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Environment, Community & the Individual; Characterising the Ecology of Aquatic Invasive Alien Plants in Ireland

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M.Sc., B.Sc. (Hons)



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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism

Signed: _____

Date: _____

This thesis is dedicated to those I love.

Some of whom I've gained over the course of this Ph.D.

Some of whom I've lost.

All of whom I could not have completed this work without.

Abstract

The Irish freshwater environment is particularly vulnerable to invasion by alien species. Given the importance of invasive alien species as drivers and passengers of ecological change it is pertinent that the mechanisms and processes involved in the invasion of Irish aquatic habitats are better understood. The overarching aim of this research was to characterise the relevant traits, of the environment, biological communities and individuals, responsible for the successful dispersal, establishment and spread of alien macrophytes. To that end, invasive alien macrophyte species (including *Elodea canadensis*, *Elodea nuttallii*, *Lagarosiphon major* and *Myriophyllum aquaticum*) were studied *in situ* and *ex situ*, combining field surveys, comparative experiments and molecular techniques. Hypotheses relating to propagule pressure, habitat disturbance, resource availability, community invasibility, stress tolerance and cryptic invasions were tested. It was found that the occurrence and distribution of invasive alien species in standing waterbodies in Ireland is substantially greater than previously recorded. 54% of surveyed waterbodies were found to be invaded. The intensity of human amenity use was the best predictor of the occurrence of invasive alien species and there was a positive association between nutrient concentrations and the occurrence and abundance of some invasive species. The influence of alien species was additive to the native community, increasing the complexity of native assemblages in terms of richness, biomass, diversity and function of invaded ecosystems. It was found that those native species that were excluded by alien species tended to be morphologically similar. Most invasive species studied had high capacity to tolerate fragmentation and desiccation indicating their capacity to overcome barriers to reproduction, dispersal and colonization. Using a combination of lab and field based experiments, *M. aquaticum* was shown to utilise phenotypic plasticity in its tolerance of saline conditions, and was capable of colonising and invading brackish waters. A molecular investigation of alien *Myriophyllum* spp. in Ireland revealed genetic diversity amongst and between populations of the clonal species *M. aquaticum*. The discovery of multiple cryptic taxa (*M. heterophyllum* and *M. sp. "red 1"*) originating in the horticultural trade is of particular concern. Implications for the management of aquatic invasive species in Ireland are discussed.

Chapter 1

Concepts of Invasion Ecology in the Context of Irish Freshwater Environments

"For there are queer things in lakes"

Lady Augusta Gregory

Introduction

For millennia humans have been transporting biological material and living organisms throughout the earth. Human migration, commercial trade and transport are continuing to disperse an ever-increasing number of species across environmental barriers such as oceans, rivers, mountain ranges, and climatic zones which were previously insurmountable (Mack *et al.*, 2000; Hulme, 2009). It has been suggested that, globally, few habitats remain free of species introduced by human activity (Mack *et al.*, 2000). Non-native (alien) species can be introduced either intentionally or accidentally using a wide variety of human activity as vectors (Hulme *et al.*, 2008). As global travel and trade increases, the rate of increase of non-native species introductions has accelerated worldwide (Mack *et al.*, 2000) and is likely to continue (Levine & D'Antonio, 2003). This is a trend that has been expressed in an Irish context, particularly in recent decades (Reynolds, 2002).

Freshwater ecosystems are said to be of particular risk of invasion (Shea & Chesson, 2002) and alien species are very common and widespread in freshwater systems (Strayer, 2010) with inventories estimating hundreds of alien species in some locations. It is estimated that 24% of the world's most invasive plants are wetland plants (Zedler & Kercher, 2004). Important aquatic plant invaders cover all of the major groups of aquatic plants, including riparian species, emergent plants, submerged species, floating-leaved species, and free floating plants (Strayer, 2010).

What is an Invasive Species?

Introduction of organisms from all taxa seem to be ubiquitous, however only a small proportion of introduced organisms survive to establish naturalised, self-sustaining populations and a lesser proportion of these again become invasive and expand in their new range and potentially threaten or negatively influence native biodiversity, economy or human health (Mack *et al.*, 2000). The 'tens rule' (Williamson & Fitter, 1996a) implies that 10% of non-native species imported into a region will subsequently appear in the wild, of these only 10% will establish a population which is self-sustaining, and only 10% of these self-sustaining species will become invasive.

Many authors have proposed definitions to describe the stages from importation to invasion (Davis & Thompson, 2000; Richardson *et al.*, 2000; Davis & Thompson, 2001; Colautti & MacIsaac, 2004; Richardson & Pyšek, 2004; Valéry *et al.*, 2008) often with

disagreement on the definition of certain terms. Much of the disagreement lies with the use of the word “invasive” as it evokes an emotive (mostly negative) response from a human reader (Davis & Thompson, 2001) and has been interpreted differently by various authors (Valéry *et al.*, 2008). Valéry *et al.* (2008) highlight further discrepancy in the use of the term ‘invasive’, stating “ambiguities probably depend in part on the lack of pertinence of both of the main criteria currently in use: the geographic (or biogeographic) criterion and the impact criterion”. Valéry *et al.*’s (2008) observation highlights that authors may use the term ‘invasive’ to refer to (a.) the expansion of a native species into a previously unoccupied range or habitat (b.) The naturalization and spread of a non-native species in its new range, (c.) the impact (positive or negative) that a species may have in the ecosystem to which it spreads. Valéry *et al.* (2008) further recognised that biological invaders defined by (a.) and (b.) are likely to share similar ecological traits.

In general, it is accepted that the process from importation to invasion involves a number of stages or phases. Richardson *et al.*, (2000) conceptualized the phases of invasion by considering the “limiting factors that restrict the spread of introduced taxa in a region as a series of ‘barriers’” similar to those used in connection with biological migration. According to Richardson *et al.* (2000) ‘introduction’ refers to the organism overcoming a major geographical barrier, by means of a human agent. Many introduced taxa survive as ‘casuals’. Such ‘casuals’ may reproduce sexually or asexually, “but fail to maintain their populations over longer periods. Casuals therefore must rely on repeated introduction for their persistence” (Richardson *et al.*, 2000). It is only when environmental and biological barriers no longer prevent the survival and regular self-sustaining reproduction of a population that ‘naturalization’ occurs (Richardson *et al.*, 2000). According to Richardson *et al.*, (2000) ‘invasion’ occurs only once the naturalized population overcomes barriers to dispersal and survival in the wider region and can spread into areas away from the sites of introduction.

It is interesting to note that Richardson *et al.*’s definition of invasion does not include any reference to the impact of invaders. This is in contrast to the widely used definition which requires an impact factor. For example, the IUCN (2000) defined invasive species as “alien species which becomes established in natural or seminatural ecosystems or habitat, is an agent of change, and threatens native biological diversity”. Similarly, the U.S. Executive Order on ‘invasive species’ (Executive Order No. 13112) defined invasive species as “alien species whose introduction does or is likely to cause economic or environmental harm or

harm to human health” (Clinton, 1999). This definition extends the impact factor beyond ecological impacts, and also includes socioeconomic impacts and human well being. Davis & Thompson (2000), like Richardson *et al.*, (2000) agree that there are varying degrees of colonization. In contrast, however, they distinguish between ‘colonizers’ and ‘invaders’ based on large, usually undesirable, impacts on their new environments (Davis & Thompson, 2000). They do not, however, offer a means of determining what constitutes a “large impact”.

A critical comparison of both definitions (Daehler, 2001) argues that both definitions will “generally point to the same set of species”, but that the Richardson *et al.*, (2000) definition is preferable because of the subjectivity and dependence on human values of Davis & Thompson’s (2000) definition. Daehler (2001) further argues that based on what is known about ecological systems, direct and indirect interactions between species, 80-100% of Richardson *et al.*,’s invaders are likely to have ecological impacts “perceived to be great by someone”.

More recently, Gurevitch *et al.*, (2011) noted four defining characteristics of invasion which differ from both the stages of invasion and the impacts of invasion. They suggested that “rapid local population increase, the establishment of local dominance or monocultures and/or rapid range expansion” and in some cases “major ecosystem alteration” may identify an alien species as an invasive one. Though not referring specifically to impacts or stages of spread, similarities between the definitions are visible as both the rate of spread of the alien and its effect on the receiving ecosystem are referred to.

Factors Accounting for Invasion Success

In 1958 Elton first hypothesised on the factors which account for the success of invasion by alien species. The subsequent, but related, ‘ideal weed’ theory (Baker, 1965) hypothesised that the “life history, characteristics and traits of the invading species facilitate invasion by enabling them to outcompete indigenous species” (Catford *et al.*, 2009). This hypothesis suggests that biological traits of the invasive species such as life history, reproductive strategies, high levels of phenotypic and genotypic plasticity, and growth rate may give a competitive advantage over the native species. Since Elton’s (1958) defining works on invasion ecology many more hypothesis have been suggested which extend into broader ecological contexts, these include: The ‘disturbance’ hypothesis (Sher & Hyatt, 1999) the

‘fluctuating resource availability’ hypothesis (Davis *et al.*, 2000) and the much cited ‘enemy release’ hypothesis (Keane & Crawley, 2002).

It is accepted that the success of invasion depends on success at each of the stages of the invasion process as described by Richardson *et al.*, (2000) and success at each stage is likely to be dependent on a suite of varying factors. Examining about thirty different hypotheses for invasion success, Catford *et al.*, (2009), identified four major factors that underpin invasion hypotheses. Mediated by some form of human activity “invasion is essentially a function of propagule pressure (P), the abiotic characteristics of the invaded ecosystem (A) and the characteristics of the recipient community and invading species (biotic characteristics, B), and reflects positions in time and space” (Catford *et al.*, 2009). Propagule pressure is defined as the “number of individuals introduced in an event multiplied by the temporal frequency of these events” and includes dispersal and geographic constraints. Accompanying the propagule pressure hypothesis is considerations of a ‘lag phase’, genetic diversity of propagules, and chance of introduction to a favourable environment (Catford *et al.*, 2009). A number of pathways to invasion have been identified within the ‘PAB’ approach, and the biological characteristics of the invader play a great role in almost in all pathways (Catford *et al.*, 2009).

Indeed, there appears to be three reoccurring themes throughout the literature which combine to facilitate invasion success: (1) Ecosystem properties, both biotic and abiotic, (2) the traits of the alien species and (3) propagule pressure and dispersal rates, (Lonsdale 1999). Further, Gurevitch *et al.*, (2011) demonstrated that the “invasiveness of species and invasibility of communities both vary with context and across temporal and spatial scales”. Additionally, they argue that fundamental ecological and evolutionary components, can control whether a particular individual or population of a colonizing species matches to a suitable, novel habitat (Gurevitch *et al.*, 2011).

According to Strayer (2010), freshwater invasions are not random, with respect to taxonomic identity and biological traits of the invaders and the ecological characteristics of the ecosystems that are invaded. When the various mechanisms of invasiveness or invasibility are considered in the context of aquatic ecosystems it is easy to identify that the same processes are involved as in terrestrial environments (Fleming & Dibble 2015),

namely; (1) abiotic and biotic characteristic of aquatic ecosystems, (2) traits of aquatic alien species and (3) propagule pressure and dispersal rates.

Ecosystem Properties

The concept of 'invasibility' attempts to characterise ecosystems, communities or habitats which may be particularly vulnerable or resistant to invasion (Alpert *et al.*, 2000; Milbau *et al.*, 2009); in theory, such knowledge would assist in the management of habitats for prevention of invasion. Alpert *et al.*, (2000) maintain that it is easier to identify differences in the invasability of habitats than it is to identify specific traits of invasiveness. A number of factors have been identified to account for invasibility in habitats, these include: community structure (including species diversity and species interactions), disturbance and resource availability (Alpert *et al.*, 2000).

Community composition, richness and diversity are hypothesised to play a role in habitat invasability. Elton (1958) suggested a negative relationship between native species diversity and community invasability. It is expected that species poor communities do not completely utilize available resources; Thus, in essence Elton's hypothesis is based in the theoretical idea that in less diverse communities there are probably empty niches available (Richardson & Pyšek, 2006). Species rich communities are also more likely to contain species that are capable of resisting or thwarting invasion, through various forms of competition or interactions (Richardson & Pyšek, 2006). High species diversity has been linked with higher primary productivity, which in turn leads to greater competition for limited resources; in theory this should result in less available niches and lower invasability as "a potential invader must be able to survive, grow, and reproduce by using the resources left unconsumed by established species" (Tilman, 2004). The literature is unclear as to the validity of this suggestion (Richardson & Pyšek, 2006). It appears that the spatial scale at which invasability is considered influences whether the 'Species diversity hypothesis' is true. Studies which show a positive correlation between native species diversity and invasability tend to be at large spatial scales with high degrees of habitat heterogeneity; this is in contrast with the studies conducted at finer scales, which negatively correlate invasability as a function of species diversity (Richardson & Pyšek, 2006; Milbau *et al.*, 2009).

Much of the evidence collected to support the 'community invasability'/'biotic resistance' hypothesis is based on terrestrial ecosystems such as grasslands and forests; however

freshwater systems typically have simpler communities with fewer species (Capers *et al.*, 2007; Fleming & Dibble, 2015). Where habitats contain emergent or floating leaved plants one would expect competition between species, especially with those that occur in the same structural group or those beneath them. However, it has been suggested that competition may be less important among submerged plants (McCreary, 1991; Capers *et al.*, 2007). A study of 103 lakes in Connecticut was unable to show that greater native species richness lead to resistance to invasion, however denser communities showed some evidence of resistance toward invasion (Capers *et al.*, 2007). This indicates that freshwater systems may have more open niches than terrestrial habitats where competition is known to structure communities, and resistance has been documented at smaller spatial scales. Capers *et al.* (2007) believe that the availability of open niches in freshwater ecosystems is a result of high levels of disturbance.

The 'disturbance' hypothesis argues that it is "critical for a successful model for invasions to incorporate both environmental and species traits" (Sher & Hyatt, 1999). In this case the authors differentiated between integral disturbances which are naturally part of ecosystem functioning such as fires and floods, highlighting that disturbances that alter historical ecosystem patterns or flux of resources are often associated with plant invasions. The 'disturbance' hypotheses has been echoed in many other studies concerning invasion success, identifying ecosystem perturbation as a means for potentially invasive species to gain a foothold when competitive native communities are weakened. The 'fluctuating resource availability' hypothesis theorises that a "plant community becomes more susceptible to invasion whenever there is an increase in the amount of unused resources" (Davis *et al.*, 2000). This theory assumes that a species invading an area will be limited by the resources available and will have greater success as an invader if competition for resources such as light, water and nutrients is limited. This assumption is based on the theory that the intensity of competition is inversely correlated with the availability of unused resources (Davis *et al.*, 2000). The resource availability theory and disturbance theory are complemented by the earlier work of Grime (1974, 1988). Grime's work showed that in recently disturbed environments competition is less important (Davis *et al.*, 2000). This is because after disturbance the native plant community is unlikely to be utilizing all available resources, thus allowing new species the opportunity to colonize. Sher and Hyatt (1999) further acknowledge the novel or excess provision of resources to what is normal (such as eutrophication) as a disturbance event, even if the disturbance is long term or

persistant. Importantly, based on resource availability and disturbance Davis *et al.* (2000) stated that a “community's susceptibility to invasion is not a static or permanent attribute, but a condition that can fluctuate over time”.

In both standing and running water systems disturbances are frequent, varied and many. Aquatic habitats are repeatedly subject to physical, chemical and biological disturbances, some of which are natural and some of which are anthropogenic in nature. Physiochemical disturbances vary spatially and temporally (Trémolières, 2004) depending on season, climate, position in the landscape and surrounding landuse. Natural physical disturbances include currents and waves (Keddy, 1985), fluctuating water levels, flooding events and sedimentation (Lake, 2008). Such disturbances can be exasperated by human activity including water extraction, reservoir activities and alteration of the surrounding landscape and catchment. Flooding events can scour out vegetative communities, change channel morphology and open previously occupied space in the habitat (Lake, 2008). Likewise, water drawdown (Wilcox & Meeker, 1991; Barrat-Segretain & Cellot, 2007) or sedimentation (Madsen *et al.*, 2001) may alter communities and open previously occupied niches. Hydrologic disturbances such as flooding and drought, and the subsequent changes in macrophyte communities have been linked to the expansion of invasive species (Kercher & Zedler, 2004)

Because freshwater ecosystems are not static there are often chemical disturbances and fluctuations in resource availability. This is particularly true in anthropogenic landscapes, both rural and urban. Eutrophication of aquatic systems as a result of both diffuse and point source pollutants is considered to be one of the greatest threats to freshwater habitats (Ansari *et al.*, 2010). Eutrophication though linked with increases in plant biomass (Ansari *et al.*, 2010) may cause an excess of nutrient resources that the native community are unable to effectively use, thus providing an opportunity for invasive species to establish (Davis & Thompson 2001; Thiébaud, 2005; Thiébaud, 2006). Other chemical disturbances which may alter aquatic plant communities include fluctuations in biological oxygen demand, pH (Thiébaud & Muller, 1999), or toxins such as metals (Zhou *et al.*, 2008) which may enter aquatic systems through natural runoff or anthropogenic input.

Physical disturbance can also be a result of direct human activity in aquatic habitats. These include waterway navigation by boats, weed cutting, dredging and canalisation, all of which

have been linked with altering aquatic plant communities and sometimes promote the occurrence of invasive species (Känel & Uehlinger, 1998; Ehrenfeld, 2008). Motor boat disturbance has been shown to alter macrophyte biomass and dominant lifeforms in canals and lakes, through direct physical disturbance and indirect disturbance such as generation of turbidity and waves (Murphy & Eaton 1983; Asplund & Cook, 1997; Mosisch & Arthington, 1998).

Linked to community composition, the 'enemy release' hypothesis states that alien plant species, when introduced to a new biogeographical region, "experience a decrease in regulation by herbivores and other natural enemies, resulting in a rapid increase in distribution and abundance" (Keane & Crawley, 2002). This hypothesis has its foundations in the assumption that plant populations are regulated by natural enemies, that native species are more greatly impacted by enemies than alien species and that in the absence of enemy regulation plants can 'capitalize' resulting in increased population growth (Keane & Crawley, 2002). It has also been argued that because a species does not have to invest in defence more resources are theoretically free to use competitively. In contrast, Keane & Crawley (2002) also noted that an alien species may fail to capitalize on release from enemy pressure if resources are limited and competition for said resources is high, thus acknowledging again the role of resource availability and disturbance in invasion. Darwin (1859) first observed that the relatedness of an alien to native species influences the likelihood of establishment and spread (Dawson, 2009), this observation has been extended to the enemy release hypothesis by Mack (1996) and Parker & Gilber (2004); indicating that alien species more closely related to the native residents may acquire enemies from them as herbivores and pathogens often display a strong phylogenetic signal (Parker and Gilbert, 2007).

The enemy release hypothesis has been investigated in freshwater systems to mixed results. A Chinese study found that a generalist aquatic snail showed preference for 20 native species of macrophyte over seven alien species (Xiong *et al.*, 2008). In contrast, a N. American study found that generalist, native crayfish showed preference for alien macrophytes (Parker & Hay. 2005). A number of studies have also shown that aquatic plants may not be limited by herbivores, even in their native range, thus reducing the likelihood that the enemy release hypothesis explains successful aquatic plant invasions globally (Fleming & Dibble, 2015).

Biological Traits of Invasive Plants

Numerous attempts have been made to compile lists of common traits shared by successful invaders, in an attempt to predict the identity of future invaders. Some groups of invaders do appear to have traits in common, but generally lists of traits are only applicable for small groups of species, and exceptions to the rule are plentiful (Mack *et al.*, 2000). It has been observed, however, that useful generalisations on invasive traits are sometimes difficult to make because data on failed invasions are not usually available, thus not allowing a comparison between traits of successful and unsuccessful invasions (Rejmánek & Richardson, 1996). Alternatively, alien and native invaders can be compared as these species are largely functionally similar so are likely to possess common traits (Thompson *et al.*, 1995; Valéry *et al.*, 2008).

The 'ideal weed' theory (Baker, 1965) hypothesised that weedy species would possess certain traits compared to their non-weedy congeneric relatives. Baker (1965) compiled a list of such traits common to all weeds. The characteristics of such 'weeds' have been adopted throughout the literature on invasive plants, hypothesising that life history, characteristics and traits of the invading species facilitate invasion by enabling them to outcompete species native to that range (Catford *et al.*, 2009). A 2004 review of pair-wise experiments between invaders and native plant species supported the general perception that invaders are good competitors compared to their native counterparts, under experimental conditions (Vila & Weiner, 2004). However, it has been argued that no one invader possesses all the traits of an 'ideal weed' and that many successful invaders possess "remarkably few" of those features (Mack, 1996). More recently summaries of broad characteristics of plant invasiveness have been compiled (Booth *et al.*, 2003; Rejmánek *et al.*, 2005).

Competitive Traits:

Plants that are able to maintain general fitness homeostasis over a range of environments are thought to have an advantage with respect to invasion potential (Rejmanek, 2000; Booth *et al.*, 2003; Rejmánek *et al.*, 2005). This fitness can be maintained by either phenotypic plasticity or genotypic variation. Rejmanek (2000) warns that, in contrast to that of the individual, "population fitness homeostasis is not a readily quantifiable variable". A meta-analysis of the phenotypic plasticity of 150 species found that invasive species were almost always more plastic in their responses to greater resource availability

than non-invasives, but that this did not always result in a benefit to fitness (Davidson *et al.*, 2011); in contrast, when resources were limited, non-invasive species maintain a greater fitness homeostasis. Similarly, Daehler (2003) concluded that invaders were often more plastic than natives in both growth and physiological responses, but that it did not necessarily indicate a performance advantage; this was explained by a presumed greater genetic variation among native species. For freshwater plants, many of which are clonal in their invasive range, phenotypic plasticity is likely to be the primary adaptive strategy (Geng *et al.*, 2007; Ward *et al.*, 2008; Riis *et al.*, 2010)

Invasive plants are often described as having generally better performance indicators, allowing them to outcompete native competitors; indeed, multiple comparative studies support this hypothesis. Daehler's (2003) summary of comparative experiments stated, however, that there was "no clear evidence that invaders grow faster than co-occurring natives". It appears that fast growth rates are dependent on the growth condition, as demonstrated by experimental work which investigated growth responses under multiple conditions (Daehler, 2003). Such observations were made with respect to a number of widely used performance indicators such as RGR, biomass, height, root traits and photosynthetic activity. Similarly, in a Canadian study of over 300 species, invasive plant species were found to grow significantly taller than their noninvasive congeners, but height was found to be a poor predictor of invasiveness when applied to a predictive model (Goodwin *et al.*, 1999). In contrast to Daehler's (2003) summary, a meta-analysis of over 320 species found that invasive species performed significantly better in all performance-related traits including photosynthetic activity, leaf area allocation, shoot allocation, growth rate and fitness (Van Kleunen *et al.*, 2010). Interestingly that study also found that for "comparisons between invasive species and native species that themselves are invasive elsewhere, no trait differences were significant" (Van Kleunen *et al.*, 2010). Van Kleunen *et al.* (2010) thus argue that the meta-analysis approach is more powerful than the summary approach used by Daehler (2003). In a review which was confined to congeneric comparisons (Daehler's wasn't) growth rate, specific leaf area and photosynthetic capacity were positively associated with invasiveness, and tissue investment was negatively correlated with invasiveness (Pyšek & Richardson, 2008). The two characteristics related to growth performance that Daehler (2003) did identify as consistent among invasive species were larger leaf size and lower tissue construction cost. Competitive strength in the context of invasion is thus likely to be context dependent.

