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Sciomyzidae (Diptera) assemblages in constructed and natural wetlands: implications for constructed wetland design

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Abstract

Wetlands constructed primarily for the treatment of wastewaters have been shown to have a role in enhancing biodiversity. However, while most biodiversity studies of constructed wetlands focus on the larger, more iconic animal groups, there is a paucity of information on the aerial phases

of wetland invertebrate species associated with constructed wetlands. This study compares Sciomyzidae (Diptera) assemblages, established indicators of wetland dipteran communities, in Irish constructed and natural wetlands, in addition to determining the impacts of water quality and surrounding habitats on Sciomyzidae community structure. Natural wetlands had significantly greater species richness, abundances and diversity (measured as Shannon's entropy) of sciomyzid flies than constructed wetlands. Nevertheless, although concentrations of nitrogen and phosphorus in waters were significantly greater in constructed wetlands, seven of the eight constructed wetlands examined hosted species of Sciomyzidae listed as scarce or threatened in Britain. In addition, sciomyzid species richness increased as areas of semi-natural habitat immediately surrounding constructed and natural wetlands increased. Composition of Sciomyzidae assemblages in both natural and constructed wetlands were analysed. The results of this study demonstrate that constructed wetlands can be important contributors to biodiversity particularly in the context of current losses of natural wetlands worldwide. The importance of habitats immediately surrounding constructed wetlands also highlights the need for relatively simple design recommendations (e.g. wet grassland creation or judicious planting of wetland trees) that could enhance the biodiversity of existing and future constructed wetlands.

Key words:

Snail-killing flies, community structure, constructed wetlands, natural wetlands.

Introduction

Constructed wetlands (CWs) are artificial wetlands used to treat water pollution (Scholz, 2005) among other reasons, and with tens of thousands of CWs across the globe, they are rapidly gaining in popularity for the treatment of municipal, industrial (Vymazal 2011) and agricultural wastewaters (Healy et al., 2007). Advantages of CWs include the lower operation and maintenance costs than that of conventional wastewater treatment systems (Zhang et al. 2009). Not surprisingly, studies on the design of CWs are primarily based on their pollutant removal efficacies and the enhancement of their wastewater treatment capabilities (Kadlec and Wallace 2009). However, CWs also play a role in the provision of habitats for plants and animals (Knight 1997) and have been described by Greenway (2005) as multifunctional ecological systems which can assist in the restoration of aquatic flora and fauna. Nevertheless, the biodiversity of CWs has received relatively little attention to date. Those studies which address the biodiversity of CWs frequently focus on larger iconic groups such as birds (Hsu et al. 2011; Fleming-Singer and Horne 2006), mammals (Stahlschmidt et al. 2012) and amphibians (Schulse et al. 2010; Mulkeen et al. 2017). Despite invertebrates being recognised as essential components of wetlands and for their high diversity in wetland habitats (Wu et al. 2009), less is known about them in CWs. The exception to this are the aquatic phases of freshwater invertebrates which have long been used as biomonitors of water quality in CWs (Andersen and Vondracek 1999; Wallace et al. 1996; Jurado et al. 2009; Jurado et al. 2010; Spieles and Mitsch 2000; Streever et al. 1996). However, wetland environments offer a wide variety of niches for many other invertebrates (Kadlec & Wallace 2009) which are known to have significant ecosystem functions including acting as a food source for wildlife (de Szalay et al. 1997), influencing nutrient cycles (Wallace and Webster 1996), and assisting in the decomposition of litter (Murkin and Wrubleski 1988). Nevertheless, there is a paucity of knowledge regarding the aerial phases of wetland invertebrate species associated with CWs and consequently, the full biodiversity potential of CWs has yet to be revealed (Jurado et al. 2014).

Although true flies (Order Diptera) have been described as sensitive indicators of habitat change (Rivers-Moore and Samways 1996), they are often excluded from ecological studies of wetlands due to challenges associated with sampling and a requirement for specialist taxonomic expertise (Keiper et al. 2002). However, seventeen families of the Order Diptera are commonly associated with wetland habitats, with many of them achieving greatest abundances and species richness in a wetland environment (Keiper et al. 2002). Furthermore, sampling of the adult phases can provide additional data for the more terrestrial component of wetland insects which can then be used to monitor colonisation events (Keiper et al. 2002). One dipteran family in particular i.e. the Sciomyzidae (marsh / shade flies) which are predominantly wetland specialists, have been shown to be suitable bioindicators of wetland habitats (Carey et al. 2015; Carey et al. 2017a). Sciomyzid larvae are almost exclusively obligate natural enemies of molluscs (Knutson and Vala 2011), with adult flies tending to move infrequently within and between macrohabitats (Murphy et al. 2012). This is supported by Williams et al. (2010) who found that marked sciomyzid adults travelled a maximum of only 23 m in wet grasslands, thereby suggesting low levels of movement within habitats (Williams et al. 2010). More recently, Carey et al. (2017a), who compared dipteran families such as the Sciomyzidae which display limited movement in comparison to the more mobile Syrphidae, found Sciomyzidae to be more indicative of changes in wider dipteran community structure at small spatial scales. Given that some CWs are relatively small-scale (often less than 500 m²) and are either isolated or occur in urban landscapes, using Sciomyzidae for biodiversity studies is a logical choice given their microhabitat specificity and their potential as bioindicators of wider diversity of dipterans in wetland habitats. In addition, little information currently exists relating to water quality and the abundance / diversity of Sciomyzidae.

In this paper, we focus specifically on the aerial (adult) stage of Sciomyzidae through Malaise trap collections, though we supplement this with emergence trap data. Emergence traps are a definitive record of a breeding population at a particular site and have been used successfully in the past (Staunton et al. 2008) to collect new records for Britain and Ireland. It may appear preferable to sample larval or puparial Sciomyzidae, given that this is the stage that is most likely to be impacted by water quality metrics. However, there remains large taxonomic impediments to the identification of larvae and puparia. Knutson and Vala (2011) provide a global key to larvae and pupae, but this is limited to genera and one must look in many scattered publications for adequate keys (if even at all available) for specific identification. Also, given the rather sedentary nature of Sciomyzidae (Carey et al. 2017a; Williams et al. 2010), inferring larval micro-habitats from adult macro-habitats may be reasonable.

This study will, for the first time, compare the composition of Sciomyzidae (known bioindicators of dipteran communities of wetland habitats) of CWs with natural wetlands (NWs), in addition to determining the impacts (if any) of water quality on sciomyzid community structure. The influence of habitats surrounding both CWs and NWs on Sciomyzidae assemblages will also be quantified for the first time. The results of this study will be used to inform the future design and siting of CWs to enhance their value to biodiversity without impeding their primary function in wastewater treatment. This is particularly important in the context of CWs playing an increasingly important role in the provision of wetland ecosystem services (including biodiversity) given the decline of NWs worldwide (Zedler 2003).

Materials and Methods

Site descriptions

Eight CWs, built for the tertiary treatment of municipal wastewater, were selected in counties Mayo, Galway, Leitrim and Roscommon in the west of Ireland. Each CW consisted of a surface flow reed-bed treating municipal wastewater and had been constructed and operational since the 1990s. Eight NWs containing reed-beds (areas of Reed and Large Sedge Swamp (Fossitt 2000)) were selected for comparison. The NWs were located within 20 km of each CW and were selected on the basis of: (1) the presence of reed-beds; and (2) the proximity to the CWs, thereby reducing the influence of weather conditions on invertebrate catches. Locations of each of the CWs and NWs is shown in Fig. 1. Areas of all wetlands studied are shown in Table 1.

Invertebrate sampling

Sciomyzidae were sampled at all CWs and NWs using Malaise (black nylon Townes design; Townes 1972) and bottom-less emergence traps (designed to catch emerging adults based on Owen 1989). Malaise traps, which required firm ground to ensure stability, were positioned on the north-eastern edge of the reed beds (CW and NW) since the prevailing winds in Ireland are between the south and west. Emergence traps were positioned directly on the reed-beds of the CWs and NWs to capture emerging adult Sciomyzidae. Trap collection heads containing a 70% ethanol solution, faced in a south-westerly direction (Speight et al. 2000). Malaise traps were activated on 21st May 2014 with samples collected approximately every three weeks until 29th October 2014, and emergence traps were in place from April 2015 until October 2015 and samples collected monthly. Collections were removed to the laboratory and Sciomyzidae were identified to species level using Rozkošný (1987) and Vala (1989).

