rest, or inspecting potential nectar sources and foodplants. Females in flight often showed more rapid wing beats and a hovering flight action, covering only short distances at a time (often from one potential foodplant to the next). When this hovering action was observed near a potential foodplant, the female was deemed to be in 'egg laying mode'. In many cases females appeared to be larger than males, although this characteristic could only be used as a guide and not as a rule for separation. In figure 21 on page 59, the female *L. reali* appears whiter on the underside of the forewings when at rest than the male. This is due to the angle each holds their wings while at rest, adding to the overall larger appearance of females. Furthermore, females could often be distinguished by their swollen abdomens.

Behaviour of females during egg laying

When following females and observing multiple egg laying events, a pattern of behaviour emerged. Once a female was found in egg laying mode, it was usual for an egg to be laid followed by an extended period of nectaring, followed by a short period of rest, before beginning the search for a suitable place to lay her next egg. Many of the multiple egg laying events were brought to an end or interrupted by males making courtship attempts and the females either accepting or flying away. The following is an account taken from field notes that illustrates the typical behaviour of a *L. reali* female:

> **24th May, Craigavon Lakes:**
> *1 egg observed being laid on MV, and then courtship display recorded –female flapping wings, male side to side with proboscis. Time 6 minutes 32 seconds. 2 other males chased the courting male away.*
> *Same female then nectared on TV then BFT, then followed to roost on grass stem, and then laid further egg on MV before disappearing from view. All same female from 13:37 – 13:55.*

4. DISCUSSION

4.1 COMPARISON OF RESULTS TO OTHER *L. REALI* STUDIES

This study offers several opportunities to compare and contrast my results with those from similar studies on the ecology of *L. reali* across Europe. As this study appears to be the first of its kind on this species in the UK or RoI, there is little information with which to compare my results within these countries, other than those stated in the introduction. Table 16 shows some of the key comparisons that can be made between the results of the present study and those from a selection of studies from the Continent.

Table 16: *Comparison of a selection of results with other studies*

	This study	**Friberg *et al* (2008[3])**	**Freese & Fiedler, (2002; 2004)**	**Wiklund, (1977[2])**	**Thompson & Nelson, (2006)**
Did *L. reali* prefer *L. pratensis* for oviposition?	Yes	Yes	Yes	Yes	N/A
% Eggs laid on *L. pratensis* in choice tests	72%	92% (+/-6.6)	40.4%	N/A	N/A
% Eggs laid on *L. pratensis* from field observations	79%	90%	N/A	87.3%	N/A
% Of courtships not resulting in mating	100%	N/A	88%	N/A	N/A
Maximum courtship time	7 minutes 51 seconds	N/A	9 minutes 53 seconds	30 minutes	N/A
Average courtship time	2 minutes 51 seconds	N/A	7 minutes 12 seconds	N/A	N/A
Egg hatch time	13 days	N/A	N/A	N/A	10 days

N/A= not applicable, area not covered by this study

Friberg *et al* (2008[3]) reports that the propensity of *L. reali* to oviposit on *L. pratensis* did not differ greatly between the field and the 'laboratory'. This was also evident in the current study. Field observations and flight cage choice tests showed that *L. reali* favoured *L. pratensis* as its host plant, with 79% of eggs from field observations and

72% of eggs from flight cages preferring this plant for oviposition. This demonstrates that little difference in propensity occurred between the two techniques.

Freese and Fielder (2002) experimentally studied the response of Swedish *L. reali* towards 4 potential foodplants including *L. pratensis*, *L. corniculatus* and *V. cracca.* Choice tests showed that *L. reali* favoured *L. pratensis*. Results showed substantial individual variation in egg laying preferences. Nine *L. reali* females laid more than 50% of their eggs on *L. pratensis*, 5% on *L. corniculatus* and 2% on *V. cracca.* This compares favorably with the present study, where 9 out of 15 females laid eggs exclusively on one plant species (8/9 on *L. pratensis* and 1/9 on *L. corniculatus*).

My results also differ from Freese and Fiedler (2002) who found that of the 752 eggs laid by 25 experimental *L. reali* females 40.4% were on *L. pratensis*, 33.9% were on *L. corniculatus* and 18.2% were laid on *V. cracca*. Compare this to the results of the present study, where, of the 113 eggs laid by 15 flight cage females, 71.7% were found on *L. pratensis*, 26.5% were on *L. corniculatus* and only 1.8% were laid on *V. sepium*. This suggests that *L. reali* from NI (from at least 2 sites) may show a stronger preference towards *L. pratensis* than those from Sweden. However, Wiklund (1977[2]) offers a different view of assumed Swedish *L. reali* (at the time, the study was believed to involve *L. sinapis*, but has subsequently been confirmed as representing *L. reali* by Lorkovic (1993)) by observing 87.3% of eggs being laid on *L. pratensis*, a result more in keeping with the findings of my own study.

Interestingly, approximately 6% of eggs from the Freese and Fielder (2002) study were laid on non-plant substrates such as glass, filter paper or gauze. This is something I managed to avoid, using the methodology of the present study.

More recently, a study by Friberg *et al* (2008[3]) provides further field and experimental evidence that *L. reali* strongly prefers *L. pratensis* as its host plant. Field observations showed that 6 out of 10 *L. reali* laid exclusively on *L. pratensis* (compared to 8/15 of flight cage females in the present study) and 90% of *L. reali* eggs were laid on *L. pratensis* (compared to 79% in the present study), while no other foodplant hosted more than 4% of the eggs (22% of eggs from my field observations at Craigavon and 6% of eggs from Oxford Island were laid on *L. corniculatus*). Laboratory experiments

(Friberg *et al.*, 2008[3]) showed that on average 92% (+/- 6.6) of *L. reali* eggs were laid on *L. pratensis* (compared to 72% in the present study). Overall the results provided by Friberg *et al* (2008[3]) compare favorably with my own.

Freese and Fiedler (2002) also report that, in mating experiments, 88% of *L. reali* courtships did not result in mating. This comes as no surprise, since 100% of observed *L. reali* courtships (*n*=11), from the present study, did not result in mating. Wiklund (1977[2]) reports that male courtship can last up to 30 minutes, while the maximum courtship duration found by Freese and Fiedler (2004) was 9 minutes 53 seconds, with courtship trials providing an average duration of 7 minutes and 12 seconds. The average courtship time from field observations in the present study provided an average time of 2 minutes 51 seconds, with a maximum duration of 7 minutes 51 seconds. This average duration is much shorter than found by Freese and Fiedler (2004).

It is important to point out that Freese and Fiedler (2002) and Friberg *et al* (2008[3]) used alternative methodologies to those of my own study, to test for oviposition preference. Freese and Fiedler (2002) placed field-caught females singly into 2 litre glasses, covered with gauze and lined with moist filter paper. A small container of sucrose solution was added for nourishment. The glasses were then placed in an environmental chamber under controlled temperature and light regimes. Females were then offered small bunches of each foodplant and egg laying was recorded for each experimental female until her death.

Friberg *et al* (2008[3]) used small egg laying cages (0.5m x0.5m x0.5m) and positioned butterflies on the foodplants and again used a specific light and temperature regime. Females were only used “after termination of copula”. This relates to a difficulty I encountered in my flight cage experiments, where 5/20 females failed to lay any eggs at all, suggesting that they had already expelled all their eggs in the field, or had not ‘copulated’ before being placed in the flight cage.

Another key difference to point out, is that Friberg *et al* (2008[3]) positioned each female on the host plants once every 20-45 minutes using cotton tips, changing the foodplant on which they placed the females, each time. These techniques appear to provide a much larger return of eggs (12 females laid 543 eggs, Friberg *et al* (2008[3]) and 25 females laid 752 eggs, Freese and Fiedler (2002) compared to 15 females laying 113 eggs in the present study). However I wonder about the consequences this may have on so-called 'choice' experiments, when one considers that approximately 6% of eggs in the Freese and Fiedler (2002) study were laid on non-plant substrates and that only the first egg laid by each female is statistically independent, particularly as *L. reali* are believed to lay eggs singly on foodplants (Thompson & Nelson, 2006). The females in the present study were placed in large flight cages (2m x1m x1m) and left to their own devices and were entirely free to decide where and when to oviposit. Furthermore eggs were counted and females returned to their site of origin after only 24 hours and were not studied until their death.

Thompson and Nelson (2006), www.ukbutterflies.co.uk and www.habitas.uk.org provide the most up to date ecological information on the Northern Irish population of *L. reali*, which is relevant to this study. Eggs are believed to be laid singly on the upper parts of foodplants and hatch after around 10 days. The present study showed that the large majority (72%) of eggs are laid singly on the 2 uppermost fronds of the foodplant. In certain circumstances *L. reali* oviposit multiple eggs, not only on the same plant, but also on the same leaf (see field observations section below). This study also provides an average hatch time for eggs of 13 days (*n*=14), which compares favorably to what was previously described.

The present study also clears up some of the confusion over *L. reali* foodplants in NI outlined in the introduction. Female *L. reali* in choice tests and in the field laid the majority of their eggs on *L. pratensis*, although some preferred *L. corniculatus* for oviposition. Eggs were also recorded in very small numbers on *L. pedunculatus* and *V. sepium* but no eggs were recorded on *V. cracca*.

