“Ghost ponds” : Resurrecting lost ponds and species to assist aquatic biodiversity conservation

Thesis submitted for the degree of Doctor of Philosophy

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“ I took in February three table-spoonfuls of mud from three different points, beneath water, on the edge of a little pond; this mud when dry weighed only 6 and ¾ ounces; I kept it covered up in my study for six months, pulling up and counting each plant as it grew; the plants were of many kinds, and were altogether 537 in number; and yet the viscid mud was all contained in a breakfast cup!”

Excerpt from: Charles Darwin. “The Origin of Species by Means of Natural Selection / Or, the Preservation of Favoured Races in the Struggle for Life, 6th Edition.”

Thesis declaration

Abstract

Over the last century, around half of all ponds and small wetlands have been lost globally, predominantly from agricultural regions. These losses present a major conservation challenge for aquatic biodiversity and habitat connectivity. While the creation of new ponds is widely advocated as a means of restoring some of these lost habitats, this thesis presents an alternative approach – the resurrection of 'ghost ponds', historic ponds in-filled during agricultural intensification. Many aquatic organisms have evolved dormant propagules for surviving periods of habitat desiccation, and can remain viable for centuries buried in wetland sediments. While such long-term viability is well established in extant aquatic habitats, the fate of propagules buried within ghost ponds has remained unexplored. If their viability was proven, ghost ponds could present a crucial overlooked conservation resource.

Here, I examine the potential conservation value of ghost pond resurrection within a typical agricultural UK landscape. First, historic declines in pond numbers and pond density are examined for this landscape, and the potential implications of these changes for aquatic biodiversity are explored using a space-for-time replacement approach. The conservation value of ghost ponds is then explored through a series of lab and field studies, that demonstrate the viability of propagules representing at least eight aquatic macrophyte species, after laying dormant for 50 – 150 years. Once resurrected, ghost ponds were quickly (<6 months) re-colonised by a diverse aquatic vegetation. With ghost ponds representing abundant, dormant time capsules for aquatic species in agricultural landscapes, these findings open up exciting new possibilities for aquatic habitat and biodiversity restoration on a global scale.

Acknowledgements

Firstly, I would like to thank my supervisors Carl Sayer and Jan Axmacher for their continued support and patience, and their invaluable contribution to the ideas explored in this thesis.

I would like to express my extreme gratitude to all of the people who have helped me in the field, in particular during the gruelling excavation and set-up of the study ponds and on-site mesocosms. Particular thanks go to Dom Arnold for his excellent digger-work, and to Ian Patmore, Phill Brown, Carl Sayer, Emily Smith, Phoebe Lewis, Bernard Dawson, and Henry Crawley, without whose tireless dedication to pond restoration and creation, this study would not have been possible. Thanks also go to Geoff Nobes for his assistance with the water beetle surveys, to Steve Lambert for his assistance with the microcosm experiment, and to Rachael Davies at the Millennium Seed Bank for her help with seed viability testing.

Special thanks go to the landowners and managers who allowed me to dig up their fields, all in the name of pond science! To Nick Anema, the Jensen family, and Ross Haddow & the Stody Estate, a big thank you for taking part in this study. I also wish to thank the Natural Environment Research Council (NERC), Norfolk Biodiversity Partnership, and ENSIS Ltd. for funding this research.

Finally, I would like to thank my friends and family, not just for their love and support, but because most of them also helped out in the field. Special thanks go to uncle-Phill for his exceptional botany skills, to my dad John for helping on numerous survey trips, and to Sarah Morgan and Dan Denis for their emotional support, both in the field and at home.

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Chapter 1 An introduction to ponds

1.1 What is a pond?

At present, there is no universally applied definition of a 'pond'. Natural and man-made ponds exist in all biogeographical regions of the globe, from deserts to the Arctic tundra (Céréghino *et al.* 2014), and as such the definition of what constitutes a 'pond' rather than a 'puddle' 'pool' or 'shallow lake', is highly variable. Definitions may be based on physical or functional characteristics, or may be deliberately broad in scope (*Table 1*).

Table 1 – Commonly cited definitions of a 'pond'

Definition type	Definition	Reference
Broad	*"Small and shallow standing waters that permanently or temporarily contain water".*	De Meester *et al*. (2005)
Physical	"*Water bodies between 1m^2 and 2ha in area which may be permanent or seasonal, including both man-made and natural water bodies".*	Biggs *et al.* (2005)
Physical	"*A body of water between 25 m^2 and 2ha in area which usually holds water for at least 4 months of the year*".	Williams et al. (2010)
Functional	*"…Due to their small size and lack of wind mixing, ponds are prone to chemical stratification of the water…in larger shallow waterbodies this chemical separation of the water does not usually occur."*	UCL Pond Restoration Research Group (2014)
Functional & physical	"*A water body with a maximum depth of no more than 8m, offering water plants the potential to colonise almost the entire area of the pond".*	Oertli *et al.* (2005)

While some features are consistent across definitions – that a pond is a body of stillwater, which can be natural or artificial – aspects such as area, depth, salinity, and permanence of a 'pond' vary greatly. Some definitions remain deliberately broad to encompass the wide diversity of ponds (De Meester *et al.* 2005), while others attempt to set specific physical (area, depth, hydroperiod), or ecological (vegetation structure) cut-off points, to aid classification (Biggs *et al.* 2005; Williams *et al.* 2007). In the UK, most waterbodies are relatively small and permanent, and the definitions most commonly used for 'ponds' reflect this (Biggs *et al.* 2005; Williams *et al.* 2007).

While purely physical definitions make it easy to differentiate 'ponds' from other small waterbodies, they are based on arbitrary cut-off points which may not be appropriate across all regions / climates, and have little meaning in defining what is *functionally* different about a pond vs. any other small waterbody. More functional definitions of a 'pond' (*Table 1*), for example those used by the Norfolk Ponds Project or Oertli *et al.* (2005), attempt to address some of the functional differences between a pond and a shallow lake. These relate to the interaction between physical and ecological components of the pond, for example wind mixing and chemical stratification of the water column (used in the Pond Restoration Research Group definition), or complete light penetration through the water column (Oertli *et al.* 2005), resulting in complete macrophyte coverage. This last definition in *Table 1* requires some careful interpretation, with regards to whether aquatic plants have the *potential* to colonise almost the entire area of the pond. Not all ponds will support aquatic plants (perhaps due to environmental constraints such as excessive numbers of waterfowl, pollution, water turbidity, or unsuitable sediment). In these instances, the word *potential* is key – if environmental constraints were removed, could aquatic plants colonise almost the entire pond? This may not be easy to judge!

For the purposes of this thesis, I define a pond as:

"A small (permanent or temporary), body of still water, less than 100m across and with an average depth less than 2m, in which aquatic plants (submerged or free-floating), have the potential to colonise almost the entire area of the pond, and where chemical stratification of the water column often occurs".

This definition incorporates physical, functional, and ecological components in order to encompass the broad range of pond types, while distinguishing these from small lakes, which tend not to undergo stratification of the water column or provide potential for complete macrophyte coverage. It is worth bearing in mind that while each of these characteristics of a pond, when considered individually, might apply to other aquatic habitats, when all are considered collectively, they provide a good definition of what makes a pond.

Evidently, some degree of flexibility is required in defining what constitutes a 'pond' in any given region / climate (*Figure 1*). A pond can have an area less than 1m^2 to several hundred square meters, and a depth between a few centimetres to over a metre. A pond may be permanent, occasionally dry, or only present in exceptionally wet years. Ponds can be fed by spring or groundwater, rainfall, or by man (such as a garden or irrigation pond). They can be natural or artificial in origin, and might be used for a wide range of purposes (fish farming, irrigation, recreation, conservation). Ponds can be hydrologically isolated, or connected to other water courses via pipes, ditches, streams, or flood events.

Figure 1 - An illustration of the diversity of 'ponds'. Top row (L to R): a garden pond (*www.telegraph.co.uk/news/earth/wildlife/7703235/Tap-water-is-culprit-for-polluting-garden-ponds.html*); a tundra pond in the arctic circle (*www.panoramio.com/photo/56905161*); a roadside drainage pond (*www.geograph.org.uk/photo/1817340*). Bottom (L to R): a farmland pond (*author's photo*); a temporary pond in the temperate zone (*http://aqualandpetsplus.com/Amphibian,%20Toad%20Tadpole.htm*); farmland ponds in Norfolk (*photo courtesy of Carl Sayer*).

Despite this huge variety, there are some features common to all ponds. All ponds are standing water bodies, and their relatively small size leads to them having a high aquatic-terrestrial contact zone (De Meester *et al.* 2005). Ponds have relatively small catchment areas, influencing both the nutrient and pollution inputs to these habitats (Williams *et al.* 2004; Davies *et al.* 2008a). Ponds tend to show greater biotic and environmental amplitudes than other waterbodies, due to their small size and isolation (Jeffries 1989; Williams *et al.* 2004; De Meester *et al.* 2005; Céréghino *et al.* 2008b). These factors are important in creating the high habitat heterogeneity and biodiversity associated with ponds (Jeffries 1989; Oertli *et al.* 2002; Williams *et al.* 2004; Scheffer *et al.* 2006; Céréghino *et al.* 2008a; Hill & Wood 2014; Sayer 2014).

1.2 Why are ponds important? - understanding an undervalued aquatic habitat

Historically, ecological research has tended to focus on large habitats, often following the assumptions of island biogeographic theory, that “bigger is better” (MacArthur & Wilson 1967). This has been the case in both terrestrial and aquatic ecology, with the later tending to focus on large river systems, wetlands, and lakes, while generally neglecting smaller systems like streams, ditches or ponds (De Meester *et al.* 2005; Davies *et al.* 2008b). Ponds may historically have received little scientific attention, as freshwater biologists have tended “*to ignore ponds completely, or regard them simply as smaller versions of larger lakes*” (Céréghino *et al.* 2008a). Ponds have also been neglected from water quality legislation, such as the Water Framework Directive (WFD), which effectively excludes standing waters less than 50ha in area from any of the directive’s improvement and monitoring programs (Davies *et al.* 2008b).

However, interest in small aquatic systems (especially ponds), has been growing, with a tripling in the number of papers on *pond biodiversity* published per year, over the last decade (Céréghino *et al.* 2014). Ponds are now widely recognised as important hotspots for biodiversity (Scheffer & Van Geest 1999; Williams *et al.* 2004; Davies *et al.* 2008b; Céréghino *et al.* 2014; Hill & Wood 2014), and as useful model systems for exploring ecological theories such as metapopulations, metacommunites, and migration corridors (De Meester *et al.* 2005). Ponds are also an important habitat type due to their sheer abundance; globally, there are an estimated 277+ million ponds smaller than 1ha (Downing *et al.* 2006), making up a large proportion of total global standing waterbodies.

1.2.1 Ponds & ecosystem services

Ponds provide a range of ecosystem services, include water quality improvement, flood control and carbon sequestration. Ponds contribute to both flood abatement and water quality improvement by increasing water retention time, thus reducing the runoff of sediment, nutrients, and pollution into other aquatic systems (Yin & Shan 2001; Heathwaite, Quinn & Hewett 2005; Ockenden *et al.* 2012). This is particularly beneficial in farmland, where strategically placed ponds can be an effective method for trapping sediment and nutrients (Yan, Yin & Tang 1998; Ockenden *et al.* 2012). Urban detention ponds can act in a similar way, providing an effective system for trapping sediment and heavy-metal runoff (Martin 1989). In both cases, creating ponds with deep water areas (which act as silt traps), combined with shallow water areas for macrophyte establishment, results in the greatest improvement in water quality (Martin 1989; Ockenden *et al.* 2012). Such 'dual-purpose' wetlands can make a significant contribution to aquatic biodiversity, while providing a valuable ecological service (Thiere *et al.* 2009; Dicks *et al.* 2013). Ponds also play an important but largely overlooked role in the global carbon cycle (Downing 2010). Due to their abundance, combined with high biological productivity, rates of organic carbon sequestration per unit area of sediment may be at least an order of magnitude higher in ponds & small lakes, compared to large lakes (Downing *et al.* 2008; Boyd *et al.* 2010; Downing 2010; Moore & Hunt 2012).

Ponds created to address water management issues, such as trapping agricultural or urban runoff, can also make important contributions to biodiversity, and are not necessarily of poor ecological quality (Hansson *et al.* 2005; Céréghino *et al.* 2014). Indeed, despite highly eutrophic conditions, farmland ponds can still support diverse aquatic assemblages (Rosset *et al.* 2014), and make significant contributions to regional aquatic biodiversity (Williams *et al.* 2004), while improving the conservation function of urban storm-water ponds may also improve their function in pollution control (Woodcock, Monaghan & Alexander 2010).

1.2.2 What makes ponds so biodiverse?

As research interest in small aquatic habitats has grown, previously held assumptions that aquatic biodiversity was mainly held in larger rivers and lakes (Davies et al., 2008), have been revised. The high biodiversity value of ponds, whether located in ‘pristine’ habitats such as nature reserves (Biggs *et al.* 2005; Williams, Whitfield & Biggs 2008), or in intensive farmland (Beja & Alcazar 2003; Davies *et al.* 2008b; Sayer *et al.* 2012), has now become widely recognised. Ponds have been found to contribute disproportionately to regional aquatic biodiversity, containing more species, more unique species, and more scarce species than rivers, lakes or streams within the same environment (Williams *et al.* 2004; Davies *et al.* 2008b). Key to this high biodiversity is the high heterogeneity between ponds, which are extremely variable in characteristics including hydroperiod, trophic structure, levels of disturbance, and levels of shading (Biggs *et al.* 2005; De Meester *et al.* 2005; Sayer *et al.* 2012; Scott *et al.* 2012). As such, species turnover (β- diversity), between ponds is often high, in turn contributing to high regional (γ-) diversity (Williams *et al.* 2004; Céréghino *et al.* 2008a; Davies *et al.* 2008b; Bosiacka & Pienkowski 2012). In a comparison of four different types of aquatic habitat (streams, ditches, rivers and ponds), all situated in a lowland agricultural landscape in Southern England, Williams et al. (2004), found that ponds contributed more to regional (γ-) diversity than the other aquatic habitats. This was true for both aquatic macrophyte and macroinvertebrate diversity. While individual river sites were often the richest in terms of species number, they tended to be uniform in their species composition. In contrast, ponds were more variable in their species richness, but overall supported a wider diversity of species (*Figure 2*).

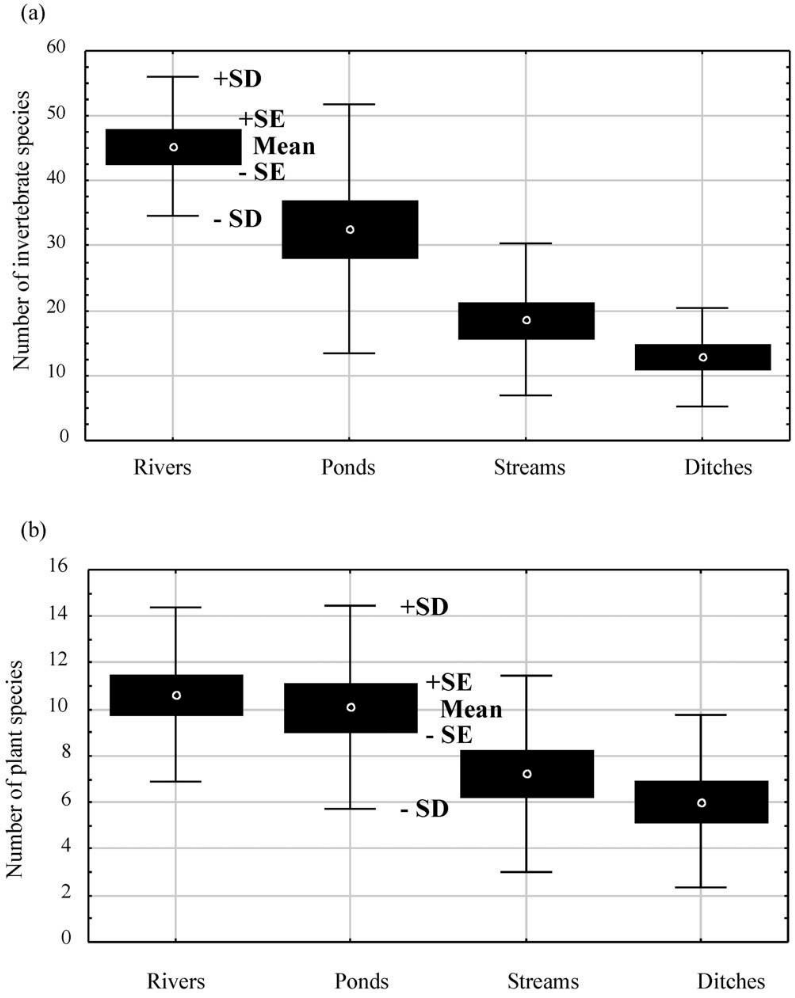
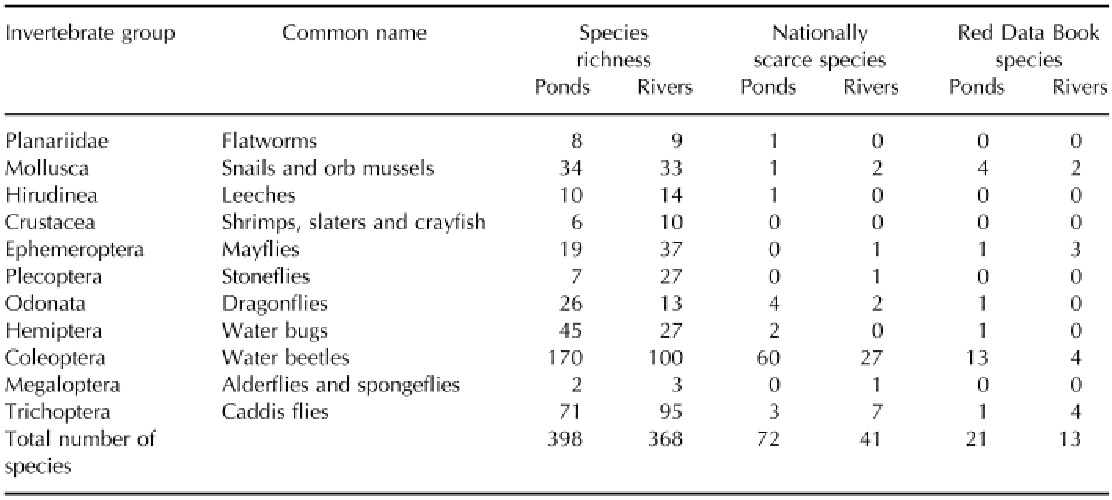


Figure 2 – Comparative biodiversity of lowland rivers, ponds, streams and ditches. Species richness of aquatic invertebrates (a), and aquatic macrophytes (b), in four different lowland freshwater habitats, (From Williams et al. 2003).

Other studies from the UK have produced similar results, with ponds supporting a greater richness, and a greater number of scarce and rare species, than other aquatic habitats (Wood, Greenwood & Agnew 2003; Pond Conservation 2008) (*Table 2*).

These biodiversity trends appear to be consistent across Europe, with ponds consistently outperforming rivers, lakes, ditches, and streams in terms of both macrophyte and macroinvertebrate diversity (Davies *et al.* 2008b).

Table 2 - Comparisons in species richness between ponds and rivers in Europe (from Wood et al. (2003), modified from Williams et al. (1998)).



Invertebrate group	Common name	Species richness		Nationally scarce species		Red Data Book species	
		Ponds	Rivers	Ponds	Rivers	Ponds	Rivers
Planariidae	Flatworms	8	9	1	0	0	0
Mollusca	Snails and orb mussels	34	33	1	2	4	2
Hirudinea	Leeches	10	14	1	0	0	0
Crustacea	Shrimps, slaters and crayfish	6	10	0	0	0	0
Ephemeroptera	Mayflies	19	37	0	1	1	3
Plecoptera	Stoneflies	7	27	0	1	0	0
Odonata	Dragonflies	26	13	4	2	1	0
Hemiptera	Water bugs	45	27	2	0	1	0
Coleoptera	Water beetles	170	100	60	27	13	4
Megaloptera	Alderflies and spongeflies	2	3	0	1	0	0
Trichoptera	Caddis flies	71	95	3	7	1	4
Total number of species		398	368	72	41	21	13

Many physical, chemical and biological factors contribute to the high heterogeneity of ponds. Factors most frequently found to be significantly related to pond biodiversity include pond area, pond isolation, and macrophyte cover. The relationship between pond size and biodiversity has received particular attention in the literature, as although the ‘species-area relationship’ (MacArthur & Wilson 1967) generally holds for large waterbodies and lakes (Browne 1981; Murphy 2002), ponds present more complex patterns. The small size of ponds (relative to other waterbodies), has been suggested to contribute to their high biodiversity value, with many small habitats providing greater heterogeneity (Williams *et al.* 2004; De Meester *et al.* 2005) and thus supporting greater biodiversity (Gee *et al.* 1997; Oertli *et al.* 2002) than fewer large ones. Additionally, a pond’s small catchment areas combined with high macrophyte productivity and coverage, may make them more resilient to eutrophication than larger waterbodies, potentially contributing to their high biodiversity value (Davies *et al.* 2008a; Rosset *et al.* 2014).

While the small size of ponds likely contributes to their high biodiversity value at a regional scale, relationships between within pond (α-) diversity and pond size may vary. Generally, larger habitats support larger populations, resulting in lower risk of stochastic extinction, and higher genetic diversity and population stability (MacArthur & Wilson 1967; Shaffer 1981). In lakes, these factors have been shown to be especially important for dispersal limited organisms, including plants (Murphy 2002) and zooplankton

(Hoffmann & Dodson 2005). However, in ponds the relationships between area and diversity appear to be more complex.

In some studies, positive correlations between pond area and diversity have been found, for taxa including Gastrapoda (Brönmark 1985), Odonata (Oertli *et al.* 2002), and aquatic and emergent macrophytes (Møller & Rørdam 1985; Biggs *et al.* 2005; Bosiacka & Pienkowski 2012), while taxa such as amphibians (Joly *et al.* 2001) have been found to show a negative correlation with pond area. Other studies have found no relationship between pond size and biodiversity across multiple taxa (Hassall, Hollinshead & Hull 2011). The relationship between pond area and biodiversity may also vary with pond hydroperiod; Beja & Alcazar (2003), found that while temporary ponds show a positive correlation between area and biodiversity, permanent ponds show the opposite, attributed to the presence of fish in these sites (Beja & Alcazar 2003; Scheffer *et al.* 2006). Scheffer *et al*. (2006) suggest that the presence of fish in larger ponds and lakes is detrimental to diversity of other taxa due to increased competition, predation, and destruction of aquatic macrophytes.

In addition to pond area, two other variables most frequently associated with high pond biodiversity are abundance / coverage of macrophytes, and high density of neighbouring ponds / wetlands. Macrophyte coverage has been found to be significantly correlated with invertebrate diversity across a number of pond studies, with greater coverage and diversity of macrophyte species creating a more heterogeneous habitat for invertebrates. Greater coverage of floating and submerged macrophytes has been associated with greater diversity across multiple invertebrate taxa (Gledhill, James & Davies 2008; Hassall *et al.* 2011; Raebel *et al.* 2012a), as well as being positively associated with the presence of amphibians (da Silva, Candeira & Rossa-Feres 2012).

High pond density in the neighbouring landscape is another factor often associated with higher pond biodiversity, which will be covered further in *Chapter 4*. For many taxa, distances between ponds may be more important than individual pond area, as dispersal allows populations to persist across a number of smaller habitats (Delettre & Morvan 2000; Fortuna, Gómez-Rodríguez & Bascompte 2006; Shulman & Chase 2007; Raebel *et al.* 2012a).

1.2.3 Biodiversity value of farmland ponds

The biodiversity contribution of ponds is especially important in agricultural landscapes, which can otherwise be a rather homogenous sea of poor quality habitat (Benton, Vickery & Wilson 2003; Declerck *et al.* 2006; Casas *et al.* 2012; Raebel *et al.* 2012b; De Marco *et al.* 2014). However, farmland ponds are largely overlooked in discussions of agricultural biodiversity and conservation, and are offered very little legislative protection (Céréghino *et al.* 2008a; Sayer *et al.* 2013b). Agricultural land covers approximately 40% global land surface (Foley 2005), and this is likely to increase with growing food demand. Finding solutions which support biodiversity in the face of increasing demand for food production and agricultural intensification is a major conservation challenge (Tscharntke *et al.* 2005). Biodiversity in agricultural landscapes depends largely on the intensity of land use, with many protected habitat types including hay meadows, wet grassland, and heathland, relying on traditional agricultural practices for their conservation (Reidsma *et al.* 2006; Henle *et al.* 2008). The global intensification of agriculture has led to widespread declines across multiple taxa (Donald, Green & Heath 2001; Benton *et al.* 2003; Flynn *et al.* 2009), including many (such as pollinating and predatory insects), which are of importance for agricultural productivity.

Farmland ponds and their surrounding margins can provide a network of habitat ‘islands’ within the agricultural landscape, supporting both aquatic and terrestrial biodiversity. Heterogeneity between sites is again an important factor in the biodiversity value of farmland ponds, and even when diversity at individual sites is low, at a regional level farmland ponds make an important contribution to biodiversity (Céréghino *et al.* 2008b).. The majority of research on the value of farmland ponds has come from the UK and Europe (Beja & Alcazar 2003; Declerck *et al.* 2006; Downs & Racey 2006; Raebel *et al.* 2012b; Sayer *et al.* 2012). In the UK, managed farmland ponds are important habitats for aquatic macrophyte, macroinvertebrate, odonate, and amphibian diversity, although neglect has led to large numbers of these habitats becoming over-shaded, reducing their biodiversity value (Sayer *et al.* 2012). Ponds are the obligate habitat for at least 35% of British odonate species (Raebel *et al.* 2012b), and good quality farmland ponds (high in emergent and submerged macrophytes), support a high diversity of odonate species (Raebel *et al.* 2012a; b). Similar patterns in the contribution of farmland ponds to regional biodiversity have come from Brazil (De Marco *et al.* 2014), where species turnover between cattle watering ponds is a major component of regional biodiversity.

Farmland ponds can provide important hotspots for aquatic biodiversity, both in areas where man-made ponds provide the only wetland habitats (Céréghino *et al.* 2008b), and areas where they increase connectivity between larger natural wetlands (Casas *et al.* 2012). In the agricultural landscape of south-western France, artificial ponds provide the only stillwater habitats. These ponds contain large numbers of species not found in running waters in the region, potentially making a high contribution to regional freshwater biodiversity (Céréghino *et al.* 2008b). In other regions, artificial ponds located in low-intensity farmland can complement natural stillwaters, providing alternative habitats which can support comparable habitat complexity and species diversity to natural wetlands (Fuentes-Rodríguez *et al.* 2012). In intensively managed farmland, ponds tend to be less biodiverse and provide lower habitat complexity, but can still provide important 'stepping stones' across the agricultural landscape (Casas *et al.* 2012). This effect may be particularly important for dispersal limited taxa like amphibians (Beja & Alcazar 2003; Casas *et al.* 2012).

Farmland ponds and their surrounding margins also provide important habitat for terrestrial species. The high densities of insects emerging from open-water ponds provide an important food source for bats (Downs & Racey 2006) and farmland birds (Bradbury & Kirby 2006; Davies 2014), with the latter also benefiting from nesting sites provided by pond margins. The impact of ponds on wider terrestrial linkages and food webs has been under researched (Knight *et al.* 2005). However, by contributing to landscape heterogeneity (Benton *et al.* 2003), and providing terrestrial buffer zones of benefit for agriculturally important taxa, ponds are likely to benefit certain agricultural biodiversity services, including pollination and the control of insect pests (Gardiner & Neal 2009; Power 2010).

With 75% of the UK, and 40% of global land area under agriculture (Foley 2005; Raebel *et al.* 2012b), farmland ponds are a key resource for maintaining regional biodiversity. Despite their abundance and proven ecological value, farmland ponds remain relatively under-researched and under-valued. The view that farmland ponds, and indeed farmland more generally, is of little biodiversity value remains widespread (Biggs *et al.* 2005; Tscharntke *et al.* 2005). Farmland ponds are offered little legislative protection (Céréghino *et al.* 2008a; Sayer *et al.* 2013b), and are poorly covered by agri-environment schemes aimed at preserving farmland biodiversity (Langton, Beckett & Foster 2001; Publications 2008).

While farmland ponds can potentially be extremely biodiverse (Williams *et al.* 2004; Sayer *et al.* 2012), the biological value of these habitats is often compromised by a variety of pressures, in particular; agricultural pollution and run-off, modifications to morphology and hydroperiod, and abandonment or in-filling for land reclamation. However, if managed and restored correctly, farmland ponds can make a significant contribution to both regional biodiversity and habitat connectivity. This thesis focuses on novel ways of restoring these key habitats to the farm landscape, while understanding the implications their historic loss has had for regional and local biodiversity.

1.3 Factors threatening the biodiversity value of ponds

While many of the threats experienced by ponds are similar to those of other aquatic systems (nutrient and pollution run-off, acidification, invasive species), a pond's small size and volume can often exacerbate these issues (Williams *et al.* 2007). Some of the most common and severe threats to ponds can be divided into five broad categories (which will be considered with particular reference to farmland ponds):

- *1.3.1* *Threats to water quality*, including urban/agricultural run-off, nutrient enrichment, acidification.
- *1.3.2* *Modification of the physical structure/form of a pond*, including abstraction, alterations to hydroperiod, and bankside modifications.
- *1.3.3* *Invasive species threats*, including alteration of habitat structure, water quality, and genetic 'pollution' of rare species.
- *1.3.4* *Abandonment & terrestrialisation*, including over-shading and siltation.
- *1.3.5* *Pond loss*, including both the loss of individual ponds (often due to in-filling for land reclamation), and the resulting fragmentation of the pond landscape.

1.3.1 Threats relating to pond water quality

Threats to pond water quality are highly dependent on both the location and type of pond in question. In the case of urban ponds, pollution from heavy metals, detergents and salt from roads can be key pressures (Hassall 2014). In more ‘natural’ environments, away from direct human impacts, pond water quality can still be impacted by acid rain, and long range transportation of pollutants, including PCBs and heavy metals (Larsson 1989; Chambers *et al.* 2013).

One of the most frequently highlighted threats to pond water quality is the input of excessive amounts of nutrients. While the small catchment size of ponds can make them more robust to nutrient pollution (Davies *et al.* 2008a), various studies have demonstrated a negative effect of elevated nutrients on aquatic macrophyte abundance and diversity in ponds (Akasaka *et al.* 2010; Véronique *et al.* 2013; Hassall 2014). This can have a knock-on effect on other taxa, including macroinvertebrates and amphibians, the diversity of which are often related to the diversity of aquatic macrophytes (Oertli *et al.* 2002; da Silva *et al.* 2012). In 2007, the Countryside Survey conducted a census of lowland ponds across the UK. In England, 38% of ponds were found to have concentrations of soluble reactive phosphorous (SRP), exceeding the threshold at which a pond is considered to be ‘highly polluted’. In addition, 20% of lowland ponds had nitrogen levels above the ‘highly polluted’ threshold, and water turbidity was often high, with only 49% of lowland ponds considered to have ‘clear’ or ‘moderately clear’ water.

Although nutrient enrichment can be a considerable threat to ponds, the small catchment size of these habitats (Davies *et al.* 2008a), means that they can be relatively easily buffered against surface run-off (*Figure 3*). In such cases, these ponds may be under greater threat from more physical changes, especially abandonment and over-shading (see section 1.3.4).

Figure 3 - A well buffered farmland pond. Even in the middle of a field, a farmland pond can have good water quality, and support a high diversity of plants and invertebrates, provided that it is surrounded by a buffer strip (preferable 6+ meters).

1.3.2 Threats relating to modification of a pond's structure, shape, or permanence

The biodiversity value of a pond is often highly dependent upon physical attributes such as pond size (Brönmark 1985; Oertli *et al.* 2002), hydroperiod (Pechmann *et al.* 1989; Schneider 1994), and bank slope and stability (Wood *et al.* 2003). These characteristics can effect pond biodiversity directly, for example by limiting the area or time period available for aquatic macrophyte establishment, and more indirectly, through influencing water chemistry. Pond hydroperiod and bank characteristics are routinely altered by human activities, such as modifying ponds for field drainage or irrigation, and watering livestock.

Pond hydroperiod, or the amount of time over which the pond holds water, can be particularly significant for pond biodiversity, as permanent and ephemeral ponds are often important for different taxa. While ponds with a longer hydroperiod tend to be more biodiverse overall (Pechmann *et al.* 1989; Brooks 2000), ephemeral ponds and ponds with fluctuating water levels are important for a number of rare plant and invertebrate species (Schneider 1994; Brooks 2000; Lott 2001; Beja & Alcazar 2003; Scott *et al.* 2012). As such, a network of ponds with different hydroperiods is often desirable from a biodiversity perspective (Williams *et al.* 2008). Pond hydroperiod is easily disrupted by human modifications and changes in land use. Abstraction for irrigation is common in agricultural ponds (Beja & Alcazar 2003), and can lead to reduced hydroperiods – a particular problem for amphibians (Pechmann *et al.* 1989; da Silva *et al.* 2012). Conversely, converting ephemeral ponds to permanent reservoirs can be detrimental to species which rely on fluctuations in hydroperiod (Beja & Alcazar 2003). As well as the direct impacts of changing the volume and permanence of a pond, hydroperiod alterations can have a knock-on effect on pond biodiversity, through effects on water quality and the susceptibility of a pond to invasive species (Zacharias *et al.* 2007; Usio *et al.* 2013).

Changes to the shape, slope, or stability of the banks around a pond can also be detrimental to biodiversity. Shallow and gently sloping banks are important for the establishment of aquatic and emergent macrophytes, as well as accessibility for amphibians (Wood *et al.* 2003). The greater habitat complexity and food availability in shallow bank areas in turn leads to higher invertebrate abundance and diversity in these microhabitats. For example, aquatic Coleoptera diversity and abundance is often highest along pond banksides (Fairchild *et al.* 2003). These shallow habitats can be threatened by human modifications, including their removal / steepening when natural ponds are converted to reservoirs (Beja & Alcazar 2003). Pond banksides are also often threatened by disturbance, especially in farmland. Poaching by livestock can significantly reduce the number of macrophyte taxa and structural diversity of the macrophyte community along pond banks, while also negatively impacting water quality (Declerck *et al.* 2006). Having said this, in some instances low to intermediate levels of bankside disturbance can be beneficial to pond biodiversity (Oertli *et al.* 2009), and can be important in re-activating dormant seedbanks (Abernethy & Willby 1999).

1.3.3 Invasive species threats to ponds

Although invasive species are problematic for all waterbodies, the small size of ponds means that they can quickly become overrun (*Figure 4),* while the abundance of ponds in human modified landscapes (urban areas, farmland, roadsides), often exposes them to greater risk of invasion. The aquarium and horticultural trades are a major source of invasive species in ponds, with unwanted plants, fish and other organisms being dumped by naive hobbyists, or escaping from garden ponds (Maki & Galatowitsch 2004; Padilla & Williams 2004). The occurrence of invasive species in ponds is often highly related to their accessibility – for example, the occurrence of invasive goldfish in Epping Forest is significantly related to the distance of a pond from a public road or footpath (Copp, Wesley & Vilizzi 2005).

The 2007 Countryside Survey of lowland ponds in the UK found that 10% of surveyed ponds contained invasive macrophyte species. These can threaten a ponds' biodiversity value by out-compete native macrophytes (Villamagna & Murphy 2010; Strayer 2010; Ewald 2014), and reducing habitat complexity for invertebrates (Fairchild, Faulds & Matta 2000; Gioria *et al.* 2010), and amphibians (Strayer 2010).

Figure 4 - Invasive species in ponds. The aquatic macrophyte *Crassula helmsii* (pictured left), is a common problem for UK ponds, where it can quickly smother entire sites. *Image from: www.herefordhart.org*. Common goldfish another problematic invasive, altering habitat structure and endangering native species through competition or hybridization (pictured right, a crucian carp / goldfish hybrid caught in Norfolk – photo courtesy of Carl Sayer).

While invasive macrophytes can have a 'bottom-up' effect on pond ecosystems, influencing both habitat (*Figure 4*), and trophic structure, invasive fish often exert a 'top-down' influence. Invasive fish can have a devastating effect on pond biodiversity, both through altering predator / prey interactions, and through habitat disturbance and destruction (Zambrano, Scheffer & Martinez-Ramos 2001). In UK ponds, non-native cyprinid species such as the common carp *Cyprinus carpio*, and goldfish *Carassius auratus*, are particularly common, and particularly destructive. The feeding behaviour of these species can destroy a pond's macrophyte community, triggering a switch from a clear to turbid state (Zambrano *et al.* 2001). In addition to impacting upon the trophic and habitat structure within a pond, invasive carp species also threaten the existence of one of the UK's rarest native fish – the crucian carp *Carassius carassius*. Closely related to both the common carp and ornamental goldfish, crucian carp readily hybridise with both these invasive species (Wheeler 2000; Sayer et al. 2013). This, along with loss of pond habitats, has led to the sharp decline in this protected species. Extinction through hybridisation with non-native and invasive species is of concern for other pond taxa, including amphibians (Brown 1971; Riley et al. 2003), and odonata (Sánchez-Guillén et al. 2013).

1.3.4 Pond loss through abandonment and terrestrialisation

Most farmland ponds are man-made in origin, and without regular management quickly become over grown and begin to terrestrialise. Routine pond management, in particular the removal of trees and shrubs from pond margins, continued in some areas of the UK until as recently as the 1960s. Some ponds were still in use for watering livestock or providing field irrigation, while others were kept clear as a way of reducing brown rat habitat, or simply to provide labour for farm workers during quiet periods in the agricultural calendar (Sayer *et al.* 2012). There are now few agricultural reasons to manage or maintain farmland ponds, and those too large to have been infilled for agricultural intensification have largely been overgrown to succession (*Figure 5*). While succession is a natural component of habitat development, it is problematic for farmland ponds, with ‘early succession ponds’ (new or restored open water ponds), rarely being replaced. This can lead to a very homogenous landscape of late-successional ponds, with limited biodiversity value (Sayer *et al.* 2012, 2013a).

Figure 5 - Late successional farmland pond in Norfolk, UK (author’s photographs). Without management, many farmland ponds succeed to a willow-dominated state, with a highly anoxic water column and very low biodiversity value.

Pond succession and terrestrialisation has a negative impact on aquatic biodiversity through a number of mechanisms (Hassall *et al.* 2011; Sayer *et al.* 2012). First, there is the direct impact of shading from trees, which reduces the amount of light entering the water column, impairing aquatic macrophyte growth. Reduced macrophyte cover and diversity has a knock-on effect on multiple taxa, through a reduction in habitat, food, and impaired water quality (Carpenter & Lodge 1986; Engelhardt & Ritchie 2001; Akasaka *et al.* 2010). Over-shading also impacts pond water quality by reducing wind mixing of the water column (*Figure 6*). This, in combination with the loss of aquatic macrophytes, reduces dissolved oxygen in the water column, negatively impacting aquatic invertebrate diversity.

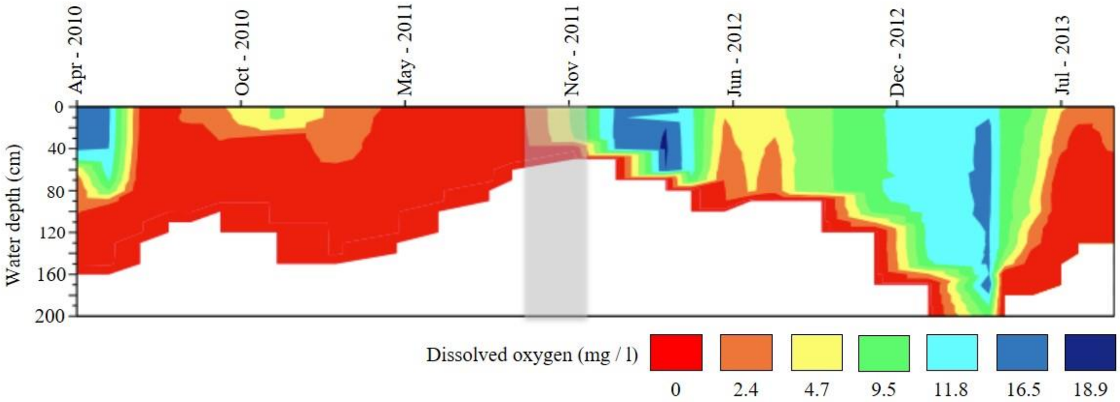


Figure 6 - Oxygen profile of a farmland pond before and after restoration. Oxygen profile for pond "SABA", north Norfolk, before and after restoration (shaded grey area). Pre-restoration, oxygen levels throughout the water column are close to zero. Post-restoration, reduced tree cover allows seasonal wind mixing. Diagram adapted from H. Greaves' PhD thesis (UCL).

Overgrown ponds also experience high inputs of leaf matter, the decomposition of which further reduces dissolved oxygen in the water column. Once broken down, this highly organic and anoxic sediment forms an unstable and chemically hostile environment, preventing the establishment of aquatic macrophytes. Eventually evapotranspiration from overhanging trees, combined with high siltation rates, can result in complete terrestrialisation of a pond.

While it has been suggested that highly overgrown ponds might provide important habitats for some rare species (Biggs, Corfield & Walker 1994), there is limited evidence to support this notion (Hassall *et al.* 2011; Sayer *et al.* 2012). Furthermore, at present highly overgrown ponds dominate the farmland pond-landscape, while open water ponds are progressively disappearing. This trend has been observed across the UK, in areas including Norfolk (Prince 1962) and the north-west of England (Boothby & Hull 1997), where less than half of surveyed ponds had "substantial areas of open water". The homogeneity of overgrown ponds poses a substantial threat to their biodiversity value, both for aquatic (Sayer *et al.* 2012) and terrestrial taxa, such as birds and bats, which may experience food shortages as a result of reduced invertebrate abundances (Downs & Racey 2006).

It is generally recognised that for maximum biodiversity benefit, a mosaic of ponds at different successional stages, and of different types, is desirable (Wood et al. 2003). While the creation of new ponds has been suggested as a way of achieving this habitat mosaic (Davies *et al.* 2004; Williams *et al.* 2008), this approach is not always sustainable, requiring the constant creation of new ponds to replace those being lost through succession. Outside of nature reserves, this approach is often not economically or practically feasible. An alternative approach is to manage our existing ponds (Sayer *et al.* 2012, 2013a), periodically re-setting succession in some sites, in order to achieve the same habitat-mosaic effect. This is a more feasible solution; restoring existing ponds does not create competition with other land uses, and is considerably cheaper and easier than new pond creation. Pond management can restore historic connectivity across the pond landscape, as opposed to creating new patterns in connectivity through new pond creation.

While the effects of pond management on biodiversity are now receiving more research interest, the *mechanisms* behind the re-establishment of managed ponds have so far not been studied. In particular, the role of the historic seedbank in re-colonisation of restored ponds is of great interest. Aquatic macrophyte seedbanks from habitats including lakes, and temporary and permanent wetlands, have been shown to remain viable for decades (De Winton, Clayton & Champion 2000), to centuries (Stobbe, Gregor & Röpke 2014). These historic seedbanks can act as a source for the re-establishment of local species (Vivian-Smith & Handel 1996; Beltman & Allegrini 1997; Weyembergh, Godefroid & Koedam 2004), and have the potential to restore / maintain genetic diversity within small, isolated populations (Uesugi *et al.* 2007; Honnay *et al.* 2008). As such, the historic seedbank may be important in both the restoration of overgrown ponds, and in the 'resurrection' of ponds previously filled-in during land-use intensification. The potential role of the historic seedbank as a source of species and genetic diversity is covered in more detail in chapter six.

1.3.5 Pond loss through deliberate in-filling

Arguably the most pressing threat to pond biodiversity has been the widespread destruction of these habitats, largely due to in-filling for agricultural intensification (Davidson 2014). In the Prairie Pothole region of the US, 95% of wetland losses between 1997 – 2009 occurred as a result of agricultural development (Dahl 2014), with the greatest losses occurring in small wetlands and ponds (Dahl 2014; Serran & Creed 2016). Across Europe, pond loss has increased dramatically since the Second World War, as growing pressure to increase food production led to the removal of hedgerows, ponds, and other semi-natural farmland habitats (Rackham1986). It has been estimated that around half of the ponds in western Europe were lost between 1900 – 1990, many from agricultural land (Hull 1997; Curado, Hartel & Arntzen 2011). In the UK, similar extents of agricultural pond loss have been reported across Cheshire (Boothby & Hull 1997), Essex (Heath & Whitehead 1992), and Northumberland (Jeffries 2012). Such extensive pond loss has consequences for both pond biodiversity (Møller & Rørdam 1985; Gledhill *et al.* 2008; Bosiacka & Pienkowski 2012) and connectivity (Boothby 1997; Oertli *et al.* 2009) across pond landscapes. These issues are covered in greater detail in chapters three and four.

1.4 A brief introduction to habitat fragmentation – structural connectivity

Habitat fragmentation has been a central theme in conservation biology since the field began (Harrison & Bruna 1999), and has been widely studied both theoretically and empirically. Most research on the effects of fragmentation has focused on terrestrial habitats, from which the theories of island biogeography (MacArthur & Wilson 1967), and later metacommunity dynamics (Wilson 1992), emerged. Both theories assert that the population of an insular habitat is determined by distance-dependent colonisation, and area-dependent extinction (Harrison & Bruna 1999). In altering both the distances between habitat patches, and the size of these patches, fragmentation effects both core processes of colonisation and extinction, which in turn effect population size, genetic structure, and community and species diversity (*Figure 7*). While these purely structural components of habitat connectivity are of key importance, overall landscape connectivity involves both these structural features of habitat connectivity (structural connectivity), and the degree to which organisms are able to move between habitat patches, based on their dispersal capabilities and habitat requirements (functional connectivity - Watts and Handley, 2010). Structural connectivity between habitat patches is relatively easy to measure, and has historically been the focus of most studies of habitat fragmentation. Functional connectivity is more difficult to assess, and is discussed in more detail in *Section 1.5*.

Theoretical models of habitat fragmentation generally assume that the smaller and more isolated a habitat, the fewer species it will support. This is due to the increased extinction risk for isolated populations, which may be vulnerable to sudden environmental changes, reduced adaptability as a result of inbreeding, or inability to disperse to more suitable habitat as a result of their isolation (Young, Boyle & Brown 1996; Harrison & Bruna 1999; Fahrig 2003). Empirical studies however have produced mixed results, with most of the studies which fit theoretical expectations of fragmentation tending to be simple, small scale experiments, often using arthropods (Harrison & Bruna 1999; Debinski & Holt 2000). Larger scale studies using more complex habitats and communities have produced much more varied results. Population size and density are not only influenced by habitat area and isolation, but also by inter-specific and intra-specific interactions between organisms. In some instances, fragmentation can promote increased regional or γ- diversity by providing refuges from predation or competition (Scheffer & Van Geest 1999; Debinski & Holt 2000; Jost 2007), or can increase population sizes through crowding effects (Debinski & Holt 2000). Fragmentation can even have a positive effect on population genetic structure due to specialisation and local adaptation, although problems associated with inbreeding and reduced adaptability in the face of environmental change are more common outcomes (Harrison & Bruna 1999; Jump & Peñuelas 2005; Lopez *et al.* 2009).

Another complication for the empirical study of fragmentation is the difficulty in separating the purely spatial component (i.e. increased distances between habitats), from the confounding problem of overall habitat loss (Fahrig 1997). While it is almost impossible to separate these two effects in the field, Fahrig (1997) examined the two processes independently in a theoretical model. Simulation results suggested that even under an unrealistically conservative model for dispersal (no exchange between habitat patches), habitat loss had a much larger effect than habitat fragmentation on population extinction. Empirical studies have produced mixed results concerning the relative importance of fragmentation versus habitat loss, in particular because the functional connectivity of a fragmented landscape differs for taxa with different dispersal abilities and habitat requirements (Harrison & Bruna 1999; Debinski & Holt 2000; Joly, Morand & Cohas 2003; Krauss *et al*. 2004; Watts & Handley 2010).

Some of the key theoretical processes and consequences, relating to changes in structural connectivity associated with habitat fragmentation, are illustrated *Figure 7*. Fragmentation is broadly divided into three key components; the reduction of habitat size, reduction in habitat quality, and increase in habitat isolation. Over time, these key components can lead to multiple possible outcomes and feed-back loops within population dynamics. For example, fragmentation may lead to reduced dispersal between populations, resulting in reduced genetic diversity and higher risk of extinction in the face of sudden environmental change. Alternatively, reduced dispersal between populations due to fragmentation could potentially result in higher specialisation and local adaptation, inferring greater community resilience to invasive or competitive species (Scheffer & Van Geest 1999).

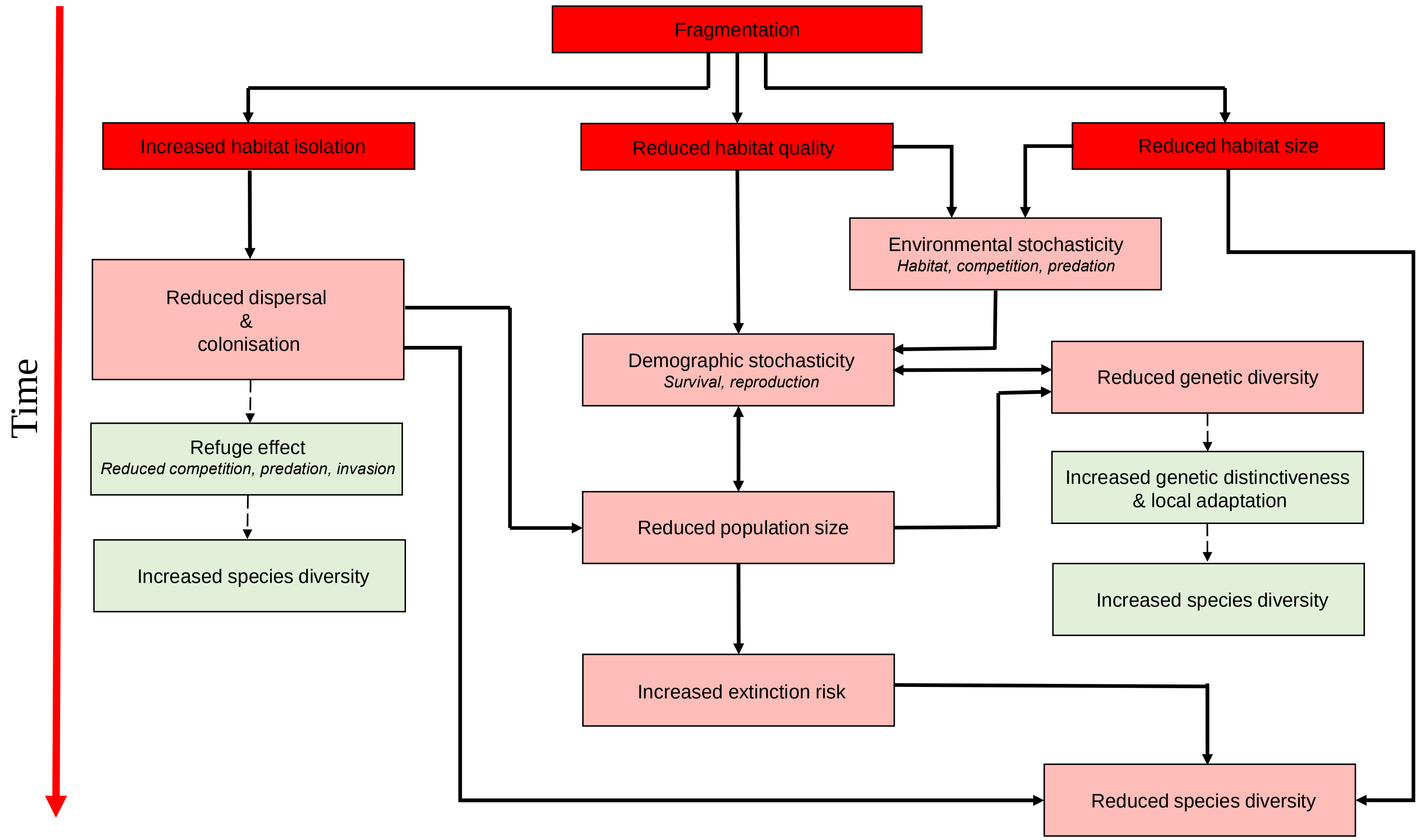


Figure 7 – Schematic diagram of some of the key components of habitat fragmentation, and how these change over time. Solid arrows indicate common consequences, dotted arrows less common consequences. Negative consequences of fragmentation are in red boxes, positive consequences are in green.

1.5 Functional connectivity, and an organism's view of the landscape

Theories of habitat fragmentation based on ideas from island biogeography (as explored in *section 1.4*), have often led to fragmented habitats being viewed as islands within an inhospitable matrix. However, the matrix surrounding most habitat patches does not represent a completely impassable barrier, but is instead more or less permeable to different species (Kupfer, Malanson & Franklin 2006). As such, understanding the effects of fragmentation requires a consideration of both landscape characteristics (structural connectivity), and aspects of the mobility of the organism (functional connectivity), particularly its ability / willingness to cross the 'matrix' in between habitat patches.

The permeability of the matrix in between habitat patches depends largely on the habitat quality provided by the matrix, the presence of suitable habitat stepping-stones or corridors through the matrix, and the gap-crossing willingness of the species in question (Kupfer *et al.* 2006). The permeability of the matrix (and thus functional connectivity of the landscape), is not only species specific, but can vary between individuals, and in relation to the process under consideration (Adriaensen *et al.* 2003). For example, the functional connectivity of a landscape for short-range dispersal during foraging, vs. long-range dispersal for breeding, will likely differ.

Incorporating functional connectivity into models of habitat fragmentation presents a considerable challenge. One commonly used approach for assessing the permeability of the matrix around habitat patches is 'least cost' modelling. This approach calculates the route of least resistance between two habitat patches, considering structural connectivity (actual distance between patches), combined with information on the geography of the matrix (land cover), and the behaviour of the organism in question (Adriaensen *et al.* 2003). Least-cost modelling requires the definition of different land cover classes (which should be done from the perspective of the organism in question), followed by setting resistance values for the 'cost' of crossing these different land cover classes (Adriaensen *et al.* 2003). This cost should reflect both the energy expenditure and the mortality risk of crossing a given area (Murphy & Lovett-Doust 2004). Little is published on the ecological assumptions underlying the use of least cost analysis, and defining both land cover class and resistance values requires detailed ecological knowledge of the species in question, which is often lacking (Adriaensen *et al.* 2003; Murphy & Lovett-Doust 2004). Setting quantifiable resistance values for different land use types is particularly problematic; for example, an amphibian may be very capable of dispersal across woodland (low resistance), and completely incapable of dispersal across a building (impassable barrier). In between these extremes, cropland may present greater resistance to dispersal than meadow (Joly *et al.* 2001; Murphy & Lovett-Doust 2004), but by how much? There is also the issue that modelling functional connectivity based on least cost pathways assumes that an organism always seeks out the path of least resistance, which may not be the case.

In some respects, ponds represent 'fragmented' habitats; aquatic islands within a terrestrial matrix. For purely aquatic organisms incapable of overland dispersal (for example fish), ponds truly follow the assumptions of island biogeography, with a distinct habitat boundary surrounded by an inhospitable matrix (Murphy & Lovett-Doust 2004). For other organisms, ponds are not entirely isolated, but linked by overland dispersal pathways which facilitate species exchange. The functional connectivity of the pondscape thus differs greatly between taxa. For example, while fish experience complete dispersal limitation imposed by the terrestrial matrix surrounding ponds, amphibians are able to cross this matrix to some extent (although their dispersal capabilities are strongly limited by both aquatic and terrestrial habitat components; Ray, Lehmann & Joly 2002; Fortuna *et al.* 2006), and flying insects such as aquatic Coleoptera and Odonata may cross the matrix with relative ease, experiencing pondscape connectivity at a much larger spatial scales (Fairchild *et al.* 2003; Raebel *et al.* 2012b; Coccia *et al.* 2016). As such, ponds can provide a useful system for understanding the ecological consequences of fragmentation across multiple spatial scales (Harrison & Bruna 1999; De Meester *et al.* 2005).

With pond loss due to agricultural land intensification highlighted as a key threat to pond habitats and biodiversity, developing effective methods to counteract these losses is imperative. While new pond creation and the restoration of overgrown ponds are two approaches to the problem, this thesis focuses on another, completely novel, approach. This is the resurrection of ponds formerly lost to agricultural land intensification – or 'ghost ponds'.

1.4 What is a 'ghost pond'?

A 'ghost pond' is a former pond which has been subjected to agricultural land reclamation. While ponds may be filled-in for a variety of reasons, including urban expansion and road construction, ghost ponds are specifically those lost from agricultural land, most commonly due to agricultural intensification. The term 'ghost pond' was coined by Dr. Carl Sayer to describe these lost aquatic habitats, as they often remain visible in the agricultural landscape as a damp depression or change in vegetation or soil colour (

Figure 8) – an almost 'ghostly' shadow of the habitat which used to be there. The name is also in fitting with the hypothesis that these habitats might be able to be 'resurrected' from the historic propagule banks within their buried sediments.

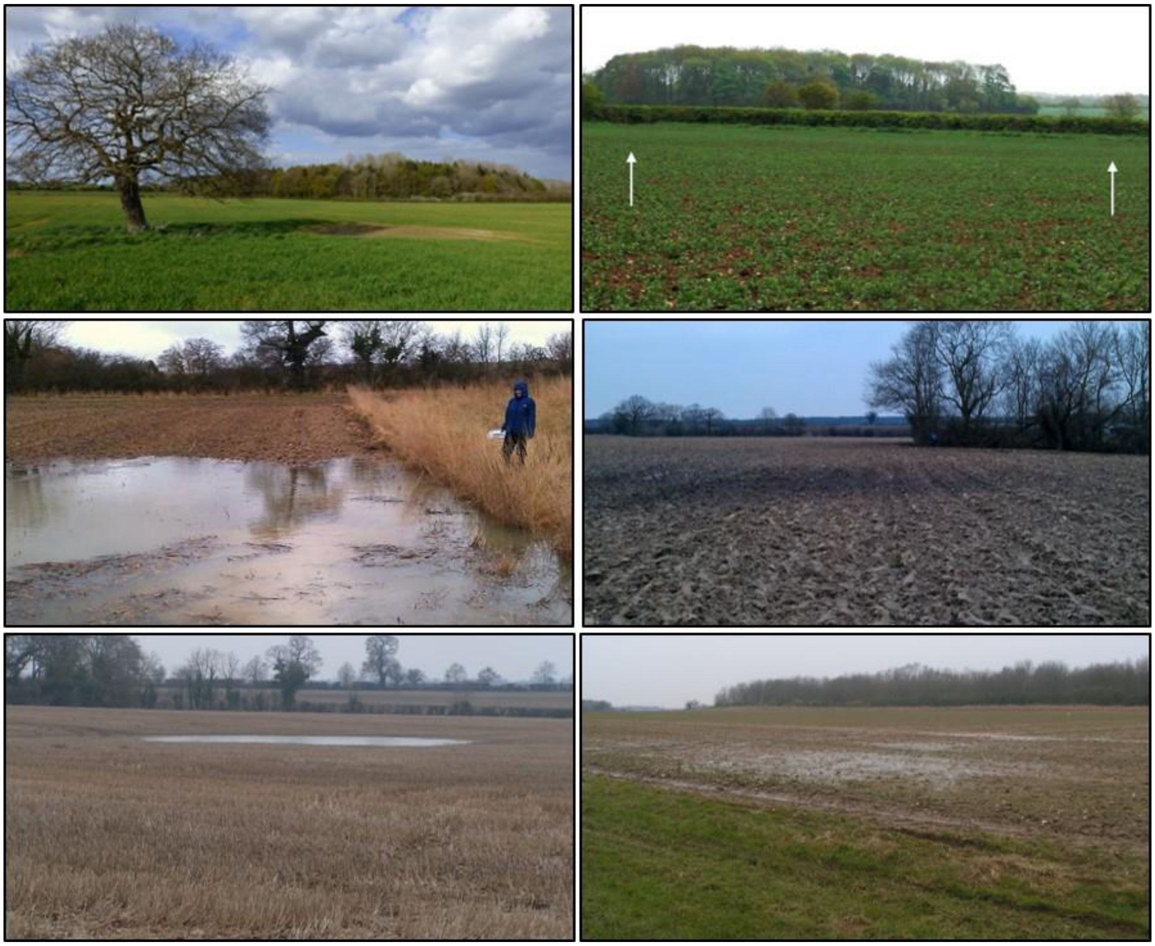


Figure 8 - Ghost ponds in north Norfolk farmland. A selection of photos of buried ghost ponds across north Norfolk. Ghost ponds may be visible as patches of poor crop production (top), or as damp depressions (middle and bottom), in agricultural fields.

With some areas of the UK having lost up to 90% of their ponds (Wood *et al.* 2003), and levels of pond loss of 50 – 60% common across European agricultural regions (Boothby & Hull 1997; Céréghino *et al.* 2008b), ghost ponds are an abundant landscape feature. Resurrecting these ghost ponds could re-create past aquatic network connectivity along historic pathways, seemingly an appropriate way of targeting pond creation to optimally improve pond landscape connectivity (Boothby 1997; Davies et al. 2004). Additionally, the resurrection of ghost ponds, which are often marked by areas of wet ground and poor crop production, could benefit conservation with minimal impact upon farming practice and productivity.

Perhaps the most intriguing and potentially important feature of ghost ponds however is the historic sediment bank stored within these sites. During land intensification, the pond sediments containing the seeds and eggs of past macrophyte and invertebrate communities, are buried under topsoil. Work in the field of resurrection ecology shows that the seeds and eggs of some aquatic organisms remain viable on decadal to centennial timescales (De Winton *et al.* 2000; Bonis & Grillas 2002; Stobbe *et al.* 2014), and it is therefore possible that the sediments of ghost ponds could act as inert time capsules for genetic and species diversity. If this hypothesis is true, it could give ghost ponds a number of advantages over new ponds, in terms of biodiversity and habitat restoration. If viable, the in-situ local propagule bank of ghost ponds could accelerate colonisation by native species, reducing the risk of invasive species establishment (Freshwater Habitats Trust 2015). The longevity of many aquatic seeds and eggs also offers the potential to resurrect locally lost or rare species (Beltman & Allegrini 1997; Kaplan *et al.* 2014), and restore lost genetic diversity to fragmented populations (Uesugi *et al.* 2007; Honnay *et al.* 2008). These exciting possibilities have never before been investigated.

1.5 Thesis aims and objectives

Pond are widely recognised as important biodiversity hotspots (Céréghino *et al.* 2008a; Davies *et al.* 2008b), and as key features in aquatic landscape connectivity (Boothby 1997; Boix *et al.* 2012). As such, the restoration and enhancement of these habitats is of great concern for aquatic biodiversity conservation. However, our current knowledge of the relationships between pond connectivity and biodiversity, how pond loss has impacted upon biodiversity, and how best to restore biodiversity across agricultural pond landscapes, is lacking. This thesis examines some of the key issues surrounding pond loss and its potential implications for biodiversity, and novel approaches for pond conservation. This thesis aims to:

A1 – Improve understanding of pond loss processes, and how historic pond loss has influenced the structural connectivity of the pondscape across a typical agricultural region.

A2 – Examine how changes in the pondscape arising as a result of historic pond loss (reduced pond density and increased distance between ponds), influence the diversity and occurrence of multiple aquatic taxa.

A3 – Assess the potential role that the resurrection of ghost ponds and restoration of overgrown ponds could play in pond conservation, focusing on the role of the historic seed bank for macrophyte recolonisation.

Each of these overall aims is addressed in order; A1 is covered in *Chapter 3*, A2 in *Chapter 4*, and A3 in *Chapters 5 & 6*. In order to fulfil these aims, each chapter of this thesis has the following research objectives:

Chapter 2 Study sites

This chapter provides background on the geology and history of ponds within the study region, and the locations and selection criteria for the study areas. The objectives for this chapter are:

1) Describe the characteristics of ponds in the study region.
2) Outline the different study scales and experimental approaches used throughout the thesis.

Chapter 3 Changes in an agricultural 'pondscape' – a history of pond loss and habitat fragmentation.

Pond loss and the fragmentation of pond landscapes or 'pondscapes' (Boothby 1997), have been highlighted as major threats to aquatic biodiversity (Boothby 1997; Joly *et al.* 2003; Wood *et al.* 2003). However, very few comprehensive studies of pond loss have been conducted. This chapter examines the current literature on pond loss and pondscape fragmentation, leading into a detailed analysis of these process across the study region of Norfolk, East Anglia. Pondscape changes are examined at a regional (whole county), and local (3 km^2) scale, using historic and modern Ordinance Survey (OS) maps. The advantages and limitations of this map-based approach are evaluated, and the implications for pond conservation are discussed. The objectives for this chapter are:

1) Evaluate the reliability and accuracy of the map-based approach for measuring historic and present-day pond numbers.
2) Determine the extent of pond loss across the study region of Norfolk, and identify areas with the greatest pond losses, and the types of pond most affected.
3) Examine how the rate of pond loss varied before and after WW2, in relation to the drive for increased agricultural productivity after the war.
4) Assess the extent to which historic pond loss has resulted in the fragmentation of the pondscape, and how this has influenced pond density across the study region.

Chapter 4 The ecological consequences of pond loss and pondscape fragmentation – a space for time approach.

Pond loss and pondscape fragmentation are considered major threats to aquatic biodiversity for several reasons; more isolated habitats generally support fewer species (MacArthur & Wilson 1967; Møller & Rørdam 1985; Beisner *et al.* 2006), put populations at greater risk of both deterministic and stochastic extinction (MacArthur & Wilson 1967; Tscharntke *et al.* 2005), and experience lower species turnover due to dispersal limitation (Williams *et al.* 2008). As such, it is expected that historic loss of ponds is likely to have had a detrimental effect on pond biodiversity (Heath & Whitehead 1992; Boothby 1995; Wood *et al.* 2003). In the absence of long-term datasets of pond biodiversity in relation to changes in pond numbers, a space-for-time approach is often employed, studying the relationships between modern pond density and biodiversity to answer these questions. Although pond density is often invoked as a determinant of aquatic biodiversity (Boothby 1995; Hill & Wood 2014), there have been relatively few large-scale studies, covering a wide range of pond densities, conducted. This chapter examines the relationships between pond density, and the diversity and occurrence of a range of aquatic taxa (macrophytes, Coleoptera and amphibians). Analysis is conducted across a range of spatial scales, using data collected from 121 agricultural ponds located in different 'pond density landscapes'. The objectives for this chapter are:

1) Assess the extent to which pond density influences α- and β- diversity of aquatic macrophytes and Coleoptera, independently of environmental factors.
2) Establish whether the diversity / occurrence of the different taxa (macrophytes, Coleoptera and amphibians), are influenced by pond density at different spatial scales, reflecting their different dispersal abilities.
3) Determine the magnitude and direction of effect that pond density has on α- and β- diversity of aquatic macrophytes and Coleoptera.
4) Examine whether pond density influences the occurrence of the four native amphibian species occurring in this study, to different extents, or at different spatial scales.

