1 Indicators of Diptera diversity in wet grassland habitats are influenced by environmental variability, 2 scale of observation, and habitat type. 3 4 John G.J. Carey^a*, Shane Brien^a, Christopher D. Williams^b and Michael J. Gormally^a ^a Applied Ecology Unit, School of Natural Sciences, National University of Ireland Galway, Galway, 5 6 Ireland. 7 ^b School of Natural Sciences and Psychology, Liverpool John Moores University, Liverpool, United 8 Kingdom. 9 *Author for correspondence (jgjcarey@gmail.com) 00353 91 492719 10 **Highlights** 11 Fine spatial scale analysis is required to investigate dipteran diversity in 12 13 agri-ecosystems The effectiveness of dipteran surrogate indicators is determined by wet grassland 14 15 category 16 The scale at which invertebrate diversity is assessed on farmland needs further 17 investigation Marsh fly and hoverfly communities are highly congruent with nine other dipteran 18 19 families 20 21 1. Abstract In low intensity agri-ecosystems such as wet grassland habitats, the inclusion of invertebrates in 22 23 conservation assessments and monitoring is usually limited to charismatic groups such as bees or 24 butterflies. However, wet grasslands support a wide range of inveterate groups, some of which may 25 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 26 suggested as a possible means of including these overlooked invertebrates (such as Diptera) in 27 28 conservation planning within these habitats. Based on collections made by Malaise trap, we utilized 29 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 30

31 habitats. We examined the role of environmental variability, spatial scale and habitat type on patterns

32 of cross-taxon congruence for all three assemblages. Both environmental correlation and community

33 congruence were significantly stronger among assemblages when examined at low spatial scales; 34 highlighting the need to examine dipteran groups at scales untypical of current agri-environmental 35 assessments; namely field and farm level. Furthermore, when wet grasslands were differentiated into 36 two habitat categories (Sedge and Rush dominated grasslands), the significance of the community 37 congruence increased markedly. This correlation was particularly strong between Sciomyzidae and 38 PUs which demonstrated similar differentiation based on habitat type-implying that assemblages 39 which exhibit comparable ecological partitioning are more likely to be useful surrogates of one 40 another. Correlations between richness, abundance and Shannon's diversity were highly variable among groups, suggesting compositional analysis as the most appropriate examination of dipteran 41 42 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 43 44 examination of assemblages differentiated into appropriate habitat categories. The results illustrate the 45 need to investigate invertebrate biodiversity surrogates at scales appropriate to the indicator groups 46 and examine congruence among assemblages within specific habitat categories. Such an approach has 47 the potential to maximise gamma diversity in areas where wet grasslands are under threat of intensification or abandonment. 48

49 Keywords: Biodiversity; Diptera; Sciomyzidae; Syrphidae; Parataxonomy; Community Congruence

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51 **2.** Introduction

52 European wet grassland habitats are typically low-intensity agricultural systems with semi-natural 53 habitats which support a rich mosaic of plant and animal communities (Bignal, E.M. & McCracken, 54 1996; Bignal and McCracken, 2000; Billeter et al., 2008). While much of the conservation of lowland 55 wet grassland is driven by botanical or ornithological interests, wet grasslands also sustain a high 56 diversity of invertebrates (Drake, 1998; Hayes et al., 2015; Joyce and Wade, 1998; Maher et al., 57 2014). However, the difficulties associated with collecting comprehensive invertebrate data from 58 habitats have contributed towards the continued limited inclusion of invertebrate groups in broad scale 59 conservation planning and for monitoring conservation objectives (Cardoso et al., 2011). This is 60 particularly relevant in a European agricultural context, where intensification and abandonment of traditional farming practices in areas such as wet grasslands is threatening biodiversity (Henle et al., 61 62 2008). The use of invertebrates in the designation and management of agricultural areas considered as being of a high nature value is usually limited (if included at all) to a few well known, easily 63 64 identifiable, and often iconic groups such as butterflies or bees (Andersen et al., 2004). Other 65 invertebrate groups such as Diptera are largely overlooked despite contributing significantly to the 66 overall biodiversity of such habitats (Keiper et al., 2002).

