



BIODIVERSITY AND PERFORMANCE OF CONSTRUCTED WETLANDS; A  
COMPARISON WITH NATURAL WETLANDS

by

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## **Declaration**

I, the undersigned, hereby declare that this thesis, entitled '*Biodiversity richness and performance of constructed wetlands; a comparison with natural wetlands*', is entirely my own work. The thesis has not been submitted in whole or in part to any other University or Institution. All sources used have been acknowledged and referenced in the text.

Collette J. Mulkeen

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## **Abstract**

Natural wetlands (NWs) are one of the most economically and ecologically important habitats on Earth, currently comprising about 6% of the world's surface area. They provide many ecosystem services including the supply of fresh water, food and building materials, water quality improvement, biodiversity support and flood mitigation. The loss of NWs (> 50%) globally over the past two centuries has, however, greatly reduced their facilitation in water purification and wastewater treatment. For this reason, various types of artificial wetlands (constructed wetlands; CWs) have been designed to assist in the removal of a range of pollutants from wastewaters, and accordingly, improve water quality.

Constructed wetlands have several advantages in comparison to conventional wastewater treatment systems. They are a sustainable, green system requiring lower operation and maintenance costs. In addition, the vegetation in CWs assists in many important pollutant removal mechanisms including sedimentation, filtration and plant uptake of metals and nutrients. However, a paucity of information exists on metal and nutrient accumulations within vegetation in CWs, with many studies focusing on concentrations only. As a result, best practices for the harvesting of CW vegetation as a means of metal / nutrient removal, is lacking. This study addresses this significant knowledge gap in CW performance via biomass harvesting of CW vegetation. The additional benefits to biodiversity provided by CWs have received comparatively less attention than their capacity in wastewater treatment. This provides the incentive for the second aim of this study where the biodiversity value of CWs in comparison to that of NWs is assessed. For the first time, the suitability of terrestrial habitats surrounding CWs and NWs for the protected smooth newt is compared, with a view to recommending newt-friendly changes to existing and future CW design. Similarly, marsh flies (Diptera: Sciomyzidae) which are recognised bioindicators of wetlands, are used to quantify, for the first time, the value of CWs to aerial invertebrate diversity. This study also assesses the impacts of water quality and the habitats surrounding CWs on marsh flies since no systematic study has examined this to date.

The results of the study show that the concentrations and accumulations of metals and nutrients in CW vegetation follow contrasting seasonal patterns. Some metals and nutrients measured in the belowground (BG) biomass were greater than 80% of the

more commonly measured aboveground (AG) biomass suggesting that analysis of emergent shoots only may significantly underestimate the metal and nutrient uptake capacity of CW vegetation. Based on the results of the study, it is important to schedule harvesting at specific times of the year to coincide with maximum accumulations of specific metals and nutrients in CW plants. The study also shows that CWs present an opportunity to compliment biodiversity in the locations in which they are placed. The results of a Habitat Suitability Index (HSI) whereby each CW and NW received a score, concluded that appropriate management of the areas immediately surrounding CWs can provide habitat for the protected smooth newt and recommendations to improve new and existing CWs as newt-friendly habitat were crafted. Marsh fly assemblages are similarly influenced by habitats surrounding CWs. In addition, the potential value of CWs to marsh fly conservation is evidenced by over one third of the Irish sciomyzid fauna being represented in the eight CWs in this study, including four species listed as scarce or threatened in the UK.

In conclusion, the results of this study have reinforced the notion which suggests that CW treatment performance is better when plants are present, due to the uptake capacity and accumulation of metals and nutrients into the CW vegetation. The results have elucidated the seasonal patterns of metals and nutrients in AG and BG biomass in a temperate oceanic climate, and provide recommendations on removal via vegetation harvesting, which could prevent potential pollution events in receiving waters. In addition, CWs can now be viewed as crucial in providing habitat to species of conservation concern such as the smooth newt, and scarce and threatened sciomyzid flies, that may be otherwise absent in the surrounding landscape in which CWs are placed. Extensive recommendations to include minor modifications to the future design and management of CWs for smooth newts and marsh flies are provided, which can also be applied to enhance CWs for other wildlife groups and species of conservation concern.

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## Nomenclature

AG	Aboveground
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BOD <sub>5</sub>	Biological oxygen demand
BG	Belowground
Cav.	Cavanilles
Cd	Cadmium
CO <sub>2</sub>	Carbon dioxide
COD	Chemical oxygen demand
Cr	Chromium
Cu	Copper
CWs	Constructed wetlands
EPA	Environmental Protection Agency
ESRI	Environmental Systems Research Institute
FCW	Farm Constructed Wetland
FWS	Free water surface
g	Gram
GIS	Geographic Information Systems
GLM	General Linear Model
HCl	Hydrochloric acid
HNO <sub>3</sub>	Nitric Acid
HSI	Habitat Suitability Index
ICP	Inductively Couple Plasma
ICW	Integrated Constructed Wetland
ISA	Indicator Species Analysis
IUCN	International Union for the Conservation of Nature
kg	Kilogram
L.	Linnaeus
LOD	Limit of detection
<i>Log</i>	Logarithm
m	meter
mg	Milligram

MRPP	Multi-response Permutation Procedures
N	Nitrogen
N <sub>2</sub>	Nitrogen gas
N <sub>2</sub> O	Nitrous oxide
NH <sub>4</sub>	Ammonium
Ni	Nickel
NIST	National Institute of Standards and Technology
NMS	Non-metric Multidimensional Scaling
NO <sub>2</sub>	Nitrite
NO <sub>3</sub>	Nitrate
NW <sub>s</sub>	Natural wetlands
P	Phosphorus
PERMANOVA	Permutational Analysis of Variance
PO <sub>4</sub>	Orthophosphate
Pb	Lead
QC	Quality Control
SPSS	Statistical Package for the Social Sciences
SS	Suspended solids
SSHF	Sub-surface horizontal flow
SSVF	Sub-surface vertical flow
TN	Total nitrogen
TP	Total phosphorus
Trin.	Trinius
UK	United Kingdom
WWTP	Wastewater treatment plant
Zn	Zinc

# 1. Introduction

## 1.1 Background

Natural wetlands (NWs) are one of the most economically and ecologically important habitats on Earth (Staunton et al., 2014) and can be described as transitional environments, occurring between terrestrial and aquatic ecosystems (Lehner and Döll, 2004). Natural wetlands provide vital ecosystem services such as acting as a source of fresh water and food, water purification, flood control, and climate change mitigation. In addition, NWs have a rich biodiversity supporting extensive numbers of bird, mammal, fish, invertebrate, reptile and amphibian species. Despite these benefits, NWs have been considered a hindrance towards agricultural and urban development, and this attitude has led to the loss of over 50% of the global area of NWs in the last 200 years (Van Meter and Basu, 2015). As NWs also perform important functions in water purification and wastewater treatment, this reduction in the areas of NWs has significantly decreased their ability to deliver this service (Millennium Ecosystem Assessment, 2005). In more recent years, wetlands are being constructed specifically to tackle the treatment of wastewater and water pollution (Harrington et al., 2013).

Constructed wetlands (CWs) are human-made wastewater treatment systems which are gaining in popularity due to their acceptance as economical, green, and efficient wastewater treatment systems (Mustafa, 2017) requiring little operation and maintenance (Zhang et al., 2009). Numerous studies to date have concluded that CWs planted with vegetation perform better than unplanted systems (Kadlec and Wallace, 2009). Wetland vegetation, or macrophytes, are capable of element accumulation and are effectively used for phytoremediation techniques (Bonanno and Vymazal, 2017). An understanding of the seasonal variation in the standing stock of metals and nutrients in emergent vegetation of CWs, as well as the amounts present in aboveground (AG) and belowground (BG) biomass, is crucial to their design, including plant species selection, and future management. However, relatively little information currently exists on accumulation and standing stocks in biomass in CWs (Vymazal and Březinová, 2016). If the use of CWs is to increase, the seasonal

variations of metals in vegetation, and the management of the vegetation, must be first of all understood.

The biodiversity of CWs (Ghermandi et al., 2008), an ancillary benefit, has received relatively little attention to date. Those studies addressing biodiversity have focused largely on birds, mammals, and freshwater invertebrates within CWs. However, the landscapes in which CWs are situated may also have a role to play in the conservation of animals with bi-phasic, life-cycle requirements such as the smooth newt (*Lissotriton vulgaris* [Linnaeus, 1758]). The smooth newt which is the sole native species of newt found in Ireland is known to use a variety of aquatic habitats during the breeding season. After breeding, smooth newts tend to move short distances into terrestrial habitats on land (Griffiths, 1984). However, drainage and infilling (Staunton et al., 2014; 2015), and the eradication of vegetation surrounding the CWs (King et al., 2011), remain a threat to smooth newt populations. Given that smooth newts are known to occupy ponds in CWs in Ireland (Scholz et al., 2007), the suitability of the terrestrial habitats around CWs has yet to be examined in detail.

Constructed wetlands are also important habitats for aerial invertebrates, including the marsh flies (Diptera: Sciomyzidae) which are predominantly wetland specialists. Marsh fly species are known bioindicators, found in almost all wetland types, making them a useful group to assess the wider dipteran community in wetlands (Carey et al., 2017). However, no systematic study has yet been undertaken on the value of CWs to marsh fly assemblages, nor has any investigation yet been undertaken on the impacts of either water quality in CWs or the habitats surrounding CWs on sciomyzid species richness, abundance and diversity.

In this thesis, constructed wetlands are examined from two perspectives. Firstly, from a wastewater treatment perspective and in particular, the role played by CW vegetation in metal and nutrient removal from wastewater. Secondly, it examines the role of CWs in the provision of biodiversity in comparison with NWs, with particular reference to amphibians and marsh flies (Diptera: Sciomyzidae).

## 1.2 Knowledge gaps addressed

The study aims to address the following key knowledge gaps:

- While much attention has previously focused on the wastewater treatment capabilities and nutrient removal in CWs, there is a paucity of information on the removal of metals by CWs, particularly in north-western European countries.
- Little research to date exists on vegetation management in CWs, including best practices for harvesting vegetation as a means of nutrient and metal removal.
- In terms of harvesting vegetation in CWs, knowledge of the seasonal variations, accumulation and standing stocks of metals and nutrients in the biomass is lacking.
- As the importance of NWs to the continued survival of animal species becomes more apparent, CWs in the landscape may also have a role to play in the conservation of threatened wildlife.
- In comparison to the many studies which have focused on the water treatment capabilities of CWs, the biodiversity of CWs has attracted relatively little attention.
- The suitability of terrestrial habitats surrounding CWs for the terrestrial phase of the smooth newt life-cycle has yet to be addressed.
- Definitive guidelines for engineers regarding the design of CWs and their surroundings, which incorporate features to support the conservation of the smooth newts, is currently lacking.
- While many studies on invertebrate diversity in CWs focus on aquatic invertebrates as indicators of water quality, much less is known about the aerial phase of invertebrate species, including the Sciomyzidae which are known indicators of wetland aerial invertebrates in general.
- The influence of habitats surrounding CWs and NWs, and the impacts of water quality on sciomyzid assemblages has yet to be addressed.

### **1.3 Research aims**

The first aim of this study was to investigate the performance of the vegetation in a CW in relation to nutrient and metal removal.

The specific objectives to achieve this aim were to:

- evaluate metal and nutrient uptake and accumulation by the vegetation in a CW over three seasons.
- investigate the efficacy of metal and nutrient removal via harvesting of the vegetation, in addition to identifying an optimal period for harvesting.

The second aim of this study was to assess the biodiversity value of CWs in comparison to that of NWs, with a specific focus on the smooth newt and sciomyzid flies.

The specific objectives to achieve this aim were to:

- identify a range of CWs and NWs with similar vegetation types, and within close proximity to each other, to carry out biodiversity studies.
- carry out a habitat suitability assessment of CWs for the smooth newt in comparison to NWs.
- determine the impacts of water quality at CWs and NWs on sciomyzid assemblages, well-known bioindicators of wetland habitat.
- quantify, in comparison with NWs, the influence of surrounding habitats on sciomyzid communities in CWs.
- develop guidelines for engineers on design which support the conservation of smooth newt and scarce or threatened invertebrate species.

### **1.4 Structure of the dissertation**

Chapter 2 consists of a review of the economic and ecological benefits, and the conservation of NWs worldwide. The primary function of CWs in wastewater treatment is discussed along with an ancillary benefit of CWs, the potential contribution to biodiversity enhancement, in particular to smooth newts and Diptera: Sciomyzidae.

Chapter 3 investigates the seasonal patterns of metals and nutrients in the vegetation of a CW for municipal wastewater treatment. It addresses the first aim of the thesis i.e. an examination of the accumulation of metals and nutrients in the AG and BG parts of the vegetation. An optimal period for biomass harvesting in CWs is identified which will be crucial to the design and management of CWs in the future.

Chapter 4 examines the suitability of terrestrial habitats at CWs for the smooth newt, in comparison to NWs. It addresses the second aim of the thesis. The application of a Habitat Suitability Index (HSI) is used to assess the likelihood of the presence of smooth newts and recommendations for CWs (both new and existing), to enhance their usefulness as newt-friendly habitats, are provided.

Chapter 5 examines the sciomyzid assemblages of CWs and NWs. It also addresses the second aim of the thesis. The influence of water quality and habitats surrounding CWs and NWs on sciomyzid community structure is quantified for the first time. In addition, suggestions for the future design and siting of CWs are presented.

Finally, in Chapter 6, the conclusions from the thesis are presented, in addition to recommendations for future research.

## **1.5 Contribution to existing knowledge**

### **1.5.1 Journal Papers (Published)**

Mulkeen, C.J., Gibson-Brabazon, S., Carlin, C., Williams, C.D., Healy, M.G., Mackey, P., Gormally, M.J. 2017. Habitat suitability assessment of constructed wetlands for the smooth newt (*Lissotriton vulgaris* [Linnaeus, 1758]): A comparison with natural wetlands. *Ecological Engineering* 106: 532 - 540.

Mulkeen, C.J., Williams, C.D., Gormally, M., Healy, M.G. 2017. Seasonal patterns of metals and nutrients in *Phragmites australis* (Cav.) Trin. ex Steudel in a constructed wetland in the west of Ireland. *Ecological Engineering* 107: 192 - 197.

Maher, C.A., Carey, J.G.J., Mulkeen, C.J., Williams, C.D., Knutson, L.V., Healy, M.G., Gormally, M.J. 2018. Diagnostic definitions and figures of male and female *Tetanocera punctifrons* and *T. latifibula*, new records of *T. punctifrons* in Ireland, and notes on biology (Diptera, Sciomyzidae). *Dipterists Digest* 25.

The published journal papers are provided in Appendix A, B and C.

### **1.5.2 Journal Paper (submitted)**

Mulkeen, C.J., Williams, C.D., Healy, M.G. and Gormally, M.J. (2018). Sciomyzid (Diptera) assemblages in constructed and natural wetlands: implications for constructed wetland design. (submitted to *Journal of Insect Conservation and Diversity*).

### **1.5.3 International conference oral presentations**

Mulkeen, C.J., Healy, M.G., Gormally, M.J., Williams, C.D. 2014. Biodiversity richness and water quality in natural and constructed wetlands: what can Sciomyzids tell us? 8th International Congress on Dipterology, August 10 – 15, Potsdam, Germany.

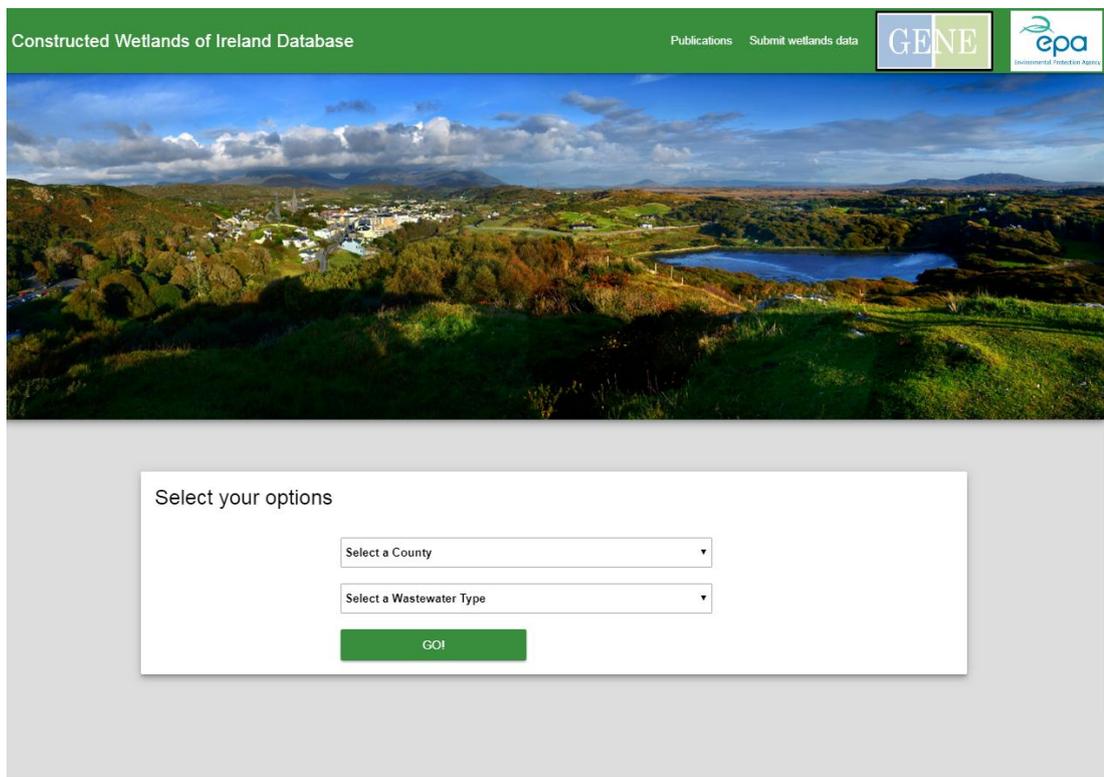
Mulkeen, C.J., Gibson-Brabazon, S., Carlin, C., Mackey, P., Healy, M.G. and Gormally, M.J. 2016. Habitat suitability assessment of constructed wetlands for the smooth newt (*Lissotriton vulgaris*); a comparison with natural wetlands. Inaugural Conference on Natural and Constructed Wetlands, June 21 – 22, NUI, Galway, Ireland.

Mulkeen, C.J., Healy, M.G., Williams, C.D., Gormally, M.J. 2017. The power of plants in constructed wetlands. 14th International Phytotechnologies Conference. September 25 – 29, Montreal, Canada.

### **1.5.4 Constructed wetlands of Ireland database**

As part of this project, the need for a coherent, comprehensive and up-to-date database of CWs, and their performances, across Ireland was identified. In the past, various endeavours to compile a database of CWs in Ireland have been attempted (Babatunde et al., 2008; Healy and O' Flynn, 2011). During this PhD project, a database was created at NUI, Galway capturing CW locations across Ireland for the

first time. Constructed wetland performance data were gathered from a mixture of published and unpublished data from local authorities, Irish Water, private companies and the Environmental Protection Agency (EPA). This synthesized dataset of CW performances is essential in helping to develop specific design criteria, guidelines and methodology for CWs in Ireland. Until now, CWs have been designed in accordance with empirical equations that were developed for climates quite different to the Irish climate. The information gathered in the database may inform design modifications to CWs to optimize their performance under Irish climatic conditions. There are currently over 100 CWs in the database and the website ([www.wetlands.nuigalway.ie](http://www.wetlands.nuigalway.ie)) (Figure 1.1), allows users to submit or download performance data of CWs, or record additional CW locations. The database aims to provide an evidence-based reference point for CW designers, engineers, scientists and researchers, and perhaps activate future implementation of CW technology throughout Ireland. Hereafter, the database will be managed by Dr Mark Healy, Civil Engineering, NUI, Galway.



*Figure 1.1 Constructed Wetlands of Ireland Database homepage*

## **2. Literature Review**

### **2.1 Overview**

Wetlands are one of the most important ecosystems on Earth. Traditionally viewed as convenient waste disposal sites, wetlands have been destroyed over time at alarming rates in the developing and developed worlds. However, the value of wetlands is increasingly being recognised in more recent times, leading to the heightened awareness of the protection and conservation of wetlands across the globe. Today, the use of artificial wetlands, commonly referred to as CWs, is now preferred for the treatment of wastewaters. By virtue of resembling NWs, CWs have the potential to play multi-functional roles including wastewater treatment and enhancement of biodiversity.

This chapter discusses the functions, ecological and economical values, and conservation of NWs. In addition to this, the role of CWs in wastewater treatment and their performance is discussed. The potential contribution of CWs to biodiversity, in particular to the smooth newt and invertebrates, is elucidated. In addition, gaps in the existing knowledge of CWs are identified, thereby providing a route map for future research.

### **2.2 Natural wetlands**

Natural wetland environments have been recognised as a natural resource throughout human history (Scholz & Lee, 2005) and continue to sustain human societies across the globe (Mitsch & Gosselink, 2007). Natural wetlands have been described as ‘transitional environments’ occurring between terrestrial and aquatic systems (Lehner & Döll, 2004). Highly variable in appearance and species composition, NWs have one shared characteristic – inundation by water (most often, freshwater) (Keddy, 2010). This unique environment plays a major role in the health of our planet by providing ecosystem functions including biodiversity support, water quality improvement, flood abatement (Zedler, 2000), and sequestration / long term storage of carbon dioxide (CO<sub>2</sub>) (Mitsch et al., 2013). However, water containing biodegradable organic matter, inorganic and organic chemicals, toxins and disease-causing pathogens, are frequently

discharged without prior treatment into aquatic environments such as oceans, rivers, lakes and wetlands (Kivaisi, 2001). In addition, in our failure to recognise the ecosystem services provided by NWs, there has been widespread conversion of NWs for agriculture and urban settlements (He et al., 2015). As a result, it is estimated that 50% of the Earth's original NWs have been destroyed (Mitsch & Gosselink, 2007) and in Ireland alone, between 1990 and 2012, wetland areas decreased by 2.95% due to the extraction of peat and agricultural drainage (EPA, 2016).

### **2.2.1 Conservation of natural wetlands**

The value we place on NWs has increased in recent decades since the *Ramsar Convention*, an intergovernmental treaty, was signed in Ramsar, Iran, in 1971. The mission of the *Ramsar Convention* is the conservation and wise use of all wetlands, through local and national actions along with international co-operation (Millenium Ecosystem Assessment, 2005). The *Ramsar Convention* is seen as an outstanding step towards the sustainable use and conservation of wetland habitats globally (Kasoar et al., 2015). The convention came into force in 1975 and since its implementation, it has been successful with currently over 2, 200 designated sites of protection on the territories of 169 countries across the globe today, covering 2.1 million square kilometres of wetlands (Millenium Ecosystem Assessment, 2005). In Ireland, there are currently 45 sites designated as *Ramsar* protected sites covering almost 70,000 hectares across the country which include habitats such as lakes, peatlands, estuaries, bays and beaches, river catchments, mountain, woodland and fen habitats (Ramsar, 2018).

### **2.2.2 Functions of natural wetlands**

Natural wetlands are considered as one of the most economically and ecologically important habitats on earth (Staunton et al., 2014). The earliest of human civilisations were first established around NWs (river edges and floodplains) and these NWs continue to produce many benefits for humans today including fertile soils suitable for agriculture (Keddy, 2010). Ecosystem services are ecosystem properties which are recognised, utilized and valued by humans (Moor et al., 2015) and NWs are biologically productive ecosystems, providing a range of physical / hydrological, chemical and biological functions, in addition to functions of secondary importance (Williams, 1993).

### **2.2.2.1 Physical / hydrological functions**

The physical functions of NWs include atmospheric and climate control, flood control and sediment trapping. These are detailed below.

#### **2.2.2.1.1 Atmospheric and climate control**

Natural wetlands are significant sinks of carbon (C) (Mitsch et al., 2013). Soils in NWs are known to contain 200 times more C than the associated wetland vegetation (Garnett et al., 2001). Natural wetlands such as peatlands have enormous importance in protecting the Earth from higher temperatures by acting as a C store, which would otherwise be released to the atmosphere as CO<sub>2</sub> (Keddy, 2010). Fortunately, the restoration of previously destroyed NWs can, in time, once again make NWs a sink of atmospheric CO<sub>2</sub> (Lal, 2008).

#### **2.2.2.1.2 Flood control and mitigation**

The potential of NWs and floodplains to reduce flooding is widely recognised (Watson et al., 2016). Floodplains are the lands adjacent to rivers, formed from their lateral migration (Acreman et al., 2003). Natural wetlands, including floodplains, provide flood control by gradually storing and slowing the rate of floodwaters (Mitsch and Gosselink, 2007). For this reason, not all floodwaters reach the main channel at the same time, in turn protecting downstream localities from flooding (Williams, 1993).

#### **2.2.2.1.3 Sediment trapping**

Suspended sediments in water have a strong tendency to absorb substances such as nutrient, metals, pesticides and other toxins, which are detrimental to water quality (Williams, 1993). Natural wetlands serve as sinks (Mitsch and Gosselink, 2007) and the velocity of flowing water decreases dramatically in NWs in comparison to rivers and streams (Mitsch et al., 2014). The sedimentation process in these sinks is greatest as the water moves slowly, and the entrapment of sediments and substances are enhanced by the vegetation, or they may undergo slow decomposition in NWs (Williams, 1993).

### **2.2.2.2 Chemical functions**

One of the most valued ecosystem services of NWs is associated with water purification. The chemical functions of NWs include removal of pollutants and toxic residues in water.

#### **2.2.2.2.1 Pollutant removal**

Natural wetlands have an influential role to play in removing nitrogen (N) and phosphorus (P) from nutrient-rich waters (Williams, 1993). The removal of pollutants such as N, P and metals, is accomplished by uptake by vegetation, adsorption onto plant detritus, and in particular aerobic and anaerobic processes which promotes nitrification and denitrification, and chemical precipitation (Williams, 1993) (See Sections 2.3.5.1, 2.3.5.2 and 2.3.6).

### **2.2.2.3 Biological functions**

Natural wetlands play crucial biological functions such as high primary production and supporting biological diversity.

#### **2.2.2.3.1 Productivity**

Natural wetlands are an enormous producer of human demands such as food including fish, rice and crustaceans, in addition to fuel – timber, and building materials. The production of animal biomass in NWs has direct economic values for example, in fisheries (Keddy, 2010). Many NW plants are perennials and are constant, powerful converters of solar energy (photosynthesis) (Williams, 1993). Due to their higher rates of biological activity in comparison to other ecosystems, NWs have the ability to transform many pollutants occurring in wastewaters into harmless by-products or essential nutrients that can be used for additional biological productivity within the wetland system (Kadlec & Wallace, 2009).

#### **2.2.2.3.2 Supporting biological diversity**

Frequently inhabited by many plants, NWs also provide a home to 100,000 animal species which require freshwater habitats (Lévêque et al., 2005). Extensive numbers of these animals are often entirely dependent on wetland habitats (Zedler & Kercher, 2005) and include a multitude of animal groups such as birds, invertebrates, reptiles,

fish, amphibians and mammals, often uncommon in other ecosystems (Kadlec & Wallace, 2009). In particular, NWs are well known for supporting waterfowl abundance (Mitsch & Gosselink, 2007) and provide year-round habitat, breeding grounds, and wintering sites for numerous species of waterfowl and migratory birds.

#### **2.2.2.4 Functions of secondary importance**

Due to their ecological diversity, NWs are visually and educationally rich environments (Mitsch and Gosselink, 2007) and have become economically important and the focus of much ecotourism (Fernando & Shariff, 2015). For example, safaris to African swamps such as the Okavanga Delta in Botswana to view wildlife brings in much hard currency to the country (Williams, 1993).

Natural wetlands have been used as wastewater discharge sites since sewage was first collected (Kadlec & Wallace, 2009). However, very often, the NWs were considered as convenient disposal sites, rather than for their wastewater treatment capabilities (Vymazal, 2011). It is only in relatively recent times that NWs worldwide have been recognised for their wastewater treatment capabilities (Vymazal, 2011) and have been constructed *de novo* specifically for the purposes of treating wastewater. Since then, CWs have been designed to intercept wastewater after conventional treatment processes and to remove a range of pollutants before discharging into natural water bodies (Hsu et al., 2011).

### **2.3 Constructed wetlands**

The availability of clean water in Europe has become a topic of great concern as the Water Framework Directive (WFD) is putting pressure on European Union (EU) Member States to improve water quality at catchment scale and provide water quality of a high standard throughout the Union (EU, 2000; EU, 1991). Additional relevant European legislation promoting good water quality include the Nitrates Directive (ND) promoting good agricultural practices, the Urban Waste Water Treatment Directive (UWTT) and the licensing of industrial facilities (IPCC Directive) (O'Boyle et al., 2016). An effective method of tackling water pollution problems is the use of CWs (Harrington et al., 2013). This concept has emerged since the first experiments using wetland plants or macrophytes to improve water quality were carried out in the

1960s (Biswas et al., 2017). Constructed wetlands are man-made systems designed to emphasise the unique characteristics of NW ecosystems for improved water treatment capacity (Kadlec & Wallace, 2009). These are engineered wastewater treatment systems and operate in a controlled setting, utilizing the various biological, physical and chemical processes which also occur in NW vegetation, soils, and microbial assemblages (Vymazal, 2005). Increasingly recognised as a relatively low-cost method for treating wastewaters (Campbell & Ogden, 1999), CWs require minimal operation and maintenance (Zhang et al., 2009). In comparison to conventional wastewater treatment systems, CWs are favourably accepted as efficient, low-tech, green, economical and sustainable wastewater treatment systems (Mustafa, 2017). In addition to their wastewater treatment capabilities, CWs can also provide habitat for a wide diversity of plants and animals. Today, CWs are gaining in popularity for the treatment of municipal (Vymazal, 2011) and industrial wastewaters, including, *inter alia*, landfill leachate (Bulc, 2006; Białowiec et al., 2012), tannery industry wastewaters (Calheiros et al., 2012), highway runoff (Gill et al., 2014), effluents from wineries (Grismer et al., 2003), aquaculture wastewater (Lin et al., 2005), mine wastewater (O’Sullivan et al., 2004), wastewaters containing estrogens, androgens and hormones (Cai et al., 2012; Vymazal et al., 2015), and pharmaceutical and personal care products (Matamoros et al., 2009). Tens of thousands of applications of CW technology currently exist worldwide today (Vymazal, 2011), with approximately 140 sites recorded in the latest inventory of CWs in Ireland (Babatunde, 2008). The possibility of their establishment in small communities or sparsely populated areas (Brix & Schierup 1989), such as those in rural areas of Ireland, also has obvious advantages. Despite this, the application of CW technology in Ireland to date is still in its infancy in comparison to North America and Europe (Healy & Cawley, 2002).

### **2.3.1 Types of Constructed wetlands**

There are two types of CWs: Free water surface (FWS) and sub-surface CWs (Healy et al. 2007). Free water surface CWs consist of areas of open water with floating or emergent vegetation (macrophytes) and are similar in appearance to natural marshes (Kadlec & Wallace, 2009). Sub-surface flow CWs consist of gravel or soil beds planted with emergent vegetation (Mustafa, 2017) and do not often contain standing water (Scholz & Lee, 2005). Sub-surface flow CWs may be configured as sub-surface

horizontal flow (SSHF) CWs, whereby the wastewater flows horizontally through the substrate, or as subsurface vertical flow (SSVF) CWs, whereby the wastewater is dosed intermittently onto the surface of filters, allowed to drain through filter media and collected in a drain at the base (Healy et al., 2007) (Fig. 2.1). A combination of SSHF and SSVF CWs, known as hybrid wetlands, can also be employed (Saeed & Sun, 2012). Many European countries currently use SSHF CWs, as less land area is required in comparison to FWS CWs, and are more popular in North America (Mustafa, 2017).

The application of Integrated Constructed Wetlands (ICWs) originated in Ireland (Harrington et al., 2005) and developed from work started in the late 1980s and early 1990's (Harrington et al., 2007). At a landscape scale, ICWs are ecologically engineered systems and consist of FWS CWs, the design of which is based on the holistic use of land to control water quality (Scholz et al., 2007). Fundamental to the design of ICWs is water quality improvement, landscape fit (designing the ICW to fit into the topography of the surrounding landscape), as well as the provision of ecological habitat (Dunne et al., 2005). The ICW approach has successfully been applied to the treatment of wastewater sources such as domestic sewage, industrial wastewaters, landfill leachates, mining waste, and urban storm water (Harrington et al., 2013). A recent modification to the ICW are Farm Constructed Wetlands (FCWs), which are designed specifically to help manage farmyard run-off and farm effluents, reducing the impact of potential pollution incidents from farms (Carty et al., 2008). Both ICWs and FCWs typically have greater land requirements than conventional FWS CWs in order to provide for other ecological services and habitats in the surrounding areas.

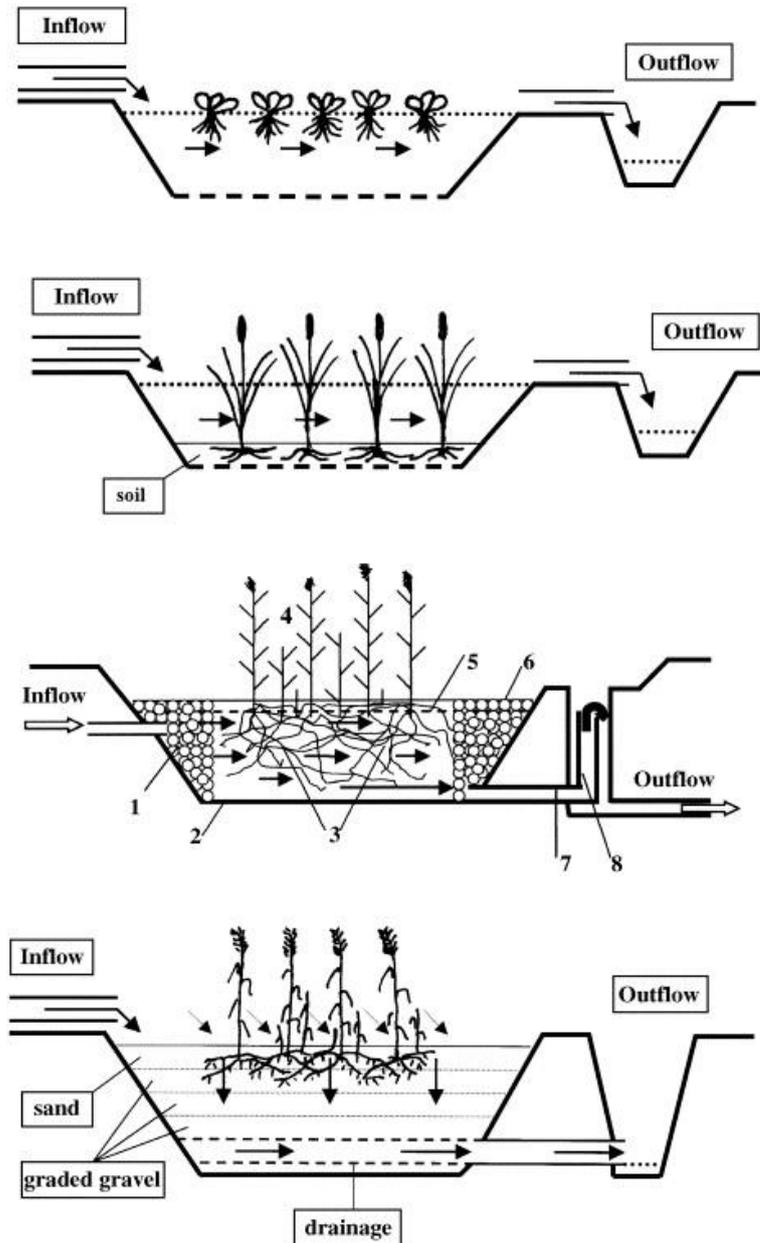


Fig. 2.1 Constructed wetlands for wastewater treatment (from top to bottom): CW with free water surface flow and floating vegetation (FWS); CW with free water surface flow and emergent macrophytes (FWS); CW with horizontal sub-surface flow (HSSF); and CW with vertical sub-surface flow (VSSF) (Vymazal, 2007)

### 2.3.2 Removal mechanisms in CWs

Constructed wetlands have three major components: the *water component* which includes the influent, effluent, water column within the CW, and any additional pollutants; the *fixed component* which includes the vegetation, substrate, accumulated

litter and microbial biofilms; and the *atmospheric component* which regulates the movement of gases into and out of the water column (Wallace & Knight, 2006). Constructed wetlands are exposed to fluctuating quantities of different pollutants such as N, P, metals and coliforms depending on the source of wastewater. The main contaminant removal mechanisms in CWs are an array of physical, chemical and biological removal processes (Mustafa, 2017).

#### **2.3.2.1 Physical removal processes**

The dominant physical removal mechanisms taking place in CWs include sedimentation, volatilisation and diffusion. Once wastewater enters a CW, its velocity is greatly reduced since the surface area of the CW is very large in comparison to that of the incoming stream of wastewater and, in addition to the dense network of emergent macrophytes, suspended solids (SS) and particles are allowed to settle out due to gravity (sedimentation) (Wallace and Knight, 2006). Volatilisation is a significant removal mechanism for organic compounds with significant vapour pressures (also known as volatile organic compounds; VOCs), which vaporise and escape to the atmosphere (Hansen et al., 1998). The diffusion process occurs when dissolved substances are physically moved from areas with higher concentrations to areas with lower concentrations (Moshiri, 1993). In CWs, these distances are short, as the three main components – water, atmosphere and sediments – are within close proximity to each other (Wallace and Knight, 2006). This results in the diffusion of oxygen from the atmosphere into the water column, resulting in a thin layer of near-saturated dissolved oxygen (DO) at the top of the water column (Mustafa, 2107). Dissolved oxygen is the driver for aerobic decomposition and nitrification in CWs and is critical for the survival of fish and other aquatic organisms, and for the general health of receiving water bodies (Kadlec and Wallace, 2009) (Fig. 2.2)

#### **2.3.2.2 Chemical removal processes**

The dominant chemical removal mechanisms in CWs include adsorption, chemical precipitation and ultraviolet (UV) radiation. Adsorption occurs when chemical constituents attach or sorb onto solids (Moshiri, 1993) such as the substrate or the accumulated plant detritus in CWs. Organic compounds can be microbially degraded when adsorbed onto solids, which results in the renewal of sorption sites (Mustafa, 2017). If the adsorbed material cannot be degraded by microbes, as is the case with P,

the sorption sites will eventually become saturated, leading to a termination of removal via this mechanism (Wallace and Knight, 2006). The process of chemical precipitation arises when reactions within the CW result in the formation of insoluble compounds. Hydroxide and sulphide precipitation drives the removal of metals such as iron (Fe), copper (Cu) and nickel (Ni), which can result in the secondary removal of pollutants such as P which can bind to the precipitate (Wallace and Knight, 2006). Ultraviolet radiation enters the CW water column from direct sunlight, triggering a number of chemical reactions such as the breakdown of soluble organic molecules, as well as affecting the viability of pathogens and other organisms (Wallace and Knight, 2006) (Fig. 2.2).

### **2.3.2.3 Biological removal processes**

Constructed wetlands are home to a large diversity of micro-organisms including bacteria, fungi and other organisms. This microbial biomass is a major sink and repository for organic carbon and many nutrients (Moshiri, 1993). Microorganisms are responsible for the breakdown and consumption of organic matter (such as biological oxygen demand (BOD) in influent wastewater), in addition to the uptake and transformation of nutrients such as N (Wallace and Knight, 2006). Nutrients, metals and hydrocarbons (pesticides, herbicides and insecticides) are also taken up by wetland plants (Fig. 2.2).

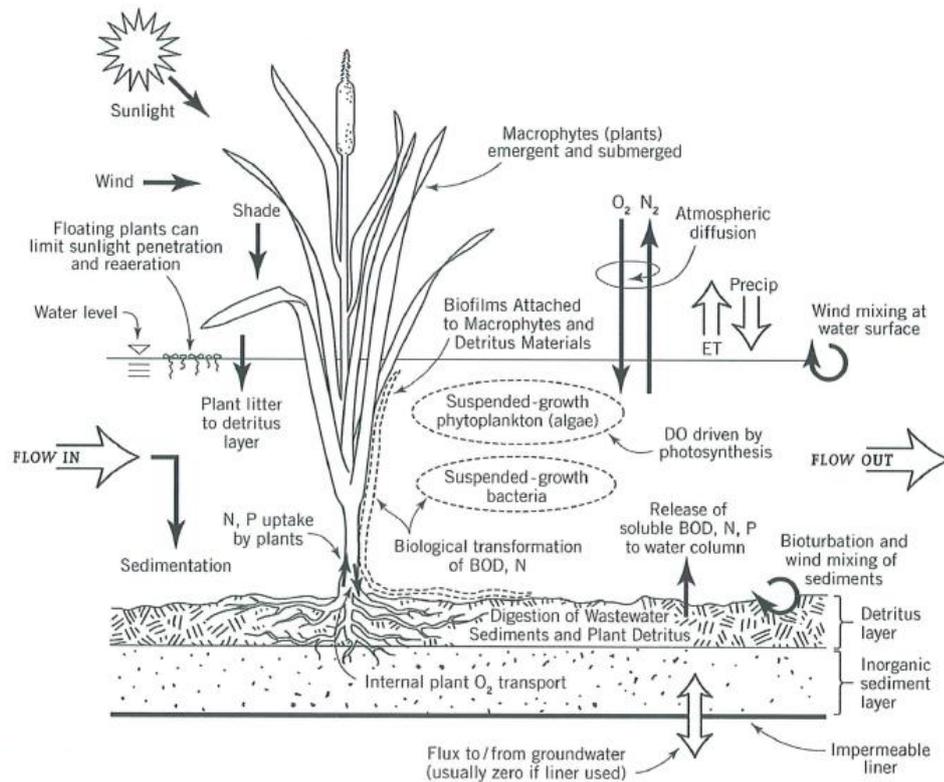


Fig 2.2 Constructed wetland components and major ecological processes in FWS CWs (Wallace and Knight, 2006)

### 2.3.3 Wastewater treatment by constructed wetlands

After half a century of research and implementation, CWs are now recognized as a reliable wastewater treatment technology and a useful solution for the treatment of many wastewater types (Vymazal, 2011). This attention, along with an increasing public demand for more stringent water quality standards and more cost-effective treatment methods, encouraged considerable research and development in the subject (Brix & Schierup 1989), with the main areas of research being water quality, nutrients, vegetation, and flow rates in CWs (Zhi and Ji, 2012). Constructed wetlands are known to reduce many pollutants in wastewater including organics like BOD and chemical oxygen demand (COD) (Vymazal and Kröpfelová, 2008), SS, N, P, trace metals and pathogens (Vymazal et al., 1998).

Table 2.1 shows the performance of CWs across a range of wastewater types and locations. In comparison to NWs, CWs are very nutrient rich due to the high N and P

loadings in wastewater. While high removal rates for BOD, SS and bacteria are commonly achieved by CWs, ammonium ( $\text{NH}_4\text{-N}$ ) removal efficiencies by nitrification / denitrification are variable and depend on the design of the CW, oxygen supplies and retention time (Moshiri, 1993). The results achieved in Table 2.1 show good BOD and SS removal (> 90%) with the exception of Calheiros et al. (2009), where 77% removal BOD was achieved. Typically, the removal efficiency in a CW system for SS is in excess of 90% (Wallace and Knight, 2006). The organic load is measured in terms of BOD and COD mass loading onto a CW, and despite the high COD and BOD concentrations in industrial influents (Calheiros et al., 2009; Table 2.1), reductions in excess of 77% were achieved by the SSHF CW. Organic contaminant removal was also greater than 80% in the hybrid CW treating domestic wastewater in Spain (Ávila et al., 2015; Table 2.1). This CW system also had the highest efficiency in removing total nitrogen (TN) (95% removal). The hybrid CW incorporates the strengths and weaknesses of SSHF and SSVF systems and therefore, when combined, it is possible to obtain effluents with low TN concentrations (Vymazal, 2007). Total P removal ranged from 0.2% (Brix and Arias, 2005; Table 2.1) to approximately 50% (Ávila et al., 2015; Table 2.1). Removal of P from all types of CWs is generally low, unless special substrates with high sorption capacity are used (Vymazal, 2007).

