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**Taxonomy, Parataxonomy, and Metabarcoding:  
an investigation of invertebrate diversity in  
High Nature Value Wet Grasslands.**



A thesis submitted to the National University of Ireland for the degree of  
Doctor of Philosophy

September 2017

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“The last word in ignorance is the man who says of an animal or plant, "What good is it?" If the land mechanism is good, then every part is good, whether we understand it or not. If the biota, in the course of aeons, has built something we like but do not understand, then who but a fool would discard seemingly useless parts? To keep every cog and wheel is the first precaution of intelligent tinkering.”

— Aldo Leopold, *Round River: From the Journals of Aldo Leopold*

“We should not rush in headlong and so firmly take our stand on one side that, if further progress in the search of truth justly undermines this position, we too fall with it.”

—St. Augustine, *De Genesi ad litteram*



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## **Declaration**

I, John G.J. Carey, hereby verify that this thesis is all my own work and that I have not obtained a degree in this university or elsewhere on the basis of this work

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## Structure of the thesis

The aim of this thesis was to study and enhance the existing knowledge of aerial invertebrate diversity in High Nature Value Wet grassland within the context of conservation assessments, planning, and monitoring. Specifically, I sought to examine the following:

1. To determine the most effective means of capturing Diptera: Sciomyzidae in wet grassland habitats
2. The role that habitat heterogeneity and temporal turnover plays in maintaining dipteran diversity in wet grasslands
3. The effects of spatial scale of observation, environmental variability, and habitat type on invertebrate biodiversity surrogate groups
4. The use of DNA metabarcoding to investigate aerial invertebrate diversity and temporal dynamics in wet grassland habitats

Aerial invertebrates were captured from wet grassland habitats located in the west of Ireland (see Appendix 1) and identified using three methodologies: Taxonomic, Parataxonomic, and molecular (DNA-based) identification. Taxonomic identification was focused on two dipteran families (Sciomyzidae and Syrphidae) of which 34 and 72 species respectively were determined to species level (see Appendices 2 and 3). The thesis is structured into chapters following an article-based format. Three of the chapters (2, 3 and 4) are currently available as peer-reviewed publications in International journals. The research chapters are preceded by a general introduction (Chapter 1) and concluded with a general discussion, conclusions, and recommendations for future research (Chapter 6).

Along with these research chapters, the thesis also contains a first record of *Dorylomorpha anderssoni* Albrecht, 1979; A species of Big-headed fly (Diptera: Pipunculidae) new to Ireland, which was discovered during this investigation (See Appendix 4). This record is published in the Irish Naturalists Journal.

The sampling sites and methodology of identification and analysis is maintained throughout the study. There is therefore, a level of overlap amongst some sections of the individual chapters

## **Abstract**

Wet grassland habitats characterised by seasonal inundation and low-intensity agricultural practices support a rich mosaic of plant and animal communities. Whilst most of the conservation strategies aimed at protecting these areas of High Nature Value focus on botanical and/or ornithological interests, these areas also harbour a significant diversity of invertebrate species. However, the inclusion of invertebrates in conservation planning is normally limited to iconic species such as bees or butterflies with the majority of other invertebrate groups overlooked. This exclusion is driven by factors related to a deficiency of information on distributions, and difficulties associated with the identification of hyper-diverse orders. The need to develop means of including a broader range of invertebrates in the assessments of such High Nature Value (HNV) wet grasslands and generating new information on invertebrate diversity within these areas provided the incentive for this study. Chapter one gives an introductory overview of wet grasslands as HNV areas and identifies solutions to including invertebrate diversity in the selection and monitoring of these habitats.

Chapter two investigates the capture of insects in wet grassland habitats, with a specific focus on one potential bioindicator group, namely Diptera: Sciomyzidae. Sciomyzid capture in two wet grassland habitat types with markedly different vegetation structure (rush dominated and sedge dominated wet grassland) was examined using three different sampling methods: Sweep net, Malaise Trap, and Emergence trap. The results illustrated that sweep nets were not effective at capturing Sciomyzidae from sedge dominated wet grassland. Species collections differed according to capture method with a marked variation between sweep nets and Malaise traps in rush dominated wet grassland. However, no capture method comprehensively collected all species observed in the short-term pilot study. The results imply that multiple sampling methods may be required to produce comprehensive inventories of aerial invertebrates where assessments are conducted over restricted time periods. The investigation also

suggests that synecological studies of Sciomyzidae in wet grasslands should include the use of Malaise traps which are effective in both long and short vegetation.

Chapter three examines the role that two habitat categories of wet grassland (rush and sedge dominated wet grasslands) play in maintaining the diversity of two Diptera families (Sciomyzidae [marsh flies] and Syrphidae [hoverflies]). Using analysis that considered spatial, temporal, and spatiotemporal partitioning of diversity, the results highlight that both habitat types significantly contribute towards the maintenance of the overall gamma diversity of both dipteran families. This is despite rush dominated wet grassland being largely considered ecologically and productively inferior to sedge dominated areas. Managing and maintaining wet grassland habitat heterogeneity in wet grasslands, characterised by different vegetation types, is crucial to conserving these two dipteran families. The results of this work illustrate that wet grassland with abundant rush cover has a significant role to play in the conservation of wet grassland invertebrate species and should be considered as an ecologically important habitat.

In chapter four, the use of two dipteran families (Sciomyzidae and Syrphidae) were investigated as surrogates of broader dipteran diversity; a potential strategy for the inclusion of Diptera in conservation objectives. The study examined community congruence among three dipteran assemblages (Sciomyzidae, Syrphidae, and nine other dipteran families identified as Parataxonomic units [PUs]) whilst accounting for environmental variability, scale of observation, and habitat type. Sciomyzidae and PUs demonstrated strong patterns of congruence at low spatial scales (individual traps) and displayed similar community differentiation based on habitat type. When wet grasslands were further differentiated into two habitat categories (rush and sedge dominated), the significance of community congruence among groups also increased markedly, suggesting a need to examine congruence of communities within categorized units such as habitat types. These results also imply that invertebrate assemblages which exhibit comparable ecological partitioning and similar responses to spatial scale are more likely to be useful surrogates of one another. Correlations between the richness, abundance, and Shannon's diversity of groups were observed as highly variable and inconsistent. The results demonstrate compositional analysis of Diptera as an effective means of including this group in biodiversity surrogacy studies

and highlights the need to consider invertebrate diversity at spatial scales untypical of current assessments at farm or field level.

The penultimate chapter of the thesis investigated the potential use of DNA metabarcoding (MBC) technology as a means of examining wholesale aerial invertebrate diversity in wet grassland habitats. Using taxonomic and parataxonomic datasets for comparison with the MBC data, the richness and diversity of two contrasting wet grassland habitats were investigated; namely rush and sedge dominated areas. The results indicated that rush dominated wet grasslands were significantly more diverse based on the MBC data; highlighting its potential importance to maintaining invertebrate diversity. Rush wet grassland also exhibited a significantly higher Local Contribution to beta diversity based on MBC sequence data, suggesting that at a molecular level these habitats have higher numbers of uncommon assemblages of invertebrates. Using all three datasets, the study also examined successional changes in the community structure of wet grassland invertebrates which is largely unreported. The results illustrate novel findings into aerial invertebrate community turnover which exhibits a series of distinct compositions that follow a cyclical pattern of succession. Finally, the taxonomic and parataxonomic groups examined in the study proved useful as surrogates of the overall community (as represented by MBC data) in terms of community congruence; illustrating for the first time that the use of selected *a priori* groups to monitor wholesale invertebrate diversity is feasible.

Chapter six summarizes the main outcomes of the four preceding chapters through the establishment of key points and examines the potential of including Diptera and wider aerial invertebrate diversity in conservation strategies. The discussion also examines the implications of the findings within the context of the current conservation framework, and highlights areas in need of further research.



## **Chapter 1:**

### **General Introduction**



Wet grassland habitat as defined by Fossitt's Guide to the Habitats of Ireland (2000) near Lough Hackett, Co. Galway Ireland (J.G.J. Carey, June 2015)

## **General Introduction**

### **1.1. Scope and Objectives**

The inclusion of invertebrates in conservation planning and monitoring is largely curtailed by a lack of data concerning the distribution and status of invertebrates along with the difficulties of including such a diverse group in typical conservation assessments (Cardoso et al., 2011). Owing to these impediments, much of the conservation of invertebrates focuses heavily on protected species such as those listed on Annex II and IV of the European Communities (Natural Habitats) Regulations, 1997, or alternatively, on species with elevated public appeal such as bees or butterflies. Despite being considered as farmland areas with a potentially High Nature Value, relatively little information is available concerning invertebrate diversity in lowland wet grasslands in Ireland. This has contributed towards the value of these habitats being determined by their plant species richness and to some extent their utilization by birds. The aim of this thesis was to investigate invertebrate communities sampled across a temporal gradient from two distinctive wet grassland habitat types (rush dominated and sedge dominated), and to contribute towards a greater understanding of the invertebrate diversity within these areas. Along with this, the project aimed to inform strategies associated with conservation planning and monitoring of invertebrates. The objectives of the study were:

1. Determine the most effective means of sampling Diptera: Sciomyzidae from wet grassland habitats
2. Examine the contribution of habitat heterogeneity to invertebrate diversity in lowland wet grasslands
3. Investigate the use of two well-known dipteran families (Sciomyzidae, Syrphidae) as surrogates for wider dipteran diversity
4. Compare and contrast DNA metabarcoding data of wholesale aerial invertebrate diversity (obtained from Malaise traps) with more traditional datasets (taxonomic/parataxonomic) within the context of conservation assessments and monitoring, along with providing novel ecological insights.

## 1.2. Literature review

### 1.2.1 Lowland wet grasslands

Agricultural landscapes within Europe, especially those with low-intensity production systems and enduring semi-natural habitat, contribute towards the maintenance of species diversity (Bignal and McCracken 2000; Bignal and McCracken 1996). One such semi-natural habitat is lowland wet grassland, which often supports a rich mosaic of plant and animal communities. These wet grasslands are typically defined as being in close proximity to water bodies, subject to some level of seasonal inundation, and contain a range of characteristic plant species (Joyce and Wade, 1998). Despite the fact that wet grassland is largely considered as having a High Nature Value owing to its natural or semi-natural state (Billetter et al., 2008), changes in land use practices such as intensification, commercial afforestation, and abandonment have seen many wet grasslands throughout Europe badly degraded or lost (Habel et al., 2013; Tschardt et al., 2005).

Within an Irish context, wet grassland is typically classified as having >50% cover of grass, sedge, or rush species with a significant proportion of drier grassland plants but not dominated by broadleaf herbs or reeds (Fossitt, 2000). Broadleaf plants which generally indicate wet grassland in Ireland include Creeping Buttercup (*Ranunculus repens*), Marsh Thistle (*Cirsium palustre*), Silverweed (*Potentilla anserina*), Meadowsweet (*Filipendula ulmaria*), Water Mint (*Mentha aquatica*), Common Marsh-bedstraw (*Galium palustre*), Devil's-bit Scabious (*Succisa pratensis*), Lesser Spearwort (*Ranunculus flammula*) and Cuckooflower (*Cardamine pratensis*) (Fossitt, 2000)<sup>1</sup>. These criteria are broad enough to include many types of wet grassland with markedly different dominant vegetation (Fig. 1), which has led to the ecological quality of wet grasslands usually being determined by their plant species richness (Sullivan et al., 2010).

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<sup>1</sup> Plant taxonomy follows Webb's An Irish Flora (Parnell and Curtis, 2012)

Strategies to conserve wet grassland habitats that are actively utilized for agriculture include the development of agri-environmental schemes, the most recent incarnation being the Green, Low-Carbon, Agri-Environment Scheme (GLAS) (Department of Agriculture Food and the Marine, 2015a). Such policy incentives to maintain and/or restore wet grasslands are largely based on the presence of protected habitats or species associated with wet grasslands; in particular Annex II habitats or Annex IV species as listed on the EU Habitats Directive (1997). Drake (1998) underlines the fact that much of the conservation of lowland wet grassland is driven by botanical or ornithological interests, with these habitats being important refugia for many species of breeding waders (Vickery et al., 2001). However, the environmental heterogeneity associated with wet grasslands can also sustain a high diversity of invertebrates; many of which are scarce or threatened (Joyce and Wade, 1998). Features associated with wet grasslands such as damp hollows (Kirby, 1992), temporary pools (Nicolet et al., 2004), drainage ditches (Verdonschot et al., 2011), and isolated trees and hedgerows (Drake, 1998) have all proven to be important to invertebrate conservation in agricultural landscapes. However, it is increasingly evident that the conservation of invertebrate biodiversity in such areas requires attention to small-scale habitat features which may be overlooked in conservation planning (Kirby, 1992).

### **1.2.2. Including invertebrates in conservation planning and monitoring**

The use of invertebrates in the designation and management of agricultural areas considered as being of High Nature Value is usually limited (if included at all) to a few well known, easily identifiable, and often iconic groups such as butterflies or bees (Andersen et al., 2004). In Ireland, only one insect, the Marsh Fritillary Butterfly (*Euphydryas aurinia* Rottemburg, 1775), is afforded special protection under EU law (European Communities [Natural Habitats] Regulations, 1997).



**Figure 1. a)** Sedge (*Carex spp.*) dominated wet grassland being grazed by cattle. **b)** Rush (*Juncus spp.*) dominated wet grassland which offers little forage value to grazers. Both habitats are classified as Wet Grassland by Fossitt (2000) although they have markedly different vegetation cover (J.G.J. Carey *pers. Obs.*)

However, it remains to be seen whether the effective conservation of an insect species which persists as a highly mobile metapopulation, and has a narrow trophic requirement at the larval stage (Warren, 1994), is a sufficient umbrella species for wider invertebrate diversity. Other invertebrate groups which are intractable to biodiversity assessments due to a combination of hyper-abundance and hyper-diversity are overlooked in favour of species which are considered priority in terms of policy frameworks and legislative responsibilities. This has effectively led to the wholesale exclusion of the vast majority of invertebrates from conservation policy. Groups such as the true flies (Diptera), and the Hymenoptera (with the exception of bees) are routinely overlooked in conservation strategies despite contributing significantly to the diversity and biomass of freshwater wetland habitats (Keiper et al., 2002) including associated wet grasslands. Diptera, of which there are over 3,300 species in Ireland (Chandler et al., 2008) are especially neglected. Reasons for this lack of inclusion in conservation planning include:

- 1) The abundance and diversity of the group makes it difficult to include them in the narrow time-frames and limited resources usually allocated to conservation assessments and monitoring and
- 2) A paucity of information regarding the populations and distribution of species in comparison to more iconic groups such as bees and butterflies renders wholesale assessments difficult.

However, the need to include a broader range of invertebrates in conservation strategies is increasingly important considering their contribution to biodiversity and ecosystem services in general. Inclusion of overlooked invertebrate groups such as Diptera in conservation policy largely depends on the use of strategies that conform to the timeframes and scales of existing assessments and monitoring. These vary from focusing on a few well-known and easily identifiable species (which may be representative of the wider diversity) to the identification of a broader range of species using rapid biodiversity assessment techniques (Moreno and Sánchez-Rojas, 2007).

### **1.2.3. Biodiversity surrogates**

One approach to the rapid assessment of invertebrates is the use of selected invertebrate groups as biodiversity surrogates for a broader range of taxa (Anderson et al., 2011; Duelli et al., 1999; Duelli and Obrist, 2003; Hayes et al., 2015). A predetermined measure of the diversity of the selected surrogate is used to reflect the diversity of similar or sometimes different taxa (McGeoch, 1998). One of the most frequently utilized measures of diversity in such cross-taxon evaluations is species richness or alpha diversity (Duelli and Obrist, 2003; Moreno and Sánchez-Rojas, 2007; Prendergast, 1997). In effect, the biodiversity surrogates' species richness is either positively or negatively correlated with the species richness of other taxa. In theory, the monitoring of the surrogate is representative of the status of a broader range of taxa. However, patterns of congruence between the richness of groups are highly unpredictable and the use of species richness correlations between groups does not readily inform conservationists about species identity or complementarity<sup>2</sup> in given areas (Su et al., 2004).

Alternative approaches to cross-taxon congruence evaluations include the use of community similarity (also referred to as community congruence) between assemblages (Rooney and Bayley, 2012; Su et al., 2004). For examination of dipteran assemblages in particular, changes in assemblage composition is considered the most reliable approach (Hughes et al., 2000). Therefore, community analysis may be the most useful method of investigating wholesale invertebrate diversity in habitats such as wet grasslands where Diptera may represent a large proportion of the aerial invertebrate diversity. Such examinations may also provide more comprehensive insight into the role of wet grasslands in the maintenance of overall invertebrate diversity across the landscape (i.e. gamma diversity).

### **1.2.4. Selecting suitable biodiversity indicators**

A broad definition of a bioindicator is a species (or group of species) that reflects the biotic or abiotic condition of the environment under observation; characterises the

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<sup>2</sup> Complementarity can be defined as the degree to which the addition of an area adds unrepresented attributes to a system as a whole e.g. how much an area contributes to gamma (overall) diversity within a landscape (McGeoch, 1998; Vane-Wright et al., 1991)

impact of environmental change upon a habitat, community or ecosystem; or is indicative of the overall biodiversity within the area of investigation (McGeoch, 1998). McGeoch (1998) divides bioindicators into three classes: ecological, biodiversity and environmental. The basic demarcation being that environmental and ecological indicators are used to detect changes in the environment, while biodiversity indicators reflect the diversity of a subset of taxa, or wholesale diversity within the area sampled (Rainio and Niemelä, 2003). These divisions are not considered mutually exclusive and some species or groups of species may reflect one or several of the indicator classes.

Lindenmayer et al., (2000) further refine these two broad classes with detailed definitions:

Biological diversity indicators:

- A species which indicates the presence of a set of other species and the absence of which indicates the absence of that entire set of species
- A keystone species, which is a species whose addition to or loss from an ecosystem leads to major changes in abundance or occurrence of at least one other species
- A dominant species that provides much of the biomass or number of individuals in an area

Indicators of abiotic conditions and/or changes in ecological processes:

- A species the presence of which indicates human-created abiotic conditions such as air or water pollution
- A species that indicates particular environmental conditions such as certain soil or rock types
- A species thought to be sensitive to change, thereby serving as an early warning indicator of environmental changes
- A management indicator species, which is a species that reflects the effects of a disturbance regime or the efficacy of efforts to mitigate disturbance effects

The recommendations provided in McGeoch et al., (1998) and Lindenmeyer et al., (2000) have been applied to test the suitability of certain well known terrestrial insects many of which are associated with wet grassland habitats e.g. Carabidae (Rainio and Niemelä, 2003) and Hymenoptera (Anderson et al., 2011). Other groups such as the Sciomyzidae (marsh flies) and Syrphidae (hoverflies) have been suggested as possible indicators of wetland habitats including wet grasslands (Murphy et al., 2012; Speight, 1986). There are several defined prerequisites that a useful indicator should have (Cassola and Pearson, 1992; Pearson, 1994) and the selection of suitable bioindicators for environmental and conservation planning has been subject to several reviews (Favreau et al., 2006; McGeoch, 1998; Noss, 1990). In a tabularized summary, McGeoch (1998) identifies 32 criteria based on a review of publications and their pertinence to the relevant category of bioindication, the consensus being that a good bioindicator should display the following traits:

- Stable and well-known taxonomy
- Practical and easily identified
- Wide geographic range within a range of habitat types (not obligatory)
- Well understood life history and biology characteristics
- Readily observed in the field and easily captured
- Each population or species tends to be specialized within a narrow habitat
- Patterns observed in the indicator taxon are reflected in other related and unrelated taxa

However, the characteristics of a good indicator are almost entirely dependent on the objectives of the environmental issue being addressed, and suitable and effective bioindicators must also be evaluated on a cost-effective and logistic level (McGeoch, 1998). In terms of indicators of biodiversity or bioindicators in general, considerations such as the use of well-known groups which can be easily captured and identified may well supersede those groups which might function as excellent indicators but are problematic to analyse owing to taxonomic constraints i.e. difficult to identify

(Pearson, 1994). It has also been suggested that some very well-known groups should be considered as ‘foundation groups’ for analysis in all habitats whilst others known as ‘auxiliary groups’ would be utilized in specific areas (Speight, 1986). There is also a default tendency to utilize rare or endangered taxa as biodiversity indicators due to the legal obligations although these species may not actually be suitable indicators of broader diversity at all (Pearson, 1994).

#### **1.2.5. Shortcuts in biodiversity evaluation-Parataxonomy**

One of the main obstacles facing the inclusion of invertebrates in conservation planning and monitoring is the lack of data on their spatial and temporal distributions which is often exacerbated by their vast abundance and specious nature (Cardoso et al., 2011). While the need to include invertebrates in conservation plans is increasingly urgent, decisions must also be based on robust data obtained through efficient measures of biodiversity (McGeoch et al., 2002). However, the identification of complex and diverse groups of invertebrates to species level usually requires expertise and time which is not readily available in the context of typical diversity assessment timeframes (Moreno and Sánchez-Rojas, 2007).

One of the most frequently utilized means of including broader invertebrate diversity in conservation assessments includes attempting to identify several groups of invertebrates using less traditional methods (Oliver et al., 2016). One such identification technique is parataxonomy as described by Oliver et al., (1993) which utilizes an approach whereby individuals with similar external morphological traits are grouped together as typological units or Parataxonomic Units (PUs). Originally referred to a ‘*Morphospecies*’ or ‘*Recognisable taxonomic units (RTUs)*’ the term ‘*Parataxonomic Unit*’ was introduced by Krell (2004) as a more valid term to describe biological units that are subjectively assigned. However, a search of Google Scholar illustrates that the use of the term ‘*Morphospecies Invertebrates*’ is still extensively employed (10,300 results, 1,850 since 2015) whereas ‘*Parataxonomy Invertebrates*’ (509 results, 87 since 2015) is less commonly utilized. However, search results illustrate that the method is widely in use, and in terms of nomenclature both terms are largely interchangeable.

The parataxonomic process involves the sorting of bulk samples of invertebrates into morphologically similar units or PUs. Generally, the sorter is given a basic introduction to the taxonomy of the groups by an expert. The work is usually carried out by individuals with minimal taxonomic training, often undergraduate students, and possibly even through public participation such as citizen science (Casanovas et al., 2014). As the PUs are assigned to newly encountered specimens a reference collection is established and distinguishing characteristics noted (Oliver and Beattie, 1993a). This reference collection is maintained as a series of voucher specimens but the use of digital photography to collate samples has also been used effectively (Basset et al., 2000).

After the initial sorting and PU allocation, two sets of sorted specimens are usually examined. The first set is the original voucher specimens which are determined to species level. The second set is a random sub-set of <30 individuals from each PU allocation. Both sets of samples are then identified to species level by experts. The examination of the voucher specimens identifies where taxonomic species were ‘split’ into two or more PUs. The examination of the other sub-set of samples determines (to a degree) where taxonomic species were ‘lumped’ into the same PU allocation. These verifications of sample subsets are regarded as an effective method of accounting for the over or under-estimation of PU richness and produces a rate of error (Oliver and Beattie, 1996b). However, in cases where there is a vast number of PUs allocated, the examination of 30 individuals from each PU may not be considered feasible within the framework of available resources. For example, if a study allocated 200 PUs, then 6,000 individuals would need to be identified to identify the degree of ‘lumping’. In effect, this process undermines the concept of rapid biodiversity assessment.

In wet grassland habitats, the parataxonomic method has previously been employed to determine the relationship between the hyper-diverse Order Diptera (True flies) and environmental variables (Ryder et al., 2005) as well as a measure of Diptera richness (Hayes et al., 2015) for biodiversity assessments. These studies of Diptera PUs in wet grasslands adapted the basic parataxonomic approach and concentrated on sorting the entirety of flies collected by first identifying them to family level using taxonomic keys and then allocating them into PUs without any further taxonomic verification i.e.

no analysis of under or over-estimation. Whilst this derivative of the process proves very useful for comprehensive sorting of all flies, and for determining the family richness, the level of sorting to PU level is inhibited by the sheer number of individuals, often resulting in low numbers of PUs per family. In cases where a family is particularly speciose and contains many sibling genera or species which are morphologically similar, and time is a restricting factor in the sorting process, the PUs are usually not sorted to a high resolution and are often underestimated. An example of this would be the allocation of three PUs to the family Dolichopodidae in the Ryder et al., (2005) study, though the species pool was likely considerably higher (a total of 158 species are recorded in Ireland, with wet grassland being a favoured habitat [Chandler et al., 2008]). Though useful patterns can be derived from such data, especially in terms of family richness and abundances, a more in-depth investigation of a few families of Diptera using more prolonged sampling and intensive sorting methods might be more insightful (Frouz, 1999).

Though the parataxonomic method is subject to continuing discussions regarding its effectiveness (Krell, 2004; Ward and Stanley, 2004), when executed with caution, and subject to some taxonomic verification, it can be utilized to give ecologically relevant outcomes (Cotes et al., 2009; Obrist and Duelli, 2010; Oliver et al., 2016). In the most comprehensive review of the method, Krell (2004) concludes that while the usefulness of parataxonomic data is limited, in studies where it can (and perhaps must) be utilized, it provides valuable information, particularly of the alpha diversity of areas. However, while parataxonomy continues to be applied as a measure of species richness in biodiversity investigations, its use as a primary source of information for setting conservation objectives should be considered with a degree of caution (Goldstein, 1997). The reason for this caveat stems from the fact that when utilized in isolation, parataxonomy is principally useful for detecting estimates of the number of species and not the 'quality of the species' (Krell, 2004). As such, for determining complementarity and the prioritisation of areas for conservation, taxonomic (species level) data is considered the only accurate method for comparing sites and areas (Krell, 2004).

### 1.2.6. A molecular approach to invertebrate diversity assessments

The increasingly lower costs of DNA analysis has created a new avenue for the rapid, reliable, and comprehensive assessments of invertebrates (Yu et al., 2012). Thousands of specimens can now be examined in a single assessment using advanced high-throughput sequencing, allowing entire communities of invertebrates to be assessed simultaneously and at a large scale. By isolating short gene sequences which can be used to identify species e.g. the mitochondrial cytochrome c oxidase subunit I gene (*mtDNA COI* which is referred to as the Barcode gene [Hebert et al., 2003]), conservationists can examine the species composition of bulk samples without having to sort and manually identify individuals. The process of analysing bulk samples is carried out by trapping invertebrates *en masse* (as is generally the case in both taxonomic and parataxonomic studies), homogenising them together (through freeze-drying with liquid nitrogen and crushing), and extracting genomic DNA from the subsequent ‘insect soup’ (Yu et al., 2012). The *COI* gene, or a suitable sub-section of it, is amplified using Polymerase Chain Reaction (PCR) and the PCR product is analysed using high throughput sequencing such as an Illumina MiSeq platform. Bioinformatics tools are then utilized to examine the diversity of genes within the sample, yielding information on the composition of species or patterns of variation between genes from within the original bulk sample (Yu et al., 2012). This combination approach is generally referred to as *Metabarcoding* (MBC) (Yu et al., 2012).

Analysis of bulk arthropod samples assemblages under laboratory conditions using mock communities with known species compositions have consistently been shown to be reliable ways of determining the composition of these mixed samples (Yu et al., 2012; Zhou et al., 2013). However, studies also report that not every species within the sample is always recovered, and ecological patterns derived from such data may not always match exactly with those from standard datasets owing to this loss of species (Ji et al., 2013). This ‘dropout’ of species, whereby species expected in the results were absent, is one of the principal challenges facing the widespread use of MBC in biodiversity assessments. Interestingly, studies also report that MBC can

unveil morphologically cryptic species which standard taxonomy has failed to recognise (Janzen et al., 2005).

In spite of this, reliable biodiversity monitoring can be carried out using MBC as exemplified by Ji et al., (2013) who compared wholesale metabarcoding analysis of samples with species level (standard) data-sets. They concluded that the MBC data produced similar policy conclusions for two conservation applications: restoration ecology and systematic conservation planning, despite species dropout. In the aforementioned study, the community composition of three indicator groups (Ants, Spiders and Carabidae) obtained through pit-fall trapping was compared to overall trap content analysed using MBC. Community compositions were significantly co-structured using Mantel R tests illustrating the role that specific indicator groups can play in exemplifying whole scale arthropod diversity.

The continued use and improvement of MBC technology represents a possible rapid and effective means of including more comprehensive invertebrate diversity in assessments of habitats such as wet grasslands. Though the technique may be limited owing to database deficiency, which in turn makes species level assessments difficult, MBC analysis presents itself as the most practical approach to assessing invertebrates at the entire community level. Passive trapping methods such as Malaise traps produce an inordinate level of non-target organism capture which often remain unverified and represents a significant loss of potential information on invertebrate diversity (Buchholz et al., 2011; Oxbrough et al., 2009). A multifaceted approach whereby target groups are examined using taxonomy and parataxonomy and by-catch is examined using MBC technology could provide comprehensive and novel information on invertebrate communities. Such complementary analytics may well be the marriage between traditional taxonomy and molecular technology that most benefits invertebrate conservation.

## 1.4 References

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## Chapter 2:

### Observations concerning the sampling of Sciomyzidae (Diptera) in High Nature Value wet grassland habitats: caveats to consider

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Malaise and Emergence traps in rush dominated (left) and sedge dominated (right) wet grassland habitats (J.G.J Carey, May 2015)

## **Observations concerning the sampling of Sciomyzidae (Diptera) in High Nature Value wet grassland habitats: caveats to consider**

### **2.1 Abstract**

1. High Nature Value (HNV) farmland is increasingly important within Europe and effective sampling strategies that collect invertebrates in agricultural wet grassland are vital to gauge the biodiversity in these pockets of high species richness.
2. One group of potential bioindicators of HNV farmland particularly suited to wet grasslands are the marsh or snail-killing flies (Diptera: Sciomyzidae). Their relatively sedentary adult habit and terrestrial – aquatic habitats make them ideal for fine-grain resolution of biodiversity within farms.
3. In this short communication we demonstrate that using malaise trapping, emergence trapping and sweep-netting in isolation can result in incomplete species lists in addition to which we relate capture efficacies of the methods employed with reference to previous studies and the biology of the species concerned.
4. Furthermore, we demonstrate that sweep net captures are more effective at crepuscular periods (dawn and dusk) than during the middle of the day.

## 2.2. Introduction

The recognition of High Nature Value Farming (HNV) is a pressing conservation issue within both the EU and at national levels (Andersen et al., 2004). While recent studies indicate that wet grasslands represent some of the most plant species-rich grasslands remaining in western Europe (Sullivan et al., 2010), less is known about the associated invertebrate assemblages (Jefferson and Grice, 1998). Nevertheless, knowledge of the value of different types of wet grasslands for invertebrates (as well as plants) is essential if the overall biodiversity of wet grasslands is to be recognised, protected and monitored. Most Sciomyzidae (Diptera) are wetland habitat specialists with sedentary adults which permit their use as both qualitative and quantitative bioindicators of wetlands (Knutson and Vala, 2011; Murphy et al., 2012; Keiper et al., 2002). Sciomyzidae have also recently been shown to be both ubiquitous and diverse in wet grassland habitats with assemblages responding to hydrological and management changes (Maher et al., 2014; Williams et al., 2009a). Phenological differentiation within the family may also be linked to habitat type with univoltine species preferring ephemeral wetlands (Berg et al., 1982; Williams et al., 2009b). Such ecological patterns could yield important information on the future management of HNV wet grasslands which harbour small-scale wetland features important for wetland insect communities. However, Williams et al., (2009a) suggest the possibility that determination of sciomyzid communities may be influenced by artefacts of the most widely utilized capture method, namely sweep-netting. Speight (2001, 2004) also highlights different sciomyzid catch assemblages in farmed grasslands using Malaise and emergence traps. In addition, Vala (1984) has demonstrated improved sciomyzid catches at sunrise and sunset using a sweep-net under Mediterranean conditions but this sampling strategy has not been examined in temperate climates.