Reproductive Traits:

Characteristics of reproduction and dispersal were particularly highlighted as indicators of plant invasiveness in a number of summaries of plant invasiveness (Booth *et al.*, 2003; Rejmánek *et al.*, 2005). The reproductive assurance of species capable of self-pollinating in the early stages of invasion, while populations are small was highlighted as a particular advantage for maximizing fertility (Richardson & Pyšek 2006). Additionally, when compared to similar native species, plant invaders often have higher relative fitness including high dispersal rates, seed longevity and early reproductive maturity (Roy, 1990). Rejmánek & Richardson (1996) observed that invasive tree species shared a common reproductive r-strategy; Comparing species of the *Pinus* genus an underlying r-K selection continuum was observed, along which invasive-noninvasive pine species were situated. This strategy was later confirmed to be linked to invasion success in other coniferous species (Richardson & Rejmánek, 2004). Such studies suggest that plants with shorter lifespans, greater fecundity and earlier reproductive maturity are more likely to become invasive than their counterparts with less but larger seeds and later maturity. Similar reproductive advantages have been observed in some herbaceous species with respect to seed production and germination (Daehler, 2003) though this advantage is not consistent and is often context dependant.

In a review paper Pysek & Richardson (2008) suggested that there are two characteristic groups of aliens that use different reproductive strategies to successfully invade: “aliens that are more K-strategists (long-lived, tall, and with big seeds) than native K-strategists, and those that are more r-strategists than native r-strategists (small, rapidly maturing, species that soon succumb to interspecific competition during secondary succession). Facon *et al.*, (2006) agree with this idea, explaining that “species investing too much in competitive ability (i.e. K strategists) are poor colonists... Species that are more r selected will invade faster and/or more often provided they remain above the competition threshold set up by residents.”

The capacity to reproduce asexually is also linked to many successful invasions (Kolar & Lodge, 2001; Booth *et al.*, 2003; Rejmánek *et al.*, 2005) For example, a study of British, Irish and Dutch flora found that in Britain and Ireland alien invaders were more likely to be perennial, clonal plants compared to invasive natives (Thompson *et al.*, 1995). Similarly, in the Czech Republic, an analysis of 132 species alien found that vegetative reproduction

favoured invasion success in semi-natural habitats (Pyšek *et al.*, 1995). In a Danish study, asexual reproduction played an important role in some 56% of species invading semi-natural habitats (Andersen, 1995). Asexually reproducing species can be said to be r-strategists as they are capable of reproducing early and have a high fecundity (Facon *et al.*, 2006); Capability to reproduce asexually provides reproductive assurance of initially small populations, or even single individuals, since even one viable propagule is sufficient to start an entire new colony (Ashton, 1989). However, if adaptive change is required for invasion success, asexual reproduction may be a limiting trait, unless the individual has high levels of phenotypic plasticity (Facon *et al.*, 2006).

As with many other plant traits, the role of vegetative reproduction in invasion success depends on the context; clonal invaders are more common in colder wetland habitats, for example (Thompson *et al.*, 1995; Pyšek, 1997). Most aquatic plant species exhibit clonal reproductive traits in some form (Santamaría, 2002) and the aquatic environment provides optimal conditions for the production of propagules at low cost (Grace, 1993). One distinct advantage of clonal propagules such as vegetative fragments is that fragments are capable of continued uptake nutrients from the surrounding water, while being dispersed (Santamaría, 2002). In terrestrial environments, compared to plants dispersed by seed, clonality is only important with respect to short distance dispersal (Rejmánek, 1996), though Pyšek (1997) observed that this may be compensated for by the fact that humans are more likely disperse clonal plants. In contrast, in the aquatic environment, clonal plants have a distinct advantage, and are dispersed long distances, aiding invasion and spread (Rejmánek, 1996). Once established, clonal plants appear to be rather competitive and persistent, effectively dominating communities (Pyšek, 1997).

Native Distribution:

It has been hypothesised that a large native range is an indicator of potential invasiveness (Booth *et al.*, 2003; Rejmánek *et al.*, 2005). In Britain, invasive plants that form dominant populations have been identified to have much wider geographical distributions in their native range, in contrast to those that occur as scattered individuals (Williamson & Fitter, 1996b). Similarly, a Canadian study found that the size of a species native range predicted the invasability of 70% of the alien species tested (Goodwin *et al.*, 1999). Additionally, European plants of the families Fabaceae, Gramineae and Compositae which have successfully naturalised N. America have significantly larger native latitudinal ranges than those that never successfully colonised N. America (Rejmánek, 1996). Likewise, a German

study identified native habitat range, as one of only two variables that could reliably account for invasion success (Küster *et al.*, 2008).

It appears that a large native range is a good predictor of invasion for two reasons; (1) The likelihood of agent mediated dispersal increases with large range, and (2) adaptation to a greater range of environmental conditions is more likely facilitating survival in new abiotic environments dispersal (Goodwin *et al.*, 1999; Booth *et al.*, 2003) It is suggested that the same biological traits that allow species to spread across native continents and climatic zones may also facilitate their invasion of new continents (Roy *et al.*, 1991). Kuster *et al.* (2008) thus propose that combinations of traits, relating to habitat and range requirements, determine invasion success, but that this is not a primary biological trait in itself, but a secondary trait as a result of environment. Additionally, Booth *et al.*, (2003) issues a caution with respect to this predicting factor however, as species with confined ranges due to enemy control may become invasive when released from enemy pressure.

It has been said that aquatic plants tend to have a broader distribution than their terrestrial counterparts and that there is a high proportion of widely distributed taxa among aquatic plants (Santamaría, 2002). Local endemic aquatic species are rare, with the exception of the tropics; this is in contrast with terrestrial species which show a larger level of endemism and lower proportion of broadly distributed species (Santamaría, 2002). In terrestrial environments climatic factors often limit the distribution of plants, however in aquatic systems climatic factors seem to be of limited importance except when it comes to large climatic differences such as tropical 'v' temperate. It is often believed that the supposed uniformity and buffering capacity of water to climatic variations may facilitate aquatic plants to occupy large ranges, however, Santamaria (2002) argues that such speculation is dependent on the spatio-temporal scale of observation.

Desirability to Humans:

As introduction to a new range, by human activity, is one of the primary sources of invasive species, it is interesting to consider the traits which make plants desirable to humans. Species and cultivars which are selected for horticultural purposes are often, unintentionally, chosen because they possess traits that relate to the invasiveness of a plant (Anderson *et al.*, 2006; Dawson, 2009) Plants introduced and bred for aesthetic horticulture, agricultural or silvicultural purposes are often selected for fast growth rates, early maturity, high reproductive output, and tolerance of a wide range of environmental

conditions (Dawson, 2009). For example, nurseries regularly supply species which are cheaper because they are easier to propagate more often than species that propagate less easy and may therefore be less capable of establishing invasive populations outside of cultivation (Dehnen-Schmutz & Touza, 2008).

Each year the aquarium trade supports a \$25billion industry, globally and it continues to grow. One third of the world's worst aquatic invasive species are of aquarium or ornamental origin (Padilla & Williams, 2004). Interestingly every aquatic plant which has been listed as a noxious weed in the USA is available to purchase online (Kay & Hoyle, 2001). One Canadian study found that thousands of invasive plant propagules were introduced to aquatic habitats each year in Montreal through the aquarium trade alone (Cohen *et al.*, 2007)

Predicting Invasiveness Using Traits

There appears to be a certain level of scepticism throughout the literature about the existence of a suite of traits with which invasive species can be generalised. Despite this a number of studies and meta-analyses have shown that invasive plant species generally exhibit higher performance related traits (Van Kleunen *et al.*, 2010). Many authors argue that ability of most invaders to outperform co-occurring or congeneric natives is context dependent. Characteristics of invasive alien plant species are strongly dependent on habitat and time, which may explain why various investigations seemingly contradict each other on occasion. As Thompson *et al.*, (1995) correctly observed, it is not possible for any species to simultaneously maximise growth rate, competitive ability and reproductive output. In fact, it is regularly argued that the success or failure of invasions is not limited to a single factor, rather it is more likely to be a combination of multiple factors (Facon *et al.*, 2006; Catford *et al.*, 2009; Gurevitch *et al.*, 2011). For example, Facon *et al.*, (2006) stated “there is neither a definitive list of characters that define a good invasion strategy nor a general predictor of community invasability.

The Irish Context

In Ireland a number of authors have accepted the approach that the naturalization and invasion process occurs in stages and that alien species can be described as being at different stages of the naturalization process (Reynolds, 2002; Milbau & Stout, 2008; Williamson *et al.*, 2008; Stout, 2011). For example, Reynolds (2002) generated a catalogue of alien plants in Ireland, describing to what extent the species had become naturalized.

Reynolds (2002) uses the impact factor to define an invasive species, stating that “it appears that the majority of alien plants have little or no adverse impact”. More recently Milbau & Stout (2008), Williamson *et al.*, (2008) and Stout (2011) have accepted Richardson *et al.*,’s (2000) definitions when discussing alien plants in Ireland.

Comparing the definitions and findings of Reynolds (2002), Milbau & Stout (2008) and Stout (2011) one might come to the same conclusion as Daehler (2001) (that both definitions generally point to the same set of species) when considering alien plants in an Irish context. Reynolds (2002) found that of an alien flora of 645 species “currently” (1987-2001) in Ireland, 45% are casuals, 25% are found only as persisting relics of prior cultivation, and 30% (c. 200 species) are established. Of the c. 200 established species only 94 (15% of the total) species are naturalized (have spread into) in natural or seminatural habitats “of which only a small number are invasive”. Similarly, Milbau & Stout (2008), described the status of 716 taxa of alien plant recorded in Ireland since 1970. Of these 52% were found to be casuals. A further 44% (347 taxa) are well established, “of which 65 were considered invasive and 282 naturalized”. With regards distribution throughout Ireland; Reynolds (2002) found that 55% of alien species were rare and 18% common. Similarly, Milbau & Stout (2008) found that 65% were rare and only 16% were common throughout Ireland (that is occurring in more than half of the 10x10km grid squares). This indicates that in general both definitions point to largely the same set of species in an Irish context. Following the advice of Daehler (2001) it would be wise to exercise caution and adopt the less subjective approach used by Richardson *et al.* (2000), and Milbau & Stout (2008), as this approach identifies more species as invaders and may act as a forewarning to identifying invaders with potentially negative impacts.

Interestingly, policy makers, state and semi-state bodies in Ireland appear to adopt the ‘impact’ approach to invasive species. For example, a recent EPA STRIVE report (Maguire *et al.*, 2011) stated that when “non-native species become established in existing ecosystems and threaten biodiversity and/ or result in economic damage, they are referred to as ‘invasive alien species’”. Similarly, Invasive Species Ireland (a joint venture between the NPWS and NIEA) define ‘invasive species’ according to the Convention on Biological Diversity (UNEP, 2002) as “species that have been introduced (deliberately or accidentally) by humans and have a negative impact on the economy, wildlife or habitats of Ireland and Northern Ireland” (Invasive Species Ireland, 2013) as does the “Invasive Alien Species

Strategy for Northern Ireland” (Dept. of Env. N.I., 2013). O’Flynn and Lysaght (2011), however, highlighted the importance of early detection and prevention of spread of “potentially invasive species” in another recent EPA STRIVE report. This shows that Irish agencies and policy makers recognise the link between the Richardson *et al.*, ‘spread’ definition and the potential to cause ecological impacts.

At present there are nearly as many non-native plant species recorded in Ireland as there are native species (Reynolds, 2002). Though evidence shows that plant species have been introduced to the Irish flora for millennia (Williamson *et al.*, 2008) almost two thirds of alien plant taxa in Ireland have been recorded in the last three decades (Reynolds, 2002). The majority of these (70%) have been introduced as a result of cultivation; either for horticultural purposes (80%) or as crop plants (Reynolds, 2002). However, not all alien plant introductions to Ireland become invasive. Spreading into areas away from sites of introduction, nor do many of them have a significant impact on Irish ecosystems or economy (Reynolds, 2002; Milbau & Stout, 2008). Despite the fact that few alien plant species in Ireland are considered invasive, Stout (2011) noted that about 30% of alien species found in Ireland are considered to be invasive elsewhere in the world. Some authorities warn against relying on the experience of neighbouring regions to predict potentially invasive species, rather they recommend “predicting future invasions from the traits of alien species and from the characteristics of already invaded systems” (Nijs *et al.*, 2009).

Comparing the traits of hundreds of alien plant species in Ireland Milbau & Stout (2008) tested the hypothesis that “the likelihood of an alien plant species of becoming naturalized and subsequently invasive depends on a series of parameters (related to invasion history, native distribution, and biological traits...)”. They found that alien plants were more likely to become naturalised in Ireland if they “showed clonal growth, had a high moisture-indicator score, a low nitrogen-indicator score, a small native range, and an early first record date”. Additionally, it was found that alien plant species were likely to become invasive if they were “introduced for ornamental reasons, had hermaphrodite flowers, were not pollinated by means of animals or selfing, were invasive elsewhere, started flowering later in the year, preferred wetter habitats, had a small native range, and were already in Ireland for a long time”. This indicates that alien plants with affinity to wetter habitats are likely to naturalize and successfully become invaders. It also shows that clonal

reproduction will allow an alien plant species to establish a naturalized population more easily, but not necessarily provide the level of dispersal and spread required to become a successful invader (Milbau & Stout, 2008); It is thus logical to assume that in order for a clonal plant to become a successful invader it requires a medium, such as water, if not an animal or human vector to disperse propagules. Similarly plants that are not self or animal pollinated (i.e. water or wind pollinated) are more likely to become invasive (Milbau & Stout, 2008). Finally, it shows that the longer an alien plant has existed in Ireland, the more likely it is to become invasive. The evidence thus suggests that aquatic and riparian plant species pose a particular threat as invasive species in Ireland.

Freshwater Invasive Plants in Ireland

Kelly *et al.*, (2013) performed risk assessments of 377 alien species of plant, animal and other taxa currently found in Ireland. The risk assessment used species traits, distribution data and invasive status elsewhere to identify alien species at “high risk” of becoming invasive and having an impact in an Irish context. The risk assessment identified some 46 high risk species, 16 of which were plants (Kelly *et al.*, 2013). Of the 16 plant species, nine are aquatic (including one marine species); *Hydrocotyle ranunculoides* L.f., *Myriophyllum aquaticum* (Vell.) Verdc, *Crassula helmsii* (Kirk) Cockayne, *Nymphoides peltata* (S.G. Gmel.) Kuntze, *Elodea canadensis* Michx., *Elodea nuttallii* (Planch.) H. St. John, *Lagarosiphon major* (Ridl.) Moss, *Spartina anglica* C. E. Hubb. (Table 1). A further four are typical of riparian habitats; *Impatiens glandulifera* Royle, *Heracleum mantegazzianum* Somm. et Lev., *Fallopia japonica* (Houtt.) Ronse Decr., *Fallopia sachalinensis* (F. Schmidt) Ronse Decr. and their hybrids. A further two plant species not currently recorded in the wild in Ireland, but identified to be of particular risk, were identified. Both are aquatic; *Ludwigia grandiflora* (Michx.) Greuter & Burdet and *Ludwigia peploides* (Kunth) P.H. Raven. Interestingly Maguire *et al* (2011) included *Lemna minuta* Kunth and *Azolla filiculoides* Lam. as high risk invasive species in their assessment of aquatic invaders. *A. filiculoides* has also been assessed as a moderate to major risk in Ireland by Millane & Caffrey (2014). These risk assessments are based on a tool developed by the European and Mediterranean Plant Protection Organisation and adapted for Ireland. Discussing the findings of the risk assessments O’Flynn *et al.*, (2014) noted that since 1980 the greatest rate of increase in alien introductions has been to the freshwater environment and it is the only environment where more there were more species of high risk than medium risk found; indicating that freshwater alien species are more likely to become high risk invaders where they are introduced.

In 2011, a statutory instrument (S.I. No. 477/2011) to address deficiencies in Ireland's implementation of the E.U. Birds Directive (Council Directive 2009/147/EC) and E.U. Habitats Directive (Council Directive 92/43/EEC) was signed into law. This regulation imposes restrictions on the importation, dispersal, propagation and sale of listed alien plant and animal species. These blacklisted alien species are considered to pose a threat to the conservation status of Irish habitats and species. Included on this blacklist are thirteen freshwater alien plant taxa, nine of which are known to already occur in Ireland (Table 1). More recently, restrictions have been imposed throughout the E.U. on the importation and sale of invasive alien species of union concern and member states are required to take measures for their early detection, rapid eradication and/or management (Regulation (EU) 1143/2014). Included on the list of invasive plant species of union concern are ten aquatic plant species (Table 1), five of which have already been recorded in Ireland, namely *E. nuttallii*, *L. major*, *M. aquaticum*, *H. ranunculoides* and *L. peploides* (Tsiamis *et al.*, 2017).

Table 1: Regulated and/or high risk (Kelly *et al.*, 2013; Maguire *et al.*, 2007) freshwater invasive alien plant species and their presence in Ireland (as recorded by the National Biodiversity Data Centre, 2018).

Species	Common Name	Present	S.I. No. 477	E.U. Reg. 1143	Kelly <i>et al.</i> (2013)	Maguire <i>et al.</i> (2007)
<i>Alternanthera philoxeroides</i>	Alligator weed			✓		
<i>Aponogeton distachyos</i>	Cape pondweed	✓	✓			
<i>Azolla filiculoides</i>	Water fern	✓	✓			✓
<i>Cabomba caroliniana</i>	Fanwort		✓	✓		
<i>Crassula helmsii</i>	N.Z. pigmyweed	✓	✓		✓	✓
<i>Eichhornia crassipes</i>	Water hyacinth			✓		
<i>Elodea (all species)</i>	Waterweeds	✓	✓			
<i>Elodea canadensis</i>	Canadian waterweed	✓			✓	
<i>Elodea nuttallii</i>	Nuttall's waterweed	✓		✓	✓	✓
<i>Hydrocotyle ranunculoides</i>	Floating pennywort	✓	✓	✓	✓	✓
<i>Lagarosiphon major</i>	Curly waterweed	✓	✓	✓	✓	✓
<i>Lemna minuta</i>	Least duckweed	✓				✓
<i>Ludwigia (all species)</i>	Water-primrose	*	✓			
<i>Ludwigia grandiflora</i>	Water-primrose	*		✓		✓
<i>Ludwigia peploides</i>	Floating primrose			✓		✓
<i>Myriophyllum aquaticum</i>	Parrot's feather	✓	✓	✓	✓	✓
<i>M. heterophyllum</i>	Broadleaf watermilfoil			✓		
<i>Nymphoides peltata</i>	Fringed water-lily	✓	✓		✓	✓
<i>Pistia stratiotes</i>	Water lettuce		✓			
<i>Trapa natans</i>	Water chestnut		✓			

**Ludwigia grandiflora* is not currently recorded in the wild in Ireland, but is recorded in two garden ponds (NBDC, 2018).

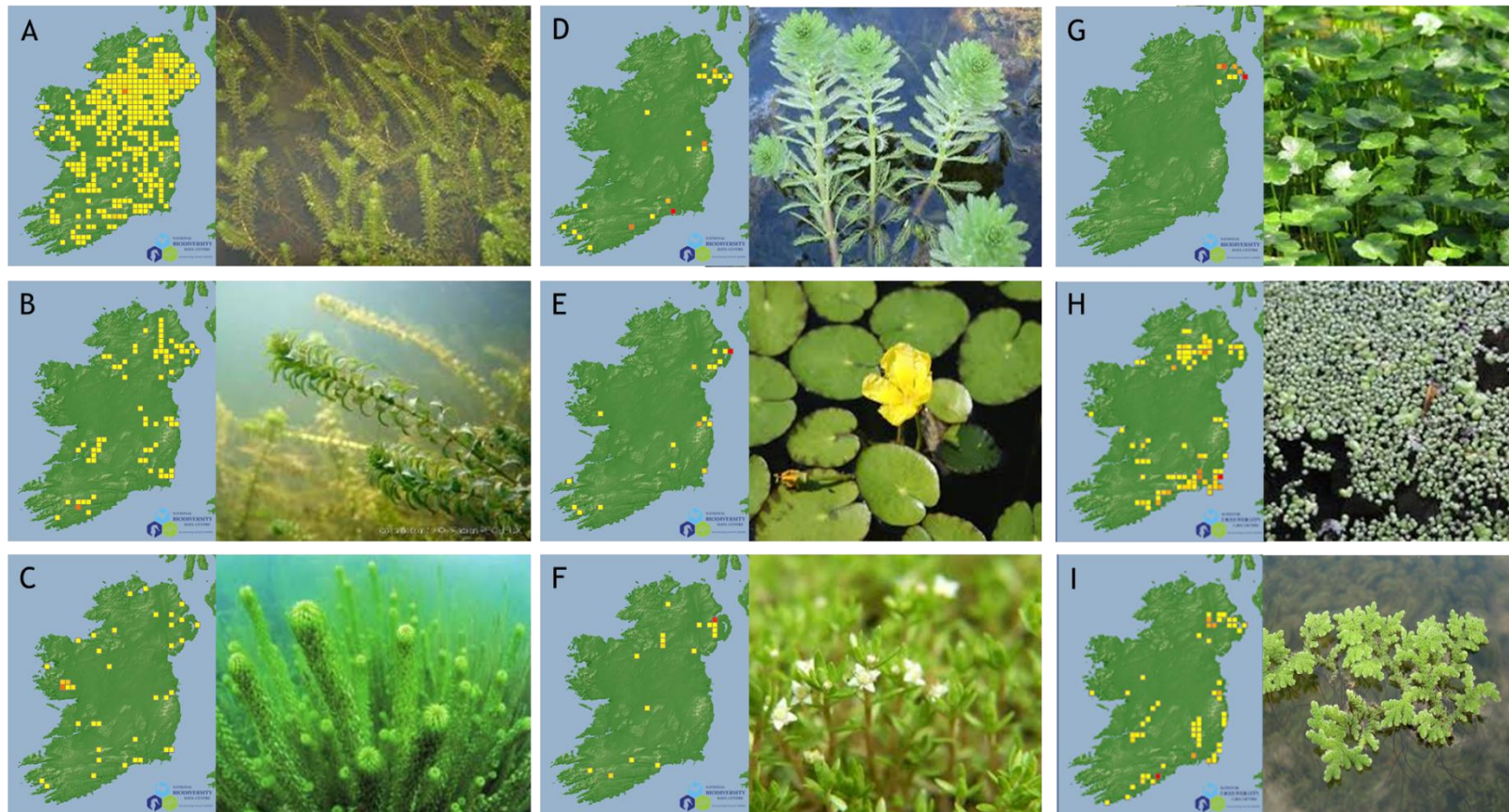


Figure 1: Recorded distribution of nine high risk aquatic invasive plant species present in the wild in Ireland at the commencement of this research in 2014: A. *Elodea canadensis*, B. *Elodea nuttallii*, C. *Lagarosiphon major*, D. *Myriophyllum aquaticum*, E. *Nymphaeodes peltata*, F. *Crassula helmsii*, G. *Hydrocotyl ranunculoides*, H. *Lemna minuta* and I. *Azolla filiculoides*. Distribution maps are from the National Biodiversity Data Centre (2014) and photographs of plants were retrieved from Google Image

Aims & Objectives

Investigation of the patterns of success and failure of species and their interactions with the ecosystems within which they occur is central to the concept of ecology. Understanding the ecological envelopes in which species exist and the biological mechanisms that allow them to thrive there is essential for the management of pests, provision of ecosystem services and conservation of biodiversity. Given the importance of invasive alien species as drivers and passengers of ecological change it is pertinent that the mechanisms and processes involved in the invasion of Irish aquatic habitats are better understood.

The overarching aim of this research was to characterise the ecological traits, of the environment, biological communities and individuals, responsible for the successful dispersal, establishment and spread of alien macrophytes. To that end hypotheses relating to propagule pressure, habitat disturbance, resource availability, community invasibility, stress tolerance and cryptic invasions were tested. Invasive alien macrophyte species were studied in situ and ex situ, combining field survey, comparative experiments and molecular techniques to meet the five specific objectives of this research.

The specific objectives of this research were to

1. Understand how the biotic, abiotic and anthropogenic characteristics of aquatic ecosystems are related to the occurrence, distribution, abundance and impact of an invasive alien species (Chapter 2, 3 & 5).
2. Identify the potential vectors of invasive species dispersal and determine the mechanisms by which propagules overcome barriers to dispersal and establishment (Chapter 2 & 4)
3. Elucidate the capacity of invasive alien plant species to tolerate and persist in stressful conditions (Chapter 4 & 5)
4. Investigate how molecular tools can be used to advance knowledge of the occurrence and distribution of cryptic aquatic invaders in Ireland (Chapter 6).