#### **2.3.4 Role of vegetation in wastewater treatment**

The earliest experiments employing wetland plants to treat wastewaters were undertaken by Käthe Seidel in the 1950s in Germany (Vymazal, 2011). In the decades following Seidel's initial research, considerable interest grew regarding the capacity of aquatic plants to control pollution and treat municipal and industrial wastewater (Brix & Schierup, 1989). Wetland vegetation forms the dominant structural element of most CWs (Kadlec & Wallace, 2009). Numerous studies to date measuring wetland treatment performance, with and without vegetation, have concluded almost invariably, that wetland performance is better when plants are present (Kadlec and Wallace, 2009). The vegetation in CWs must have the ability to tolerate high concentrations of nutrients and metals, as well as to accumulate them in their plant tissues (Stottmeister et al., 2003). Therefore, the selection of plant species for new

Table 2.1 Data from constructed wetlands treating various wastewater types

Wetland type <sup>a</sup>	Wastewater type	Pre-treatment	Location	Loading rate	Influent quality (mg L <sup>-1</sup> )							Effluent quality (mg L <sup>-1</sup> )							Ref
					BOD	COD	SS	TN	NH <sub>4</sub> -N	NO <sub>3</sub> -N	TP	BOD	COD	SS	TN	NH <sub>4</sub> -N	NO <sub>3</sub> -N	TP	
ICW	Domestic	-	Ireland	-	768	1,279	2,184	-	32	4.8	-	5	39	12	-	0.3	0.3	-	<sup>1</sup>
Hybrid	Municipal	Mechanical	Czech republic	246 - 510 1 d <sup>-1</sup>	102	241	65	32	26	-	4	8	39	3	7	2.9	-	2.8	<sup>2</sup>
Hybrid	Domestic	Screening/ sand & grease removal	Spain	-	320	405	212	40	25.5	-	5.9	4	43	3	2.2	0.6	-	3.1	<sup>3</sup>
SSHF	Grey water	Screening	Japan	60 g BOD d <sup>-1</sup>	44	77	4.9	7.1	-	-	0.8	3.5	11	0.29	3.9	-	-	0.5	<sup>4</sup>
FWS	Domestic	-	Morocco	-	-	-	-	20	7.6	10.3	2	-	-	-	10.3	3.8	4.8	0.95	<sup>5</sup>
SSHF	Industrial	Equalisation & sedimentation tank	Portugal	242-1925 kg COD ha <sup>-1</sup> d <sup>-1</sup>	706	1598	80	-	-	-	0.41	159	252	9	-	-	-	0.27	<sup>6</sup>
SSVF	Domestic				320	-	124	30	18	-	4.6	2	-	4	9	0.4	-	4.5	<sup>7</sup>

<sup>a</sup> ICW = Integrated constructed wetland; SSHF = Subsurface horizontal flow constructed wetland; SSVF = Subsurface vertical flow constructed wetland; FWS = Free-water surface flow constructed wetland

<sup>1</sup> Kayranli et al., 2010; <sup>2</sup> Vymazal and Kröpfelová, 2015; <sup>3</sup> Ávila et al., 2015; <sup>4</sup> Laaffat et al., 2015; <sup>5</sup> Abe et al., 2014; <sup>6</sup> Calheiros et al., 2009; <sup>7</sup> Brix and Arias, 2005

CWs require careful consideration, as the vegetation must be capable of surviving any potential toxic effects of wastewater and its variability (Maine et al., 2009).

The type of wetland plants used in a CW system is often related to the wetland design employed (Tanner 1996): FWS CWs often employ a combination of free-floating or emergent macrophytes, whereas SSF CWs are limited to emergent macrophytes (Mitsch & Gosselink, 2007). In general, a large group of wetland plants may be used in CWs. However, only a few species of plants are commonly used (Vymazal & Kröpfelová, 2005), as field experience has shown that only relatively few plants actually flourish in the high nutrient, high BOD wastewaters in CWs (Mitsch & Gosselink, 2007). The common reed, *Phragmites australis*, (Cav.) Trin. ex Steudel, is a flood-tolerant perennial grass with an extensive rhizome system and is used worldwide for the treatment of domestic and industrial wastewaters in CWs (Du Laing et. al, 2003). Other common wetland species used in CWs include *Phalaris arundinacea* (reed canarygrass), *Glyceria maxima* (sweet managrass), *Typha* spp. (cattails) and *Scirpus* spp. (bulrush) (Vymazal & Kröpfelová, 2005). *Phragmites australis* is the most common wetland plants found in CWs worldwide (Fig. 2.3) and provides many ecosystem services relating to habitat function and biodiversity support (Kiviat, 2013). However, *P. australis* is not favoured in North American CWs, where it is known for its invasive behaviour (Mitsch & Gosselink, 2007).

Wetland plants are highly productive organisms and possess several functions in relation to wastewater treatment (Brix, 2003) such as flow resistance and particulate trapping (Kadlec and Wallace, 2009), nutrient uptake (Shelef et al., 2013) and insulation, particularly in colder climates. The most important mechanisms by which plants contribute to CW treatment processes are the physical effects of the root structure assisting with particulate trapping combined with aeration (Shelef et al., 2013). Due to their high biomass production and fast growth rates, wetland plants have high remediation potential for macronutrients and heavy metals (Bragato et al., 2006). Investigations of the uptake and seasonal variations in storage capacities of nutrients in *P. australis* and other plants such as *Typha latifolia* L. have been undertaken in CWs (Healy et al., 2007; Mustafa and Scholz, 2011; Bragato et al.,

2006). However, a paucity of information exists on metal cycling and accumulation by vegetation, in particular in CWs of North Western European countries.



*Fig. 2.3 Constructed wetland at Hollymount, Co. Mayo planted with Phragmites australis*

### **2.3.5 Nutrient removal in CWs**

#### **2.3.5.1. Nitrogen**

Nitrogen compounds in wastewater are one of the principal constituents of concern due to their role in eutrophication and effect on oxygen content in receiving waters (Kadlec and Wallace, 2009). Nitrogen exists in various forms including organic matter,  $\text{NH}_4$ , nitrate ( $\text{NO}_3$ ), nitrite ( $\text{NO}_2$ ), or nitrogen gas, depending on the oxidation/reduction conditions of the CW (Wallace & Knight, 2006). Removal mechanisms of N from CWs include ammonia volatilization, denitrification, uptake by vegetation followed by biomass harvesting, and ammonia adsorption (Vymazal, 2007). Other processes occurring in CWs such as ammonification [organic N is converted to  $\text{NH}_4^+$  as the wetland organic matter is decomposing or degrading (Mitsch

& Gosselink, 2007)] and nitrification [a process mediated by microbes which is an important mechanism to reduce the concentration of ammonia (Mustafa, 2017)], are responsible for converting N to various forms, but do not remove N from wastewaters (Vymazal, 2007). However, nitrification coupled with denitrification [a temperature-dependant process which is also dependant on the availability of organic C, in which the oxidised N compounds,  $\text{NO}_3$  or  $\text{NO}_2$ , are reduced to the N gases -  $\text{N}_2$  or nitrous oxide ( $\text{N}_2\text{O}$ ) (Mustafa, 2017)], appears to be a major N removal mechanism in CWs (Vymazal, 2007).

#### **2.3.5.2 Phosphorus**

Similar to N, P is a nutrient required for plant growth. There are three principal processes for P removal in CWs: (1) soil sorption (2) uptake by biota, including bacteria and macrophytes, whereby maximum capacity is limited and provides only initial removal or short-term storage, and (3) a sustainable mechanism, accretion, which has no capacity limit (IWA, 2000). The direct settling and trapping of particulate P contributes to the accretion process (Wallace and Knight, 2006) and 10 to 20% is permanently stored in residual form from the decomposition process (Kadlec and Wallace, 2009).

#### **2.3.6 Heavy metal removal in CWs**

Heavy metals are non-biodegradable, and water pollution by heavy metals is a serious environmental problem which is difficult to solve (Keng et al., 2014). The main difficulty in treating wastewaters containing heavy metals is that metals cannot be degraded or destroyed (Galletti et al., 2010). In CWs, metals tend to accumulate in the sediments as well as in the plants (Březinová & Vymazal, 2015). Phytoremediation is considered to be an effective, low-cost, biological and environmentally friendly clean-up method in contaminated areas (Weis & Weis, 2004). However, metal content in the roots and shoots of wetland vegetation varies from season to season and there has been no attempt to explain this variability, or to determine optimum conditions for metal uptake by plants in CWs to date (Vymazal and Březinová, 2016). In the context of how CWs are managed, the seasonal variations of metals in macrophytes must be first of all understood, if it is intended to expand the use of CWs for treating effluents containing metals in the future.

Maximum recorded heavy metal, and N and P concentrations, from international studies in AG and BG biomass of *P. australis* are presented in Table 2.2. Macrophytes are known to take up metals from the environment but largely accumulate these in the BG organs - the roots and rhizomes (Peeverly et al., 1995). The generally lower concentrations of metals in AG organs of macrophytes (stems and leaves) may be attributable to metal tolerance, and it has been suggested that macrophytes limit high metal concentrations in the photosynthetic organs of the plant (Bragato et al., 2006). The levels of metals in AG organs may vary seasonally in response to plant growth dynamics, metal levels and availability in the surrounding waters (Larsen & Schierup, 1981; Schierup & Larsen, 1981) and do not follow the well-known pattern of nutrient levels (Vymazal & Březinová, 2015). The possibility of harvesting of the AG vegetation as a means of wetland management and removal of metals from the system has previously been suggested (Bragato et al., 2006; Březinová & Vymazal, 2015). Harvesting of the AG vegetation in CWs may be important in the future design and operation, particularly when the efficacy of CWs regarding nutrient and heavy metal removal from wastewaters is being assessed.

Table 2.2. Heavy metal and nutrient concentrations ( $\text{mg kg}^{-1}$ ) in aboveground and belowground biomass of *Phragmites australis* in constructed and natural wetlands.

Element	Aboveground					Belowground				
	Max value <sup>1</sup>	Country	Wetland type <sup>2</sup>	Wastewater type	Reference	Max value <sup>1</sup>	Country	Wetland type <sup>2</sup>	Wastewater type	Reference
<b>Cd</b>	2.1	Greece	NW		<sup>3</sup>	1.21	Denmark	NW		<sup>7</sup>
<b>Cr</b>	118	Italy	CW	Municipal	<sup>4</sup>	6.97	Italy	NW		<sup>5</sup>
<b>Cu</b>	14.98	Italy	NW		<sup>5</sup>	230	UK	CW	Mine water	<sup>9</sup>
<b>Ni</b>	60	Italy	CW	Municipal	<sup>4</sup>	9.12	Italy	NW		<sup>5</sup>
<b>Pb</b>	39	China	CW	Mine water	<sup>6</sup>	>2,000	China	CW	Mine water	<sup>6</sup>
<b>Zn</b>	217	Denmark	NW		<sup>7</sup>	>1,000	China	CW	Mine water	<sup>6</sup>
<b>N</b>	26,500	Italy	CW	Municipal	<sup>4</sup>	19,100	Czech Republic	CW	Municipal	<sup>8</sup>
<b>P</b>	2,200	Czech Republic	CW	Municipal	<sup>8</sup>	2,700	Czech Republic	CW	Municipal	<sup>8</sup>

<sup>1</sup> Maximum values are based on the maximum concentration values reported in the papers reviewed throughout this study

<sup>2</sup> NW = natural wetland; CW = constructed wetland

<sup>3</sup>Obolewski et al. (2011); <sup>4</sup>Bragato et al. (2006) ; <sup>5</sup>Bonanno & Giudice (2010); <sup>6</sup> Deng et al. (2004); <sup>7</sup>Schierup & Larsen (1981); <sup>8</sup>Vymazal & Kröpfelová (2008); <sup>9</sup>Ye et al. (2003)

### **2.3.7 Macrophyte management in CWs (in relation to nutrient and metal removal)**

The management of CW vegetation has been a controversial topic (Thullen et al. 2002), with some promoting the idea that CWs be allowed to follow their natural course by allowing “self-design” – the natural recolonisation of species (Mitsch & Wilson, 1996). While management tools such as burning and harvesting in CWs has previously been proposed, an understanding of the seasonal variations in the standing stock of metals and nutrients in emergent vegetation is crucial to the management of CWs. The total storage of a substance in a plant part is called standing stock (Vymazal & Březinová, 2015) and is calculated by multiplying the concentration in the plant by the biomass per unit area. Vymazal & Březinová (2015) suggest that knowledge of concentrations alone does not provide any information of the translocation or accumulation of metals in a plant without knowing the biomass. A dearth of information currently exists on macrophyte management in CWs, including best practices for harvesting of CW vegetation. Results of experiments involving the burning of vegetation as a management tool proved to be a temporary (1 year) method of curtailing CW vegetation (Thullen et al., 2002) and harvesting of CW vegetation has a pronounced effect on growth and nutrient uptake rates (Healy et al., 2007). Biomass harvesting is a labour and time-consuming operation, and therefore a paucity of information exists on the accumulation and standing stocks in AG biomass in CWs. In a literature review of metals in AG biomass of *P. australis* by Vymazal & Březinová (2016), the authors theorize that in order to obtain correct accumulation values in a plant, it is necessary to include the biomass values.

### **2.3.8 Biodiversity of CWs**

By virtue of resembling NWs which are known to support biological diversity, CWs have the potential to play multi-functional roles encompassing wastewater treatment and biodiversity (Jurado et al. 2012). While CWs are established primarily with the main goal of improving water quality, they can support other functional values, and the development of wildlife or habitats associated with CWs is often a welcome and desired aspect (Mitsch & Gosselink, 2007). However, very few CWs have been specifically designed to contribute to wildlife conservation (Rousseau et al., 2008). Nevertheless, macrophytes, particularly *Phragmites* spp., planted abundantly in CWs, provide, *inter alia*, food and foraging sites, nesting sites and materials, protection of

wildlife from predators, and shelter from weather (Kiviat 2013). As stated by Greenway (2005), CWs can also act as multifunctional ecological systems assisting in the restoration of aquatic flora and fauna, yet, in comparison to the many studies which have focused on the water treatment capabilities of CWs in the last half century, the biodiversity, an ancillary benefit of CWs, has attracted relatively little attention to date. Some studies, however, have addressed the biodiversity of CWs by focusing on iconic groups such as birds (Hsu et al., 2011); Fleming-Singer and Horne, 2006), mammals (Stahlschmidt et al., 2012) and amphibians (Schulze et al., 2010). Freshwater invertebrates have also been studied to determine water quality and therefore, functionality of CWs (Spieles and Mitsch, 2000; Jurado et al., 2010). However, these studies have generally focussed on the CW itself and not on the surrounding habitats in which the CW is situated, although the latter are often critical for fauna, such as amphibians, with biphasic life cycle requirements. As a result, there is a paucity of information regarding the incorporation of biodiversity features in the design and construction of new CWs and their surroundings. As the value of CWs to endangered animal species has long been recognised (Chovanec, 1994), CWs across the landscape may have a role to play in the conservation of threatened species.

#### **2.3.8.1 Birds in CWs**

Constructed wetlands for wastewater treatment provide a reasonable alternative habitat and a valuable resource for waterfowl and birds (Murray and Hamilton, 2010). In addition, many predatory birds such as falcon and kite are attracted to CWs to prey on the small birds, mammals, amphibians and reptiles within CWs (Greenway and Simpson, 1996). While birds provide an important visual feature in CWs and are attractive to birdwatchers and hunters, some waterfowl, particularly geese, may be problematic by grazing intensively on newly planted seedlings and transplants (Kadlec and Wallace, 2009). Since FWS CWs provide a sanctuary for wading birds and waterfowl (Mitsch & Gosselink, 2007), the question of their contribution to nutrient loadings on the system has been investigated with the conclusion that bird-use of a CW does not lead to a significant reduction in wastewater treatment performance, despite the fact that, in one case, bird numbers peaked at 12,000 individuals per day in a CW in the USA (Andersen et al., 2003).

### **2.3.8.2 Mammals in CWs**

Kadlec & Wallace (2009) identify rodents as being the largest group of mammals associated with CWs. Small mammals such as mice and voles are herbivorous species grazing on plants and seeds, and are prey to wading birds and raptors (Kadlec and Wallace, 2009). Over two thirds of all bat species exhibit insectivorous feeding behaviour (Kunz et al., 2011), and with high densities of aerial insects occurring in wetlands (Wu et al., 2009), bats using CWs for foraging may benefit greatly (Park & Cristinacce 2006). Constructed wetlands (in Ireland) are known as foraging sites for otters (*Lutra lutra*, Brünnich, 1771) breeding in the River Tolka (Dublin City Council, 2008). In addition, muskrat (*Ondatra zibethicus*, Linnaeus, 1766) (Kadlec et al., 2007), mink (*Neovision vision*, Schreber, 1777) and nutria (*Myocaster coypus*, Molina, 1782) are also known to inhabit CWs (Knight, 1992).

### **2.3.8.3 Amphibians in CWs**

Amphibians typically require terrestrial and aquatic environments to complete their semi-aquatic life cycle (Dodd & Cade, 1998), and the importance of terrestrial habitats and microhabitats for amphibian breeding site selection has been highlighted by Marnell (1998). However, amphibians are currently experiencing striking global declines (Beebee & Griffiths 2005) due, in part, to the destruction of wetland habitats (Stuart et al., 2004) and fungal disease (Voyles et al., 2009). Frogs are well represented in CWs (Dublin City Council, 2008; Simon et al., 2009; Schulse et al., 2010) and play an important role in devouring large numbers of insects as well as being a source of prey for fish and birds (Kadlec and Knight, 1996). The presence of newts in CWs treating wastewaters (Scholz et al., 2007) suggest that CWs can also support breeding by newts. The smooth newt (*Lissotriton vulgaris* [Linnaeus, 1758], is the focus of Chapter 4 of this thesis, further details regarding its life-cycle and ecology are given below (Section 2.4)).

### **2.3.8.4 Invertebrates in CWs**

Invertebrates, which have been described as essential components of wetlands, are known for their high diversity in wetland habitats (Wu et al., 2009). Wetland environments offer a wide variety of niches for many invertebrates (Kadlec & Wallace, 2009) which are known to perform significant ecosystem functions

including influencing nutrient cycles (Wallace and Webster, 1996), and assisting in the decomposition of litter (Murkin and Wrubleski, 1988). Invertebrates are also critical to the energy dynamics in a CW, acting as a food source for many vertebrates (Greenway and Simpson, 1996; de Szalay, et al., 1997).

A reasonable body of knowledge exists regarding the aquatic phases of invertebrates of CWs as bioindicators of water quality (Jurado et al., 2009; Jurado et al., 2010; Spieles and Mitsch, 2000; Streever et al., 1996; Andersen and Vondracek, 1999; Wallace et al., 1996). Water beetles are also one of the main macroinvertebrate orders which have been studied in CWs (Jurado et al., 2014). However, considerably less research has been undertaken on the aerial / terrestrial 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).

After a review of the literature on previous biodiversity studies of CWs, two animal groups were selected in order to assess the biodiversity value of CWs. Firstly, the iconic smooth newt was chosen, which was already known to inhabit some CWs (Scholz et al., 2007). However, it was not clear why some CWs support smooth newt populations and others do not. In addition, the smooth newt is a species which is in the public domain in terms of its conservation (Meehan, 2013), and due to its popularity, the development of recommendations for CW design in the future (in association with designers and engineers) may be more accomplishable for such a well-known species. Secondly, a family of invertebrates, the marsh flies, Diptera:Sciomyzidae, were selected for investigation in the study to further assess the biodiversity value of CWs. Although Diptera:Sciomyzidae may not be as familiar in the public domain, these insects occur in almost all wetlands and have previously been shown to be good indicators of invertebrate diversity in wetland habitats at small spatial scales (Carey, 2017). Since NWs are in decline, it is crucial to determine the possible role of CWs in supporting biodiversity. Further details regarding the ecology of marsh flies are given below (Section 2.5).

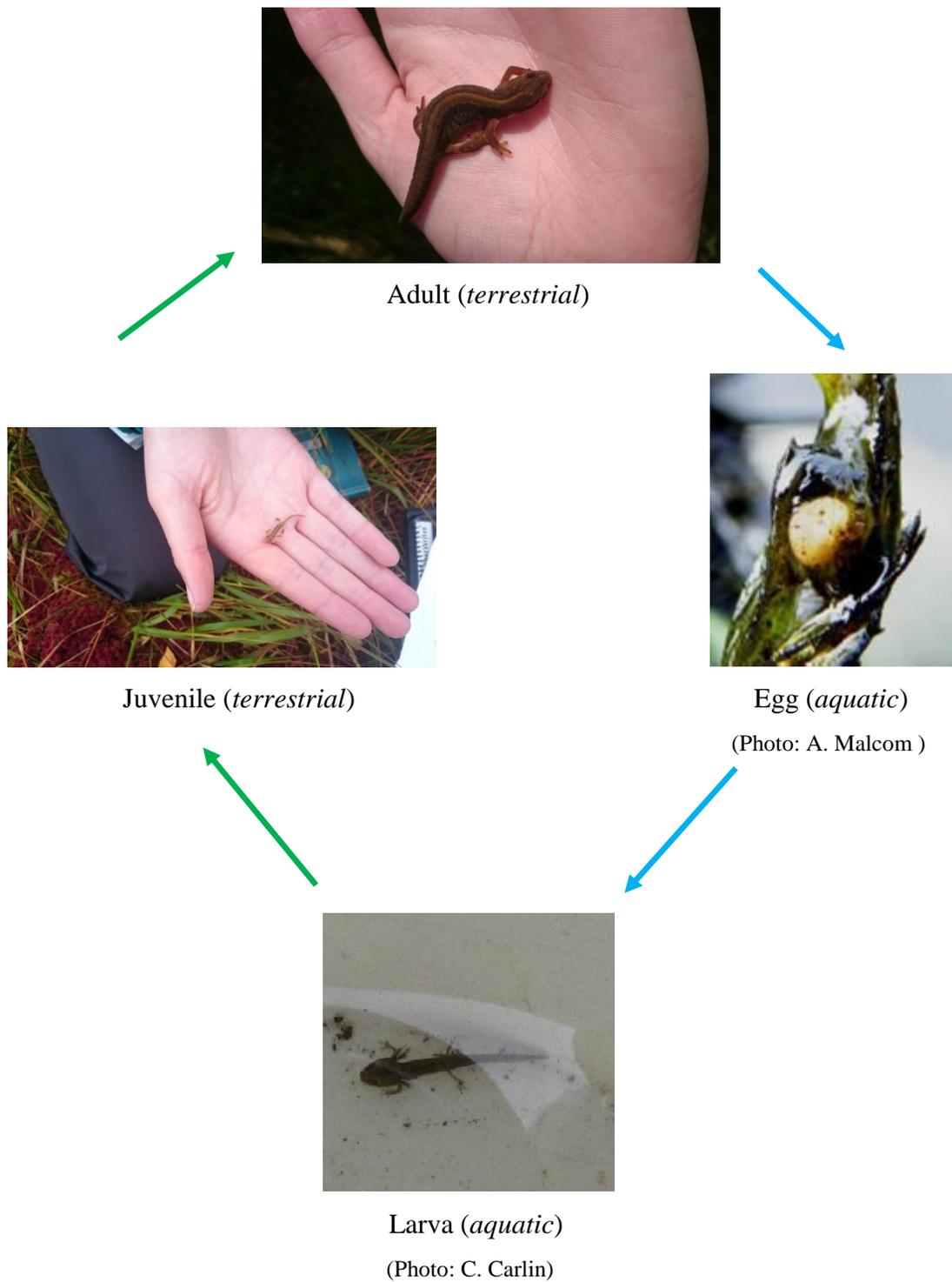
#### **2.4 The Smooth Newt**

The smooth newt (*L. vulgaris*) the sole native species of newt found in Ireland (Meehan, 2013), is widespread across most of Europe. Breeding takes place annually

in water during spring, and sometimes extending into early summer, after which the adults return to land (Bell, 1977). After metamorphosis, the juveniles are solely terrestrial, spending several years on land, before reaching maturity between the ages of three and seven years (Bell, 1977) (Figure 2.3), at which stage they return to water bodies to breed. Smooth newts are known to use a variety of water bodies during the breeding season which include lakes, natural ponds, garden ponds and slow-moving drainage ditches (Meehan, 2013), with larvae rarely being found in running water (Bell & Lawton, 1975). Even water bodies with a surface area of no more than 400 m<sup>2</sup> (considerably smaller areas than many CWs for wastewater treatment) have been known to support up to 1,000 individual adult smooth newts (Bell & Lawton, 1975) and the presence of smooth newts has already been documented in some CWs (Scholz et al., 2007).

The smooth newt life cycle has complex requirements. Adults require aquatic habitats for breeding as well as terrestrial habitats for foraging and overwintering, although adults have been found to overwinter in ponds in Italy (Fasola & Canova, 1992). In some cases, larvae have even been recorded in water bodies during the winter, but this is usually the result of a combination of factors such as late egg production, high population densities, competition for food resources and low water temperatures in countries such as England (particularly northern parts), Poland and Montenegro (Jehle et al., 2011). While juveniles leaving the waterbody for the first time can travel further on land (Joly et al., 2001), adult smooth newts generally move towards favourable habitat patches in the vicinity (Malmgren, 2002).

Although terrestrial behaviour of smooth newts is still not fully understood, diverse structural habitats (Vuorio et al., 2015), in addition to climatic and landscape factors (Joly et al., 2001), may drive patterns of movement (Pittman et al., 2014) and survival (Griffiths et al., 2010). Smooth newts tend to travel in straight lines on land since movement here is slower and requires more energy than movement in water, where the newt is buoyed up by the surrounding medium (Griffiths, 1996). Once on land, suitable refuges must be sought from predation, desiccation and temperature extremes (Griffiths, 1984).



*Figure. 2.4 Life cycle of the smooth newt (Lissotriton vulgaris)*

Habitats that provide shelter and protection such as scrub and woodland (both deciduous and coniferous), unimproved grassland and gardens are considered newt-friendly habitats (Oldham, 2000) (Table 2.3). Although acidic habitats such as

peatland (Marnell, 1998) and water bodies containing fish are thought to be less suitable for smooth newts in the UK (Aronsson & Stenson, 1995) and Lombardy, Italy (Ficetola & de Bernardi, 2004), it appears that habitat selection in smooth newts may be limited by barriers and competition. In Ireland, for example, where the smooth newt is at the most westerly edge of its range, and it lacks competition for habitats from other newt species, it has a tendency towards a wide niche occupation including lakes of a considerable size containing fish, in addition to acid peatland pools (Meehan, 2013). In addition, microhabitats such as dead wood and stone features can be important in amphibian breeding site selection (Marnell, 1998), while roads and rivers adjacent to the breeding water body have been shown to interfere with newt migration (Oldham, 2000; Matos et al., 2017).

The movement of adult smooth newts on land, which tends to be short distances from breeding water bodies (Griffiths, 1984), has been described as philopatric, i.e. individuals remain or return to relatively few permanent hiding places throughout the year and/or on an annual basis (Dolmen, 1981; Sinsch & Kirst, 2015). Although individuals of smooth newt have been found in terrestrial habitats at distances exceeding 500 m from water bodies (Kovar, et al. 2009), this is likely to be the exception rather than the rule. Bell (1977) found that over forty times more smooth newts were captured in pitfall traps within 5 m of a wetland edge compared with pitfalls placed 50 m from the wetland edge. In addition, Bell (1977) released sixty-one marked smooth newt juveniles 22.5 m from a pond edge and recaptured over 50% within 10 m from the point of release thirty-five days later. In another study, Dolmen (1981) observed that no recaptured smooth newts ventured further than 7.5 m from the original capture point on land, suggesting that adult smooth newts tend to settle close to the water body in which they were born (Bell, 1977). Most smooth newts will remain relatively close to the breeding pond, provided that habitat quality immediately surrounding the breeding water body is optimal and connectivity is excellent. Terrestrial habitats surrounding wetlands can, therefore, serve as wildlife corridors, and are important in the conservation and management of semi-aquatic species such as amphibians (Semlitsch & Bodie, 2003) including smooth newts.

Table 2.3. Terrestrial habitats identified in the literature as suitable for the terrestrial phase of *Lissotriton vulgaris* (L., 1758)

<i>Terrestrial habitat</i>	<i>Reference</i>
Meadows / long grass	Marnell, 1998; Oldham et al., 2000; Flood, 2012; Meehan, 2013
Rough grassland	Oldham et al., 2000
Hedgerows	Oldham et al., 2000
Scrub	Marnell, 1998; Oldham et al., 2000; Flood 2012
Woodland	Oldham et al., 2000; Flood, 2011; Meehan, 2013
Gardens	Oldham et al., 2000
Damp woodland	Flood, 2011
Bogland	Flood, 2011
Dense vegetation in water/lake margins	Meehan, 2013

#### 2.4.1 Smooth newt conservation

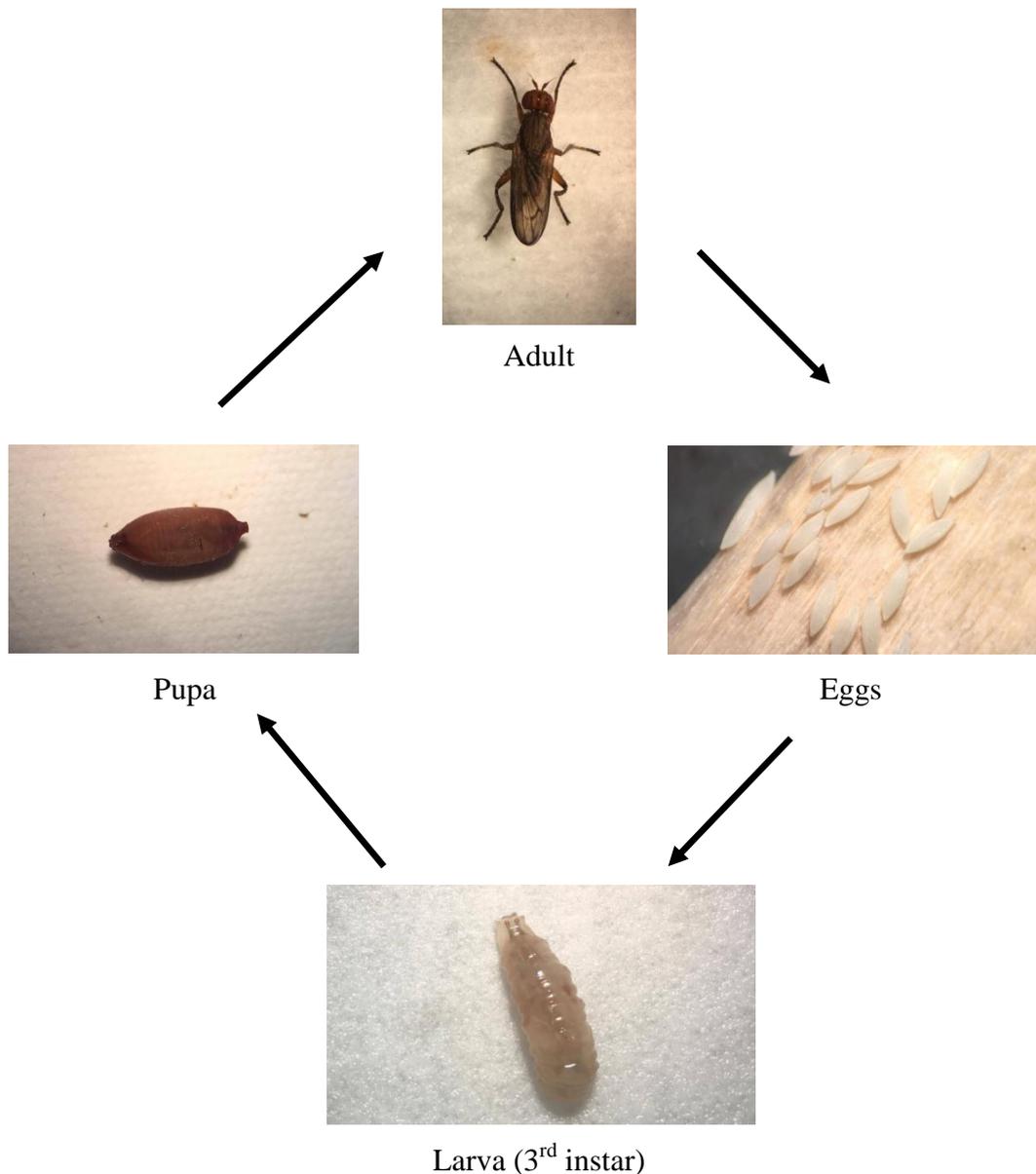
In Ireland, drainage and infilling of NWs (Staunton et al., 2015), in conjunction with excessive clearing of vegetation around breeding sites, remain a threat to smooth newt populations (King et al., 2011). *Lissotriton vulgaris* is currently on the International Union for the Conservation of Nature (IUCN) Red list of threatened species in Ireland (King et al., 2011) and loss of suitable terrestrial habitats for overwintering or refuge remains a concern. While the value of CWs as a conservation strategy for amphibians has been highlighted by previous studies (Denton & Richter, 2013), the suitability of terrestrial habitats surrounding CWs for the terrestrial phase of the smooth newt life-cycle has yet to be addressed.

#### 2.5 Diptera: Sciomyzidae

Although true flies (Order Diptera) have been described as sensitive indicators of habitat change (Rivers-Moore and Samweys, 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 habitat, 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 more data for the more terrestrial component of wetland insects, which can then be used to monitor colonisation events (Keiper et al., 2002).

One of the best-known dipteran families are the Sciomyzidae (marsh/shade flies). This moderately sized family of flies has a worldwide distribution (Vala et al., 2012). Sciomyzid flies are well known inhabitants of marshes, wet grasslands, swamps and lake margins (Knutson and Vala, 2011), and occur in climates ranging from polar to tropical (Knutson and Berg, 1971). During their life cycle, sciomyzid flies pass through three typical larval instars (or pre-pupal stages), a pupal stage, adult and egg stage (Knutson and Vala, 2011) (Figure 2.4). Also known as snail-killing flies, sciomyzid larvae are almost exclusively obligate natural enemies of molluscs (Knutson & Vala, 2011) with most species restricted to feeding on non-operculate freshwater, semi-terrestrial or terrestrial snails (Murphy et al., 2012). However, a few species are known to feed on fingernail clams (Mollusca: Sphaeridae), while others are known to attack oligochaetes, slugs, operculate snails, snail eggs and snail species of brackish waters (Murphy et al., 2012). Multivoltine species of Sciomyzidae breed continuously throughout the Spring and Summer in temperate climates, primarily overwintering in the puparium or as adults, while the univoltine species are known for overwintering as embryonated eggs, partly-grown larvae or pupae (Berg et al., 1982). Multivoltine life cycles are considered by far the most common phenology exhibited by sciomyzid flies (Berg et al., 1982).



*Figure 2.5 Life cycle of Diptera: Sciomyzidae (Photo credit: K.D.Ahmed)*

As wetland specialists, sciomyzid flies have been shown to be suitable bioindicators of wetland habitats (Speight, 1986; Carey et al., 2015) with adult flies tending to move infrequently within and between habitats (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 of a seasonal karstic lake (turlough), thereby suggesting low levels of movement by sciomyzid flies within habitats (Williams et al., 2010). More recently, Carey et al. (2017), who tested the differences between Diptera displaying limited movement such as the Sciomyzidae

and the more mobile Syrphidae, found that sciomyzids were 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 m<sup>2</sup>) and are either isolated or occur in urban landscapes, using local-scale invertebrate wetland specialists such as sciomyzids for biodiversity studies of CWs is a logical choice. In addition, while sciomyzids have been highlighted for their microhabitat specificity and their potential as bioindicators of wetland habitats, little information currently exists relating to water quality and abundance / diversity of Sciomyzidae. This is particularly important in the context of CWs playing an ever-increasing role in the provision of wetland ecosystem services (including biodiversity), given the worldwide decline of NWs (Zedler, 2003).

## **2.6 Methodologies chosen**

To date, research has mainly focused on the wastewater treatment capabilities of CWs. However, there is a dearth in the literature regarding the removal of metals and nutrients by vegetation, the impact of biomass harvesting, and in particular best practices for harvesting. To address these knowledge gaps, a study of the seasonal patterns and accumulations of metals and nutrients in *P. australis* was conducted in a CW. Above ground and BG biomass was collected monthly, washed, dried and analysed for metals and nutrients to determine the seasonal patterns over three seasons. Best practices of biomass harvesting to achieve maximum metal and nutrient removal were then elucidated.

Many of the biodiversity studies in the literature focus on the CW itself, and not on the surrounding habitats in which the CW is situated. The areas surrounding CWs are critical for fauna, such as amphibians with aquatic and terrestrial life-cycle requirements such as the smooth newt (*Lissotriton vulgaris*). The aim of this study was to compare the suitability of terrestrial habitats around CWs and NWs for the smooth newt. Habitat mapping of terrestrial areas around eight CWs and eight NWs was conducted. Notable features of importance (wood and stone) to the smooth newt were mapped and the areas of all habitats calculated. A HSI for newts, detailed in Chapter 4, was applied to all CWs and NWs, whereby each wetland was given a

score. Based on the scores received by each CW or NW, recommendations to improve new and existing CWs as newt-friendly habitats were then crafted.

Also under-represented in the literature are the invertebrates in CWs, in particular the aerial invertebrate fauna. Malaise and emergence trapping was used to capture aerial invertebrates at eight CWs and eight NWs. Sciomyzid flies are wetland specialists and known biological indicators of wetland habitat and Dipteran diversity (Carey et al., 2017). Upon capture, they were identified to species level. The influence of surrounding habitats and the water quality impacts of CWs and NWs on Sciomyzidae were investigated. The results of the study will be used to inform the future design and biological diversity enhancement of CWs, without impeding their primary function of wastewater treatment.

## **2.7 Statistical approaches chosen**

In order to examine the differences between CWs and NWs, a wide range of statistical techniques were used in the study. Univariate analysis is the simplest form of analysing data and was carried out on SPSS version 24.0. SPSS is an effective tool for carrying out hypothesis testing and reporting, and ad-hoc analysis. Multivariate analysis involves complex analysis of more than one statistical variable at a time and was chosen to analyse the water quality and surrounding habitat variables with Sciomyzidae community dynamics in the study. This was carried out on PC-Ord (version 6.0).

In Chapter 3, a Two-way ANOVA was used to test if there were any significant differences anywhere within the data. Two factors were considered here. Factor one was month of the year which had eight levels (eight months), and factor two was the AG versus BG (two levels). The Tukey (HSD) post hoc test ( $P < 0.05$ ) was used to determine among which levels of the significant factors the significant differences lay, ie. if results for some months were significantly different from each other.

In order to test for normal distribution in Chapter 4, a Kolmogorov-Smirnov test was first performed. As the residuals were found to be normally distributed, a Pearson's correlation was then carried out to test for correlations between area of the wetland

and the number of habitats present. The General Linear Model (GLM) tests for any significant effects of wetland type (CW or NW) and area, on the habitat richness.

In Chapter 5, Pearson's correlations and Spearman Rank correlations performed using SPSS, 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. In order to test for any correlations between the areas of reed bed, or areas of semi-natural habitat with Sciomyzidae species richness, a linear regression was also performed. Significant differences in water quality variables between CWs and NWs were tested using Mann-Whitney U-tests and independent samples t-tests depending on whether the residuals conformed to parametric assumptions (homoscedasticity and normality) or not. Independent samples t-tests were also used to test for differences between CWs and NWs with regard to Sciomyzidae abundance, richness and Shannon's entropy.

Non-metric multi-dimensional scaling (NMS) is an ordination technique, which does not rely on assumptions of multivariate normality and so is more appropriate for ecological studies. NMS is an iterative procedure which seeks to reduce the stress between the distance among sampling locations in ordination space and the distance (dissimilarity) between the same locations in n-dimensional species-space. Significance of axes are determined by permutation of the species matrix. NMS displays sites in species-space and can be overlaid by species centroids and environmental variables displayed as vectors, to determine what drives compositional changes in community dynamics.

A PERMANOVA was used to test for significant differences in species composition between CWs and NWs. In addition, an Indicator Species Analysis (ISA) tested for the significant fidelity of any particular Sciomyzidae species to CWs or NWs. The Multi-response permutation procedure (MRPP) tested if either CWs or NWs had an effect on Sciomyzidae species composition. Non-metric multi-dimensional scaling (NMS) is an ordination technique which does not rely on assumptions of multivariate normality and so is more appropriate for ecological studies.

## **2.8 Summary**

In this chapter the background of the research is presented. An introduction to NW functions, values and the conservation of NWs was presented. Constructed wetlands for wastewater treatment were introduced to include the types, removal mechanisms and performances. This was followed by a discussion on the role of vegetation and management practices in CWs, and the removal of nutrients and metals in wastewater by CWs. Finally, the biodiversity of CWs, an ancillary benefit, was introduced along with the main animal groups known to inhabit CWs to date. Many biodiversity studies in CWs focus on the CW itself and not on the surrounding terrestrial habitats which are critical for semi-aquatic species of conservation concern, including the smooth newt. The types and importance of terrestrial habitats in the life cycle of the smooth newt, and the conservation of this red listed species were highlighted. In addition, the ecology of snail-killing flies, Diptera:Sciomyzidae, was introduced along with the potential of these insects as bioindicators in wetland habitats.

In the following chapter (Chapter 3), the seasonal patterns of metals and nutrients in the vegetation of a CW is described, and the optimal time for biomass harvesting is determined in temperate oceanic climatic conditions.

### **3. Seasonal patterns of metals and nutrients in *Phragmites australis* (Cav.) Trin. ex. Steudel in a constructed wetland in the west of Ireland**

#### **3.1 Overview**

The aim of this chapter is to evaluate the seasonal variations of metals and nutrients in AG and BG biomass of *Phragmites australis* (Cav.) Trin. ex Steudel in a CW receiving municipal wastewater with a view to (1) investigating the efficacy of metal and nutrient removal via biomass harvesting of AG vegetation, and (2) identifying an optimal period for biomass harvesting.

The study has been published in Ecological Engineering (Mulkeen, C.J., Williams, C.D., Gormally, M.J., Healy, M.G., 2017. Seasonal patterns of metals and nutrients in *Phragmites australis* (Cav.) Trin. ex. Steudel in a constructed wetland in the west of Ireland. Ecological Engineering 107: 192 – 197. doi: 10.1016/j.ecoleng.2017.07.007).

#### **3.2 Introduction**

*Phragmites australis* is one of the most common plants found in wetland ecosystems and it has the ability to withstand extreme environmental conditions including the presence of toxic pollutants and metals (Bonanno & Giudice, 2010; Schierup & Larsen, 1981). Given the widespread use of *P. australis* for the treatment of wastewaters with elevated levels of metals, such as tannery industry wastewaters (Calheiros et al., 2007), landfill leachates (Bialowiec et al., 2012) and highway runoff (Gill et al., 2014), an understanding of the seasonal patterns and accumulations of metals present in the AG and BG biomass of *P. australis*, is crucial. However, the seasonal patterns of metals in plant biomass vary considerably and do not follow the well-known pattern for nutrients (Vymazal & Brezinova, 2015). In addition to this, knowledge of metal concentrations alone does not provide information about the accumulation or translocation in the vegetation when the plant biomass is unknown. In order to obtain correct accumulation values in the vegetation, it is necessary to include plant biomass values (Vymazal & Brezinova, 2016).

As a means of CW management, the harvesting of wetland vegetation has been suggested as a method for nutrient and metal removal from CW systems (Bragato et

al., 2006; Vymazal & Brezinova 2015). However, information on macrophyte management and best practices for harvesting is lacking. Given that the harvesting of vegetation in CWs is a labour and time-consuming operation, a paucity of information currently exists on the metal and nutrient accumulation and standing stocks in plant biomass in CWs, in Ireland and the north west of Europe. To address this knowledge gap, this chapter examines the seasonal patterns of metals and nutrients in *P. australis* in a CW treating municipal wastewater, with a view to identifying an optimal time for biomass harvesting of the AG vegetation. The results of this study may inform how a wetland treating industrial wastewaters or effluents with higher concentrations of metals may be managed in the future. We focus on a north western European context, but many of our suggestions may be suitable for other environmental contexts.

### **3.3. Materials and methods**

#### **3.3.1 Site description**

The FWS CW investigated in this study is located in Fenagh, Co. Leitrim, Ireland (54°1'2"N; 7°49'43"W) (Fig. 3.1). This CW was designed and constructed to cater for a population equivalent (PE) of 400 in 2004, but currently receives wastewater with a PE of 132 (Table 3.1) and is operated by Leitrim County Council. Wastewater enters the treatment works at the primary settlement tank, flows by gravity to a rotating biological contactor before entering the CW, where the wastewater undergoes tertiary treatment. The CW has a surface area of 400 m<sup>2</sup>, and is lined with a high-density polyethylene liner. The wetland was originally planted with a monoculture of *P. australis*. Vegetation cover in the wetland is 100%, with some occasional bramble (*Rubus fruticosus* agg.), nettle (*Urtica dioica* L.) and willow scrub (*Salix* spp. L.) encroaching onto the reed bed.