Chapter 5 Pond restoration and resurrection - the importance of the historic propagule bank.

Intensive agriculture has contributed to the destruction of numerous wetland habitats through drainage and infilling (Davidson 2014; Gibson *et al.* 2015). Given the significant contribution of small agricultural wetlands and ponds towards regional biodiversity (Declerck *et al.* 2006; Davies *et al.* 2008b), their widespread disappearance from agricultural landscapes represents a significant loss of aquatic biodiversity and reduction in aquatic habitat connectivity, therefore posing a considerable challenge for biodiversity conservation. However, many aquatic organisms have evolved life-cycle strategies for surviving habitat desiccation as dormant propagules. These propagules comprise aquatic macrophyte seeds (De Winton *et al.* 2000; Aponte *et al.* 2010), oospores (Beltman & Allegrini 1997) and cladoceran 'resting eggs' (Hairston 1996) that remain viable for centuries (Hairston 1996; Stobbe *et al.* 2014) and support species re-establishment following habitat improvement (Beltman & Allegrini 1997; Kaplan *et al.* 2014). While the long-term viability of propagules is well established for extant aquatic habitats (Bakker *et al.* 1996; Hairston 1996; Beltman & Allegrini 1997; De Winton *et al.* 2000), their fate in ponds in-filled for agricultural land consolidation has remained unexplored. This chapter assesses the viability of the historic propagule bank of ghost and overgrown ponds prior to their restoration, in order to assess whether long term viability is possible under the stresses of intensive agriculture. Germination trials conducted in outdoor microcosms are used to assess the potential for macrophyte re-establishment from the historic propagule bank. The objectives for this chapter are:

1) Assess the potential for macrophyte re-establishment from the historic propagule bank, using microcosm germination trials conducted under natural conditions.
2) Compare the germination success and species composition occurring from the sediments of ghost and overgrown ponds.
3) Determine the longevity of the buried propagule bank of ghost ponds, using germination trials and seed viability testing for propagule banks buried for 45, 50 and 150 years.
4) Examine the viability of seeds and oospores extracted from ghost pond sediments, to determine whether germination success is limited by seed viability of by environmental conditions during the germination trials.

Chapter 6 *Pond restoration and resurrection – recolonisation from the seed bank vs. dispersal pathways.*

Following on from the investigation into propagule viability within the sediments of ghost and overgrown ponds, this chapter examines the influence that the historic propagule bank has over macrophyte establishment post-restoration. While newly dug ponds rely on dispersal of aquatic organisms for colonisation, which may limit the biodiversity of isolated sites (Biggs *et al.* 2005; Williams *et al.* 2008), the colonisation of ghost and restored ponds may benefit from the historic propagule bank buried within these sites. If this is the case, resurrected and restored ponds could benefit from an accelerated rate of macrophyte re-establishment, and a more diverse initial macrophyte assemblage. Additionally, re-establishment from the historic propagule bank could potentially restore rare species (Weyembergh *et al.* 2004; Kaplan *et al.* 2014), and genetic diversity (Uesugi *et al.* 2007), ostensibly lost from the wider landscape. This chapter describes the initial colonisation by aquatic macrophytes and Coleoptera in the three restored and three ghost ponds, over the first 22 months after their excavation. The relative importance of the historic propagule bank vs. dispersal of aquatic macrophytes is examined using on-site mesocosms. These mesocosms contain either sterile substrate (relying on dispersal for colonisation), or historic pond sediment (relying on the propagule bank for colonisation), in both open and closed treatments. The objectives for this chapter are:

1) Assess the relative importance of the historic propagule bank vs. dispersal mechanisms for macrophyte colonisation, using a 4x4 mesocosm design.
2) Compare the colonisation rates of ghost pond and restored pond mesocosms, evaluating any differences in macrophyte establishment between the two.
3) Evaluate the extent to which the species composition of macrophytes in the seed viability and germination trials (chapter 5), and in the outdoor mesocosms (chapter 6), represents the species composition occurring in the study sites.
4) Explore any differences in macrophyte species composition, and colonisation rate, between the three ghost ponds and three restored ponds.
5) Assess the extent to which the outdoor mesocosms provide a realistic representation of processes occurring in the study ponds, comparing their water chemistry, macrophyte species composition, and colonisation by aquatic Coleoptera.

Chapter 7 Summary, conclusions and future research directions

This chapter summarises the findings of this thesis, evaluating the potential role that the resurrection of ghost ponds and restoration of overgrown ponds could have in aquatic conservation. The objectives for this chapter are:

1) Evaluate the conservation value of ghost and restored ponds, both in terms of the abundance of these habitats within agricultural landscapes, and the potential contribution of the historic propagule bank.
2) Consider how pond restoration and resurrection might be targeted to maximise biodiversity value, in the context of the findings of *chapter 4 – the ecological consequences of pond loss*.
3) Discuss future research directions and pathways to impact.

Chapter 2 Study sites

2.1 The ponds of Norfolk

The county of Norfolk, east England, has one of the highest densities of ponds found in the UK, with an average of 6.4 ponds / km^2 recorded in the 1920s (Rackham 1986). Smaller patches of high pond density can be seen in other areas in England, including parts of Lancashire, Cheshire and Essex (*Figure 9*), as well as in mainland Europe, including south-west France (Céréghino *et al.* 2008b), Denmark (Møller & Rørdam 1985) and Portugal (Beja & Alcazar 2003). Although significant pond loss over the past century will likely have reduced the pond densities shown in *Figure 9*, the relative differences between regions have remained, with pond loss having occurred at similar rates right across Europe (Heath & Whitehead 1992; Wood *et al.* 2003; Curado *et al.* 2011; Bosiacka & Pienkowski 2012).

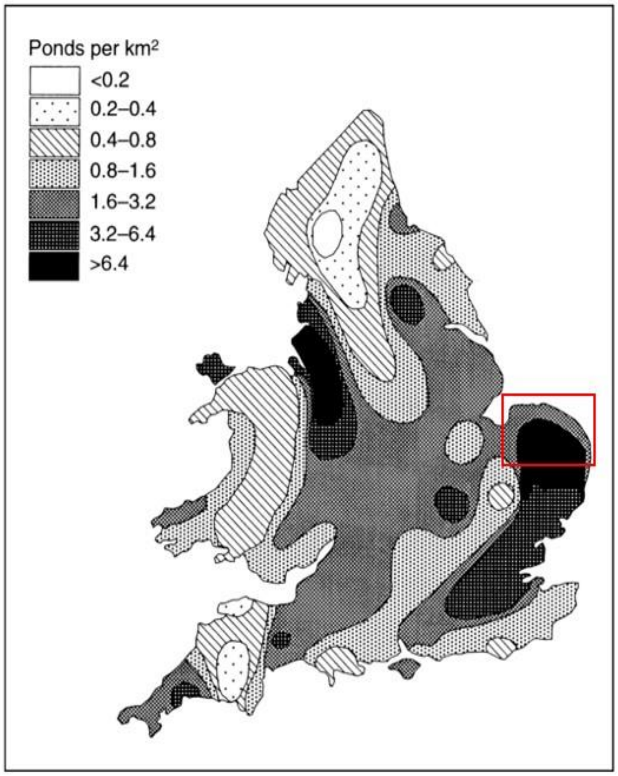


Figure 9 – Pond density across England and Wales during the 1920s. From Wood et al. (2003), adapted from Rackham (1986). Norfolk is outlined in red.

While Norfolk still contains a high density of ponds, it has experienced significant pond loss throughout the 20th century (*Chapter 3*), comparable to many other agricultural regions of the UK (Heath & Whitehead 1992; Boothby & Hull 1997; Wood *et al.* 2003), Europe (Agger & Brandt 1988; Beja & Alcazar 2003; Céréghino *et al.* 2008b; Curado *et al.* 2011), and the USA (Dahl 2014; Serran & Creed 2016). The historically high densities of ponds seen in Norfolk and in some other areas of the UK (particularly Cheshire), are related to the agricultural practice of 'marling', in which marl clay dug from the sub-soil was spread on the fields to improve soil texture, and balance acidity (Prince 1962; Brian *et al.* 1987). This practice created large numbers of marl pits, which once abandoned filled with rain water and became ponds. Marl pit ponds are common (although often in lower densities), across the agricultural areas of the UK and Europe. As such, the pondscape of Norfolk provides a good representative study region, having similar land use, pond densities, and historic pond losses to many agricultural regions in Europe. The large number of extant ponds in Norfolk allows for a landscape-wide study of pond biodiversity and connectivity, while the abundance of ghost ponds provides a wide choice of study sites for examining the restoration potential of buried propagule banks.

Pond distribution across Norfolk closely matches the underlying geology of the county (*Figure 10*). Most ponds are found where the cover of glacial drift is more than 1m thick (Prince 1962), with pond density varying between soil regions. The ponds of Norfolk have a variety of origins, although the vast majority are man-made and found within agricultural fields. Prince (1962) recorded just over 27,000 ponds across Norfolk, based on Ordinance Survey maps at the time. He noted that the highest densities of small, permanent ponds are associated with the central districts, where the chalky boulder clay subsoil is thickest, while larger but less numerous ponds are associated with lighter soils, in particular the sandstone regions (*Figure 10*).

In many instances, the location and shape of a pond give an indication of its origin. Sand, gravel, and brick pits are common throughout Norfolk, and most of the medieval villages in the county would have had several such pits for providing building materials. While some of these pits were later filled-in or used as rubbish dumps, others were simply abandoned, filling with water to become ponds (Prince 1962). Such pits are particularly common along roadsides, where they provided both the materials to repair the road surface

(Rackham 1986), and afterwards could be used for watering horses. The origins of some of these ponds are recorded on Ordinance Survey maps, where either the name of the pond or the road running adjacent to it, may describe the pond's origin; "chalk pit", "clay pit pond", and "brick kiln lane" are all examples seen in north Norfolk.

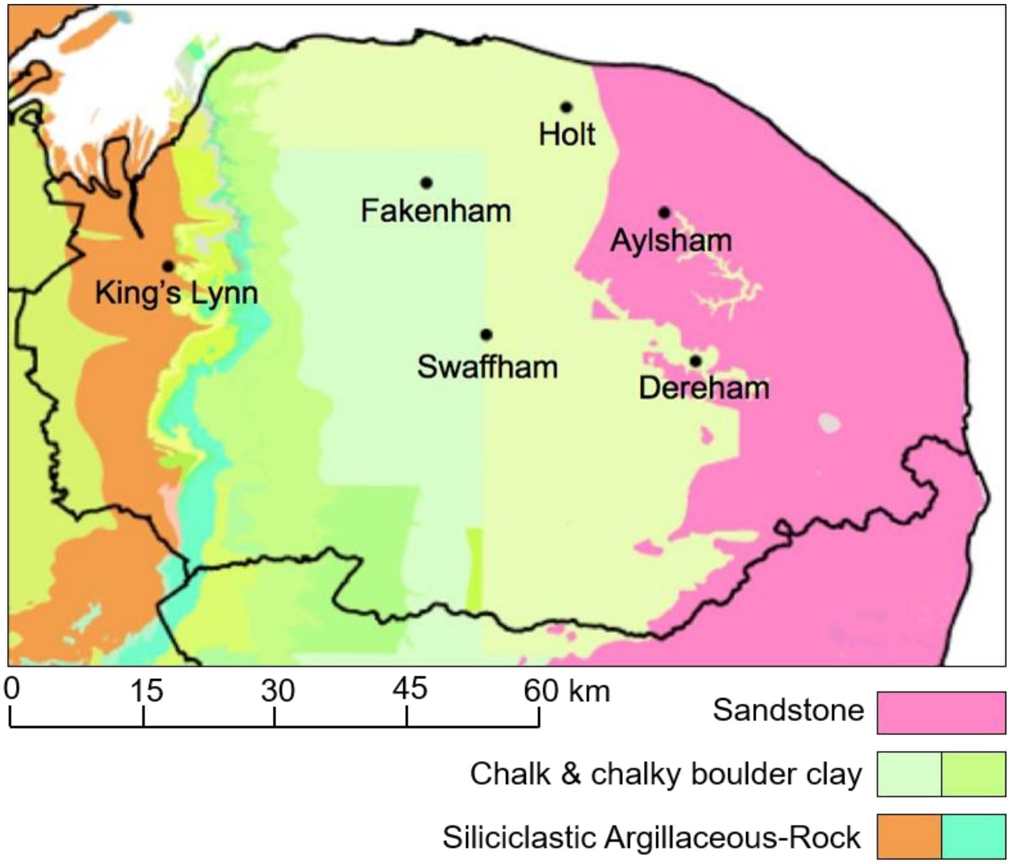


Figure 10 – Geological map of Norfolk.

Other common origins for ponds across Norfolk include fish ponds from the middle ages, moats, decoy ponds for duck shooting, and crater ponds from the Second World War (Rackham 1986). A smaller number of ponds, particularly those found in uncultivated land or woodland, likely have natural origins. In the south of Norfolk, several areas of pingo ponds (formed by glaciers during the last ice age), can be found, while in chalky regions sinkholes may provide another natural source of ponds (Rackham 1986). Although a diverse array of pond types can be found across Norfolk, the greatest densities of ponds are associated with the chalky boulder clay central region, where the majority of ponds are located in arable fields, often far away from roads or farm buildings. These ponds were excavated for ‘marling’ – the practice of combining the subsoil and surface soil to correct soil acidity and improve soil texture (Prince 1962; Rackham 1986). This practice was common across Norfolk up until the First World War, and resulted in the creation of numerous small pits, sometimes several per field, across the clay rich central region of the county (Prince 1962). Once the marl had been extracted, the vast majority of these pits were abandoned, naturally filling with water to form the numerous small agricultural ponds seen today. These marl pit ponds have a characteristic shape (*Figure 11*), with a gentle slope at one end where carts full of marl would be hauled over, and a steep rounded edge at the other, where the marl was being cut (Prince 1962; Boothby 1995; Jeffery 2008).

Marl pit ponds are common in several other areas of the UK. In Cheshire, the extraction of marl is purported to be the most common origin for ponds, with 41,000 ponds on the 1870s OS maps being attributed to marling (Boothby 1995). Marl pits are also common in Northamptonshire and Cambridgeshire (Upex 2004), and in West Sussex (Jeffery 2008).

Figure 11 – Marl pit ponds. Many of the ponds of central Norfolk originated as marl pits, dug to extract the soft white marl (pictured left), used for improving surface soil texture and acidity. Once the marl had been collected, abandoned pits would fill with water, creating marl pit ponds (pictured right). This photograph is of a Suffolk marl pit pond c.1914 (salthousehistory.co.uk).

Because the majority of Norfolk’s agricultural ponds originated as marl pits, they are reasonably uniform in terms of substrate, shape / depth, water chemistry, and surrounding land use (agricultural, usually with a small buffer zone around the pond). Having very large numbers of comparable study sites, all with similar origins and physio-chemical properties, is another feature that makes the pondscape of Norfolk an ideal study region.

2.2 Study site selection

Three scales of study region are examined in this thesis:

Regional scale – This covers the entire county of Norfolk (*Figure 13*). The regional scale study addresses the research hypothesis of Chapter 3, exploring patterns in pond loss and pondscape fragmentation at a geographical scale rarely studied.

Landscape scale – This covers nine discrete 'pond density regions' (blue regions in *Figure 13*), each containing a different density of ponds, ranging between 4 - 30 ponds/km^2. The landscape scale study addresses the research hypothesis of *Chapter 4,* using a space-for-time approach to examine how historic pond loss and changes in pond density may influence the diversity and occurrence of aquatic macrophytes, Coleoptera, and amphibians. In total, 121 ponds across the nine pond density regions were surveyed between May – June 2013. Initially, it was intended that pond density regions should be at least 2km apart. However, problems arose with land access permissions, resulting in some pond density regions being closer together or even overlapping (Godwick and Tittleshall).

Local scale – This covers three ghost and three restored ponds (locations marked by black triangles in *Figure 13*), which were excavated between September – October 2013. The local scale study addresses the research hypothesis of *Chapters 5 & 6,* examining the viability of the buried propagule banks of ghost and restored ponds, and the relative importance of the propagule bank vs. dispersal mechanisms in pond colonisation. The six study ponds were monitored between September 2013 – June 2015. Paired study sites (one ghost and one restored pond), are located on each of the farms in Guestwick, Stody, and Westfield (*Table 3*). Study sites were selected based on landowner responses to an advertisement, calling for volunteers who owned land containing both a ghost pond and an overgrown pond in need of restoration, who would be willing to have these sites excavated.

Table 3 – Study site names – ghost and restored ponds

	Guestwick	Stody	Westfield
Ghost pond	GP_{50}	GP_{45}	GP_{150}
Restored pond	GURE	STRE	WERE

*Sub-script number for ghost ponds refers to the approx. number of years ago that they were in-filled.

The six study ponds in the local scale study were monitored in detail, using four complementary approaches (*Figure 12*):

i) Field study monitoring macrophyte and Coleoptera colonisation of the three resurrected ghost ponds, and three restored ponds, between September 2013 – June 2015.

ii) Field study monitoring mesocosm experiments set up next to the study ponds, comparing macrophyte colonisation of treatments (4x4) open and closed to dispersal, and containing either sterile or historic pond sediments.

iii) Germination trials conducted in outdoor sealed microcosms (6x6), to confirm the role of the historic seed bank in recolonisation of the study ponds and associated mesocosms.

iv) Seed viability testing (tetrazolium chloride staining), of propagules extracted from sediments of the three ghost ponds, to examine whether germination success in the microcosms and mesocosms represents the viable portion of the historic seed bank, or whether germination success may be limited by environmental factors.

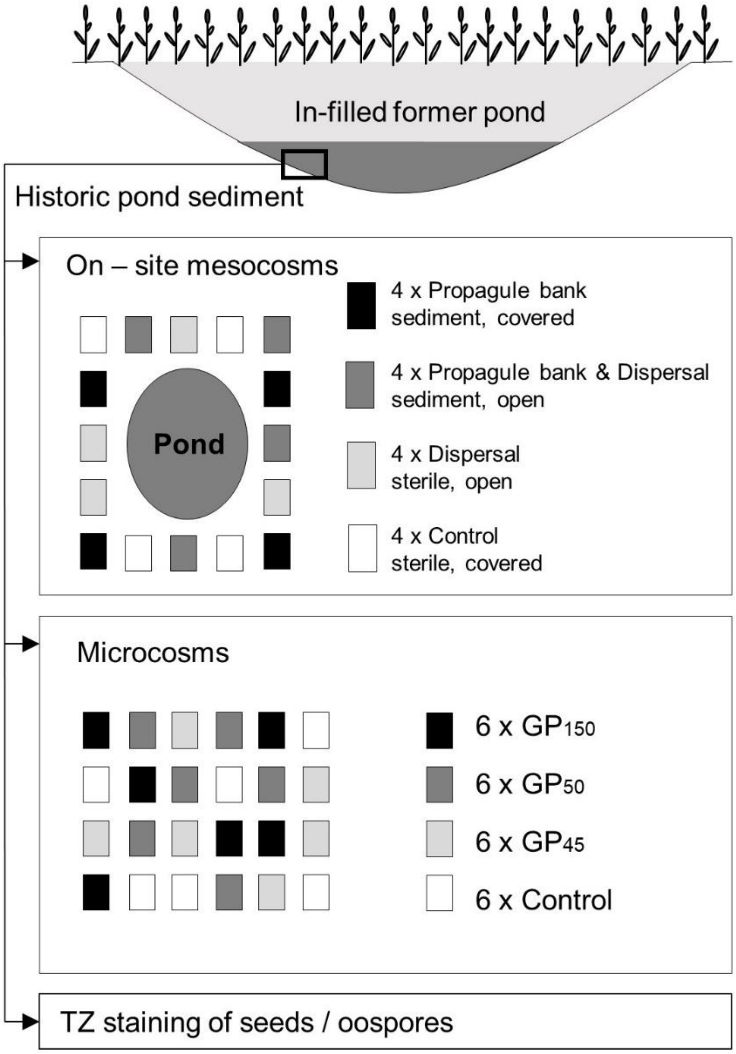


Figure 12 – Study design and experimental treatments for investigating ghost pond seed bank viability. From (Alderton *et al.* 2017). Historic sediment from the three ghost ponds (GP_{45}, GP_{50} and GP_{150}) provided the aquatic propagule material for three different experimental treatments; on-site mesocosms, sealed microcosms, and viability testing using tetrazolium chloride (TZ) stain.

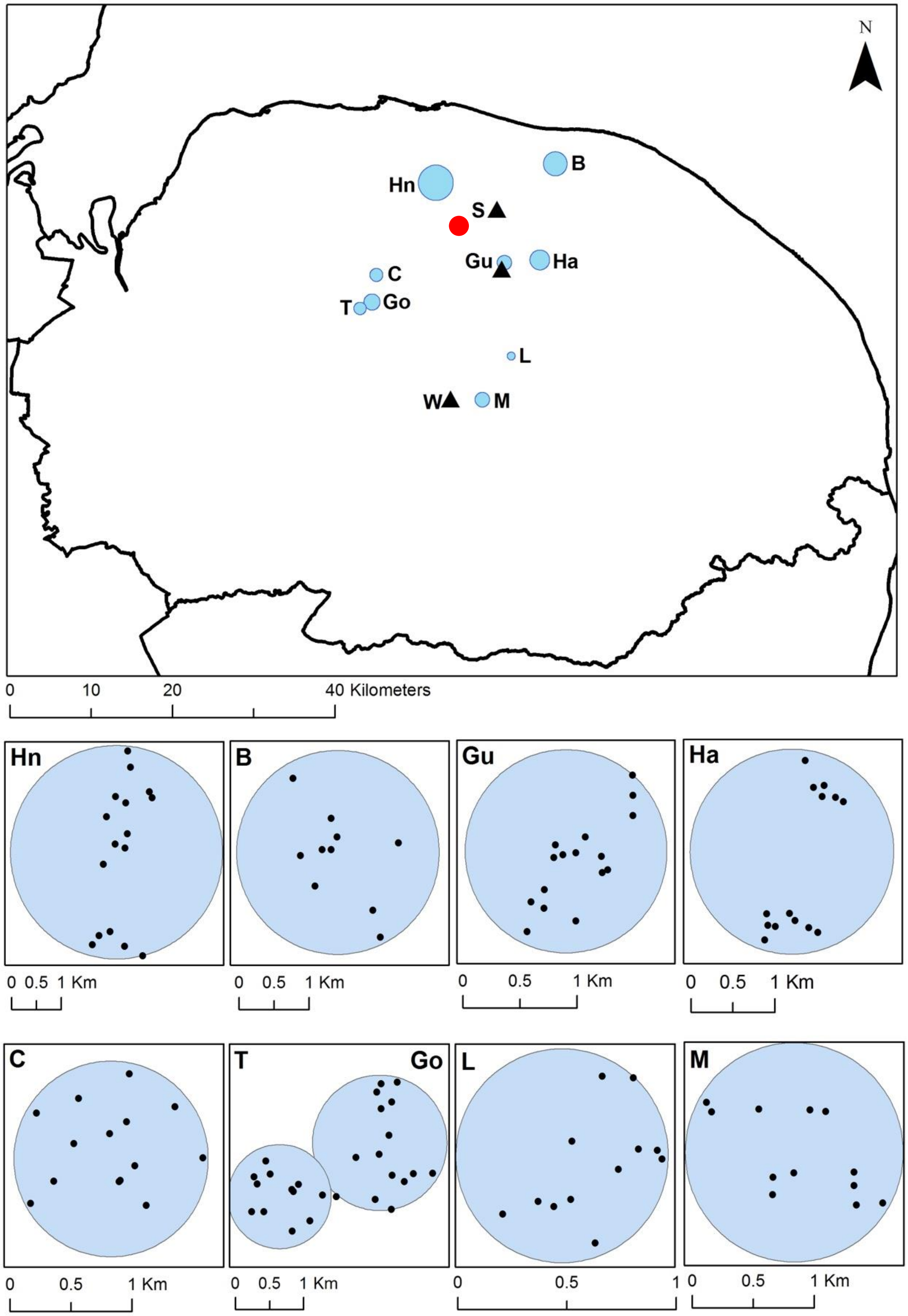


Figure 13- Location map showing the regional (whole county), landscape (blue circles), and local (black triangles), scale study regions. The red circle marks the location of the ground-truthing study (Chapter 3). Landscape scale study regions are centred around the villages of Hindringham (**Hn**), Bodham (**B**), Guestwick (**Gu**), Haydon (**Ha**), Colkirk (**C**), Tittleshall (**T**), Godwick (**Go**), Lyng (**L**), and Mattishall (**M**). Local scale study regions are located in the villages of Stody (**S**), Guestwick (**Gu**), and Westfield (**W**).

Chapter 3 Changes in an agricultural 'pondscape' – a history of pond loss and pondscape fragmentation.

3.1 Introduction

Agricultural pond loss is part of a wider global pattern of wetland loss due to agricultural intensification. It has been frequently stated that the world has lost 50% of its wetlands since 1900 AD, with agricultural land use identified as a major cause (Davidson 2014; Gibson *et al.* 2015). Although the origins of this "50%" figure remain obscure (Zedler & Kercher 2005; Davidson 2014), recent studies of global (Dixon *et al.* 2016), regional (Serran & Creed 2016), and local (Heath & Whitehead 1992) scale wetland losses suggest that it is broadly correct.

Global wetland loss is difficult to quantify due to inconsistent data availability, in particular poor historic records of wetland extent in Africa and Asia (Dixon *et al.* 2016). Additionally, the omission of small wetlands from most wetland indices (Serran & Creed 2016), may lead to an underestimation of the extent of wetland loss. Using a meta-analysis approach, Davidson (2014) reports average global wetland losses of between 54 – 57% since 1700, with the rate of loss being 3.7 times faster since 1900. The extent and rate of wetland loss was found to vary between regions, slowing in North America and Europe since the 1980s, but remaining high in the neo-tropics and Asia. Davidson reports that overall long-term wetland losses since 1700 have been highest in Europe (56.3% wetlands lost, *n=26*) and North America (56%, *n=7*), with smaller losses having occurred in Asia (45.1%, *n=7*), Africa (43%, *n=3*), and Oceania (44.3%, *n=6*). Using a different approach, Dixon *et al*. (2016) examined time-series data on global wetland extents, creating a 'WET' index based upon the sub-region in which a wetland was located, and the class of wetland (marine, inland, and human-made). The index is weighted to account for inconsistent data. The results from this study suggest a 31% loss of wetlands globally between 1970 and 2008, with the greatest decline occurring in Europe (50%), and least in Oceania (17%).

Accurate records of current and historic wetland extent are predominantly limited to Europe and North America, and as such most of the available literature focuses on these regions. In the United States, Hefner & Brown (1984) used random sampling of aerial photographs from the 1950s and 1970s to assess changes in wetland extent. Over an area of ~14,500 square miles, they found that of 215 million hectares of wetland recorded in the 1950s, only 99 million hectares remained by the 1970s. Nearly all of the palustrine wetland losses could be attributed to agricultural development (Hefner & Brown 1984).

Studies from both Europe and the US suggest that ponds are particularly threatened by agricultural intensification, as their small size and lack of statutory protection make these habitats easy to fill-in, compared to larger natural wetlands (Gibbs 2000; Wood *et al.* 2003; Serran & Creed 2016). In the Prairie Pothole Region of North America, an estimated 70% wetlands have been lost or degraded, with 95% of these losses having occurred as a result of agricultural development (Dahl 2014). Serran & Creed (2015) combined aerial photographs and a light detection and ranging (LiDAR) digital elevation model, to better capture changes in ponds and small wetlands (<0.04 ha), within the Prairie Pothole Region. This approach suggested a preferential loss of small wetlands, which had been overlooked by previous lower resolution map-based studies. In Europe, Agger & Brandt (1988) reported on a preferential loss of small, wet ‘biotopes’ (small uncultivated areas), from the agricultural landscape of Denmark, compared to larger waterbodies or terrestrial biotopes such as hedgerows and thickets. They suggest this could reflect both the preferential in-filling of small ponds, combined with the abandonment and subsequent succession of remaining ponds, into dry thickets (which showed a slight increase in number). Similarly, work by Curado *et al.* (2011), found that across an agricultural region of northern France, small man-made ponds were more frequently lost from the landscape than larger, semi-natural ponds.

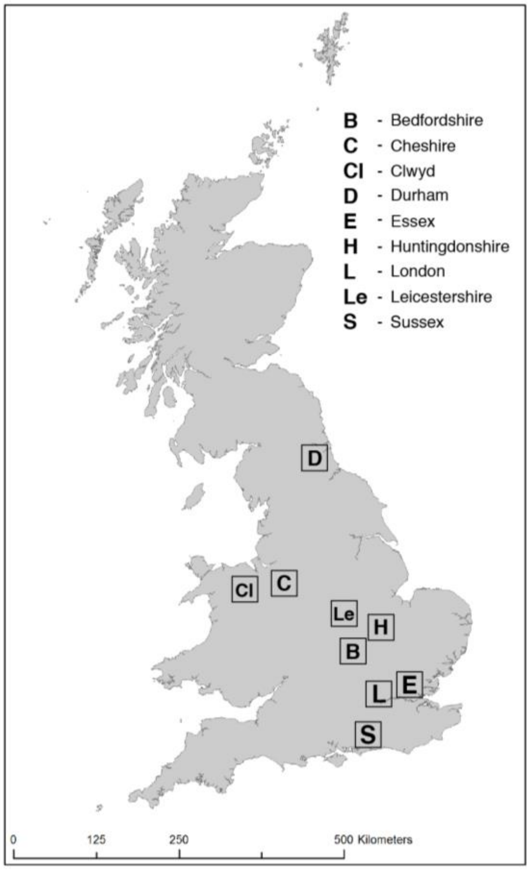
Studies of pond loss have predominantly been conducted in Europe, with an emphasis on agricultural landscape change since the Second World War. Farming practices have become increasingly more intensive over the post-war period, resulting in dramatic reductions in landscape heterogeneity (Robinson & Sutherland 2002; Benton *et al.* 2003). The widespread loss of non-cropped areas, including hedgerows, field margins and ponds, has been reported across Europe, and pond loss in particular has been the subject of a number of detailed studies from the UK.

Across England and Wales, it is estimated that pond numbers decreased by around three-quarters during the 20th Century, from an estimated 800,000 ponds in the late 19th century, to around 200,000 by the 1980s (Rackham 1986; Biggs *et al.* 2005; Williams *et al.* 2007). In Essex, an estimated 55% of ponds recorded in 1870 had disappeared by 1960, reducing the average pond density from 3 ponds / km^2 to 1.5 ponds / km^2. The greatest loss of ponds occurred between 1920 and 1960 (Heath & Whitehead 1992). Similarly, in Cheshire, 61% of ponds recorded *ca.* 1870 had disappeared by the early 1990s, reducing the average pond density from 17.8 ponds / km^2 to 3.3 ponds / km^2. In Ireland, map-based analysis of pond numbers revealed the mean number of ponds / km^2 to have decreased by 51%, from 0.87 ponds / km^2 in 1887, to 0.4 ponds / km^2 in 2005 (Reid *et al.* 2014). Other regional studies of pond loss in the UK are outlined in Wood *et al.* (2003) and Hassall (2014), and have reported pond losses of 21% - 90% between the early 1900s and 1990s (*Figure 14*). The majority of these pond losses are attributed to agricultural intensification, although in some areas (particularly London), urban development has played a significant role in pond loss. Having said this, garden ponds (particularly in enclosed urban gardens), are poorly recorded, but may represent a considerable resource; Hassall (2014) suggests that garden ponds in the UK may number between 2.5 – 4.5 million, although little is known about how the number of garden ponds has changed over time.

Few studies of UK pond numbers have been conducted since the 1990s, although the 2007 Ponds Report (Williams *et al.* 2007) suggested that between 1998 and 2007, pond numbers in Great Britain in fact increased by 12.5% (based on a random sample of 591 1km x 1km grid squares).

Similar levels of pond loss have been reported from other agricultural regions across Europe. In Denmark, an average of 69% agricultural ponds were lost between the mid-1880s and late 1970s, although the preferential loss of smaller ponds means that overall mean pond area has been reduced by only 12% (Agger & Brandt 1988). As in the UK, the extent of pond loss in Denmark varies between regions; Møller & Rørdam (1985) report levels of pond loss of 16% and 41% for two different study regions between 1950 - 1985. In the Pas-de-Calais region of north-western France, 57% of ponds recorded in the 1970s had disappeared by 2006 (Curado *et al.* 2011). These substantial pond losses have significant implications for landscape scale biodiversity.

In some cases, agricultural pond loss disproportionately affects certain pond types. In a Mediterranean region of southwest Portugal, 56% temporary ponds were lost from farmland between 1991 – 2009 (Ferreira & Beja 2013). While the majority of these ponds were filled-in during agricultural intensification (68.7%), some were deepened to create permanent farm ponds (16.8%), which had lower amphibian diversity, and were less frequently used for breeding than semi-natural temporary ponds.



Bedfordshire	-82%	(1910 – 1981)
Cheshire	-61%	(1870 – 1993)
Clwyd	-32%	(1840 – 1990)
Durham	-41%	(1840 – 1990)
Essex	-68%	(1840 – 1990)
Huntingdonshire	-56%	(1890 – 1980)
London	-90%	(1870 – 1984)
Leicestershire	-60%	(1840 – 1990)
Sussex	-21%	(1977 – 1996)

Figure 14 – Regional pond loss in the UK. From data in Wood et al. (2003) and Hassall (2014).

Most studies of pond loss have focused on changes in the total number of ponds across a region, with the concurrent change in pond density receiving less attention. However, the destruction of pond habitats due to agricultural (or other) land use change has three structural components; the straightforward loss of habitat, increased fragmentation of remaining habitat, and reduced quality of the remaining habitat (Wood *et al.* 2003). For many species, in particular dispersal limited taxa such as amphibians, the terrestrial matrix of farmland surrounding a pond is an important component of the 'pondscape' (Boothby

1997). Changes in the connectivity and distances between ponds may have as significant an impact upon population persistence of dispersal limited taxa as actual pond loss (Gibbs 2000; Curado *et al.* 2011), influencing rates of species dispersal, extinction and population exchange (Shaffer 1981; Taylor *et al.* 1993). The impacts of habitat fragmentation & dispersal limitation upon pond biodiversity are covered in more detail in *Chapter 4*.

While pond loss has been widely examined across the UK (Heath & Whitehead 1992; Boothby & Hull 1997; Wood *et al.* 2003), and to a lesser extent Europe (Møller & Rørdam 1985; Curado *et al.* 2011; Casas *et al.* 2012), and the USA (Serran & Creed 2016), the implications for structural connectivity of the pondscape have received less attention (Boothby 1997). To address this important component of pond loss, this chapter takes a novel approach in mapping both changes in pond numbers and pond density. Ponds are additionally categorised by land-use 'type' (such as roadside ponds, woodland ponds, and hedgerow ponds), following the approach of Heath & Whitehead (1992). Surrounding land use can strongly influence the ecological value of a pond, potentially effecting both organism dispersal (functional connectivity), and pond water quality (Lehtinen, Galatowitsch & Tester 1999; Downs & Racey 2006). Considering changes in pond type, alongside changes in pond numbers, density, and configuration, is therefore important for better understanding pondscape change.

Longitudinal studies of pondscape change, in which pond numbers and (sometimes) pond biodiversity are monitored in real-time over the course of many years, are rare. One exception is a study of pond loss and amphibian diversity in northern France, in which ponds were surveyed in 1975 and again in 2006 (Curado *et al.* 2011). Given the logistic difficulties of such long running studies, most research on pond loss is based on analysis of historic maps or aerial photographs (Heath & Whitehead 1992; Boothby & Hull 1997; Wood *et al.* 2003; Serran & Creed 2016). Detailed historic maps exist for most regions of Europe and the US, providing a means of examining landscape changes across centennial timescales. However, the accuracy of historic and modern maps for recording small features like ponds is often unknown, and may vary between regions and over time. As such, most map-based studies of pondscape change include some validation of the methodology, comparing maps to either aerial photographs (Serran & Creed 2016), or a ground-truthed sample region (Reid *et al.* 2014).

This chapter examines changes in pond numbers, pond density, and pond types across the county of Norfolk, eastern England. The study region is predominantly agricultural, with large numbers of man-made ponds, similar to many other agricultural areas around the globe (Boothby & Hull 1997; Beja & Alcazar 2003; Edvardsen & Økland 2006; Céréghino *et al.* 2008a; Dahl 2014). The abundance of ponds with similar origins, geology, and surrounding land use (see *Chapter 2* for further details), make Norfolk an ideal study region. Pondscape changes are assessed using historic and modern Ordinance Survey (OS) maps from the early 1900s, 1950s, and present day. A critical evaluation of this map-based approach is conducted, using a ground-truthed sample area to assess the accuracy with which ponds are recorded on both the historic and modern OS maps.

3.2 Chapter hypotheses

H1) Assessment of pondscape changes based on historic and modern OS maps provides a reliable and accurate means of assessing pond loss, with a high proportion of both existing and ghost ponds marked on OS maps visible in the field.

H2) Pond loss between the 1950s – present day was greater than that during the previous time period (1900s – 1950s), reflecting changing agricultural practice and increased agricultural intensification.

H3) High levels of pond loss between the 1950s – present day have resulted in both fewer ponds, and a reduction in mean pond density, at the landscape scale.

H4) Historic changes in pond numbers and pond density across the studied pondscape (Norfolk, UK), are comparable to those reported for other regions of the UK and Europe.

H5) Pond loss has disproportionately affected certain types of agricultural pond, in particular those associated with hedgerows. This reflects the widespread removal of hedgerows during agricultural intensification, and subsequent in-filling of ponds which had been associated with these habitats.

3.3 Methodology

For this study, a desk-based analysis of pond numbers, pond locations, and pond density was conducted in ArcGIS 10.2, using Ordnance Survey (OS) maps. OS map tiles covering the entire county of Norfolk, eastern England, were downloaded from Edina Digimap (digimap.edina.ac.uk). Norfolk was chosen as a suitable study region as the county contains a large number of ponds of similar origin and surrounding land use (see *Chapter 2*), with a comparable pond density to many other agricultural areas around the globe. Additionally, OS map coverage of the county is fairly complete, with only a few gaps in the historic records.

OS map editions used were the modern (2013 – 2014), 3rd Revision (1953-1957), and 1st Edition (1899-1904) OS county series maps (1: 10,560), hereafter referred to as the 2014, 1955 and 1900 maps. These map editions were selected as they span a comparable time period to other studies of pond loss (Rackham 1986; Heath & Whitehead 1992; Boothby & Hull 1997; Wood *et al.* 2003; Curado *et al.* 2011; Hassall 2014), and cover a key tipping-point in agricultural land use, namely the widespread intensification of food production after World War 2. They also provided the most complete coverage of the county. Pond density analysis was conducted using the Spatial Analyst toolbox in ArcGIS.

3.3.1 Ground truthing the Ordinance Survey maps

To assess the validity of the map-based approach, a ground truthing exercise covering 10 x 1 km^2 grid-squares was conducted in February 2013. This allowed for an assessment of the accuracy with which the historic and modern OS maps record small ponds across different land-use types. The area selected for ground-truthing (*Figure 16*), surrounds the villages of Briston and Melton Constable, north Norfolk. This area was selected for ground-truthing because a) the landowners were known to us, and access was easy to arrange; b) the area was considered highly representative (in terms of land use), of agricultural areas across both Norfolk and the UK (Heath & Whitehead 1992; Boothby & Hull 1997; Wood *et al.* 2003); c) the area contained a wide range of pond types, which allowed for a better assessment of the reliability of OS pond mapping across different land uses. The ground-truthing exercise identified both existing and ghost ponds, providing an indication of the accuracy of the historic OS maps, as well as the modern 2014 edition. Ground-truthing was also key in determining the number of ghost ponds which remained visible on the ground after decades (and sometimes centuries), of burial. This was an important consideration for both the selection of study sites, and the wider suitability of the approach for identifying ghost ponds for 'resurrection'; sites which could still be identified on the ground would be much easier to excavate accurately than sites which could only be identified on a map.

3.3.2 Assessing changes in pond numbers

To assess changes in pond numbers and pond density, ponds were initially counted manually across three 'local scale' study regions (*Figure 13*). These were centred around the paired ghost and restored study ponds (*Chapters 5 & 6*) in Guestwick, Stody and Westfield. A 3 km search radius was drawn around each pair of ponds (giving an area of 28.27 km^2), and all of the ponds within these areas were counted on the 2014, 1955 and 1900 OS maps. A 3 km search radius was used as this provided three discrete, non-overlapping study areas, which contained large numbers of ponds across all three time periods.

While the focus of this chapter is on changes in the number and density of farmland ponds (which make up the clear majority of standing waters in Norfolk), all standing water bodies marked on the OS maps were counted, and classified as shown in *Table 4*. Water body classifications are based upon those used by Heath & Whitehead (1992), but have been adapted to better describe the range of ponds present in Norfolk.

The chosen classifications reflect potentially ecologically meaningful differences between ponds, for instance the level of connectivity between the pond and terrestrial habitat matrix (higher for a woodland, rough ground or hedgerow pond, lower for an open field pond), and proximity to direct sources of pollution (roadside or farmyard pond). These factors will be covered in more detail in the discussion. In addition to the three 'local scale' regions analysed in detail in this chapter, pond numbers at the same three time periods (1st Edition, 3rd Revision and modern OS maps), were also counted within a 1km search radius (3.14km^2 area), around the 'landscape scale' study regions used in *Chapter 4* (*Figure 13*).

The smaller search radius was necessary to keep the ‘landscape scale’ regions from overlapping.

Table 4 – Pond type classifications used in GIS analysis.

Water body type	Definition
Open field pond	Pond located in the middle of an agricultural field.
Hedgerow pond	Pond adjoining a hedgerow.
Roadside pond	Pond adjoining a road.
Woodland pond	Pond located in woodland.
Farmyard pond	Pond adjacent to a farm building / farmyard.
Hydrologically connected pond	Pond marked as being hydrologically connected to a ditch, stream, or river (blue line on modern OS map, black line on historic OS maps)
Rough ground pond	Pond located on uncultivated ground, other than a woodland ie. scrub, heathland or marsh areas.
Ornamental pond	Pond which is likely to be heavily modified for ornamental purposes, including ponds located on golf courses, estate gardens, and village green ponds.
Moat	Water body marked as ‘moat’ on the OS map.
Gravel pit	Pond marked as a flooded gravel pit / sand working.
Farm reservoir	Water body marked as “Res.” on OS map, or small, square waterbody located in farmland.
Fishery pond*	Pond marked as fisheries on the modern OS map.
Lake**	Large body of water, greater than 100m across.

** Fisheries were only marked on the modern OS map. Fishery ponds were excluded from comparisons of pond numbers between time periods.*

*** See definition of a pond in Chapter 1.*

Based on the initial assessment of pond numbers and density across the three ‘local scale’ study regions, subsequent pondscape analysis for the whole of Norfolk was conducted on the 1955 and 2014 county series maps. These map editions were selected because they capture the period of rapid post-war (~1950 onwards) agricultural intensification, with the local scale study suggesting that pond loss between the 1955 - 2014 was far greater than pond loss between 1900 - 1955 (*Figure 19*). The 1900 (1st Edition) OS map was also excluded from regional scale analysis due to the difficulty in accurately identifying small ponds, in particular those on hedgerows, on these earlier map editions (*Figure 15*).

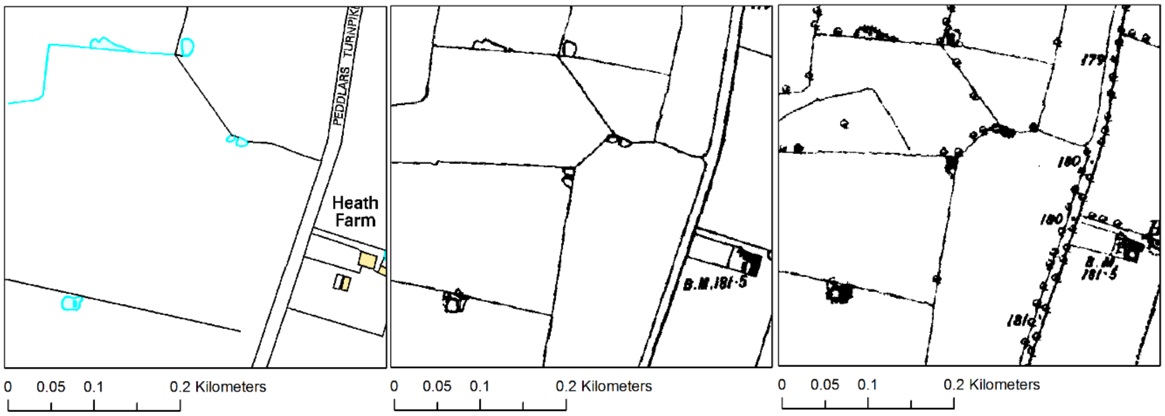


Figure 15 – Comparison of map drawing quality on the OS county series. From left to right: Current Edition (2013 – 2014), 3rd Revision (1953 – 1957), and 1st Edition (1899 – 1904).

Ponds were most easily identified on the 2014 colour OS map, and so a shapefile of modern pond locations was created first. This shapefile was then super-imposed over the 1955 map, allowing for rapid identification of ponds present during both time periods. New point features were then added to the shapefile to mark ponds present on the 1955 map, but which were not drawn on the 2014 map. In cases where a feature was unclear, for instance distinguishing between ponds and dry pits on the black and white 1955 map, switching between maps from the three different time periods, and cross-referencing with aerial photographs, could usually resolve this issue.

3.3.3 Assessing changes in pond density

Pond density analysis was conducted using the ‘point density’ tool in the Spatial Analyst toolbox of ArcGIS. This calculates the magnitude-per-unit-area (set to km^2) of point features (ponds) within a given search radius (Esri 2016). In this chapter, pond density is measured at the 1 km^2 level (search radius 564.19 m), consistent with the majority of the published literature on pond loss and pondscape change (Heath & Whitehead 1992; Boothby & Hull 1997; Wood *et al.* 2003; Biggs *et al.* 2005).

Pond density is presented in this chapter in two different ways; from the ‘landscape perspective’ and from the ‘pond perspective’. Pond density is most commonly presented from the ‘landscape perspective’, i.e. the mean number of ponds within a given area of the landscape. Because some areas will contain no ponds, pond density can have a range from 0 upwards. In this study, pond density from the ‘landscape perspective’ represents the mean number of ponds / km^2, calculated for every 100 m x 100 m area of Norfolk. This was calculated by creating a pond density raster layer (cell size 100 x 100 m), across the entire study area, and extracting the pond density value of each raster cell. This measure of pond density is used to compare the findings of this study to the existing literature on pond loss (Heath & Whitehead 1992; Boothby 1995; Wood *et al.* 2003), answering *Hypothesis 4* (H4) of this chapter.

Pond density can alternatively be measured from the ‘pond perspective’, i.e. the number of ponds within a given radius of any other pond. This measure of pond density is perhaps more biologically relevant for understanding the dispersal of pond organisms. Pond density from the ‘pond perspective’ was calculated using the same pond density raster layer as the ‘landscape perspective’, from which raster cell values were then extracted for each pond. This measure of pond density is used to answer all other chapter hypothesis, and unless otherwise stated, all analysis is conducted using pond density from the ‘pond perspective’. As well as being more ecologically meaningful, density measured from the ‘pond perspective’ provided more reasonable sample sizes for statistical analysis (Curado *et al.* 2011).

Local scale changes in pond density (measured over three time points at three locations), were analysed using profile analysis conducted in SPSS. Profile analysis is a multivariate technique which can be used to test one dependent variable measured at different times, or several different dependent variables measured at the same time. The test is an extension of a repeated measures ANOVA, but avoids multiple comparisons where data are analyzed across more than two time points. Profile analysis is robust to both small sample sizes and violations of normality (Von Ende 2001; Tabachnick & Fidell 2007).

Regional scale changes in pond density (measured over two time points across one area), were analysed in R, using a t-test for comparing two means (Field, Miles & Field 2013). For comparison to the existing literature, regional scale changes in pond density were additionally presented from the ‘landscape perspective’, using the raster calculator tool in ArcGIS 10.2. Throughout this chapter (and entire thesis), statistical significance is assumed at the $p = 0.05$ level. Where p-values are lower than this ($p = 0.01$, $p < 0.001$), these values are quoted, but the threshold for significance was consistently $p = 0.05$.

Although the functional connectivity of a pondscape will differ between taxa (Beisner *et al.* 2006; De Bie *et al.* 2012), and between habitat patches (Joly *et al.* 2003), the simple measure of pond density / km^2 is useful in detecting general patterns and changes in pondscape structural connectivity. Pond density has been shown to be a significant predictor of both biodiversity (Bosiacka & Pienkowski 2012; Hassall 2014; Jeliazkov *et al.* 2014) and species occurrence & composition (Delettre & Morvan 2000; Joly *et al.* 2001; Scribner *et al.* 2001), and is an important component of the overall functional connectivity of the pondscape. Indeed, some authors have suggested that given the difficulty in trying to accurately model functional diversity in highly heterogeneous landscapes (With, Gardner & Turner 1997; Adriaensen *et al.* 2003), the proportion and density of habitat patches is nearly as important as more complex measures of connectivity, as it effectively determines the probable range of many other characteristics (Murphy & Lovett-Doust 2004). As such, this chapter focuses on changes in pondscape structural connectivity (pond density and inter-pond distances), with a more detailed analysis of how these factors influence pond biodiversity and community composition provided in *Chapter 4*.

3.3.4 Future potential – Adjusting analysis to incorporate spatial patterns and spatial autocorrelation in pondscape change.

At present, the methods presented in this chapter focus on overall changes in pond numbers and pond density, while the spatial patterns in pondscape change are only briefly considered. Given that pond loss is largely attributed to agricultural intensification, and that this tends to vary between farms, estates, and regions, it is likely that pondscape fragmentation will display strong spatial patterning. While this is explored to some degree in the maps depicting local (*Figure 23*), and regional (*Figure 29*) pond density change, it has not been analysed quantitatively, and the potential for spatial autocorrelation (see *section 4.3.7* for details), in pond density changes has not been accounted for in statistical analysis.

In order to address these issues, spatial autocorrelation (SAC) in pondscape change could be assessed using a global Moran's *I* test, which can be conducted within ArcGIS (Esri 2017). During the analysis of regional pond density change (*Figure 26, Figure 27 & Figure 29*), each modern-day pond marked on the 2014 OS map was assigned a 'density change' value, measuring the number of neighbouring ponds (within 1 km^2), either lost or gained since 1955. Using a suitable distance threshold (120 km, covering the furthest distance between study ponds), the Moran's *I* test could be used to detect whether there is SAC occurring in the pond density changes. It is hypothesised that positive SAC is likely to be present, with changes in pondscape density likely to be clustered in relation to land use. In addition, ArcGIS provides a number of tools for detecting clustering (in this case, among pond density change values), across a range of distances. Future analysis could be expanded to include such measures, providing more detail on the spatial scale(s) at which pondscape fragmentation has been most pronounced. This could be used in combination with known dispersal thresholds for key taxa, in order to better understand how fragmentation may influence the functional connectivity of the pondscape.

3.4 Results

3.4.1 Ground truthing study

Across the ten 1 km^2 grid squares surveyed during the ground truthing exercise, a total of 107 ponds were marked on the modern OS map. All of the marked ponds were identified in the field, in addition to 14 ponds which had been omitted from the OS map. The highest number of omitted ponds were found in woodland dominated grid square 5 (*Figure 17*). As such, the modern OS map seems to slightly underestimate the number of ponds, particularly those located in woodland.

Of 29 ghost ponds recorded on the OS maps (ponds which appeared on the 1955 map, but not on the 2014 map), 23 were identified in the field as visible depressions, or changes in crop / soil colour (*Figure 18*). The ghost pond marked in the centre of the field in grid square 3 was later used as one of the study sites in *Chapters 5 & 6*. Six ghost ponds, which were marked on the OS maps, could not be found in the field (‘Invisible’ ghost ponds). These sites may represent ponds which were filled-in particularly effectively, or which experienced heavier land management after being filled. Alternatively, they could represent error in the drawing or interpretation of the 1955 OS map.

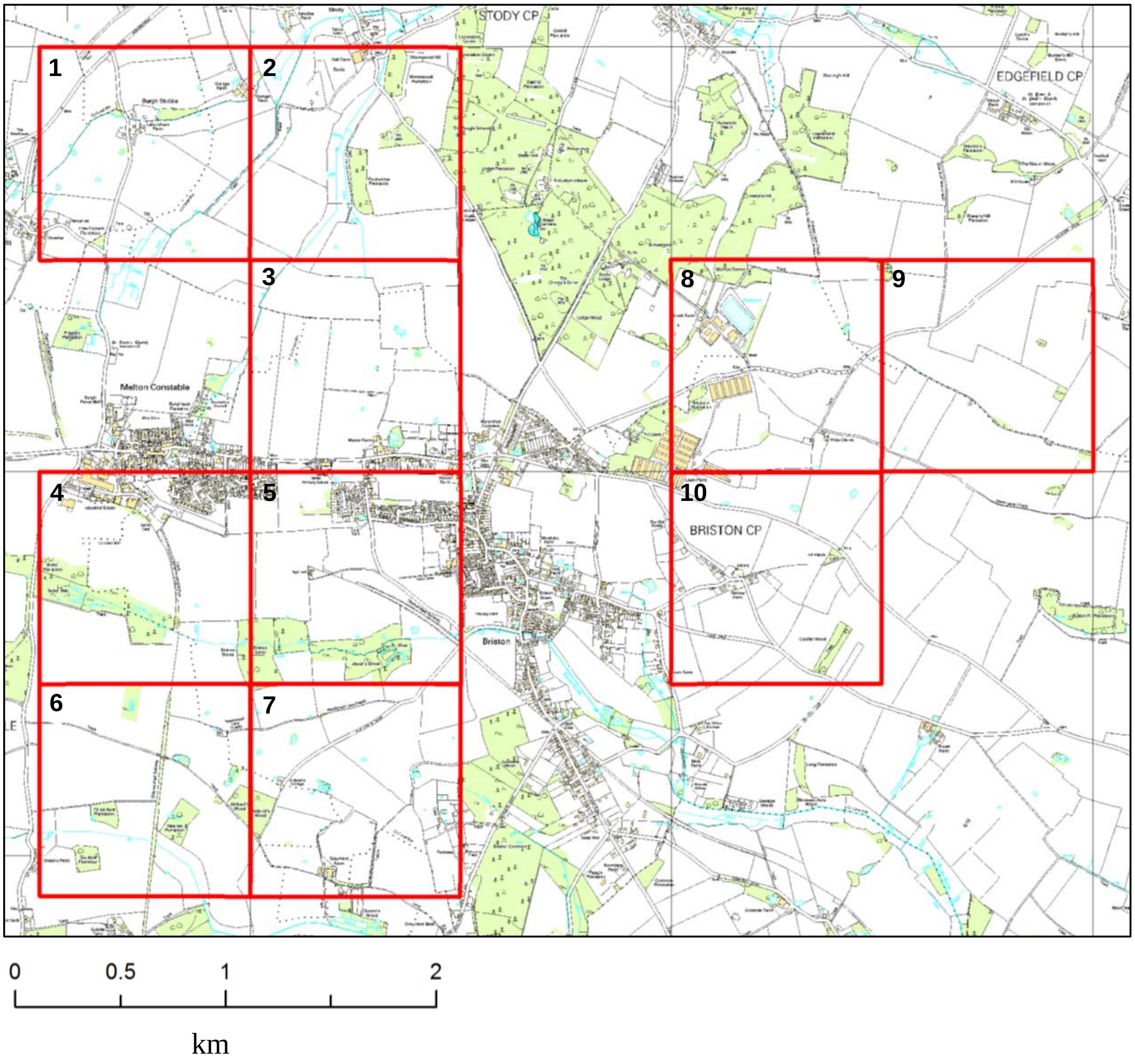


Figure 16 - Location of the 10 x 1 km^2 ground-truthing squares.

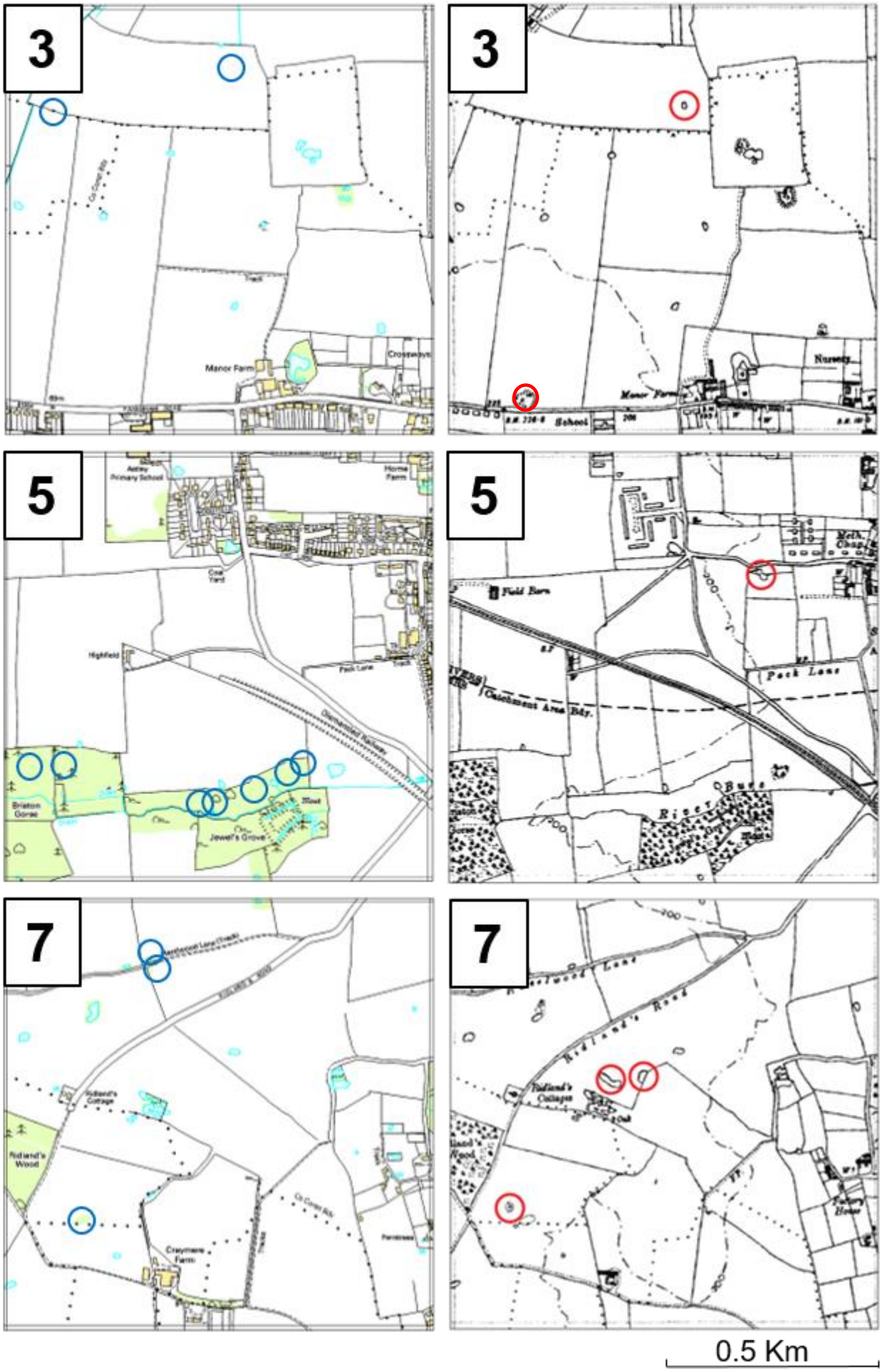


Figure 17 - Selected ground-truthing squares from the 2014 and 1955 OS maps. On the 2014 map squares (left), ***blue circles*** *mark existing ponds which had been missed off the 2014 map. On the 1955 map squares (right),* ***red circles*** *mark ghost ponds, filled-in between 1955 - 2014. These ghost ponds were still visible in the field.*

Table 5 – Ponds recorded on the 1955 and 2014 OS maps, and identified in the field during ground-truthing.

Grid square	Ponds recorded on 2014 map	Ponds identified in the field	Ponds omitted from 2014 map	Ghost ponds on 1955 map	Ghost ponds identified in the field	'Invisible' ghost ponds
1	11	11	0	2	2	0
2	7	8	1	1	0	1
3	17	19	2	2	2	0
4	2	2	0	5	5	0
5	17	24	7	1	0	1
6	15	16	1	8	6	2
7	25	28	3	4	3	1
8	3	3	0	1	1	0
9	6	6	0	3	2	1
10	4	4	0	2	2	0
Total	107	121	14	29	23	6

Figure 18 – Ghost ponds identified in the field during the ground truthing exercise. Numbers refer to the grid square in which the ghost pond is located.

3.4.2 Local scale changes in pond numbers

Local scale changes in pond numbers and pond density were measured across the three study regions in Westfield, Guestwick, and Stody (*Figure 13*). In all three regions, the greatest change in pond numbers occurred between 1955 – 2014, with pond losses of between 17.1 – 47.5%, compared to a relatively small increase in pond numbers of between 2.9% – 14% during the previous period (1900 – 1955) - *Table 6*. The Stody region showed the smallest change in pond numbers (217 ponds in 1900, 195 ponds in 2014), and historically had fewer ponds than either Guestwick or Westfield (444 and 452 ponds in 1900, respectively – *Figure 19*). Both Guestwick and Westfield experienced similar levels of pond loss between 1955 – 2014, with 45.1% and 47.5% ponds lost, respectively (*Table 6*). Despite a net gain of ponds between 1900 – 1955, pond losses still occurred during this time period, with old ponds being filled in while new ponds were created (*Table 6*).

Table 6 - Local scale changes in the total number of ponds, and ghost ponds (Red), between 1900–1955 and 1955–2014.

Westfield		Guestwick		Stody	
1900 - 1955	1955 - 2014	1900 - 1955	1955 - 2014	1900 - 1955	1955 - 2014
+2.9%	-47.5%	+14.0%	-45.1%	+7.8%	-17.1%
49	**240**	**32**	**243**	**24**	**123**

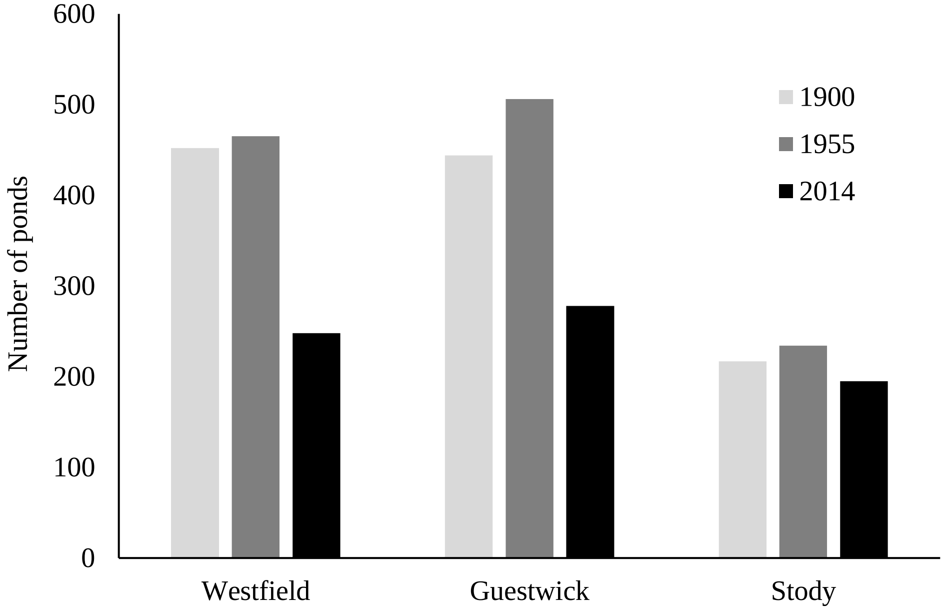


Figure 19 - Total number of ponds in 1900, 1955, and 2014, at the three local scale study areas.

Examining these local scale changes in pond numbers by pond type (*Table 7, Figure 20*), it is apparent that the greatest levels of pond loss occurred among hedgerow ponds, with losses of 52.2 – 64.8 % between 1955 – 2014. Other pond types also show a declining trend, with the exception of woodland ponds, which show an increase over time of between 55.6 – 142.9% across the three regions (*Table 7, Figure 20*). Across Westfield and Guestwick, hedgerow ponds were the most abundant pond type at all three time periods, followed by open field, and roadside ponds. In contrast, Stody had greater numbers of open field than hedgerow ponds in both 1900 and 2014, but greater numbers of hedgerow than open field ponds in 1955.

Table 7 - Local scale changes in pond numbers between 1900, 1955, and 2014.