Cognisant that “there is neither a definitive list of characters that define a good invasion strategy nor a general predictor of community invasability” (Facon *et al.*, 2006) the concluding chapter provides a synthesis of the research findings and describes their implications for the early detection, eradication and management of aquatic invasive alien plant species in Ireland.

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Chapter 2

Abiotic Traits & Amenity Value Associated with the Invasion of Freshwaters

Introduction

Globally, the introduction and spread of alien invasive species are thought to be one of the leading causes of biodiversity decline (IUCN, 2000; Gallardo *et al.*, 2016) with known impacts on ecosystem services, socioeconomic activity and human wellbeing (Vilà *et al.*, 2010; Pimentel *et al.*, 2001; Pejchar & Mooney, 2009). The ecological impacts of invasive alien species are not restricted to direct biological interactions with the native community but may also occur indirectly by alteration of an ecosystem's abiotic conditions (Gallardo *et al.*, 2016). Freshwater ecosystems are of particular risk of invasion by non-native species (Moyle & Light, 1996; Gherardi, 2007) and it is estimated that 24% of the world's most invasive plants are wetland plants (Zedler & Kercher 2004). Excessive growth of freshwater invasive plant species have been demonstrated to negatively affect native communities of aquatic flora and fauna, alter the physicochemical composition and hydrological function of waterbodies and impact upon navigation, fisheries, recreational activity and water extraction (Gallardo *et al.*, 2015; Hussner, 2012). Indeed, in Ireland, since 1980 the greatest rate of increase in alien introductions has been to the freshwater environment (Kelly *et al.*, 2013; O Flynn *et al.*, 2014).

In Europe, at least 96 alien aquatic plant (macrophyte) species have become established in freshwater ecosystems, of which 18 have been identified as being highly invasive (Hussner, 2012). Recent risk assessments by EU member states have identified a number of 'High Risk' alien macrophytes of national concern (Kelly *et al.*, 2013). Invasive macrophytes of priority concern already occurring in Irish waters include *Azolla filiculoides* Lam., *Crassula helmsii* (Kirk) Cockayne, *Elodea canadensis* Michx., *Elodea nuttallii* (Planch.) H. St. John, *Hydrocotyle ranunculoides* L.f., *Lagarosiphon major* (Ridl.) Moss, *Lemna minuta* Kunth, *Ludwigia grandiflora* (Michx.) Greuter & Burdet, *Myriophyllum aquaticum* (Vell.) Verdc. and *Nymphoides peltata* (S.G. Gmel.) Kuntze (Kelly *et al.*, 2013; Maguire *et al.*, 2011; NBDC, 2018). More recently, restrictions have been imposed throughout the European Union on the importation and sale of invasive alien macrophytes and member states are required to take measures for their early detection, rapid eradication and/or management (Regulation (EU) 1143/2014).

Biological invasion processes involve the introduction of alien species to a novel location outside of their native range, the establishment of that alien species and the subsequent growth and spread of a population in its new range (Richardson *et al.*, 2000). The dispersal of an alien species into a new habitat can be intentional or unintentional. The frequency of

propagule introduction and the abundance of propagules per introduction event is termed propagule pressure (Lockwood *et al.*, 2005; Simberloff, 2009). In freshwater ecosystems, as elsewhere, propagule pressure is intrinsically linked to anthropogenic activity and the intensity of exploitation of the waterbody (Copp *et al.*, 2007; Tamayo & Olden, 2014). Key vectors of alien plant species into the freshwater environment include recreational and commercial fisheries, waterway navigation and escapes/discards from ornamental horticulture/landscaping activity (MacIsaac *et al.*, 2004; Cohen *et al.*, 2007; Thomaz *et al.*, 2015a; Thomaz *et al.*, 2015b).

When newly introduced to a new environment, the invasiveness of a plant species is partially dependent on the species' intrinsic biological traits, its competitive ability, its response to local environmental conditions, and the availability of resources (Richardson & Pyšek, 2006; Catford *et al.*, 2009; Colautti *et al.*, 2014). The success of a newly introduced alien species is also partially dependent on the invasibility of the ecosystem to which it has been introduced. A number of factors have been identified to account for the invasibility of habitats, including: community structure (discussed in more detail in Chapter 3), disturbance and resource availability (Alpert *et al.*, 2000; Davis *et al.*, 2000). Freshwater ecosystems are often subject to natural and anthropogenically induced disturbances and fluctuations in resource availability. This is particularly true in anthropogenic landscapes, both rural and urban. Eutrophication of aquatic systems as a result of both diffuse and point source pollution is considered to be one of the greatest threats to freshwater habitats (Ansari *et al.*, 2010). Such disturbance events (e.g. nutrient enrichment) can provide additional resources to an environment and provide opportunities for alien species to invade vacant ecological niches. Depending on the extent of the disturbance, availability of resources and number of open niches, certain habitats or regions may be at risk of multiple invasions by different species (Ibanez *et al.*, 2009).

Describing the distribution, habitat preferences and responses of aquatic invasive species to environmental variables and human activity is vital for the monitoring and management of invasive freshwater macrophytes (June-Wells *et al.*, 2013). Determining habitat preferences and/or suitability can be used to define the known distribution of invasive macrophytes, predict the likelihood of occurrence elsewhere or to identify habitats which are vulnerable to invasion (Buchan & Padilla, 2000; Thum & Lennon, 2010; Hoffmann & Raeder, 2016; Liu *et al.*, 2018). Quantifying the performance (e.g. abundance, percentage cover or biomass) of invasive species where they occur provides valuable information on

the optimal and sub-optimal conditions within the tolerated range of an environmental variable (Kostrakiewicz-Gierałt & Zajac, 2014). Taking this approach allows practitioners to not only predict where an invasive species will occur and persist but also where it will perform best, potentially growing into an ecological threat.

Kelly (2012) and Kelly *et al.*, (2014) previously used detailed environmental niche models to predict suitable range for eight freshwater invasive plant species in Ireland species (*A. filiculoides*, *C. helmsii*, *E. canadensis*, *E. nuttallii*, *L. major*, *L. minuta*, *M. aquaticum* and *N. peltata*). These models were based on known distribution of the species throughout Ireland and environmental data harvested from databases held by public agencies. Model projections of suitable ranges under current conditions were greater than the currently recorded distribution for all study species. Kelly (2012) concluded that these species had the capacity to colonise much larger ranges than those currently recorded and that the distribution of those species in Ireland are probably under recorded. However, these distribution models were not ground-truthed and did not attempt to describe the performance of invasive species growing under varying abiotic conditions within their tolerated environmental range in Ireland.

This study investigated the distribution and environmental envelope of invasive macrophytes in an agricultural/urban landscape within a 50km radius of Cork City, SW Ireland. The aims were to elucidate the relationships between anthropogenic activity, environmental traits and the distribution, richness and abundance of invasive macrophytes. We also wished to determine the extent to which currently uninvaded freshwater habitats were vulnerable to invasion by alien macrophytes. The aims of this study were achieved by testing the following hypotheses:

H₁. The frequency and distribution of freshwater invasive plant species in Cork is greater than currently recorded.

H₂. The environmental characteristics of waterbodies invaded by alien macrophytes do not differ from uninvaded waterbodies.

H₃. The ecological characteristics and human use of the waterbody are related to the occurrence, distribution, richness and abundance of freshwater invasive species.

H₄. The most frequently occurring invasive alien macrophytes have different environmental envelopes.

Methods

Site Selection

The risk assessments for all ten species of priority invasive macrophyte in Ireland were consulted to determine in which habitat types the species were likely to occur. Slow-moving and standing waters were identified as the likely habitats for all ten species. The study was thus restricted to such slow-moving and standing waterbodies as lakes, ponds, reservoirs, canals, and backwaters. Flowing watercourses such as rivers, streams and drainage ditches were excluded from the study. Fossit (2000) was consulted to assist determination of suitable habitats where necessary. It should be noted that Fossit (2000), which bases habitat classification on vegetation, does not make a distinction between lakes and ponds. Standing backwaters were included in this study as they are widely acknowledged to support macrophyte communities similar to those of other standing water habitats such as lakes or ponds (Willby & Eaton, 1996; Sayer, 2014). Artificial lakes and ponds with semi-natural vegetation were also included as they have been shown to represent “reference condition macrophyte assemblages” and are important habitats for aquatic plant diversity (Sayer *et al.*, 2008; Bubíková & Hrivnák, 2018). Gioria *et al.*, (2010) previously showed that temporary ponds in the Irish landscape do not support the type of aquatic plant communities of interest to this study. Thus, only permanent waterbodies associated with seminatural-natural vegetation assemblages (Fossit, 2000) were included for detailed survey. Brackish or saline ponds and small private garden ponds were also excluded at this stage in the study.

Potential standing waterbodies within a 50km radius of University College Cork were identified using the “OSI Rivers & Lakes” and “New Lake Waterbodies” datasets downloadable from the Environmental Protection Agency website and supplemented with smaller waterbodies identified from freely available on-line satellite imagery. Practicality, accessibility and field safety were also taken into account when selecting the waterbodies for this study. Permission to access and survey a waterbody was obtained from the landowner, where appropriate. If permission could not be obtained the site was excluded from the study. For safety reasons, waterbodies located in active quarries were excluded from the study. The impounded reservoir of the River Lee was excluded from this study due to its size and logistical sampling problems, although a number of distinct backwaters and ponds associated with the River Lee and its floodplain were included.

Scoping of selected waterbodies occurred in April 2014. No prior knowledge of the macrophyte communities at each site was assumed prior to visitation. In total 82 standing waterbodies were included in the study, the distribution of which is shown in Figure 1. Field surveys to collect environmental and botanical data took place in July & August 2014.

Environmental & Geographical Characteristics

Four sampling locations were established at each waterbody, at the cardinal points (N,S,E and W) around the perimeter of the pond. Deviation from the cardinal points was necessary in areas which were not safely accessible or where conditions were not suitable for plant communities (e.g. exposed concrete substrates, reservoir heads, livestock access points etc.). At each sampling point, the pH and conductivity was measured *in situ* using a portable multi-meter (Wissenschaftlich Technische Werkstätten Meter). Based on these measurements the average pH and conductivity were calculated per waterbody.

At each waterbody, one 500ml water sample was collected in previously unused polyethylene sampling bottle. Bottles were pre-rinsed using water from the waterbody in question. Water samples were collected from 10cm below the surface by inverting the bottle while placing it beneath the surface before allowing it to fill. Water samples were kept on ice in a cooler-box until return to the lab and frozen at -40°C on the same day (APHA, 2005). Analysis for total nitrogen (mg/l) and total phosphorus (mg/l) was conducted at the Aquatic Services Unit laboratory at University College Cork.

The waterbody was assessed for evidence of fishing and boating activity, including active participation, boat houses, jetties, equipment, discarded fishing tackle and/or signage etc. Evidence of gardening activity, landscaping or ornamental horticulture activity was also recorded. These included landscaped surfaces, lawns, ornamental planting etc. in, or immediately adjacent, to the waterbody and its riparian zone. Land owners and passers-by were consulted when available. An amenity value of 0 to 3 was assigned to each waterbody, based on the cumulative presence of these activities.

Latitude and longitude of the approximate centre of each water body was recorded using the interactive mapping tools provided by mymaps.google.com. The surface area of the waterbody was calculated by tracing a polygon around the perimeter of the waterbody, using the satellite imagery and interactive mapping tools available at mymaps.google.com. The perimeter of each waterbody was traced three times and the average value recorded.

Macrophyte Communities

A detailed description of macrophytes survey techniques is given in Chapter 3. Two survey methods were employed to record the occurrence and abundance of non-native macrophytes species at each waterbody. A comprehensive species list was compiled for macrophytes communities at each site by searching the perimeter of the waterbody and scanning the water's surface for visible plants. Detailed searches of the nearshore communities were conducted by wading up to a depth of 1.1m along a 2m stretch of the shoreline either side of the established cardinal sampling points. All species observed were recorded as present. The biomass of macrophytes at each cardinal sampling point was quantified using a rake sampling system modified from Free *et al.*, (2006) for nearshore use. Data from all four sampling points were then averaged to calculate the biomass of each species per sampling unit. Vascular plant identification follows Parnell *et al.*, (2012) and Haslam *et al.*, (1976).

To investigate whether invasive alien plant species distributions were under recorded in Co. Cork existing databases were consulted. The interactive species mapping tools provided by the National Biodiversity Data Centre (NBDC, 2018) was used to check all 82 waterbodies surveyed in this study for previous records of aquatic invasive species. Data from Paolacci (2016) who investigated the distribution of *L. minuta* and *A. filiculoides* in Cork was also consulted for prior records of both species. Records and locations of invasive species which had been recorded in study sites prior to the commencement of this study were noted for comparison to the records made by this study.

Biosecurity

In order to limit any accidental spread of invasive species and/or pathogens a number of biosecurity precautions were taken when moving between waterbodies. Protocol followed Inland Fisheries Ireland biosecurity protocol for field survey work (Inland Fisheries Ireland, 2010). Prior to leaving a field site, all equipment including waders, grapnel and rope were checked for mud and visible biological material, which was removed if present. The grapnel, rope and external portion of the waders were then washed thoroughly and soaked in a 10% bleach solution for 15 minutes when moving between water bodies. Upon returning from the field all equipment was hung in a heated glasshouse and allowed to dry thoroughly before returning to the field. Any plant material that was returned to the lab for identification purposes was destroyed by autoclave and disposed of as lab waste.

Data Analysis

Waterbodies were classified as 'Invaded' or 'Uninvaded' based on the presence or absence of an invasive species in the aquatic plant community. Waterbodies that did not possess an macrophyte community were classified as 'Vacant'. Vacant waterbodies were excluded from all further analysis. The pre-existing records for invasive alien species in the surveyed waterbodies were compared to those collected in this study. Mann-Whitney U tests were run to determine if invaded and uninvaded waterbodies differed in amenity value, area and physicochemical parameters.

Principal component analysis (PCA) was used as a means of data reduction to account for the variance in geographic location, surface area, amenity value, pH, conductivity, TN, TP, native species richness and native species biomass in surveyed waterbodies that contained an macrophyte community. The suitability of PCA was assessed prior to analysis. Inspection of the correlation matrix showed that all variables had at least one correlation coefficient greater than ± 0.3 but were not multicollinear as they did not exceed a correlation coefficient greater than ± 0.7 . Bartlett's test for sphericity was statistically significant ($p < 0.001$) indicating that the data was likely factorizable. The invasion status of the waterbody was overlaid on the plot in order to interpret the plot.

To test the hypothesis that there was a relationship between the location, area, amenity value physicochemical variables, the native plant community and the likelihood that a waterbody contained an invasive species, a forward selection binomial logistic regression was employed. Here variable entry testing was based on the significance of the score statistic and removal testing based on the probability of a likelihood-ratio statistic based on conditional parameter estimates. Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell (1962) procedure. A Wald test was used to determine statistical significance for each of the independent predictor variables in the regression.

A Spearman's rank-order correlation was run to assess the relationship between invasive species richness and the area, amenity value, physicochemical and biological variables in this study. A Spearman's rank-order correlation was also run to assess the relationship between invasive species biomass and the area, amenity value, physicochemical and biological variables in this study. Preliminary analysis showed the relationships in question to be monotonic, as assessed by visual inspection of a scatterplot. The strength of the relationship was described as per Cohen (1988).

In order to test the relationship between *Elodea* spp. occurrence and biomass genus specific and species specific investigations were also conducted where *E. canadensis* and *E. nuttallii* were treated cumulatively and independently. PCA, forward selection binomial logistic regression and Spearman's rank order correlations were run as previously described to test the relationships between the amenity value, geographic, physicochemical and biological variables and the presence and/or biomass of *Elodea* spp. In order to determine whether *E. canadensis* and *E. nuttallii* occupy different environmental envelopes in Co. Cork the physicochemical traits of waterbodies invaded by *E. canadensis* were compared to those occupied by *E. nuttallii* using Mann-Whitney U tests where abiotic data could not be normalised and independent sample t-tests where data could be normalised using LN transformation.

Results

Frequency of Invasive Alien Species

Of the 82 waterbodies surveyed for this study, 23 (27%) had previously had at least one invasive macrophyte recorded in them (Fig. 2.A). 57 (67%) of waterbodies had not previously had invasive macrophytes recorded in them. In five waterbodies invasive species had previously been recorded adjacent to the waterbody in question but the exact location of occurrence was ambiguous; such records were classed as indefinite (Fig. 2.A). *E. canadensis*, *E. nuttallii* and *A. filiculoides* were the most frequently recorded invasive macrophyte by the NBDC in the study region (Fig. 3), occurring 13, 9 and 5 times respectively in the existing datasets.

In this study, seven different priority invasive macrophytes were found to occur throughout the surveyed waterbodies. These were *A. filiculoides*, *E. canadensis*, *E. nuttallii*, *L. major*, *L. minuta*, *M. aquaticum* and *N. peltata*. Waterbodies were classified as 'Invaded' or 'Uninvaded' based on the presence or absence of an invasive species in the aquatic plant community. One or more aquatic invasive plant species were recorded as present in 44 (54%) of the surveyed waterbodies. Native species only were observed in 29 (35%) of surveyed waterbodies. Nine (11%) of the surveyed waterbodies did not have a macrophyte community and were classified as 'Vacant' (Fig. 1 & Fig. 2). The distribution of invaded, uninvaded and vacant waterbodies is displayed in Fig. 1.

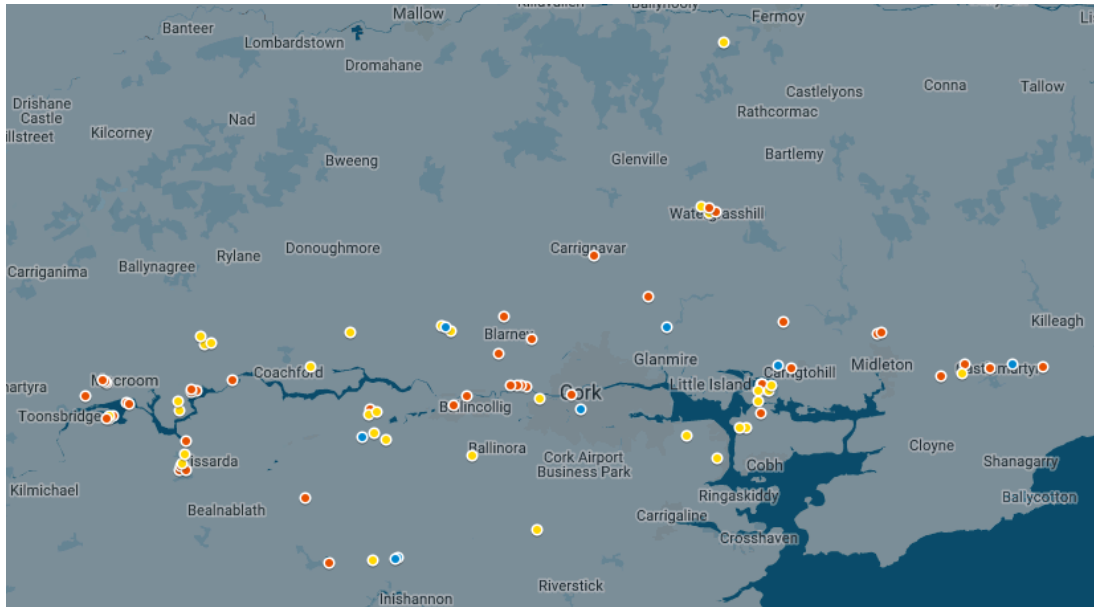


Figure 1: The distribution and invasion status of 82 surveyed waterbodies in Co. Cork.
(Red=Invaded, Yellow=Uninvaded, Blue=Vacant).

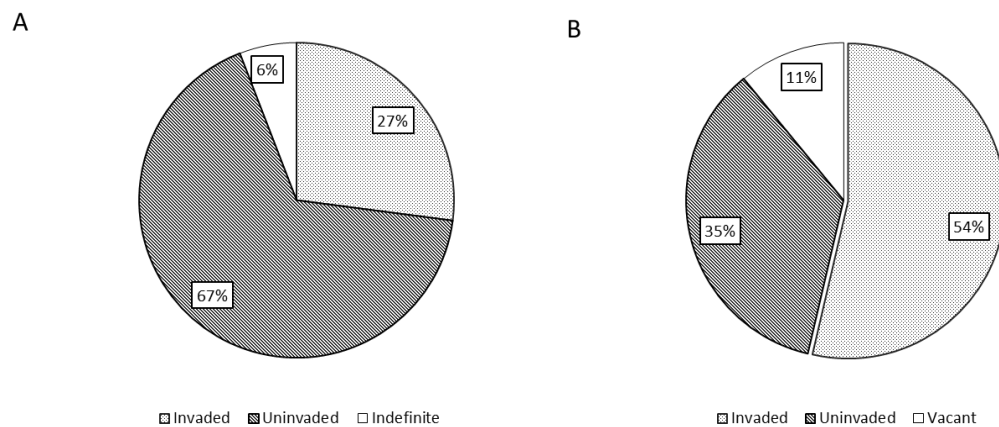


Figure 2: A. The percentage of 82 surveyed waterbodies classified as 'Invaded', 'Uninvaded' and 'Indefinite' based on records of alien aquatic plant species lodged with the National Biodiversity Data Centre prior to the commencement of this study; B. The percentage of 82 surveyed waterbodies classified as 'Invaded', 'Uninvaded' and 'Vacant' on records of alien aquatic plant species in this study.

The number of different invasive species observed in a single invaded waterbody ranged from 1-4. More than one invasive species was observed in 15 out of 44 invaded waterbodies. Ballyhonock Lough, the most easterly waterbody surveyed, was the only waterbody where four invasive species were recorded and thus possessed the richest alien aquatic flora. The frequency of occurrence for each invasive species is depicted in Fig. 3. The most frequently occurring aquatic invasive plant species was *E. canadensis* which was observed to occur in 23 waterbodies. The second and third most frequently occurring invasive species were *E. nuttallii* which was recorded in 15 waterbodies and *L. minuta* which was recorded in 12 waterbodies. *N. peltata*, *A. filiculoides*, *M. aquaticum* and *L. major* occurred in 6, 5, 1 and 1 waterbodies respectively.

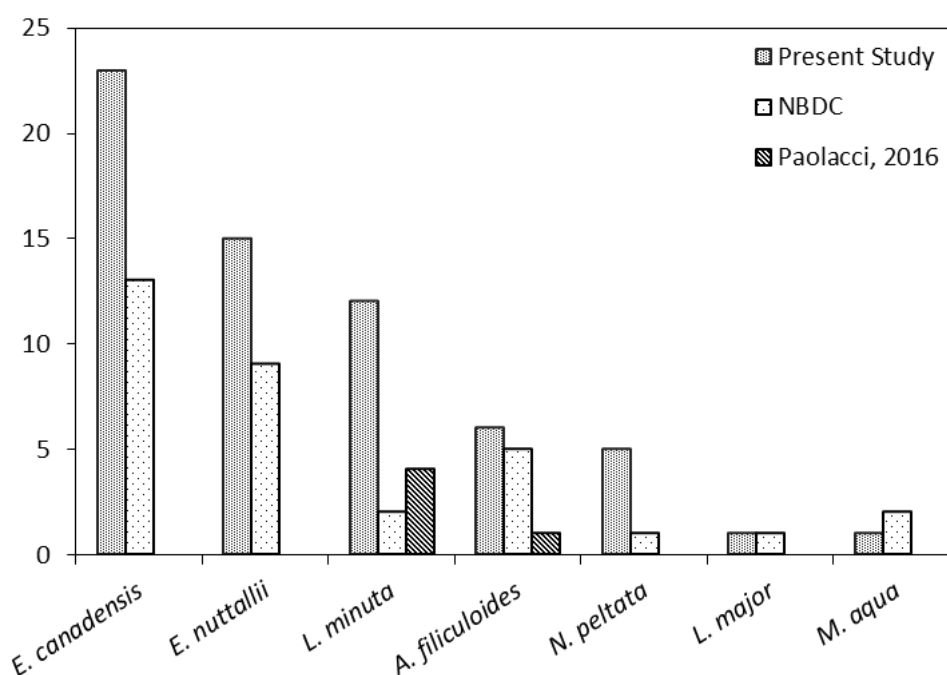


Figure 3: The frequency of occurrence of seven aquatic invasive species at 82 surveyed waterbodies, comparing the present study to data obtained from the National Biodiversity Data Centre and Paolacci (2016).