This investigation used the three most widely employed adult sciomyzid capture methods (Malaise trap, emergence trap and sweep net), to compare the species composition of each method in two distinct wet grassland types in the west of Ireland i.e. a wet grassland dominated by *Carex* species and a *Juncus* dominated wet grassland. We also examined the difference between sweep net catch returns at crepuscular times compared to catches made during the middle of the day.

## **2.3 Materials and Methods**

### **2.3.1. Site description**

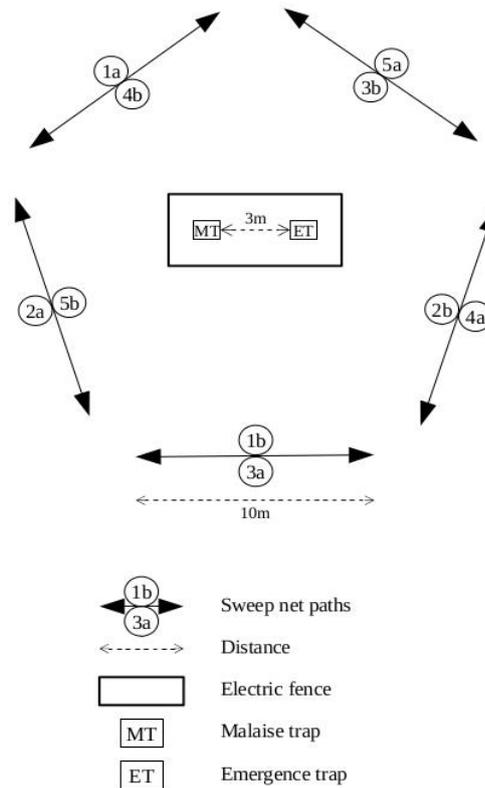
Sampling was undertaken in June and July (2014) at two adjacent (~100 metres apart) wet grassland sites in Menlo, north-east of Galway City in the west of Ireland (53°17'57.39"N; 9° 4'35.35"W). Site 1 was situated in a habitat with tall vegetation (mean 66.85cm ± 37.35 S.D.; n = 50) dominated by the rush species *Juncus conglomeratus* (L.) and *Juncus acutiflorus* (Ehrh. Ex Hoffm.). Site 2 was characterised by a relatively homogenous sward of shorter vegetation (mean 32.12cm ± 21.94 S.D.; n = 50) dominated by *Carex nigra* (L.) Reichard and occasionally interspersed with some taller plants.

### **2.3.2. Sampling strategy**

A standard Townes Malaise trap and emergence trap (165cm x 115cm x 110cm) were positioned three meters apart (high point south) in homogenous vegetation within each site and protected from livestock by a portable electric fence. Traps were placed a minimum distance of 10m from linear or man-made features e.g. ditches, hedgerows. Samples were collected weekly for five weeks between June 12<sup>th</sup> and July 16<sup>th</sup> (2014) with the months of June and July (2014) having the lowest total rainfalls in the region from May to August (2014) (Met Eireann, 2014). While consistent dry weather conditions were required to compare sweep net samples, it is important to note that a number of early emerging species may not have been captured during this restricted sampling period. Sweep netting was undertaken weekly at dawn (05:30hrs), mid-morning (09:30hrs), solar noon (13:30hrs), mid-afternoon (17:50hrs) and dusk (22:00hrs).

Five sweep net sample paths (10m long) were defined in the immediate vicinity (~7.5m) of the Malaise/emergence enclosures. The sweep net sampling plan (Fig. 2) ensured that each path was sampled once per week on the same day e.g. during week one, samples were taken from sweep paths 1a/1b (05:00hrs); 2a/2b (09:30hrs); 3a/3b (13:30hrs); 4a/4b (17:50hrs); 5a/5b (22:00hrs). Each sweep path was separated by approximately 1.0-1.5m. Weather conditions were comparable every time sampling was undertaken and specimens which were dispatched in a kill jar with ethyl acetate,

stored in 70% alcohol, and subsequently determined in the laboratory using Rozkošný (1987).



**Figure 2.** Sampling strategy

#### 2.4. Results and Discussion

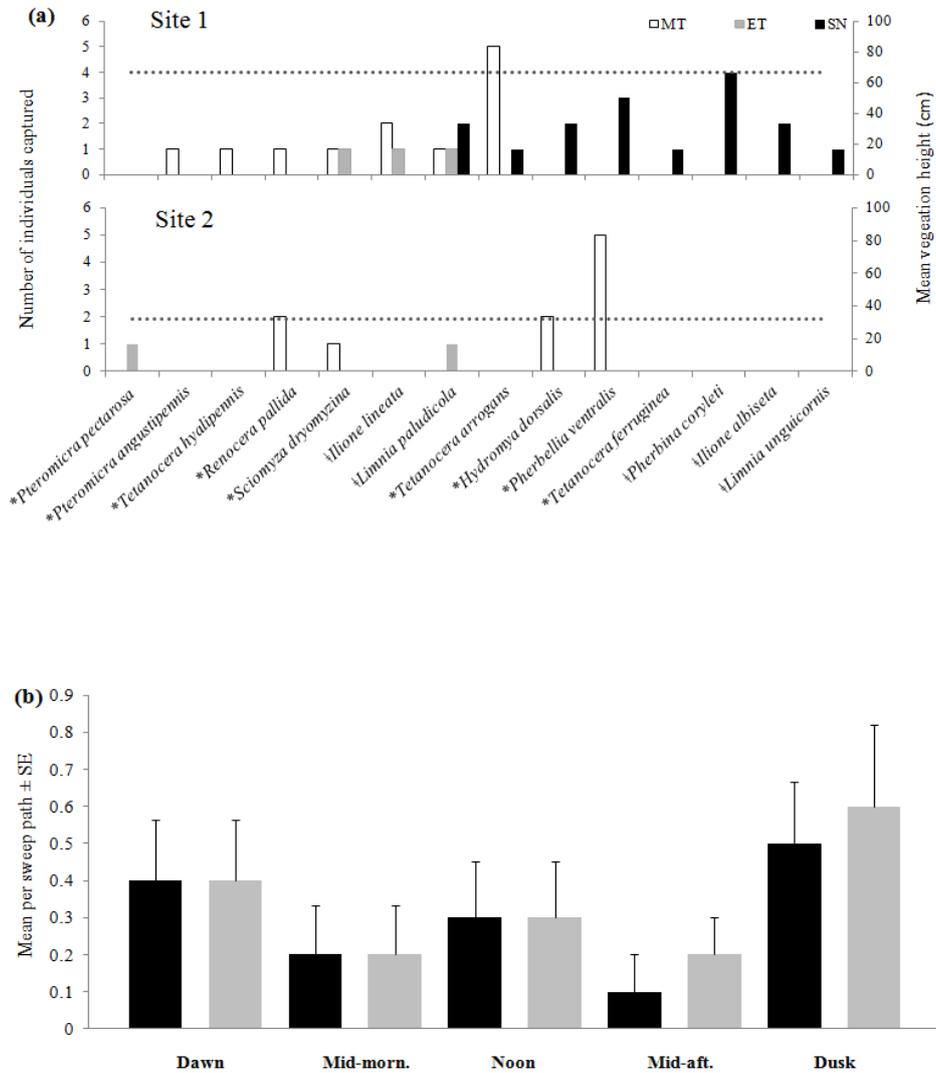
A total of 43 individual sciomyzids comprising 14 different species were captured during the five-week period. Wind speed ( $p=0.53$ ), luminosity ( $p=0.13$ ), temperature ( $p=0.24$ ), humidity ( $p=0.19$ ) and nebulosity ( $p=0.59$ ) were measured during sweep netting sampling but showed no significant correlation (Spearman rank) with captures. Thirty-one individuals (13 species) were captured at Site 1 with 16 (eight species), 12 (seven species) and 3 (three species) individuals trapped using the sweep-net, Malaise trap and emergence trap, respectively. Six species at Site 1 were unique

to the sweep-net catches and three were unique to the Malaise trap with no unique species being caught using emergence traps (Fig 3a).

Site 1 captures comprised eight univoltine and five multivoltine species suggesting ephemeral inundation (Berg et al., 1982). In contrast, only 12 individuals (six species) were captured at Site 2 with no sciomyzids caught using sweep-nets compared to ten (four unique species) and two (two unique species) individuals caught using the Malaise trap and emergence trap, respectively (Fig 3a). However, Site 2 produced similar numbers of individual captures in the Malaise trap as Site 1, and capture comprised of six multivoltine species compared to only one univoltine species, suggesting a more permanently wet habitat. It is interesting to note that had sweep netting been the only sampling method employed, just 61% of species would have been recorded at Site 1 and 0% at Site 2. Similarly, if sampling were restricted to Malaise traps, just 54% of species would have been recorded at Site 1 and 67% at Site 2. While no additional species were captured using emergence traps at Site 1, one third (albeit with small numbers) of species would have been missed had emergence traps not been employed at Site 2.

Sweep-netting appears as a standard collection method in many articles related to the qualitative collection of sciomyzid adults for faunistic accounts (McDonnell et al., 2010) and ecological investigations (Williams et al., 2009a; Williams et al., 2009b; Maher et al., 2014). Speight and Chandler (1995) referred to the difficulty of capturing the small secretive *Pteromicra* genus using a sweep-net which concurs with our findings since *Pteromicra* was limited to Malaise traps and emergence traps in our study. Emergence traps have previously been suggested as potentially better for the capture of *Pherbellia ventralis* (Knutson and Vala, 2011; Speight, 2001) but our study finds them limited to Malaise (Site 2) and sweep-net (Site 1) captures. Individuals of the genus *Sciomyza* have been captured using all three sample methods (Gittings and Speight, 2010; Staunton, et al., 2008) but were limited to Malaise and emergence traps in our study. All the species captured by sweep net in our study have previously been captured at different sites using this method (McDonnell et al., 2010). However, other species readily captured using sweep net e.g. *Ilione lineata* (Fallén), *Tetanocera*

*hyalipennis* (Roser) and *Renocera pallida* (Fallén) were limited to Malaise and emergence trap captures at our sites.



**Figure 3.** a) Species and individuals captured using three sampling techniques: Site 1 (dominated by *Juncus*); Site 2 (dominated by *Carex*). Mean vegetation height shown on the secondary y axis (dotted line) b) Mean species richness (black bars) and mean abundance (grey bars)  $\pm$  standard error per sweep net sample in each time period. (MT) Malaise trap, (ET) emergence trap, (SN) sweep net, (\*) Multivoltine species, (≠) Univoltine species

Within the *Juncus* dominated habitat (Site 1), a distinct difference in sciomyzid species composition was observed dependent on the capture method (Fig 3a). Six species were exclusively captured in this habitat using the sweep-net, and five species were exclusively captured using the combination of Malaise and emergence traps. Twelve ♂♂ and only four ♀♀ were captured using the sweep method in this site compared to three ♂♂ and 12 ♀♀ in the Malaise/emergence traps. This differentiation may signify some resource partitioning of sciomyzids within longer vegetation stands. Male dominance in the upper vegetation layers (more prone to sweeping) may also reinforce the assault mating strategy suggested by Foote (1977). The *Carex* dominated habitat (Site 2) produced no sciomyzid captures using the sweep net – most likely due to the overall shorter vegetation at the site (Southwood and Henderson, 2000).

Of the 16 individuals captured using a sweep-net at Site 1, ten of these were captured during the crepuscular time periods (four unique species) and six during the period between mid-morning and mid-afternoon (one unique species). Mean ( $\pm$  SE) captures during dawn and dusk were  $0.40 \pm 0.16$  and  $0.60 \pm 0.22$  individuals per 10 metre sweep, respectively. Mean captures ( $\pm$  SE) during the mid-morning, noon and mid-afternoon periods were  $0.20 \pm 0.13$ ,  $0.30 \pm 0.15$  and  $0.10 \pm 0.10$  individuals per 10 metre sweep respectively. Vala (1984) showed a marked variation between temperature/time of day and the number of Sciomyzidae individuals captured in a Mediterranean forest biotope. A similar trend was illustrated in our findings with an increase in capture rates during the crepuscular periods (Fig 3b).

Future investigations of sciomyzid assemblages should acknowledge the potential sampling errors of specific sampling methods in addition to recognising the importance of time of day in reporting sweep net captures. Through more comprehensive sampling, Sciomyzidae may provide further insights into small scale invertebrate habitats routinely overlooked.

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### Chapter 3:

#### **Spatiotemporal variation of Diptera changes how we evaluate High Nature Value (HNV) wet grasslands**

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Images of the Sciomyzid *Ilione albisetata* (left) and the Syrphid *Playcheirus rosarum* (right) kindly provided by permission of Steven Falk.

## **Spatiotemporal variation of Diptera changes how we evaluate High Nature Value (HNV) wet grasslands**

### **3.1. Abstract**

Proposed strategies to protect biodiversity within agricultural systems are often based on botanical criteria with plant species richness generally considered the prime indicator of conservation potential. While wet grasslands dominated by rushes (*Juncus* spp.) are commonly considered to be of lesser ecological value than those which are more botanically diverse (e.g. *Carex* dominated wet grasslands), their value for invertebrates such as Diptera has not yet been fully explored. Data from two Diptera families (Sciomyzidae and Syrphidae) were examined at spatial, temporal, and spatiotemporal scales to determine the contribution of two different (*Juncus* and *Carex* dominated) wet grassland habitats towards the maintenance of Diptera diversity. The two habitats were significantly different in terms of community structure for both families and temporal variation was a significant component of dipteran diversity. Spatiotemporal analysis showed that species turnover between habitats at different times made the most significant contribution to overall Diptera diversity. Temporal variation of both families suggests that the relative importance of each habitat type to overall diversity fluctuates depending on sampling period, with both habitats supporting diversity at different times. Our results indicate that lowland wet grasslands characterised by *Juncus* cover need to be recognised as ecologically important for the maintenance of dipteran diversity. We discuss the possible implications for the diversity of Diptera in wet grasslands if these commonly perceived marginal areas (both agriculturally and ecologically) are ignored in conservation strategies. The necessity of recognising spatiotemporal variation when evaluating habitats using invertebrates as indicators is also discussed.

### **3.2. Introduction**

The loss of habitat heterogeneity in agricultural systems is considered a major factor in the overall decrease of farmland biodiversity. Simplification of agricultural land through the removal of less productive areas such as field boundaries and sward species with low grazing value contributes significantly towards the reduction in habitat diversity and overall spatial heterogeneity (Fahrig et al., 2011; Henle et al., 2008; Sullivan et al., 2013). This reduction in habitat variability can have impacts at temporal scales by limiting the availability of alternative habitats where resources become available at different times (Benton et al., 2003). The resulting habitat loss may be even more pronounced for insects, many of which rely on variability at spatial and temporal scales generally not considered in conservation strategies (Haslett, 2001).

Approaches to maintaining spatial heterogeneity within agricultural areas include the identification and preservation of areas of High Nature Value (HNV) farmland which is typically characterised by mosaics of semi-natural habitat (Andersen et al., 2004; European Environment Agency, 2009; Paracchini et al., 2008). In the west of Ireland, much of this HNV farmland is contained in areas of wet grassland (Sullivan et al., 2010) which are typified by extensive grazing regimes and periodic or seasonal flooding. Wet grassland in Ireland is classified as having >50% cover of grass, sedge or rush species with a significant proportion of drier grassland plants but not dominated by broadleaf herbs or reeds (Fossitt, 2000). This criterion is broad enough to include many types of wet grassland with markedly different dominant vegetation, which has led to the ecological quality of wet grasslands usually being determined by their plant species richness (Sullivan et al., 2010).

While botanical (and ornithological) interests are the principal drivers of wet grassland conservation, wet grasslands can also contain a high diversity of insect species including many scarce or threatened species (Drake, 1998). Features of wet grasslands such as damp hollows (Kirby, 1992), temporary pools (Nicolet et al., 2004), and drainage ditches (Verdonschot et al., 2011) have all proven to be important to invertebrate conservation in these agricultural landscapes. Though often the focus of conservation strategies in grasslands, increased plant species richness is not always a

reliable indicator of invertebrate diversity (Vessby et al., 2002; Billeter et al., 2008; Maher et al., 2014). Features such as vegetation structure have been shown to be more important factors contributing towards the diversity of insects in grasslands (Krueß and Tschardtke, 2002a, 2002b). In wet grasslands in particular, certain dipteran species have been shown to respond more positively to characteristics such as the length of vegetation but not necessarily the plant species richness (Ryder et al., 2005; Williams et al., 2009a; Maher et al., 2014).

Patches of wet grassland frequently become dominated by rush species such as *Juncus effusus* (L.) and *Juncus conglomeratus* (L.) lowering the grazing potential of the sward and potentially reducing the overall plant species richness. In general, plant ecologists and farmers often place little value on fields with extensive *Juncus* cover but for different reasons. The former regard extensive *Juncus* cover as being of poor ecological value and the latter an indication of poor agricultural productivity. The control and removal of rushes from farmland using mechanical and chemical methods is actively encouraged in Ireland, even by agri-environmental schemes. A dense cover of rushes within any particular field can have financial consequences for farm subsidy schemes and is currently seen as a breach of Good Agricultural and Environmental Condition (GAEC) (Department of Agriculture Food and the Marine, 2015a, 2015b). While extensive rush cover offers limited grazing potential when compared to more intensively managed grass pastures, its environmental role is poorly understood, particularly in terms of its contribution to insect diversity.

Wet grasslands have previously been noted for their invertebrate species richness (Hayes et al., 2015; Joyce and Wade, 1998) and the temporal variations associated with wet grasslands, particularly inundation, are likely to have significant effects on invertebrate diversity (Maher et al., 2014). Organisms such as insects generally require combinations of spatial and temporal variation to complete their lifecycles. An adult insect, for example, may need to move to a different habitat patch to obtain resources if those resources are not available within its larval habitat (spatial variation). Alternatively, an insect may be able to complete its lifecycle within a single habitat patch if that patch undergoes seasonal changes thereby providing the required resources on a temporal basis (temporal variation). Some species of Diptera which are

considerably mobile such as Syrphidae (Hoverflies) can move more freely between habitats in search of resources (Burgio and Sommaggio, 2007; Sommaggio, 1999). Other dipteran families are less vagile in nature and may be more reliant on temporal variation within habitats to complete their lifecycles. Many species of Sciomyzidae (Marshflies) for example, require periodic inundation for larvae to feed on stranded aquatic snails but equally require dry periods where vegetation is accessible for adults to forage for food and reproduce (Knutson and Vala, 2011).

By utilizing these two well-known families of Diptera which have contrasting mobility and life strategies, we examined the role that two different wet grassland vegetation types play in maintaining dipteran diversity in lowland wet grasslands. Both families are considered potentially good bioindicators of wetland habitats (Speight, 1986) and meet the criteria for suitable bioindicators outlined in McGeoch et al., (2002) and Lindenmayer et al., (2000) in that they are easily captured and identified; are well understood biologically; and are taxonomically stable and ubiquitous within the habitat of investigation. There is a paucity of information regarding the insect diversity of wet grasslands within Europe, particularly Diptera, and this study, for the first time, examines the role that different wet grassland habitat types play in maintaining these communities.

Through an intensive and continuous sampling regime using Malaise traps we investigated:

- 1) The role that two different wet grassland vegetation types contribute towards maintaining dipteran diversity
- 2) The contribution and significance of temporal variation to the species richness of our two target Diptera families within wet grasslands
- 3) The importance of considering spatiotemporal variation when making decisions regarding the protection of sites for biodiversity

To achieve these goals, our objectives were to compare Sciomyzidae and Syrphidae diversity at different scales through partitioning diversity and to examine the role each habitat plays in harbouring specific species.

### **3.3. Materials and Methods**

#### **3.3.1. Study area**

Given that the west of Ireland is considered one of the most likely places in Ireland to contain HNV farmland (European Environment Agency 2009), we selected ten independent grassland sites (classified as wet grassland according to Fossitt [2000]) located in north County Galway and south County Mayo within a 100km<sup>2</sup> area east of Lough Corrib (the largest lake in the Republic of Ireland). Sites were selected using ortho-corrected aerial photographs and ground-truthed to ensure they conformed to the criteria outlined in Fossitt (2000). Plant species cover and vegetation length were recorded using five 50cm x 50cm quadrats randomly placed at each trap location to differentiate areas based on dominant vegetation type. Sites were subsequently classed as either *Juncus* or *Carex* habitats based on which vegetation type contributed most to overall percentage cover. Five each of the *Juncus* or *Carex* habitats were selected, with some sites in close proximity to one another i.e. adjacent fields ~100m apart. All 10 of the sampling sites were grazed by livestock (Cattle or Horses) and none was subject to extensive cutting or fertilizer application. Within each wet grassland site, two black nylon Malaise traps of Townes design (Townes, 1972) were placed 20m apart with the collection head facing in a southerly direction (Speight et al., 2000). A 70% ethanol solution was used in the Malaise Trap collecting bottles to kill and preserve the specimens. Traps were positioned a minimum of five metres away from any obvious flight line features such as wet flushes, hedgerows, ditches and obvious ecotones such as marked differences in vegetation types to facilitate invertebrate collection within each vegetation type. This method was employed to maximise the collection of insect species within the site rather than those utilizing the area as a corridor between habitats (Speight et al., 2000).

#### **3.3.2. Sample collection and determination**

A 5 x 5 metre area around each trap was excluded from livestock (Cattle and Horses) using a portable electric fence to protect the traps from damage. Malaise traps were activated on 1<sup>st</sup> May (2014) with samples subsequently collected from the traps every 14 days until 4<sup>th</sup> September (2014), creating a total of nine successive sampling periods, hereafter referred to as “periods”. All 20 sampling traps had equal sampling

intensity and all samples were collected on the same day. Each sample was removed to the laboratory where the two dipteran families (Sciomyzidae and Syrphidae) were determined to species level using Rozkošný (1987) and Vala (1989) for sciomyzids and Ball and Morris (2013) and Stubbs and Falk (2002) for syrphids. It should be noted that seven of the Syrphid ‘species’ were identified to group level only owing to difficulties associated with the determination of females e.g. *Sphaerophoria* species (Stubbs and Falk, 2002).

### **3.3.3. Data analysis**

Statistical analyses were carried out using PAST for univariate analysis (Hammer et al., 2001) and PC-Ord version 6.0 for multivariate analysis (McCune and Mefford, 2011). Species accumulation curves, constructed using PC-Ord version 6.0, were used to assess the adequacy of sampling. Accumulation curves were made using each trapping event as a sample (n=180).

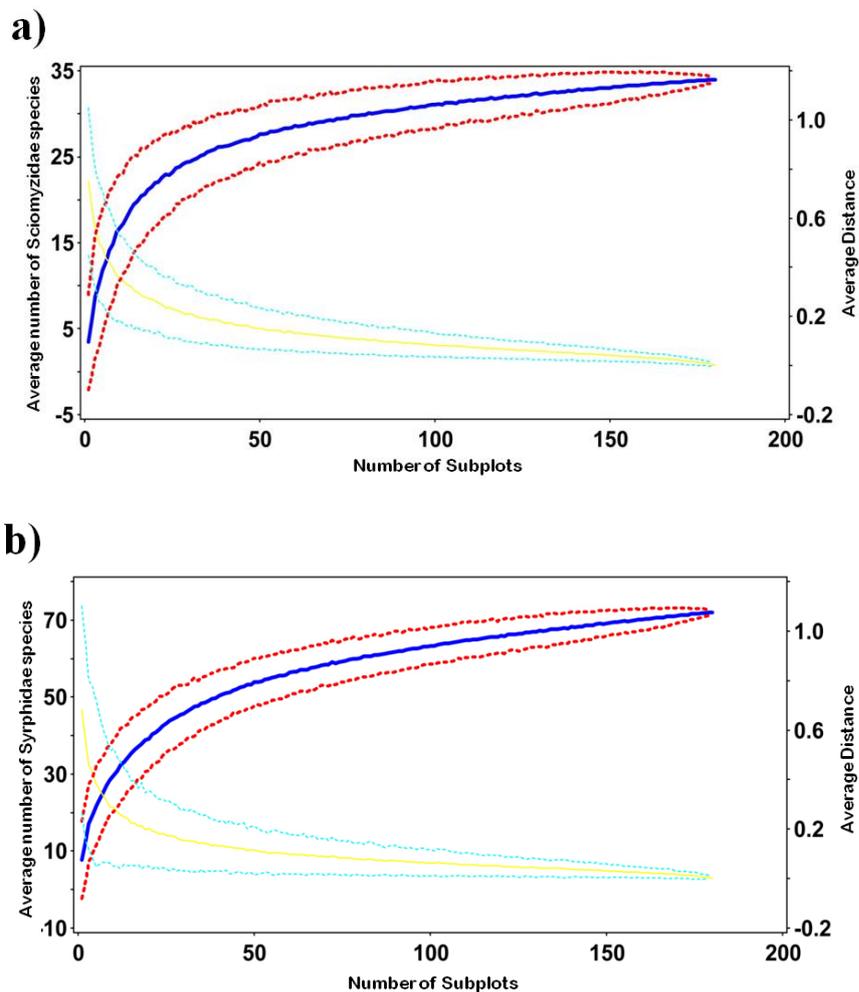
Non-metric multi-dimensional scaling ordinations (NMS) of untransformed trap data in species-space were performed using PC-Ord version 6.0. The ordination used the Sørensen distance measure and 250 real data runs. NMS ordinations do not assume linear relationships and allow the use of distance measures suited to data distributions considered non-normal (McCune and Mefford, 2011). Ordinations with stress values of <10 are considered reliable for interpretation of ecological data (McCune and Mefford, 2011). NMS was used to investigate patterns and differentiation in assemblage composition between habitats based on spatial and temporal configurations. A second NMS ordination of pooled samples from habitats based on period of capture ordinated in species-space was also performed e.g. all samples from *Carex* from period one were pooled and referred to as C1. This was considered a spatiotemporal ordination as both temporal variation and spatial variation (in the form of habitat type) were examined. Sequential vectors were utilized to illustrate the relationship between spatiotemporal samples. A non-parametric multivariate analysis of variance (PerMANOVA) was used to test the significance of differentiation between habitats using both untransformed and  $\log_{10}(x+1)$  transformed data. An examination of  $\log_{10}(x+1)$  transformed data was used to down-weight dominant species.

Additive and multiplicative partitioning of species richness along with Shannon's and Simpsons diversity was performed using PARTITION 3.0 (Veech and Crist, 2009). By performing the procedure outlined by Lande (1996) we were able to examine the contribution of each of the alpha and beta diversity components to overall (gamma) diversity (Veech et al., 2002). This methodology has previously been shown to be effective for analysing the role of spatial scale in species diversity. For spatial partitioning, data from each site were pooled across the sampling periods (n=10); for temporal partitioning, trap data were pooled together based on sampling period and habitat type (n=18); for spatiotemporal partitioning, data from each site at each time period were considered a replicate (n=90). Comparisons were made between the observed diversities and 10,000 individual based randomisations to account for any possible autocorrelation. Spatial diversity was partitioned into  $\alpha$  (within sites),  $\beta_1$  (among sites), and  $\beta_2$  (between habitats). Temporal diversity was partitioned into  $\alpha$  (within periods based on habitat type),  $\beta_1$  (among periods based on habitat type) and  $\beta_2$  (between habitats). Spatiotemporal diversity was partitioned into  $\alpha$  (within samples),  $\beta_1$  (among samples) and  $\beta_2$  (among samples from different habitats at different periods).

Indicators species analysis (ISA) based on Dufrene and Legendre (1997) was carried out on the spatiotemporal dataset (n=90) with groups defined as habitat types and period as a blocking variable (McCune and Mefford, 2011). This methodology is suited to an experiential set-up where blocks can be considered as temporal analogues to a traditional randomised complete block design where they would be spatial (McCune and Mefford, 2011). The blocked ISA assesses group indicators based on the relative frequency and abundance within each group by examining relativized data from within each block (period). Monte Carlo permutation tests based on 4,999 permutations give an estimated *P*-value for the percentage of perfect indication, or indicator value (IV). In this instance it was used to identify species with fidelity to particular habitat types.

### 3.4. Results

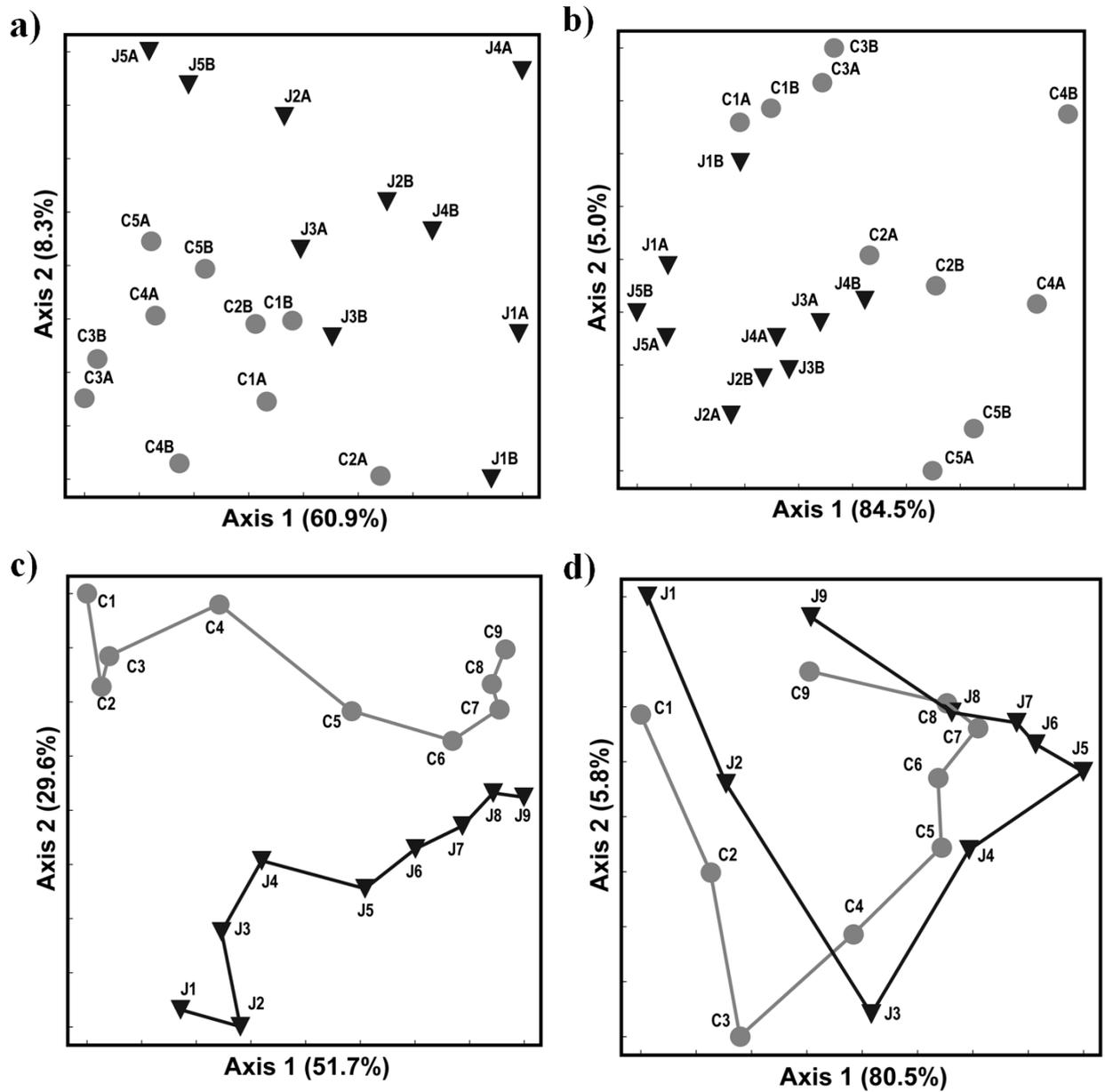
A total of 180 samples were collected from 20 Malaise traps over an 18-week period, with this sampling effort yielding 34 sciomyzid species (2,589 individuals) and 72 syrphid species (9,567 individuals). Species accumulation curves for both families approach an asymptote based on first order jackknife estimates (Figs. 4a and 4b). Rare species (singleton and doubletons) accounted for 24% of Sciomyzidae and 31% of Syrphidae.