4.2 RECOMMENDATIONS FOR MANAGEMENT

General

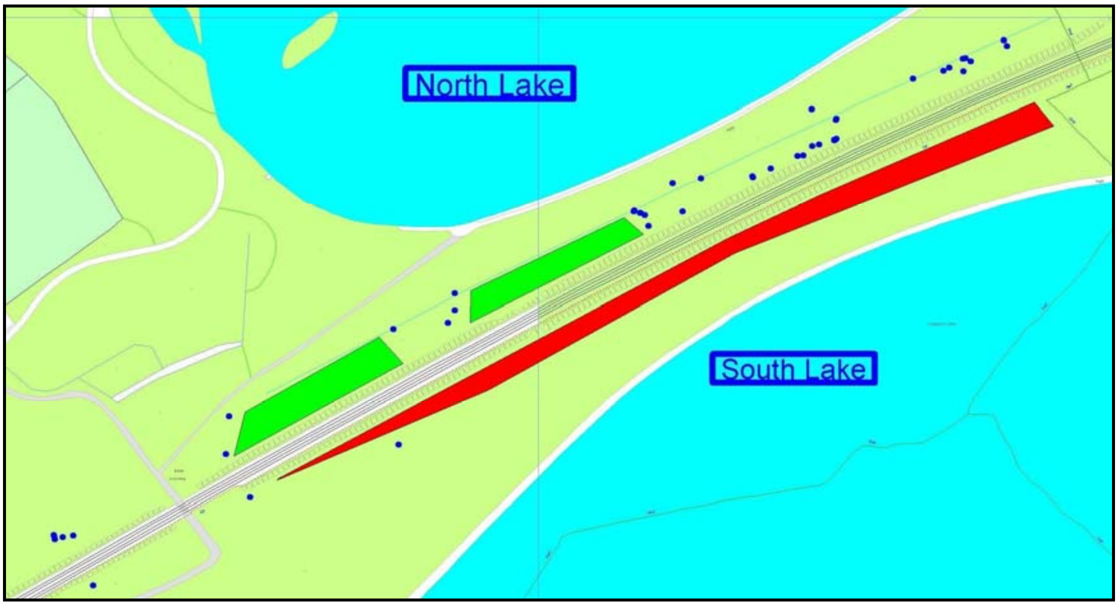
Results show that management of sites for *L. reali* should aim to establish an average sward height of approximately 38cm (+/-10cm) and it is recommended that this ideal sward height is present by the first emergence of adults. It is also demonstrated that the presence of thick tussocks of grass (that create hollows) should not be discouraged from sites, as these offer the microclimatic conditions required for oviposition. Furthermore the close proximity of oviposition sites to scrub and larger trees, particularly at Craigavon Lakes, suggests that scrub should be encouraged, but kept under control.

The sward should contain a mixture of nectar source and food plant species, but these do not appear to be required to cover large percentages of the ground. Typically when females chose to oviposition on the preferred foodplant *Lathyrus pratensis,* the average percentage ground cover of this species was 13%. Interestingly, when females decided to oviposition on *Lotus corniculatus* the average percentage ground cover of this species was 24%, suggesting that this plant is only preferred for egg laying in areas where this species is dominant. It is recommended that sites maintain at least 10% ground cover of both these species, with the addition of *Vicia sepium,* the most widely used nectar source.

Craigavon Lakes

Figure 20 shows the distribution of all 49-egg locations observed at Craigavon Lakes. The areas highlighted green are cut annually as wildflower meadows under current management and the area in red represents a large area of recent path improvement and scrub removal work. This figure shows that no eggs were laid in either area. The wildflower meadows had a short sward and little scrub, with *Dactylorhiza fuchsii* and *Vicia cracca* as dominant species. It is recommended that if this site is to be managed solely for *L. reali*, then cutting the vegetation to promote a short sward should cease. If cutting continues in these locations, then it is recommended that it takes place at least 8 weeks after the last adult butterfly has been recorded. This will allow any eggs laid in these meadows sufficient time to develop into caterpillars, grow and to pupate.

Figure 20: *Map showing the locations of all observed eggs at Craigavon Lakes. Areas marked in green are managed as wildflower meadows and those in red have undergone recent path improvement and scrub removal work.*



Map produced by Fiona Barbour, CBC with permission from OSNI

The area of path rebuilding and scrub removal, shown in red, was found to be left with a thick covering of topsoil, which can be seen in image A in appendix 5. Perhaps this work will improve the habitat for *L. reali*, on the southern side of the railway line over time, but the ground conditions found here during the present study meant that very little suitable habitat remained for *L. reali*, other than on the banks of the railway track itself. It was no surprise that only 3 oviposition events were recorded on this side of the tracks. It is recommended that future path improvement work and/or scrub removal across this site are more closely monitored and managed in a more sensitive way to *L. reali*. This would include removing any spoil from path creation from site and not spreading it over a large area (as was found in the present study and illustrated in appendix 5).

Overall much of the northern section of this site represents ideal *L. reali* habitat and it is recommended that management should monitor this site to ensure current conditions are maintained. Continued monitoring of the *L. reali* population through BMS will act as an early warning sign of population decline and the encroachment of undesirable species in this area, including the further spread of willow (*Salix*) saplings, should be discouraged.

Oxford Island NNR

The large majority of the *L. reali* population at this site was found in Kinnegoe Meadows, with a small population (maximum 8 adults) occurring on the Western shore (picture B appendix 5). It is therefore fundamentally important that Kinnegoe Meadows is managed in a way that offers *L. reali* the best possible conditions to thrive. This includes having an adequate sward height in the meadow at the time of first adult emergence (in this case 5th May), which under ideal weather conditions could be estimated as the 1st May each year. This is necessary to provide suitable nectar and potential foodplant sources from the very beginning of the flight period.

Unfortunately, this was not the case during this study. Kinnegoe Meadow is not solely managed for *L. reali* and is also considered a fire risk during the summer months (M. Malley, pers. comm.). This makes winter grazing of the area necessary. However, I was dismayed to discover that cattle remained on this site until 16th May, 11 days after the first butterfly emerged. This resulted in *L. reali* only being recorded outside the managed fenced area until 21st May. Consequently the first 6 egg laying events at this location were all observed outside the managed fenced area. The first observed egg to be laid in Kinnegoe Meadows itself was recorded on 2nd June, more than 2 weeks after the cattle were removed from the site and almost a month since the first adults emerged at the site. It is recommended that grazing cattle are removed from Kinnegoe Meadows by 14th April every year, to give sufficient time for growth in the meadows before *L. reali* begin to emerge. It is also believed that 2008's BMS counts were negatively skewed, since a large majority of *L. reali* would have been missed by taking a transect straight through the middle of Kinnegoe Meadows and ignoring the perimeter.

Image C in appendix 5 shows the heavily grazed meadows on the 7th May, by which time at least 7 *L. reali* were on site and were observed searching beyond the managed area for nectar and food plants. Image F shows the much-improved condition of Kinnegoe Meadows by the 2nd June.

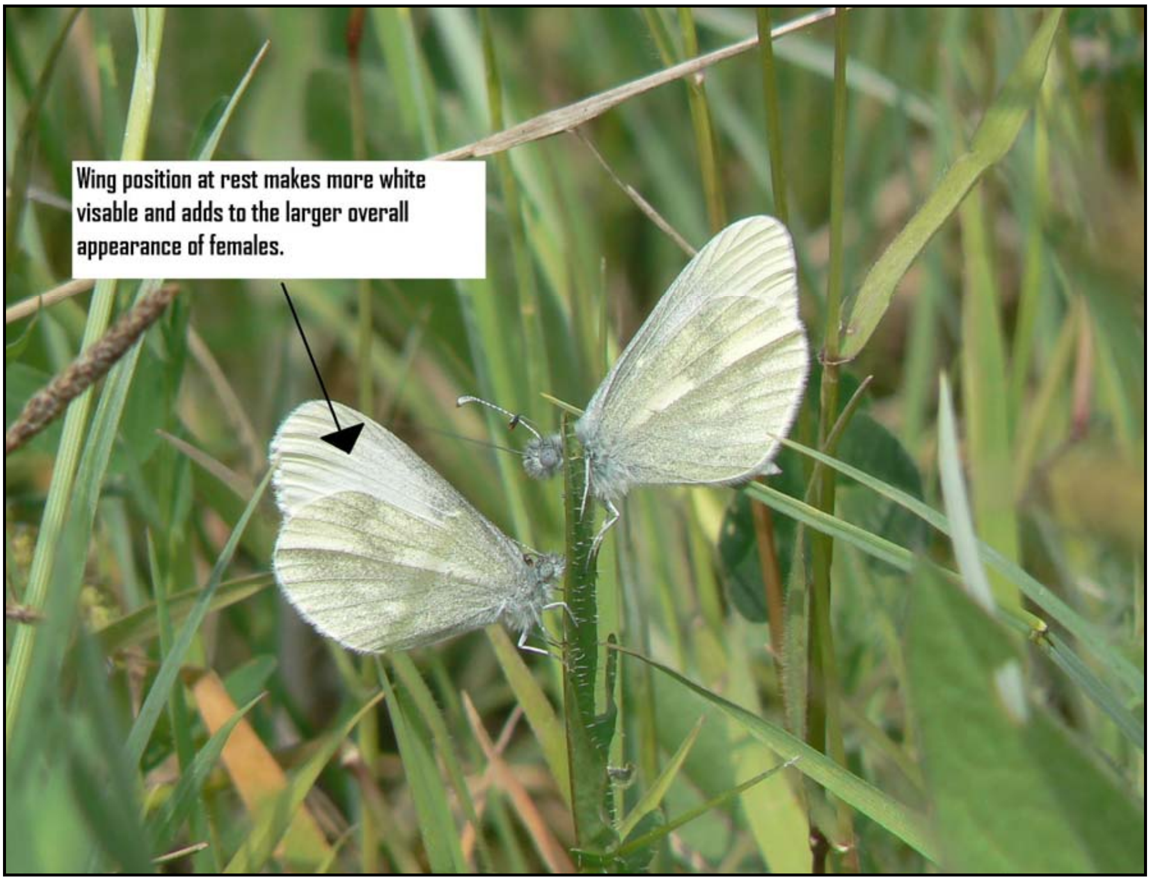
It is also recommended that the Western shore area, which currently supports a small population of *L. reali*, is considered for inclusion in future winter grazing schemes. It is believed that a combination of appropriate management, a reduction in rank grasses and a shorter sward, could see a much larger number of *L. reali* supported in this area.

4.3 FIELD OBSERVATIONS

Courtship position and display

Separation of the sexes could be confirmed during courtship displays and in particular through the position each sex took up during these events. On every occasion that a courtship display was observed, the males took up a position at an angle above the female. Figure 21 shows the typical position of *L. reali* during a courtship display.

Figure 21: *Image showing* L. reali *during courtship. Male top right and female bottom left*



Separation between *Leptidea* species can also be confirmed by adult behaviour during courtship. *L. sinapis* males perform a number of irregular wingbeats during courtship, while *L. reali* express the opposite, keeping their wings in a rested position throughout (Friberg *et al.*, 2008[1]).

During courtship the behaviour of *L. reali* matched that described in Friberg *et al* (2008[1]). Males either followed females until they came to rest or approached females sitting in the vegetation and started to display by beating their head and proboscis from side to side. The male's wings remained in a closed position throughout the display and the female often gave a number of frantic wingbeats either at the beginning of the display as a deterrent to the male, or as a signal that she was about to fly away. Unfortunately, no observed courtship event led to the female producing her abdomen as an acceptance to mate. Video footage of *L. reali* courtship display is available from the author on request.