67 While the need to include a wider suite of invertebrates in conservation strategies for wet grasslands 68 makes ecological sense, the sheer abundance and diversity of groups such as Diptera are perceived as 69 barriers to their inclusion in routine habitat assessments. Alternative approaches such as the use of 70 selected invertebrate groups as biodiversity surrogates for a broader range of taxa has been suggested 71 as a possible means of including invertebrates in conservation and monitoring programs (Anderson et 72 al., 2011; Duelli et al., 1999; Duelli and Obrist, 2003; Hayes et al., 2015). Such biodiversity indicators 73 generally include a well-studied taxon or group of taxa which are ubiquitous within the habitat of 74 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 75 similar, or sometimes different, taxa. One such approach has been the examination of species richness 76 77 congruence between indicator groups and the taxa they are deemed to represent (Duelli and Obrist, 78 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 79 80 investigation and it provides little insight into overall species representation and composition (Su et 81 al., 2004).

82 More recently, patterns of congruence derived from community similarity and/or the examination of 83 similarity of community responses to environmental variability have been utilized as approaches to 84 biodiversity surrogacy (Larsen et al., 2012; Paszkowski and Tonn, 2000; Rooney and Azeria, 2015; 85 Rooney and Bayley, 2012; Su et al., 2004). A potential caveat with this method, however, is the effect 86 of spatial scale of observation and habitat differentiation on congruence patterns. Invertebrate 87 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 88 89 changes across small scales that can have a marked effect on community structures (Cole et al., 2010). 90 In wet grassland habitats, this may be further exacerbated by temporal changes such as periodic 91 inundation in combination with grazing patterns (Carey et al., 2017; Maher et al., 2014; Ryder et al., 92 2005). Thus, the examination of invertebrate communities may need to be assessed at spatial scales 93 untypical of those employed in conventional biodiversity assessments which are often linked to 94 agri-environmental scheme evaluations at farm or field-level scales.

95 The differentiation of habitats into categorical groups has also been shown to affect congruence 96 patterns with anthropogenic disturbance and ecoregion having a noticeable influence on congruence 97 measures (Ekroos et al., 2013; Myšák and Horsák, 2014; Rooney and Azeria, 2015; Rooney and 98 Bayley, 2012). The selection of invertebrate biodiversity surrogates, therefore, needs to consider carefully determinants such as the distribution of the indicator taxa relative to the scale of the 99 100 observation, response of the indicator to ecological variance, and possible ecological relationships 101 between the indicator and the wider community it is chosen to represent (McGeoch, 1998; Paoletti, 102 1999).

103 In wet grassland habitats, adult Marshflies (Diptera: Sciomyzidae) are considered as potential 104 bioindicators owing to their ubiquity and ease of capture (Carey and LeRoy et al., 2015; Knutson and 105 Vala, 2011). However, they are known to have highly localised habitat fidelity and exhibit a markedly 106 limited movement (Williams et al., 2010); factors which may restrict their usefulness as surrogates for 107 broader dipteran diversity if the scale of observation utilized is greater than that which accurately 108 reflects their distribution. In contrast to this, adult Hoverflies (Diptera: Syrphidae) are considered 109 suitable bioindicators in agricultural systems (Burgio and Sommaggio, 2007) but are vagile in nature 110 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 111 112 response to factors such as spatial scale and habitat differentiation relative to the broader invertebrate 113 diversity for which they are selected to be a proxy