	Westfield			Guestwick			Stody		
	1900	1955	2014	1900	1955	2014	1900	1955	2014
Hedgerow	277	295	133	250	267	94	73	115	55
Open field	74	84	64	112	153	85	81	63	71
Roadside	60	56	25	36	49	38	19	8	3
Woodland	3	9	14	4	14	34	17	23	53
Farmyard	19	11	6	17	18	21	5	5	6
Rough ground	12	5	0	15	2	0	15	17	0
Ornamental	3	0	0	10	3	3	4	1	2
Connected	4	5	2	0	0	3	3	2	4
Total	452	465	248	444	506	278	217	234	195

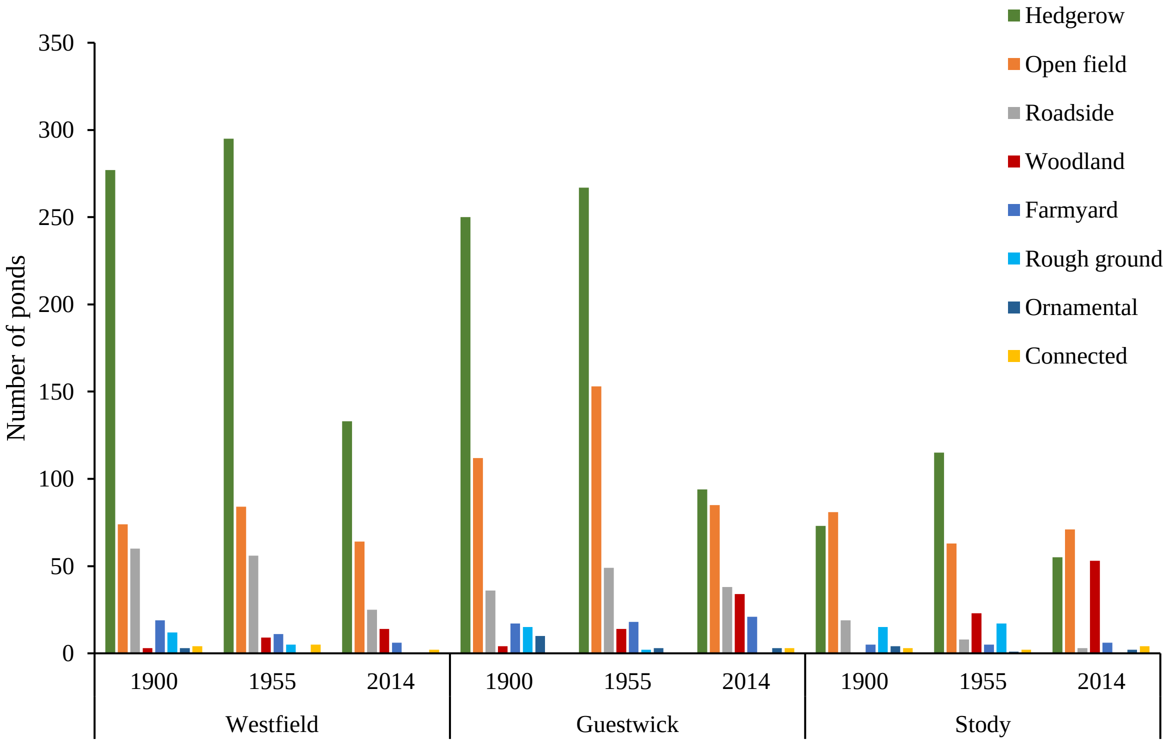


Figure 20 – Local scale changes in pond types between 1900, 1955, and 2014.

3.4.3 *Landscape scale changes in pond numbers*

Changes in pond numbers at the nine 'landscape scale' regions varied between locations. All locations except Bodham experienced pond loss between 1955 – 2014. Changes in pond numbers between 1900 – 1955 were more varied, with five locations (Hindringham, Bodham, Tittleshall, Heydon, Mattishall) experiencing pond loss, and four locations (Colkirk, Godwick, Guestwick, Lyng), experiencing pond gain. As with the three 'local scale' study regions, overall change in pond numbers was far greater between 1955 – 2014 than between 1900 – 1955.

Table 8 – Landscape scale changes in pond numbers between 1900, 1955 and 2014.

	Hindringham	Bodham	Colkirk	Tittleshall	Godwick	Guestwick	Heydon	Lyng	Mattishall	**Total**
1900	31	22	43	46	32	67	66	58	74	**439**
1955	27	18	45	44	35	77	56	63	72	**437**
2014	20	25	37	34	26	61	42	43	44	**332**

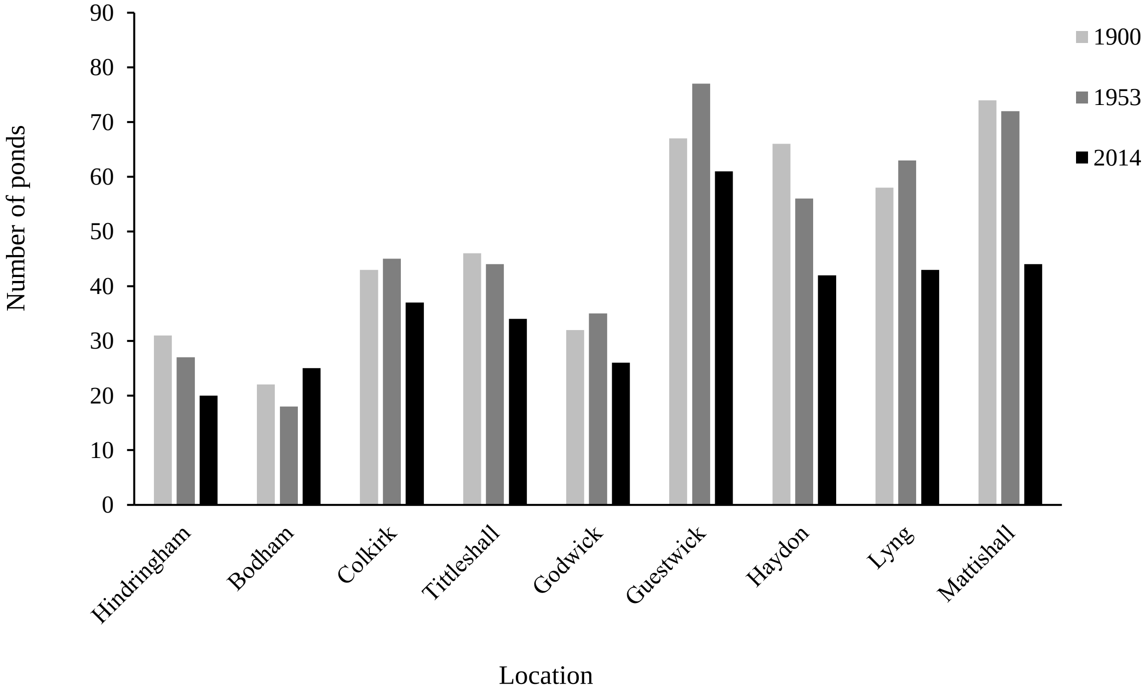


Figure 21 – Landscape scale changes in pond numbers between 1900, 1955 and 2014.

3.4.4 *Local scale changes in pond density*

Changes in pond density (from the pond perspective), across the three local scale study regions mirror the changes in pond numbers, with a significant effect of date (F(2,1456) = 7212.3, $p < 0.001$), location (F(2,728) = 136.3, $p < 0.001$), and date-location interaction (F(4,1456) = 567.9, $p < 0.001$). The greatest changes in pond density occurred between 1955 – 2014, in particular in Westfield and Guestwick, while smaller changes in pond density occurred in Stody (*Table 9* & *Figure 22*). Westfield showed the greatest change in maximum pond density, from 41 ponds / km^2 in 1955, down to 24 ponds / km^2 in 2014.

Table 9 – Local scale changes in pond density (ponds / km^2), between 1900, 1955, and 2014, from the 'pond perspective'.

		1900	1955	2014
Westfield	Min	1	2	1
	Max	39	41	24
	Mean	17.6	19.2	12.5
	Median	16	18	13
	Mode	14	18	15
	SD	6.6	6.5	4.9
Guestwick	Min	3	4	1
	Max	32	40	39
	Mean	16.7	20.7	13.7
	Median	17	20	12
	Mode	17	17	13
	SD	5.7	6.9	7.5
Stody	Min	1	1	1
	Max	28	33	27
	Mean	10.5	12.6	10.2
	Median	10	12	9
	Mode	9	14	8
	SD	5.5	6.2	5.6

Across all three local regions, median pond density increases between 1900 – 1955, before decreasing below 1900 levels by 2014 (*Table 9*). Maximum pond density in all three regions also increased slightly between 1900 – 1955, before decreasing by 2014.

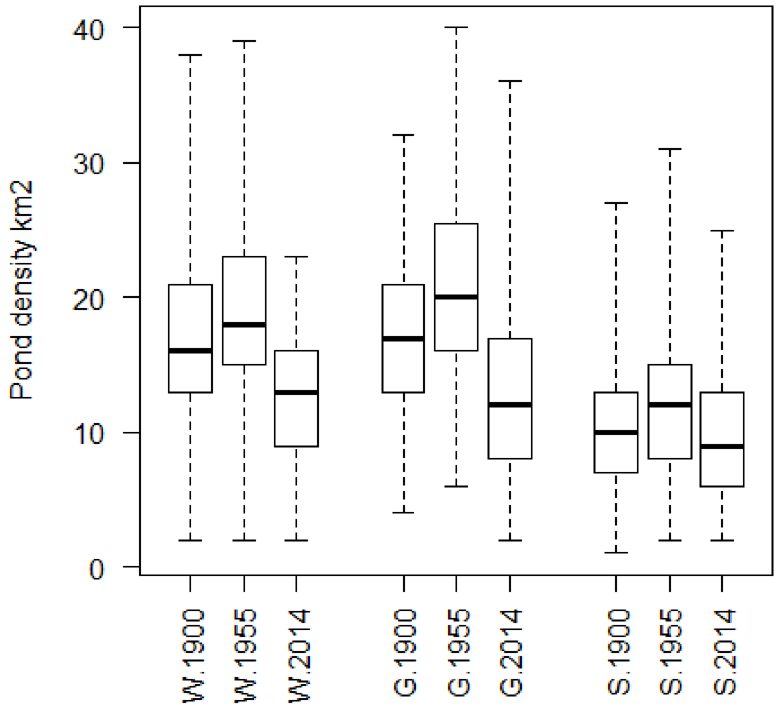


Figure 22 – Local scale changes in pond density / km^2, between 1900, 1955, and 2014, from the 'pond perspective'. W- Westfield, G- Guestwick, S- Stody. Boxes show the median, and upper and lower quartiles in pond density / km^2, while whiskers show the upper and lower range limit in pond density / km^2.

The impact of these changes in pond density for the connectivity of the pondscape is illustrated in *Figure 23*, which shows pond density / km^2 for the three local scale study regions. In Stody, the general pattern of connectivity across the pondscape changed little between 1900 – 2014, with a single patch of high pond density (21 – 30 ponds / km^2) being maintained across the three time points. The central 'corridor' of mid pond density declined from 11 – 20 ponds / km^2 in 1955, to 6 – 15 ponds / km^2 in 2014, but continued to support a higher density of ponds than the surrounding landscape. In contrast, the pondscape of Guestwick shows more pronounced changes in structure, with the large, continuous area of high pond density in 1955 (21 – 40 ponds / km^2), becoming fragmented into two smaller, discrete patches by 2014. Large patches of the Guestwick pondscape have a pond density of fewer than 6 ponds / km^2 in 2014, a lower level of pond density than observed anywhere in the region in 1955. In Westfield, the overall density of ponds across the 28.27 km^2 region was considerably lower in 2014 than 1955; while in 1955 over half the region had pond densities of 16+ ponds / km^2, by 2014 over half of the Westfield region had pond densities lower than 10 ponds / km^2, with a thinner band of high pond density remaining in the south-east of the region. From the local scale maps, there are clear patterns in pond density change, with a tendency for entire pond clusters to be lost, rather than an even distribution of random pond loss.

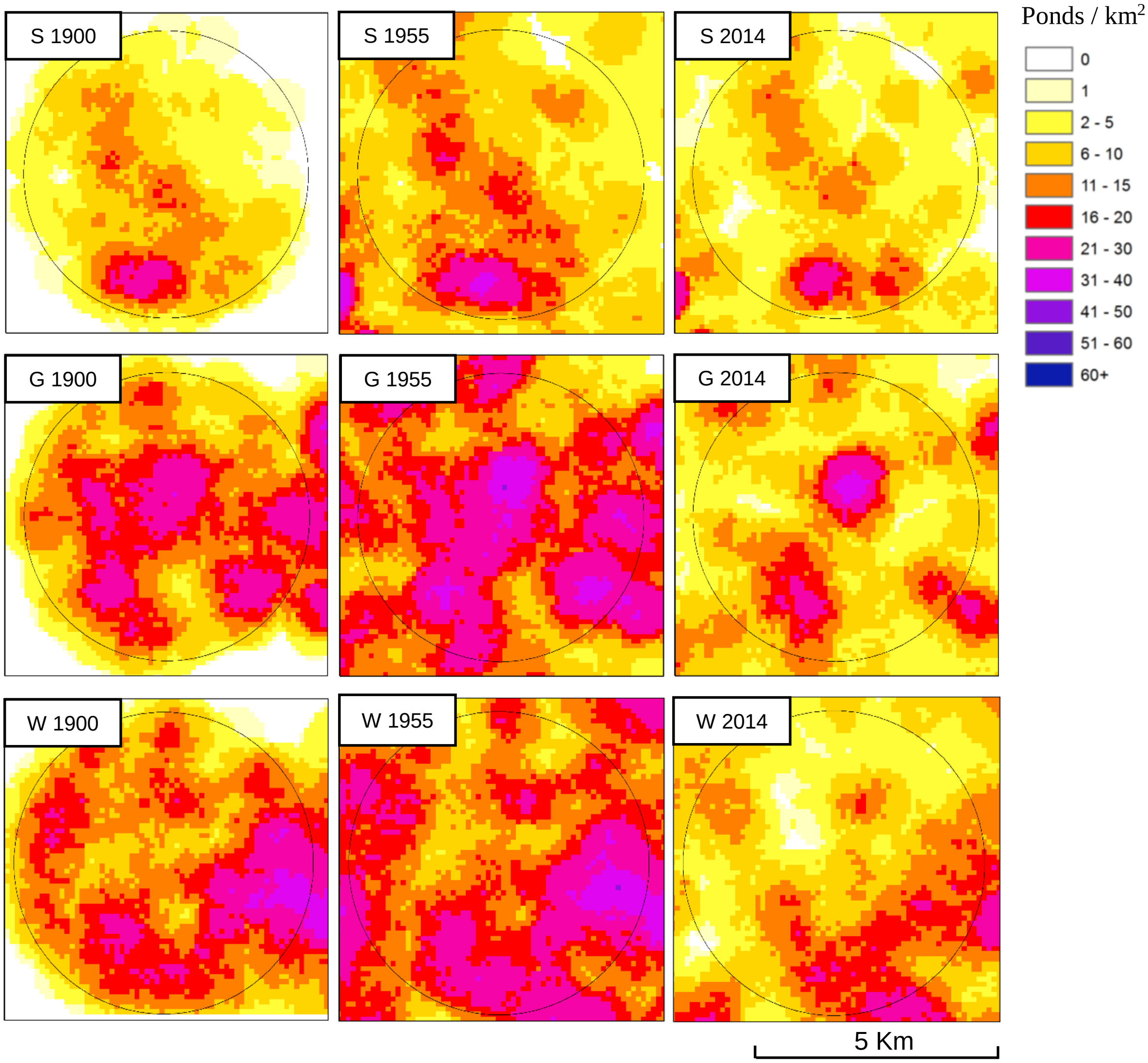


Figure 23 - 'Local scale' changes pondscape connectivity, measured as ponds / km^2, between 1900, 1955 and 2014. S – Stody, G – Guestwick, W – Westfield.

3.4.5 Regional scale changes in pond numbers

Desktop analysis of the 2014 OS maps revealed that 21,697 ponds, or 23,109 water bodies (when lakes, gravel pits and reservoirs are included), are currently present in Norfolk. The highest densities of ponds are found across central Norfolk (*Figure 24*), on heavy boulder clay subsoil, which was extensively excavated for marl (see *Chapter 2*). Lower densities of ponds are found to the west of the county, associated with lighter sandy soils (see geology map, *Figure 10*). Patches of lower pond density also occur to the east of the county, around the urban centres of Norwich and Great Yarmouth, and around the Norfolk Broads (much larger bodies of water which originated as medieval peat workings).

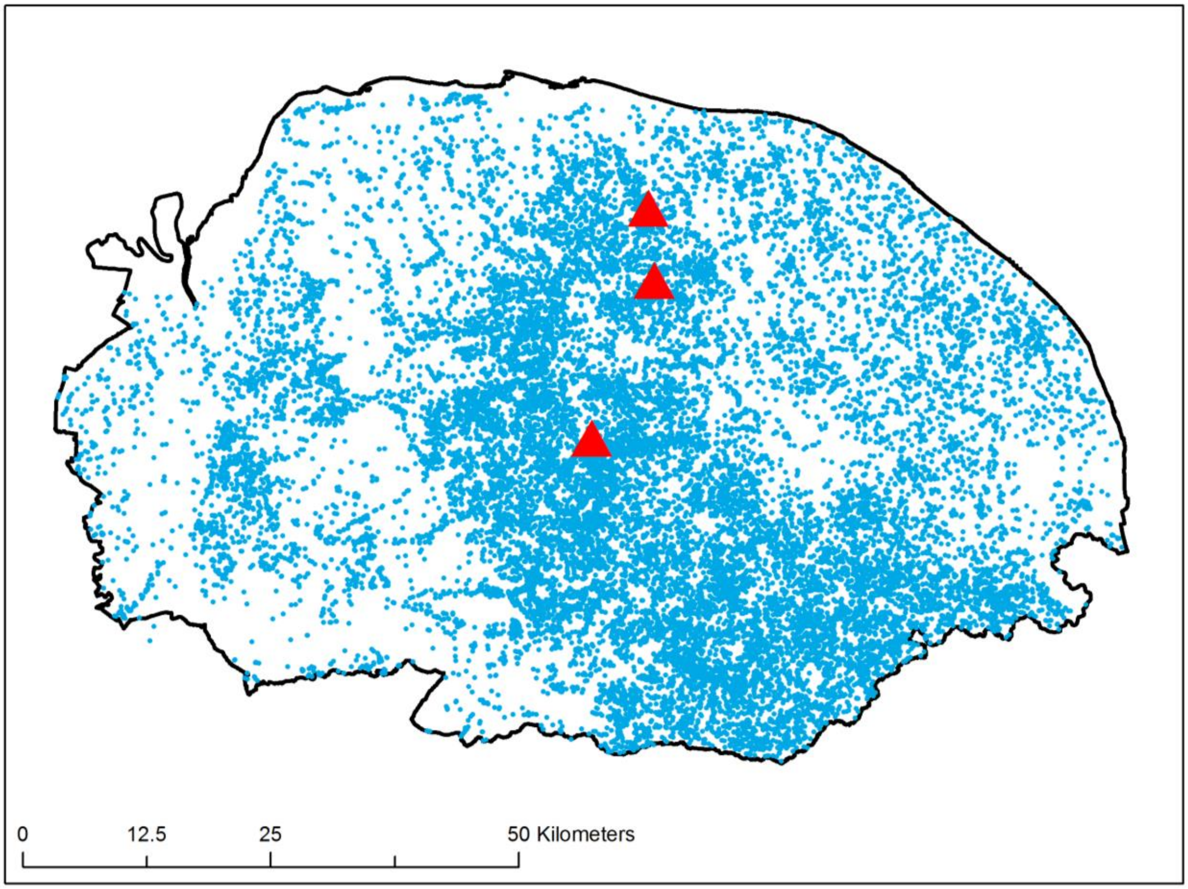


Figure 24 - Present-day distribution of ponds across Norfolk, from the 2014 OS map. Red triangles mark the three ‘local scale’ study areas examined in this chapter. From north to south; Stody, Guestwick and Westfield.

Across the county, pond numbers decreased by 27.8% between 1955 and 2014 (*Table 10*), from 30,088 ponds in 1955, to 21,697 ponds in 2014. Regional patterns in pond loss were similar to local scale patterns, with hedgerow ponds experiencing the highest rate of loss (- 50.4%), followed by open field ponds (- 29.6%). Regionally, woodland (+ 44.2%), and hydrologically connected ponds (+ 64.5%), increased in numbers between 1955 and 2014.

Table 10 – Regional scale changes in pond numbers between 1955 – 2014.

Pond type	1955	2014
Hedgerow	13,198	6,545
Open field	7,573	5,331
Roadside	3,180	2,256
Woodland	2,369	3,417
Farmyard	1,405	1,453
Rough ground	662	200
Ornamental	459	444
Connected	1,242	2,051
Total	30,088	21,697

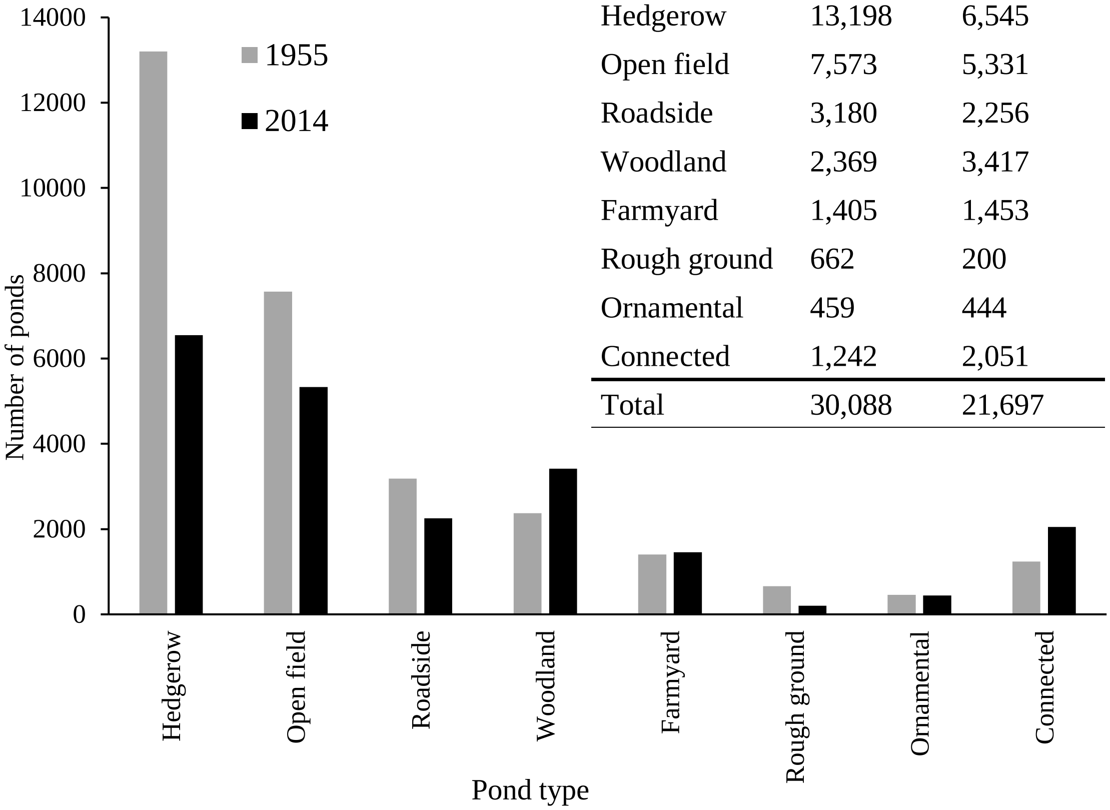


Figure 25 – Regional scale changes in pond numbers between 1955 – 2014.

3.4.6 Regional scale changes in pond density

At the regional scale, significant differences in pond density were evident from both the 'landscape perspective' and the 'pond perspective'. From the 'landscape perspective', mean pond density across Norfolk decreased slightly, from 5.6 ponds / km^2 in 1955, to 4.2 ponds / km^2 in 2014. From the 'pond perspective', the mean number of neighbouring ponds within 1 km^2 of a focal pond decreased significantly from 13.3 ponds / km^2 in 1955, to 10.7 ponds / km^2 in 2014 (t (54,815) = 35.9, $p < 0.001$, $d = 0.3$). Further details of these changes are given in *Table 11*.

Sixty one percent of ponds experienced a reduction in pondscape density between 1955 – 2014, with 31% ponds experiencing losses of between 2 – 5 neighbouring ponds within 1 km^2 (*Figure 26*). Over a quarter (28%), ponds experienced an increase in pondscape density over this same time period, while 11% experienced no change in pondscape density at the 1km^2 level. Areas of pond loss and gain across Norfolk are illustrated in *Figure 29*, which shows how areas of pond gain trend to be small and local, (small blue 'spots' across the county), while areas of pond loss are larger and more continuous.

Table 11 – Regional scale changes in pond density from the 'landscape perspective' and 'pond perspective', between 1955 – 2014.

	'landscape perspective'		'pond perspective'	
	1955	2014	1955	2014
Min	0	0	1	1
Max	95	83	95	83
Mean	5.6	4.2	13.3	10.7
Median	.	.	12	9
SD	6.4	5	8.4	8.3
N	550,330	550,330	31,688	23,129

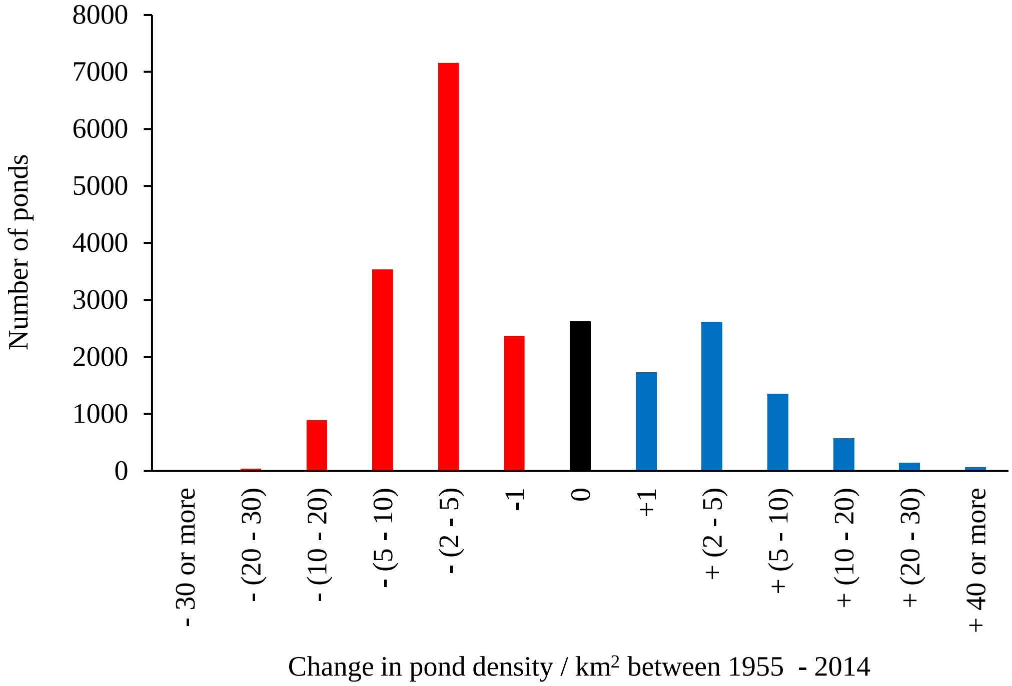


Figure 26 - Regional scale changes in pond density (ponds / km^2), between 1955 – 2014, from the 'pond perspective'.

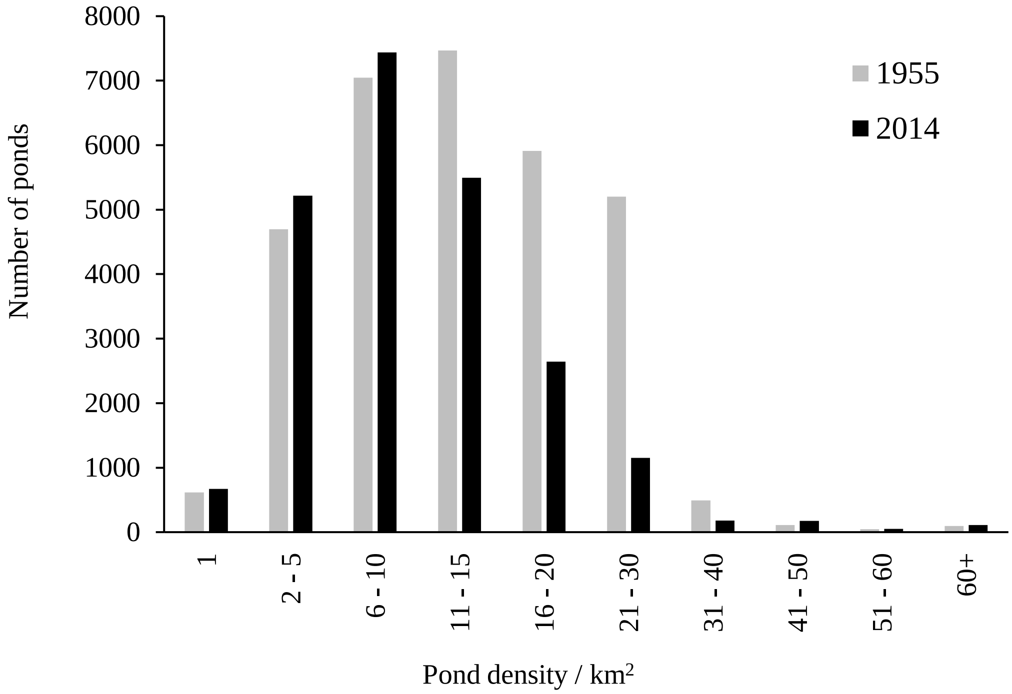


Figure 27 - Regional scale changes in pond density (ponds / km^2), between 1955 – 2014, from the 'pond perspective'.

The general shift towards a lower density pondscape between 1955 – 2014 can be seen in *Figure 26 – Figure 29*. *Figure 27* shows the distribution of ponds across the 10 density categories used during pond density mapping. In 1955, the pond density category of 11 – 15 ponds / km^2 contained the greatest number of ponds, with a gradual decline in pond numbers in the higher density categories (16 – 20 ponds / km^2, and 21 – 30 ponds / km^2), and a sharp drop in pond numbers in the density categories exceeding 31 ponds / km^2. In contrast, by 2014 the most common pond density category was 6 – 10 ponds / km^2, with a pronounced decline in pond numbers occurring in the higher density categories. These changes in pond density are mapped in *Figure 29*, which shows how pond losses of 2 – 5 ponds / km^2 occurred widely across the entire county between the 1950s and present day. More intensive pond loss (11 or more ponds lost between 1955 – 2014), occurred across central Norfolk, where pond densities in the 1950s had been highest, and in a smaller patch in the north-west of the county, associated with the urban area of King's Lynn. Increases in pond density between 1955 – 2014 are concentrated towards the north-east of the county, with small, localised 'hot-spots' of increased pond density (gains of 21+ ponds / km^2), occurring sporadically across the rest of the county.

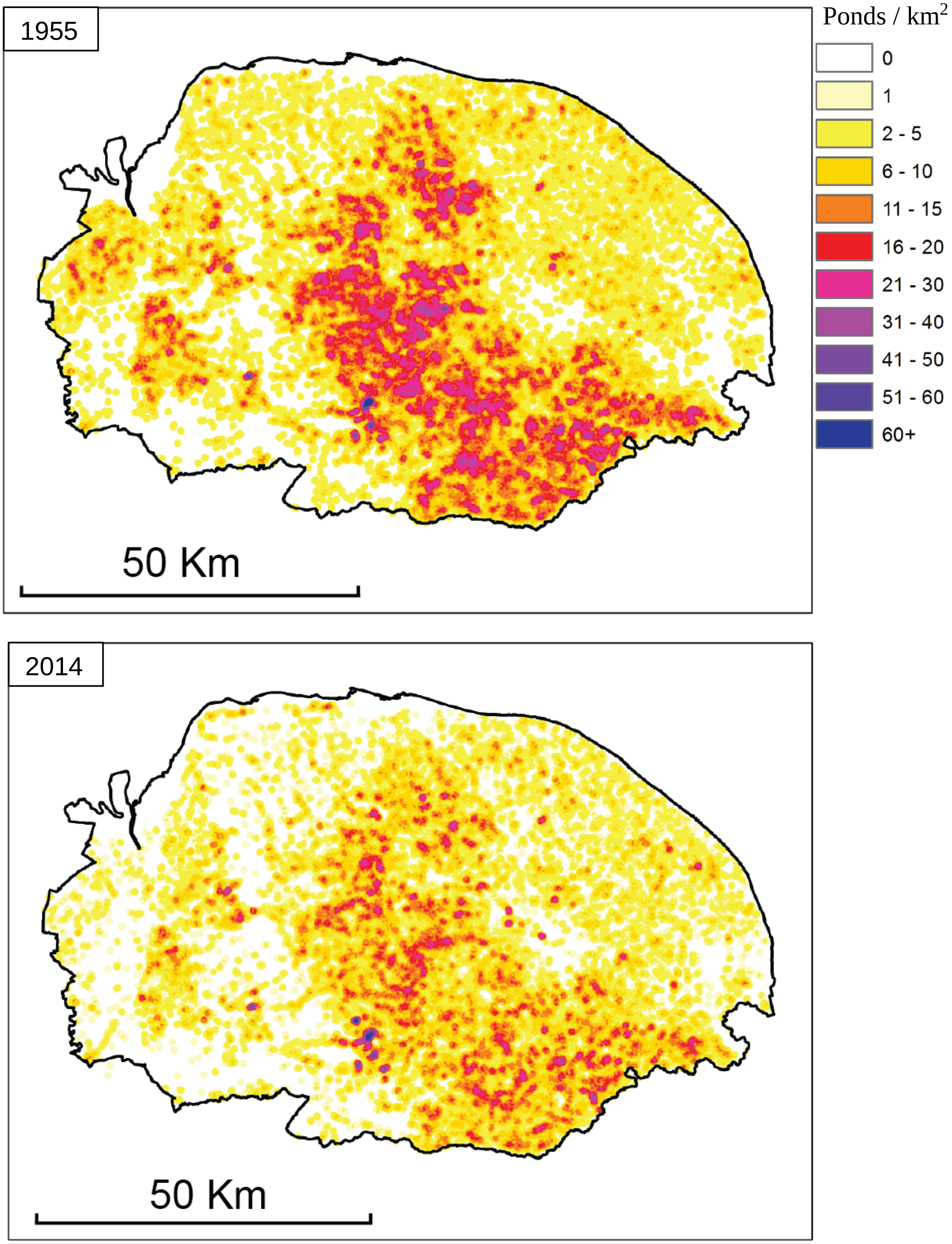


Figure 28 - Pond density (ponds / km^2*), across Norfolk in 1955 and 2014.*

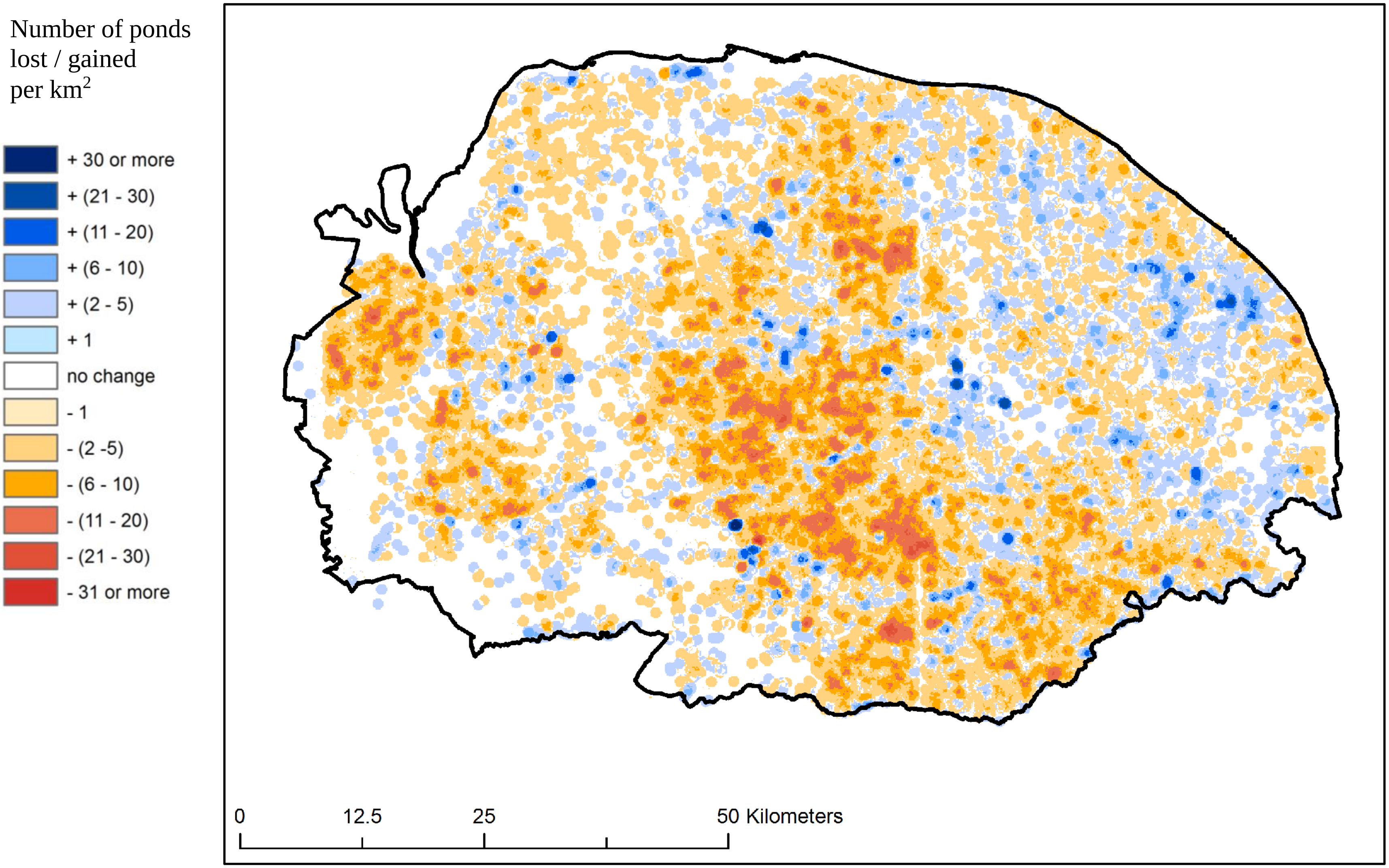


Figure 29 – Change in pond density / km² across Norfolk, between 1955 and 2014.

3.5 Discussion

3.5.1 Ground truthing study

Ordnance Survey (OS) maps record topographical landscape features, including even small ponds, in great detail (Seymour 1980; Boothby & Hull 1997). As such they have been widely used for studies of pond numbers and pond loss across the UK (Heath & Whitehead 1992; Boothby & Hull 1997; Wood *et al.* 2003; Jeffries 2012; Hassall 2014). The results from this ground truthing study however suggest that while the OS maps record the location of small agricultural ponds in open areas with high accuracy (*Table 5*), woodland ponds in particular may be under-recorded (*Figure 17*). This is likely due to the low visibility of these ponds from the air, with OS map editions from the 3rd Revision onwards relying largely on aerial photography for mapping small landscape features (Seymour 1980). This may also lead to the omission of some small hedgerow ponds from the OS maps (for example seen in grid square 3, *Figure 17*). In some instances, ponds which had contained water at the time of the ground truthing survey (conducted in February 2013), had dried out by the summer of 2013. These ephemeral ponds might not appear on the OS maps, depending on the timing of aerial surveys.

All of the ponds marked on the modern OS map were identified in the field. Additionally, of the 29 ghost ponds which were marked on the 1955 map, but not on the 2014 map, 23 were visible in the field as damp depressions or changes in crop cover (*Figure 18*). This suggests that the majority of ghost ponds identified from OS maps can be easily pin-pointed in the field, making the excavation of these 'extinct' habitats highly feasible.

Results from the ground truthing survey suggest that pond estimates obtained from OS maps may slightly underestimate actual pond numbers, particularly for woodland or seasonal ponds. This could disproportionately affect pond estimates obtained by scaling-up pond counts across small areas (Heath & Whitehead 1992), compared to estimates based on larger survey regions (Boothby & Hull 1997). However, given the reasonably high accuracy and similarity in survey methods used to draw both the historic and modern OS maps (Seymour 1980), there is unlikely to be significant variation in pond-mapping accuracy between editions. Any under-estimation in pond numbers is likely to occur across all map editions, and therefore not significantly affect estimates of pond loss. Given the apparent underestimation of woodland ponds on OS maps, it is possible that in areas where woodland planting (as a result of agri-environment schemes or conservation work), has been conducted, the number of ponds contained within these new woodlands may be underestimated.

3.5.2 Changes in pond numbers

Between the 1950s and present day, pond numbers in Norfolk declined by 27.8%, from 30,088 ponds recorded on the 3rd Revision OS map (drawn between 1953 – 1957), to 21,697 recorded on the modern map. This level of pond loss is lower than reported from other regions of the UK and Europe; across England and Wales, pond numbers are thought to have decreased by around 75% during the 20th century (Rackham 1986; Biggs *et al.* 2005; Williams *et al.* 2007), with estimates of pond loss of between 40 – 60% reported from agricultural regions including Essex (55% - Heath & Whitehead 1992), Cheshire (61% - Boothby & Hull 1997), and Leicestershire (60% - Wood *et al.* 2003). It is worth bearing in mind however that the current study of the Norfolk pondscape focused only on the latter half of the 20th century, rather than the entire 20th century period examined for other counties. This was due to difficulties in reading earlier map editions, and because results from the local-scale study suggested that there was little pondscape change during the early 20th century. As other studies have also suggested that pond loss was far greater in the latter half of the 20th century (Boothby 1995; Curado *et al.* 2011; Jeffries 2012), focusing on this time period should provide a reasonable measure of pondscape changes.

It is surprising that Norfolk appears to have experienced lower levels of pond loss than other agricultural regions of the UK and Europe. East Anglia is one of the most productive agricultural regions in Europe, often described as ‘England’s breadbasket’ (Cole 2009), and has played a major role in agricultural development throughout the 20th century (Riches 1967). In some cases, this discrepancy could in part be an effect of scale. Most of the existing pond-loss literature has focused on small study regions, and as such may not account as well for local variation in pond loss. It is evident from the three ‘local scale’ and nine ‘landscape scale’ study regions examined in this chapter, that such local variation in pond loss is high; for example, while the 28.27 km^2 areas around Guestwick and Westfield experienced pond losses of 45.1% and 47.5% respectively (between the 1950s and present day), Stody experienced a much lower rate of pond loss of 17.1%.

Only two other studies have examined pond loss at a similar scale to this chapter; Jarvis and Marshall (1987), cited in Heath & Whitehead (1992), examined pond loss across the entire county of Hertfordshire, where 48.7% of ponds present in 1882 had disappeared by 1978. Similarly, Boothby & Hull (1997) examined pond loss across the county of Cheshire, and reported pond losses of 61% between *ca*.1870 and 1993, leaving a total of 16,728 in the county. With both large and small scale studies suggesting rates of pond loss in excess of 40% for many areas of the UK, it appears that while pond loss in Norfolk has been significant, it is perhaps less pronounced than in other agricultural regions of Europe. This could perhaps be an effect of focusing only on the latter half of the 20th century, and thus missing pond loss occurring before this point. However, results from the local scale study (*Figure 19*), suggest little change in pond density during the first half of the century. Alternatively, it is possible that recent increases in pond numbers within the last decade (Williams *et al.* 2007), have resulted in the modern pondscape of this study being better connected than that of previous studies conducted in the 1980s (Jarvis and Marshall 1987), and early 1990s (Heath & Whitehead 1992; Boothby 1995).

One unexpected aspect of pondscape change revealed by the ‘local scale’ study is the *increase* in pond numbers and pond density between the 1900s and 1950s. While some areas of the UK and Europe have experienced an increase in pond numbers in recent years due to conservation efforts (Williams *et al.* 2007; Jeffries 2012), possible reasons for the historic increase in pond numbers (between the 1900s – 1950s), in Norfolk are unclear. Part of the explanation could be error in interpreting the 1st Edition (1900) OS map, with small ponds potentially mistaken for trees or other landscape features (*Figure 15*). However, considerable care was taken to avoid such errors, with unclear features cross-referenced between different map editions and / or aerial photographs. Another possibility is that an improvement in mapping techniques, in particular a shift towards greater use of aerial photography between the drawing of the 1st Edition and 3rd Revision OS maps, could have led to a greater number of ponds being recorded on the later edition. While this is likely to account for some of the apparent increase in pond numbers, it should not have affected the data to the extent observed in the 'local scale' study.

To determine whether the local scale increases in ponds numbers observed between 1900 – 1955 (*Figure 20*), are likely to reflect regional scale patterns, or whether they are simply local variation across the small sample size, pond numbers were counted across nine smaller 'landscape scale' study regions, for 1900, 1955, and 2014 (*Figure 13*). This revealed extensive local variation in pond numbers, with five of the nine regions experiencing pond loss between 1900 – 1955, and four of the nine regions experiencing pond gain. This suggests that the unexpected increase in pond numbers between 1900 – 1955 experienced by the three 'local scale' study regions could be due to local land use changes, and not reflect regional scale changes. Both the 'local scale' and 'landscape scale' results suggest that pond loss was greater and more consistent across the region between 1955 – 2014, than between 1900 – 1955. This most likely reflects changing agricultural practices and increasing agricultural intensification during the second half of the 20th century (Robinson & Sutherland 2002; Benton *et al.* 2003; Wood *et al.* 2003). This disparity in the levels of pond loss when measured at the local scale highlights a major problem for studies which calculate regional level pond loss based on replicate samples across small areas (Heath & Whitehead 1992).

Although the apparent increase in pond numbers between 1900 – 1955 appears to be a result of local variation, it does suggest that a considerable number of agricultural ponds in Norfolk are more recent in origin than sometimes assumed. Prince (1962), suggested that most of Norfolk's ponds originated as mineral workings or marl pits. While this is undoubtedly true of many older ponds, the practice of marling had ceased by the end of the 19th century (Prince 1962), and as such cannot explain the local increases in pond numbers between 1900 – 1955. Rackham (1986) disputes Prince's suggestion that most of the ponds of Norfolk originated as marl pits, saying that this is "*not a sufficient explanation for the very large numbers of ponds in East Anglia*", although he proposes no alternative origin for these ponds. In the south of Norfolk, large numbers of pits were dug for the extraction of 'clay lump', used in traditional buildings. As these pits filled with water, they would often be re-purposed to serve for livestock watering, fishing, and cooling of early agricultural machinery (Norfolk Ponds Project 2015). While this may have contributed to increasing pond numbers between 1900 – 1955, there are many other potential origins for ponds; local changes in farming practice could lead to ponds being created for watering livestock, cooling machinery, or providing field drainage, while in some areas bomb-craters which subsequently filled with water have contributed to the pond landscape.

While there was considerable local and landscape-scale variation in pond numbers between 1900 – 1955, the large-scale pond loss occurring between 1955 – 2014 was consistent at local and regional scales. Hedgerow ponds suffered the greatest losses at both local (*Figure* 20) and regional (*Figure 25*) scales, reflecting the widespread removal of hedgerows which occurred after WW2 (Rackham 1986; Robinson & Sutherland 2002). Many of the smaller hedgerow ponds would have been filled-in as the hedge was removed, with stumps and wood from the hedgerow being used to fill-in the pond. This has been evidenced from the excavation of a number of ghost ponds which were formerly located on hedgerows, and which contained large burnt stumps and branches. Some of the larger ponds located along hedgerows would have been too difficult to fill-in, and as such were left behind to becoming open field ponds.

Although most pond types experienced a decline in numbers between 1955 – 2014, woodland ponds increased in numbers both locally (increases of between 55.6% – 142.9%), and regionally (increase of 44.2%). This is mirrored by an increase in the area of woodland marked on the 2014 OS map, relative to the 1955 map, which could be a result of woodland planting agri-environment schemes. Organisations including the Forestry Commission, Countryside Stewardship scheme, and Woodland Trust all provide funding and subsidies for tree planting on agricultural land, most of which began in the 1970s – 1980s. Hydrologically connected ponds also appear to increase in numbers between 1955 – 2014. In the case of nature reserves and restored river reaches, this increase is likely the result of conservation efforts. In agricultural fields however, the apparent increase is more likely an artefact of mis-identification of other linear features on the black and white 1955 map, combined with improved mapping accuracy between the 1955 and 2014 colour edition.

3.5.3 Ecological implications of the loss of different pond types

The preferential loss / gain of certain pond types not only influences the overall connectivity of the pondscape, but could also have taxon-specific ecological consequences. For example, the increase in woodland pond numbers (and concurrent increase in woodland), could potentially be beneficial for amphibians, which often use woodland ponds for foraging (Laan & Verboom 1990; Joly *et al.* 2001, 2003). However, woodland ponds tend to be over-shaded with limited aquatic macrophyte diversity (Sayer *et al.* 2012, and *Chapter 4* of this thesis), making them poor breeding habitat for amphibians, which may instead rely on ponds *near* but not *within* woodland. Ponds located on uncultivated patches within the agricultural matrix (classified as 'rough ground' ponds), could potentially provide more suitable habitat for amphibians. These ponds might be expected to experience lower shading than woodland ponds, while maintaining high connectivity to suitable foraging habitat. Ponds in this category declined in numbers at both a local and regional scale between 1955 – 2014, likely due to a combination of pond loss, and conversion of rough ground into more profitable arable fields or agri-environment scheme woodland.

The disproportionate loss of hedgerow ponds could have significant consequences for the connectivity of the pondscape – hedgerows are often considered to be 'habitat corridors' (Laan & Verboom 1990; Scribner *et al.* 2001; Downs & Racey 2006), which may be used by dispersing amphibians (Scribner *et al.* 2001; Reid *et al.* 2014), and by more mobile taxa such as the Chironomidae, which benefit from having high densities of hedgerows close to emergence ponds (Delettre & Morvan 2000). Hedgerow ponds may not only be important for aquatic organism dispersal, but they may also be better buffered against agricultural run-off than open field ponds (Heathwaite *et al.* 2005; Dorioz *et al.* 2006).

Ornamental ponds (-3.3%), and farmyard ponds (+3.4%) experienced the least change in numbers between 1955 – 2014, both at the local and regional scale. Both pond types are generally located on ground where the incentive to fill them in is reduced, either because they are decorative or because they provide drainage for a farmyard, which likely explains their consistent numbers. Unfortunately, these types of pond are often of low ecological value. Many farmyard ponds receive high levels of sediment run-off, resulting in low water quality, while ornamental ponds often contain invasive plant and fish species, or large numbers of waterfowl, which destroy macrophytes and reduce water quality (Copp *et al.* 2005; Hill & Wood 2014).

Including some of these ecological factors into GIS based analysis would be one way of beginning to incorporate the functional connectivity, rather than the purely structural connectivity, of changing pondscapes. While precise models of functional pondscape connectivity would be very difficult to achieve for highly heterogeneous agricultural areas (With *et al.* 1997; Adriaensen *et al.* 2003), including some measure of key landscape features could be helpful in better identifying the most ecologically ‘valuable’ pond clusters. For example, Joly et al., (2001), describe a simple approach of measuring the width of the uncultivated sector (any uncropped land within a given radius of a focal pond), as an indicator of pond suitability for newts. This sort of approach would be less computationally intensive than species-specific least cost modelling (see *Introduction - section 1.5*), and could be used to provide a better indication of pond suitability for certain taxa, once pond density and inter-pond distance have been accounted for.

The patterns in pond loss observed in this study differ considerably from those of Heath & Whitehead (1992), the only other study found which also categorised ponds by surrounding land use. Their study examined pond loss in Essex, England, between the 1st edition (*ca.* 1870), 3rd Revision (*ca.* 1921), and 1960’s pathfinder series OS maps. Despite the Essex study only examining pond loss up until the early 1960s, which falls in the middle of the period of most rapid pond loss for Norfolk, Heath & Whitehead (1992) report higher rates of pond loss for all comparable pond categories. In Essex, 59% of farmland field ponds were lost between 1870 – 1960, compared to 42% loss for Norfolk between 1955 – 2014. Pond loss in Essex was also greater than that in Norfolk for Farmyard ponds (57.9% loss in Essex, 3.4% gain in Norfolk), Roadside ponds (62.5% loss in Essex, 29% loss in Norfolk), and Woodland ponds (53.6% loss in Essex, 44.2% gain in Norfolk). Given that agricultural intensification appears to be the main cause of pond loss in both counties (Prince 1962; Heath & Whitehead 1992), these differences are surprising. This may be an effect of sample size, as while this chapter examines pond loss across the whole county of Norfolk, with a change in pond numbers from 30,088 to 21,697 ponds between 1955 - 2014, the Essex pond loss study covers 12 smaller areas, with a change in pond numbers from 952 to 416 ponds between 1870 – 1960.

3.5.4 Ecological implications of changes in pond density and fragmentation of the pondscape

It is evident from this study that while pond loss across Norfolk may have been lower than other parts of the UK, considerable fragmentation of the pondscape and reduction in pond densities has occurred. Although the change in mean pond density across the county seems fairly low (5.6 – 4.2 ponds / km^2 measured from the landscape perspective, or 13.3 – 10.7 ponds / km^2 from the pond perspective), *Figures 28 & 29* show how pond loss tends to be clustered, with local networks of ponds often experiencing far greater levels of pond loss. Areas with formerly high density pond networks (>20 ponds / km^2), seem to have suffered disproportionately, showing the greatest change in pond density and loss of structural connectivity between ponds. These changes in structural connectivity are likely to have ecological consequences for aquatic communities, which will experience both a reduction in habitat availability and increase in habitat isolation (Harrison & Bruna 1999; Lehtinen *et al.* 1999; Krauss *et al.* 2004). These factors can have detrimental effects on biodiversity and population stability (Møller & Rørdam 1985; Krauss *et al.* 2004), although the extent to which the pondscape has been fragmented will depend on the functional connectivity experienced by different taxa (Watts & Handley 2010). For dispersal limited amphibians, even relatively small reductions in pond density may be detrimental to population stability (Laan & Verboom 1990; Ray *et al.* 2002), while for more mobile taxa such as aquatic Coleoptera, the modern pondscape of Norfolk may still be reasonably well connected.

At the local scale, significant variation in pond density occurred over time and between locations, indicating local-scale variability in pondscape fragmentation. While Guestwick and Westfield experienced similar levels of pond loss (45.1% and 47.5% respectively), they show quite different patterns in pondscape fragmentation (*Figure 23*). For example, the pondscape in Guestwick fragments into 2 - 3 discrete ‘islands’ of high pond density, each maintaining a similar density level to that once seen across most of the region. In contrast, the pondscape in Westfield experiences less fragmentation, and instead shows a more ubiquitous loss of ponds, resulting in a ‘corridor’ of lower pond density to the south of the region. These different patterns in pondscape structural connectivity may have different effects on aquatic communities, depending on how different taxa perceive the resulting functional connectivity of the pondscape.

From the ‘landscape perspective’, regional pond density decreased from 5.6 ponds / km^2 in 1955, to 4.2 ponds / km^2 in 2014. This figure falls within the range of previous estimates of pond density within Norfolk; > 6.4 ponds / km^2 (Rackham 1986), and 3.9 ponds / km^2 (Prince 1962), both of which were based on smaller sample sizes. The modern pond density in Norfolk appears to exceed that reported from agricultural areas of Essex (1.5 ponds / km^2), Cheshire (3.3 ponds / km^2), and northern France (3 ponds / km^2) (Essex – Heath & Whitehead 1992, Cheshire – Boothby & Hull 1997, France - Céréghino *et al.* 2008b), and could reflect the lower rate of pond loss which seems to have occurred in Norfolk, compared to these other regions.

Of greater ecological significance for aquatic taxa is the change in pond density from the ‘pond perspective’, which reflects the change in inter-pond distances and pond density from the perspective of a dispersing organism. Sixty-one percent of ponds experienced a reduction in pondscape density between 1955 – 2014, with the majority experiencing losses of between 2 – 5 neighbouring ponds within 1km^2 (*Figure 26*). These changes in pond density can be seen on the map of Norfolk in *Figure 29*, which shows how pond losses of 2 – 5 ponds / km^2 occurred widely across the entire county between the 1950s and present day. Areas experiencing greater levels of pond loss are focused towards the centre of the county, where the pondscape of the 1950s was historically densest. Small, localised patches of increased pond density (*Figure 29*), are most often associated with small-scale conservation projects in nature reserves, or along river restorations. These tend to be concentrated towards the north-east of the county, between the Broads and the coastline. Other apparent hot-spots represent commercial fisheries, which have little ecological value. Local variation in pond loss and structural connectivity (*Figures 23 & 29*), may be due to several factors. Different estates or farms may have taken different approaches to pond management, depending on their chosen farming practice (pastoral being perhaps less likely to fill-in ponds than arable), scale of commercial operation (large scale intensive farms being more likely to fill-in ponds than smaller more extensive farms), and landowner interest in 'traditional' vs. 'modern' farming values. Such considerations not only relate to the original decision in maintaining or filling-in ponds, but are now relevant in the discussion of where to potentially restore or 'resurrect' lost ponds.

It is perhaps unsurprising that the greatest pond losses have occurred in the central regions of the county, where pond densities were historically higher, yet these findings could have considerable implications for aquatic diversity. *Figure 28* shows the fragmentation of the once large, continuous, high-density pondscape of central Norfolk (where many ponds in the 1950s experienced densities of 16 + ponds / km^2), into smaller islands of high density, surrounded by a lower pond density landscape (2 - 10 ponds / km^2). There is also a considerable increase in areas which contain no ponds within 1km^2 (shown in white), particularly in the west of the county, resulting in increased isolation of the remaining ponds in this area.

Overall, this study reveals that pond loss has been widespread across the county of Norfolk, and has resulted in both a significant reduction in pond numbers, and a significant reduction in pond density, at the local and regional level. Agricultural ponds, particularly those located along former hedgerows, have experienced the greatest reduction in numbers, reflecting both their abundance in the landscape and their vulnerability to changing farming practices and agricultural intensification.

Connectivity between ponds is an important factor in population persistence across multiple taxa. Ponds are stochastic environments, with any given pond being susceptible to short term (seasonal drying), and long term (succession) extinction (Fortuna *et al.* 2006; Hassall, Hollinshead & Hull 2012). As such, high connectivity across pond networks is essential for allowing both individuals and populations to disperse into new, more suitable habitats. Pond density and inter-pond distances have been shown to significantly affect diversity and population dynamics across multiple taxa, including macrophytes (Møller & Rørdam 1985; Gledhill *et al.* 2008; Bosiacka & Pienkowski 2012), invertebrates (Gledhill *et al.* 2008; Raebel *et al.* 2012a), and amphibians (Joly *et al.* 2003; Fortuna *et al.* 2006; Curado *et al.* 2011). As such, this study provides a useful starting point in understanding pondscape fragmentation, with components of structural connectivity (reduced numbers and density of ponds, and increased isolation), being both easy to measure, and important factors in determining wider functional connectivity (Murphy & Lovett-Doust 2004). The current study could be expanded and improved upon by incorporating other determinants of functional connectivity i.e. the relative mobility of different taxa of interest. This presents several challenges in the context of highly heterogeneous agricultural pondscapes;

1) Determining the ‘permeability’ of the terrestrial matrix in between ponds requires detailed knowledge of both the dispersal capabilities, and habitat preferences, of the target organism. While reasonable estimates of dispersal distance might be established for some easily tracked species, for example amphibians, even relatively well studied species have proved difficult; Murphy & Lovett-Doust (2004) state that maximum dispersal distance between breeding and foraging grounds for the common toad (“*one of the most studied amphibian species*”), have been estimated anywhere between a few meters and 3 km, and this does not consider variation in dispersal distance across different land use categories. Other pond organisms may be even more difficult to establish optimal / maximum dispersal thresholds for, particularly small flying insects which are difficult to track.

When the ability or willingness of an organism to cross different land use types within the agricultural matrix is also considered, the question becomes yet more complicated. Least cost models of functional connectivity often rely on ‘expert judgement’ in assigning relative costs for crossing different land use categories. For many taxa, this knowledge is simply lacking (Boothby 1997; Oldham *et al.* 2000; Ray *et al.* 2002; Lin 2008; Kärnä *et al.* 2015). While it is often possible to establish relative preferences (for example, amphibians may easily cross woodland, less easily cross meadow, and struggle to cross arable fields), quantifying the actual cost (energy expenditure and mortality risk), of different habitat types is very difficult. Such modelling approaches to functional connectivity also assume that the organism in question will always choose the path of least resistance, which may not be the case (Murphy & Lovett-Doust 2004).

2) Delineating matrix permeability is further complicated for taxa which require other habitats in addition to ponds (for example, amphibians using woodland patches for foraging). While a basic level of functional connectivity could be estimated by including the density / distance of other landscape components into the GIS model (Ray *et al.* 2002; Murphy & Lovett-Doust 2004), these requirements may vary seasonally, or with the life stage of the organism.

3) The matrix surrounding pond habitats is not static, and will change throughout the year (with crop cycles), and on longer time scales. Different crops may be more or less suitable for different taxa to cross, and changes in planting regime may change the functional connectivity of the pondscape on a regular basis.

4) In the current GIS based study, it is assumed that all ponds marked on the map are potentially suitable habitat. However, it is evident from *Chapter 4* that this is far from true, with many existing ponds being heavily over-shaded, providing little or no suitable habitat for aquatic macrophytes, Coleoptera or amphibians. Ponds which appear to be in highly connected patches on the map may in fact be ecologically isolated; Denoël & Lehmann (2006) comment on this in their study of pond occupancy in newts, in which newt occurrence was negatively associated with overall pond density, but positively associated with the density of inhabited ponds.

Although there are various challenges in expanding the current study to include not only structural connectivity, but also functional connectivity, there are a number of possibilities which could be incorporated with relative ease. Incorporating aerial photography (for example from Google maps), could provide a rough indication of whether available pond habitats are completely shaded (and therefor poor habitat for most species), or have some open water visible (more likely to be suitable habitats), as a means of estimating which ponds represent viable habitat patches. In addition, land use maps and aerial photographs could be used to provide an estimate of the suitability of the surrounding terrestrial matrix, particularly for amphibians, for which habitat requirements are better understood. A crude but suitable measure might be the width of uncultivated sector (Joly *et al.* 2001), or % cover of uncultivated land, within the mean dispersal distance for the species of interest. For highly mobile taxa, for which the matrix in between ponds may be less of a barrier to connectivity (aquatic Coleoptera, Odonata, waterfowl), a simple adjustment of the buffer distance (to match the mean dispersal distance of the organism of interest), could make the current GIS model more species-specific.

By incorporating species-specific dispersal distances, habitat preferences, and weighted measures of matrix permeability, changes in the functional connectivity of the pondscape from the perspective of different key taxa could be modelled. Such an approach would be extremely useful for the conservation of pond-dependant species. However, the simple structural connectivity approach used in the current study provides a good starting point, highlighting areas where the greatest changes in pond density have occurred, where the highest densities of ghost ponds could be found, and which types of pond have been most affected by pond loss.

3.6 Conclusions

This study has revealed wide-spread pond loss across the predominantly agricultural county of Norfolk. While the county appears to have experienced lower levels of pond loss (28% ponds present in the 1950s lost by 2014), than reported from other areas of the UK and Europe, this still represents a considerable reduction in these valuable habitats. Hedgerow ponds have been disproportionately affected, reflecting the widespread removal of hedges during agricultural intensification. Pond loss has not only resulted in a reduction in pond numbers across the entire county, but also a significant reduction in pond density – two key components of habitat structural connectivity. This fragmentation of the pond landscape or ‘pondscape’ has reduced previously continuous areas of high pond density into smaller, more isolated hotspots. Patterns in pond loss and pondscape fragmentation were observed at both the local and regional scale, although local and landscape scale analysis revealed the patchy nature of these processes. In accordance with the published literature, local and landscape scale analysis suggested that rates of pond loss accelerated in the latter half of the 20th century, while changes in pond numbers between 1900 – 1955 were both smaller and less consistent between regions.

Historic pond loss is widely assumed to have had a negative impact upon pond biodiversity (Heath & Whitehead 1992; Boothby 1997; Wood *et al.* 2003). However, the relative importance of pond connectivity versus pond quality has been shown to vary widely between taxa and between land-use types (Brönmark 1985; Brose 2001; Gledhill *et al.* 2008; Bosiacka & Pienkowski 2012). Understanding how the historic pond loss and pondscape fragmentation observed in this study may have impacted upon pond biodiversity is important for understanding the threats to agricultural pond biodiversity, and for informing the selection of suitable sites for pond creation, restoration or resurrection. These issues are addressed in the following chapter, which uses a space-for-time approach to examine how density might affect biodiversity in fragmented agricultural landscapes, and whether this effect is independent of pond quality.

Chapter 4 The ecological consequences of pond loss and pondscape fragmentation – a space for time substitution approach.

4.1 Introduction

The literature on the effects of habitat fragmentation on biodiversity is extensive and diverse (Fahrig 2003), and although habitat fragmentation is generally thought to have strong negative effects on biodiversity (Fahrig 2003; Tscharntke *et al.* 2005; Krauss *et al.* 2010), the significance and magnitude of this effect varies greatly between habitat types and between taxa. The fragmentation of 'natural' habitats by agricultural expansion has been highlighted as a major cause of extinction and declines in the ecological fitness and abundance of fragmented, small and isolated populations (Tscharntke *et al.* 2005), including farmland birds (Donald *et al.* 2001), invertebrates (Benton *et al.* 2002), and plants (Robinson & Sutherland 2002). Fragmentation has been widely linked to declines in biodiversity (Fahrig 2003; Krauss *et al.* 2004, 2010; Tscharntke *et al.* 2005), with habitat loss and increasing habitat isolation as key components of fragmentation both influencing biodiversity at local and regional levels. Since habitat fragmentation commonly involves both changes in the spatial arrangement of habitat patches and changes in the overall area of available habitat (Fahrig 1997, 2003), analysing the independent contributions of these different components towards diversity declines can be problematic (Fahrig 2003; Krauss *et al.* 2004). In this respect ponds provide a useful model for studying habitat fragmentation, as they provide multiple, discrete habitat patches (De Meester *et al.* 2005), where the effect of habitat isolation and connectivity can be examined independently of habitat area.

Considering the spatial component of habitat fragmentation manifesting itself by an increased distance and reduced connectivity between habitat patches, there are a number of theoretical predictions which can be made about how fragmentation might affect α-, β- and therefore also γ-diversity. At the patch scale, increasing distances between habitat patches would usually be expected to result in lower α- diversity. This is due to reduced species exchange between patches leading to reduced species diversity at any given patch (Fahrig

2003). In contrast, β- diversity (or turnover / dissimilarity between patches), may be expected to increase with increasing distance between habitat patches, as reduced species exchange results in more dissimilar assemblages (Jost 2007). At the regional or γ-diversity level, the effect habitat fragmentation has upon biodiversity may vary, depending on the relative contributions of the α- and β- components. Generally, the negative effects of fragmentation on biodiversity are far stronger than the positive effects (Fahrig 2003). This has been demonstrated in several studies of pond biodiversity, where habitat isolation is negatively associated with γ- diversity, due to the decrease in α- diversity far out-weighing the increase in β- diversity (Zealand & Jeffries 2009; Akasaka & Takamura 2012). Occasionally however, the β- diversity contribution to overall regional (γ-) diversity can be high, with pond isolation potentially having a positive influence on biodiversity at the regional level (De Marco *et al.* 2014). Understanding the interactions between the different components of habitat fragmentation, and the different components of biodiversity, makes the study of habitat fragmentation a complex problem (Fahrig 2003).