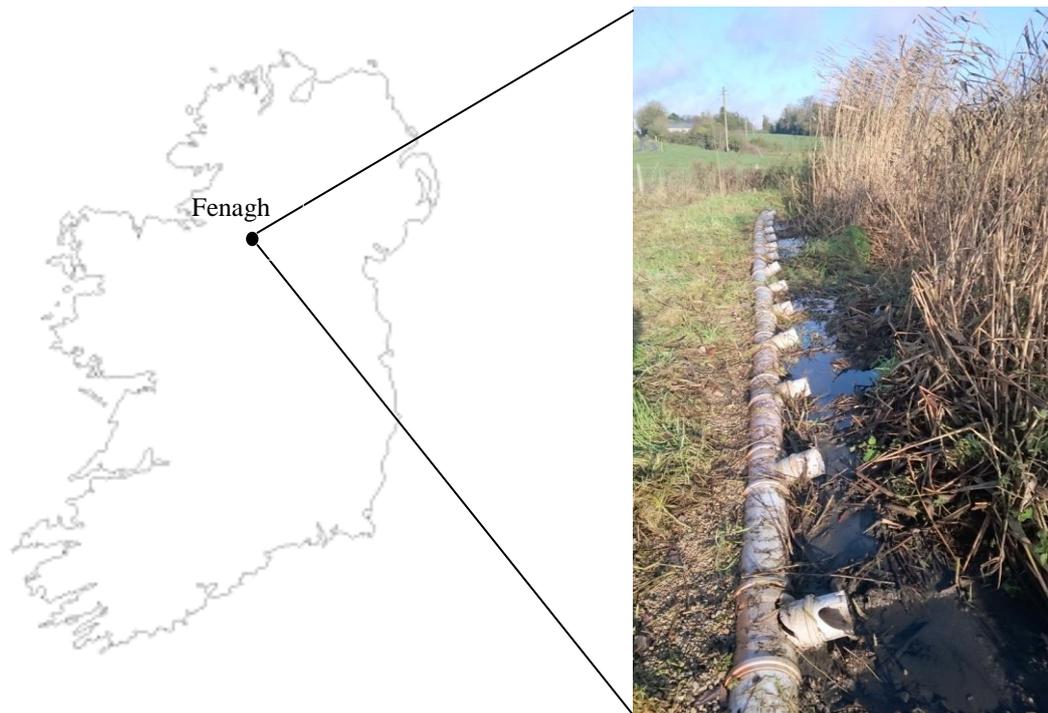


Figure 3.1 Study location: free water surface constructed wetland at Fenagh WWTP, Co. Leitrim planted with *Phragmites australis*

Table 3.1 Details of site characteristics

			Area (m <sup>2</sup> )	PE	Volume (m <sup>3</sup> )	Hydraulic retention time (d)*	Hydraulic loading rate (m d <sup>-1</sup> )*
<b>Reed bed dimensions</b>							
<i>Length</i>	<i>Width</i>	<i>Depth</i>					
(m)	(m)	(m)					
20	20	0.5	400	400	200	7.3	0.068*

\*Based on a mean flow of 27.3m<sup>3</sup> per day

### 3.3.2 Vegetation sampling regime

Sampling and analysis of vegetation was undertaken between April and November 2015 (covering four seasons in an Irish climate). Aboveground and BG biomass of *P. australis* were sampled monthly in the inlet and outlet zones (5 m from the inlet and outlet edges) of the CW. During each sampling time, four 0.25 m<sup>2</sup> quadrats were placed into each of the inlet and outlet zones of the wetland using a randomized block design. All shoots (living and dead) were clipped at ground level within each of the eight quadrats (Fig. 3.2).



*Figure 3.2* Quadrat (0.25 m<sup>2</sup>) place within constructed wetland from which aboveground and belowground biomass was removed

The BG biomass was completely dug out to a depth of 0.3 m from within the same quadrats. This depth was chosen as an appropriate depth for the vegetation study as it reflects the depths of roots and rhizomes of *P. australis* in CWs (J. Vymazal *pers. comm.*). Upon delivery to the laboratory, the BG samples were thoroughly washed with potable water to remove all sediment and gravel. The washing was performed in large containers to minimize loss of hairy roots. The AG biomass consisted of stems,

leaves and flowers combined, and the BG biomass consisted of roots and rhizomes combined. All samples of AG and BG biomass were then dried in a 70°C oven (after Vymazal et al., 2010) until samples reached constant weight, and the total dry biomass was calculated ( $\text{g biomass m}^{-2}$ ) (Fig. 3.3). Aboveground and BG samples were then ground in a mill and a subsample was tested in the laboratory. This process was repeated monthly.

### 3.3.3 Laboratory analysis

Nitrogen testing was carried out by combustion analysis using a Carla Erba nitrogen analyser following the Association of Official Analytical Chemists (AOAC) method 990.03 (2005). The instrument was calibrated daily with an atropine standard. Quality control (QC) [National Institute of Standards and Technology (NIST)] tomato leaf check samples were run throughout analysis (every ten samples). Phosphorus, Cu and zinc (Zn) were digested using nitric acid and hydrogen peroxide in a CEM Mars microwave system and analysed using a Thermo 65 Duo ICP following P4.3 “Soil, Plant and Water Reference methods for the Western Region” (Gavlak et al., 2003). Check samples were run through the ICP system every 50 samples. Cadmium (Cd), chromium (Cr), Ni and lead (Pb) were analysed using Inductively Coupled Plasma (ICP) mass spectrometry after digestion with *aqua regia* (1:3  $\text{HNO}_3$ :  $\text{HCl}$ ) at 110°C for three hours. Similarly, calibration standards and QC samples were run initially followed by blank, spiked and matrix spiked samples throughout the analysis (every ten samples) for verification purposes. Using these data, the AG and BG biomass and nutrient and metal content for each sampling section were obtained. Standing stocks were calculated as follows: standing stock ( $\text{g m}^{-2}$ ) = concentration ( $\text{g kg}^{-1}$ )  $\times$  dry matter ( $\text{kg m}^{-2}$ ).



Figure 3.3 Aboveground (left) and belowground (right) biomass samples of *Phragmites australis*

### 3.3.4 Statistical analysis:

A full factorial (i.e. including first order interaction) Two-way ANOVA and Tukey (HSD) post hoc tests ( $P < 0.05$ ) were used for statistical analysis of biomass along with metal and nutrient concentration of *P. australis*. The two independent variables were month and AG versus BG with dependent variables being various metal and nutrient concentrations, and biomass. All significant values were reported at alpha  $P < 0.05$ . All data analysis was conducted on SPSS version 24.

## 3.4. Results

### 3.4.1 Aboveground and belowground biomass

The average dry AG and BG biomass harvested during the study is presented in Fig. 3.4. Maximum recorded AG biomass in the study was recorded in August ( $1,636 \text{ g m}^{-2}$ ), while biomass was lowest in June ( $835 \text{ g m}^{-2}$ ). Belowground biomass which ranged from  $523 \text{ g m}^{-2}$  to  $872 \text{ g m}^{-2}$  represented 53% to 62% of the AG biomass, respectively. There was a statistically significant ( $P = 0.002$ ) interaction between AG and BG biomass and month of the year.

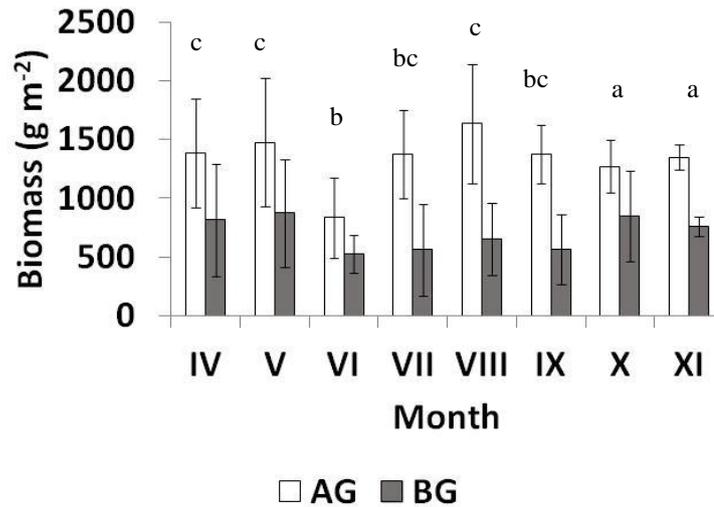


Figure 3.4 Average amounts of aboveground (AG) and belowground (BG) biomass (inlet and outlet zones combined) in the wetland vegetation during the period of April – November, 2015. Error bars represent the standard deviation. Different letters indicate significant differences between the monthly means at  $P < 0.05$ .

### 3.4.2 Seasonal pattern of metal concentrations and accumulations

Average Cd and Pb concentrations in the influent wastewater were below the limit of detection (LOD) during the study (Table 3.2), and likewise were not detected in either the AG or BG biomass. Both Cr and Ni concentrations were lower in AG than BG, or were below the LOD (Fig. 3.5). Belowground values for both peaked in August (12.7 mg kg<sup>-1</sup> for Cr and 4 mg kg<sup>-1</sup> for Ni). The BG organs cumulatively held > 80% of the total Ni and Cr in the plant as a whole. The interactions between AG versus BG, and month of the year was significant ( $P < 0.05$ ), with respect to the concentrations of both Ni and Cr in the biomass of *P. australis*.

Table 3.2. Average concentrations of heavy metals in inflow wastewater entering the constructed wetland at Fenagh during the study period (April – November, 2015) (n = 3)

Metals (total)	Limit of Detection (LOD)	Average result (n = 3)	Units	Limits in surface water ( $\mu\text{g L}^{-1}$ ) <sup>1</sup>
Cadmium <sup>3</sup>	0.3	<0.3	$\mu\text{g L}^{-1}$	1
Chromium	3.0	<0.3	$\mu\text{g L}^{-1}$	50
Copper	3.0	7.0	$\mu\text{g L}^{-1}$	1,000
Lead <sup>3</sup>	0.9	<0.9	$\mu\text{g L}^{-1}$	50
Nickel	1.5	1.9	$\mu\text{g L}^{-1}$	4 <sup>2</sup>
Zinc	10	17	$\mu\text{g L}^{-1}$	1,000

<sup>1</sup>From Subsidiary Legislation 549.21, 28<sup>th</sup> June, 2002

<sup>2</sup>From Directive 2013/39/EU, 12<sup>th</sup> August 2013

<sup>3</sup>Cadmium and lead consistently reported below the LOD

The average influent Cu concentration measured during the study was  $7 \mu\text{g L}^{-1}$  (Table 3.2). Belowground concentrations of Cu ranged from  $17.6 \text{ mg kg}^{-1}$  to  $28.5 \text{ mg kg}^{-1}$ , and were always higher than AG concentrations, which ranged from  $7.1 \text{ mg kg}^{-1}$  to  $16.7 \text{ mg kg}^{-1}$  (Fig. 3.5). Aboveground standing stock of Cu was highest early in the growing season in April ( $15.4 \text{ mg m}^{-2}$ ). No significant ( $P > 0.05$ ) interactions occurred between months and AG versus BG, for the concentration of Cu in the biomass. Zinc concentrations were highest in AG organs in September and November ( $165.2 \text{ mg kg}^{-1}$  and  $165.6 \text{ mg kg}^{-1}$ ). Zinc standing stocks were also highest during these months ( $233.9 \text{ mg m}^{-2}$  and  $224.3 \text{ mg m}^{-2}$ ). The highest monthly concentration of Zn was measured in BG organs in September ( $187 \text{ mg kg}^{-1}$ ), and the lowest was measured in May ( $77.1 \text{ mg kg}^{-1}$ ). There was no significant ( $P > 0.05$ ) interaction between AG versus BG, and month of the year for the concentration of Zn in *P. australis* biomass throughout the study (Fig. 3.5).

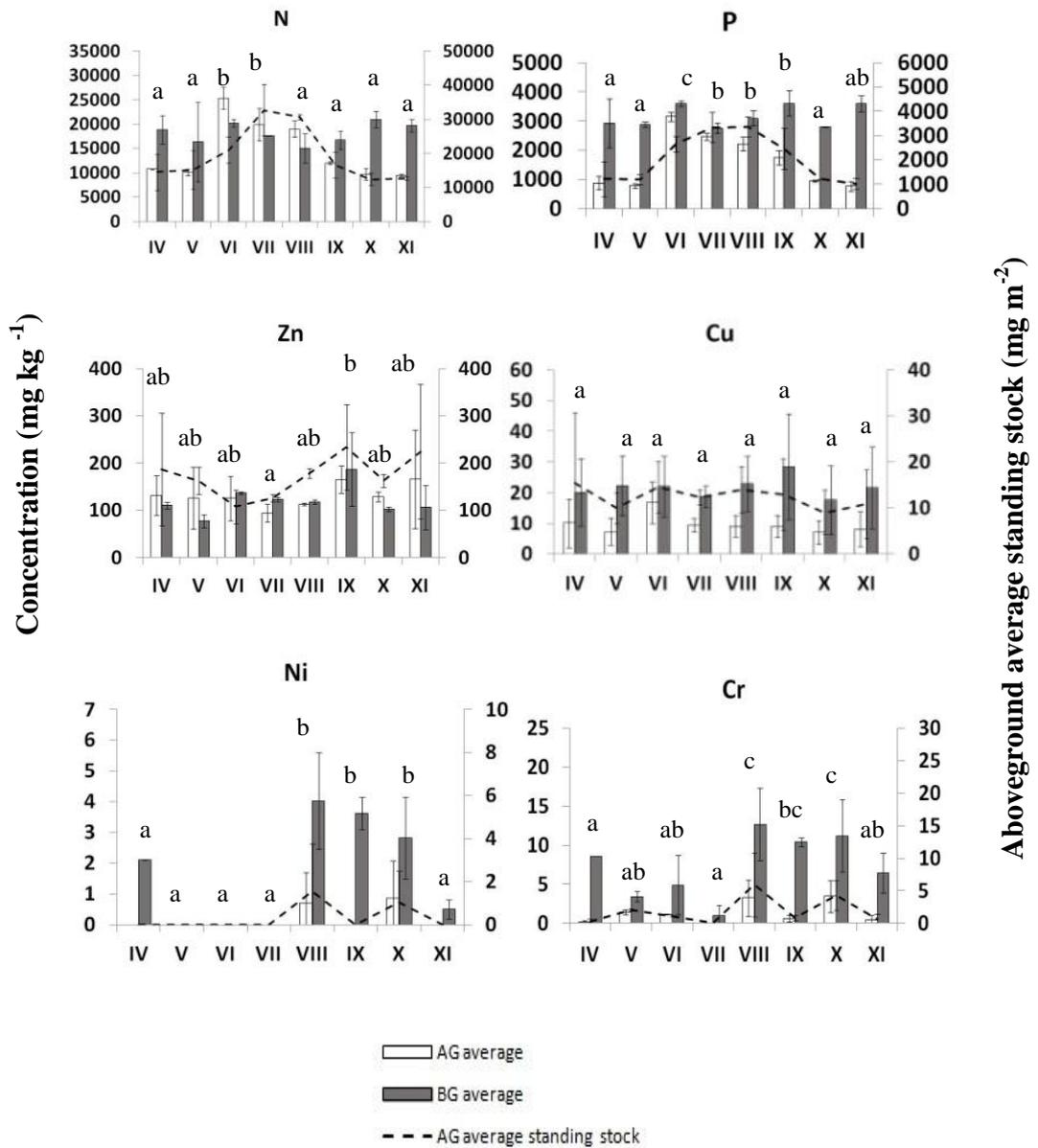


Figure 3.5 Comparison of the seasonal variation in aboveground (AG) and belowground (BG) concentrations of nutrients (nitrogen and phosphorus) and metals (zinc, copper, nickel and chromium) (mg kg<sup>-1</sup>) and aboveground standing stocks (mg m<sup>-2</sup>) in biomass of *Phragmites australis* during the study. Error bars represent the standard deviation. Different letters indicate significant differences between the monthly means at  $P < 0.05$ .

### 3.4.3 Seasonal pattern of nutrient concentrations and accumulations

Concentrations and AG standing stocks of N and P are presented in Fig. 3.5. Nitrogen concentrations in the AG tissues peaked in June (25,338 mg kg<sup>-1</sup>), the early growing season in Ireland, and declined from then to its lowest concentration of 9,463 mg kg<sup>-1</sup> in November. Nitrogen was lowest in the BG tissues in August (15,000 mg kg<sup>-1</sup>) and highest in October (20,975 mg kg<sup>-1</sup>). The maximum nitrogen AG standing stock (32.6 g m<sup>-2</sup>) was measured in July. The AG biomass cumulatively contained almost half (44%) of the total N accumulated in the CW. The interaction between AG versus BG and month of the year was significant ( $P < 0.05$ ) with respect to the concentration of N in the biomass of *P. australis*.

Concentrations AG of P peaked in June (3156 mg kg<sup>-1</sup>) and steadily declined throughout the study until November (768 mg kg<sup>-1</sup>). Belowground values for P ranged from 2755 mg kg<sup>-1</sup> in July to 3605 mg kg<sup>-1</sup> in September. Belowground biomass cumulatively accounted for two thirds of the total P accumulated within the wetland. The highest AG standing stock of P was recorded in July and August (3.3 g m<sup>-2</sup> and 3.4 g m<sup>-2</sup>, respectively) and lowest in November (1 g m<sup>-2</sup>). Similar to N, there was a significant interaction ( $P < 0.05$ ) between AG versus BG and month of the year for P concentrations in the study.

### 3.5 Discussion

Heavy metals enter the environment from natural and anthropogenic sources, and are non-biodegradable, accumulate in the environment, and pose a threat to the environment and human health (Ali et al., 2013). Studies examining the ability of emergent vegetation in CWs to uptake metals and nutrients have commonly examined AG vegetation only or concentrations only. However, the findings of the current study suggest that analysis of only the emergent shoots or concentrations only, may significantly underestimate the metal and nutrient uptake of the plant. Metal accumulation in the AG biomass relative to the total amount entering the system (Table 3.2) over the eight-month study period ranged from 0.02% Cu to 1.22% Zn. With the exception of Zn and N, there were higher concentrations of metals and nutrients in the BG organs of the plant during each month of analysis. Overall, Zn concentrations were cumulatively higher in AG biomass (52%) during April, May,

October and November, whereas N concentrations in AG biomass were higher during June, July and August (the typical growing season for *P. australis*). The findings of higher concentrations in BG biomass was similar to other studies (Pevery et al., 1995; Mays & Edwards, 2001; Bragato et al., 2009), and indicates that *P. australis* is prevalently a root bioaccumulator species (Bonanno, 2011). The roots and rhizomes are the immediate points of uptake in plants and, consequently, the concentrations are usually greater in roots in comparison to leaves and other AG organs (Vymazal et al., 2007). The lower concentrations in AG organs in the current study is in agreement with the speculation that plants restrict the movement of metals into their AG plant tissues to avoid the potential toxic effects of high metal concentrations on their photosynthetic organs (Bragato et al., 2006). The reduction of N and P in AG parts in October and November, is known to occur in rhizomatous plants such as *P. australis*, where the nutrients are translocated to and stored in BG organs during winter, and are ready to initiate growth the following season (Chapin III et al., 1990). The concentrations of N and P at the beginning of the study (April and May) are similar to concentrations at the end of the study (October and November), therefore it may be assumed that nutrients are overwintered in BG organs.

The current study was carried out in a lightly loaded system with a small PE (Table 3.1). Previous studies have suggested that uptake by plants in AG and BG organs, is significant only under low loading conditions (Brix, 1997), similar to that of the CW in the current study. Zinc was the only metal to be present in higher concentrations in AG biomass during some months of the study which was similar to Pevery (1995) and Schierup and Larsen (1981), where higher concentrations of Zn were found in AG plant parts and stems. Zinc plays an essential role in plant nutrition and enzymatic processes (Bonanno & Giudice, 2010). The higher concentrations of Zn in AG tissues may have occurred due to its essential function in the formation of indole acetic acid, a plant hormone which is manufactured in the stems of plants (Schierup and Larsen, 1981). Unlike Zn, which is essential to plant growth, Ni and Cr are regarded as elements which are toxic to plants (Bonanno & Giudice, 2010). Nickel was only detected in August and October in the AG biomass (Fig. 3.5), and at levels lower than 5 mg kg<sup>-1</sup>. However, *P. australis* has the potential to store up to 60 mg kg<sup>-1</sup> of Ni (Bragato et al., 2006). Chromium content has previously been recorded at 4,825 mg kg<sup>-1</sup> and 827 mg kg<sup>-1</sup> in the roots and shoots of *P. australis* in a pot study using

tannery wastewater (Calheiros et al., 2008) and values found in this study were significantly lower than this threshold level. Significant quantities of N were detected in the AG tissues of *P. australis* (up to 25,338 mg kg<sup>-1</sup>). Nitrogen removal from a CW is greatly facilitated by the plant uptake through the root system of *P. australis*. June, July and August are the growing season for *P. australis* in Ireland; therefore, higher quantities of N were found in the AG biomass during these months. In addition to this, AG biomass was lowest in June (Fig. 3.4), the typical early growing season for *P. australis* in Ireland. At this point, the majority of dead plant growth from the previous year has fallen away and new shoots are appearing. The AG biomass values in April and November are similar (1,384 g m<sup>-2</sup> and 1,346 g m<sup>-2</sup>, respectively), which leads us to believe that these values may be typical of the biomass values throughout the winter season. However, further studies are needed to verify this.

Common reed is a traditional building material which is widely used in roofs, and insulation blocks made from reed are highly valued in eco-friendly construction (Maddisson et al., 2009). With this in mind, harvesting of the AG biomass of macrophytes has been suggested by many researchers as an option for nutrient and metal removal in CWs (Bragato et al., 2006; Vymazal et al., 2010; Vymazal & Březinová, 2015). In order to maximise removal, the harvesting process needs to take place during a period of maximum content of the targeted element in the plant. However, based on the results of this study, under temperate maritime climatic conditions, metals and nutrients follow different seasonal patterns, and it is difficult to identify an optimum time for harvest to obtain maximum removal of all nutrients and metals at the same time based on the concentrations only. Therefore, if harvesting is to be considered as an option, it will be necessary to prioritise between maximising the removal of specific nutrients and metals. Furthermore, the effect of frequent harvesting on the regrowth success of *P. australis* also needs to be evaluated (Maddisson et al., 2009). However, the results of standing stocks of each metal and nutrient measured in the study, would suggest a harvest in Autumn (late August or September) which may capture the maximum contents of most nutrients and metals in the AG biomass. This could result in the removal of between 0.6 g (Ni) and 71.2 g (Zn) based on a harvest in August. While these removal values are representative of this CW which is treating municipal wastewater, values may be greater in a CW vegetation treating higher quantities of heavy metals and further studies are needed to

verify this. However, the ability of *P. australis* to accumulate metals and nutrients in AG biomass under such climatic conditions provides strong encouragement for CW applications in industrial settings. Further work is needed to investigate the translocation and accumulation of metals to the AG tissues, and the implications of harvesting in terms of regrowth success in CWs treating industrial wastewaters.

### **3.6 Conclusions**

Plant uptake and accumulation is one method of metal and nutrient removal from CWs. With the exception of Zn and N during some months of the study, BG biomass of *P. australis* predominantly contained higher concentrations of metals and nutrients than AG biomass. In order to remove maximum quantities of metals and nutrients, the harvesting process must take place during the period of maximum content of the targeted element in the plant. Knowledge of the concentrations alone does not provide information on the translocation or accumulation of elements in the plants. In order to maximise the removal of metals and nutrients in CWs, a harvest should take place during the period of maximum accumulation in AG biomass. With this in mind, a harvest in Autumn of AG biomass is suggested based on the results of this study.

### **3.7 Summary**

This chapter examined the seasonal variations of metals and nutrients in *P. australis* in a CW, and identified an optimal time for biomass harvesting for metal and nutrient removal. Chapter 4, investigates the habitat suitability of CWs for the smooth newt (*Lissotriton vulgaris*, [Linnaeus, 1758]), in comparison to NWs.

#### **4. Habitat suitability assessment of constructed wetlands for the Smooth Newt (*Lissotriton vulgaris* [Linnaeus, 1758]): a comparison with natural wetlands**

##### **4.1 Overview**

This chapter compares the suitability of CWs and NWs to the terrestrial phase of the life cycle of the smooth newt (*Lissotriton vulgaris*, [Linnaeus, 1758]) with the aim of developing recommendations for both new and existing CWs to enhance their usefulness as newt-friendly habitats.

This study is published in Ecological Engineering (Mulkeen, C.J., Gibson-Brabazon, S., Carlin, C., Williams, C.D., Healy, M.G., Mackey, P., Gormally, M.J., 2017. Habitat suitability assessment of constructed wetlands for the Smooth Newt (*Lissotriton vulgaris* [Linnaeus, 1758]): a comparison with natural wetlands. Ecological Engineering 106: 532 – 540. doi: 10.1016/j.ecoleng.2017.06.005).

##### **4.2 Introduction**

Given the current decline in NWs worldwide and the consequent negative impacts on amphibians, wetlands constructed for the treatment of wastewater have the potential to play a role in the protection of these animals. Studies to date have mainly focused on the aquatic phase of the amphibian life-cycle in CWs which supports breeding. However, the surrounding habitats in which the CW is situated, are essential for amphibians to complete the terrestrial phase (protection, feeding and hibernation) of their biphasic life-cycle.

The smooth newt, *Lissotriton vulgaris*, is the sole native species of newt to be found in Ireland and uses a variety of aquatic habitats during the breeding season including lakes, natural ponds, garden ponds and slow-moving drainage ditches (Meehan, 2013). The presence of smooth newt is also recorded at CWs for wastewater treatment (Scholz et al., 2007). After breeding in aquatic habitats, smooth newts spend the remainder of the year in the terrestrial areas surrounding wetlands, and provided that habitat quality is good, will remain close to breeding water bodies. For this reason, the terrestrial areas surrounding wetlands are important in the conservation of this red-

listed newt species. However, in Ireland, the drainage and infilling of wetlands (Staunton et al., 2014; 2015), in conjunction with excessive clearing of vegetation around breeding sites, remains a threat to smooth newt populations (King et al., 2011).

This study aims to compare, for the first time, the terrestrial habitats of NWs and CWs as refuges for the smooth newt. A HSI was applied to assess the likelihood of the presence of smooth newts and to grade CWs and NWs in the study. The results are discussed in the context of providing definitive guidelines for engineers regarding the design of CWs which incorporate features that support the conservation of the species.

### **4.3 Materials and Methods**

#### **4.3.1 Site descriptions**

Eight CWs and eight NWs were selected in counties Mayo, Galway, Roscommon and Leitrim in the west of Ireland (Figure 4.1). Each CW, built for the tertiary treatment of municipal wastewater, consisted of a surface flow reed bed planted with either *Phragmites australis* (Cav.) Trin. ex Steud. or *Typha latifolia* L. Natural wetlands, containing areas of *P. australis* and / or *T. latifolia*, within 20 km of each CW, were selected for comparison (Table 4.1). Since individuals of *L. vulgaris* have been recorded 500 m from breeding ponds (Kovar et al., 2009), habitats such as hedgerows, scrub, drainage ditches, woodland or grasslands, which have been described as newt-friendly habitats (Oldham, 2000), were found to be occurring within 500 m of each wetland. Habitats such as these could be used as connecting habitats across the landscape for smooth newts during the terrestrial stages of the life cycle.

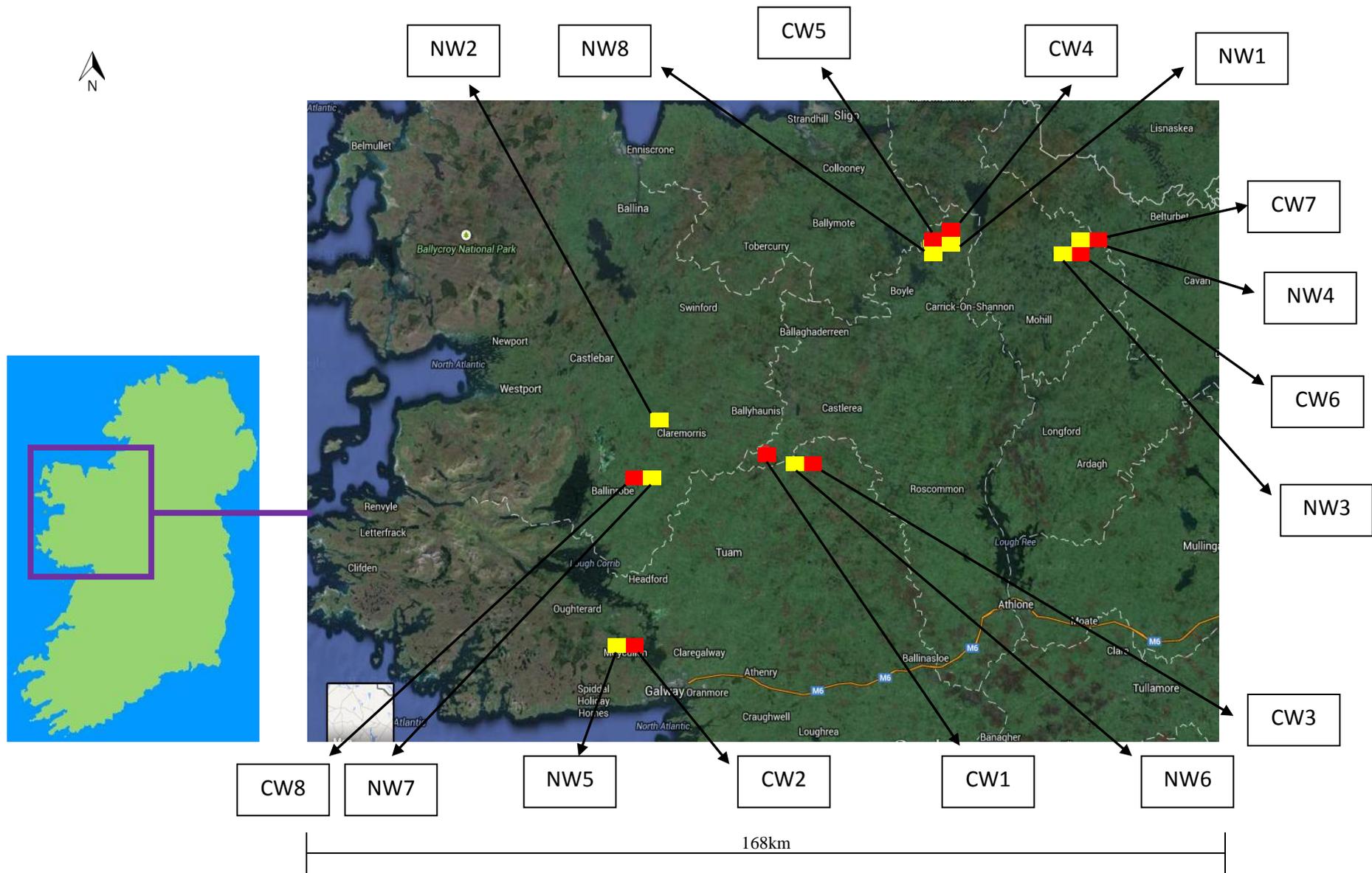


Figure 4.1 Locations of constructed (■) and natural (■) wetlands in the west of Ireland

Table 4.1 Constructed and natural wetland sites and site codes numbered from largest to smallest for each wetland type

Site code	Constructed wetland	Size (m <sup>2</sup> )	Site code	Natural wetland	Size (m <sup>2</sup> )
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 Lake	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	Clooncrufter	8,086

### 4.3.2 Habitat mapping

Between August and October 2015, habitats were mapped at all sites. A colour orthoimage, sourced from ArcGIS (Release Version 10.3; Environmental Systems Research Institute [ERSI], California, USA) and produced in 2012, was printed for each wetland at a scale of 1:2650. Given that a minimum mappable polygon size of 400 m<sup>2</sup> is recommended by Smith et al. (2011) for small-scale field mapping, orthoimages were printed with a 20 m × 20 m grid superimposed on the image to aid with mapping habitats in the field. The photograph was used as a base map in which habitats were recorded. All habitats within 40 m of the water's edge were documented since most of the *L. vulgaris* population will confine normal intra-habitat wanderings to short distances from a pond (Griffiths, 1984).

Habitats were identified, described and classified according to a standard habitat classification scheme used in Ireland covering terrestrial, freshwater and marine environments (Fossitt, 2000). This classification scheme is hierarchical and operates at three levels comprising eleven broad habitat groups at Level 1; thirty habitat sub-

groups at Level 2; and 117 individual habitats at Level 3 e.g. “Grassland and marsh” (Level 1) → Semi-natural grassland (one of three sub-groups at Level 2) → “wet grassland” (one of seven habitats at Level 3).

During the surveys of terrestrial habitats, it was noted that grasslands which would normally be classified as “improved agricultural grassland” under Fossitt’s classification (Fossitt, 2000) often consisted of poorly drained fields which supported abundant *Juncus* species. For the purposes of the current study, such sites were classified as “improved agricultural grassland with abundant *Juncus* spp.” to separate them from truly improved fields i.e. “intensively managed or highly modified agricultural grassland” with rye grasses (*Lolium perenne* L.) usually abundant (Fossitt, 2000). Notable features of importance to smooth newts such as wood or stone features (Marnell, 1998) were recorded as present or absent for each 20 m × 20 m grid square. Wood features referred to tree stumps, dead/decaying/fallen branches, fallen trees and stone features referred to boulders and loose rock.

Field survey recorded data were later digitised using ArcGIS 10.3 (Appendix C) and the areas for each habitat calculated. Wood and stone features were recorded as point features. Linear features such as treelines, hedgerows and drains were assigned an arbitrary width of 1 m (reflecting the minimum width of linear habitats encountered) so that areas of different habitats could be compared. As the total areas for each wetland varied, the wetlands in this study have been numbered consecutively from the largest to the smallest for each wetland type i.e. CW1 – CW8 and NW1 – NW8 (Table 4.1). Maps were created using ArcGIS 10.3 and the extent of all habitats was determined. Using a HSI for the great crested newt in the UK, CWs and NWs were scored and ranked in order of their potential value to the smooth newt.

#### **4.3.3 Habitat Suitability Index**

The HSI, first developed by Oldham et al. (2000) in Britain (and later modified by the National Amphibian & Reptile Recording Scheme, 2007), is used by Natural England, Natural Resources Wales and the Department of Environment, Food and Rural Affairs (UK) to assess the likelihood of the presence of the great crested newt (*Triturus*

*cristatus* [Laurenti, 1768]) in a given area in the UK (Department of Environment, Food and Rural Affairs, 2016) (Table 4.2). The great crested newt is larger than the smooth newt and has been found to travel further from ponds (> 200 m and > 500 m) (Stoefer & Schneeweiss, 2001; Kinne, 2004; Redgrave, 2009). Within their range, great crested newts have been recorded with smooth newts more than other newt species (Jehle et al., 2011). Both species also seem to have similar requirements in terms of the variety of the terrestrial habitats surrounding water bodies for dispersal (Malmgren, 2002; Griffiths, 1996) and the presence of *T. cristatus* in ponds in the UK usually seems to be a good indicator for the presence of *L. vulgaris* (Griffiths, 1996), although *L. vulgaris* can be found in a wider range of localities (Skei et al., 2006). Given the absence from Ireland of the great crested newt, *L. vulgaris* occupies a similar range of habitats, in addition to which there is considerable overlap in the timing of seasonal and diel activities (Griffiths & Mylotte, 1987) and environmental responses (Vuorio et al., 2015). For these reasons, the UK HSI for *T. cristatus* was adopted during this study as an initial starting point to assess habitat suitability in Ireland for *L. vulgaris* at a landscape-scale and prioritise areas for action. Wetlands at the lower end of the scale are evaluated and recommendations on how their suitability can be improved are proposed.

#### **4.3.4 Statistical analysis**

A Kolmogorov - Smirnov test was performed to test for normal distribution of the residuals. A General Linear Model (GLM) was used to test whether there was a significant effect of area and wetland type on habitat richness. A Pearson's Correlation was used to test whether there was any correlation between area of the wetland and the number of habitats present

*Table 4.2. Great Crested Newt (Triturus cristatus [Laurenti, 1768]) Habitat Suitability Index used for scoring terrestrial habitats around ponds (from National Amphibian & Reptile Recording Scheme, 2007)*

<b>Category</b>	<b>SI</b>	<b>Criteria</b>
Good	1	Extensive area of habitat that offers good opportunities for foraging and shelter completely surrounds pond (e.g. rough grassland, scrub or woodland).
Moderate	0.67	Habitat that offers opportunities for foraging and shelter, but may not be extensive in area and does not completely surround pond.
Poor	0.33	Habitat with poor structure that offers limited opportunities for foraging and shelter (e.g. amenity grassland).
None	0.01	Clearly no suitable habitat around pond (e.g. centre of large expanse of bare habitat).

#### **4.4 Results**

A total area of 2.25 km<sup>2</sup> (including open water) was mapped across sixteen CW and NW sites. Areas of open water and surrounding terrestrial habitats mapped at CWs range from 0.008 km<sup>2</sup> to 0.020 km<sup>2</sup>, while those of the generally larger NWs range from 0.008 km<sup>2</sup> – 1.45 km<sup>2</sup> (Table 4.1). Using Level 1 (Fossitt, 2000), “freshwater” habitats dominated the NWs overall (74%) compared to only 13% at the CWs, where “grassland & marsh” dominated (54%) (Figure 4.2). This is not surprising, given that a more in-depth analysis of freshwater habitats at Level 3 (Fossitt, 2000) revealed that the open water of the NWs (primarily lakes) is reflected by the dominance (82% cover) of “mesotrophic lakes” compared to the, not unexpected, dominance of “reed & large sedge swamp” (74%) at the CWs, represented at the NWs by a cover of just 16%. “Woodland & scrub” had similar percentage covers of 13% and 15% at the NWs and CWs, respectively (Figure 4.2) but “exposed rock & disturbed ground” and “cultivated and built land”, a total of < 2% combined at the NWs, had a cover of 8% and 10%, respectively, at the CWs.

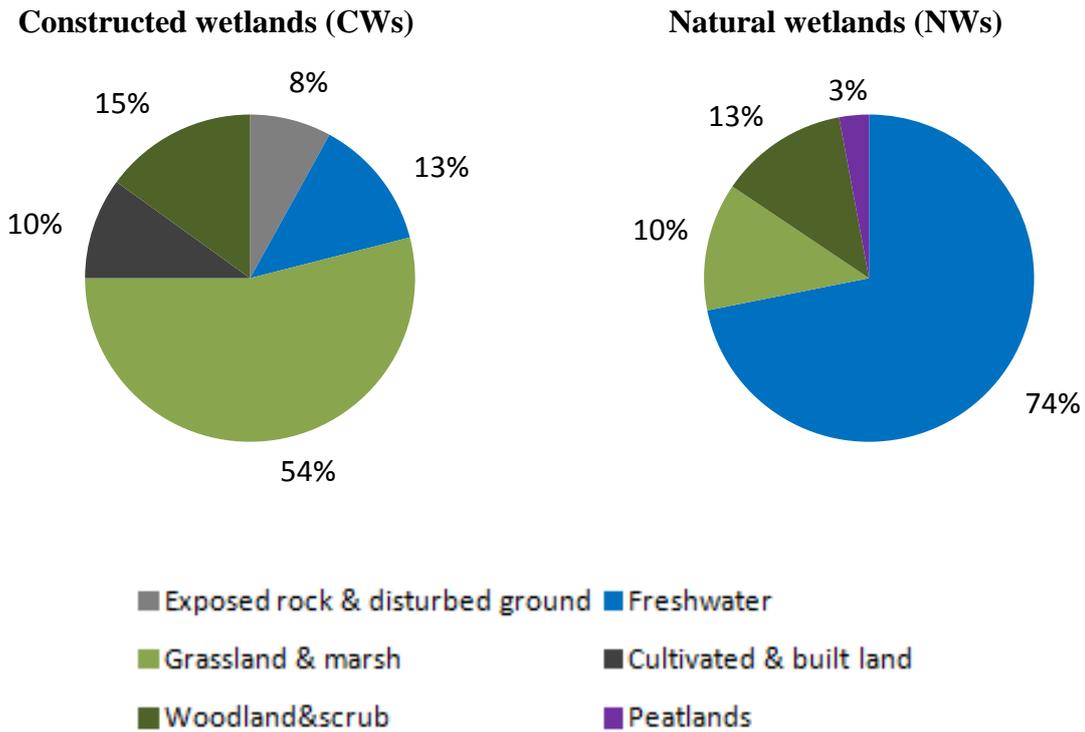


Figure 4.2 Percentage cover of terrestrial and aquatic habitats at constructed and natural wetlands (Level 1) (Fossitt, 2000) (percentages rounded to nearest whole number)

Given that the focus of this chapter is the terrestrial phase of the smooth newt which spends less than 50% of the year (generally March – July) (Bell, 1977) in still water for breeding, suitable terrestrial habitats were examined in more detail since they form an essential component of the newt life cycle (Denoël & Lehmann, 2006). With this in mind, less optimal habitats for newts from August to February (i.e. the “freshwater” habitats above with the exception of “freshwater swamps”) were removed from the analysis to examine the remaining habitats in detail for suitability for newts. “Freshwater swamps” were included in the analysis because these are not areas of fully open water, but generally occupy a zone at the transition from open water to terrestrial habitats (Fossitt, 2000). An examination of the order of dominance of terrestrial habitats (Figure 4.3) at Level 1 (Fossitt, 2000) revealed a similar pattern to those in Figure 4.2, with the exception that the percentage cover of “freshwater swamp” at the NWs was almost co-dominant with “woodland & scrub” (32% and 33%, respectively). In the CWs, “freshwater swamp” had the same percentage cover

as “cultivated and built land” (Figure 4.3) which along with “exposed rock & disturbed ground”, had overall percentage covers of 10% and 9% respectively. In NWs, both categories, along with “heath & dense bracken”, had an overall combined percentage cover of < 2%.

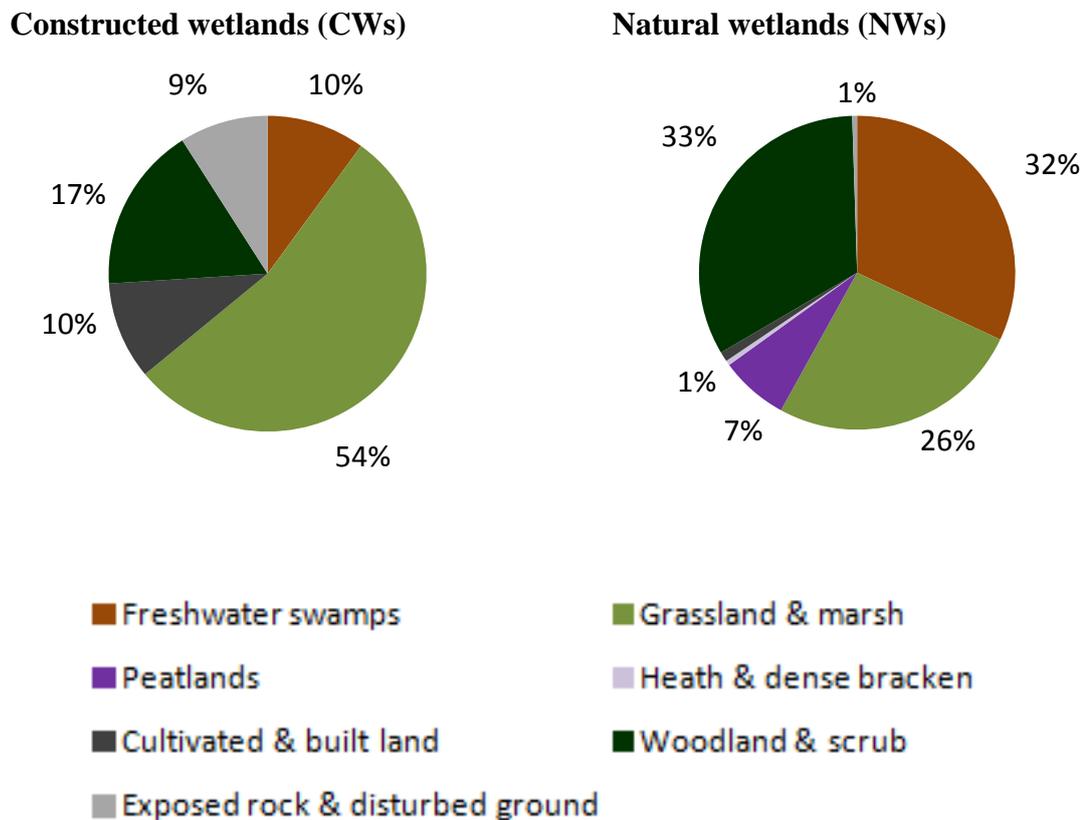


Figure 4.3 Percentage cover of terrestrial habitats (Level 1) (Fossitt, 2000) at constructed and natural wetlands excluding freshwater habitats (with the exception of freshwater swamps). (Percentages rounded to nearest whole number).