Observations of multiple egg-laying on single leaves of foodplants

It was interesting to note just how often females placed their eggs on the same leaf of the same plant, as it is thought that *L. reali* eggs are laid singly on the foodplant (Thompson & Nelson, 2006 & www.habitas.org.uk) The bulk of these observations came, as one might expect, from the flight cage work (34/113 or 30%), but a considerable number came from field observations at Craigavon Lakes (7/49 or 14%). In figure 22, picture A shows how females used in flight cages would often choose a particular leaf and place multiple eggs there; in this instance 5 eggs were laid close together. But this phenomenon was not restricted to the flight cages, as shown in picture B, when up to 3 eggs were observed in the field being laid on the same leaf, by the same female one after another, or by different females at different times.

Figure 22: *Multiple eggs laid on the same leaf, from a flight cage female (A) and field observed female (B).*

(A)

(B)

This phenomenon could suggest that there is insufficient suitable habitat or foodplants at a particular site to sustain the entire butterfly population. It may be construed as a call for help, as populations are struggling to find suitable conditions for oviposition. This would certainly be the case in flight cages where the amount of suitable or preferred foodplant is restricted, which therefore requires females to lay multiple eggs on any suitable plant or leaf available. However, the fact that this is also happening in the field is alarming and may suggest that the population at Craigavon Lakes is not doing as well as it might. Alternatively, maybe these observations are no cause for concern and simply relate to an aspect of *L. reali* ecology not previously described.

Personal observations from Murlough NNR

Personal observations over the same time period at Murlough NNR, County Down, allowed a number of key comparisons. Firstly, Murlough is an ancient sand dune system, which has little or no *L. pratensis,* yet the population of *L. reali* on this 285-hectare site numbered in the hundreds. *L. reali* were observed here throughout the *L. corniculatus* dominated fore dunes (during BMS transects, pers. obs.) and were recorded egg laying on this species on the 17th May and 1st June. Results from the present study suggest that when *L. reali* oviposit on *L. corniculatus,* they do so when the ground covering of this species is above 24%. This observation, combined with personal observations at Murlough, suggest that populations of *L. reali* in coastal sand dune systems are able to thrive without *L. pratensis,* where large carpets of *L. corniculatus* exist. This raises the question; is larval survival higher on *L. corniculatus* or *L. pratensis*? Freese and Fiedler (2002) make an important distinction between larval host plants and larval foodplants. Results showed that *L. reali* prefers *L. pratensis* for oviposition but selected *L. corniculatus* as the final instar (fully grown caterpillar) foodplant.

Secondly, Murlough dunes have little or no *V. sepium,* with the most abundant vetch on site being *Vicia sativa.* This suggests that a completely different array of nectar sources is used here. For instance several *L. reali* were observed nectaring on *Potentilla erecta* and *L. corniculatus* on the 11th May (pers. obs.). The former species is common around the margins of Kinnegoe Meadows but *L. reali* at this location seemed to avoid it.

The habitat characteristics of an egg laid on the 17th May at Murlough NNR provide a stark contrast to those found in the present study. The egg was laid on the top frond, 8cm up a *L. corniculatus* plant, in an area with an 80% ground covering of this species (in a 1m quadrat). The average sward height was 15 cm and the egg was laid on a SE facing slope. No other known foodplants were available. These elements combine to suggest that it would have been interesting to compare this site, and a number of others, with those used in the present study.

5. CONCLUSIONS

5.1 KEY FINDINGS

The following outlines the main findings of this study. It is hoped that some of the information given will influence future conservation management of *L. reali* in NI.

- In flight cage choice experiments *L. reali* laid 80% of first eggs and 72% of all eggs on *Lathyrus pratensis*. *L. reali* shows a preference towards *L. pratensis* (Chi squared = 15.6, $p<0.05$) and an observed frequency of 12 against an expected frequency of 5, further confirms that *Lathyrus pratensis* is the foodplant of choice.

- Field observations of oviposition show a preference by *L. reali* towards *Lathyrus pratensis,* with 78% of all eggs recorded on this species. 18% of eggs were found on *Lotus corniculatus* and 2% each on *Vicia sepium* and *Lotus pedunculatus*.

- The flight period at both sites lasted approximately 8 weeks.

- 73% of all eggs were laid on the top two fronds of the foodplant.

- Observations of multiple egg laying events gave an average time to lay an egg of 4 minutes 6 seconds.

- Eggs hatched into 1st instar larvae in an average time of 13 days.

- 3 eggs kept in captivity took 14 days to hatch into 1st instar larvae, 27 days to resemble adult caterpillars, 41 days to become fully grown and 48 days to pupate.

- 81% of all nectaring events involved female butterflies.

- Nectaring was recorded on *Vicia sepium, Lathyrus pratensis, Veronica chamaedrys, Lotus corniculatus, Trifolium pratense, Vicia cracca* and *Lychnis flos-cuculi. L. reali* preferred *Vicia sepium* on 57% of occasions.

- The average duration of a courtship display was 2 minutes 51 seconds.

- 6 specimens were confirmed as *L. reali* by dissection of genitalia.

5.2 LIMITATIONS OF THIS STUDY

One of the main limitations of the study was that time constraints meant that only one flight period could be observed. This meant that 2nd generation *L. reali* could not be searched for. In addition, any annual population trends and the reasons for such fluctuations could not be analysed. I was also restricted by labour constraints. Working alone for most of the study meant that there was not enough time to make daily checks of all eggs for presence/absence or changes in morphology.

Another limiting factor was the choice, location and number of study sites. Using only two study sites in County Armagh meant that while the findings of this research may be true for these locations, it may not necessarily be true across Northern Ireland. To remedy this would require similar research across several sites in each county.

It would also have been valuable to control certain variables in the flight cage tests, such as daylight and temperature. However, an environmental chamber was not available in the present study. Other variables that would have benefited this study include an estimate of plant species abundance, to compare with nectar source choice.

5.3 FUTURE RESEARCH

One of the most important aspects of studying the ecology of any butterfly is to repeat the methodology over a number of flight periods to demonstrate if any natural cycles in populations occur and, if so, for what reason. This was not an option in the present study, but future research may wish to conduct a study over the course of at least 3 flight periods. It would also be beneficial to conduct intensive searches for 2nd brood adults during each of these years. Similarly, as mentioned earlier, it would also be valuable to conduct future research at a greater number of sites across Northern Ireland that better represent the broad range of habitats where *L. reali* can be found.

Another aspect I would like to see studied in more depth is the ecological differences between populations of *L. reali* from Northern Ireland and populations of *L. sinapis* found mostly in the Burren region of the Republic of Ireland. What differences might be observed between these species when used in flight cage experiments of foodplant choice? Are the measurements of genital apparatus significantly different? Where does genetic analysis place butterflies from these populations? It would also be interesting to perform capture, release and recapture experiments on the two populations, such as those used by Friberg *et al* (2008[2]) in Sweden, to see if individuals from each species are found to associate more closely with certain habitats or food plants.

Freese and Fiedler (2002) make an important distinction between larval host plants and larval foodplants, with results showing *L. reali* prefers *L. pratensis* for oviposition but selects *L. corniculatus* as the final instar (fully grown caterpillar) foodplant. This suggests that similar research is needed in NI to discover if eggs are mostly laid on *L. pratensis* but then require *L. corniculatus* to reach final instar. This relates to the earlier discussion of the *L. reali* population at Murlough NNR, where *L. pratensis* is scarce and *L. corniculatus* is abundant.

The present study will supply 23 mid-leg samples to be analysed genetically by an undergraduate student at Queen's in 2009. This research will be the first of its kind in the UK and will help support species descriptions of the *Leptidea* butterfly populations at Craigavon Lakes and Oxford Island NNR. These samples may also be used to supplement future genetic analysis of populations throughout Ireland.

In conclusion this study has highlighted several important aspects of *L. reali* ecology in Northern Ireland, including its habitat requirements, foodplant preferences and choice of nectar sources. Through a range of field observations and flight cage tests, it has illustrated a number of ecological and behavioural traits not previously noted in Northern Ireland. It is hoped that this research and its recommendations for site management and future research, will help to ensure that the 'magical metamorphosis' of *Leptidea reali* continues in all its splendour in Northern Ireland.

6. REFERENCES

Asher, J.; Brereton, T.; Roy, D.; Warren, M Fox, R.. (2006) *The state of butterflies in Britain and Ireland.* Newbury, Nature Bureau.

Austin A. (2004) *The Lough Neagh Wetlands: A habitat survey of six areas within Craigavon Borough*. MSc Environmental Resource Management thesis, University College Dublin.

Beneš, J., Konvika, M., Vrabec, V., & Zámečník, J. (2003) Do the sibling species of small whites, *Leptidea sinapis* and *L. reali* (Lepidoptera, Pieridae) differ in habitat preferences. *Biologia Bratislava.* 58, 943-951.

Davis, E (2006). *Long-term trends in butterfly populations in grasslands at Oxford Island National Nature Reserve*. BSc Zoology dissertation, Queen's University Belfast.

Descimon, H. & Mallet, J. (2007). Bad species in European butterflies. In: *Ecology of Butterflies in Europe* (eds. Settele, J., Konvicka, M., Shreeve, T., Dennis, R. & Van Dyck, H.). Cambridge University Press. (in press).

Feltwell, J. (1995). *The Conservation of Butterflies in Britain past and present*. Wildlife Matters, Battle, England.

Francis J and Dixie G. (1996). *Planting Mixes based on the National Vegetation Classification system*. H.V Horticulture LTD, UK.

Freese, A. (1999): Die Weißlinge *Leptidea sinapis* und *L. reali* - wirklich zwei getrennte Arten? (Lepidoptera: Pieridae). Diplomarbeit, Universität Bayreuth, 128 S. URL http://www.univie.ac.at/population-ecology/people/kf/diploma%20theses/afreese.htm

Freese, A. and Fiedler, K**.** (2002). Experimental evidence for specific distinctness of the two wood white taxa, *Leptidea sinapis* and *Leptidea reali (Pieridae). Nota lepidopterologic*. 25, 39-59.

Freese, A. and Fiedler, K**.** (2004). Unterschiedungsmerkmale von *Leptidea sinapis* (Linnaeus, 1758) und *Leptidea reali* Reissinger, 1989 (Lepidoptera, Pieridae) und ihre

Eignung zur Artbestimmung. *Nachr. entomol. Ver. Apollo, N.F.* 25: 65-77 [Character differentiations between *Leptidea sinapis* (LINNAEUS, 1758) and *Leptidea reali* REISSINGER, 1989 (Lepidoptera, Pieridae) and their value for species identification.