Habitat fragmentation has a temporal dimension, with landscape changes occurring continuously over short and long time scales. Very few studies have examined the effects of fragmentation in 'real time', and these have generally been limited to small-scale manipulations of microcosms or mesocosms (Harrison & Bruna 1999; Debinski & Holt 2000). At a larger scale, some studies have considered past habitat connectivity extracted from historic maps, as a variable in the analysis of present-day biodiversity (Krauss *et al.* 2010; Curado *et al.* 2011). Most taxa have a delayed response or 'extinction debt' in relation to habitat fragmentation (Lindborg & Eriksson 2004; Krauss *et al.* 2010), with mobile taxa showing more immediate responses than sessile ones (Krauss *et al.* 2010). Most commonly however, inferences about the consequences of habitat fragmentation are made through examining the relationships between present-day habitat connectivity and biodiversity, comparing biodiversity between areas with different densities and spatial arrangements of habitats. This 'space for time' replacement approach is employed in this chapter, to investigate the relationships between pond connectivity and pond biodiversity in different pondscape settings.

While pondscapes provide a useful system for studying spatial patterns in biodiversity, providing numerous discrete habitat patches (De Meester *et al.* 2005), there remain a number of complications. The effects of fragmentation upon the pond landscape or pondscape will be experienced at different spatial scales by taxa with different dispersal abilities (Tscharntke *et al.* 2005; Beisner *et al.* 2006). For all taxa, the pondscape includes not just the ponds themselves, but also aspects of the terrestrial matrix surrounding the ponds (Boothby 1997). While fully aquatic taxa may experience fragmentation of the pondscape as simple increases in pond-to-pond distances (structural connectivity), other taxa with partly terrestrial life stages such as amphibians, dragonflies, and water beetles, may be more strongly influenced by the terrestrial matrix, and the relative permeability of different habitats within this (functional connectivity). For example, amphibian diversity and abundance in ponds in the Netherlands and the USA (Laan & Verboom 1990; Marsh & Trenham 2001), has been found to be more strongly related to the distance between a pond and woodland habitats than to inter-pond distance. Among other taxa, both inter-pond distance and distance to terrestrial habitat components can influence species diversity and abundance at a site. Delettre & Morvan (2000) found that for adult Chironomidae emerging from agricultural waterbodies, large-scale patterns in diversity and species occurrence were significantly related to inter-pond distance, while local scale patterns were significantly related to hedgerow proximity and quality.

Understanding the ecological and biological consequences of pondscape fragmentation is further complicated by the complex and varied relationships between community structure and habitat isolation. In some instances, the relative isolation of ponds contributes to their high habitat heterogeneity, which in turn is associated with high γ- (regional) diversity (Scheffer *et al.* 2006). High pond connectivity (and hence increased rates of dispersal), may lead to homogenisation of pond communities, and an overall reduction in biodiversity at a regional scale. This trend has been observed in some studies; for example in Zealand & Jeffries' (2009), examination of pond snail diversity across ponds in northern England, and by Pedruski & Arnott's (2011) study of freshwater invertebrate diversity across artificial pond mesocosms. In both studies, a homogenising effect of pond connectivity led to lower species turnover (β- diversity), that resulted in overall lower regional (γ-) diversity, despite a weak positive effect of pond connectivity on within-pond (α-) diversity. Increasing pond isolation can also create a 'refuge effect' for prey taxa (*Figure 30*), with dominant predatory species such as fish often absent from more isolated ponds, leading to an overall positive effect of pond isolation (Scheffer *et al.* 2006).

Alternatively (and more commonly), high densities of ponds with high connectivity between sites can help to maintain genetic diversity and population stability, increasing biodiversity at the local / pond level, and thus resulting in higher biodiversity at a regional scale (Fortuna *et al.* 2006; Raebel *et al.* 2012a; Jeliazkov *et al.* 2014). This positive association between pond connectivity and pond biodiversity has been observed across multiple taxa (Møller & Rørdam 1985; Shulman & Chase 2007; Gledhill *et al.* 2008; Akasaka & Takamura 2012; Bosiacka & Pienkowski 2012; Raebel *et al.* 2012a; Jeliazkov *et al.* 2014). For aquatic macrophytes, Møller & Rørdam (1985) found that ponds which were closer together held significantly more species per unit area, when comparing two pond 'districts'. Gledhill et al. (2008), found pond density to be a significant determinant in both aquatic macrophyte, and aquatic invertebrate species richness, in a series of 37 urban ponds. Both groups were more strongly influenced by pond densities at larger (>1km) than smaller (<500m) spatial scales. Conversely, Bosiacka & Pieńkowski (2011), found a positive relationship between pond proximity and macrophyte species richness, but only at a local scale.

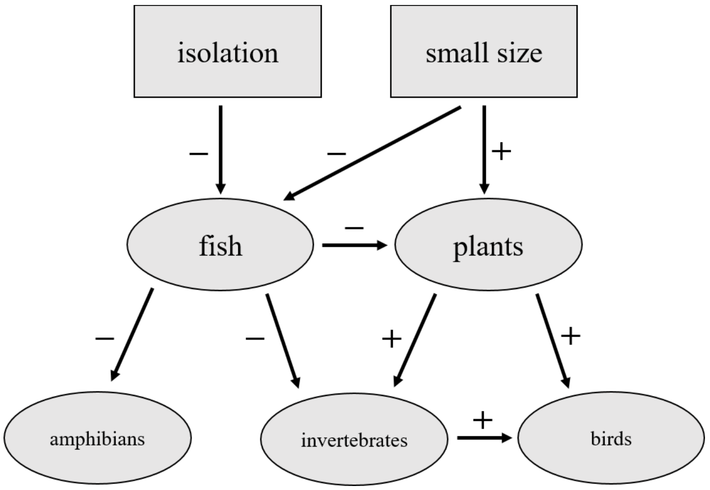


Figure 30 – Schematic diagram of proposed relationships between waterbody size and isolation, and abundance and diversity of different groups of organisms. The sign of the pathways represents their relative positive / negative influence over diversity and abundance of each group of organisms. From Scheffer et al. (2006).

It should be kept in mind that most studies of pond density and biodiversity have focused on structural connectivity of the pondscape (inter-pond distances and pond density), with little consideration of the functional connectivity of these habitats (determined by both the dispersal capabilities and habitat preferences of the organism in question). While components of functional connectivity have received some attention in the study of amphibian (Joly *et al.* 2001, 2003; Watts & Handley 2010) and reptile (Pereira, Segurado & Neves 2011) populations, they have tended to be overlooked in multi-taxa studies, where the same landscape may have many possible connectivity measures based on the different habitat requirements and dispersal capabilities of different species (Watts & Handley 2010).

In the case of macrophyte diversity, the spatial scale at which species richness is influenced by pondscape connectivity is likely to vary with the dispersal mechanism of macrophyte seeds / propagules. For species dispersed by wind, the functional connectivity of the pondscape is likely to be highly related to its structural connectivity, with distances between ponds being the strongest determinant of community structure. For many macrophyte species however, dispersal is largely attributed to the movement of waterfowl (DeVlaming & Vernon 1968; Wade 1990; Green & Figuerola 2005; Scheffer *et al.* 2006; Soons *et al.* 2016). While waterfowl are capable of dispersal over large distances, they may experience the functional connectivity of the pondscape differently, being attracted disproportionately to clusters of higher density ponds (Guadagnin & Maltchik 2007), or to more isolated ponds (Sebastián-González & Green 2014), depending on species, surrounding land use, and time of year. This influence over the dispersal pathways for aquatic macrophytes is likely to affect the strength and spatial scale of relationships between macrophyte richness and pond density. However, given little knowledge of the habitat preferences, or relative importance for seed dispersal of different waterfowl species, these components of functional connectivity would be difficult to measure.

Studies of macroinvertebrate diversity and inter-pond distances have produced similar results to those for macrophyte diversity, and again have tended to focus on the structural connectivity of the pondscape. This may be appropriate for strong flying insects, which perhaps experience little difficulty in crossing the terrestrial matrix between ponds. Shulman & Chase (2007) found that in a series of mesocosms located at varying distances from a central pond, macroinvertebrate diversity declined with isolation, with predator richness declining more rapidly than prey richness. Species richness and invertebrate community composition may be influenced by pond density / inter-pond distances at both the local and regional spatial scale; Raebel et al. (2012), found that the species richness of Odonata exuviae decreased with increasing distance between ponds, falling by more than 40% for distances over 100m. In other studies, relationships between invertebrate species richness and pond connectivity have been observed only at large spatial scales (>1km), attributed to the high dispersal capabilities of many invertebrate taxa (Briers & Biggs 2005; Florencio *et al.* 2014). Overall, most studies which have considered macrophyte and / or macroinvertebrate species richness, have identified a significant positive relationship with pond structural connectivity, although the spatial scale at which this relationship exists appears to vary with species. In the case of macroinvertebrates, this likely reflects the different functional connectivity experienced by stronger vs. weaker flying species, and different habitat suitability of the terrestrial matrix for different species.

Fragmentation of the pondscape is likely to be especially detrimental to dispersal-limited taxa, and has perhaps been most widely studied with regard to amphibians. Pond density has been found to significantly increase both amphibian diversity (Jeliazkov *et al.* 2014), and frequency of occurrence (Joly *et al.* 2001) in agricultural landscapes, although, as already mentioned above, the availability of terrestrial habitat may be equally if not more influential (Laan & Verboom 1990; Marsh & Trenham 2001). Similar to macrophytes and macroinvertebrates, the spatial scale at which pond connectivity is considered is an important factor for amphibian diversity and occurrence. Laan & Verboom (1990) found that some amphibian species only occurred in ponds within 1000 m of another pond containing the same species, while for other species the required nearest-neighbour pond distance was as low as 500 m.

Overall, understanding the implications of pond loss and pondscape fragmentation for biodiversity and community composition requires consideration of both structural and functional connectivity of the pondscape. Given the importance of structural connectivity in habitat fragmentation, combined with the difficulty of trying to accurately predict functional connectivity for multiple taxa across highly heterogeneous pondscapes (see *Section 3.5.4*), examining the influence of structural connectivity across different spatial scales provides a good starting point in understanding the ecological consequences of pond loss. Globally, there have been extensive losses of small agricultural wetlands (Wood *et al.* 2003; Dahl 2011; Ferreira & Beja 2013). With this trend predicted to continue (Semlitsch & Bodie 1998), improving our understanding of how to maintain species diversity and population viability across increasingly fragmented pondscapes is a major conservation concern.

This chapter examines the relative importance of environmental and spatial variables in determining the community structure, biodiversity, and occurrence of three groups of organisms in agricultural ponds; macrophytes (passive dispersers with high dispersal ability), aquatic Coleoptera (active dispersers with high dispersal ability), and amphibians (active dispersers with lower dispersal ability). Subsequently, the implications these findings have for understanding the effects of pondscape fragmentation, and for targeting pond restoration and management, are discussed.

4.2 Chapter Hypotheses

1) Community structure among aquatic macrophytes and Coleoptera are influenced by both environmental and spatial (pond density) variables.

2) Pond density is of secondary importance for species diversity, with local environmental variables, in particular pond shading, having a stronger influence upon pond biodiversity.

3) Once local environmental variables have been accounted for, pond density has a significant, independent effect upon macrophyte and aquatic Coleoptera diversity, and occurrence of amphibians.

4) Lower pond density (representing a more fragmented pondscape), is associated with lower within-pond (α-) diversity of both macrophytes and water beetles, and a higher likelihood that amphibians will be absent from lower density ponds.

5) Lower pond density is associated with higher between-pond (β-) diversity of both macrophytes and water beetles.

6) The effects of pond density are experienced at different spatial scales by the different taxa, with macrophyte and aquatic Coleoptera diversity more strongly influenced by pond density at a large scale (>1 km), while amphibian occurrence is more strongly influenced by pond density at a local scale (<1 km).

4.3 Methodology

4.3.1 Study site selection

Study ponds were selected to cover a wide range of different pond densities, identified during the pondscape mapping in chapter one. Nine ‘landscape-scale pond regions’, with different mean pond densities (*Table 12*), were chosen based on the following criteria;

1) Pond density: landscapes representing low, medium or high pond densities.
2) Pond type: landscapes containing comparable pond types (all agricultural ponds associated with marl subsoil, located in north Norfolk).
3) Ease of obtaining landowner access permissions.

Pond density was chosen as the measure of pond connectivity for several reasons. Pond density provides a simple measure of the key structural changes associated with pondscape fragmentation (increased isolation of individual ponds), and has been widely used in other studies of the spatial patterning of pond biodiversity (Gledhill *et al.* 2008; Raebel *et al.* 2012a; Florencio *et al.* 2014; Sebastián-González & Green 2014). Pondscape connectivity evidently involves other, more complex landscape features in addition to pond density, including the relative importance of different terrestrial habitat features (Laan & Verboom 1990; Scribner *et al.* 2001), relating to the different permeability of different land use types and habitats for different dispersing taxa (Lehtinen *et al.* 1999; Ray *et al.* 2002). However, attempting to include all possible aspects of pondscape connectivity requires a detailed understanding of taxa-specific dispersal requirements, land-use changes, and environmental factors, which are beyond the scope of this study.

Table 12 – The nine pond regions selected for the study.

Area	Mean pond density (ponds / km^2)	Range pond density (ponds / km^2)	Ponds surveyed / area	Ponds surveyed / density category
Hindringham	6.8	4 - 10	16	45
Bodham	8.2	1 - 13	15	
Godwick	9.4	4 - 15	14	
Heydon	12.1	8 - 15	14	39
Tittleshall	12.4	9 - 16	13	
Mattishall	14.6	10 - 20	12	
Colkirk	15.7	8 - 20	9	37
Lyng	19	12 - 25	12	
Guestwick	26.3	11 - 34	16	

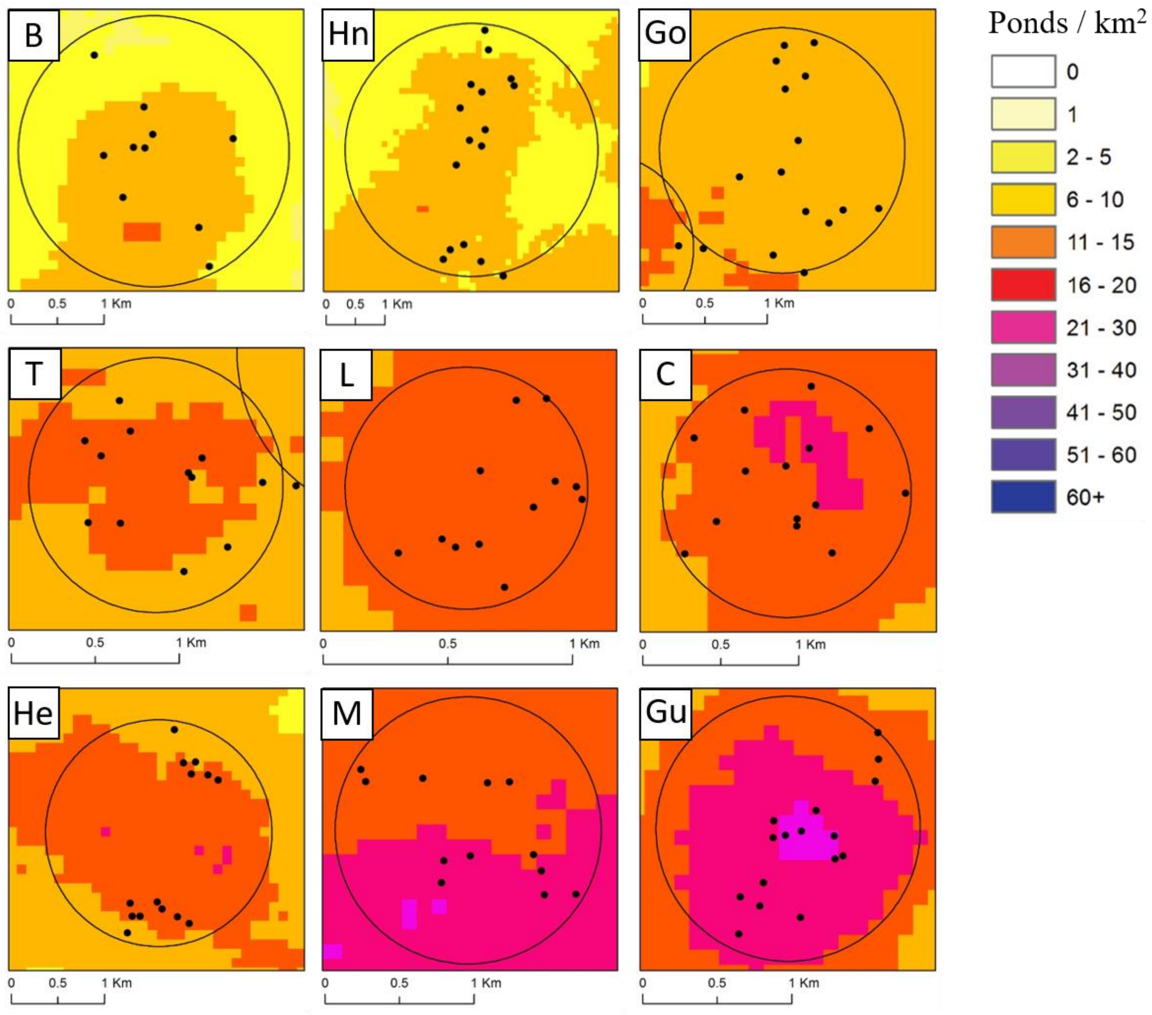


Figure 31 – Pond density / km^2 across the nine study regions. Black dots mark the study pond locations. B – Bodham, Hn – Hindringham, Go – Godwick, T – Tittleshall, L – Lyng, C – Colkirk, He – Heydon, M – Mattishall, Gu – Guestwick.

4.3.2 Field survey

A total of 121 ponds were surveyed between 21st May – 6th June 2013. Parameters recorded for each pond can be divided into three classes of variables; spatial variables (relating to pond density and inter-pond distance), environmental variables (relating to pond water chemistry or physical pond attributes), and biological variables (macrophyte species richness and cover, species richness of aquatic Coleoptera, and presence / absence of four native amphibian species). At each site, the following environmental variables were measured:

Physical parameters – *width and type of buffer strip, connection between pond and any ditch or pipe, slope of banks, % pond surface overhung by trees, % pond surrounded by trees, % pond surface covered by aquatic macrophytes, pond area* (m^2).

Water chemistry parameters – *Dissolved* O_2 ($\mu g\ L^{-1}$)*, pH, water temperature, conductivity* ($mS\ cm^{-1}$)*, alkalinity* ($mg\ CaCO_3\ L^{-1}$)*, turbidity (cm visibility), chlorophyll a concentration* ($\mu g\ L^{-1}$)*.* Dissolved O_2, pH, water temperature, and conductivity were measured on-site using a HACH multi-parameter meter. Alkalinity was measured on-site using a field titration kit. Turbidity was measured on-site using a turbidity tube. Water filtration (for chlorophyll *a* analysis) was conducted on-site, and filter papers were frozen on the day of collection for subsequent analysis.

Physical and water quality parameters were selected based on prior ecological knowledge of the key environmental determinants of pond biodiversity and community structure. Pond shading is anticipated to have a particularly strong impact upon pond biodiversity, with shading known to have a significant negative influence on biodiversity across multiple taxa (Boothby & Hull 1997; Mokany, Wood & Cunningham 2008; Hassall *et al.* 2012; Sayer *et al.* 2012). Pond shading was estimated by eye using two measures; *% pond surrounded by trees*, and *% pond surface overhung by trees*, assuming mid-day, direct sunlight. As both measures were highly correlated, subsequent analysis used *% pond surface overhung by trees* as the measure of pond shading. The number of heavily shaded ponds in the agricultural landscape has increased in parallel with agricultural pond loss (Boothby & Hull 1997; Sayer *et al.* 2012), with most ponds that escaped in-filling tending to be abandoned to succession and terrestrialisation. As such, pond shading is anticipated to be a major confounding variable in the analysis of the relationship between pond density and pond biodiversity.

Pond area has been found in some studies to be significantly positively related to pond biodiversity across various taxa (Brönmark 1985; Oertli *et al.* 2002; Beja & Alcazar 2003). However, under some circumstances the opposite trend may be true, in particular where larger ponds contain fish negatively influencing macrophyte and macroinvertebrate diversity (Scheffer *et al.* 2006). Physical parameters, including the presence / width of the buffer strip surrounding a pond, and whether the pond is connected to any ditches, pipes or drains, were included in the survey as they relate to agricultural run-off and pond water quality (Biggs *et al.* 1994; da Silva *et al.* 2012; Raebel *et al.* 2012a). The percent of the pond surface covered by aquatic macrophytes was recorded at each site, as this has been shown to be significantly related to the diversity and occurrence of both invertebrate and amphibian taxa (Bloechl *et al.* 2010; da Silva *et al.* 2012; Raebel *et al.* 2012a; Florencio *et al.* 2014).

Water chemistry parameters selected for the survey replicate those used in other studies of pond biodiversity (Angélibert *et al.* 2004; Hassall *et al.* 2011; Sayer *et al.* 2012; Hill & Wood 2014). In particular, dissolved O_2 often shows significant correlations with macroinvertebrate and amphibian diversity and occurrence (Gledhill *et al.* 2008; Rannap, Lõhmus & Briggs 2009; Sayer *et al.* 2012). Chlorophyll *a* (chl *a*) concentration was included as an indicator of algal productivity, and proxy for nutrient status of the ponds. Although nutrient status can have a strong influence over aquatic communities in oligotrophic and mesotrophic systems (Jeppesen *et al.* 1997, 2000; Biggs *et al.* 2005), these variables rarely have a significant influence in small farmland ponds (Linton & Goulder 2000; Hassall *et al.* 2011; De Marco *et al.* 2014; Rosset *et al.* 2014).

In addition, the following biological variables were recorded at each pond:

Aquatic macrophytes – all macrophyte species associated with wetland conditions, including submerged, emergent, and marginal species, were recorded and their abundance scored using the DAFOR scale, as described by Palmer, Bell & Butterfield (1992).

‘aquatic macrophytes’ were defined as plants listed in the National Pond Survey methods guide (Biggs *et al.* 1998). Common terrestrial or arable weed species which occurred within the pond margins were not recorded. Submerged aquatic macrophytes were surveyed using a double-headed rake thrown from the bank. Each pond was surveyed until no new species were found. All macrophytes were identified in the field to species level, with the exception of the Characeae, which were preserved in 75% isopropyl alcohol and later identified to species level using a dissecting microscope at x 20 – 40 magnification.

Aquatic Coleoptera – Surveys for adult aquatic Coleoptera were conducted at each pond following the invertebrate sampling protocol recommended by Biggs *et al.* (2005). Using a 1 mm mesh standard pond net, a three-minute sampling time was divided evenly between all pond mesohabitats. This was followed by 30 minutes ‘live-picking’ time, in which all adult water beetles collected within this time frame were preserved in 75% Isopropyl alcohol, for later identification. Identification was carried out to species level by Geoff Nobes. Unfortunately, this survey method produced very small sample sizes, causing problems for statistical analysis. While a longer survey time may have improved sample sizes, subsequent field trips with Geoff suggested that the trade-off between search time and sample size increase would be low. For heavily overgrown ponds, even searching for up to an hour would sometimes result in a single beetle being found.

Amphibians – A short amphibian survey (approx. 15-minute search time), was conducted at each study pond, with a search for eggs, larvae, and adults of the four native species found in north Norfolk; the great crested newt *Triturus cristatus*, smooth newt *Lissotriton vulgaris,* common toad *Bufo bufo* and the common frog *Rana temporaria*. Larvae and adults were recorded as present if they were found during the macrophyte or aquatic Coleoptera sampling. A visual search (lasting 15 minutes), was then made for frog or toad spawn, and the leaves of submerged aquatic vegetation were checked for eggs of great crested and smooth newt. Although the palmate newt *Lissotriton helveticus* has been recorded in some ponds in Norfolk, it is extremely uncommon, and as such all ‘small newt’ type eggs were recorded as smooth newt. Because only one survey was conducted at each pond, ‘absence’ of amphibians does not necessarily represent true absence, but rather an indication of the suitability of each site for amphibians. Since more suitable ponds will host a higher density of amphibians, linked with a higher detection probability, data was comparable between all ponds surveyed.

4.3.3 Analysis – wetland macrophytes

Relationships between wetland macrophyte diversity and pond density were examined through multiple linear regression (MLR), an approach widely used to examine species diversity patterns in relation to multiple predictor variables (Brönmark 1985; Krauss *et al.* 2004; Gledhill *et al.* 2008; Akasaka & Takamura 2011; Bosiacka & Pienkowski 2012). MLR was conducted in SPSS Statistics 22, with environmental and spatial variables added in two separate blocks; block one contained significant environmental variables, while block two contained significant spatial variables. This approach allows for any significant, independent effect of the spatial variables to be identified from the effect of the environmental variables, as a change in R^2 between the two models (Tabachnick & Fidell 2007). Similar approaches for incorporating spatial and environmental variables into a ML model have been used by Ficetola & Bernardi (2004); Gledhill *et al.* (2008); Bosiacka & Pieńkowski (2012).

Environmental variables (from the list in section 4.2.3), were selected for inclusion in regression analysis based on ecological relevance, and statistical significance in an exploratory correlation matrix (Tabachnick & Fidell 2007). Selection of only the significant environmental and spatial variables for inclusion in the MLR was necessary to reduce the number of extraneous predictor variables in the model, and to improve its statistical power (Ficetola & De Bernardi 2004; Tabachnick & Fidell 2007). All environmental variables were transformed to Z values to improve normality (Linton & Goulder 2000; Tabachnick & Fidell 2007; Gledhill *et al.* 2008), and were screened for univariate & multivariate outliers, normality of residuals, linearity, co-linearity (correlation > 0.9), and homoscedasticity (Tabachnick & Fidell 2007).

Pond density within a given search radius around each study site (200m, 500m, 1000m, 2000m, 5000m), provide the spatial predictor variables used in the model. Pond densities were calculated in ArcGIS 10.2. Pond densities at the local (200 – 500m), to the broader landscape scale (2000 – 5000m), were included in the analysis, to examine the different spatial scales at which pond density might influence taxon diversity (Tscharntke *et al.* 2005; Beisner *et al.* 2006; Gledhill *et al.* 2008). All spatial variables were included in the exploratory correlation matrix, and those which were significant (or if none were significant, those with the highest R^2 value), were then included in subsequent regression analysis. Spatial variables were screened for normality, normality of residuals, linearity, and co-linearity, prior to analysis.

α - diversity within each pond was measured as species richness of wetland macrophytes. Species richness was used as a measure of macrophyte diversity for consistency with the existing literature (Oertli *et al.* 2002; Edvardsen & Økland 2006; Gledhill *et al.* 2008; De Marco *et al.* 2014; Hill & Wood 2014), and for consistency between the analysis of α- and β- diversity. Species richness was also deemed the most appropriate measure of plant α-diversity in the context of understanding how pond density and connectivity influence diversity independently of environmental factors – species richness is more strongly influenced by spatial determinants of colonisation and dispersal, and less influenced by subsequent differences in species dominance and establishment success, than diversity indices incorporating species evenness and abundance (Tuomisto 2010a).

β - diversity of wetland macrophytes was calculated as the mean pairwise Jaccard dissimilarity for each pond site, as suggested by Whittaker (1972), Tuomisto (2010), and Chen (2013). Macrophyte incidence data were Hellinger transformed (R package 'vegan'), so that dissimilarity values became more ecologically meaningful (Borcard *et al.* 2004; Beisner *et al.* 2006; Legendre & De Cáceres 2013). A Jaccard dissimilarity matrix was then constructed in R package 'vegan', and the mean dissimilarity for each site was used in MLR in SPSS, following the same procedures for data screening and selection of variables as for analysis of α- diversity.

4.3.4 Analysis – aquatic Coleoptera

It was intended that analysis of aquatic Coleoptera diversity would be conducted in the same way as for wetland macrophytes. However, a large number of study sites produced no adult Coleoptera within the standard 3-minute survey, and overall Coleoptera diversity was extremely low (44 of the 121 ponds surveyed contained no water beetles, and only 12 ponds contained more than 5 species). As such, exploratory correlations based on incidence data were used to describe broad patterns in Coleoptera occurrence. Analysis was then conducted on Coleoptera incidence data grouped by ‘pond density region’ (*Table 12*), in order to allow large enough sample sizes for analysis. These nine pond density regions were classified by mean pond density (measured at different spatial scales), as either ‘below average’ or ‘above average’ pond density (*Table 13*), as suggested by Boothby (1997). Independent ANOVAs were conducted to check for significant differences in pond density between the ‘below average density’ and ‘above average density’ categories, and for any significant differences in environmental variables, which could confound the results.

Table 13 – The nine pond regions and their associated mean pond density, measured at five spatial scales. Regions within the top part of the table have above average pond densities, and those within the bottom part of the table have below average pond densities.

Mean ponds within 200m		Mean ponds within 500m		Mean ponds within 1000m		Mean ponds within 2000m		Mean ponds within 5000m	
Hindringham	1.4	Hindringham	5.5	Hindringham	18.4	Bodham	59.6	Bodham	280.7
Bodham	2.0	Godwick	7.6	Bodham	21.2	Hindringham	66.9	Hindringham	349.1
Godwick	2.1	Bodham	8.8	Godwick	26.0	Tittleshall	94.5	Heydon	423.4
Colkirk	2.3	Heydon	9.1	Tittleshall	32.0	Godwick	100.2	Tittleshall	492.2
Tittleshall	2.5	Tittleshall	9.7	Heydon	32.3	.	.	.	.
Heydon	2.7	Mattishall	12.0	Lyng	39.4	Colkirk	109.8	Colkirk	510.2
Mattishall	3.2	Colkirk	12.3	Colkirk	41.7	Heydon	111.5	Lyng	570.6
Lyng	3.6	Lyng	14.8	Mattishall	48.8	Lyng	119.0	Godwick	637.9
Guestwick	4.4	Guestwick	21.9	Guestwick	56.9	Guestwick	121.0	Guestwick	664.4
.	.	.	.	.	.	Mattishall	181.7	Mattishall	785.3

Unlike wetland macrophytes, for which a relatively complete census of each pond was possible, the aquatic Coleoptera survey provides an incomplete sample of species richness at each pond, with different relative sampling effort between ponds of different sizes and types. As such it is necessary to apply a species richness estimator to the data (Colwell & Coddington 1994; Hortal, Borges & Gaspar 2006). Because data were grouped by region (making each pond within a region a replicate), the ICE species richness estimator was used (R package ‘fossil’), which estimates sample coverage in a set of replicated incidence samples (Gotelli & Colwell 2011). The ICE species estimator is a robust estimator of minimum species richness across multiple scales (Hortal *et al.* 2006; Gotelli & Colwell 2011), and has been used in other studies of pond macroinvertebrate diversity (Davies *et al.* 2008b). All species estimators are strongly influenced by sample coverage (Hortal *et al.* 2006), and given the very low diversity recorded across the majority of ponds in this survey, results should be interpreted with caution.

β - diversity of aquatic Coleoptera was examined using chord normalised expected species shared (CNESS), between the same pond regions used for analysis of α- beetle diversity. Coleoptera incidence data were Hellinger transformed in R package 'Vegan' (Borcard *et al.* 2004; Beisner *et al.* 2006; Legendre & De Cáceres 2013). CNESS results were then calculated using the software COMPAH. CNESS index calculates pair-wise similarity, from the expected species shared between two random draws of *m* individuals from two samples. As such, the index can be made more or less sensitive to rare species by adjusting *m*, the number of individuals being randomly drawn from each sample (Trueblood, Gallagher & Gould 1994). A smaller *m* will strongly weight changes in the dominant species, while a large *m* will make the index more sensitive to changes in rarer species. Three levels of CNESS score were selected for this analysis:

$m = 1$: Dominant species weighting.
$m = 7$: Intermediate weighting, using the highest richness of aquatic Coleoptera species common to all nine regions.
$m = 31$: Rare species weighting, using the highest richness of aquatic Coleoptera species common to eight of the nine regions (Lyng is omitted from this analysis due to low richness of aquatic Coleoptera species).

4.3.5 Analysis – amphibians

Occurrence of the four native amphibian species found across the study area was assessed through logistic regression, a robust method for analysing data with a dichotomous dependant variable (presence / absence of amphibians), and continuous predictor variables (Tabachnick & Fidell 2007). The approach is widely used in the analysis of amphibian occurrence (Gulve 1994; Scribner *et al.* 2001; Ficetola & De Bernardi 2004; Rannap *et al.*

2009). Independent t-tests were conducted to select significant predictor variables for inclusion in the logistic regression (Tabachnick & Fidell 2007). Environmental and spatial variables were then added to the model as separate blocks (Ficetola & De Bernardi 2004), in the same manner as for the analysis of macrophyte diversity, with model 1 containing significant spatial variables, and model 2 containing significant spatial + environmental variables. Logistic regression was conducted in STATA.

4.3.6 Analysis – aquatic macrophyte and Coleoptera community structure

To further explore patterns in community structure of aquatic macrophytes and Coleoptera, a series of ordinations were performed in CANOCO 5. All environmental variables were standardised (Z scores), and species data were Hellinger transformed (Leps & Smilauer 2003; Legendre & Legendre 2012). Rare species were down-weighted, and only significant environmental variables (*Table 16* and *Table 17*), were included in the analysis, thus avoiding co-linear predictors. Environmental variables across the study ponds were explored through principle component analysis (PCA). Constrained ordinations (CCA) were then conducted separately for macrophytes and Coleoptera, to examine the variation in species composition explained by the environmental variables. To correct for spatial autocorrelation (see further details in *section 4.3.7*), partial CCA were also conducted for macrophyte and Coleoptera data, using the spatial coordinates of the ponds as co-variables (Legendre & Fortin 1989; ter Braak & Verdonschot 1995; Leps & Smilauer 2003). To see if there was any relationship between macrophyte and Coleoptera community structure, the significant principle components from a PCA of macrophyte species (PC1 & PC2), were included as predictor variables in a constrained ordination (CCA), of aquatic Coleoptera community structure.

In addition to the partial CCAs, which essentially provide a way of removing the ‘nuisance’ effect of spatial autocorrelation from the community structure, variation partitioning was also conducted. This method provides a way of examining the amount of variation in community structure which is due to spatial processes (including spatial autocorrelation), and that due to environmental processes. This method is described in detail in the paper and supporting material of De Bie *et al.* (2012). The approach essentially consists of conducted two canonical ordinations, one constrained by environmental variables, the other constrained by spatial variables, and then using these to 'partial out' the variance in community structure (Borcard, Legendre & Drapeau 1992). First, an environmental model (matrix) is constructed, based on a parsimonious combination of significant environmental variables (selected by forward selection). Next, a spatial model is constructed using significant principle components (PCNMs), extracted from a Euclidean distance matrix of the locations of the sample sites. Variation partitioning then seperates out the variation in the species data which is uniquely explained by the environmental model, that explained uniquely by the spatial model, and that explained by the environmental / spatial interaction (De Bie *et al.* 2012). Variation partitioning was conducted in R version 3.2.3, using packages "vegan" and "packfor", following the R code provided by De Bie *et al.* (2012). Species presence / absence data were Hellinger transformed, and environmental variables converted to Z scores, prior to analysis. Significant environmental and spatial variables were selected for the model by applying forward selection, following the procedure of De Bie *et al.* (2012).

4.3.7 Adjusting the current statistical analysis to account for spatial autocorrelation

What is spatial autocorrelation?

Spatial autocorrelation (SAC) occurs when values of variables sampled at nearby locations are not independent from one another (Legendre & Fortin 1989; Dormann *et al.* 2007). This can be both an interesting functional feature of ecological data, which should be studied for its own sake, but it can also be a problem, because structure or autocorrelation violates many assumptions made by standard statistical tests (Legendre 1993; Borcard & Legendre 2002; Briers & Biggs 2005; Guillot & Rousset 2013).

In ecological data, SAC tends to form patches or gradients, and is often found across wide spatial scales. Patterns in SAC reflect both the dispersal of organisms (often resulting in patches at different spatial scales), and environmental gradients between sampling sites (Legendre 1993). Species data frequently shows positive SAC, where sites close together are more similar in species composition than those further apart (Legendre & Fortin 1989;

Borcard *et al.* 1992; Briers & Biggs 2005). Sometimes however the opposite may be true, for example among solitary animals, where avoidance can lead to negative SAC. Alternatively, both positive and negative SAC may occur within the same data set, but over different distance classes. This can happen in environmental data measured along a gradient, where positive autocorrelation at short distance classes will be coupled with negative autocorrelation at long distance classes (Legendre and Fortin, 1989). Positive SAC can lead to increased type 1 errors in standard statistical tests (Legendre & Fortin 1989), and can alter the apparent explanatory power of different environmental variables (Keitt *et al.* 2002). As such, methods to detect and then remove or account for the effects of SAC, are an important consideration in ecological analysis.

At present, SAC is not properly accounted for in this thesis. To remedy this, several different approaches for detecting and accounting for SAC are available. These are discussed in the following sections, and those deemed most appropriate for use with the data presented in this thesis are highlighted for future re-analysis.

Detecting and quantifying SAC

In order to correctly account for SAC within ecological analysis, it is first necessary to establish whether the data exhibit SAC, and if so, where this is occurring. SAC may occur in the explanatory variables, the dependant variable(s), or in the residuals of a non-spatial model. If model residuals display SAC, they are not independent of one another, violating the assumptions of standard statistical tests (Legendre & Fortin 1989; Dormann *et al.* 2007). This can be tested by calculating Moran's *I* or Geary's *c* coefficients (Legendre & Fortin 1989). The Moran's *I* test for SAC is one of the most common approaches used in ecological analysis, and can also be applied to individual variables to identify which components exhibit SAC (Legendre & Fortin 1989; Dormann *et al.* 2007). The test is easily conducted in R, package "spdep". Moran's *I* is calculated by first creating a spatial weights matrix. This essentially defines the distances between all samples, weighting this so that samples that are closer together have more influence over one another than samples further apart. The researcher is required to define the distance limit for the test, which should cover the largest distance between samples, without going too far beyond this. Setting the distance limit much higher than the spatial scale used in the study can influence the significance of the test, and could miss spatial structuring at the smaller spatial scale (Dray, Saïd & Débias 2008). Once the spatial weights matrix has been created for calculating Moran's *I*, the eigenvectors from this same matrix can be used to correct for SAC using spatial filtering (Griffith 2000; Getis & Griffith 2002), which is described below.

If SAC is present in the data (significant Moran's *I*), structure functions, particularly correlograms, can be used to visualise the patterns and extent of SAC. Correlograms (for single variables), or Mantel correlograms (for multivariate data), plot SAC values against distance, with the shape of the resulting plot describing the type and scale of spatial structure occurring in the data (Legendre & Fortin 1989; Legendre 1993).

An alternative approach for testing for SAC is the Mantel test (Mantel 1967), which tests the significance of correlation between two square distance matrices (Briers & Biggs 2005; Guillot & Rousset 2013). The partial Mantel test was later proposed by Smouse et al. (1986), to test the dependence between two distance matrices, while controlling for the effects of a third distance matrix (i.e. testing the dependence between a species dissimilarity matrix and environmental matrix, while controlling for the spatial distance matrix). While Mantel and partial Mantel tests remain popular in ecological analysis (Briers & Biggs 2005; Zealand & Jeffries 2009; Capers, Selsky & Bugbee 2010), various authors have raised concerns over inflated type I error rates and low power (Guillot & Rousset 2013). In particular, Guillot and Rousset (2013) note that the Mantel test is valid only in cases where both matrices are stationary (the direction and magnitude of SAC is constant across all sites), and one of the matrices is not autocorrelated. However, this is rarely the case in ecological data. As such, the Moran's *I* test, which is essentially a 'special case' of the Mantel test (Legendre, 2000), is often more appropriate.

Future re-analysis: The Moran's I test for SAC can be conducted in R, package "spdep". For the data presented in Chapter 4, a suitable distance limit would be 30 km, representing the greatest distance between study ponds. Moran's I should be conducted to test for SAC in the residuals of the regressions. For the mesocosm data in Chapter 5, a smaller neighbourhood distance of around 50 m should be used for both testing for SAC, and adjusting the statistical analysis (if necessary). For the pond density data presented in

Chapter 3, Moran's I could be calculated within ArcGIS using a neighbourhood distance of 120 km, to detect SAC in pond density change across the county.

Accounting for SAC in statistical analysis

Not only can SAC be a statistical 'nuisance', but the spatial organisation of both predictor and dependant variables is also a key function of ecological systems (Legendre 1993; Borcard & Legendre 2002). Different statistical methods are available for analysing SAC, some of which aim to remove the problem of autocorrelation between samples, and others which explicitly aim to quantify this spatial component. Broadly speaking, these approaches can be split into two groups; those which use a linear combination of geographic coordinates to express spatial structure, and those that represent spatial structure through a matrix of geographic distances among samples (Legendre 1993). Both types of approach can be incorporated into canonical or regression-based analysis.

SAC and canonical analysis – ordinations of community structure

Community structure in ecological datasets is commonly assessed using ordination techniques. This family of multivariate methods extract synthetic environmental gradients from ecological data-sets, which can more succinctly describe and visualise the different habitat preferences of taxa (ter Braak & Verdonschot 1995; Legendre & Gallagher 2001). Various approaches exist for correcting for SAC within ordinations. One of the simplest options is to conduct a partial constrained ordination, in which the coordinates of the study sites are included as covariables (Legendre & Fortin 1989; ter Braak & Verdonschot 1995; Leps & Smilauer 2003). *This is the approach used in the current study (section 4.4.4).* This method is suitable for removing the 'nuisance' effect of SAC among the data, revealing the relative importance of the environmental variables once SAC has been accounted for. However, examining the pattern of SAC, and how this influences the dependant variable(s), is often also of interest to ecologists, especially in studies of species dispersal and distribution patterns.

Where SAC in the dataset is of explicit interest, the extraction of spatial eigenvectors (sometimes known as spatial eigenvector mapping; Dormann et al., (2007), may be a suitable approach. This method requires first creating a distance matrix, which describes the Euclidean distances between all pairs of sample sites. From this matrix, the significant eigenvectors which best describe the spatial data, i.e. those that maximise Moran's Index of autocorrelation, can be extracted using forward selection procedures in MEM analysis (De Bie et al., 2012), also known as PCNM analysis (Borcard *et al.* 2004). These principal coordinates can then be incorporated directly as explanatory variables in ordinations, or in regression analysis (Dray *et al.* 2008; Borcard, Gillet & Legendre 2011). Incorporating spatial eigenvectors into regression analysis is known as 'spatial filtering', and is described in more detail in the next section – *SAC and regression-based analysis*.

Using spatial eigenvectors in canonical analysis controls for the effects of SAC in much the same way as using the raw coordinates of sample locations. However, it has an advantage in that the wavelength structure of the principal coordinates can be analysed, revealing patterns in the spatial scale(s) over which SAC is occurring (Borcard & Legendre 2002; Borcard *et al.* 2004; Dray, Legendre & Peres-Neto 2006; De Bie *et al.* 2012). In addition, the eigenvectors extracted from the spatial distance matrix can be used in variation partitioning analysis (Beisner *et al.* 2006; De Bie *et al.* 2012). Spatial eigenvectors can be extracted in R package "spdep".

To examine the spatial structure in the community data presented in Chapter 4, two different approaches that account for SAC have been used. First, partial constrained ordinations (partial CCA), were conducted to explore the relative importance of the environmental variables, once SAC had been accounted for. Next, variation partitioning was conducted for both the macrophyte and aquatic Coleoptera data, examining the amount of variation in community structure which was explained by the environmental variables, and that explained by the spatial arrangement of ponds.

SAC and regression-based analysis

There are a wide range of methods available for incorporating SAC into regression-based analysis. Dormann et al., (2007) provide a good review of some of the most common approaches, along with some of their advantages / disadvantages. Here I discuss two of the most common approaches used in ecological analysis; spatial autoregression (which is based on generalised least squares regression), and spatial filtering (based on ordinary least squares regression). For the data presented in this thesis, the spatial filtering approach is deemed most suitable, as it can be directly incorporated into the existing ordinary least squares regression model. The future application of this method is described at the end of this section.

Autoregressive models, which are closely related to generalised least squares regression (Quinn *et al.* 2001; Dormann *et al.* 2007), have proved a popular method for dealing with SAC in ecological data. Similar to other approaches for dealing with SAC, autoregressive models start with a distance matrix, describing the distances between all pairs of sample sites. These could be Euclidean distances, or some other measure of connectivity, such as least cost distances (Adriaensen et al., 2003). This distance matrix is used to provide an additional term in the model that accounts for variation in the variables (or residuals), which is related to the values recorded at neighbouring locations, rather than to the local environmental variables (Keitt *et al.* 2002; Dormann *et al.* 2007). Different types of autoregressive model exist for accounting for SAC in the dependant variables (lagged-response model), dependant and independent variables (lagged-mixed model), or for when SAC occurs in the error term only (spatial error model). The Moran's *I* test can be conducted to detect where SAC is occurring in the data, and if more than one model is suitable, the Lagrange multiplier test can be performed to select the best fitting model (Denoël & Lehmann 2006; Dormann *et al.* 2007).

Studies which have made use of spatial autoregressive models include those of Kühn, (2007), who re-examined data on plant species richness in relation to environmental correlates in Germany, and Keitt et al., (2002), who present a range of case studies analysed using autoregressive models. In both cases, the authors demonstrated that autoregressive models provided a significantly better fit than non-spatial models, and that omitting SAC from analysis could alter the relative importance of environmental predictors, even to the extent that an inverse trend was erroneously observed (Kühn 2007).

Although autoregressive models are becoming more widely used in ecological analysis, they have a number of limitations. Autoregressive models almost exclusively assume normality, spatial isotropy (processes causing SAC are in the same direction across all regions), and stationarity (SAC constant across whole region). Where SAC results at least in part from organism dispersal, these assumptions are often violated. Stationarity is often violated by patch dynamics and dispersal limitation, while isotropy may be violated by directional dispersal, for example with wind or water direction (Dormann *et al.* 2007). In addition, autoregressive models are related to generalised least squares regression, and as such require different analysis and test statistics from the more commonly used ordinary least squares regression (Getis & Griffith 2002; Dormann *et al.* 2007).

In contrast, the spatial filtering approach can be directly incorporated into traditional linear regression (Getis & Griffith 2002), such as that currently used in *Chapter 4*. Spatial filtering is a relatively new approach in ecological analysis, which involves using spatial eigenvectors (as described in the previous section – *SAC and canonical analysis*), as covariates within the regression model (Dormann *et al.* 2007). Essentially, spatial filtering techniques convert variables that are spatially autocorrelated, into spatially independent variables in an ordinary least squares regression framework (Getis & Griffith 2002). This approach has a number of advantages over other methods:

1) Spatial filtering can use the same eigenvectors extracted for partial constrained ordination or variance partitioning analysis (Keitt *et al.* 2002; Griffith & Peres-Neto 2006), meaning that canonical and regression analysis can be conducted using the same spatial components.

2) The wavelength structure of distance based eigenvectors (extracted through MEM analysis), provides information on the spatial scale of SAC (De Bie *et al.* 2012), which can be useful in understanding dispersal and distribution patterns. Spatial filtering is suitable for capturing SAC across a wide range of geographic scales (Griffith & Peres-Neto 2006).

3) Spatial filtering does not assume spatial isotropy or stationarity, making it more flexible than other spatial approaches (Griffith & Peres-Neto 2006), including autoregressive methods (Getis & Griffith 2002).

Once suitable eigenvectors have been selected for use in spatial filtering, for example using forward selection procedures (Beisner *et al.* 2006; De Bie *et al.* 2012), the chosen eigenvectors can then be used as covariates within a linear regression model. The residuals of this new model should now be free from SAC, which can be checked using the Moran's *I* test (Griffith & Peres-Neto 2006). Likelihood ratio tests can be used to compare the goodness of fit of the original spatially autocorrelated model, and the new corrected model, or to compare the goodness of fit between two models in a hierarchical regression, both of which have been corrected for SAC using spatial filtering.

Perhaps due to the newness of the technique, there are relatively few empirical studies of ecological analysis which have employed spatial filtering. At one end of the geographic scale of SAC, Diniz-Filho and Bini, (2005), use spatial filtering to evaluate patterns in bird species richness across the whole of South America, correcting for SAC. The authors selected eigenvectors for spatial filtering by performing multiple regressions, fitting species richness successively to each eigenvector, and selecting those that made a significant contribution for spatial filtering. Selection of eigenvectors is important to avoid 'overcorrecting' for SAC (Getis & Griffith 2002; Diniz-Filho & Bini 2005). At the other end of the scale, dealing with predominantly short geographic distances, Griffith and Peres-Neto, (2006) employ spatial filtering in their analysis of oribatid mite species data, collected over an area of 10 x 2.5m. Their analysis found spatial filtering to be a suitable method for removing SAC in the model residuals, and one which performed better than autoregressive models.

Future re-analysis: Spatial filtering and the extraction of significant spatial eigenvectors can be conducted in R, package "spdep". For the analysis presented in Chapter 4, it is suggested that spatial eigenvectors should be included as covariates in the hierarchical regression for macrophyte species richness, and likelihood ratio tests used to compare the fit of the two models (after correction for SAC). Similarly, spatial filtering using significant eigenvectors as covariates, should be conducted for the logistic regression of amphibian occurrence, and ANCOVA of aquatic Coleoptera richness (Dormann *et al.* 2007).

4.4 Results

Screening of the spatial and environmental variables prior to analysis indicated the presence of a small number (5), of univariate outliers within the variables; conductivity, chlorophyll a concentration, pond area, and number of ponds within 200m of the study site. Once these outliers were removed from the dataset, no multivariate outliers remained. After transformation to Z scores, all environmental and spatial variables were within acceptable limits of linearity and normality of residuals (Tabachnick & Fidell 2007). No variables, including the measures of pond density (*Table 14*), exhibited multicollinearity. Descriptive statistics for all spatial, environmental, and biological variables considered in the analysis are given in *Table 15.*

Table 14 - Table of correlations for pond density measured at different spatial scales. ** significant at the 0.01 level.

200 m	200 m				
500 m	0.643**	500 m			
1000 m	0.552**	0.797**	1000 m		
2000 m	0.357**	0.390**	0.724**	2000 m	
5000 m	0.368**	0.443**	0.699**	0.864**	5000 m

Table 15 – Descriptive statistics for the spatial and environmental variables.

Spatial variables	N	Min	Max	Mean	SD
Ponds within 200m	121	1	8	2.69	1.54
Ponds within 500m	121	1	29	11.40	6.19
Ponds within 564m	121	1	34	14.02	7.08
Ponds within 1000m	121	4	65	35.48	13.74
Ponds within 2000m	121	43	199	106.94	35.33
Ponds within 5000m	121	218	829	526.40	157.58
Nearest neighbor	121	1	600	155.85	102.17
Environmental variables	N	Min	Max	Mean	SD
Pond area (m^2)	121	35.00	1140.00	306.01	227.67
Chl a	121	0.31	263.12	27.57	50.82
O_2 (μg L^{-1})	121	0.80	20.06	8.26	4.32
pH	121	6.51	9.31	8.05	0.49
Conductivity (mS cm^{-1})	121	46.00	1798.00	643.38	251.93
Alkalinity (mg $CaCO_3$ L^{-1})	121	50.00	520.00	240.82	89.19
% surface overhung by trees	121	0.00	100.00	51.09	37.77
Macrophyte biological variables	N	Min	Max	Mean	SD
Macrophyte species richness	121	0.00	21.00	6.20	5.28
Macrophyte Jaccard dissimilarity	105	1.76	2.80	2.04	0.27
Macrophyte % cover	121	0.00	100.00	31.32	35.87
Coleoptera biological variables	N	Min	Max	Mean	SD
Coleoptera ICE	9	1.00	28.00	13.00	10.75
Coleoptera CNESS m=1	9	0.91	1.03	0.95	10.75
Coleoptera CNESS m=7	9	0.85	0.98	0.89	0.38
Coleoptera CNESS m=31	9	0.73	0.82	0.77	0.37

4.4.1 *Wetland macrophyte diversity*

In total, 70 species of aquatic macrophyte were identified during the survey, including one UK Biodiversity Action Plan (BAP) priority species, *Oenanthe fistulosa*, which was recorded in a single pond. Two invasive species, *Crassula helmsi* and *Fallopia japonica* were also recorded, each in a separate pond. The most species rich pond surveyed contained 21 aquatic macrophyte species, compared to the mean of 6.2 species. Sixteen ponds contained no aquatic macrophytes.

Variables showing significant relationships with macrophyte species richness in an exploratory correlation matrix (*Figure 33*), were included in subsequent MLR models. The two broadest scale measures of pond density (number of ponds within 2000 m and 5000m of study site), showed a significant negative correlation with macrophyte species richness. Pond density measured at all other spatial scales was a non-significant predictor. The environmental variables pond shading, alkalinity, and conductivity, showed significant negative correlations with macrophyte species richness, while dissolved oxygen and pond area showed significant positive correlations with macrophyte species richness.

Significant predictor variables were added to the MLR in two blocks; Model 1 contained significant environmental predictors, while Model 2 contained significant environmental + spatial predictors (*Table 16*). Model 1 (environmental predictors), was significant ($p < 0.001$), and explained 29% variance in macrophyte α – diversity. In this model, shade ($p < 0.001$) and pond area ($p < 0.1$) independently predicted α – diversity, with increasing shading having a negative influence, and increasing pond area a positive influence. Adding spatial variables to the model (Model 2), significantly improved variance explained ($p < 0.01$), suggesting that pond density at broad spatial scales influences macrophyte α – diversity. Overall, environmental + spatial variables explained 33% variance in macrophyte α – diversity, with pond shading, pond density at 5,000 m and 2,000 m, and pond area, significantly predicting macrophyte α – diversity. Pond shading was the strongest determinant of macrophyte α – diversity, with heavily shaded ponds associated with lower macrophyte species richness (*Figure 32*).

Although pond density at 2,000 m and 5,000 m was significantly correlated with macrophyte α – diversity (*Figure 33*), in the MLR model neither variable significantly predicted α – diversity. This may be because the density measures were highly correlated ($r = 0.86$, see *Table 14*), and therefor in the regression neither variable predicted α – diversity independently of the other. When the analysis was re-run using pond density at 2,000 m and 5,000 m in separate models, both predicted macrophyte α – diversity independently of environmental variables (*Table 16*).

Table 16 – MLR aquatic macrophyte α - diversity . ***p < 0.001, ** p < 0.01, * p < 0.05.

	Model 1			Model 2		
		95% CI			95% CI	
	β	lower	upper	*β*	lower	upper
Shade	-1.9 ***	-2.85	-0.96	-1.93***	-2.85	-1.01
Area	1.15 **	0.37	1.97	1.10**	0.30	1.90
Alkalinity	-1.07	-2.25	0.10	-0.91	-2.06	0.23
O_2	0.059	-0.80	0.92	-0.23	-1.12	0.66
Conductivity	0.025	-1.16	1.21	-0.17	-1.33	1.00
Pond density 5,000 m	-	-	-	-1.08**	-1.90	-0.27
Pond density 2,000 m	-	-	-	-1.00*	-1.84	-0.17
F (5, 115)	10.85***			10.67***		
Adj R^2	0.29			0.33		
R^2 change	6.98**					

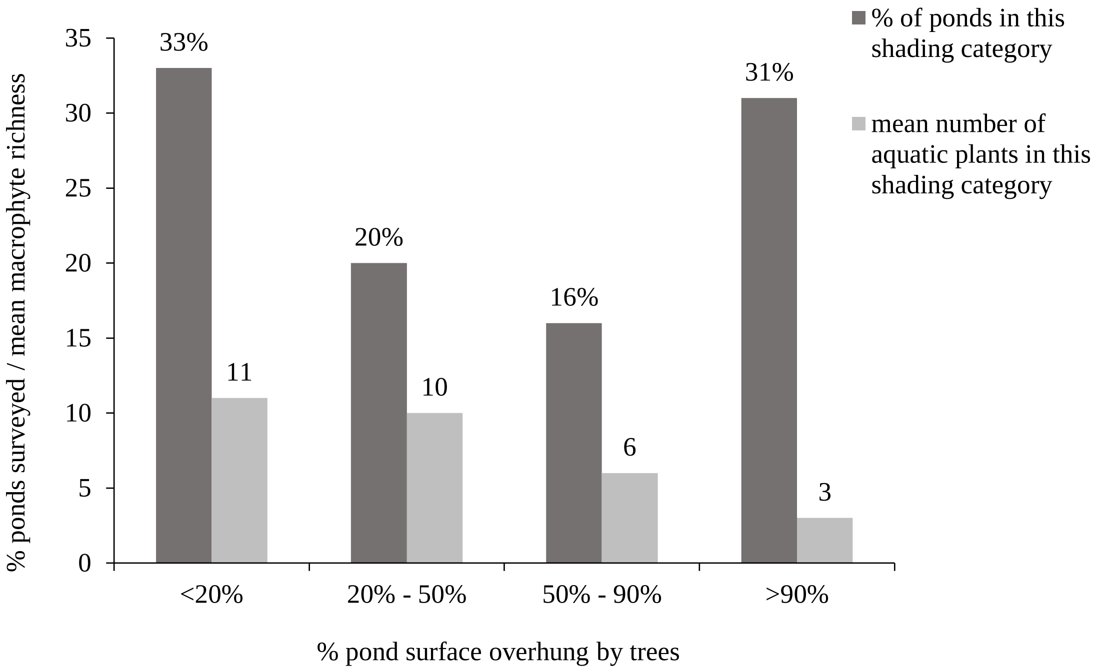


Figure 32 – Percentage of sampled ponds within each of four shading categories, and their associated macrophyte species richness (across all pond density regions).

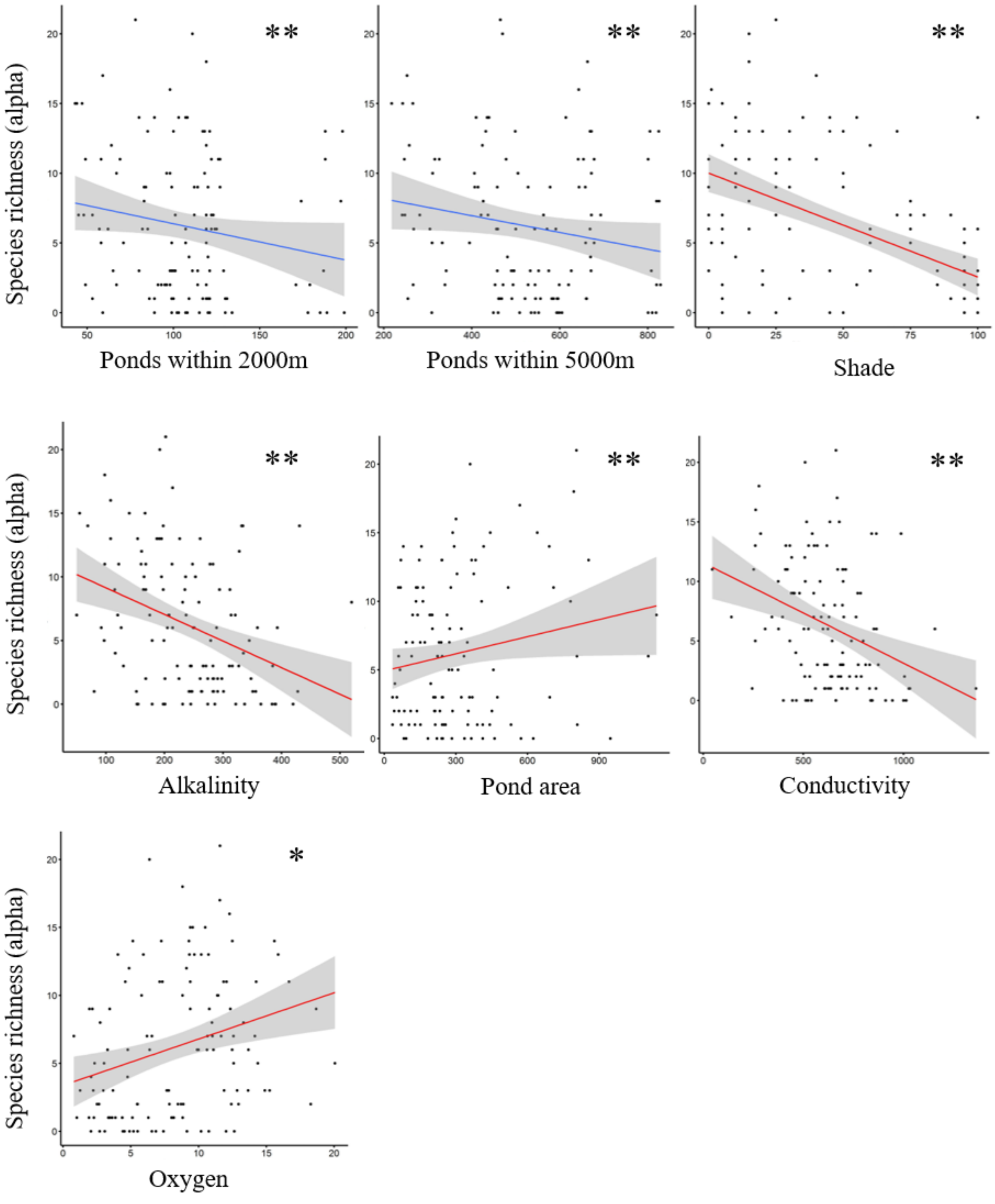


*Figure 33 – Significant correlations with macrophyte species richness. ** $p < 0.01$, * $p < 0.05$. Blue lines are used for pond density variables, red for environmental variables.*

Macrophyte β- diversity was significantly positively correlated with the environmental variables alkalinity, conductivity, and pond shading, and significantly negatively correlated with chlorophyll *a* and dissolved O_2 (*Figure 34*). No spatial variables were significantly correlated with β- diversity, but for consistency with the macrophyte α- diversity analysis, ponds within 2,000 m and 5,000 m were again used in Model 2 (environmental + spatial model).

Model 1 (environmental predictors), was significant ($p < 0.001$), and explained 26% variance in macrophyte β- diversity. In this model, shade ($p < 0.001$) was the only variable that independently predicted β- diversity. Adding spatial variables to the model (Model 2), did not significantly improved variance explained, suggesting that pond density does not influences macrophyte β- diversity. Overall, environmental + spatial variables explained 27% variance in macrophyte β- diversity, with pond shading being the only significant predictor of macrophyte β- diversity.

Table 17 – MLR aquatic macrophyte β - diversity. ***$p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

	Model 1			Model 2		
		95% CI			95% CI	
	β	lower	upper	*β*	lower	upper
Shade	0.10***	0.05	0.15	0.10***	0.05	0.16
Chl *a*	-0.04	-0.09	0.01	-0.04	-0.09	0.05
O_2	-0.03	-0.08	0.01	-0.04	-0.09	0.01
Conductivity	0.05	-0.03	0.12	0.05	-0.02	0.13
Alkalinity	-0.02	-0.09	0.05	-0.03	-0.10	0.05
Pond density 5,000 m	-	-	-	0.08	0.00	0.17
Pond density 2,000 m	-	-	-	-0.07	-0.16	0.02
F (5, 99)	8.24***			6.49***		
Adj R^2	0.26			0.27		
R^2 change	0.03					

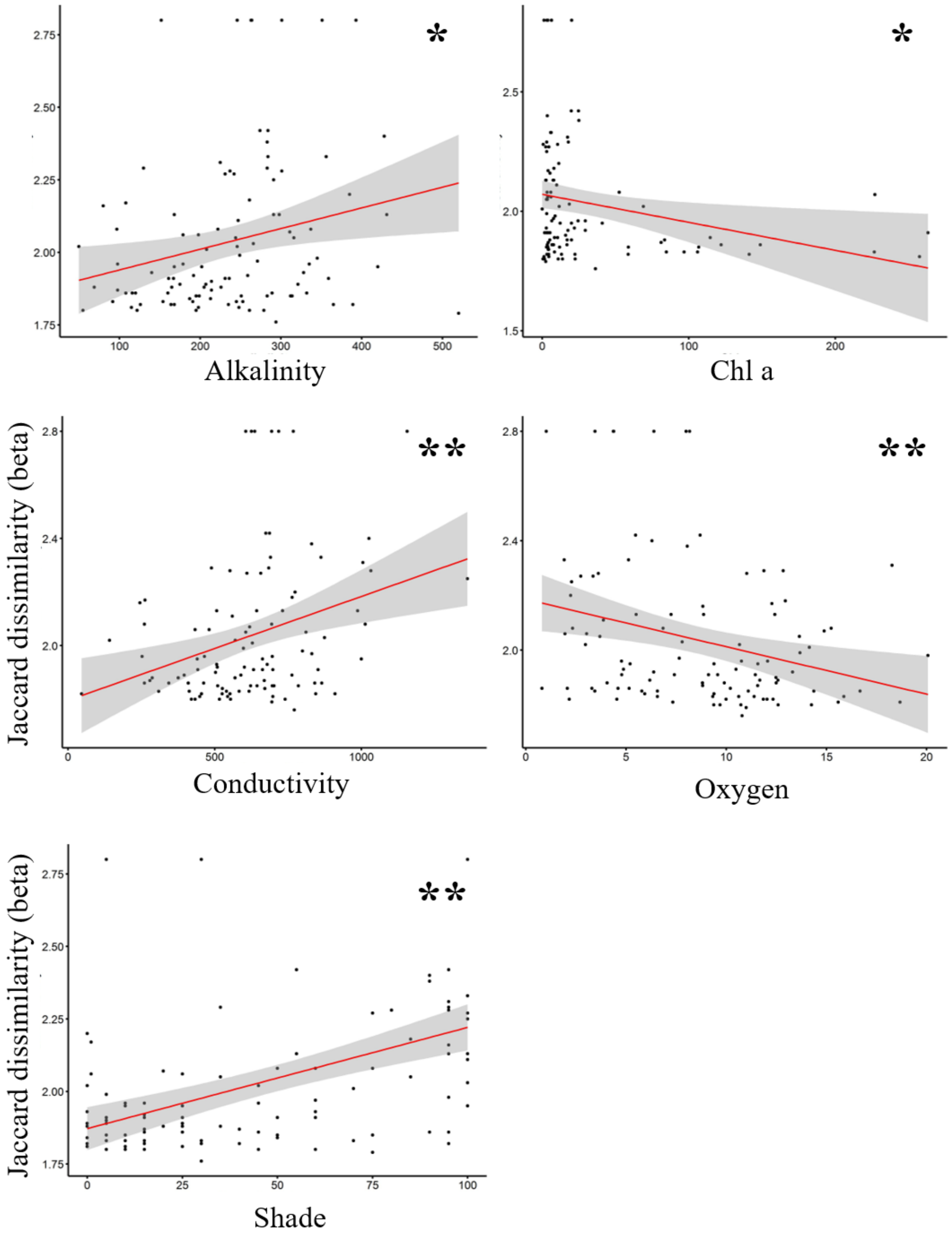


*Figure 34 - Significant correlations with macrophyte β- diversity (mean pairwise Jaccard dissimilarity). ** $p < 0.01$, * $p < 0.05$.*

4.4.2 Aquatic Coleoptera diversity

In total, 46 species of aquatic Coleoptera were recorded from the 121 study ponds. Sample size, both in terms of the number of individuals ($N_{ind.}$), and the number of species ($N_{sp.}$) collected from each pond, varied greatly between regions (*Table 19*). Lyng produced the smallest sample of water beetles, with a total of only 7 water beetles collected across 12 ponds. The most species-rich pond in Lyng produced only two species of aquatic Coleoptera. Godwick produced the largest sample size, with 113 individuals collected across 14 ponds. The most species rich pond in Godwick produced 14 species of aquatic Coleoptera during the survey. All nine study regions contained some ponds in which no water beetles were found.