**Figure 4.** Sciomyzidae **a)** and Syrphidae **b)** species accumulation curve and distance (dissimilarity) decay curve for all samples. Dotted lines represent  $\pm 2$  SDs. First Order Jackknife estimates of total species richness were 38.97 (Sciomyzidae) and 87.91 (Syrphidae).

### 3.4.1. Spatial and Temporal differentiation

The ordination of traps in species-space resulted in two-dimensional solutions for both dipteran families explaining 69.2% of the variance for Sciomyzidae (Stress 12.73) and 89.5% of the variance for Syrphidae (Stress 9.16) (Figs. 5a and 5b). Habitats were significantly different for both families based on a PerMANOVA of traps in species space using both untransformed and transformed data (Table 1). In the Sciomyzidae data, *Juncus* habitats showed more marked variance among traps with an average Sørensen distance measure of 0.71 compared to variance between traps from *Carex* habitats which had an average distance measure of 0.58. Syrphidae data showed that traps from *Juncus* were less varied having an average distance measure of 0.40 compared with *Carex* which had an average distance measure of 0.53. Ordinations of samples from habitats pooled according to period of capture (Spatiotemporal analysis) resulted in a three-dimensional solution for Sciomyzidae (stress 4.80) explaining 86.7% of the variance (Figure 5c [axis one and two are illustrated]). The same ordination for Syrphidae resulted in a two-dimensional solution explaining 86.3% of the variance with a stress of 5.22 (Figure 5d). Sequential vectors for both families showed different temporal trajectories with Sciomyzidae having a linear pattern and Syrphidae showing a more cyclical pattern whereby early and late temporal samples were similar. Habitats were significantly different for Sciomyzidae in the spatiotemporal ordination but only significantly different for Syrphidae based on transformed data (Table 1).



**Figure 5.** Non-metric multi-dimensional (NMS) scaling of traps in **a)** Sciomyzidae species-space and **b)** Syrphidae species-space. Habitat type is denoted by grey circles (*Carex* habitat [C]) and black triangles (*Juncus* habitat [J]). Letters (A/B) associated with traps refer to pair-wise counterparts from the same sites e.g. C1A and C1B are from the same sites. NMS ordination of pooled spatiotemporal samples in **c)** Sciomyzidae species-space and **d)** Syrphidae species-space. Numbers associated with habitats refer to the sampling period e.g. C1 are the pooled samples from *Carex* habitats from period one. Grey and black lines linking habitat-period samples are successional vectors.

**Table 1.** Results of PerMANOVA based on Bray-Curtis dissimilarities of Diptera assemblages between two habitat types. Spatial groups are based on samples from sites; Spatiotemporal groups are based on samples collected from the same habitat at the same time period. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

	Untransformed data			Transformed data		
	Df	Pseudo F value	$P$	Df	Pseudo F value	$P$
<b>Spatial</b>						
Sciomyzidae	1	3.738	0.0017**	1	3.468	0.0010**
Syrphidae	1	5.957	0.0001***	1	2.065	0.0280*
<b>Spatiotemporal</b>						
Sciomyzidae	1	3.6704	0.0050**	1	3.6704	0.0050**
Syrphidae	1	1.0943	0.3320	1	0.8349	0.0480*

### **3.4.2. Partitioning of Diversity**

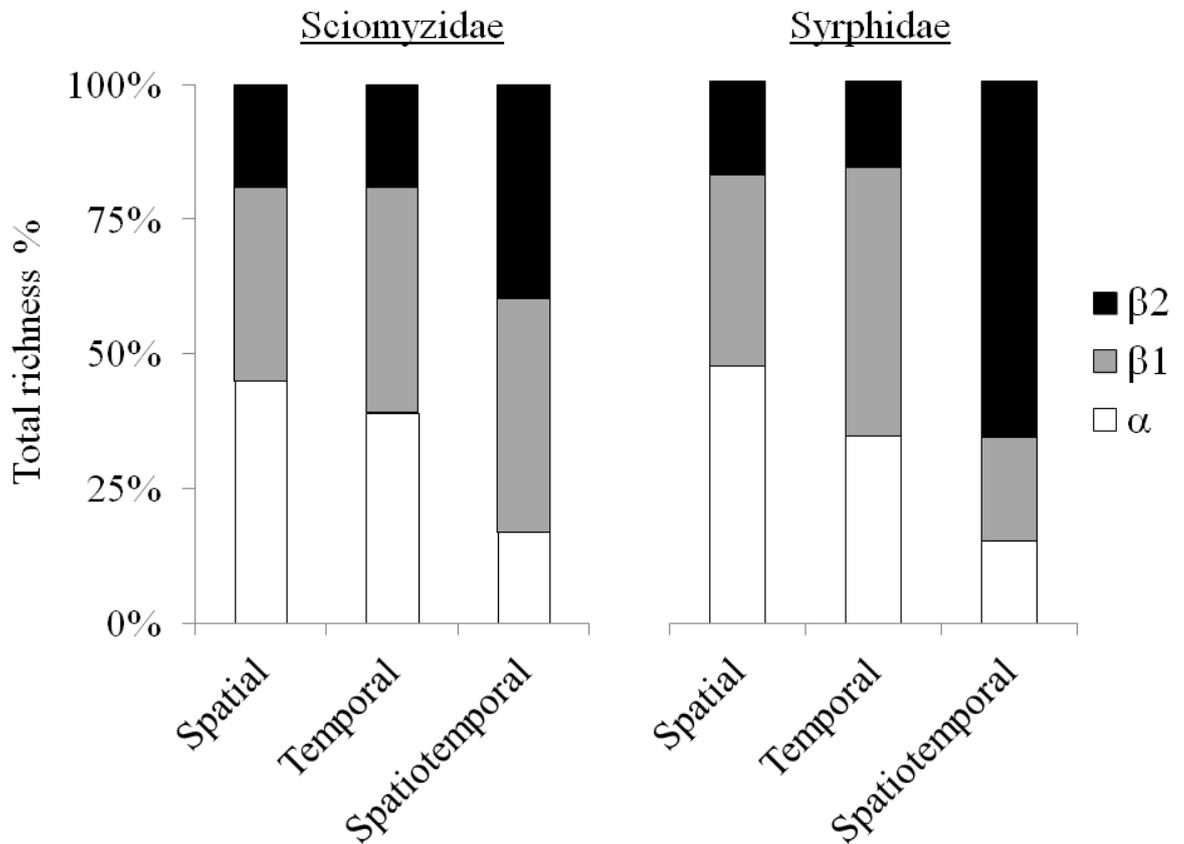
In terms of spatial scale, within and among site diversity contributed the most to overall diversity for both families (Table 2) although they are not significantly higher or lower than expected. A similar pattern is seen in temporal partitioning with within period and among period contributing the most to overall diversity (Table 2). In both of these scales, the contribution of habitat type was  $\leq 20\%$  of the total diversity, though in both scales the contribution of habitat ( $\beta_2$ ) type is significantly higher than expected across all diversity measures (with the exception of Simpsons diversity in spatial scale and multiplicative diversity in temporal scale for Syrphidae). When diversity was partitioned using spatiotemporal replicates, which compare sites and habitats at different times, the contribution of habitat type markedly increases for both families (Fig. 6) and is significantly higher than expected by chance (Table 2).

### **3.4.3. Indicator species analysis**

Blocked indicator species analysis (Table 3) highlighted a total of seven species indicative of *Carex* habitats and 12 for *Juncus* habitats. Both habitats have similar numbers of sciomyzid indicators; however, *Juncus* habitats had six Syrphidae indicators in comparison to two in the *Carex* habitats.

**Table 2.** Partitioning of additive, multiplicative species richness and Shannon's and Simpsons diversity between / among grouping variables. Figures followed by no asterisk were not significantly different from a random distribution based on 10000 individual-based iterations. \* $P < 0.05$  (higher than expected by chance). Null values in parentheses

	Additive species richness			Multiplicative species richness ( $q = 0$ )			Shannon's diversity (exp H) ( $q = 1$ )			Simpsons diversity (1/D) ( $q = 2$ )		
	$\alpha$	$\beta_1$	$\beta_2$	$\alpha$	$\beta_1$	$\beta_2$	$\alpha$	$\beta_1$	$\beta_2$	$\alpha$	$\beta_1$	$\beta_2$
<b>Spatial (n=10)</b>												
Sciomyzidae	15.30	12.20	<b>6.50*</b> (4.05)	15.30	1.80	<b>1.24*</b> (1.14)	7.70	1.47	<b>1.11*</b> (1.01)	4.91	1.44	<b>1.12*</b> (1.00)
Syrphidae	34.10	25.40	<b>12.50*</b> (9.44)	34.10	1.74	<b>1.21*</b> (1.15)	7.03	1.12	1.03* (1.00)	3.46	1.07	1.00
<b>Temporal (n=18)</b>												
Sciomyzidae	13.20	14.22	<b>6.50*</b> (3.42)	13.28	2.07	<b>1.24*</b> (1.11)	5.45	1.77	<b>1.25*</b> (1.01)	2.99	1.79	<b>1.41*</b> (1.00)
Syrphidae	24.78	35.72	<b>11.50*</b> (9.92)	24.78	2.44	1.19	7.76	1.14	<b>1.08*</b> (1.00)	4.34	0.93	<b>1.08*</b> (1.00)
<b>Spatiotemporal (n=90)</b>												
Sciomyzidae	5.74	14.76	<b>13.50*</b> (2.19)	5.74	3.57	<b>1.66*</b> (1.07)	3.12	3.58	<b>2.85*</b> (1.15)	2.26	3.35	<b>3.92*</b> (1.20)
Syrphidae	10.87	13.80	<b>47.33*</b> (42.75)	10.87	2.27	<b>2.92*</b> (2.46)	4.47	1.67	<b>1.81*</b> (1.14)	2.85	1.46	<b>1.69*</b> (1.03)



**Figure 6.** Percentage of total species richness (additive partitioning) explained by alpha and beta components of diversity. Beta diversity is partitioned among/between two spatial scales: sites ( $\beta_1$ ) and habitats ( $\beta_2$ ). Temporal beta diversity is partitioned among 18 samples based on habitat type and time ( $\beta_1$ ) and between habitat type ( $\beta_2$ ). Spatiotemporal beta diversity is partitioned into among samples from sites at different times ( $\beta_1$ ) and between habitats at different times ( $\beta_2$ ).

**Table 3.** Significant ( $P < 0.05$ ) Sciomyzidae and Syrphidae indicator species for wet grassland habitat type based on dominant vegetation cover (*Carex* habitat or *Juncus* habitat).

	<i>Carex</i> habitat			<i>Juncus</i> habitat	
	IV (% perfect indication)	<i>P</i> -value		IV (% perfect indication)	<i>P</i> -value
<u>Sciomyzidae</u>			<u>Sciomyzidae</u>		
<i>Pherbellia ventralis</i> (Fallén, 1820)	49.8	0.0006	<i>Tetanocera ferruginea</i> Fallén, 1820	36.7	0.0084
<i>Ilione albisetata</i> (Scopoli, 1763)	46.9	0.0058	<i>Renocera pallida</i> (Fallén, 1820)	35.1	0.0122
<i>Sciomyza testacea</i> Maquart, 1835	24.4	0.0008	<i>Tetanocera arrogans</i> Meigen, 1830	34.3	0.0224
<i>Tetanocera fuscinervis</i> (Zetterstedt, 1838)	23.9	0.0070	<i>Tetanocera robusta</i> Loew, 1847	30.3	0.0016
<i>Colobaea bifasciella</i> (Fallén, 1820)	18.2	0.0254	<i>Pherbellia argyra</i> Verbeke, 1967	17.8	0.0048
			<i>Tetanocera elata</i> (Fabricius, 1781)	16.5	0.0174
<u>Syrphidae</u>			<u>Syrphidae</u>		
<i>Platycheirus clypeatus</i> (Meigen, 1822)	64.9	0.0040	<i>Platycheirus granditarsus</i> (Forster, 1771)	55.9	0.0080
<i>Platycheirus fulviventris</i> (Macquart, 1829)	14.0	0.0410	<i>Melanostoma scalare</i> (Fabricius, 1794)	39.9	0.0280
			<i>Platycheirus rosarum</i> (Fabricius, 1787)	36.2	0.0302
			<i>Neoascia tenur</i> (Harris, 1780)	27.7	0.0314
			<i>Chrysotoxum bincinctum</i> L., 1758	23.9	0.0260
			<i>Volucella pellucens</i> L., 1758	10.4	0.0350

### 3.5. Discussion

Our results show that both types of wet grassland contribute towards the maintenance of Diptera diversity. An overall trend within the data indicates that diversity is spatially structured among sites and habitats with temporal turnover also an important factor. The resulting spatiotemporal variation between wet grassland habitats with different vegetation types is a significant contributor to species diversity in these areas. The results indicate that areas of wet grassland dominated by *Juncus* vegetation which are normally associated with low ecological and agricultural value play an important role in maintaining the diversity of Sciomyzidae and Syrphidae. Further to this, our analysis shows that the role each habitat contributes to dipteran diversity is dependent on temporal considerations, with each habitat harbouring important levels of species at different times. It is only through the comprehensive spatiotemporal analysis of these sites that the ecological importance of *Juncus* dominated wet grasslands can be considered in terms of the diversity of these two Diptera families.

Dominant vegetation type significantly differentiated the Sciomyzidae and Syrphidae communities; a pattern previously noted in investigations of Diptera from different habitat types (Hughes et al., 2000; Savage et al., 2011). Spatial NMS analysis in this study suggests that this differentiation is a combination of the relative abundance of certain species within each habitat type and the preference of particular species for certain habitats as seen in the ISA. The species indicators of *Carex* habitats all have relatively similar ecological preferences whereas the species indicative of *Juncus* habitats are more multifarious in their larval and adult habitat preferences. The sciomyzid species most significantly associated with *Carex* habitats are all predators of aquatic or semi-aquatic snails which become stranded or exposed during fluctuations in water levels (*Pherbellia ventralis* [Fallén, 1820], *Ilione albiseta* [Scopoli, 1763], *Sciomyza testacea* Maquart, 1835, *Tetanocera fuscinervis* [Zetterstedt, 1838], *Colobaea bifasciella* [Fallén, 1820]) (Knutson and Vala, 2011; Speight and Knutson, 2012). This is consistent with the hydrology of the *Carex* sites examined in this study which are prone to winter flooding but are largely dry during the summer months which would facilitate the feeding strategy of the larvae of these indicator species. The two Syrphidae indicator species of these habitat types are largely

associated with the combination of grassland and lush marsh vegetation typical of wet grasslands, as well as having larvae that can tolerate the inundation that is typical within the *Carex* habitats (*Platycheirus clypeatus* [Meigin, 1822] and *Platycheirus fulviventris* [Macquart, 1829] (Speight et al., 2000). *Platycheirus clypeatus*, in particular, is known to prefer unshaded, open grassy areas that are not subject to very heavy grazing (Stubbs and Falk, 2002); both characteristic features of the *Carex* habitats in this study. The sciomyzids associated with *Juncus* habitats range in trophic strategy between predators of aquatic snails (*Tetanocera robusta* Loew, 1847, *Tetanocera ferruginea* Fallén, 1820), generalised predators (*Pherbellia argyra* Verbeke, 1967), specialist predators of pea mussels (*Renocera pallida* [Fallén, 1820]) and terrestrial predators (*Tetanocera arrogans* Meigen, 1830 and *Tetanocera elata* [Fabricius, 1781]) (Knutson and Vala 2011; Speight and Knutson 2012). This broad range of trophic guilds is also evident in the spatial NMS ordination which shows high variability between *Juncus* areas and illustrates that these sites may have assemblage types which are variable and site-specific. This idiosyncratic mixture of species is also evident in the range of Syrphidae indicators of the habitat. Some of the *Juncus* syrphid indicator species such as *Platycheirus granditarsus* (Forster, 1771), *Platycheirus rosarum* (Fabricius, 1787) and *Neoascia tenur* (Harris, 1780) are typically associated with wet grassland habitat features such as marshy vegetation and standing water (Speight et al., 2000; Stubbs and Falk, 2002). The remaining indicator species i.e. *Melanostoma scalare* (Fabricius, 1794) and *Chrysotoxum bincinctum* L., 1758 are associated with more sheltered grasslands, whereas *Volucella pellucens* L., 1758 is typically found in woodland and copses (Stubbs and Falk, 2002). The long robust vegetation that typifies the *Juncus* habitats may well act as a refuge for certain syrphid species in the absence of significant shelter belts such as woodland or hedgerow features within these grasslands (Sarhou et al., 2005). In this respect, the dominance of *Juncus* in these areas may be beneficial to Hoverflies.

The spatiotemporal NMS results illustrate a cyclical successional pattern of Syrphidae and temporal differentiation between the habitat types. Syrphidae are exceptionally mobile and may move freely between habitat types in response to perturbations or resource requirements (Sommaggio, 1999; Stubbs and Falk, 2002). Several of our

sampling sites were in close enough proximity (adjacent fields which were 100-400m apart) to one another and had no significant barriers to hoverfly movement (Wratten et al., 2003). It is not unreasonable to suggest that this would have allowed species to move between habitat types in response to temporal changes in environmental condition e.g. seeking out newly available floral resources, shelter from predators or poor weather, or lekking sites (Haenke et al., 2014; Sommaggio, 1999; Sutherland et al., 2001). This dynamic shifting of species between habitat types at different times is evident in the large contribution of beta diversity between habitats at different times to the overall diversity of Syrphidae. This suggests that Syrphidae may utilize certain *Juncus* sites at particular times and possibly relocate to *Carex* habitats in response to resource availability, disturbance in the form of grazing or a combination of both. A reduction in the availability of *Juncus* dominated wet grasslands could therefore have a negative effect on the diversity of Syrphidae at a landscape level. In contrast to the vagile nature of Syrphidae, Sciomyzidae are relatively sedentary, having a marked habitat fidelity (Williams et al., 2010) and have previously shown very high site-specificity (Maher et al., 2014). The contribution of spatiotemporal partitioning to overall Sciomyzidae diversity is not solely an artefact of differences between habitats at different times but also the diversity among sites at different times as seen in Figure 3. Sciomyzidae are unlikely to move between habitat types so changes in community composition are possibly a result of phenological shifts in community structure at site level whereby species with different over-wintering and reproductive strategies dominate sites at different times. Berg and Knutson (1978) remark that seasonal mortality or a hiatus in reproduction may limit the population of multivoltine Sciomyzidae in ephemeral wetlands such as wet grasslands. This interruption may enable univoltine species to compete with multivoltine species within these habitats. The univoltine species *I. albiseta* was a strong and significant indicator of *Carex* habitats which typically follow a predictable pattern of winter flooding followed by dry summer as proposed by Berg and Knutson (1978). Based on the wide range of species indicative of the *Juncus* habitats, it would appear that these habitats have a broader scale of hydrological variability which accounts for their support of sciomyzids that range from fully aquatic larvae e.g. *T. robusta*, to fully terrestrial larvae e.g. *T. elata*. The singular nature of each of the *Juncus* sites in terms of

sciomyzid composition, therefore, contributes significantly to the overall species diversity, at a landscape scale, within these wet grasslands.

Patterns of differentiation between *Carex* and *Juncus* dominated grasslands have previously been reported for Sciomyzidae with *Juncus* dominated wet grasslands often supporting a wider range of species (Carey and LeRoy et al., 2015). It is also interesting to note that spatiotemporal habitat differentiation decreased in the Sciomyzidae samples at the later stages of the collection (Periods 7-9). Variations in flood depth and duration are known to benefit sciomyzid species richness (Maher et al., 2014) as well as molluscs (Ilg et al., 2009). It is possible that as sites started to become inundated towards the end of the collection period (Period 9), mollusc prey became more readily available and competition between species was reduced allowing for a more spatiotemporally stable fauna to co-exist within sites.

Several environmental factors including hydrological regime, vegetation structure and composition have all been shown to influence community structure and species richness of Sciomyzidae and Syrphidae (Carey, LeRoy et al., 2015; González-Megías et al., 2011; Maher et al., 2014; Ryder et al., 2005; Williams et al., 2009b). Various fluctuations of each of these variables, which themselves are subject to changes across temporal scales, may exist within each sample site and by reason within each habitat. Vegetation composition and structure, for example, continuously alters throughout the growing season and wet grasslands generally begin to inundate in the autumn and winter. It is likely that the contribution of temporal turnover to species diversity is related to a combination of these perturbations, the availability of resources associated with them, and the phenology of the species within each of the families. In our study, spatial variability appears to act as a safeguard for Syrphidae allowing them to exploit different habitats at different times, whereas Sciomyzidae communities within wet grasslands are especially site specific and rely on temporal variation within sites to complete their life cycles.

The maintenance of habitat heterogeneity is largely considered an elixir for the conservation of biodiversity within agricultural areas (Benton et al., 2003; Fahrig et al., 2011; Tews et al., 2004), and the broad scale beta diversity associated with

increased habitat heterogeneity is known to significantly increase the diversity of insects at spatial (Gering et al., 2003), temporal (González-Megías et al., 2011; Zamora et al., 2007) and spatiotemporal scales (Sobek et al., 2009a; Sobek et al., 2009b; Tylianakis et al., 2005). It should also be noted that previous studies in regions with more extreme seasonality such as the Mediterranean have determined that temporal changes have a major influence of insect diversity (González-megías et al., 2011; Zamora et al., 2007). Our results suggest that even in a temperate climate such as the west of Ireland, these processes have similar influences on the gamma diversity and should be considered an integral component of wet grassland Diptera diversity.

### **3.6. Conclusions**

Where conservation objectives continuously focus on one particular outcome such as maintaining or increasing plant species richness in wet grasslands, a consensus can (inadvertently) be formed that such aims are a suitable panacea for biodiversity protection in general. Few studies have examined the contribution of *Juncus* dominated wet grassland to biodiversity owing to its general lack of botanical diversity. However, when invertebrate groups such as Diptera are considered in evaluations, it is apparent that *Juncus* dominated wet grasslands play a vital role in maintaining the diversity of these groups. Our study demonstrates that both spatial and temporal turnover is a significant factor in dipteran diversity and needs to be considered in the evaluation of habitats for conservation potential. The spatiotemporal variation between habitat types in lowland wet grasslands was a significant contributor to Sciomyzidae and Syrphidae in our study. While the work presented in this investigation is particular to the location of the study, similar spatiotemporal evaluations of habitats, normally not considered as ecologically important, may yield significant insights into the roles that such areas play in maintaining the diversity of overlooked groups such as Diptera. We suggest that in lowland wet grassland habitats, areas of dominant *Juncus* cover maintain highly varied communities of Sciomyzidae and provide resources on a wider scale for Syrphidae; therefore, they should be regarded as ecological focus areas. The loss of *Juncus* dominated areas of lowland wet grasslands could have serious implications for the conservation of Sciomyzidae and Syrphidae in wet grassland ecosystems.

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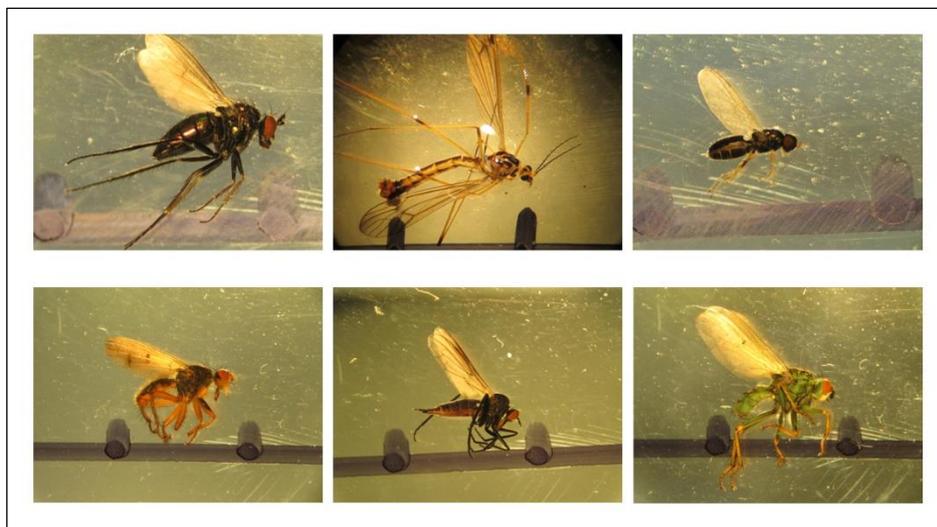
## Chapter 4:

### **Indicators of Diptera diversity in wet grassland habitats are influenced by environmental variability, scale of observation, and habitat type**

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Images of various Diptera voucher specimens which were allocated into parataxonomic units for use in this study (J.G.J. Carey, May 2015)

## **Indicators of Diptera diversity in wet grassland habitats are influenced by environmental variability, scale of observation, and habitat type**

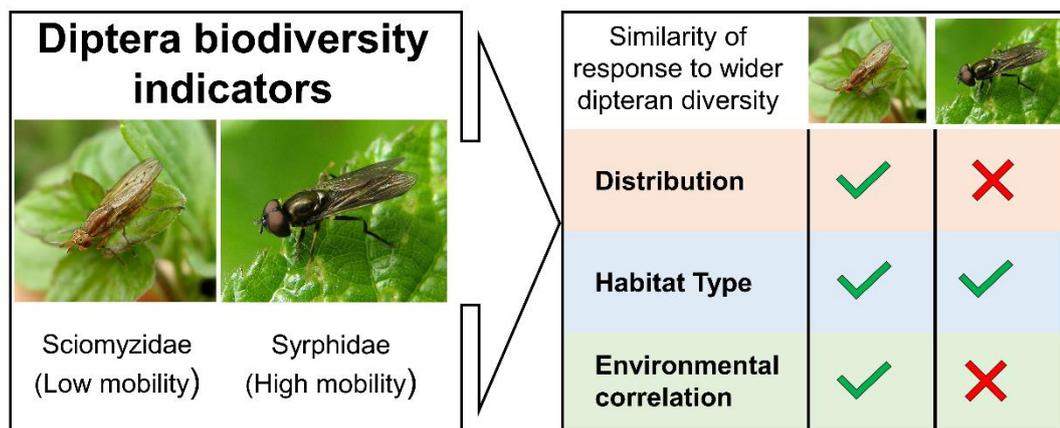
### **4.1. Abstract**

In low intensity agri-ecosystems such as wet grassland habitats, the inclusion of invertebrates in conservation assessments and monitoring is usually limited to charismatic groups such as bees or butterflies. However, wet grasslands support a wide range of invertebrate groups, some of which may exhibit limited movement not generally represented by more mobile groups such as those typically examined. The use of surrogate species which exemplify broader invertebrate diversity has been suggested as a possible means of including these overlooked invertebrates (such as Diptera) in conservation planning within these habitats. Based on collections made by Malaise trap, we utilized two families of Diptera (Sciomyzidae and Syrphidae) as indicators of a wider range of dipteran diversity (nine Diptera families identified to Parataxonomic unit level [PUs]) in wet grassland habitats.

We examined the role of environmental variability, spatial scale, and habitat type on patterns of cross-taxon congruence for all three assemblages. Both environmental correlation and community congruence were significantly stronger among assemblages when examined at low spatial scales, highlighting the need to examine dipteran groups at scales untypical of current agri-environmental assessments; namely field and farm level. Furthermore, when wet grasslands were differentiated into two habitat categories (sedge and rush dominated grasslands), the significance of the community congruence increased markedly. This correlation was particularly strong between Sciomyzidae and PUs which demonstrated similar community differentiation based on habitat type, implying that assemblages which exhibit comparable ecological partitioning are more likely to be useful surrogates of one another. Correlations between richness, abundance, and Shannon's diversity were highly variable among groups, suggesting compositional analysis as the most appropriate examination of dipteran diversity for surrogacy studies. The results indicate that cross-assemblage congruence of Diptera is influenced by similarity of response to environmental variability, scale of observation, and examination of assemblages differentiated into

appropriate habitat categories. The results illustrate the need to investigate invertebrate biodiversity surrogates at scales appropriate to the indicator groups and examine congruence among assemblages within specific habitat categories. Such an approach has the potential to maximise gamma diversity in areas where wet grasslands are under threat of intensification or abandonment.

#### 4.2. Graphical abstract



The Graphical abstract is a feature of the International Journal ‘*Ecological Indicators*’ where this chapter has been published.

### **4.3. Introduction**

European wet grassland habitats are typically low-intensity agricultural systems containing semi-natural habitats which support a rich mosaic of plant and animal communities (Bignal and McCracken, 1996, 2000; Billeter et al., 2008). While much of the conservation of this lowland wet grassland is driven by botanical or ornithological interests, wet grasslands also sustain a high diversity of invertebrates (Drake, 1998; Hayes et al., 2015; Joyce and Wade, 1998; Maher et al., 2014). Although some invertebrate groups have previously been utilized as indicators within wet grasslands, e.g. spiders (Oxbrough et al., 2007) and Carabidae (Luff et al., 1992; Williams et al., 2014), comprehensive invertebrate data from these habitats is limited. Such a lack of information has contributed towards the omission of invertebrate groups in broad scale conservation planning and for monitoring conservation objectives (Cardoso et al., 2011). This is particularly relevant in a European agricultural context, where intensification and abandonment of traditional farming practices in areas such as wet grassland threatens biodiversity (Henle et al., 2008). The use of invertebrates in the designation and management of agricultural areas considered as being of a High Nature Value is usually restricted (if included at all) to a few well known, easily identifiable, and often iconic groups such as butterflies or bees (Andersen et al., 2004). Other invertebrate groups such as Diptera are largely overlooked despite contributing significantly to the overall biodiversity of such habitats (Keiper et al., 2002).