Habitat mapping

Between August and October 2015, habitats were mapped at all CWs and NWs. Similar to the habitat mapping methods used in Mulkeen et al. (2017), a colour orthoimage produced in 2012 and sourced from ArcGIS (Release Version 10.3; Environmental Systems Research Institute [ESRI], California, USA) was printed for each wetland at a scale of 1:2650. Orthoimages were printed with 20 m × 20 m grids (based on Smith et al. [2011] who recommend a minimum mappable polygon size of 400 m² for small scale field mapping) superimposed onto the image to assist with mapping habitats in the field. Habitats within 25 m of the malaise trap were documented to reflect current knowledge that Sciomyzidae exhibit extreme philopatry and limited movement as adults (Williams et al. 2010). All habitats were identified, described and classified according to a standard habitat classification scheme used in Ireland (Fossitt 2000). This classification scheme operates at three levels and comprises eleven broad habitat groups at Level 1 (e.g. Freshwater, Grassland and Marsh, Heath and dense bracken, Peatlands etc.); thirty habitat sub-groups at Level 2 (e.g. Lakes and ponds, Watercourses, Springs, Swamps, Improved grassland, Semi-natural grassland, Freshwater marsh etc.); and 117 individual habitats at Level 3 (e.g. Dystrophic lakes, Acid oligotrophic lakes, Dry calcareous and neutral grassland, Wet heath, Montane heath, Upland blanket bog etc.). Field survey recorded data and maps were created using ArcGIS 10.3 and the areas for each habitat calculated. As the overall total area for each wetland in the study varied, the wetlands are numbered consecutively from the largest to the smallest for each wetland type i.e. CW1 – CW8 and NW1 – NW8.

Water quality sampling and analysis

At CWs, a water sample was taken at the inflow and the outflow approximately every three weeks during the malaise trapping study. During the same period, a water sample was collected at the NWs in the littoral zone of the lake / wetland where a river or stream entered, and another water sample was collected in the littoral zone near the out-flowing river or stream. Water samples were taken at a similar depth and distance from the shore during each sampling occasion. All water samples were collected in acid-washed bottles, stored in a cooler box and transported to the laboratory for analysis. Water samples were tested for pH using a pH probe (WTW, Germany) and for suspended solids (SS) using vacuum filtration through Whatman GF/C (pore size 1.2 μm) filter paper. Subsamples were filtered through 0.45 μm filters and analysed for ammonium (NH_4), nitrate (NO_3), nitrite (NO_2) and ortho-phosphorus (PO_4) using a Konelab nutrient analyzer (Konelab 20, ThermoClinical LabSystems, Finland). Unfiltered samples were tested for total nitrogen (TN) and total phosphorus (TP) using a BioTector analyzer (BioTector Analytical Systems Ltd., Cork, Ireland), and for chemical oxygen demand (COD) and biological oxygen demand (BOD). All water quality parameters were tested in accordance with the standard methods (APHA 2005).

Statistical analysis

Univariate analysis was carried out on SPSS version 24.0. This included Pearson's correlations and Spearman Rank correlations, which were used to test whether there was a significant effect of habitat richness, semi-natural habitat richness or habitat Shannon's entropy on Sciomyzidae richness, abundance or Shannon's entropy. A linear regression was used to test whether there was any correlation of areas of reed-beds or semi-natural habitat with species richness of Sciomyzidae.

The residuals of Sciomyzidae abundance, species richness and Shannon's entropy were tested for homogeneity and variance, and normality by Levene's test for equality of variance and the Kolmogorov-Smirnov test, respectively. Following this, Sciomyzidae abundance, species richness and Shannon's entropy (raw data, not residuals) were tested for differences between CWs and NWs by the independent samples t-tests. Paired t-tests (by pairing sites based on geographical location) were not considered appropriate given the short distances that Sciomyzidae fly (Williams et al. 2010).

Multivariate statistical analyses were performed on the data to assess factors such as water quality and surrounding habitat richness on community composition using PC-Ord (version 6.0) (McCune and Mefford 1999; McCune and Grace 2002). Non-metric multidimensional scaling (NMS) ordinations of Sciomyzidae samples, in the primary matrix, with water quality and habitat variables in the secondary matrix, were undertaken using the Sørensen distance measure and a two-dimensional NMS solution was chosen based on stress curves. An Indicator Species Analysis (ISA) which relies on relative frequency and relative abundance (Dufrene and Legendre 1997) was used to assess the fidelity of Sciomyzidae species to a particular level of grouping variable, which was wetland type (i.e. CWs versus NWs). We also used the extension to Indicator Species

Analysis, Multilevel Pattern Analysis (De Cáceres et al. 2012). This identifies combinations of species as indicators. Multi-response permutation procedure (MRPP), a non-parametric test, was used to test whether there was any effect of wetland type on species composition.

We also used a model-based approach to analyse multivariate abundance data. A multivariate GLM with a poisson error family was fitted to the data on species abundance using R v.4.3.1 (R Core Team, 2023), RStudio v2023.6.1.524 (Posit team, 2023) and the 'mvabund' package (Wang *et al.*, 2022). All water quality and habitat measures were initially included as factors in the maximal model. The model was simplified in stepwise fashion to improve model fit (as indicated by AIC), and remove factors that were not significant predictors of species abundance or were highly collinear with other factors. The minimal adequate model included habitat type, H⁺ ion concentration (derived from pH), TN, SS, semi-natural habitat Shannon's entropy, and habitat richness as predictors. The minimal adequate model was assessed by analysis of deviance with 1000 bootstrap iterations, and likelihood ratio tests to determine statistical significance of each predictor.

Residuals of water quality variables were tested for normality (Kolmogorov-Smirnov test) and equality of variance (Levene's test). COD, BOD, SS, TN, NH₄ and PO₄ were found to be non-normal ($P < 0.05$) and therefore a Mann-Whitney U-test was used to test for significant differences between CWs and NWs on raw data, not residuals. pH and TP residuals were found to be normally distributed ($P > 0.05$) and of equal variance ($P > 0.05$), and so were subjected to an independent samples t-test.

Results

Over half the known Irish Sciomyzidae fauna (Chandler et al., 2008; Staunton et al., 2008; Gittings and Speight, 2010) i.e. thirty-two species (654 individuals) were captured in Malaise traps at CWs and NWs during the study. Over two-thirds of total abundances were captured at NWs (69%; 451 individuals), while 31% (203 individuals) of the total abundance was captured at CWs (Fig. 2a). Species richness was also greatest at NWs (29 species) in comparison to 23 species at CWs (Appendix 1). Twenty-eight percent of the total number of species captured (32) were found exclusively at NWs, 9% (3 species) were exclusive to CWs, while 63% (20 species) of species captured were common to both wetland types (Fig. 2b).

An ISA which assessed the fidelity of Sciomyzidae species to either CWs or NWs, revealed that no particular species was significantly faithful to either wetland type, despite some species being captured exclusively in CWs and in NWs. In contrast to this, Multilevel Pattern Analysis with 999 permutations, identified two significant indicators of NWs. They were: *Tetanocera ferruginea* 97.4% $P = 0.001$ and *T. freyi* 89.4% $P = 0.005$. Residuals of Sciomyzidae abundance, species richness and Shannon's entropy were all normally distributed and of equal variance as tested by Levene's test and the Kolmogorov-Smirnov test ($P > 0.05$ in each case). Independent samples t-tests revealed that Sciomyzidae species richness, abundance and Shannon's entropy were significantly greater in NWs than CWs. In all cases, the mean value at NWs was greater than that of CWs (Fig. 3). Species richness at CWs ranged from just two species at CW2 to fourteen species at CWs 4 and 5 (Fig. 4a). At NWs, species richness ranged from nine species at NW2 to twenty species at NW4 (Fig. 4a). The abundances of Sciomyzidae at CWs were lowest (3) at CW7, in

comparison to 93 individuals at CW4. Abundances at the NWs ranged from 16 at NW7 to 89 individuals captured at NW3. Shannon's entropy (Shannon-Wiener), a species diversity measure (Ellison 2010), was greatest at NW4 and lowest at CW2 (Fig. 4b).