Environmental Characteristics of Invaded and Uninvaded Habitats

The surface area, pH, conductivity, total phosphorus and total nitrogen of each waterbody was measured. Amenity value was assigned to the waterbody based on the cumulative presence or absence of fishing, boating and/or horticultural landscaping activity. The range and median of amenity value and each environmental variable in invaded and uninvaded waterbodies are summarised in Table 1 and visualised in Fig. 4.

Mann-Whitney U tests were run to determine if invaded and uninvaded waterbodies differed in the ranges of abiotic variables. There were no statistically significant differences in the median surface area of invaded (0.29ha) and uninvaded (0.28ha) waterbodies. The median pH of invaded (7.75) and uninvaded (7.94) waterbodies did not differ significantly. The median TP concentrations of invaded (0.06mg/l) and uninvaded (0.04mg/l) waterbodies were not statistically significantly different either. Median TN concentrations were statistically significantly higher in invaded ponds (2.26mg/l) than in uninvaded ponds (1.13mg/l), $U=801$, $z=2.038$, $p=0.042$. TN concentrations in uninvaded and invaded waterbodies ranged from 0.53-6.45mg/l and 0.55-7.58 respectively. Waterbody conductivity in uninvaded and invaded ponds ranged from 61.9-681.67 μ S/cm and 96.43-1300 μ S/cm respectively. The median conductivity of the water was statistically significantly higher in invaded (238.17 μ S/cm) than in uninvaded waterbodies (198 μ S/cm), $U=828$, $z=2.562$, $p=0.01$.

Table 1: The median value, minimum value and maximum value of abiotic variables measured in invaded and uninvaded waterbodies in Co. Cork.

Invasion Status		Amenity Value	Area (ha)	pH	Conductivity (μ S/cm)	TP (mg/l)	TN (mg/l)
Uninvaded	Median	1	0.28	7.94	198.00	0.06	1.31
	Minimum	0	0.03	7.11	61.90	0.01	0.53
	Maximum	3	2.52	9.70	681.67	0.78	6.45
Invaded	Median	1	0.30	7.76	238.17	0.04	2.26
	Minimum	0	0.02	6.80	96.43	0.01	0.55
	Maximum	3	12.85	9.96	1300.00	0.50	7.58

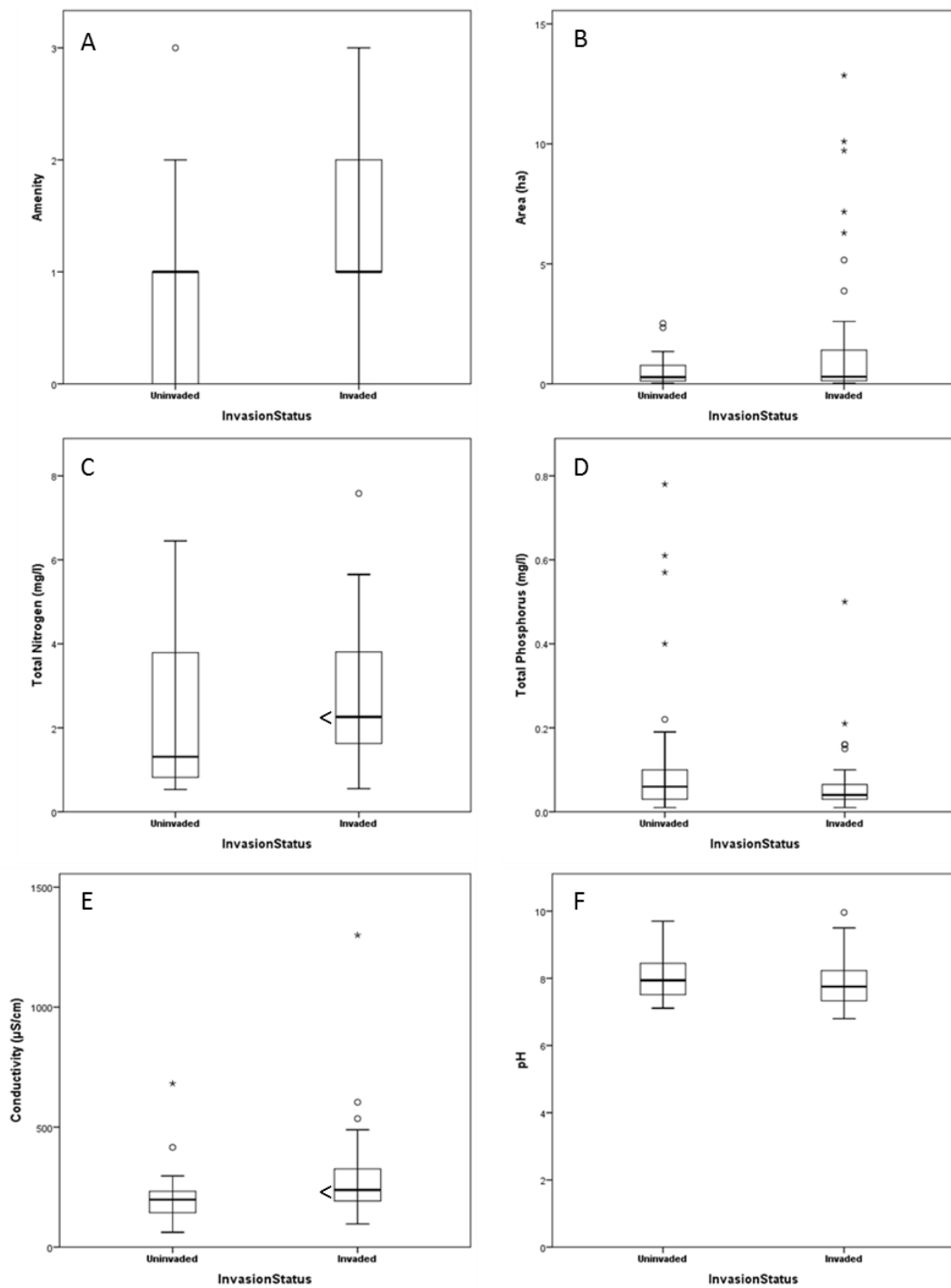


Figure 4: The range in amenity value and environmental variables measured in invaded and uninvaded waterbodies in Co. Cork. A. Amenity value (Presence of fishing + boating + horticultural landscaping), B. Surface area (ha), C. Total nitrogen concentration (mg/l), D. Total phosphorus concentration (mg/l), E. Conductivity ($\mu\text{S}/\text{cm}$) and F. pH. Where ° is an insignificant outlier in the data, * is an extreme outlier and < indicates a median significantly greater than its counterpart.

Factors Controlling the Occurrence, Richness & Abundance of Alien Species

Geographical variables (surface area, latitude and longitude), environmental variables (TN, TP, conductivity, pH), biological variables (Native species richness and biomass) and amenity use of a waterbody may influence the likelihood of occurrence of an invasive species in a waterbody. A principal component analysis (PCA) was run to reduce the geographical, environmental, biological and amenity variables of all surveyed waterbodies with a macrophyte community to their principal components. PCA revealed five components that had eigen values greater than one which respectively explained 23.9%, 16%, 13.9%, 11.4% and 10.7% of the total variance in the data. The first two components (visualised in Fig. 5) cumulatively explained 39.9% of total variance. Principal component 1 (PC1) was comprised primarily of native species richness, surface area and amenity value. Principal component 2 (PC2) was comprised primarily of pH followed by longitude and latitude. The distribution of waterbodies in the PCA plot (Fig. 5) show uninvaded waterbodies distributed primarily at the lower end of PC1 while the upper end of PC1 is dominated by invaded waterbodies.

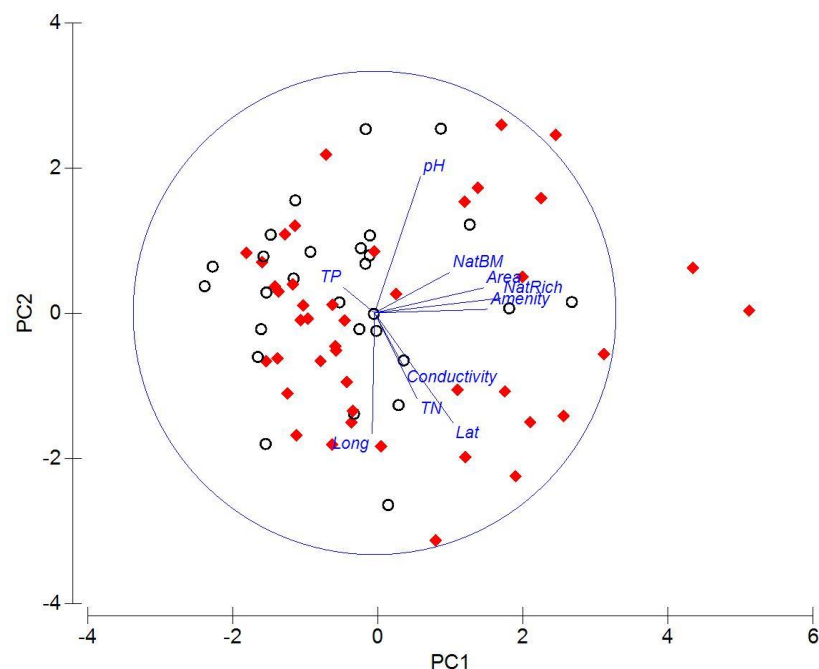


Figure 5: Principal Component Analysis of the first two principal components which cumulatively explain 39.9% of variance in surveyed waterbodies. Where red diamonds represent invaded waterbodies and circles represent uninvaded waterbodies.

A forward selection binomial logistic regression was performed to ascertain the effects of abiotic variables (location, surface area, pH, conductivity, TN and TP), biological variables (native species richness and native species biomass) and waterbody amenity value (scored by the cumulative presence of fishing, boating and/or horticultural/landscaping activity) on the likelihood of waterbodies containing an invasive alien species. Step one of the forward selection method determined that of the eight potential predictor variables just one was an appropriate fit, namely amenity value. The logistic regression model was statistically significant, $\chi^2(1)=9.246$, $p=0.002$. The model explained 17% (Nagelkerke R^2) of the variance and correctly classified the invasion status of 67.1% of ponds. Increasing amenity value was statistically significantly ($p=0.005$) associated with an increased likelihood of waterbodies being colonized by an invasive species. Waterbodies had a 2.3 times higher odds to possess an aquatic invasive alien plant species with every additional amenity activity undertaken at the waterbody.

Invasive species richness data did not meet the assumptions of linear regression or Poisson distribution regression analysis. Nonparametric Spearman's rank-order correlations were thus run to assess the relationship between invasive species richness and waterbody size, physicochemical variables and amenity value. Invasive species richness was positively correlated with waterbody amenity value ($r_s(73)=0.295$, $p=0.011$) and conductivity ($r_s(71)=0.321$, $p=0.006$) (Fig. 6). Spearman's rank-order correlations were also run to determine if the number of invasive species in invaded habitats were associated with native species richness, functional richness, native diversity or native biomass. No biological variables were found to be correlated with invasive species richness.

The chemical composition of a waterbody is known to control the productivity of the plant species that live within. Spearman's rank-order correlations were run to determine whether cumulative invasive species biomass was associated with the chemical composition of invaded waterbodies in this study. No significant correlations were identified between TN, TP, conductivity or pH and cumulative invasive species biomass.

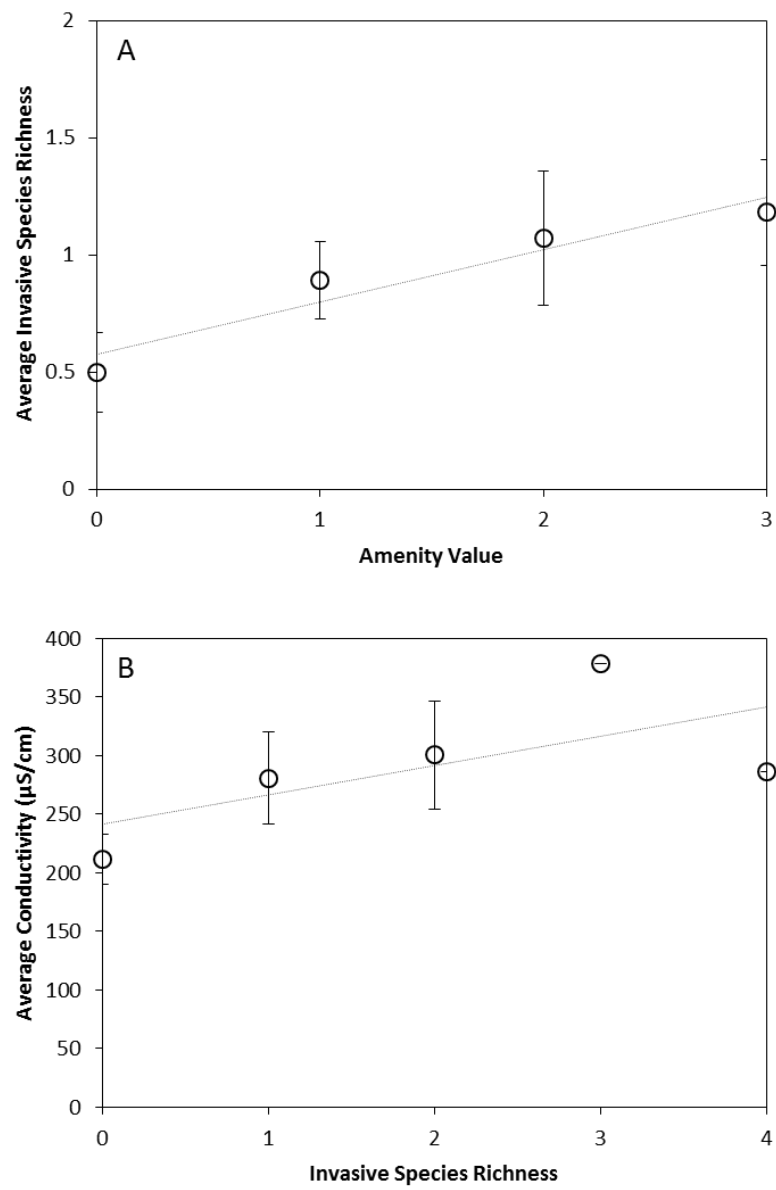


Figure 6: The significant correlation between invasive species richness and A. waterbody amenity value and B. waterbody conductivity.

Species Specific Relationships

The species specific median, minimum and maximum values recorded for each invasive species throughout the study are presented in table 2, however only the two most frequently occurring species provided enough data for meaningful analysis of relationships between the abiotic environment and their occurrence and abundance. The two most frequently occurring invasive species were *E. canadensis* and *E. nuttallii* which occurred in 23 and 15 waterbodies respectively, including six waterbodies where they cooccurred.

Table 2: The species specific median and range of abiotic variables recorded in waterbodies where aquatic invasive species occurred.

Species		Amenity	Area (ha)	pH	Conductivity (µS/cm)	TP (mg/l)	TN (mg/l)
<i>A. filiculoides</i>	Median	1	1.06	7.85	320.00	0.04	2.17
	Minimum	1	0.03	7.30	230.33	0.02	.77
	Maximum	3	2.52	8.96	378.67	0.08	4.79
<i>E. canadensis</i>	Median	2	0.69	7.95	238.17	0.04	2.32
	Minimum	0	0.05	6.80	167.67	0.01	1.19
	Maximum	3	12.85	9.96	489.00	0.09	5.65
<i>E. nuttallii</i>	Median	1	0.18	7.61	197.17	0.03	2.19
	Minimum	0	0.04	6.80	125.67	0.01	.55
	Maximum	3	2.60	8.37	603.67	0.50	5.20
<i>L. major</i>	Median	2	0.8	8.18	360.67	0.02	5.60
	Minimum	2	0.8	8.18	360.67	0.02	5.60
	Maximum	2	0.8	8.18	360.67	0.02	5.60
<i>L. minuta</i>	Median	1	0.33	7.33	267.67	0.09	2.16
	Minimum	0	0.02	6.85	96.43	0.01	.93
	Maximum	3	10.1	8.96	603.67	0.50	7.58
<i>M. aquaticum</i>	Median	1	0.21	8.25	535.25	0.05	1.28
	Minimum	1	0.21	8.25	535.25	0.05	1.28
	Maximum	1	0.21	8.25	535.25	0.05	1.28
<i>N. peltata</i>	Median	1	0.34	8.32	293.33	0.06	1.55
	Minimum	1	0.1	7.68	203.83	0.04	1.23
	Maximum	3	10.1	8.96	535.25	0.16	2.11

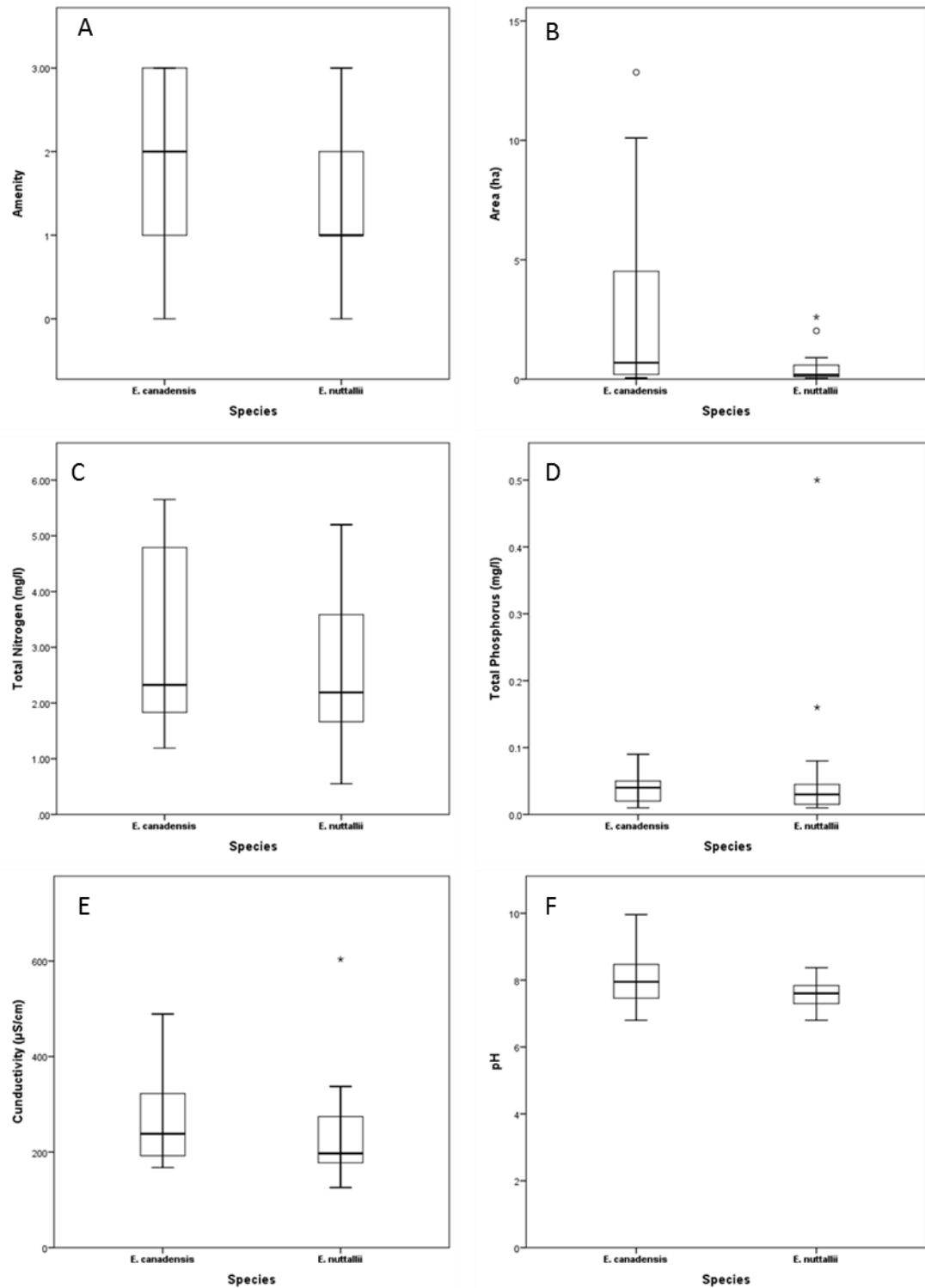


Figure 7: The range in amenity value and abiotic variables in waterbodies invaded by *E. canadensis* and *E. nuttallii*. A. Amenity value (Presence of fishing + boating + horticultural landscaping), B. Surface area (ha), C. Total nitrogen concentration (mg/l), D. Total phosphorus concentration (mg/l), E. Conductivity (µS/cm) and F. pH. Where ° is an insignificant outlier in the data, * is an extreme outlier.

The medians and ranges of amenity and abiotic variables in waterbodies occupied by *Elodea* spp. are visualised in Fig. 7. The average area, pH, conductivity and TP (conductivity and TP were LN transformed) of waterbodies occupied by *E. canadensis* and *E. nuttallii* were compared using independent sample t-tests. TN values were not normally distributed, thus a Mann Whitney U test was employed to compare TN concentrations between waterbodies occupied by *E. canadensis* and *E. nuttallii*. On average, *E. canadensis* ($2.79\text{ha} \pm 3.85$) occupied waterbodies with significantly larger surface areas than *E. nuttallii* ($0.53\text{ha} \pm 0.76$), $t(2.727)=24.629$, $p=0.012$ (Fig. 7. B). The average pH of ponds occupied by *E. canadensis* (8.08 ± 0.82) was significantly higher than that of ponds occupied by *E. nuttallii* (7.55 ± 0.46), $t(2.195)=34$, $p=0.035$ (Fig. 7. F). There was no significant difference in amenity value, conductivity, TP concentrations or TN concentrations of waterbodies occupied by either species.

Waterbodies that contained *E. canadensis* and *E. nuttallii* were plotted against the previously established principal components (Fig. 8). Waterbodies that contained *E. canadensis* were primarily distributed toward the upper end of PC1, while waterbodies that contained *E. nuttallii* were distributed vertically along PC2. Forward selection binomial logistic regressions were performed to determine whether location, amenity value, abiotic variables and/or biological variables could predict the likelihood of an *Elodea* species occurring in a waterbody. The binomial logistic regressions largely agreed with the distribution of *Elodea* spp. in the principal component analysis.

In the case of *E. canadensis* the best fit model with only one variable, area, was determined on step 1 of the forward selection method. There were two outliers with studentized residuals of 3.09 and 5.53 which were kept in the analysis. The logistic regression model was statistically significant, $\chi^2(1)=12.505$, $p<0.005$. The model explained 23% (Nagelkerke R^2) of the variance and correctly classified the occurrence of *E. canadensis* in 75.7% of ponds. Increasing surface area was significantly ($p=0.016$) associated with an increased likelihood of waterbodies containing *E. canadensis*. For every hectare increase in surface area waterbodies had 1.8 times higher odds that *E. canadensis* would occur within that waterbody.