114 Along with these considerations, the identification of multiple and diverse groups such as Diptera to 115 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 116 attempting to rapidly identify several groups of invertebrates using less traditional taxonomic methods 117 118 (Cardoso et al., 2011). Rapid biodiversity assessment techniques such as parataxonomy 119 (morphospecies) as described by Oliver et al. (1993) utilizes an approach whereby individuals with 120 similar external morphological traits are grouped together as typological units or Parataxonomic Units 121 (PUs) without the use of taxonomic keys. This work can be carried out by individuals with minimal 122 taxonomic training and possibly even through public participation initiatives such as citizen science 123 (Casanovas et al., 2014). Though the method is subject to debate regarding its effectiveness (Thorsten-Krell, 2004; Ward and Stanley, 2004), when executed with caution, and subject to some 124 125 level of taxonomic verification, it can be utilized to give ecologically relevant outcomes (Cotes et al., 126 2009; Obrist and Duelli, 2010; Oliver and Beattie, 1996; Ward and Stanley, 2004). Studies of Diptera in wet grasslands which have utilized the two approaches (taxonomic and parataxonomic) have 127 generally focused on richness correlations of all Diptera and not examined community similarity 128 129 (Hayes et al., 2015; Ryder et al., 2005). Though useful patterns can be derived from such data, 130 especially in terms of family richness and abundances, a more in-depth investigation of a smaller 131 number of dipteran families using more prolonged sampling and intensive sorting methods might be more insightful (Frouz, 1999). 132

Given that Sciomyzidae and Syrphidae fulfil the criteria for suitable bioindicators as outlined by McGeogh (1998), we compared measures of their diversity with a broader assemblage of nine Diptera families identified using parataxonomy. By examining agreement among groups in terms of environmental responses we were able to identify the role that environmental factors play in determining community structure of different dipteran assemblages. We 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. We 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.

143 Our principal objectives were to:

- Examine patterns of environmental correlation between the groups at two spatial scales (Trap
 level and Patch level)
- 146 2. Determine whether cross-taxon congruence among groups was affected by scale of147 observation
- 148 3. Investigate the role that habitat type plays in contributing to patterns of cross-assemblage149 congruence

150 The results of this investigation are discussed in the context of selecting suitable invertebrate151 biodiversity indicators within high nature value agri-ecosystems such as wet grasslands.

3. Materials and Methods

153 **2.1 Study area**

154 This investigation was undertaken in the west of Ireland in wet grassland habitats defined according to 155 Fossitt (2000). For inclusion in this classification, grass, rush or small sedge cover needs to exceed 156 50% and broadleaf herbs, reeds and larger sedges should not dominate i.e. must be <50%. The 157 broadleaf herb component should also be relatively evenly divided between drier grassland and 158 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 159 grasslands dominated by sedges (Family Cyperaceae). All wet grassland sites in this study were 160 161 actively managed for livestock grazing and were not subject to intensive cutting regimes or 162 application of fertilizers.

163 **2.2 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 20m 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 as a result 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 170 positioned in a southerly direction and were collected every 14 days. A portable electric fence was 171 operated to protect the traps from interference by livestock, and vegetation within the enclosure was 172 intermittently shortened and removed to maintain trap efficacy and replicate conditions outside the 173 fenced area. Eleven families of adult Diptera were selected for analysis based on their ease of 174 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 the 175 176 Dolichopodidae, Empididae, Hybotidae, Limoniidae, Pipunculidae, Scathophagidae, Sciomyzidae Stratiomyidae, Syrphidae, Tabanidae, and Tipulidae. 177

178 Sciomyzidae and Syrphidae were utilized as the principal biodiversity indicators and identified to species level using Rozkošný (1987) and Vala (1989) for sciomyzids and Ball & Morris (2013) and 179 Stubbs & Falk (2002) for syrphids. The remaining nine families were identified using parataxonomy, 180 181 a rapid biodiversity assessment method. Non-specialist individuals (undergraduate students hereafter 182 referred to as parataxonomists) utilized a simplified character key derived from Oosterbroek (2007) 183 and Unwin (1981) to assist them in the removal of the remaining nine families from bulk samples. Subsequent to initial sorting to family level, the specimens were categorised into parataxonomic units 184 185 (PUs) based on their external morphological features without the use of keys (Oliver and Beattie, 186 1996, 1993). Each newly assigned PU was digitally photographed and the image inserted into a shared 187 data base allowing each parataxonomist access to the image for referral. The initial voucher specimen 188 was preserved in 100% ethanol for determination to species level by taxonomists. These voucher 189 specimens were utilized to determine the level of 'splitting' whereby a taxonomic species was split 190 into two or more PUs. Oliver and Beattie (1996) 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 191 192 more taxonomic species are classed within the same PU. Our study opted to exclude this verification 193 as it would have entailed the identification of over 3000 individual specimens by taxonomists. Instead, only the original voucher specimens were identified by specialists and, therefore, only species 194 splitting and not lumping was accounted for. Omitting the 'lumping' protocol due to time constraints 195 196 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 197 effectively maximised taxonomic input by minimising effort; an important consideration due to the 198 199 increasingly limited availability of taxonomic specialists (Cardoso et al., 2011) and requirement for 200 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 utilized 201 202 for analysis. The percentage of splitting error for each PU family was reported according to Oliver 203 and Beattie (1996).