The number of newt friendly terrestrial habitats recorded at Level 3 (Fossitt, 2000) varied within each wetland type, with those in NWs ranging from 17 at the largest NW1 to seven at NW5 and from 12 habitats at CW3 to six at CW8. To test for normal distribution, a Kolmogorov – Smirnov test was used ( $P > 0.05$ ) indicating that the data are not significantly different from a normal distribution (CW area = 0.690, CW number of habitats = 0.473; NW area = 0.808, NW number of habitats = 0.598). A

Pearson’s correlation confirmed that the correlation between area of CWs and number of habitats present was not significant ( $P > 0.05$ , R squared = 0.602) in comparison to the correlation between area of NWs and number of habitats present which was significant ( $P < 0.05$ , R squared = 0.898). Using a GLM, there was a significant effect of both area and wetland type on habitat richness. The GLM displays a positive relationship between number of habitats and the covariate area and NWs had significantly more habitats than CWs (Table 4.3).

*Table 4.3. General Linear Model (GLM) of the effect of wetland type and area on habitat richness*

**Tests of Between – Subjects Effects**

Dependant variable: Number of habitats

<b>Source</b>	<b>Type III Sum of squares</b>	<b>df</b>	<b>Mean square</b>	<b>F</b>	<b>Sig.</b>
Model	1580.473 <sup>a</sup>	3	526.824	132.916	.000
Total area	82.223	1	82.223	20.745	.001
Wetland type	830.759	2	415.380	104.799	.000
Error	51.527	13	3.964		
Total	1632.000	16			

<sup>a</sup> R squared = .968 (Adjusted R squared = .961)

Given that “grassland & marsh” represented over a quarter of the cover of terrestrial habitats at both wetland types (26% and 54% for NWs and CWs, respectively) and that long grass and rough grassland are among those considered as some of the best habitats for the terrestrial phase of newts, these were examined in more detail at Level 3 (Fossitt, 2000) (Figure 4.4; Table 4.3). Nine different “grassland & marsh” habitat types were found in the current study. “Wet grasslands” represented more than half (52%) of the cover of the “grassland & marsh” habitats at the NWs, but less than a

quarter (24%) at CWs, where “improved agricultural grassland” was dominant (44%). “Improved agricultural grassland with abundant *Juncus* spp.” represented 13% and 22% cover at NWs and CWs, respectively, while “freshwater marsh”, present at the NWs (6%), was absent from the CWs (Figure 4.4; Table 4.4).

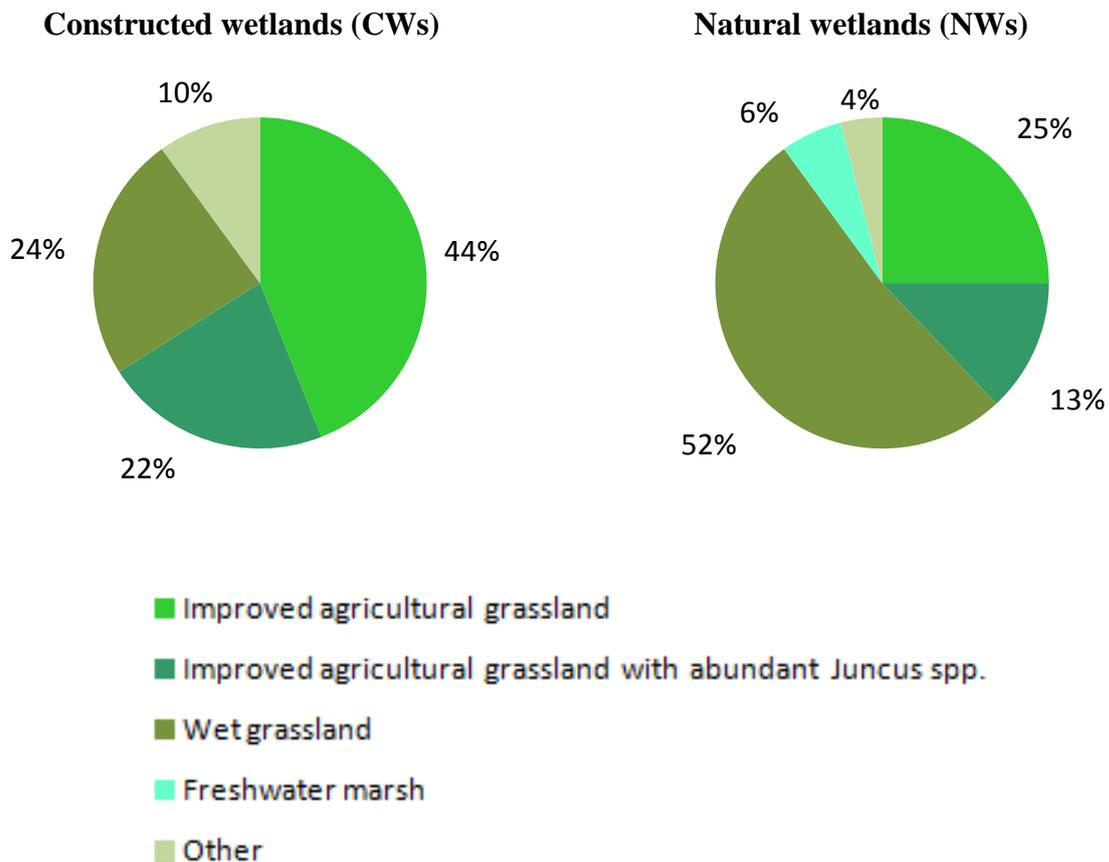


Figure 4.4. Percentage cover of “grassland & marsh” habitats ( $\geq 5\%$  cover) at constructed and natural wetlands (Level 3) (Fossitt, 2000). The breakdown of “grassland & marsh” habitats (Fossitt, 2000) with which had less than 5% cover and represented as “Other”, is presented in Table 4.4.

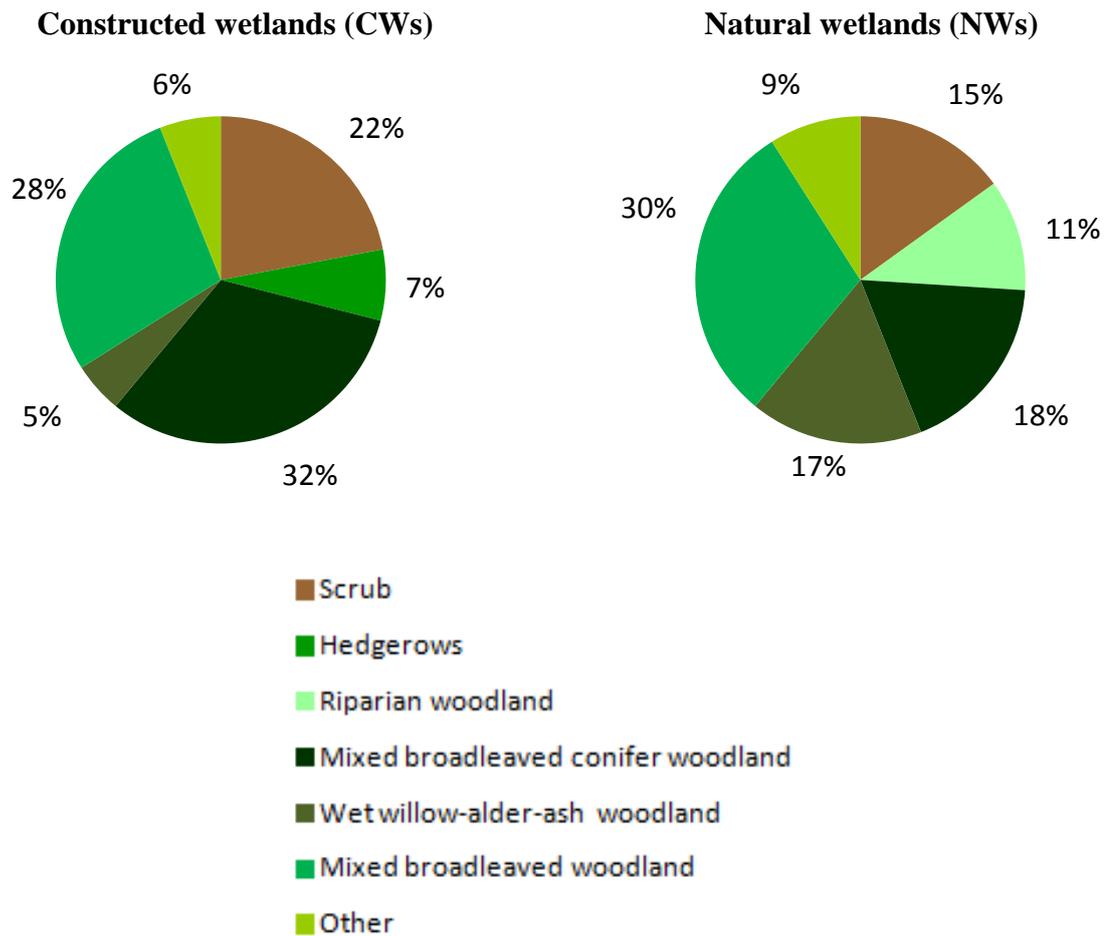
Since woodland, damp woodland, scrub and hedgerows are also considered excellent terrestrial habitats for smooth newts (Table 2.2), these were examined further (Figure 4.5; Table 4.4) at Level 3 (Fossitt, 2000). Altogether, twelve “woodland and scrub” habitat types were present at CWs and NWs.

Table 4.4 Breakdown of “grassland & marsh” and “woodland & scrub” habitats with < 5% cover (presented as “Other” in Figure 4.4 and Figure 4.5)

Habitat (Fossitt, 2000)	% cover at CWs	% cover at NWs
<b>Grassland &amp; marsh</b>		
Amenity grassland	5	2
Dry-humid acid grassland	3	0
Dry meadows & grassy verges	2	0
Dry calcareous and neutral grassland	<1	0
Wet grassland/scrub	0	2
<b>Woodland &amp; scrub</b>		
Treeline	4	<1

“Mixed broadleaved woodland” and “mixed broadleaved conifer woodland” cover combined, dominated both wetland types with 48% and 60% cover at the NWs and CWs, respectively (Figure 4.5; Table 4.3). These were followed by “wet willow-alder-ash” (17%) and “scrub” (15%) at the NWs and “scrub” (22%) and hedgerows (7%) at the CWs. “Riparian woodland” and “bog woodland” were exclusive to NWs with 13% cover in total.

Given that, regardless of habitat type, barriers to movement by newts play a pivotal role in newt survival, these were also examined at the CW and NW sites. These barriers include roads and rivers which are classed as serious barriers to newt migration (Oldham, 2000; Matos et al., 2017). Other barrier habitats (directly bordering breeding sites) identified include “buildings & artificial surfaces”, “improved agricultural grassland”, “exposed sand, gravel & till”, and “spoil & bare ground”. Forty-four percent of the total perimeter of the CW sites in this study constituted potential barriers to newt migration compared to < 2% at NW sites. While six out of eight CWs had barriers of some kind, only one of eight NWs had barriers.



*Figure 4.5 Percentage cover of “woodland and scrub” habitats ( $\geq 5\%$  cover) at constructed and natural wetlands (Level 3) (Fossitt, 2000). Breakdown of “woodland & scrub” habitats with  $<5\%$  cover (Other) is presented in Table 4.4*

The significance of terrestrial microhabitats or features such as wood and stone which can act as potential refuges for newts, can contribute significantly to amphibian conservation when selecting breeding sites (Marnell, 1998). Twenty-eight percent of the 20 m  $\times$  20 m grids surrounding the NWs which were surveyed in this study contained features compared to just 18% for the CWs. Habitats such as “mixed broadleaved woodland” and “mixed broadleaved conifer woodland” accounted for the greatest percentage frequencies (5 – 11%) of features at both wetland types, with “wet willow-alder-ash woodland” within the same range for NWs only (Table 4.5). Features present within a range of 1 – 4% frequency (Table 4.5), included “riparian

woodland” at the NWs, and “recolonising bare ground”, “improved agricultural grassland” and “wet willow-alder-ash-woodland” at CWs.

*Table 4.5 Percentage frequency of occurrence of features (wood and stone) in habitats at constructed and natural wetlands*

<b>Habitat code (Level 3) (Fossitt, 2000)</b>	<b>% frequency CWs</b>	<b>% frequency NWs</b>
(Mixed) broadleaved woodland	5.3	10.3
Mixed broadleaved conifer woodland	5.3	6
Recolonising bare ground	1.8	0.04
Improved agricultural grassland	1.1	0.1
Wet willow-alder-ash woodland	1.1	6.2
Dry-humid and acid grassland	0.4	0
Wet grassland	0.4	0.4
Scrub	0.4	0.1
Rich fen and flush	0	0.1
Reed and large sedge swamps	0	0.7
Marsh	0	0.2
Hedgerows	0	0.1
Riparian woodland	0	3
Cutover bog	0	0.05
Conifer plantation	0	0.1
Bog woodland	0	0.3
Recently-felled woodland	0	0.05
Exposed sand, gravel or till	0	0.2
Treelines	0	0.05
Improved agricultural grassland with abundant <i>Juncus</i> spp	0	0.1

Using the HSI, only two out of the eight CWs received the highest score of 1 (*Good*), while seven of the eight NWs received a *Good* score (1), in that there were no barriers present (Table 4.6). One hundred percent of the perimeter lines of all CWs and NWs

which received *Good* scores, contained extensive areas of habitat with good opportunities for foraging and shelter completely surrounding the wetland. One CW (CW4) received a *Moderate* score of 0.67, where 17% of the perimeter line of the CW is made up of “buildings & artificial surfaces”, while one NW (NW4) received a *Moderate* score (0.67) due to the presence of “buildings & artificial surfaces” (0.4% of the perimeter) directly bordering the lake. Five of the CWs received *Poor* scores (0.33) while none of the NWs received a *Poor* score.

*Table 4.6 Constructed and natural wetlands and their potential value to the terrestrial phase of the life cycle of the smooth newt using the Great Crested Newt Habitat Suitability Index (Table 4.2) (National Amphibian & Reptile Recording Scheme, 2007)*

<b>Constructed wetland</b>	<b>Score</b>	<b>Natural Wetland</b>	<b>Score</b>
CW1	1	NW1	1
CW2	0.33	NW2	1
CW3	0.33	NW3	1
CW4	0.67	NW4	0.67
CW5	1	NW5	1
CW6	0.33	NW6	1
CW7	0.33	NW7	1
CW8	0.33	NW8	1

#### **4.5 Discussion**

The results of this study indicate that the NWs had significantly more terrestrial habitat types than CWs and that the number of terrestrial habitat types present in NWs was significantly correlated with the size of the area containing the terrestrial habitats. Both NWs and CWs were selected on the basis of: (1) the presence of reed and large sedge swamps (2) their location i.e. paired CWs and NWs  $\leq 20$  km apart; and 3) the presence of newt friendly terrestrial habitats within 500 m of the wetland. Nevertheless, given that most of the NWs were lakes (Table 4.1), the generally larger

size of aquatic habitats, including open water, resulted in comparatively larger areas of terrestrial habitats being surveyed within 40 m of the water's edge than in the smaller CWs. In addition, while similar woodlands at both wetland types were most likely to contain features of benefit to newts, almost twice as many grids (20 m × 20 m minimum mappable areas) in the terrestrial habitats of NWs contained features compared to those of CWs. Furthermore, “wet grassland” dominated the grasslands around NWs while “improved agricultural grassland” dominated the grasslands around CWs. The latter grasslands, which are generally managed through intensive grazing regimes, cutting and the application of fertilizer / herbicides, may result in the absence of structural diversity such as that of rough grassland and meadows – habitats which can offer cover and foraging for the terrestrial phase of the newt (Oldham, 2000). “Wet grassland” (often occurring on sloping ground with poorly drained soils) with abundant rushes, tall grasses and a high broadleaved herb component (Fossitt, 2000) may, in comparison to “improved agricultural grassland”, offer more potentially suitable terrestrial habitats. Areas of “marsh” unique to NWs in this study (along lake shores), can also offer good structural habitats, particularly for immature newts, given the presence of high moss cover in conjunction with rushes (*Juncus* spp.), sedges (*Carex* spp.) and a high proportion of broadleaved herbs. This is reflected in the HSI scores, where seven of the eight NWs, but only two of the eight CWs, received a “good” score. A number of CWs received lesser scores primarily because of the presence of a barrier to movement which could potentially impact on the migration of the newt from aquatic to terrestrial habitats. This is reflected by almost one fifth of the surface area of the CWs examined in this study consisting of “cultivated & built land” and “exposed rock & disturbed ground”, some of which is necessary for machinery access to the site.

Previous studies have emphasized the value of using CWs as a conservation strategy for amphibians and the need for future research and monitoring in these areas (Denton & Richter, 2013). While our study focused on suitable terrestrial habitats for newts and did not involve a survey of smooth newt abundance, a single adult specimen of the species was recorded on the edge of one CW during the study (Mulkeen & Gibson-Brabazon, *pers. obs*). The presence of newts in CWs in Ireland (Scholz et al., 2007) also suggests that water quality in CWs treating wastewaters, at least in some cases, is not an issue and can support breeding by newts. In addition, newts have been

recorded in natural ponds and wetlands as small as 25 m<sup>2</sup> (Skei et al., 2006) and with up to 1,000 individuals recorded in ponds less than 400 m<sup>2</sup> (Bell & Lawton, 1975). Regardless of waterbody size, if aquatic and terrestrial conditions are favourable for breeding, shelter, food and overwintering, it is likely that newts may colonise and breed in these areas. However, small changes to the design of new CWs, and the management of the lands surrounding both new and existing CWs, could enhance their dual role as water treatment systems and suitable habitats for the newt and other amphibian species.

In the design of new CWs, the overall size of the site should be considerably larger than the actual wetland itself to ensure that the area surrounding the wetland is of sufficient size to provide adequate refuges for the terrestrial phase of the newt. While lands outside the CW fence may provide suitable refuges for the newt when the CW is being constructed, there is no guarantee that this area will not be lost to development at some time in the future. As a guideline, and based on the evidence observed by previous authors of smooth newt migration distances (Bell, 1977; Dolmen, 1981), it is desirable that a buffer zone around a CW be incorporated within the site. By way of example, the inclusion of 20 m minimum buffer zone (providing suitable terrestrial habitats for smooth newts) around a 20 m × 20 m (400 m<sup>2</sup>) CW would result in the purchase of just an additional 0.32 ha. However, the width of the buffer zone may be amphibian species specific (Rothermal, 2004) with Calhoun et al. (2014) recommending a buffer zone of 300 m of forested areas surrounding vernal pools to favour the persistence of amphibian species such as wood frog and salamander in the USA (Calhoun et al., 2014). While buffer zones wider than 20 m could also accommodate juveniles who appear to travel greater distances during dispersal, further research is required to substantiate this. Large areas of open habitat offering little cover can act as a barrier during newt migrations to and from water bodies for breeding. Habitats such as “amenity grassland”, “improved agricultural grassland”, “spoil & bare ground” and “buildings & artificial surfaces”, offer little cover, shelter, hibernation, foraging or overwintering sites for newts. By their very nature, CWs built for the tertiary treatment of wastewater also contain areas covered with artificial surfaces such as tarmac or concrete, built structures for wastewater treatment and unpaved areas for access points and driveways. These should, however, be reduced to a minimum, particularly immediately adjacent to the edge of the CW. If hard surfaces

are required adjacent to the CW, they should ideally be at one side only, leaving the other three sides with direct access to terrestrial habitats.

Prior to construction taking place, a habitat survey should be undertaken to determine the value of existing habitats to newts. The proximity of the proposed construction to the nearest NWs should also be considered as suggested by Drayer & Richter (2016), which may strengthen connectivity across the landscape (Calhoun et al., 2014). In particular, habitats identified in this study such as “mixed broadleaved woodland”; “mixed broadleaved conifer woodland”, “wet willow-alder-ash woodland” and scrub should be retained where possible, as should “wet grassland” and “improved agricultural grassland with abundant rushes”. In sites undergoing construction, judicious planting with suitable trees and shrubs and / or the creation of wet grassland using membranes beneath the soil surrounding the CW would also be beneficial. In particular, the availability of terrestrial cover around breeding sites in the form of logs and deadwood was found to be an important habitat parameter in discriminating between sites used or unused by the smooth newt during its life cycle (Marnell, 1998). Therefore, the addition of features such as stones or wood to all types of existing habitats around CWs would also enhance these areas as newt refuges. Skei et al. (2006), Marnell (1998) and Oldham (2000) suggest that woodland and scrub offer smooth newts suitable terrestrial habitats to complete the terrestrial phase of the life cycle. By their very nature, woodland and scrub habitats usually present a highly structured habitat, which could offer shelter and refuge in the form of large amounts of deadwood, often in the form of tree stumps, fallen branches or logs. At existing CWs, less frequent mowing of “improved” or “amenity grasslands” would encourage the growth of a greater proportion of tall, coarse or tussocky grasses, and a broadleaved herb component which could offer suitable refuge or foraging areas for newts. Even a reduction in the management (cutting and herbicide applications) of unpaved surfaces or gravel would facilitate the colonisation of plants over time which also decreases site maintenance costs. Therefore, without compromising the vital function of access to the CW and wastewater treatment areas, these unconsolidated surfaces with plant cover may also assist smooth newts during their migrations from aquatic to terrestrial habitats.

An indication of the variability of CWs vis-à-vis their suitability for smooth newts can be seen in the contrasting HSI scores for two CWs, one scoring “good” and one scoring “poor”. The CW which received a “good” score is completely surrounded by favourable terrestrial habitats, which provide good structure for the smooth newt during migrations (scrub; earth bank; treeline; and dry meadows & grassy verges). No barriers were identified on the wetland edge and despite it being located in an urban area, an adult specimen of the smooth newt was recorded on the edge of the wetland within the “scrub” habitat under a wood feature during the study (Mulkeen & Gibson-Brabazon, *pers. obs*). The CW which received a “poor” score is surrounded by an unsuitable terrestrial habitat for newts i.e. “spoil & bare ground” which could act as a barrier to newt migration. “Spoil & bare ground” includes areas of bare ground due to ongoing disturbance or maintenance, unconsolidated surfaces which are regularly trampled or driven over, and areas which are largely unvegetated (<50% cover) (Fossitt, 2000). Areas such as these are open and provide little structure or protection for the smooth newt during migrations from the wetland to favourable terrestrial habitats. The relocation (where possible) of bare ground or unconsolidated surfaces with trampling activities, away from the edge of a CW, along with the creation of a grassland / woodland (with a diversity of structures) plus the simple addition of wood and/or stone features could, at minimal cost, support successful newt migrations from aquatic to terrestrial habitats.

#### **4.6 Conclusions**

Natural wetlands have significantly more terrestrial habitat types than CWs and the size of NWs is significantly correlated with the number of surrounding terrestrial habitat types. Seven of the eight NWs received a “good” score using the HSI in comparison to two of the eight CWs. Constructed wetlands received lower scores primarily because of the presence of unsuitable habitat types or barriers which could potentially impact the migration of the newt from aquatic to terrestrial habitats. Therefore, in the future design of new CWs, it is important that the overall size of the site be larger than the actual CW itself. The inclusion of an additional 20 m buffer zone (at the very minimum) around new CWs, containing no barrier habitats, is recommended for CW designers during the design stage. Larger areas of buffer zones could have the capacity to provide a supplementary range of newt-friendly habitats and refuges. The buffer zones should facilitate the incorporation of newt-friendly

terrestrial habitat which is immediately adjacent to the edge of the CW and consist of an abundance of wood and stone features to act as refuges for smooth newts. Appropriate management of the areas surrounding CWs, could also enhance these areas for smooth newts and other amphibian species.

The HSI used in the current study assessed the likelihood of the presence of smooth newts and did not involve a survey of smooth newt abundance. However, based on the results of this study, appropriate next steps should include field investigations which would be useful in confirming newt presence or absence and would benefit site maintenance staff in their attempts to conserve the species.

#### **4.7 Summary**

This chapter compared the terrestrial habitats surrounding CWs and NWs as refuges for the smooth newt. The recommendations for the design and management of new and existing CWs as newt-friendly habitats may help efforts targeted to conserve the species. Of similar conservation concern are insects, of which only 0.12% of insect species are currently protected by law in Europe (Leandro et al., 2017). Equally under-studied in CWs are terrestrial insects, in spite of the vital ecosystem services they provide such as wildlife dietary needs and the decomposition of litter. Chapter 5 investigates the Diptera: Sciomyzidae assemblages in CWs and NWs and further discusses the implications for CW design.

## **5. Sciomyzid (Diptera) assemblages in constructed and natural wetlands: implications for constructed wetland design**

### **5.1 Overview**

The aim of this chapter is to compare sciomyzid flies (known bioindicators of dipteran communities of wetland habitats) of CWs and NWs, and determine the impacts of water quality and the habitats surrounding CWs and NWs on sciomyzid community structure.

### **5.2 Introduction**

Natural wetland environments offer a variety of niches to many invertebrate species (Kadlec & Wallace, 2009) which are recognised as essential components of NWs and are known for their high diversity within NW habitats (Wu et al., 2009). Significant ecosystem functions carried out by invertebrates are critical to the energy dynamics in NWs (Greenway and Simpson, 1996) and include assisting in the decay of wetland litter (Murkin and Wrubleski, 1988), and acting as a food source for other wildlife (de Szalay et al., 1997). A reasonable body of knowledge exists regarding the aquatic phases of freshwater invertebrates in CWs (Jurado et al., 2009; 2010). However, there is a paucity of knowledge regarding the aerial phases of wetland invertebrate species associated with CWs. Consequently, the full biodiversity potential of CWs has yet to be revealed (Jurado et al., 2014).

While the true flies (Diptera) are frequently associated with NWs, they have commonly been excluded from ecological surveys due to perceived difficulties in sampling in NWs and the requirement for specialist taxonomic skills (Keiper et al., 2002). However, sampling of the aerial phases of dipterans can provide useful data on the terrestrial phase of aerial insects (Keiper et al., 2002). The predominantly wetland specialist dipteran family, the Sciomyzidae (marsh / shade flies), are known bioindicators of dipteran communities in wetland habitats (Carey et al., 2017). Using sciomyzids for biodiversity studies in CWs is, therefore, a logical choice given their microhabitat specificity (Williams et al., 2010) and their potential as bioindicators of wetland habitats (Carey et al., 2015). In addition, the impacts of water quality on the

abundance / diversity of Sciomyzidae which has not heretofore been studied, has yet to be addressed.

This study will, for the first time, compare sciomyzid assemblages of CWs and 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 sciomyzid assemblages will also be quantified for the first time. The results of this study will be used to inform the future design and optimum location of CWs to enhance their value to biodiversity.

### **5.3 Materials and Methods**

#### **5.3.1 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 (Figure 4.1). Each CW consisted of a surface flow reed bed treating municipal wastewater. Eight NWs containing areas of Reed and Large Sedge Swamp (Fossitt, 2000) were selected for comparison and all NWs had an inlet and outlet stream. 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. All NWs had an inflowing stream or river, and an outflowing stream or river for water sampling collections.

#### **5.3.2 Invertebrate sampling**

Sciomyzids were sampled at all CWs/NWs using Malaise (black nylon Townes design; Townes, 1972) and emergence (modified Townes design) traps (Figure 5.1). 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 (Met Eireann, 2017). Emergence traps were positioned directly on the reed beds of the CWs and NWs to capture emerging adult sciomyzids. Trap collection heads containing a 70% ethanol solution, faced in a south-westerly direction (Speight et al., 2000). Malaise traps were activated on 21<sup>st</sup> May 2014 with samples collected approximately every three weeks until 29<sup>th</sup> 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 sciomyzid flies were identified to species level using Rozkošný (1987) and Vala (1989).



*Figure 5.1 South-westerly facing malaise trap in operation at CW4, Keadue, Co. Leitrim (2014)*

### **5.3.3 Habitat mapping**

Between August and October 2015, habitats were mapped at all CW and NW sites. Similar to the habitat mapping methods used in Chapter 4, 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 m<sup>2</sup> 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 sciomyzids exhibit limited movement (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; thirty habitat sub-groups at Level 2; and 117 individual habitats at Level 3. 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 (Table 4.1).

### **5.3.4 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 outflowing 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. As some parameter values may change during storage and transport of water samples, the time between sampling and analysis was kept to a minimum, and samples were refrigerated until analysis began (a maximum of 48 hours later in some cases).

Water samples were tested for pH using a pH probe (WTW, Germany) and for SS using vacuum filtration through Whatman GF/C (pore size 1.2  $\mu\text{m}$ ) filter paper. Subsamples were filtered through 0.45 $\mu\text{m}$  filters and analysed for  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$  and ortho - phosphorus ( $\text{PO}_4\text{-P}$ ) using a Konelab nutrient analyzer (Konelab 20, ThermoClinical Labsystems, Finland). Unfiltered samples were tested for TN and total phosphorus (TP) using a BioTector analyzer (BioTector Analytical Systems Ltd., Cork, Ireland), and for COD and BOD. All water quality parameters were tested in accordance with the standard methods (APHA, 2005).

### **5.3.5 Statistical analysis**

Univariate analysis was carried out on SPSS version 24.0. This included Pearson correlations and Spearman Rank correlations - 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 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 entropy 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).

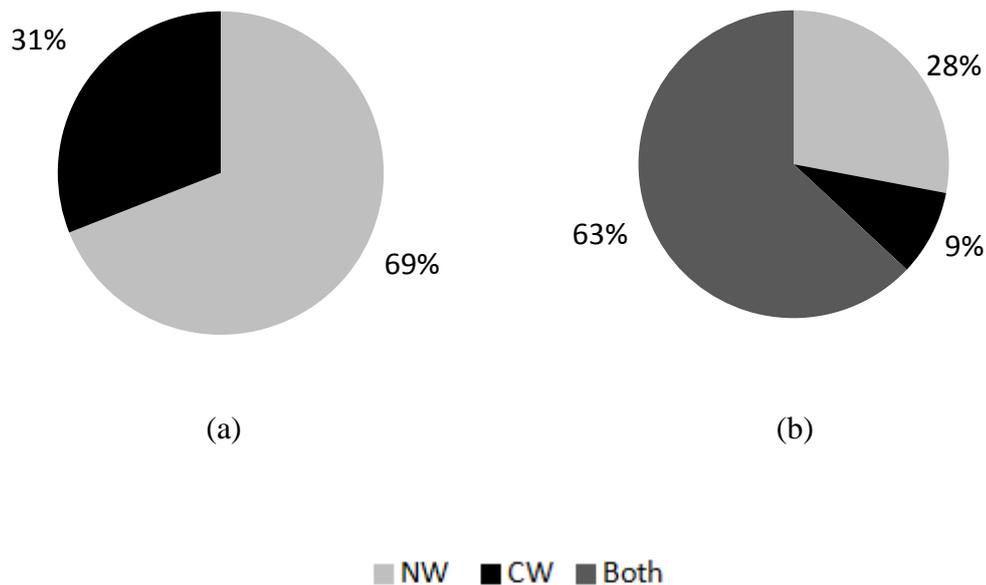
Multivariate statistical analyses were performed on the data to assess factors such as water quality and surrounding habitat richness on community dynamics using PC-Ord (version 6.0). Non-metric multidimensional scaling (NMS) ordinations of sciomyzid samples, in the primary matrix, with water quality and habitat variables in the secondary matrix, were undertaken using the Sørensen distance measure and a two-dimensional NMS solution was chosen. An Indicator Species Analysis (ISA) which relies on relative frequency and relative abundance was used to assess the fidelity of sciomyzid species to a particular level of grouping variable, which was wetland type (i.e. CWs versus NWs). Multi-response permutation procedure (MRPP), a non-parametric test, was used to test whether there was any effect of wetland type on species composition. A PERMANOVA, a multivariate analogue of the univariate ANOVA, was also used to test whether there were any significant differences in species composition between CWs and NWs.

Residuals of water quality variables were tested for normality (Kolmogorov-Smirnov test) and equality of variance (Levene's test). COD, BOD, SS, TN NH<sub>4</sub> and PO<sub>4</sub>-P 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. 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.

#### **5.4 Results**

Over half the known Irish sciomyzid 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%), while 31% of the total abundance was captured at CWs (Figure 5.2a). Species richness was also greatest at NWs (29 species)

in comparison to 23 species at CWs (Appendix D). Twenty-eight percent of the total number of species captured (32) were found exclusively at NWs, 9% were exclusive to CWs, while 63% of species captured were common to both wetland types (Figure 5.2b).



*Figure 5.2 (a) Percentage abundance of sciomyzids captured in Malaise traps at constructed and natural wetlands (n = 654); and (b) percentage of sciomyzid species captured at constructed, natural and both wetland types (n = 32)*

An ISA which assessed the fidelity of sciomyzid 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. Residuals of sciomyzid 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 sciomyzid 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 (Figure 5.3).

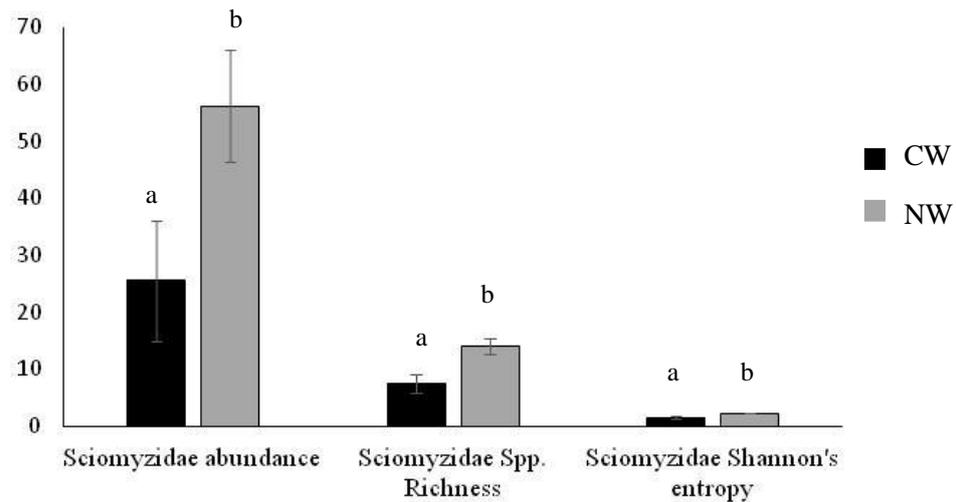


Figure 5.3 Mean ( $\pm$  S.E.) sciomyzid abundance, species richness, and Shannon's entropy on CWs and NWs. Different superscripts indicate significant differences ( $P < 0.05$ ) between CWs and NWs for each category as tested by the Independent samples *t*-tests.

Species richness at CWs ranged from just two species at CW2 to fourteen species at CWs 4 and 5 (Figure 5.4a). At NWs, species richness ranged from nine species at NW2 to twenty species at NW4 (Figure 5.4a). A PERMANOVA also revealed that overall there was a significant quantitative difference ( $P = 0.003$ ) in species composition between CWs and NWs. The abundances of sciomyzids 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-Weiner), a species diversity measure (Ellison, 2010), was greatest at NW4 and lowest at CW2 (Figure 5.4b).

Non-metric multidimensional scaling (NMS) ordinations resulted in two significant axes (Fig. 5.5), 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.

Water quality variables, which were more strongly correlated with Axis 1, indicate that poorer water quality (i.e. greater levels of nitrogen, phosphorus, COD and BOD) was more linked to CWs than NWs. In all cases, water quality values for TN, NH<sub>4</sub>, TP and PO<sub>4</sub>-P were significantly ( $P < 0.05$ ) greater (i.e. more polluted) in the CWs than in the NWs.

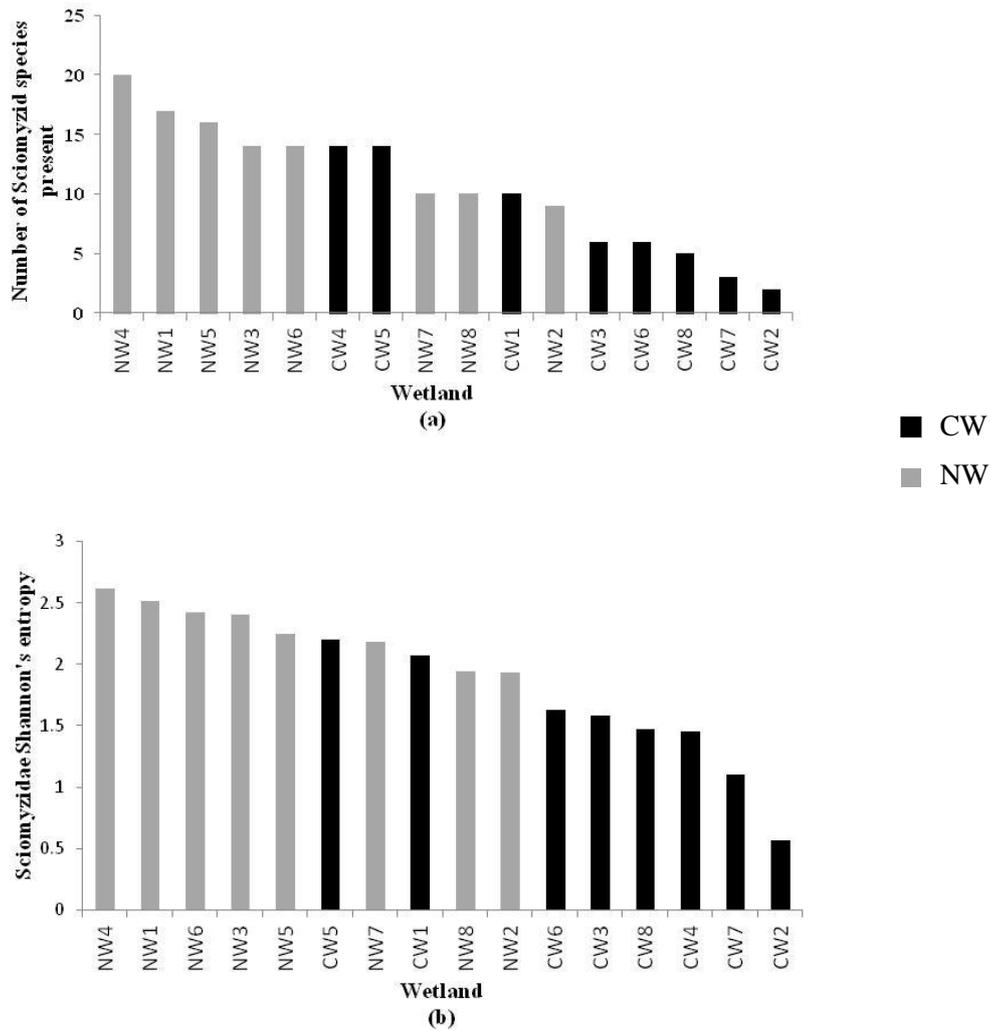


Figure 5.4 (a) Sciomyzid species richness; (b) Shannon's entropy at constructed and natural wetlands

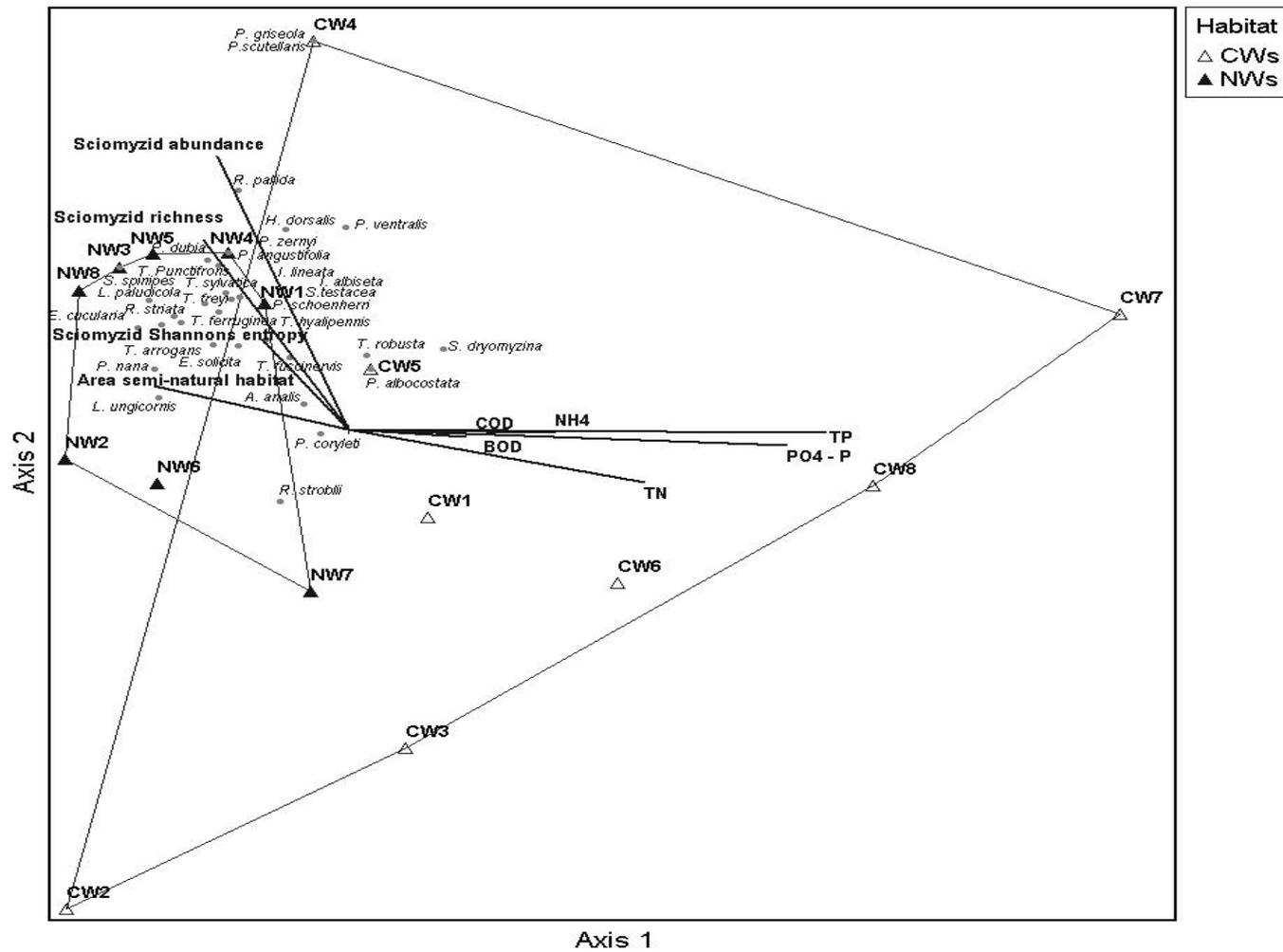


Figure 5.5 Non-metric multidimensional scaling plot of constructed and natural wetlands with sciomyzid species overlaid with water quality variables and habitat area and type. Axes 1 and 2 account for 41.2% and 46.9% of the variation, respectively.

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 (Figure 5.5).

*Renocera pallida* Fallén, 1820 was the most commonly captured species in CWs, followed by *Tetanocera hyalipennis* Roser, 1840 and *Sciomyza dryomyzina* Zetterstedt, 1846 (Figure 5.6). At NWs, *T. arrogans* Meigen, 1830 was most common followed by *R. pallida* Fallén, 1820 and *T. ferruginea* Fallén, 1820 (Figure 5.6). Over half of the sciomyzid species captured overall during the study were multivoltine species, while more than one quarter were univoltine species.

To investigate the influences of habitats mapped in the study, sciomyzid total abundance, species richness, and sciomyzid 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 sciomyzid diversity, richness and total abundance at CWs and NWs (Table 5.1). A linear regression investigating the effects of *Log* reedbed area on *Log* sciomyzid species richness at CWs and NWs also revealed that there was no effect of area of reed bed on sciomyzid species richness. However, a linear regression between *Log* area of semi-natural habitats within 25 m of the Malaise traps and *Log* sciomyzid species richness at CWs and NWs combined, revealed a significant ( $P = 0.021$ ) relationship (Figure 5.7).