While the need to include a wider suite of invertebrates in conservation strategies for wet grasslands makes ecological sense, the sheer abundance and diversity of groups such as Diptera are perceived as barriers to their inclusion in routine habitat assessments. Alternative approaches such as the use of selected invertebrate groups as biodiversity surrogates for a broader range of taxa has been suggested as a possible means of including invertebrates in conservation and monitoring programs (Anderson et al., 2011; Duelli et al., 1999; Duelli and Obrist, 2003; Hayes et al., 2015). These biodiversity indicators generally include a well-studied taxon or group of taxa which are ubiquitous within the habitat of interest and can be easily collected and identified (Lindenmayer et al., 2000; McGeoch et al., 2002). A predetermined measure of the diversity of the selected indicator is then used to reflect the diversity of similar, or

sometimes different, taxa. One such approach has been the examination of species richness congruence between indicator groups and the taxa they are deemed to represent (Duelli and Obrist, 2003; Moreno and Sánchez-Rojas, 2007; Prendergast, 1997). However, the species richness approach is considered as having variable outcomes due to its dependency on the pairs of taxa under investigation having direct interactions e.g. predator/parasitoid, as well as providing little insight into overall species representation and composition (Su et al., 2004).

More recently, patterns of congruence derived from community similarity and/or the examination of similarity of community responses to environmental variability have been utilized as approaches to biodiversity surrogacy (Larsen et al., 2012; Paszkowski and Tonn, 2000; Rooney and Azeria, 2015; Rooney and Bayley, 2012; Su et al., 2004). However, a potential caveat with this method is the effect of spatial scale of observation and habitat differentiation on congruence patterns. Invertebrate diversity may respond to spatial scales not typically considered in conservation strategies (Haslett and Salzburg, 1997; Weaver, 1995), and community composition can be influenced by microhabitat changes across small scales that can have a marked effect on community structures (Cole et al., 2010). In wet grassland habitats, this may be further exacerbated by temporal changes such as periodic inundation in combination with grazing patterns (Carey et al., 2017; Maher et al., 2014; Ryder et al., 2005). Thus, the examination of invertebrate communities may need to be assessed at spatial scales untypical of those employed in conventional biodiversity assessments which are often linked to agri-environmental scheme evaluations at farm or field-level scales (Anderson et al., 2011; Boyle et al., 2015).

The differentiation of habitats into categorical groups has also been shown to affect congruence patterns with anthropogenic disturbance and ecoregion having a noticeable influence on congruence measures (Ekroos et al., 2013; Myšák and Horsák, 2014; Rooney and Azeria, 2015; Rooney and Bayley, 2012). Therefore, the selection of invertebrate biodiversity surrogates needs to carefully consider determinants such as the distribution of the indicator taxa relative to the scale of the observation, response of the indicator to ecological variance, and possible ecological relationships between

the indicator and the wider community it is chosen to represent (McGeoch, 1998; Paoletti, 1999).

In wet grassland habitats, adult marsh flies (Diptera: Sciomyzidae) are considered as potential bioindicators owing to their ubiquity and ease of capture (Carey and LeRoy et al., 2015; Knutson and Vala, 2011; Murphy et al., 2012). However, they are known to have highly localised habitat fidelity and exhibit a markedly limited movement (Williams et al., 2010); factors which may restrict their usefulness as surrogates for broader dipteran diversity if the scale of observation utilized is greater than that which accurately reflects the distribution of broader diversity. In contrast to this, adult hoverflies (Diptera: Syrphidae) are considered suitable bioindicators in agricultural systems (Burgio and Sommaggio, 2007) but are vagile in nature with adults capable of foraging over long distances (Sommaggio, 1999). The use of either of these groups as invertebrate biodiversity surrogates is therefore dependent on the similarity of their response to factors such as spatial scale and habitat differentiation relative to the broader invertebrate diversity for which they are selected to represent.

Along with these considerations, the identification of multiple and diverse groups such as Diptera to species level usually requires expertise and time which is not readily available in the context of typical designation or monitoring timeframes. Suggested alternatives to this impediment include attempting to rapidly identify several groups of invertebrates using less traditional taxonomic methods such as non-taxonomic sorting of species or molecular identification methodologies (Cardoso et al., 2011; Emerson et al., 2016; Oliver and Beattie, 1996a; Yu et al., 2012). Rapid biodiversity assessment techniques such as parataxonomy (morphospecies) as described by Oliver and Beattie (1993) utilises an approach whereby individuals with similar external morphological traits are grouped together as typological units or Parataxonomic Units (PUs) without the use of taxonomic keys. This work can be carried out by individuals with minimal taxonomic training and possibly even through public participation initiatives such as citizen science (Casanovas et al., 2014).

The main advantage of using PUs in assessments is that it is relatively quick and permits the analysis of a broader suite of groups in limited timeframes. Nevertheless,

PU allocation is especially difficult for non-specialists when sibling species are encountered within samples. This can lead to many outwardly similar individuals being erroneously labelled as the same species (lumping), or conversely, the separating out of the same species into two PUs (splitting) (Krell, 2004). Though the method is subject to debate regarding its effectiveness (Krell, 2004; Ward and Stanley, 2004), when executed with caution and subject to some level of taxonomic verification, it can be utilized to give ecologically relevant outcomes (Cotes et al., 2009; Obrist and Duelli, 2010; Oliver and Beattie, 1996 a, b; Ward and Stanley, 2004). Previous studies of Diptera in wet grasslands which have utilised the two approaches (taxonomic and parataxonomic) have generally focused on richness correlations of all Diptera and not examined community similarity (Hayes et al., 2015; Ryder et al., 2005). Though useful patterns can be derived from such data, especially in terms of family richness and abundances, a more in-depth investigation of a smaller number of dipteran families using more prolonged sampling and intensive sorting methods might be more insightful (Frouz, 1999).

Given that Sciomyzidae and Syrphidae fulfil the criteria for suitable bioindicators as outlined by McGeoch (1998), we compared measures of their diversity with a broader assemblage of nine Diptera families identified using parataxonomy; Dolichopodidae, Empididae, Hybotidae, Limoniidae, Pipunculidae, Scathophagidae, Stratiomyidae, Tabanidae, and Tipulidae. By examining agreement among groups in terms of environmental responses, this study was able to identify the role that environmental factors play in determining community structure of different dipteran assemblages. The investigation also conducted a hierarchical sampling regime from two wet grassland habitat types (rush dominated and sedge dominated wet grasslands) based on samples from individual traps or samples from traps from the same sample patch pooled together. The study investigated the role that spatial scale and habitat type played in determining patterns of congruence among the three assemblages (Sciomyzidae, Syrphidae, and dipteran PUs) using a range of tests.

The principal objectives were to:

1. Examine patterns of environmental correlation between the groups at two spatial scales (trap level and patch level)
2. Determine whether cross-taxon congruence among groups was affected by scale of observation
3. Investigate the role that habitat type plays in contributing to patterns of cross-assembly congruence

The results of this investigation are discussed in the context of selecting suitable invertebrate biodiversity indicators within High Nature Value agri-ecosystems such as wet grasslands.

#### **4.4. Materials and methods**

##### **4.4.1. Study area**

This investigation was undertaken in the west of Ireland in wet grassland habitats defined according to Fossitt (2000). For inclusion in this classification, grass, rush, or small sedge cover needs to exceed 50% and broadleaf herbs, reeds, and larger sedges should not dominate (i.e. must be <50%). The broadleaf herb component should also be relatively evenly divided between drier grassland and wetland species. We selected five each of two sub-categories of wet grassland based on their dominant vegetation type, i.e. wet grasslands dominated by rushes (Family Juncaceae) and wet grasslands dominated by sedges (Family Cyperaceae). In total, ten wet grassland patches were examined with two traps placed in each patch (n = 20 overall). All wet grassland sites in this study were actively managed for livestock grazing and were not subject to intensive cutting regimes or application of fertilisers, herbicides, insecticides, or molluscicides.

##### **4.4.2. Diptera sampling and determination**

Diptera sampling was undertaken between May 1st and September 4th 2014 using black Malaise traps of Townes design (Townes, 1972). Two traps were placed 20 m

apart in homogeneous patches of vegetation and away from obvious topographical features such as drainage ditches, wet flushes, hedgerows, etc. This method was employed to maximise Diptera collections from within the sample patch rather than because of movement between habitats or due to the presence of any obvious ecotonal changes (Carey et al., 2017). Collection heads containing a 70% ethanol solution were positioned in a southerly direction and were collected every 14 days. A portable electric fence was operated to protect the traps from interference by livestock. Vegetation within the enclosure was intermittently shortened using a hand-shears and removed to maintain trap efficacy and replicate conditions outside the fenced area. Eleven families of adult Diptera were selected for analysis based on their ease of identification to family level, ubiquity within the habitat, and previous recommendations for use as bioindicators of wetland habitats (Hayes et al., 2015; Speight, 1986). These families were Dolichopodidae, Empididae, Hybotidae, Limoniidae, Pipunculidae, Scathophagidae, Sciomyzidae, Stratiomyidae, Syrphidae, Tabanidae, and Tipulidae. Sciomyzidae and Syrphidae were utilised as the principal biodiversity indicators and identified to species level using Rozkošný (1987) and Vala (1989) for sciomyzids and Ball and Morris (2013) and Stubbs and Falk (2002) for syrphids. The remaining nine families were identified using parataxonomy; a rapid biodiversity assessment method.

Non-specialist individuals (undergraduate students hereafter referred to as parataxonomists) utilised a simplified character key derived from Oosterbroek (2007) and Unwin (1981) to assist them in the removal of the remaining nine families from bulk samples (See Appendix 5). All samples were rechecked by taxonomists to ensure that no specimens were overlooked. Subsequent to the initial sorting to family level, the specimens were categorised into Parataxonomic Units (PUs) based on their external morphological features such as body shape, body length, distinguishing colouration, and antennal/appendage features. PU allocation was made without the use of keys (Oliver and Beattie, 1996a/b, 1993). Each newly assigned PU was digitally photographed, and the image inserted into a shared database allowing each parataxonomist access to the image for referral. The initial voucher specimen was preserved in 100% ethanol for determination to species level by taxonomists. These

voucher specimens were utilised to determine the level of ‘splitting’ whereby a taxonomic species was split into two or more PUs. Oliver and Beattie (1996a, b) recommend that a random subset of ~30 individuals from each PU is also maintained (as vouchers) to determine the degree of ‘lumping’ (i.e. when two or more taxonomic species are classed within the same PU). Our study opted to exclude this verification as it would have entailed the identification of over 3,000 individual specimens by taxonomists. Instead, only the original voucher specimens were identified by specialists and therefore, only species ‘splitting’ and not ‘lumping’ was accounted for. Omitting the ‘lumping’ protocol due to time constraints is likely to have led to underestimations of species richness but was countered by the speed of the species determination by specialist taxonomists who needed to verify ~30 times less specimens. This effectively maximised taxonomic input by minimising effort; an important consideration due to the increasingly limited availability of taxonomic specialists (Cardoso et al., 2011) and requirement for rapid assessments of biodiversity in line with typical conservation strategies. The original inventory was then subjected to changes based on the species determinations and the corrected PU data utilised for analysis. The percentage of splitting error for each PU family was reported according to Oliver and Beattie (1996a/b).

#### **4.4.3. Environmental data**

A series of environmental variables was recorded at each site throughout the collection period and mean values calculated. This included vegetation height along with the percentage cover of grasses, sedges, rushes, moss, moribund vegetation, broadleaf herbs, and bare ground. Each of the variables was estimated using five 50× 50 cm quadrats placed randomly within 10 m of each Malaise trap location and within the homogeneous vegetation patch. Soil samples were extracted using a soil auger from the same random sampling areas and mean pH, soil carbon content, and soil moisture were analysed (Anon, 1990). The depth of standing water at each site was recorded in the October following trap removal and prior to any extensive winter inundation which would have rendered water depth measurements unsafe.

#### 4.4.4. Data analysis

Prior to multivariate analysis invertebrate abundance data was  $\log_{10}(x + 1)$  transformed to reduce the influence of numerically dominant species and to approximate multivariate normality. Species which were considered outliers ( $> 2.0$  standard deviations) using the Sørensen distance measure were also removed (McCune and Mefford, 2011). Environmental data (with the exception of soil pH) was arcsine square root transformed or  $\log_{10}(x + 1)$  transformed to improve linearity and to approximate normality (McCune and Mefford, 2011).

Non-metric multidimensional scaling (NMS) ordinations (McCune and Grace, 2002) of samples were undertaken using the Sørensen distance in PC-Ord v.6 (McCune and Mefford, 2011). Samples which were identified as extreme outliers with standard deviations  $> 3.0$  using the Sørensen distance measure were removed prior to NMS ordination as they may impart undue influence over ordination results (McCune and Mefford, 2011). We utilised 250 runs of real data to 250 runs with randomised data to determine the number of significant axes. An orthogonal principal axis output was selected for each NMS to illustrate maximum community variation along axis 1. Environmental data were utilised as a second explanatory matrix and variables with Pearson  $R^2$  values  $> 0.2$  overlain as a bi-plot (McCune and Mefford, 2011). Multi-Response Permutation Procedures (MRPP), which are non-parametric procedures for the testing the hypothesis of no difference between two groups, were utilized to examine for significant differentiation between habitat types based on the species/PU composition of each assemblage (McCune and Mefford, 2011).

A Principal Components Analysis (PCA) with a cross products matrix based on correlation was utilised to evaluate the environmental data. MRPP was also utilised to examine habitat differentiation based on PCA results. The PC1 score obtained from this analysis was used to rotate the NMS ordination of each assemblage to ensure a standard alignment for comparisons between community structure and NMS axes. Such rotation does not alter the relative position of each sample in species space and allows for comparison between assemblages (McCune and Mefford, 2011; Rooney and Bayley, 2012). Subsequent to rotation, we examined the response of the three assemblages to environmental variables by comparing the Pearson correlation

coefficients of each significant axis derived from the NMS using Spearman Rank correlations in PAST (Hammer et al., 2001). All responses were considered at trap scale (each individual Malaise trap [n= 20]) and patch scale (where data from pairwise traps from the same vegetation patch were combined [n =10]).

We compared changes in assemblage structure within fields (i.e. between pair-wise traps) using the Sørensen similarity as a measure of differentiation between pair-wise traps. Similarity scores obtained for each assemblage were compared using linear correlation (Pearson's R) to investigate whether patterns of differentiation between pairwise traps was congruent between assemblages. For each assemblage, we also tested the relationship between the level of community similarity between pairwise traps and the differentiation of environmental variables between pair-wise traps using Spearman Rank correlations in PAST (Hammer et al., 2001). Community congruence between indicator groups was tested using Partial Mantel tests controlling for geographical and environmental autocorrelation using Sørensen distance measures for species/PU data and Euclidean distance measure for control matrices (McCune and Mefford, 2011). Partial Mantel tests were carried out across all samples, at two spatial scales (patch and field), and within habitat types at trap scale using PC-Ord v.6. Sciomyzidae, Syrphidae, and PU richness from each sample was calculated as a proportion of the total richness of each assemblage across all sample sites (Finch and Löffler, 2009). Shannon's entropy (previously known as Shannon's diversity) was utilised as a measure of the diversity of each assemblage (Ellison, 2010; Jost, 2007), along with raw abundance values. We utilised Spearman rank correlations to investigate patterns of cross-taxon congruence of each of these values using PAST (Hammer et al., 2001). Correlations were investigated at two spatial scales (trap scale [n= 20], patch scale [n= 10]) and within habitat types (rush or sedge dominated sites) at trap scale (n = 10).

## 4.5. Results

### 4.5.1. General results

A total of 105,666 individuals from eleven families of Diptera were collected from the sampling sites and subjected to taxonomic or parataxonomic identification. Sciomyzidae (1,975 individuals) and Syrphidae (9,568 individuals) were determined to species level with 34 and 72 species identified, respectively. This represents 53% of Sciomyzidae and 40% of Syrphidae from the Irish fauna (Chandler et al., 2008). First order Jackknife estimates of richness were 38.97 for Sciomyzidae and 87.91 for Syrphidae (see Carey et al., 2017).

A total of 105 PUs were identified from the nine remaining Diptera families. Once splitting had been accounted for, this was reduced to 85 with an overall percentage splitting error of 24% (Table 4). Based on this dataset of 85 PUs, first order Jackknife estimates of inventory completeness was 89.90 for PUs. Dolichopodidae (long-legged flies) represented the most abundant family (45,337) with Stratiomyidae (soldier flies) contributing the least number of individuals (685). Scathophagidae (dung flies) showed the highest percentage splitting error of PU allocation owing to the markedly different body size and colouration attributed to the yellow dung fly (*Scathophaga stercoraria* L. 1758). Pipunculidae (big-headed flies) showed the lowest level of splitting error, though it is likely that ‘lumping’ of species occurred in the allocation of PUs to this family owing to marked similarity among sibling species.

**Table 4.** Number of individuals, species, and Parataxonomic units (PUs) per Diptera family. The percentage splitting error refers to the proportion of splitting within each family whereby a species was classified as more than one PU by parataxonomists.

Family (Individuals)	Number of species	Number of PUs	% splitting error
Dolichopodidae (45,337)	15	17	13
Limoniidae (13,796)	14	15	7
Empididae (11,987)	15	18	20
Scathophagidae (8,933)	7	14	100
Hybotidae (6,098)	12	13	8
Pipunculidae (3,129)	4	4	0
Tabanidae (2,820)	3	4	33
Tipulidae (1,338)	5	8	60
Stratiomyidae (685)	10	12	20
Overall	85	105	24

#### 4.5.2. Response to environmental variability

NMS ordinations of samples in species/PU-space produced three dimensional solutions which explained >80% of the variation for each assemblage (Sciomyzidae 86.0%, Syrphidae 84.3%, PUs 80.4%). Environmental variables with a Pearson  $R^2$  score of >0.2 are shown as bi-plots (Fig. 7). Stress levels for each ordination were all <11.5 with values of ~10 are considered suitable for reasonable interpretation (McCune and Mefford, 2011). MRPP analysis showed significant differences between the two habitat types for all three assemblages using the Sørensen distance measure. PUs showed the most significant differentiation between habitat types ( $A= 0.080$ ,

$P=3\times 10^{-5}$ ), followed by Sciomyzidae ( $A = 0.062$ ,  $P=6\times 10^{-4}$ ) and then Syrphidae ( $A = 0.030$ ,  $P = 0.027$ ).

PCA of environmental variables showed that PC1 explained 35.4% of the variance between samples (Fig. 8). MRPP analysis confirmed that habitats were significantly different from one another based on environmental data ( $A= 0.249$ ,  $P=2\times 10^{-5}$ ). The scores from PC1 were utilized to rotate the NMS ordinations for examination of cross-assembly agreement based on environmental correlations. All three assemblages showed significant correlations based on NMS axis one irrespective of spatial scale of observation (Table 5). At trap scale ( $n =20$ ), Sciomyzidae and Syrphidae showed significant correlations across all axes. This was reduced to the first two axes at patch scale. PUs showed significant correlations with the other groups only on axis one. It is worth noting that the negative correlation between Syrphidae and PUs is likely an artefact of the rotation of the NMS using only PC1 scores (Rooney and Bayley, 2012).

#### **4.5.3. Community similarity between pairwise traps**

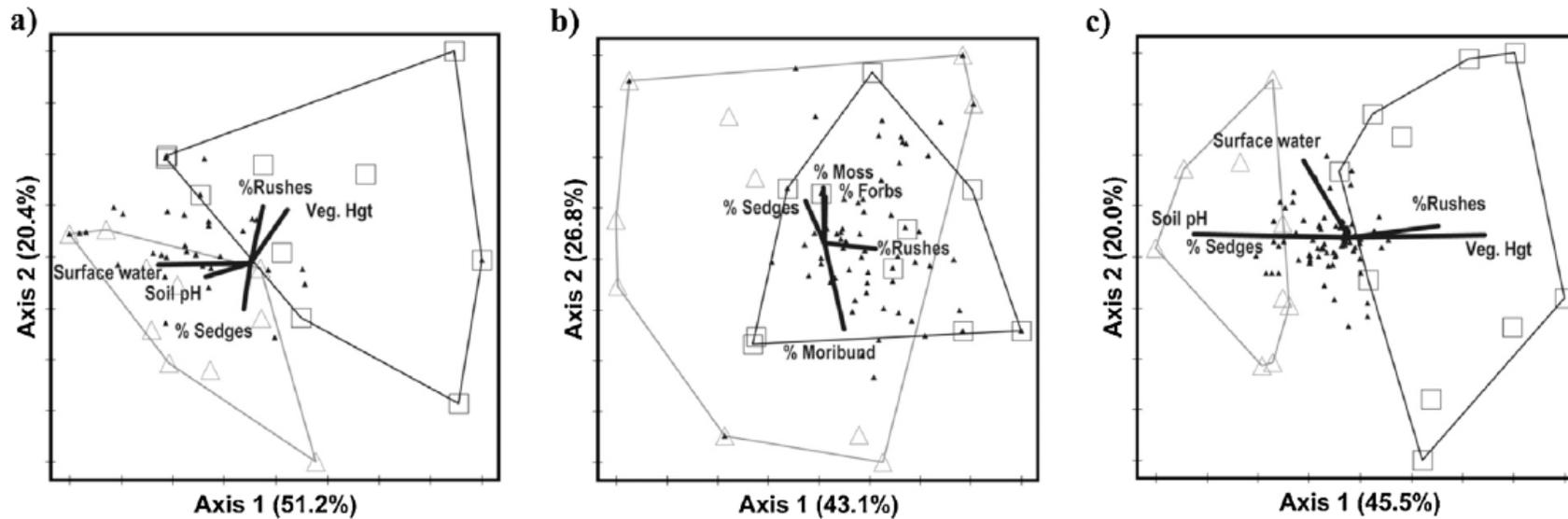
Sciomyzidae and PUs showed a significant relationship in terms of how their assemblages responded to differentiation between pairwise traps, i.e. increased dissimilarity between pairwise traps was congruent among both assemblages ( $r^2 0.84$ ,  $P = 0.002$ ) (Fig. 9). None of the measured environmental variables were significantly correlated with changes in community similarity of Sciomyzids or PUs between pairwise traps, i.e. none of the measured variables (examined in isolation) appeared to significantly affect differences in community structure between traps from the same patch. Syrphidae were not significantly correlated with Sciomyzidae ( $r^2 -0.23$ ,  $P = 0.470$ ) or PUs ( $r^2 -0.17$ ,  $P= 0.063$ ) in terms of similarity of community differentiation between pairwise traps. However, differentiation of Syrphidae between pairwise traps was significantly correlated with differences in the percentage cover of broadleaf herbs between pairwise traps (Spearman's  $R 0.82$ ,  $P = 0.004$ ), a relationship that was not significant for Sciomyzidae or PUs. In essence, patches of habitat in close proximity that had similar percentage cover of broadleaf herbs had similar community structures of Syrphidae.

#### **4.5.4. Cross-assembly congruence**

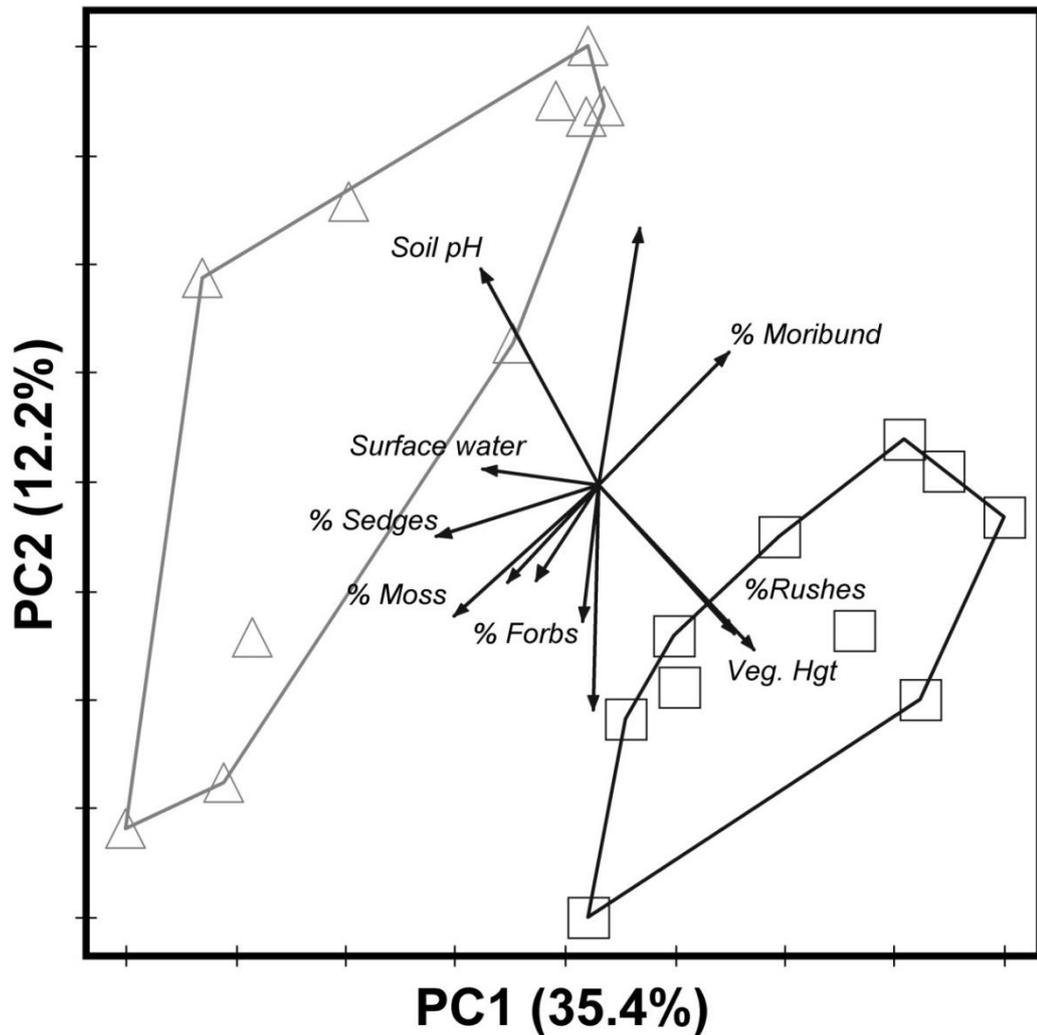
Mantel R values were weak but significant between Sciomyzidae and PUs across all samples but only at trap level (Table 5). This relationship was maintained even when geographic and environmental autocorrelation was controlled for. There was also a significant relationship between Syrphidae and PUs at trap scale but this was not maintained when either geographic or environmental autocorrelation was controlled for. There were no significant correlations between any of the three assemblages at patch level. When correlations among assemblages were examined within habitat types, Sciomyzidae and PUs were significantly congruent within both habitats (Table 6) even when geographic and environmental autocorrelation were controlled for. Sciomyzidae were also significantly correlated with Syrphidae in both habitats, however, in the sedge habitats this was a result of geographic and/or environmental co-response, i.e. trap proximity or similarity of environmental variables at the trap locations appeared to influence congruence. Syrphidae were congruent with PUs in rush dominated wet grasslands but not in sedge dominated habitats.

#### **4.5.5. Cross-taxon congruence of richness, abundance and Shannon's entropy**

Patterns of cross-taxon congruence were not significant for richness among any of the groups (Table 7). Sciomyzidae and PU abundance was significantly positively correlated across all samples at trap scale ( $n = 20$ ) but not at patch scale ( $n = 10$ ) or within habitat types. Syrphidae and PU abundance was significantly positively correlated, but only within rush dominated wet grasslands. There was also a significantly positive relationship between Shannon's entropy of Syrphidae and PUs across all samples but only at trap scale ( $n = 20$ ).



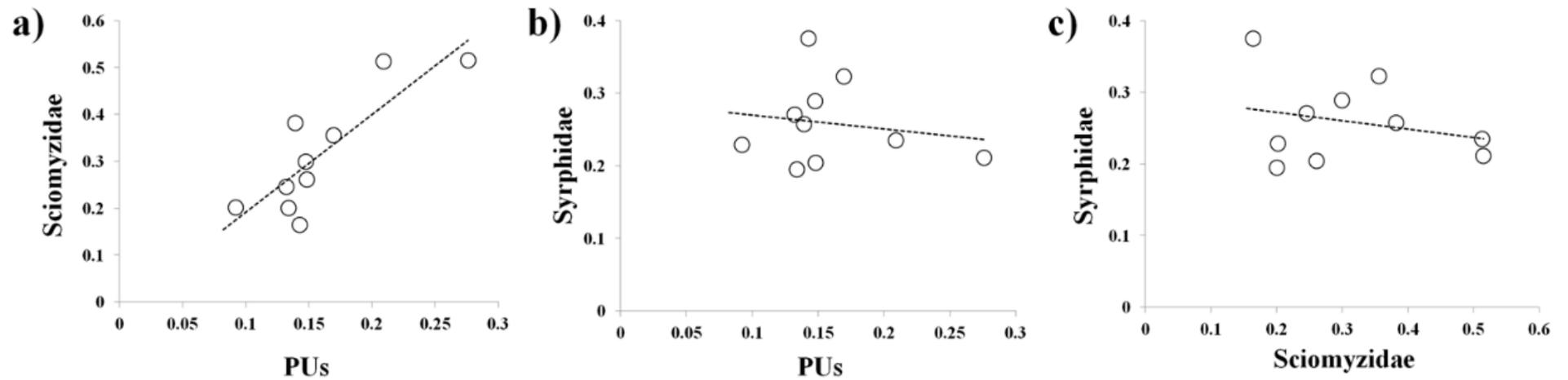
**Figure 7.** NMS ordinations of samples in species/PU space. Environmental variables with  $r^2$  values  $>0.2$  are shown as bi-plots. The principal orthogonal axes are shown with the percentage of variation associated with each axis. Samples are shown as open squares (rush dominated wet grassland) and open triangles (sedge dominated wet grassland), species/PUs as black triangles. **a)** Sciomyzidae, **b)** Syrphidae and **c)** PUs. Only axes 1 and 2 are illustrated which explain a cumulative variation of: Sciomyzidae (71.6%), Syrphidae (69.9%); PUs (65.5%). Habitat types were significantly different using MRPP analysis for all three groups **a)**  $A=0.062$ ,  $P=6 \times 10^{-4}$  **b)**  $A=0.030$ ,  $P=0.02700$  **c)**  $A=0.080$ ,  $P=3 \times 10^{-5}$ .



**Figure 8.** Principal Components Analysis of sampling patches showing the environmental variables most strongly associated with PC1. Samples are differentiated into habitat type (rush wet grassland: open squares; sedge wet grassland: open triangles). Habitat types were significantly different based on MRPP analysis ( $A=0.249$ ,  $P=0.00002$ ). Arrows with no associated environmental variable were associated with PC2.