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2.3 Environmental data

205 A series of environmental variables was recorded at each site throughout the collection period and 206 mean values calculated. These included vegetation height as well as the percentage cover of grasses, 207 sedges, rushes, moss, moribund material, broadleaf herbs and bare ground. Each of the variables was 208 estimated using five 50 x 50 cm quadrats placed randomly within 10m of each Malaise trap location 209 and within the homogeneous vegetation patch. Soil samples were extracted from the same random 210 sampling areas and mean pH, soil carbon content, and soil moisture were analysed (Anon, 1990). The 211 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. 212

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2.4 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 was 220 undertaken using the Sørensen distance in PC-Ord v.6 (McCune and Mefford, 2011). Samples which 221 222 were identified as extreme outliers with standard deviations >3.0 using the Sørensen distance measure were removed prior to NMS ordination. We utilized 250 runs of real data to 250 runs with randomised 223 224 data to determine the number of significant axes. An orthogonal principal axis output was selected for 225 each NMS to illustrate maximum community variation along axis 1. Environmental data were utilized as a second explanatory matrix and variables with Pearson r^2 values >0.2 overlain as a bi-plot 226 227 (McCune and Mefford, 2011). Multi-Response Permutation Procedures (MRPP), which are 228 non-parametric procedures for the testing the hypothesis of no difference between two groups, were 229 utilized to examine for significant differentiation between habitat types based on the species/PU 230 composition of each assemblage (McCune and Mefford, 2011).

231 A Principal Components Analysis (PCA) with a cross products matrix based on correlation was utilized to evaluate the environmental data. MRPP was also utilized to examine habitat differentiation 232 233 based on PCA results. The PC1 score obtained from this analysis was used to rotate the NMS 234 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 235 236 space and allows for comparison between assemblages (McCune and Mefford, 2011; Rooney and 237 Bayley, 2012). Subsequent to rotation, we examined the response of the three assemblages to 238 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 239

responses were considered at trap scale (each individual Malaise trap [n=20]) and patch scale (where data from pair-wise 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 pair-wise traps was congruent between assemblages. For each assemblage, we also tested the relationship between the level of community similarity between pair-wise 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 utilized as a measure of the diversity of each assemblage (Ellison, 2010; Jost, 2007), along with raw abundance values. We utilized 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).

261 **5. Results**

262 **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).

A total of 105 parataxonomic units 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 1). Dolichopodidae (Long-legged flies) represented the most abundant family (45,337) with Stratiomyidae (Soldierflies) 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.

- 274 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.
- 277 Table 1: Number of individuals, species, and Parataxonomic units (PUs) per Diptera family. The
- 278 percentage splitting error refers to the proportion of splitting within each family whereby a species
- 279 was classified as more than one PU by parataxonomists.

Family (Individuals)	Number of species	Number of PUs	% splitting error		
Dolichopodidae (45337)	15	17	13		
Limoniidae (13796)	14	15	7		
Empididae (11987)	15	18	20		
Scathophagidae (8933)	7	14	100		
Hybotidae (6098)	12	13	8		
Pipunculidae (3129)	4	4	0		
Tabanidae (2820)	3	4	33		
Tipulidae (1338)	5	8	60		
Stratiomyidae (685)	10	12	20		
Overall	85	105	24		

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281 **5.2.** Response to environmental variability

NMS ordinations of samples in species/PU-space produced three dimensional solutions which 282 explained >80% of the variation for each assemblage (Sciomyzidae 86.0%, Syrphidae 84.3%, PUs 283 80.4%). Environmental variables with a Pearson r^2 score of >0.2 are shown as bi-plots (Figure 1). 284 Stress levels for each ordination were all <11.5 with values of ~10 are considered suitable for 285 reasonable interpretation (McCune and Mefford, 2011). Multi-response permutation-procedure 286 287 (MRPP) analysis showed significant differences between the two habitat types for all three 288 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 289 290 then Syrphidae (A=0.030, P=0.027).