Friberg, M. Vongvanich, N. Borg-Karlsson, A.K. Kemp, D.J & Wiklund, C. (2008[1]). Female mate choice determines reproductive isolation between sympatric butterflies. *Behavioral Ecology and Sociobiology*, 2008. Published online 8th December 2007.

Friberg, M. Bergman, M. Kullberg, J. Wahlberg, N & Wiklund, C. (2008[2]). Niche separation in space and time between two sympatric sister species-a case of ecological pleiotropy. *Evolutionary Ecology.* 22, 1-18.

Friberg, M, Olofsson, M, Berger, D, Karlsson, B and Wiklund, C. (2008[3]). Habitat choice precedes host plant choice-niche separation in a species pair of a generalist and a specialist butterfly. *Oikos* 117: 1337_1344, 2008.

Friberg, M & Wiklund, C. (2007) Generation-dependent female choice: behavioral polyphenism in a bivoltine butterfly. *Behavioral Ecology*. 18, 758-763.

Goulson, D. (1993). Variation in the genitalia of the butterfly *Maniola jurtina* (Lepidoptera: Satyrinae). *Zool. J. Linn. Soc.* 107, 65-71.

Heal, H.G. (1965). The wood white *Leptidea sinapis L*. and the railways. *Ir. Nat. J*. 15, 8-13.

Herman, P. (1999). Taxonomie a rozsireni druhu *Leptidea* (Lepidoptera, Pieridea). Diploma thesis. Prirodovedna fakulta Univerzity Karlovy, Praha, 97 pp.

Jeffcoate, S. (2006). Seasonal variation in the use of vegetation resources by *Leptidea sinapis* (Linnaeus, 1758) (Lepidoptera: Pieridae), a multivoltine species in southern Britain: implications for its conservation at the edge of its range and in the context of climate change. *Entomologist's Gazette.* 57, 69-82.

Karsholt, O. (1999)**.** Dagsommerfugleslægten *Leptidea* Billberg i Danmark. *Lepidoptera.* 7,237-249.

Lorković, Z. (1993). *Leptidea reali* Reissinger 1989 (=lorkovicii Réal 1988), a new European species (Lepid,. Pieridae). *Natura Croatica.* 2, 1-25.

Martin, J-F. (1997)**.** Deux espèces jumelles de Lépidoptères *Leptidea sinapis* et *L. reali* Reissinger. Systématique et Génétique moléculaire. Mémoire de D.E.A. Laboratoire de Systématique évolutive, UPRES Biodiversité, Université de Provence.

Martin, J.-F., Gilles, A., & Descimon, H. (2003). *Species Concepts and Sibling Species: The Case of* Leptidea sinapis *and* Leptidea reali*. In Butterflies - Ecology and Evolution - Taking Flight* (eds C.L. Boggs, W.B. Watt & P.R. Ehrlich), pp. 459-476. University of Chicago Press, Chicago.

Mazel, R. and Leestmans, R**.** (1996). Séparation biométrique des *Leptidea sinapis* L., morsei FENTON et *reali* REISSINGER. Linneana Belgica 17, 46-52.

Monti, L. Baylac, M & Lalanne-Cassou, B. (2001). Elliptic Fourier analysis of the form of genitalia in two *Spodoptera* species and their hybrids (Lepidoptera: Noctuidae). *Biol. J. Linn*. *Soc* 72, 391-400.

Nelson, B., Hughes, M., Nash, R. and Warren, M. (2001). *Leptidea reali* Reissinger 1989 (Lep.: Pieridae): a butterfly new to Britain and Ireland. *Entomologist's Record & Journal of Variation.* 113, 97-101.

Pollard, E., (1977). A method for assessing change in the abundance of butterflies. *Biological Conservation.* 12, 115–134.

Pollard, E. (1982). Monitoring butterfly abundance in relation to the management of nature reserves. *Biological Conservation.* 24, 317-328.

Réal, P. (1988). Lepidoptéres nouveaux principalement Jurassiens. *Mémoires du Comité de Liaison pour les Recherches Ecofaunistiques dans le Jura*. 4, 17.

Reissinger, E. (1989). Checklist Pieridae DUPONCHEL, 1835 (Lepidoptera) der Westpaläarktis (Europa, Nordwestafrika, Kaukasus, Kleinasien). *Atalanta.* 20, 149-185.

Rippey, I. (1986). The Butterflies of Northern Ireland. *Irish Naturalists' Journal.* 22, 133-144.

Rodwell, J. S. (1998). *British plant communities, volume 3: Grasslands and montane communities*. Cambridge University Press.

Thompson, R. S. & Nelson, B., 2003 (Oct 2). *[In] The Butterflies and Moths of Northern Ireland.* URL http://www.ulstermuseum.org.uk/lepidoptera/species.asp?item=430856

Thompson, R & Nelson, B. (2006). *The Butterflies and Moths of Northern Ireland.* Blackstaff Press, National Museums Northern Ireland, Belfast.

Verovnik, R & Glogovcan, P. (2007). Morphological and molecular evidence of a possible hybrid zone of *Leptidea sinapis* and *L. reali* (Lepidoptera: Pieridae). *European Journal of Entomology*. 104, 667-674.

Warren, M.S. (1984). The biology and status of the wood white butterfly, *Leptidea sinapis* (L.) Lepidoptera: Pieridae), in the British Isles. *Entomologists' Gazette*. 35, 207-223.

Warren, M.S. (1985). The influence of shade on butterfly numbers in woodland rides, with special reference to the Wood White *Leptidea sinapis*. *Biological Conservation.* 33, 147-164.

Warren, M.S. Pollard, E. Bibby, T.J, (1986). Annual and long-term changes in a population of the Wood White butterfly *Leptidea sinapis*. *J. Anim. Ecology.* 55, 707-719.

Warren, M.S & Bourn, N.A.D. (1998). Species Action Plan: Wood White *Leptidea sinapis*. Butterfly Conservation, Wareham.

Wiklund, C. (1984). Egg-laying patterns in relation to their phenology and the visual apparency and abundance of their host plants. *Oecologia*. 63, 23-29.

Wiklund, C. (1977[1]). Oviposition, feeding and spatial separation of breeding and foraging habitats in a population of *Leptidea sinapis* (Lepidoptera). *Oikos.* 28, 56-68.

Wiklund, C. (1977[2]). Courtship behaviour in relation to female monogamy in *Leptidea sinapis* (Lepidoptera). *Oikos.* 29, 275-283.

Wiklund, C. Eriksson, T. Lundbergh, H. (1979). The wood white butterfly *Leptidea sinapis* and its nectar plants: a case of mutualism or parasitism? *Oikos.* 33, 358-352.

***(Reference citations presented in *Biological Conservation* journal format).**

INTERNET RESOURCES

L. reali and *L. sinapis* UK and Ireland distribution maps

http://www.ukbutterflies.co.uk/distribution.php?vernacular_name=Réal's%20Wood%20White

http://www.ukbutterflies.co.uk/distribution.php?vernacular_name=Wood%20White

Leptidea genitalia diagram

http://www.toyen.uio.no/norlep/pieridae/leptidea_gen.html

Distribution of *L. reali* in Northern Ireland

http://www.habitas.org.uk/moths/map.asp?item=430856

Habitat requirements

http://www.habitas.org.uk/priority/species.asp?item=430857

http://www.ukbutterflies.co.uk/species.php?vernacular_name=Réal's%20Wood%20White

http://www.butterfly-conservation.org/Butterfly/17/Butterfly.html?ButterflyId=50&Country=&Size=&Colour=&Features=&Family=Whites%20and%20yellows

Life cycle

http://www.habitas.org.uk/priority/species.asp?item=430857

Life cycle table (figure 4)

http://www.ukbutterflies.co.uk/species.php?vernacular_name=Réal's%20Wood%20White

Research needed in NI proposed by BCNI

http://www.irishmoths.fsnet.co.uk/. NB now obsolete.