The presence at CWs of species such as *Antichaeta analis* (Rare), *Tetanocera freyi* (Rare), *Sciomyza dryomyzina* (Vulnerable) and *Pherbellia griseola* (Notable) (Table 3) as classified in Britain by Falk (1991), suggest that CWs can act as important sites for the conservation of scarce and threatened flies. Seven of the eight CWs were found to contain one or more species from this list.

Non-metric multidimensional scaling (NMS) ordinations resulted in two significant axes (Fig. 5), one of which accounted for 41.2% of the variation (Axis 1) and the other accounting for 46.9% of the variation (Axis 2). Natural wetland sites were generally clustered together on the ordination with Sciomyzidae species plotting more towards the NWs due to greater abundances in NWs. Compositionally, the CWs were more dissimilar from each other than were the NWs, with community metrics of the Sciomyzidae (richness, total abundance and Shannon's entropy) more strongly correlated with the secondary axis of composition (i.e. NMS axis 2). The area of semi-natural habitats is negatively correlated with Axis 1, i.e. there is generally a greater area of semi-natural habitats surrounding NWs compared to CWs. Sciomyzidae total abundance tends to correlate well with NWs – the vector of this variable lying close to the cluster of NWs and away from the CWs.

Water quality variables, which were more strongly correlated with Axis 1, indicate that poorer water quality (i.e. greater levels of TN, TP, COD and BOD) was more linked to CWs than NWs. In all cases, water quality values for TN, NH₄, TP and PO₄-P were significantly ($P < 0.05$) greater (i.e. more polluted) in the CWs than in the NWs. Appendix 2 shows summary data for the water quality metrics.

An MRPP revealed that there was a significant, but weak, effect of wetland type (CW or NW) on species composition. Approximately 7% of the differences in species composition can be explained by differences in wetland type. This effect may have been stronger, were it not for the outlier CW4 on the ordination, which clusters closer to NWs rather than CWs (Fig. 5). For the model-based approach, the minimal adequate model fitted the data significantly better than the null model (LR = 831, $p < 0.001$), and analysis of deviance showed that species abundance was significantly affected by each of the factors in the model: habitat (LR=208.37, $p=0.006$) – see Fig 6, $[H^+]$ (LR=99.99, $p=0.033$), TN (LR=130.24, $p=0.004$), SS (LR=197.89, $p=0.001$), semi-natural habitat Shannon's entropy (LR=106.30, $p=0.002$), and habitat richness (LR=88.07, $p=0.003$). Due to the large number of statistical tests, the correction for multiple comparisons meant that an extremely large effect size was needed to show significant deviance for each species among explanatory variables. The p values for these significant deviances were as follows: *T. ferruginea* Habitat (CW versus NW) $p=0.008$, *R. pallida* suspended solids $p=0.001$ and semi-natural habitat Shannon's entropy $p=0.0024$, *T. freyi* suspended solids $p=0.026$, *E. cucularia* Habitat richness $p=0.025$.

Renocera pallida Fallén, 1820 was the most commonly captured species in CWs in Malaise traps, followed by *Tetanocera hyalipennis* Roser, 1840 and *Sciomyza dryomyzina* Zetterstedt, 1846 (Fig. 7). At NWs, *Tetanocera arrogans* Meigen, 1830 was most common, followed by *R. pallida* Fallén, 1820 and *Tetanocera ferruginea* Fallén, 1820 (Fig. 7). Not all emergence traps functioned effectively due to a weakness in the fabric of some of the traps resulting in tears occurring, which meant that it was not possible to determine whether species captured hatched from a pupa within the wetland under study. However, one individual of *Pherbellia dubia* Fallén 1820 was captured in an intact trap at CW4, and *Renocera*

pallida (1), *P. dubia* (1), *Pteromicra angustipennis* Staeger, 1845 (2) and *T. ferruginea* (1) were captured in intact traps at NW1, NW4 and NW6, respectively.

Notwithstanding the fundamental differences between the CWs and NWs, there is, nevertheless, considerable overlap in Sciomyzidae species composition (63%) between the two wetland types. The CWs were found to be much more variable than their NW counterparts in that some had low Sciomyzidae species richness (e.g. CWs 2 and 7) while others (CWs 4 and 5) had greater species richness than some NWs (NWs 2, 7 and 8). These NWs were found to contain some “peatland” and “heath and dense bracken” habitats, which are not known to support many Sciomyzidae species, possibly contributing to the lower species richness at these NWs. Areas surrounding NW8, for example, contained over 40% cover of these habitat types. On the other hand, NW4, which had the greatest species richness (20), was surrounded predominantly (97% cover) by “improved agricultural grassland with abundant *Juncus* spp.”; “wet willow-alder-ash woodland”; and “reed and large sedge swamp”. Natural wetlands 1, 3, 5 and 6, comprised between 14 and 17 Sciomyzidae species, and also comprised areas between 62% and 90% of semi-natural habitat with suitable wetland-type habitats for Sciomyzidae. These areas of semi-natural habitat are likely to account for the greater Sciomyzidae species richness at these NWs.

To investigate the influences of habitats mapped in the study (see Appendix 3 for a list of habitats and habitat maps for each wetland), Sciomyzidae total abundance, species richness, and Sciomyzidae Shannon’s entropy were correlated with habitat richness, semi-natural habitat richness and habitat Shannon’s entropy and semi-natural habitat Shannon’s entropy at CWs and NWs. There was no relationship between surrounding habitat richness /diversity and Sciomyzidae diversity, richness and total abundance at CWs and NWs (Table 2). A linear regression investigating the

effects of *Log* reed-bed area on *Log* Sciomyzidae species richness at CWs and NWs also revealed that there was no effect of area of reed-bed on Sciomyzidae species richness. However, a linear regression between *Log* area of semi-natural habitats within 25 m of the Malaise traps and *Log* Sciomyzidae species richness at CWs and NWs combined, revealed a significant ($P = 0.021$) relationship (Fig. 8).

Discussion

This study reveals, for the first time, that despite the major physical differences (particularly in size and water quality) between the NWs and CWs, a majority of Sciomyzidae species captured were common to both wetland types and a small number (3 species) were found in CWs only. While the results of this study indicate that Sciomyzidae species richness, abundance and diversity (Shannons's entropy) were significantly greater in NWs than in CWs, this appears to be dependent on the area of semi-natural habitat immediately surrounding the wetland i.e. the greater the area of surrounding semi-natural habitat, the greater the Sciomyzidae species richness. Given that the main focus of CWs is the treatment of urban wastewaters, domestic effluent or wastes from intensive farming practices, many CWs are frequently placed in urban or intensive agricultural landscapes where semi-natural habitat area is often diminished. In addition, CW sites have been found to frequently contain considerable areas (up to one fifth) of disturbed ground or artificial surfaces such as tarmac or concrete and driveways, often necessary for machinery access (Mulkeen et al. 2017). In spite of this, CWs appear to provide habitat for invertebrates such as Sciomyzidae that might otherwise be absent from the surrounding landscape and in this study harboured almost a third of the known Sciomyzidae fauna in Ireland. All four of the rare/threatened species found in CWs (*A. analis*, *T. freyi*, *S. dryomyzina* and *P. griseola*) have a requirement for wetland habitat (Falk 1991; Knutson and Vala 2011) and

three of the seven CWs (CW1, CW3 and CW8) in which they were found did not contain any wetland habitats in the areas surrounding the malaise traps apart from the CW reed-bed itself. The habitats immediately adjacent to the malaise traps at these three CWs could be described as non-wetland (*dry*) habitats and made up, on average, 67% of the surrounding habitats. These habitats included dry areas of “scrub”, “improved agricultural grasslands”, “earth banks”, “hedgerows”, “flower beds & borders”, “buildings & artificial surfaces”, “ornamental / non-native shrub”, “recolonising bare ground” and “dry meadows & grassy verges”. In CW7, the presence of an adjacent, fast flowing drainage ditch was unlikely to have contributed to Sciomyzidae catches, since marsh flies are associated primarily with lentic rather than lotic habitats (Knutson and Vala 2011). Nevertheless, despite CW7 being situated in an intensive agricultural grassland / village location, it still presented with three Sciomyzidae species (albeit in low numbers), one of which (*S. dryomyzina*) is classed as a vulnerable species in Britain by Falk (1991). This highlights the potential of CWs across the landscape to support scarce and threatened species. Given that recent research has also found that adult Sciomyzidae are strongly correlated with other dipteran assemblages (Carey et al. 2017a) and parataxonomic units of diptera (Hayes et al. 2015) in wetlands, CWs are likely to play an important role in the protection and conservation of other dipteran species.