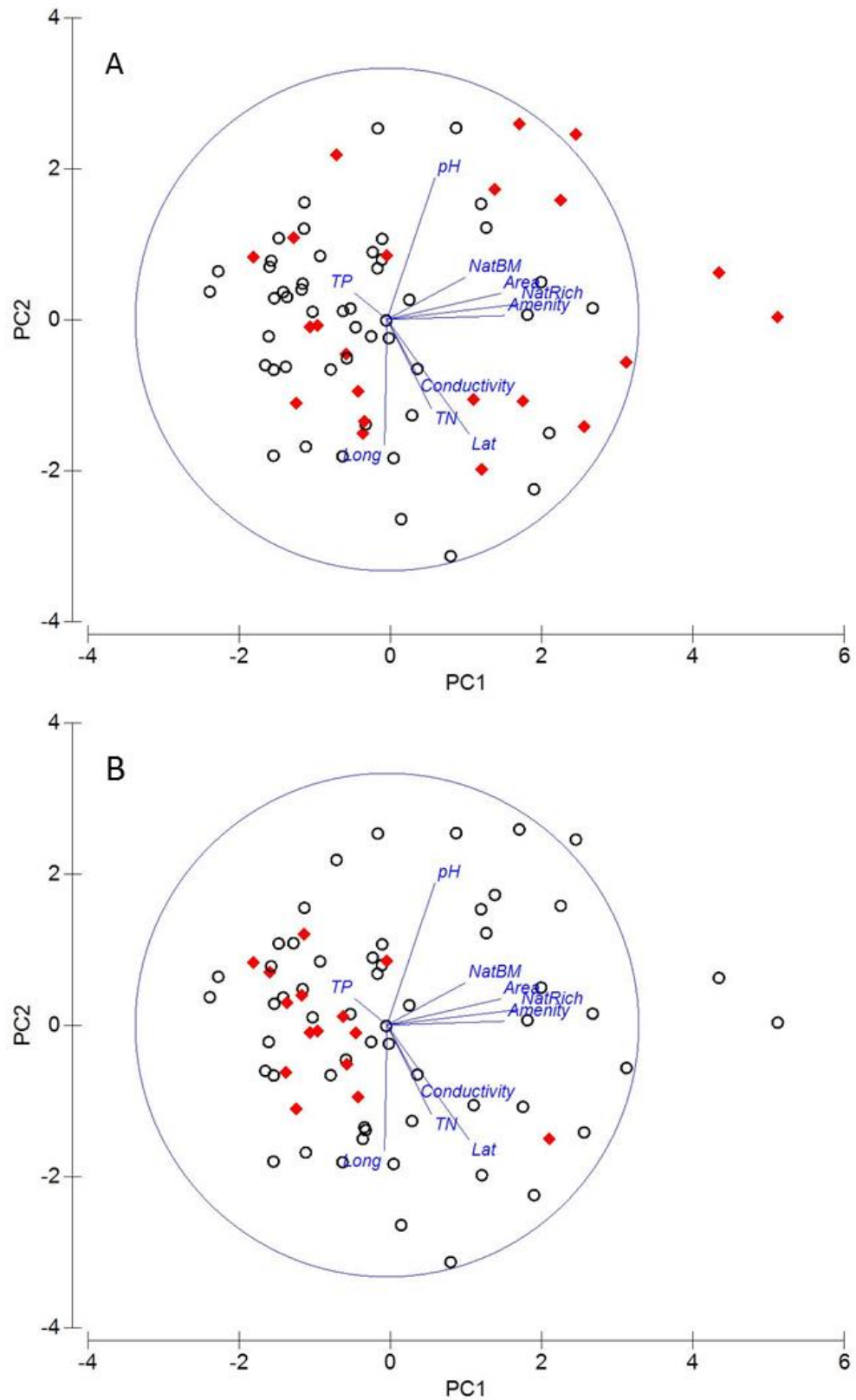


Figure 8: Principal Component Analysis of the first two principal components which cumulative explain 39.9% of variance in surveyed waterbodies. Where red diamonds represent waterbodies invaded by *E. canadensis* (A) and *E. nuttallii* (B) and circles represent uninvaded waterbodies.

In the case of *E. nuttallii* the most appropriate model, which included pH and latitude, was identified at step 2 of the forward selection process. The logistic regression model was statistically significant, $\chi^2(2)=19.023$, $p<0.001$. The model explained 37.6% (Nagelkerke R^2) of the variance and correctly classified the occurrence of *E. nuttallii* in 85.7% of ponds. Waterbodies with lower pH ($p=0.025$) and lower latitudes ($p=0.003$) were significantly more likely to contain *E. nuttallii*.

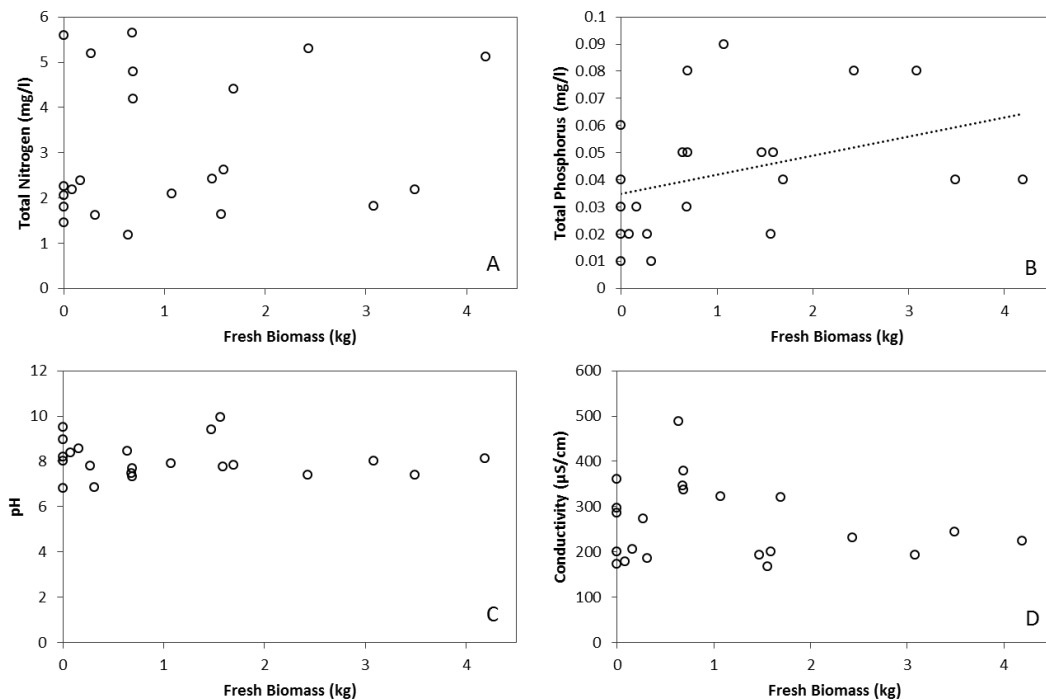


Figure 9: The relationship between the biomass of *E. canadensis* and A. Total nitrogen, B., Total phosphorus, C., pH, D. Conductivity of waterbodies where it occurs. The presence of a trend line indicates a statistically significant correlation.

The chemical composition of a waterbody may control the productivity of plant species growing within. Spearman's rank order correlations were used to determine whether the biomass of *Elodea* species was related to any of the measured chemical variables in waterbodies where *Elodea* species had been confirmed. No statistically significant correlations were observed between cumulative *E. canadensis* and *E. nuttallii* biomass and any of the environmental variables. The biomass of *E. canadensis* (Fig. 9) was significantly moderately correlated with TP concentrations ($r_s(22)=0.452$, $p=0.035$) in waterbodies where it was recorded. In waterbodies where *E. nuttallii* (Fig. 10) occurred no significant correlations were observed between *E. nuttallii* biomass and any of the environmental variables. The maximum biomass exhibited by *E. nuttallii* (12.3kg) was almost three times

greater than the maximum biomass exhibited by *E. canadensis* (4.2kg) despite being at a lower TP concentrations (0.028mg/l and 0.043mg/l respectively).

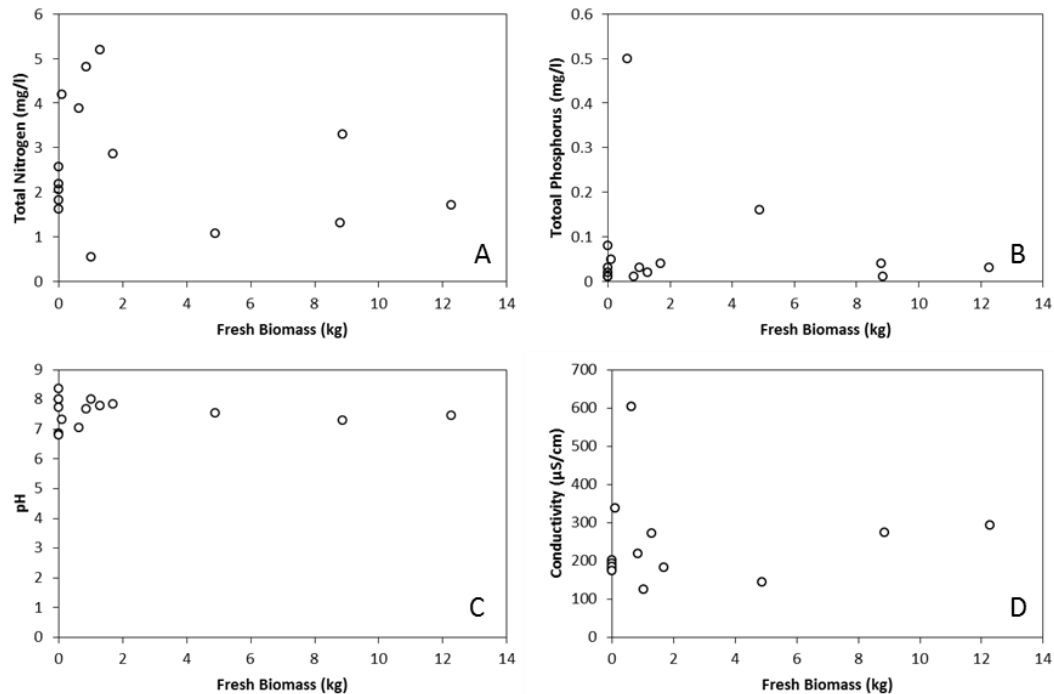


Figure 10: The relationship between the biomass of *E. nuttallii* and A. TN, B., TP, C., pH, D. Conductivity of waterbodies where it occurs. There were no significant correlations.

Discussion

Frequency and Distribution of Freshwater Invasive Alien Species

It is evident that frequency of occurrence and distribution of invasive alien macrophytes in Co. Cork is under recorded. Twice as many waterbodies than previously confirmed were determined to contain at least one invasive alien species in their macrophyte communities. Assuming that Co. Cork is not unusual, it is likely that invasive alien macrophytes are under recorded nationwide. The most frequently recorded invasive macrophytes in the study region prior to this study were *E. canadensis*, *E. nuttallii* and *A. filiculoides* (NBDC, 2018). *E. canadensis* and *E. nuttallii* were the most frequently recorded invasive macrophytes in this study, and an additional ten and six waterbodies respectively were confirmed and added to its known distribution. *L. minuta* replaced *A. filiculoides* as the third most frequently

occurring alien macrophyte. Paolacci (2016) previously showed that *L. minuta* occurred more frequently and persistently than *A. filiculoides* in freshwater habitats surrounding Cork City. Island-wide, at the 10km² resolution, *E. canadensis*, *E. nuttallii*, *A. filiculoides* and *L. minuta* were found to be the most frequently occurring invasive macrophytes in that order (Kelly, 2012). In the past in Ireland, the recorded distribution of invasive alien aquatic plant and animal species has tended to be incomplete until detailed species-specific investigations are undertaken (Maguire *et al.*, 2011; Minchin, 2007a; Minchin, 2007b; Kelly, 2012; Kelly *et al.*, 2014; Caffrey *et al.*, 2008). This study confirmed this under-recording of alien invasive macrophytes species, despite systematic surveys of aquatic plant communities throughout Ireland as part of the monitoring process for the E.U Water Framework Directive.

The least frequently recorded alien macrophyte in this study was *Lagarosiphon major*, recorded in an urban pond in Carrigtohill, Co. Cork; this is a new record for the species. *L. major* was previously recorded in Fitzgerald's Park, Cork City in 1966, but despite extensive searches was not found at this site during the present study. Paolacci (2016) showed that the occurrence of the free-floating macrophytes *A. filiculoides* and *L. minuta* in freshwater habitats in Cork changed from year to year, with species being present one year and absent the next. Indeed, some ponds which did not possess *L. minuta* or *A. filiculoides* in the present study had previously been dominated by those species in the past (Paolacci, 2016). *Myriophyllum aquaticum* was recorded in a single waterbody in the present study, although it previously occurred at a second site, which was deemed vacant in this study. Personal communication with landowners revealed that *M. aquaticum* at this location had been managed for eradication. This study thus highlights the importance of continued monitoring of invaded habitats in order to effectively report on the distribution and changes in range of invasive species in Ireland.

Of the invasive macrophytes which occurred in this study, *E. canadensis* was introduced earliest into Ireland and first recorded in Co. Down in 1836. The length of time that *E. canadensis* has been in the country likely explains the fact that it is also the most frequently occurring alien macrophyte. Residence time post-introduction has been linked with the opportunity for secondary dispersal and successful spread (Pysek *et al.*, 2009; Wilson *et al.*, 2007; Pysek & Jarosik, 2005). Indeed, Milbau & Stout (2008) previously showed that alien plants were more likely to have become naturalised and invasive in Ireland if they had an early first record date and were already in Ireland for a long time.

Variation in the Environmental Envelopes of Invaded and Uninvaded Freshwaters

There was little clear difference in the surface area, amenity value, pH or TP concentrations between invaded and uninvaded waterbodies. TN concentrations and conductivity were however significantly higher in invaded than uninvaded ponds. Conductivity and nitrates are commonly correlated in Ireland (Dodkins *et al.*, 2005) because N ions such as nitrate increase specific conductivity (Toivonen & Huttunen, 1995). Indeed, TN and conductivity were correlated in the PCA in this study. The eutrophication of freshwater environments leads to the exclusion of nutrient-sensitive plant species and a reduction in diversity, as the chemical disturbance occurs at a faster rate than plant adaptations to higher nutrients (Bedford *et al.*, 1999; Byers, 2002; Holdredge *et al.*, 2010). Nutrient enrichment also selects for macrophyte species that are strongly competitive at high nutrient levels (Blumenthal, 2005; Green & Galatowitsch, 2002). Thus, in eutrophic freshwater systems, the disturbance, resource availability and vacant niche hypotheses are all likely to be at play in the context of habitat invasibility (Davis *et al.*, 2000; Alpert *et al.*, 2000; Byers, 2002). Further, it has been suggested that many wetland invasive macrophytes species have high nutrient thresholds and are better adapted to excessive nutrient availability than native macrophyte communities (Green & Galatowitsch, 2001; Green & Galatowitsch, 2002; Zedler & Kercher, 2010; Madsen 1998). In an Irish context, Milbau and Stout (2008) found that plants with an affinity for high nitrogen were among those species most likely to become successful invaders. All invasive macrophytes species recorded in this study have been demonstrated to tolerate, grow well and often favour, nutrient enriched environments (Landolt, 1986; Paolacci *et al.*, 2016; James *et al.*, 2006; Ratray *et al.*, 1994; Reddy, 1987; Xie *et al.*, 2010; Njambuya *et al.*, 2011; Darbyshire & Francis, 2008; Marion & Paillisson, 2003; Hussner *et al.*, 2009; Wersal & Madsen, 2011; Penning *et al.*, 2008).

Factors Related to the Occurrence, Richness and Abundance of Invasive Alien Macrophytes

The first principal component analysis of the characteristics of studied waterbodies showed that amenity value, size and native species richness were correlated. Larger lakes provide more opportunity for recreational activity such as boating and fishing, greater public access, and tend to have more adjacent property owners (Anderson & West, 2006; Reed-Anderson *et al.*, 2000, Bossenbroek *et al.*, 2001). Similarly, larger lakes tend to be more species-rich because they have greater levels of habitat heterogeneity and provide greater

habitat diversity and niche opportunity for a variety of native species (Bronmark, 1985; Heino, 2000; Oertli *et al.*, 2002; Rørslett, 1991; Sondergaard *et al.*, 2005.). The PCA analysis also showed that larger waterbodies with greater amenity value and higher species richness were more frequently invaded, while smaller waterbodies with lower amenity value were more frequently uninvaded. Regression analysis also identified amenity value as the trait most likely to indicate the occurrence of an invasive alien species. With each additional amenity activity taking place at a location, waterbodies were more than twice as likely to contain an invasive alien macrophyte. The amenity activity recorded in this study was a cumulative value based on the occurrence of boating activity, fishing activity and/or horticultural/ornamental landscaping. All three of the included amenities have been identified as sources of either intentional or unintentional introduction of alien species as well as subsequent vectors of secondary dispersal and spread (Johnson *et al.*, 2001; Kilian *et al.*, 2012; Anderson *et al.*, 2015; Reichard & White, 2001; Cohen *et al.*, 2007). High levels of anthropogenic activity, amenity use and tourism (and by default, larger surface area) of waterbodies have thus been linked to the likelihood of occurrence and distribution of invasive alien species of many taxa (Capers *et al.*, 2009, Anderson *et al.*, 2015; Copp *et al.*, 2005; Thum & Lennon, 2009; Buchan & Padilla, 2000, Kelly *et al.*, 2014; Miro & Ventura, 2013). This link is inherently one of propagule pressure combined with disturbance, because with increased human activity comes increased likelihood and frequency of propagule introduction (intentional and/or unintentional) and increased disturbance of the natural environment (Davis & Pelsor, 2001; Leung & Mandrak, 2007; Drake & Mandrak, 2014, Thomaz *et al.*, 2015b).

Invasive alien macrophytes frequently co-occurred with other invasive alien species in this study. It has been suggested that propagule pressure as a result of human activity is a likely driver of invasive species richness in lakes (Capers *et al.*, 2009; Lockwood *et al.*, 2009). Davis and Pelsor, (2001) found that plant invasions most commonly occur when high propagule pressure coincides with periods of high invasibility as a result of disturbance and fluctuating resources. The theory of 'invasional meltdown' proposes that cooccurring non-native species may interact to facilitate further invasion (Simberloff, 2006) however there is insufficient evidence in the present data to suggest invasional meltdown has occurred in waterbodies that are particularly alien species rich.

Species-Specific Relationships with Abiotic Traits of Freshwaters

No relationship was identified between the cumulative biomass of invasive alien macrophytes and the physical/chemical characteristics of the freshwater environment. Due to the variation in size, function and growth form of invasive macrophytes in this study, it is probable that any trends in biomass with respect to environment were likely to be species-specific. *Elodea canadensis* and *E. nuttallii* were the two most frequently occurring invasive alien species in this study. These two members of the family Hydrocharitaceae are morphologically very similar (Herault *et al.*, 2008), exhibit high plasticity and have a sympatric native distribution throughout North America (St. John, 1965; Thiebaut & Di Nino, 2009). *Ex situ* experimental evidence has shown that both species often respond similarly in terms of tolerance, regeneration, growth rate and physiological responses to a wide range of environmental variables (pH, oxygen, dissolved organic carbon, nutrient enrichment) (James *et al.*, 1999; Jones *et al.*, 2000; Barrat-Segretain *et al.*, 2002; James *et al.*, 2006). Herault *et al.*, (2008) concluded that *E. canadensis* and *E. nuttallii* occupied a similar enough ecological niche in France to render them ecologically redundant. In this study, no difference was found in the ranges in conductivity, TP, or TN between waterbodies occupied by *E. canadensis* or *E. nuttallii*. However, waterbodies occupied by *E. nuttallii* had a lower median pH than those occupied by *E. canadensis*. Kelly *et al.*, (2015), however, found no relationship between pH and the occurrence of *E. nuttallii* in rivers and lakes in Northern Ireland.

Several authors have found that *E. nuttallii* has a preference for alkaline habitats (Robach *et al.*, 1996; Thiebaut & Muller, 1999; Zehndorf *et al.*, 2015). *E. nuttallii*, however, is also capable of increasing the pH of its surroundings (Jones *et al.*, 2000; Szabo *et al.*, 2009). The majority of occurrences in the present study were in locations with a pH greater than 7 and the maximum value recorded in this study exceeds upper end of the range previously recorded within stands of *E. nuttallii* in Ireland (Kelly *et al.*, 2015). Why then does the present study highlight lower pH as an important predictor of *E. nuttallii* occurrence? In the present study the relationship between *E. nuttallii* and pH must be interpreted in the context of geographic location as the PCA demonstrated that pH was negatively related to longitude and the regression model determined that pH and location combined best explained the species' occurrence. The majority (14/15) of waterbodies that contained *E. nuttallii* in this study occurred in the western half of the study area and ten of these occur within the floodplain of the River Lee, while four others are in the catchments of tributaries

to the River Lee. The circumneutral pH in surface waters of this region is likely to be linked to landscape characteristics such as sandstone bedrock (Porter, 1902; Lehanne *et al.*, 2004). The apparent relationship between *E. nutalli* and pH may thus simply be due to the geographical location of the species, rather than any true chemical association. Kelly *et al.* (2014) warned that models based on data from the known distribution of an aquatic alien species may underestimate the suitability of currently uninvaded habitats because uncolonized habitats are treated as absences by the model. It should thus not be assumed that *E. nuttallii* only has capacity to occupy waterbodies at the lower end of the pH range in this study. Rather this model should be interpreted as a description of the species current distribution in the study area.

Waterbodies containing *E. canadensis* had a higher median surface area than those containing *E. nutalli*. The relationship between *E. canadensis*, amenity and surface area is most likely explained by the relationship between waterbody size, amenity (horticulture, fishing and boating) and propagule pressure. *E. canadensis* is a popular species amongst aquarium and garden pond enthusiasts (Halford, 2011). Asexual propagules are also easily dispersed unintentionally by fishing and boating activity (Barnes *et al.*, 2013). It has had a longer residence time in Ireland than *E. nuttallii* (Reynolds, 2002) and thus had greater opportunity for repeated introductions and secondary dispersal by anthropogenic activity. In Ireland, Milbau and Stout (2008) have demonstrated that ornamental plants with long residence times are amongst the most likely to have become invasive.

There was no relationship between the biomass of *E. nuttallii* and the physical/chemical characteristics of the waterbodies in this study. The biomass of *E. canadensis* was, however, weakly correlated with TP concentrations. The relationship between the two *Elodea* species and phosphorus concentrations is a subject of ongoing study. Both species are capable of uptake of phosphorus from the sediment through their roots and from the water through their shoots (Zehndorf *et al.*, 2015). They are thus capable of adapting their phosphorus uptake depending on the sources available (Angelstein & Schubert, 2008). Further, *Elodea* spp. can function as 'phosphorus pumps' and can release biologically available phosphorus to the water column through leakage, decomposition and herbivory (Angelstein & Schubert, 2008). The exact relationship between large stands of *Elodea* spp. and the biogeochemical cycling of phosphorus is thus unclear but likely to be important. Free *et al.*, (2006) previously noted that although *E. canadensis* occurred in oligotrophic, mesotrophic and eutrophic lakes in Ireland, it tended to dominate communities at higher

TP concentrations. James *et al.*, (2006) demonstrated that *E. nuttallii* and *E. canadensis* did not differ in their negative growth response to phosphorus enrichment from eutrophic to hypertrophic. Contrastingly, Barrat-Segretain (2004) found that the dry weight relative growth rate (RGR) of *E. nuttallii* increased with phosphorus enrichment to eutrophic levels while the RGR of *E. canadensis* did not change. In the present study, despite not being correlated with TP, the maximum biomass exhibited by *E. nuttallii* was almost three times greater than the maximum biomass exhibited by *E. canadensis* despite being at a substantially lower TP concentration. In the wild, *E. nuttallii* may be better able to utilise available phosphorus resources in the sediment and water column.

Conclusions

This study aimed to describe the current frequency and distribution of invasive alien plant species in the freshwater environments of Co. Cork. It further aimed to describe the environmental envelopes occupied by said alien species and elucidate factors, (anthropogenic and abiotic) which are related to the occurrence, richness and abundance of invasive alien species. Finally, it aimed to determine if these relationships were species specific.

The study found that the occurrence and distribution of invasive alien species in standing waterbodies in Ireland is substantially greater than previously recorded. In fact, waterbodies invaded by at least one invasive alien species outnumbered those with none. Invaded waterbodies were richer in total nitrogen and had higher conductivity but the intensity of human amenity use was the best predictor of the occurrence of invasive alien species. Invasive alien species often co-occurred, with up to four alien species simultaneously occupying the same habitat. This study thus provides evidence to support the hypothesis that propagule pressure, anthropogenic disturbance and resource availability interact to control the occurrence, richness and distribution of invasive alien macrophytes in Irish freshwater environments.