Table 18 – Table of correlations between Coleoptera species richness and pond environmental and spatial variables. ** $p < 0.01$, * $p < 0.05$.

	Coleoptera richness
Shade	-0.47**
Macrophyte % cover	0.40**
Conductivity	-0.21*
O_2	0.21*
Macrophyte richness	0.12
Area	-0.50
Chl a	0.01
pH	0.02
Alkalinity	-0.13
Pond density 200 m	-0.25**
Pond density 500 m	-0.14
Pond density 1,000 m	-0.18*
Pond density 2,000 m	-0.11
Pond density 5,000 m	-0.71

Exploratory correlations (*Table 18*), should be interpreted with caution due to the small sample sizes involved. However, correlations suggest that species richness of aquatic Coleoptera was significantly related to the environmental variables pond shading, macrophyte % cover, conductivity, and dissolved O_2. Coleoptera species richness also showed a significant negative association with pond density at both a local and medium spatial scale (200m and 1,000m).

The relationship between Coleoptera species richness and pond density was explored further, grouping incidence data by pond region in order to provide larger sample sizes (*Table 12*). Independent ANOVAs comparing the 'below average' and 'above average' pond density categories showed there to be a significant difference ($p < 0.05$) between the two, for density measured at all spatial scales (200m, 500m, 1000m, 2000m and 5000m). There were no significant differences in environmental variables (pond shading, pond area, pH, conductivity, alkalinity, % macrophyte cover), between the 'above average' and 'below average' density categories. Results from α- diversity (ICE species estimator), and β- diversity (mean CNESS score), analysis are given in *Table 19*.

Despite the trend suggested by the exploratory correlations (*Table 18*), there was no significant difference in aquatic Coleoptera α- diversity (ICE) between the 'above average' and 'below average' pond density categories, at any spatial scale (*Table 19*). There was also no significant difference in β- diversity, regardless of dominant / rare species weighting of the CNESS score. Dissimilarity in species composition of aquatic Coleoptera between all nine study regions was high, although caution is required in interpreting both the α- and β- diversity results, due to the very small sample sizes.

Table 19 – Number of individuals and species of aquatic Coleoptera collected from each pond region, with aquatic Coleopteraα- diversity (ICE), and β- diversity (mean CNESS score).

Region	$N_{ind.}$	$Range_{ind.}$	$N_{sp.}$	$Range_{sp.}$	ICE	mean CNESS (m=1)	mean CNESS (m=7)	mean CNESS (m=31)
Hindringham	76	0 - 21	21	0 - 10	15	1.00	0.92	0.77
Bodham	62	0 - 18	19	0 - 8	28	0.93	0.87	0.75
Godwick	113	0 - 45	27	0 - 14	10	0.91	0.85	0.76
Tittleshall	50	0 - 16	19	0 - 6	3	0.96	0.89	0.82
Heydon	53	0 - 11	22	0 - 6	21	0.94	0.88	0.73
Lyng	7	0 - 2	6	0 - 2	1	1.03	0.98	N/A
Colkirk	37	0 - 11	21	0 -8	28	0.95	0.90	0.81
Mattishall	31	0 - 9	17	0 - 5	1	0.93	0.86	0.73
Guestwick	43	0 - 16	19	0 - 7	10	0.93	0.89	0.81

$N_{ind.}$ – Number of individual beetles collected from the region.

$Range_{ind.}$ – Range in the number of individual beetles collected from a single pond within the region.

$N_{sp.}$ – Number of beetle species collected from the region.

$Range_{sp.}$ – Range in the number of beetle species collected from a single pond within the region.

4.4.3 Amphibian occurrence

In total, 82 of the 121 ponds surveyed supported amphibian populations. Of these, 37 ponds contained frogs, 31 contained toads, 23 contained smooth newts, and 20 contained great crested newts (GCN). The four different species responded differently to both density (spatial), and environmental variables (*Table 21*). Two variables were found to independently predict occurrence of smooth newt, which were found to be negatively associated with pond shading (Odds ratio (OR) = 0.46, $p < 0.01$), and with pond density at 2,000 m (OR = 0.49, $p < 0.05$). GCN were negatively associated with pond density at 5,000 m (OR = 0.13, $p < 0.01$), and positively associated with macrophyte species richness (OR = 1.76, $p < 0.05$). The occurrence of frogs showed a diverging trend in spatial relationships, with frog occurrence positively associated with pond density at 200 m (OR = 1.93, $p < 0.05$), and positively associated with macrophyte species richness (OR = 1.88, $p < 0.01$). While toad occurrence was significantly predicted by the overall environmental model ($Chi^2 = 18.64$, $p < 0.001$) no variables independently predicted toad occurrence.

*Table 20 – Descriptive statistics for significant variables identified in preliminary t-tests. * p < 0.05, ** p < 0.01, *** p < 0.001. These were the variables used in subsequent logistic regression.*

Variable	GCN present		GCN absent		
	Mean	SD	Mean	SD	Sig.
Pond density 2000m	92.7	36.3	109.8	34.6	*
Pond density 5000m	425.4	163.8	546.4	149.2	***
Macrophyte % cover	67.8	109.4	29.2	36.1	**
Macrophyte richness	9.4	5.5	5.6	5	**

Variable	Smooth newt present		Smooth newt absent		
	Mean	SD	Mean	SD	Sig.
Pond density 2000m	88.6	23.8	111.3	36.3	**
Shade	28.8	34.9	56.3	36.6	***

Variable	Frog present		Frog absent		
	Mean	SD	Mean	SD	Sig.
Pond density 200m	3.3	2	2.4	1.2	**
Pond density 500m	13.2	7.5	10.6	5.4	*
Conductivity	575.2	202.5	673.4	266.4	*
Macrophyte richness	8.3	5.8	5.3	4.8	**

Variable	Toad present		Toad absent		
	Mean	SD	Mean	SD	Sig.
Conductivity	542.7	176.7	678.1	265.1	**
Alkalinity	207.7	91.5	252.2	86	*
Shade	29.5	27.8	58.5	38	***
Macrophyte % cover	55.8	92.4	28.6	35	*
Macrophyte richness	9	5.2	5.2	5	***

Table 21 – Logistic regression results for smooth newt, GCN, frog, and toad occurrence. In each case, Model 1: significant environmental predictor variables, Model 2: significant environmental + spatial variables. Odds ratios (OR) ***p < 0.001, ** p < 0.01, * p < 0.05.

Smooth newt	Model 1			Model 2		
		95% CI			95% CI	
	OR	lower	upper	OR	lower	upper
Shade	0.43**	0.25	0.75	0.46**	0.26	0.80
Pond density 2,000 m	-	-	-	0.49*	0.27	0.88
Chi^2 =	10.67***			17.45***		

GCN	Model 1			Model 2		
		95% CI			95% CI	
	OR	lower	upper	OR	lower	upper
Macrophyte % cover	1.60	0.92	2.77	1.61	0.79	3.31
Macrophyte richness	1.86*	1.12	3.07	1.76*	1.01	3.07
Pond density 2,000 m	-	-	-	3.18	0.95	10.70
Pond density 5,000 m	-	-	-	0.13**	0.07	0.25
Chi^2 =	12.29**			21.34***		

Frog	Model 1			Model 2		
		95% CI			95% CI	
	OR	lower	upper	OR	lower	upper
Conductivity	0.75	0.46	1.21	0.84	0.52	1.35
Macrophyte richness	1.66	1.09*	2.52*	1.88**	1.20	2.95
Pond density 200 m	-	-	-	1.93*	1.10	3.39
Pond density 500 m	-	-	-	1.03	0.25	0.60
Chi^2 =	10.08**			19.87***		

Toad	Model 1			Model 2		
		95% CI			95% CI	
	OR	lower	upper	OR	lower	upper
Shade	0.54*	0.30	0.95	-	-	-
Macrophyte % cover	1.17	0.77	1.78	-	-	-
Macrophyte richness	1.57	0.98	2.51	-	-	-
Chi^2 =	18.64***					

4.4.4 Aquatic macrophyte and Coleoptera community structure

Further to the regression analysis of species richness of aquatic macrophytes and Coleoptera, patterns in community structure were explored through ordination techniques and variation partitioning.

Ordinations

PCA ordination was conducted for the same significant environmental variables used in regression analysis. Separate PCA ordinations were conducted for the ponds used in aquatic macrophyte and Coleoptera analysis. For ponds used in aquatic macrophyte analysis, the first four axis explained 86.2% cumulative variance in the predictor variables. Axis 1 was strongly related to pond shading and alkalinity, while axis 2 was related to pond density (measured at the 5km level, as used in regression analysis). Study ponds were distributed pretty evenly across the ordination space (with the exception of CHEST1), with no obvious clustering of sites along environmental gradients (*Figure 35*). PCA of environmental variables for ponds used in aquatic Coleoptera analysis (*Figure 36*), explained 88.4% variance in predictor variables in the first four axis. Axis 1 was strongly related to % cover of aquatic macrophytes. Axis 2 was less well defined, but appeared most strongly associated with conductivity. Again, study ponds did not display any obvious clusters across the environmental gradients, and tended to be fairly evenly distributed across the ordination space.

Constrained (CCA) and partial constrained (partial CCA) ordinations were conducted to examine relationships between predictor variables and community structure, before and after accounting for SAC. Plots from both analysis for aquatic macrophytes are presented below, to illustrate how failing to account for SAC can distort the apparent importance of the predictor variables. Subsequent analysis focuses only on the partial CCAs, as SAC present in the CCA is a violation of statistical independence (Dormann *et al.* 2007).

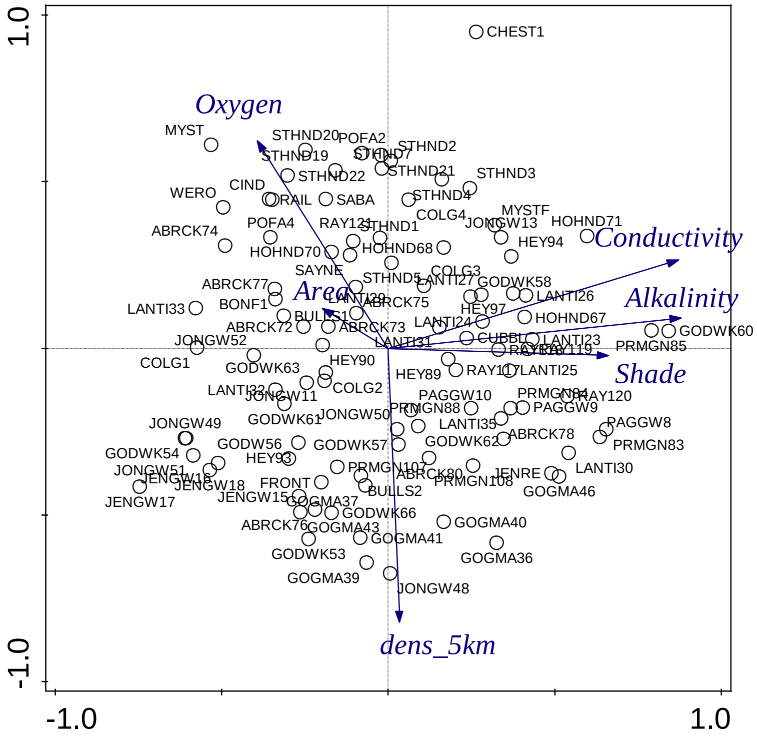


Figure 35 – PCA predictor variables for study sites used in analysis of aquatic macrophytes.

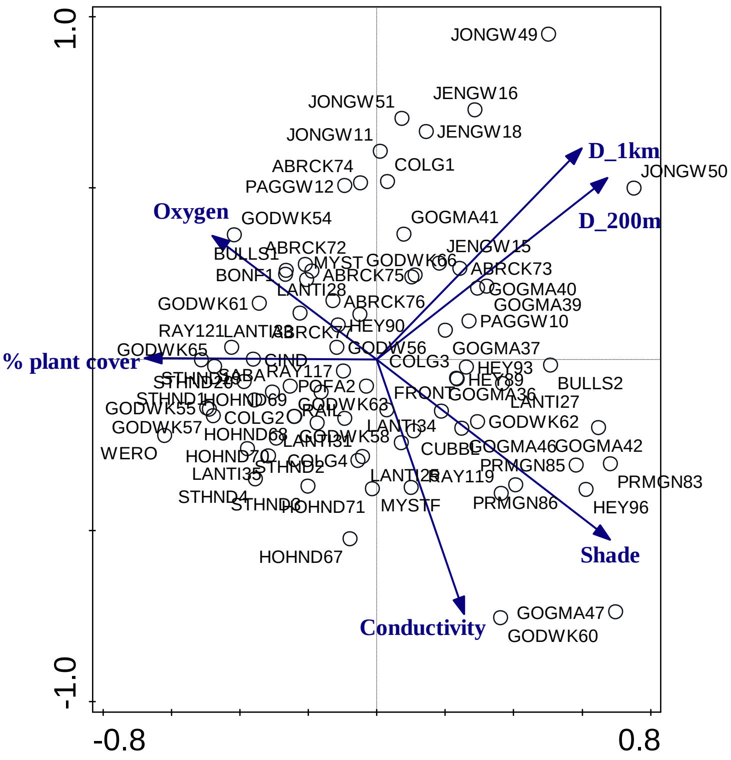


Figure 36 – PCA predictor variables for study sites used in analysis of aquatic Coleoptera.

It is apparent when comparing the CCA (*Figure 37*) and partial CCA (*Figure 38*) plots for aquatic macrophyte species, that controlling for SAC alters the relative contributions of the predictor variables. The importance of pond density within 5km (d_5km), is greatly reduced in the partial CCA, as might be expected given that pond density is inherently linked to SAC. Accounting for SAC between study ponds also changes the apparent relationships between some of the environmental variables; axis 1 can be seen to be less strongly defined by pond shading than previously suggested, while the influence of pond area and dissolved oxygen over axis 2 is greatly increased. However, the overall distribution of species across the ordination space shows little change, likely due to the environmental variables having little explanatory power over community structure (1.6% variance explained). This low explanatory power was true for both macrophytes and Coleoptera, with the predictor variables explaining 1.6% and 0.6% adjusted variation, respectively.

Macrophyte species assemblages showed a broad continuum across the environmental gradients, although there appear to be some associations between particular species and environmental variables (*Figure 38*). Submerged aquatic species including the Potamogeton spp. (*PotmBerSL*, *PotmNatn*, *PotmCris*), *Ceratophyllum* spp. (*CertDemr, CertSubm*), *Callitriche* spp. (*CallBrut*, *CallPlat*, *CallStag*), and Oenanthae spp. (*OenFisSL*), tend to be associated with lower shading. *Carex* spp. (*CarxAcut*, *CarxFlex, CarxRemt*, *CarexPend*), and emergent and generalist species including *Typha angustifolia* (*TyphAngs*), *Solanum dulcamara* (*SolnDulc*), and *Scrofula nodosa* (*ScrpNods*), appear to be more tolerant of higher shading. Charophytes (*CharaSp*) appear to be associated with larger ponds with higher conductivity, as does the fine leaved pondweed *Zannichellia palustris* (*ZannPals*). However, given the very low variance explained by the predictor variables (1.6%), these patterns have little meaning.

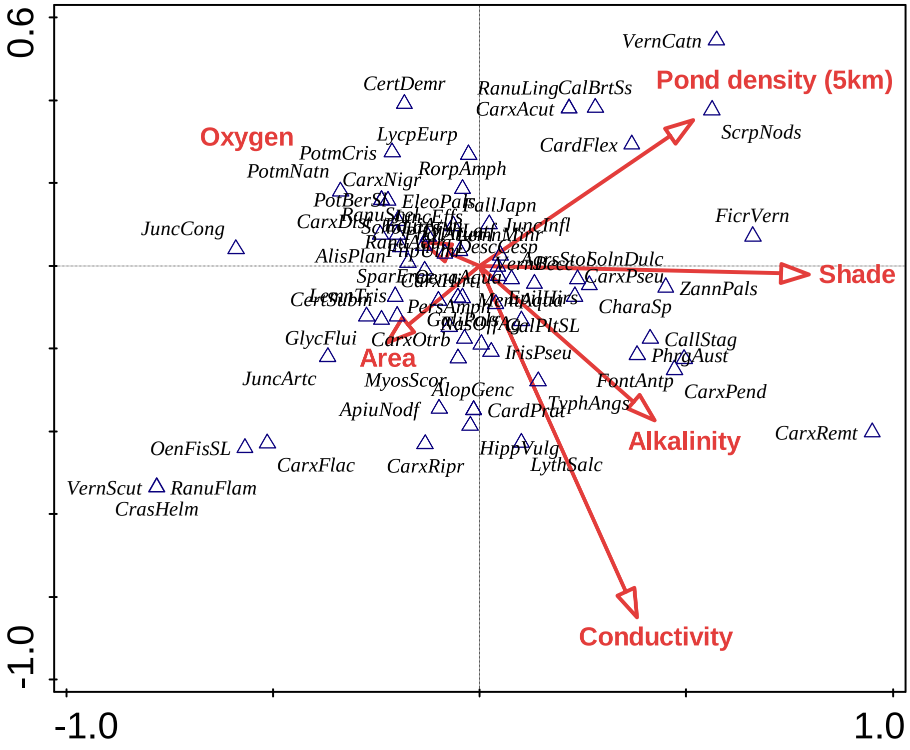


Figure 37 – CCA of macrophyte species against predictor variables.

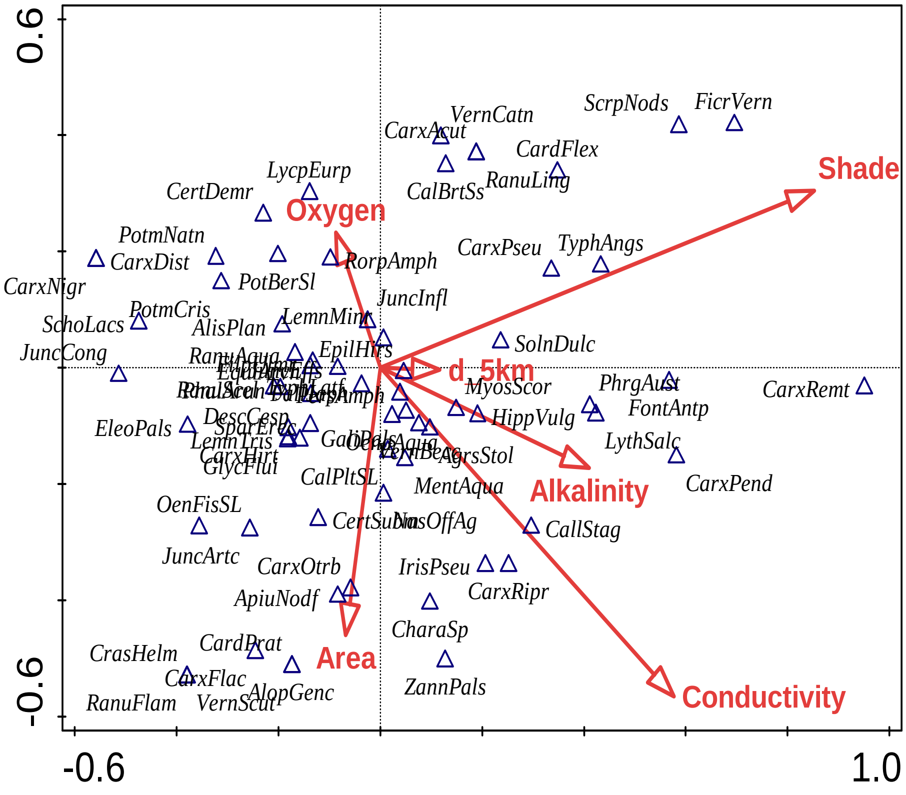


Figure 38 – partial CCA of macrophyte species against predictor variables (controlling for SAC).

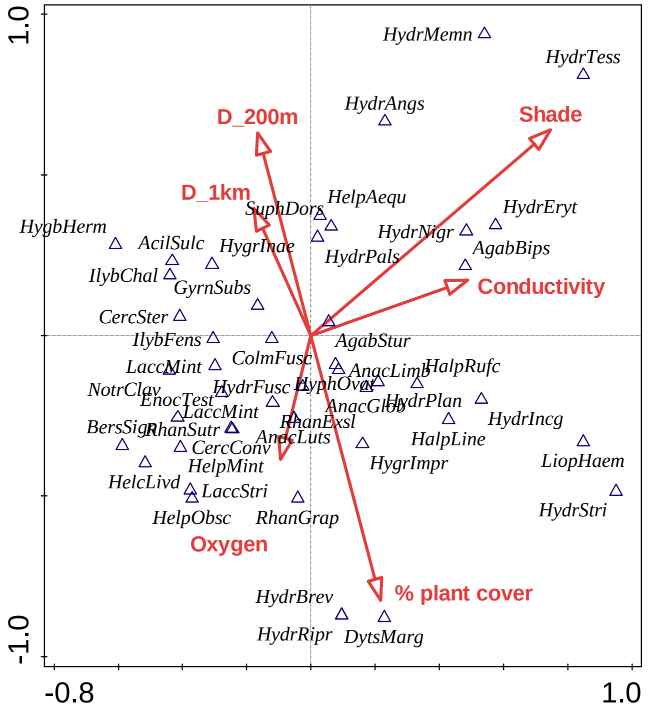


Figure 39 – CCA of Coleoptera species against predictor variables.

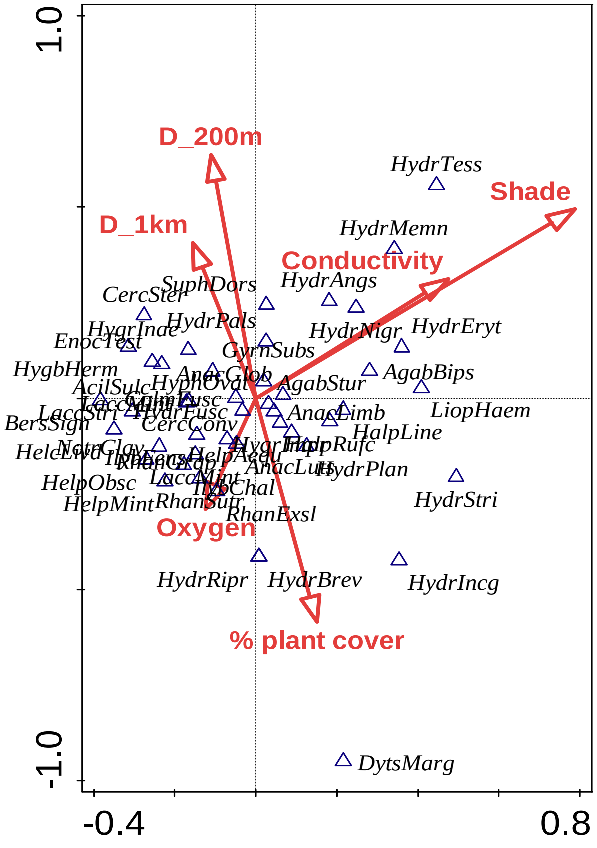


Figure 40 – partial CCA of aquatic Coleoptera species against predictor variables (controlling for SAC).

For the aquatic Coleoptera data, correcting for SAC with a partial CCA had a less noticeable effect on the predictor variables. In both the initial CCA (*Figure 39*), and subsequent partial CCA (*Figure 40*), neither axis was particularly well defined by specific predictor variables. Examining the partial CCA, it appears that the majority of Coleoptera species cluster towards the low shade side of the ordination. There is a broad continuum in Coleoptera assemblage, similar to that observed for macrophyte species, with no distinct patterns or separate clusters forming along the environmental gradients. Several species within the genus *Hydroporus* appear to be more shade-tolerant (*HydrTess, HydrMemn, HydrEryt, HydrNigr, HydrAngs*), while the species *Hydrochus brevis* (*HydrBrev*), *Hydroporus incognitus* (*HydrIncg*), *and Hydraena riparia* (*HydrRipa*), appear to be associated with lower shading, and high aquatic macrophyte cover. Again however, given the extremely low variance explained by the predictor variables (0.6%), these patterns are most likely due to chance.

To examine potential relationships between macrophyte and Coleoptera community structure, the first two PCs from a PCA of macrophyte species data, were included as predictor variables in a partial CCA of Coleoptera community structure. Again, overall variance explained by the first four axis was extremely low (0.3%), and macrophyte PCs did not appear to be strongly associated with the ordination axis (*Figure 41*).

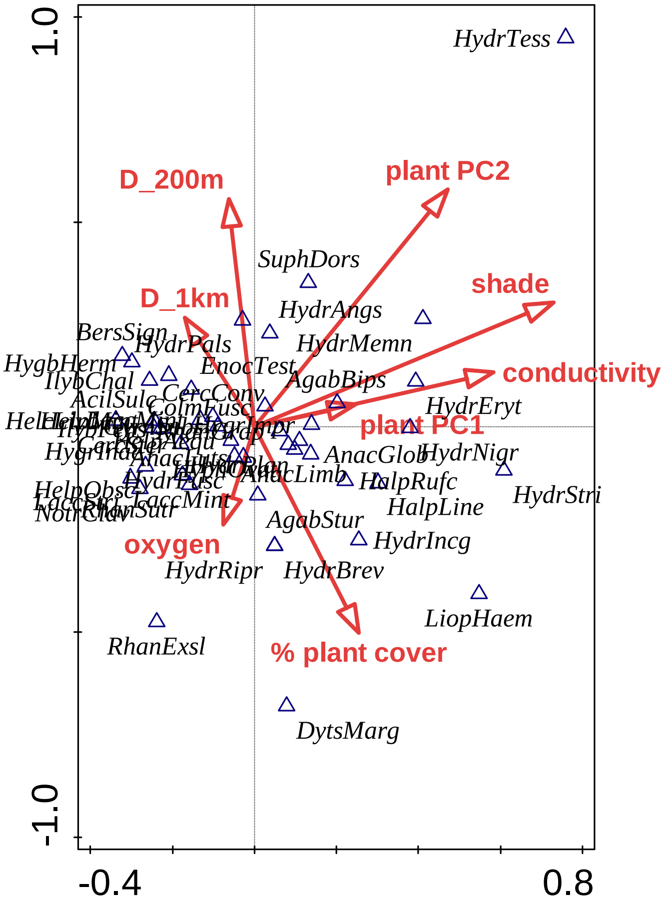


Figure 41 – partial CCA of aquatic Coleoptera species against predictor variables, including the first two principal components from a PCA of plant species data.

Variation partitioning analysis

To better examine the relative importance of environmental and spatial processes in structuring macrophyte and Coleoptera communities, a variation partitioning approach was used (Beisner *et al.* 2006; Santi *et al.* 2010; De Bie *et al.* 2012). For aquatic macrophytes, community variation was significantly explained by both variation in the environment (adj. $R^2 = 0.03$, $p = 0.001$), and variation in spatial patterns (adj. $R^2 = 0.04$, $p = 0.001$) - *Figure 42*. Although both the environmental and spatial models were significant, each explained only a small amount of variation in community structure (3% and 4% respectively). The environmental model (E) was composed of the significant environmental variables pond shading, conductivity, and pond area. The spatial model (S) was composed of three significant PCNMs, of a total of 22: PCNM12, PCNM2, and PCNM18. The total number of PCNMs (22), describe the spatial structure at all scales encompassed by the data matrix, with the lowest numbered PCNMs representing long distance spatial patterning, and the highest numbers representing short distance patterns (Bellier *et al.* 2007; De Bie *et al.* 2012). As such, the significant PCNMs suggest spatial patterning of macrophyte community structure is occurring across multiple spatial scales, from broad (PCNM2), to fine scale (PCNM18).

For aquatic Coleoptera, only variation in spatial patterns made a significant contribution to variation in community structure (adj. $R^2 = 0.03$, $p = 0.001$), while variation in the local environment was not significant. Only one environmental variable (percentage cover of aquatic macrophytes), was chosen through forward selection, for inclusion in the environmental model (E). The spatial model (S) was composed of four significant PCNMs, of a total of 19: PCNM14, PCNM8, PCNM3, and PCNM6. The significant PCNMs suggest spatial patterning of Coleoptera community structure occurs most at medium to broad spatial scale. Again however, the overall variance explained by the spatial model is low (3%).

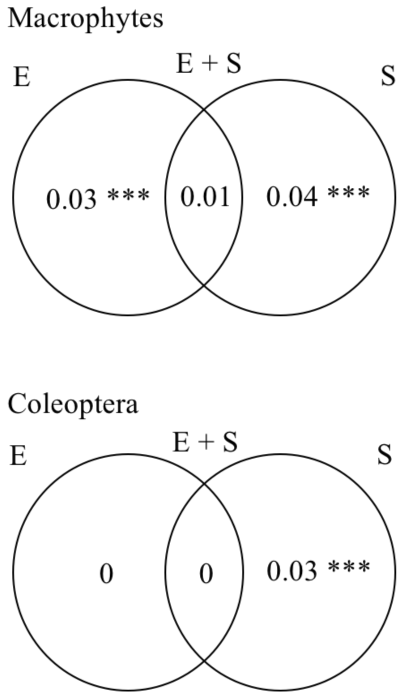


Figure 42 – Variation partitioning of aquatic macrophyte and Coleoptera community structure. E – purely environmental component, S – purely spatial component, E + S – environmental and spatial interaction.

4.5 Discussion

Increasing agricultural intensification and the associated loss of habitat heterogeneity have been widely blamed for the decline of farmland biodiversity across multiple taxa (Benton *et al.* 2003; Tscharntke *et al.* 2005; Declerck *et al.* 2006). Pond loss and pondscape fragmentation have been one consequence of these wider changes in agricultural land use (Robinson & Sutherland 2002; Wood *et al.* 2003), and have been highlighted as particular threats to aquatic biodiversity and connectivity (Wood *et al.* 2003; Curado *et al.* 2011; Sayer 2014). Much research has been conducted on the relationships between pondscape connectivity and pond biodiversity, using a similar space-for-time replacement approach to this study, with pond density or inter-pond distance used as a measure of pondscape 'connectivity' (Møller & Rørdam 1985; Shulman & Chase 2007; Gledhill *et al.* 2008; Akasaka & Takamura 2012; Bosiacka & Pienkowski 2012; Raebel *et al.* 2012a; Jeliazkov *et al.* 2014).

This study found mixed relationships between pond biodiversity and pond density. For aquatic macrophytes, α- diversity showed a significant negative relationship with pond density at the broad spatial scale (2,000 m and 5,000 m), while β- diversity showed no significant relationships with pond density. For aquatic Coleoptera, no significant relationships between α- or β- diversity and pond density were detected. For amphibians, smooth and great crested newt were found to be significantly negatively associated with higher pond densities at the broad spatial scale (2,000 m and 5,000 m), while frogs showed a diverging trend, being positively associated with pond density at the local scale (200 m).

4.5.1 Aquatic macrophyte diversity

Both α- and β- diversity of aquatic macrophytes were found to be significantly predicted by pond shading (% pond surface overhung by trees). Macrophyte α- diversity was significantly predicted by pond shading, pond area, and pond isolation (at the broad spatial scale). These variables explained 33% of the variance in macrophyte species richness. Both pond area (Møller & Rørdam 1985; Oertli *et al.* 2002; Edvardsen & Økland 2006; Bosiacka & Pienkowski 2012), and pond shading (Hassall *et al.* 2011; Sayer *et al.* 2012) have been identified as key determinants of aquatic macrophyte diversity in other studies.

However, the significant negative relationship with pond density at the broad spatial scale (2,000 m and 5,000 m) was unexpected, as it goes against observations reported in the existing literature (Gledhill *et al.* 2008; Akasaka *et al.* 2010). Macrophyte β- diversity showed no significant relationship with pond density at any spatial scale. The only environmental variable which independently predicted macrophyte β- diversity was pond shading (*Table 17*), with more heavily shaded ponds being more dissimilar in macrophyte community composition.

The unexpected negative association between macrophyte α- diversity and pond density at the broad spatial scale has several possible explanations. First, it is possible that this trend relates to the passive dispersal mechanism of aquatic macrophytes, many of which rely on dispersal by waterfowl (DeVlaming & Vernon 1968; Wade 1990; Soons *et al.* 2016). Sebastián-González & Green (2014), found in their study of bird use of 96 temporary ponds that ducks and large birds (key dispersal vectors for pond macrophytes), preferred more isolated ponds, and suggest that isolated ponds effectively have a greater catchment area for birds flying overhead. Under these conditions, it is possible that this behaviour of the dispersal vector (waterfowl), could influence macrophyte community structure and diversity in the way described in the results.. Another possible explanation for these results is that any initial difference in species dispersal success may be quickly modified by competitive ability during colonisation (Bosiacka & Pienkowski 2012). If more isolated ponds experience fewer colonisation events, this may promote the establishment and persistence of less competitive species. While areas with higher densities of ponds and greater dispersal between sites might initially support higher macrophyte α- diversity, they may also be more likely to become dominated by just a few common species.

Alternatively, the counter-intuitive relationship between species richness and pond density may be an artefact resulting from SAC in the data. In future analysis, this should be tested using Moran's *I* test (see *section 4.3.7* on spatial autocorrelation). Keitt *et al.* (2002) and Kühn (2007) both found that failure to account for SAC in ecological data could dramatically affect the results, even resulting in inverted associations between the independent and dependent variables.

While no other studies appear to have found a negative relationship between pond density and macrophyte diversity, some have found no, or only very weak, relationships (Edvardsen & Økland 2006; Bosiacka & Pienkowski 2012). Edvardsen & Økland (2006) suggested that this may be related to the high number of confounding variables acting simultaneously to influence macrophyte diversity, resulting in farmland ponds acting as islands in the agricultural landscape that accumulate species more or less individualistically. It is worth noting that although the addition of pond density into the MLR model resulted in a significant improvement in model fit, the variance explained by this environmental + spatial model was only 4% higher than that explained by the environmental model only (*Table 16*). Pond shading remained the dominant predictor of macrophyte α- diversity, as was hypothesised at the start of this chapter (H1). The majority of ponds within the study region experienced high levels of shading (*Figure 32*), with mean pond shading being 50% pond surface covered by overhanging tree canopies. Almost 1/3 of the study sites experienced extremely high shading of over 90% pond surface overhung. This level of shading is considerable higher than the means reported from other studies detecting a pond density / inter-pond distance effect on macrophyte diversity; Møller & Rørdam (1985) report a mean shading of 16%, Gledhill *et al.* (2008) a mean of 15% and 30% for two study areas, Thiere *et al.* (2009) a mean shading of 40%, and Hassall *et al.* (2011) a mean shading of 23%. The levels of pond shading recorded in this study are also considerably higher than average for ponds in England, which the 2007 Countryside Survey estimated at 27% (Williams *et al.* 2007). As pond shading is known to have a particularly strong influence upon macrophyte diversity (Hassall *et al.* 2011; Sayer *et al.* 2012), it is likely that the low suitability of many of these heavily shaded ponds would out-weigh any biodiversity effects of pond density.

4.5.2 Aquatic Coleoptera diversity

Aquatic Coleoptera species richness estimates (ICE) suggest overall low beetle diversity across the nine pond density regions (*Table 19*). This is likely to be an effect of both the high number of heavily shaded ponds found across all sample regions, combined with the small sample sizes obtained. Exploratory correlations (*Table 18*) suggested that Coleoptera species richness was significantly related to both pond shading and macrophyte % cover, as has been observed in other studies (Gledhill *et al.* 2008; Bloechl *et al.* 2010;

Gioria *et al.* 2010; Hassall *et al.* 2011; Sayer *et al.* 2012). As such, it is likely that the high number of heavily shaded ponds with low macrophyte diversity and cover, has a greater effect upon aquatic Coleoptera diversity than pond density. The absence of any pond density effect over Coleoptera species richness goes against the hypothesis H2 and H5 outlined at the start of this chapter.

Dissimilarity in aquatic Coleoptera species composition between the nine study regions was relatively high, regardless of the weighting of CNESS scores towards dominant or rare species (*Table 19*). This high dissimilarity between sample regions, combined with the absence of a pond density effect upon species richness or dissimilarity, suggests that within the study landscape aquatic Coleoptera are more strongly influenced by local environmental factors than by pond density and dispersal limitation. This could be due to the relatively high pond densities observed in this study, which may be above the threshold for which dispersal limitation of adult Coleoptera would occur. Many species of aquatic Coleoptera are strong fliers (Fairchild *et al.* 2003; Bilton 2014), and as such may not be limited by pond availability within the study landscape. Alternatively, it is possible that any spatial patterning in aquatic Coleoptera composition is happening at a larger scale than accounted for in this study (Florencio *et al.* 2014), again due to the relatively high density of ponds relative to the taxa's dispersal ability. Although the small sample sizes obtained for aquatic Coleoptera mean that the results of this study should be interpreted with caution, they do suggest that pond density within the study region has little effect upon aquatic Coleoptera species compositions. The extremely low abundance of aquatic Coleoptera found in overgrown ponds in this study may reflect both the unusually high levels of pond shading (ponds unsuitable for Coleoptera), combined with the high density of ponds within the study region (many alternative habitats to unsuitable ponds).

4.5.3 Amphibian occurrence

This study found that of the four native amphibian species recorded, responses to pond density varied between species. While newts showed a negative association with pond density at the broad spatial scale, frogs showed a positive association at the local spatial scale, and toads did not appear to be influenced by pond density (*Table 21*). Other studies of amphibians have generally found a positive relationship between pond density and amphibian occurrence (Lehtinen *et al.* 1999; Joly *et al.* 2001; Ficetola & De Bernardi 2004), with pond density / inter-pond distance at small spatial scales having the strongest influence (Joly *et al.* 2001; Fortuna *et al.* 2006; Gustafson 2011). As such, the apparent negative effect of pond density upon newt occurrence was unexpected. Denoël & Lehmann (2006) found a similar negative relationship between pond density and the abundance of newts in an agricultural landscape in southern France, which they attributed to the low suitability of many ponds for newts. When their analysis was adjusted to include only ponds which supported newt populations, they found a positive relationship between pond density and newt occurrence. It is possible that a similar effect is occurring in this study, with the negative relationship between macrophyte species richness and pond density at the broad spatial scale driving patterns in newt occurrence. Newt occurrence has been shown to be positively related to macrophyte cover and diversity (Joly *et al.* 2001; Sztatecsny *et al.* 2004; Gustafson, Pettersson & Malmgren 2006), and submerged leaves are required for egg-laying in all newt species (Marco *et al.* 2001). While frogs and toads may benefit from the habitat provided by a diverse aquatic macrophyte community, they do not require submerged vegetation for breeding, and so may be less strongly influenced by the relationship between aquatic macrophytes and pond density. This could explain why the occurrence of frogs was positively associated with pond density at the local scale, as was hypothesised for amphibians more generally (H5), but newt occurrence showed the opposite trend, being more strongly driven by macrophyte availability. There may also be an effect of measurement error due to newt eggs being more difficult to detect than those of frogs or toads, which could lead to an underestimation of newt occurrence, especially at low abundances.

Although pond density showed some unexpected relationships with amphibian occurrence, other environmental variables showed more expected patterns. Occurrence of GCN, frogs, and toads, was positively associated with aquatic macrophyte species richness (*Table 20*), although these variables were only significant predictors in the regression models for GCN and frogs (*Table 21*). GCN and toad occurrence were positively associated with % cover of aquatic macrophytes, although this variable was not a significant predictor in the regression model. Both smooth newt and toad occurrence were negatively associated with pond shading, but this was only a significant predictor for smooth newt occurrence (*Table 21*). Although several environmental variables were significantly associated with toad occurrence (conductivity, alkalinity, pond shading, and macrophyte richness and cover), none independently predicted toad occurrence in the logistic regression model. This could indicate that within the study landscape, toads are more robust to local environmental factors than the other amphibian species studied.

Amphibian occurrence can be strongly influenced by other environmental variables which were not included in this study, in particular those associated with the suitability of the terrestrial habitat matrix (Oldham *et al.* 2000; Joly *et al.* 2001; Marsh & Trenham 2001; Denoël & Lehmann 2006; Gledhill *et al.* 2008). Although constructing species-specific habitat matrices was beyond the scope of this study, the omission of variables relating to the terrestrial habitat could be influencing the findings presented above. Future work on amphibian occurrence in agricultural landscapes should be expanded to include density variables relating to both aquatic and terrestrial habitat components. Additionally, for an accurate survey of amphibian occurrence, multiple site visits and survey methods (bottle trapping, torch-light searches at night, egg surveys), should be employed (Buskirk 2005; Curado *et al.* 2011). The current survey should not be regarded as a definitive measure of amphibian occurrence, but rather an indication of likely occurrence, which is comparable between sites but is not exhaustive.

4.5.4 Aquatic macrophyte and Coleoptera community structure

The partial CCA ordinations of aquatic macrophyte and Coleoptera community composition suggest that variation in species assemblages were poorly explained by the measured variables. This could suggest that either; i) the environmental variables most important in driving community structure were overlooked by the survey, or; ii) that macrophyte and Coleoptera community structure are not strongly determined by environmental variables. Similar conclusions were reached by Edvardsen and Økland, (2006), who suggest that ponds accumulate species more or less individualistically, with local environmental variables playing little role in the community structure. Jeffries (1998) found similarly weak relationships between species composition and environmental variables in ponds in Northumberland, while Sayer *et al.* (2012) observed the same for aquatic macrophytes in ponds in Norfolk. Coleoptera species composition appeared even more poorly defined by environmental variables, which explained only 0.3% variation in community structure. The extremely low contribution of environmental variables in determining community structure was corroborated by variation partitioning analysis (*Figure 42*), which found environmental variables alone explained 3% variation for macrophytes, and 0% for aquatic Coleoptera. It is not entirely clear why the chosen environmental variables, which other studies have found to be important in community structure (Angélibert *et al.* 2004; Hassall *et al.* 2011; Sayer *et al.* 2012; Hill & Wood 2014), have so little influence within the study ponds. One factor might be that the majority of species surveyed (both macrophytes and Coleoptera), are very common, with wide environmental tolerances, resulting in species composition at any given pond being largely random. The results may also be confounded by the extremely high number of heavily shaded ponds within the survey, which tended to contain very low numbers of macrophyte and Coleoptera species. For Coleoptera in particular, the small sample sizes may strongly influence patterns in species composition.

Variation partitioning showed that spatial patterns explained slightly more variation in community composition than environmental variables, for both macrophytes and Coleoptera. For aquatic macrophytes, a small but significant amount of community variation was explained uniquely by the environmental model (3%), and uniquely by the spatial model (4%). For aquatic Coleoptera, only the spatial model had a significant influence on species composition (3% variance explained). While the level of variance explained by both spatial and environmental models is low, it does not differ dramatically from the findings of De Bie *et al.* (2012), who examined lake communities, or Fuentes-Rodríguez *et al.* (2012), who examined pond communities. In their cross-taxa study, De Bie *et al.* (2012), found variance explained by environmental and spatial models to be; aquatic macrophytes - 7% variance explained by spatial model, 4% by environmental model; aquatic Coleoptera - 2% variance explained by spatial model, 3.5% by environmental model. Their study found that spatial patterns were less pronounced for more mobile taxa, and that taxa with relatively pronounced spatial patterns were less controlled by the environment. This agrees with the variation partition analysis of this study, in which aquatic macrophytes showed slightly more pronounced spatial patterns than aquatic Coleoptera, although the difference was not as great as that observed in the lake communities examined by De Bie *et al.* (2012).

4.6 Conclusions

Pond loss and pondscape fragmentation are widely considered to be a significant threat to aquatic biodiversity (Heath & Whitehead 1992; Boothby 1997; Wood *et al.* 2003; Gledhill *et al.* 2008). This study found evidence that the spatial arrangement and density of ponds had a small but significant influence over macrophyte and Coleoptera community structure, aquatic macrophyte α- diversity, and the occurrence of some amphibian species. Aquatic Coleoptera α- and β- diversity, and aquatic macrophyte β- diversity, were not influenced by pond density. Overall it seems that spatial patterns, including pond density, have a relatively weak influence over the pond communities studied.

Pond density effects for aquatic macrophytes, and for the two newt species *T. cristatus* and *L. vulgaris*, were in the opposite direction to those observed by other studies (Møller & Rørdam 1985; Shulman & Chase 2007; Gledhill *et al.* 2008; Akasaka & Takamura 2012; Bosiacka & Pienkowski 2012; Raebel *et al.* 2012a; Jeliazkov *et al.* 2014). Several factors have been identified which may contribute to these findings. Higher macrophyte α-diversity in more isolated ponds could be due to behaviour of the key dispersal vector (waterfowl), which may show a preference for more isolated sites. Alternatively these patterns could be due to the 'refuge effect', in which more isolated ponds potentially support a higher diversity of less competitive macrophyte species, due to the limited immigration of stronger competitors. This negative effect of pond density on macrophyte species richness may in turn be influencing the occurrence of smooth and great created newts, which showed a negate association with pond density at the same spatial scale.

Even for taxa for which a significant effect of pond density was detected, pond shading remained the strongest determinant of species richness and occurrence. Pond shading within the study region was considerable higher than has been reported from other areas of the UK and Europe (Møller & Rørdam 1985; Williams *et al.* 2007; Gledhill *et al.* 2008; Thiere *et al.* 2009). Almost half of the ponds surveyed had over 50% of their water surface overhung by trees, with 1/3 having over 90% of their water surface overhung. Other studies have found negative biodiversity effects to occur at much lower levels of shading than those commonly observed across this study region (Møller & Rørdam 1985; Gee *et al.*

1997; Hassall *et al.* 2011). As such the overwhelming influence of pond shading is likely to impose a far greater limitation on aquatic biodiversity than pond density or inter-pond distance. Such high levels of pond shading are in fact another symptom of pondscape fragmentation. Abandonment of ponds to succession and terrestrialisation has occurred alongside the active process of pond in-filling for land reclamation (Boothby & Hull 1997; Boothby 2003; Sayer *et al.* 2012), resulting in modern pondscapes containing not only fewer ponds in lower density networks, but a greater proportion of over-shaded and poor quality ponds. These findings suggest that in order to address the biodiversity losses associated with historic pondscape fragmentation, a greater consideration of pond quality (in particular pond shading), needs to be made, before issues of pond connectivity / pond density are addressed.

Improving pondscape connectivity across agricultural landscapes should remain a priority for conservation; the highly temporal nature of ponds means that sites of high conservation value now may not remain that way (Hassall *et al.* 2012), and as such highly connected pondscapes are essential for the migration of populations. However, a highly connected pondscape in which all ponds are in the late stages of succession will have very limited biodiversity value. Conservation measures which improve pondscape connectivity, while creating a matrix of different pond types, are necessary. In landscapes which contain high densities of overgrown ponds, restoration though the removal of trees and sediments can be an extremely effective means of improving the pondscape (Sayer *et al.* 2012; Davies *et al.* 2016). In landscapes where pond densities are lower, and aquatic diversity may be more limited by pond availability than by pond quality, pond creation may be of higher conservation value.

In addition to these two approaches lies a third option – the ‘resurrection’ of ghost ponds, which had formerly been filled-in for agricultural land reclamation. Combining all three approaches could provide an effective means of tackling both the reduced quality and connectivity of agricultural pondscapes. Because aquatic diversity in the study landscape appears to be more strongly limited by pond shading than by inter-pond distances, the primary benefit of ghost pond resurrection is likely to be the creation of new open-water ponds, with improved pondscape connectivity a secondary benefit. The resurrection of ghost ponds has several potential advantages over the creation of new ponds (*section 1.3*). In particular, it is possible that the historic seedbank buried within ghost ponds could remain viable, and contribute to the re-colonisation of these sites. While it has been suggested that this mechanism likely contributes to the re-colonisation of restored ponds after surface sediment removal (Weyembergh *et al.* 2004; Sayer *et al.* 2012), there is little experimental evidence confirming this, and the potential for the same mechanism to occur in buried ghost ponds remains unexplored.

Chapter 5 Pond restoration and resurrection - the importance of the historic seedbank.

5.1 Introduction

Many aquatic organisms have evolved life-cycle strategies for surviving in temporary or variable environments, remaining dormant in the sediment as seeds (De Winton *et al.* 2000; Aponte *et al.* 2010), oospores (Beltman & Allegrini 1997; Stobbe *et al.* 2014), or resting eggs (Hairston 1996). Many of these ‘resting propagules’ can remain viable for centuries (Hairston 1996; Stobbe *et al.* 2014), and support species re-establishment following habitat improvement (Beltman & Allegrini 1997; Kaplan *et al.* 2014). Seeds and seedbanks have a particularly long history of research, with some of the earliest work in this field conducted by Darwin (1859). Since then, a large body of research covering the longevity, viability, and ecological consequences of seedbanks has been generated, with aquatic environments being extensively covered (van der Valk & Davis 1978; Bonis, Lepart & Grillas 1995; Bakker *et al.* 1996; Hairston 1996; Beltman & Allegrini 1997; De Winton *et al.* 2000; Aponte *et al.* 2010). While much is known about the importance of seedbanks for the seasonal re-vegetation of temporary ponds and wetlands (Bonis *et al.* 1995; Aponte *et al.* 2010), there has been little research into the long-term seedbanks of small waterbodies, and none into the persistence of these seedbanks in in-filled ghost ponds.

This chapter explores our current knowledge of aquatic seedbanks, their role in population persistence and restoration following habitat improvements, and the environmental factors that potentially limit their long-term viability. A detailed examination of the viability and germination success of the seedbanks of restored and ghost ponds is made, using a combination of seed viability staining and microcosm based germination trials.

5.1.1 The seedbank as a means of temporal dispersal for plant populations

A ‘seedbank’ can be defined as “*an aggregation of ungerminated seeds potentially capable of replacing adult plants*” (Leck, Parker & Simpson 1989). Seedbanks are broadly classified as *transient* - seeds which germinate within one year, or *persistent* - seeds germinating after one year (Leck *et al.* 1989). Some of the earliest observations of soil seedbanks were made by Darwin (1859):

> *“I took in February three table-spoonfuls of mud from three different points, beneath water, on the edge of a little pond; this mud when dry weighed only 6 and 3/4 ounces; I kept it covered up in my study for six months, pulling up and counting each plant as it grew; the plants were of many kinds, and were altogether 537 in number; and yet the viscid mud was all contained in a breakfast cup!”*

Seedbanks are important for both year-to-year and long term survival of plant communities. Transient seedbanks can maintain populations through short periods of unfavourable environmental conditions, or allow species to exploit seasonal gaps (Thompson & Grime 1979), while persistent seedbanks are important for maintaining populations through longer periods of environmental stress or disturbance (Brown & Venable 1986). As such, persistent seedbanks are characteristic of environmentally challenging or changeable environments, from deserts (Guo, Rundel & Goodall 1999; Daws *et al.* 2007) to ephemeral wetlands (Casanova & Brock 1990; Bissels *et al.* 2005; Aponte *et al.* 2010).

Theoretical work on seedbanks suggests that a trade-off exists between seed dormancy and seed dispersal ability (Brown & Venable 1986; Venable 1989). Both strategies reduce variability in success, with dispersal averaging across space, and dormancy averaging across time (Venable & Brown 1988; Hulme 1998). The optimal strategy for any particular plant then depends largely on its environment, with dispersal favoured in spatially unpredictable and rare habitats, and seed dormancy and seedbank development favoured in temporally unpredictable habitats (Bakker *et al.* 1996). In habitats which are both spatially rare and temporally short-lived, such as small ephemeral ponds, species may have evolved both seed persistence and dispersal strategies (Bakker *et al.* 1996).

Seedbanks reduce variance in success in several ways. The overlapping generations in the seedbank magnify the effect of favourable years over poor ones, reducing the risk of extinction from a poor year (Bonis *et al.* 1995). This 'storage effect' not only protects species from extinction due to environmental change, but also promotes the co-existence of species, indefinitely delaying competitive exclusion as different species emerge from the seedbank at different points in time (Bonis *et al.* 1995; Hulme 1998; Faist, Ferrenberg & Collinge 2013). In the field, the seedbank storage effect is often observed as low levels of similarity between the seedbank and standing vegetation composition (Thompson & Grime 1979; Hopfensperger 2007). The storage effect has also been observed in germination trials, for example the staggered germination of different species from the seedbank of a South African floodplain, following two artificial 'flood' events (Brock & Rogers 1998). In frequently disturbed habitats, similarity between the seedbank and standing vegetation can be higher, as the seedbank acts as a source for recolonisation after disturbance. This trend has been observed in experimentally disturbed patches of alluvial wetland (Touzard *et al.* 2002), riverine floodplains that experience regular flood and mechanical disturbance (Abernethy & Willby 1999), and the emergence of arable weeds from the seedbank after agricultural disturbance (Thompson & Grime 1979).

The storage effect of seedbanks not only maintains species level diversity, but also genetic diversity within populations (Leck *et al.* 1989; Levin 1990; Honnay *et al.* 2008). This can effectively increase the local population size, buffering isolated populations against extinction (Honnay *et al.* 2008). Overlapping generations in the seedbank provide a genetic 'memory' of past selective conditions, which can act as an evolutionary filter, dampening the selective importance of individual years (Templeton & Levin 1979; Levin 1990). This can be particularly important following extreme disturbances such as fire. Dolan *et al.* (2007) demonstrated the importance of seedbank genetic diversity for the recovery of the perennial shrub *Hypericum cumulicola*, after a fire which destroyed nearly all above ground plants. Following the fire, recovery from the seedbank resulted in an increase in heterozygosity, and marked shifts in allele frequencies, in comparison to the old vegetation. The post-fire cohort contained alleles new to the study, and one new to the species (Dolan, Quintana-Ascencio & Menges 2008).

The ability of a seedbank to maintain species and genetic diversity in the long term is highly dependent upon seed longevity, and thus the number of generations and different genotypes accumulated in the soil (McGraw, Vavrek & Bennington 1991; Honnay *et al.* 2008). Seed dormancy and seedbank longevity vary between environments, species, and between individual populations (Leck *et al.* 1989; Baskin & Baskin 2001).

5.1.2 Seed dormancy & seedbank longevity

The first requirement for the establishment of a persistent seedbank is that of seed dormancy, commonly defined as;

> "*the failure of a seed to germinate although environmental conditions including water, temperature, light and gasses are favourable for germination"* (Baskin & Baskin 2001).

Dormancy and germination are complex traits controlled by a multiple interacting genes, and are affected by both developmental and environmental factors (Koornneef, Bentsink & Hilhorst 2002). Dormancy is important in regulating the timing of germination, both so that environmental conditions are favourable for seedling survival, and so that not all seeds germinate at the first opportunity, but some are maintained in the seedbank for future colonization (Baskin & Baskin 2001). Seed dormancy and germination depend on seed structures surrounding the embryo, including the seed coat, and on factors affecting the growth of the embryo, including compounds imported from the mother plant and produced by the embryo itself (Koornneef *et al.* 2002). Although a complex process, there are two key hormones involved in seed dormancy, influencing both embryo growth and the break-down of physical barriers within the seed; gibberellin (GA), and abscisic acid (ABA) (Koornneef *et al.* 2002; Finch-Savage & Leubner-Metzger 2006). While ABA acts to maintain seed dormancy, GA induces dormancy release and germination. In species where dormancy is in part due to a physical structure, such as the seed coat or endosperm preventing germination, GA promotes a weakening of these structures. GA also promotes embryo maturation and growth (Finch-Savage & Leubner-Metzger 2006). As such, dormant seeds are characterised by a high ABA : GA ratio, while germinating seeds have a high GA : ABA ratio.

Increase in GA synthesis, and thus the breaking of seed dormancy, can be triggered by a range of environmental factors. Temperature is particularly important in breaking physical dormancy, with many species requiring periods of hot or cold stratification before germination will occur (Baskin & Baskin 2001; Bissels *et al.* 2005; Finch-Savage & Leubner-Metzger 2006; Hay, Probert & Dawson 2008). In some species, chemical factors including nitrate and leachate from leaf litter can effect dormancy breaking (Finch-Savage & Leubner-Metzger 2006). Once physical dormancy has been broken, light is often required for triggering germination (Baskin & Baskin 2001). For the seeds of species inhabiting variable aquatic environments, other environmental factors may be required to break dormancy. Seeds of aquatic plants will often germinate in response to submergence, while shore-line species germinate when floodwaters recede. Seeds are able to detect and respond to oxygen levels in the soil, with aquatic species germinating at low oxygen levels. These germination triggers often act in combination with temperature, ensuring germination at the right time of year, and when environmental conditions are optimal (Baskin & Baskin 2001).

In order for dormant seeds to form a persistent, long-lived seedbank, they have to become buried at a sufficient depth to inhibit germination. Generally, the most persistent seeds tend to be small and compact, as these more easily work their way into the deeper soil layers, and are less likely to suffer predation (Thompson & Grime 1979; Hulme 1998; Baskin & Baskin 2001). Environmental conditions also have to be favourable to both maintain seed dormancy and prevent seed death. Wetlands have been found to have particularly diverse and long-lived persistent seedbanks; cool, wet, anoxic conditions help prevent seed decay and dehydration, while promoting seed dormancy (Skoglund & Hytteborn 1990; Bakker *et al.* 1996; Hopfensperger 2007). The sterility of some acid wetlands, such as sphagnum bog, may also promote seed survival in these habitats (Leck *et al.* 1989). As well as providing suitable conditions for seed survival, the often temporary or variable nature of wetland environments means that may species inhabiting them have evolved persistent seedbanks. Bet-hedging theory predicts seeds of temporary or variable environments will have greater longevity, as plants in which only a fraction of one year's seed output germinates immediately will be favoured by natural selection (Venable & Brown 1988; Thompson *et al.* 1998). As such, both transient and persistent seedbanks are a common feature of wetland habitats.

5.1.3 Egg banks: a means of temporal dispersal in invertebrates

Although the focus of this chapter is on the seedbank, and its role in macrophyte re-establishment in restored ponds, the egg bank provides an analogous strategy of temporal dispersal for many species of freshwater zooplankton (Hairston 1996; Langley *et al.* 2001; Brendonck 2003). Diapausing eggs allow populations to persist in the sediment through periods of environmental stress (Hairston 1996), while overlapping generations in the egg bank perform the same ecological functions of maintaining genetic diversity (Weider *et al.* 1997) and species coexistence (Decaestecker *et al.* 2007), as seeds in a seedbank. The long term viability of zooplankton egg banks has been widely used in the field of resurrection ecology - the hatching of dormant eggs and study of the resulting populations (Kerfoot, Robbins & Weider 1999; Kerfoot & Weider 2004; Orsini *et al.* 2013). Many of the methods used in the study of egg banks (such as mesocosms to compare the role of sediment vs. dispersal mechanisms in pond colonisation (Langley *et al.* 2001; Cáceres & Soluk 2002), are also applicable in the study of seedbanks.

5.1.4 Seed & egg banks of aquatic and wetland environments

Wetland and aquatic environments typically contain very dense seedbanks (Keddy 1976; Grillas *et al.* 1993; Bonis *et al.* 1995), which are important for population survival in these often variable habitats. Seedbanks have been widely studied in ephemeral aquatic habitats including temporary marsh (Grillas *et al.* 1993), ponds (Weyembergh *et al.* 2004; Faist *et al.* 2013), and lakes (Casanova & Brock 1990), as well as in larger permanent water bodies (De Winton *et al.* 2000; Nishihiro, Nishihiro & Washitani 2006).

In permanent waterbodies, the ‘storage effect’ of seed and egg banks provides a genetic and species reservoir, from which different species can re-establish under different environmental conditions. Emergence from the seed / egg bank may vary seasonally with temperature, or with water level fluctuations. For example, the seedbank of prairie glacial marshes in the USA is key for macrophyte establishment at different phases in water level (van der Valk & Davis 1978; Van Der Valk & Davis 1979). Typically, these habitats cycle through a dry phase dominated by emergent species, regeneration phase in which submerged species return with rising water levels, and a lake phase dominated by submerged and floating leaved species. Experimental flooding of sediments collected from the same area of one marsh (Eagle Lake), triggered germination of different species, depending on depth of submergence. Species occurring in experimental flooding were analogous to those occurring in the marsh at an equivalent water depth (van der Valk & Davis 1978; Van Der Valk & Davis 1979). Similarly, studies of the Great Lakes have revealed the importance of the seedbank in maintaining vegetation diversity during different water level phases (Keddy & Reznicek 1986). In this habitat, regular fluctuations in water level are important for killing-off dominant emergent species (primarily *Typha* sp.), opening up gaps which can be exploited by aquatic (in the case of increased water level), or mudflat species (in the case of lowered water levels). A dense and diverse surface seedbank is key for enabling lake shore species to exploit these habitat gaps (Keddy & Reznicek 1986; De Winton *et al.* 2000; Nishihiro *et al.* 2006). The egg banks of invertebrates provide a similar storage effect in permanent water bodies, with different species emerging in response to different environmental conditions. Sediment samples collected from Loch Leven, Scotland, were found to contain the site's complete species diversity of rotifers, as resting eggs. From these sediment samples, different seasonal species assemblages could be hatched by following different temperature treatments (May 1986).

In temporary waterbodies, seed and egg banks may need to survive much longer periods of environmental unsuitability, before repopulating the habitat. Work on temporary Mediterranean marshes has shown that the seedbank contains on average 15% of total plant mass (Grillas *et al.* 1993), demonstrating the high investment macrophytes make into the seedbank. In these temporary marshes, angiosperm species which recolonised from the seedbank every year after flooding showed high similarity between the seedbank and above ground vegetation. For charophyte species, which tended to yield abundant growth only in particularly favourable years, similarity between the oospore bank and above ground vegetation was low, reflecting the storage effect of the oospore bank (Grillas *et al.* 1993; Bonis & Grillas 2002). Low similarity between the oospore bank and standing charophyte vegetation has also been observed in an Australian temporary lake, where only a small portion of apparently viable oospores germinated during germination trials (Casanova & Brock 1990). Further experimental evidence of the storage effect of wetland seedbanks comes from germination trials of flooding of sediments collected from an ephemeral floodplain in South Africa (Brock & Rogers 1998). Sediments were dried, wetted, dried, and re-wetted, with different species assemblages germinating in the first and second wetting period (Brock & Rogers 1998). Similar patterns of staggered emergence can be seen in zooplankton egg banks, helping to maintain a diverse reservoir of overlapping generations within the egg bank, while reducing inter-specific competition between adults (Brendonck 2003). Staggered emergence also ensures persistence of part of the egg bank through extremely short-lived wetting events, which otherwise might trigger hatching, but not allow enough time for a full life cycle to be completed (Brock *et al.* 2003). In general, the staggered hatching of zooplankton egg banks is due largely to morphological differences in the shells of dormant eggs, while the staggered germination of macrophyte seedbanks is due to differences in burial depth (Gilbert 1995; Brock *et al.* 2003).

In temporary wetlands, staggered germination and hatching are important for population persistence, and dissimilarities between the dormant seed and egg bank and existing vegetation and zooplankton assemblages, are often high (Casanova & Brock 1990; Brock & Rogers 1998; Brock *et al.* 2003). In extreme cases, these dissimilarities can become so great that rare species which have been lost above ground, may persist within the seedbank. These ostensibly ‘extinct’ species may be returned to the landscape if habitat conditions become more favourable. This ‘resurrection’ of macrophyte species has been observed in the seedbanks of Mediterranean temporary ponds (Aponte *et al.* 2010), restored vernal pools in the USA (Faist *et al.* 2013), and restored woodland pools in the Czech Republic (Kaplan *et al.* 2014). In some instances, these rare species may have been absent from above ground vegetation for several decades (Weyembergh *et al.* 2004; Kaplan *et al.* 2014). These cases provide some of the best evidence for the longevity of seedbanks under natural conditions (as oppose to artificial storage or burial experiments), and demonstrate the great potential that these buried resources hold for conservation and habitat restoration.

5.1.5 Persistence and longevity of seed & egg banks

While there have been relatively few field studies of long-term viability of seed and egg banks, a number of lab-based studies have examined the extreme longevity possible in seed and egg banks, which are capable of providing an evolutionary archive spanning many decades to centuries. This ‘evolutionary time machine’ has been widely researched in the field of resurrection ecology, with respect to the resting eggs of zooplankton (Hairston & Kearns 2002; Brendonck 2003; Orsini *et al.* 2013). Eggs can be dated and hatched, and the resulting populations studied for phenotypic traits, genetic differentiation with respect to modern populations, and micro-evolutionary responses to stress (Orsini *et al.* 2013). For example, Decaestecker et al. (2007) resurrected past populations of *Daphnia* from different sediment depths in a Belgian pond, in order to examine the surprisingly rapid co-evolution between these zooplankton and their micro-parasites. Population responses to environmental shifts can also been observed by hatching zooplankton eggs of different ages. Weider *et al*. (1997) demonstrated how the genetic loci of *Daphnia galeata* changed over the course of several decades, in response to alterations in lake phosphorous content in Lake Constantine, central Europe. As many species of zooplankton are capable of producing resting-eggs with viabilities ranging from 15 to over 112 years (Hairston 1996), they provide an extensive record of population and environmental changes within a waterbody.

Although seeds often exhibit even greater longevity than zooplankton eggs, this ‘resurrection’ approach to studying historic genetic and phenotypic shifts has not been widely applied to seedbanks. Exceptions include the work of Uesugi et al. (2007), who compared the allelic diversity of remnant populations of a rare aquatic plant *Nymphoides peltata* (from Lake Kasumigaura, Japan), to that of plants germinated from the lake-bottom seedbank. Plants germinated from the historic seedbank showed significantly different genetic variation from the remnant population, including several alleles which had been lost from the above ground vegetation. These findings suggest that methods used in the study of egg bank resurrection ecology may also be applicable to seedbanks, and that historic seedbanks could potentially provide a reservoir of genetic novelty (Levin 1990; England, Whelan & Ayre 2003), for restoring rare or fragmented populations (Uesugi *et al.* 2007; Honnay *et al.* 2008).