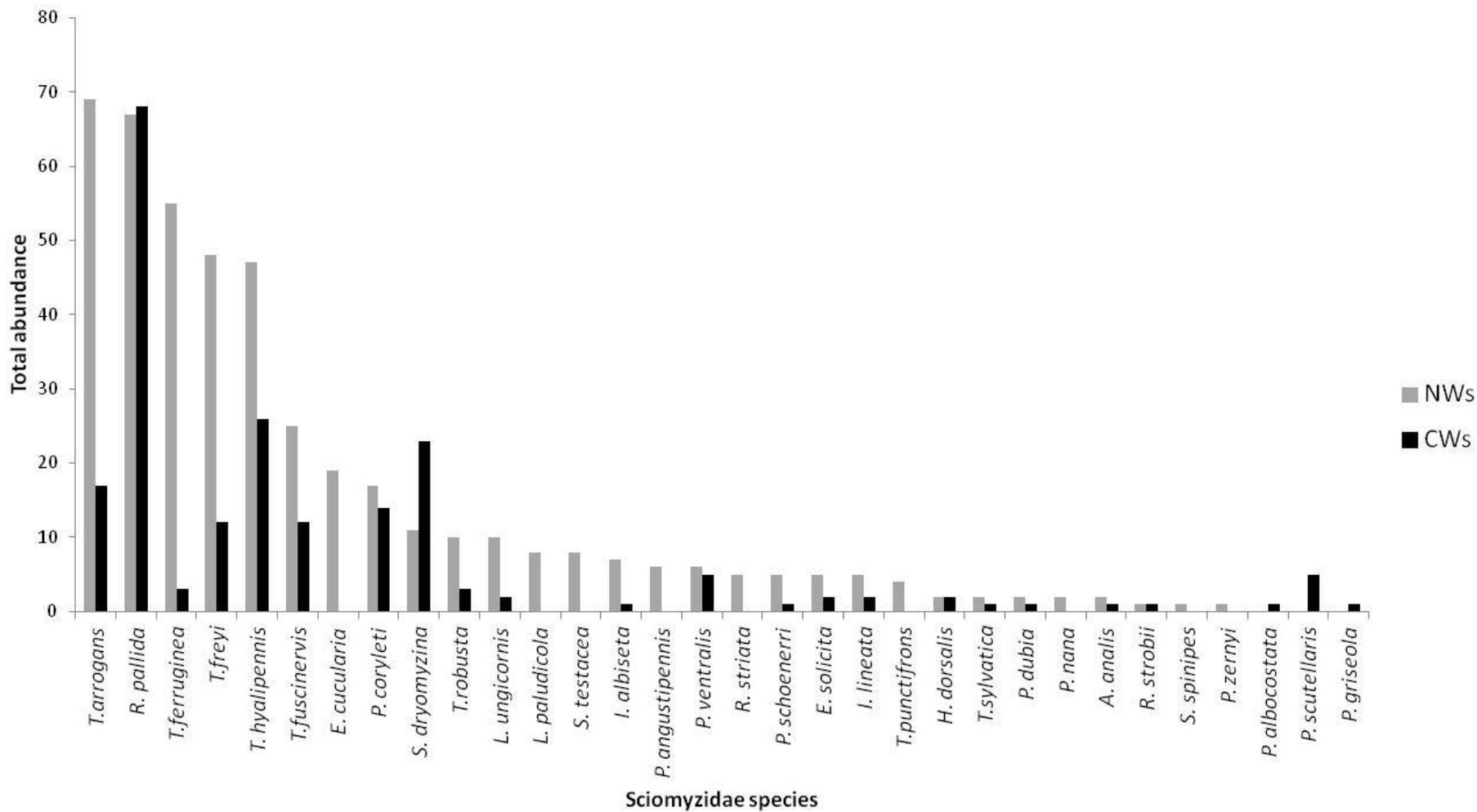


Figure 5.6 Total abundances of species captured in Malaise traps at constructed and natural wetlands

Table 5.1. Relationships between surrounding habitat and semi-natural habitat richness / diversity (Shannon's entropy) and sciomyzid diversity (Shannon's entropy), richness and 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	<b>Sciomyzidae total abundance</b>	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	
	<b>Sciomyzidae species richness</b>	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	
	<b>Sciomyzidae Shannon's entropy</b>	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	<b>Sciomyzidae total abundance</b>	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
		<b>Sciomyzidae species richness</b>	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	
<b>Sciomyzidae Shannon's entropy</b>		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	

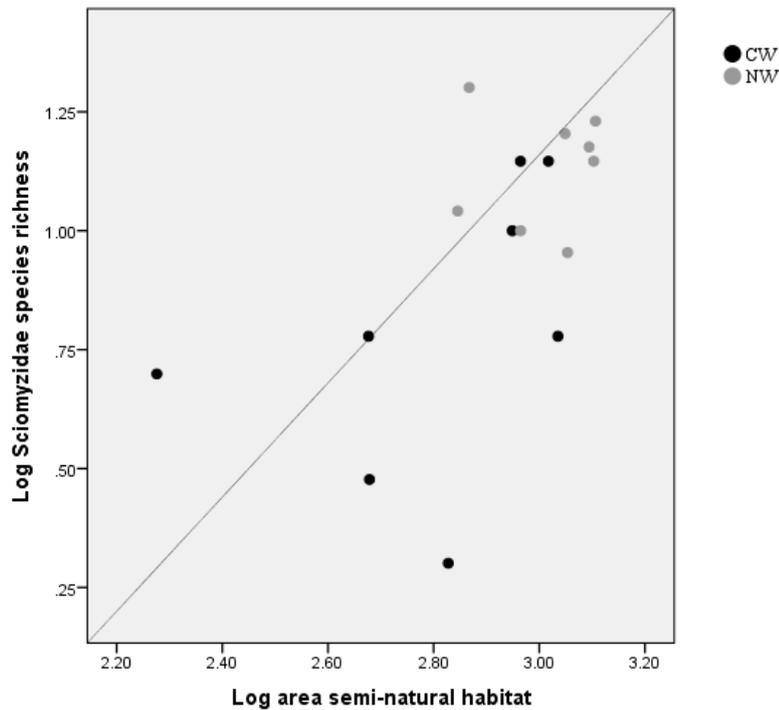


Figure 5.7 Linear regression of Log area of semi-natural habitat within 25 m of malaise traps and Log sciomyzid species richness at constructed and natural wetlands

A total of six individuals of four species were captured in the study using the emergence traps in 2015. Two individuals of *Pherbellia dubia*, Fallén, 1820 emerged during the month of April – one at CW4 and another at NW6. The individual at CW4 was the sole individual to be captured in emergence traps at CWs. *Pherbellia dubia* is a multivoltine species which overwinters as a pupa. At NW6, an individual of the multivoltine species, *T. ferruginea*, was collected from emergence traps during May. *T. ferruginea* is also known to overwinter as a pupa. One individual of *R. pallida* was found at NW1 and two individuals of *Pteromicra angustipennis* Staeger, 1845 were found at NW4 during June. Both *R. pallida* and *P. angustipennis* are multivoltine species which overwinter as pupae.

Seven species captured across both trap types during the course of this study are mentioned by Falk (1991) in The Scarc and Threatened Flies of Great Britain Review (Table 5.2).

Table 5.2 Sciomyzid species collected during the study at constructed and natural wetlands and listed in *The Scarce and Threatened Flies of Great Britain Review* (Falk, 1991) (Knutson & Vala, 2011)

Species	Status	Habitat	Ecology	Recorded in present (malaise trap) study
<i>Antichaeta analis</i> (Meigen, 1830)	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> (Fallen, 1820)	Notable	Fens, bogs, dune slacks, damp woods. Requirement for standing water	Parasitoid of aquatic snails. Multivoltine	Constructed wetlands
<i>Pherbellia nana</i> (Fallen, 1820)	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> (Mayer, 1953)	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> (Meigen, 1830)	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> (Rondani, 1868)	Notable	Damp woodland, riverside, damp heathland, coastal marsh	Larvae predatory or parasitoid of gastropod molluscs	Natural wetlands (Appendix C)
<i>Tetanocera freyi</i> (Stackelberg, 1963)	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> (Zetterstedt, 1846)	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; **Vulnerable:** Taxa believed likely to move into the Endangered category in the near future if the causal factors continue operating; **Rare:** Taxa with small populations that are not at present in endangered or vulnerable but are at risk; **Notable:** Species which are estimated to occur within the range of sixteen to one hundred modern 10km squares

## 5.5 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, 63% of Sciomyzidae species captured were common to both wetland types and 9% 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. What is more, the presence at CWs of species such as *Antichaeta analis* (Rare), *Tetanocera freyi* (Rare), *Sciomyza dryomyzina* (Vulnerable) and *Pherbellia griseola* (Notable) (Table 2) 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. All four of these listed species 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., 2017) 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., 2017) 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 habitats surrounding the malaise trap (within 25m) for sciomyzids. 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.

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. 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. 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. 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, NH<sub>4</sub>, TP and PO<sub>4</sub>-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.

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 exceptionally 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.

## **5.6 Conclusions**

Constructed wetlands enhance biodiversity in the locations in which they are placed. The results of the study show that NWs have significantly greater species richness, abundances and diversity of sciomyzid 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 sciomyzid was present at CWs. Moreover, seven of the eight CWs hosted species of sciomyzid that are listed as “scarce” and “threatened” by Falk (1991). In terms of raising the public awareness of CWs, outreach and public engagement activities such as workshops and presentations could be carried out to inform local communities of the biodiversity value of CWs for these and other rare species. In addition, field visits from schools and universities as well as setting up information boards featuring CWs for their environmentally friendly wastewater treatment capabilities, would also create an appreciation for these systems.

The results of this study show that CWs are critical in providing a habitat to invertebrates such as sciomyzid flies, habitats that may be otherwise absent from the

surrounding landscape in which CWs are commonly situated. However, sciomyzid species richness was shown to increase as the surrounding area of semi-natural habitat increased. Sciomyzid flies are not considered a pest species and are not a health risk to humans, pets or livestock. 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 sciomyzid assemblages, which are known bioindicators of dipteran communities in wetlands.

## 6. Conclusions and Recommendations

### 6.1 Overview

Natural wetlands, one of the most important ecosystems on Earth, are continuously being destroyed across the globe despite the many ecosystem services they deliver. The capacity of NWs to carry out important services such as wastewater treatment and water purification is therefore significantly reduced. In more recent times, the construction of wetlands for the treatment of various types of wastewater is becoming increasingly accepted as a sustainable, green and efficient method for wastewater treatment. In comparison to the wastewater treatment capabilities of these CWs, their potential for the enhancement of biodiversity, an ancillary benefit, has received relatively little attention to date.

In this thesis, CWs were examined from the perspectives of wastewater treatment and biodiversity enhancement. In relation to wastewater treatment, the role of vegetation in the removal of metals and nutrients from wastewaters was investigated. In relation to biodiversity enhancement, the role of CWs in comparison to NWs in the provision of biodiversity, with particular reference to the smooth newt and sciomyzid flies, was investigated.

### 6.2 Conclusions

The main conclusions from the study are as follows:

- Constructed wetland vegetation has the capacity to uptake and store varying levels of metals and nutrients. This study showed that *P. australis*, which is native to Ireland, can accumulate metals and nutrients in both the aboveground and belowground parts of the plant. The maximum concentrations and accumulations of metals and nutrients varied throughout the year, and the mass contained in the belowground parts of the plant was up to 80% of that which was contained in the aboveground parts. This indicates that traditional testing methodology, which mainly measures the emergent shoots, may

significantly underestimate the metal and nutrient uptake capacity of the plant and therefore the importance of its role in wastewater treatment.

- Results from the study showed that if vegetation harvesting as a means of metal and nutrient removal is to be considered, a harvesting schedule should be put in place to target specific metals or nutrients of concern. These may not necessarily reach their maximum accumulations in above and belowground parts of the plant contemporaneously. Therefore, harvesting of emergent vegetation may need to be conducted at different times in the year with due regard to the potential impacts on biodiversity.
- In light of the historical and current losses of NWs and their associated biodiversity worldwide, evidence from this study has shown that CWs are important contributors to biodiversity and may present an opportunity to enhance biodiversity greatly in the often relatively species-poor locations in which they are placed. In addition, the presence of less common species of Sciomyzidae suggests that poorer water quality is not an issue, at least for these species.
- The application of a Habitat Suitability Index to CWs and NWs classified the NWs and their surrounds, as providing better quality terrestrial habitats in comparison to the CWs for the smooth newt. Similarly, NWs had significantly greater species richness, abundance and diversity of sciomyzid flies than CWs. However, this study found that CWs can now be viewed as critical in providing an appropriate habitat to species of conservation concern such as the smooth newt, and scarce and threatened sciomyzid flies, that may be otherwise absent in the surrounding landscape in which CWs are placed.
- No standard for CW design to optimize their performance currently exists in Ireland. Traditionally, most Irish CWs have been designed in accordance with empirical equations that have been developed for climates dissimilar to that of Ireland and therefore there is the possibility that some CWs may not be optimally sized. The performance database of CWs in Ireland created during this project, will over time provide an evidence-based picture of the performance of CWs in Ireland for CW designers, engineers and scientists to develop a standard for Ireland and countries with temperate oceanic climates. It is recommended that the biodiversity enhancement proposals outlined for the future design of CWs, and the

management recommendations for new and existing CWs, be publicized using the Constructed Wetlands of Ireland database.

- Adjustments to the future design of CWs may enhance biodiversity without hampering their primary function of wastewater treatment. The following specific adjustments could be considered in the design of new constructed wetlands:
  - A reduction in the amount of hardened surfaces at sites should be considered during the design stage. Hardened surfaces may be necessary for machinery access to sites. However, these areas should consist of artificial surfaces such as stone which can readily colonise with herbaceous plants. This would allow some plant-cover for migrating amphibians as well as a source of food for insects. These surfaces are preferable to artificial surfaces such as concrete or tarmac which provide no advantages to migrating amphibians or insects.
  - The addition of suitable newt-friendly habitats such as grassland or scrub immediately adjacent to the CW should be considered during the design stage. As newts tend to stay close to breeding sites after leaving water bodies (provided that suitable newt-friendly habitat is available), a minimum buffer zone of 20 m is recommended around the CW to provide a source of food and shelter to newts and amphibians during the terrestrial phase of the life cycle. The 20 m buffer zone should incorporate scrub and grassland areas. Judicious planting of native trees is also recommended. The addition of wooden logs and tree branches, stones and rocks, would also provide a selection of areas of refuge and food sources in the buffer zone during the terrestrial phase. These habitats and features are also beneficial for invertebrates and other species groups at CWs.
- In existing CWs, food and shelter requirements can also be accommodated with the simple addition of these stone and wooden features adjacent to the CW and around the site in all habitat types.

- The following future management recommendations should be considered in new and existing CWs:
  - A reduction in herbicide applications around CW sites would allow the recolonisation of plants which are beneficial to amphibians and invertebrates.
  - Less frequent mowing of the grasslands would encourage the growth of tall and coarse grasses, as well as a more broadleaved component in the grasslands. This will enhance CWs for amphibians, invertebrates and other animal groups in comparison to frequently mowed amenity grasslands.

### **6.3 Recommendations for future work**

- Results from this study indicated that emergent vegetation has good potential for metal removal. This suggests that they could be useful in the treatment of waters with a high metal concentration, such as landfill leachate. Currently, only around 23% of landfill leachate is treated on site, with the remaining part being treated at municipal wastewater treatment plants. The use of constructed wetlands for landfill leachate treatment should be further investigated.
- Further studies comparing metal and nutrient removal capabilities in other commonly-used CW vegetation types such as *Typha* spp. L. and *Phalaris* spp. L., would be useful in determining the most appropriate vegetation types for metal uptake in CWs in Irish climatic conditions.
- Growth and metal/nutrient uptake rates in young vegetation stands are quite high relative to older vegetation stands. Therefore, harvesting of emergent vegetation may enhance removals in CWs, but this aspect of wetland management requires more research. In addition, the impact of harvesting and vegetation removal on the biodiversity of the CW should be addressed by conducting biodiversity surveys before, during and after harvesting operations.
- In this study, a HSI assessed the likelihood of the presence of smooth newts at CWs and NWs. A field investigation of smooth newts, such as egg searches, torching and refugia searches for smooth newts, would be useful in confirming

newt presence or absence at sites. This information would benefit site maintenance staff in their efforts to help conserve the species by reflecting appropriate management activities across the site.

- An investigation of the impacts of water quality at CWs on a full range of cursorial and aerial invertebrates which have some requirement for aquatic habitat to complete their life cycle may provide a comprehensive analysis of the value of CWs to invertebrate conservation.
- While this study examined the aerial phases of sciomyzid flies, additional studies examining the aquatic / semi-aquatic larval and pupal phases are recommended to determine the effects of water quality on these life-cycle stages.
- As the larval stages of sciomyzid flies are natural enemies of molluscs such as freshwater, semi-terrestrial or terrestrial snails, slugs and fingernail clams, it would be beneficial to carry out mollusc surveys at CWs to determine whether the generally more polluted waters in CWs result in any negative effects on host prey.

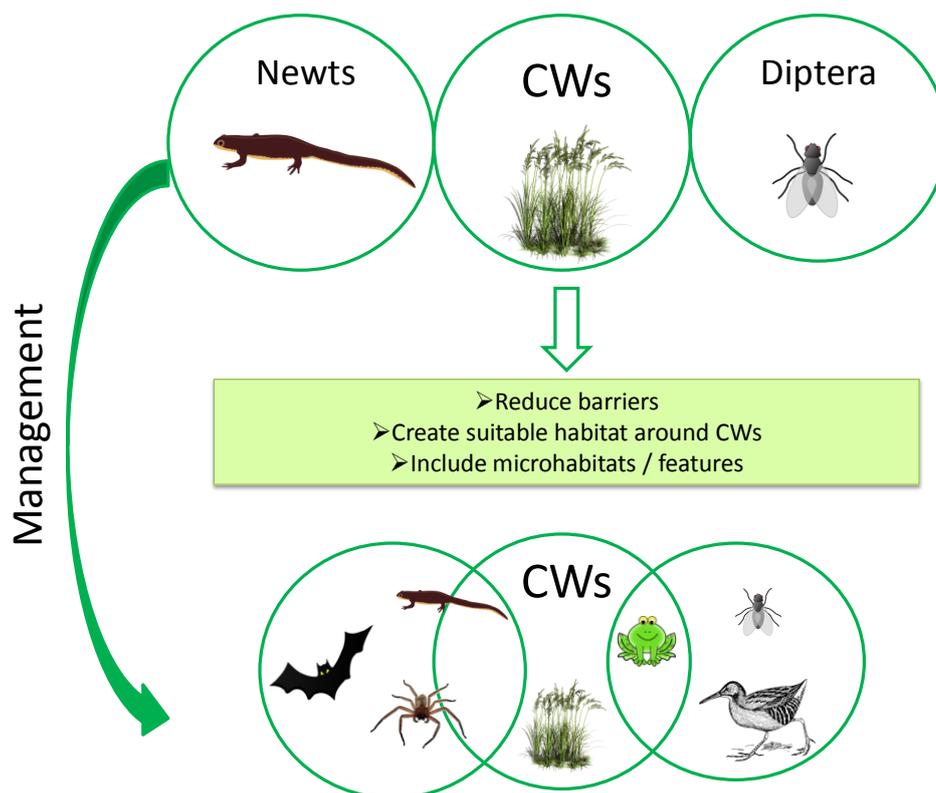
#### **6.4 Concluding remarks**

Constructed wetlands are acknowledged as economical, low-maintenance systems for the treatment of various types of wastewater. The first aim of this study was to investigate the performance of the vegetation in a CW in relation to nutrient and metal removal. The results of the study concluded that metal and nutrient accumulations in the plant biomass followed contrasting seasonal patterns. In the context of how CWs are managed with a view to metal and nutrient removal via harvesting of vegetation, this study suggests taking cognisance of plant biomass in the identification of an optimal time for vegetation harvesting. This information may be used in the design of management protocols for wetland managers.

In relation to the second aim of the study, which investigated the biodiversity value of CWs in comparison to NWs, it was concluded that despite many CWs being based in urban centres or areas of intensive agriculture, they provide habitats not otherwise available in the surrounding landscape. In fact, CWs may be viewed as critical systems in providing habitats to species of conservation concern such as newts and threatened sciomyzid flies. Adjustments to the future design of CWs such as a

reduction of barriers or hardened surfaces at CW sites, the creation of suitable habitats around CWs, and the addition of microhabitats or features (Figure 6.1), may enhance the biodiversity value of CWs, without hampering their primary function of wastewater treatment. These recommendations, coupled with appropriate management practices of CWs and their surrounding habitats, will enhance CWs for inter alia amphibian, invertebrate, bird and mammal species.

While it is unlikely that CWs could ever replace the ecosystem functions of a healthy NW, they do have a role to play in the conservation of threatened wildlife, which can be easily enhanced with minor modifications to existing and future CWs and their immediate surrounds.



*Figure 6.1. Summary of management recommendations for enhancement of constructed wetlands for biodiversity.*

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## **Appendix A**

### **Seasonal patterns of metals and nutrients in *Phragmites australis* (Cav.) Trin. ex Steudel in a constructed wetland in the west of Ireland**

Mulkeen, C.J., Williams, C.D., Gormally, M.J, Healy, M.G.

*Article associated with Chapter 3*



## Seasonal patterns of metals and nutrients in *Phragmites australis* (Cav.) Trin. ex Steudel in a constructed wetland in the west of Ireland



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### ABSTRACT

An understanding of the seasonal variation in the standing stock of metals and nutrients in emergent vegetation of constructed wetlands (CWs), as well as the amounts present in aboveground (AG) and belowground (BG) biomass, is crucial to their design and management. Given that biomass harvesting is a labour and time consuming operation, a paucity of information currently exists on accumulation and standing stocks in biomass in CWs, in particular in North Western European countries. To address this knowledge gap, this paper examined the seasonal variations of metals and nutrients in *Phragmites australis* (Cav.) Trin. ex Steudel in a CW treating municipal wastewater, with a view to identifying an optimal time for biomass harvesting of the AG vegetation. Although the AG biomass was greatest in August ( $1636 \pm 507 \text{ g m}^{-2}$ ), the maximum concentrations and accumulations of metals and nutrients occurred at different times throughout the duration of the study (April to November). Furthermore, with the exception of zinc and nitrogen, metals and nutrients measured in BG biomass ranged from 66% (phosphorus) to greater than 80% (nickel and chromium) of the AG biomass. This indicates that analysis of only the emergent shoots may significantly underestimate the metal and nutrient uptake and capacity of the plant. In order to effectively target the bulk of metals and nutrients, an AG harvest in late August or September is suggested.

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### 1. Introduction

Constructed wetlands (CWs) are gaining in popularity for the treatment of municipal (Vymazal, 2011) and industrial wastewaters, including, *inter alia*, landfill leachate (Bulc, 2006; Białowiec et al., 2012), tannery industry wastewaters (Calheiros et al., 2012), highway runoff (Gill et al., 2014), effluents from wineries (Grismer et al., 2003), aquaculture wastewater (Lin et al., 2005), mine wastewater (O'Sullivan et al., 2004), wastewaters containing estrogens, androgens and hormones (Cai et al., 2012; Vymazal et al., 2015), and pharmaceutical and personal care products (Matamoros et al., 2009). Numerous studies measuring wetland treatment performance with and without vegetation have concluded almost invariably, that wetland performance is better when plants are present (Kadlec and Knight, 1996). Wetland macrophytes are highly productive plants and possess several functions in relation to wastewater treatment (Brix, 2003) such as flow resistance and

particulate trapping (Kadlec and Wallace, 2009), nutrient uptake (Shelef et al., 2013), and insulation, particularly in colder climates. In addition to this, the vegetation in CWs has the ability to tolerate high concentrations of nutrients and metals, as well as to accumulate them in their plant tissues (Stottmeister et al., 2003).

The selection of plant species for CWs requires careful consideration, as the vegetation must be capable of surviving the potential toxic effects of wastewater and its variability (Maine et al., 2009). The Common Reed, *Phragmites australis*, (Cav.) Trin. ex Steudel, is used worldwide for the treatment of domestic and industrial wastewaters in CWs (Du Laing et al., 2003). Investigations of the uptake and seasonal variations in storage capacities of nutrients in *P. australis* and other plants such as *Typha latifolia* L. have been undertaken in CWs under Irish climatic conditions (Healy et al., 2007; Mustafa and Scholz, 2011). However, a paucity of information exists on metal cycling and accumulation by vegetation, in particular in CWs of North Western European countries. Such information is important in the future design and operation of CWs, particularly when the efficacy of CWs regarding nutrient and metal removal from wastewaters is being assessed.

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Metals are non-biodegradable, and water pollution by metals is a serious environmental problem which is difficult to solve (Keng et al., 2014). In CWs, metals tend to accumulate in the sediments as well as in the plants (Březinová and Vymazal, 2015). While metals in CWs are removed through physical (settling and sedimentation) and chemical (sorption and adsorption) mechanisms, metal uptake by plants has also been identified as the principal removal mechanism for some pollutants, particularly in lightly loaded systems (Březinová and Vymazal, 2015). However, metal content in the roots and shoots of wetland vegetation varies from season to season and there has been no attempt to explain this variability, or to determine optimum conditions for metal uptake by plants in CWs to date (Vymazal and Březinová, 2016). In the context of how we manage CWs, the seasonal variations of metals in macrophytes must be first of all understood, if we intend to expand the use of CWs for treating effluents containing metals in the future.

Maximum recorded metal concentrations from international studies in above and belowground (BG) biomass of *P. australis* are presented in Table 1. Macrophytes are known to take up metals from the environment but largely accumulate these in the BG organs, such as the roots and rhizomes (Peeverly et al., 1995). The generally lower concentrations of metals in aboveground (AG) organs of macrophytes (stems and leaves) may be attributable to metal tolerance, where it has been suggested that macrophytes limit high metal concentrations in the photosynthetic organs of the plant (Bragato et al., 2006). The levels of metals in AG organs may vary seasonally in response to plant growth dynamics, metal levels and availability in the surrounding waters (Larsen and Schierup, 1981; Schierup and Larsen, 1981). The possibility of harvesting of the AG vegetation as a means of wetland management and removal of metals from the system has previously been suggested (Bragato et al., 2006; Březinová and Vymazal, 2015). However, a dearth of information currently exists on macrophyte management in CWs, including best practices for harvesting.

The total storage of a substance in a plant part is called standing stock (Vymazal and Březinová, 2015) and is calculated by multiplying the concentration by biomass per unit area. Vymazal and Březinová (2015) suggest that knowledge of concentrations alone does not provide any information of the translocation or accumulation of metals in a plant without knowing the biomass. In a literature review of metals in AG biomass of *P. australis* by Vymazal and Březinová (2016), the authors theorize that in order to obtain correct accumulation values in a plant, it is necessary to include the biomass values. Biomass harvesting is a labour and time consuming operation, and therefore a paucity of information exists on accumulation and standing stocks in AG biomass in CWs.

With this in mind, the current study aims to evaluate the seasonal variations of metals as well as nutrients (nitrogen (N) and phosphorus (P)) in AG and BG biomass of *P. australis* in a CW receiving municipal wastewater in a temperate oceanic climate in the west of Ireland, with a view to: (1) investigating the efficacy of metal and nutrient removal via biomass harvesting of AG vegetation; and (2) identifying an optimal period for biomass harvesting. The results of this study may inform how a wetland treating industrial wastewaters or effluents with high concentrations of metals may be managed in the future. We focus on a north western European context, but many of our suggestions may be suitable for other environmental contexts.

## 2. Materials and methods

### 2.1. Site description

The free-water surface constructed wetland (FWS CW) investigated in this study is located in Fenagh, Co. Leitrim, Ireland

(54°1'2"N; 7°49'43"W). This CW was designed and constructed to cater for a population equivalent (PE) of 400 in 2004, but currently receives wastewater with a PE of 132 (Table 2). Wastewater enters the treatment works at the primary settlement tank, flows by gravity to a rotating biological contactor before entering the CW, where the wastewater undergoes tertiary treatment. The CW has a surface area of 400 m<sup>2</sup>, and is lined with a high-density polyethylene liner. The wetland was originally planted with a monoculture of *P. australis*. Vegetation cover in the wetland is 100%, with some occasional bramble (*Rubus fruticosus* agg.), nettle (*Urtica dioica* L.) and willow scrub (*Salix* spp. L.) encroaching onto the reed bed.

### 2.2. Vegetation sampling regime

Sampling and analysis of vegetation was undertaken between April and November 2015. Aboveground and BG biomass of *P. australis* were sampled monthly in the inlet and outlet zones (5 m from the inlet and outlet edges) of the CW. During each sampling time, four 0.25 m<sup>2</sup> quadrats were placed into each of the inlet and outlet zones of the wetland using a randomized block design. All shoots were clipped at ground level within each of the eight quadrats. The BG biomass was completely dug out to a depth of 0.3 m from within the same quadrats. Upon delivery to the laboratory, the BG samples were thoroughly washed with potable water to remove all sediment and gravel. The washing was performed in large containers to minimize loss of hairy roots. The AG biomass consisted of stems, leaves and flowers combined, and the BG biomass consisted of roots and rhizomes combined. All samples of AG and BG biomass were then dried in a 70°C oven (after Vymazal et al., 2010) until samples reached constant weight, and the total dry biomass was calculated (g biomass m<sup>-2</sup>). Aboveground and BG samples were then ground in a mill and a subsample was tested in the laboratory. This process was repeated monthly.

### 2.3. Laboratory analysis

Nitrogen testing was carried out by combustion analysis using a Carlo Erba nitrogen analyzer following the Association of Official Analytical Chemists (AOAC) method 990.03 (2005). The instrument was calibrated daily with an atropine standard. Quality control (QC) [National Institute of Standards and Technology (NIST)] tomato leaf check samples were run throughout analysis (every ten samples). Phosphorus, aluminium (Al), boron (B), iron (Fe), manganese (Mn), magnesium (Mg), potassium (K), copper (Cu), zinc (Zn), sulphate (S) and calcium (Ca) were digested using nitric acid and hydrogen peroxide in a CEM Mars microwave system and analysed using a Thermo 65 Duo ICP following P4.3 "Soil, Plant and Water Reference methods for the Western Region" (Gavlak et al., 2003). Check samples were run through the ICP every 50 samples. Cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb) were analysed using Inductively Coupled Plasma (ICP) mass spectrometry after digestion with aqua regia (1:3 HNO<sub>3</sub>: HCl) at 110°C for three hours. Similarly, calibration standards and QC samples were run initially followed by blank, spiked and matrix spiked samples throughout the analysis (every ten samples) for verification purposes. Using these data, the AG and BG biomass and nutrient and metal content for each sampling section were obtained. Standing stocks were calculated as follows: standing stock (g m<sup>-2</sup>) = concentration (g kg<sup>-1</sup>) x dry matter (kg m<sup>-2</sup>).

### 2.4. Statistical analysis

A full factorial (i.e. including first order interaction) Two-way ANOVA and Tukey (HSD) post hoc tests (P < 0.05) were used for statistical analysis of biomass along with metal and nutrient concentration of *P. australis*. The two independent variables were

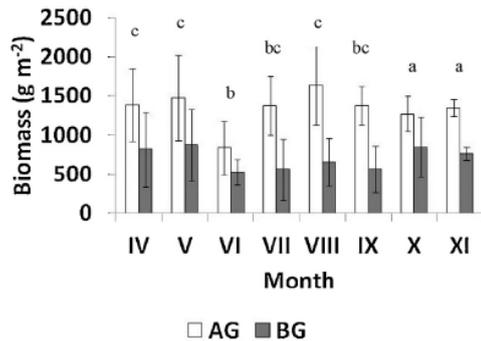
**Table 1**Metal and nutrient concentrations ( $\text{mg kg}^{-1}$ ) in aboveground and belowground biomass of *Phragmites australis* in natural and constructed wetlands from previous studies.

Element	Aboveground					Belowground				
	Max value <sup>a</sup>	Country	Wetland type <sup>b</sup>	Wastewater type	Reference	Max value <sup>a</sup>	Country	Wetland type <sup>b</sup>	Wastewater type	Reference
Cd	2.1	Greece	N		c	1.21	Denmark	N		g
Cr	118	Italy	C	Municipal	d	6.97	Italy	N		e
Cu	14.98	Italy	N		e	230	UK	C	Mine water	i
Ni	60	Italy	C	Municipal	d	9.12	Italy	N		e
Pb	39	China	C	Mine water	f	>2000	China	C	Mine water	f
Zn	217	Denmark	N		g	>1000	China	C	Mine water	f
N	26,500	Italy	C	Municipal	d	19,100	Czech Republic	C	Municipal	h
P	2200	Czech Republic	C	Municipal	h	2700	Czech Republic	C	Municipal	h

<sup>a</sup> Maximum values are based on the maximum concentration values reported in the papers reviewed throughout this study.<sup>b</sup> N = natural wetland; C = constructed wetland.<sup>c</sup> Obolewski et al. (2011).<sup>d</sup> Bragato et al. (2006).<sup>e</sup> Bonanno and Giudice (2010).<sup>f</sup> Deng et al. (2004).<sup>g</sup> Schierup and Larsen (1981).<sup>h</sup> Vymazal and Kröpfelová (2008).<sup>i</sup> Ye et al. (2003).**Table 2**

Details of site characteristics.

Reed bed dimensions			Area ( $\text{m}^2$ )	PE	Volume ( $\text{m}^3$ )	Hydraulic retention time (d) <sup>a</sup>	Hydraulic loading rate ( $\text{m d}^{-1}$ ) <sup>a</sup>
Length (m)	Width (m)	Depth (m)					
20	20	0.5	400	400	200	7.3	0.068 <sup>a</sup>

<sup>a</sup> Based on a mean flow of  $27.3\text{m}^3$  per day.

**Fig. 1.** Average amounts of aboveground (AG) and belowground (BG) biomass (inlet and outlet zones combined) in the wetland vegetation during the period of April–November 2015. Error bars represent the standard deviation. Different letters indicate significant differences between the monthly means at  $P < 0.05$ . (For the significance of above versus below ground and the above versus below ground x month interaction, see the text of the results section).

month and AG versus BG with dependent variables being various metal and nutrient concentrations, and biomass. All significant values were reported at alpha  $P < 0.05$ . All analyses were conducted on SPSS version 24.

### 3. Results

#### 3.1. Aboveground and belowground biomass

The average dry AG and BG biomass harvested during the study is presented in Fig. 1. Maximum recorded AG biomass in the study was recorded in August ( $1636\text{g m}^{-2}$ ), while biomass was lowest in June ( $835\text{g m}^{-2}$ ). Belowground biomass which ranged from  $523\text{g m}^{-2}$  to  $872\text{g m}^{-2}$  represented 53% to 62% of the AG biomass respectively. There was a statistically significant ( $P = 0.002$ ) interaction between AG and BG biomass and month of the year.

#### 3.2. Seasonal pattern of metal concentrations and accumulations

Average Cd and Pb concentrations in the influent wastewater were below the limit of detection (LOD) during the study (Table 3), and likewise were not detected in either the AG or BG biomass. Both Cr and Ni concentrations were lower in AG than BG, or were below the LOD (Fig. 2). Belowground values for both peaked in August ( $12.7\text{mg kg}^{-1}$  for Cr and  $4\text{mg kg}^{-1}$  for Ni). The BG organs cumulatively held > 80% of the total Ni and Cr in the plant as a whole. The interactions between AG versus BG, and month of the year were significant ( $P < 0.05$ ), with respect to the concentrations of both Ni and Cr in the biomass of *P. australis*.

The average influent Cu concentration measured during the study was  $7\mu\text{g L}^{-1}$  (Table 3). Belowground concentrations of Cu ranged from  $17.6\text{mg kg}^{-1}$  to  $28.5\text{mg kg}^{-1}$ , and were always higher than AG concentrations, which ranged from  $7.1\text{mg kg}^{-1}$  to  $16.7\text{mg kg}^{-1}$ . Aboveground standing stock of Cu was highest early in the growing season in April ( $15.4\text{mg m}^{-2}$ ). No significant ( $P > 0.05$ ) interactions occurred between months and AG versus BG, for the concentration of Cu in the biomass.

Zinc concentrations were highest in AG organs in September and November ( $165.2\text{mg kg}^{-1}$  and  $165.6\text{mg kg}^{-1}$ ). Zinc standing stocks were also highest during these months ( $233.9\text{mg m}^{-2}$  and  $224.3\text{mg m}^{-2}$ ). The highest monthly concentration of Zn was measured in BG organs in September ( $187\text{mg kg}^{-1}$ ), and the lowest was measured in May ( $77.1\text{mg kg}^{-1}$ ). There was no significant ( $P > 0.05$ ) interaction between AG versus BG, and month of the year for the concentration of Zn in *P. australis* biomass throughout the study.

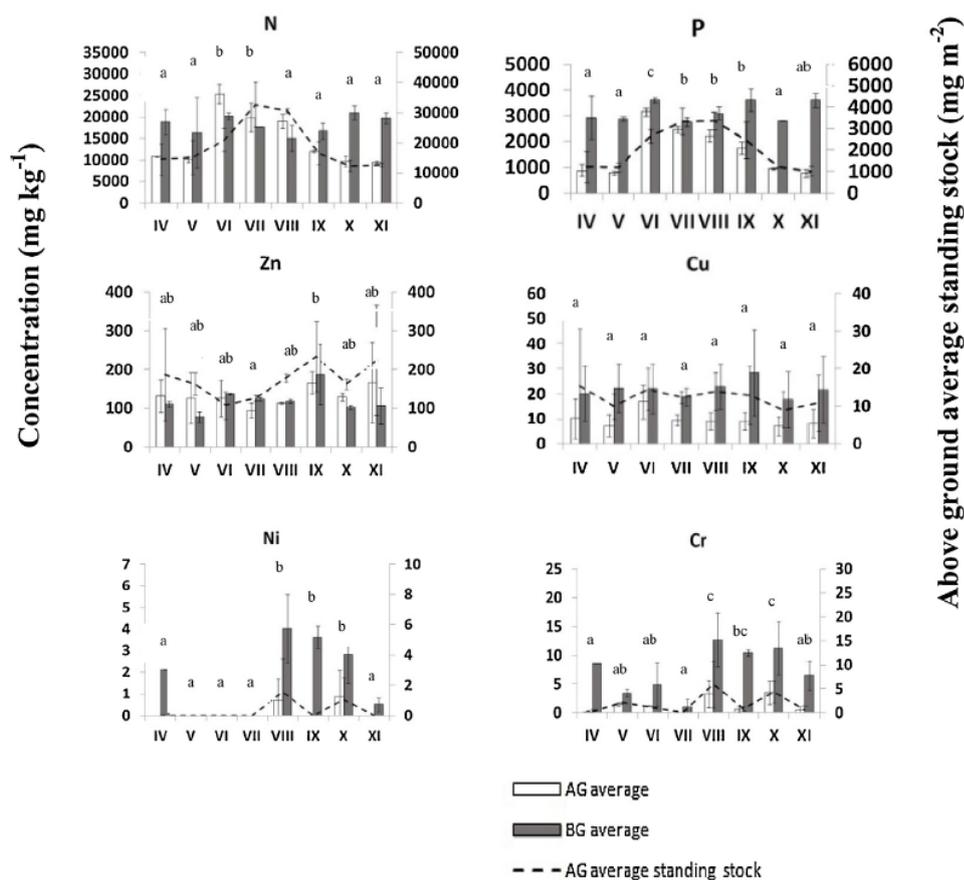
#### 3.3. Seasonal pattern of nutrient concentrations and accumulations

Concentrations and AG standing stocks of N and P are presented in Fig. 2. Nitrogen concentrations in the AG tissues peaked in June ( $25,338\text{mg kg}^{-1}$ ), the early growing season in Ireland, and declined from then to its lowest concentration of  $9463\text{mg kg}^{-1}$  in November. Nitrogen was lowest in the BG tissues in August ( $15,000\text{mg kg}^{-1}$ ).

**Table 3**

Average concentrations of metals in inflow wastewater entering the constructed wetland at Fenagh during the study period (April–November 2015) (n=3).

Metals (total)	Limit of Detection (LOD)	Average result (n=3)	Units	Limits in surface water ( $\mu\text{g L}^{-1}$ ) <sup>a</sup>
Cadmium <sup>b</sup>	0.3	<0.3	$\mu\text{g L}^{-1}$	1
Chromium	3.0	<0.3	$\mu\text{g L}^{-1}$	50
Copper	3.0	7.0	$\mu\text{g L}^{-1}$	1000
Lead <sup>b</sup>	0.9	<0.9	$\mu\text{g L}^{-1}$	50
Nickel	1.5	1.9	$\mu\text{g L}^{-1}$	
Zinc	10	17	$\mu\text{g L}^{-1}$	1000

<sup>a</sup> From Subsidiary Legislation 549.21, 28th June 2002.<sup>b</sup> Cadmium and lead consistently reported below the LOD.

**Fig. 2.** Comparison of the seasonal variation in aboveground (AG) and belowground (BG) concentrations of nutrients (nitrogen and phosphorus) and metals (zinc, copper, nickel and chromium) ( $\text{mg kg}^{-1}$ ) and aboveground standing stocks ( $\text{mg m}^{-2}$ ) in biomass of *Phragmites australis* during the period April–November 2015. Error bars represent the standard deviation. Different letters indicate significant differences between the monthly means at  $P < 0.05$ . (For the significance of above versus below ground and the above versus below ground x month interaction, see the text of the results section).

$\text{mg kg}^{-1}$ ) and highest in October ( $20,975 \text{ mg kg}^{-1}$ ). The maximum nitrogen AG standing stock ( $32.6 \text{ g m}^{-2}$ ) was measured in July. The AG biomass cumulatively contained almost half (44%) of the total N accumulated in the CW. The interaction between AG versus BG and month of the year was significant ( $P < 0.05$ ) with respect to the concentration of N in the biomass of *P. australis*.

Concentrations AG of P peaked in June ( $3156 \text{ mg kg}^{-1}$ ) and steadily declined throughout the study until November ( $768 \text{ mg kg}^{-1}$ ). Belowground values for P ranged from  $2755 \text{ mg kg}^{-1}$  in July to  $3605 \text{ mg kg}^{-1}$  in September. Belowground biomass cumulatively accounted for two thirds of the total P accumulated within the wetland. The highest AG standing stock of P was recorded in July and August ( $3.3 \text{ g m}^{-2}$  and  $3.4 \text{ g m}^{-2}$ , respectively) and lowest in November ( $1 \text{ g m}^{-2}$ ). Similar to N, there was a significant inter-

action ( $P < 0.05$ ) between AG versus BG and month of the year for P concentrations in the study.

#### 4. Discussion

Metals enter the environment from natural and anthropogenic sources, and are non-biodegradable, accumulate in the environment, and pose a threat to the environment and human health (Ali et al., 2013). Studies examining the ability of emergent vegetation in CWs to uptake metals and nutrients have commonly examined AG vegetation only or concentrations only. However, the findings of the current study suggest that analysis of only the emergent shoots or concentrations only, may significantly underestimate the metal and nutrient uptake of the plant. Metal accumulation in the AG biomass

relative to the total amount entering the system (Table 3) over the eight-month study period ranged from 0.02% (for Cu) to 1.22% (for Zn). With the exception of Zn and N, there were higher concentrations of metals and nutrients in the BG organs of the plant during each month of analysis. Overall, Zn concentrations were cumulatively higher in AG biomass (52%) during April, May, October and November, whereas N concentrations in AG biomass were higher during June, July and August (the typical growing season for *P. australis*). The findings of higher concentrations in BG biomass was similar to other studies (Pevery et al., 1995; Mays and Edwards, 2001; Bragato et al., 2009), and indicates that *P. australis* is prevalently a root bioaccumulator species (Bonanno, 2011). The roots and rhizomes are the immediate points of uptake in plants and, consequently, the concentrations are usually greater in roots in comparison to leaves and other AG organs (Vymazal et al., 2007). The lower concentrations in AG organs in the current study is in agreement with the speculation that plants restrict the movement of metals into their AG plant tissues to avoid the potential toxic effects of high metal concentrations on their photosynthetic organs (Bragato et al., 2006). The reduction of N and P in AG parts in October and November, is known to occur in rhizomatous plants such as *P. australis*, where the nutrients are translocated to and stored in BG organs during winter, and are ready to initiate growth the following season (Chapin III et al., 1990). The concentrations of N and P at the beginning of the study (April and May) are similar to concentrations at the end of the study (October and November), therefore it may be assumed that nutrients are overwintered in BG organs.