The two most commonly occurring alien macrophytes, *E. canadensis* and *E. nuttallii*, occupied similar habitats and had similar environmental envelopes, which differed only in terms of pH and surface area of the waterbody. However, these differences were likely to be related to geographic location and amenity intensity, rather than a real difference in water chemistry preference. Neither species showed a strong positive relationship

between TP concentrations and species-specific biomass. The two species thus showed considerable niche overlap, although *E. nuttali* attained a higher maximum biomass than *E. canadensis*.

This study thus illustrates that the success of an invasive species and the invasibility of a habitat are inherently interlinked. Invasibility of a habitat can fluctuate depending on the degree of anthropogenic activity it is subjected to and the fluctuation of resource availability within. Similarly, the success of the invasive species is dependent on the frequency with which it can be dispersed into new habitats, its ability to tolerate the abiotic environment and its capacity to utilise the available resources in the new habitat competitively. When tackling the problem of invasive species, prevention of introduction is essential and the invasibility of vulnerable habitats must be reduced. This can be achieved by the enforcement of good biosecurity measures to reduce propagule pressure, the reduction of anthropogenic nutrient enrichment, and the restoration of water quality to reference conditions.

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Chapter 3

Richer, Poorer and None the Better; Native Macrophyte Communities in the Context of Alien Invasion

Introduction

Standing freshwaters play an important role as habitats for biodiversity and provide many valuable ecosystem services (Dudgeon *et al.*, 2006; Balian *et al.*, 2008; Postel & Carpenter, 1997). The biodiversity value of lentic habitats can be particularly pronounced in landscapes which are dominated by anthropogenic activity, where they can act as reservoirs of biodiversity adding substantially to the total biodiversity of a region. In that context, lakes, reservoirs, artificial ponds and canals have all been identified as important for biodiversity conservation (Gioria *et al.*, 2010; Hassall, 2014; Colding *et al.*, 2009; Chester & Robson, 2013; Dudgeon *et al.*, 2006). The role of macrophytic communities in such freshwater systems is particularly important (Engelhart & Richie, 2001). They are amongst the dominant primary producers and play critical roles in biogeochemical cycling of nutrients and carbon (Hargeby *et al.*, 1994; Mitsch & Gosselink, 1993). Macrophytes can produce large amounts of biomass, acting as food sources for organisms at higher trophic levels and detritivores alike (Cremona *et al.*, 2008; Lodge, 1991). Occupying the euphotic zone, riparian edges and the transitional zone between land and water, macrophytes vary widely in structure, form and function (McAbendroth *et al.*, 2005;). Variation in physical form can increase habitat heterogeneity providing microhabitats and niche space for epiphyton, invertebrates, fish and birds (Lalonde & Downing 1991; McAbendroth *et al.*, 2005; Valley & Bremigan, 2002; Jeppesen *et al.*, 2012, Wolcox & Meeker, 1992; Hinojosa-Garro *et al.*, 2000). In Ireland a number of rare and threatened plant species are associated with freshwater systems while others are protected by national and international law (Wyse Jackson *et al.*, 2016). In recognition of the value of freshwater ecosystems many wetland habitats have been designated for the conservation of biodiversity. These designated sites are protected by national law (Natural Heritage Areas) and international laws and agreements (Special Areas of Conservation, Special Protection Areas, Ramsar sites (NPWS, 2013)). Further, Ireland is committed to achieving 'good ecological status' in its surface waters under the E.U. Water Framework Directive (Donohue *et al.*, 2006). Improving knowledge of the causes of wetland biodiversity decline, and the threats to native macrophytes communities is thus essential for the management and conservation of freshwater ecosystems in Ireland.

The anthropogenic introduction of alien species to ecosystems is thought to be a significant threat to global and Irish biodiversity alike (Stout, 2011; Gallardo *et al.*, 2016; Powell *et al.*, 2011). Many introductions have led to the establishment, excessive spread and dominance

of an alien species to the extent that they have been associated with declines in local biodiversity, perturbation of ecosystem function and ecosystem services and even species extinction (Powell *et al.*, 2011; Clavero & Garia-Berthou, 2005; Pejchar & Mooney, 2009; Vilá *et al.*, 2011). The perceived impact of an alien species in its new range is generally the means by which an alien species becomes defined as invasive (Colautti & MacIsaac, 2004). Alternatively, an alien introduction will either fail to become established or, if successfully established, become naturalised without any noticeable influence on the communities they occupy (Richardson *et al.*, 2000). Based on their impacts on biodiversity and ecosystem functioning in their non-native range a number of alien freshwater macrophytes species (*Azolla filiculoides* Lam., *Crassula helmsii* (Kirk) Cockayne, *Elodea canadensis* Michx., *Elodea nuttallii* (Planch.) H. St. John, *Hydrocotyle ranunculoides* L.f., *Lagarosiphon major* (Ridl.) Moss, *Lemna minuta* Kunth, *Ludwigia grandiflora* (Michx.) Greuter & Burdet, *Myriophyllum aquaticum* (Vell.) Verdc. and *Nymphoides peltata* (S.G. Gmel.) Kuntze) are considered to be invasive species of concern in Ireland (Kelly *et al.*, 2013, Maguire *et al.*, 2011; Tsiamis *et al.*, 2017).

The negative effect of invasive alien species in freshwater systems has been repeatedly demonstrated (Gallardo *et al.*, 2016; Strayer, 2010). The reduction in biodiversity as a result of outcompeting and replacement of native flora and fauna is regularly recorded (Michelan *et al.*, 2010; Stiers *et al.*, 2011, Boylen *et al.*, 1999 Brendonck *et al.*, 2003). Also recorded are implications for the abiotic environment, food webs and trophic interactions (Kelly & Hawes, 2005; Vander Zanden *et al.*, 1999; Baxter *et al.*, 2004; Bunn *et al.*, 1998; Urban *et al.*, 2006) The complete extirpation of a species from freshwater systems, resulting in local extinction, appears to be restricted to multitrophic antagonistic interactions such as the unprecedented predation of a native species by an introduced alien species (Moyle & Light, 1996), but positive relationships between multiple non-native species have on occasion been demonstrated in freshwater systems, where one alien species facilitates the establishment and spread of another (invasion meltdown), resulting in negative impacts on native assemblages (Ricciardi, 2001; Britton *et al.*, 2010). Despite the mounting evidence, the concept of biodiversity loss due to invasion of the freshwater environment has also been challenged (Thomas & Palmer, 2015). Some researchers have found that the invasion of freshwaters has had a neutral effect on local community composition, while others have demonstrated the relationship between alien species and native communities to be positive (Kolada & Kutyla, 2016; Smith & Buckley, 2015). However, the perceived impact of an introduced species, positive or negative, is not the same at all trophic levels because the

alteration of a native community at one trophic level can have cascading influence on other trophic levels in the same ecosystem (Langdon *et al.*, 2004, Kelly *et al.*, 2015, Kelly & Hawes, 2005). Further it is evident that the relationship between invasive species and native community richness and diversity is scale dependent, with negative relationships occurring at smaller scales (within habitat) and the opposite occurring at larger scales (whole habitat to landscape) (Capers *et al.*, 2007).

The theory of biotic resistance (Elton, 1958) proposes that a community with higher species diversity is less likely to become invaded than one with lower diversity. The mechanisms underlying biotic resistance are those of efficient use of resources by communities competitively adapted to local condition, reduced availability of vacant niches, and the likelihood that diverse native communities will possess potential enemies (e.g. pathogens or predators) capable of suppressing establishment and spread (Fleming & Dibble, 2015). The occupation/vacancy of the functional and ecological niche by native species is thus central to the concept of biotic resistance.

However, in anthropogenic landscapes the ubiquitous eutrophication of the freshwater environment confounds the theory of biotic resistance. Eutrophication disturbances can lead to the exclusion of nutrient-sensitive plant species and a reduction in diversity, as the chemical disturbance occurs at a faster rate than native plants can adapt to nutrient enrichment (Bedford *et al.*, 1999; Byers, 2002; Holdredge *et al.*, 2010). Nutrient enrichment also selects for plant species that are strongly competitive at high nutrient levels and reduces community complexity (Blumenthal, 2005; Green & Galatowitsch, 2002). In eutrophic systems, the underlying concepts of Elton's biotic resistance theory are compromised because diversity is reduced, excess resources become available, and previously occupied niches become vacant (Davis *et al.*, 2000; Alpert *et al.*, 2000; Byers, 2002).

Regardless of direction (positive, negative or neutral), the relationships that introduced alien species have with native communities are a function of direct and indirect interactions. Direct interactions include predation, herbivory or competition for the same suite of resources (Fleming & Dibble, 2015). The outcome of a competitive interaction can be species specific, with some species being strong competitors while others are vulnerable to displacement because they are naturally scarce or unable to compete (Moyle & Light, 1996). In a given environment, highly competitive macrophytes can possess high growth rates, superior resource efficiency/uptake, high stress tolerance or allelopathic capacity for

example (Rejmánek & Richardson, 1996). Meanwhile indirect interactions may include alteration of the abiotic environment or ecosystem function. In the case of invasive macrophytes this might include light exclusion, sediment retention, alteration of chemical components of the water column, or carbon sequestration etc.

Without detailed knowledge of macrophyte communities prior to an invasion event it is difficult to unequivocally distinguish cause and effect of biodiversity decline (MacDougall & Turkington, 2005). However, declines in richness, diversity and abundance of native plant assemblages are recognised as indicators of ecosystem deterioration (Xu *et al*, 2001). It is therefore appropriate for research to investigate the patterns of association between native and alien macrophytes as indicators of ecological status. If negative associations are detected the ecological mechanisms behind such patterns can be further investigated. Alternatively, knowledge of reference conditions for similar uninvaded habitats, or habitats considered to be of 'good ecological status' can be used to inform narratives as to the putative relationships between invasive alien species and native macrophyte communities (Penning *et al.*, 2008). The implications of invasive species in the management of wetland habitats for the conservation of biodiversity can in this way be elucidated.

Using standing waterbodies in a landscape dominated by human activity as a study system, this study investigated the associations between invasive alien macrophytes and native macrophyte communities. This study aimed to describe the association between invasive alien species and native macrophytes communities and their implications for wetland management for conservation. To this end the following hypotheses were tested.

H₁. The occurrence and richness of invasive alien plant species in freshwater ecosystems is associated with the traits of native macrophyte community.

H₂. The characteristics of native macrophyte communities differ between invaded and uninvaded habitats.

H₃. The associations between invasive alien species and native macrophyte communities are density dependant.

H₄. The association between alien macrophytes and the native community is species specific for the most frequently occurring non-native species, *E. canadensis* and *E. nuttallii*.

H₅. That habitats legally designated for the conservation of biodiversity are not immune to invasion by freshwater alien plant species.

Methods

Site Selection and Abiotic Characteristics

82 standing waterbodies with aquatic plant communities were selected for survey using the methods described in Chapter 2. No prior knowledge of the aquatic plant community of a waterbody was assumed prior to the commencement of the study. The environmental characteristics of the waterbodies were characterised as described in Chapter 2.

Macrophyte Communities

The botanical communities of freshwater habitats tend to exhibit zonation. Vegetation composition changes with the transition from terrestrial (terrestrial species), through periodically inundated/partially wetted areas (helophytes) to completely aquatic zones (hydrophytes) (Denny, 1985). Vegetation classification systems typically differentiate between helophyte and hydrophyte plant communities (Fossit, 2000; Rodwell, 1998) as do biodiversity studies of aquatic habitats (Sayer *et al.*, 2012). As all invasive alien plant species of interest to this study are obligate aquatic plants, only the hydrophyte communities were surveyed. A list of aquatic plants for inclusion in this study was obtained from Dodkins & Rippey (2007).

Two survey methods were employed to record hydrophyte species at each waterbody. A comprehensive species list was compiled for each waterbody by searching the perimeter of the waterbody and scanning the water's surface for visible plants. Detailed searches of the nearshore communities were conducted by wading up to a depth of 1.1m along a 2m stretch of the shoreline either side of the established cardinal sampling points. All submerged, floating leaved and free floating plant species observed during this search were recorded. The duration of the search varied according to habitat complexity and size.

To estimate the abundance of macrophytes species a rake sampling system based on Free *et al.*, (2006) was modified for nearshore use. By wading beyond the emergent helophyte vegetation it was ensured that mainly submerged and floating leaved species were sampled. A 1kg, double-headed rake, 35cm in width, with 14 teeth either side was thrown perpendicular to the shore at each sampling point. Average throw distance was 8m. The rake was allowed to come to rest and then hauled back to the operator, sampling the vegetation as it moved. It was observed that the rake occasionally became saturated before returning to the operator and thus did not always sample the nearshore effectively.

The decision was thus made to combine a long throw (8m) and a short throw (4m) in order to effectively sample all vegetation along the 8m belt transect created by the rake sampling unit (approximately 2.8m² on average). Vegetation from both hauls were combined and weighed. The percentage species composition was then estimated and recorded. Data from all four sampling points were then averaged to calculate the abundance of each species per sampling unit.

Vascular plant identification follows Parnell *et al.*, (2012) and Haslam *et al.*, (1976). Taxa of *Callitriche* spp., *Ranunculus* spp. were identified to genus level because of the difficulty of identification in the absence of flowering and/or fruiting structures. Bryophyte species were identified according to Atherton *et al.*, (2010). Charophyte species were not distinguished from each other and filamentous algae were excluded from the study.

Native species were assigned to a functional/structural group. These groups were the free floating species (Lemnids), floating leaved species (Nymphaeids), submerged canopy forming species (Elodeids), submerged low-growing species (Isoetids), unrooted submerged species (Ceratophyllids), bryophytes and charophytes. Richness, biomass and relative abundance of each functional group was calculated per waterbody.

Data Analysis

Comprehensive species lists were produced for each waterbody by combining species recorded in rake hauls with species otherwise observed through searching and wading (Ray *et al.*, 2001). A waterbody was classified as invaded or uninvaded based on the presence or absence of an invasive species. Species richness was calculated as the total number of different aquatic plant species recorded. Shannon-Wiener Diversity (H') was calculated for entire aquatic plant communities at each waterbody and for native species in invaded and uninvaded communities. Only species recorded by rake haul were included in the calculation of diversity indices (Ray *et al.*, 2001).

Shannon-Wiener Diversity Index (H') was calculated as follows:

$$H = - \sum_{i=1}^S (p_i * \ln p_i)$$

Where p_i is the fraction of the entire population made up of species i and S is the total number of species recorded.

To test the hypothesis that there was a relationship between the occurrence of an alien species and the characteristics of the native community, a forward selection binomial logistic regression was employed using native species richness and native species biomass as predictor variables. Native species diversity and the diversity of functional groups were excluded from the analysis to avoid the effects of multicollinearity. Here variable entry testing was based on the significance of the score statistic and removal testing based on the probability of a likelihood-ratio statistic based on conditional parameter estimates. Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell (1962) procedure. A Wald test was used to determine statistical significance for each of the independent predictor variables in the regression.

In order to determine how similar botanical communities in this study were hierarchical cluster analysis was conducted based on the presence/absence data of 24 native taxa. Sorensen (Bray-Curtis) similarity was employed as a distance measure and clusters were assigned based on group average and plotted as a dendrogram. Non-metric multidimensional scaling (NMDS) was employed to visualise the distance between plant communities and cluster groups identified by the cluster analysis. NMDS was run based on the Sorensen (Bray-Curtis) distance between communities with a minimum stress value of 0.01 and 50 restarts. The NMDS plot was overlain with the groups generated by the hierarchical cluster analysis. Hierarchical cluster analysis and NMDS were conducted using the software package Primer v6.

Richness, diversity, evenness and biomass of native plant communities was compared between invaded and uninvaded communities using Mann-Whitney U tests (Mann & Whitney, 1947) due to the non-parametric nature of the data. The additional alien species were then added to the invaded community data and differences Mann-Whitney U tests repeated. Distribution of the data was visually inspected for similarity between categories and medians or mean ranks were reported as appropriate.

The relationship between invasive species biomass and native species richness (at the habitat scale and average rake sampling unit scale), native species biomass, native species diversity, native functional richness and native functional diversity was explored for invaded communities using Spearman's rank-order correlation. Preliminary analysis showed the relationships in question to be monotonic, as assessed by visual inspection of a scatterplot. The strength of the relationship was described as per Cohen (1988). Spearman's rank-order correlations were also used to determine if there was a relationship

between the richness of alien species and the richness, biomass, diversity and function of the native macrophyte community.

The relationship between native plant communities and *Elodea* spp. were explored in more detail with respect to native species richness, native species biomass, native species diversity, native functional richness and native functional diversity. *Elodea* spp. were initially treated cumulatively and the community traits of habitats where either *Elodea* species had been recorded were compared to uninvaded habitats using a Mann-Whitney U test. *E. canadensis* and *E. nuttallii* were then treated independently and the traits of the native plant communities in invaded and uninvaded habitats were compared using Kruskal-Wallis tests. Pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons.

Spearman's rank-order correlations were used to explore the relationship between *Elodea* abundance (in the form of biomass per rake sampling unit) and native plant community traits. Communities which contained either *Elodea* spp. were first tested cumulatively. Communities which contained either *E. canadensis* or *E. nuttallii* were then tested independently and sites where both species co-occurred were excluded from the analysis. Sites where *N. peltata*, *M. aquaticum* and *L. major* occurred were also excluded from the analysis. Exclusion of such sites eliminated any confounding effects other invasive species may have. In order to elucidate any potential differences in community composition associated with the abundance of *Elodea* spp. it was decided to investigate in further detail the relationship between *Elodea* biomass and the functional traits of the native community. Spearman's rank order correlations were run to elucidate the association between the richness, biomass and relative abundance of native functional groups and *Elodea* biomass. Because both *E. canadensis* and *E. nuttallii* co. occur frequently and are part of the same functional group (the Elodeids) their biomass was treated cumulatively in this context.

Results

Community Characteristics Associated with Invasion

A total of 24 different native taxa were recorded throughout this study. This was comprised of 21 native aquatic vascular plant taxa, two bryophyte species and at least one charophyte taxa (Table 1). Native species richness ranged from 0-10 in surveyed waterbodies. Six of the native species were only recorded in invaded communities. None of the native species were restricted to uninvaded communities. The most frequently recorded native taxa in

both invaded and uninvaded communities were *Potamogeton natans* L., *Lemna minor* L. and *Callitriche* spp. which occurred at 43, 39 and 33 waterbodies respectively. The least frequently recorded native taxa were *Potamogeton obtusifolius* Mert. & Koch, and the aquatic liverwort *Riccia fluitans* L. which were each recorded on only one occasion. The waterbodies with the richest native community were Lough Gal and Ballyhonock Lough, both of which had ten native species occurring in the aquatic plant community. No invasive species were recorded at Lough Gal, but four invasive species were recorded at Ballyhonock Lough making it the richest community of alien species observed in this study. On average (rounded to the nearest integer) native species richness was 4 species in invaded communities and 3 species in uninvaded communities (Table 2). Table 2 summarises the native species richness, native functional richness, native biomass and native species diversity of uninvaded and invaded communities. Invasive alien species of macrophyte recorded in this study, in order of frequency, were *E. canadensis*, *E. nuttallii*, *L. minuta*, *A. filiculoides*, *N. peltata*, *M. aquaticum* and *L. major*. (Refer to Chapter 2 for a detailed discussion of the frequency of alien species in this study).

Table 1: Native aquatic plant species recorded in 73 waterbodies surveyed in Co. Cork, their frequency of occurrence overall and in habitats invaded and uninvaded by alien aquatic plant species.

Species	Frequency	Invaded	Uninvaded
<i>Potamogeton natans</i> L.	40	26	14
<i>Lemna minor</i> L.	37	22	15
<i>Callitriche</i> spp.	33	19	14
<i>Potamogeton pectinatus</i> L.	21	12	9
<i>Potamogeton crispus</i> L.	18	10	8
<i>Apium inundatum</i> (L.) Rchb. f.	17	10	7
<i>Myriophyllum spicatum</i> L.	14	10	4
<i>Nymphaea alba</i> L.	13	11	2
<i>Ranunculus</i> sp.	8	6	2
<i>Myriophyllum alterniflorum</i> DC.	6	2	4
<i>Fontanalis</i> spp.	6	5	1
<i>Ceratophyllum demersum</i> L.	5	5	0
<i>Littorella uniflora</i> (L.) Asch.	4	4	0
<i>Nuphar lutea</i> (L.) Sm.	4	4	0
<i>Potamogeton berchtoldii</i> Fieber	4	2	2
<i>Potamogeton perfoliatus</i> L.	4	2	2
<i>Lemna trisulca</i> L.	3	3	0
<i>Potamogeton polygonifolius</i> Pourr.	3	1	2
<i>Utricularia</i> Sp	3	1	2
<i>Spirodela polyrhiza</i> (L.) Schleid.	2	2	0
<i>Chara</i> sp.	2	1	1
<i>Riccia fluitans</i> L.	1	1	0

Table 2: The mean, median, minimum and maximum values for the species richness, functional richness, biomass and species diversity in uninvaded and invaded native macrophytes communities.

Invasion Status		Native Richness	Functional Richness	Native Biomass	Native Diversity
Uninvaded	Mean	3	1.7	2.26	0.24
	Median	2	2.00	2.07	0.02
	Minimum	1	0	0.00	0.00
	Maximum	10	3	7.69	1.18
Invaded	Mean	3.7	1.9	1.70	0.30
	Median	3.5	2	1.15	0.08
	Minimum	0	0	0.00	0.00
	Maximum	10	4	6.99	1.22

A forward selection binomial logistic regression was performed to ascertain the effects of native community richness and biomass on the likelihood of waterbodies containing an invasive alien species. Neither native species richness nor native biomass improved the model from the constant, demonstrating no relationship between community traits and the occurrence of invasive macrophytes. Spearman's rank-order correlations were also run to identify any relationships between invasive species richness and native community traits, but no significant relationships were found.

Hierarchical cluster analysis and non-metric multidimensional scaling was used to group native plant communities based on the presence of native species (Fig. 1). Cluster analysis produced four main groups of species at the 30% similarity level but overlap in species composition occurred in the two most frequent groups. At the 15% resemblance level all but three of the native communities fall within the same group. This indicates that the native plant communities in this study are comparable in species composition. Invaded communities, represented by the number 1 in figure 1 are distributed throughout the MDS plot indicating that there is overlap in native species composition of the plant communities in invaded and uninvaded habitats.