While the ecologies and habitat requirements of some Sciomyzidae species are still unknown, 75% of the species captured across both NWs and CWs in this study are known to require water or wetland-type habitats. Of the twenty-three species captured in CWs, more than half are dependent on wetland habitat. Those CWs with the highest species richness were CWs 4 and 5, both with 14 species present. Of all CWs studied, these two CWs had the greatest percentage cover of surrounding wetland habitat (65% and 50% cover for CW4 and CW5, respectively). These wetland habitats included not only the CW reed-bed itself but also “improved agricultural grassland with abundant *Juncus* spp.” and “wet willow-alder-

ash woodland”. The additional presence of “depositing / lowland rivers” and “drainage ditches” both of which were fast flowing, was unlikely to have contributed significantly to the sciomyzid catch overall. However, fields with *Juncus* spp. “improved agricultural grassland with abundant *Juncus* spp.” are known to support Sciomyzidae species (Carey et al., 2017a) as are wet woodland habitats (“wet willow-alder-ash woodland”). It is likely that the greater diversity and larger area of these wetland habitats surrounding CWs 4 and 5, complemented the Sciomyzidae assemblages adding to the greater species richness at both CWs.

Of the remaining CWs i.e. CW2 and CW6, which had a species richness of two and six, respectively, surrounding wet habitats apart from the CW reed bed itself included “drainage ditches” and “canals” at CW2, and “drainage ditches” and “wet grassland” at CW6. Both CWs contained areas of 66% and 57%, respectively, of unsuitable adjacent habitats for Sciomyzidae. The higher species richness (6) and abundance (12) at CW6 in comparison to just four individuals of two species at CW2 may be a result of the additional area (12%) of “wet grassland” habitat adjacent to CW6. It appears that in an environment containing habitats which would otherwise be seen as unsuitable for Sciomyzidae, CWs themselves in the landscape can support Sciomyzidae assemblages. The addition of areas of wetland habitats such as “wet grasslands” adjacent to CWs, could further enhance Sciomyzidae and other dipteran communities. With the areas of reed-beds at six CWs making up between only 15% and 46% of adjacent habitats, in an environment which would otherwise be seen as unsuitable to support Sciomyzidae, it is rational to assume that the CW itself is supporting the Sciomyzidae communities in these areas.

In ten turloughs (temporary, ground-water-fed, Winter lakes) in the west of Ireland, Williams et al. (2009) and Williams et al. (2010) collected, with sweep-net surveys, between two and ten species of Sciomyzidae from a single vegetation zone (*Carex nigra*). Across twelve river floodplain

sites in the Shannon callows, Maher et al. (2014) collected, again with sweep-net surveys, twelve species of Sciomyzidae in the distal zone, 15 in the median zone and 20 in the proximal zone. In Malaise trap surveys from 20 traps deployed in rush (*Juncus* sp.) and sedge (*Carex* sp.) dominated wet grassland habitats, Carey et al. (2017b) collected 34 Sciomyzidae species. Given these results in the literature from Ireland, it appears as though NW collections in the present study were representative of other NWs on the island of Ireland.

The NMS ordination showed that area of semi-natural habitats surrounding CWs and NWs was correlated with compositional changes in Sciomyzidae associated with Axis 1 of the ordination, and this variable may be important in explaining compositional as well as Sciomyzidae species richness changes. However, the NMS ordination also showed that this axis was strongly correlated with poorer water quality (higher nutrient values). With such multicollinearity i.e. simultaneous changes in macro-habitat (areas of surrounding semi-natural habitats) and micro-habitat (water quality) variables, it is impossible to determine which is having the greater effect. Micro-habitat water quality variables are likely to affect larvae and mollusc host / prey communities, whereas macro-habitats are likely to affect the wider-dispersing adult stage. Williams et al. (2013) highlight the importance of water temperature, conductivity and pH to the larvae and puparia of *Colobaea* spp. The MRPP also confirmed that there was a significant, but weak, effect of wetland type on Sciomyzidae species composition. This effect may have been stronger were it not for CW4, which on the NMS ordination appears to cluster closer to the NWs due to high abundances and species richness at this particular site.

The emergence traps while providing limited data, do furnish direct evidence of sciomyzid flies emerging directly from within the wetlands. The single record of *P. dubia* at CW4 is definitive evidence of a CW supporting breeding populations of this species. Low numbers of emerging Sciomyzidae adults in the NWs suggests that single emergence traps in each wetland type may not have been sufficient to detect the full

complement of emerging species, though it should be noted that emergence traps in other NWs have given rise to new records to Britain and Ireland~~the British Isles~~ (Staunton et al. 2008), presumably by sampling species with a cryptic life-history. Given the relatively small size of the emergence traps, it is likely that multiple emergence traps would need to be deployed at individual sites in future studies.

In the current study, the main purpose of CWs (wastewater treatment) is also reflected in their poorer water quality in comparison to the NWs. At all CWs, water quality values for TN, NH₄, TP and PO₄-P were significantly ($P < 0.05$) greater (i.e. more polluted) than in the NWs. It is possible that these elevated water quality variables or pollution events were having either a direct negative effect on some Sciomyzidae larvae or pupae or else negatively affecting their hosts / prey (molluscs), which resulted in the significantly greater species richness, abundances and diversity at NWs. However, the presence of 23 species of Sciomyzidae at CWs, including those listed as scarce and threatened (Falk 1991), suggest that water quality is not a major issue for these species and further studies are required to clarify this. To the authors' knowledge, the only water quality analysis done in relation to Sciomyzidae communities may be found in Williams et al. (2013).. In the analysis of species deviances in the model-based approach, of the significant variables tested only suspended solids in two species (*R. pallida* and *T. freyi*) had a significant effect, whereas habitat type (CW versus NW), semi-natural habitat Shannon's entropy and habitat richness were significant for three species.

In the construction of new CWs, the size of the proposed site should be large enough to incorporate some areas of semi-natural habitats which would encourage Sciomyzidae and associated dipteran fauna. Without compromising the primary functions of wastewater treatment at CWs, artificial surfaces should be kept to a minimum. As proposed in Mulkeen et al. (2017), the creation of wet grassland habitat by extending the high-density polyethylene liner beneath the soil surrounding the CW, would be beneficial to Sciomyzidae fauna which are known bioindicators of wet

grassland habitats and reflect dipteran families such as, Dolichopodidae, Hybotidae, Limoniidae, Empididae, Pipunculidae, Scathophagidae, Stratiomyidae, Tabanidae, Tipulidae and Syrphidae, which are also present at wet grassland habitats (Carey 2018). In addition, the judicious planting of suitable wetland trees in these areas would benefit any species of Sciomyzidae associated with woodland-type habitats. As Sciomyzidae travel short distances (< 25 m), the creation of areas of semi-natural habitats, such as wetland-type habitats immediately adjacent to the CW or within 25 m, is advised. In order to support Sciomyzidae and other aerial invertebrates in new and existing CWs, the relocation (where possible) of “buildings and artificial surfaces” or bare ground away from the edges of the CW should be given due consideration to allow for wetland-type habitat creation. Clearly, situating CWs close to existing wetland habitats would enhance the biodiversity value of CWs although caution is advised as a CW should not be built on the site of an existing wetland with biodiversity value. However, the creation of suitable habitat linkages between CWs situated in urban / intensive agricultural grasslands and suitable wetland habitats is another option which is likely to enhance their biodiversity and is worthy of further exploration.

Conclusions

Constructed wetlands enhance biodiversity in the locations in which they are placed. The results of the present study show that NWs have significantly greater species richness, abundances and diversity of Sciomyzidae flies than CWs. However, although the N and P concentrations were significantly greater in CWs than in NWs, over one third of Irish species of Sciomyzidae was present at CWs. Moreover, seven of the eight

CWs hosted species of Sciomyzidae that are listed as “scarce” and “threatened” in Britain by Falk (1991). This conclusion is based only on Irish CWs, but there is no reason to believe that the general trends may not be applicable globally to CWs in general.