The current study was carried out in a lightly loaded system with a small PE (Table 2). Previous studies have suggested that uptake by plants in AG and BG organs, is significant only under low loading conditions (Brix, 1997), similar to that of the CW in the current study. Zinc was the only metal to be present in higher concentrations in AG biomass during some months of the study which was similar to Pevery et al. (1995) and Schierup and Larsen (1981), where higher concentrations of Zn were found in AG plant parts and stems. Zinc plays an essential role in plant nutrition and enzymatic processes (Bonanno and Giudice, 2010). The higher concentrations of Zn in AG tissues may have occurred due to its essential function in the formation of indole acetic acid, a plant hormone which is manufactured in the stems of plants (Schierup and Larsen, 1981). Unlike Zn, which is essential to plant growth, Ni and Cr are regarded as elements which are toxic to plants (Bonanno and Giudice, 2010). Nickel was only detected in August and October in the AG biomass (Fig. 2), and at levels lower than  $5 \text{ mg kg}^{-1}$ . However, *P. australis* has the potential to store up to  $60 \text{ mg kg}^{-1}$  of Ni (Bragato et al., 2006). Chromium content has previously been recorded at  $4825 \text{ mg kg}^{-1}$  and  $827 \text{ mg kg}^{-1}$  in the roots and shoots of *P. australis* in a pot study using tannery wastewater (Calheiros et al., 2008) and values found in this study were significantly lower than this threshold level. Significant quantities of N were detected in the AG tissues of *P. australis* (up to  $25,338 \text{ mg kg}^{-1}$ ). Nitrogen removal from a CW is greatly facilitated by the plant uptake through the root system of *P. australis*. June, July and August are the growing season for *P. australis* in Ireland; therefore, higher quantities of N were found in the AG biomass during these months. In addition to this, AG biomass was lowest in June (Fig. 1), the typical early growing season for *P. australis* in Ireland. At this point, the majority of dead plant growth from the previous year has fallen away and new shoots are appearing. The AG biomass values in April and November are similar ( $1384 \text{ g m}^{-2}$  and  $1346 \text{ g m}^{-2}$ , respectively), which leads us to believe that these values may be typical of the biomass values throughout the winter season. However, further studies are needed to verify this.

Common reed is a traditional building material which is widely used in roofs, and insulation blocks made from reed are highly valued in eco-friendly construction (Maddison et al., 2009). With this

in mind, harvesting of the AG biomass of macrophytes has been suggested by many researchers as an option for nutrient and metal removal in CWs (Bragato et al., 2006; Vymazal et al., 2010; Vymazal and Březinová, 2015). In order to maximise removal, the harvesting process needs to take place during a period of maximum content of the targeted element in the plant. However, based on the results of this study, under temperate maritime climatic conditions, metals and nutrients follow different seasonal patterns, and it is difficult to identify an optimum time for harvest to obtain maximum removal of all nutrients and metals at the same time based on the concentrations only. Therefore, if harvesting is to be considered as an option, it will be necessary to prioritise between maximising the removal of specific nutrients and metals. Furthermore, the effects of frequent harvesting on the regrowth success of *P. australis* also needs to be evaluated (Maddison et al., 2009). However, the results of standing stocks of each metal and nutrient measured in the study, would suggest a harvest in Autumn (late August or September) may capture the maximum contents of most nutrients and metals in the AG biomass. This could result in the removal of between 0.6 g (Ni) and 71.2 g (Zn) based on a harvest in August. The ability of *P. australis* to accumulate metals and nutrients in AG biomass under such climatic conditions provides strong encouragement for CW applications in industrial settings. Further work is needed to investigate the translocation and accumulation of metals to the AG tissues, and the implications of harvesting in terms of regrowth success in CWs treating industrial wastewaters.

## 5. Conclusions

Plant uptake and accumulation is one method of metal and nutrient removal from CWs. With the exception of Zn and N during some months of the study, BG biomass of *P. australis* predominantly contained higher concentrations of metals and nutrients than AG biomass. In order to remove maximum quantities of metals and nutrients, the harvesting process must take place during the period of maximum content of the targeted element in the plant. Knowledge of the concentrations alone does not provide information on the translocation or accumulation of elements in the plants. In order to maximise the removal of metals and nutrients in CWs, a harvest should take place during the period of maximum accumulation in AG biomass. With this in mind, a harvest in Autumn of AG biomass is suggested based on the results of this study.

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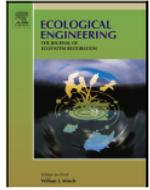
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Appendix B

**Habitat suitability assessment of constructed wetlands for the Smooth Newt  
(*Lissotriton vulgaris* [Linnaeus, 1758]): a comparison with natural wetlands**

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*Article associated with Chapter 4.*



# Habitat suitability assessment of constructed wetlands for the smooth newt (*Lissotriton vulgaris* [Linnaeus, 1758]): A comparison with natural wetlands



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## ABSTRACT

Given the current decline of natural wetlands worldwide and the consequent negative impacts on amphibians, wetlands constructed for the treatment of wastewaters have the potential to play a role in the protection of these animals. However, there is a paucity of information regarding the value of constructed wetlands (CWs) to amphibians, particularly relating to the terrestrial phase of their life-cycle. This study compares the terrestrial habitats of natural wetlands (NWs) and CWs as refuges for the smooth newt (*Lissotriton vulgaris*, [L., 1758]) with the aim of developing recommendations for CWs (both new and existing) to enhance their usefulness as newt-friendly habitats. Terrestrial habitats surrounding NWs and CWs were mapped using ArcGIS. Potential barriers to newt movement in addition to the presence of features such as wood or stone which could act as potential newt refuges were also mapped. Natural wetlands had significantly more terrestrial habitat types than CWs and while woodlands at both wetland types were most likely to contain features of benefit to newts, terrestrial habitats of NWs contained more features compared to those of CWs. The application of a Habitat Suitability Index, which assesses the likelihood of the presence of newts, resulted in seven of eight NWs compared to only two of eight CWs receiving “good” scores, the lower scores for CWs being due primarily to the presence of a barrier to newt movement. Recommendations for enhancing the design and management of CWs for smooth newts include less intensive ground maintenance, reduction of barriers to newt movement, judicious planting of suitable trees or shrubs and the provision of additional refuges such as wood or stone.

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## 1. Introduction

Natural wetlands (NWs), one of the most important ecosystems on earth (Mitsch and Gosselink, 2007), have been described as ‘transitional environments’ occurring between terrestrial and aquatic systems (Lehner and Döll, 2004). The ecosystem services provided by NWs include biodiversity support, water quality improvement, flood abatement (Zedler, 2000) and sequestration/long term storage of carbon dioxide (Mitsch et al., 2013). In addition, extensive numbers of bird, mammal, fish, amphibian and invertebrate species are entirely dependent on NW habitats across the globe (Zedler and

Kercher, 2005). It is estimated that 50% of the Earth’s original NWs have been destroyed (Mitsch and Gosselink, 2007) and in Ireland alone, areas covered by NWs decreased by almost 2.5% between 2000 and 2006 (Corine, 2006).

While NWs have been used as convenient wastewater discharge sites since sewage was first collected (for at least 100 years in some locations) (Kadlec and Wallace, 2008), it is only in the last fifty years (approximately) that wetlands worldwide have been recognised for their wastewater treatment capabilities (Vymazal, 2011). Since then various types of artificial wetlands (constructed wetlands; CWs) have been designed to intercept wastewater (after conventional treatment processes) and remove a range of pollutants before discharging into natural water bodies (Hsu et al., 2011). Constructed wetlands are increasingly recognised as a relatively low-cost method for treating wastewaters such as sewage, agricultural/industrial wastewaters and storm water runoff (Campbell

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and Ogden, 1999), requiring minimal operation and maintenance (Zhang et al., 2009). While most attention has been paid to the waste water treatment capabilities of CWs, relatively little attention has been given to the incorporation of biodiversity features in the design and construction of CWs and their surroundings. As stated by Greenway (2005), CWs can also act as multifunctional ecological systems assisting in the restoration of aquatic flora and fauna, and a number of studies have been undertaken on the biodiversity of existing CWs including studies on freshwater invertebrates (Spieles and Mitsch, 2000; Jurado et al., 2010), amphibians (Korfel et al., 2010), birds (Andersen et al., 2003; Fleming-Singer and Horne, 2006) and mammals (Kadlec et al., 2007). However, these studies have generally focussed on the CW itself and not on the surrounding habitats in which the CW is situated, although the latter are often critical for fauna, such as amphibians, with biphasic life cycle requirements.

Amphibians typically require terrestrial and aquatic environments to complete their semi-aquatic life cycle (Dodd and Cade, 1998) and the importance of terrestrial habitats and microhabitats for amphibian breeding site selection has been highlighted by Marnell (1998). However, amphibians are currently experiencing striking global declines in recent decades due, in part, to the destruction of wetland habitats (Stuart et al., 2004) and fungal disease (Voyles et al., 2009). *Lissotriton vulgaris*, while widespread across most of Europe, is the sole native species of newt found in Ireland (Meehan, 2013), with breeding invariably taking place in water during spring, and sometimes extending into early summer. After metamorphosis, juveniles of *L. vulgaris* are solely terrestrial, spending several years on land, before reaching maturity between the ages of three and seven years (Bell, 1977), at which stage they return to water bodies to breed. Smooth newts are known to use a variety of water bodies during the breeding season including lakes, natural ponds, garden ponds and slow-moving drainage ditches (Meehan, 2013), with larvae rarely being found in running water (Bell and Lawton, 1975). Even water bodies with a surface area of no more than 400 m<sup>2</sup> (considerably smaller areas than many CWs for wastewater treatment) have been known to support up to 1000 individual adult smooth newts (Bell and Lawton, 1975). The smooth newt life cycle has complex requirements. Adults require aquatic habitats for breeding as well as terrestrial habitats for foraging and overwintering, although adults have been found to overwinter in ponds in Italy (Fasola and Canova, 1992). In some cases larvae have even been recorded in water bodies during the winter, but this is usually the result of a combination of factors such as late egg production, high population densities, competition for food resources and low water temperatures in countries such as Northern England, Poland and Montenegro (Jehle et al., 2011). While juveniles leaving the waterbody for the first time can travel further on land (Joly et al., 2001), adult smooth newts generally move towards favourable habitat patches in the vicinity (Malmgren, 2002). Although terrestrial behaviour of smooth newts is still not fully understood, diverse structural habitats (Vuorio et al., 2015) in addition to climatic and landscape factors (Joly et al., 2001) may drive patterns of movement (Pittman et al., 2014) and survival (Griffiths et al., 2010). Smooth newts tend to travel in straight lines on land since movement here is slower and requires more energy than movement in water, where the newt is buoyed up by the surrounding medium (Griffiths, 1996). Once on land, suitable refuges must be sought from predation, desiccation and temperature extremes (Griffiths, 1984). Habitats that provide shelter and protection such as scrub and woodland (both deciduous and coniferous), unimproved grassland and gardens are considered newt-friendly habitats (Oldham et al., 2000) (Table 1). Although acidic habitats such as peatland (Marnell, 1998) and water bodies containing fish are thought to be less suitable for smooth newts in the UK (Aronsson and Stenson, 1995) and Lombardy, Italy (Ficetola and De Bernardi, 2004), it appears that habitat

selection in smooth newts may be limited by barriers and competition. In Ireland, for example, where *L. vulgaris* is at the most westerly edge of its range, and it lacks competition for habitats from other newt species, it has a tendency towards a wide niche occupation including lakes of a considerable size containing fish in addition to acid peatland pools (Meehan, 2013). In addition, microhabitats such as dead wood and stone features can be important in amphibian breeding site selection (Marnell, 1998), while roads and rivers adjacent to the breeding water body have been shown to interfere with newt migration (Oldham et al., 2000; Matos et al., 2017).

The movement of adult smooth newts on land, which tends to be short distances from breeding water bodies (Griffiths, 1984), has been described as philopatric i.e. individuals remain or return to relatively few permanent hiding places throughout the year and/or on an annual basis (Dolmen, 1981) (Sinsch and Kirst, 2015). Although individuals of *L. vulgaris* have been found in terrestrial habitats at distances exceeding 500 m from water bodies (Kovar et al., 2009), this is likely to be the exception rather than the rule. Bell (1977) found that over forty times more smooth newts were captured in pitfall traps within 5 m of a wetland edge compared with pitfalls placed 50 m from the wetland edge. In addition, Bell (1977) released sixty-one marked *L. vulgaris* juveniles 22.5 m from a pond edge and recaptured over 50% within ten meters from the point of release thirty-five days later. In another study, Dolmen (1981) observed that no recaptured smooth newts ventured further than 7.5 m from the original capture point on land, suggesting that adult smooth newts tend to settle close to the water body in which they were born (Bell, 1977). Most smooth newts will remain relatively close to the breeding pond, provided that habitat quality immediately surrounding the breeding water body is optimal and connectivity is excellent. Terrestrial habitats surrounding wetlands can, therefore, serve as wildlife corridors and are important in the conservation and management of semi-aquatic species such as amphibians (Semlitsch and Bodie, 2003), including *L. vulgaris*.

The Habitat Suitability Index (HSI), first developed by Oldham et al. (2000) in Britain (and later modified by the National Amphibian & Reptile Recording Scheme, 2007), is used by Natural England, Natural Resources Wales and the Department of Environment, Food and Rural Affairs (UK) to assess the likelihood of the presence of the great crested newt (*Triturus cristatus* [Laurenti, 1768]) in a given area in the UK (Department for Environment, Food and Rural Affairs, 2016) (Table 2). This species, which is larger than the smooth newt, has been found to travel further from ponds (>200 m and >500 m) (Redgrave, 2009; Kinne, 2004; Stoefer and Schneeweiss, 2001). Within their range, great crested newts have been recorded with smooth newts more than other newt species (Jehle et al., 2011). Both species also seem to have similar requirements in terms of the variety of the terrestrial habitats surrounding water bodies for dispersal (Malmgren, 2002; Griffiths, 1996) and the presence of *T. cristatus* in ponds in the UK usually seems to be a good indicator for the presence of *L. vulgaris* (Griffiths, 1996), although *L. vulgaris* can be found in a wider range of localities (Skei et al., 2006). Given the absence from Ireland of the great crested newt, *L. vulgaris* occupies a similar range of habitats, in addition to which there is considerable overlap in the timing of seasonal and diel activities (Griffiths and Mylotte, 1987) and environmental responses (Vuorio et al., 2015). For these reasons, the UK HSI for *T. cristatus* was adopted by the authors of this article as an initial starting point to assess habitat suitability in Ireland for *L. vulgaris* at a landscape-scale and prioritise areas for action.

In Ireland, drainage and infilling of NWs (Staunton et al., 2015), in conjunction with excessive clearing of vegetation around breeding sites, remains a threat to smooth newt populations (King et al., 2011). *Lissotriton vulgaris* is currently on the International Union for the Conservation of Nature (IUCN) Red List of threatened species in Ireland (King et al., 2011), and loss of suitable terrestrial habitats for

**Table 1**Terrestrial habitats identified in the literature as suitable for the terrestrial phase of *Lissotriton vulgaris* (L., 1758).

Terrestrial habitat	Reference
Meadows/long grass	Marnell (1998), Oldham et al. (2000), Flood (2012), Meehan (2013)
Rough grassland	Oldham et al. (2000)
Hedgerows	Oldham et al. (2000)
Scrub	Marnell (1998), Oldham et al. (2000), Flood (2012)
Woodland	Oldham et al. (2000), Flood (2011), Meehan (2013)
Gardens	Oldham et al., (2000)
Damp woodland	Flood (2012)
Bogland	Flood (2012)
Dense vegetation in water/lake margins	Meehan (2013)

**Table 2**Great Crested Newt (*Triturus cristatus* [Laurenti, 1768]) Habitat Suitability Index used for scoring terrestrial habitats around ponds (from National Amphibian & Reptile Recording Scheme, 2007).

Category	SI	Criteria
Good	1	Extensive area of habitat that offers good opportunities for foraging and shelter completely surrounds pond (e.g. rough grassland, scrub or woodland).
Moderate	0.67	Habitat that offers opportunities for foraging and shelter, but may not be extensive in area and does not completely surround pond.
Poor	0.33	Habitat with poor structure that offers limited opportunities for foraging and shelter (e.g. amenity grassland).
None	0.01	Clearly no suitable habitat around pond (e.g. centre of large expanse of bare habitat).

overwintering or refuge remains a concern. While the value of CWs as a conservation strategy for amphibians has been highlighted by previous studies (Denton and Richter, 2013), the suitability of terrestrial habitats surrounding CWs for the terrestrial phase of the smooth newt life-cycle has yet to be addressed.

The aim of this study was to compare, for the first time, the suitability of terrestrial habitats surrounding CWs and NWs for *L. vulgaris*. The results are discussed in the context of providing definitive guidelines for engineers regarding the design of CWs which incorporate features that support the conservation of the species.

## 2. Methods & materials

### 2.1. Site descriptions

Eight CWs and eight NWs were selected in counties Mayo, Galway, Roscommon and Leitrim in the west of Ireland (Fig. 1). Each CW, built for the tertiary treatment of municipal wastewater, consisted of a surface flow reed bed planted with either *Phragmites australis* (Cav.) Trin. ex Steud. or *Typha latifolia* L. Natural wetlands, containing areas of *P. australis* and/or *T. latifolia*, within 20 km of each CW, were selected for comparison (Appendix A in Supplementary material). Suitable newt friendly habitats such as hedgerows, scrub, drainage ditches, woodland or grasslands occurred within 500 m of each wetland.

### 2.2. Habitat mapping

Between August and October 2015, habitats were mapped at all sites. A colour orthoimage, sourced from ArcGIS (Release Version 10.3; Environmental Systems Research Institute [ERSI], California, USA) and produced in 2012, was printed for each wetland at a scale of 1:2650. Given that a minimum mapable polygon size of 400 m<sup>2</sup> is recommended by Smith et al. (2011) for small-scale field mapping, orthoimages were printed with a 20 m × 20 m grid superimposed on the image to aid with mapping habitats in the field. The photograph was used as a base map in which habitats were recorded. All habitats within 40 m of the water's edge were documented since most of the *L. vulgaris* population will confine normal intra-habitat wanderings to short distances from a pond (Griffiths, 1984).

Habitats were identified, described and classified according to a standard habitat classification scheme used in Ireland covering terrestrial, freshwater and marine environments (Fossitt, 2000). This classification scheme is hierarchical and operates at three levels

comprising eleven broad habitat groups at Level 1; thirty habitat sub-groups at Level 2; and 117 individual habitats at Level 3 e.g. "Grassland and marsh" (Level 1) → Semi-natural grassland (one of three sub-groups at Level 2) → "wet grassland" (one of seven habitats at Level 3).

During the surveys of terrestrial habitats, it was noted that grasslands which would normally be classified as "improved agricultural grassland" under Fossitt's classification (Fossitt, 2000) often consisted of poorly drained fields which supported abundant *Juncus* species. For the purposes of this study, such sites were classified as "improved agricultural grassland with abundant *Juncus* spp." to separate them from truly improved fields i.e. "intensively managed or highly modified agricultural grassland" with rye grasses (*Lolium perenne* L.) usually abundant (Fossitt, 2000). Notable features of importance to smooth newts such as wood or stone features (Marnell, 1998) were recorded as present or absent for each 20 m × 20 m grid square. Wood features referred to tree stumps, dead/decaying/fallen branches, fallen trees; and stone features referred to boulders and loose rock.

Field survey recorded data were later digitised using ArcGIS 10.3 and the areas for each habitat calculated. Wood and stone features were recorded as point features. Linear features such as treelines, hedgerows and drains were assigned an arbitrary width of 1 m (reflecting the minimum width of linear habitats encountered) so that areas of different habitats could be compared. As the total areas for each wetland varied, the wetlands in this study have been numbered consecutively from the largest to the smallest for each wetland type i.e. CW1–CW8 and NW1–NW8 (Appendix A in Supplementary material). Maps were created using ArcGIS 10.3 and the extent of all habitats was determined. Using the UK HSI for the great crested newt, CWs and NWs were scored and ranked in order of their potential value to the smooth newt. Those at the lower end of the scale are evaluated and recommendations on how their suitability can be improved are proposed.

### 2.3. Statistical analysis

A Kolmogorov – Smirnov test was performed to test for normal distribution of the residuals. A General Linear Model (GLM) was used to test whether there was a significant effect of area and wetland type on habitat richness. A Pearson's Correlation was used to test whether there was any correlation between area of the wetland and the number of habitats present.

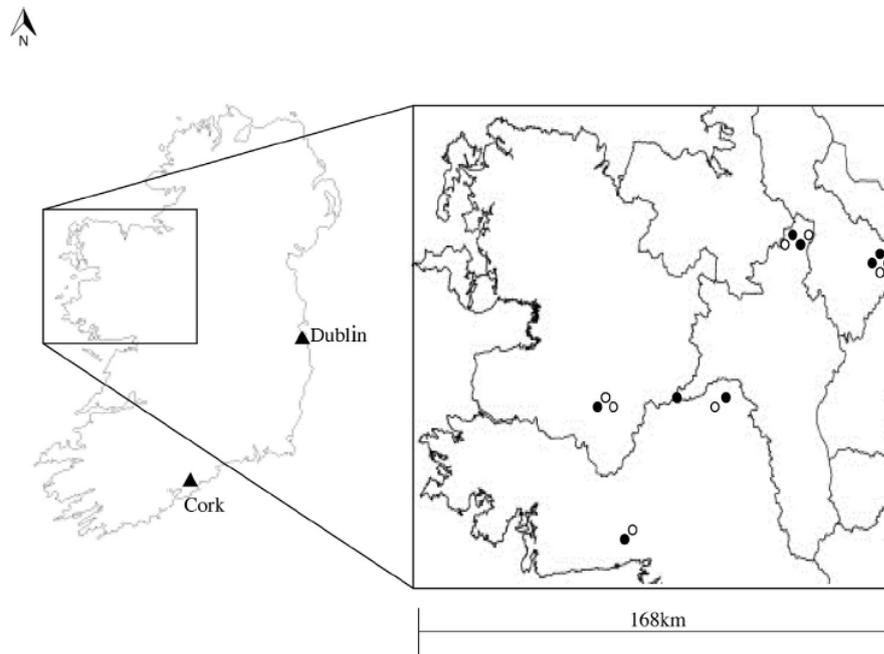


Fig. 1. Locations of constructed (●) and natural (○) wetlands in the west of Ireland (see Appendix A in Supplementary material).

### 3. Results

A total area of 2.25 km<sup>2</sup> (including open water) was mapped across sixteen CW and NW sites. Areas of open water and surrounding terrestrial habitats mapped at CWs range from 0.008 km<sup>2</sup> to 0.020 km<sup>2</sup>, while those of the generally larger NWs range from 0.008 km<sup>2</sup>–1.45 km<sup>2</sup> (Appendix A in Supplementary material). Using Level 1 (Fossitt, 2000), “freshwater” habitats dominated the NWs overall (74%) compared to only 13% at the CWs, where “grassland & marsh” dominated (54%) (Fig. 2). This is not surprising, given that a more in-depth analysis of freshwater habitats at Level 3 (Fossitt, 2000) revealed that the open water of the NWs (primarily lakes) is reflected by the dominance (82% cover) of “mesotrophic lakes” compared to the, not unexpected, dominance of “reed & large sedge swamp” (74%) at the CWs, represented at the NWs by a cover of just 16%. “Woodland & scrub” had similar percentage covers of 13% and 15% at the NWs and CWs respectively (Fig. 2) but “exposed rock & disturbed ground” and “cultivated and built land”, a total of <2% combined at the NWs, had a cover of 8% and 10% respectively, at the CWs.

Given that the focus of this paper is the terrestrial phase of the smooth newt which spends less than 50% of the year (generally March–July) (Bell, 1977) in still water for breeding, suitable terrestrial habitats were examined in more detail since they form an essential component of the newt life cycle (Denoël and Lehmann, 2006). With this in mind, less optimal habitats for newts from August to February (i.e. the “freshwater” habitats above with the exception of “freshwater swamps”) were removed from the analysis to examine the remaining habitats in detail for suitability for newts. “Freshwater swamps” were included in the analysis because these are not areas of fully open water, but generally occupy a zone at the transition from open water to terrestrial habitats (Fossitt, 2000). An examination of the order of dominance of terrestrial habitats (Fig. 3) at Level 1 (Fossitt, 2000) revealed a similar pattern to those in Fig. 2, with the exception that the percentage cover of “freshwater swamp” at the NWs was almost co-dominant with “woodland & scrub” (32% and 33%, respectively). In the CWs, “fresh-

Table 3

General Linear Model (GLM) of the effect of wetland type and area on habitat richness.

Tests of Between – Subjects Effects					
Dependant variable: Number of habitats					
Source	Type III Sum of squares	df	Mean square	F	Sig.
Model	1580.473 <sup>a</sup>	3	526.824	132.916	.000
Total area	82.223	1	82.223	20.745	.001
Wetland type	830.759	2	415.380	104.799	.000
Error	51.527	13	3.964		
Total	1632.000	16			

<sup>a</sup> R squared = .968 (Adjusted R squared = .961).

water swamp” had the same percentage cover as “cultivated and built land” (Fig. 3) which along with “exposed rock & disturbed ground”, had overall percentage covers of 10% and 9% respectively. In NWs, both categories, along with “heath & dense bracken”, had an overall combined percentage cover of <2%.

The number of newt-friendly terrestrial habitats recorded at Level 3 (Fossitt, 2000) varied within each wetland type, with those in NWs ranging from 17 at the largest NW1 (Appendix A in Supplementary material) to seven at NW5 and from 12 habitats at CW3 to six at CW8. To test for normal distribution, a Kolmogorov – Smirnov test was used ( $P > 0.05$ ) indicating that the data are not significantly different from a normal distribution (CW area = 0.690, CW number of habitats = 0.473; NW area = 0.808, NW number of habitats = 0.598). A Pearson’s correlation confirmed that the correlation between area of CWs and number of habitats present was not significant ( $P > 0.05$ , R squared = 0.602) in comparison to the correlation between area of NWs and number of habitats present which was significant ( $P < 0.05$ , R squared = 0.898). Using a General Linear Model (GLM), there was a significant effect of both area and wetland type on habitat richness. The GLM displays a positive relationship between number of habitats and the covariate area and NWs had significantly more habitats than CWs (Table 3).

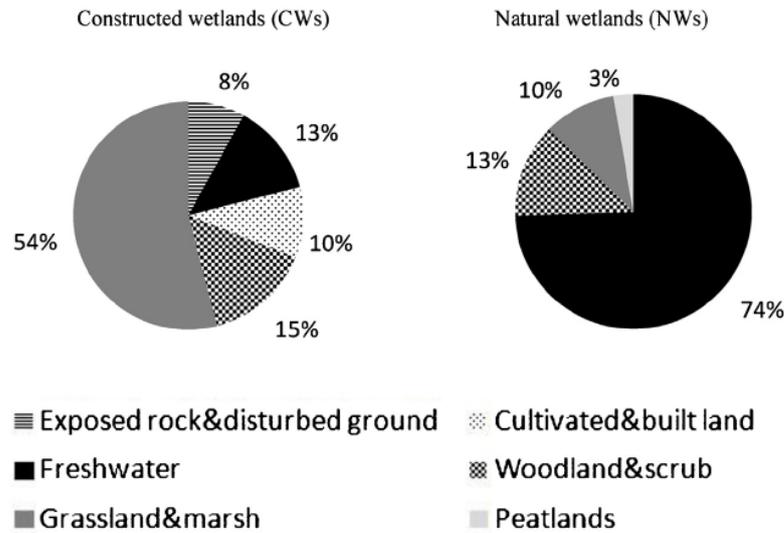


Fig. 2. Percentage cover of terrestrial and aquatic habitats at constructed (CW) and natural (NW) wetlands (Level 1) (Fossitt, 2000) (percentages rounded to nearest whole number).

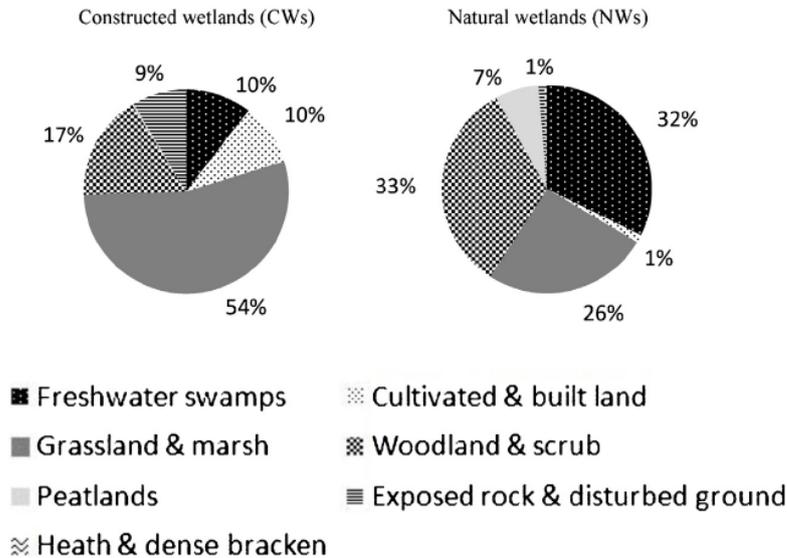


Fig. 3. Percentage cover of terrestrial habitats (Level 1) (Fossitt, 2000) at constructed (CW) and natural (NW) wetlands excluding freshwater habitats (with the exception of freshwater swamps). (Percentages rounded to nearest whole numbers).

Given that “grassland & marsh” represented over a quarter of the cover of terrestrial habitats at both wetland types (26% and 54% for NWs and CWs respectively) and that long grass and rough grassland are among those considered as some of the best habitats for the terrestrial phase of newts (Table 1), these were examined in more detail at Level 3 (Fossitt, 2000) (Fig. 4; Appendix B in Supplementary material). Nine different “grassland & marsh” habitat types were found in the current study. “Wet grasslands” represented more than half (52%) of the cover of the “grassland & marsh” habitats at the NWs, but less than a quarter (24%) at CWs, where “improved agricultural grassland” was dominant (44%). “Improved agricultural grassland with abundant *Juncus* spp.” represented 13% and 22% cover at NWs and CWs, respectively, while “freshwater marsh”, present at the NWs (6%), was absent from the CWs (Fig. 4; Appendix B in Supplementary material).

Since woodland, damp woodland, scrub and hedgerows are also considered excellent terrestrial habitats for smooth newts (Table 1), these were examined further (Fig. 5; Appendix B in Supplementary material) at Level 3 (Fossitt, 2000). Altogether, twelve “woodland and scrub” habitat types were present at CWs and NWs. “Mixed broadleaved woodland” and “mixed broadleaved conifer woodland” cover combined, dominated both wetland types with 48% and 60% cover at the NWs and CWs, respectively (Fig. 5; Appendix B in Supplementary material). These were followed by “wet willow-alder-ash” (17%) and “scrub” (15%) at the NWs and “scrub” (22%) and hedgerows (7%) at the CWs. “Riparian woodland” and “bog woodland” were exclusive to NWs with 13% cover in total.

Given that, regardless of habitat type, barriers to movement by newts play a pivotal role in newt survival, these were also examined at the CW and NW sites. These barriers include roads and rivers which are classed as serious barriers to newt migration (Oldham

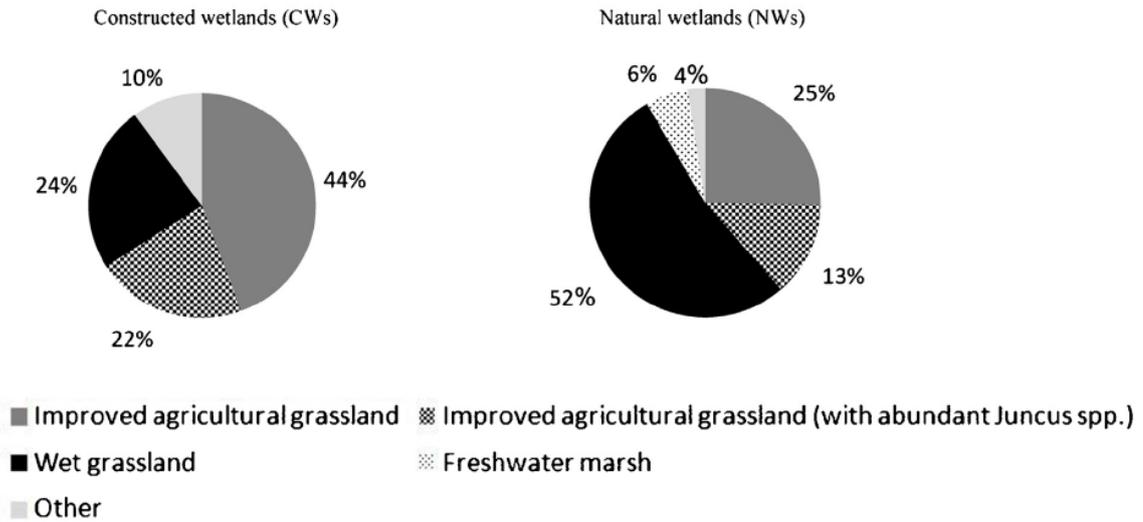


Fig. 4. Percentage cover of "grassland & marsh" habitats ( $\geq 5\%$  cover) at constructed (CW) and natural (NW) wetlands (Level 3) (Fossitt, 2000). Breakdown of "grassland & marsh" habitats with  $< 5\%$  cover (Other) is presented in Appendix B in Supplementary material.

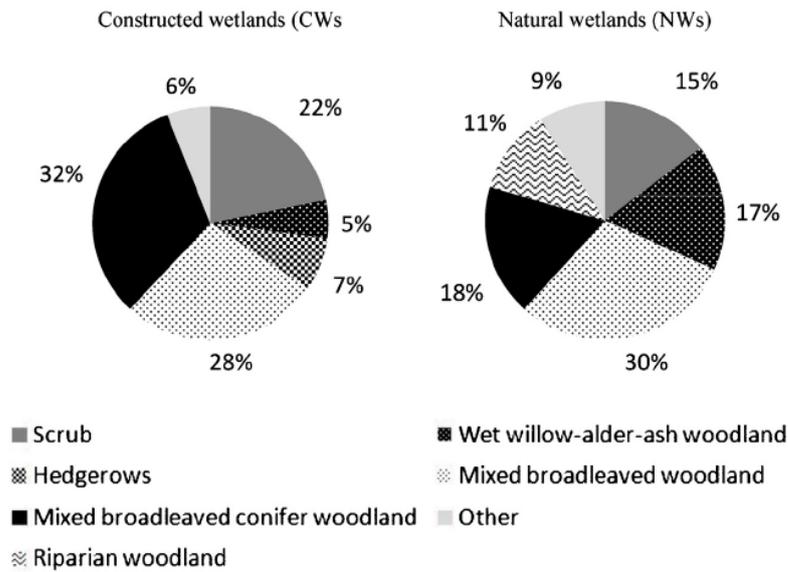


Fig. 5. Percentage cover of "woodland and scrub" habitats ( $\geq 5\%$  cover) at constructed (CW) and natural (NW) wetlands (Level 3) (Fossitt, 2000). Breakdown of "woodland & scrub" habitats with  $< 5\%$  cover (Other) is presented in Appendix B in Supplementary material.

et al., 2000; Matos et al., 2017). Other barrier habitats (directly bordering breeding sites) identified include "buildings & artificial surfaces", "improved agricultural grassland", "exposed sand, gravel & till", and "spoil & bare ground". Forty-four percent of the total perimeter of the CW sites in this study constituted potential barriers to newt migration compared to  $< 2\%$  at NW sites. While six out of eight CWs had barriers of some kind, only two out of eight NWs had barriers at the edge of the water body.

The significance of terrestrial microhabitats or features such as wood and stone which can act as potential refuges for newts, can contribute significantly to amphibian conservation when selecting breeding sites (Marnell, 1998). Twenty-eight percent of the  $20\text{ m} \times 20\text{ m}$  grids surrounding the NWs which were surveyed in this study contained features compared to just 18% for the CWs. Habitats such as "mixed broadleaved woodland" and "mixed broadleaved conifer woodland" accounted for the greatest per-

centage frequencies (5–11%) of features at both wetland types, with "wet willow-alder-ash woodland" within the same range for NWs only (Table 4). Features present within a range of 1–4% frequency (Table 4), included "riparian woodland" at the NWs, and "recolonising bare ground", "improved agricultural grassland" and "wet willow-alder-ash-woodland" at CWs.

Using the HSI (Table 2), only two out of the eight CWs received the highest score of 1 (*Good*) (Appendix C in Supplementary material), while seven of the eight NWs received a *Good* score (1), in that there were no barriers present (Table 5). One hundred percent of the perimeter lines of all CWs and NWs which received *Good* scores, contained extensive areas of habitat with good opportunities for foraging and shelter completely surrounding the wetland. One CW (CW4) received a *Moderate* score of 0.67, where 17% of the perimeter line of the CW is made up of "buildings & artificial surfaces", while one NW (NW4) received a *Moderate* score (0.67)

**Table 4**

Percentage frequency of occurrence of features (wood and stone) in habitats at constructed and natural wetlands.

Habitat code (Level 3)	% frequency CWs	% frequency NWs
(Mixed) broadleaved woodland (WD1)	5.3	10.3
Mixed broadleaved conifer woodland (WD2)	5.3	6
Recolonising bare ground (ED3)	1.8	0.04
Improved agricultural grassland (GA1)	1.1	0.1
Wet willow-alder-ash woodland (WN6)	1.1	6.2
Dry-humid and acid grassland (GS3)	0.4	0
Wet grassland (GS4)	0.4	0.4
Scrub (WS1)	0.4	0.1
Rich fen and flush (PF1)	0	0.1
Reed and large sedge swamps (FS1)	0	0.7
Marsh (GM1)	0	0.2
Hedgerows (WL1)	0	0.1
Riparian woodland (WN5)	0	3
Cutover bog (PB4)	0	0.05
Conifer plantation (WD4)	0	0.1
Bog woodland (WN7)	0	0.3
Recently-felled woodland (WS5)	0	0.05
Exposed sand, gravel or till (ED1)	0	0.2
Treelines (WL2)	0	0.05
Improved agricultural grassland with abundant <i>Juncus</i> spp	0	0.1

**Table 5**

Constructed and natural wetlands and their potential value to the terrestrial phase of the life cycle of the smooth newt using the Great Crested Newt Habitat Suitability Index (Table 2) (National Amphibian and Reptile Recording Scheme, 2007).

Constructed wetland	Score	Natural Wetland	Score
CW1	1	NW1	1
CW2	0.33	NW2	1
CW3	0.33	NW3	1
CW4	0.67	NW4	0.67
CW5	1	NW5	1
CW6	0.33	NW6	1
CW7	0.33	NW7	1
CW8	0.33	NW8	1

due to the presence of “buildings & artificial surfaces” (0.4% of the perimeter) directly bordering the lake. Five of the CWs received *Poor* scores (0.33) (Appendix D in Supplementary material) while none of the NWs received a *Poor* score.

#### 4. Discussion

The results of this study indicate that the NWs had significantly more terrestrial habitat types than CWs and that the number of terrestrial habitat types present in NWs was significantly correlated with the size of the area containing the terrestrial habitats. Both NWs and CWs were selected on the basis of: a) the presence of reed and large sedge swamps; b) their location i.e. paired CWs and NWs  $\leq 20$  km apart; and c) the presence of newt friendly terrestrial habitats within 500 m of the wetland. Nevertheless, given that most of the NWs were lakes (Appendix A in Supplementary material), the generally larger size of aquatic habitats, including open water, resulted in comparatively larger areas of terrestrial habitats being surveyed within 40 m of the water's edge than in the smaller CWs. While similar woodlands at both wetland types were most likely to contain features of benefit to newts, more grids (20 m  $\times$  20 m minimum mapable areas) in the terrestrial habitats of NWs contained features compared to those of CWs. Furthermore, “wet grassland” dominated the grasslands around NWs while “improved agricultural grassland” dominated the grasslands around CWs. The latter grasslands, which are generally managed through intensive grazing regimes, cutting and the application of fertilizer/herbicides, may result in the absence of structural diversity such as that of rough grassland and meadows – habitats which can offer cover and foraging for the terrestrial phase of the newt (Oldham et al., 2000). “Wet grassland” (often occurring on sloping ground with

poorly drained soils) with abundant rushes, tall grasses and a high broadleaved herb component, (Fossitt, 2000) may, in comparison to “improved agricultural grassland”, offer more potentially suitable terrestrial habitats. Areas of “marsh” unique to NWs in this study (along lake shores), can also offer good structural habitats, particularly for immature newts, given the presence of high moss cover in conjunction with rushes (*Juncus* spp.), sedges (*Carex* spp.) and a high proportion of broadleaved herbs. This is reflected in the HSI scores, where seven of the eight NWs, but only two of the eight CWs, received a “good” score. A number of CWs received lesser scores primarily because of the presence of a barrier to movement which could potentially impact on the migration of the newt from aquatic to terrestrial habitats. This is reflected by almost one fifth of the surface area of the CWs examined in this study consisting of “cultivated & built land” and “exposed rock & disturbed ground”, some of which is necessary for machinery access to the site.

Previous studies have emphasized the value of using CWs as a conservation strategy for amphibians and the need for future research and monitoring in these areas (Denton and Richter, 2013). While our study focused on suitable terrestrial habitats for newts and did not involve a survey of smooth newt abundance, a single adult specimen of the species was recorded on the edge of one CW during the study (Mulkeen & Gibson-Brabazon, pers. obs). The presence of newts in CWs in Ireland (Scholz et al., 2007) also suggests that water quality in CWs treating wastewaters, at least in some cases, is not an issue and can support breeding by newts. In addition, newts have been recorded in natural ponds and wetlands as small as 25 m<sup>2</sup> (Skei et al., 2006) and with up to 1000 individuals recorded in ponds less than 400 m<sup>2</sup> (Bell and Lawton, 1975). Regardless of waterbody size, if aquatic and terrestrial conditions are favourable for breeding, shelter, food and overwintering, it is likely that newts may colonise and breed in these areas. However, small changes to the design of new CWs, and the management of the lands surrounding both new and existing CWs, could enhance their dual role as water treatment systems and suitable habitats for the newt and other amphibian species.

In the design of new CWs, the overall size of the site should be considerably larger than the actual wetland itself to ensure that the area surrounding the wetland is of sufficient size to provide adequate refuges for the terrestrial phase of the newt. While lands outside the CW fence may provide suitable refuges for the newt when the CW is being constructed, there is no guarantee that this area will not be lost to development at some time in the future. As a guideline, and based on the evidence observed by previous

authors of smooth newt migration distances (Bell, 1977; Dolmen, 1981), it is desirable that a buffer zone around a CW be incorporated within the site. By way of example, the inclusion of 20 m minimum buffer zone (providing suitable terrestrial habitats for smooth newts) around a 20 m × 20 m (400 m<sup>2</sup>) CW, would result in the purchase of just an additional 0.32 ha. However, the width of the buffer zone may be amphibian species specific (Rothermel, 2004) with Calhoun et al. (2014) recommending a buffer zone of 300 m of forested areas surrounding vernal pools to favour the persistence of amphibian species such as wood frog and salamander in the USA (Calhoun et al., 2014). While buffer zones wider than 20 m could also accommodate juveniles who appear to travel greater distances during dispersal, further research is required to substantiate this. Large areas of open habitat offering little cover can act as a barrier during newt migrations to and from water bodies for breeding. Habitats such as “amenity grassland”, “improved agricultural grassland”, “spoil & bare ground” and “buildings & artificial surfaces”, offer little cover, shelter, hibernation, foraging or overwintering sites for newts. By their very nature, CWs built for the tertiary treatment of wastewater also contain areas covered with artificial surfaces such as tarmac or concrete, built structures for wastewater treatment and unpaved areas for access points and driveways. These should, however, be reduced to a minimum, particularly immediately adjacent to the edge of the CW. If hard surfaces are required adjacent to the CW, they should ideally be at one side only, leaving the other three sides with direct access to terrestrial habitats.