Lough Neagh statistics

http://www.jncc.gov.uk/default.aspx?page=2073

http://www.ni-environment.gov.uk/area_interest_sitesview?SiteNo=ASSI030

Separating males from females

http://www.butterfly-conservation.org/Butterfly/32/Butterfly.html?ButterflyId=50

7. APPENDICES

Appendix 1: Craigavon Lakes raw data

Nectaring events

Date	*Nectaring event number*	*Plant*	*Sex*		*Date*	*Nectaring event number*	*Plant*	*Sex*		*Date*	*Nectaring event number*	*Plant*	*Sex*
08-May	1	Bush Vetch	F		21-May	21	Bush Vetch	M		25-May	41	Bush Vetch	F
08-May	2	Bush Vetch	F		21-May	22	Bush Vetch	F		25-May	42	Bush Vetch	F
08-May	3	Bush Vetch	F		21-May	23	Bush Vetch	F		28-May	43	Bush Vetch	M
12-May	4	Bush Vetch	M		21-May	24	Bush Vetch	F		28-May	44	Bush Vetch	F
12-May	5	Bush Vetch	M		21-May	25	Bush Vetch	F		28-May	45	Bush Vetch	F
12-May	6	Bush Vetch	M		21-May	26	Bush Vetch	F		28-May	46	Tufted Vetch	M
12-May	7	Bush Vetch	F		24-May	27	Bush Vetch	F		30-May	47	Bush Vetch	F
12-May	8	Bush Vetch	F		24-May	28	Bush Vetch	F		30-May	48	Bush Vetch	F
14-May	9	Bush Vetch	M		24-May	29	Bush Vetch	F		30-May	49	Bush Vetch	F
15-May	10	Bush Vetch	F		24-May	30	Bush Vetch	F		02-Jun	50	Birds-foot Trefoil	F
15-May	11	Bush Vetch	F		24-May	31	Bush Vetch	F		02-Jun	51	Bush Vetch	F
15-May	12	Bush Vetch	M		24-May	32	Bush Vetch	F		02-Jun	52	Birds-foot Trefoil	F
15-May	13	Bush Vetch	M		24-May	33	Bush Vetch	F		02-Jun	53	Birds-foot Trefoil	F
15-May	14	Red Clover	M		24-May	34	Bush Vetch	F		02-Jun	54	Birds-foot Trefoil	F
19-May	15	Bush Vetch	M		24-May	35	Meadow Vetchling	F		02-Jun	55	Birds-foot Trefoil	F
19-May	16	Germander Speedwell	M		24-May	36	Meadow Vetchling	F		02-Jun	56	Birds-foot Trefoil	F
19-May	17	Bush Vetch	M		24-May	37	Tufted Vetch	F		02-Jun	57	Birds-foot Trefoil	F
19-May	18	Bush Vetch	F		25-May	38	Birds-foot Trefoil	F		02-Jun	58	Birds-foot Trefoil	F
19-May	19	Bush Vetch	M		25-May	39	Meadow Vetchling	M		02-Jun	59	Birds-foot Trefoil	F
21-May	20	Bush Vetch	M		25-May	40	Tufted Vetch	F		02-Jun	60	Birds-foot Trefoil	F
Date	*Nectaring event number*	*Plant*	*Sex*		*Date*	*Nectaring event number*	*Plant*	*Sex*		*Date*	*Nectaring event number*	*Plant*	*Sex*
02-Jun	61	Birds-foot Trefoil	F		06-Jun	81	Birds-foot Trefoil	F		09-Jun	101	Birds-foot Trefoil	F
02-Jun	62	Bush Vetch	F		06-Jun	82	Birds-foot Trefoil	F		09-Jun	102	Birds-foot Trefoil	F
02-Jun	63	Birds-foot Trefoil	F		06-Jun	83	Birds-foot Trefoil	F		09-Jun	103	Meadow Vetchling	F
02-Jun	64	Bush Vetch	F		06-Jun	84	Birds-foot Trefoil	F		10-Jun	104	Meadow Vetchling	F
02-Jun	65	Tufted Vetch	F		06-Jun	85	Meadow Vetchling	F		10-Jun	105	Meadow Vetchling	F
02-Jun	66	Red Clover	M		06-Jun	86	Meadow Vetchling	F		12-Jun	106	Meadow Vetchling	F
02-Jun	67	Red Clover	F		06-Jun	87	Meadow Vetchling	F		12-Jun	107	Meadow Vetchling	F
02-Jun	68	Bush Vetch	F		06-Jun	88	Red Clover	F		12-Jun	108	Meadow Vetchling	F
02-Jun	59	Bush Vetch	F		06-Jun	89	Meadow Vetchling	M		12-Jun	109	Meadow Vetchling	F
02-Jun	70	Bush Vetch	F		06-Jun	90	Red Clover	F		23-Jun	110	Meadow Vetchling	F
02-Jun	71	Bush Vetch	F		06-Jun	91	Bush Vetch	F					
02-Jun	72	Bush Vetch	F		06-Jun	92	Bush Vetch	F					
02-Jun	73	Bush Vetch	F		06-Jun	93	Bush Vetch	F					
02-Jun	74	Tufted Vetch	F		06-Jun	94	Bush Vetch	F					
02-Jun	75	Bush Vetch	M		06-Jun	95	Tufted Vetch	F					
03-Jun	76	Bush Vetch	F		06-Jun	96	Birds-foot Trefoil	F					
03-Jun	77	Bush Vetch	F		06-Jun	97	Tufted Vetch	F					
03-Jun	78	Tufted Vetch	M		06-Jun	98	Birds-foot Trefoil	F					
03-Jun	79	Bush Vetch	F		06-Jun	99	Birds-foot Trefoil	F					
06-Jun	80	Bush Vetch	F		09-Jun	100	Birds-foot Trefoil	F					

Multiple egg laying events, courtship displays, weekly log and egg hatch data

Date	***Multiple egg laying event No.***	***No. eggs***	***Total time***	***Average time per egg***		***Date***	***Courtship display No.***	***Duration (secs)***	***Site (C/O)***
21-May	1	4	1318	330		19-May	1	15	C
24-May	2	3	1080	360		19-May	2	37	C
30-May	3	3	600	200		19-May	3	327	C
02-Jun	4	3	1140	380		21-May	4	60	C
03-Jun	5	2	420	210		24-May	5	9	C
06-Jun	6	2	405	203		24-May	6	392	C
06-Jun	7	4	187	62		02-Jun	7	123	C
12-Jun	8	2	452	226		06-Jun	8	301	C
				246		06-Jun	9	471	C
				4 mins 6 secs		06-Jun	10	100	C
						20-Jun	11	45	O
							Average	171	**2 minutes 51 seconds**
WEEKLY LOG	(Field)								
Week Number	No. of site visits Craigavon (Inc)	Average peak count	Average count per hour	No. of necatring events	Total No. of new eggs	Average Weekly AST	Total adults per week		
1	3	0	0	0	0	13.7	0		
2	4	3	2	3	0	18.8	12		
3	4	39	24	16	1	17.3	155		
4	4	38	13	27	14	16.2	153		
5	4	57	21	53	28	19.0	226		
6	5	9	6	10	6	16.0	45		
7	4	2	2	1	0	15.1	5		
	28								
Egg hatch number	Time (days)	No. of days hatched	length (mm)	State					
1	18	1	1.5	1st instar					
2	14	8	5	Caterpillar					
3	14	14	9	Caterpillar					
4	14	21	13	Caterpillar					
5	14	26	17	Caterpillar					
6	11	29	20	Caterpillar					
7	11	35	21	Pupa					
8	12								
9	13								
10	13								
11	13								
12	14								
13	9								
14	9								
Average	**13 days**								

Ovipostion events: field observations

Date	*Egg Number*	*Foodplant*	*Observed/Found*	*Ht. On foodplant*	*Height Category*	*Average ht of sward*	*Ratio*	*Other foodplants available*	*% MV*	*%BV*	*% BFT*	*% TV*	*Aspect*	*Prom plant?*	*Air temp at egg*	*Frond*	*Dist to larger veg*	*Latitude*	*Longitude*
19-May	C1E	Meadow Vetchling	Observed	38	4	41	1.08	No	5	0	0	0	Flat	No	19.1	2	2	305103	357897
21-May	C2E	Bush Vetch	Found	24	3	37	1.54	Yes	5	10	0	0	NE	No	*	2	1	305085	357894
21-May	C3E	Meadow Vetchling	Observed	24.5	3	60	2.45	Yes	35	5	2	0	Flat	No	19.5	3	10	304697	357669
21-May	C4E	Meadow Vetchling	Observed	15	2	26	1.73	Yes	5	5	0	0	Flat	No	23.9	2	5	304704	357670
21-May	C5E	Meadow Vetchling	Observed	16	2	25	1.56	No	20	0	0	0	Flat	No	22.7	2	5	304692	357668
21-May	C6E	Meadow Vetchling	Observed	21	3	42	2.00	Yes	10	5	0	0	Flat	No	22.2	2	5	304692	357669
21-May	C7E	Meadow Vetchling	Observed	28.5	3	47	1.65	No	10	0	0	0	Flat	No	23.4	1	7	304691	357670
24-May	C8E	Meadow Vetchling	Observed	30	3	36	1.20	Yes	10	2	0	0	Flat	No	26.3	1	10	305069	357867
24-May	C9E	Birds-foot Trefoil	Observed	23.5	3	32	1.36	No	0	0	40	0	Flat	No	27.6	2	3	305067	357874
24-May	C10E	Meadow Vetchling	Observed	14	2	24	1.71	Yes	5	5	0	2	N	No	25.3	2	1	305091	357826
24-May	C11E	Meadow Vetchling	Observed	21	3	38	1.81	Yes	10	0	5	0	N	No	21.6	3	1	305187	357922
24-May	C12E	Meadow Vetchling	Found	13	2	38	2.92	Yes	10	0	5	0	N	No	*	3	1	305187	357922
24-May	C13E	Meadow Vetchling	Found	13	2	38	2.92	Yes	10	0	5	0	N	No	*	3	1	305187	357922
24-May	C14E	Meadow Vetchling	Found	13	2	38	2.92	Yes	10	0	5	0	N	No	*	3	1	305187	357922
25-May	C15E	Meadow Vetchling	Observed	17.5	2	35	2.00	Yes	30	0	0	2	Flat	No	21.7	2	3	304803	357745
30-May	C16E	Meadow Vetchling	Observed	26	3	35	1.35	Yes	20	5	0	0	Flat	No	30.7	5	8	305297	357981
30-May	C17E	Meadow Vetchling	Observed	17	2	40	2.35	Yes	10	5	2	0	Flat	Yes	28.7	3	5	305295	357985
30-May	C18E	Meadow Vetchling	Observed	25	3	38	1.52	Yes	10	10	0	0	S	No	28.9	1	6	305295	357985
30-May	C19E	Birds-foot Trefoil	Observed	12	2	30	2.50	Yes	20	0	30	0	Flat	No	31.5	2	9	305257	357922
30-May	C20E	Birds-foot Trefoil	Observed	12	2	30	2.50	Yes	20	0	30	0	Flat	No	31.5	2	9	305257	357922
02-Jun	C21E	Meadow Vetchling	Observed	16.5	2	26	1.58	Yes	25	0	5	0	Flat	No	30.4	1	15	305270	357965
02-Jun	C22E	Meadow Vetchling	Observed	20	2	36	1.80	Yes	5	2	0	15	S	No	29.7	2	5	305238	357960
02-Jun	C23E	Meadow Vetchling	Observed	25	3	47	1.88	Yes	10	0	30	0	N	No	29.2	4	3	305188	357922
02-Jun	C24E	Meadow Vetchling	Observed	7.5	1	25	3.33	Yes	10	0	2	0	Flat	No	33.5	1	4	305178	357919
02-Jun	C25E	Meadow Vetchling	Observed	22	3	40	1.82	Yes	25	0	15	0	N	No	30.2	2	3	305168	357912
02-Jun	C26E	Meadow Vetchling	Observed	17	2	30	1.76	Yes	5	0	2	0	Flat	No	31.9	3	3	305164	357911
02-Jun	C27E	Meadow Vetchling	Observed	27	3	45	1.67	No	20	0	0	0	Flat	No	35.3	2	2	304911	357727
03-Jun	C28E	Meadow Vetchling	Observed	16	2	30	1.88	Yes	15	0	5	0	Flat	Yes	19.9	2	1	305274	357971
03-Jun	C29E	Birds-foot Trefoil	Observed	18	2	35	1.94	Yes	20	0	25	0	Flat	No	27.8	1	2	305269	357973
03-Jun	C30E	Meadow Vetchling	Observed	22	3	38	1.73	Yes	5	0	15	2	Flat	No	24.3	3	7	305173	357918
03-Jun	C31E	Meadow Vetchling	Observed	14	2	32	2.29	No	25	0	0	0	SW	No	23.2	2	3	304816	357694
06-Jun	C32E	Meadow Vetchling	Found	11.0	2	32	2.91	Yes	25	0	10	0	S	No	*	5	2	305271	357974
06-Jun	C33E	Birds-foot Trefoil	Observed	19	2	45	2.37	Yes	15	0	25	0	Flat	No	19.9	1	1	305188	357935
06-Jun	C34E	Meadow Vetchling	Observed	16	2	30	1.88	No	10	0	0	0	Flat	No	20.5	1	12	305173	357941
06-Jun	C35E	Birds-foot Trefoil	Found	14	2	45	3.21	Yes	15	0	25	0	Flat	No	*	2	1	305189	357935
06-Jun	C36E	Birds-foot Trefoil	Observed	28	3	36	1.29	No	0	0	30	0	N	No	20.7	1	3	305147	357904
06-Jun	C37E	Meadow Vetchling	Found	22	3	35	1.59	Yes	5	0	5	0	N	No	*	3	3	305136	357898
06-Jun	C38E	Meadow Vetchling	Found	24	3	35	1.46	Yes	5	0	5	0	N	No	*	2	3	305135	357898
06-Jun	C39E	Birds-foot Trefoil	Found	18	2	35	1.94	Yes	5	0	5	0	N	No	*	2	3	305136	357898
06-Jun	C40E	Meadow Vetchling	Observed	21	3	38	1.81	Yes	10	0	0	2	S	No	19.9	2	2	305060	357876
06-Jun	C41E	Meadow Vetchling	Observed	18	2	35	1.94	Yes	10	0	0	2	S	No	20.6	1	1	305060	357877
06-Jun	C42E	Birds-foot Trefoil	Observed	19	2	32	1.68	Yes	2	0	20	2	Flat	No	21.9	1	1	305064	357875
06-Jun	C43E	Birds-foot Trefoil	Observed	21	3	32	1.52	Yes	2	0	20	2	Flat	No	19.9	2	1	305064	357875
09-Jun	C44E	Meadow Vetchling	Observed	15.5	2	36	2.32	Yes	5	2	0	0	S	No	24.9	2	1	304907	357801
09-Jun	C45E	Birds-foot Trefoil	Observed	20	2	55	2.75	Yes	5	5	15	0	Flat	No	27.6	1	4	304801	357721
10-Jun	C46E	Meadow Vetchling	Observed	25	3	49	1.96	No	10	0	0	0	S	No	21.4	2	0	304716	357638
12-Jun	C47E	Meadow Vetchling	Observed	5.5	1	20	3.64	No	15	0	0	0	Flat	No	20	4	1	304942	357805
12-Jun	C48E	Meadow Vetchling	Observed	16	2	22	1.38	No	15	0	0	0	Hollow	No	19.2	2	1	304946	357813
12-Jun	C49E	Meadow Vetchling	Observed	16	4	33	2.06	Yes	10	5	0	0	S	No	22.2	1	2	304946	357824
Average				**17.4**		**33.1**	**1.91**		**11.0**	**1.0**	**7.8**	**0.6**			**24.0**		**3.5**		