These results show that CWs are critical in providing a habitat to invertebrates such as Sciomyzidae flies, habitats that may be otherwise absent from the surrounding landscape in which CWs are commonly situated. However, Sciomyzidae species richness was shown to increase as the surrounding area of semi-natural habitat increased. Therefore, in the future design of CWs, the incorporation of areas of semi-natural habitats such as wet grasslands and wet woodland habitats immediately adjacent to the CWs is advised to enhance Sciomyzidae assemblages which are known bioindicators of dipteran communities in wetlands.

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We dedicate this paper to the memory of Dr Lloyd V. Knutson who sadly passed away on the tenth of January 2018. He was the doyen of Sciomyzidae biology, a friend, colleague and mentor. May he Rest in Peace

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Statements & Declarations

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Data Availability: Data available from corresponding author on reasonable request.

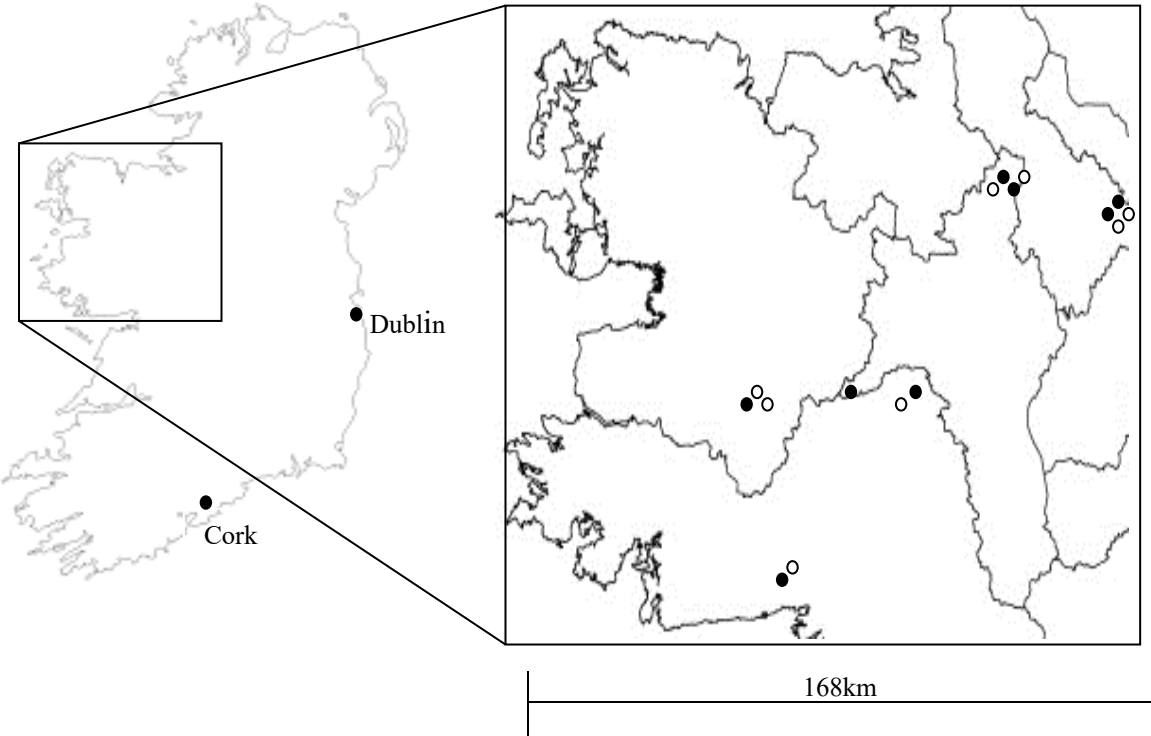


Fig. 1 Locations of constructed (●) and natural (○) wetlands in the west of Ireland

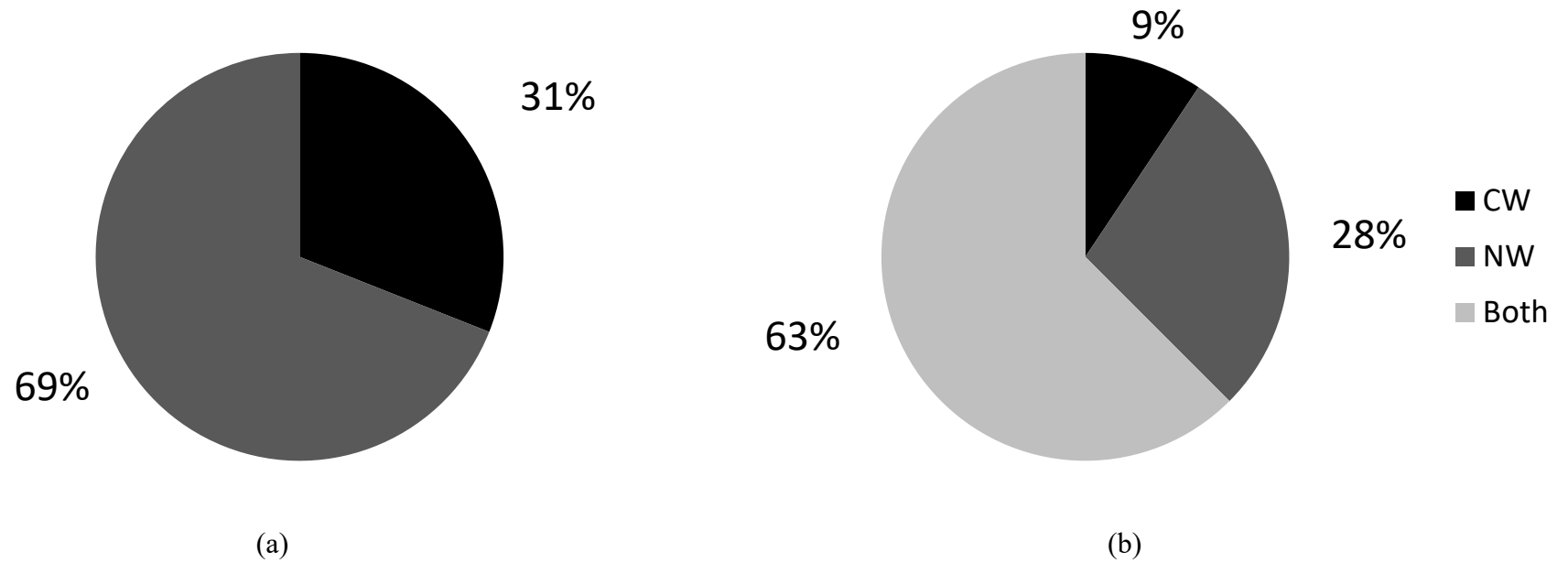


Fig. 2 (a) Percentage abundance of Sciomyzidae flies captured in Malaise traps at constructed and natural wetlands (n = 654) and (b) percentage of Sciomyzidae species captured at constructed, natural and both wetland types (n = 32)

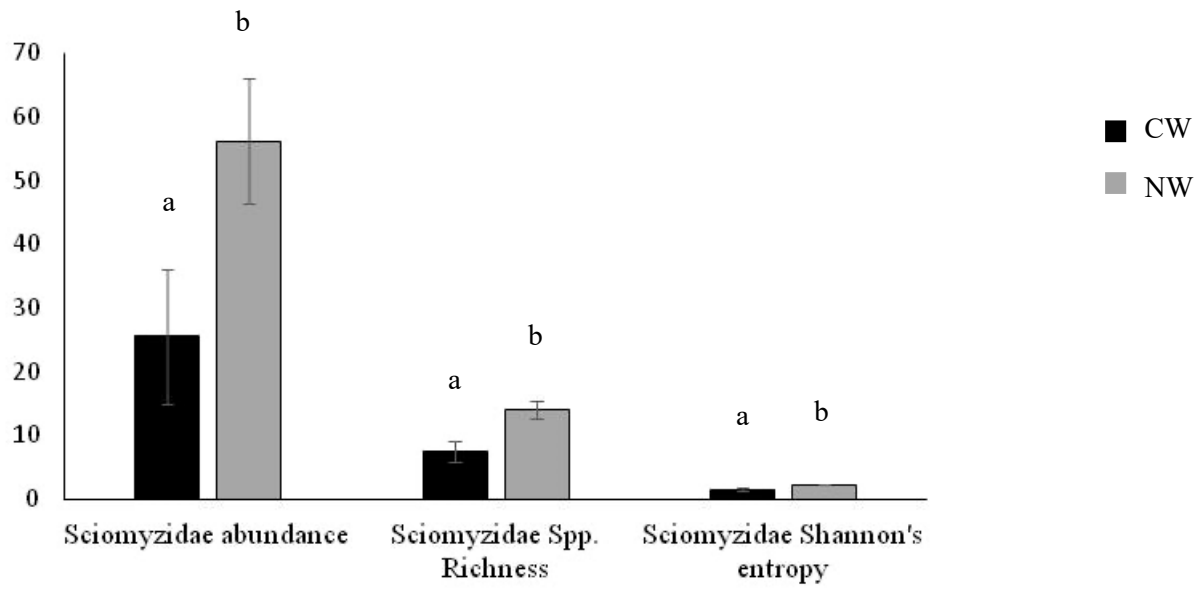


Fig. 3 Mean (\pm S.E.) Sciomyzidae abundance, species richness and Shannon's entropy at CWs and NWs. Different superscripts indicate significant differences ($P < 0.05$) between CWs and NWs for each category as tested by the Independent samples t-tests.