Prior to construction taking place, a habitat survey should be undertaken to determine the value of existing habitats to newts. The proximity of the proposed construction to the nearest NWs should also be considered as suggested by Drayer and Richter (2016), which may strengthen connectivity across the landscape (Calhoun et al., 2014). In particular, habitats identified in this study such as “mixed broadleaved woodland”; “mixed broadleaved conifer woodland”, “wet willow-alder-ash woodland” and scrub should be retained where possible, as should “wet grassland” and “improved agricultural grassland with abundant rushes”. In sites undergoing construction, judicious planting with suitable trees and shrubs and/or the creation of wet grassland using membranes beneath the soil surrounding the CW would also be beneficial. In particular, the availability of terrestrial cover around breeding sites in the form of logs and deadwood was found to be an important habitat parameter in discriminating between sites used or unused by the smooth newt during its life cycle (Marnell, 1998). Skei et al. (2006), Marnell (1998) and Oldham et al. (2000) suggest that woodland and scrub offer smooth newts suitable terrestrial habitats to complete the terrestrial phase of the life cycle. By their very nature, woodland and scrub habitats usually present a highly structured habitat, which could offer shelter and refuge in the form of large amounts of deadwood, often in the form of tree stumps, fallen branches or logs. At existing CWs, less frequent mowing of “improved” or “amenity grasslands” would encourage the growth of a greater proportion of tall, coarse or tussocky grasses, and a broadleaved herb component which could offer suitable refuge or foraging areas for newts. The addition of features such as stones or wood to all types of existing habitats would also enhance these areas as newt refuges. Even a reduction in the management (cutting and herbicide applications) of unpaved surfaces or gravel would facilitate the colonisation of plants over time. Therefore, without compromising the vital function of access to the CW and wastewater treatment areas, these unconsolidated surfaces with plant cover may also assist smooth newts during their migrations from aquatic to terrestrial habitats.

An indication of the variability of CWs vis-à-vis their suitability for smooth newts can be seen in the contrasting HSI scores for two CWs, one scoring “good” and one scoring “poor” (Appendix C and D in Supplementary material). The CW which received a “good” score

(Appendix C in Supplementary material) is completely surrounded by favourable terrestrial habitats, which provide good structure for the smooth newt during migrations (scrub; earth bank; treeline; and dry meadows & grassy verges). No barriers were identified on the wetland edge and despite it being located in an urban area, an adult specimen of the smooth newt was recorded on the edge of the wetland within the “scrub” habitat under a wood feature during the study (Mulkeen & Gibson-Brabazon, *pers. obs.*). The CW which received a “poor” score (Appendix D in Supplementary material) is surrounded by an unsuitable terrestrial habitat for newts i.e. “spoil & bare ground” which could act as a barrier to newt migration. “Spoil & bare ground” includes areas of bare ground due to ongoing disturbance or maintenance, unconsolidated surfaces which are regularly trampled or driven over, and areas which are largely unvegetated (<50% cover) (Fossitt, 2000). Areas such as these are open and provide little structure or protection for the smooth newt during migrations from the wetland to favourable terrestrial habitats. The relocation (where possible) of bare ground or unconsolidated surfaces with trampling activities, away from the edge of a CW, along with the creation of a grassland/woodland (with a diversity of structures) plus the simple addition of wood and/or stone features could, at minimal cost, support successful newt migrations from aquatic to terrestrial habitats.

## 5. Conclusions

Natural wetlands have significantly more terrestrial habitat types than CWs and the size of NWs is significantly correlated with the number of surrounding terrestrial habitat types. Seven of the eight NWs received a “good” score using the HSI in comparison to two of the eight CWs. Constructed wetlands received lower scores primarily because of the presence of unsuitable habitat types or barriers which could potentially impact the migration of the newt from aquatic to terrestrial habitats. Therefore, in the future design of new CWs, it is important that the overall size of the site be larger than the actual CW itself to facilitate the incorporation of newt friendly terrestrial habitat which is immediately adjacent to the edge of the CW. Appropriate management of the areas surrounding new and existing CWs along with the addition of stone or wood features, could also enhance these areas for smooth newts and other amphibian species.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecoleng.2017.06.005>.

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Appendix C

**Diagnostic definitions and figures of male and female *Tetanocera punctifrons* and *T. latifibula*, new records of *T. punctifrons* in Ireland, and notes on biology (Diptera, Sciomyzidae)**

Maher, C. A., Carey, J.G.J., Mulkeen, C.J., Williams, C.D., Knutson, L.V., Healy, M.G. and Gormally, M.J.

*Article associated with Chapter 5.*

**Diagnostic definitions and figures of male and female  
*Tetanocera punctifrons* and *T. latifibula*, new records of *T.  
punctifrons* in Ireland, and notes on biology (Diptera,  
Sciomyzidae)**

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**Summary**

New records of *Tetanocera punctifrons* Rondani, 1868 in Ireland are presented. Distinguishing characters from the very similar *T. latifibula* Frey, 1924 are discussed. The female abdomens of both species are described for the first time. The distributions of both species are summarised. The history of type examinations and of taxonomists' conceptions of the two species is tracked, especially the relative reliability of published figures of diagnostic features. We emphasise the need for such analyses of rare and closely related species, even if apparently disjunct in distribution. Habitats of *T. punctifrons* and *T. latifibula* are described, and the biology and morphology of the immature stages are compared.

## Introduction

Collection of the Palaearctic *Tetanocera punctifrons* Rondani, 1868 in Ireland has led us to analyse the features of the adults of that species and the closely related Holarctic *T. latifibula* Frey, 1924.

Considering the importance of *Tetanocera* Duméril, 1800 to the study of cladistics and behavioural evolution of Sciomyzidae (snail-killing flies), we believe it is useful to thoroughly document the range extensions and identities of such relatively poorly known and similar species. We also describe the habitats and summarise the biology and morphology of the two species in the hope of expediting further studies.

*Tetanocera* is one of the best known genera of Sciomyzidae. Extensive biological information is available on 26 of the 39 species, in most cases complete life cycles (Foote 1961, 1996a, b, 1999, 2008, 2011; Knutson 1963; Knutson *et al.* 1965; Rozkošný 1965, 1967; Trelka and Berg 1977; Trelka and Foote 1970). The morphology of the immature stages has been described for 21 species and, in most cases, all stages (Knutson 1963; Foote 2013; Rozkošný 1965, 1967). Some of the information on biology and immature stages of European species, along with adult taxonomy, has been presented in regional studies by Rivosecchi (1992: Italy), Rozkošný (2002: Central Europe) and Vala (1989: Mediterranean Europe). The larvae range from overt predators of snails in open water to predators of shoreline or otherwise exposed aquatic snails to parasitoid-predators of Succineidae, slugs, or terrestrial snails. *Tetanocera ferruginea* Fallén, 1820, is one of the best known species in the family, a result of extensive laboratory experimental studies on development, overwintering, competition, food consumption, fecundity, survival, etc. (reviewed by Knutson and Vala, 2011 and Foote 1996a). Recently Chapman *et al.* (2006) used phylogenetic methods, including molecular and larval morphological data, in exploring morphological adaptations of North American *Tetanocera* species to both aquatic and terrestrial habitats, one of the first attempts to do so within a dipteran lineage. In a subsequent publication, Chapman *et al.* (2012) built “on those findings by examining feeding behaviour evolution, as feeding behaviours are dependent on both larval morphological adaptations to different environments and specific requirements related to finding and subduing different prey species.” *Tetanocera latifibula*, but not *T. punctifrons*, was included in those studies.

*Tetanocera* is the fourth-largest genus in the family Sciomyzidae [12 Holarctic species, 8 Palearctic species (with *T. chosenica* Steyskal, 1951 ranging from Japan and Korea to Yunnan, Kwangsi, and Fukien China in the Oriental Region); 18 Nearctic species (with *T. plumosa* Loew, 1847 extending from Alaska to Venezuela); and one strictly Oriental species, *T. nigrostriata* Li, Yang & Gu, 2001 (Yunnan)].

All species of Sciomyzidae occurring in Ireland were included in Rozkošný (1987) and Vala (1989). Stephenson and Knutson (1970) listed 26 species in 13 genera of Sciomyzidae from Ireland. They included seven species of *Tetanocera*, by counties, but without detail, based on their review of only some of the literature, some collections, and records provided by 22 colleagues in the British Isles (*T. ferruginea*, *T. fuscinervis* (Zetterstedt, 1838) [as *T. unicolor* Loew, 1847], *T. phyllophora* Melander, 1920, *T. elata* Fabricius, 1781, *T. hyalipennis* Roser, 1840; *T. punctifrons* and *T. silvatica* Meigen, 1830]. Chandler (1972) provided a much more detailed summary of the distribution of 40 species in 17 genera in Ireland, including six of the species of *Tetanocera* listed by Knutson & Stephenson (1970) but omitting *T. silvatica* and adding *T. freyi* Stackelberg, 1963 and *T. arrogans* Meigen, 1830. In Chandler (1972) a female *T. punctifrons* from Cratloe, County Clare, 1895 (Dublin Museum) served as the first detailed record of the species from Ireland; it was noted that the presence of this species in Ireland needed confirmation. The Holarctic *T. silvatica* was reinstated by Speight and Nash (1977). Speight (2001, 2004) reported collecting 1 male and 1 female of *T. punctifrons* (and 6 other *Tetanocera* species) in County Cork, but without discussion of identifying features. Speight (2007) added *T. montana* Day, 1881 to the Irish fauna with a detailed comparison of the adult to the related *T. arrogans* and including the geographical and habitat distribution of *T. montana*. Recent extensive collections of *Tetanocera* species in Ireland have been documented fully in Speight (2004: County Cork) and Williams *et al.* (2007: County Clare, County Galway, County Mayo, County Roscommon, and County Westmeath). Speight (1979) provided a list of 45 species in 19 genera, without details, and subsequently published records of six additional species. The most recent list was by McLean (1998), including 51 species. Six additional species were recorded recently (Staunton *et al.* 2008). Despite recent extensive collecting in a few areas, the Irish Sciomyzidae are still not well known; major range extensions of Sciomyzidae in the

Palaearctic are being reported. Currently Ireland has 60 recorded species of Sciomyzidae in 19 genera.

We report here collection of adults of *T. punctifrons* by C. Maher, C. Mulkeen, and J. Carey in Ireland (Table 1). Identifications were confirmed by LVK. The specimens, in perfect condition, were transferred from alcohol and glued to a pin; the abdomens were removed, processed in NaOH and subsequently in slightly acidic alcohol, and then placed in a microvial of glycerine pinned below the rest of the specimen. They are deposited in the Natural History Museum, Dublin.

**Table 1. Date-locality and collection data for the specimens of *Tetanocera punctifrons* (Rondani, 1868) reported in the present paper.**

Locality	Co-ordinates	No./sex	Date	Collector	Collection method	Depository
Skealaghan turlough	53°36'35"N. -9°8'29"W.	1♀	5/8/2005	C. Maher	Sweep-net	LVK collection, Gaeta
Annagh East	53°24'29"N. -9°2'45"W.	1♀	4/9/2014	J. Carey	Malaise trap	Natural History Museum Dublin
Corgar Lough	54°3'38"N. -7°45'38"W.	1♀	1/7/2014	C. Mulkeen	Malaise trap	Natural History Museum Dublin
Corgar Lough	54°3'38"N. -7°45'38"W.	1♀	7/8/2014	C. Mulkeen	Malaise trap	Natural History Museum Dublin
Lough Down	53°20'57"N. -9°10'58"W.	1♂	2/7/2014	C. Mulkeen	Malaise trap	Natural History Museum Dublin
Lough Meelagh	54°3'24"N. - 8°9'3"W.	1♂	7/8/2014	C. Mulkeen	Malaise trap	Natural History Museum Dublin

### Identification and distribution

Ostensibly, with only two of the Western European species of *Tetanocera* – the central and southern *T. punctifrons* and the northern *T. latifibula* – sharing the very distinctive feature of a single strong seta on the posterior surface of the mid femur, near the apex, one might think that it should be relatively easy to confirm the identity of *T. punctifrons* in Ireland. However, some of the features traditionally used for distinguishing *T. punctifrons* from *T. latifibula* are either variable or difficult to evaluate, especially when a series of specimens of both species are not at hand for comparison, as noted below.

Rozkošný and Knutson (2011) recorded *T. punctifrons* from Ireland (based on Speight 1979), across Wales, Scotland, and England to Denmark and southern Sweden, then across central and southern Europe to Spain, Italy, Poland, Romania, Bulgaria, and Turkey and across Central European Russia, but it was absent from Norway and central and northern Sweden and Finland. They recorded *T. latifibula* from northernmost Sweden and Finland, through north-east, north-west, and Central European Russia (Kaliningrad region) and eastwards across Mongolia and Siberia, but it was absent from England to Belgium to France to Denmark and south. Foote (1999) mapped the distribution of *T. latifibula* in North America, showing it ranging from coastal and north-central Alaska just below the Arctic Circle south in mountainous areas to north-eastern California, northern Utah, central Colorado, and northern-most New Mexico as well as into the plains of southern-most Manitoba to north-western Iowa.

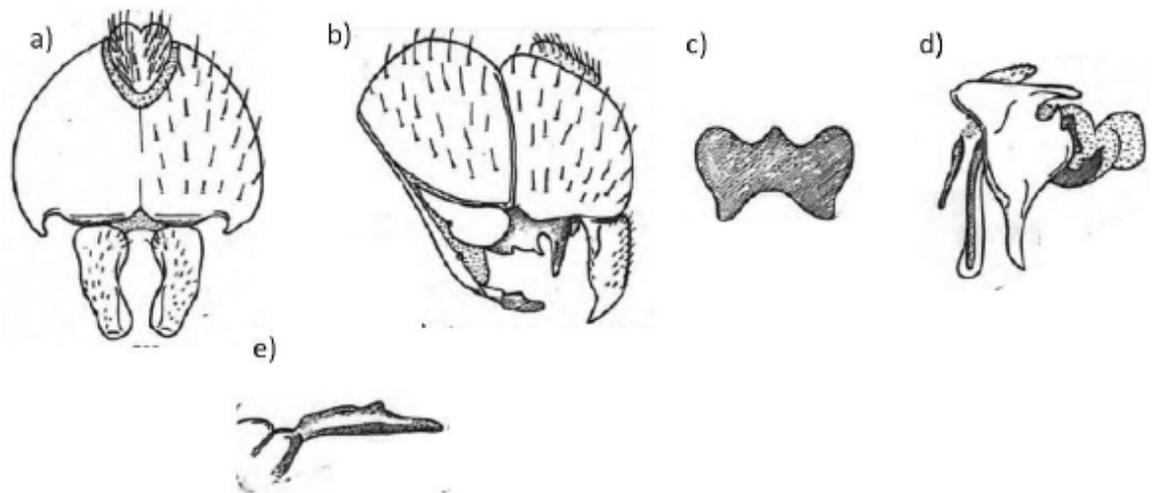
*Tetanocera punctifrons* and *T. latifibula* can be placed with about 11 other, mainly Nearctic, *Tetanocera* species in which the surstylus is quite similar in lateral view (scoop-like and more or less short) and somewhat similar in ventral view. Eight of the Nearctic species also have a pre-apical seta on the posterior surface of the mid femur, whereas all other species lack this seta. The shape of the posterior surstylus varies within some of the Nearctic species; there are few figures of them other than in Steyskal's (1959) taxonomic revision and in regional studies by Fisher and Orth (1983: California), Foote *et al.* (1999: Alaska), and Foote and Keiper (2004: Ohio). It cannot be excluded that *T. punctifrons* is the senior synonym of a species in North America.

When documenting the presence of rare species, especially in the Palearctic where many of the earlier described species were inadequately described and where there are often many synonyms within a genus, it can be useful, primarily for the sake of future

researchers, to refer to studies of type specimens and to track the record of examination of characters and understanding of the species concepts by the leading taxonomists. We do this here for *T. punctifrons* and *T. latifibula*, the only species of this group of *Tetanocera* likely to be confused in Western Europe.

***Tetanocera punctifrons* Rondani, 1868 (Fig. 1, a-e; from Rozkošný 1984, figs 536-540)**

Rondani's type specimens of *T. punctifrons* (two males and one female from Parma, Italy) in the Florence Museum were studied by Verbeke (1964), who designated a male (No. 1512) as "type" (= lectotype). He figured the antenna and a posterior view of the surstyli of a cotype male (from slide preparations; these slides probably were made in Verbeke's laboratory and perhaps are in the Institut royal des Sciences naturelles de Belgique, Brussels). Verbeke (1964) also described other characters of the species and stated that Sack (1939) had correctly interpreted Rondani's concept of the species. Sack included both *T. punctifrons* and *T. latifibula* but did not mention the setae on posterior surface of the mid femur (Sack's publication was used extensively for identification of Palearctic Sciomyzidae until Rozkošný's 1987 monograph). Under *T. punctifrons*, Verbeke (1964) synonymised *T. collarti* Verbeke, 1948 (from Belgium) and, with a question mark, *T. marginella* Robineau-Desvoidy, 1830 (from France), noting that Robineau-Desvoidy's specimens had been destroyed. Collin (1960) commented on the confused history of the name *T. marginella* (listed as a synonym of *T. elata* Fabricius by Hendel 1900) and used that name for what we consider to be *T. punctifrons* in his key to nine British species of *Tetanocera* based in part on presence of one pre-apical seta on the posterior surface of the mid femur. For further clarification of Collin (1960), Verbeke (1968) placed *T. elegans* Collin as a synonym of *T. phyllophora* Melander, 1920. Rozkošný and Elberg (1984) listed *T. marginella* as a "doubtful species". Verbeke (1964) noted in his detailed description – based on the three syntype specimens and 29 other specimens from England, Wales, Belgium, Luxembourg, Austria, Italy, Denmark, and Bulgaria as one of the "principaux caractères" of *T. punctifrons* the presence of a posterior pre-apical seta on the mid femur, a character which "existe également chez *T. latifibula*." Subsequent authors followed this interpretation of the species and have presented figures of the male genitalia and other features.



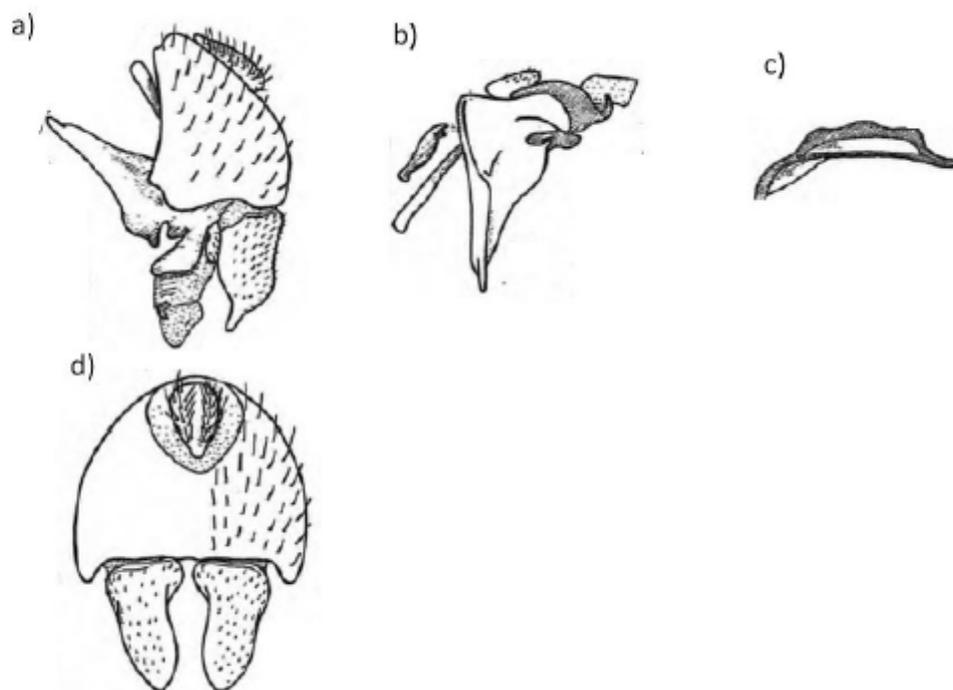
**Fig. 1. Male genitalia of *Tetanocera punctifrons* (after Rozkošný 1984).**

Mayer (1953), in his key to 19 species of *Tetanocera* in the Palearctic region (in German), included *T. punctifrons* and *T. latifibula* easily separated by the length of the arisal hairs and thickness of the hind femur, and with characters of the fronto-orbital setae, frons, hind femur setae, and wing in the terminal couplets, but without figures. Rivosecchi and Santagata (1979) presented poor outline figures of the surstylus and hypandrium. Rozkošný's (1984, 1987) figures of the surstylus agree well with specimens we have seen except that in lateral view the anterior margin is more evenly and gently excavated in our specimens. Vala's (1989) figures agree with our specimens except that one of his two lateral views of the surstylus (his fig. 119 l) shows the posterior margin as slightly indented in the upper two-thirds, not straight as in our specimens. Vala (1989) and Rivosecchi (1992) presented figures of the sixth sternum of *T. punctifrons*. Vala showed two approximate protuberances on the right side, with a median protuberance; Rivosecchi showed three weak protuberances. Our specimens have a right and a left protuberance, with a weakly sclerotised median protuberance. Rozkošný's (1984) figure of *T. latifibula* and our specimens of *T. latifibula* display three equally separated, sclerotised protuberances. Rozkošný (1984, 1987) and Vala (1989) figured the "ix sternum" (epandrium) with a straight ventral margin in *T. punctifrons* and a slightly inwardly curved ventral margin in *T. latifibula*; in our specimens the margin is only slightly curved inwards in both species. Rivosecchi (1992) provided figures of the

surstylus and other characters of specimens of *T. punctifrons* from Lazio, central Italy; those of the surstylus and antenna generally agree with Verbeke's (1964) figures. However, as with many of Verbeke's published figures, most of Rivosecchi's figures were made from slide preparations and thus include considerable distortion.

***Tetanocera latifibula* Frey, 1924 (Fig. 2, a-d; from Rozkošný 1984, figs. 519-522)**

*Tetanocera latifibula* was proposed by Frey (1924) for three males and four females from Munio and Enontekis, Finland and from "Beresow", western Siberia; he presented a few characters of *T. latifibula* in his key to 14 *Tetanocera* species and a lateral, outline view of the epandrium and surstylus of the male. His only reference to *T. punctifrons* was in a list of four species, "...not known to me but to all appearances are probably distinct." Sack (1939) gave a slightly more detailed description, without figures. Of subsequent authors, apparently only Stackelberg (1963) and Rozkošný (1984) studied the syntype specimens. Steyskal (1959) did not recognise *T. latifibula* from North America, but he described *T. hespera* from Alberta, Canada and from Alaska, Utah, and South Dakota, U.S.A. on the basis of a few characters, presented outlines of the posterior and lateral views of the postabdomen, and (1965) synonymised it under *T. latifibula*. It cannot be discounted that *T. hespera* is a valid species. Verbeke (1964) mentioned a few characters of *T. latifibula* and presented a posterior view of the epandrium and surstylus (specimen: "T4: Asie, Altai, Ularak", not part of the type series) drawn from a slide preparation that apparently has been lost. Stackelberg (1963) reproduced Frey's (1924) figure of *T. latifibula*, included it with additional characters in his key, and recorded specimens from the Kola Peninsula and from Leningrad, Russia. Fisher and Orth (1983) – an overlooked source by most European authors, of excellent figures of Holarctic species – figured the male and female postabdomens of specimens from California, U.S.A. and mentioned other characters (noted below). Apparently only Rozkošný (1984) subsequently examined Frey's (1924) type specimens; he (1984, 1987) figured the male genitalia in detail.



**Fig. 2. Male genitalia of *Tetanocera latifibula* (after Rozkošný 1984).**

Although it is difficult to reconcile some differences in the drawings (there are no photographs) of the male postabdomen in the publications noted above (the best are in Rozkošný [1964, 1987] and Verbeke [1964]), the shape of the surstylus in lateral view seems to be the best feature for distinguishing males of the two species. The surstylus of *T. latifibula* seems to be shorter than that of *T. punctifrons*, is slightly curved inward in the upper half posteriorly, whereas that of *T. punctifrons* is straight, and the anterior margin of *T. latifibula* is deeply excavated, whereas that of *T. punctifrons* is more shallowly and gently excavated towards the apex. The other characters traditionally used to separate the species, and a few other characters, seem to vary somewhat and are difficult to compare without a series of both species in hand.

We have seen the following 45 specimens:

*T. punctifrons*: Ireland, 2♂ 4♀; Belgium, 4♂ 2♀; France, 1♂; Spain, 1♂; Italy, 2♂; Bulgaria, 1♂; Denmark, 4♂ 4♀.

*T. latifibula*: Sweden, 1♂, 1♀ plus 1♀ (laboratory reared, F<sup>1</sup> pinned with puparium); Finland, 2 specimens; Mongolia, 1 specimen; Siberia, 1 specimen; Canada, Northwest

Territories, 1♂; British Columbia, 1♂; Manitoba, 3♂ 3♀; Alberta, 1♂; U.S.A., Alaska, 1♂; Colorado, 2♂; Nebraska, 1♂; Washington, 1♂.

We have focused above on the more recent literature of primary importance concerning these two species. However, we have also surveyed other major, older publications, e.g., by H. Loew (1841-1876), F. Hendel (1900-1938), etc. *Tetanocera punctifrons* appeared as a valid species in Hendel's (1903) key; he did not mention *T. latifibula*. Becker (1902), in his review of Meigen's collection in Paris and Vienna, did not mention *T. punctifrons*. Becker, in Becker *et al.* (1905), listed only the original description of *T. punctifrons*. Séguy (1934) did not include either species, but included *T. marginella*, which he confused with *T. elata* or *T. phyllophora*; he did not use the character of a seta on the posterior surface of the mid femur.

#### **Additional comments on external features**

- a. Plumosity of arista: a fairly reliable character, well-figured for *T. punctifrons* by Verbeke (1964) and Rivosecchi (1992) from slide preparations. Recorded as broader than pedicel in *T. punctifrons*, narrower in *T. latifibula*. In addition, the plumosity is less dense and more brownish in *T. latifibula*.
- b. As noted by Rozkošný (1984), in *T. latifibula* the pedicel is usually distinctly longer than half the length of the basal flagellomere (= postpedicel) but is slightly shorter in *T. punctifrons*.
- c. Rozkošný (1984) also pointed out that in *T. latifibula* the second aristal segment is slightly longer than broad, whereas in *T. punctifrons* it is, at most, as long as wide.
- d. We could see no differences between the species in extent of facial hairs or colour of antennae at insertion of arista, as noted by Fisher and Orth (1983) in distinguishing *T. latifibula* from other species in California.
- e. Orbito-antennal spot: one of four key characters used in separating the two species by Rozkošný (1984, 1987) but apparently an unreliable character in these species. Traditionally regarded as present in *T. punctifrons* but present or absent in our Irish specimens; absent in *T. latifibula* but present or weak in our three specimens from Sweden.

- f. Hind femur anterodorsal setae: traditionally recorded as two in *T. punctifrons*, and 3–4 in *T. latifibula*, as in our specimens.
- g. As noted by Verbeke (1964), the antero- and posteroventral setae on the hind femur are very strong in the female of *T. punctifrons*; we noted that they are weaker and more sparse in *T. latifibula*.

**Female abdomen:** Characters of the female abdomen have not been used extensively in taxonomic studies of Sciomyzidae; the relatively few published descriptions have been reviewed by Knutson and Vala (2011) and Murphy *et al.* (in prep). With regards to *Tetanocera*, on the basis, in part, of study of the female terminal abdominal segments, Verbeke (1964) resolved the status of several Palearctic names. Fisher and Orth (1983) figured the abdominal sterna of 10 species of *Tetanocera*, including *T. latifibula*, from California. Rivosecchi (1992) figured and described various features for seven species of *Tetanocera* from Italy, including, for *T. punctifrons*, the habitus, head, antenna, mid femur, abdominal terminal segments and spermathecae of the female, male postabdomen – sternite 6, ventral and lateral views, internal genitalia, and wing.

In a cladistic analysis and taxonomic revision of the related genus *Renocera* Hendel (Knutson, Mathis and Chapman, in prep.) of the eight genera in their outgroup, the following characters and character states of the female abdomen have been provisionally recognised as the most important:

1. Sterna 7 and 8 broadly to narrowly separated by membrane (plesiomorphic); fused (apomorphic).
2. Sternum 8 a single, transverse plate (plesiomorphic); a pair of hemispherical lobes (apomorphic).
3. Hypoproct a single, transverse, setose lobe-like plate (plesiomorphic); separated medially by membrane into two lateral lobes (apomorphic state 1); a single lobe, setose posteriorly, with anterior portion a bare concave plate with tricuspid anterior margin (apomorphic state 2).
4. Spermathecae without an apical appendage (plesiomorphic); with an apical appendage (apomorphic).

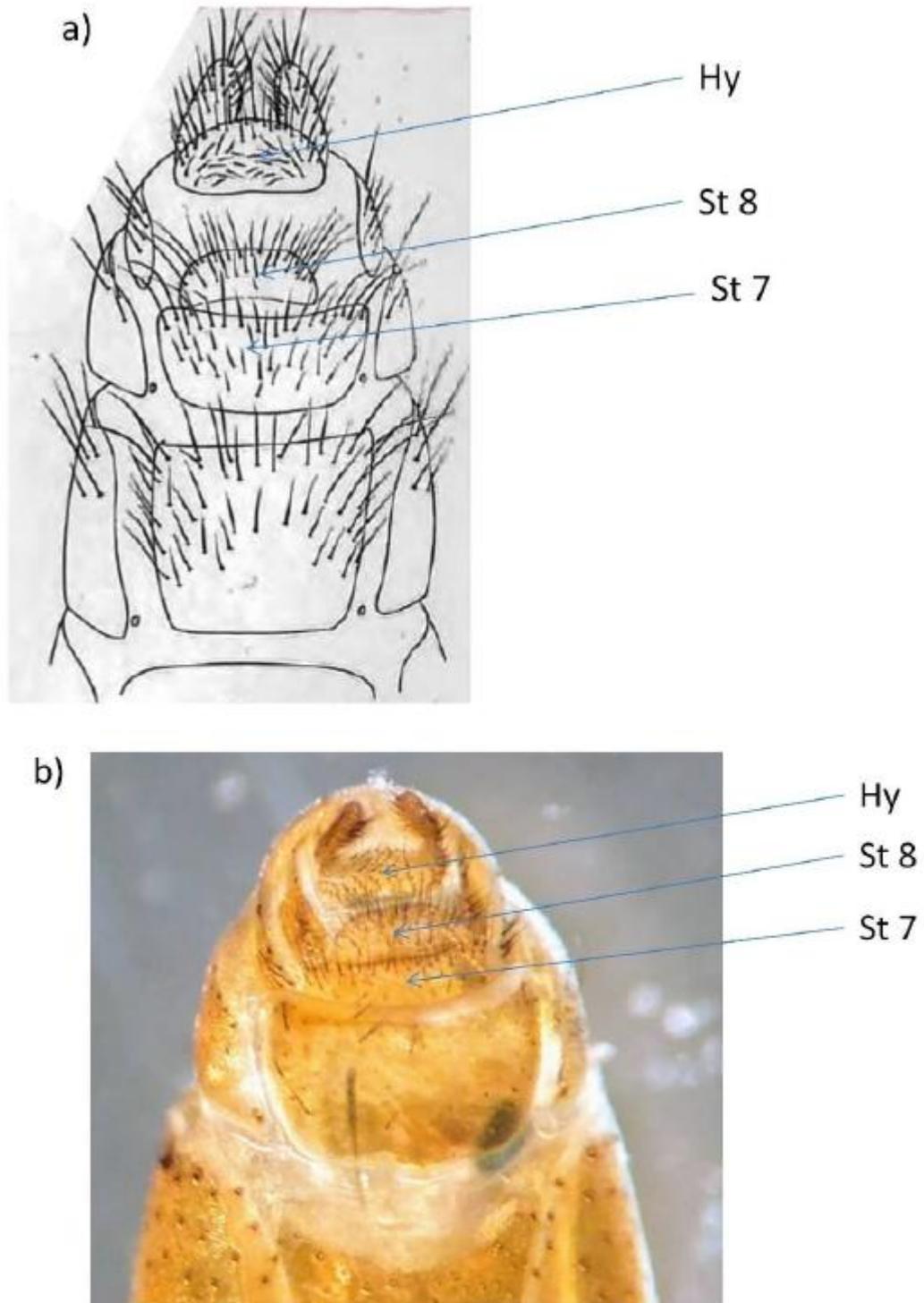
Other fine details also have been distinguished in the female abdomen. Following is a description of features common to both *T. punctifrons* and *T. latifibula* (specimens examined: *T. punctifrons*, 3♀, Ireland; *T. latifibula*, 2♀, Sweden). We note that it is important to view the sterna not only in ventral view (in which view sterna 7 and 8 may appear fused), but also with the abdomen tipped upward posteriorly (in which view any membrane separating sterna 7 and 8 can be seen more clearly).

Spiracles 6 and 7 in the extreme anterolateral corner of terga 6 and 7 (as figured for *T. plebeja* Loew by Knutson [1987]). Fisher and Orth (1983) figured these spiracles in the terga for seven of the species they studied but in the membrane for *T. latifibula*. Abdomen without mid-dorsal dark stripe. Sterna 6 and 7 broadly separated by membrane. Epiproct a minute, lightly sclerotised plate, with about four setulae. Two spermathecae (viewed at 70x) hemispherical, surface smooth, base flattened, stem not sclerotised.

The following diagnoses reveal very significant differences between females of the two species, not previously described.

*T. latifibula* (Fig 3a, from Fisher and Orth 1983): Terga 2, 3, and 4 without a trace of mid-dorsal dark stripe. Setae near posterior margin of terga 3, 4, and 5 strongest, especially laterally (note: the posterior-most row of so-called “posterior marginal tergal setae” are not on the ultimate posterior margin of the terga; there is a rather broad, bare, somewhat more lightly sclerotised posterior marginal extension to terga 3-7, which is especially strong on tergum 4 [well illustrated for *T. plebeja* in Knutson 1987]). Sterna 7 and 8 broadly separated by membrane). Hypoproct a densely setose, semi-circular plate. Cerci slightly broadened apically in lateral view. Spermathecae without apical process.

In Figs 3a and b, St 7 and 8 = Sterna 7 and 8.



**Fig. 3. Female genitalia: a, *Tetanocera latifibula* (after Fisher and Orth, 1983); b, *Tetanocera punctifrons* (photograph by J. Carey).**

*T. punctifrons* (Fig. 3b, photograph by J. Carey): Terga 2, 3, and 4 with faint to strong mid-dorsal dark stripe. Setae near posterior margin of terga 4, 5, and 6 strongest, much stronger than in *T. latifibula*. Sterna 7 and 8 appearing fused in ventral view but in posterior view barely but distinctly separated by membrane. Hypoproct a transversely rectangular plate, in some specimens very narrowly separated by median membrane on posterior margin, posterior margin only slightly curved. Cerci not broadened apically in lateral view. Spermathecae with minute but distinct apical process (note: this apical process also is figured for *T. punctifrons* by Rivosecchi 1992).

**Other characters:** Other characters used by various authors in separating other species of *Tetanocera* were not found to be useful in separating our specimens of *T. punctifrons* and *T. latifibula*, but they may be worth checking further. These characters included positions of fronto-orbital setae relative to anterior margin of frons and anterior ocellus; colour of face, parafacies, and genae; width of gena relative to eye height; extent of hairs on parafacies; length of hairs on anterior margin of frons; basal flagellomere concave or straight above (but more often more deeply concave in *T. punctifrons* than in *T. latifibula*); colour of occipital spot; colour of thoracic dorsum; scutellum flat or convex; scutellum with or without an anterior ridge; colour of fore tarsus; curvature of posterior cross-vein; cross-veins infumated or not; and colour of stigma.

**Key:** The following key is in a format that may be of broader use than is the traditional format. That is, first we present characters that we have found to be the most reliable. Second, we include, in parentheses, characters that have been used by other students of the two species but for which there is disagreement or doubt as to their reliability. Thus we first guide the user to the so-called reliable distinctions and then provide other characters that may prove important.

1. Aristal hairs moderately dense, black, longer than width of pedicel; in lateral view pedicel at most as long as broad. Hind femur with two anterodorsal setae beyond mid length and rarely with a third, short, anterior-most seta. Surstylus long, in lateral view posterior ventral margin straight, anterior ventral margin gently and evenly excavated in apical half. (Second arisal segment at most as long as wide.

Ventral margin of epandrium straight. In female, ventral setae of hind femur strong and numerous) ..... *T. punctifrons*

- Arista hairs less dense, brownish black, shorter than width of pedicel; in lateral view pedicel slightly longer than broad. Hind femur with three or four anterodorsal setae. Surstylus shorter, in lateral view posterior ventral margin slightly excavated in basal half, anterior ventral margin more deeply and abruptly excavated in apical half. (Second arista segment slightly longer than broad. Ventral margin of epandrium slightly excavated. In female, ventral setae of hind femur weaker and sparser) ..... *T. latifibula*

The only other *Tetanocera* species in Ireland that might be confused with *T. punctifrons* or *T. latifibula* is *T. robusta* Loew, which ranges from Ireland to Kamchatka and which is widespread in the Nearctic. Males of *T. robusta* are readily recognised by the conical projection (even in dry specimens) on the left side of the epandrium. Notably, *T. robusta* is the only species of *Tetanocera* (both males and females) with a setose prosternum (posterior portion). Furthermore, whereas *T. punctifrons* and *T. latifibula* have one strong seta before the apex on the posterior surface of the mid femur, *T. robusta* usually has one strong seta and two to three weaker setae in this area. If, as a result of collection or preparation procedure, female specimens of *T. robusta* have lost the prosternal setae and the setae on the posterior surface of the mid femur (but sockets should still be visible) or if they show unusual variation (we have seen one female *T. robusta* from Ireland with setulae on only one side of the prosternum), it might be useful to note that *T. robusta* and *T. latifibula* lack a mid-dorsal dark stripe on the abdomen (present in *T. punctifrons*). In *T. robusta*, the postpedicel is longer than wide, with the dorsal and ventral margins almost parallel, as in *T. latifibula* (not almost square as in *T. punctifrons*), and the arista setulae are sparse but long as in *T. punctifrons* (not shorter and more dense as in *T. latifibula*).

### Habitat

Many recent and on-going ecological studies of Sciomyzidae in Ireland have focused on the use of sciomyzids as ecosystem service providers and bioindicators. In a study of 10 turloughs (temporary lakes practically unique to the west of Ireland), Williams *et al.* (2009a) showed a negative relationship between the abundance of the dominant species [*Ilione albiseta* (Scopoli)] and its prey when factors such as hydrology and vegetation structure were controlled. *Tetanocera arrogans*, *T. ferruginea*, and *T. robusta* were significant indicators of particular turloughs, but *T. punctifrons* was not collected in this study. One of the specimens of *T. punctifrons* noted in the present paper came from Skealaghan Turlough (Co. Mayo) during a separate study. Despite an intensive study of a transect at this turlough, Williams *et al.* (2009b) failed to collect *T. punctifrons*.

Other recent work in Ireland has included a detailed study of the Sciomyzidae of the Shannon Callows, the largest unregulated river flood plain in Europe. Maher *et al.* (2014) delineated hydrological niches for 22 species of Sciomyzidae in Ireland, including six species of *Tetanocera*. Whereas Williams *et al.* (2009a) demonstrated a quadratic relationship between Sciomyzidae species richness and soil moisture, Maher *et al.* (2014) showed a linear relationship between species richness and hydroperiod. More recent work on Sciomyzidae in Ireland has focused on wet grasslands. In a detailed study of temporal and spatial partitioning of Sciomyzidae and Syrphidae on often ecologically overlooked wet grasslands, Carey *et al.* (2017a) found that, "Spatiotemporal analysis showed that species turnover between habitats at different times made the most significant contribution to overall Diptera diversity." Carey *et al.* (2017b) showed significant correlations between parataxonomic unit abundance and co-structure of nine families of Diptera and Sciomyzidae abundance and co-structure, making them useful bioindicators of Diptera in general. Whereas Williams *et al.* (2009a) could find no support for co-structure between Sciomyzidae communities and Mollusca, Carey (*pers. obs.*) did find a relationship between his Malaise trap collections and soil-sieved Mollusca.

Mulkeen collected four *T. punctifrons* from Malaise traps as part of an on-going study to investigate the biodiversity-supporting functions of constructed wetlands as compared to those of natural wetlands. This study has involved the use of both Malaise and emergence traps at selected sites.

### *Habitat of T. punctifrons*

Beaver (1972) collected a few adults of "*T. punctifrons*" from marshy dune slacks and a lake margin in north-western Wales. These specimens have been destroyed, but as noted above, the distribution data would seem to support the identification. Rozkošný (1984) described the habitat of this species throughout its range as "mesic woods, alongside running water, and also in the mountains." Vala (1989) stated that adults are found at higher altitudes as well as in plains, along canals, and in dry woods. Rivosecchi (1992) recorded adults from various types of heavily vegetated habitats near water in Italy. A male collected on 14 July 1994 in France (Thoranc, Alpes Maritime, J.P. Haenni, and C. Dufour, Mus. Neuchâtel, Switzerland) is labelled "Jones, laïches, massettes, russeau, parte marécageus." In a summary of the macrohabitats of the 81 species of Sciomyzidae known from the Atlantic zone of Europe, Speight and Knutson (2012) noted, for *T. punctifrons*, "wetland / open ground; tall-herb open areas in *Alnus incana* alluvial forest; montane fen and stream-sides in seasonally-flooded, lightly grazed, humid, unimproved grassland."

Most of the extensive collecting of Sciomyzidae in Ireland has been conducted in turloughs and other seasonal or permanent, aquatic to semi-aquatic habitats. However, Speight (2001, 2004) carried out a detailed analysis of sectors (primarily infrastructure, disused, productive, plus set-aside) of a 41-ha. case-study farm in Riverstick, County Mayo. A 27-Malaise-trap survey of Syrphidae and Sciomyzidae was conducted from April through September. Of the 182 specimens of 17 species of Sciomyzidae collected by Malaise traps (23 species were collected from the farm by use of sweep-net, Malaise, and emergence traps; six other species were collected by use of sweep-net and emergence trap), one male and one female *T. punctifrons* were collected in an acidic fen-like habitat in one of the 10 disused sectors, the male "from an acid fen/seasonally flooded, oligotrophic *Molinia* grassland" and the female "from a grassy field margin beside a permanently-flowing streamlet backed by a hedge." Speight (2004) tested the predicted occurrence of Sciomyzidae in the total of 21 different kinds of habitats in the three main sectors by intensive emergence surveys from April to September 2000-2003 inclusive (total of 1,316 trapping units where 1 unit equalled 1 sq. m trapped for 1 month). *Tetanocera punctifrons* was not recovered among the 18 species of Sciomyzidae

recovered from three productive land habitats, four infrastructure habitats, and five disused habitats.

One of our female specimens was collected at Skealaghan Turlough, County Mayo, in the west of Ireland, by C. Maher. Turloughs are temporary wetlands that develop on karstified limestone; they are found primarily in the west of Ireland. The specimen was caught by sweep-net within an 8 x 8 m enclosure (Moran 2005) where no grazing had taken place for four years, in a vegetation zone dominated by the sedge *Carex nigra*. This vegetation zone is situated in one of the wetter areas of the turlough where flooding takes place for an average of six months each year (Moran *et al.* 2008). Other species of Sciomyzidae caught with this specimen of *T. punctifrons* included *Pherbina coryleti* (Scopoli, 1763), *Ilione albiseta* (Scopoli, 1763), and *Sepedon sphaea* (Fabricius, 1775). A female specimen of *T. punctifrons* was collected by J. Carey in a Malaise trap positioned in a dense but relatively small stand of the rush species *Juncus effusus* in close proximity to a small, permanent pond in semi-improved wet grassland at Annagh East, County Galway (53°24'28.95"N -09°02'44.90"W) approximately 350 m from the nearest large water body (Lough Corrib). The vegetation was subject to very light grazing by cattle but was generally undisturbed. Both the *Juncus* stand and the pond were located in wet grassland. This Malaise trap was part of a larger invertebrate biodiversity study being carried out in wet grassland habitats. It was operated continuously from 1 May 2014 to 4 September 2014. Other Sciomyzidae species captured with *T. punctifrons* from this location included *Colobaea bifasciella* (Fallén, 1820), *Elgiva cucularia* (Linnaeus, 1767), *Hydromya dorsalis* (Fabricius, 1775), *Ilione albiseta*, *Ilione lineata* (Fallén, 1820), *Limnia unguicornis* (Scopoli, 1763), *Pherbellia argyra* (Verbeke, 1967), *Pherbellia s. schoenherri* (Fallén, 1826), *Pherbellia ventralis* (Fallén, 1820), *Pherbina coryleti*, *Pteromicra angustipennis* (Staeger, 1845), *Pteromicra pectorosa* (Hendel, 1902), *Renocera pallida* (Fallén, 1820), *Tetanocera arrogans*, *T. elata*, *T. ferruginea*, *T. freyi*, *T. hyalipennis* and *T. robusta*.