Daily log of ambient shade temperature, number of adults, number of oviposition events and related Chi-squared calculations

DAILY LOG	(Field)				
Date	*Ambient Shade Temperature*	*No. visible Adults*	*Average per hour*	*Ovipositioning Events*	
22-Apr-08	14.2	0	0	0	
28-Apr-08	13.2	0	0	0	
30-Apr	13.7	0	0	0	
01-May	14.2	0	0	0	
05-May	19	3	2	0	
07-May	20.3	3	3	0	
08-May	21.8	6	4	0	
12-May	19.5	26	20	0	
14-May	15.7	32	24	0	
15-May	17.8	46	31	0	
19-May	16.2	51	19	1	
21-May	15.9	36	10	6	
24-May	14.4	52	12	7	
25-May	20.1	48	21	1	
28-May	14.3	17	7	0	
30-May	20.3	79	32	5	
02-Jun	21.1	78	20	7	
03-Jun	18.6	30	13	4	
06-Jun	16	39	20	12	
09-Jun	21.5	19	13	2	
10-Jun	16.8	20	11	1	
12-Jun	14.2	4	4	3	
13-Jun	13.7	1	1	0	
16-Jun	13.7	1	1	0	
19-Jun	14.5	1	1	0	
20-Jun	14.5	2	2	0	
23-Jun	15.5	2	2	0	
25-Jun	16	0	0	0	
CHI SQUARED TESTS					
Week Beginning	*Numbers from BMS*	*Average weekly peak count*	*Average weekly count per hour*		*Median weekly peak number*
05/05/2008	6	4	3		3
12/05/2008	43	39	24		39
26/05/2008	42	48	20		48
02/06/2008	60	42	17		39
09/06/2008	11	9	6		4
		P value	P Value		P Value
		0.178	**0.031**		**0.128**
			Significant at 95% confidence level		
			reject null hypothesis		

Appendix 2: Oxford Island NNR raw data. **Nectaring events, multiple egg laying events and courtship displays.**

Date	Nectaring event number	Plant	Sex		Date	Nectaring event number	Plant	Sex
14-May	1	Bush Vetch	M		06-Jun	22	Bush Vetch	F
16-May	2	Bush Vetch	M		06-Jun	23	Bush Vetch	F
25-May	3	Bush Vetch	F		09-Jun	24	Meadow Vetchling	F
25-May	4	Bush Vetch	F		09-Jun	25	Ragged Robin	F
25-May	5	Bush Vetch	M		09-Jun	26	Bush Vetch	F
26-May	6	Bush Vetch	F		09-Jun	27	Ragged Robin	M
26-May	7	Bush Vetch	F		12-Jun	28	Meadow Vetchling	F
26-May	8	Bush Vetch	F		12-Jun	29	Meadow Vetchling	F
26-May	9	Bush Vetch	F		12-Jun	30	Meadow Vetchling	F
26-May	10	Bush Vetch	M		12-Jun	31	Meadow Vetchling	F
02-Jun	11	Bush Vetch	M		12-Jun	32	Ragged Robin	M
02-Jun	12	Bush Vetch	F		12-Jun	33	Bush Vetch	M
06-Jun	13	Bush Vetch	F		16-Jun	34	Meadow Vetchling	F
06-Jun	14	Bush Vetch	F		16-Jun	35	Meadow Vetchling	F
06-Jun	15	Bush Vetch	F		16-Jun	36	Meadow Vetchling	F
06-Jun	16	Bush Vetch	F		16-Jun	37	Meadow Vetchling	F
06-Jun	17	Bush Vetch	F		19-Jun	38	Meadow Vetchling	F
06-Jun	18	Bush Vetch	F		19-Jun	39	Tufted Vetch	F
06-Jun	19	Bush Vetch	F		20-Jun	40	Meadow Vetchling	F
06-Jun	20	Bush Vetch	F		20-Jun	41	Red Clover	F
06-Jun	21	Bush Vetch	F					
Date	**Multiple egg laying event No.**	**No. eggs**	**Total time**	**Average time per egg**		**Date**	**Courtship display No.**	**Duration**
26-May	1	4	1594	399		20-Jun	1	45 secs
02-Jun	2	3	414	138				
19-Jun	3	3	600	200				
				246				
				4 mins 6 secs				

Oviposition data

Date	Egg Number	Foodplant	Observed/Found	Ht. On foodplant	Height Category	Ratio	Average ht of sward	Other foodplants available	% MV	%BV	% BFT	% TV	%GBFT	Aspect	Prom plant?	Air temp at egg	Frond	dist to larger veg	Latitude	Longitude
25-May	O1E	Meadow Vetchling	Observed	51	6	1.2	59	Yes	5	5	0	0	0	S	No	22.9	2	1	304522	361873
25-May	O2E	Meadow Vetchling	Observed	9.5	1	3.7	35	Yes	2	2	0	0	0	Flat	No	26.5	2	5	305191	361421
26-May	O3E	Meadow Vetchling	Observed	17.5	2	2.9	50	No	15	0	0	0	0	Hollow	No	24.5	4	2	305489	361410
26-May	O4E	Meadow Vetchling	Observed	22	3	2.1	46	Yes	15	0	5	0	0	Hollow	No	23.7	3	1	305490	361409
26-May	O5E	Meadow Vetchling	Observed	21	3	2.7	57	No	20	0	0	0	0	Flat	No	23.5	1	10	305492	361413
26-May	O6E	Birds-foot Trefoil	Observed	4	1	5.8	23	Yes	10	2	5	0	0	Flat	No	25.9	2	10	305497	361412
02-Jun	O7E	Meadow Vetchling	Observed	39	4	1.3	50	No	5	0	0	0	0	W	No	35.2	1	25	305393	361343
02-Jun	O8E	Meadow Vetchling	Observed	18	2	1.8	33	Yes	2	0	0	15	0	W	No	33	1	30	305396	361343
02-Jun	O9E	Meadow Vetchling	Observed	18	2	1.8	33	Yes	2	0	0	15	0	W	No	33	1	30	305396	361343
02-Jun	O10E	Meadow Vetchling	Observed	29	3	1.7	50	Yes	10	0	0	10	0	W	No	31.8	4	30	305397	361340
02-Jun	O11E	Meadow Vetchling	Observed	17	2	1.9	32	Yes	5	0	0	2	0	SW	No	28.6	1	30	305403	361347
03-Jun	O12E	Meadow Vetchling	Observed	14.5	2	1.8	26	Yes	5	0	0	2	2	SW	No	30.1	3	12	305214	361408
06-Jun	O13E	Meadow Vetchling	Observed	55	6	1.2	66	Yes	5	2	0	0	0	Flat	No	26.7	1	9	305288	361344
16-Jun	O14E	Meadow Vetchling	Observed	23	3	2	44	Yes	10	0	0	0	2	Flat	No	20.5	1	12	305294	361358
19-Jun	O15E	Meadow Vetchling	Observed	22	3	1.7	37	Yes	10	0	0	2	0	S	No	28.3	2	15	305396	361333
19-Jun	O16E	Greater Birds-foot trefoil	Observed	20	2	2.4	47	Yes	2	0	0	5	15	S	No	32.6	2	30	305385	361341
19-Jun	O17E	Meadow Vetchling	Observed	17	2	2.6	45	Yes	5	0	0	2	2	S	Yes	29	4	20	305385	361360
Averages				**23.4**		**2.3**	**43.1**		**7.5**	**0.6**	**0.6**	**3.1**	**1.2**			**28.0**		**16.0**		