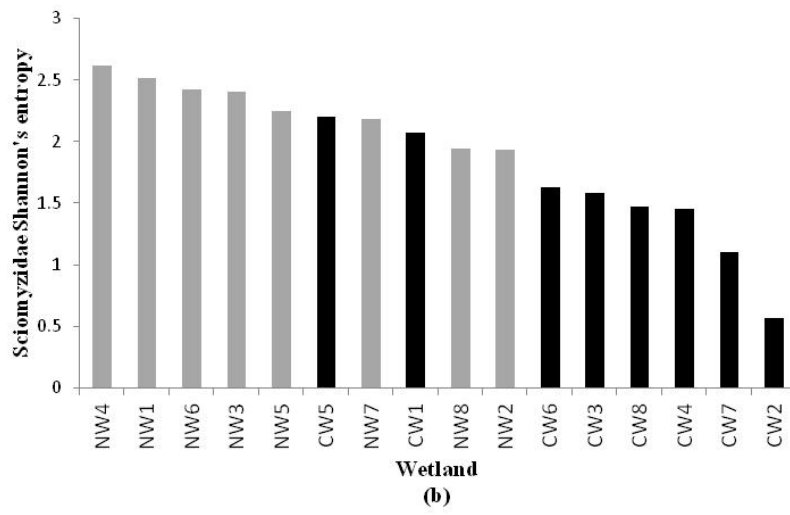
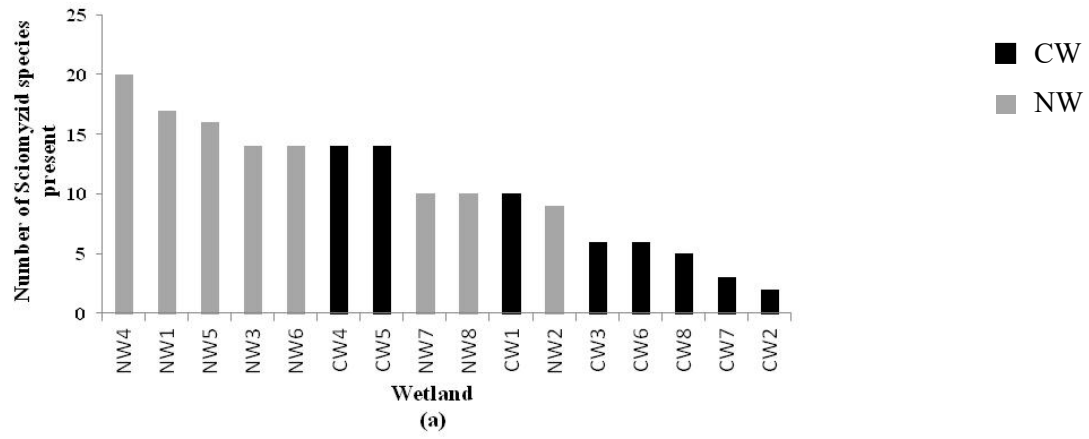
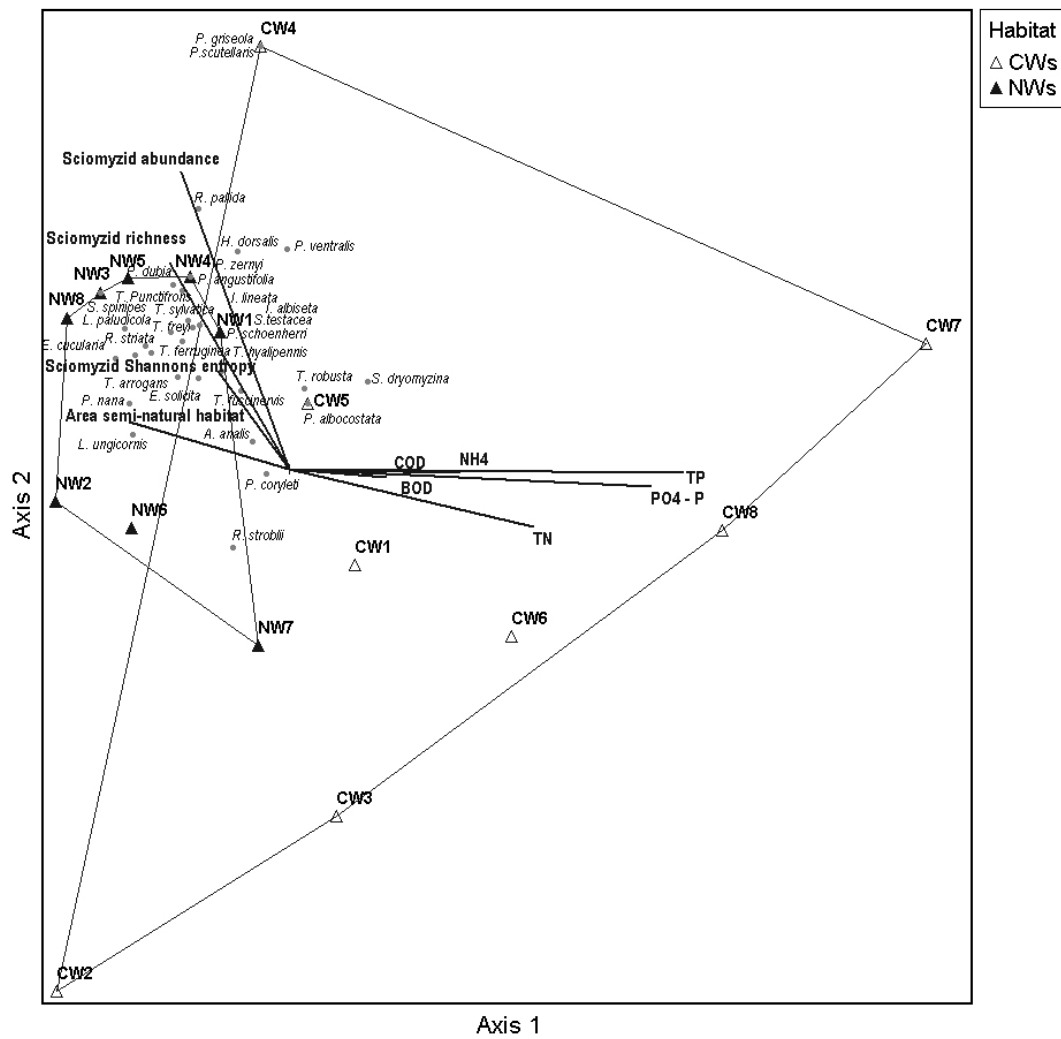
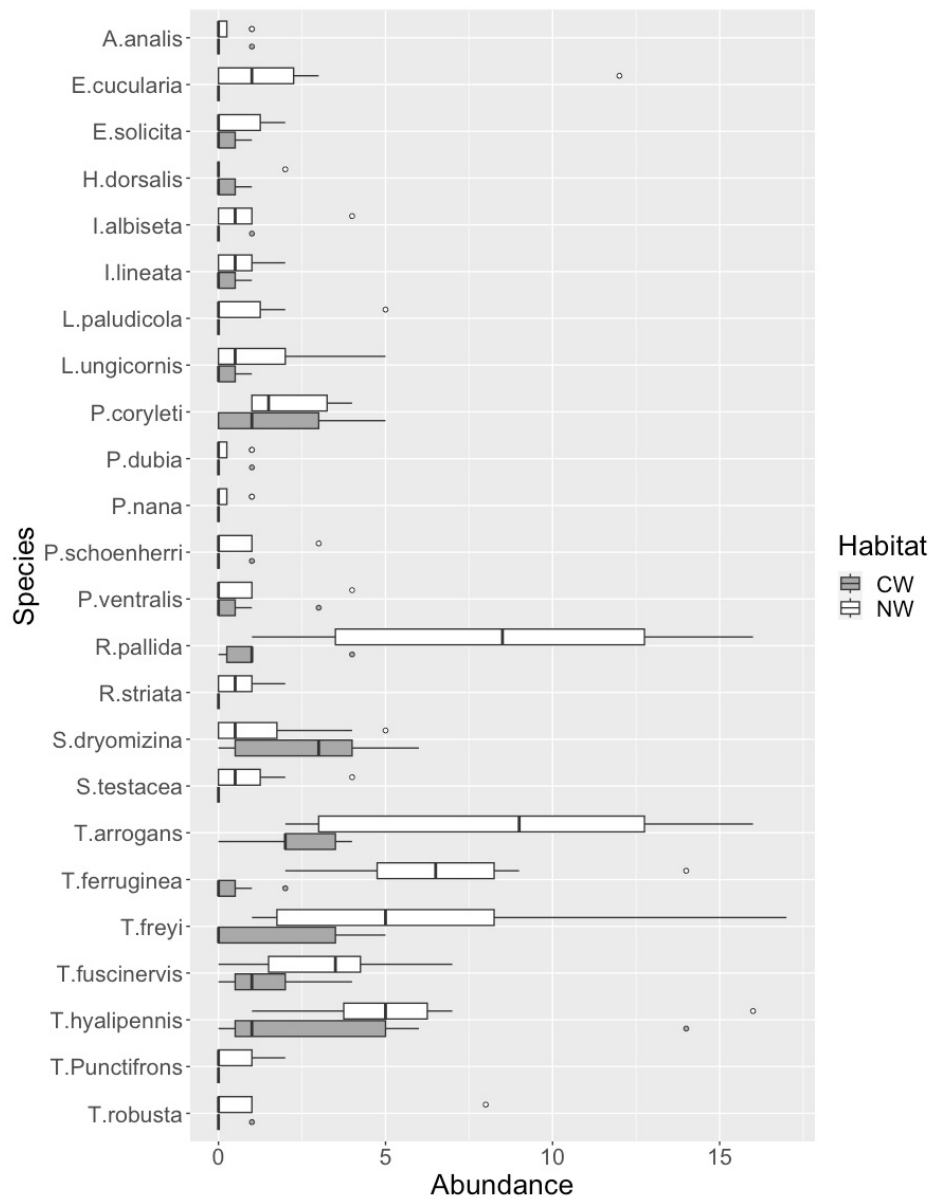