While the study of evolutionary and genetic changes has been the focus of much research on zooplankton egg banks, seedbank research has tended to focus on maximum longevity of different species under different conditions. Artificial burial experiments provide a controlled means of measuring seed longevity under semi-natural conditions, although few have been run over long time-spans. One exception is “Dr. Beal’s seed viability experiment”, which began with the burial of seeds from 23 species common in the USA in 1879. 20 up-turned bottles, each containing 50 seeds of 23 species, were buried in sandy ground in Michigan, USA, and exhumed after 5, 13, 15, 20, 40, 50, 80 and 120 years. The most recently exhumed bottle contained viable seeds (after 120 years of burial), from three species; two species of *Verbascum*, and the small mallow *Malva rotundiflora* (Telewski & Zeevaart 2002). Such precise seed viability estimates are rare, although close approximations can often be made by other means, for example when viable seeds collected from habitats where the species has been ‘extinct’ above ground for a known period of time (Beltman & Allegrini 1997; De Winton *et al.* 2000; Weyembergh *et al.* 2004; Kaplan *et al.* 2014), or by carbon-dating seeds (Shen-Miller *et al.* 1995; Daws *et al.* 2007; Stobbe *et al.* 2014).

Decadal-scale longevity has been observed across many aquatic macrophyte species, both in lab-based germination trials and in the field. Some examples include germination trials using sediment collected from 15 New Zealand lakes, which revealed dense species-rich seedbanks, even in two lakes which had been de-vegetated for 17 and 23 years (De Winton *et al.* 2000). Similar seedbank regeneration, in habitats which had not contained the species above ground for many years, has been observed across other wetland types. Beltman & Allegrini (1997) found that newly dug turf ponds in a Dutch fenland were rapidly colonised by a range of charophyte species, which had been absent from the area for over 20 years. Kaplan *et al*. (2014) observed the re-appearance of an ‘extinct’ pondweed *Potamogeton coloratus* in fenland ponds in the Czech Republic. The species had been declared extinct within the Czech Republic over 30 years ago, but germinations appeared in both a restored pond, and in a new pond which had been ‘seeded’ with historic sediment from the restored site. While this strongly suggests that species reestablishment occurred from the seedbank, no *P. coloratus* seeds were found in sediment samples collected from the restored pond, and as such no further testing of seed viability was possible.

Evidence of seed viabilities in excess of 80 -100 years is scarce, although a few confirmed cases do exist. Studies of aquatic seedbanks sampled through sediment cores have found that seed density dramatically declines with sediment depth in many environments (Van Der Valk & Davis 1979; Bonis & Grillas 2002; Beas *et al.* 2013). There have been few studies in which viable seeds have been accurately dated, with estimates often based instead on sedimentation rate and depth of burial. This tends to produce broad estimates of seed age, which may be confounded by processes such as bioturbation, which could result in seeds of different sizes being moved to different depths (Jauhiainen 1998; Rodrigo, Alonso-Guillén & Soulié-Märsche 2010; Rodrigo & Alonso-Guillén 2013; Stobbe *et al.* 2014). There have however been a few notable exceptions, where viable seeds have been accurately dated to over a century. Sediment samples taken from 13 lakes in the Trans-Urals have yielded germinable oospores of *Nitella mucronata*, which radio-carbon dating revealed to be over 300 years old (Stobbe *et al.* 2014). Although other studies have provided anecdotal evidence of centennial-scale viability in *Chara* and *Nitella* oospores, none have been reliably dated, instead estimating oospore age from burial depth and sedimentation rate.

Given optimal environmental conditions for seed preservation (such as extreme dryness or freezing temperatures), even greater extremes of seed longevity are possible. Shen-miller *et al.* (1995) collected four germinable seeds of the sacred lotus *Nelumbo nucifera* from a dry lake bed in China, which carbon-dating revealed to be 1,350 ± 220 years old. Perhaps the greatest confirmed longevity for viable plant tissue comes from a study by Yashina *et al.* (2012), who obtained viable fruit tissue of the narrow-leaved campion *Silene stenophylla* from within fossil squirrel burrows in the Siberian permafrost. These fruits were dated to around 30,000 years old, and were able to produce whole fertile plants after tissue culture and clonal micropropagation. While such extremes in seed longevity are rare, they illustrate that given the right environmental conditions persistence for several centuries is possible, and persistence for several decades is relatively common.

5.1.7 The impact of agriculture and drainage on wetland seedbanks

While long-term propagule viability is possible under certain environmental conditions, those associated with intensive agriculture can be extremely detrimental. Drainage, compaction (Wienhold & van der Valk 1989; Brown 1998; Stroh *et al.* 2012), and possibly fertiliser inputs (Bekker *et al.* 1998), all significantly reduce wetland propagule viability. This has led some researches to conclude that wetland propagule banks which have been subject to agricultural intensification could not contribute to wetland restoration (Baastrup-Spohr *et al.* 2016). However, even where extremely low densities of viable propagules have been found underneath agricultural land, these can be sufficient for macrophyte re-establishment (Wienhold & van der Valk 1989; Middleton 2003; Beas *et al.* 2013), and could contribute to habitat restoration post-agriculture.

In many parts of the globe, significant wetland losses have occurred as a result of drainage for agricultural intensification (Tscharntke *et al.* 2012; Davidson 2014; Gibson *et al.* 2015). Attempts at restoring lost agricultural wetlands from their historic seedbanks have generally been of limited success, with very low seedbank densities, consisting of just a few species, managing to survive prolonged drainage and burial. In a study of Finish boreal mires drained ~ 40 years ago, Jauhiainen (1998) found only six species of vascular plant in the viable seedbank. Similarly low seed survival rates are reported for drained prairie potholes in the USA, where both the number of wetland species and density of the seedbank declined dramatically over time, with nearly 60% wetland species lost from the seedbank after 20 years of drainage (Wienhold & van der Valk 1989). Even relatively short periods of drainage can impact heavily upon the seedbank, many decades after wetland restoration. Stroh *et al.* (2012) found that the seedbank of a restored fen (located in Cambridgeshire, UK), was heavily impacted by very short periods of drainage (~ 6 years), even after 60 + years or restoration and re-wetting.

Despite the low numbers of viable species surviving in the seedbanks of drained wetlands, it should be noted that some species *are* able to survive even the longest periods of drainage and agricultural cultivation. Long-term seedbank viability within drained agricultural wetlands varies with specific drainage conditions, herbicide use, and the types of cultivation and crops sown over the wetland (Wienhold & van der Valk 1989; Budelsky

& Galatowitsch 1999), with less thoroughly drained wetlands containing higher densities of viable seeds. Although seedbank viability in drained and cultivated wetlands is relatively low, it is anticipated that within undrained ghost ponds, a sufficient proportion of the seedbank is likely to remain viable and contribute to the revegetation of these sites. As such, revegetation of ghost ponds is expected to follow a similar trajectory to that observed after sediment removal in overgrown ponds.

This chapter examines the restoration potential of the seedbank in three ghost ponds, and three overgrown ponds scheduled for restoration (tree and sediment removal). Germination trials were conducted under semi-natural conditions, to examine the potential for macrophyte re-colonisation from the seedbank of both ghost and overgrown ponds. Seed viability testing was subsequently conducted on the ghost pond seedbanks, to further examine the relative viabilities of different macrophyte species, and to check whether seedbank germination was limited by availability of viable seeds, or by some other factor during the germination trials.

5.2 Chapter hypothesis

H1 – Sediments of both ghost ponds and overgrown ponds contain a viable aquatic seedbank, from which germination of multiple species can be triggered by exposing the propagules contained in the historic pond sediment to water.

H2 – Germination from the historic seedbank of both ghost ponds and overgrown ponds occurs rapidly (i.e. weeks / months), as has been suggested by the rapid revegetation of formerly overgrown ponds following restoration.

H3 – Germination trials of seedbanks from ghost ponds and overgrown ponds will produce similar aquatic species compositions, over a similar time-frame, reflecting the similarity of the seedbank conditions in the two pond types.

H4 – The germination success from ghost pond sediments is most strongly influence by the availability of viable seeds (examined using seed staining), rather than environmental influences during the germination trials – i.e. germination trials and seed viability staining results reflect the viability of the same set of aquatic species.

5.3 Methodology

Chapters 5 and 6 of this thesis explore the potential role that the resurrection of ghost ponds, and restoration of overgrown ponds, could have in aquatic conservation across agricultural landscapes. These questions are addressed in two parts; *Chapter 5* examines the viability of the propagule bank through germination trials and seed viability testing. *Chapter 6* applies these findings in the field, using outdoor mesocosms to compare colonisation from the propagule bank vs. colonisation by dispersal, and assess how these process contribute to the restoration of ghost and overgrown ponds (
Figure 12). The following methodology covers the microcosm germination trials and seed viability testing (tetrazolium chloride staining) which are presented in this chapter.

5.3.1 Microcosm germination trials

There are many different methods for assessing seed bank viability and composition, and selection of the most suitable methodology will vary depending on the aims of the study. In the case of germination trials, the aim is often to achieve maximum possible germination from the seedbank. This requires detailed knowledge of species-specific germination triggers, such as cold stratification, temperature fluctuations, or fluctuations in soil moisture / hydroperiod (Leck *et al.* 1989; Budelsky & Galatowitsch 1999; Mcfarland & Shafer 2011). When dealing with mixed species assemblages, or species for which little is known about their germination requirements, more general approaches may be used to try and maximise germination success (Mcfarland & Shafer 2011). Some studies have suggested that sieving seeds prior to germination trials on artificial substrate, may improve germination success (Ter Heerdt *et al.* 1996; Bernhardt *et al.* 2008). For submerged aquatic species, there is some suggestion that germination trials conducted under waterlogged conditions may produce better results than the more common approach using damp substrate (Britton & Brock 1994; Boedeltje, ter Heerdt & Bakker 2002). To further maximise the number of germinations from the seedbank, seedlings are usually removed once they have been identified, to reduce competition and shading (Casanova & Brock 1990; Brown 1998; La Peyre *et al.* 2005).

For this study, germination trials were designed to mimic conditions in the field as closely as possible, rather than to maximise the number of germinations from the seed bank. This was because the main aim of the study was to establish the potential for recolonisation from the seed bank under natural conditions in the field. As such, germination trials were conducted outdoors in sealed microcosms, using the pond sediment collected from the study sites prior to their resurrection / restoration. Seedlings were not removed from the microcosms, so as not to cause disturbance to the sediment, and more closely mimic processes in the study ponds.

Sealed microcosms were set up in March 2014 (*Figure 43*), using sediment collected from the study sites prior to their restoration / resurrection (September 2013). Sediment was collected from each ghost or overgrown pond from multiple points within the pond basin, to reduce the effects of patchiness in the seed bank. Bulk sediment samples were then homogenised, and kept in cold storage (5˚C) inside airtight black plastic bags, closely mimicking sediment conditions within the ghost and overgrown ponds prior to excavation. Sediment collected from each study pond was distributed between 6 plastic microcosms (30 x 20 x 19 cm^3). Each microcosm was filled with 1 L of sediment. Six additional ‘control’ microcosms were filled with a 50 / 50 mix sterile potting soil and builder’s sand (Boedeltje *et al.* 2002; Neill *et al.* 2009). All microcosms were filled with filtered rainwater, covered with cling-film, and positioned on outdoor pallets. A frame was constructed to support a thin polythene cover (December - February), or shade netting (June - July), to protect the microcosms from damaging temperature extremes, while allowing for a natural light regime. Aside from protecting the microcosms from winter frost damage and high summer temperatures, we allowed for a wide variation in temperature to break dormancy across species with a range of likely germination triggers (Proctor 1967; Bernhardt *et al.* 2008; Hay *et al.* 2008). Over the course of the study, microcosms experienced a water temperature range of 0 - 28°C. Microcosms were surveyed for germinations between May 2014 and May 2015 (weeks 8, 13, 18, 22, 29, 37, 47, and 62). Since microcosms were sealed, their filtered rainwater was replaced at weeks 13, 18, 22 and 37 to reduce the effects of stagnation and subsequent anoxia on germination. At each survey, germinations were counted for each species. In order to reduce sediment disturbance, and more closely mimic conditions in the on-site mesocosms and ponds, seedlings were not removed from the microcosms.

Profile analysis (conducted in SPSS), was used to test for significant differences between the number and rate of germinations occurring in ghost and restored pond microcosms. This multivariate technique can be used to test one dependent variable measured at different times, or several different dependent variables measured at the same time. The test is an extension of a repeated measures ANOVA, but avoids multiple comparisons where data are analyzed across more than two time points. Profile analysis is robust to both small sample sizes and violations of normality (Von Ende 2001; Tabachnick & Fidell 2007). Specifically, profile analysis of the microcosm data compared three components of the response curves of each sediment group (treatment); the overall level of each curve (levels – whether there is a significant effect of treatment), the shape of the response curves relative to each other (parallelism – whether treatments differ from each other at different time points), and whether each curve has an average slope different from zero (flatness – the effect of time, irrespective of treatment). Only *chara* spp. and *Potamogeton natans* germinations were included in this analysis, as these were the only macrophytes which germinated in sufficient numbers for statistical analysis.

5.3.2 Seed viability testing

Because viability results obtained through germination trials can be influenced by a range of factors – for example, whether all dormancy-breaking triggers for all species were met during the trial, or whether some viable seeds were overlooked because they were buried too deeply or not exposed to the correct triggers – germination trials are commonly followed by a direct analysis of seed viability. This could involve extracting seeds from the sediments used in the germination trials, or from replicate sediment samples, and assessing these for viability using one of several methods. Some studies have used a simple visual inspection to estimate seed viability, counting all un-damaged seeds (La Peyre *et al.* 2005), or seeds which appear to have a healthy endosperm and embryo when dissected (Ter Heerdt *et al.* 1996), as viable. Alternatively, the 'seed crush test' can be used as a test for seed turgidity, with seeds that resist crushing deemed viable (Bonis & Lepart 1994; Borza, Westerman & Liebman 2007). All of these methods however have received criticism for likely over-estimating seed viability (Leck *et al.* 1989; Mcfarland & Shafer 2011). Another commonly used assessment for seed viability is tetrazolium chloride (TZ) staining, which detects hydrogen ions produced during respiration (Peters & Lanham 2005; Mcfarland & Shafer 2011; Schirtzinger 2011; Millennium Seedbank, Proofed & Section 2013). This form of testing can work on both dormant and non-dormant seeds (Mcfarland & Shafer 2011), with the TZ stain forming a pink / red precipitate when in contact with viable seed tissue (Millennium Seedbank *et al.* 2013).

In order to corroborate the results from the microcosm germination trials, and assess whether further viable seeds remained in the sediment, TZ staining was conducted on seed bank samples collected from the three ghost ponds. Time constraints meant that only the ghost pond seed banks were assessed using TZ staining, which was deemed sufficient to answer the key research question of whether ghost pond seed banks could potentially contribute to the resurrection of these habitats.

TZ staining was conducted at the Millennium Seed Bank at Wakehurst Place, Royal Botanic Gardens, Kew. Sediment collected from the ghost ponds (September 2013), was kept in cold storage over 27 months. Two months prior to TZ staining, seeds were extracted from 200 ml of sediment (4 x 50 ml homogenised samples) from each ghost pond. An additional 450 ml bulk sample from GP_{150} was processed, giving a total of 650 ml of sampled sediment, in order to maximise the number of examined propagules from this oldest site. Sediment was passed through 355 µm and 125 µm sieves to remove seeds and oospores, which were transferred to vials of distilled water, and returned to cold storage (5°C) for two months before further processing. During this period, fungal growth was removed periodically from seeds and oospores with a fine, soft brush. Imbibed cleaned seeds and oospores then underwent an X-ray assay to assess numbers of full, insect infested and empty seeds. Seeds were subsequently prepared for the TZ assay. Imbibed seeds were kept for 2 - 4 days at 20°C to initiate metabolic processes. *Potamogeton natans* seeds were bisected laterally, the cut being made slightly off-centre, allowing the embryo and seed coat to be removed without damage. *Ranunculus aquatilis* seeds were also bisected laterally, but no healthy seeds were found. Oospores from *Chara* spp. were left intact. Seed embryos and oospores were incubated in 1% TZ solution at 30°C for 36 and 48 hours respectively; the longer time period allowing penetration of the TZ solution through the oospore wall. TZ staining was finally assessed under a light microscope at x 10 - 20 magnification. *P. natans* embryos were assessed externally, and then bisected to assess internal staining. *Chara* spp. oospores were cut with a micro needle to assess internal staining (*Figure 45*). Seeds / oospores were classed as 'viable' if they exhibited complete red staining, or 'maybe viable' if the staining was slightly patchy, or more pink than red in colour. Seeds / oospores were classed as 'non-viable' if they did not stain, or exhibited very patchy or pale pink staining.

5.4 Results

5.4.1 Microcosm germination trials

In total, six aquatic macrophyte species germinated in microcosms containing ghost pond sediments, and four species germinated from overgrown pond sediments (*Table 23*). Germinations began to appear within both the ghost and overgrown pond microcosms in May 2014, two months after the experiment was started. From the ghost pond sediments, the pondweeds *Potamogeton natans* (GP_{45}, GP_{50}, GP_{150}), and *Potamogeton trichoides* (GP_{150}) were the first to germinate, followed by germinations of charophytes one month later. Initially, a more diverse species assemblage germinated from the overgrown pond sediments, with the pondweeds *P. natans*, *P. trichoides,* and *Zanichellia palustris* germinating from WERE sediments, and the charophyte *Chara vulgaris* from APS sediments (*Table 22 & 23*)

Table 22 – Species list of microcosm germinations occurring from each study pond.

Ghost ponds			Overgrown ponds		
GP_{45}	GP_{50}	GP_{150}	STRE	GURE	WERE
N. flexilis agg. *P. natans*	Aquatic moss *C. contraria* *C. globularis* *P. natans*	*C. virgata* *P. natans* *P. trichoides*	*C. vulgaris* *P. natans*	*P. natans*	*C. vulgaris* *P. natans* *P. trichoides* *Z. palustris*

The oldest ghost pond (GP_{150}) produced at least 13 microcosm germinations, representing three macrophyte species (*Table 22, Figure 43*), GP_{45} produced 4 germinations representing two macrophyte species, and GP_{50} produced at least 56 germinations representing four species. From the overgrown pond sediments, WERE produced at least 32 germinations representing four species, STRE produced 12 germinations representing two species, and GURE produced 1 germination representing one species.

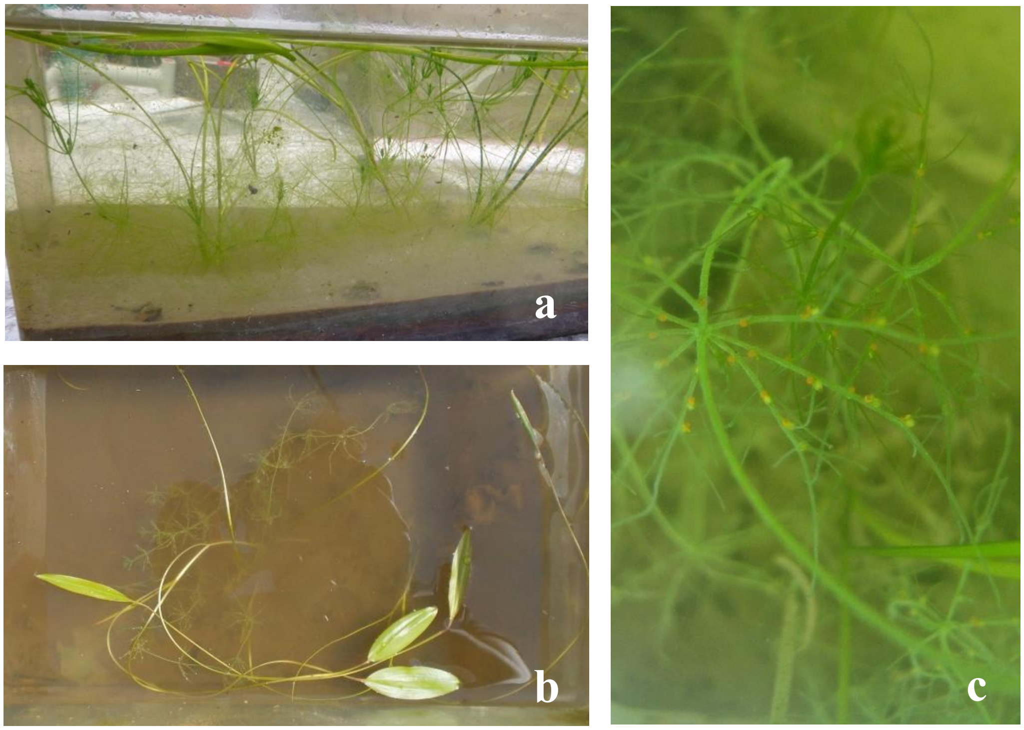


Figure 43 - Germinations occurring in experimental microcosms. a) P. *natans* and charophyte germinations in GP$_{150}$ microcosm (Feb. 2015). b) *P. natans* and charophyte germinations in GP$_{50}$ microcosm (Jul. 2014). c) Oospore development on a charophyte in a GP$_{150}$ microcosm (Aug. 2014).

Table 23 – Microcosm germinations from ghost and overgrown pond sediments. Charophytes are listed by species (where known), and by family (includes sporelings which could not be identified to species level). Sporeling counts followed by a ‘+’ indicate the minimum number of individual sporelings, after which distinguishing between individuals and clonal shoots became difficult.

		May 2014	Jun. 2014	Jul. 2014	Aug. 2014	Sept. 2014	Nov. 2014	Feb. 2015	May 2015
Ghost ponds	Characeae	-	1	5	5	12	20+	20+	40+
	C. globularis	-	-	1	-	1	10	8	10+
	C. contraria	-	-	2	4	10	10+	10+	10+
	C. virgata	-	1	-	-	-	-	-	-
	N. flexilis agg.	-	-	2	-	-	-	-	4
	P. natans	13	17	20	15	14	22	19	17
	P. trichoides	3	3	1	-	-	-	-	-
Overgrown ponds	Characeae	3	14	7	7	7	10+	10+	10
	C. vulgaris	3	8	7	7	7	10+	10+	10+
	P. natans	3	3	1	1	1	1	1	1
	P. trichoides	6	2	-	-	1	-	1	-
	Z. palustris	1	1	2	1	1	-	1	2

Across all microcosms, only charophytes (grouped by family), and the pondweed species *P. natans* germinated in sufficient numbers for conducting profile analysis. Both showed different patterns in seedling / sporeling numbers between ghost and overgrown ponds. For *P. natans*, ghost pond sediments produced a significantly greater (F(1) = 6.7, p < .05) number of germinations than overgrown pond sediments (*Figure 44b*). There was no significant effect of time. For charophytes (*Figure 44c*), there was no significant difference between the number of germinations from ghost and overgrown pond sediments, but there was a significant effect of time (F(7) = 3.0, p = .05), and of time / group interaction (F(7) = 2.2, p < .05). Charophyte germinations tended to occur earlier in the overgrown pond microcosms than in the ghost pond microcosms, but after 5 months (Sept. 2014), more overall germinations had occurred in the ghost pond microcosms than the overgrown pond microcosms. Overall numbers of germinations across all aquatic macrophytes (*Figure 44a*) did not differ significantly between ghost and overgrown pond microcosms.

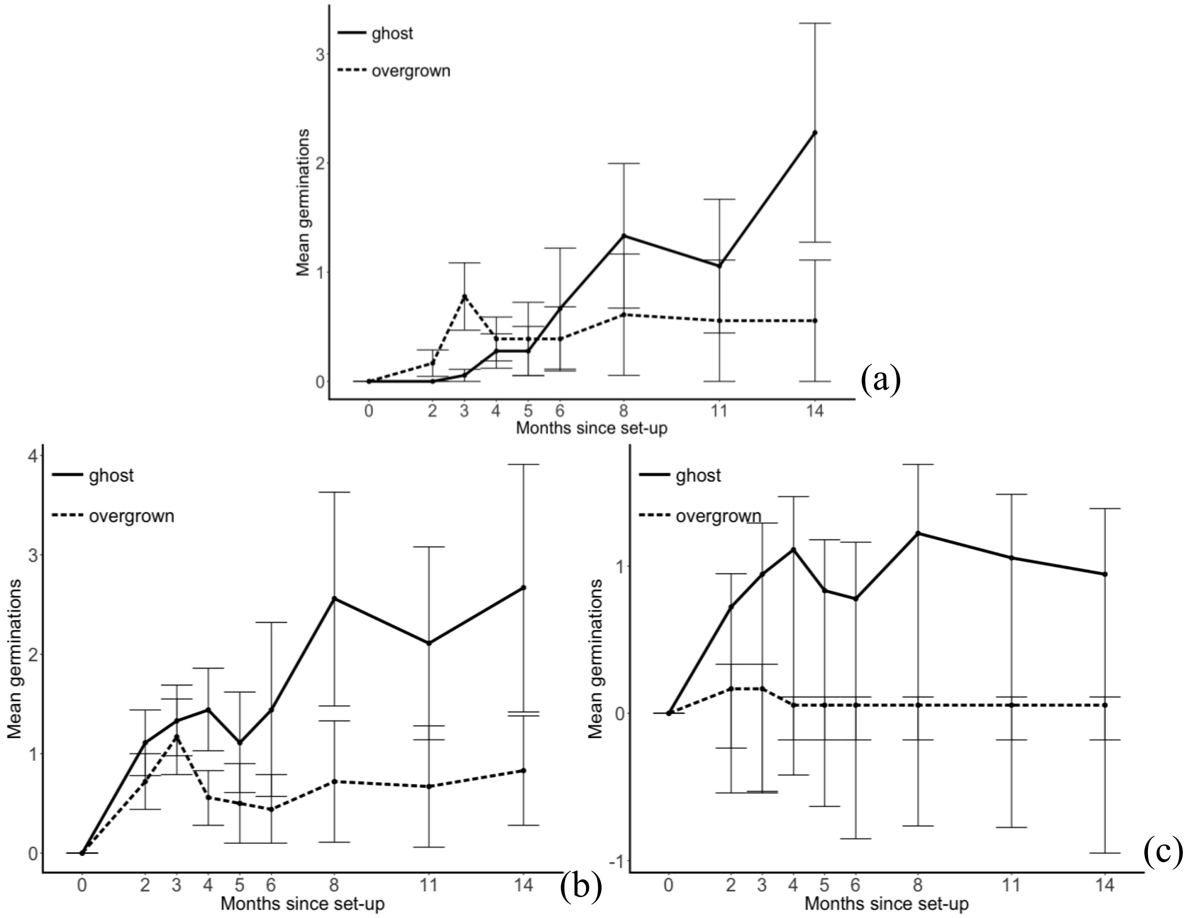


Figure 44 – Mean numbers of germinations in ghost and overgrown pond microcosms. a) Total number of germinations b) *P. natans* germinations c) charophyte germinations. Grey error bars show SEM. Month 0 represents experimental set-up (March 2014), and month 14 is the experiment end (May 2015).

5.4.2 Seed viability testing

The results of the tetrazolium chloride (TZ) seed viability test (*Table 24*) indicate that the sediment of the oldest ghost pond (GP_{150}) contained viable propagules of at least four aquatic macrophytes; *Chara virgata, P. natans, R. aquatilis* and *Juncus* sp. (*Table 24*). When stained, 20% of charophyte oospores from GP_{150} appeared viable, and a further 20% were classified as 'potentially viable' (*Figure 45*). Many oospores, and individual seeds of *Juncus* sp. (*Figure 46*) and *R. aquatilis* furthermore germinated from GP_{150} sediment in cold storage (5°C) prior to viability testing. Seeds of *Mentha aquatica* and *Persicaria amphibia* recorded in GP_{150} sediment were shown not to be viable. These species did not germinate in any experimental treatment, but quickly colonised resurrected pond GP_{150}.

Table 24 – Ghost pond seed viability. 'micro' – number of germinations occurring in the microcosms, 'store' – number of germinations occurring during propagule storage in water, 'exam' – number of propagules examined for viability / total number collected from sediment, 'maybe' – number of propagules showing partial or light staining, which might indicate viability, 'viable' – number of propagules showing complete staining, which are likely viable.

	GP$_{45}$					GP$_{50}$					GP$_{150}$				
Species	micro	store	exam	maybe	viable	micro	store	exam	maybe	viable	micro	store	exam	maybe	viable
A. plantago aquatica	.	.	.	.	.	.	.	7 / 7	.	.	.	.	41 / 75	.	.
Characeae	2	.	.	.	.	40+	.	17 / 17	1	.	1	70 / 740	160 / 740	32	32
E. hirsutum	.	.	.	.	.	.	.	.	.	.	.	.	62 / 62	.	.
Juncus sp.	.	.	.	.	.	.	.	.	.	.	.	1 / 22	21 / 22	.	.
L. trisulca	.	.	.	.	.	.	.	35 / 35	.	.	.	.	62 / 62	.	.
M. aquatica	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
P. amphibia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
P. berchtoldii	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
P. natans	1	.	.	.	.	22	.	69 / 69	5	4	9	.	5 / 5	.	.
P. trichoides	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
R. aquatilis	.	.	.	.	.	.	.	1 / 1	.	.	.	1 / 143	142 / 143	.	.
T. latifolia	.	.	.	.	.	.	.	.	.	.	.	.	30 / 30	.	.
Z. palustris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

While the propagule bank of GP$_{150}$ was dominated by charophyte oospores, analysis of 200 ml sediment from GP$_{50}$ showed the propagule bank of this pond to be dominated by viable *P. natans* seeds (*Table 24* and *Figure 45*), and non-viable *Lemna trisulca* seeds, with only one viable charophyte oospore identified during TZ staining. Both *P. natans* and the charophyte species *C. globularis* and *C. contraria* germinated in GP$_{50}$ microcosms, suggesting that low densities of viable charophyte oospores were present in the sediment of GP$_{50}$.

Only empty charophyte oospores and seed cases of *A. plantago-aquatica, L. trisulca, Potamogeton* spp. and *R. aquatilis* were found in 200 ml of sediment from GP$_{45}$. However, *P. natans* and *Nitella flexilis* agg. germinated in both the microcosms and study pond, again suggesting low densities of viable propagules from these species in GP$_{45}$ sediment.

Figure 45 – Photographs of the TZ staining of seeds and oospores. From left to right: non-viable chara oospore and *P. natans* embryo from GP$_{150}$, potentially viable chara oospore and *P. natans* embryo from GP$_{50}$, and viable chara oospore (GP$_{150}$), and *P. natans* embryo (GP$_{50}$).

Figure 46 – Germinating Juncus sp. seed from GP$_{150}$ sediment.

5.5 Discussion

While the long-term viability of wetland seed banks is well established for extant aquatic habitats (Britton & Brock 1994; Brock & Rogers 1998; De Winton *et al.* 2000; Weyembergh *et al.* 2004; Nishihiro *et al.* 2006; Kaplan *et al.* 2014; Stobbe *et al.* 2014), this study provides the first evidence for long term propagule viability within buried ghost ponds and overgrown agricultural ponds. Germination trials and seed viability testing reveal that multiple aquatic macrophyte species maintain viable, germinable propagules, even after burial under intensively managed agricultural land for over 150 years. Although the species diversity of viable propagules in both ghost and overgrown ponds appears to be low, the results demonstrate that:

a) Long-term propagule viability, even under the harsh conditions of intensive agriculture, is possible.
b) The historic sediments of ghost and overgrown ponds could be used as a source of viable propagules for habitat restoration.

Results from the microcosm trials (*Table 22 & 23, Figure 44*), identify multiple aquatic species for which rapid germination of propagules (within 2 months of the experiment beginning), could be triggered under natural light and temperature regimes. These findings could have important consequences within the field of aquatic habitat restoration, as they suggest that even historic propagule banks which have been buried for over a century, could act as an early source for native species propagules. The ability of a range of species to germinate quickly under semi-natural conditions, without requiring any special treatment of the sediment or propagules, suggests that habitat restoration using ghost pond and overgrown pond sediments could be highly successful and cost efficient conservation tool.

In general, the number and rate of germinations from ghost pond sediments appears to be higher than that of overgrown pond sediments (*Figure 44*), although these differences are not significant, likely due to the small sample sizes of this study. There is also an indication that different aquatic macrophyte species in ghost and overgrown pond seed banks respond differently to restoration. The number of *P. natans* germinations (*Figure*

44b) was higher in ghost pond sediments than in overgrown pond sediments across all time points, with most germinations occurring within the first four months after experimental set-up (March – July 2014). In contrast, overgrown pond sediments initially produced more charophyte germinations than ghost pond sediments (*Figure 44c*), but after 5 months (August 2014), germinations from ghost pond sediments overtook those from overgrown pond sediments.

Germination trials revealed considerable variation in the number of germinable propagules obtained from each study site. In the case of the ghost ponds, the observed differences in aquatic propagule viability suggest that pond conditions pre-burial may have a stronger effect upon propagule viability than the length of burial. In two of the study sites of similar age (buried for 50 and 45 years respectively), GP_{50} was filled-in while still wet, while GP_{45} was buried after it had dried out during a particularly dry year. This may account for lower propagule viability in GP_{45}, with desiccation having been reported to negatively impact aquatic propagule viability in drained wetlands (Brown 1998; Stroh *et al.* 2012). Nonetheless, even very low viable propagule densities can enable macrophyte re-establishment, given the large volumes of sediments commonly contained within ghost ponds (Kaplan *et al.* 2014), and this could explain the rapid recolonisation of aquatic macrophytes in all three resurrected ghost ponds.

Germination trials using sediment from the three overgrown ponds also showed considerable variation between sites. Pond WERE produced the greatest number and species diversity of germinations (32+ germinations representing at least 4 species), followed by pond STRE (12+ germinations representing 2 species), and pond GURE (1 germination). The reasons for these differences are unclear, as sediments used in the experimental treatments were collected from close to the marl base of each study pond, such that burial conditions were roughly similar across the three sites. It could be that pond conditions prior to restoration may influence the viability of buried propagules. Pond WERE, which had the highest germination success in microcosm trials, experienced the lowest shading (25% water surface overhung by trees), highest macrophyte diversity (14 species), and highest level of dissolved oxygen (10.32 µg L^{-1}), prior to restoration. Pond GURE, which had the lowest germination success in microcosm trials, experienced the highest shading (85% water surface overhung by trees), lowest macrophyte diversity (3 species), and lowest level of dissolved oxygen (3.69 µg L^{-1}), prior to restoration. However, it is unclear how surface pond conditions might influence the viability of propagules buried several meters down, and further investigation into the different sediment conditions of overgrown ponds is needed to understand factors influencing historic seed bank viability.

Results from the seed viability testing (*Table 24*), support the findings of the germination trials. All species identified as having viable propagules in the TZ staining also produced germinations in the microcosm trials, suggesting that the germination trials were a good measure of propagule viability. Microcosm germination trials had several other advantages over TZ staining; conducting the germination trials directly from the sediment of ghost and overgrown ponds provided a good indication of which species could be expected to germinate under field conditions. Additionally, the microcosm approach was far less labour intensive than the extraction and identification of seeds / oospores required for TZ staining, resulting in far larger volumes of sediment being included in the analysis. This provided a more complete survey of the historic propagule bank, as indicated from the microcosm and TZ staining results for GP_{45} (*Table 24*). While TZ staining failed to detect any viable seeds / oospores from this site, microcosm germination trials identified two viable species. While both germination trials and TZ staining indicated low number of viable propagules within the historic sediment of both ghost and overgrown ponds, these could be sufficient for re-establishing historic populations in restored sites.

5.6 Conclusions

For ghost ponds to serve as a viable conservation resource, long term dormancy or ‘quiescence’ of propagules is required. Extreme examples of plant propagule longevity have been observed in various habitats already, and include a viable 1,300 year-old seed of the Sacred Lotus (*Nelumbo nucifera*) from a dry Chinese lake bed (Shen-Miller *et al.* 1995) and a ~ 30,000 year-old fruit tissue of the Narrow-leaved Campion (*Silene stenophylla*) preserved in Siberian permafrost (Yashina *et al.* 2012). Even under less extreme environmental conditions, long-term dormancy of propagules appears widespread in species associated with ephemeral wetland habitats, and can facilitate ‘temporal dispersal’ over hundreds of years (Bakker *et al.* 1996; Daws *et al.* 2007). Such temporal dispersal of propagule banks in extant aquatic habitats has been shown to also act as a valuable source for the re-establishment of aquatic macrophyte species of conservation interest (Beltman & Allegrini 1997; Weyembergh *et al.* 2004). This study, however, is the first to demonstrate at least centennial-scale survival of aquatic plants buried in ‘extinct’ aquatic habitats widely invisible beneath intensively cultivated agricultural fields.

In contrast to the generally observed loss of viable propagules from wetlands during drainage (Brown 1998; Stroh *et al.* 2012), the rapid burial of ghost pond sediments during in-filling appears to effectively conserve long-term propagule viability. Similarly, conditions within overgrown ponds appear to conserve buried propagule longevity, although the reasons for the considerable variation between sites needs further exploration. Several of the taxa surviving prolonged burial within ghost and overgrown ponds are keystone species; charophytes provide complex habitat structure and promote water clarity (Schneider *et al.* 2015), but have become increasingly scarce in agricultural landscapes (Lambert & Davy 2011), and floating-leaved species like *P. natans* strongly enhance the diversity of Odonata (Raebel *et al.* 2012a).

The findings of this chapter demonstrate that propagules within the sediments of ghost and overgrown ponds are capable of remaining viable, even after 150 years or more of burial. These findings demonstrate the potential for resurrection of historic macrophyte populations from ghost and overgrown ponds, which could benefit the restoration of these habitats. To establish how the historic propagule bank might contribute to pond restoration in agricultural landscapes, a detailed examination of the colonisation processes in ghost and restored ponds is required.

Chapter 6 Pond restoration and resurrection – recolonisation from the seed bank vs. dispersal pathways.

6.1 Introduction

Considering our current knowledge of aquatic propagule banks, it seems likely that the environmental conditions found within both overgrown and ghost ponds should favour long term propagule viability. The historic sediments of both environments provide cool, dark, damp, anaerobic conditions, beneficial for both seed survival (Jauhiainen 1998) and maintaining seed dormancy (Stobbe *et al.* 2014). Although intensive agricultural land use around / above the buried propagule banks is likely to have detrimental impacts on viability and longevity, it is anticipated that high propagule densities will result in sufficient numbers of viable propagules surviving to contribute to the re-vegetation of the ponds. Having demonstrated in the previous chapter that propagules from ghost and overgrown ponds are able to survive and germinate, even after 150 years or more of burial, there is strong evidence that the historic propagule bank of these sites could contribute to their resurrection / restoration, potentially returning populations or even species ostensibly lost form the agricultural landscape.

Although there has been considerable research into the longevity and restoration potential of propagule banks in both agricultural (Middleton 2003; Beas *et al.* 2013) and semi-natural (Beltman & Allegrini 1997; Kaplan *et al.* 2014) environments, pond research has continually emphasised the importance of dispersal for colonisation, and the need for highly connected pond networks to facilitate the exchange of species (Boothby 1997; Williams *et al.* 2008). While dispersal is undoubtedly an important component of pond colonisation, the restoration potential of historic pond propagule banks should not be overlooked, especially in fragmented agricultural landscapes where dispersal may be limited (Boothby 1997; Soomers *et al.* 2013).

This chapter examines the relative importance of the historic propagule bank and dispersal mechanisms for pond colonisation, using outdoor mesocosms to represent new, restored, and resurrected pond conditions. The colonisation by aquatic macrophytes and Coleoptera in three resurrected ghost ponds, and three restored (formerly overgrown) ponds, is also examined, and discussed in relation to the colonisation of the outdoor mesocosms and microcosms.

6.1.1 Restoration using propagule banks

Although relatively unexplored in the restoration of buried aquatic habitats, seed and propagule banks have been widely used in the restoration of degraded, extant aquatic habitats. Aquatic propagule banks can be used for both *in situ* and *ex situ* restoration. In the restoration of severely impacted habitats in which the *in situ* propagule bank may have been damaged or destroyed, translocation of healthy propagule banks from neighbouring habitats can be used for restoration. This could involve the translocation of seeds collected from standing vegetation at the donor site (Patzelt, Wild & Pfadenhauer 2001), or the translocation of sediment containing the propagule bank (Vivian-Smith & Handel 1996; Beltman & Allegrini 1997; Brown & Bedford 1997). The translocation of freshly harvested seeds is quite frequently used in the restoration of hay meadows and flood meadows. For example, Patzelt *et al.* (2001) examined the restoration of drained fenland in southern Germany, where the study site had been under intensive agriculture for over 200 years. Topsoil was removed from the study site, and hay harvested from local donor sites was spread over the exposed ground. This resulted in 70% of the species occurring in the donor sites becoming established in the study site. It was assumed that due to the long period of drainage and agricultural land use, no viable seed bank had survived at the study site, and that all fenland species which germinated were from translocated seeds.

The translocation of entire propagule banks, containing mixed generations of seeds / oospores, provides an alternative approach for restoring degraded aquatic habitats. Brown & Bedford (1997), conducted both small and large scale experimental manipulations of propagule bank translocations from small extant wetlands, to a series of drained wetlands around New York and Lake Ontario, USA. Experimental plots consisted of controls (no translocated propagule bank), transplanted soil blocks (containing seeds as well as rhizomes and roots), and transplanted sieved soil (containing seeds, but no roots or rhizomes). Both transplanted treatments had higher species richness and wetland vegetation cover after one and two years than control plots, with transplanted soil blocks performing better than sieved soil. Soil translocation was also applied in a large scale study across an entire wetland basin. This resulted in significantly higher species richness and wetland vegetation cover, and reduced establishment of invasive *Typha* spp., compared to mowing and ploughing treatments with no seedbank translocation. A similar wetland propagule bank translocation was conducted in New Jersey, USA, to restore an overgrown sand mine. Analysis of the mine propagule bank prior to restoration found it to have low species richness, dominated by rushes and non-obligate wetland plants. The donor site, a wetland due to be destroyed for road construction, contained a propagule bank rich in obligate wetland species, with significantly higher seed / oospore densities than the recipient mine propagule bank. After translocation, the donor propagule bank made a major contribution to species richness at the restored mine site, with 23% species occurring in the restored site also occurring in greenhouse germination trials (Vivian-Smith & Handel 1996).

Once established, restored wetland sites may in turn be used as donor sites for future restorations. McKnight (1992), reports on the translocation of sediment from a 13 year old restored wetland in east Texas, which was subsequently used as a donor site to re-establish vegetation in a newly created wetland basin. In such cases, monitoring the impact sediment removal has on the donor site is important; in this study, excavating thin, widely-spaced strips of sediment from the donor site was found to have little impact upon vegetation cover or species composition in the subsequent growing season (McKnight 1992).

As well as consideration of the effect propagule bank removal may have on donor sites, there are other potential issues with propagule bank translocations; thorough analysis of the donor propagule bank is required to ensure invasive or non-native species are not being transferred during restoration. Unless donor and recipient sites are located very close together, there is a risk of introducing species or genetic variants which would not historically have been found at the recipient site, which could compromise the uniqueness of any remnant propagule bank. Additionally, the labour and costs involved in large scale propagule bank translocations can be high.

An alternative restoration approach is using *in situ* propagule banks to restore historic communities to habitats which had previously been degraded. This approach has been applied across a range of habitat types, with success varying greatly with habitat type and environmental conditions. Jefferson & Usher (1987), found that the historic seedbank of disused chalk quarries in Yorkshire contained a number of rare species, absent from the above ground vegetation. Rotational scraping to disturb the historic seedbank was sufficient to restore these species to the above ground community.

In aquatic environments, the *in situ* propagule bank may contribute to habitat restoration after anthropogenic stressors have been removed. For example, Nishihiro *et al.* (2005) examined the potential for sediments collected from Lake Kasumigaura, Japan, to facilitate the restoration of lakeshore vegetation which had been lost as a result of eutrophication and water regime shifts. Lake sediments were spread over artificial substrates along the lake shores, restoring 180 plant species to these areas. Similarly, aquatic propagule banks may contribute to the restoration of habitats which have been undergone succession. Beltman & Allegrini (1997) demonstrated the ability of historic turf pond propagule banks to re-populate restored sites after the removal of late-successional floating fen vegetation, while Kaplan *et al.* (2014) found the same to be true of fenland propagule banks, which restored early successional stage vegetation to a desilted pool. This mechanism has also been implicated in the restoration of late successional, overgrown agricultural ponds, where removal of trees and surface sediment from the pond has been shown to be a highly effective means of restoration (Sayer *et al.* 2012). While the role of the historic propagule bank in the restoration of agricultural ponds is strongly implicated by field data, analysis of the historic sediments has not yet been conducted to corroborate these results.

6.1.2 The role of dispersal in new pond colonisation, and dispersal limitation in agricultural landscapes

While the use of historic and modern seed banks for aquatic restoration has been widely studied in lakes and larger wetlands, the application of these techniques in small wetlands and ponds has received little attention. In these habitats, dispersal is considered the main mechanism behind colonisation, and this has driven the emphasis on the importance of pond connectivity for biodiversity (Biggs *et al.* 2005; Williams *et al.* 2008; Céréghino *et al.* 2014). Traditionally, the wide distribution of many aquatic plant species was considered evidence that this group experienced little dispersal limitation (Darwin 1859; Capers *et al.* 2010). However, strong genetic differentiation between populations (Gornall, Hollingsworth & Preston 1998; Nies & Reusch 2005), and studies of the regional distribution of aquatic macrophyte species (Møller & Rørdam 1985; Linton & Goulder 2003; Jeffries 2008), suggest that the ability to disperse long distances occasionally may only be weakly related to the distribution of plants on a smaller geographical scale (Capers *et al.* 2010).

Studies of macrophyte and macroinvertebrate colonisation in newly created ponds highlight three key dispersal mechanisms: dispersal by wind, water, or birds. For many aquatic macrophytes, long-distance dispersal of seeds and oospores within the digestive tracts of birds is a key dispersal mechanism (Proctor & Malone 1965; Soons *et al.* 2016). Gut contents analysis has revealed that a wide range of aquatic macrophyte species produce seeds / oospores which can survive, and even benefit, from passage through the digestive tract of waterfowl. Studies by Proctor and Malone (1965), and DeVlaming & Proctor (1968), found that a range of charophyte and algal species (Proctor & Malone 1965), as well as angiosperm species (DeVlaming & Vernon 1968), are able to survive passage through the intestines of ducks. A recent review of the literature (Soons *et al.* 2016) suggests that over 445 macrophyte species occur in the diet of dabbling ducks, with many of these producing seeds / oospores which are likely to survive passage through the intestines. The importance of waterfowl as a dispersal vector for many aquatic macrophytes can subsequently influence their distribution, with waterfowl generally showing a preference for larger, and possibly more isolated sites (Frisch *et al.* 2012; Sebastián-González & Green 2014).

Aquatic macrophytes can also disperse between habitats via wind or water. Dispersal via these mechanisms is likely to be more restricted in pond landscapes, where the small size and isolation of habitats makes colonisation by passive dispersal less likely. However, hydrochorous dispersal (dispersal by water), can be important when ponds are hydrologically connected, either permanently by ditches or channels (Akasaka & Takamura 2012; Soomers *et al.* 2013), or seasonally by flooding events (Jeffries 2008). In these instances, hydrochorous dispersal of propagules can influence macrophyte biodiversity (Akasaka & Takamura 2012), and community distributions (Jeffries 2008).

Despite the range of potential dispersal pathways exhibited by aquatic macrophytes, dispersal limitation between ponds is common, even over relatively short distances. Various studies have found macrophyte diversity and species composition to be significantly related to that found in neighbouring ponds (Barnes 1983; Linton & Goulder 2003; Jeffries 2008), reflecting generally short dispersal distances of aquatic macrophytes. In a small study of six ponds, Linton & Goulder (2003) found macrophyte species richness to be related to that of neighbouring ponds, and suggested that the study sites had a ‘baseline richness’ which reflected long distance propagule dispersal, with local dispersal between ponds then influencing macrophyte diversity above the ‘baseline’ species.

Studies using a greater number of sites have suggested similarly short distances for dispersal limitation. Jeffries (2008) monitored a series of 30 newly dug ponds covering an area of 30 x 30m, over a 10-year period. Even over these small distances and long time span, there was significant spatial patterning of communities in 8 out of 10 years. Similar findings have come from a study of the colonisation of 10 ball-clay ponds (created by open-cask mining), over a period of 15 years (Barnes 1983). Macrophyte colonisation was found to follow a predictable pattern between sites and years, with close agreement in the number and identity of species occurring in consecutive years. The majority of macrophyte species colonising new ball-clay ponds had potential sources within 500m of the colonised site, suggesting short-distance dispersal. Additionally, 7 species found nearby (within 1km), did not colonise any of the study ponds during the 15-year study period, most likely due to dispersal limitation. In more isolated ponds, initial community composition was less predictable, as limited dispersal was more likely to result in the chance colonisation and dominance of just one species. Overall, 64% macrophyte species found within the local area colonised the study ponds within 15 years, compared to 96% insects, and 64% non-insect invertebrates.

Evidence for strong dispersal limitation of pond communities has also come from work on cladocerans and copepods. Similar to aquatic macrophytes, these taxa are mostly dispersed as resting eggs via water or within the digestive tracts of birds (Green & Figuerola 2005;

Allen 2007), and as such experience similar patterns in dispersal limitation. Frisch *et al.* (2012) found that copepod and cladoceran populations experienced strong spatial influences in their colonisation of 96 experimental ponds, with hydrological connectivity and over-land distance both significantly influencing dispersal. In contrast the smaller rotifers, which are more easily dispersed by wind, did not show significant spatial patterning during colonisation.

Given the evidence for strong dispersal limitation of aquatic macrophytes, even over relatively short distances, it is likely that dispersal limitation is a major impairment to habitat restoration within fragmented agricultural landscapes. For example, Stroh *et al.* (2012) found that in the restoration of a wet fen in Cambridgeshire, there was very little natural recovery of vegetation, even though the restored site was in close proximity to intact species-rich fenland. As such it is possible that the historic propagule bank of buried agricultural wetlands, even if this has been significantly impaired by drainage and cultivation, could make an important contribution to colonisation of these sites post-restoration.

6.1.3 Aquatic restoration and the importance of the propagule bank vs. dispersal

Although aquatic propagule banks buried under agriculture often show considerably reduced species diversity and seed density (Middleton 2003; Stroh *et al.* 2012; Beas *et al.* 2013), the combination of both propagule bank and dispersal from neighbouring sites could benefit aquatic restoration. Few studies have compared the contribution of the aquatic propagule bank to that from dispersal, but those which have suggest a generally weak contribution from the propagule bank.

In riverine system in the UK, Gurnell *et al.* (2006) compared the composition of the seed bank and seed rain (collected on seed traps), to the composition of the final established vegetation along a newly dug stretch of river. Final vegetation composition was found to be more similar to that of the seed traps than the seed bank samples. Of 145 taxa, 50% occurred in both in the final vegetation, and depositional or seed bank samples, with 30 species occurring in the final vegetation absent from the seed bank. Low similarity between the seed bank and final vegetation composition has also been reported from a study of backwaters along navigation canals in the Netherlands. Although seven submerged aquatic species germinated from sediment samples collected prior to the restoration of the backwaters, none occurred in the backwaters after restoration, suggesting low potential for restoration from the propagule bank (Boedeltje, Bakker & Ter Heerdt 2003). The relatively low contribution of the seed bank to the final vegetation in these studies is perhaps not surprising, given the high potential for dispersal along hydrologically connected systems. However, similarly low seed bank contribution to standing vegetation has been observed in restored agricultural playas wetlands (Beas *et al.* 2013), suggesting these habitats may rely primarily on seed dispersal by waterfowl for colonisation.

Despite the generally low diversity and density of propagule banks buried under agricultural land, it is possible that these could make a small but important contribution to aquatic habitat restoration. In a recent restoration of a Danish lake (Lake Fil), which had been buried under intensive agriculture for 60 years, rapid aquatic macrophyte establishment occurred within two years of re-flooding (Kaplan *et al.* 2014). The majority of the 40 aquatic macrophyte species occurring in the new lake most likely arrived by dispersal from the near-by (50m) Lake Fidde, which shared 35 species in common with the newly created lake, or from the canals feeding the new lake, which shared 10 species in common. However, eight aquatic macrophyte species appeared in the new Lake Fil, which were not found anywhere else in the local area. These included one species, *Baldellia repens*, which was formerly extinct in Denmark but formed 13 new populations in Lake Fil, and one species, *Elatine hydropiper*, which is extremely rare (Baastrup-Spohr *et al.* 2016). The authors attributed these occurrences to dispersal, and did not assess the historic seed bank because they regarded it as negligible, nonetheless it is quite possible that this was in fact the source of these species. Kaplan *et al.* (2014) report on the re-establishment of two populations of *Potamogeton coloratus* in restored fenland ponds in the Czech Republic, where the species had been extinct for over 30 years. In addition, two other rare native plants established in the same ponds, and their viable seeds were identified in sediment samples collected prior to restoration. Although no seeds of *P. coloratus* were found in the same sediments, it is likely that this was due to extremely low propagule density. Similar findings for the potential contribution of historic propagule banks have come from work on restored turf ponds in the Netherlands, where dense beds of charophytes re-colonised restored sites, after being absent from the region for around 80 years (Beltman & Allegrini 1997).

Having demonstrated in the previous chapter that a number of aquatic macrophyte species remain viable in the sediments of ghost and overgrown ponds, this chapter examines the colonisation processes in these habitats after their resurrection / restoration. The relative importance of the historic propagule bank vs. dispersal processes for the colonisation of the study sites is investigated through a series of outdoor mesocosms. The establishment of aquatic and wetland macrophytes in the study ponds, over the first year of colonisation, is discussed in relation to the findings from the mesocosms, microcosms, and seed viability testing.

Although the main focus of this chapter is the contribution of the historic propagule bank to macrophyte establishment in ghost and restored ponds, data was also collected on the colonisation by aquatic Coleoptera of both the study ponds and outdoor mesocosms. Aquatic Coleoptera have been suggested as a good indicator group for general invertebrate diversity (Briers & Biggs 2003; Gioria *et al.* 2010), and as particularly sensitive bio-indicators (Fairchild *et al.* 2000). As strong-flying active disperses, Coleoptera are usually one of the first taxa to colonise new ponds (Bloechl *et al.* 2010; Coccia *et al.* 2016), and their assemblages are often related to aquatic vegetation characteristics (Gioria *et al.* 2010). As such, the species composition of Coleoptera in both the ghost and restored ponds and the open outdoor mesocosms, was monitored at three time points throughout the first year of colonisation. Monitoring was conducted in order to assess whether; i) there were any differences in species composition between ghost and restored ponds, ii) there were any differences in species composition between open mesocosms and the study ponds, providing some measure of the ‘ecological validity’ of the mesocosms in replicating real pond conditions, and iii) there were any differences in species composition between different mesocosm treatments, most likely related to difference in macrophyte colonisation.

6.2 Chapter hypothesis

H1 – ‘Propagule bank’ treatment mesocosms, which contain historic pond sediment, are colonised more rapidly by aquatic macrophytes than sterile ‘dispersal’ treatment mesocosms.

H2 – ‘Propagule bank’ treatment mesocosms contain a greater diversity of aquatic macrophyte species than ‘Dispersal’ treatment mesocosms, throughout the first year of colonisation.

H3 – Macrophytes germinating in the ‘Propagule bank’ treatment mesocosms are a sub-set of the species occurring in the study ponds, and include the species found to be viable in the microcosm and seed viability testing (*Chapter 5*).

H4 – Macrophyte species richness and colonisation rate are similar for both ghost and restored ponds, reflecting their shared environment and similar propagule bank viabilities.

H5 – Water chemistry parameters and aquatic Coleoptera colonisation are similar between ghost and restored ponds, and between study ponds and open mesocosms.

6.3 Methodology

6.3.1 Locating and excavating ghost and overgrown ponds

Ghost ponds were identified using historic UK Ordinance Survey (OS) maps (EDINA Digimap 2013), or local tithe maps (Norfolk County Council Map Explorer). Landowners with a ghost pond on their land were contacted to see whether they also had an overgrown pond in need of restoration, so that study sites could be paired across the different locations. In addition, an advertisement was placed in Farmers Weekly calling for landowners who knew that they had a ghost pond on their land, and who would be happy to have it re-excavated. Pairs of study sites were then chosen based on their suitability and landowner access permission. Suitable study sites had to have both a ghost pond and an overgrown pond within close proximity to each other, both located within intensive arable land use. For the three ghost ponds, time since burial was estimated from the most recent map demarcation of a pond, and from landowner knowledge of historic pond in-filling. The oldest selected ghost pond, GP_{150}, was buried between 1839 and 1883. GP_{50} was filled-in during the late 1960s, and GP_{45} during the early 1970s. All three ponds were located in north Norfolk. Directly prior to excavation, GP_{150} was situated under a hedgerow grass buffer, while both GP_{50} and GP_{45} were located in the middle of arable fields. Once ghost pond locations were established based on the presence of a field contour depression, or by changes in vegetation and soil colour (*Figure 47a*), a trench was excavated through their centre (*Figure 47b*). Top soil was removed using a 360° excavator until dark historic pond sediment, with its fine silty texture, was reached. Bulk samples (approx. 30 litres), of the historic sediments were collected by hand from multiple locations around the old pond basin, and stored in dark air-tight bags for 2-3 days, before being used in the outdoor mesocosms (*Figure 49*). Additional sediments were kept in long-term cold storage at 5°C, and used in the microcosm and seed viability testing experiments presented in *Chapter 5*. Each ghost pond was then fully excavated following the profile of the historic sediment layer, to closely resemble the size and depth of the historic pond (*Figure 47d*). The spoil was spread across the surrounding arable field, and a buffer of at least 6m width was established around each resurrected pond.

Overgrown ponds were surveyed for aquatic macrophytes in April – May 2013, prior to their restoration in September 2013. At each site, trees and large shrubs were removed from the pond basin and roughly ¾ of the pond edge, opening the pond up to light (*Figure 48*). Large logs were stacked around the pond margin to provide hibernacula for amphibians and reptiles, while scrub was removed by the farmer or left in a brash heap on the pond margin (at least 6m wide). Following tree removal, the top layers of unstable, anoxic sediment which had built up during the ponds' overgrown phase were removed by 360° excavator. Sediment was removed until the oldest layers of firm, stable sediment were reached, close to the interface with the original marl pond basin (*Figure 48*). Bulk sediment samples (approx. 30 litres), were collected from the lowest sediment layers reached in each pond, and stored for use in mesocosms, microcosms and seed viability tests. As with the ghost ponds, spoil from the restored ponds was spread across the surrounding arable field, avoiding any established pond margin or floristically rich grass areas.

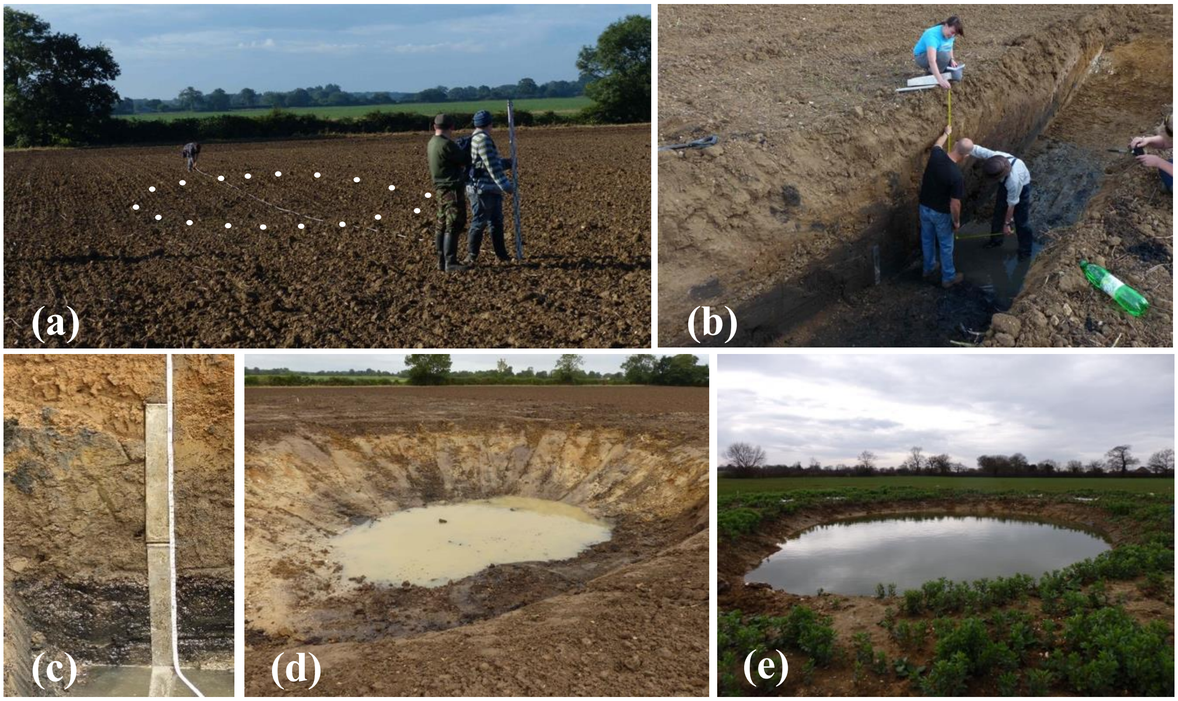


Figure 47 – ‘Resurrection’ of GP$_{50}$ in Sept. 2013. (a) Marking out the perimeter of the depression (highlighted in the photograph with white dots), which indicates the location of GP$_{50}$. (b) Excavation of a trench through GP$_{50}$ to identify and sample the black layer of historic pond sediment, buried underneath the brown top-soil. (c) Sampling a cross section of the historic sediment layer and overlying top soil. (d) Final profile of the newly excavated GP$_{50}$, showing the dark pond sediments left within the pond basin to provide a source of propagules. (e) GP$_{50}$ in Jan. 2014, having filled with water over the winter.

Figure 48 – Restoration of STRE in Oct. 2013. (a) Inside the pond, prior to restoration. (b) Beginning tree clearance. (c) Removing the surface layers of unstable, anoxic sediment, which had built-up during the overgrown pond phase. (d) Final profile of the restored pond. The lower layers of dark pond sediment were left in the basin to provide a source of propagules. The white marl clay, which forms the pond base, has been exposed in some areas, as can be seen towards the back of the pond.

6.3.2 On-site mesocosms

Sixteen PVC-lined mesocosms measuring 40 x 30 x 30 cm^3 were established around each of the study ponds (*Figure 49*). For each pond, eight mesocosms were prepared with 2 L of historic pond sediment. Four of these mesocosms were kept open ('Propagule bank & Dispersal'), while 4 ('Propagule bank') were covered with 0.25 mm diameter mesh to prevent contamination by dispersing plant seeds and oospores. The remaining eight mesocosms were prepared with 2 L of a 50 / 50 mix of steam-treated potting soil and builder's sand (Skoglund & Hytteborn 1990; Boedeltje *et al.* 2002); with 4 of these again left open ('Dispersal') and 4 covered with 0.25 mm mesh ('Control'). Despite their small size, the positioning of the mesocosms adjacent to the study ponds meant that waterfowl, a key dispersal vector for aquatic macrophyte seeds (Cook 1990; Brochet *et al.* 2010; Soons *et al.* 2016), accessed both ponds and open mesocosms in an equivalent manner. All mesocosms were filled with filtered (53 µm mesh) rainwater and surveyed for aquatic macrophytes at the same time intervals as the study ponds (*Table 25*). However, after week 40 (Jul 2014), closed 'Control' and 'Propagule bank' mesocosms at two of the three study locations (Westfield and Guestwick), were found open and damaged (likely due to trampling by deer), with likely contamination. As such, results are presented up until week 40, and the causes of contamination and limitations of the method are discussed.

Figure 49 – Mesocosm set-up. (a) Open treatment mesocosms around GP_{150}. (b) Open and closed treatment mesocosms around STRE.

6.3.3 Monitoring and data collection

Ghost and restored ponds were excavated in September 2013, and naturally filled with water over the winter months. On-site mesocosms were established at the same time, but were filled by hand with filtered rain water. Both ponds and mesocosms were surveyed for aquatic macrophytes throughout the first year of colonisation (*Table 25*), at weeks 5 (autumn), 16 (winter), 28, 34 (spring), 40, 44 and 50 (summer). Restored ponds were additionally surveyed in April 2013, prior to their restoration. Submerged aquatic macrophytes were surveyed by eye with the assistance of a double-headed rake for surveying deep water areas. Marginal and emergent macrophytes were surveyed by eye, and identified in the field where possible. Charophytes were identified as far as possible in the field, using a x10 - 20 eye-piece. Additional samples were collected and preserved in alcohol, and later identified to species level using x10 – 40 binocular microscope, with reference to the identification guide by Moore (2005). A sub-sample of the charophytes collected from each study pond were sent to Nick Stewart (BSBI referee and National recorder), for ID confirmation. Species abundances within the study ponds were recorded on the DAFOR scale (Palmer *et al.* 1992), although statistical analysis of the mesocosms was based on species presence / absence data.

Water chemistry parameters were recorded in both the study ponds and mesocosms at weeks 16 (winter), 28 (spring), 40 and 50 (summer). Four key water chemistry parameters which are known to influence macrophyte germination, and which could be quickly recorded across a large number of field sites, were monitored using a portable HACH multi-parameter probe; water temperature, dissolved oxygen ($\mu g\ L^{-1}$), pH, and conductivity ($mS\ cm^{-1}$). Water chemistry within the mesocosms was monitored to check whether differences in macrophyte colonisation were due to the propagule bank, or whether this might be confounded by chemical differences between treatments. Differences in water chemistry between the mesocosms and study ponds were also of interest for determining how well the mesocosms represented conditions within the study ponds, and whether differences in key water chemistry parameters might influence macrophyte colonisation in the two different environments.

Study ponds and open treatment mesocosms were surveyed for adult aquatic Coleoptera at weeks 5, 28 and 50, providing data on species colonisation within the first month after pond excavation (October), during the peak dispersal and breeding period for Coleoptera (April – May), and one year after pond excavation (September). Surveys were conducted by Geoff Nobes (Norfolk County recorder), with species identified in the field and then released back into the study ponds. Study ponds were surveyed exhaustively using a BSBI standard pond net, until no new species were being found. Mesocosms were surveyed using a smaller hand net, with 4 – 5 ‘sweeps’ made through the mesocosm.