Additional invertebrate surveys were taking place concurrently at natural and constructed wetlands in the west of Ireland between May and October 2014. During this study, two female specimens of *T. punctifrons* were captured in a south-westerly facing Malaise trap on the edge of a reed and large sedge swamp (Fossitt 2000) on the shores of Corgar Lough. The habitat was dominated by tall stands of *Phragmites australis* with occasional

*Typha latifolia* and *Equisetum fluviatile*. Other habitats in the area include improved agricultural grassland and scrub (Fossitt 2000). Additional Sciomyzidae species captured at this site included *Hydromya dorsalis*, *Pherbellia ventralis*, *Renocera pallida*, *Sciomyza dryomyzina* (Zetterstedt, 1846), *T. arrogans*, *T. hyalipennis*, and *T. robusta*.

One of the male specimens of *T. punctifrons* was captured during the same study in a south-westerly facing Malaise trap on the edge of a reed and large sedge swamp (Fossitt 2000) on the shores of Lough Down. The habitat was also dominated by tall stands of *Phragmites australis* with a mixture of broadleaved herbs such as *E. fluviatile*, *Mentha aquatica*, *Filipendula ulmaria*, and *Menyanthes trifoliata*. Neighbouring habitats include wet grassland, improved agricultural grassland, and rich fen and flush (Fossitt 2000). Some other sciomyzid species captured at Lough Down included *Renocera pallida*, *T. arrogans*, and *T. hyalipennis*. The second male specimen of *T. punctifrons* was captured in a Malaise trap on the edge of a reed and large sedge swamp at Lough Meelagh. Other habitats immediately surrounding the collection point include tall-herb swamps, hedgerows and wet grassland (Fossitt 2000).

#### *Habitat of T. latifibula*

Fisher and Orth (1983) collected an unusually large number of specimens (110 females, 226 males) (1949-1974) in north-eastern California in “open, unshaded or sparsely shaded grassy meadows and marshes,” at 1334-1783 m, 7 June–21 September, primarily with a suction machine (this huge, unique resource of specimens would have been useful for further study of variation in identification features, but it was discarded by the Department of Entomology, University of California–Riverside, after Fisher had died and Orth retired). Foote (1999) collected adults, “...most commonly in Idaho and Washington by sweeping emergent and shoreline vegetation bordering open, permanent ponds and lakes” e.g. “a dense stand of *Scirpus* sp. growing in about seven centimetres of water at a small, permanent lake,” but he also collected a few specimens from “unshaded vernal marshes that became dry by midsummer.” Foote *et al.* (1999), in Alaska, collected adults in “open sedge and rush marshes, road-side drainage-ditches, and marshy borders of shallow lakes and ponds. Particularly common in those fresh-water situations in which standing water disappears as summer progresses.” Knutson (unpublished)

collected adults from a marshy area on the shore of Umeå R. in northern Sweden, on 16, 18, and 23 July 1967, where 21 other species of Sciomyzidae were found.

### **Biology and Immature stages**

The life cycles of *T. punctifrons* and *T. latifibula* are in general similar to those of the other species of *Tetanocera* in Ireland that are typical predators of freshwater, non-operculate snails in truly aquatic situations, i.e. *T. ferruginea*, *T. hyalipennis*, *T. montana*, and *T. robusta* (Knutson and Vala 2011).

*T. punctifrons*: our life cycle data on *T. punctifrons* is based on a single laboratory rearing from adults collected in Belgium by J. Verbeke, reared at Cornell University by Knutson, and reported in Knutson's thesis (Knutson 1963; summarised by Vala 1989). Eggs were laid end to end on leaves of substrate vegetation during August and hatched about four weeks later. During the 20-25 days of larval life the larvae killed and ate the fresh tissues of *Gyraulus parvus* Say, *Lymnaea humilis* Say, and *Helisoma trivolvis* Say (none being natural prey) and *Physa* sp. About half of the tissues of each snail (12-18 snails, 2.0-8.0 mm. in length or diameter, attacked per larva) were consumed within a few hours, and then the larvae left the snail; only one larva pupariated, and the puparium did not produce an adult.

The rather extensive biological information on "*T. punctifrons*" in the papers by Beaver (1972, 1973, 1974) on studies in north Wales probably can be accepted as pertaining to that species, but the specimens upon which her studies were based, and the other Sciomyzidae she studied in Wales, were destroyed by an infestation of museum pests (O. Beaver, *pers. comm.* to C. Maher, 2008).

We summarise the main aspects of Beaver's data on life cycles as follows. A female (1 of 4 adults collected between June and August near Newborough, Anglesey, Wales) laid 69 eggs over a period of 7 days, with 41% hatching. The incubation period was 14-20 days. The total duration of larval life was 26-35 days, with the first stadium being 7-21 days (mean 13.0) and the third 11-18 days (mean 15.7). The duration of the puparial stage was 36-50 days (mean 43.3).

*T. latifibula*: Foote (1999) presented fragmentary results from a laboratory rearing based on a female collected on 17 August in the state of Washington, U.S.A. A few first-instar larvae dissected from eggs (37 laid on the cheese-cloth cover of the breeding jar during

late August) fed on *Physella* snails 1.4-10.0 mm in length. Only one larva pupariated, having killed and consumed 38 snails during the 35 days of larval life. The puparium, formed on 20 March on the lid of the rearing dish, produced a male on 29 March. The author concluded that *T. latifibula* has only one generation per year, with overwintering as eggs or young larvae.

Knutson (unpublished) had similar difficulties rearing *T. latifibula*. A female collected on 18 July 1967 near Umeå in northern Sweden (by LK) laid 46 eggs between 24 July and 24 August. Several eggs hatched (some having been held in a refrigerator at 7°C for 3 months), but most larvae emerged only partially from the egg membranes, as Foote (1999) also noted during his rearings. Several larvae killed and ate *Lymnaea peregra* (Müller) and *Planorbis planorbis* (Linnaeus) but refused *Bathymophalus contortus* (Linnaeus). Only one larva pupariated (23 November) after about 70 days passing through the three larval stadia; it produced a female on 11 December. Knutson concluded that *T. latifibula* has only one generation per year (as did Foote 1999), with overwintering as first-instar larvae within the egg membrane.

The morphology of the immature stages of *T. latifibula* and *T. punctifrons* is similar to those of other aquatic, predaceous species of *Tetanocera*. The integument of first-instar larvae is unpigmented, whereas that of older larvae is rather darkly pigmented, with a dark mid-dorsal stripe; integumentary papillae are lacking in both species; the body segments are tuberculate, especially laterally; the posterior end is uplifted dorsally, the posterior spiracular disc bears short, subequal dorsal and lateral lobes and much longer ventrolateral and ventral lobes, and the ventrolateral lobes have a short, broad basal portion and a narrower, longer apical portion. The ventrolateral lobes of first-instar *T. latifibula* are exceptionally long and not bipartite, similar only to the Nearctic *T. soror* Melander. Both species have well-developed float hairs between the spiracular openings on spiracular plates of the spiracular tubes, and the projecting anal proleg bears long, recurved spines. The anterior spiracles have 13-16 papillae in *T. punctifrons* and 16 in *T. latifibula*.

The puparia of both species are very similar, with the posterior end uplifted dorsally and an evident anal proleg, except that *T. latifibula* differs from *T. punctifrons* in having weaker posterior spiracular disc lobe vestiges, and a weaker mid-dorsal stripe, without

lighter-coloured borders. Finally, the integument of *T. latifibula* has a bronze cast, not present in *T. punctifrons*.

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## **Appendix D**

Summary of the habitat classification (Levels 1, 2 and 3) (Fossitt, 2000) and maps of constructed and natural wetlands associated with Chapter 4 & 5

## Summary of habitat classification

Fossitt, J.A. 2000. A Guide to Habitats in Ireland. The Heritage Council, Kilkenny, Ireland. Available at :

[http://www.heritagecouncil.ie/content/files/guide\\_to\\_habitats\\_2007\\_5mb.pdf](http://www.heritagecouncil.ie/content/files/guide_to_habitats_2007_5mb.pdf)

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### NON – MARINE

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<b>F Freshwater</b>	<b>FL</b> Lakes and ponds	<b>FL1</b> Dystrophic lakes	
		<b>FL2</b> Acid oligotrophic lakes	
		<b>FL3</b> Limestone / marl lakes	
		<b>FL4</b> Mesotrophic lakes	
		<b>FL5</b> Eutrophic lakes	
		<b>FL6</b> Turloughs	
		<b>FL7</b> Reservoirs	
		<b>FL8</b> Other artificial lakes and ponds	
	<b>FW</b> Watercourses	<b>FW1</b> Eroding / upland rivers	
		<b>FW2</b> Depositing / lowland rivers	
<b>FP</b> Springs	<b>FP1</b> Calcareous springs		
	<b>FP2</b> Non – calcareous springs		
<b>FS</b> Swamps	<b>FS1</b> Reed and large sedge swamps		
	<b>FS2</b> Tall – herb swamps		
<b>G Grassland and marsh</b>	<b>GA</b> Improved grassland	<b>GA1</b> Improved agricultural grassland	
		<b>GA2</b> Amenity grassland (improved)	
	<b>GS</b> Semi – natural grassland	<b>GS1</b> Dry calcareous and neutral grassland	
		<b>GS2</b> Dry meadows and grassy verges	
		<b>GS3</b> Dry – humid acid grassland	
		<b>GS4</b> Wet grassland	
	<b>GM</b> Freshwater marsh	<b>GM1</b> Marsh	
	<b>H Heath and dense bracken</b>	<b>HH</b> Heath	<b>HH1</b> Dry siliceous heath
			<b>HH2</b> Dry calcareous heath
<b>HH3</b> Wet heath			
<b>HH4</b> Montane heath			
<b>HD</b> Dense bracken		<b>HD1</b> Dense bracken	
<b>P Peatlands</b>	<b>PB</b> Bogs	<b>PB1</b> Raised bog	
		<b>PB2</b> Upland blanket bog	
		<b>PB3</b> Lowland blanket bog	
		<b>PB4</b> Cutover bog	

	<b>PF</b> Fens and flushes	<b>PB5</b> Eroding blanket bog <b>PF1</b> Rich fen and flush <b>PF2</b> Poor fen and flush <b>PF3</b> Transition mire and quaking bog
<b>W Woodland and scrub</b>	<b>WN</b> Semi – natural woodland  <b>WD</b> Highly modified / non – native woodland  <b>WS</b> Scrub / transitional woodland  <b>WL</b> Linear woodland / scrub	<b>WN1</b> Oak – birch – holly woodland <b>WN2</b> Oak – ash – hazel woodland  <b>WN3</b> Yew woodland <b>WN4</b> Wet pedunculate oak-ash woodland <b>WN5</b> Riparian woodland <b>WN6</b> Wet willow – alder – ash woodland <b>WN7</b> Bog woodland <b>WD1</b> (Mixed) broadleaved woodland  <b>WD2</b> Mixed broadleaved / conifer woodland <b>WD3</b> (Mixed) conifer woodland <b>WD4</b> Conifer plantation <b>WD5</b> Scattered trees and parkland <b>WS1</b> Scrub <b>WS2</b> Immature woodland <b>WS3</b> Ornamental / non – native shrub <b>WS4</b> Short rotation coppice <b>WS5</b> Recently – felled woodland <b>WL1</b> Hedgerows <b>WL2</b> Treelines
<b>E Exposed rock and disturbed ground</b>	<b>ER</b> Exposed rock  <b>EU</b> Underground rock and caves  <b>ED</b> Disturbed ground	<b>ER1</b> Exposed siliceous rock  <b>ER2</b> Exposed calcareous rock  <b>ER3</b> Siliceous scree and loose rock <b>ER4</b> Calcareous scree and loose rock <b>EU1</b> Non – marine caves <b>EU2</b> Artificial underground habitats <b>ED1</b> Exposed sand and gravel <b>ED2</b> Spoil and bare ground <b>ED3</b> Recolonising bare ground <b>ED4</b> Active quarries and mines <b>ED5</b> Refuse and other waste
<b>B Cultivated and built land</b>	<b>BC</b> Cultivated land  <b>BL</b> Built land	<b>BC1</b> Arable crops <b>BC2</b> Horticultural land  <b>BC3</b> Tilled land <b>BC4</b> Flower beds and borders <b>BL1</b> Stone walls and other stonework <b>BL2</b> Earth banks

**C Coastland****CS** Sea cliffs and islets**CS1** Rocky sea cliffs**CS2** Sea stacks and islets**CS3** Sedimentary sea cliffs**CW** Brackish waters**CW1** Lagoons and saline lakes**CW2** Tidal lakes**CM** Salt marshes**CM1** Lower salt marsh**CM2** Upper salt marsh**CB** Shingle and gravel banks**CB1** Shingle and gravel banks**CD** Sand dune systems**CD1** Embryonic dune**CD2** Marram dunes**CD3** Fixed dunes**CD4** Dune scrub and woodland**CD5** Dune slacks**CD6** Machair**CC** Coastal constructions**CC1** Sea walls, piers and jetties**CC2** Fish cages and rafts

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CW1

## Cloonfad CW



**Legend**

- Wood\_feature
- WL2\_Linear
- FW2\_Linear

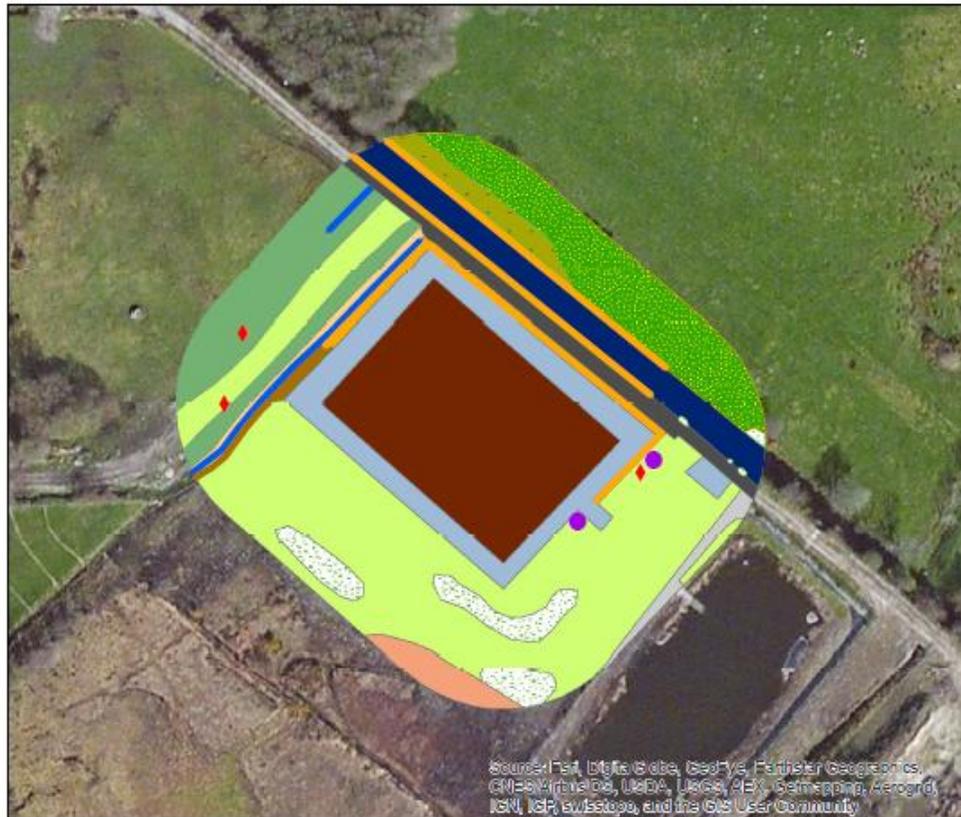
**40 m Buffer Zone**

**Habitats**

- BC4
- BL2
- BL3
- ED2
- FS1
- FW2
- GA1
- GA2
- GS2
- GS4
- WL2
- WS1



# Moycullen CW



**Legend**

- Wood\_feature
- ◆ Stone\_feature
- FW3\_Linear
- WL2\_Linear
- WL1\_Linear
- FW4\_Linear

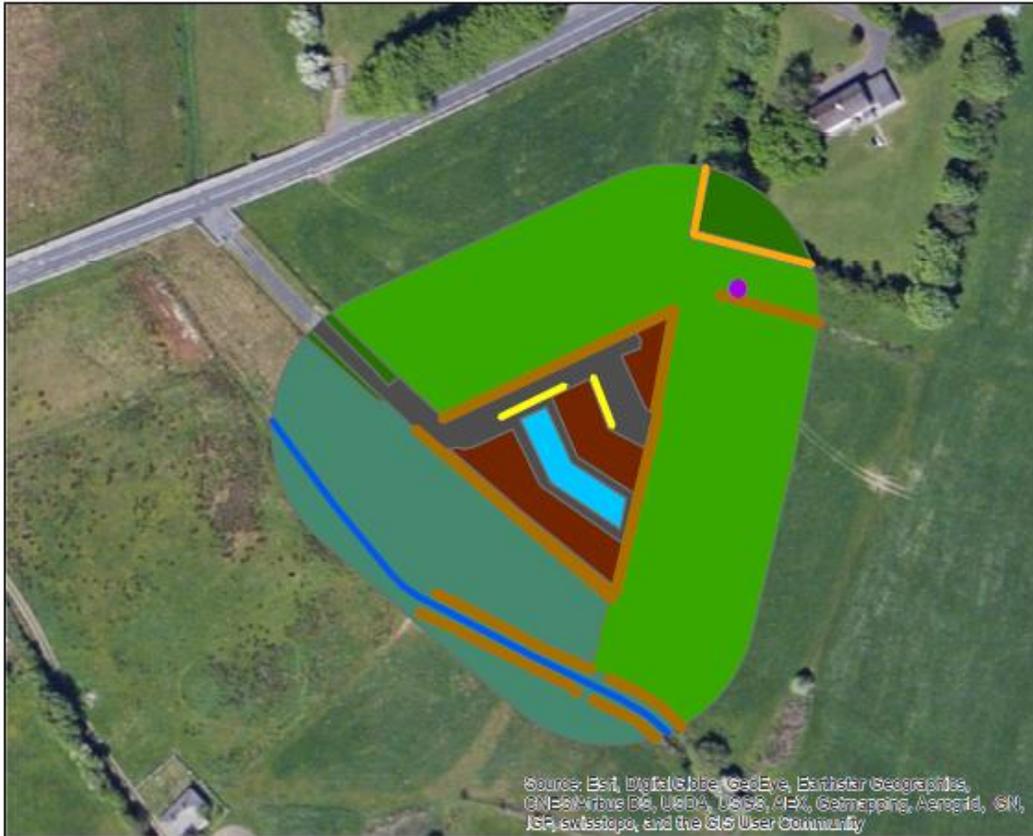
**40 m Buffer Zone**

**Habitats**

- BL2
- BL3
- ED1
- ED2
- ED3
- FS1
- FW3
- GA1 (abundant Juncus. spp)
- GS3
- PF3
- WL1
- WL2
- WN6
- WS1

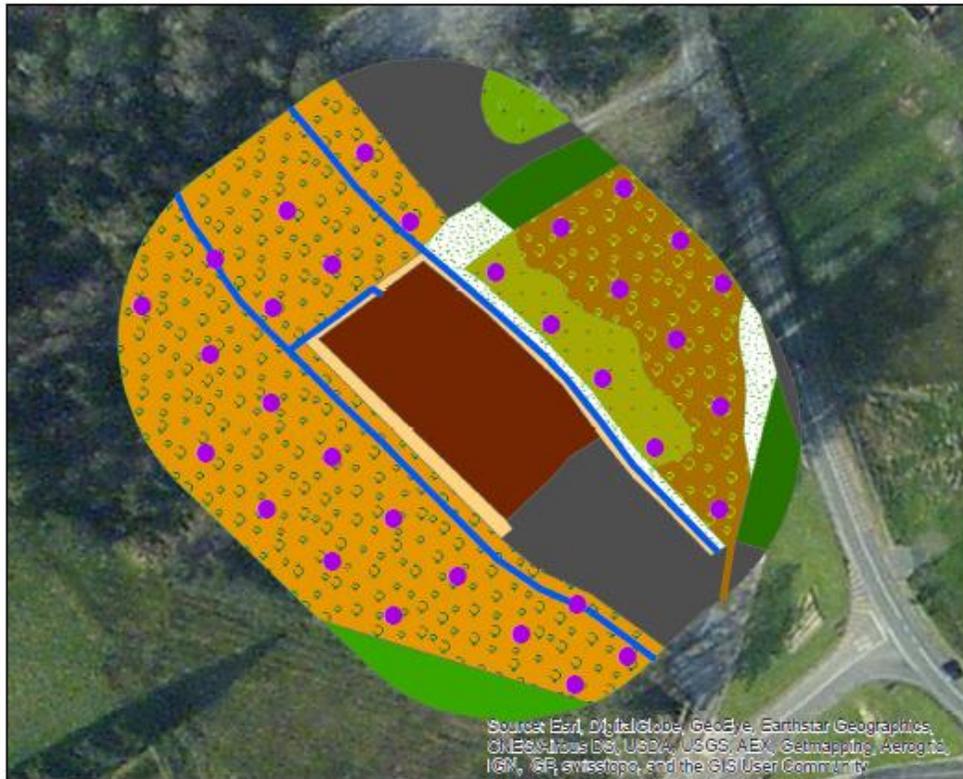
CW3

## Williamstown CW



CW4

# Keadue CW



**Legend**

- Wood\_Feature
- FW4\_Linear
- WL1\_Linear

**40 m Buffer Zone**

**Habitats**

- BL2
- BL3
- FS1
- GA1
- GA2
- WD1
- WD2
- WD5
- WN6
- WS1



CW5

## Ballyfarnon CW



### Legend

- Wood\_Feature
- WL2\_Linear
- WL1\_Linear
- FW4\_Linear

### 40 m Buffer Zone

#### Habitats

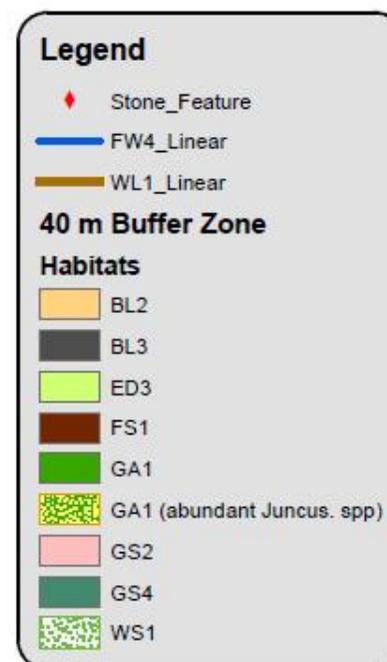
- BL2
- BL3
- FS1
- FW2
- GA1 (abundant *Juncus. spp*)
- GS4
- WD1
- WS1

CW6

## Fenagh CW

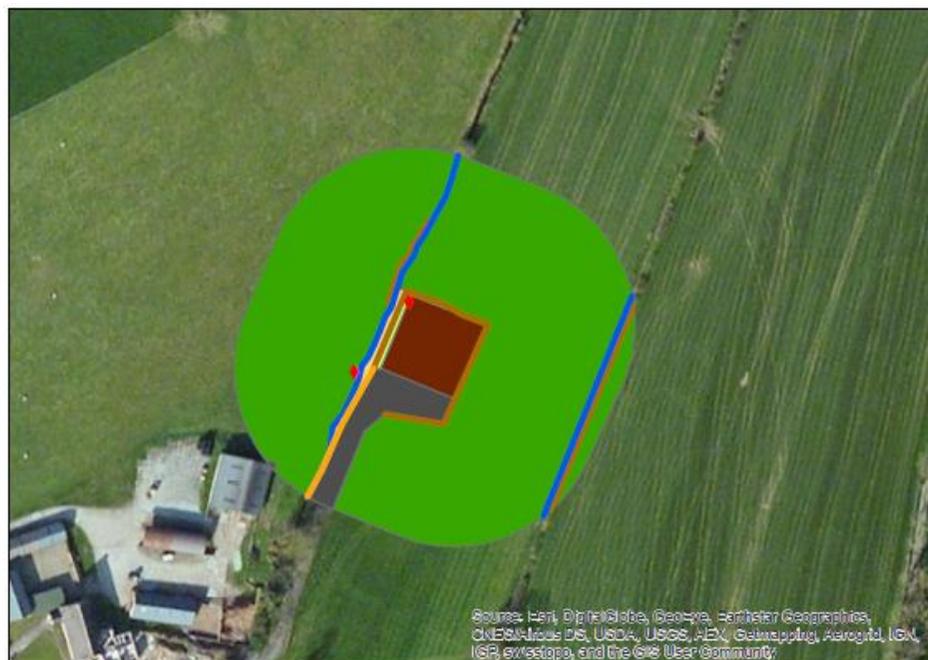


Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNR/Airbase DS, USDA, USGS, AeroGRID, IGN, IGP, swisstopo, and the GIS User Community

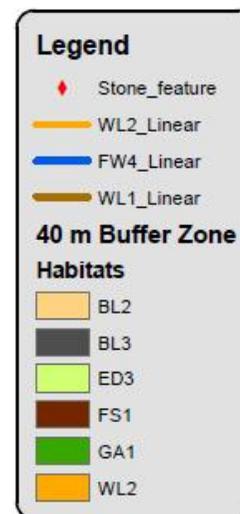


CW7

## Newtowngore CW



0 50 100 200 Meters

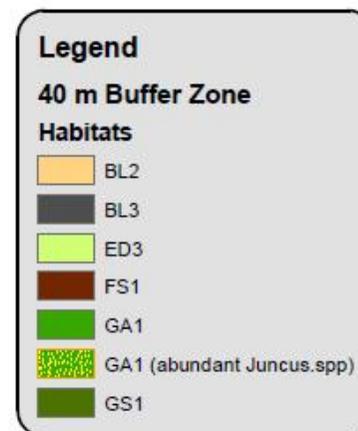


CW8

## Hollymount CW



Source: Esri, Digital Globe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, SFR, swisstopo, and the GIS User Community



NW1 (Part 1 of 4)

# Lough Meelagh (1 of 4)



0 50 100 200 Meters



**Legend**

- Wood\_feature
- Stone\_feature
- BL2\_Linear
- WL2\_Linear
- WL1\_Linear
- FW2\_Linear
- FW4\_Linear
- Fishing jetties

**40 m Buffer Zone**

**Habitats**

- BL1
- BL1 (well)
- BL3
- ED1
- FL5
- FL6
- FS1
- FS2
- FW4
- GA1
- GA2
- GM1
- GS4
- WD1
- WD2
- WD5
- WL1
- WL2
- WN5
- WN6
- WS1
- WS5

# Lough Meelagh (2 of 4)



0 50 100 200 Meters  
|-----|-----|-----|-----|

N

**Legend**

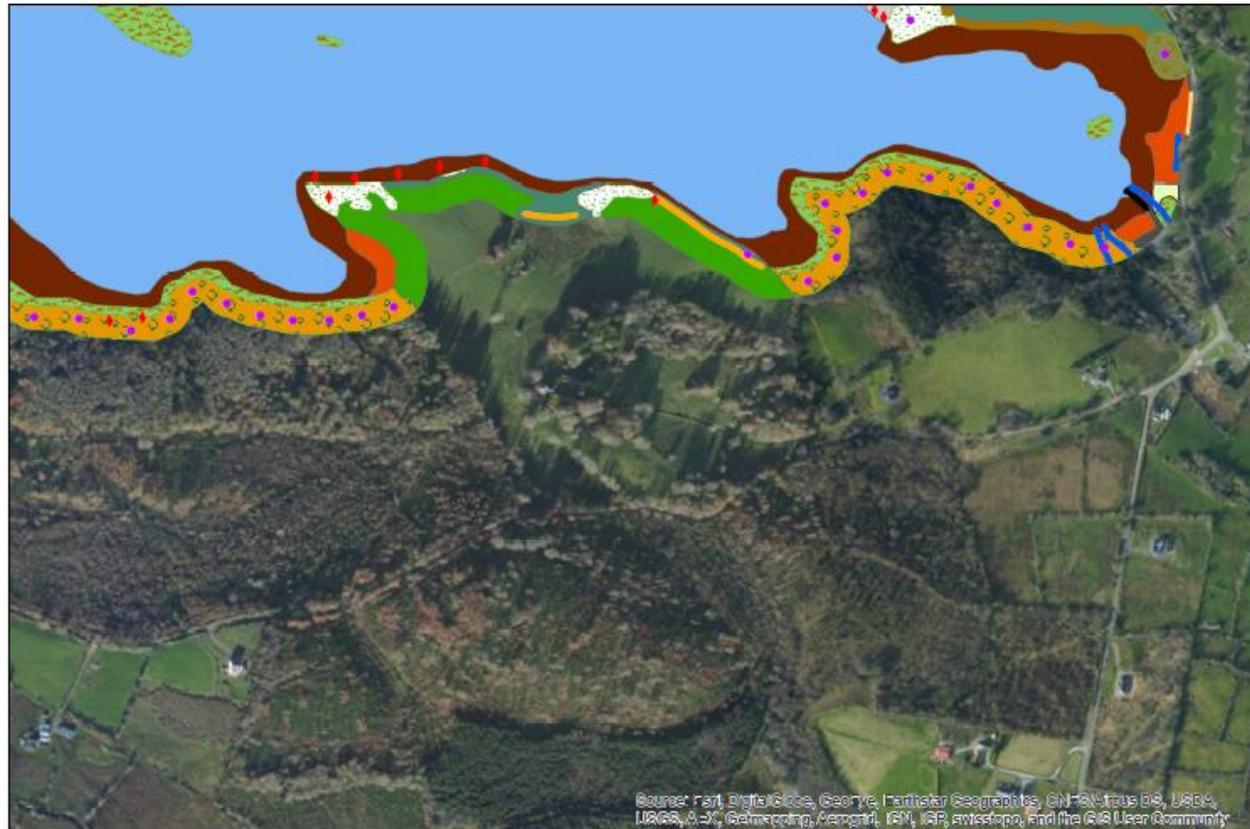
- Wood\_feature
- Stone\_feature
- BL2\_Linear
- WL2\_Linear
- WL1\_Linear
- FW2\_Linear
- FW4\_Linear
- Fishing jetties

**40 m Buffer Zone**

**Habitats**

- BL1
- BL1 (well)
- BL3
- ED1
- FL5
- FL6
- FS1
- FS2
- FW4
- GA1
- GA2
- GM1
- GS4
- WD1
- WD2
- WD5
- WL1
- WL2
- WN5
- WN6
- WS1
- WS5

# Lough Meelagh (3 of 4)



0 50 100 200 Meters  
|-----|-----|-----|



**Legend**

- Wood\_feature
- Stone\_feature
- BL2\_Linear
- WL2\_Linear
- WL1\_Linear
- FW2\_Linear
- FW4\_Linear
- Fishing jetties

**40 m Buffer Zone**

**Habitats**

- BL1
- BL1 (well)
- BL3
- ED1
- FL5
- FL6
- FS1
- FS2
- FW4
- GA1
- GA2
- GM1
- GS4
- WD1
- WD2
- WD5
- WL1
- WL2
- WN5
- WN6
- WS1
- WS5

# Lough Meelagh (4 of 4)



0 50 100 200 Meters  
|-----|-----|-----|-----|

**Legend**

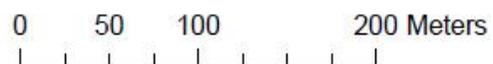
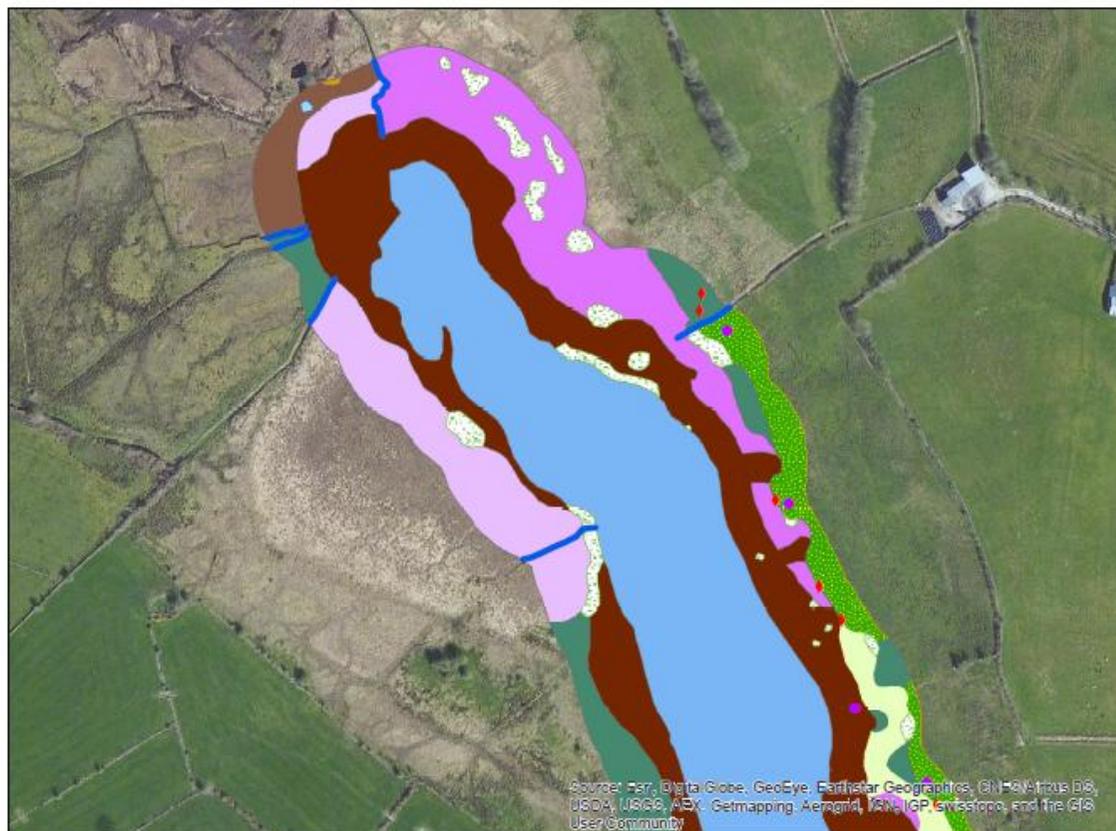
- Wood\_feature
- Stone\_feature
- BL2\_Linear
- WL2\_Linear
- WL1\_Linear
- FW2\_Linear
- FW4\_Linear
- Fishing jetties

**40 m Buffer Zone**

**Habitats**

- BL1
- BL1 (well)
- BL3
- ED1
- FL5
- FL6
- FS1
- FS2
- FW4
- GA1
- GA2
- GM1
- GS4
- WD1
- WD2
- WD6
- WL1
- WL2
- WN5
- WN6
- WS1
- WS5

# Drumady Lough (1 of 3)



**Legend**

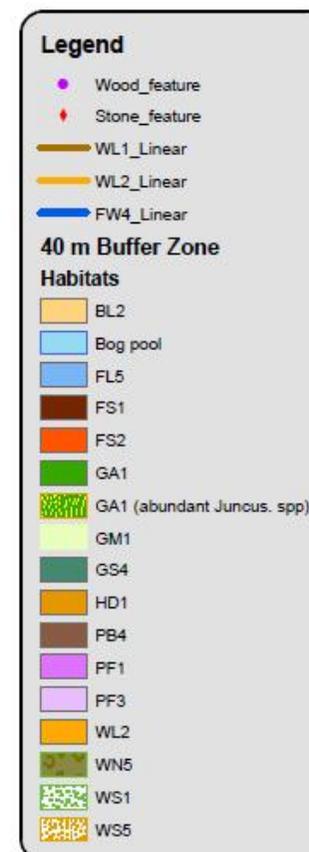
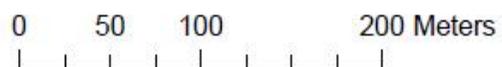
- Wood\_feature
- Stone\_feature
- WL1\_Linear
- WL2\_Linear
- FW4\_Linear

**40 m Buffer Zone**

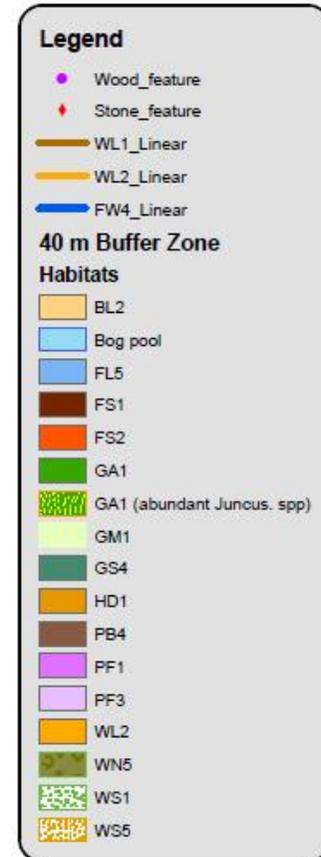
**Habitats**

- BL2
- Bog pool
- FL5
- FS1
- FS2
- GA1
- GA1 (abundant Juncus. spp)
- GM1
- GS4
- HD1
- PB4
- PF1
- PF3
- WL2
- WN5
- WS1
- WS5

## Drumady Lough (2 of 3)

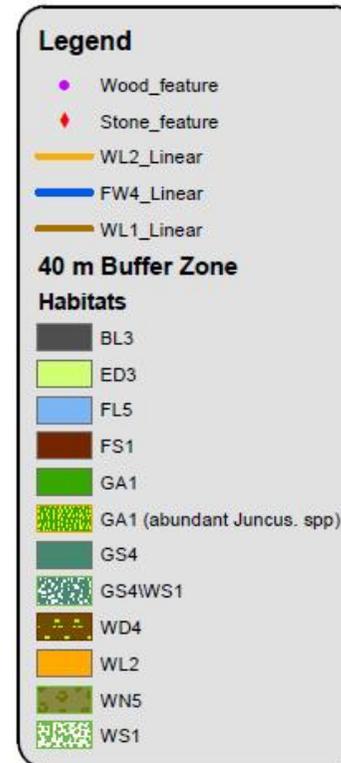


# Drumady Lough (3 of 3)



NW3

# Drumroosk Lake



NW4

# Lake Corgar



**Legend**

- Wooden footbridge
- Stone\_feature
- Wood\_feature
- FW3\_Linear
- Fishing jetties
- FW4\_Linear
- WL1\_Linear

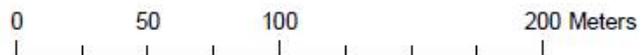
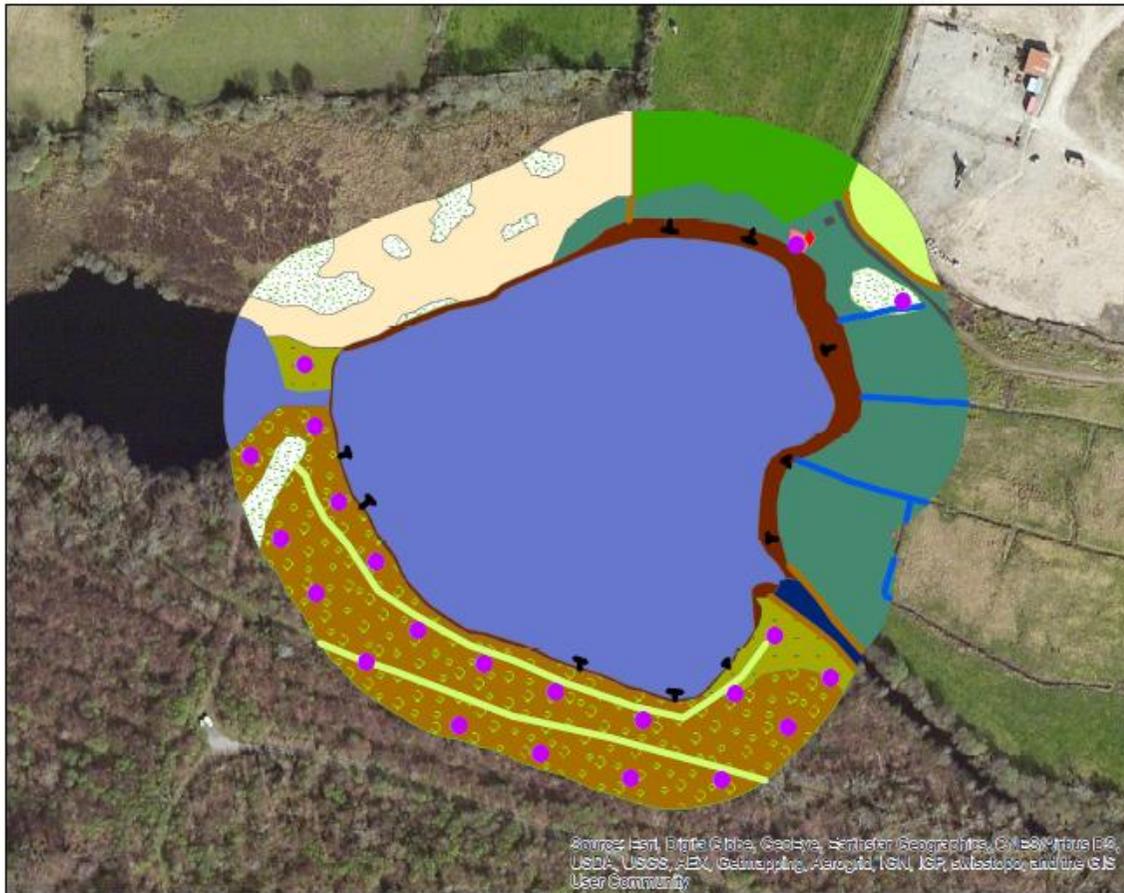
**40 m Buffer Zone**

**Habitats**

- BL3
- ED3
- FL5
- FS1
- FS1\FS2
- FS2
- GA1
- GA1 (abundant Juncus. spp)
- GS4
- WN5
- WS1

NW5

# Lough Down



**Legend**

- Wood\_feature
- Stone\_feature
- FW3\_Linear
- Fishing jetties
- ED3 Path
- WL2\_Linear
- WL1\_Linear
- FW4\_Linear

**40 m Buffer Zone**

**Habitats**

- BL3
- ED3
- ED5
- FL4
- FS1
- FW3
- GA1
- GS4
- PF1
- WD1
- WL1
- WN6
- WS1



NW6

# Corralough



**Legend**

- WL1\_Linear
- FW4\_Linear

**40 m Bufferzone**

**Name**

- BL3
- ED5
- FL5
- FS1
- GS4
- PF1
- PF3
- WS1



NW7

# Lehinch Bog



**Legend**

- Wood\_feature
- FW4\_Linear

**40 m Buffer Zone**

**Habitats**

- ED5
- FS1
- HD1
- PB4
- WD1
- WD4
- WN7
- WS1



NW8

# Clooncruffer Bog



## Appendix E

List of Sciomyzidae captured at CWs and NWs during this investigation

Sciomyzid species	Constructed wetlands	Natural wetlands
<i>Antichaeta analis</i>	✓	✓
<i>Elgiva cucularia</i>		✓
<i>Elgiva sollicita</i>	✓	✓
<i>Hydromya dorsalis</i>	✓	✓
<i>Ilione albiseta</i>	✓	✓
<i>Ilione lineata</i>	✓	✓
<i>Limnea paludicola</i>		✓
<i>Limnea unguicornis</i>	✓	✓
<i>Pherbellia albocostata</i>	✓	
<i>Pherbellia dubia</i>	✓	✓
<i>Pherbellia griseola</i>	✓	
<i>Pherbellia nana</i>		✓
<i>Pherbellia schoenerri</i>	✓	✓
<i>Pherbellia scutellaris</i>	✓	
<i>Pherbellia ventralis</i>	✓	✓
<i>Pherbina coryleti</i>	✓	✓
<i>Psacadina zernyi</i>		✓
<i>Pteromicra angustipennis</i>		✓
<i>Renocera pallida</i>	✓	✓
<i>Renocera striata</i>		✓
<i>Renocera strobii</i>	✓	✓
<i>Sciomyza dryomyzina</i>	✓	✓
<i>Sciomyza testacea</i>		✓
<i>Sepedon spinipes spinipes</i>		✓
<i>Tetanocera arrogans</i>	✓	✓
<i>Tetanocera ferruginea</i>	✓	✓
<i>Tetanocera freyi</i>	✓	✓
<i>Tetanocera fuscinervis</i>	✓	✓
<i>Tetanocera hyalipennis</i>	✓	✓
<i>Tetanocera punctifrons</i>		✓
<i>Tetanocera robusta</i>	✓	✓
<i>Tetanocera sylvatica</i>	✓	✓