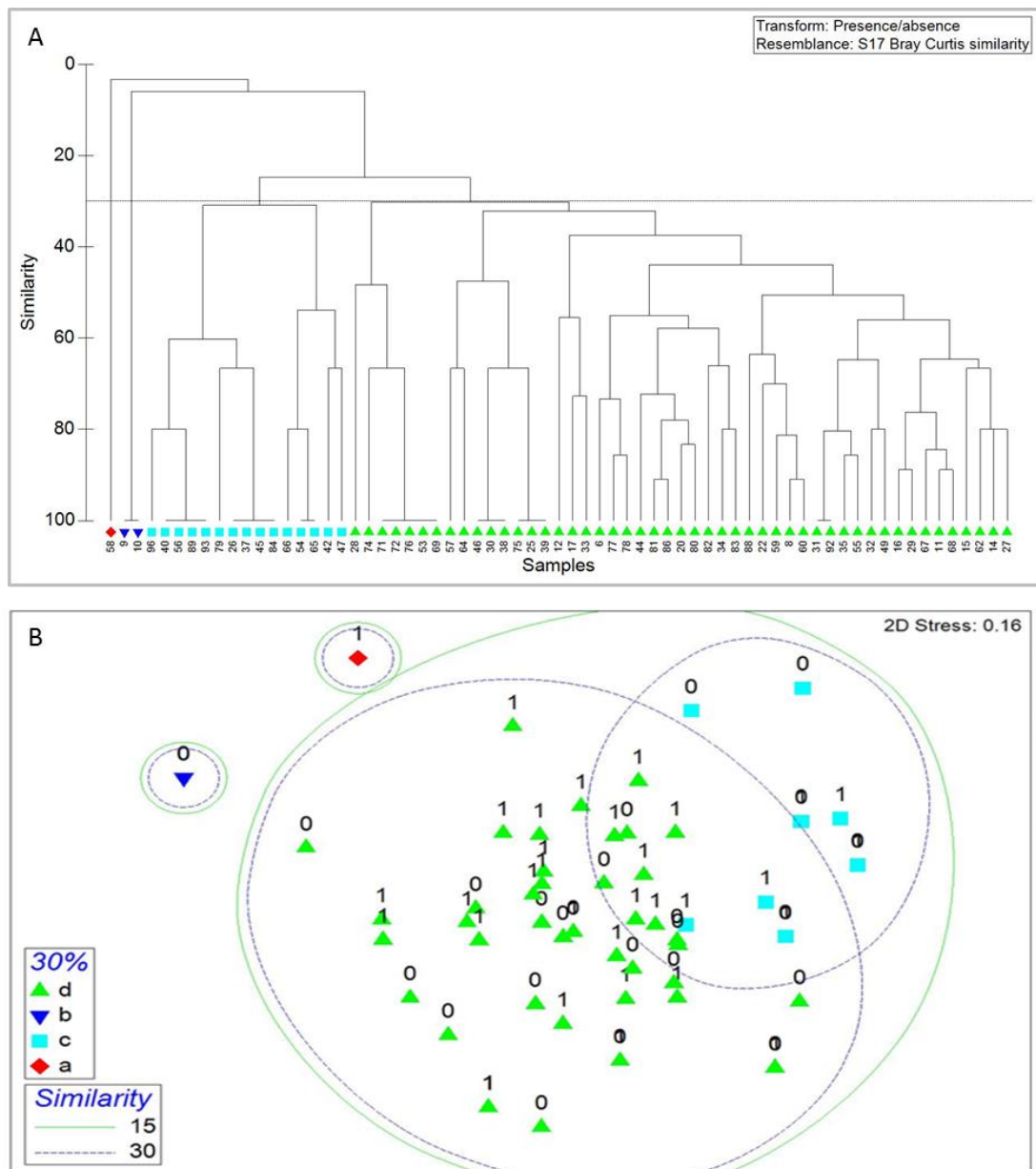


Figure 1: A. Hierarchical cluster analysis with surveyed sites grouped at the 30% similarity level using Bray Curtis similarity based on the presence or absence of 24 native taxa. B. 2-Dimensional MDS configuration with superimposed clusters from Fig. 1.A at similarity levels of 15% and 30% (Where 1=Invaded Communities and 0=Uninvaded Communities)

A Mann-Whitney U test was run to determine if there were differences in native species richness between invaded and uninvaded aquatic plant communities (Fig.2.A). The number of native species recorded ranged from 1-10 and 0-10 in uninvaded and invaded communities respectively. There was no statistically significant difference in median native species richness between invaded and uninvaded communities, $U=748.5$, $z=1.264$, $p=0.206$. In addition to the recorded native species there was, on average, 1.5 additional non-native species recorded in invaded communities. A Mann-Whitney U test was also run to determine if there were differences in total species richness between invaded and uninvaded aquatic plant communities. Median total species richness for invaded communities was statistically significantly higher than for uninvaded communities, $U=944.5$, $z= 3.488$, $p < 0.001$.

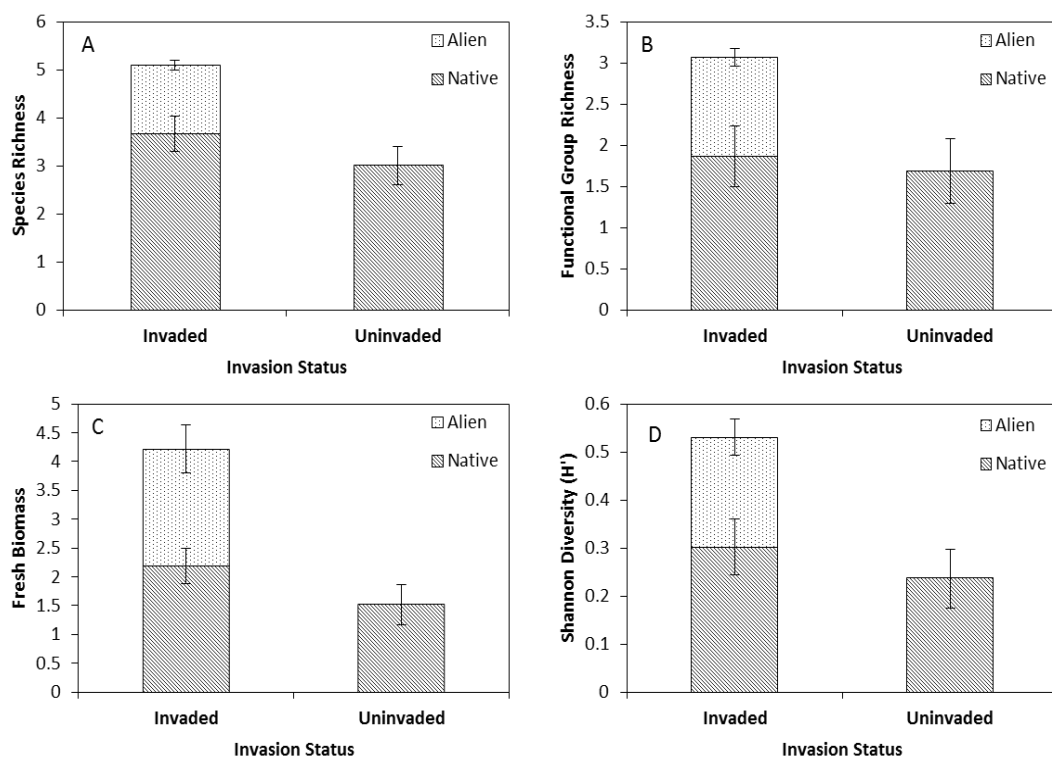


Figure 2: Mean community traits of invaded and uninvaded aquatic plant communities A. Species richness, B. Functional richness, C. Plant biomass, D. Shannon Diversity. Where there is no significant difference between native community traits, but the addition of alien species to invaded habitats significantly increases species richness, functional richness, plant biomass and Shannon Diversity.

The functional richness of native species was calculated based on the presence of functional groups in the plant community. Native species functional richness ranged from 0-3 functional groups in uninvaded habitats and 0-4 functional groups in invaded habitats (Fig.2.B). A Mann-Whitney U test was run to determine if there was a difference in the functional richness of native species in invaded and uninvaded communities, $U=675.5$, $z=0.434$, $p=0.664$. However, median functional richness for invaded communities (2) was not statistically different from that of uninvaded communities (2). However the median functional richness of alien plus native plant species in invaded habitats (3) was significantly greater than that of native only communities (2), $U=972$, $z=3.866$, $p<0.001$.

The average biomass of native species per rake sampling unit was, ranged from 0-7.69kg in uninvaded communities and 0-6.99kg in invaded communities (Fig. 2.C). A Mann-Whitney U test was used to determine if there was a significant difference in biomass. The median native biomass of invaded communities (1.15kg) was not significantly different from that of uninvaded communities (2.07kg), $U=535.5$, $z=-1.162$, $p = 0.245$. When the biomass of native and alien species was combined in the case of invaded communities the total biomass ranged from 0-16.95kg (Fig. 7). In this case total biomass for invaded communities (2.88kg) was statistically significantly higher than total biomass for uninvaded communities (1.15kg), $U=829$, $z=2.155$, $p=0.031$.

Shannon-Wiener Diversity Indices were calculated for native species recorded with the rake sampling method in invaded and uninvaded communities. Native species diversity ranged from 0-1.1 and 0-1.22 in uninvaded and invaded communities respectively. The mean native diversity of invaded and uninvaded communities was 0.23 and 0.3 respectively (Fig. 2.D). A Mann-Whitney U test was run to determine if there were differences in the Shannon-Wiener Diversity of native species in invaded and uninvaded communities. Median diversity for invaded communities (0.08) and uninvaded communities (0.02) was not statistically significantly different, $U=706.5$, $z=0.79$, $p=0.429$. Shannon-Wiener Diversity of invaded communities was also calculated using combined alien and native species recorded during rake sampling. Here the average invaded community had a mean diversity of 0.53. In this case the median diversity of invaded communities (0.54) was significantly greater than that of uninvaded communities (0.02), $U=880$, $z=2.752$, $p=0.006$.

The relationship between cumulative invasive species biomass and native species richness (at the habitat scale and average rake sampling unit scale), native species biomass, native species diversity, native functional richness and native functional diversity was explored for

invaded communities (Fig. 3). Invasive species biomass ranged from 0kg per sampling (communities where invasive species were present but not abundant and thus not sampled by rake haul) to 12.3kg per sampling unit. All six relationships were assessed using a Spearman's rank-order correlation (Fig. 3).

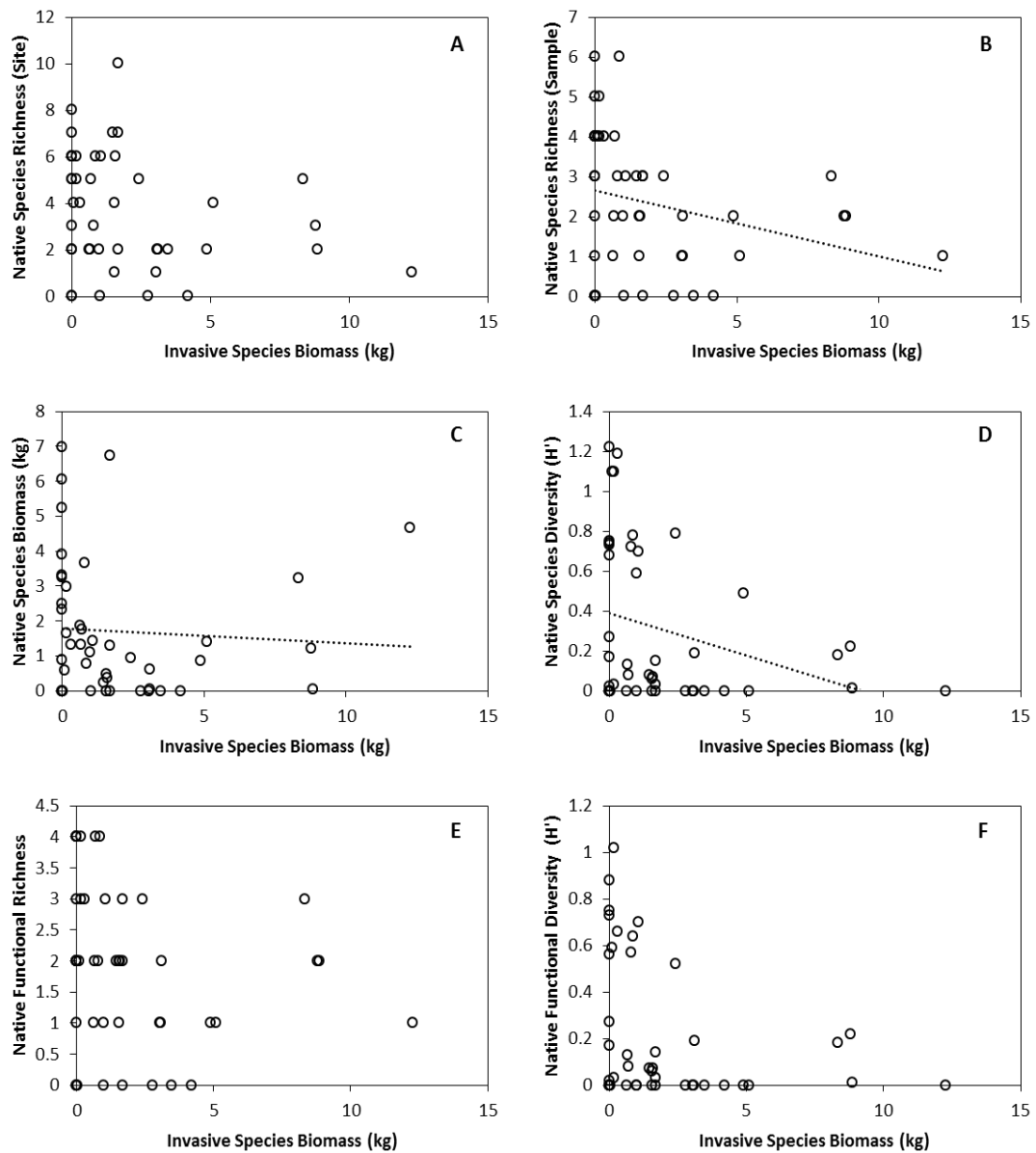


Figure 3: The relationship between invasive species biomass and community traits, A. Native species richness at the habitat scale, B. Native species richness at the rake sample scale, C. Native species biomass, D. Native species diversity, E. Native functional richness, F. Native functional diversity. Trend lines are only presented for correlations which are statistically significant.

In general, communities were found to be less rich in native species when cumulative biomass of invasive species was greater. At the habitat scale there was a small negative correlation between the biomass of invasive species and richness of native species which was approaching significance, $r_s(44) = -0.294$, $p = 0.053$. A significant moderate negative correlation was found between invasive species biomass and native species richness at the rake sampling unit scale, $r_s(44) = -0.371$, $p = 0.013$. A significant moderate negative relationship was also identified between average native species biomass and invasive species biomass, $r_s(44) = -0.325$, $p = 0.031$. This association indicates that native species are cumulatively less abundant as invasive species become more abundant. Invasive species biomass was also significantly, moderately, negatively correlated with native species diversity, $r_s(44) = -0.303$, $p = 0.046$. No significant relationship was identified between invasive species biomass and native plant functional richness or native functional diversity.

Species Specific Relationships

The range of native species richness, biomass, diversity and function of uninvaded communities and communities that contained *E. canadensis* and *E. nuttallii* are visualised in Fig 4. *Elodea* spp. were initially treated cumulatively and the community traits of habitats where either *Elodea* species had been recorded were compared to uninvaded habitats using a Mann-Whitney U test, but no significant differences were identified. *E. canadensis* and *E. nuttallii* were then treated independently and the traits of the native plant communities in invaded and uninvaded habitats were compared using Kruskal-Wallis tests. Statistically significant differences were identified in the median native species richness at the habitat scale, $\chi^2(2) = 6.594$, $p = 0.037$. Subsequently, pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. Median native species richness of habitats occupied by *E. canadensis* (4) was significantly greater ($p = 0.05$) than it was for habitats occupied by *E. nuttallii* (2) but was not significantly different from uninvaded habitats. Native species richness per rake sampling unit, native species biomass, native species diversity and native species function were not significantly different between uninvaded communities and communities occupied by *E. canadensis* or *E. nuttallii*.

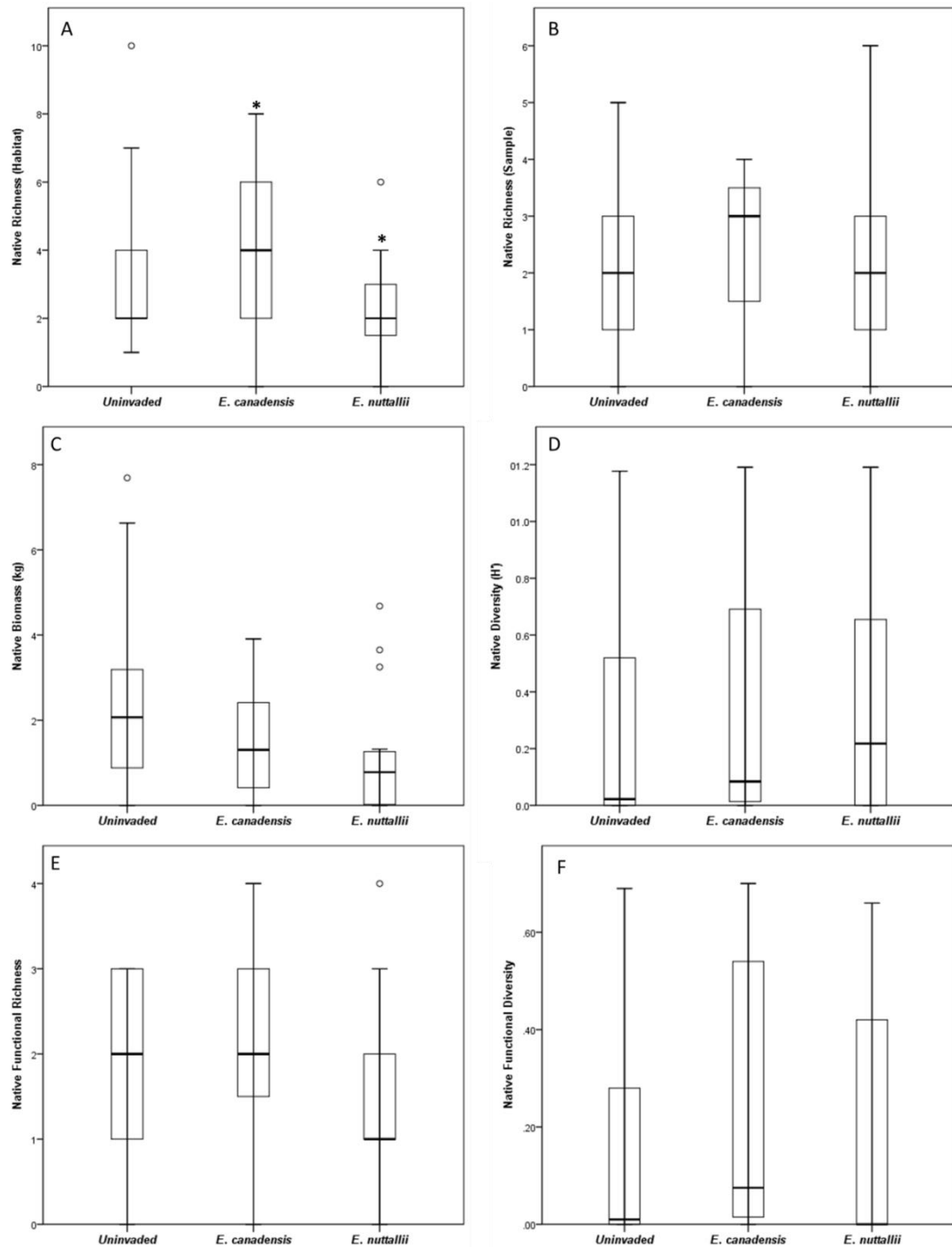


Figure 4: Native plant community traits (A. Native species richness at the habitat scale, B. Native species richness at the rake sampling unit scale, C. Native species biomass, D. Native species diversity, E. Native species functional richness, F. Native species functional diversity) in uninvaded habitats and habitats where *E. canadensis* and *E. nuttallii* occur. Where * indicates a statistically significant difference in the median, and where ° indicates an insignificant outlier in the data.

The relationship between native plant communities and *Elodea* spp. may be density dependent. Spearman's rank-order correlations were used to explore the relationship between *Elodea* abundance (in the form of biomass per rake sampling unit) and native plant community traits. Communities which contained either *E. canadensis* or *E. nuttallii* were tested independently. Sites where both species co-occurred were excluded from the analysis, along with sites where *N. peltata*, *M. aquaticum* and *L. major* also occurred. Exclusion of such sites eliminated any confounding effects that other invasive species may have. The biomass of *E. nuttallii* was not significantly correlated with any of the native community traits (Fig. 5). However, the biomass of *E. canadensis* was significantly, strongly, negatively correlated with native species richness at the habitat scale ($r_s(14)=-0.543$, $p=0.045$), native species richness per rake sampling unit ($r_s(14)=-0.732$, $p=0.003$), native species functional richness ($r_s(14)=-0.633$, $p=0.015$) and native species biomass ($r_s(14)=-0.806$, $p<0.005$) (Fig. 6).

It was decided to investigate in further detail how the abundance of *Elodea* spp. is associated with the functional traits of the native community. Spearman's rank order correlations were run to elucidate the association between the richness, biomass and relative abundance of native functional groups and *Elodea* biomass. Because both *E. canadensis* and *E. nuttallii* co-occur frequently and are part of the same functional group (the Elodeids) their biomass was treated cumulatively in this context. The biomass of *Elodea* spp. was found to be significantly, moderately, negatively correlated with the richness of native species in the Elodeid functional group ($r_s(28)=-0.377$, $p=0.048$) (Fig. 7). *Elodea* spp. biomass was significantly strongly negatively correlated with the biomass of native elodeid species ($r_s(28)=-0.558$, $p=0.002$) and moderately negatively correlated with the relative abundance of the elodeid functional group in native communities ($r_s(28)=-0.475$, $p=0.011$) (Fig. 7). No significant relationship between *Elodea* spp. biomass and any of the other functional groups was identified.

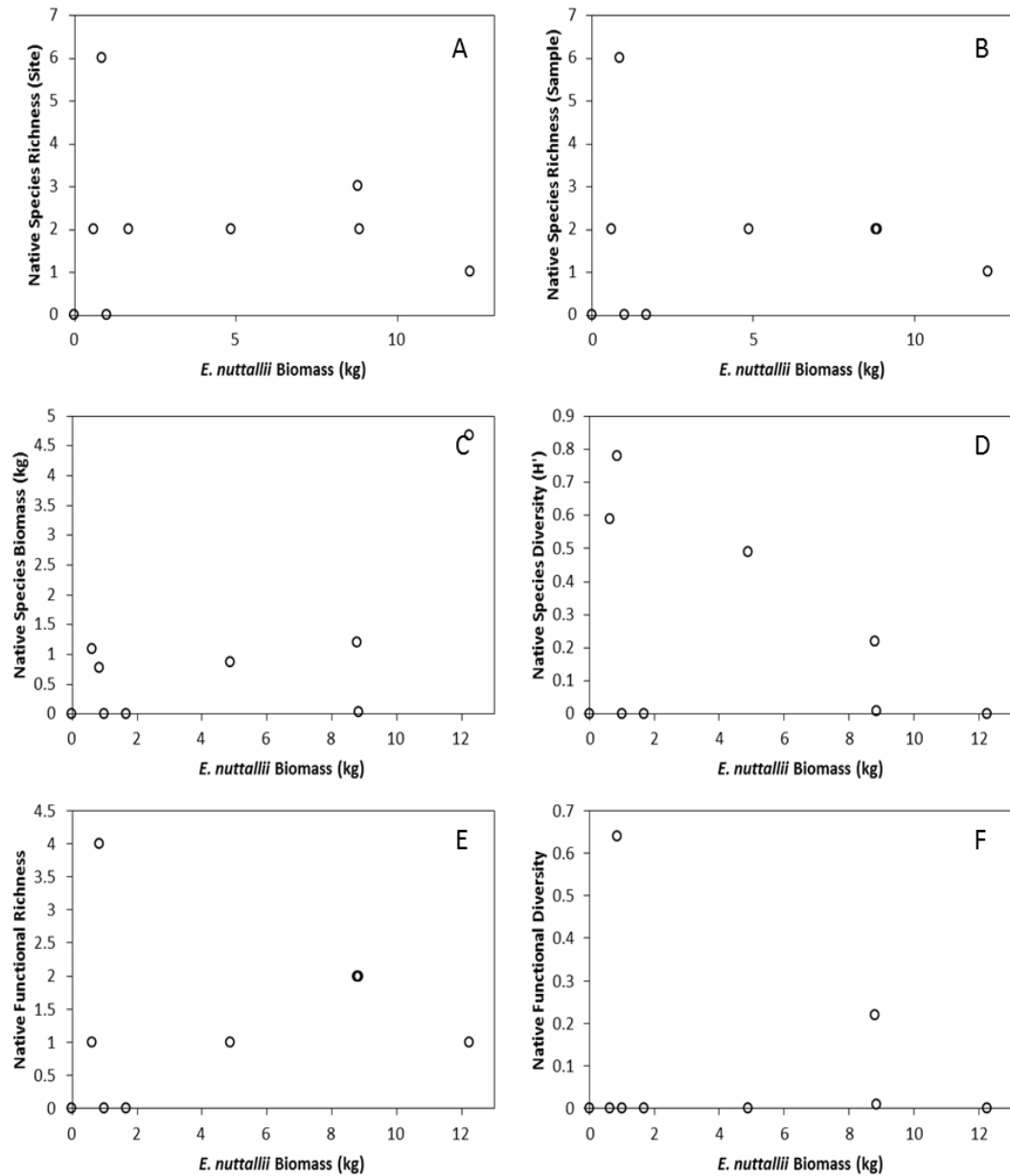


Figure 5: The relationship between native community traits (A. Native species richness at the habitat scale, B. Native species richness at the rake sampling unit scale, C. Native species biomass, D. Native species diversity, E. Native species functional richness, F. Native species functional diversity) and the biomass of *E. nuttallii*, where the presence of a trend line indicates a statistically significant correlation. Habitats that contained *E. canadensis*, *M. aquaticum*, *N. peltata* and *L. major* were excluded from the analysis.