**Table 5.** Environmental correlations (Spearman's r) between dipteran assemblages based on the axes of the NMS ordinations rotated by PCA score. (P<0.05\*, P<0.01\*\*, P<0.001\*\*\*)

	Axis 1			Axis 2			Axis 3		
	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs
<b>Trap scale (n=20)</b>									
Sciomyzidae	1			1			1		
Syrphidae	<b>0.73**</b>	1		<b>0.65*</b>	1		<b>0.73**</b>	1	
PUs	<b>0.98***</b>	<b>0.70**</b>	1	-0.01	-0.53	1	-0.38	-0.07	1
<b>Patch scale (n=10)</b>									
Sciomyzidae	1			1			1		
Syrphidae	<b>0.85***</b>	1		<b>0.79**</b>	1		0.07	1	
PUs	<b>0.76**</b>	<b>0.73**</b>	1	-0.49	<b>-0.80**</b>	1	-0.13	-0.05	1



**Figure 9.** Linear regression illustrating the congruence of changes in assemblage structure between pair-wise traps using the Sorensen distance measure. Increasing values are indicative of greater dissimilarity. a) Sciomyzidae and PUs were significantly correlated ( $r^2$  0.84,  $P=0.002$ ), b) Syrphidae and PUs ( $r^2$  -0.17,  $P=0.0630$ ) and c) Syrphidae and Sciomyzidae ( $r^2$  -0.23,  $P=0.470$ ) were not significantly correlated.

**Table 6.** Mantel R statistics of assemblage concordance at two spatial scales using the Sorensen distance measure for species/PU data and the Euclidean distance measure to control for location (geographic) and environmental variability. (P<0.05\*, P<0.01\*\*, P<0.001\*\*)

Controlled effect	None			Geographical			Environmental		
	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs
<b>Trap scale (n=20)</b>									
Sciomyzidae	1			1			1		
Syrphidae	0.08	1		0.03	1		0.06	1	
PUs	<b>0.44**</b>	<b>0.20*</b>	1	<b>0.41**</b>	0.13	1	<b>0.44**</b>	0.15	1
<b>Patch scale (n=10)</b>									
Sciomyzidae	1			1			1		
Syrphidae	-0.16	1		-0.17	1		-0.16	1	
PUs	-0.03	0.12	1	-0.03	0.09	1	-0.01	0.18	1

**Table 7.** Mantel R statistics of assemblage concordance within habitats using the Sørensen distance measure for species/PU data and the Euclidean distance measure to control for location and environmental variability. (P<0.05\*, P<0.01\*\*, P<0.001\*\*\*)

Controlled effect	None			Geographical			Environmental		
	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs
<b>Sedge grasslands (n=10)</b>									
Sciomyzidae	1			1			1		
Syrphidae	<b>0.27*</b>	1		0.15	1		0.20	1	
PUs	<b>0.71**</b>	0.04	1	<b>0.68**</b>	0.13	1	<b>0.71**</b>	0.02	1
<b>Rush grasslands (n=10)</b>									
Sciomyzidae	1			1			1		
Syrphidae	<b>0.35*</b>	1		<b>0.24*</b>	1		<b>0.36*</b>	1	
PUs	<b>0.72**</b>	<b>0.51**</b>	1	<b>0.67**</b>	<b>0.42**</b>	1	<b>0.75**</b>	<b>0.47*</b>	1

**Table 8.** Spearman Rank correlations of richness, abundance, and Shannon’s diversity (entropy) of each group. (P<0.05\*, P<0.01\*\*, P<0.001\*\*\*)

	Overall (Trap scale n=20)			Overall (Patch scale n=10)			Sedge Habitats (n=10)			Rush Habitats (n=10)		
	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs
<b>Richness</b>												
Sciomyzidae	/	-0.003	0.261	/	-0.055	0.212	/	-0.6	0.044	/	0.482	0
Syrphidae		/	0.055		/	0.024		/	0.314		/	0.360
PUs			/			/			/			/
<b>Abundance</b>												
Sciomyzidae	/	0.002	<b>0.543*</b>	/	0.018	0.045	/	-0.067	0.382	/	0.249	0.079
Syrphidae		/	0.310		/	0.503		/	0.552		/	<b>0.746*</b>
PUs			/			/			/			/
<b>Shannon’s H’ (Entropy)</b>												
Sciomyzidae	/	-0.381	-0.307	/	-0.467	-0.285	/	-0.164	-0.309	/	-0.321	-0.539
Syrphidae		/	<b>0.448*</b>		/	0.587		/	0.224		/	-0.018
PUs			/			/			/			/

#### **4.6. Discussion**

Community congruence between dipteran groups examined at low spatial scales and within district habitat types provided the most useful predictors of selected dipteran diversity. Assemblages which showed similar responses to environmental variables and exhibited shared community differentiation based on habitat type were most significantly correlated. Diptera which display limited movement (Sciomyzidae) were more indicative of changes in wider dipteran community structure (represented by PUs) than those which are generally more mobile in nature (Syrphidae). The results highlight the variation that occurs between groups when a range of statistical expressions and scales of observation are utilised in biodiversity indication.

The results also suggest compositional analysis of Diptera as the most appropriate approach to the investigation of this overlooked group as previously suggested (Hughes et al., 2000). Assessments of invertebrate cross-taxon congruence in agricultural systems may be restricted to larger spatial resolutions so that patterns can be examined at scales such as field or farm level which coordinate with administrative requirements, e.g. agri-environmental payments are allocated at such scales (Anderson et al., 2011; McMahon et al., 2012). While this is a pragmatic approach to aligning assessment and monitoring of biodiversity with policy, the distribution of certain organisms such as Diptera may occur at more restricted scales. Sciomyzidae, for example, have been shown to exhibit habitat fidelity across scales as low as 23 m (Williams et al., 2010), and Hughes et al., (2000) determined high levels of dipteran community dissimilarity (0.52 Bray-Curtis) between two Malaise traps sited 25 m apart in a meadow. Therefore, the evaluation of patterns of congruence between surrogates and wider diversity of such organisms should occur at scales appropriate to the community distribution of the target taxa (McGeoch, 1998). Subsequent monitoring of such groups which take place at fixed sampling sites can then provide accurate insights into the success of typical conservation strategies such as habitat maintenance or restoration; but inclusive of organisms that inhabit areas at lower spatial scales.

In the present study, environmental correlations between groups based on axis 1 of the NMS ordination were of most interest as they explained the largest amount of variation owing to the use of orthogonal principal outputs in the analysis. While all three dipteran assemblages showed some congruence in terms of environmental correlates, the strongest relationship was seen between Sciomyzidae and PUs at trap level. Based on the NMS output, Sciomyzidae and PU assemblages were largely influenced by the same environmental variables (% rush, % sedge, soil pH, vegetation height and surface water depth) whereas Syrphidae were influenced by a different combination of environmental factors (% rush, % sedge, % moss, % forbs, % moribund vegetation). It is unsurprising that Syrphidae communities responded primarily to variables associated with vegetation type and structure as adults are largely dependent on pollen and nectar resources for food (Ricarte et al., 2011). Sciomyzidae adults, on the other hand, have previously been shown to respond to factors related to hydrological variance such as water depth and hydroperiod as well as type and structure of vegetation (Maher et al., 2015; Williams et al., 2010, 2009a). Along with this, adult Sciomyzidae distribution may potentially be influenced by the availability of larval food sources, i.e. aquatic and semi-aquatic molluscs which often have very clustered distributions (Knutson and Vala, 2011). Their response to factors such as soil pH and water depth suggests that environmental influences that may affect their malacophagous larvae could also influence adult distributions.

The strong correlation between PU and Sciomyzidae assemblages in terms of environmental correlates at trap level not only illustrates that PUs are influenced by comparable environmental factors as Sciomyzidae, but that they also respond at similar scales. Fine scale habitat features are known to affect arthropods in grassland systems, especially more sedentary groups (Cole et al., 2010). Within wet grassland habitats, variations in hydrological regime and grazing patterns are known to have significant influences on dipteran diversity – in particular Sciomyzidae (Maher et al., 2014; Ryder et al., 2005; Williams et al., 2009a). Changes in environmental factors across small spatial scales, e.g. between pairwise traps 20 m apart, could therefore have a more marked effect on groups such as Sciomyzidae which are characterised as having limited movement. This response to small scale features could be a result of their

reduced ability to respond to disturbances or stressors, or alternatively, it may be an indication of the availability of a specific resource located at finer scales. Factors that influence dispersal of Diptera adults include larval development, sexual behaviour, and food requirements (Delettre et al., 1998). Analysis of Syrphidae community differentiation between pairwise traps within each site was not congruent with Sciomyzidae or PUs but was significantly influenced by changes in the percentage cover of broadleaf herbs. As Syrphidae adults are largely dependent on pollen and nectar as food sources (Sutherland et al., 2001), and patches of increased broadleaf herb cover within sites most likely attracted Syrphidae adults, this result is not surprising. Sciomyzidae adults, on the other hand, can be restricted to very small areas, and variables related to hydrology, vegetation type, and vegetation structure are believed to influence assemblages (Maher et al., 2015; Williams et al., 2010, 2009 a/b). Sciomyzidae community differentiation between pairwise traps was anticipated in our results but interestingly was not correlated with any of the measured environmental variables examined in this study. However, the significant correlation between Sciomyzidae and PU community structure between pairwise traps strongly suggests that the factors contributing towards community differentiation across small spatial scales on these two groups are similar.

This result may explain why highly mobile adult Syrphidae were not strongly correlated with the other dipteran assemblages in terms of environmental correlates or community congruence between pairwise traps as they likely respond to environmental heterogeneity at much broader scales (Hendrickx et al., 2007). In contrast, groups which share a decreased ability to disperse and/or have high habitat fidelity have previously been shown to have increased levels of congruence (Grenouillet et al., 2008). However, such patterns of congruence might only be recognisable at spatial scales where these taxa respond similarly to environmental stressors (McGeoch, 1998).

The partitioning of data into biogeographical units, discrete habitat types or subsets of taxa based on community structure is also known to affect congruence patterns between assemblages showing both increased and decreased correlations (Myšák and Horsák, 2014; Pawar et al., 2007; Rooney and Bayley, 2012). In the present study, all

three assemblages showed significant community differentiation between the two habitat types, and community congruence between groups within these two wet grassland habitats were markedly more significant than congruence from groups examined at overall wet grassland level. Wet grassland in Ireland is broadly defined, and generally classified as having >50% cover of grasses, small sedges or rushes, with the remaining vegetation comprised of a mixture of wetland and drier grassland herbs (Fossitt, 2000). However, dominant vegetation type is known to significantly contribute towards dipteran community structure (Hughes et al., 2008) and the results imply that assessments of dipteran diversity in wet grassland habitats should consider sub-categories based on dominant vegetation type. Selecting a biodiversity surrogate requires knowledge of the scale of distribution of the proposed surrogate within the habitat of interest and an examination of its relationship with the predicted taxa below this scale (McGeoch, 1998). In the present study, examining the wet grassland habitats as distinct habitat types increased community congruence among the groups, especially between Sciomyzidae and PUs which displayed similar differentiation between the two wet grassland habitats. Strong community congruence between groups that share similar responses to environmental variables and gradients such as habitat change has been previously found among arthropods in grassland habitats (Oertli et al., 2005).

The examination of Diptera community congruence within these habitat categories at the lowest spatial scale provided the most significant insight into relationships between potential biodiversity surrogates (predictor taxa) and the wider assemblage as represented by PUs (predicted taxa). However, while Sciomyzidae and Syrphidae were both useful surrogates of the PU assemblages in rush dominated habitats, only sciomyzids were significantly congruent with PU assemblages in sedge dominated wet grasslands. The result further emphasises the need to include multiple biodiversity surrogates in evaluations and monitoring rather than reliance on one group (Rooney and Bayley, 2012). The results also illustrate the importance of examining community congruence between taxa based on distinct partitioning of communities.

Species richness is largely utilised as a measure of the success of conservation actions (Su et al., 2004) and is a relatively straightforward value to communicate to

policymakers. However, changes in the species richness of a target group may not be indicative of the response of a wider suite of organisms, especially in agricultural systems (Billeter et al., 2008). Correlations between richness, abundance, and Shannon's entropy in this study varied between groups as is generally reported in studies investigating cross taxon congruence (Báldi, 2003; Hayes et al., 2015; Kati et al., 2004). Only three significant correlations were recorded using these measures, all of which were found when the lowest spatial scale (trap level) was examined. Hayes et al., (2015) reported a significant relationship between Sciomyzidae richness and overall Diptera PU richness in a comparative study of wet grassland conducted at sampling scales similar to the trap level utilised in this investigation; albeit using a sweep net. Though our study was limited to the use of nine families of Diptera to PU level, it is interesting to note that Sciomyzidae abundance and PU abundance was significantly correlated overall at trap level. In general, the richness and abundance of a group tends to be significantly correlated so it could have been expected that if abundance between two groups was significant, then the richness measures would also have been co-correlated. An underestimation of PU richness as a result of the conservative allocation of PUs in this study, i.e. lumping, may have contributed towards a lack of significance between the richness of PUs and Sciomyzidae. Maher et al., (2014) illustrated patterns that suggested increased richness and abundance of Sciomyzidae was indicative of decreased abundance of Syrphidae in wet grassland habitats. However, these findings were reflective of reduced flowering plant diversity due to increased hydroperiod which positively affected Sciomyzidae but negatively affected Syrphidae.

Syrphidae diversity (Shannon's entropy) and PU diversity (Shannon's entropy) were also significantly correlated, but only at trap scale. It could be considered that the most robust PU measure utilised within these correlations was abundance which was a raw value not subject to interpretation. It is therefore interesting that the most significant correlation among these abundance measures was seen between Syrphidae abundance and PU abundance in rush dominated habitats. This result suggests that within such habitats, areas of rush dominated wet grasslands with high abundances of hoverflies could be important for maintaining high abundances of other Diptera, a pattern also

reflected in the community congruence of these two groups with this habitat type. However, the results also highlight the variability of congruence when using richness, abundance, and Shannon's entropy as measures of each group and suggest compositional analysis as a measure of Diptera for surrogacy evaluation.

Agricultural landscapes such as those frequent in the west of Ireland are a mosaic of improved grasslands interspersed with pockets of wet grassland habitat. Realistically, land managers may be required to maintain a suite of wet grasslands whilst others are sacrificed to drainage and/or change of use. Dipteran diversity provides one practical way of maximising landscape-level complementarity in the assemblage conserved. The suite of sites to be preserved, which would maximise gamma diversity of the eleven families studied here, could reasonably be predicted by reference to changes in Sciomyzidae composition dissimilarities (if the sites were *Carex* or *Juncus*-dominated) or with reference to Syrphidae composition (if the sites were *Juncus*-dominated).

#### **4.7. Conclusion**

The inclusion of overlooked groups such as Diptera in conservation strategies is unlikely to reach the thresholds of more iconic invertebrate groups so the use of surrogate taxa is increasingly probable. If such action is required, the surrogates selected for representation of wider dipteran diversity need to reflect the distribution of Diptera at both habitat level and at smaller scales within those habitats. Our results demonstrate that the use of community similarity at low spatial scales and within specific habitat types appears to be the most useful surrogate of dipteran diversity in wet grasslands. However, it may well be most beneficial for conservationists to adopt several invertebrate groups should a surrogate approach be utilised to account for the range of dispersal and distribution patterns of wet grassland invertebrates. While a more extensive study of invertebrates in High Nature Value farming systems is required before a practical solution to invertebrate diversity surrogacy can be selected, in the case of Diptera within wet grassland habitats, our results suggest that both Sciomyzidae and Syrphidae meet these requirements, though the former appear to represent the predicted taxa (PUs) better in this study. In light of the increasing intensification and abandonment of wet grassland habitats, the inclusion of easily

captured, readily identified, and ubiquitous groups as seen in this study may provide essential information on the status of dipteran assemblages within these areas and inform for the selection of areas for conservation. We recommend that considering samples from low spatial scales rather than administrative units such as field or farm level in cross-taxon congruence investigations may highlight patterns of correlation among invertebrate groups which are generally overlooked.

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## Chapter 5:

### **Taxonomy, Parataxonomy and Metabarcoding: complementary approaches to invertebrate conservation and monitoring in High Nature Value wet grasslands.**

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(Left) Petri dish containing a bulk sample of arthropods captured from seasonally inundated wet grasslands (Right) using Malaise traps (J.G.J Carey, October 2017)

## **Taxonomy, Parataxonomy and Metabarcoding: complementary approaches to invertebrate conservation and monitoring in High Nature Value wet grasslands.**

### **5.1. Abstract**

Comprehensive assessment and monitoring of the diversity of hyper-diverse biological communities such as invertebrates is generally overlooked in conservation strategies. Modern high-throughput sequencing techniques such as DNA metabarcoding offers a potential tool for expanding invertebrate assessments beyond a narrow range of indicator groups and into wholesale community analysis. Using invertebrate data obtained across a temporal gradient from High Nature Value wet grassland habitats, we compared selected invertebrate families identified using taxonomy and parataxonomy with DNA metabarcoding (MBC) data obtained from bulk invertebrate samples. Comparisons of two ecologically and agriculturally contrasting wet grasslands (*Carex* and *Juncus* dominated habitats) showed no significant difference between the habitats based on richness, irrespective of the assessment type utilised. However, MBC data showed significantly greater diversity in ecologically and agriculturally overlooked *Juncus* wet grasslands, and the local contribution to beta diversity (LCBD) in *Juncus* dominated wet grassland habitats was significantly higher based on de-novo clustering of MBC sequences. The results highlight the potential idiosyncratic nature of *Juncus* grasslands and their importance in the maintenance of terrestrial invertebrate diversity. The outcomes also illustrate the differentiation between MBC data outputs using assignments made using de-novo methods (DADA2) and those made using OTU-based assignments at 97%. Each dataset provided novel and compelling insight into the temporal dynamics of the invertebrate communities, suggesting an overall temporal succession with a cyclical structure. Finally, the taxonomic and parataxonomic groups examined in the study proved useful as surrogates of the overall metacommunity in terms of community congruence; implying that the use of selected *a priori* groups to monitor wholesale invertebrate diversity is feasible. We discuss the role that MBC can play as a complementary approach to traditional (taxonomic) and rapid (parataxonomic) invertebrate assessment techniques, and the need to advance the inclusion of invertebrates in conservation strategies.

## 5.2. Introduction

Despite contributing overwhelmingly to the diversity and ecosystem functioning of most habitats, invertebrates are generally excluded from the selection and monitoring of areas of conservation interest. In a European context, the preservation of biodiversity in threatened areas such as low intensity High Nature Value (HNV) farming systems is therefore driven by interests such as birds and plants-with invertebrates routinely overlooked unless they are specifically listed as qualifying interests. The difficulties associated with the identification of specious groups of invertebrates, along with their sheer abundance, prohibits their incorporation into conservation assessments which are often limited in terms of time and resources (Cardoso et al., 2011, 2008). While some invertebrates such as Lepidoptera may be included in assessments (Andersen et al., 2004; New, 1997), many hyperdiverse groups such as Diptera or Hymenoptera (with the exception of bees) which require substantial effort and expertise to identify using traditional taxonomy are ignored (Morinière et al., 2016)..

Examination of synecological patterns of invertebrate communities such as spatial or temporal differentiation are usually restricted to groups where taxonomic information and expertise is readily available (Krell, 2004). However, for determining conservation strategies, ecological patterns, or examining the effectiveness of policy measures (e.g. assessing the value of particular habitats for invertebrate biodiversity; investigating wholesale community structure across habitual or seasonal gradients; selecting surrogates of diversity in areas of conservation interest for future monitoring), assumptions derived from a narrow range of groups may not adequately represent the community as a whole (Moreno and Sánchez-Rojas, 2007).

Invertebrate conservationists have considered numerous strategies to broaden the range of invertebrates utilized in conservation assessments beyond species with public appeal or protected through legal obligations (Pearson, 1994). These strategies range from the examination of less iconic groups that are nonetheless well known to scientists (e.g. Anderson et al., 2011; Carey et al., 2017; Gerlach et al., 2012; Pollet, 2001), through to the inclusion of a wider range of invertebrates identified using more non-traditional means such as parataxonomy (Abadie et al., 2008; Hayes et al., 2015;

Krell, 2004) or molecular methods such as DNA metabarcoding which uses high throughput sequencing technology to simultaneously sequence DNA fragments from bulk mixtures of diverse taxa (Ji et al., 2013; Morinière et al., 2016; Yu et al., 2012). However, each of these methods is subject to associated limitations: traditional taxonomic identification of a few groups, though highly accurate, is usually restricted to a smaller number of species owing to time constraints and availability of taxonomic expertise (Moreno and Sánchez-Rojas, 2007); parataxonomy is deemed useful as a preliminary sorting strategy complementary to taxonomic identification, but it is not considered sufficient for detecting important patterns of beta diversity (Krell, 2004; Ward and Stanley, 2004); DNA MBC, whilst rapidly improving in technical capability, is prone to difficulties with taxonomic bias introduced via Polymerase Chain Reaction, factors concerning the efficiency of primer sets, and the availability of reference datasets for assignment of Operational Taxonomic Units (OTUs) (Ji et al., 2013; Zhou et al., 2013).

Devising and selecting which approach to apply to invertebrate assessments at an operational level should be based on the overall objective of the study (McGeoch, 1998). In the context of evaluating and monitoring wholesale invertebrate diversity, the inclusion of as many groups as possible is required in order to generate the most comprehensive insights. In practical terms, parataxonomy or DNA metabarcoding (MBC) would allow for examination of a wider range of species compared to taxonomic identification; especially within the restricted timeframes of a typical assessment. MBC could be regarded as a more reliable methodology for assessing both alpha and beta diversity (Ji et al., 2013) compared with parataxonomy which is only considered limited in its usefulness for assessing richness values (Krell, 2004, Carey et al., 2017b). Despite the reservations associated with the effectiveness of parataxonomy as a rapid biodiversity assessment technique, it continues to be utilized as an analogue of taxonomic verification in invertebrate conservation studies owing to the consensus that multiple taxon investigations are required to deliver sustainable conservation measures (Hackman et al., 2017; Peters et al., 2016; van Schalkwyk et al., 2017). Irrespective of the continued inclusion of parataxonomy in diversity investigations, or indeed the advancements in MBC analysis, the examination and

analysis of target groups to species-level using taxonomic verification is still regarded as the most accurate methodology for assessing diversity and ecological patterns such as spatial and temporal differentiation (Moreno and Sánchez-Rojas, 2007; Krell, 2004).

Invertebrate conservation urgently requires a balanced approach that combines high quality data with expedient analysis of a more expansive range of taxa (McGeoch, 1998). A possible solution to this dichotomy is a complementary approach whereby bulk collection of invertebrates are subject to all three assessments techniques; taxonomic, parataxonomic, and MBC. In light of the fact that much of the sampling of target groups of invertebrates is indiscriminate and produces excessive levels of by-catch, especially by standardized methods such as Malaise trapping (Oxbrough et al., 2009), such an approach also represents a more responsible use of non-target invertebrate groups. Species which are of taxonomic or parataxonomic interest can be removed and identified accordingly, and the remaining bulk sample, along with representatives of the taxonomic and parataxonomic datasets could, and perhaps should, be assessed using DNA MBC. This would allow for a more holistic assessment of invertebrate diversity, generating useful insight into wholesale community dynamics, and may also assist in identifying whether the target groups are useful proxies of overall diversity i.e. they adequately represent the broader diversity of invertebrates within the samples and act as biodiversity surrogates (McGeoch, 1998).

Wet grassland habitats represent diverse mosaics of habitats and habitat features that support significant levels of biodiversity in low intensity agricultural systems throughout Europe (Joyce and Wade, 1998). In light of increasing agricultural intensification and abandonment across Europe, these areas in particular require urgent strategies to protect and maintain their associated biodiversity (Billeter et al., 2008). However, conservation strategies to maintain, protect, and restore these habitats are largely driven by non-invertebrate interests such as birds or plants (Drake, 1998), and little is known about the community structure of invertebrates within these systems. This study examined two families of well-known Diptera (Sciomyzidae; Syrphidae) which are considered bioindicators of wet grasslands (Carey and LeRoy et al., 2015a; Sommaggio, 1999; Speight, 1986) and identified them to species level using taxonomic keys. Nine other families of Diptera (Dolichopodidae, Empididae,

Hybotidae, Limoniidae, Pipunculidae, Scathophagidae, Sciomyzidae Stratiomyidae, Syrphidae, Tabanidae, and Tipulidae) which are ubiquitous within these grasslands were identified using the rapid biodiversity assessment technique known as parataxonomy (morphospecies) (Oliver and Beattie, 1996a, 1993b). DNA metabarcoding of the entirety of the captured invertebrates (including the taxonomic and parataxonomic groups) was conducted using OTU-based and de-novo assignment of DNA sequences to assess diversity at the overall community level.

The principal objectives were

- 1) To apply this three-pronged approach to a typical conservation assessment i.e. comparing the richness and diversity of aerial invertebrates using taxonomic, parataxonomic, and MBC data obtained from two contrasting habitat types namely *Carex* and *Juncus* dominated wet grasslands
- 2) To utilize these data to determine patterns of temporal beta diversity in invertebrate communities in light of a paucity of information concerning changes in invertebrate communities over time
- 3) To examine the effectiveness of taxonomic and parataxonomic subsets to act as surrogates of the overall community as represented by the MBC data

The implications of the findings are discussed in terms of the inclusion of invertebrates in conservation evaluations and monitoring, and in particular the role that MBC can play in augmenting our understanding of invertebrate communities in HNV farming habitats.

### **5.3. Materials and Methods**

Invertebrate sampling was carried out in ten wet grassland habitats as determined from Fossitt (2000) in the west of Ireland using all black Malaise traps as described in Carey et al., (2017a,b) over a period of 18 weeks (May 4<sup>th</sup> to September 2014). Five of these areas were characterised as having abundant grass and *Carex* cover (>50%), whereas the other five sites were dominated with abundant grass and *Juncus* cover (>50%). All the sample sites were actively managed for grazing by cattle and/or horses and not subject to application of fertilizer, herbicides, or pesticides.

Samples were collected from Malaise traps every 14 days yielding a total of nine sequential periodic samples from each trap. In this study, we examined samples from periods one, three, five, seven and nine, omitting the samples from periods two, four, six, and eight owing to resource constraints. In total, 100 invertebrate samples were assessed, 50 from *Carex* wet grasslands and 50 from *Juncus* wet grasslands. Eleven families of Diptera were removed from the bulk samples for taxonomic (Sciomyzidae; Syrphidae) or parataxonomic (Dolichopodidae; Empididae; Hybotidae; Limoniidae; Pipunculidae; Scathophagidae; Stratiomyidae; Tabanidae; Tipulidae) identification (see Carey et al., 2017b). Subsequent to their identification, all taxonomic and parataxonomic specimens were returned to their original bulk samples for preparation for DNA extraction.

### **5.3.1. Metabarcoding**

To ensure large bodied specimens did not dominate the mixed community DNA yield, we only used material comprised of two legs from individuals with a body-size greater than that of a honey-bee and entire bodies of anything smaller (Ji et al., 2013). Subsequently, individual MBC samples were prepared by using whole bodies of arthropods which were freeze-dried in liquid nitrogen and ground to a fine homogenous paste in a sterile mortar and pestle. Five randomly selected 50mg subsamples of the overall homogenised sample were removed, mixed again, and a final 50mg sample of this tissue was selected for DNA extraction. Tissue was lysed for 24 hours at 56° C in 180 µl Qiagen Buffer ATL and 20 µl Qiagen proteinase K (Hilden, Germany). DNA was extracted from the lysed solution following the protocol for the Qiagen DNeasy® Blood and Tissue kit. The DNA was visualised on a 1% agarose gel run at 100 volts for 45 minutes, and quality and quantity were assessed using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA).

DNA samples were amplified for sequencing at RTLGenomics (Lubbock, TX, USA) in a two-step process. The forward primer was constructed with (5'-3') the Illumina i5 sequencing primer (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG) and the *mtCOIintF* GGWACWGGWTGAAWGTWTAYCCYCC primer; a short section of the cytochrome c oxidase I mtDNA barcoding gene (Brandon-Mong et al., 2015;

Leray et al., 2013). The reverse primer was constructed with (5'-3') the Illumina i7 sequencing primer (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG) and the *HCO2198* TAAACTTCAGGGTGACCAAAAATCA primer (Brandon-Mong et al., 2015; Leray et al., 2013). Amplifications were performed in 25 µl reactions with Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, California), 1 µl of each 5 µM primer, and 1µl of template. Reactions were performed on ABI Veriti thermocyclers (Applied Biosystems, Carlsbad, California, USA) under the following thermal profile: 95°C for 5 min, then 25 cycles of 94°C for 30 secs, 54°C for 40 secs, 72°C for 1 min, followed by one cycle of 72°C for 10 min and 4°C hold.

Equimolar PCR products from the first stage amplification were added to a second PCR. Primers for the second PCR were designed based on the Illumina Nextera PCR primers as follows:

Forward

- AATGATACGGCGACCACCGAGATCTACAC[i5index]TCGTCCGCAGCGTC

Reverse

- CAAGCAGAAGACGGCATAACGAGAT[i7index]GTCTCGTGGGCTCGG.

The second stage amplification was run the same as the first stage except for 10 cycles.

Amplification products (313bp) were visualized with eGels (Life Technologies, Grand Island, New York, USA). Products were then pooled equimolar and each pool was size selected in two rounds using SPRIselect (BeckmanCoulter, Indianapolis, Indiana, USA) in a 0.7 ratio for both rounds. Size-selected pools were then quantified using the Qubit 2.0 fluorometer (Life Technologies) and loaded on an Illumina MiSeq (Illumina, Inc. San Diego, California, USA) 2x300 flow cell at 10pM and sequenced at RTLGenomics.

## 5.3.2. Bioinformatics

### 5.3.2.1. Creation of reference database

All sequences for 5' region of the mitochondrial gene COI for the phylum *Arthropoda* along with their annotations were downloaded from the BOLDSYSTEMS v3.0 database [<http://www.boldsystems.org/>]. A custom workflow was utilized to filter out sequences that shared the same IDs, and a reference database and taxonomy file was generated (a total of 3,717,139 sequences).

### 5.3.2.2. Operational Taxonomic Units (OTUs) based approach

Abundance tables were generated by constructing OTUs (a proxy for species) as follows: filtered paired-end reads were trimmed using Sickle v1.200 [N.A. Joshi and J.N. Fass. Sickle: A sliding-window, adaptive, quality-based trimming tool for fastq files. Version 1.21: Available at <https://github.com/najoshi/sickle>., 2011.] by applying a sliding window approach and trimming regions where the average base quality drops below 20. A 10bp length threshold was applied to discard reads that fell below this length. BayesHammer (Nikolenko et al., 2013) from the Spades v2.5.0 assembler was utilized to error correct the paired-end reads followed by pandaseq v(2.4) with a minimum overlap of 50bp to assemble the forward and reverse reads into a single sequence spanning the entire region. The above choice of software was as a result of recent work (Schirmer et al., 2015) where it was shown that this strategy results in substitution errors reduced by 77-98% with an average of 93.2% for MiSeq datasets. Having obtained the consensus sequences from each sample, UPARSE (v7.0.1001) pipeline (All these steps documented in UPARSE.pdf located at <https://bitbucket.org/umerijaz/amplimock/src/2015>) were used for OTU construction. The approach used was as follows: reads from different samples were pooled together and barcodes added to keep an account of the samples these reads originated from. Reads were then dereplicated and sorted by decreasing abundance and singletons discarded. Reads were then clustered based on 97% similarity, with any reads that were shorter than 32bp discarded. Although the cluster\_otu command in usearch removed reads that have chimeric models built from more abundant reads, a few chimeras may have been missed, especially if they had parents that are absent from the reads or are

present with very low abundance. Therefore, in the next step, a reference-based chimera filtering step using a gold database ([http://drive5.com/uchime/uchime\\_download.html](http://drive5.com/uchime/uchime_download.html)) that is derived from the ChimeraSlayer reference database in the Broad Microbiome Utilities (<http://microbiomeutil.sourceforge.net/> 2011) was utilised. The original barcoded reads were then matched against clean OTUs with 97% similarity (a proxy for species level separation) to generate the OTU table.