Fig. 4 (a) Sciomyzidae species richness; (b) Shannon's entropy at CWs and NWs



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 2 Fig. 5 Non-metric multidimensional scaling plot of constructed and natural wetlands with
 3 Sciomyzidae species overlaid with water quality variables and habitat area and type. Axes 1
 4 and 2 account for 41.2% and 46.9% of the variation, respectively.



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6 Fig. 6 Box and whisker plot of abundance for each species measured at CW and NW sites.
 7 Species that were not found at any sites have been excluded from the plot. To make it easier
 8 to visualise differences, a single data point for *R. pallida* of 61 individuals counted at one CW
 9 site has also been omitted from the plot.

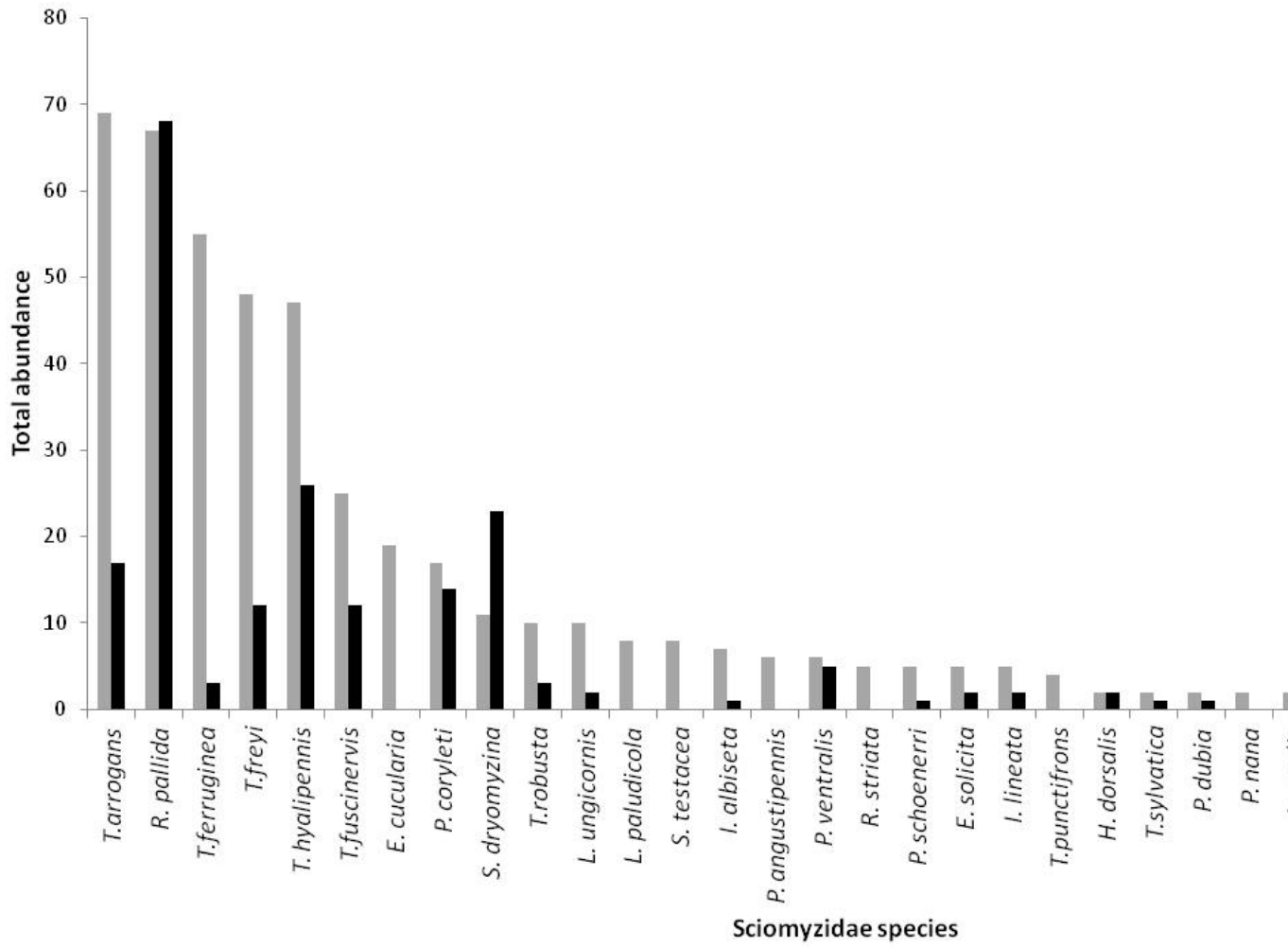
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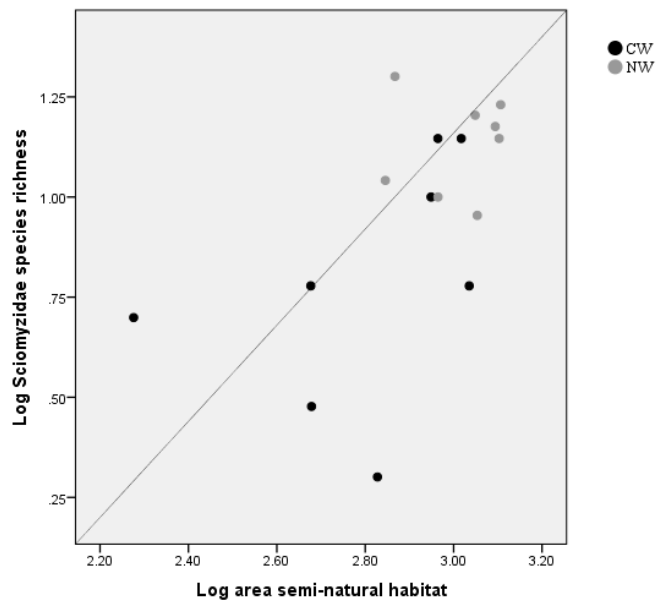