Weekly log and chi-squared test data

WEEKLY LOG	(Field)						
Week Number	**No. of site visits Oxford Island**	**Average peak count**	**Average count per hour**	**No. of nectaring events**	**Total No. of new eggs**	**Average Weekly AST**	**Total adults per week**
1	3	0	0	0	0	14	0
2	3	3	2	0	0	19.5	8
3	3	13	11	2	0	16.9	38
4	3	18	15	8	6	16.8	85
5	4	25	23	17	7	21.1	98
6	3	6	6	12	4	15.7	25
7	3	1	1	2	0	16.9	4
	22						
CHI SQUARED TESTS							
Week Beginning	**BMS number**	**Average weekly peak count**	**Average weekly count per hour**				
19/05/2008	8	28	15				
02/06/2008	10	27	24				
09/06/2008	10	13	13				
		P Value	P Value				
		0.064	**0.063**				

Appendix 3: List of abbreviations

AST Ambient Shade Temperature
BAP Biodiversity Action Plan
BC Butterfly Conservation
BCNI Butterfly Conservation Northern Ireland
BFT Birds-foot Trefoil
BMS Butterfly Monitoring Scheme
BV Bush Vetch
CBC Craigavon Borough Council
DNA Deoxyribonucleic acid
EHS The Environment and Heritage Service of Northern Ireland
GBFT Greater Birds-foot Trefoil
GPS Global Positioning System
LNR Local Nature Reserve
MV Meadow Vetchling
NI Northern Ireland
NNR National Nature Reserve
NISAP Northern Ireland Species Action Plan
NVC National Vegetation Classification
OSNI Ordnance Survey Northern Ireland
RAPD Random polymorphic DNA
RoI Republic of Ireland
SOCC Species of Conservation Concern
TV Tufted Vetch
UK The United Kingdom of Great Britain and Northern Ireland (note that ‘Britain’ is used in the text to denote the island of England, Scotland and Wales).

Appendix 4: Common and Latin names of species mentioned in the text

Butterflies

Real’s Wood White *Leptidea reali*
Wood White *Leptidea sinapis*
Heath Fritillary *Melitaea athalia*
Essex Skipper *Thymelicus lineola*
Pearl-bordered Fritillary *Boloria euphrosyne*

Plants

Meadow Vetchling *Lathyrus pratensis*
Common Bird's-foot-trefoil *Lotus corniculatus*
Bush Vetch *Vicia sepium*
Tufted Vetch *Vicia cracca*
Greater Bird’s-foot Trefoil *Lotus pedunculatus*
Common Knapweed *Centaurea nigra*
Ribwort Plantain *Plantago lanceolata*
White Clover *Trifolium repens*
Red Clover *Trifolium pratense*
Common Yarrow *Achillea millefolium*
Selfheal *Prunella vulgaris*
Autumnal Hawkbit *Leontodon autumnalis*
Meadow Buttercup *Ranunculus acris*
Common Sorrel *Rumex acetosa*
Great Burnet *Sanguisorba officinalis*
Germander Speedwell *Veronica chamaedrys*
Ragged Robin *Lychnis flos-cuculi*
Oxeye Daisy *Leucanthemum vulgare*
Meadowsweet *Filipendula ulmaria*
Common Spotted-Orchid *Dactylorhiza fuchsii*
Common Vetch *Vicia sativa*
Tormentil *Potentilla erecta*

Trees and Shrubs

Common Hawthorn *Crataegus monogyna*
Willow sp *Salix*
Bramble *Rubus fruticosus*
Rowan *Sorbus aucuparia*
Whitebeam *Sorbus aria*
Wild Cherry *Prunus avium*
Alder *Alnus glutinosa*
Common Oak *Quercus robur*
Ash *Fraxinus excelsior*

Grasses

Crested Dog’s-tail *Cynosurus cristatus*
Red Fescue *Festuca rubra*
Yorkshire Fog *Holcus lanatus*
Cocksfoot *Dactylis glomerata*
Common Bent *Agrostis capillaris*
Sweet Vernal-grass *Anthoxanthum odoratum*
Perennial Ryegrass *Lolium perenne*
False Oat-grass *Arrhenatherum elatius*
Tufted Hair-grass *Deschampsia cespitosa*
Meadow Foxtail *Alopercurus pratensis*

Appendix 5: Site photographs

A) South-east study area, Craigavon Lakes- extensive removal of gorse and creation of a new path (22nd April 2008).

B) Western Shore habitat in early spring, Oxford Island NNR (22nd April 2008).

C) Kinnegoe Meadows in mid-spring, Oxford Island NNR (7th May 2008).

D) North-west section of Craigavon Lakes, with *L. corniculatus* in flower (19th May 2008).

E) Kinnegoe East, Oxford Island NNR, with egg laying locations visible in the foreground (6th June 2008).

F) Kinnegoe Meadow, Oxford Island (2nd June 2008).

All photos © Neal Warnock

Appendix 6: Flight cage raw data

Daily Log		(Flight Cage Exp)											
									Per Food plant				
Date	**Flight cage number**	**Female code**	**Egg number**	**Food plant**	**Frond**	**Height**	**Height Category**	**First egg plant**	**BFT**	**MV**	**BV**	**Total No. per female**	**Notes**
26-May	1	O4O1EFC1										0	
30-May	2	C5FC2	1	Birds-foot Trefoil	2	8	1	Birds-foot Trefoil	4	5	0	9	
30-May	2	C5FC2	2	Birds-foot Trefoil	2	8	1						
30-May	2	C5FC2	3	Birds-foot Trefoil	1	9.5	1						
30-May	2	C5FC2	4	Birds-foot Trefoil	1	10	1						
30-May	2	C5FC2	5	Meadow Vetchling	1	9.5	1						
30-May	2	C5FC2	6	Meadow Vetchling	2	14	2						
30-May	2	C5FC2	7	Meadow Vetchling	5	6.5	1						
30-May	2	C5FC2	8	Meadow Vetchling	2	15.5	2						
30-May	2	C5FC2	9	Meadow Vetchling	2	15	2						
02-Jun	3	CFC3C23,24,25E										0	Died
02-Jun	4	C7FC4	10	Meadow Vetchling	2	28	3	Meadow Vetchling	0	17	0	17	
02-Jun	4	C7FC4	11	Meadow Vetchling	2	28	3						
02-Jun	4	C7FC4	12	Meadow Vetchling	2	28	3						
02-Jun	4	C7FC4	13	Meadow Vetchling	2	28	3						
02-Jun	4	C7FC4	14	Meadow Vetchling	1	30	3						
02-Jun	4	C7FC4	15	Meadow Vetchling	3	25	3						
02-Jun	4	C7FC4	16	Meadow Vetchling	2	10	1						
02-Jun	4	C7FC4	17	Meadow Vetchling	Stem								
02-Jun	4	C7FC4	18	Meadow Vetchling	1	23	3						
02-Jun	4	C7FC4	19	Meadow Vetchling	1	23	3						
02-Jun	4	C7FC4	20	Meadow Vetchling	1	23	3						
02-Jun	4	C7FC4	21	Meadow Vetchling	1	33	4						
02-Jun	4	C7FC4	22	Meadow Vetchling	2	29	3						
02-Jun	4	C7FC4	23	Meadow Vetchling	3	27	3						
02-Jun	4	C7FC4	24	Meadow Vetchling	3	27	3						
02-Jun	4	C7FC4	25	Meadow Vetchling	3	27	3						
02-Jun	4	C7FC4	26	Meadow Vetchling	4	25	3						
03-Jun	5	CFC5C30E										0	
03-Jun	6	C6FC6C28,29E	27	Birds-foot Trefoil	1	8	1	Meadow Vetchling	1	1	0	2	
03-Jun	6	C6FC6C28,29E	28	Meadow Vetchling	3	14	2						
06-Jun	7	O5FC7O13E	29	Meadow Vetchling	1	16	2	Meadow Vetchling	0	6	0	6	
06-Jun	7	O5FC7O13E	30	Meadow Vetchling	1	16	2						
06-Jun	7	O5FC7O13E	31	Meadow Vetchling	1	16	2						
06-Jun	7	O5FC7O13E	32	Meadow Vetchling	1	16	2						
06-Jun	7	O5FC7O13E	33	Meadow Vetchling	1	16	2						
06-Jun	7	O5FC7O13E	34	Meadow Vetchling	2	14	2						
06-Jun	8	C8FC8C36E	35	Meadow Vetchling	3	21	3	Meadow Vetchling	0	1	0	1	
07-Jun	9	CFC9	36	Meadow Vetchling	2	27	3	Meadow Vetchling	2	7	0	9	
07-Jun	9	CFC9	37	Meadow Vetchling	2	27	3						
07-Jun	9	CFC9	38	Meadow Vetchling	2	17	2						
07-Jun	9	CFC9	39	Meadow Vetchling	1	22	3						
07-Jun	9	CFC9	40	Meadow Vetchling	1	22	3						
07-Jun	9	CFC9	41	Meadow Vetchling	1	23	3						
07-Jun	9	CFC9	42	Meadow Vetchling	1	25	3						
07-Jun	9	CFC9	43	Birds-foot Trefoil	1	7	1						
07-Jun	9	CFC9	44	Birds-foot Trefoil	1	7	1						
07-Jun	10	CFC10										0	
08-Jun	11	C9FC11C45E	45	Meadow Vetchling	1	19.5	2	Meadow Vetchling	13	6	1	20	
08-Jun	11	C9FC11C45E	46	Meadow Vetchling	2	18	2						
08-Jun	11	C9FC11C45E	47	Meadow Vetchling	1	33	4						
08-Jun	11	C9FC11C45E	48	Meadow Vetchling	1	30	3						
08-Jun	11	C9FC11C45E	49	Meadow Vetchling	4	12	2						
08-Jun	11	C9FC11C45E	50	Meadow Vetchling	1	33	4						
08-Jun	11	C9FC11C45E	51	Bush Vetch	Stem								
08-Jun	11	C9FC11C45E	52	Birds-foot Trefoil	1	12.5	2						
08-Jun	11	C9FC11C45E	53	Birds-foot Trefoil	2	12.5	2						
08-Jun	11	C9FC11C45E	54	Birds-foot Trefoil	2	10	1						
08-Jun	11	C9FC11C45E	55	Birds-foot Trefoil	2	10	1						
08-Jun	11	C9FC11C45E	56	Birds-foot Trefoil	2	10	1						
08-Jun	11	C9FC11C45E	57	Birds-foot Trefoil	3	7.5	1						
08-Jun	11	C9FC11C45E	58	Birds-foot Trefoil	3	7.5	1						
08-Jun	11	C9FC11C45E	59	Birds-foot Trefoil	1	11	1						
08-Jun	11	C9FC11C45E	60	Birds-foot Trefoil	4	5.5	1						
08-Jun	11	C9FC11C45E	61	Birds-foot Trefoil	1	13	2						
08-Jun	11	C9FC11C45E	62	Birds-foot Trefoil	1	13	2						
08-Jun	11	C9FC11C45E	63	Birds-foot Trefoil	4	9.5	1						
08-Jun	11	C9FC11C45E	64	Birds-foot Trefoil	4	9.5	1						
09-Jun	12	C10FC12	65	Meadow Vetchling	1	22	3	Meadow Vetchling	0	11	0	11	
09-Jun	12	C10FC12	66	Meadow Vetchling	1	22	3						
09-Jun	12	C10FC12	67	Meadow Vetchling	1	22	3						
09-Jun	12	C10FC12	68	Meadow Vetchling	1	22	3						
09-Jun	12	C10FC12	69	Meadow Vetchling	1	22	3						
09-Jun	12	C10FC12	70	Meadow Vetchling	1	22	3						
09-Jun	12	C10FC12	71	Meadow Vetchling	1	22	3						
09-Jun	12	C10FC12	72	Meadow Vetchling	2	20	2						
09-Jun	12	C10FC12	73	Meadow Vetchling	1	19.5	2						
09-Jun	12	C10FC12	74	Meadow Vetchling	1	19.5	2						
09-Jun	12	C10FC12	75	Meadow Vetchling	1	19.5	2						
10-Jun	13	C11FC13C46E	76	Birds-foot Trefoil	1	26.5	3	Birds-foot Trefoil	1	4	0	5	
10-Jun	13	C11FC13C46E	77	Meadow Vetchling	3	27	3						
10-Jun	13	C11FC13C46E	78	Meadow Vetchling	3	27	3						
10-Jun	13	C11FC13C46E	79	Meadow Vetchling	3	27	3						
10-Jun	13	C11FC13C46E	80	Meadow Vetchling	1	27.5	3						
10-Jun	14	C12FC14	81	Meadow Vetchling	1	20	2	Meadow Vetchling	0	1	0	1	
12-Jun	15	C14FC15C47,48E										0	
12-Jun	16	C13FC16C49E	82	Birds-foot Trefoil	2	14	2	Meadow Vetchling	3	11	1	15	
12-Jun	16	C13FC16C49E	83	Birds-foot Trefoil	1	13	2						
12-Jun	16	C13FC16C49E	84	Birds-foot Trefoil	1	13	2						
12-Jun	16	C13FC16C49E	85	Meadow Vetchling	Stem								
12-Jun	16	C13FC16C49E	86	Meadow Vetchling	1	26	3						
12-Jun	16	C13FC16C49E	87	Meadow Vetchling	2	25	3						
12-Jun	16	C13FC16C49E	88	Meadow Vetchling	1	24.5	3						
12-Jun	16	C13FC16C49E	89	Meadow Vetchling	3	21	3						
12-Jun	16	C13FC16C49E	90	Meadow Vetchling	4	20	2						
12-Jun	16	C13FC16C49E	91	Meadow Vetchling	4	20	2						
12-Jun	16	C13FC16C49E	92	Meadow Vetchling	2	19	2						
12-Jun	16	C13FC16C49E	93	Meadow Vetchling	2	19	2						
12-Jun	16	C13FC16C49E	94	Meadow Vetchling	2	19	2						
12-Jun	16	C13FC16C49E	95	Meadow Vetchling	1	21	3						
12-Jun	16	C13FC16C49E	96	Bush Vetch	3	19	2						
16-Jun	17	O6FC17O14E	97	Meadow Vetchling	1	15	2	Meadow Vetchling	0	1	0	1	
16-Jun	18	C15FC18	98	Meadow Vetchling	2	14	2						
16-Jun	18	C15FC18	99	Meadow Vetchling	2	14	2						
16-Jun	18	C15FC18	100	Meadow Vetchling	3	10	1	Meadow Vetchling	0	3	0	3	
19-Jun	19	C16FC19	101	Birds-foot Trefoil	1	16	2	Birds-foot Trefoil	6	0	0	6	
19-Jun	19	C16FC19	102	Birds-foot Trefoil	1	16	2						
19-Jun	19	C16FC19	103	Birds-foot Trefoil	2	19	2						
19-Jun	19	C16FC19	104	Birds-foot Trefoil	2	19	2						
19-Jun	19	C16FC19	105	Birds-foot Trefoil	3	11	2						
19-Jun	19	C16FC19	106	Birds-foot Trefoil	1	14	2						
19-Jun	20	O7FC20O15-17E	107	Meadow Vetchling	1	17	2	Meadow Vetchling	0	7	0	7	
19-Jun	20	O7FC20O15-17E	108	Meadow Vetchling	2	14	2						
19-Jun	20	O7FC20O15-17E	109	Meadow Vetchling	3	12	2						
19-Jun	20	O7FC20O15-17E	110	Meadow Vetchling	4	12	2						
19-Jun	20	O7FC20O15-17E	111	Meadow Vetchling	2	16	2						
19-Jun	20	O7FC20O15-17E	112	Meadow Vetchling	1	15.5	2						
19-Jun	20	O7FC20O15-17E	113	Meadow Vetchling	2	13.5	2						
					2	**18**			**30**	**81**	**2**	**113**	