### **5.3.2.3. OTUs free approach**

The DADA2 algorithm (Callahan et al., 2016) which can resolve real variants differing by as little as one nucleotide without the need of clustering the sequences at 97% similarity was utilized for the OTU-free approach. The method is built on a model of the errors in Illumina-sequenced reads, and assigning an "abundance p-value" to unique sequences (with associated abundance) as goodness-of-fit criteria to the Poisson density function. Starting with placing all the sequences in a single partition, the algorithm iterates by forming new partitions (with their associated Poisson density function) whenever the criteria were not met, and allowing unique sequences to join the partition most likely to have produced them. When the algorithm has converged, all the partitions represent unique variants the central most abundance sequence of each partition as representative sequence. The standard workflow for DADA2 given at <http://benjjneb.github.io/dada2/tutorial.html> was utilised. This workflow learns the error model from the data first, de-replicates the reads and then runs the DADA2 algorithm separately on both forward and reverse reads. Finally, the sequence variants are reduced by merging the overlapping reads from both forward and reverse reads to give unique sequences (henceforth referred to as SEQs), which were then used to create the sequence table for different samples.

The representative OTUs/SEQs were then taxonomically classified against the custom database derived from BOLDSYSTEMS v3.0 with `assign_taxonomy.py` script from the Qiime (Caporaso et al., 2010) workflow. To find the phylogenetic distances between OTUs/SEQs, we first multisequence aligned the OTUs/SEQs against each other using `mafft v7.040` (Katoh and Standley, 2013) and then used `FastTree v2.1.7`

(Price et al., 2010). Finally `make_otu_table.py` from Qiime workflow was employed to combine abundance table with taxonomy information to generate biome files for both OTUs and SEQs.

### **5.3.3. Statistical analysis**

#### **5.3.3.1. Alpha diversity**

Statistical analyses were performed in R using the tables and data generated as above as well as the metadata associated with the study. For community analysis (including alpha and beta diversity analyses) the R package `vegan` was used (Oksanen et al., 2015). The following alpha diversity measures: *Richness*, estimated number of species per sample and *Shannon*, a commonly used index to characterise species diversity were examined. `vegan`'s `aov()` was employed to calculate pair-wise ANOVA p-values which were illustrated with alpha diversity figures.

#### **5.3.3.2. Beta diversity**

Unifrac distances (that account for phylogenetic closeness) were calculated using the `phyloseq` package (McMurdie and Holmes, 2013). Nonmetric Multidimensional Scaling (NMDS) plots of community data (OTUs/SEQs) were examined using different distance measures (`vegan`'s `metamds()` function): *BrayCurtis*, considers the species abundance count; *Unweighted Unifrac*, considers the phylogenetic distance between the branch lengths of OTUs/SEQs observed in different samples without taking into account the abundances; and *Weighted Unifrac*, unweighted unifrac distance weighted by the abundances of OTUs/SEQs. The samples were grouped based on temporal periods and the mean ordination value and spread of points was illustrated (ellipses were drawn using `vegan`'s `ordiellipse()` function that represent the 95% confidence interval of the standard errors).

To understand multivariate homogeneity of groups dispersions (variances) between multiple conditions, `vegan`'s `betadisper()` function as multivariate analogue of Levene's test for homogeneity of variances was used. Non-euclidean distances between objects and group centroids were handled by reducing the original distances (*BrayCurtis*, *Unweighted Unifrac*, or *Weighted Unifrac*) to principal coordinates and

then performing ANOVA on them. Vegan's `adonis()` was utilized for analysis of variance using distance matrices (BrayCurtis/Unweighted Unifrac/Weighted Unifrac) i.e., partitioning distance matrices among sources of variation (both qualitative and quantitative information). This function, henceforth referred to as PERMANOVA, fits linear models (e.g., factors, polynomial regression) to distance matrices and uses a permutation test with pseudo-F ratios.

To find OTUs/SEQs that are significantly different between different conditions, the `DESeqDataSetFromMatrix()` function from DESeq2 (Love et al., 2014) package was used with a significance value cut-off of 0.01 and one log fold cut-off of 1. This function allows negative binomial GLM fitting (as abundance data from metagenomic sequencing is overdispersed) and Wald statistics for abundance data. After performing multiple testing corrections, it reports OTUs/SEQs that have log-fold changes between multiple conditions. The abundance table was relative log transformed and the subset of OTUs/SEQs from the previous step retained, along with this a random forest classifier (using the `randomForest()` function from the `randomForest` package (Liaw and Wiener, 2002) was trained. From the classifier, to get the relative importance of the differentially-expressed OTUs/SEQs, statistics such as *Mean Decreasing Accuracy* and *Mean Gini Index* using the `importance()` function were extracted.

Local Contribution to Beta Diversity (LCBD) analysis (Legendre and DeCaceres, 2013) was performed by using Hellinger transformation to compute the total sum of squares of the species composition for all samples from which the sample-wise local contributions to beta diversity could be derived as a proportion of the total beta diversity. These values were then plotted as bubbles under stacked bar plots to indicate samples that differ markedly in their species composition. ANOVA of LCBD values between different treatment groups was also performed.

### **5.3.3.3. Community congruence**

For community congruence analysis (between OTUs, SEQs, taxonomic, and parataxonomic tables), the `mantel()` function (with 10,000 permutations) from the `ecodist` package (Goslee and Urban, 2007) as well as symmetric procrustes analysis using the `protest()` function from the `vegan` package were used. In the latter case, the

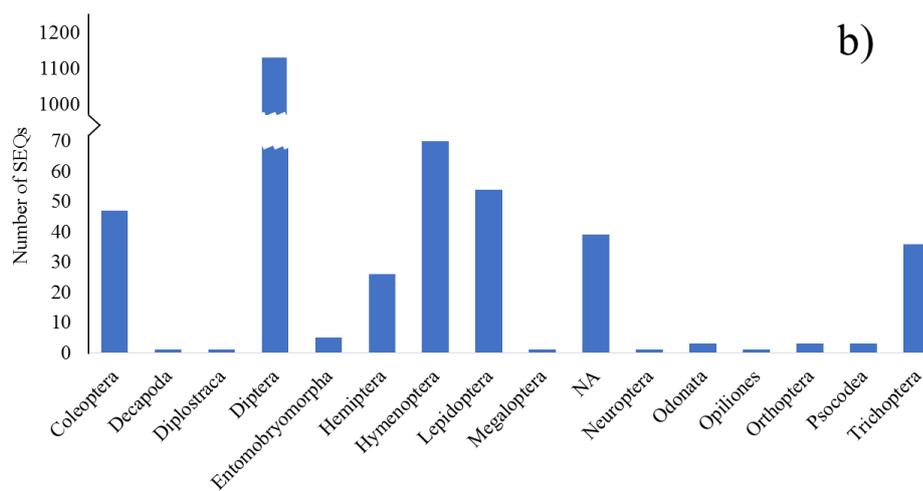
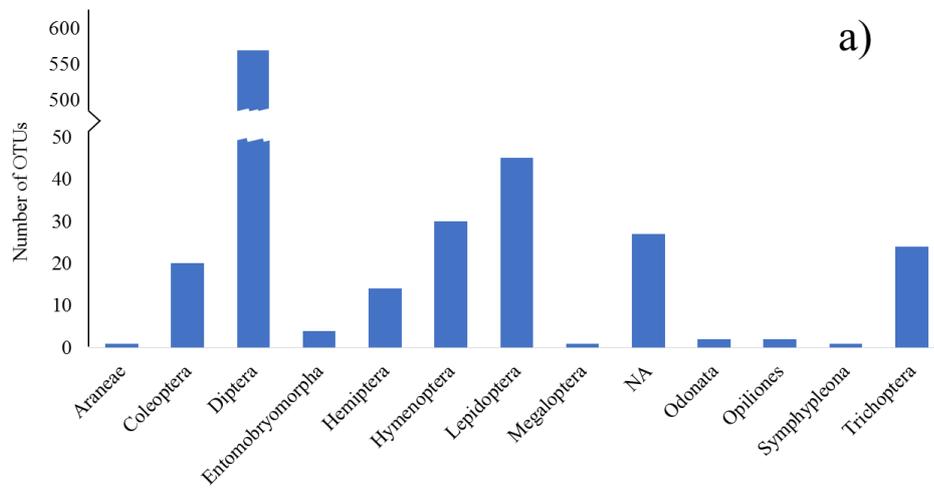
ordination was rotated (using `monoMDS()` function from `vegan`) of one data table to the maximum similarity of the ordination (again using `monoMDS()` function) of another data table. Both methods give a correlation value between the two tables along with their significances. The statistical scripts and workflows for all above can be found at <http://userweb.eng.gla.ac.uk/umer.ijaz#bioinformatics> 2011

## **5.4. Results**

### **5.4.1. Invertebrate diversity**

Taxonomic data was comprised of 92 species in total; 31 species of *Sciomyzidae* and 61 species of *Syrphidae*. Parataxonomic data was comprised of 85 parataxonomic units derived from the nine target dipteran families. OTU-based assignment of MBC data identified 37 OTUs from the taxonomic families (60% underestimate) and 42 OTUs from the parataxonomic families (53% underestimate). The OTU-free assignment of MBC data identified 111 SEQs from the taxonomic families (21% overestimate) and 59 from the parataxonomic families (34% underestimate).

A total of 741 OTUs belonging to 12 orders and 82 families were observed in the OTU-based clustering (Fig. 10a) with 29 OTUs not taxonomically assigned to any order. This increased to 1430 SEQs belonging to 15 orders and 85 families based on OTU-free assignment (Fig 10b) with 36 SEQs not taxonomically assigned to order level. Two arthropod orders, *Decapoda* and *Symphyleona* were solely observed in OTU-based assignment whereas five orders, *Decapoda*, *Diplostraca*, *Neuroptera*, *Orthoptera* and *Trichoptera* were solely observed in OTU-free assignment. *Diptera* (561 [76%]) and *Lepidoptera* (45 [6%]) were the most frequently observed orders from OTUs while *Diptera* (1139 [80%]) and *Hymenoptera* (70 [5%]) were the most frequently observed orders from SEQs.



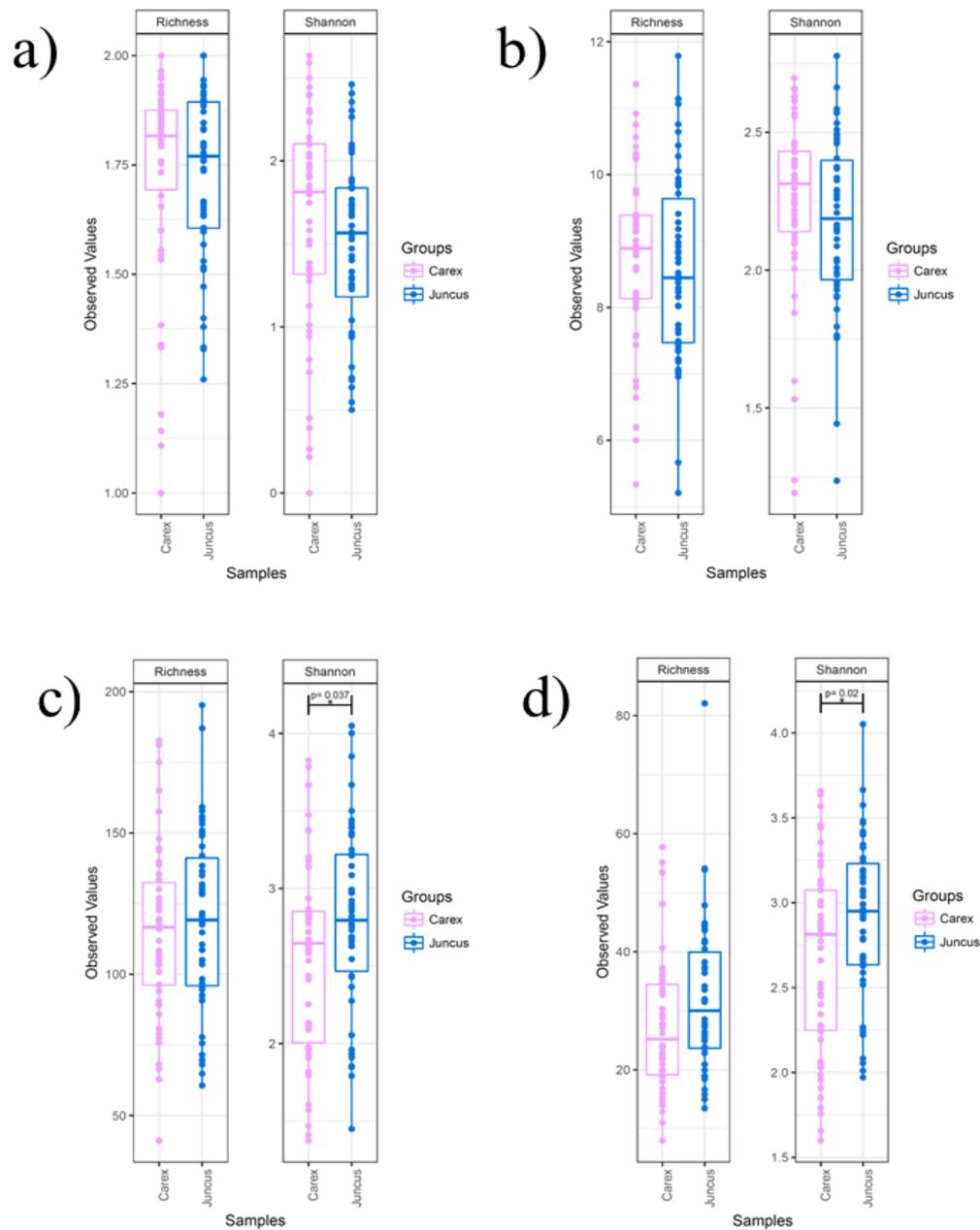
**Figure 10.** Number of **a)** OTUs and **b)** SEQs per arthropod Order based on MBC. NA refers to no taxonomic assignment

#### **5.4.2. Habitat comparisons**

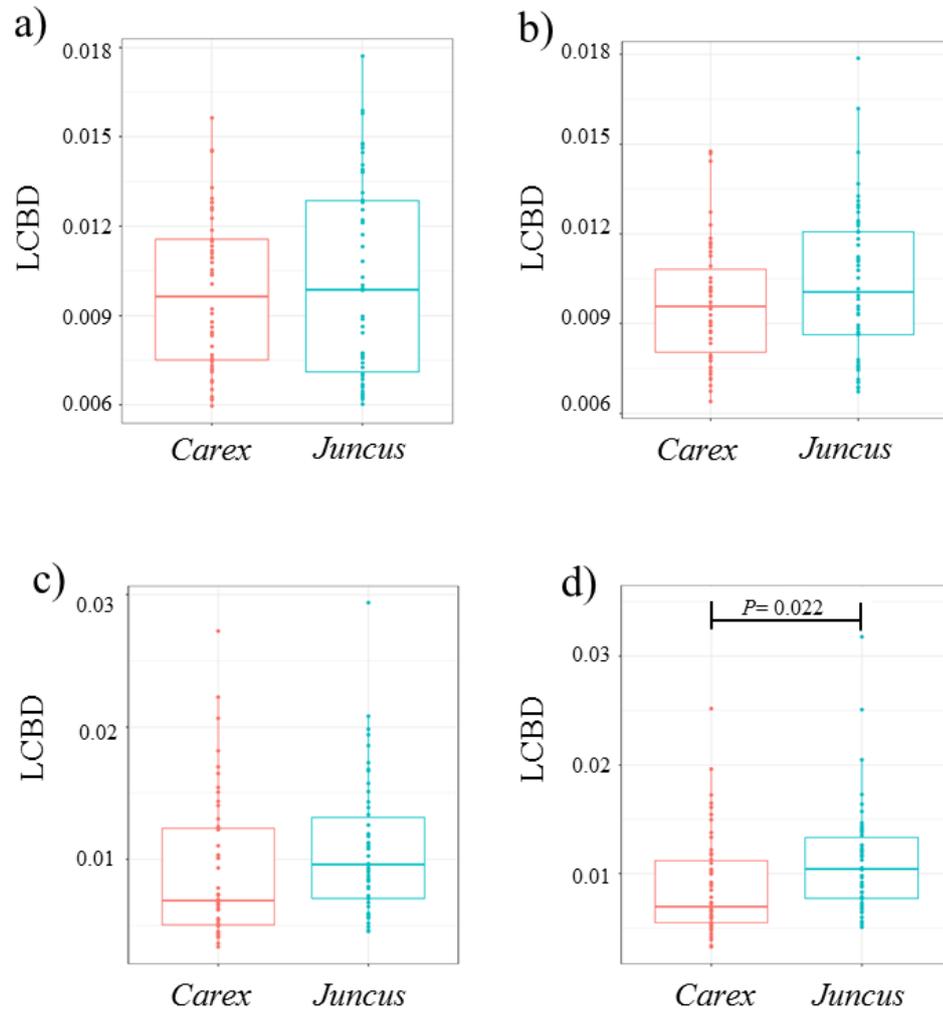
Comparisons of samples from *Carex* dominated (n=50) and *Juncus* dominated (n=50) wet grasslands were made using ANOVA of richness and Shannon's entropy (H'). There were no significant differences between the richness or diversity of the wet grassland habitat types based on taxonomic or parataxonomic datasets (Fig. 11a and 11b). Richness was not significantly different between the habitat types based on OTU or SEQ data (Fig. 11c and 11d), however, both OTUs ( $P=0.037$ ) and SEQs ( $P=0.020$ ) were significantly greater in terms of diversity (Shannon's H) in *Juncus* dominated wet grasslands. Local contribution to beta diversity (LCBD), which determines the uniqueness of samples from the different habitat types, showed similar values based on taxonomic, parataxonomic, and OTU data. However, the *Juncus* dominated wet grassland had significantly higher LCBD than *Carex* dominated wet grassland based on SEQ data (Fig. 12).

#### **5.4.3. Patterns of Temporal beta diversity**

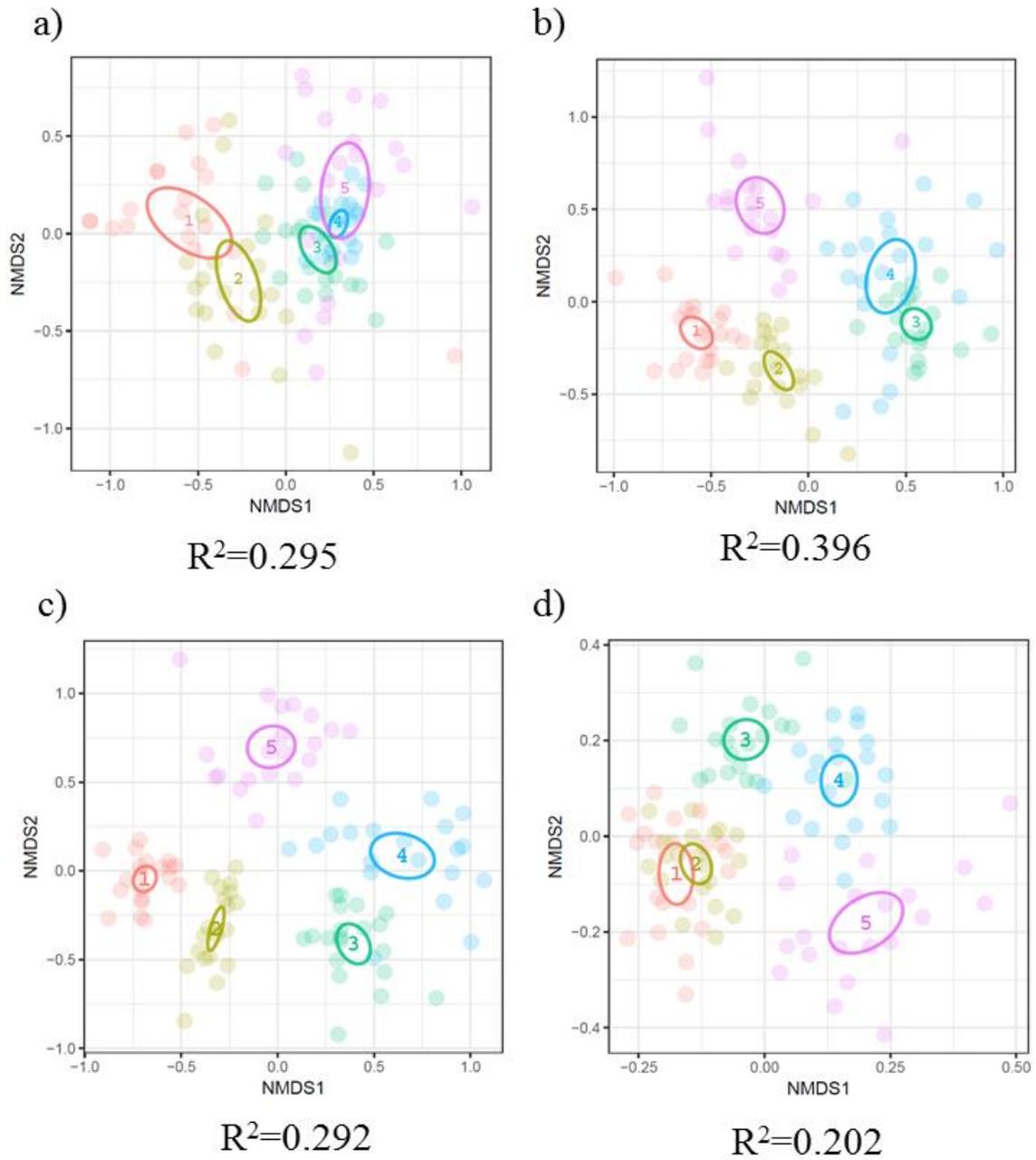
NMDS of each dataset revealed clear and comparable patterns of temporal distribution of invertebrate communities between time periods (Fig. 13). Within each dataset, differentiation can be seen between sequential time periods and the temporal composition of each of the assessed communities was significantly different from one another based on PERMANOVA (Table 9). The pattern observed within the NMDS ordinations was most comparable between the parataxonomic and MBC datasets, all of which display strong cyclical arrangements.



**Figure 11.** Comparison of Richness and Shannon's diversity for *Carex* and *Juncus* dominated wet grasslands using data obtained from **a)** Taxonomic groups, **b)** Parataxonomic groups, **c)** OTUs assigned at 97%, and **d)** SEQs assigned using de-novo clustering. Significant differences are illustrated above boxplots.



**Figure 12.** Comparison of Local contribution to beta diversity (LCBD) of samples from *Carex* and *Juncus* wet grasslands based on **a)** Taxonomic groups, **b)** Parataxonomic groups, **c)** OTUs assigned at 97%, and **d)** SEQs assigned using de-novo clustering. Significant differences are illustrated above boxplots.



**Figure 13.** NMDS of temporal community structure of **a)** taxonomic groups, **b)** parataxonomic groups, **c)** OTUs, and **d)** SEQs. Numbers represent sequential temporal samples.

**Table 9.** Results of PERMANOVA of temporal community composition based on Bray-Curtis dissimilarity for four different invertebrate datasets.

Group	Df	Pseudo F value	<i>P</i>
Taxonomic	4	9.70	0.002
Parataxonomic	4	15.53	0.002
OTUs	4	9.81	0.002
SEQs	4	6.03	0.002

#### 5.4.5. Biodiversity surrogacy

Analysis of community congruence between taxonomic data and parataxonomic data using Mantel tests and Procrustes analysis was utilised to examine the potential of the sub-groups (taxonomic/parataxonomic) to act as surrogates for the invertebrate metacommunity. Mantel tests of taxonomic and parataxonomic data and the MBC datasets (OTUs/SEQs) showed significant correlations between both sub-groups and overall invertebrate diversity as represented by MBC data (Table 10). Parataxonomic units showed greater R values with MBC data-sets based on Mantel tests compared with the taxonomic groups. Similarly, Procrustes analysis indicated significant correlations for both sub-groups and MBC data, with the greatest correlations between parataxonomic data and SEQ datasets (Table 10).

**Table 10.** Community congruence of MBC data sets (OTUs/SEQs) with taxonomic and parataxonomic groups.

MBC	Taxonomic groups		Parataxonomic Groups	
	Mantel R	<i>P</i>	Mantel R	<i>P</i>
OTUs	0.347	0.0001	0.524	0.0001
SEQS	0.272	0.0001	0.414	0.0001
MBC	Procrustes R	<i>P</i>	Procrustes R	<i>P</i>
OTUs	0.654	0.0010	0.668	0.0010
SEQs	0.547	0.0010	0.710	0.0010

## 5.5. Discussion

Analysis of invertebrate wholesale community structure or diversity is scarce, especially within the context and limitations of conservation strategies. This study has illustrated that the use of DNA metabarcoding can augment the knowledge and understanding of wholesale invertebrate diversity in wet grassland habitats which is generally derived from limited taxonomic and parataxonomic collections. Data from each of our assessments has illustrated that while the *a priori* indicators groups utilised in this study (taxonomic and parataxonomic) adequately represented overall invertebrate richness in two ecologically and productively contrasting wet grassland habitats, diversity in *Juncus* habitats was significantly higher based on MBC data; highlighting the potentially significant role of *Juncus* dominated wet grassland for maintaining invertebrate diversity. Similarly, LCBD values were significantly higher

in *Juncus* wet grassland based on non-OTU based sequence analysis; suggesting unique invertebrate community compositions within these habitats. Our study also revealed novel insights into the temporal turnover of the invertebrate community in High Nature Value wet grassland. The results illustrate a cyclical temporal turnover and significant differentiation of community compositions between temporal periods. Finally, because most conservation assessments are resource limited, this investigation also exhibited the role that selected sub-groups of invertebrates can play in acting as surrogates of wholesale diversity. A strategy of monitoring these groups could be utilised to exemplify changes in the broader community, and for the selection of habitats to ensure the maintenance of gamma diversity in agri-ecosystems.

#### **5.5.1. Assessment of habitat types**

Wet grasslands classified according to the most widely used system in the Republic of Ireland (Fossitt, 2000) can vary considerably in their dominant vegetation type and their conservation value is often determined by plant species richness (Sullivan et al., 2010). Where areas of *Juncus* wet grassland are included in conservation designations or planning it is generally through their fortuitous proximity to wet grasslands or associated habitats that are of more botanical interest. In reality, wet grasslands which are dominated by *Carex* (or other small sedges) would be considered superior both ecologically and in terms of potential agricultural production (grazing value) to those with abundant *Juncus* cover (Carey et al., 2017a). In fact, the wholesale removal of *Juncus* from grasslands is actively encouraged in Ireland (Department of Agriculture Food and the Marine, 2015b). Despite this, our analysis has shown that both grassland categories have comparable levels of invertebrate richness, but *Juncus* dominated wet grasslands were significantly more diverse based on MBC analysis. This is further reflected in the significantly greater LCBD of *Juncus* grasslands when compared to *Carex* using non OTU-based clustering of DNA sequences.

Previous studies of the taxonomic and parataxonomic groups utilized in this study have shown that they have significantly different community structures within these two wet grassland types and therefore maintaining such habitat heterogeneity is crucial to protecting invertebrate diversity (Carey et al., 2017a). However, while studies which utilize vouched taxonomic specimens of target groups, can provide compelling insight

into the role of such habitats in maintaining gamma diversity (e.g. Carey 2017a), the question remains concerning the degree of representativeness of these outcomes to the overall invertebrate community. Based on the results presented here, we can comprehensively illustrate that both wet grassland habitats have comparable levels of overall richness and diversity. In effect, the results illustrate that *Juncus* dominated wet grasslands are not just important for the conservation of certain groups of invertebrates such as Sciomyzidae, Syrphidae, or other dipteran groups (as represented by PUs), but have an important role to play in the maintenance of wholesale invertebrate diversity.

It is particularly interesting to note the significantly increased diversity of OTUs and SEQs in the *Juncus* habitats based on the MBC data. *Juncus* dominated wet grassland is likely to be idiosyncratic in nature with its distribution in agricultural areas possibly deriving from the presence of remnant wetland or peatland features, or resulting from excessive disturbance of other wet grassland vegetation allowing competitive *Juncus* species such as *Juncus effusus* (L.) to dominate (Grimes et al., 1990). Contrary to this, *Carex* dominated wet grasslands tend to be more homogeneous in provenance with their distribution in wet grassland habitats normally associated with seasonal inundation and underlying alkaline geology (Fossitt, 2000; Maher et al., 2015). It is possible, that the invertebrate MBC data in this study reflects the underlying and diverse nature of these *Juncus* habitats, which in turn, support a wider diversity of invertebrates owing to their latent and varied ecology. This is also evident in the significantly greater LCBD value associated with *Juncus* areas based on the SEQ (non OTU-based) dataset. Increased LCBD values may be indicative of high priority habitats with uncommon species combinations or alternatively, degraded sites with poor species richness (Legendre and DeCaceres, 2013). Our results would suggest that some *Juncus* wet grasslands are comprised of valuable associations of invertebrates comparable to more ecologically favoured *Carex* wet grasslands. Previous indicator species analysis of the taxonomic groups utilized in this study also support this suggestion with *Juncus* wet grasslands harbouring a wide range of life histories and ecological requirements for both Sciomyzidae and Syrphidae (Carey et al., 2017a), including a number of species listed as scarce or threatened in the UK; *Pherbellia*

*dorsata* Zetterstedt 1846, *Pherbellia argyra* Verbeke 1967, *Tetanocera punctifrons* Rondani 1868, *Neoscia geniculata* Meigen 1822 *Neoscia obliqua* Coe 1940, *Parhelophilus consimilis* Malm 1863. The MBC results also imply that *Juncus* wet grasslands may harbour a greater diversity of invertebrate genetic variation in comparison to *Carex* wet grasslands.

### **5.5.2. Temporal beta diversity**

Information relating to the temporal turnover of terrestrial invertebrate groups is generally poor, with much of the existing knowledge of the temporal beta diversity of invertebrates derived from changes in species richness (García-López et al., 2010) or limited to variation within a few families (Carey et al., 2017a; García-López et al., 2010; Sobek et al., 2009; Tylianakis et al., 2005). The significant differentiation between temporal groupings as seen in our results could be driven by a variety of processes such as temporal, spatial, or trophic partitioning, which are known to limit niche overlap amongst wetland Diptera (Keiper et al., 2002); the most prominent invertebrate Order within this investigation.

Increased ecological productivity (biomass) has been linked to compositional turnover and cyclical community states in ecosystems (Steiner and Leibold, 2004). It has also been suggested that invertebrates might exploit predictably inundated habitats such as wet grasslands through a form of cyclic colonization (Batzer and Wissinger, 1996; Florencio et al., 2011; Wissinger, 1997). Within this colonisation strategy, species utilize local or long-distance dispersion of adults or timed metamorphosis of larvae to exploit the habitat as resources become available. The cyclic pattern observed in the results of this study is likely to be a combination of both mechanisms (dispersal and adult emergence from larval stages) as invertebrate mobility can vary considerably and some groups are more adapted to inundation than others e.g. Syrphidae are highly mobile and many species are wholly terrestrial (Sommaggio, 1999) while Sciomyzidae are limited in their movement with most larval stages reliant to some degree on aquatic habitats or features (Williams et al., 2010).