Figure 1: NMS ordinations of samples in species/PU space. Environmental variables with r² values 292 >0.2 are shown as bi-plots. The principal orthogonal axes are shown with the percentage of variation 293 associated with each axis. Samples are shown as open squares (rush dominated wet grassland) and 294 295 open triangles (sedge dominated wet grassland), species/PUs as black triangles. a) Sciomyzidae, b) Syrphidae and c) and PUs. Only axes 1 & 2 are illustrated which explain a cumulative variation of: 296 Sciomyzidae (71.6%), Syrphidae (69.9%); PUs (65.5%). Habitat types were significantly different 297 using MRPP analysis for all three groups **a**) A=0.062, $P = 6 \times 10^{-4}$ **b**) A=0.030, P = 0.02700 **c**) 298 A=0.080, P=3 x 10⁻⁵.0 299

300 Principal components analysis of environmental variables showed that PC1 explained 35.4% of the variance between samples (Figure 2). MRPP analysis confirmed that habitats were significantly 301 different from one another based on environmental data (A=0.249, P= 2×10^{-5}). The scores from PC1 302 were utilized to rotate the NMS ordinations for examination of cross-assemblage agreement based on 303 304 environmental correlations. All three assemblages showed significant correlations based on NMS axis one irrespective of spatial scale of observation (Table 2). At trap scale (n=20), Sciomyzidae and 305 306 Syrphidae showed significant correlations across all axes. This was reduced to the first two axes at 307 patch scale. PUs showed significant correlations with the other groups only on axis one. It is worth 308 noting that the negative correlation between Syrphidae and PUs is likely an artefact of the rotation of 309 the NMS using only PC1 scores (Rooney and Bayley, 2012).



Figure 2: 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

314	MRPP analysis (A=0.249, P=0.00002). Arrows with no associated environmental variable were
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327 Table 2: Environmental correlations (Spearman's r) between dipteran assemblages based on the axes

	Axis 1			Axis 2			Axis 3			
	Sciomyzidae Syrphidae		PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	
Trap scale (n=20)										
Sciomyzidae	1			1			1			
Syrphidae	0.73**	1		0.65*	1		0.73**	1		
PUs	0.98***	0.70**	1	-0.01	-0.53	1	-0.38	-0.07	1	
Patch scale (n-10)	_									
Sciomyzidae	1			1			1			
Syrphidae	0.85***	1		0.79**	1		0.07	1		
PUs	0.76**	0.73**	1	-0.49	-0.80**	1	-0.13	-0.05	1	
328 of	the NMS ordin	nations rotate	d by PCA	score. (P<0.05*	*, P<0.01**, 1	P<0.001**	**)			
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330	5.3. Commu	nity similar	ity betwe	en pair-wise tra	aps					

331 Sciomyzidae and PUs showed a significant relationship in terms of how their assemblages responded to differentiation between pair-wise traps i.e. increased dissimilarity between pair-wise traps was 332 congruent among both assemblages (r^2 0.84, P=0.002) (Figure 3). None of the measured 333 334 environmental variables were significantly correlated with changes in community similarity of Sciomyzids or PUs between pair-wise traps i.e. none of the measured variables appeared to 335 336 significantly affect differences in community structure between traps from the same patch. Syrphidae 337 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 pair-wise traps. However, differentiation of 338 Syrphidae between pair-wise traps was significantly correlated with differences in the percentage 339 cover of broadleaf herbs between pair-wise traps (Spearman's r 0.82, P=0.004); a relationship that 340 341 was not significant for Sciomyzidae or PUs.

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Figure 3: 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) ($r^2 - 0.23$, P=0.470) and **c**) Syrphidae and Sciomyzidae ($r^2 - 0.23$, P=0.470) were not significantly correlated.