Table 25 – Sampling dates for the study ponds, mesocosms, and microcosms.

Date	Oct. 13	Feb. 14	May 14	Jun. 14	Jul. 14	Aug. 14	Sept. 14	Nov. 14	Feb. 15	May. 15
Week	5	16	28	34	40	44	50	55	68	82
Macropytes	☐	☐	☐	☐	☐	☐	☐	.	.	.
Water chemistry	.	☐	☐	.	☐	.	☐	.	.	.
Coleoptera	☐	.	☐	.	.	.	☐	.	.	.
Microcosms	.	.	☐	☐	☐	☐	☐	☐	☐	☐

6.3.4 *Macrofossil analysis*

During the collection of seeds and oospores for TZ staining (*Chapter 5*), non-viable macrophyte remains including empty seed cases and leaf fragments, were also recorded. This was to provide further context to the macrophyte species data obtained from the study ponds, which were colonised by a number of species that did not occur in the experimental treatments. Due to time constraints, macrofossils were recorded from ghost pond sediments only, as this could be conducted alongside the extraction of viable seeds for use in *Chapter 5*. For each ghost pond, 200 ml of sediment (4 x 50 ml homogenised samples), were sampled. An additional 450 ml bulk sample from GP_{150} was processed, giving a total of 650 ml of sampled sediment, in order to maximise the number of examined propagules from this oldest site. Sediment was passed through 355 µm and 125 µm sieves, using a water jet and soft brush to clean sediment from the macrofossils. Macrofossils were then pipetted into a divided petri dish (~2 mm diluted sample at a time), and examined under a x 10 – 40 dissecting microscope. Seeds and oospores which appeared full and turgid were extracted for TZ staining (see *Chapter 5 – Methodology*). Additional plant remains, including empty seed cases and leaf and stem fragments, were preserved in glycerol, and identified with reference to the UCL macrofossil catalogue and the Digital seed Atlas of the Netherlands (Cappers, Bekker & Jans 2006).

6.3.5 Statistical analysis and data presentation

Profile analysis, conducted in SPSS, was used to test for differences in macrophyte colonisation and water chemistry parameters between the four different mesocosm treatments. Additionally, species richness of aquatic Coleoptera found in the open treatment mesocosms, and ghost and restored study ponds, was also assessed through profile analysis. Profile analysis is a multivariate approach for analysing repeated measures over time, and is robust to both small sample sizes and violations of normality (Tabachnick & Fidell 2007). Prior to analysis, data screening was conducted to check for normality of variables and homogeneity of variance. Graphs were generated in R (package 'ggplot2').

Macrophyte species richness and colonisation rates within the six study ponds are presented as heat maps, showing the DAFOR score and date of first appearance for all colonising species. Macrophyte species composition of the study ponds and mesocosms, as well as the microcosms, TZ staining, and macrofossil record (*Chapter 5*), are presented and discussed. Species composition of adult Coleoptera occurring in the ghost and restored study ponds and open mesocosms are presented, and the degree of species overlap between the different treatments is discussed.

6.4 Results

6.4.1 Relative importance of the propagule bank vs. dispersal in mesocosm colonisation

During the first 40 weeks of the mesocosm experiment (September 2013 – July 2014), both ghost and restored pond mesocosms showed similar trends in macrophyte colonisation (*Figure 50*). For both pond types, germination in covered mesocosms containing pond sediment (‘Propagule bank’ treatment), was significantly higher ($p < 0.02$) and faster than in other mesocosm types.

Within ghost pond mesocosms (*Figure 50a* and *Table 26*), a total of six macrophyte species germinated in the order: *P. natans* (week 5, GP_{50} sediment), *C. vulgaris* (week 28, GP_{150} and GP_{50} sediment; week 40, GP_{45} sediment), *C. contraria* (week 28, GP_{50} sediment), *C. virgata* (week 34, GP_{150} sediment), *C. globularis* and *C. hispida* (week 40, GP_{45} sediment). Only *P. natans* (week 28, GP_{50}) and *C. vulgaris* (week 34, GP_{150} and GP_{50}) germinated in the open ‘Propagule bank & Dispersal’ treatment, and only *C. vulgaris* (week 34, GP_{150} and GP_{50}) germinated in the open ‘Dispersal’ treatment containing sterile substrate. No germinations occurred under ‘Control’ treatments. Overall, there was a significant effect of date ($F(1.8) = 15.78$, $p < 0.001$), mesocosm treatment ($F(3) = 7.95$, $p < 0.001$), and date/treatment interaction ($F(5.5) = 3.99$, $p < 0.001$), with the ‘Propagule bank’ treatment being significantly different ($p < 0.02$) from all other mesocosm treatments. There were no significant differences between the other mesocosm treatments.

Within restored pond mesocosms (*Figure 50b* and *Table 27*), a total of five macrophyte species germinated in the order: *P. trichoides* (week 5, WERE sediment), *P. natans* (week 5, GURE sediment; week 30, STRE sediment; week 40, WERE sediment), *Z. palustris* (week 16, WERE sediment), *C. vulgaris* (week 34, STRE and WERE sediment). Only *P. natans* (week 34, STRE and week 40, GURE) and *C. vulgaris* (week 40, GURE) germinated in the open ‘Propagule bank & `Dispersal’ treatment, and only *C. vulgaris* (week 40, GURE) germinated in the open ‘Dispersal’ treatment containing sterile substrate. No germinations occurred under ‘Control’ treatments. Overall, there was a significant effect of date (F(1.8) = 13.31, $p < 0.001$), mesocosm treatment (F(3) = 10.65, $p < 0.001$), and date/treatment interaction (F(5.5) = 5.05, $p < 0.001$), with the 'Propagule bank' treatment being significantly different ($p < 0.02$) from all other mesocosm treatments. There were no significant differences between the other mesocosm treatments. Between weeks 40 – 44 of the mesocosm experiment, several 'Control' and 'Propagule bank & Dispersal' mesocosms sustained damage. Germinations of *P. trichoides* (week 50, pond STRE), *C. vulgaris* (week 44, ponds WERE and GURE), and *Z. palustris* (week 44, pond WERE) had occurred in the damaged 'Control' mesocosms, and as such data collected after week 40 are not included in the analysis.

In addition to aquatic macrophyte germinations, crustaceans (*Daphnia* spp. and *Cyclops* spp.) were also observed in both the 'Propagule bank' mesocosms and sealed microcosms, indicating the presence of viable 'resting eggs' of crustacean zooplankton, although their establishment patterns were not investigated.

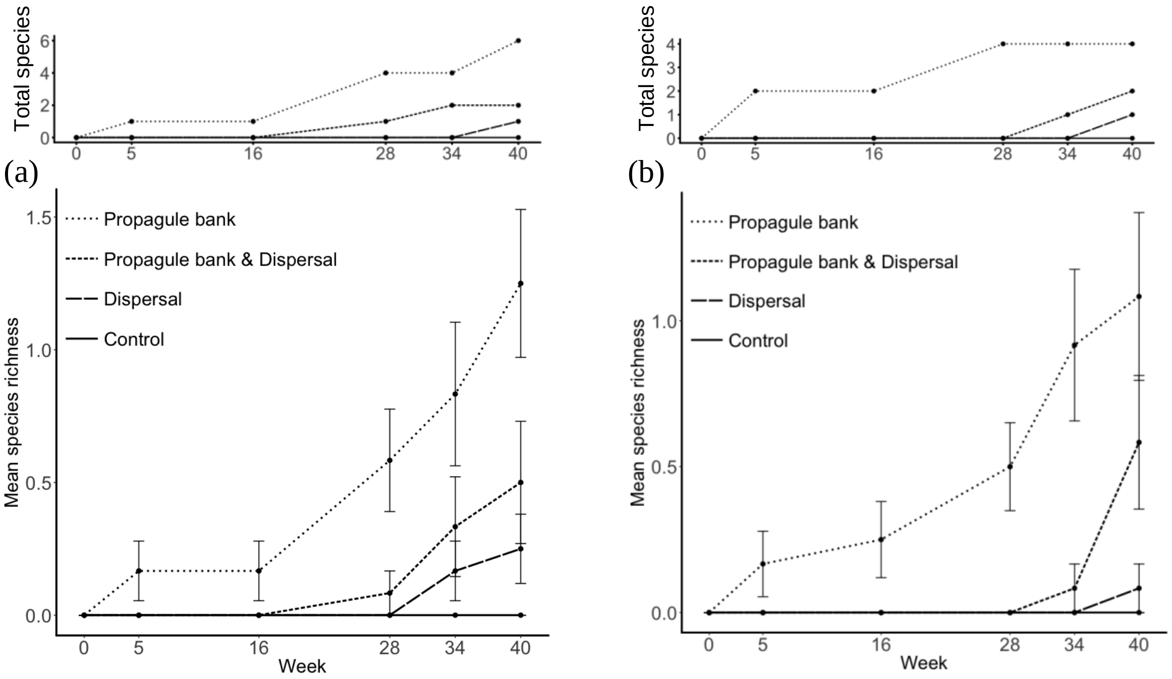


Figure 50 – Macrophyte species richness in the ghost pond mesocosms (a), and restored pond mesocosms (b). Error bars show the SEM. Top graphs show the total species richness of the different mesocosm treatments.

Table 26 – Descriptive statistics for macrophyte colonisation in the ghost pond mesocosms.

Propagule bank	Week 5	Week 16	Week 28	Week 34	Week 40
Total species richness	1	1	4	4	6
Mean species richness	0.17	0.17	0.58	0.08	1.25
SD	0.39	0.39	0.19	0.94	0.97
SE	0.11	0.11	0.08	0.27	0.28
Propagule bank & Dispersal	Week 5	Week 16	Week 28	Week 34	Week 40
Total species richness	0	0	1	2	2
Mean species richness	0	0	0.08	0.333	0.50
SD	0	0	0.29	0.65	0.80
SE	0	0	0.08	0.19	0.23
Dispersal	Week 5	Week 16	Week 28	Week 34	Week 40
Total species richness	0	0	0	1	2
Mean species richness	0	0	0	0.17	0.25
SD	0	0	0	0.39	0.45
SE	0	0	0	0.11	0.13

Table 27 – Descriptive statistics for macrophyte colonisation in the restored pond mesocosms.

Propagule bank	Week 5	Week 16	Week 28	Week 34	Week 40
Total species richness	2	2	3	4	4
Mean species richness	0.17	0.25	0.50	0.92	1.08
SD	0.39	0.45	0.52	0.90	0.99
SE	0.11	0.13	0.15	0.26	0.29
Propagule bank & Dispersal	Week 5	Week 16	Week 28	Week 34	Week 40
Total species richness	0	0	0	1	2
Mean species richness	0	0	0	0.083	0.58
SD	0	0	0	0.29	0.79
SE	0	0	0	0.08	0.23
Dispersal	Week 5	Week 16	Week 28	Week 34	Week 40
Total species richness	0	0	0	1	1
Mean species richness	0	0	0	0.83	0.83
SD	0	0	0	0.29	0.29
SE	0	0	0	0.08	0.08

6.4.2 Water chemistry parameters within the mesocosms and study ponds

Within the ghost ponds and associated mesocosms, all four water chemistry variables showed significant seasonal variation ($p < 0.01$), and a significant effect of treatment ($p < 0.05$). For conductivity, there was also a significant time / treatment interaction ($p < 0.001$). Water temperature (*Figure 51a*) showed a significant difference between the study ponds and mesocosms ($p < 0.01$), but no significant differences between mesocosms in the different treatments. Conductivity (*Figure 51b*) showed a significant difference between the study ponds and mesocosms ($p < 0.001$), and between the closed ‘Propagule bank’ treatment and open ‘Propagule bank & Dispersal’ and ‘Dispersal’ treatments ($p < 0.05$). As such, differences in conductivity appeared to be due to whether a mesocosm was closed or open, and not related to the sediment vs. sterile substrate treatments. Dissolved oxygen (*Figure 51c*) did not differ significantly between treatments, and pH (*Figure 51d*) showed a significant difference between the open ‘Propagule bank and Dispersal’ and closed ‘Control’ mesocosm treatments only ($p < 0.05$). Again, these difference appear to be related to the open / closed treatments, rather than to substrate type. Descriptive statistics for study pond and mesocosm water chemistry are presented in *Table 28.*

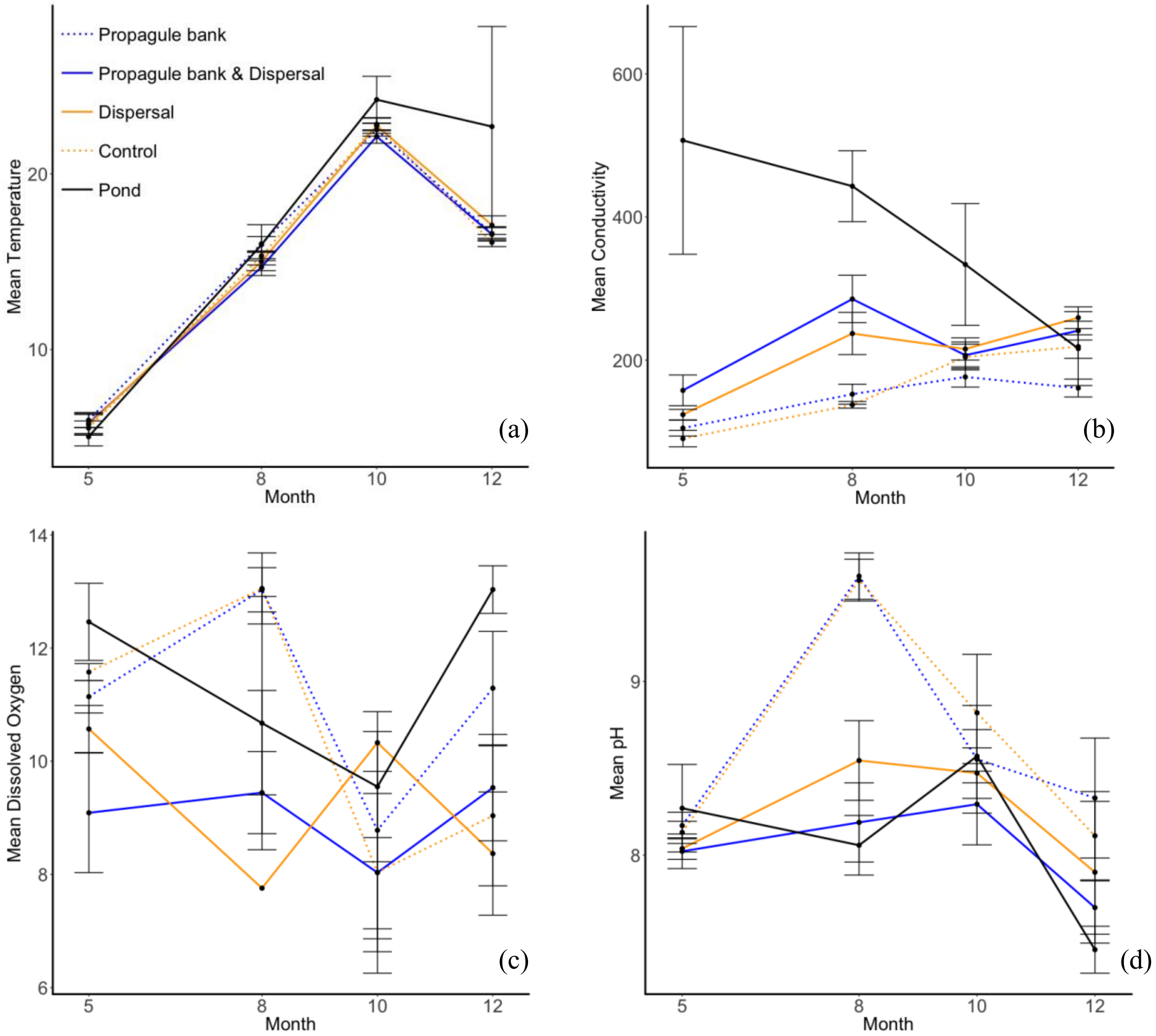


Figure 51 – Water chemistry in ghost mesocosms and study ponds. (a) mean temperature, (b) mean conductivity (mS cm^{-1}), (c) mean dissolved oxygen (μg L^{-1}), (d) mean pH. Error bars show the SEM.

Within the restored ponds and associated mesocosms, all four water chemistry variables showed significant seasonal variation ($p < 0.001$). Conductivity and pH showed a significant effect of treatment ($p < 0.001$), and dissolved oxygen, conductivity and water temperature showed a significant time / treatment interaction ($p < 0.05$). Water temperature (*Figure 52a*) and dissolved oxygen (*Figure 52c*) did not differ significantly between mesocosm treatments or study ponds. Conductivity (*Figure 52b*) showed significant differences between study ponds and all mesocosm treatments ($p < 0.001$), and between the two open 'Dispersal' and 'Propagule bank & Dispersal' and closed 'Control' and 'Propagule bank' treatments ($p < 0.05$). As with the ghost pond mesocosms, differences in conductivity appeared to be due to whether a mesocosm was closed or open, and not related to the sediment vs. sterile substrate treatments. pH (*Figure 52d*) showed significant differences between the 'Propagule bank & Dispersal' and 'Control' mesocosms only ($p < 0.05$).

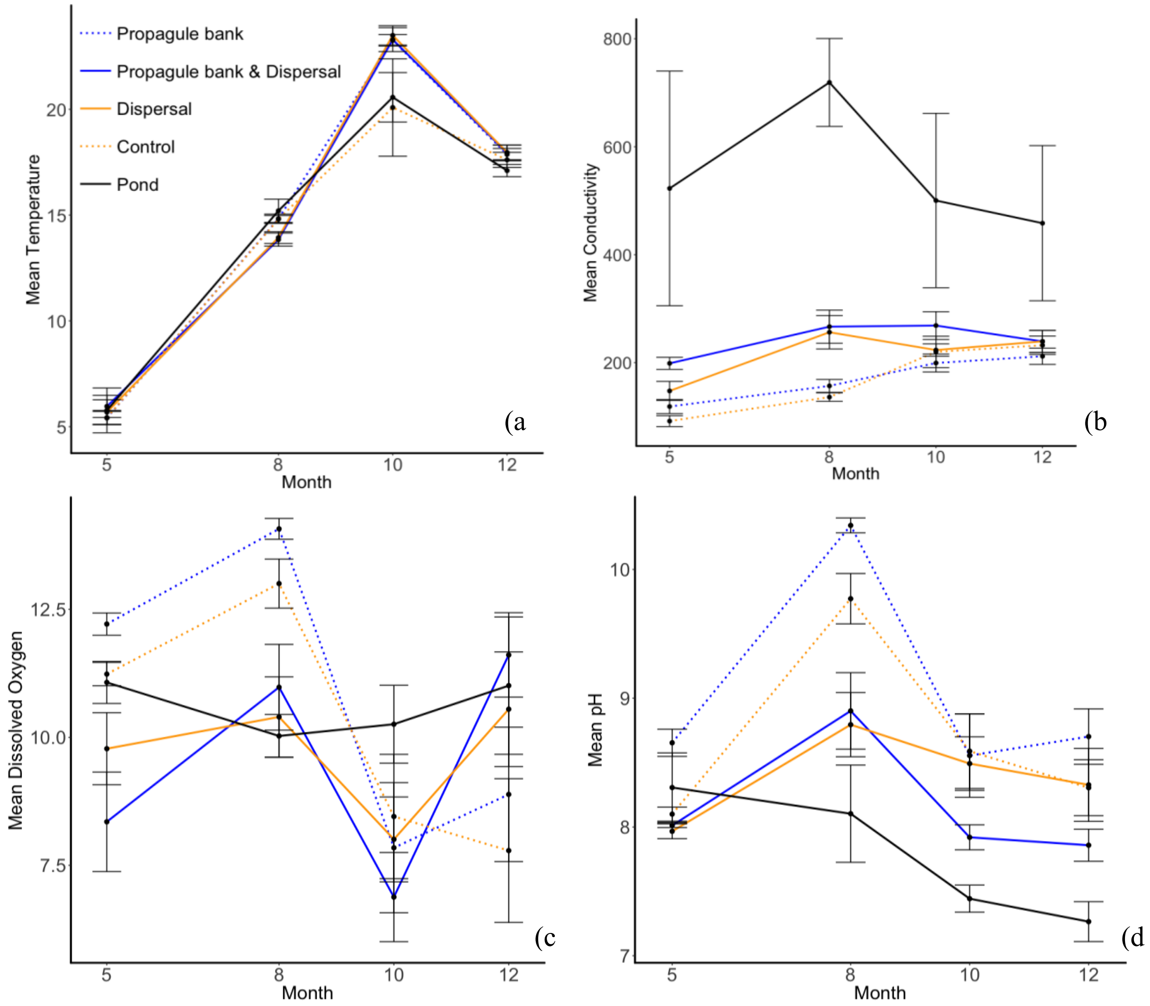


Figure 52 – Water chemistry in restored mesocosms and study ponds. (a) mean temperature, (b) mean conductivity (mS cm^{-1}), (c) mean dissolved oxygen (µg L^{-1}), (d) mean pH. Error bars show the SEM.

Overall, significant differences in water chemistry do not appear to be related to the historic sediment / sterile substrate treatments, but to whether mesocosms were open / closed. Differences in macrophyte colonisation between mesocosms containing historic pond sediments, and mesocosms containing sterile substrate, were therefor likely due to propagule bank differences, as opposed to any confounding influence of water chemistry.

Table 28 – Descriptive statistics for ghost and restored pond pH, dissolved oxygen (µg L^{-1}), conductivity (mS cm^{-1}), and water temperature.

Ghost pH

Propagule bank	Month 5	Month 8	Month 10	Month 12
Mean	8.17	9.61	8.55	8.33
SD	0.27	0.46	1.07	0.98
SE	0.08	0.13	0.31	0.35
Propagule bank & Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	8.02	8.19	8.29	7.70
SD	0.34	0.79	0.81	0.44
SE	0.10	0.23	0.23	0.15
Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	8.04	8.55	8.47	7.90
SD	0.22	0.79	0.51	1.15
SE	0.06	0.23	0.15	0.41
Control	Month 5	Month 8	Month 10	Month 12
Mean	8.13	9.58	8.82	8.11
SD	0.22	0.42	1.17	0.72
SE	0.06	0.12	0.34	0.25
Pond	Month 5	Month 8	Month 10	Month 12
Mean	8.27	8.06	8.57	7.46
SD	0.44	0.30	0.27	0.19
SE	0.25	0.17	0.15	0.14

Restored pH

Propagule bank	Month 5	Month 8	Month 10	Month 12
Mean	8.65	10.34	8.56	8.70
SD	0.38	0.20	1.12	0.61
SE	0.11	0.06	0.32	0.22
Propagule bank & Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	8.01	8.90	7.92	7.86
SD	0.05	1.03	0.32	0.33
SE	0.02	0.30	0.10	0.12
Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	7.97	8.80	8.49	8.33
SD	0.20	0.86	0.72	0.80
SE	0.06	0.25	0.21	0.28
Control	Month 5	Month 8	Month 10	Month 12
Mean	8.10	9.77	8.59	8.31
SD	0.19	0.68	1.00	0.61
SE	0.05	0.20	0.29	0.22
Pond	Month 5	Month 8	Month 10	Month 12
Mean	8.31	8.10	7.44	7.27
SD	0.47	0.65	0.18	0.22
SE	0.27	0.38	0.11	0.16

Ghost Dissolved Oxygen

Propagule bank	Month 5	Month 8	Month 10	Month 12
Mean	11.14	13.03	8.78	11.29
SD	1.08	1.35	6.04	3.47
SE	0.29	0.39	1.74	1.00
Propagule bank & Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	9.09	9.45	8.03	9.53
SD	3.66	2.51	4.84	3.25
SE	1.06	0.72	1.40	0.94
Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	10.57	10.33	7.76	8.37
SD	1.44	3.19	3.10	3.77
SE	0.42	0.92	0.89	1.09
Control	Month 5	Month 8	Month 10	Month 12
Mean	11.58	13.05	8.04	9.04
SD	0.52	2.18	6.17	4.28
SE	0.15	0.63	1.78	1.24
Pond	Month 5	Month 8	Month 10	Month 12
Mean	12.46	10.67	9.55	13.03
SD	1.18	3.87	2.30	0.73
SE	0.68	2.24	1.33	0.42

Restored Dissolved Oxygen

Propagule bank	Month 5	Month 8	Month 10	Month 12
Mean	12.21	14.07	7.84	8.89
SD	0.75	0.70	4.41	4.55
SE	0.22	0.20	1.27	1.31
Propagule bank & Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	8.35	10.98	6.88	11.61
SD	3.23	2.78	2.89	2.74
SE	0.97	0.84	0.87	0.83
Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	9.78	10.40	8.01	10.55
SD	2.44	2.72	2.87	3.88
SE	0.70	0.78	0.83	1.12
Control	Month 5	Month 8	Month 10	Month 12
Mean	11.24	13.01	8.45	7.79
SD	0.79	1.66	4.57	4.86
SE	0.23	0.48	1.21	1.40
Pond	Month 5	Month 8	Month 10	Month 12
Mean	11.07	10.03	10.26	11.01
SD	0.71	0.73	1.32	2.32
SE	0.41	0.42	0.76	1.34

Ghost Conductivity

Propagule bank	Month 5	Month 8	Month 10	Month 12
Mean	105.13	152.53	176.60	161.08
SD	38.48	48.41	49.06	43.27
SE	11.11	13.98	14.16	12.49
Propagule bank & Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	157.80	285.43	206.94	241.24
SD	74.36	114.76	63.80	45.63
SE	21.47	33.13	18.42	13.17
Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	123.95	237.25	215.61	259.34
SD	25.49	101.81	53.53	56.90
SE	7.36	29.39	15.45	15.11
Control	Month 5	Month 8	Month 10	Month 12
Mean	90.48	137.66	204.38	219.02
SD	39.38	17.68	62.28	57.00
SE	11.37	4.70	17.98	16.45
Pond	Month 5	Month 8	Month 10	Month 12
Mean	507.00	443.00	333.70	216.20
SD	275.41	85.77	147.48	89.48
SE	159.01	49.52	85.15	51.66

Restored Conductivity

Propagule bank	Month 5	Month 8	Month 10	Month 12
Mean	118.76	156.92	199.38	211.85
SD	45.15	41.30	57.66	51.93
SE	13.03	11.92	16.64	14.99
Propagule bank & Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	198.73	266.68	268.78	239.31
SD	36.85	101.64	84.78	67.11
SE	11.11	30.64	25.71	20.23
Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	147.66	256.41	223.39	239.43
SD	60.86	107.44	39.32	70.48
SE	17.57	31.02	11.35	20.35
Control	Month 5	Month 8	Month 10	Month 12
Mean	91.80	136.25	219.93	232.31
SD	35.22	27.21	100.59	59.17
SE	10.17	7.85	29.04	17.08
Pond	Month 5	Month 8	Month 10	Month 12
Mean	522.73	719.00	500.33	458.33
SD	376.35	140.90	279.66	248.81
SE	217.29	81.35	161.46	143.65

Ghost Temperature

Propagule bank	Month 5	Month 8	Month 10	Month 12
Mean	5.98	16.02	22.52	16.61
SD	1.51	1.42	1.26	1.23
SE	0.44	0.41	0.36	0.36
Propagule bank & Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	5.79	14.69	22.13	16.56
SD	1.98	1.67	1.33	1.33
SE	0.57	0.48	0.38	0.38
Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	5.72	15.01	22.75	17.08
SD	1.99	1.81	1.49	1.82
SE	0.57	0.52	0.43	0.53
Control	Month 5	Month 8	Month 10	Month 12
Mean	5.53	15.33	22.83	16.10
SD	1.41	0.91	1.31	0.82
SE	0.41	0.26	0.38	0.24
Pond	Month 5	Month 8	Month 10	Month 12
Mean	5.03	15.97	24.23	22.70
SD	0.91	1.99	2.31	9.88
SE	0.52	1.15	1.33	5.70

Restored Temperature

Propagule bank	Month 5	Month 8	Month 10	Month 12
Mean	5.41	14.83	23.26	17.87
SD	1.06	0.76	0.93	0.97
SE	0.31	0.22	0.27	0.28
Propagule bank & Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	5.95	13.84	23.29	17.94
SD	1.73	1.03	1.88	1.24
SE	0.52	0.31	0.57	0.37
Dispersal	Month 5	Month 8	Month 10	Month 12
Mean	5.68	13.93	23.49	17.96
SD	2.02	0.97	1.58	1.19
SE	0.58	0.28	0.46	0.34
Control	Month 5	Month 8	Month 10	Month 12
Mean	5.43	14.80	20.08	17.61
SD	1.17	0.60	7.97	1.23
SE	0.34	0.17	2.30	0.35
Pond	Month 5	Month 8	Month 10	Month 12
Mean	5.77	15.20	20.57	17.10
SD	1.83	0.95	2.02	0.50
SE	1.06	0.55	1.17	0.29

6.4.3 *Macrophyte colonisation and species composition in the study ponds*

Macrophyte colonisation in both the ghost and restored ponds followed similar trajectories (*Figure 53*), with ponds reaching a total macrophyte species richness of between 18 – 25 species after one year of colonisation (*Table 29*). With UK farmland ponds typically supporting between 6 – 14 aquatic macrophyte species (Davies et al. 2008; Sayer et al. 2012), the resurrected and restored study sites represent high biodiversity habitats. Within the ghost ponds, the first submerged aquatic macrophytes were recorded in January 2014, 4 months after pond excavation; *Ranunculus aquatilis* in GP_{150}, and *Potamogeton trichoides* in GP_{45}. While patterns in overall macrophyte species richness were fairly consistent between the six study sites, the order in which species occurred varied between sites. The earliest species to colonise the study ponds (October 2013), include some species that were present before pond excavation, and which most likely colonised from a modern a modern seed bank or local population. These include the species *J. effusus* and *J. inflexus* in GP_{150}, which were present in the damp hedgerow before the pond was excavated. Within the restored ponds, the first submerged macrophytes were recorded in October 2013, 1 month after pond excavation; *R. aquatilis* and *R. sceleratus* in pond WERE.

Table 29 - Macrophyte species richness (submerged and emergent), occurring in the study ponds during the first year of colonisation.

	GP_{45}	GP_{50}	GP_{150}	STRE	GURE	WERE
Oct. 13	0	0	2	2	3	5
Feb. 14	5	0	3	6	6	8
May 14	6	2	5	7	11	19
Jun. 14	9	4	9	11	14	19
Jul. 14	15	9	19	19	14	21
Aug. 14	19	17	24	22	23	22
Sept. 14	25	18	23	22	23	22

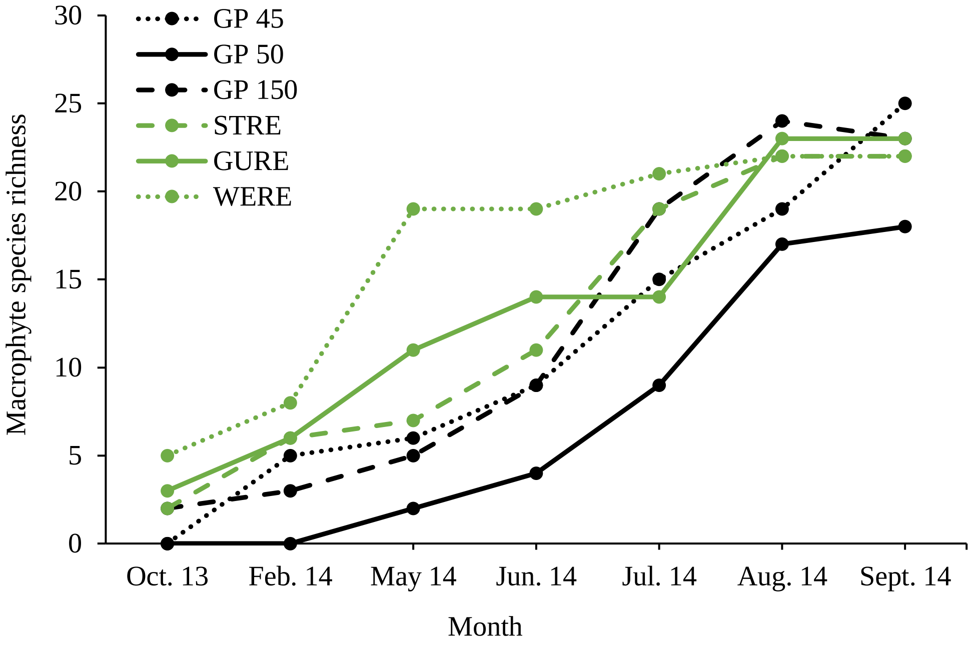


Figure 53 – Macrophyte species richness (submerged and emergent), occurring in the study ponds during the first year of colonisation.

Table 30 – GP_{45} species list, colour coded by DAFOR score (1 = rare, 2 = occasional, 3 = frequent, 4 = abundant, 5 = dominant).

Species	Oct-13	Jan-14	Feb-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14
Potamogeton trichoides	.	2	2	3	4	3	2	3
Agrostis stolonifera	.	.	1	2	2	2	3	2
Epilobium hirsutum	.	.	2	2	2	3	3	3
Juncus bufonius	.	.	2	2	2	3	3	3
Phalaris arundinacea	.	.	1	2	2	2	3	3
Persicaria amphibia	.	.	.	2	2	3	2	2
Ranunculus aquatilis	.	.	.	2	2	2	3	.
Solanum dulcamara	.	.	.	3	3	3	4	4
Juncus articulatus	.	.	.	.	2	1	3	3
Chara globularis	.	.	.	.	.	2	3	3
Chara hispida	.	.	.	.	.	2	2	3
Lemna trisulca	.	.	.	.	.	1	1	1
Nasturtium officinale agg.	.	.	.	.	.	1	.	1
Potamogeton crispus	.	.	.	.	.	2	2	3
Eleocharis palustris	.	.	.	.	.	.	2	2
Nitella flexilis agg.	.	.	.	.	.	.	1	2
Persicaria maculosa	.	.	.	.	.	.	1	1
Stachys palustris	.	.	.	.	.	.	2	3
Apium nodiflorum	.	.	.	.	.	.	.	2
Isolepis setacea	.	.	.	.	.	.	.	2
Juncus effusus	.	.	.	.	.	.	.	1
Juncus inflexus	.	.	.	.	.	.	.	1
Lemna minor	.	.	.	.	.	.	.	1
Potamogeton natans	.	.	.	.	.	.	.	2
Sparganium erectum	.	.	.	.	.	.	.	2

Table 31 – GP_{50} species list, colour coded by DAFOR score (1 = rare, 2 = occasional, 3 = frequent, 4 = abundant, 5 = dominant).

Species	Oct-13	Jan-14	Feb-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14
Chara vulgaris	.	.	.	2	2	3	3	4
Lemna minor	.	.	.	1	1	2	2	2
Ranunculus aquatilis	.	.	.	1	1	1	.	1
Chara contraria	.	.	.	.	.	2	3	3
Chara globularis	.	.	.	.	.	2	2	2
Juncus inflexus	.	.	.	.	.	2	2	2
Potamogeton crispus	.	.	.	.	.	2	3	3
Potamogeton natans	.	.	.	.	.	2	3	3
Agrostis stolonifera	.	.	.	.	.	.	1	1
Carex flacca	.	.	.	.	.	.	1	2
Epilobium hirsutum	.	.	.	.	.	.	2	2
Juncus articulatus	.	.	.	.	.	.	1	2
Juncus effusus	.	.	.	.	.	.	1	1
Persicaria maculosa	.	.	.	.	.	2	2	2
Solanum dulcamara	.	.	.	.	.	.	2	1
Sparganium erectum	.	.	.	.	.	.	1	2
Stachys palustris	.	.	.	.	.	.	1	1
Potamogeton trichoides	.	.	.	.	.	.	.	1

Table 32 – GP$_{150}$ species list, colour coded by DAFOR score (1 = rare, 2 = occasional, 3 = frequent, 4 = abundant, 5 = dominant).

Species	Oct-13	Jan-14	Feb-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14
Juncus effusus	1	1	1	1	2	2	2	2
Juncus inflexus	2	2	2	2	2	3	3	3
Ranunculus aquatilis	.	2	1	.	.	.	.	.
Juncus bufonius	.	.	2	3	3	3	3	3
Agrostis stolonifera	.	.	.	2	2	2	3	2
Chara vulgaris	.	.	.	2	3	3	3	3
Epilobium hirsutum	.	.	.	3	3	2	3	2
Mentha aquatica	.	.	.	2	2	3	2	2
Chara virgata	.	.	.	.	2	3	4	4
Persicaria amphibia	.	.	.	.	2	.	.	.
Potamogeton natans	.	.	.	.	1	1	.	2
Solanum dulcamara	.	.	.	.	2	2	3	2
Typha latifolia	.	.	.	.	1	2	2	1
Carex otrubae	.	.	.	.	.	2	3	3
Eupatorium cannabinum	.	.	.	.	.	2	2	2
Glyceria fluitans	.	.	.	.	.	2	2	2
Juncus articulatus	.	.	.	.	.	2	3	2
Persicaria maculosa	.	.	.	.	.	2	3	1
Potamogeton crispus	.	.	.	.	.	1	2	1
Sparganium erectum	.	.	.	.	.	2	1	.
Veronica anagallis-aquatica	.	.	.	.	.	1	.	.
Veronica beccabunga	.	.	.	.	.	1	.	1
Carex remota	.	.	.	.	.	.	2	2
Ranunculus sceleratus	.	.	.	.	.	.	1	.
Scrophularia auriculata	.	.	.	.	.	.	1	2
Zannichellia palustris	.	.	.	.	.	.	1	.
Carex flacca	.	.	.	.	.	.	.	1

GP$_{50}$ was colonised later in the year than the other ponds, with the first macrophyte species being recorded in May 2014. Across the ghost ponds, early colonising macrophytes tended to reach higher levels of dominance towards the end of the first year of colonisation, shown by the higher DAFOR scores and darker shading towards the top right of tables 26 – 32. The early appearance of the pondweed *P. trichoides* in January 2014 (GP$_{45}$), was unusual among the pondweeds, which appeared during the summer-time in the other study ponds; *P. trichoides* in September 2014 (GP$_{50}$), *P. crispus* in July 2014 (GP$_{45}$, GP$_{50}$ & GP$_{150}$), *P. natans* in June 2014 (GP$_{150}$) and September 2014 (GP$_{45}$), and *Z. palustris* in August 2014 (GP$_{150}$). The rushes *J. effusus* and *J. inflexus* were early colonists in GP$_{150}$, although this may have been due to a modern seed bank, closely followed by *J. bufonius* in February 2014 (GP$_{45}$ & GP$_{150}$). *J. articulatus* appeared in June 2014 (GP$_{45}$), July 2014 (GP$_{150}$), and August 2014 (GP$_{50}$). The Characeae, characteristically early colonists of aquatic habitats (Grillas *et al.* 1993; Beltman & Allegrini 1997), germinated in the ghost ponds during late spring – summer; *C. vulgaris* in May 2014 (GP$_{50}$ & GP$_{150}$), *C. virgata* in June 2014 (GP$_{150}$), *C. globularis* in Jul. 2014 (GP$_{45}$ & GP$_{50}$), *C. contraria* in July 2014 (GP$_{50}$), and *C. hispida* in July 2014 (GP$_{45}$). *Nitella flexilis* occurred in August 2014 (GP$_{45}$).

Similar patterns in species colonisation occurred in the restored ponds, despite the sites supporting different numbers of macrophyte species prior to restoration (*Table 33*). With the exception of the two Lemnid species *L. minor* and *L. trisulca*, all macrophyte species recorded in the overgrown ponds prior to restoration had re-established by the end of the first year post-restoration. Because some macrophyte species existed in the restored ponds prior to their restoration, the earliest survey (October 2013), includes a combination of newly colonising and remnant species. In pond STRE, a stand of *Iris pseudacorus* persisted through the overgrown pond phase, and was left intact during pond restoration. Similarly in pond WERE, the five species recorded in October 2013 were also present prior to pond restoration. In pond GURE, all of the species appearing in October 2013 represent new colonists, which were not present prior to restoration.

Table 33 – Macrophyte species richness within the study ponds before and after pond excavation (marked in grey).

Pond	May-13		Oct-13	May-14	Sep-14
WERE	14		5	19	23
GURE	3		3	12	20
STRE	3		3	7	23
GP$_{45}$	0		0	6	25
GP$_{50}$	0		0	2	18
GP$_{150}$	0		2	5	23

In the restored ponds, pondweeds tended to colonise slightly earlier in the year than in the ghost ponds; *P. trichoides* in May 2014 (WERE) and July 2014 (STRE), *P. natans* in June 2014 (STRE, GURE & WERE), *P. crispus* in May 2014 (GURE), and *Z. palustris* in May 14 (WERE). Rush species tended to appear around the same time of year as in the ghost ponds (except where these species were present before restoration); *J. articulatus* in May 2014 (GURE) and August 2014 (WERE), *J. effusus* in February 2014 (GURE) and July

2014 (STRE), and *J. inflexus* in February 2014 (GURE & WERE) and July 2014 (STRE). *J. bufonius* occurred in May 2014 (STRE, GURE & WERE), three months after it was first recorded in the ghost ponds. Similar to the ghost ponds, the characeae appeared earliest in the restored ponds in the late spring – early summer, appearing in the order; *C. vulgaris* in February 2014 (WERE), and May 2014 (STRE, GURE), *C. virgata* in June 2014 (WERE), *C. globularis* in June 2014 (GURE), *C. hispida* in August 2014 (STRE), *C. contraria* in June 2014 (GURE), and *N. flexilis* in May 2014 (WERE). The species *C. hispida* and *C. contraria* were location specific, with *C. hispida* occurring only in ponds GP_{45} and STRE (located in Stody), and *C. contraria* only in ponds GP_{50} and GURE (located in Guestwick). Other charophyte species occurred across ghost and restored ponds at different locations.

Table 34 – WERE species list, colour coded by DAFOR score (1 = rare, 2 = occasional, 3 = frequent, 4 = abundant, 5 = dominant).

Species	Oct-13	Jan-14	Feb-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14
Carex remota	1	1	1	3	3	3	2	3
Juncus effusus	2	2	2	.	.	2	2	2
Mentha aquatica	2	2	2	3	3	2	3	2
Nasturtium officinale agg.	1	2	2	3	3	2	3	3
Ranunculus sceleratus	1	2	2	3	3	2	2	2
Callitriche sp.	.	1	1	2	2	1	.	.
Epilobium hirsutum	.	1	2	2	4	3	3	3
Juncus inflexus	.	1	1	2	2	1	2	2
Chara vulgaris	.	.	2	4	4	4	3	4
Solanum dulcamara	.	.	2	3	3	3	2	1
Alisma plantago aquatica	.	.	.	1	3	3	3	3
Juncus bufonius	.	.	.	2	2	3	3	2
Nitella flexilis agg.	.	.	.	1	2	2	1	2
Potamogeton trichoides	.	.	.	2	4	4	4	3
Ranunculus aquatilis	.	.	.	2	2	.	.	.
Scrophularia auriculata	.	.	.	1	1	.	2	2
Sparganium erectum	.	.	.	2	3	2	3	2
Veronica anagallis-aquatica	.	.	.	3	4	3	3	3
Veronica beccabunga	.	.	.	3	3	2	3	3
Zannichellia palustris	.	.	.	2	4	3	3	2
Chara virgata	.	.	.	.	1	2	2	.
Persicaria maculosa	.	.	.	.	2	2	2	2
Potamogeton natans	.	.	.	.	2	2	.	.
Agrostis stolonifera	.	.	.	.	.	2	.	.
Glyceria fluitans	.	.	.	.	.	.	2	2
Juncus articulatus	.	.	.	.	.	.	1	2
Typha latifolia	.	.	.	.	.	.	2	1

Table 35 – STRE species list, colour coded by DAFOR score (1 = rare, 2 = occasional, 3 = frequent, 4 = abundant, 5 = dominant).

Species	Oct-13	Jan-14	Feb-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14
Iris pseudacorus	1	2	2	2	2	2	3	3
Epilobium hirsutum	.	2	2	2	2	3	4	3
Ranunculus aquatilis	.	3	3	3	4	2	2	3
Ranunculus sceleratus	.	2	2	2	3	2	2	2
Agrostis stolonifera	.	.	2	2	.	.	.	.
Solanum dulcamara	.	.	2	2	2	1	2	2
Chara vulgaris	.	.	.	2	4	4	4	4
Juncus bufonius	.	.	.	1	2	2	2	2
Sparganium erectum	.	.	.	1	3	4	4	4
Myosoties scorpoides	.	.	.	.	2	3	2	2
Potamogeton natans	.	.	.	.	2	3	3	3
Veronica catenata	.	.	.	.	2	2	2	3
Hippuris vulgaris	.	.	.	.	.	1	2	1
Juncus effusus	.	.	.	.	.	2	2	2
Juncus inflexus	.	.	.	.	.	2	2	2
Myriophyllum spicatum	.	.	.	.	.	2	3	3
Persicaria maculosa	.	.	.	.	.	2	3	2
Potamogeton berchtoldii	.	.	.	.	.	2	2	2
Potamogeton trichoides	.	.	.	.	.	2	.	.
Chara hispida	.	.	.	.	.	.	1	1
Typha latifolia	.	.	.	.	.	.	1	2
Veronica beccabunga	.	.	.	.	.	.	1	2
Apium nodiflorum	.	.	.	.	.	.	.	1

Table 36 – GURE species list, colour coded by DAFOR score (1 = rare, 2 = occasional, 3 = frequent, 4 = abundant, 5 = dominant).

Species	Oct-13	Jan-14	Feb-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14
Ranunculus aquatilis	2	3	3	3	4	3	2	2
Ranunculus sceleratus	2	3	2	3	3	2	3	2
Sparganium erectum	2	2	2	2	2	3	3	3
Agrostis stolonifera	.	.	1	2	2	2	1	.
Juncus effusus	.	.	1	1	2	2	2	2
Juncus inflexus	.	.	1	1	2	3	2	2
Chara vulgaris	.	.	.	3	4	4	4	4
Epilobium hirsutum	.	.	.	3	3	2	3	3
Juncus articulatus	.	.	.	1	.	.	2	2
Juncus bufonius	.	.	.	1	2	2	2	1
Persicaria maculosa	.	.	.	1	1	1	2	1
Potamogeton crispus	.	.	.	2	2	2	3	3
Solanum dulcamara	.	.	.	1	1	.	1	2
Chara contraria	.	.	.	.	2	2	2	2
Chara globularis	.	.	.	.	2	2	2	2
Potamogeton natans	.	.	.	.	1	2	2	3
Carex flacca	.	.	.	.	.	.	1	1
Carex pseudocyperus	.	.	.	.	.	.	2	2
Lemna minor	.	.	.	.	.	.	2	2
Lemna trisulca	.	.	.	.	.	.	2	2
Scrophularia auriculata	.	.	.	.	.	.	1	1
Typha latifolia	.	.	.	.	.	.	2	2
Oenanthe aquaticda	.	.	.	.	.	.	.	2
Senecio aquaticus	.	.	.	.	.	.	.	2

While a wide diversity of submerged, emergent, and marginal species occurred in the ghost and restored ponds, only a small sub-set of these species also germinated under the various experimental treatments (*Table 37*). These included the submerged species *P. natans,* which occurred in each study pond and their associated microcosms, and in most sealed 'Propagule bank' mesocosms (except for those containing sediment from GP_{150} and WERE). *P. trichoides* occurred in ponds only in GP_{45}, GP_{50} and STRE, but in the pond, mesocosms and microcosms for restored pond WERE. Similarly, *Z. palustris* occurred in the ponds GP_{150} and WERE, but only in the mesocosms and microcosms for WERE. This could suggest *P. natans* having higher viable seed densities than the other pondweed species, allowing *P. natans* to colonise even the experimental treatments containing relatively small volumes of sediment. Despite the early appearance of the Ranunculaceae in both restored and ghost ponds, no germinations occurred in mesocosms or microcosms, and only one viable *R. aquatilis* seed was found (GP_{150} – see *Chapter 5*). Given that these species occurred very early in both ghost and restored pond colonisation, but not in any of the open mesocosms (as might have been expected if the seeds had arrived by dispersal), it is hypothesised that these species maintained a very sparse viable seedbank, sufficient for re-establishment in the study ponds but not in the smaller volumes of sediment in the experimental treatments.

Among the Characeae, all species occurring in the study ponds also germinated in at least one experimental treatment; *C. vulgaris* occurred in the ponds, mesocosms & microcosms (STRE & WERE), ponds & mesocosms (GP_{50}, GP_{150}), and in the pond only (GURE). *C. virgata* occurred in the pond, mesocosms & microcosms (GP_{150}), and in the pond & mesocosms (WERE). *C. globularis* occurred in the pond & mesocosms (GP_{45}), pond & microcosms (GP_{50}), and pond only (GURE). *C. hispida* occurred in the pond & mesocosms (GP_{45}), and pond only (STRE). *C. contraria* occurred in the pond, mesocosms & microcosms (GP_{50}), and pond only (GURE). *N. flexilis* occurred in the pond & mesocosms (WERE), and pond & microcosms (GP_{45}).

Table 37 – Macrophyte species occurring in the ghost and restored study ponds, and associated experimental treatments. 'Meso.' – on-site mesocosm germinations, 'Micro.' – sealed microcosm germinations, 'TZ' – seeds / oospores viable according to TZ testing, 'Fossil' - seeds / oospores identified in the macrofossil record **of ghost ponds only.** '*' indicates taxa identified to species level in the field sites, but only to genus level in the macrofossil / TZ staining.

Species	Ghost	Restored	Meso.	Micro.	TZ	Fossil
Agrostis stolonifera	√	.	.	.	.	.
Alisma plantago aquatica	.	√	.	.	.	√
Apium nodiflorum	√	√	.	.	.	.
Callitriche sp.	.	√	.	.	.	.
Carex sp.	√	√	.	.	.	.
Chara sp.	√	√	√	√	√	√
Chara contraria	√	√	√	√	*	*
Chara globularis	√	√	√	√	*	*
Chara hispida	√	√	√	.	*	*
Chara virgata	√	√	√	.	*	*
Chara vulgaris	√	√	√	.	*	*
Eleocharis palustris	√	.	.	.	.	.
Epilobium hirsutum	√	√	.	.	.	√
Eupatorium cannabinum	√	.	.	.	.	.
Fontinalis antipyretica	.	√	.	.	.	.
Glyceria fluitans	√	√	.	.	.	.
Hippuris vulgaris	.	√	.	.	.	.
Iris pseudacorus	.	√	.	.	.	.
Isolepis setacea	√	.	.	.	.	.
Juncus sp.	√	√	.	.	√	√
Lemna minor	.	√	√	.	.	.
Lemna trisulca	√	√	.	.	.	√
Mentha aquatica	√	√	.	.	.	√
Myosoties scorpoides	.	√	.	.	.	.
Myriophyllum spicatum	.	√	.	.	.	.
Nasturtium officinale agg.	√	√	.	.	.	.
Nitella flexilis agg.	√	√	√	√	.	√
Oenanthe aquaticda	.	√	.	.	.	.
Persicaria amphibia	√	.	.	.	.	.
Persicaria maculosa	√	√	.	.	.	√
Phalaris arundinacea	√	.	.	.	.	.
Potamogeton birchtoldii	.	√	.	.	.	.
Potamogeton crispus	√	√	√	.	.	.
Potamogeton natans	√	√	√	√	√	√
Potamogeton trichoides	√	√	√	√	.	.
Ranunculus aquatilis	√	√	.	.	√	√
Ranunculus sceleratus	√	√	.	.	.	.
Scrophularia auriculata	√	√	.	.	.	.
Senecio aquaticus	.	√	.	.	.	.
Solanum dulcamara	√	√	.	.	.	√
Sparganium erectum	√	√	.	.	.	.
Stachys palustris	√	.	.	.	.	.
Typha latifolia	√	√	.	.	.	√
Veronica anagallis-aquatica	.	√	.	.	.	.
Veronica beccabunga	√	√	.	.	.	.
Veronica catenata	.	√	.	.	.	.
Zannichellia palustris	√	√	√	√	.	√

A number of macrophyte species occurring in the ghost ponds were also identified in the fossil record for these sites, but no germinations / viable seeds were found in the experimental treatments (*Table 37*). These included the marginal species *Epilobium hirsutum, M. aquatica, Persicaria maculosa, Solanum dulcamara* and *Typha latifolia*, as well as the free-floating *L. trisulca*. Among these species, several are readily wind dispersed (*E. hirsutum, T. latifolia, L. trisulca*), or common to damp waste ground (*P. maculosa, S. dulcamara, E.hirsutum*), and as such may be less dispersal limited than other aquatic macrophytes. This could explain their early colonisation of both the ghost and restored ponds, despite apparent lack of a viable seed bank. Alternatively, these species may have maintained a sparse historic seed bank, but may not have germinated in experimental mesocosms and microcosms due to the submerged conditions in these treatments.

6.4.4 Colonisation of study ponds and open mesocosms by adult Coleoptera

In total, 29 species of aquatic Coleoptera colonised the restored ponds, 37 species colonised the ghost ponds, and 38 species colonised the open mesocosms. Analysis of Coleoptera species richness over time revealed significant differences between the study ponds and mesocosms ($p < .05$), but no significant differences between pond types or mesocosm treatments (*Figure 54, Table 38*).

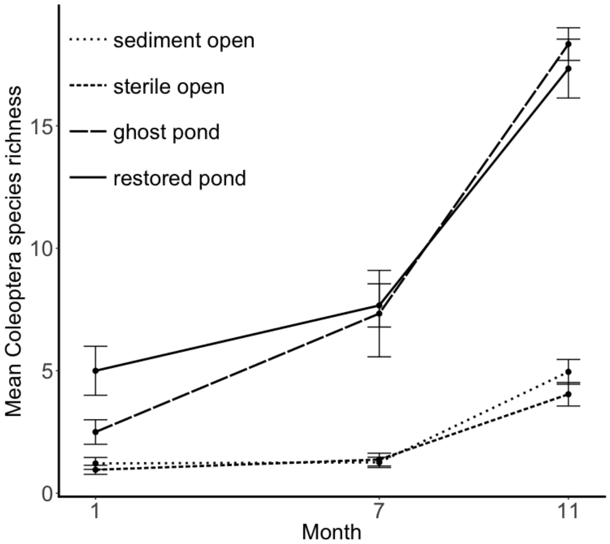


Figure 54 – Mean species richness of adult Coleoptera occurring in the study ponds and open mesocosms, over the first year of colonisation. Error bars show the SEM.

There was a significant effect of time (F(1) = 18, $p < 0.001$), group (F(4) = 5.39, $p < 0.001$), and time / group interaction (F(4) = 5.30, $p <.0001$), indicating that seasonal variation in Coleoptera richness differed between groups.

Table 38 – Descriptive statistics for species richness of adult Coleoptera in the ghost and restored ponds, and open mesocosms.

Ghost ponds	Oct. 13	Apr. 14	Sept. 14
Total species	11	15	33
Mean	2.50	7.33	18.33
SD	0.71	3.06	1.15
SE	0.50	1.76	0.67
Restored ponds	**Oct. 13**	**Apr. 14**	**Sept. 14**
Total species	9	12	27
Mean	5.00	7.67	17.33
SD	1.41	3.06	2.08
SE	1.00	1.76	1.20
Propagule & Dispersal mesocosms	**Oct. 13**	**Apr. 14**	**Sept. 14**
Total species	8	9	28
Mean	1.22	1.26	4.96
SD	1.17	1.01	2.42
SE	0.24	0.21	0.50
Dispersal mesocosms	**Oct. 13**	**Apr. 14**	**Sept. 14**
Total species	6	11	25
Mean	0.96	1.38	4.04
SD	0.91	1.28	2.35
SE	0.19	0.26	0.48

In terms of species composition, there was considerable overlap between both ghost and restored study ponds, and open mesocosms (*Table 39 & 40, Figure 55*). Across all three environments the earliest colonists were members of the Dytiscidae and Halipidae. These families include the mostly predatory diving beetles (Dytiscidae), and omnivorous crawling water beetles (Halipidae). The Dytiscidae contain many strong-flying species, which are typically early colonists of new aquatic habitats (Davy-Bowker 2002; Bilton 2014). By late spring (May 2014), several species within the Hydrophilidae (omnivorous and phytophagous beetles), had colonised the ghost and restored ponds, and open mesocosms. One species within the family Hydraenidae (*Ochthebius minimus*), was also recorded in both ghost and restored ponds in May 2014. The increasing diversity towards the end of the year of these mostly phytophagous families likely reflects increasing vegetation diversity and cover within both the ponds and mesocosms (Bloechl *et al.* 2010). Throughout the year, the Dytiscidae were the most specious family, as has been observed in a number of other pond studies (Fairchild *et al.* 2000; Bloechl *et al.* 2010; Sayer *et al.* 2012). The overall species richness of adult Coleoptera within each study pond were very similar, with 20 species in GP_{150}, 20 in GP_{50}, 23 in GP_{45}, 23 in WERE, 22 in GURE and 21 in STRE.

Table 39 – Coleoptera colonisation of ghost and restored ponds, and open mesocosms, by family.

		Oct.13	May. 14	Sept. 14
Ghost ponds	Dytiscidae	8	5	17
	Halipidae	1	.	4
	Gyrinidae	.	1	1
	Hydraenidae	.	1	2
	Hydrophilidae	.	8	8
	Hygrobiidae	.	.	1
Restored ponds	Dytiscidae	5	7	15
	Halipidae	1	.	3
	Gyrinidae	.	1	1
	Hydraenidae	.	1	2
	Hydrophilidae	1	3	6
	Hygrobiidae	.	1	1
Mesocosms	Dytiscidae	9	12	21
	Halipidae	1	1	2
	Gyrinidae	.	.	1
	Hydraenidae	.	.	3
	Hydrophilidae	.	5	8
	Hygrobiidae	.	.	.

Table 40 – Coleoptera species composition of ghost ponds, restored ponds, and open mesocosms.

Family	Species	Ghost	Restored	Meso.
Dytiscidae	*Acilius sulcatus*	√	√	√
	Agabus bipustulatus	√	√	√
	Agabus nebulosus	√	√	√
	Agabus sturmi	.	.	√
	Colymbetes fuscus	√	√	√
	Dytiscus marginalis	√	.	√
	Hydroglyphus geminus	√	√	√
	Hydroporus angustatus	√	.	√
	Hydroporus incognitus	.	.	√
	Hydroporus memnonius	√	.	√
	Hydroporus nigrita	.	.	√
	Hydroporus palustris	√	√	√
	Hydroporus planus	√	√	√
	Hydroporus pubescens	√	√	√
	Hydroporus tessellatus	.	.	√
	Hygrotus confluens	√	√	√
	Hygrotus impressopunctatus	√	.	√
	Hygrotus inaequalis	√	√	.
	Hyphydrus ovatus	.	√	√
	Ilybius ater	√	.	.
	Ilybius fuliginosus	.	√	√
	Ilybius subaeneus	.	√	.
	Laccophilus minutus	√	√	.
	Rhantus suturalis	√	√	√
	Suphrodytes figuratus	.	.	√
Gyrinidae	*Gyrinus substriatus*	√	√	√
Halipidae	*Haliplus flavicollis*	.	√	.
	Haliplus immaculatus	√	√	.
	Haliplus lineatocollis	√	√	√
	Haliplus obliquus	√	.	.
	Haliplus ruficollis	√	.	√
Hydraenidae	*Hydraena riparia*	.	.	√
	Hydraena testacea	√	√	√
	Ochthebius minimus	√	√	√
Hydrophillidae	*Anacaena globulus*	.	√	√
	Anacaena limbata	√	.	.
	Anacaena lutescens	√	√	√
	Berosus affinis	√	.	√
	Helochares lividus	√	√	√
	Helophorus aequalis	.	√	√
	Helophorus minutus	√	√	√
	Helophorus obscurus	√	.	√
	Hydrobius fuscipes	√	√	√
	Laccobius bipunctatus	.	√	.
	Laccobius colon	√	.	√
	Laccobius minutus	.	√	.
	Laccobius sinuatus	√	.	.
	Laccobius striatulus	√	√	√
Hygrobiidae	*Hygrobia hermanni*	√	√	.

As strong flying, active dispersers, it was anticipated that the species composition of adult Coleoptera would be similar across all three study pond locations (Guestwick, Stody and Westfield). While 16 species were common to all study locations, surprisingly a further 15 species were found in just one study location (*Figure 55*). Of these, 14 species were unique to a single study pond (nine unique to a single ghost pond, five unique to a single restored pond). While some of the species found in only one study pond are considered weak / moderate dispersers, including *Halipus flavicollis*, *Hydraena testacea*, and *Ilybius* sp. (Verberk, Siepel & Esselink 2008), several are good flyers, and have a generally widespread distribution across the UK. As such, the apparently 'unique' occurrence of these species is perhaps due to a combination of small sample size, and the high mobility of taxa, leading to rapid species turnover within ponds. This is perhaps especially pronounced during the first year after pond excavation, where habitat conditions within the ponds are changing rapidly.

Figure 55 – Species overlap in aquatic Coleoptera between pond types and study site locations. Study pond locations: Stody (GP_{45} / STRE), Guestwick (GP_{50} / GURE), Westfield (GP_{150} / WERE)

Considerable overlap in Coleoptera community composition occurred between pond types, and between ponds and open mesocosms, at both the family (*Table 39*) and species (*Figure 55*) level. Within the study ponds, 23 species occurred in both ghost and restored ponds, 12 species in ghost ponds only, and 8 species in restored ponds only. Of the 20 species which were recorded in only one pond type, 14 of these were only recorded once during the year, again likely reflecting the rapid species turnover during early pond colonisation. The similarity in species richness between ghost and restored ponds (*Figure 54*), and fact that those species recorded in only one pond type tended to represent single occurrences, suggests that in general both ghost and restored ponds are equally attractive to dispersing Coleoptera.

Between ponds and open mesocosms, there was an even greater overlap in species composition (*Figure 55*), with 65% species occurring in both environments. This suggests that in general, dispersing Coleoptera were attracted to both the large study ponds and adjacent small mesocosms, which provided broadly similar habitat conditions. Six species of Coleoptera were unique to the open mesocosms, and these occurrences could represent chance arrivals or habitat preferences. The species *Hydroporus tessellatus* was consistently recorded in mesocosms, but not study ponds, across all three survey dates. This species has previously been associated with highly seasonal temporary pools (Eyre, Ball & Foster 1986), and as such may be actively attracted to the smaller mesocosms. Two other *Hydroporus* spp. associated with the mesocosms (*H. incognitus* and *H. nigrita*), have been shown to preferentially occupy and oviposit in habitats without fish (Brodin, Johansson & Bergsten 2006), which could potentially lead to a preference for the smaller mesocosms over the study ponds.

6.5 Discussion

6.5.1 The use of mesocosms in pond colonisation research – strengths and limitations

Mesocosms have been widely used in the field of aquatic ecology for investigating dispersal and colonisation processes, most often among zooplankton populations (Langley *et al.* 2001; Cáceres & Soluk 2002; Brock *et al.* 2003; Shulman & Chase 2007). Aquatic macrophytes and zooplankton share many of the same dispersal pathways, both having dormant propagules capable of long-term quiescence (Leck *et al.* 1989; Hairston & Kearns 2002), and which can be transported by wind (Frisch *et al.* 2012; Soomers *et al.* 2013; Pinceel, Brendonck & Vanschoenwinkel 2016), or within the intestines of waterfowl (Proctor & Malone 1965; Green & Figuerola 2005). However, mesocosms are less often used in the study of macrophyte dispersal, meaning there was little information available to inform the design of the mesocosms used in this study. Studies of zooplankton dispersal have used mesocosms covering a broad range of sizes and designs, from relatively small (60 – 100 L) plastic tubs, which may be above ground level (Shulman & Chase 2007), or partially submerged to moderate temperature and allow access to potential dispersal vectors (Cáceres & Soluk 2002), up to much larger (several m^2), above-ground structures (Langley *et al.* 2001). While larger mesocosms have the benefit of potentially providing a greater trap for dispersing propagules, there is evidently a trade-off with the cost of purchasing / constructing large mesocosms, as well as the availability of space on which to build them. Studies using much smaller, partially submerged mesocosms have found these attract a diverse array of aquatic organisms, including visiting waterfowl (Cáceres & Soluk 2002; Shulman & Chase 2007). As such, a large number of small mesocosms were used for this study, providing a greater number of replications (Spivak, Vanni & Mette 2011), while maintain what was deemed to be a suitable size for attracting multiple taxa which could be acting as dispersal agents.

In order to provide some measure of the ‘ecological validity’ of the mesocosms in relation to the study ponds, both water chemistry parameters and colonisation by adult Coleoptera were monitored. Water chemistry parameters were monitored as they could potentially influence the germination / establishment of aquatic macrophytes, while colonisation by aquatic Coleoptera provided an indication of habitat similarity in mesocosms and study ponds.

Overall, both water chemistry parameters and colonisation by aquatic Coleoptera followed similar patterns in ghost ponds, restored ponds, and open mesocosms (H5). For water chemistry, significant differences in conductivity and pH occurred between study ponds and mesocosms, and between mesocosms in open and closed treatments (*Figure 51 & Figure 52*). The significant difference in conductivity between the mesocosms and study ponds was expected, given the much smaller volume of the mesocosms relative to the study ponds. Of greater interest were the differences between mesocosm treatments, as these could influence the reliability of between-mesocosm comparisons. The fact that significant differences in water chemistry occurred between open and closed mesocosms, and not between mesocosms containing different sediment treatments, indicates that differences in macrophyte colonisation attributed to the presence / absence of the historic propagule bank were not confounded by water chemistry effects.

Despite their small size, open mesocosms were found to attract a wide diversity of aquatic taxa, including invertebrates (Odonata, Coleoptera, Diptera, Nepomorpha), amphibians and ducks. The observation of ducks foraging in the open mesocosms could be particularly important, as water fowl provide a key dispersal vector for many aquatic macrophytes. Although ducks were observed visiting open mesocosms, it is unclear whether this would have been a sufficiently frequent occurrence for the dispersal of aquatic plant propagules. However, open mesocosms were located in close proximity to one another within the vegetated pond margins, which provide an equally attractive food source for waterfowl as the ponds themselves (Soons *et al.* 2016). A such, open mesocosms stood a reasonable chance of being visited regularly by waterfowl, and receiving aquatic propagules via this pathway.