However, predicting temporal patterns based solely on two families of Diptera is unlikely to accurately reflect the temporal turnover of the overall invertebrate

community. The NMDS ordination of the taxonomic groups utilised in this study would support this observation as, although they show significant changes in community structure along the temporal gradient, the turnover of the community is not as markedly cyclical as the other groups. In this case, it could be argued that the use of a broader suite of invertebrates with a wider range of ecological requirements is more likely to accurately represent the ecological partitioning present within the overall community; in particular amongst Diptera (Keiper et al., 2002). Parataxonomy lends itself to the rapid examination of a broader suite of invertebrate groups although it has been recommended that it should be utilized with caution in studies which examine beta diversity. This caveat is especially pertinent where no taxonomic verification is employed as PUs are not true species and patterns of turnover may be an artefact or improper sorting and allocation of PUs (Krell, 2004). The beta diversity outcomes of this study, which utilized a PU dataset that was subject to taxonomic verification (see Carey et al., 2017b), can be considered robust owing to the marked similarity between PUs, taxonomic and MBC temporal turnover; all of which display comparable cyclical patterns of temporal beta diversity.

Knowledge of such temporal patterns is crucial to understanding ecological processes within High Nature Value systems, and such fundamental data could be considered a benchmark for ecosystem health within other wet grassland habitats. For example, a sudden divergence from normal cyclical temporal patterns may be the result of an environmental perturbation or stressor to which the overall community is not naturally adapted. Such basic information on temporal community structure may also provide important insight into management strategies for these areas. Grazing patterns, for example, are known to affect invertebrates in wet grassland habitats (Ryder et al., 2005), and decisions on the timing and intensity of animal grazing may have effects on invertebrate community succession. Whilst taxonomic and parataxonomic data gives conservationists useful insight into these patterns, MBC data obtained from the overall community allows for a more comprehensive analysis.

### **5.5.3. Biodiversity surrogacy**

Invertebrate biodiversity surrogate studies are generally limited to examinations of the congruence of pair-wise groups of taxa identified to species of parataxonomic unit

level (Dollar et al., 2014; Lewandowski et al., 2010; Moreno and Sánchez-Rojas, 2007), with very few investigations into the congruence between potential predictors and the overall community. The lack of comparisons between selected indicators and wholesale invertebrate diversity stems from a lack of ability to rapidly identify the entire invertebrate community, with much of the limitation a product of a lack of expertise and time (Cardoso et al., 2011). Instead, invertebrate conservation investigations must be pragmatic in their approach to examining multiple groups and understandably focus on obtaining high quality data from the most accessible species (Moreno and Sánchez-Rojas, 2007). This is despite the fact that these target organisms may not necessarily represent wholesale invertebrate diversity (Pearson, 1994).

Metabarcoding technology could provide researchers with a possible mechanism by which to examine the structure of entire invertebrate communities (Ji et al., 2013) albeit without the verifiable taxonomic resolution of species level identification (Beng et al., 2016). However, at a compositional level, which has been suggested as the most applicable means of examining invertebrate diversity (Hughes et al., 2000; Su et al., 2004), MBC of bulk arthropod samples allows for comparisons of community congruence of potential biodiversity indicators with wholesale diversity. Despite the limitations of MBC such as factors related to species dropout or PCR bias (Ji et al., 2013; Yu et al., 2012), if the broader patterns of community structure derived from MBC analysis (in particular beta diversity as seen in this investigation) are comparable to those obtained by other assessment techniques such as taxonomic or parataxonomic sub-groups, then results can be inferred as ecologically meaningful.

Previous research has suggested Sciomyzidae and Syrphidae as possible predictors of wider dipteran diversity in wet grassland habitats owing to their ubiquity within these areas and similarity of response to habitat types and environmental correlations. (Carey et al., 2017b; Hayes et al., 2015). Within our investigation, the use of both of these families collectively showed significant congruence with the overall invertebrate community as represented by the MBC data. As the vast proportion of the OTUs and SEQs identified in the MBC analysis were from the dipteran Order, it may not be that surprising that the use of two dipteran families as surrogates showed strong levels of community congruence with the overall community. A similar outcome was observed

for PUs (nine other families of Diptera) which were also strongly congruent with the overall community. The increased congruence between PUs with the overall community could be a reflection of the wider range of life histories and ecological requirements present within the PU dataset. In turn, the PU data is likely more representative of the temporal dynamic of the community (as seen in the temporal beta diversity analysis), and wider ranging responses to environmental variability which can influence patterns of congruence (Carey et al., 2017b). It should be noted however, that the PUs utilized in this investigation were subject to a degree of taxonomic verification on the basis that they would be utilized for beta diversity analysis. Parataxonomic datasets which do not undertake rigorous allocation protocols may not be suitable for testing patterns of congruence (Krell, 2004). The similarity of response of all three assessment types (taxonomic, parataxonomic, and MBC) in terms of temporal beta diversity analysis, is reflected in the strong levels of community congruence observed between groups. The results illustrate that well-known groups of indicators such as Sciomyzidae and Syrphidae, when utilized collectively, or parataxonomic groups identified with robust protocols, are useful surrogates of wholesale aerial invertebrate diversity across wet grasslands habitats.

## **5.6. Conclusion**

This investigation has demonstrated that the assessment of habitats and subsequent monitoring of wholesale invertebrate diversity can be achieved through a three-pronged approach: utilizing *a priori* selected sub-groups which can be identified using taxonomic and/or parataxonomic methods in conjunction with overall community analysis using MBC methods. This complementary approach provides novel insights into invertebrate diversity and ecological patterns within the framework of conservation strategies and has implications for the responsible use of non-target by-catch invertebrate sampling. Though MBC data is considered ‘taxonomically more comprehensive, many times quicker to produce and less reliant on taxonomic expertise’ (Ji et al., 2013), its use in isolation from more traditional assessment techniques renders it without verifiable comparisons for examination of ecological patterns (Coward et al., 2015). In order to preserve the skills and standards associated with taxonomic identification while simultaneously broadening the scope of invertebrate assessments, we suggest that each methodology can effectively be utilized in unison.

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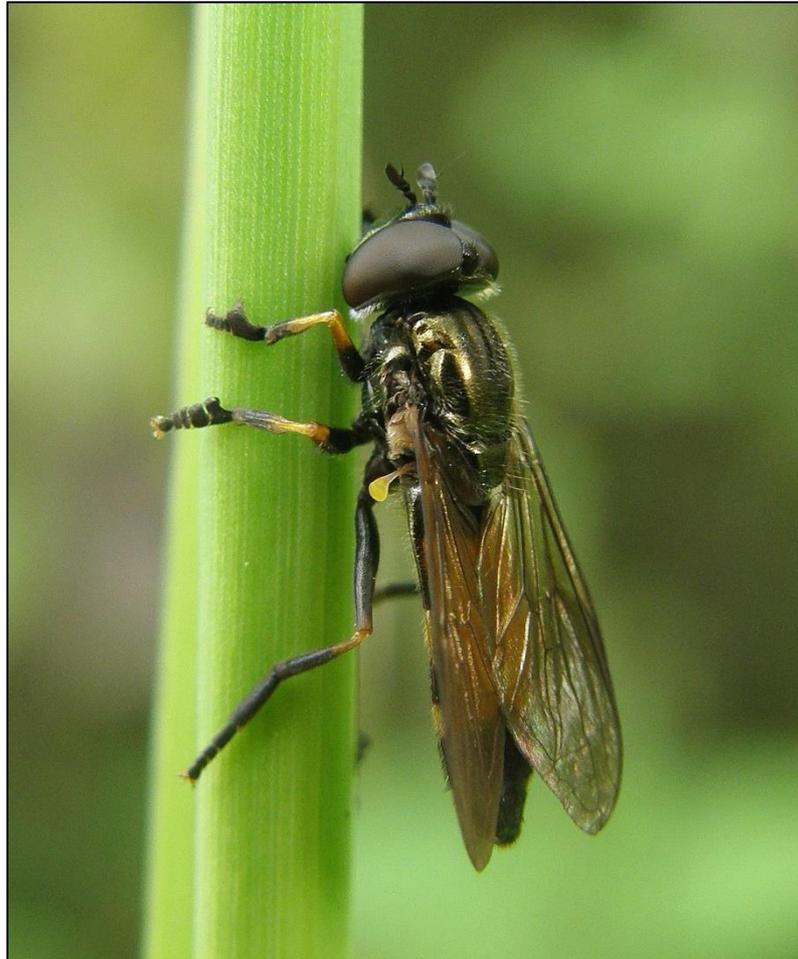
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## Chapter 6

### General Discussion



The hoverfly *Platycheirus granditarsus* (Forster, 1771), a species of Syrphidae typically associated with wet grassland habitats, and one of the most frequently encountered species in this investigation. Photograph courtesy of Steven Falk.

## **6. General Discussion**

### **6.1 Introduction**

The inherent difficulties associated with assessing invertebrate diversity has led to their omission from the selection and management of areas for nature conservation. This is despite their role in ecosystem functioning and their significant contribution towards species diversity within almost all habitat types (Cardoso et al., 2011; Kremen et al., 1993). Much of the difficulty associated with the inclusion of invertebrates in the planning, management, and monitoring of areas of High Nature Value relates to the challenges associated with invertebrate identification (Cardoso et al., 2011). Most species are small and there is considerable diversity within and among groups. In addition to these considerations, taxonomic expertise is limited, both in terms of taxonomic keys and the availability of expert verification (Cardoso et al., 2008). In reality, the majority of conservation strategies associated with areas of potential High Nature Value such as wet grasslands are driven by plants, selected vertebrates (in particular birds) and occasionally by more iconic invertebrates such as bees or butterflies (Andersen et al., 2004; Cardoso et al., 2008; Joyce and Wade, 1998). As a result of this preferential focus on a few chosen biological groups within habitats, a consensus has formed that if these interests are managed correctly they will act as suitable umbrella groups for any associated invertebrates. However, the effectiveness of such an approach is uncertain for invertebrate conservation (Roberge and Angelstam, 2004), with some studies showing a lack of correlation between bird and invertebrate species richness (Blair, 1999; Dollar et al., 2014), and overall plant species richness not necessarily acting as a surrogate for invertebrate diversity (Hayes et al., 2015)

Owing to the complex lifecycles of many species, invertebrate conservation in High Nature Value wet grasslands may require the restoration and maintenance of small scale habitat features e.g. wet flushes, ditches, earth banks, and hedgerows (Hughes et al., 2000; Kirby, 1992; Rooney and Bayley, 2012). However, the resource limitations associated with conservation strategies often means that approaches to protecting

biodiversity focus on statutory requirements which are generally applied at landscape levels. Due to this approach, less productive farm habitats (such as areas with low grazing value) which can readily be identified at these scales are lacking in data related to their role in invertebrate conservation. In turn, this lack of information means that protection of such sites is markedly difficult as the case for their retention cannot be substantiated. The maintenance and monitoring of habitats and species under which there are legal obligations also takes precedence over groups such as invertebrates where data are lacking. Until sufficient information on overlooked habitats and their constituent invertebrates is established, they will remain outside the framework of typical conservation strategies. This renders them vulnerable to degradation as an umbrella approach is unlikely to sufficiently protect groups of organisms (or habitats) of which there is no statutory requirement.

The development of efficient, reliable, and verifiable means of sampling and identifying a wide range of invertebrates from habitats of interest is required if invertebrates are to be included in conservation strategies (Cardoso et al., 2008). Along with these pragmatic considerations, there is also an urgent need to further the understanding of the structure of invertebrate communities across spatial and temporal gradients. Such high-resolution data help inform management strategies and can provide robust evidence to determine the contribution of particular habitats to overall invertebrate diversity (gamma diversity). Motivated by the urgent need to develop new means of including invertebrates in conservation policy and generate new insight into their spatial and temporal distributions, this study examined the invertebrate diversity of High Nature Value wet grasslands associated within low intensity farming in the west of Ireland. The aims of the study are to:

1. Test, for the first time, the efficacy of three sampling methods (Sweep-net; Malaise Trap; Emergence trap) for the collection of marsh flies (Diptera: Sciomyzidae), potential bioindicators of wet grassland.
2. Examine the role that two wet grassland habitat types play in the maintenance of Diptera (specifically Sciomyzidae and Syrphidae) using spatial, temporal and spatiotemporal partitioning of diversity.

3. Determine, in wet grassland habitats, the influence of environmental variability, spatial scale, and habitat type on patterns of cross-taxon congruence between two families of Diptera (Sciomyzidae and Syrphidae) and nine other Diptera families identified to parataxonomic unit level [PUs]).
4. Investigate the use of DNA MBC as a possible complementary means of rapid biodiversity assessments of invertebrates captured using Malaise traps (along with traditional taxonomy and parataxonomy) in wet grassland habitats.

## 6.2 Discussion

The use of a standardised invertebrate capture method allows for comparisons of datasets between habitats and across wider geographical areas. Although sweep netting is considered a rapid and inexpensive method for the sampling of insects, its consistency is affected by the unique action of each sweeper and the known limitations of using the net in shorter vegetation (DeLong, 1932; Southwood and Henderson, 2000; Spafford and Lortie, 2013). Bias associated with samples obtained from sweep netting in isolation from other capture methods may have implications for subsequent invertebrate community analysis. In this study, for example, there was a marked difference in sciomyzid species composition based on catches using sweep nets compared to those using Malaise traps in *Juncus* wet grassland. In addition, no sciomyzids were captured using sweep netting in shorter *Carex* vegetation.

The results of this investigation also highlighted the potential increase in sweep net capture rates of certain groups (such as Sciomyzidae) when sampling occurs at crepuscular times as previously suggested by Vala (1984). This suggests that temporal effects may also influence sweep net captures, and samples obtained by sweep nets from different times of the day may not be comparable in meta-analysis. Malaise traps are less affected by these temporal dynamics and observer efficacy as they collect continuously and passively. Despite this, had Malaise traps been utilized in isolation, 50% of the total sciomyzid fauna captured in the *Juncus* grassland would have been unrecorded. Nevertheless, the species which were only caught using sweep nets in this

study (*Hydromya dorsalis* [Fabricius, 1775], *Ilione albiseta* [Scopoli, 1763], *Limnia unguicornis* [Scopoli, 1763], *Pherbellia ventralis* [Fallén, 1820], *Pherbina coryleti* [Scopoli, 1763], *Tetanocera arrogans* [Meigen, 1830] and *Tetanocera ferruginea* [Fallén, 1820]) were all subsequently captured in *Juncus* grassland using Malaise traps in the study conducted over 18 weeks. This implies that long term studies of sciomyzids in wet grasslands that utilize Malaise traps will produce relatively comprehensive species inventories.

Sweep netting should still be considered a useful tool for the collection of live invertebrates. However, examinations of community structure would benefit from their utilisation in conjunction with passive methods such as Malaise traps or analogous passive netting procedures. Compared with other methods such as pan traps, passive nets are less at risk from weather events such as heavy rainfall or predation by birds or mammals. This is especially relevant to wet grassland habitats which are generally associated with wet climates and many sites may be characterised by short vegetation, often dominated by smaller *Carex* species.

A suitable panacea for the comprehensive assessment of invertebrate communities in wet grassland would be to utilize a wide variety of trapping mechanisms (Spafford and Lortie, 2013). However, few studies have the resources to conduct multiple simultaneous trapping techniques over long periods of time as well as undertaking the associated sorting and identification. It would therefore be pertinent to suggest that if aerial terrestrial invertebrate assessments are to be conducted over short time periods such as the five-week period examined in this pilot study, then multiple capture techniques (Malaise traps, Emergence traps, Sweep nets) could be employed simultaneously as sample sizes would be manageable. Further data concerning the long-term efficacy of sweep netting in shorter vegetation is required, but as a caveat, we suggest that sweep netting should not be utilized in isolation for short term studies of Sciomyzidae in wet grasslands.

The critical examination of invertebrate sampling techniques in habitats of conservation interest with markedly different vegetation structures is central to ensuring the reliability of invertebrate data-sets. This is especially true in Ireland,

where the broad categorisation of areas of High Nature Value such as wet grassland is based on vegetation type (Fossitt, 2000). Markedly different habitats such as sedge (*Carex*) and rush (*Juncus*) dominated grasslands are therefore considered collectively as wet grasslands. This is despite the fact that they are significantly different in terms of environmental variability, dominant plant species, and invertebrate community compositions. Grasslands with *Juncus* cover are generally treated with indifference in terms of both their agricultural productivity and contribution to biodiversity; the latter of these opinions is owing to typically poor plant species richness associated with them. Under current Irish guidelines, greater than 30% cover of *Juncus* in an area is considered a breach of Good Agricultural and Environmental Conditions (GAEC) (Department of Agriculture Food and the Marine, 2015a, 2015b) in which case farmers can be penalised by suspension or curtailment of farm subsidy payments. In wet grassland ecosystems, this has led to the wide scale removal of *Juncus* stands through cutting, spraying with herbicides, and land drainage. These wet grasslands are generally replaced with monocultures of perennial ryegrass (*Lolium perenne*) or alternatively (if the soil is considered unsuitable for grass species) it may be developed into commercial forestry (Wilson et al., 2012). These practices have led to landscapes previously characterised by wet grassland becoming increasingly homogenised despite natural habitat heterogeneity being considered as playing an important role in maintaining farmland biodiversity (Benton et al., 2003).

There is a paucity of information related to the invertebrate fauna of these *Juncus* areas and their potential role in the maintenance of biodiversity within wet grassland ecosystems is not well understood. The results of this investigation have now highlighted the significant role that *Juncus* wet grassland plays in maintaining the diversity of aerial invertebrates due to several factors:

- *Juncus* wet grasslands have significantly different invertebrate community structures compared with *Carex* habitats as illustrated by the taxonomic and parataxonomic data
- More scarce or vulnerable species of Sciomyzidae and Syrphidae are associated with *Juncus* wet grassland compared with *Carex* wet grassland

- *Juncus* wet grasslands have a significantly greater diversity of aerial invertebrates compared to *Carex* habitats

To determine the contribution of *Juncus* wet grassland to the diversity of Sciomyzidae and Syrphidae required the inclusion of temporal turnover in the analysis of diversity partitioning. The results of the taxonomic study of Sciomyzidae and Syrphidae in Chapter three showed that the two wet grassland habitat types harboured different species and community structures. The results also showed that the role of habitat differentiation in contributing to overall diversity was affected by turnover of species across time. In effect, the two habitats maintained diversity by providing resources at different times. For the more mobile Syrphidae, *Juncus* wet grassland provided a refuge and/or resource area with species moving in and out of the habitat subject to their lifecycle requirements e.g. mating, feeding, oviposition. Contrary to this, *Juncus* habitats supported specific communities of Sciomyzidae which rather than move between habitats, have communities that stay *in situ* but change over time. It was also noteworthy that the sciomyzid and syrphid indicators of *Juncus* wet grassland were much more varied in terms of their ecological requirements (ranging from species with terrestrial to aquatic larvae) compared to the *Carex* wet grasslands. This may indicate that some *Juncus* dominated wet grasslands are derived from the disturbance of wetland habitats or features and are still capable of maintaining wetland invertebrate species. The latter observation is particularly important considering that several species of wetland associated sciomyzids (*Pherbellia argyra* [Verbeke, 1967] *Pherbellia dorsata* [Zetterstedt, 1846] *Tetanocera punctifrons* [Rondani, 1868]) and syrphids (*Neoascia geniculata* [Meigen, 1822] *Neoascia obliqua* [Coe, 1940] *Parhelophilus consimilis* [Malm, 1863]) which are considered scarce or vulnerable in the UK (Falk, 1991; Speight and Knutson, 2012) were all exclusively found in *Juncus* wet grassland.

The importance of *Juncus* wet grassland to aerial invertebrate diversity was mirrored in the MBC data which showed that these areas had significantly greater Shannon's diversity compared to *Carex* dominated grasslands. The *Juncus* wet grassland also

displayed significantly greater Local Contribution to Beta Diversity (LCBD) based on non OTU-based clustering. Again, this suggests that the invertebrate diversity associated with *Juncus* wet grassland is largely driven by unique community compositions. While it could be argued that increased diversity and LCBD values were a function of the possible disturbed nature of the *Juncus* wet grassland, the presence of several notable sciomyzid and syrphid species implies that at least some of the areas are extremely important for Diptera. It is interesting to note that increased LCBD in *Juncus* was only visible at a molecular level and using non-OTU based methods. This result indicates that at a morphological level (and at 97% DNA clustering) both wet grasslands may be similar in terms of the uniqueness of invertebrate communities. However, based on de-novo clustering which highlights variation as low as a single nucleotide, the *Juncus* areas appear to have more uncommon compositions of invertebrates. A more detailed examination of this variation based on longer DNA fragments would be required to determine whether some *Juncus* habitats are a refugium for species which may be morphologically similar but genetically differentiated e.g. cryptic species. Irrespective of the unit of diversity (genetic or phenotypic) considered in this analysis, the evidence from this study strongly suggests that *Juncus* wet grasslands are an important contributor to the maintenance of terrestrial invertebrate diversity in wet grassland ecosystems.

The overall invertebrate community structure within wet grasslands was also shown to be successional, with significant community changes across a temporal gradient. It is understood that temporal turnover is a significant contributor to invertebrate diversity, but prior to this investigation, little was known about these patterns in temperate wet grasslands (Sobek et al., 2009; Zamora et al., 2007). The analysis of all three data sets (taxonomic, parataxonomic, MBC) show novel insights of this temporal turnover and reveal for the first time a cyclical community structure most likely indicative of responses to predictable inundation events (Wissinger, 1997). Knowledge of this temporal succession is crucial to informing future investigations of wet grassland invertebrates as well as establishing a baseline for monitoring the effects of management changes within such sites.

Loss or damage of wet grassland could have significant impacts on overall landscape biodiversity. This study reveals that the removal of *Juncus* dominated wet grassland, and its replacement by grass monocultures and/or commercial forestry, poses a significant threat to overall wet grassland ecosystems; especially those areas that may harbour remnant aquatic features. The loss of *Carex* wet grassland is far more unlikely owing to its typical qualification as utilizable agricultural area and thus inclusion in farming subsidy payments. Along with this, *Carex* wet grassland is generally located adjacent to water bodies, many of which are designated for conservation, and by extension it is usually afforded this extra level of protection. Contrary to this, only *Juncus* areas which happen to be in close proximity to designated conservation areas are protected although the removal of *Juncus* is not a notifiable action within designated areas and it can be cut or removed without prior consent from statutory regulators such as the National Parks and Wildlife Service. As exhibited in this study, the use of Syrphidae and Sciomyzidae species to determine the inherent ecological value of a *Juncus* wet grassland prior to its removal or reduction under GAEC policy is both practical and efficient. This is due to their ease of capture and identification, and a well-documented knowledge of their biology. However, more comprehensive national datasets and standardised capture procedures would be required prior to any application of these indicators at policy level. Theoretically, in areas where *Juncus* has outcompeted wetland plant indicators, the presence of selected species of these invertebrates (wetland specialists for example) could signify latent wetland features. These *Juncus* wet grasslands could be targeted for retention within some farming systems owing to their unique invertebrate compositions, and their likely contribution to overall diversity within the system as a whole i.e. complementarity.

It was also clear from the investigation that Sciomyzidae and Syrphidae were good proxies of wider dipteran diversity (PUs) and overall aerial invertebrate diversity as represented by the MBC data. However, the study highlighted important new insights regarding the role that habitat differentiation and scale of observation played in influencing patterns of community congruence. When *Carex* and *Juncus* grasslands were considered separately from one another, patterns of community congruence within these discrete habitat categories showed markedly higher levels of significance

than when congruence was examined at an overall wet grassland level. For example, Sciomyzidae and PUs, which showed similar responses to changes in environmental variability, had markedly higher levels of community congruence when compared to each other in *Carex* and *Juncus* independently than when compared across all wet grassland sites. It has been previously suggested that when communities have distinct biogeographical differentiation along gradients such as habitats or disturbance, their congruence with other groups should be investigated within these specific categories (Pawar et al., 2007; Rooney and Azeria, 2015; Rooney and Bayley, 2012). As *Carex* and *Juncus* wet grasslands were found to be dissimilar both environmentally and in terms of aerial invertebrate community structure, such consideration is especially relevant.

Within the context of this investigation, adult Sciomyzidae which are known to exhibit high habitat fidelity (Williams et al., 2010), were the most suitable group for monitoring the wider dipteran community structure. While the results were unclear as to which specific environmental variable had such a marked effect on Sciomyzidae and PU distribution within habitat patches, a range of factors relating to microhabitat features could be responsible e.g. hydrology, vegetation structure, microclimate. The study also confirmed that compositional analysis of dipteran communities is the most suitable method for assessment and monitoring of these overlooked invertebrates (Hughes et al., 2000). Correlations of richness, abundance and Shannon's entropy (diversity) between groups were inconsistent. This further highlights the increasing agreement that species richness in isolation does not necessarily represent the best approach to biodiversity indication as such measures fail to adequately account for changes in community composition (Su et al., 2004).

Along with this, the conventional scales of observation utilized in typical conservation assessments (namely farm or field level), which coincide with agri-environmental payment policies, were not suitable for examining patterns of congruence amongst dipteran communities. Instead, this investigation determined that owing to the general response of dipteran groups to small scale changes in environmental variables, congruence between communities is best observed at lower scales such as those from individual traps within habitats i.e. not pooled samples from the same field or patch

within a field as seen in comparative studies e.g. Anderson et al., (2011). As a possible stop-gap until more comprehensive investigations of invertebrate diversity are conducted, Sciomyzidae (and to a lesser extent Syrphidae) could feasibly be utilized to monitor invertebrate communities in wet grassland habitats. The results of the study suggest that protecting and managing wet grassland habitats that maintain the highest proportion of gamma diversity for sciomyzids and syrphids would also benefit the broader aerial invertebrate community. This in effect represents an avenue for the inclusion of invertebrates in conservation strategies associated with High Nature Value wet grasslands.

### **6.3. Key findings and conclusions**

Including invertebrates in the assessment and monitoring of High Nature Value farmland is an achievable goal within the framework of conservation strategies. The utilization of selected groups that are well-known and easily identified can significantly change how particular habitats are perceived in terms of their biodiversity value. We have shown within this research that *Juncus* dominated wet grassland, which is typically overlooked by both ecologists and land managers, is an important contributor to invertebrate diversity. However, the results of this research have highlighted several important factors that need to be considered when examining invertebrate diversity within wet grasslands:

1. Trapping method was shown to influence the capture of insects which in turn may affect synecological assumptions. For meta-analysis, Malaise traps appear to be the most consistent method for sampling aerial invertebrates in wet grasslands, especially if the wet grasslands contain areas of vegetation characterised by smaller sedges and/or short grass. However, for comprehensive inventories of invertebrates, sampling should either be carried out over the course of a typical sampling season and not limited to short timeframes.
2. Wet grasslands dominated by *Juncus* spp. may harbour remnant wetland features which support unique aerial invertebrate communities and species of

conservation interest. These areas may also act as a resource hotspot for more mobile invertebrates e.g. Syrphidae. The current GAEC guidelines which restrict *Juncus* cover to <30% and actively encourage its removal is likely contributing to the homogenisation of wet grassland ecosystems. A continued reduction in habitat heterogeneity driven by the removal of such wet grassland is likely to have negative consequences on the maintenance of aerial invertebrate biodiversity.

3. Sciomyzidae, and to a lesser extent Syrphidae, are good surrogates of wider aerial invertebrate diversity and are possible indicators of remnant wetland features within *Juncus* dominated wet grasslands. However, for these groups (particularly Sciomyzidae) to be utilized effectively as bioindicators, they need to be examined at low spatial scales such as within habitat patches and not at field or farm level to coincide with agri-environmental payment plans. Along with this, patterns of community congruence are influenced by habitat type, therefore *Carex* and *Juncus* dominated wet grasslands should be differentiated from one another for biodiversity assessments and not considered together as one wet grassland classification.
4. Evidence from this study shows that within wet grasslands, the overall terrestrial community structure is represented by a cyclical temporal succession. Future management of High Nature Value wet grassland habitats requires a sympathetic approach to this community structure. Significant alteration to the hydrology or vegetation structure of wet grasslands could reduce species recruitment and have negative effects on the sustainability of the wholesale invertebrate community.
5. The examination of wholesale invertebrate diversity using DNA MBC represents a complementary approach to morphological identification of selected invertebrates. The use of molecular analysis of non-target species as seen in this study produced novel insights regarding the overall aerial invertebrate community which otherwise would have remained unknown. The

data provided by MBC allowed for comprehensive testing of the efficacy of selected groups as overall invertebrate biodiversity surrogates. Along with this, MBC offered an opportunity to examine previously unidentified patterns of temporal turnover associated with wholesale invertebrates in wet grasslands. Finally, the potential of overlooked habitats such as *Juncus* wet grassland for maintaining selected invertebrate biodiversity can be expanded to examine diversity at a metacommunity level and provide evidence for changes in policy direction.

#### **6.4. Recommendations for further study**

- An investigation of sweep net efficacy in wet grasslands over a longer time-period e.g. 18 weeks is recommended. A more exhaustive sampling regime would also benefit from being expanded to include other Dipteran bioindicator groups such as Syrphidae. Such a study would provide comparative data to complement the work carried out in this study. An investigation of trapping efficacy comparing passive (Malaise/Emergence traps) and active (Sweep netting) capture methods should also be extended to several other habitat types including wetlands, peatlands, and wet woodlands to help determine a definitive and standardised procedure for aerial invertebrate sampling.
- While detailed and comprehensive data were collected on several groups of invertebrates, we recommend a more comprehensive investigation of the role of *Juncus* wet grassland habitats in maintaining biodiversity. A wider ranging study inclusive of ornithological, botanical, and invertebrate data, conducted across a larger number rush dominated wet grasslands may further inform agri-environmental schemes about the value of these habitats for flora and fauna. Such data would provide evidence to help align conservation strategies with current policy incentives and should seek to protect the most unique *Juncus* dominated wet grasslands from drainage and conversion to monoculture of grass or commercial forestry.

- An investigation of patterns of invertebrate temporal turnover across a management intensity gradient in grassland ecosystems is strongly encouraged and should incorporate both morphological and molecular identification. An examination of the effects of farming intensity on invertebrate community structure over time could provide an important baseline of information regarding ecosystem health. The ability of farmland to contribute to ecosystem functions such as recycling, pollination, and pest control could potentially be linked to the continued recruitment of existing species through cyclical colonisation.
- The use of a longer DNA read such as the entire *COI* gene may provide more comprehensive insights regarding genetic variation within certain habitat types. Examinations of the entire *COI* gene may highlight within species genetic variation not sufficiently determinable from shorter reads such as the amplicon used in this study. However, such a study is currently limited by the ability of high-throughput sequences to examine reads >350 base pairs. However, as technology improves, such data may help inform conservationists about the importance of overlooked areas such as *Juncus* wet grasslands for maintaining genetic and morphological diversity within groups such as invertebrates.