Appendix 7: Genitalia measurements and mid-leg sample raw data

GENITALIA MEASUREMENTS							
Specimen Number	***Unique ID code***	***Date collected***	***Sex***	***Length of Ductus bursae***	***Length of Saccus***	***Length of Aedeagus***	***Threshold Size (Freese and Fiedler, 2004)***
1	O7FC20O15-17E(g)	19.6.08	Female	0.953mm	na	na	>0.75mm
2	O6FC17O14E(g)	17.6.08	Female	0.953mm	na	na	>0.75mm
3	C15FC18(g)	17.6.08	Female	0.953mm	na	na	>0.75mm
4	C16FC19(g)	19.6.08	Female	1.089mm	na	na	>0.75mm
5	O5FC7O13E(g)	6.6.08	Female	*	na	na	>0.75mm
6	C5FC2(g)	30.5.08	Male	na	0.772mm	1.907mm	Saccus >0.77mm, Aedeagus >1.76mm
MID-LEG SAMPLES							
Number	***Unique ID code***	***Date***	***Sex***	***No. eggs laid in flightcage***			
1	C1	8.5.08	M	na			
2	C2	8.5.08	M	na		**Key**	
3	O1	8.5.08	M	na			
4	O2	8.5.08	M	na		C	From Craigavon Lakes
5	C3	14.5.08	M	na		OI	From Oxford Island NNR
6	O3	14.5.08	M	na		FC	Used in a flight cage
7	C4	21.5.08	F	na		E	Egg number
8	O4O1EFC1	26.5.08	F	0		(g)	underwent genital examination
9	C5FC2(g)	30.5.08	M	na*			
10	C6FC628,29E	3.6.08	F	2			
11	C7FC4	3.6.08	F	17			
12	O5FC7O13E(g)	6.6.08	F	6			
13	C8FC8C36E	6.6.08	F	1			
14	C9FC11C45E	8.6.08	F	20			
15	C10FC12	9.6.08	F	11			
16	C11FC13C46E	10.6.08	F	5			
17	C12FC14	10.6.08	F	1			
18	C14FC15C47,48E	12.6.08	F	0			
19	C13FC16C49E	12.6.08	F	15			
20	O6FC17O14E(g)	17.6.08	F	1			
21	C15FC18(g)	17.6.08	F	3			
22	C16FC19(g)	19.6.08	F	6			
23	O7FC20O15-17E(g)	19.6.08	F	7			
* mislabled male not used in a flight cage				More detailed information about each egg laid available upon request			

Appendix 8: Risk Assessment

FIELDWORK RISK ASSESSMENT

Activity: Butterfly fieldwork/survey

Dates: 22nd April- 25th June 2008

Locations: Craigavon Lakes and Oxford Island NNR

Participants: Neal Warnock, volunteers

NATURE OF HAZARD:	PHYSICAL AND HEALTH HAZARDS ARISING:	ESTIMATION OF RISK:	PRECAUTIONS TO TAKE:
Contact with other users: such as landowners, dog walkers, drunks/ drug users, joy riders etc.	Confrontation, assault, theft, contamination etc	Low	Avoid hazardous areas or time survey to avoid confrontation. I.e. middle of the day.
Geographical	Getting lost	Low	Sites are small and it is highly unlikely that this situation will arise. I am already familiar with both sites.
Ground conditions: uneven terrain, pot holes, slippery conditions	Trip, slip, or fall resulting in injury	Low	Proceed with care. Plan out survey work taking account of any maintenance work in the area. Always use the same route on each site visit. I will carry a mobile phone at all times.
Adverse weather conditions	Hypothermia	Low	This situation is extremely unlikely to arise, as survey work will only take place in periods of relatively high temperatures during the summer months. However, weather forecasts will be consulted before each site visit and appropriate wet weather clothing carried at all times.
	Heat-stroke or sunburn	Medium	In hot sunny weather I shall carry water, apply sun cream, and wear a sun hat.

* Template for risk assessment based on previous work with The National Trust. Permission for use received.

Insect or animal bites	Bites, stings, Lyme disease	Medium	Avoid surveying near wild animals such as cattle, keep an eye on them and be prepared to move off should it be necessary. If bites/stings persist I should wear insect repellent and ensure correct clothing is worn. Loose clothing such as t-shirts should be tucked into trousers and trouser legs tucked into socks. After finishing the activity clothing should be examined for ticks. If ticks are present on the body they should be removed using tweezers and the area cleaned with antiseptic.
Contact with animals: dogs and cattle	Minor injury	Low	Keep a safe distance from any grazing cattle and be aware of any dogs off leads.
Contact with animal faeces: dogs and cattle	Infection	Low	Be aware of where I am walking at all times and if contacted clean immediately with water and antiseptic.

FURTHER PRECAUTIONS MADE:

I have first-aid training and will carry a small First aid kit and mobile phone at all times. Any additional helpers should be given instruction as to the hazards of the fieldwork and warned about any particular places to avoid e.g. very uneven ground.

I will be in close contact with Marcus Malley (Conservation Manager, Craigavon Borough Council at the nearby Oxford Island Discovery Centre) throughout the fieldwork, so that someone is aware of my whereabouts and expected finish time. Marcus also provided me with a list of local telephone numbers to dial in the event of an emergency.

SIGNED:___________________________ DATE: _______________

* Template for risk assessment based on previous work with The National Trust. Permission for use received.