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5.4. Cross-assemblage congruence

350 Mantel R values were weak but significant between Sciomyzidae and PUs across all samples but only at trap level (Table 3). This relationship was maintained even when geographic and environmental 351 352 autocorrelation was controlled for. There was also a significant relationship between Syrphidae and 353 PUs at trap scale but this was not maintained when either geographic or environmental autocorrelation 354 was controlled for. There were no significant correlations between any of the three assemblages at 355 patch level. When correlations among assemblages were examined within habitat types, Sciomyzidae 356 and PUs were significantly congruent within both habitats (Table 4) even when geographic and 357 environmental autocorrelation were controlled for. Sciomyzidae were also significantly correlated 358 with Syrphidae in both habitats, however, in the sedge habitats this was a result of geographic and/or 359 environmental co-response i.e. trap proximity or similarity of environmental variables at the trap

360	locations appeared to influence congruence. Syrphidae were congruent with PUs in Rush dominated
361	wet grasslands but not in Sedge dominated habitats.
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370	Table 3: 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

372 (geographic) and environmental variability. (P<0.05*, P<0.01**, P<0.001***)

Controlled effect		None		Geo	ographical		Environmental				
	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs		
Trap scale (n=20)											
Sciomyzidae	1			1			1				
Syrphidae	0.08	1		0.03	1		0.06	1			
PUs	0.44**	0.20*	1	0.41**	0.13	1	0.44**	0.15	1		
Patch scale (n-10)											
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		
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385	Table 4: Mantel R statistics of assemblage concordance within habitats using the Sørensen distance
386	measure for species/PU data and the Euclidean distance measure to control for location and

387 environmental variability. (P<0.05*, P<0.01**, P<0.001***)
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Controlled effect	None			Geographical			Environmental			
	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	
Sedge grasslands (n=10)										
Sciomyzidae	1			1			1			
Syrphidae	0.27*	1		0.15	1		0.20	1		
PUs	0.71**	0.04	1	0.68**	0.13	1	0.71**	0.02	1	
Rush grasslands (n=10)										
Sciomyzidae	1			1			1			
Syrphidae	0.35*	1		0.24*	1		0.36*	1		
PUs	0.72**	0.51**	1	0.67**	0.42**	1	0.75**	0.47*	1	

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398 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 5). 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).

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424 Table 5: 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 Habitat	s (n=10)		Rush Habitats (n=10)			
Richness	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	
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			/			/			/			/	
Abundance	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	
Sciomyzidae	/	0.002	0.543*	/	0.018	0.045	/	-0.067	0.382	/	0.249	0.079	
Syrphidae		/	0.310		/	0.503		/	0.552		/	0.746*	
PUs			/			/			/			/	
Shannon's H' (Entropy)	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	
Sciomyzidae	/	-0.381	-0.307	/	-0.467	-0.285	/	-0.164	-0.309	/	-0.321	-0.539	
Syrphidae		/	0.448*		/	0.587		/	0.224		/	-0.018	
PUs			/			/			/			/	

427 **6.** Discussion

428 Community congruence between dipteran groups examined at low spatial scales and within district 429 habitat types provided the most useful predictors of selected dipteran diversity. Assemblages which showed similar responses to environmental variables and exhibited shared community differentiation 430 431 based on habitat type were most significantly correlated. Diptera which display limited movement 432 (Sciomyzidae) were more indicative of changes in wider dipteran community structure (represented 433 by PUs) than those which are generally more mobile in nature (Syrphidae). The results highlight the 434 variation that occurs between groups when a range of statistical expressions and scales of observation 435 are utilized 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 436 437 (Hughes et al., 2000).