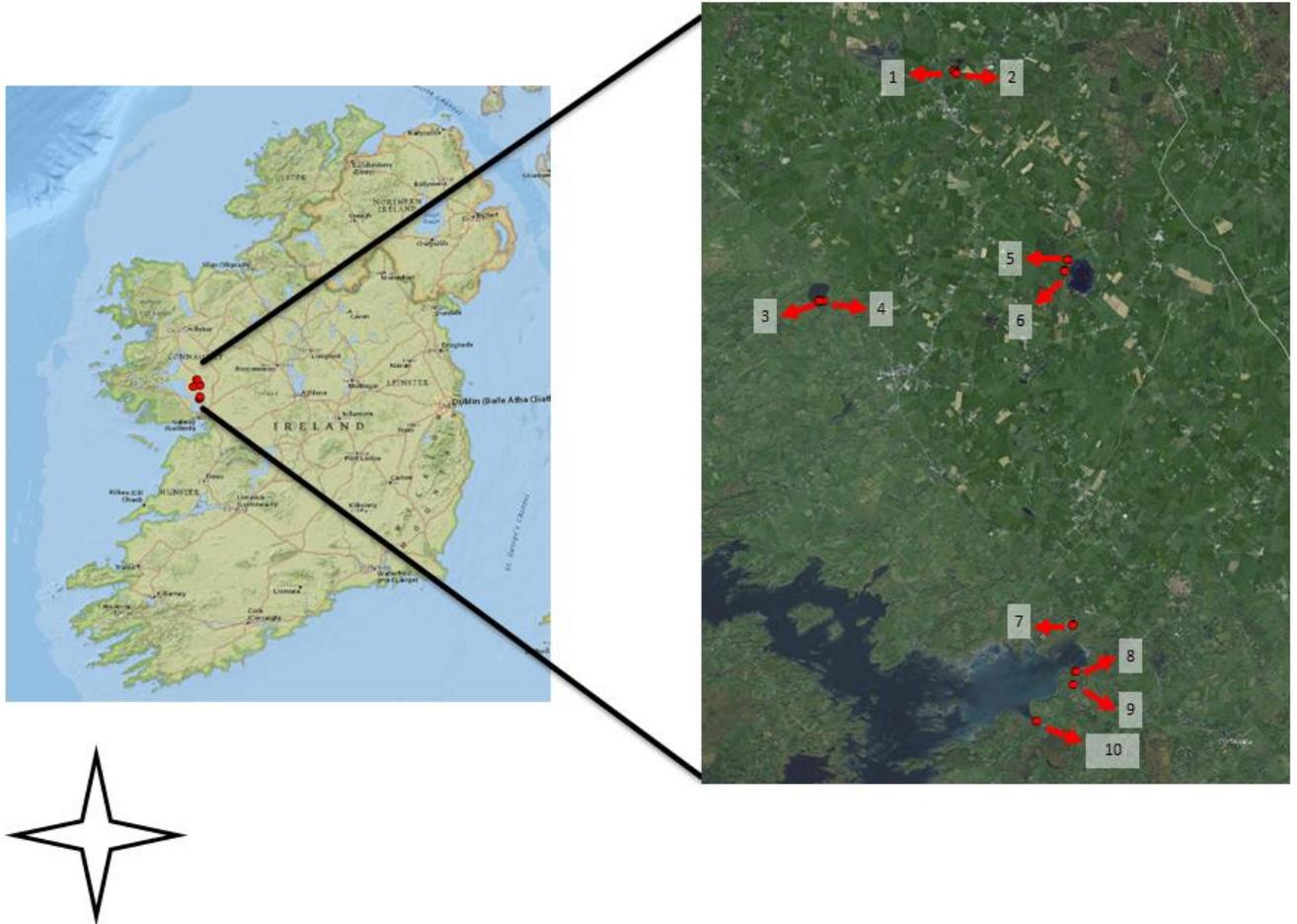
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**Appendix 1: Location of the ten wet grassland sites sampled during this study.**



## Appendix 2: List of Sciomyzidae captured during this investigation

	Overall Abundance	Abundance <i>Carex</i> wet grassland	Abundance <i>Juncus</i> wet grassland	Frequency of capture (per 20 traps [%])	Voltinism*	Behaviour**
<i>Antichaeta analis</i>	5	3	2	25	5	D
<i>Antichaeta brevipennis</i>	12	12	0	10	5	D
<i>Colobaea bifasciella</i>	26	19	7	35	1	B
<i>Coremacera marginata</i>	1	0	1	5	2	G
<i>Elgiva cucularia</i>	15	7	8	35	2	G
<i>Hydromya dorsalis</i>	53	39	14	75	1	A
<i>Ilione albiseta</i>	296	163	133	80	4	G
<i>Ilione lineata</i>	65	49	16	65	4	H
<i>Limnia paludicola</i>	8	5	3	20	5	A/E
<i>Limnia unguicornis</i>	20	9	11	50	5	A/E
<i>Pherbellia argyra</i>	22	0	22	25	1	A
<i>Pherbellia dorsata</i>	1	0	1	5	1	A
<i>Pherbellia dubia</i>	1	1	0	5	1	A
<i>Pherbellia griseola</i>	4	3	1	15	1	A
<i>Pherbellia nana</i>	10	10	0	20	1	A
<i>Pherbellia schoenherri</i>	56	44	12	55	2	C
<i>Pherbellia ventralis</i>	603	550	53	95	NA	NA
<i>Pherbina coryleti</i>	49	22	27	70	4	A
<i>Pteromicra angustipennis</i>	27	20	7	55	1	A
<i>Pteromicra pectarosa</i>	9	6	3	20	1	A
<i>Renocera pallida</i>	165	25	140	75	1	H
<i>Renocera striata</i>	7	7	0	15	1	H
<i>Sciomyza dryomyzina</i>	18	18	0	10	1	C
<i>Sciomyza testacea</i>	31	31	0	20	NA	C
<i>Sepedon sphega</i>	2	0	2	5	2	G
<i>Sepedon spinipes</i>	11	8	3	15	2	G
<i>Tetanocera arrogans</i>	176	91	85	90	1	C
<i>Tetanocera elata</i>	22	11	11	40	1	F
<i>Tetanocera ferruginea</i>	130	14	116	75	1	G
<i>Tetanocera freyi</i>	14	12	2	15	NA	NA
<i>Tetanocera fuscinervis</i>	28	22	6	45	1	A
<i>Tetanocera hyalipennis</i>	23	6	17	35	1	A
<i>Tetanocera punctifrons</i>	1	0	1	5	NA	NA
<i>Tetanocera robusta</i>	64	7	57	50	5	G

### **\*Voltinism**

Based on Berg 1982

- 1 Multivoltine OW as pupae
- 2 Multivoltine OW as adults
- 3 Univoltine OW within the egg membrane
- 4 Univoltine OW as partly grown larvae
- 5 Univoltine OW as pupae

### **\*\*Behaviour**

Based on Knutson and Vala 2011

- A. Predators/Saprophages of non-operculate freshwater snails stranded by fluctuating water levels or whilst foraging/migrating.
- B. Parasitoids/Predators associated with non-operculate freshwater snails estivating or otherwise exposed for prolonged periods in temporary freshwater habitats.
- C. Parasitoids/Predators associated with hygrophilous semi-terrestrial Succineidae snails.
- D. Obligate parasitoids/predators of exposed egg masses of freshwater Lymnaeidae or *Aplexa* or semi-terrestrial Succineidae snails during early life stages, followed by predation on juvenile to mature snails in damp or vernal situations.
- E. Predators/Saprophages opportunistic on both terrestrial snails and slugs
- F. Obligate predators/parasitoids of slugs.
- G. Predators of non-operculate snails at or just below the water's surface, just above the water on emergent vegetation, and occasionally on those exposed on moist surfaces
- H. Predators/Parasitoids of fingernail clams.

### Appendix 3: List of Syrphidae species captured during this investigation

	Overall Abundance	Abundance <i>Carex</i> wet grassland	Abundance <i>Juncus</i> wet grassland	Percentage frequency of capture (per 20 traps)
<i>Anasimyia contracta</i>	60	44	16	45
<i>Anasimyia lineata</i>	11	8	3	30
<i>Baccha elongata</i>	4	2	2	20
<i>Cheilosia albitarsis</i> [gp] ♀	20	3	17	30
<i>Cheilosia pagana</i>	10	2	8	30
<i>Cheilosia</i> spp.	8	2	6	25
<i>Chrysotoxum bicinctum</i>	41	7	34	55
<i>Chrysotoxum festivum</i>	1	1	0	5
<i>Dasysyrphus albobstriatus</i>	3	1	2	15
<i>Epistrope eligans</i>	2	2	0	5
<i>Episyrphus balteatus</i>	172	73	99	100
<i>Eristralis arbustorum</i>	6	5	1	15
<i>Eristralis horticola</i>	3	2	1	10
<i>Eristralis intricarius</i>	2	0	2	10
<i>Eristralis pertinax</i>	16	5	11	40
<i>Eristralis sepulcharis</i>	2	2	0	10
<i>Eristralis tenax</i>	20	8	12	55
<i>Eupeodes</i> ♂ [group]	2	0	2	5
<i>Eupeodes corollae</i>	12	6	6	45
<i>Eupeodes latifasciatus</i>	263	174	89	100
<i>Eupeodes luniger</i>	24	10	14	40
<i>Helophilus pendulus</i>	179	69	110	100
<i>Helophilus hybridus</i>	4	2	2	20
<i>Leucozona lucorum</i>	5	2	3	20
<i>Lejogaster metallina</i>	6	6	0	15
<i>Melanostoma</i> ♀ [melanic]	1	0	1	5
<i>Melanostoma mellinum</i>	576	283	293	100
<i>Melanostoma scalare</i>	204	92	112	100
<i>Melangyna lasiophthalma</i>	2	0	2	10
<i>Meliscaeva auricollis</i>	11	4	7	35
<i>Neoascia genticulata</i>	1	0	1	5
<i>Neoascia meticulosa</i>	3	0	3	15
<i>Neoascia tenur</i>	40	10	30	70
<i>Neoascia obliqua</i>	1	0	1	5

	Overall Abundance	Abundance <i>Carex</i> wet grassland	Abundance <i>Juncus</i> wet grassland	Percentage frequency of capture (per 20 traps)
<i>Neoascia podagrica</i>	19	4	15	40
<i>Platycheirus [scutatus]</i> ♀	99	34	65	95
<i>Platycheirus [melanic]</i> ♀	8	3	5	35
<i>Platycheirus albimanus</i>	487	208	279	100
<i>Platycheirus angustatus</i>	18	9	9	40
<i>Platycheirus clypeatus</i>	457	352	105	100
<i>Platycheirus fuliventris</i>	9	8	1	30
<i>Platycheirus granditarsus</i>	4685	1054	3631	100
<i>Platycheirus immarginatus</i>	1	1	0	5
<i>Platycheirus manicatus</i>	19	12	7	50
<i>Platycheirus occultus</i>	15	12	3	30
<i>Platycheirus perpallidus</i>	10	9	1	25
<i>Platycheirus nielsenii</i>	1	0	1	5
<i>Platycheirus ramsarensis</i>	3	3	0	10
<i>Platycheirus rosarum</i>	1276	262	1014	95
<i>Platycheirus scambus</i>	1	1	0	5
<i>Platycheirus scutatus</i> ♂	12	7	5	35
<i>Rhinga campestris</i>	262	126	136	100
<i>Scaeva pyrastris</i>	6	5	1	20
<i>Scaeva selentica</i>	2	0	2	10
<i>Sericomyia silentis</i>	10	4	6	35
<i>Sphaerophoria</i> ♀	181	67	114	65
<i>Sphaerophoria interrupta</i>	64	25	39	40
<i>Sphaerophoria loewi</i>	1	1	0	5
<i>Sphaerophoria scripta</i>	1	1	0	5
<i>Syrpitta pipiens</i>	15	3	12	30
<i>Syrphus ribesii</i>	94	53	41	85
<i>Syrphus torvus</i>	9	3	6	25
<i>Syrphus vitripennis</i>	25	14	11	50
<i>Trichopsomyia flavitarsis</i>	34	17	17	40
<i>Tropidia scita</i>	7	2	5	25
<i>Volucella bombylans</i>	11	1	10	30
<i>Volucella pellucens</i>	1	0	1	5
<i>Xylota sylvarum</i>	1	1	0	5
<i>Xylota segnis</i>	2	2	0	10

**Appendix 4: A first record of *Dorylomorpha anderssoni* Albrecht, 1979; A species of Big-headed fly (Diptera: Pipunculidae) new to Ireland.**

A record published in the Irish Naturalists Journal Vol. 35, 128-129, 2017

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Keywords: Diptera, Pipunculidae, wet grassland.

During the sampling of wet grasslands in the West of Ireland (North Co. Galway/South Co. Mayo), a species of Diptera: Pipunculidae not listed in the Irish Fauna (Chandler et al., 2008) was discovered. As part of a biodiversity study funded by the Irish Research Council, invertebrates were being collected using black Malaise traps of Townes design (Townes, 1972) with samples taken from the traps every two weeks for 18 weeks. A number of Diptera families were removed from the catches and identified; this included individuals belonging to the family Pipunculidae.

Pipunculidae are a group of small to large (2-12mm) dark flies whose most striking characteristic is their spherical heads which are almost entirely occupied by large compound eyes (Oosterbroek, 2007). The larvae are generally parasitoids of leafhoppers and planthoppers (Hemiptera: Auchenorrhyncha), with one genus (*Nephrocerus*) having been reared from adult Tipulidae (Koenig and Young, 2007). The parasitoid nature of Pipunculidae means that they can be easily overlooked as they occur at low densities and (Coe, 1966) there is a paucity of information regarding their distribution in Ireland.

The Irish fauna currently consists of 31 species (Chandler et al., 2008), ten of which were added by with Chandler *et al.*, (2002) . The genus *Dorylomorpha* has seven species recorded in Ireland (two having been added by \*Chandler *et al.*, 2002); *confusa* (Verrall, 1901), *extricata*\* (Collin, 1937), *infirmata* (Collin, 1937), *littoralis* (Becker, 1898), *maculata* (Walker, 1834), *sylvatica* (Meigen, 1824) and \**xanthopus* (Thomson,

1869). In this record we add the species *Dorylomorpha anderssoni* Albrecht, 1979, to that list from a single specimen captured in Galway in July 2014.

The genus identification was made using Coe (1966) and the species was initially identified by JC using test keys derived from Albrecht (1990). The species was verified by David Gibbs and the voucher specimen has been presented to the National Museum of Ireland.

*Dorylomorpha anderssoni* Albrecht, 1979

Galway: Lough Hackett, M303496

10 July 2014 – 24 July 2014. One ♀ caught in a black Townes design Malaise trap located in species-rich wet grassland (GS4) (Fossitt, 2000) dominated by *Carex* species, and in close proximity to stands of *Filipendula ulmaria* (L.) Maxim.

Lough Hackett is a mesotrophic lake (FL4) of ~36 hectares with a wide variety of surrounding land use ranging from improved grassland (GA1) on its north and eastern shores, to rich fen (PF1) and wet grassland (GS4) mosaic on its southern shores. The specimen was captured in an area of plant species-rich wet grassland (GS4) in the north east corner of the Lough characterised by *Carex* species. This area is privately owned by Lisdonagh House and primarily used for extensive horse grazing. Lough Hackett and its environs are not protected under and European or National conservation legislation.

This is the first record of *Dorylomorpha anderssoni* in Ireland and brings the national fauna of Pipunculidae to 32. Gibbs (2010) notes that in the United Kingdom, *D. anderssoni* is a local but not uncommon species which is associated with *Carex* swamps. This habitat preference conforms to our finding of the species in a *Carex* rich wet grassland.

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- Gibbs, D., 2010. David Gibbs Entomology and Natural History [WWW Document]. URL <http://davidgibbs.webs.com/pipunculidae.htm>
- Koenig, D., Young, C., 2007. First observation of parasitic relations between big-headed flies, *Nephrocerus zetterstedt* (Diptera: Pipunculidae) and crane flies, *tipula linnaeus* (Diptera: Tipulidae *Tipulinae*), with larval and puparial descriptions for the genus *Nephrocerus*. *Proc. Entomol. Soc. Washingt.* 109, 52–65.
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## Appendix 5: Simplified character keys utilized in parataxonomic identification

### Dolichopodidae

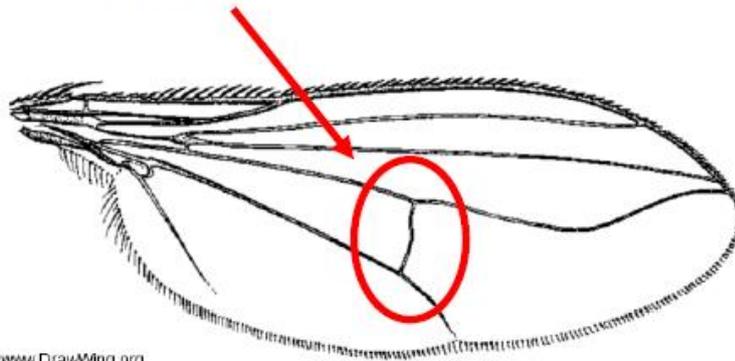


Metallic bluish/bronze or green

Head often elongate in profile

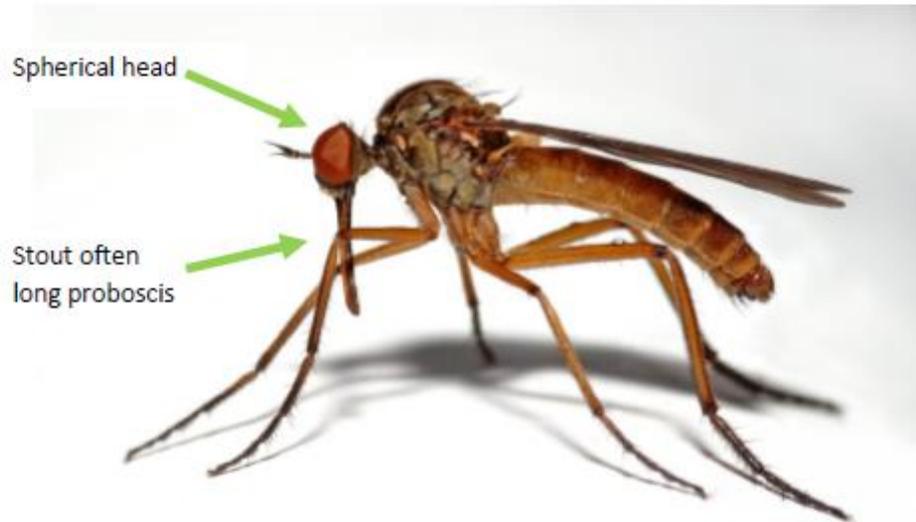


Only one cross-vein which is in middle third of wing

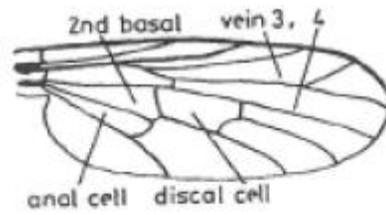


www.DrawWing.org

## Empididae

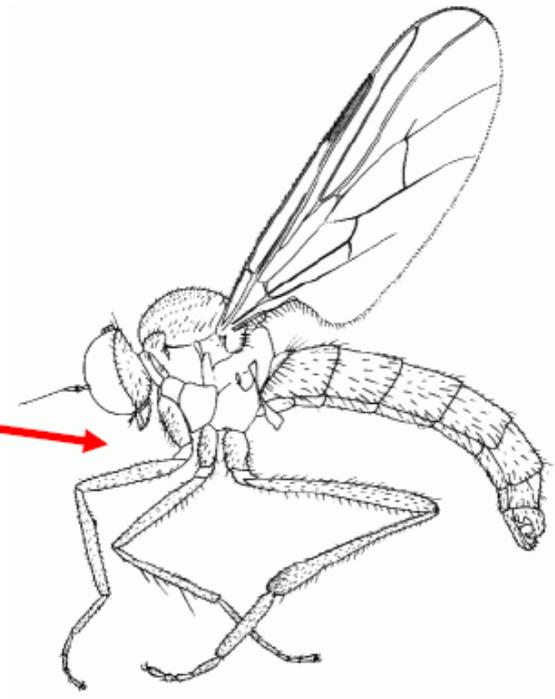


### *Dance-flies.*

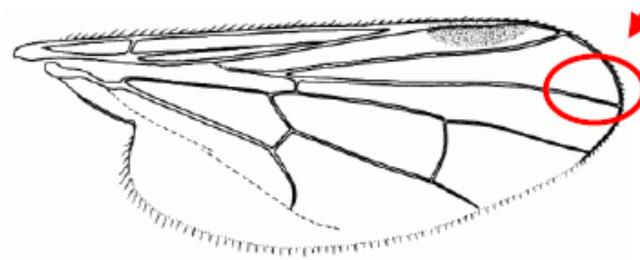


**Hybotidae**  
**(Typical)**

No long proboscis

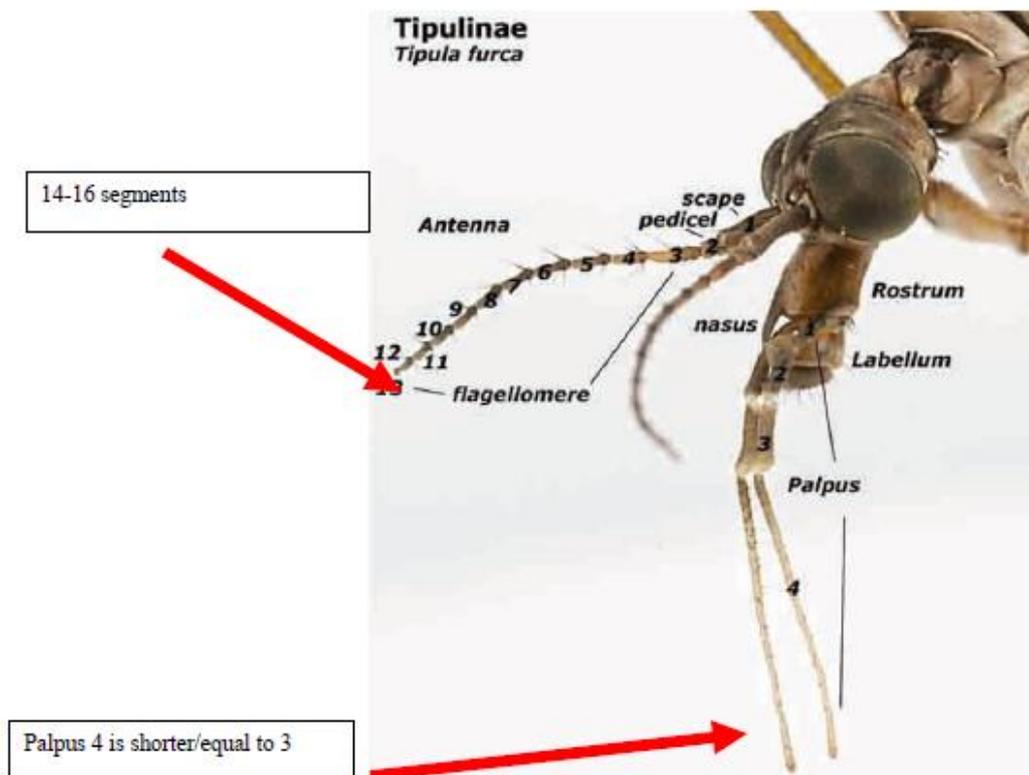


Not forked



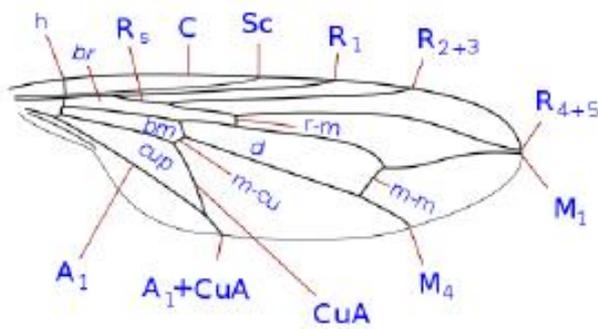
## Limoniidae vs Tipulidae

1. Terminal (fourth) segment of maxillary palpus short or subequal to third segment (elongate, longer than first three segments combined in Tipulidae)
2. antennae usually with 14 or 16 segments (13 in Tipulidae)



## Pipunculidae

Enormous head consisting almost entirely of compound eyes



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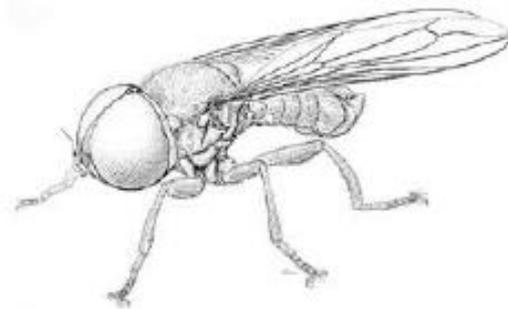


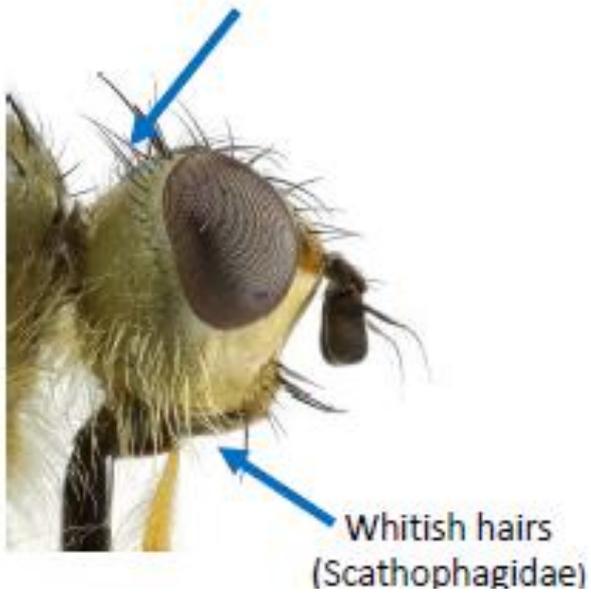
Figure 47. Family PIPUNCULIDAE

## Scathophagidae



Head rounded at the back  
(Scathophagidae)

Head flattened the back  
(NOT Scathophagidae)



## Stratiomyidae

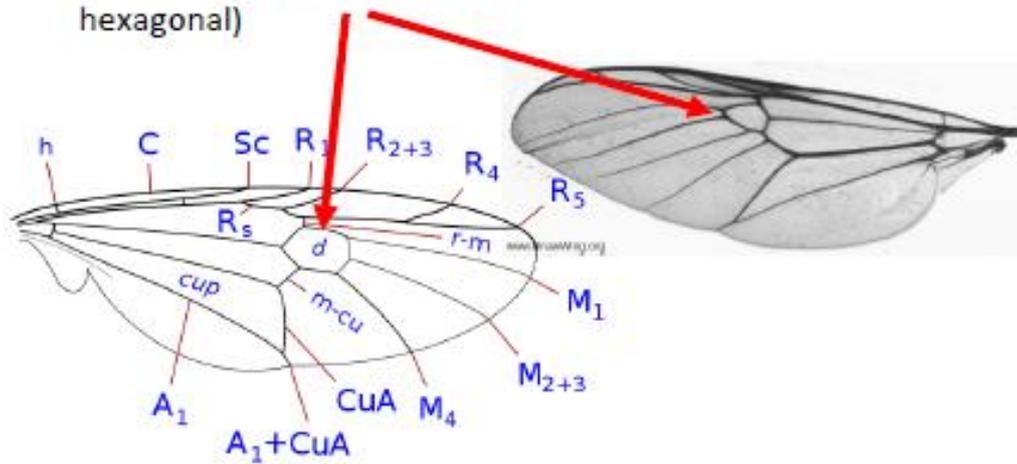


Wide variety of colours-

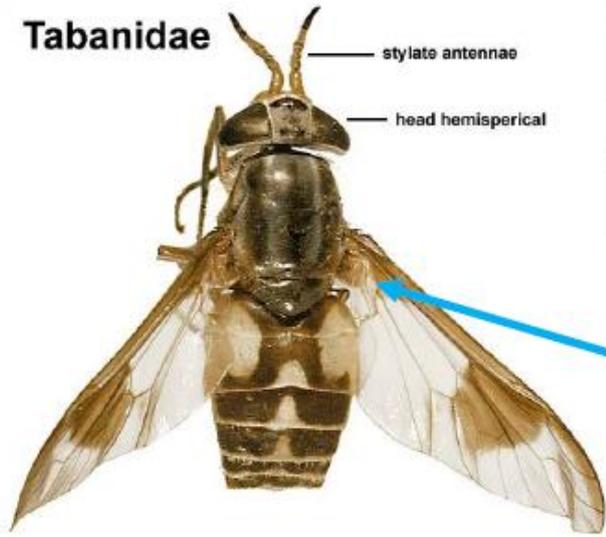
Metallic/Black/Striped

Wings folded back over the body-

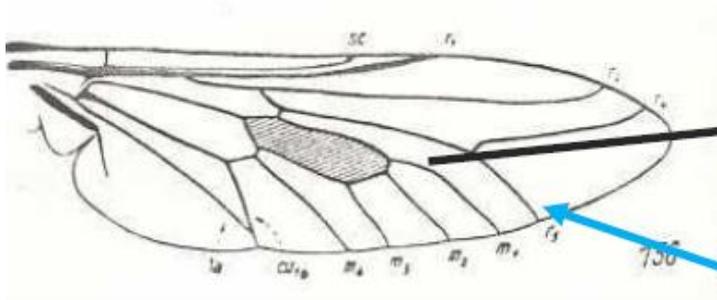
Discal cell **Diagnostic** (often hexagonal)



# Tabanidae

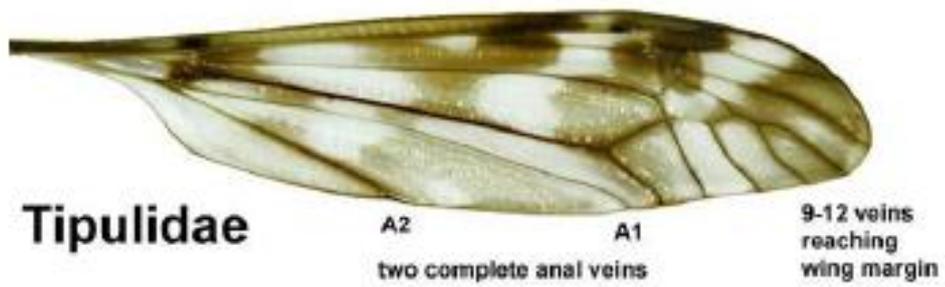
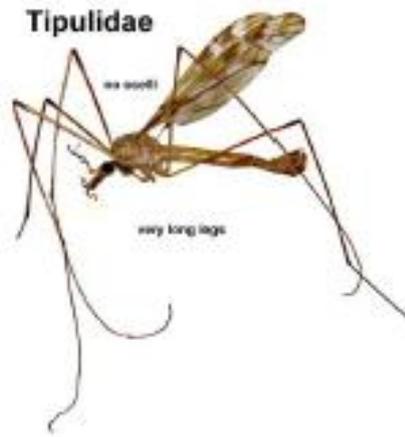


No Bristles



Wing  
Apex

Lower branch  
of fork  
**BELOW** apex



Ocelli present (NOT tipulidae)



Ocelli absent (tipulidae)