On average, open mesocosms supported a lower species richness of aquatic Coleoptera than ghost ponds or restored ponds (*Figure 54*), as might be expected from their small size and limited diversity of microhabitats. There were no differences in Coleoptera species richness between mesocosms containing sterile substrate, and mesocosms containing pond sediment, which could reflect the similar macrophyte richness (and thus habitat complexity), occurring in both mesocosm types. Despite their lower mean species richness, open mesocosms supported a similar composition of Coleoptera at both the family and species level (*Figure 55 & Table 39*), to ghost ponds and restored ponds. Although Coleoptera were only surveyed at three time points, there is some indication of community succession over the course of the first year of pond colonisation. While the early species composition in both ponds and mesocosms is dominated by strong flying and predatory species, the later composition includes a higher diversity of feeding guilds, including a number of phytophagous species. This likely reflects the increasing habitat complexity and macrophyte diversity within both mesocosms and study ponds.

Considerable overlap in the species composition of open mesocosms and study ponds (*Figure 55*), indicated that both habitats were equally attractive to actively dispersing Coleoptera. As Coleoptera provide a fair indicator of wider invertebrate diversity (Fairchild *et al.* 2000; Sánchez-Fernández *et al.* 2006), these findings suggest that pond mesocosms represent diverse aquatic habitats. In addition to the Coleoptera, invertebrate taxa including the Odonata, Diptera and Nepomorpha were found breeding in both the study ponds and open mesocosms, although the species composition of these taxa was not investigated. All four native amphibian species found in north Norfolk were recorded in both study ponds and open mesocosms, with frogs and toads breeding in both habitats.

Overall, it seems that the on-site mesocosms provided a reasonable representation of conditions within the study ponds, attracting a similarly broad diversity of taxa, and thus providing an acceptable model for colonisation processes within full-size ponds. However, there are a number of limitations associated with the mesocosms used in this study. Because of the large number of replicates (48 open mesocosms, 48 closed mesocosms), a cheap, easy to construct, and quick-to-sample design was required. In the case of closed mesocosms, it was difficult to find a solution which would reliably exclude propagules while allowing for wind mixing and oxygenation of the water column, and which could be constructed quickly in the field, and easily opened-up for monitoring. In the end, it was decided to simply peg a fine Nylon mesh cover over each mesocosm. However, this design was not as resistant to disturbance as had been hoped, and in August 2014 a number of ‘closed’ mesocosms were found open, leading to their contamination. The mesocosm design could perhaps have been improved in a number of ways; using plastic containers with clip-on lids would have provided a better barrier to propagule dispersal, but might have led to stagnation and extremes in water temperature. Constructing a wooden frame to hold the Nylon netting in place, and screwing this to the base of the mesocosms, may also have created a more reliable barrier. However, this design would have been time consuming and expensive to construct in large numbers, and may not have resulted in much more reliable results; Caceres & Soluk (2002) employed a similar design, and still had problems with contamination following storm damage to the mesocosms.

While the mesocosms provided a useful model for comparing the contribution of the propagule bank vs. dispersal under relatively controlled conditions, it is unclear how well the ‘Dispersal’ treatment mesocosms (containing sterile substrate open to dispersal), represent conditions in a newly dug pond. Ideally three newly dug ponds, located in agricultural areas which had not previously contained any aquatic habitats, would have been included in this study alongside the three ghost ponds and three restored ponds. This would have allowed for comparisons to be made between both the ‘Propagule bank’ and ‘Dispersal’ treatment mesocosms, as well as between the full-size ponds replicating these different treatment conditions. Unfortunately the creation of three additional new ponds was beyond the scope of this thesis, but would be an interesting addition to future propagule bank research.

6.5.2 The relative importance of the propagule bank vs. dispersal for new pond colonisation.

Overall, findings from the outdoor mesocosms conform to the hypothesised patterns (H1 – H5), outlined at the start of this chapter. Within both the on-site mesocosms and sealed microcosms, multiple aquatic macrophyte species germinated from the historic pond sediments. Species occurring in the experimental mesocosms and microcosms were also present in the associated study ponds (H3). The most commonly occurring species included the pondweed *P. natans*, which germinated in microcosms and mesocosms for all six study ponds, and the charophyte species *C. vulgaris*, which germinated in mesocosms and microcosms for four of the study ponds (GP_{50}, GP_{150}, STRE and WERE). Overall, a total of six aquatic macrophyte species germinated in the ghost pond mesocosms, and five species in restored pond mesocosms (*Figure 50 & Table 26*), representing a small proportion of the overall macrophyte diversity of the study ponds (*Table 29*). Despite the low aquatic macrophyte diversity occurring in the experimental mesocosms, significant differences in mean species richness and colonisation rate were detected. For both ghost pond and restored pond mesocosms, the closed 'Propagule bank' treatment had significantly higher species richness ($p < .02$), than all other mesocosm treatments (*Figure 50*). While the significantly higher species richness and earlier colonisation of 'Propagule bank' compared to the 'Dispersal' treatment was hypothesised (H1 & H2), the significant difference between the closed 'Propagule bank' and open 'Propagule bank & Dispersal' treatments was not expected. The higher germination success within the closed treatment mesocosms could be due to reduced disturbance by animals, and possibly reduced temperature fluctuations within the closed treatments. Ducks were observed foraging in the open treatment mesocosms, consuming aquatic macrophytes and causing considerable sediment disturbance, which may have significantly reduced macrophyte diversity within the open treatments.

Macrophyte development in the study ponds followed similar patterns in species composition, species richness, and colonisation rate, for both ghost ponds and restored ponds (H4). During the first half of the year, restored ponds accumulated species more quickly than ghost ponds, but by the end of the year species richness was similar in both pond types. This could be due to the historic propagule bank within restored ponds recovering more rapidly than that of ghost ponds, which have experienced greater stresses of drying and compaction. However, the early germination of aquatic macrophytes from both ghost and restored pond sediments in the mesocosms and microcosms suggests that differences between the study ponds are not related to the historic propagule bank. Instead, the higher initial diversity in restored ponds is more likely due to these sites having some remnant wetland or aquatic species, or at least a more recent propagule bank, contributing to their early colonisation.

After one year, study ponds contained between 18 – 25 macrophyte species, representing a high species diversity for agricultural ponds (Biggs *et al.* 2005; Davies *et al.* 2008b; Sayer *et al.* 2012). This high diversity of macrophytes is largely related to the reduced shading and increased dissolved oxygen in newly created and restored ponds (Williams *et al.* 2008; Mokany *et al.* 2008; Hassall *et al.* 2011; Sayer *et al.* 2012). However, the historic propagule bank could be making a significant contribution to both the rate of macrophyte establishment, and overall macrophyte diversity. Although only a limited number of species occurring in the study ponds were proven to originate from the historic propagule bank, these included a number of early colonising species known to have beneficial habitat influences. This applies particularly to the Characeae, for which viable propagules were identified within the historic sediments of all six study ponds. Charophytes can make an important contribution to improving water clarity, stabilising areas of bare sediment (Crawford 1979; Scheffer *et al.* 1994), while having an allelopathic effect on phytoplankton (Van Donk & van de Bund 2002). The early establishment of charophytes, which began to appear in the study ponds from Feb. 2014, could create conditions which in turn favour the establishment of a wide diversity of macrophyte species. The early establishment of dense chara beds also provides an attractive food source for waterfowl (Bonis & Grillas 2002), possibly increasing the arrival rate of macrophyte propagules via this dispersal pathway.

Although mesocosm, microcosm, and TZ staining experiments identified low densities of viable propagules from a limited number of species, this could be due to the relatively small volumes of sediment examined for each site. As such, it is quite possible that additional species which germinated in the study ponds could also have come from the historic propagule bank, but that very low densities of viable seeds were not detected in the experiments. For example, the Ranunculaceae were early colonists in both ghost ponds and restored ponds, despite only one single viable seed being found from the sediment sample from GP_{150}. *R. aquatilis* is commonly one of the first species to appear in ponds after restoration (Sayer *pers. obs*.), and it has been suggested that this is likely due to the historic propagule bank (Sayer *et al.* 2012). Indeed, t is possible that viable *R. aquatilis* seeds are present in very low densities within the study ponds (as indicated by the single viable seed which was found), but that given the large volume of sediment within each pond, this is sufficient for population reestablishment. Further anecdotal evidence that *R. aquatilis* may have originated from the historic propagule bank, as oppose to colonising the study ponds by dispersal, comes from the flower time of *R. aquatilis* in GP_{50}. While *R. aquatilis* populations within other ponds in north Norfolk, including those close to GP_{50}, flowered in May 2014, the population in GP_{50} flowered much later in the year, in August 2014. It is possible that this was due to disruption of the plants usual seasonal synchrony, as a result of long-term seed dormancy, or due to genetic differences between plants originating from the historic seed bank, and modern populations in neighbouring ponds.

After pond restoration, all macrophyte species recorded in the formerly overgrown study sites had re-established within one year, with the exception of the Lemnaceae. This was true even for pond WERE, which was the most diverse pond before restoration, containing 14 macrophyte species. These findings agree with the work of Sayer *et al.* (2012), and suggest that pond restoration has little negative effect on existing macrophyte populations. Aquatic and marginal macrophytes rapidly recolonise restored ponds, possibly from the historic propagule bank, remnant populations, or via dispersal. The very cautious approach to tree and sediment removal commonly promoted within pond conservation (e.g. Biggs *et al.* 1994), is likely to be unnecessary for most ponds, and may in fact be detrimental. Large scale disturbance is often required to 're-activate' historic propagule banks (Combroux, Bornette & Amoros 2002; Weyembergh *et al.* 2004; Scott *et al.* 2012), and failing to remove sufficient quantities of sediment or overhanging trees can limit the success of pond restoration.

Although this study found only a limited number of common aquatic macrophyte species which could be proven to originate from the historic propagule bank, these findings have significant implications for species and habitat conservation. The astonishing resilience of aquatic propagules, which are able to both survive long-term burial and re-populate ghost ponds after over a century of cultivation, indicates that these habitats could play a key role in restoring historic populations and species diversity to agricultural landscapes. While a number of studies have documented to re-appearance of rare or locally extinct species from historic propagule banks (Weyembergh *et al.* 2004; Scott *et al.* 2012; Kaplan *et al.* 2014), it is often assumed that this is not possible in habitats which have been subject to agricultural land intensification. Baastrup-Spohr *et al.* (2016) report on the restoration of a Danish lake, which had been drained and converted to agricultural land over 60 years ago. Once the lake was re-flooded, 40 aquatic macrophyte species re-established, including one species, *Baldellia repens*, which was presumed extinct in Denmark, and one, *Elatine hydropiper*, which was nationally red listed. Despite the appearance of 13 separate populations of the 'extinct' *B. repens*, the authors concluded that these couldn't have come from the historic seed bank, as "*intense ploughing and cultivation of fields for grain and* *potato production during several decades should hamper seed survival of aquatic plants. It is thus highly unlikely that the original lake species have survived in the soil seed bank*". (Baastrup-spohr *et al.* 2016, p. 380). However, the findings of this thesis show that aquatic macrophyte propagules can survive many decades of burial under intensive agriculture, opening up exciting possibilities for the resurrection of locally rare or extinct species.

6.5.3 The biodiversity contribution of ghost ponds and restored ponds within agricultural landscapes

One year after excavation, ghost ponds and restored ponds supported a high diversity of both aquatic macrophytes (*Table 37*) and adult Coleoptera (*Table 40, Figure 55*). Aquatic macrophyte species composition showed considerable overlap between ghost ponds and restored ponds, likely reflecting both environmental and propagule bank similarity between the study sites. Several taxa recorded in the study ponds are uncommon or under threat in agricultural areas, including the Characeae (Lambert & Davy 2011), and the species *Hippuris vulgaris, Isolepis setacea,* and *Oenanthe aquatica*. The 18 – 25 wetland macrophyte species recorded in each study ponds make these sites highly biodiverse for farmland ponds, which within the study area typically support between 6 – 14 aquatic species (Sayer *et al.* 2012), and within the UK typically fewer than 20 wetland species (Biggs *et al.* 2005). The eight species shown to survive prolonged burial thus represent a significant proportion of the expected species diversity of farmland ponds. Species found to recolonise from the historic propagule bank included floating-leaved species like *P. natans*, which strongly enhance the diversity of Odonata (Raebel *et al.* 2012a), and submerged species like the Characeae, which provide important habitat for invertebrates and promote water clarity (Crawford 1979; Van Donk & van de Bund 2002; Schneider *et al.* 2015).

In addition to reaching high levels of macrophyte diversity within just one year, there is an indication that resurrected ghost ponds and restored ponds contained many of the species which would have historically been present in these sites (*Table 37*). These include species for which no viable propagules were found, but which were present in the macrofossil record, including; *Alisma plantago-aquatica, E. hirsutum, L. trisulca, Mentha aquatica, Persicaria* sp., *S. dulcemara,* and *T. latifolia*. Of the total 52 species recorded in the study ponds, 14 appeared in the macrofossil record, indicating that these species had also been found in the study sites in the past. This will be an underestimate of past pond diversity, as not all remains were successfully identified, and not all plant species leave behind well preserved remains. The re-appearance in the study ponds of all species present in the macrofossil record suggests that pond restoration and resurrection go some way towards re-creating past pond conditions, and restoring past species diversity and composition.

The diversity of aquatic Coleoptera within the study sites was also relatively high for farmland ponds, with a total of 51 species representing 6 families being recorded (*Table 39*). Each study pond contained between 20 – 23 species, including one Nationally Scarce species, *Ilybius subaeneus* (in pond GURE), and one notable species, *Berosus affinis*, the occurrence of which in GP_{50} represents the fifth known site for this species in Norfolk. The diversity of aquatic Coleoptera within the study sites is similar to that found in near-by restored farmland ponds (Sayer *et al.* 2012), and generally comparable to agricultural and lowland ponds in other parts of Europe (Eyre *et al.* 1986; Bloechl *et al.* 2010; Hassall *et al.* 2011; Hill & Wood 2014). Species richness of Coleoptera within the ghost ponds and restored ponds was considerable higher than that reported from overgrown ponds within the same study region (Sayer *et al.* 2012), as would be anticipated given the known impacts of pond shading on both macrophyte and invertebrate diversity. Given that aquatic Coleoptera are widely recognised as early colonists of new ponds (Ruhí *et al.* 2009; Bloechl *et al.* 2010), it would be interesting to compare the colonisation rate and diversity of aquatic Coleoptera within ghost ponds, restored ponds, and newly created ponds. If the hypothesis that macrophyte colonisation and diversity within ghost ponds and restored ponds is higher than that of new ponds (as indicated by the mesocosms), this could influence the species composition of multiple invertebrate taxa, which may indirectly benefit from the historic propagule bank.

6.6 Conclusions

The findings of this chapter show that ghost ponds and restored ponds rapidly colonise with a rich diversity of both macrophytes and aquatic Coleoptera, reaching high levels of biodiversity within just one year. A significant proportion of the macrophyte species recorded in the study ponds can be shown to originate from the historic propagule bank, indicating that this could be an important source of macrophyte diversity within heavily impacted and fragmented agricultural landscapes. Although pond loss has been highlighted as a major conservation challenge across many agricultural regions around the globe, the few existing policies which offer legislative protection for these habitats focus on the creation of new ponds (Dahl 2011; Freshwater Habitats Trust 2015; Forestry Commission, Natural England & Department for Environment 2016). The success of new pond creation relies heavily upon dispersal from existing habitats (Williams *et al.* 2008; Raebel *et al.* 2012a), which may not be sufficient to restore ponds in highly modified agricultural landscapes. As such, the resurrection of ghost ponds and restoration of overgrown ponds could provide a valuable alternative for restoring lost aquatic biodiversity within agricultural landscapes.

Chapter 7 Summary, conclusions and future research directions

7.1 Introduction

Ponds are increasingly being recognised as key aquatic habitats, providing valuable ecological services (Moore & Hunt 2012; Céréghino *et al.* 2014), and contributing disproportionately to regional aquatic biodiversity (Scheffer & Van Geest 1999; Williams *et al.* 2004; Davies *et al.* 2008b). Farmland ponds are particularly valuable, providing islands of habitat heterogeneity within an otherwise homogeneous sea of agriculture (Benton *et al.* 2003; Céréghino *et al.* 2008a). However, these habitats are under threat from agricultural intensification, and globally around 50% small agricultural ponds and wetlands have been filled-in for land reclamation (Wood *et al.* 2003; Zedler & Kercher 2005; Serran & Creed 2016). While this has resulted in a considerable drive to manage and conserve ponds, many practices are largely uninformed by science (Céréghino *et al.* 2008a), and there has been a strong emphasis on new pond creation, rather than pond restoration (Dahl 2011; Freshwater Habitats Trust 2015; Forestry Commission *et al.* 2016).

The aim of this thesis was to explore the conservation value of ‘ghost ponds’, former ponds which have been subject to agricultural land reclamation. Ghost ponds are an abundant feature of agricultural landscapes, and their ‘resurrection’ could re-create past habitat connectivity, while potentially restoring lost macrophyte populations from the historic propagule banks buried within these sites. These possibilities are explored following a logical progression through the thesis; starting with an examination of the extent of pond loss and abundance of ghost ponds within a typical agricultural region, the thesis goes on to cover the possible biodiversity consequences of historic pond loss, before focusing on the potential role that the resurrection of ghost ponds and restoration of overgrown ponds could have in combating aquatic biodiversity loss in agricultural landscapes. These themes address several of the key components of habitat fragmentation, which are outlined in the thesis introduction in *Figure 7*; *Chapter 3* examines changes in pond numbers and pond density over time, addressing two of the key components of fragmentation (increase in habitat isolation, reduction in habitat size). *Chapter 4* goes on to relate all three key components of habitat fragmentation (isolation, habitat size and habitat quality), to pond biodiversity and community structure, addressing several of the theoretical consequences of fragmentation outlined in *figure 7* (reduced dispersal and colonisation, and potential species diversity consequences). Finally, *Chapters 5 & 6* explore the potential of using historic ghost and restored ponds to essentially re-set some of the effects of habitat fragmentation, 'resurrecting' both past habitats and species diversity, from a time when the pondscape was less fragmented. The key findings from the thesis are summarised below, and future research directions are discussed.

7.2 Summary

7.2.1 Historic pond loss and the abundance of ghost ponds within agricultural landscapes

Across the study region of Norfolk, eastern England, extensive pond loss has occurred, primarily as a result of agricultural land intensification. Pond loss was particularly pronounced during the latter half of the 20th century (*Chapter 3*), reflecting the increased drive in food production after the Second World War. Across Norfolk, almost 30% of ponds present in the 1950s had been lost by 2014, dramatically reducing both the number and density of ponds across the region. Pond loss was highly variable between locations, with local changes in pond numbers between 1955 – 2014 ranging from 39% pond loss, to 39% pond gain. However, at the regional scale there was a net loss of ponds between 1955 – 2014, with most ponds experiencing a loss of 2 – 5 neighbouring ponds within 1 km^2. This loss of aquatic habitats and concurrent reduction in structural connectivity is hypothesised to have had a significant negative impact upon the biodiversity value of agricultural ponds (*Chapter 4*).

7.2.2 Relationships between pond loss and biodiversity – a space-for-time replacement approach.

In order to examine the potential consequences of historic pond loss and pondscape fragmentation for aquatic biodiversity, a space-for-time replacement approach was employed (*Chapter 4*). One hundred and twenty one agricultural ponds located across a range of 'pond density landscapes' were surveyed for aquatic macrophytes, adult Coleoptera, and amphibians, in the spring of 2014. Pond density was found to have a significant negative influence on aquatic macrophyte α- diversity, and on the occurrence of three of four native amphibian species. This negative association was unexpected, and may be the result of confounding environmental or spatial process, including SAC, which was not accounted for in the current analysis. Diversity of aquatic Coleoptera was unrelated to pond density, as was toad occurrence. Only frog occurrence followed the expected pattern of being positively related to pond density. Based on these results, there was no evidence of a negative effect of pond loss and reduced pondscape density on aquatic biodiversity. However, significant negative effects of pond shading were observed for all taxa, suggesting that the high number of overgrown ponds within the study region is likely to be more detrimental to aquatic diversity than the historic reduction in pond density. As such, there is considerable conservation potential for the restoration of overgrown ponds, and the resurrection of ghost ponds, for providing new open water habitats for aquatic taxa.

7.2.3 The conservation value of the historic propagule bank

As well as restoring lost aquatic habitats to the agricultural landscape, and re-creating historic pondscape connectivity (*Chapter 3*), the restoration of overgrown ponds and resurrection of ghost ponds has the exciting potential to restore lost macrophyte populations from their historic propagule banks (*Chapter 5*). The potential viability of this novel approach to habitat ‘resurrection’ was examined using microcosm germination trials and TZ staining for seed viability. In total, eight aquatic macrophyte species were found to maintain a viable propagule bank, even after 150+ years of burial underneath intensive agricultural crop production (*Chapter 5*). The seeds / oospores of these species were not only viable, but capable of germinating from the pond sediments within just a few months, and required no special treatment for breaking of seed dormancy. Results from on-site mesocosms (*Chapter 6*), containing either historic pond sediment or a sterile substrate, indicate that the historic propagule bank can make a significant contribution to macrophyte colonisation rate and species diversity, compared to dispersal alone. These findings indicate that the historic propagule bank of both ghost and restored ponds could provide an important source of aquatic macrophyte diversity, potentially making ghost and restored ponds a more viable conservation option within agricultural landscapes than new ponds.

7.2.4 The potential role of ghost and restored ponds in aquatic conservation

In addition to the experimental investigation of ghost and restored pond propagule banks (*Chapters 5& 6*), three ghost ponds and three formerly overgrown ponds were restored, and monitored over the first year of colonisation (*Chapter 6*). Within one year, ghost and restored ponds reached high levels of biodiversity for both aquatic macrophytes and Coleoptera. Restored ponds initially had a higher macrophyte diversity than ghost ponds, but by the end of the year all ponds had reached comparable levels of diversity, supporting between 18 – 25 aquatic macrophyte species. Although the eight viable species identified in the historic propagule bank represent only a portion of the total macrophyte diversity of the study ponds, they include species which provide key habitat components within the ponds. More importantly, the persistence of viable propagules within the sediments of ghost and restored ponds demonstrates the potential for these habitats to act as 'time capsules' for past species diversity. This has exciting implications for ghost or restored ponds in which locally rare or extinct species have previously been recorded, or for ponds found within the past habitat range for such species. Work by Kaplan *et al*. (2014) has demonstrated the potential for restoring extinct aquatic macrophyte species from historic seedbanks, indicating that similar results could be achieved from ghost pond sediments (Alderton *et al.* 2017). Ghost ponds and restored ponds could thus play a key role in re-connecting fragmented pondscapes, creating new open water habitats for multiple aquatic taxa, and restoring diverse aquatic macrophyte communities from historic propagule banks.

7.3 Future research directions for ghost ponds

Since the earliest observations made by Darwin (1859), seed and propagule banks have been the subject of considerable research. Within conservation biology, this has tended to focus on seed longevity (Shen-miller *et al.* 1995; Telewski & Zeevaart 2002), the role of propagule banks for population persistence in variable habitats (Bonis *et al.* 1995; Guo, Rundel & Goodall 1998), and the potential for using in-situ and ex-situ propagule banks in the restoration of degraded habitats (Middleton 2003; Beas *et al.* 2013). While some studies have tentatively suggested the potential for restoring rare or 'extinct' species from historic propagule banks (Weyembergh *et al.* 2004; Kaplan *et al.* 2014), the application of this approach within agricultural landscapes has been largely dismissed, due to the generally poor viability of propagules within these habitats (Budelsky & Galatowitsch 1999; Middleton 2003; Beas *et al.* 2013). Within resurrection biology, the genetics of historic propagule banks has been the main focus of research, with resurrected zooplankton populations used to study evolutionary processes (Decaestecker *et al.* 2007; Frisch *et al.* 2014), and model past environmental changes (Kerfoot *et al.* 1999; Angeler 2007). There has generally been little overlap between these fields, and future research could strongly benefit from combing aspects from both disciplines.

Ghost ponds provide a completely novel research environment, and as such the possibilities for future research directions are extremely broad. First and foremost, the viability and species diversity of the historic propagule bank of ghost ponds needs examining on a much larger scale, as the results presented in this thesis come from just three study sites, all located within north Norfolk. While these study sites have provided a wealth of novel information, many important questions remain unanswered; do all ghost ponds support viable buried propagule banks? If not, what factors determine the viability and richness of the propagule bank? What is the maximum longevity of propagules buried within ghost ponds? Can rare or locally extinct species be resurrected from ghost ponds? What about rare or locally extinct genetic variations, which could contribute to the genetic diversity of fragmented populations? Does the buried propagule bank of ghost ponds provide a significant advantage for pond restoration, compared to new pond creation?

7.4 Pathways to impact

To address some of the key questions outlined above, and further the research and practical implications of this thesis, a number of key pathways should be explored. These are addressed in chapter order:

Chapter 3 - Changes in an agricultural 'pondscape'.

The detailed GIS analysis of existing and historic pond locations is already being put to practical use by the UCL Pond Restoration Research Group and Norfolk FWAG, who have been using this information to identify ghost ponds suitable for 'resurrection'. Future research should focus on building ecologically meaningful measures of functional connectivity (see discussion of *Chapter 3*), into the existing GIS project. This would create a more useful tool for selecting the most suitable ghost ponds for resurrection, or the most suitable existing ponds for management and restoration. Some simple indices which could be included in the GIS might be area of uncultivated land within 1km (or dispersal distance of species of interest), of each pond, distance to nearest woodland, or length of hedgerow within 1km of pond. More detailed measures, which might be used in least cost modelling, would require additional surveys to better define the habitat and dispersal requirements of the species of interest.

Another avenue for future research highlighted by *Chapter* 3 is the large discrepancy between pond loss in Norfolk, compared to other parts of the UK. For areas where historic surveys suggest much higher rates of pond loss than observed in Norfolk, re-analysis using the same GIS approach applied in this study may help uncover the reasons for this discrepancy; if apparent differences are due to the technique used to count ponds, or the area of land surveyed, this should be easy to detect by repeated surveys.

Chapter 4 – Ecological consequences of pond loss and fragmentation

The large scale pond survey conducted for this chapter provides a good base for future research, and for informing practical pond conservation. Although some re-analysis of the data is needed to account for SAC, the findings from this survey highlight a couple of key aspects which should inform future pond conservation practice;

1) Spatial patterns and pond density are poor predictors of farmland pond quality and species richness. Relatively isolated ponds, completely surrounded by agricultural land, were just as likely to provide high quality habitats rich in macrophytes, aquatic Coleoptera, and amphibians, as ponds located in denser networks. Pond conservation practice should recognise the potential value of even isolated farmland ponds, which are in danger of being overlooked in favour of protecting ‘pond networks’.

2) Of all the environmental and spatial variables measured in this study, pond shading had by far the greatest negative impact on pond biological value. Heavily shaded ponds contained very few species, and contrary to the findings of other studies (Biggs *et al.* 2005), did not support any of their own rare or unique species. Worryingly, a high proportion of the ponds surveyed were extremely shaded, and had received little or no management for decades. Given the high number of ponds that already exist in Norfolk, pond conservation advice should focus on the restoration and management of the existing pond resource, rather than the creation of new ponds.

The results of the pond survey also highlight several areas for future research. At present, the survey methods employed for aquatic Coleoptera and amphibians are not sufficient to provide reliable data on these taxa. Future research should use a repeated survey for amphibians, and a longer search time for aquatic Coleoptera, to obtain a more complete measure of species occurrences. It would also be informative to include other key taxa in future surveys, particularly those of conservation priority in the UK, such as Odonata and birds. In addition, future research should include more measures relating to the terrestrial habitat matrix surrounding the study ponds, particularly in relation to understanding amphibian occurrence. This point ties in with the future research suggestions for *Chapter 3*.

Chapter 5 – The importance of the historic seedbank.

The results from this chapter provide important and novel findings, demonstrating for the first time the ability of aquatic seeds to survive, even after the destruction of their habitat for agricultural intensification. Future research should focus on a number of key areas;

1) Given the small sample size in the current study, the first objective of future research should be to replicate the findings. This could be achieved, at least initially, through small scale lab-based experiments. Cores could be collected from a large number of ghost ponds relatively quickly and cheaply, causing minimal disturbance to farm practice. Historic sediments collected in this way

could be used in large scale germination trials and seed staining, following the methodology outlined in *Chapter 5*.

2) Using long core sampling, future paleolimnological work could look at reconstructing past ghost pond communities through time, improving upon the simple bulk-sample approach used in the current study. Core-based work could also be important in identifying any unwanted or invasive species hidden in ghost ponds, thus avoiding 'resurrecting' these sites.

3) Coring of ghost ponds located on land formerly of conservation interest (for example, drained fen meadow converted to agriculture), or in areas where rare species have previously been recorded, could be used to inform targeted ghost pond resurrection.

4) The potential genetic contribution of the historic propagule bank could be investigated, using seeds / germinations from core samples, for comparison with macrophyte populations of existing ponds. While some studies have considered buried propagule banks as a source of genetic novelty for plant populations (Levin 1990; Uesugi *et al.* 2007), exploring this question within the 'extinct' habitat of ghost ponds would be very interesting. Because ghost ponds were suddenly filled-in during land reclamation, the propagule bank buried within these habitats represents an unaltered genetic 'time capsule', abruptly cut-off from any genetic shifts occurring in above ground populations. This could provide a useful source of material for studying the genetics of past populations, as well as the conservation potential of this buried genetic resource for restoring fragmented macrophyte populations above ground.

The techniques employed in this chapter for testing the viability of ghost pond seedbanks (germination trials, tetrazolium chloride staining), should be put to further practical use in selecting suitable sites for resurrection. These techniques could help identify sites which contain seeds from threatened, rare, or even extinct species, or sites which contain no viable seed bank, targeting resources to focus on the most valuable ghost ponds.

Chapter 6 – The role of ghost and restored ponds in pond conservation

The findings of *Chapter 6* have a number of important research and practical implications for future pond conservation. The key topic for future research, which unfortunately was not tested in the field during the current study, is to determine whether resurrected ghost ponds (and restored ponds) provide a significantly more diverse habitat than newly created ponds. While it is hypothesised that the role of the historic seed bank may be particularly important for colonisation and establishment in isolated farmland ponds, this requires further testing in the field. Longitudinal studies comparing aquatic macrophyte colonisation and diversity within ghost ponds, restored ponds, and new ponds (excavated on ground which does not contain an aquatic propagule bank) could be conducted, to establish whether; a) ghost / restored ponds provide a significantly more diverse habitat than new ponds, and b) whether these differences are maintained past initial colonisation. In addition, in areas where ghost pond excavation is not possible but core samples suggest a diverse and viable historic propagule bank, translocation experiments could be conducted to explore the potential for ‘seeding’ newly created ponds with the sediments of historic sites. Future research may also look into ways of more accurately locating and excavating ghost ponds, for example using DGPS in combination with historic maps to more accurately locate the old pond basin (this is currently being investigated by Helene Burningham and Carl Sayer, both at UCL).

A number of practical implications for pond conservation also arise from the findings of the current study. Ghost ponds and restored ponds were both colonised rapidly, and supported a similarly high diversity of aquatic macrophytes and aquatic Coleoptera, suggesting both habitats could be equally valuable resources for rolling back freshwater habitat loss. This study found that ghost ponds could easily be identified in the field, and excavated following the contours of the historic sediment layer, without requiring any specialist equipment (bar the excavator), or training. As such, excavating ghost ponds for conservation purposes is well within the abilities, and budget, of many farmers or small conservation groups. Indeed, a number of ghost ponds have since been excavated in Norfolk, following the methods developed in this study.

This thesis demonstrates the exciting conservation potential of ghost ponds, a newly described habitat which could play a significant role in aquatic biodiversity conservation. The resurrection of ghost ponds could contribute to restoring pondscape connectivity, improving aquatic biodiversity within agricultural landscapes, and provide a novel research environment for studying past aquatic communities.

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Appendix

1. Pond locations – *Chapter 4* – pond density / biodiversity survey.

Pond code	Grid reference	lat	long	Easting (UTM)	Northing (UTM)
ABRCK72	tf9083625792	52.79593	0.829071	353631.5	5851779
ABRCK73	tf9071625673	52.7949	0.827224	353503.6	5851669
ABRCK74	tf9070925662	52.79481	0.827114	353495.8	5851658
ABRCK75	tf9093225467	52.79298	0.830304	353704.7	5851448
ABRCK76	tf9139525854	52.79629	0.837388	354193.4	5851802
ABRCK77	tf9079126540	52.80266	0.828841	353638.6	5852528
ABRCK78	tf9063026051	52.79833	0.826171	353444.1	5852052
ABRCK79	tf9077026148	52.79915	0.828301	353590.4	5852139
ABRCK80	tf9116526272	52.80012	0.834225	353993	5852235
BONF1	tg1095126778	52.7973	1.127594	373760.5	5851365
BULLS1	tg1126728319	52.81101	1.133269	374182.6	5852880
BULLS2	tg1110828326	52.81113	1.130918	374024.5	5852898
CHEST1	tg1352439541	52.91084	1.17405	377213.2	5863914
CIND	tg1090828756	52.81507	1.128233	373855	5853341
COLG1	tg1044826942	52.79897	1.120251	373270.3	5851564
COLG2	tg1042626632	52.79619	1.119726	373226.8	5851256
CUBBL	tg1211939359	52.90977	1.153071	375799.5	5863830
FRONT	tg0727116000	52.70197	1.06629	369342.7	5840873
GODW56	tf9003321706	52.75953	0.814807	352546.9	5847759
GODWK53	tf9028322058	52.7626	0.818712	352820.7	5848093
GODWK54	tf9046121964	52.76169	0.821292	352991.7	5847987
GODWK55	tf9059322079	52.76268	0.823312	353131.4	5848093
GODWK57	tf9026821562	52.75815	0.818202	352771.3	5847599
GODWK58	tf9087522088	52.76266	0.827491	353413.3	5848082
GODWK59	tf9012223026	52.77135	0.81689	352727.3	5849070
GODWK60	tf9027523126	52.77219	0.819213	352886.9	5849159
GODWK61	tf9005623267	52.77353	0.816052	352678.3	5849315
GODWK62	tf9011823388	52.7746	0.81704	352748.5	5849431
GODWK63	tf9035723412	52.77473	0.820593	352988.5	5849438
GODWK64	tf9023322639	52.76783	0.818308	352811.2	5848676
GODWK65	tf8975322318	52.76512	0.811017	352310.2	5848389
GODWK66	tf9009222365	52.76542	0.816062	352651.5	5848413
GOGMA36	tg0338710952	52.65813	1.005776	365119.3	5836108
GOGMA37	tg0393310934	52.65776	1.013826	365662.6	5836052
GOGMA38	tg0380510947	52.65793	1.011944	365535.9	5836074
GOGMA39	tg0367210433	52.65336	1.009664	365367.6	5835570
GOGMA40	tg0350310400	52.65313	1.007148	365196.8	5835549
GOGMA41	tg0349910256	52.65184	1.007001	365182.8	5835406

GOGMA42	tg0440010190	52.65091	1.02026	366076.8	5835278
GOGMA43	tg0418410174	52.65085	1.017061	365860.3	5835277
GOGMA44	tg0416710330	52.65225	1.016907	365854.1	5835433
GOGMA45	tg0416710439	52.65323	1.016974	365861.7	5835542
GOGMA46	tg0299910932	52.65809	1.000036	364731	5836115
GOGMA47	tg0295611009	52.6588	0.999448	364693.5	5836195
COLG4	tg1046226806	52.79774	1.120371	373274.8	5851427
HEY89	tg1100828437	52.81217	1.129508	373932.5	5853016
HEY90	tg1113328459	52.81232	1.131374	374058.7	5853029
HEY93	tg1136328268	52.81051	1.134658	374274.8	5852823
HEY94	tg1105726722	52.79676	1.129128	373862.3	5851302
HEY96	tg1078626862	52.79812	1.125205	373601.8	5851461
HEY97	tg1072026945	52.79889	1.124281	373541.7	5851548
COLG3	tg1055326790	52.79756	1.121708	373364.4	5851405
HOHND67	tf9782835498	52.88055	0.938512	361279.4	5860973
HOHND68	tf9760535418	52.87992	0.935154	361051.4	5860909
HOHND69	tf9747035233	52.8783	0.933039	360903.9	5860734
HOHND70	tf9849035008	52.87591	0.948037	361905.5	5860439
HOHND71	tf9811935197	52.87774	0.942647	361548.7	5860653
JENGW15	tg0648226742	52.7987	1.061387	369301.4	5851640
JENGW16	tg0620327013	52.80124	1.057426	369042	5851929
JENGW17	tg0620126852	52.7998	1.057295	369028.8	5851769
JENGW18	tg0608626906	52.80033	1.055626	368917.9	5851831
JENRE	tg0605226649	52.79803	1.05496	368866.1	5851577
JONGW11	tg0670627303	52.80365	1.065059	369563.7	5852184
JONGW13	tg0671127164	52.8024	1.065045	369559.1	5852045
JONGW48	tg0636627319	52.80393	1.060033	369225.8	5852223
JONGW49	tg0628627293	52.80373	1.058832	369144.2	5852203
JONGW50	tg0630027406	52.80473	1.05911	369166	5852315
JONGW51	tg0648127335	52.80403	1.061747	369341.6	5852231
JONGW52	tg0656227474	52.80524	1.063034	369432	5852364
LANTI23	tf8843222265	52.76511	0.791433	350988.9	5848428
LANTI24	tf8849322077	52.7634	0.792228	351036.7	5848236
LANTI25	tf8830421932	52.76216	0.789347	350838.1	5848105
LANTI26	tf8825622034	52.76309	0.788695	350797.4	5848210
LANTI27	tf8881421851	52.76126	0.796849	351341.2	5847989
LANTI28	tf8883221821	52.76098	0.797098	351357.1	5847957
LANTI29	tf8890721928	52.76192	0.79827	351439.3	5848059
LANTI30	tf8907521396	52.75708	0.80045	351569.9	5847517
LANTI31	tf8881521244	52.75581	0.796514	351300.1	5847383
LANTI32	tf8840021529	52.75851	0.790536	350905.9	5847696
LANTI33	tf8822221530	52.75858	0.787902	350728.5	5847710
LANTI34	tf8925821770	52.76037	0.803374	351778.4	5847877
LANTI35	tf8946121748	52.76011	0.806366	351979.4	5847841
MYST	tg1255839443	52.91038	1.159637	376242.7	5863886

MYSTF	tg1243039444	52.91041	1.157744	376115.5	5863893
PAGGW10	tg0697728013	52.80992	1.069522	369883.3	5852873
PAGGW12	tg0676227187	52.80259	1.065815	369611.5	5852064
PAGGW8	tg0698327661	52.80676	1.069389	369864.8	5852521
PAGGW9	tg0698127835	52.80832	1.069469	369874.9	5852695
POFA2	tg1315938583	52.90239	1.168003	376782.6	5862984
POFA4	tg1326638196	52.89887	1.169337	376862.4	5862590
PRMGN107	tg0695415667	52.69911	1.061396	369003.5	5840563
PRMGN108	tg0711515726	52.69957	1.063812	369168.1	5840610
PRMGN109	tg0718715702	52.69933	1.064861	369238.3	5840581
PRMGN110	tg0737815536	52.69777	1.067578	369417.2	5840403
PRMGN81	tg0755216288	52.70445	1.070624	369642.9	5841140
PRMGN82	tg0740916296	52.70458	1.068515	369500.9	5841158
PRMGN83	tg0748415872	52.70074	1.069356	369546.3	5840730
PRMGN84	tg0757615963	52.70153	1.070773	369644.3	5840815
PRMGN85	tg0768315919	52.70109	1.072327	369748	5840763
PRMGN86	tg0766315958	52.70145	1.072056	369730.7	5840804
PRMGN88	tg0726515735	52.6996	1.066034	369318.4	5840609
RAIL	tg1232938923	52.90577	1.155904	375978.6	5863381
RAY117	tf9033625970	52.79771	0.821768	353145.2	5851991
RAY118	tf9037526340	52.80101	0.822561	353209.8	5852357
RAY119	tf9003026221	52.80007	0.817381	352857.4	5852263
RAY120	tf9017225666	52.79503	0.819162	352960.5	5851699
RAY121	tf8998325484	52.79347	0.816257	352759.4	5851531
SABA	tg1264439622	52.91192	1.161037	376341.3	5864056
SAYNE	tg1256139892	52.91438	1.159982	376277.3	5864331
STHND1	tf9792837257	52.89631	0.941065	361501.4	5862721
STHND19	tf9793638206	52.90482	0.941762	361575.4	5863666
STHND2	tf9817837463	52.89806	0.944902	361765.1	5862909
STHND20	tf9814938081	52.90362	0.944848	361779.1	5863527
STHND21	tf9823938800	52.91004	0.946623	361918.9	5864238
STHND22	tf9818239125	52.91298	0.945974	361884.6	5864566
STHND3	tf9812737178	52.89552	0.943972	361694.4	5862628
STHND4	tf9769236845	52.89269	0.937312	361237.4	5862326
STHND5	tf9868038188	52.90439	0.952797	362316.1	5863597
STHND6	tf9862238303	52.90544	0.952007	362266.3	5863715
STHND7	tf9775537805	52.90129	0.93883	361367	5863279
WERO	tg1201440460	52.91969	1.15223	375771.4	5864935

2. Pond locations – *Chapter 5 & 6* – ghost and restored pond locations.

Pond code	Grid reference	lat	long	Easting (UTM)	Northing (UTM)
GP_{45}	TG 05550 33776	52.8622	1.0520107	605550	333776
STRE	TG 05404 34063	52.864829	1.0500205	605404	334063
GP_{50}	TG 06136 26503	52.79669	1.0561144	606136	326503
GURE	TG 06068 26640	52.797948	1.0551918	606068	326640
GP_{150}	TF 99743 10711	52.657318	0.95183551	599743	310711
WERE	TF 99857 10587	52.656166	0.95344484	599857	310587

3. Correlation matrices for macrophyte diversity analysis – *Chapter* 4

Macrophyte alpha diversity analysis

		No of Plant Species	Density 200m	Density 564m	Density 1000m	Density 2000m	Density 5000m	Distance to nearest pond	Area	Chl a	Oxygen	pH	Conductivity	Alkalinity	Shade
No of Plant Species	Pearson Correlation	1	-.059	-.042	-.166	-.241**	-.241**	.125	.311**	.129	.192*	-.008	-.326**	-.387**	-.481**
	Sig. (2-tailed)		.519	.649	.068	.008	.008	.173	.001	.159	.035	.932	.000	.000	.000
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Density 200m	Pearson Correlation	-.059	1	.584**	.552**	.357**	.368**	-.503**	-.087	.173	-.104	-.050	-.085	-.153	.180*
	Sig. (2-tailed)	.519		.000	.000	.000	.000	.000	.343	.058	.255	.586	.351	.094	.048
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Density 564m	Pearson Correlation	-.042	.584**	1	.815**	.392**	.470**	-.502**	-.024	.127	-.071	-.033	-.162	-.085	.144
	Sig. (2-tailed)	.649	.000		.000	.000	.000	.000	.794	.167	.438	.722	.075	.351	.114
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Density 1000m	Pearson Correlation	-.166	.552**	.815**	1	.724**	.699**	-.422**	-.055	.138	-.137	-.070	-.113	-.047	.160
	Sig. (2-tailed)	.068	.000	.000		.000	.000	.000	.551	.130	.133	.443	.217	.607	.080
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Density 2000m	Pearson Correlation	-.241**	.357**	.392**	.724**	1	.864**	-.319**	-.048	.074	-.317**	-.091	.018	.093	.109
	Sig. (2-tailed)	.008	.000	.000	.000		.000	.000	.602	.418	.000	.322	.844	.309	.235
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Density 5000m	Pearson Correlation	-.241**	.368**	.470**	.699**	.864**	1	-.308**	-.062	.061	-.233*	-.029	-.019	.076	.051
	Sig. (2-tailed)	.008	.000	.000	.000	.000		.001	.497	.508	.010	.756	.839	.408	.576
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Distance to nearest pond	Pearson Correlation	.125	-.503**	-.502**	-.422**	-.319**	-.308**	1	.149	.006	.209*	-.020	.040	-.014	-.163
	Sig. (2-tailed)	.173	.000	.000	.000	.000	.001		.102	.948	.022	.825	.661	.882	.073
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Pond Area	Pearson Correlation	.311**	-.087	-.024	-.055	-.048	-.062	.149	1	.170	.055	.143	-.106	-.169	-.164
	Sig. (2-tailed)	.001	.343	.794	.551	.602	.497	.102		.063	.549	.118	.249	.063	.072
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Chl a	Pearson Correlation	.129	.173	.127	.138	.074	.061	.006	.170	1	.167	-.014	-.039	.034	-.166
	Sig. (2-tailed)	.159	.058	.167	.130	.418	.508	.948	.063		.068	.876	.672	.707	.070
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Oxygen	Pearson Correlation	.192*	-.104	-.071	-.137	-.317**	-.233*	.209*	.055	.167	1	.599**	-.233*	-.225*	-.344**
	Sig. (2-tailed)	.035	.255	.438	.133	.000	.010	.022	.549	.068		.000	.010	.013	.000
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
pH	Pearson Correlation	-.008	-.050	-.033	-.070	-.091	-.029	-.020	.143	-.014	.599**	1	-.050	-.061	-.010
	Sig. (2-tailed)	.932	.586	.722	.443	.322	.756	.825	.118	.876	.000		.582	.504	.915
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Conductivity	Pearson Correlation	-.326**	-.085	-.162	-.113	.018	-.019	.040	-.106	-.039	-.233*	-.050	1	.715**	.444**
	Sig. (2-tailed)	.000	.351	.075	.217	.844	.839	.661	.249	.672	.010	.582		.000	.000
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Alkalinity	Pearson Correlation	-.387**	-.153	-.085	-.047	.093	.076	-.014	-.169	.034	-.225*	-.061	.715**	1	.408**
	Sig. (2-tailed)	.000	.094	.351	.607	.309	.408	.882	.063	.707	.013	.504	.000		.000
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121
Shade	Pearson Correlation	-.481**	.180*	.144	.160	.109	.051	-.163	-.164	-.166	-.344**	-.010	.444**	.408**	1
	Sig. (2-tailed)	.000	.048	.114	.080	.235	.576	.073	.072	.070	.000	.915	.000	.000	
	N	121	121	121	121	121	121	121	121	121	121	121	121	121	121

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Macrophyte beta diversity analysis

Correlation matrix for all variables

		Plant beta diversity	Density 200m	Density 500m	Density 564m	Density 1000m	Density 2000m	Density 5000m	Distance to nearest pond	Estimated Area	Zscore: Chl a	Oxygen	pH	Conductivity	Alkalinity	Shade
Plant beta diversity	Pearson Correlation	1	-.007	-.063	.006	.025	.060	.101	-.083	-.082	-.235*	-.284**	.017	.318**	.242*	.485**
	Sig. (2-tailed)		.943	.522	.954	.799	.544	.304	.402	.408	.016	.003	.860	.001	.013	.000
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Density 200m	Pearson Correlation	-.007	1	.691**	.632**	.608**	.411**	.411**	-.537**	-.139	.201*	-.065	-.010	-.114	-.124	.166
	Sig. (2-tailed)	.943		.000	.000	.000	.000	.000	.000	.158	.040	.507	.917	.247	.207	.091
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Density 500m	Pearson Correlation	-.063	.691**	1	.903**	.831**	.438**	.490**	-.434**	-.022	.160	-.038	-.049	-.156	-.092	.057
	Sig. (2-tailed)	.522	.000		.000	.000	.000	.000	.000	.821	.103	.701	.621	.112	.353	.561
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Density 564m	Pearson Correlation	.006	.632**	.903**	1	.836**	.424**	.506**	-.494**	-.016	.149	-.046	-.017	-.187	-.098	.104
	Sig. (2-tailed)	.954	.000	.000		.000	.000	.000	.000	.868	.129	.643	.865	.056	.322	.292
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Density 1000m	Pearson Correlation	.025	.608**	.831**	.836**	1	.724**	.707**	-.440**	-.055	.140	-.108	-.063	-.153	-.060	.150
	Sig. (2-tailed)	.799	.000	.000	.000		.000	.000	.000	.580	.155	.274	.524	.120	.540	.127
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Density 2000m	Pearson Correlation	.060	.411**	.438**	.424**	.724**	1	.852**	-.369**	-.033	.089	-.291**	-.094	-.032	.089	.111
	Sig. (2-tailed)	.544	.000	.000	.000	.000		.000	.000	.738	.368	.003	.341	.742	.364	.258
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Density 5000m	Pearson Correlation	.101	.411**	.490**	.506**	.707**	.852**	1	-.355**	-.036	.073	-.194*	-.008	-.060	.080	.044
	Sig. (2-tailed)	.304	.000	.000	.000	.000	.000		.000	.716	.457	.047	.939	.541	.418	.659
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Distance to nearest pond	Pearson Correlation	-.083	-.537**	-.434**	-.494**	-.440**	-.369**	-.355**	1	.141	-.016	.171	-.055	.084	-.005	-.108
	Sig. (2-tailed)	.402	.000	.000	.000	.000	.000	.000		.151	.875	.081	.580	.392	.956	.273
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Estimated Area	Pearson Correlation	-.082	-.139	-.022	-.016	-.055	-.033	-.036	.141	1	.176	.033	.140	-.039	-.143	-.151
	Sig. (2-tailed)	.408	.158	.821	.868	.580	.738	.716	.151		.072	.735	.155	.695	.145	.123
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Zscore: Chl a	Pearson Correlation	-.235*	.201*	.160	.149	.140	.089	.073	-.016	.176	1	.151	-.017	.004	.060	-.122
	Sig. (2-tailed)	.016	.040	.103	.129	.155	.368	.457	.875	.072		.125	.866	.964	.546	.215
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Oxygen	Pearson Correlation	-.284**	-.065	-.038	-.046	-.108	-.291**	-.194*	.171	.033	.151	1	.589**	-.193*	-.212*	-.310**
	Sig. (2-tailed)	.003	.507	.701	.643	.274	.003	.047	.081	.735	.125		.000	.049	.030	.001
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
pH	Pearson Correlation	.017	-.010	-.049	-.017	-.063	-.094	-.008	-.055	.140	-.017	.589**	1	-.052	-.075	.019
	Sig. (2-tailed)	.860	.917	.621	.865	.524	.341	.939	.580	.155	.866	.000		.599	.445	.851
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Conductivity	Pearson Correlation	.318**	-.114	-.156	-.187	-.153	-.032	-.060	.084	-.039	.004	-.193*	-.052	1	.778**	.476**
	Sig. (2-tailed)	.001	.247	.112	.056	.120	.742	.541	.392	.695	.964	.049	.599		.000	.000
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Alkalinity	Pearson Correlation	.242*	-.124	-.092	-.098	-.060	.089	.080	-.005	-.143	.060	-.212*	-.075	.778**	1	.437**
	Sig. (2-tailed)	.013	.207	.353	.322	.540	.364	.418	.956	.145	.546	.030	.445	.000		.000
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Shade	Pearson Correlation	.485**	.166	.057	.104	.150	.111	.044	-.108	-.151	-.122	-.310**	.019	.476**	.437**	1
	Sig. (2-tailed)	.000	.091	.561	.292	.127	.258	.659	.273	.123	.215	.001	.851	.000	.000	
	N	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

4. Pond density groupings used in analysis of aquatic Coleoptera – *Chapter 4*

density at 200m

Tests of Between-Subjects Effects

Dependent Variable: Low or high density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.511[a]	6	1.252	6.374	.000
Intercept	107.146	1	107.146	545.547	.000
d_200m	7.511	6	1.252	6.374	.000
Error	22.390	114	.196		
Total	283.000	121			
Corrected Total	29.901	120			

a. R Squared = .251 (Adjusted R Squared = .212)

density at 500m

Tests of Between-Subjects Effects

Dependent Variable: Low or high density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	17.431[a]	27	.646	4.805	.000
Intercept	141.893	1	141.893	1056.107	.000
d_500m	17.431	27	.646	4.805	.000
Error	12.361	92	.134		
Total	285.000	120			
Corrected Total	29.792	119			

a. R Squared = .585 (Adjusted R Squared = .463)

density at 1000m

Tests of Between-Subjects Effects

Dependent Variable: Low or high density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	240.894[a]	47	5.125	1.800	.012
Intercept	262.526	1	262.526	92.203	.000
d_1000m	240.894	47	5.125	1.800	.012
Error	207.850	73	2.847		
Total	763.000	121			
Corrected Total	448.744	120			

a. R Squared = .537 (Adjusted R Squared = .239)

density at 2000m

Tests of Between-Subjects Effects

Dependent Variable: Low or high density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	24.418[a]	66	.370	3.723	.000
Intercept	211.565	1	211.565	2128.786	.000
d_2000m	24.418	66	.370	3.723	.000
Error	5.367	54	.099		
Total	325.000	121			
Corrected Total	29.785	120			

a. R Squared = .820 (Adjusted R Squared = .600)

density at 5000m

Tests of Between-Subjects Effects

Dependent Variable: Low or high density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	29.285[a]	104	.282	9.011	.000
Intercept	257.005	1	257.005	8224.164	.000
d_5000m	29.285	104	.282	9.011	.000
Error	.500	16	.031		
Total	325.000	121			
Corrected Total	29.785	120			

a. R Squared = .983 (Adjusted R Squared = .874)

Difference in environmental variables between low and high density groupings

Pond shading

Tests of Between-Subjects Effects

Dependent Variable: Low or high density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.767[a]	20	.238	.948	.529
Intercept	151.824	1	151.824	604.074	.000
shade	4.767	20	.238	.948	.529
Error	25.133	100	.251		
Total	283.000	121			
Corrected Total	29.901	120			

a. R Squared = .159 (Adjusted R Squared = -.009)

Pond area

Tests of Between-Subjects Effects

Dependent Variable: Low or high density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	26.916[a]	108	.249	.944	.598
Intercept	239.045	1	239.045	905.856	.000
area	26.916	108	.249	.944	.598
Error	3.167	12	.264		
Total	289.000	121			
Corrected Total	30.083	120			

a. R Squared = .895 (Adjusted R Squared = -.053)

Chlorophyll a

Tests of Between-Subjects Effects

Dependent Variable: Low or high density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	26.833[a]	105	.256	1.179	.377
Intercept	239.729	1	239.729	1106.440	.000
chl_a	26.833	105	.256	1.179	.377
Error	3.250	15	.217		
Total	289.000	121			
Corrected Total	30.083	120			

a. R Squared = .892 (Adjusted R Squared = .136)

pH

Tests of Between-Subjects Effects

Dependent Variable: Low or high density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	22.833[a]	85	.269	1.297	.196
Intercept	221.211	1	221.211	1067.916	.000
ph	22.833	85	.269	1.297	.196
Error	7.250	35	.207		
Total	289.000	121			
Corrected Total	30.083	120			

a. R Squared = .759 (Adjusted R Squared = .174)

Conductivity

Tests of Between-Subjects Effects

Dependent Variable: Low or high density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	26.916[a]	109	.247	.858	.681
Intercept	246.747	1	246.747	857.123	.000
conductivity	26.916	109	.247	.858	.681
Error	3.167	11	.288		
Total	289.000	121			
Corrected Total	30.083	120			

a. R Squared = .895 (Adjusted R Squared = -.148)

Alkalinity

Tests of Between-Subjects Effects

Dependent Variable: Low or high density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	25.083[a]	99	.253	1.064	.457
Intercept	227.770	1	227.770	956.635	.000
alkalinity	25.083	99	.253	1.064	.457
Error	5.000	21	.238		
Total	289.000	121			
Corrected Total	30.083	120			

a. R Squared = .834 (Adjusted R Squared = .050)

5. CNESS dissimilarity matrices used in analysis of aquatic Coleoptera – *Chapter 4*

Dissimilarity matrix m=1

	Hind.	Bod.	Godw.	Tittle.	Hey.	Colk.	Matt.	Guest.	Lyng	Mean
Hind.	0.000	0.952	1.230	1.100	1.100	1.150	1.200	1.060	1.280	1.008
Bod.	0.952	0.000	0.892	1.210	1.150	1.140	0.853	0.941	1.240	0.931
Godw.	1.230	0.892	0.000	0.926	1.050	0.971	0.967	1.040	1.110	0.910
Tittle.	1.100	1.210	0.926	0.000	1.090	0.911	1.190	1.120	1.090	0.960
Hey.	1.100	1.150	1.050	1.090	0.000	0.993	1.000	0.996	1.120	0.944
Colk.	1.150	1.140	0.971	0.911	0.993	0.000	1.160	1.160	1.030	0.946
Matt.	1.200	0.853	0.967	1.190	1.000	1.160	0.000	0.812	1.220	0.934
Guest.	1.060	0.941	1.040	1.120	0.996	1.160	0.812	0.000	1.210	0.927
Lyng	1.280	1.240	1.110	1.090	1.120	1.030	1.220	1.210	0.000	1.033

Dissimilarity matrix m=7

	Hind.	Bod.	Godw.	Tittle.	Hey.	Colk.	Matt.	Guest.	Lyng	Mean
Hind.	0.000	0.941	1.120	0.950	0.996	1.050	1.060	0.962	1.170	0.917
Bod.	0.941	0.000	0.835	1.100	1.060	1.040	0.748	0.933	1.180	0.871
Godw.	1.120	0.835	0.000	0.841	0.983	0.922	0.865	1.020	1.080	0.852
Tittle.	0.950	1.100	0.841	0.000	0.972	0.953	1.090	1.070	1.020	0.888
Hey.	0.996	1.060	0.983	0.972	0.000	0.968	0.912	0.928	1.090	0.879
Colk.	1.050	1.040	0.922	0.953	0.968	0.000	1.090	1.100	0.942	0.896
Matt.	1.060	0.748	0.865	1.090	0.912	1.090	0.000	0.785	1.160	0.857
Guest.	0.962	0.933	1.020	1.070	0.928	1.100	0.785	0.000	1.170	0.885
Lyng	1.170	1.180	1.080	1.020	1.090	0.942	1.160	1.170	0.000	0.979

Dissimilarity matrix m=31 **WITHOUT LYNG DUE TO LOW SAMPLE SIZE**

	Hind.	Bod.	Godw.	Tittle.	Hey.	Colk.	Matt.	Guest.	Mean
Hind.	0.000	0.901	0.928	0.875	0.855	0.913	0.811	0.849	0.767
Bod.	0.901	0.000	0.791	0.996	0.875	0.787	0.695	0.957	0.750
Godw.	0.928	0.791	0.000	0.841	0.812	0.923	0.783	1.010	0.761
Tittle.	0.875	0.996	0.841	0.000	0.821	0.992	0.985	1.040	0.819
Hey.	0.855	0.875	0.812	0.821	0.000	0.958	0.761	0.781	0.733
Colk.	0.913	0.787	0.923	0.992	0.958	0.000	0.919	1.000	0.812
Matt.	0.811	0.695	0.783	0.985	0.761	0.919	0.000	0.858	0.727
Guest.	0.849	0.957	1.010	1.040	0.781	1.000	0.858	0.000	0.812

6. Aquatic macrophyte and Coleoptera species codes used in ordinations - *Chapter 4*.

Aquatic macrophytes:

Code	Species	Code	Species
AgroStol	*Agrostis stolonifera*	*JuncCong*	*Juncus conglomeratus*
AlisPlan	*Alisma plantago-aquatica*	*JuncEffu*	*Juncus effusus*
AlopGeni	*Alopecurus geniculatus*	*JuncInfl*	*Juncus inflexus*
ApiuNodf	*Apium nodiflorum*	*LemnMinr*	*Lemna minor*
CallBrut	*Callitriche brutia Ssp. hamulata*	*LemnTris*	*Lemna triscula*
CallPlat	*Callitriche platycarpa s.l.*	*LycoEuro*	*Lycopus europaeus*
CallStag	*Callitriche stagnalis*	*LythSali*	*Lythrum salicaria*
CardFlex	*Cardamine flexuosa*	*MentAqua*	*Mentha aquatica*
CardPrat	*Cardamine pratensis*	*MyoScor*	*Myosotis scorpioides*
CarxAcut	*Carex acutiformis*	*NastOffAgg*	*Nasturtium officinale agg.*
CarxDist	*Carex disticha*	*NiteFlex*	*Nitella flexilis*
CarxFlac	*Carex flacca*	*OenAqua*	*Oenanthe aquatica*
CarxHirt	*Carex hirta*	*OenFisSL*	*Oenanthe fistulosa s.l.*
CarxNigr	*Carex nigra*	*PersAmph*	*Persicaria amphibia*
CarxOtru	*Carex otrubae*	*PhalArun*	*Phalaris arundinacea*
CarxPend	*Carex pendula*	*PhrgAust*	*Phragmites australis*
CarxPseu	*Carex pseudocyperus*	*plants*	*plants*
CarxRemt	*Carex remota*	*PotmBerSL*	*Potamogeton berchtoldii sl*
CarxRipr	*Carex riparia*	*PotmCris*	*Potamogeton crispus*
CertDemr	*Ceratophyllum demersum*	*PotmNatn*	*Potamogeton natans*
CertSubm	*Ceratophyllum submersum*	*RanuAqua*	*Ranunculus aquatilis*
CharaSp	*Chara sp.*	*RanuFlam*	*Ranunculus flam*
CrasHelm	*Crasula helmsei*	*RanuLing*	*Ranunculus lingua*
DescCesp	*Deschampsia cespitosa*	*RanuScel*	*Ranunculus sceleratus*
EleoPals	*Eleocharis palustris*	*RorpAmph*	*Rorippa amphibia*
EpilHirs	*Epilobium hirsutum*	*SchoLacu*	*Schoenoplectus lacustris*
EquiArve	*Equisetum arvense*	*ScrpNods*	*Scrophularia nodosa*
FallJapo	*Fallopia japonica*	*SolnDulc*	*Solanum dulcamara*
FicrVern	*Ficaria verna*	*SpargErre*	*Sparganium erectum*
FiliUlma	*Filipendula ulmaria*	*TyphAngs*	*Typha angustifolia*
FontAntp	*Fontinalis antipyretica*	*TyphLati*	*Typha latifolia*
GaliPalu	*Galium palustre*	*VernBecc*	*Veronica beccabunga*
GlycFlui	*Glyceria fluitans*	*VernCatn*	*Veronica catenata*
HippVulg	*Hippuris vulgaris*	*VernScut*	*Veronica scutelaria*
IrisPseu	*Iris pseudacorus*	*ZannPals*	*Zannichellia palustris*
JuncArtc	*Juncus articulatus*		

Aquatic Coleoptera:

code	Coleoptera species
AcilSulc	*Acilius sulcatus*
AgabBipu	*Agabus bipustulatus*
AgabSturm	*Agabus sturmii*
AnacGlob	*Anacaena globulus*
AnacLimb	*Anacaena limbata*
AnacLute	*Anacaena lutescens*
BeroSign	*Berosus signaticollis*
CercConv	*Cercyon convexiusculus*
CercSter	*Cercyon sternalis*
ColyFusc	*Colymbetes fuscus*
DytiMarg	*Dytiscus marginalis*
EnocTest	*Enochrus testaceus*
GyriSubs	*Gyrinus substriatus*
HaliLine	*Haliplus lineatocollis*
HaliRufi	*Haliplus ruficollis*
HeloBrev	*Helophorus brevipalpis*
HeloLivi	*Helochares lividus*
HeloMinu	*Helophorus minutus*
HeloObsc	*Helophorus obscurus*
HelpAequ	*Helphorus aequalis*
HydrAngs	*Hydroporus angustatus*
HydrBrev	*Hydrochus brevis*
HydrEryt	*Hydroporus erythrocephalus*
HydrFusc	*Hydrobius fuscipes*
HydrIncg	*Hydroporus incognitus*
HydrMemn	*Hydroporus memnonius*
HydrNigr	*Hydroporus nigrita*
HydrPalu	*Hydroporus palustris*
HydrPlan	*Hydroporus planus*
HydrRipa	*Hydraena riparia*
HydrStri	*Hydroporus striola*
HydrTess	*Hydroporus tesselatus*
HygoHerm	*Hygobia hermanni*
HygrImpr	*Hygrotus impressopunctatus*
HygrInae	*Hygrotus inaequalis*
HyphOvat	*Hyphydrus ovatus*
IlybChal	*Ilybius chalconatus*
IlybFene	*Ilybius fenestratus*
LaccMinu	*Laccobius minutus*
LaccMinu	*Laccophilus minutus*
LaccStria	*Laccobius striatellus*
LiopHaem	*Liopterus haemorrhoidalis*
NoteClavi	*Noterus clavicornis*
RhanExso	*Rhantus exsoletus*
RhanGrapi	*Rhantus grapii*
RhanSutu	*Rhantus suturalis*
SuphDors	*Suphrodytes dorsalis*

7. R code used for variation partitioning – *Chapter 4*

```
#set working directory and load vegan & packfor packages.
data.environ=read.table("plants_env_no_zeros.csv", header=T, sep=",", row.names=1)
data.plants=read.table("plants_p.a_no_zeros.csv", header=T, sep=",", row.names=1)
data.xy=read.table("plants_locations_no_zeros.csv", header=T, sep=",", row.names=1)
#Hellinger transform species data:
data.organism.hel=decostand(data.plants,method="hellinger")
# Transforming Euclidean distances into matrix;
distance = dist(data.xy, method="euclidean")
dist_pcnm = pcnm(distance)
#PCNM into data frame
data.pcnm=as.data.frame(dist_pcnm$vectors)
# Significant linear trend in community data?
trend.out = rda(data.organism.hel, data.xy)
anova(trend.out, step=1000, perm.max=1000)
#If so, compute detrended residuals of the response variables:
data.lm=lm(as.matrix(data.organism.hel)~as.matrix(data.xy))
data.resid=residuals(data.lm)
# Forward selection of significant environmental variables:
data.env.sel = forward.sel(data.organism.hel,data.environ)
# Forward selection of significant spatial variables:
data.pcnm.sel = forward.sel(data.resid, data.pcnm)
# construct environmental model with forward selected variables
environmental.model=data.environ[,c("shade","conductivity")]
# construct spatial model with forward selected variables - AND, if linear trend
#in the community data, include X and Y (lat lon, as well as PCNMs)
spatial.model=read.table("spatial_model_no_zeros.csv", header=T, sep=",", row.names=1)
#Species data by environment and spatial model (both reduced to forward selected
variables). Gives ven-diagram with a, c, and b (intercept)
organism.part=varpart(data.organism.hel, environmental.model,spatial.model)
organism.part
plot(organism.part)
#RDA test significance of fractions of interest (a and c):
# test constraining matrix (environmental model), partialling out spatial model.
rda.result.a = rda(data.organism.hel,environmental.model, spatial.model)
anova(rda.result.a, step=1000, perm.max=1000, model="reduced")
# test constraining matrix (spatial model), partialling out environmental model.
rda.result.c = rda(data.organism.hel,spatial.model, environmental.model)
anova(rda.result.c, step=1000, perm.max=1000, model="reduced")
```