438 Assessments of invertebrate cross-taxon taxon congruence in agricultural systems may be limited in 439 spatial resolution so that patterns can be examined at scales such as field or farm level which 440 coordinate with administrative requirements e.g. agri-environmental payments are allocated at such 441 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 442 443 Diptera may occur at more restricted scales. Sciomyzidae, for example, have been shown to exhibit 444 habitat fidelity across scales as low as 23m (Williams et al., 2010), and Hughes et al. (2008) 445 determined high levels of dipteran community dissimilarity (0.52 Bray-Curtis) between two Malaise 446 traps sited 25m 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 452 453 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 454 congruence in terms of environmental correlates, the strongest relationship was seen between 455 456 Sciomyzidae and PUs at trap level. Based on the NMS output, Sciomyzidae and PU assemblages were 457 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 458 environmental factors (% Rush, %Sedge, % Moss, %Forbs, % Moribund). It is unsurprising that 459

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 on the other hand are known to respond to factors such as soil moisture, hydroperiod and vegetation type (Maher et al., 2014; Williams et al., 2009b) and exhibit very limited movement within wet grassland habitats (Williams et al., 2010). 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.

467 The strong correlation between PU and Sciomyzidae assemblages in terms of environmental 468 correlates at trap level not only illustrates that PUs are influenced by comparable environmental 469 factors as Sciomyzidae, but that they also respond at similar scales. Fine scale habitat features are 470 known to affect arthropods in grassland systems, especially more sedentary groups (Cole et al., 2010). 471 Within wet grassland habitats, variations in hydrological regime and grazing patterns are known to 472 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 473 474 scales e.g. between pair-wise traps 20m apart, could therefore have a more marked effect on groups 475 such as Sciomyzidae which are characterised as having limited movement. This response to small 476 scale features could be a result of their reduced ability to respond to disturbances or stressors, or 477 alternatively, it may be an indication of the availability of a specific resource located at finer scales. 478 Factors that influence dispersal of Diptera adults include larval development, sexual behaviour and 479 food requirements (Delettre et al., 1998). Analysis of Syrphidae community differentiation between 480 pair-wise traps within each site was not congruent with Sciomyzidae or PUs but was significantly 481 influenced by changes in the percentage cover of broadleaf herbs. As Syrphidae adults are largely 482 dependent of pollen and nectar as food sources (Sutherland et al., 2001) and patches of increased 483 broadleaf herb cover within sites most likely attracted Syrphidae adults, this is not surprising. 484 Sciomyzidae adults, on the other hand, can be restricted to very small areas characterised by dominant 485 vegetation type (Williams et al., 2010) and their distribution may potentially be influenced by the 486 availability of larval food sources i.e. aquatic and semi-aquatic molluses which often have very clustered distributions (Knutson and Vala, 2011). Sciomyzidae community differentiation between 487 488 pair-wise traps was anticipated in our results but interestingly was not correlated with any of the 489 measured environmental variables examined in this study. However, the significant correlation 490 between Sciomyzidae and PU community structure between pair-wise traps strongly suggests that the 491 factors contributing towards community differentiation across small spatial scales for these two 492 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 pair-wise 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 500 501 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 502 503 Bayley, 2012). In the present study, all three assemblages showed significant community 504 differentiation between the two habitat types, and community congruence between groups within 505 these two wet grassland habitats were markedly more significant than congruence from groups 506 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 507 508 comprised of a mixture of wetland and drier grassland herbs (Fossitt, 2000). However, dominant 509 vegetation type is known to significantly contribute towards dipteran community structure (Hughes et 510 al., 2008) and our results imply that assessments of dipteran diversity in wet grassland habitats should 511 consider sub-categories based on dominant vegetation type. Selecting a biodiversity surrogate requires 512 knowledge of the scale of distribution of the proposed surrogate within the habitat of interest and an 513 examination of its relationship with the predicted taxa below this scale (McGeoch, 1998). In the 514 present study, examining the wet grassland habitats as distinct habitat-types increased community congruence among the groups, in particular between Sciomyzidae and PUs which displayed similar 515 516 differentiation between the two wet grassland habitats. Strong community congruence between groups 517 that share similar responses to environmental variables and gradients such as habitat change has been 518 previously found among arthropods in grassland habitats (Oertli et al., 2005).