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16 Fig. 7 Total abundances of Sciomyzidae species captured in Malaise traps at CWs and NWs

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20 Fig. 8 Linear regression of Log area of semi-natural habitat within 25 m of Malaise traps and
 21 Log Sciomyzidae species richness at CWs and NWs

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38 Table 1. Names and sizes (areas in m²) of the Constructed (CWs) and Natural (NWs)
 39 wetlands in the present study

Site code	Constructed wetland	Size (m ²)	Site code	Natural wetland	Size (m ²)
CW1	Cloonfad WWTP	20,363	NW1	Lough Meelagh	1,449,027
CW2	Moycullen WWTP	17,164	NW2	Drumady Lough	234,663
CW3	Williamstown WWTP	17,115	NW3	Drumroosk Lough	180,930
CW4	Keadue WWTP	12,940	NW4	Lake Corgar	153,058
CW5	Ballyfarnon WWTP	12,124	NW5	Lough Down	54,141
CW6	Fenagh WWTP	9,560	NW6	Corralough	45,210
CW7	Newtowngore WWTP	9,384	NW7	Lehinch	19,145
CW8	Hollymount WWTP	7,507	NW8	Clooncruffer	8,086

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55 Table 2. Relationships between surrounding habitat and semi-natural habitat richness /
 56 diversity (Shannon's entropy) and sciomyzid diversity (Shannon's entropy), richness and
 57 total abundance at constructed and natural wetlands

Wetland type		Habitat richness	Semi-natural habitat richness	Habitat Shannon's entropy	Semi-natural habitat Shannon's entropy	
Constructed wetlands	Sciomyzidae total abundance	Spearman Rank = -0.169 <i>P</i> = 0.689	Spearman Rank = 0.346 <i>P</i> = 0.402	Spearman Rank = 0.488 <i>P</i> = 0.220	Spearman Rank = 0.390 <i>P</i> = 0.339	
		Pearson correlation = -0.070 <i>P</i> = 0.870	Pearson correlation = 0.421 <i>P</i> = 0.298	Pearson correlation = 0.441 <i>P</i> = 0.274	Pearson correlation = 0.492 <i>P</i> = 0.215	
	Sciomyzidae species richness	Spearman Rank = 0.063 <i>P</i> = 0.883	Spearman Rank = 0.402 <i>P</i> = 0.323	Spearman Rank = 0.446 <i>P</i> = 0.268	Spearman Rank = 0.446 <i>P</i> = 0.268	
		Pearson correlation = 0.050 <i>P</i> = 0.906	Pearson correlation = 0.409 <i>P</i> = 0.314	Pearson correlation = 0.533 <i>P</i> = 0.174	Pearson correlation = 0.522 <i>P</i> = 0.184	
	Sciomyzidae Shannon's entropy	Spearman Rank = 0.124 <i>P</i> = 0.770	Spearman Rank = 0.193 <i>P</i> = 0.647	Spearman Rank = 0.214 <i>P</i> = 0.610	Spearman Rank = 0.310 <i>P</i> = 0.456	
		Pearson correlation = -0.003 <i>P</i> = 0.995	Pearson correlation = 0.115 <i>P</i> = 0.786	Pearson correlation = 0.243 <i>P</i> = 0.563	Pearson correlation = 0.206 <i>P</i> = 0.625	
	Natural wetlands	Sciomyzidae Total abundance	Spearman Rank = 0.049 <i>P</i> = 0.907	Spearman Rank = 0.217 <i>P</i> = 0.606	Spearman Rank = 0.286 <i>P</i> = 0.493	Spearman Rank = 0.548 <i>P</i> = 0.160
			Pearson correlation = 0.088 <i>P</i> = 0.836	Pearson correlation = 0.350 <i>P</i> = 0.396	Pearson correlation = 0.248 <i>P</i> = 0.553	Pearson correlation = 0.689 <i>P</i> = 0.058
Sciomyzidae species richness		Spearman Rank = 0.074 <i>P</i> = 0.862	Spearman Rank = 0.192 <i>P</i> = 0.650	Spearman Rank = 0.524 <i>P</i> = 0.183	Spearman Rank = 0.333 <i>P</i> = 0.420	
		Pearson correlation = -0.245 <i>P</i> = 0.559	Pearson correlation = 0.032 <i>P</i> = 0.939	Pearson correlation = 0.133 <i>P</i> = 0.754	Pearson correlation = 0.476 <i>P</i> = 0.233	
Sciomyzidae Shannon's entropy		Spearman Rank = 0.445 <i>P</i> = 0.270	Spearman Rank = -0.140 <i>P</i> = 0.740	Spearman Rank = 0.238 <i>P</i> = 0.570	Spearman Rank = 0.119 <i>P</i> = 0.779	
		Pearson correlation = -0.640 <i>P</i> = 0.087	Pearson correlation = 0.363 <i>P</i> = 0.377	Pearson correlation = 0.248 <i>P</i> = 0.554	Pearson correlation = 0.195 <i>P</i> = 0.644	

61 Table 3. Sciomyzid species collected during the study at constructed and natural wetlands and
 62 listed in The Scarce and Threatened Flies of Great Britain Review (Falk, 1991) (Knutson &
 63 Vala, 2011)

Rare species	Status	Habitat	Ecology	Recorded in present (malaise trap) study
<i>Antichaeta analis</i>	Rare	Fens, marshes, margin of <i>Phragmites</i> swamp, wet meadow, wet ditches	Eggs and feeding larvae found in egg capsules of <i>L. truncatula</i> . Multivoltine – Overwinter as pupae	Constructed and natural wetlands
<i>Pherbellia griseola</i>	Notable	Fens, bogs, dune slacks, damp woods. Requirement for standing water	Parasitoid of aquatic snails. Multivoltine	Constructed wetlands
<i>Pherbellia nana</i>	Notable	Open marsh, deeply shaded forest pools, lake margins. <i>Phragmites</i> may be preferred. Permanent & temporary water bodies used.	Parasitoid of aquatic snails – <i>Planorbis</i> , <i>Physa</i> , <i>Lymnaea</i> , <i>Aplexa</i> and terrestrial snails – <i>Succinea</i> , <i>Hygromia</i> , <i>Helicella</i> . Multivoltine – Overwinter as pupae	Natural wetlands
<i>Psacadina zernyi</i>	Vulnerable (extremely rare southern species)	Wetlands, fens, standing water probably a requirement	Parasitoid on aquatic snails such as <i>Lymnaea</i> & <i>Physa</i> Multivoltine – Overwinter as adults	Natural wetlands
<i>Renocera striata</i>	Notable	Riverside fen and marsh. Upland areas	Larvae possibly develop as parasitoids of aquatic molluscs eg. <i>Sphaeriidae</i>	Natural wetlands
<i>Tetanocera punctifrons</i>	Notable	Damp woodland, riverside, damp heathland, coastal marsh	Larvae predatory or parasitoid of gastropod molluscs	Natural wetlands
<i>Tetanocera freyi</i>	Rare	Wetlands, unclear though some base enrichment may be required.	Larvae predatory or parasitoid of gastropod molluscs	Constructed and natural wetlands
<i>Sciomyza dryomyzina</i>	Vulnerable	Wetlands, exact preferences unclear. Mainly inland.	Very low population levels at sites. Has not been reared. Parasitoid of <i>Oxyloma</i> in N. America. (<i>O. pfeifferi</i> is terrestrial in Great Britain)	Constructed and natural wetlands

Endangered: Taxa in danger of extinction and whose survival is unlikely if causal factors continue operating

66 **Vulnerable:** Taxa believed likely to move into the Endangered category in the near future if the causal factors continue
67 operating
68 **Rare:** Taxa with small populations that are not at present in endangered or vulnerable but are at risk
69 **Notable:** Species which are estimated to occur within the range of sixteen to one hundred modern 10km squares
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