519 The examination of Diptera community congruence within these habitat categories at the lowest 520 spatial scale provided the most significant insight into relationships between potential biodiversity 521 surrogates (predictor taxa) and the wider assemblage as represented by PUs (predicted taxa). 522 However, while Sciomyzidae and Syrphidae were both useful surrogates of the PU assemblages in 523 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 524 525 surrogates in evaluations and monitoring rather than reliance on one group (Rooney and Bayley, 526 2012). The results also illustrate the importance of examining community congruence between taxa 527 based on distinct partitioning of communities.

528 Species richness is largely utilized as a measure of the success of conservation actions (Su et al., 529 2004) and is a relatively straightforward value to communicate to policy makers. However, changes in 530 the species richness of a target group may not be indicative of the response of a wider suite of 531 organisms, especially in agricultural systems (Billeter et al., 2008). Correlations between richness, 532 abundance, and Shannon's entropy in this study varied between groups as is generally reported in 533 studies investigating cross taxon congruence (Báldi, 2003; Hayes et al., 2015; Legakis et al., 2004). 534 Only three significant correlations were recorded using these measures, all of which were found when 535 the lowest spatial scale (trap level) was examined. Hayes et al. (2015) reported a significant 536 relationship between Sciomyzidae richness and overall Diptera PU richness in a comparative study of 537 wet grassland conducted at sampling scales similar to the trap level utilized 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 538 is interesting to note that Sciomyzidae abundance and PU abundance was significantly correlated 539 540 overall at trap level. In general, the richness and abundance of a group tends to be significantly 541 correlated so it could have been expected that if abundance between two groups was significant, then 542 the richness measures would also have been co-correlated. However, an underestimation of PU 543 richness as a result of the conservative allocation of PUs in this study i.e. lumping, may have 544 contributed towards a lack of significance between the richness of PUs and Sciomyzidae. Maher et al. 545 (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 546 547 were reflective of reduced flowering plant diversity due to increased hydroperiod which positively 548 affected Sciomyzidae but negatively affected Syrphidae.

549 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 utilized 550 551 within these correlations was abundance which was a raw value not subject to interpretation. It is 552 therefore interesting that the most significant correlation among these abundance measures was seen 553 between Syrphidae abundance and PU abundance in rush dominated habitats. This result suggests that 554 within such habitats, areas of rush dominated wet grasslands with high abundances of Hoverflies 555 could be important for maintaining high abundances of other Diptera; a pattern also reflected in the 556 community congruence of these two groups with this habitat type. However, the results also highlight 557 the variability of congruence when using richness, abundance and Shannon's entropy as measures of 558 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 559 560 grasslands interspersed with pockets of wet grassland habitat. Realistically, land managers may be 561 required to maintain a suite of wet grasslands whilst others are sacrificed to drainage and/or change of 562 use. Dipteran diversity provides one practical way of maximising landscape-level complementarity in 563 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 564 Sciomyzidae composition dissimilarities (if the sites were Carex or Juncus-dominated) or with 565 566 reference to Syrphidae composition (if the sites were Juncus-dominated).

567 **7.** Conclusion

The inclusion of overlooked groups such as Diptera in conservation strategies is unlikely to reach the 568 thresholds of more iconic invertebrate groups so the use of surrogate taxa is increasingly probable. If 569 such action is required, the surrogates selected for representation of wider dipteran diversity need to 570 reflect the distribution of Diptera at both habitat level and at smaller scales within those habitats. Our 571 results demonstrate that the use of community similarity at low spatial scales and within specific 572 habitat types appears to be the most useful surrogate of dipteran diversity in wet grasslands. However, 573 574 it may well be most beneficial for conservationists to adopt several invertebrate groups should a 575 surrogate approach be utilized to account for the range of dispersal and distribution patterns of wet 576 grassland invertebrates. While a more extensive study of invertebrates in high nature value farming 577 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 578 Syrphidae meet these requirements; though the former appear to represent the predicted taxa (PUs) 579 better in this study. In light of the increasing intensification and abandonment of wet grassland 580 581 habitats, the inclusion of easily captured, readily identified, and ubiquitous groups as seen in this 582 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 583 spatial scales rather than administrative units such as field or farm level in cross-taxon congruence 584 585 investigations may highlight patterns of correlation among invertebrate groups which are generally 586 overlooked.

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