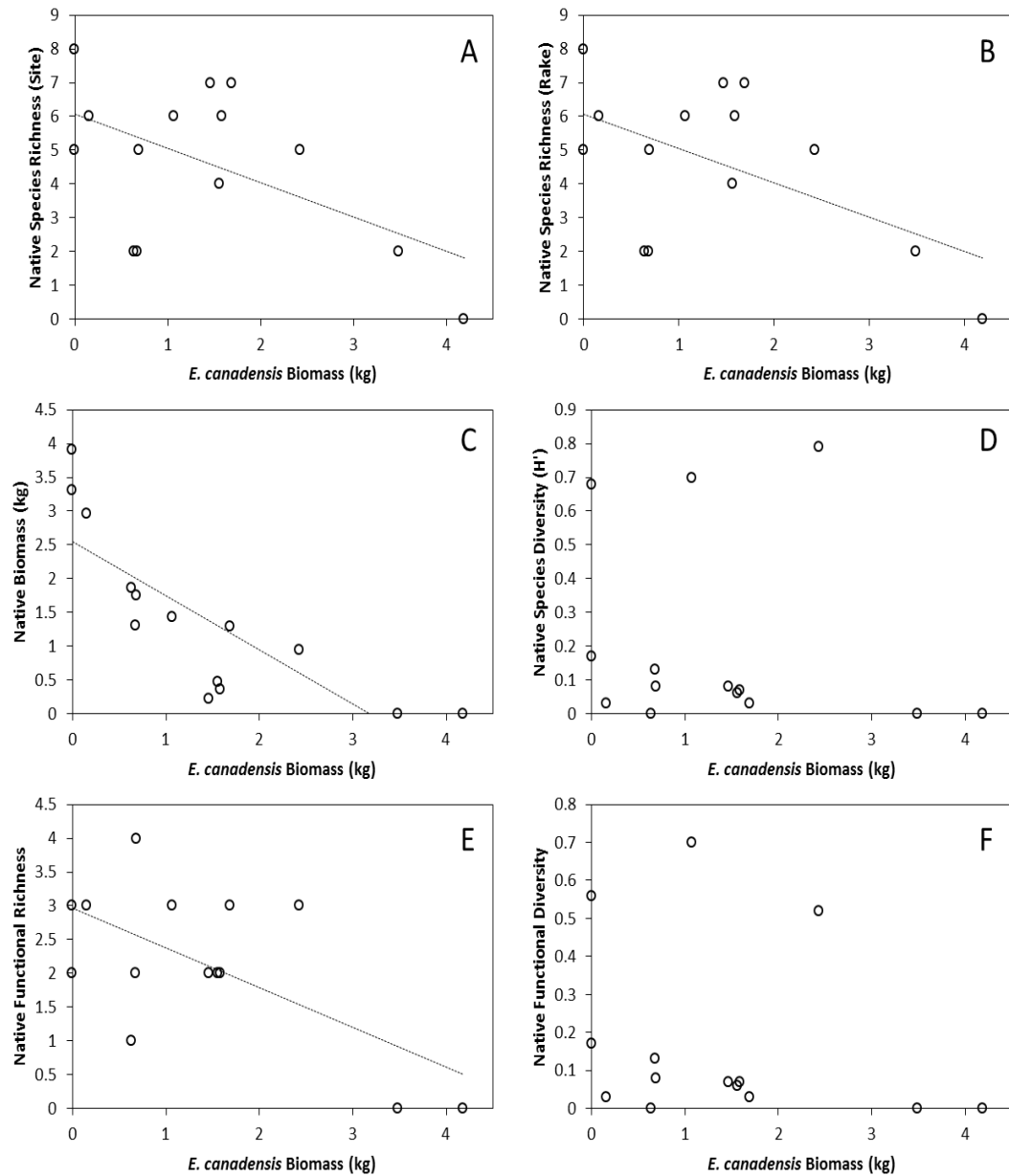


Figure 6: The relationship between native community traits (A. Native species richness at the habitat scale, B. Native species richness at the rake sampling unit scale, C. Native species biomass, D. Native species diversity, E. Native species functional richness, F. Native species functional diversity) and the biomass of *E. canadensis* per rake sampling unit, where the presence of a trend line indicates a statistically significant correlation. Habitats that contained *E. nuttallii*, *M. aquaticum*, *N. peltata* and *L. major* were excluded from the analysis.

Waterbodies Designated for the Conservation of Biodiversity

Sixteen of the surveyed waterbodies are located within the boundaries of nine different areas afforded legal designation for the conservation of biodiversity. All nine areas are Natural Heritage Areas protected by national law (Table 3). In addition, one area, The Gearagh, is designated as a Special Area of Conservation and Special Protection Area under EU Natura 2000 law. Table 3 provides a summary of these protected areas and the occurrence of aquatic alien species in surveyed waterbodies within their boundaries. At least one aquatic invasive plant species was recorded within the boundaries of six out of nine protected areas. *E. canadensis* occurred most frequently within protected areas and was recorded in five different protected areas. Seven of the species occurrence records were for species which had not previously been recorded within that protected area. The earliest existing record for the occurrence of aquatic invasive species within the boundary of a protected area is for *E. canadensis* and *E. nuttallii* which were both recorded in The Gearagh in 1987 and again in this study.

Table 3: Areas designated for the conservation of biodiversity and the occurrence of aquatic invasive species in waterbodies located within protected areas.

Site Name	Legal Designation	NPWS Site Code	Water-bodies Surveyed	Water-bodies Invaded	Invasive Species Observed	Earliest Available Record (NBDC)
Ardamadane Wood	NHA	001799	1	1	<i>A. filiculoides</i>	New Record
Blarney Bog	NHA	001857	1	1	<i>A. filiculoides</i> <i>E. canadensis</i> <i>L. minuta</i>	1995 2005 New Record
Blarney Lough	NHA	001798	1	1	<i>E. canadensis</i> <i>L. minuta</i> <i>N. peltata</i>	New Record 1993 New Record
Clasharinka Pond	NHA	001183	1	0	-	-
Cork Lough	NHA	001081	1	0	-	-
Lee Valley	NHA	000094	2	2	<i>E. canadensis</i> <i>E. nuttallii</i>	New Record New Record
Lough Gal	NHA	001067	2	0	-	-
Loughs Aderry & Ballybutler	NHA	000446	2	2	<i>E. canadensis</i>	1992
The Gearagh	NHA SPA SAC	000108	5	3	<i>E. canadensis</i> <i>E. nuttallii</i> <i>L. minuta</i>	1987 1987 New Record

NHA = Natural Heritage Area (Wildlife Act 2000), SPA = Special Protection Area (EU Birds Directive 2009), SAC = Special Area of Conservation (EU Habitats Directive 1992)

Discussion

Community Characteristics Associated with Invasion

Macrophyte species identified in this study correspond well to those identified in recent studies of waterbodies in a similar Irish landscape, as does the average number of hydrophytic species per waterbody (Gioria *et al.*, 2010). Macrophyte assemblages in this study were generally depleted in terms of species richness and averaged between 3 and 4 species in uninvaded and invaded communities, respectively. In Ireland, hydrophytic macrophyte assemblages in reference condition communities of high water quality environments are typically more speciose than those of mesotrophic and eutrophic conditions. The most commonly occurring native species, *P. natans*, *L. minor*, *Calitriche* spp. are considered to be generalists which tolerate a wide range of trophic conditions, but are typically associated with assemblages of mesotrophic to eutrophic waters. (Heegaard *et al.*, 2001; Fossit, 2000; Weekes *et al.*, 2014; Weekes *et al.*, 2018). A regionally uncommon species, *R. fluitans* was recorded at one location (Atherton *et al.*, 2010). This was confirmed by Dr. Rory Hodd to be the first record of the species in Co. Cork. This observation of *R. fluitans* confirms that despite their depleted macrophyte diversity, standing waters in human dominated landscapes remain of high importance to local biodiversity. This study did not assess the importance of these waterbodies for faunal communities, however in a survey of similar waterbodies in the Irish agricultural landscape Gioria *et al.*, (2010) recorded the presence of an IUCN red listed beetle species of global importance.

Seven alien macrophyte species were recorded to occur in this study, in varying frequencies and abundances. Native species richness and abundance were not associated with the likelihood of occurrence of an alien species or the richness of alien species in a community, nor was there a detectable difference in the measured ecological traits of invaded and uninvaded communities. Indeed, NMDS analysis demonstrated that there was no differentiation between the species composition of invaded and uninvaded communities either. These results indicate that the presence of an invasive alien species can elicit a neutral response from native assemblages, meaning that the occurrence of an alien species does not always have a negative influence on native communities. Smith and Buckley (2015) recently demonstrated that, in England, wetlands that contained *Crassula helmsii* were no less rich than those that did not. Here, our data are based on the qualitative assessment of presence or absence of alien species in an ecosystem. Gooden *et al.*, (2009) investigated whether invasion by alien plants initiates a linear or non-linear response from

native species. The authors speculated that if a non-linear relationship existed a threshold beyond which a negative impact is detected may exist. In such a scenario small populations of an invasive species may have a disproportionately low influence on native assemblages. Thus, in the context of occasional occurrences of an alien species in a large lake it is unlikely that there are cascading effects on local communities. However, when alien species increase in abundance beyond a certain threshold potential effects on native macrophytes will become more evident.

The addition of alien species to a community was demonstrated to increase overall macrophyte richness, biomass, diversity and variety of plant structural groups, without negatively influencing the native community, a trait which has previously been observed in wetland habitats (Stohlgren *et al.*, 2003). This informs us that the native communities were not saturated in terms of species diversity, structure and function (Stachowicz & Tilman, 2005). It is evident that unoccupied niches were available prior to the introduction of alien species. The previous chapter demonstrated that studied habitats were predominantly eutrophic and that nutrient resource availability was high throughout. As previously discussed, in eutrophic freshwater systems disturbance increases resource availability and vacant niches vulnerable to invasion become available (Davis *et al.*, 2000; Alpert *et al.*, 2000; Byers, 2002). Macrophyte communities such as those described in this study, despite being unaffected by the presence of an alien species, cannot be described as biologically resistant, as they do not meet the requirements of Elton's (1958) hypothesised resistant communities.

The addition of non-native species increased the total species richness, total biomass and structural diversity within a community. Such alterations to an aquatic habitat cannot be ignored and should not automatically be perceived as positive. Increased biomass will result in denser macrophyte standing stock within a given habitat, with implications for other trophic groups such as invertebrates and fish (Schultz & Dibble, 2011). Excessive standing stock can in some cases make habitats inhospitable for specialist fauna, disrupt decomposition and alter the physiochemical conditions of waterbody (Schultz and Dibble, 2011). Alternatively, the addition of structure and biomass can increase habitat heterogeneity providing previously unavailable niche space for fauna to occupy (Thomas & da Cunha, 2010; Schultz & Dibble, 2011). Modification of the structure or function of habitats in such a way can subsequent effects throughout the foodweb.

Figure 8 illustrates the extent of invasive plant biomass in some of the studied waterbodies. The density dependent exclusion of native species by the cumulative biomass of alien species was observed at the rake sample scale. Native species biomass and native species diversity were negatively related to the alien species biomass at the rake sample scale. No significant negative relationships were observed at the habitat scale. Scale dependent impacts of invasive species are well documented in the literature (Capers *et al.*, 2007). The mechanism behind such scale dependent associations is related to the rarity with which any species will occupy all available space in a given habitat, e.g. entire habitats are rarely exclusively monocultures, but stands of vegetation within a habitat can be frequently be entirely composed of a single species, native or otherwise.



Figure 8: Images depicting the extent of alien macrophyte invasions at selected locations from this study. A. *N. peltata* at Blarney Lough. B. *L. minuta* in the Lee Valley and C. *M. aquaticum* at Fota.

The Relationship Between *Elodea* spp and Native Communities

At the habitat scale, those habitats occupied by *E. canadensis* are not different from uninvaded habitats but they were more speciose than those occupied by *E. nuttallii*. The previous chapter showed that *E. canadensis* occupied waterbodies with a greater surface area than *E. nuttallii*. The present results may thus be an artefact of the positive relationship between waterbody size and species richness. The fact that community traits at the rake sampling scale were not different between alien species supports this hypothesis.

Having had an extended residence time many authors have suggested that *E. canadensis* has become a naturalised member of the European flora (Simpson, 1984). On mainland

Europe in particular *E. canadensis* has been demonstrated to be a benign occupier of waterbodies, having no discernible influence on native macrophyte communities (Mjelde *et al.*, 2012; Kolada & Kutyla, 2016). *E. nuttallii* on the other hand is recognised as an invasive species of E.U. concern (Tsiamis *et al.*, 2017) because of its perceived negative impacts and its capacity to outcompete and replace *E. canadensis* (Simpson, 1990). In this study, communities occupied by *E. canadensis* or *E. nuttallii* were no different from each other or from uninvaded communities, as seen for rake samples. However, the present study suggests that in Ireland increased levels of *E. canadensis* can dominate a habitat and have inverse density dependent effects on local communities. Indeed, density dependent, negative relationships between *E. canadensis* and the native community were detected at both the habitat and sample scale. Communities with more *E. canadensis* biomass were less species rich, less structurally rich and had lower native plant biomass. No such relationship was identified in communities occupied by *E. nuttallii*. Contrary to the present study, Kelly *et al.*, (2015) found that the cumulative abundance of *E. canadensis* and *E. nuttallii* was positively related to native species richness in river and lakes in Northern Ireland.

The previous chapter highlighted the fact that the biomass of *E. canadensis* was positively related to the availability of nutrients in the freshwater environment. Similarly, Free *et al.*, (2006) observed that as waterbodies transitioned from oligotrophic to eutrophic conditions *E. canadensis* became the most dominant species in lentic communities. Whether the localised depletion of native species in these scenarios is a result of competitive exclusion by *E. canadensis* or a result of selection by deterioration of water quality remains unanswered. MacDougall and Turkington (2005) concluded that the relative abundance of invasive and native species was determined by trade-offs relating to environmental conditions rather than ability to compete for resources (Didham *et al.*, 2005). Nevertheless, macrophytes sharing similar morphology to *Elodea* spp. were identified as those at greatest risk of being excluded in incidences of high biomass of the alien species. Species with the elodeid growth form are most likely to be in direct competition with the *Elodea* spp., and no impact was observed for other structural groups. This might suggest that the mechanisms controlling the decline in diversity with increasing *E. canadensis* is competitively controlled rather than environmentally controlled.

Given that no process in an ecosystem exists in isolation, and that competition occurs in the context of an environment, it is very probable that the localised loss of species in

invaded eutrophic habitats is the result of a double edged sword. Indeed, Byers & Noonburg (2003) and others have demonstrated that relationships between community traits and alien species are the product of the interaction between native diversity and resource availability in the context of scale.

Invasive Species in Habitats Managed for Biodiversity Conservation.

Regardless of the mechanism, negative relationships between native communities and alien species are indicators, as demonstrated, of ecological degradation (Pysek & Richardson, 2010; Didham *et al.*, 2005). Ireland is obliged to take action towards the prevention, control and eradication of putative and established invasive species under international law (Genovesi & Shine, 2004; Genovesi *et al.*, 2015; Tsiamis *et al.*, 2017). Furthermore, freshwater invasive species have been integrated into the assessment of 'good ecological status' in the context of the Water Framework Directive and are considered undesirable under Natura 2000 legislation (Cardoso & Free, 2008). The present study showed that invasive species occupied wetlands within most of the protected habitats in the study region. Indeed, many freshwater habitats designated for the conservation of biodiversity have been subject to invasion by multiple alien species and are particularly vulnerable to future invasion (Kelly, 2012). Kelly's (2012) study was based on existing records, and the present study showed that the extent of invasion in protected wetlands is greater than previously recorded. The number of designated areas subject to the influence of invasive species in Ireland may thus be substantially greater than previously thought. The frequency with which protected habitats are invaded is alarming for two reasons; firstly because of the threat invasive species can pose to diversity and function of ecosystems and secondly because they may represent an indicator of anthropogenic disturbance and environmental degradation, as demonstrated by the previous chapter.

Conclusions

Many conflicting opinions and theories exist with respect to mechanisms which drive relationships between alien invasion and native communities. Positive, negative and neutral relationships have previously been observed in freshwater systems, but the mechanism controlling such relationships are not well understood. This study aimed to describe the nature of the relationship between invasive alien species and native

macrophyte communities in an Irish anthropogenic landscape. It was found that habitats designated for the conservation of biodiversity were frequently invaded by multiple alien species and this gives cause for alarm given the negative relationship between alien and native macrophytes in the study region.

The likelihood of occurrence of an alien species was not influenced by native species richness or abundance, and the measured characteristics of native communities of invaded communities did not differ from uninvaded communities. This trend was evident for alien species as a whole and in the species specific relationships of *E. canadensis* and *E. nuttallii* with native communities. Rather, the influence of alien species was additive to the native community, increasing total species richness, biomass, diversity and structure of invaded communities.

Such neutral responses from the native macrophyte assemblages were only evident at the habitat scale, while at the sample scale density dependent, negative relationships between the characteristics of the native community and alien species biomass were recorded. The density dependent pattern of reduced native community complexity in terms of richness, diversity, biomass and structure was observed for both cumulative alien biomass and *E. canadensis* biomass. This confirms the hypothesis that the degree to which the impact of invasive species can be perceived is scale dependent and is more discernible at sub-habitat scales. The native species excluded in habitats occupied by *E. canadensis* were deemed to be those that shared the same growth form as the alien species. Competition is greatest between macrophytes of similar growth forms as they occupy largely the same niche.

Arguments were made as to the cause and effect of the observed negative relationships between invasive species and community traits. It was acknowledged that in the context of nutrient enrichment environmental drivers may best explain invaded assemblages, however the reduction of species within the elodeid growth form in *E. canadensis* dominated communities suggests that some competitive exclusion is occurring. It was concluded that given the dynamic nature of freshwater ecosystems, the observed relationship between native and alien macrophytes in this study is most likely to be a result of a double edged sword; where species richness is depleted due to the interaction between environmental degradation and exclusion/replacement by invasive alien species. In order to unequivocally confirm the drivers of biodiversity depletion, further longitudinal data of macrophyte assemblages and environmental conditions prior to and during invasion are required.

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Chapter 4

Fragmentation and Desiccation Tolerance of Aquatic Invasive Plants

Introduction

Human migration, commercial trade and other transport activities are continuing to disperse an ever-increasing number of species across environmental barriers such as oceans, rivers, mountain ranges, and/or climatic zones which were previously insurmountable (Hulme, 2009; Mack *et al.*, 2000). It has been suggested that, globally, few habitats remain free of species introduced by human activity (Mack *et al.*, 2000). Non-native (alien) species can be introduced either intentionally or accidentally using a wide variety of human activities as vectors (Hulme *et al.*, 2008). As global travel and trade increase, the rate of increase of non-native species introductions has accelerated worldwide (Mack *et al.*, 2000) and is likely to continue to do so (Levine and D'Antonio, 2003; Seebens *et al.*, 2017). This trend has been expressed in an Irish context, particularly in recent decades (Reynolds, 2002).

Freshwater ecosystems are said to be at particular risk of invasion (Shea and Chesson, 2002). Alien species are very common and widespread in freshwater systems (Strayer, 2010) with some inventories estimating hundreds of alien species occurring in some locations in extreme cases. In Ireland, since 1980 the greatest rate of increase in alien introductions has been to the freshwater environment and it is the only environment where more invasive species of high risk than medium risk are found (O'Flynn *et al.*, 2014). It is estimated that 24% of the world's most invasive alien plants are wetland plants (Zedler and Kercher, 2004). Aquatic plant invaders include all of the major groups of aquatic plants, including riparian species, emergent plants, submerged species, floating-leaved species, and floating plants (Strayer, 2010).

Invasive success of alien species is, amongst other things, a function of the number of propagules which have been introduced to a new area. The number of individuals introduced in a single event multiplied by the number of discrete introduction events makes up what is known as 'propagule pressure'. As the number of introduction events and/or the number of individuals released increases, propagule pressure also increases (Lockwood *et al.*, 2005). Increase in propagule pressure increases the likelihood of viable propagules colonizing an environment. The number of individuals released in each introduction event and the rate of introduction events occurring may depend on the mode of reproduction and dispersal of the species in question (Thomaz *et al.*, 2015). Following colonisation the spread of an alien species in its new range is what defines its invasion

success. With invasive plants, post-colonization spread requires successful growth, reproduction and the dispersal of individuals throughout the species' new range.

Most aquatic plant species exhibit clonal reproductive traits in some form (Santamaría, 2002) and it can be the almost exclusive mode of reproduction for some species, especially in invasive ranges where only a single gender of the species is present e.g. *Elodea canadensis* Michx., *Elodea nutallii* (Planch.) H. St. John, *Lagarosiphon major* (Ridl.) Moss and *Myriophyllum aquaticum* (Vell.) Verdc. in Ireland. Aquatic plant clones can be produced from a variety of plant parts, including rhizomes, stolons, tubers, turions or simple stem fragments (Riis *et al.*, 2009).

The production of stem fragments occurs in two main ways. Self-induced abscission of shoot fragments, known as autofragmentation, has been documented for a number of species, e.g. *Myriophyllum spicatum* L. and *Alternanthera philoxeroides* (Mart.) Griseb (Clements *et al.*, 2012; Xie & Yu, 2011). Autofragments form during the growing season when a layer of partially lignified cells is produced near a node causing abscission of a shoot. Such autofragments typically possess an apical tip and form roots at the nodes (Xie & Yu, 2011). In contrast allofragments are fragments of shoot generated by disturbances such as breakages of the stem by water flow, animal or human activity such as disturbance by boats etc. (Riis *et al.*, 2009). The management of invasive macrophytes may lead to the production of allofragments. Chemical control methods are often restricted in or near aquatic environments, thus, control methods are often limited to mechanical cutting and removal of plants from aquatic habitats. Such mechanical cutting activity can produce large amounts of unspecialised allofragments (Hoffmann *et al.*, 2014) capable of dispersal and regeneration into entirely new plants.

Unspecialised allofragments can substantially contribute to asexual reproduction because they can be produced outside of the growing season and are well adapted for transport and dispersal by water (hydrochory) over great distances and long periods of time (Barrat-Segretain, 1996; Riis and Sand-Jensen, 2006). Vegetative propagules possess aerenchyma tissue which provides buoyancy for several weeks in some cases (Barrat-Segretain, 1996). The capability to float and be dispersed by moving water may be essential to the invasion success of alien macrophytes. However, most dispersal of fragments by water is unidirectional, i.e. downstream and there is little hydrochoric dispersal to waterbodies outside of that catchment (Barrat-Segretain, 1996). Indeed, some waterbodies, such as

ponds and lakes, are said to be “islands” in terrestrial landscapes due to lack of natural connectivity (Browne, 1981).

Dispersal of vegetative fragments by human activity such as boating or fishing activities is well documented (Johnson *et al.*, 2001; Mosisch and Arthington, 1998; Rothlisberger *et al.*, 2010; Wittmann *et al.*, 2015). Such dispersal may occur within or between waterbodies, providing some connectivity between otherwise isolated ‘island’ waterbodies. Human mediated dispersal of vegetative fragments between waterbodies typically involves overland transport. In such scenarios fragments are exposed to desiccation stress during transport and may suffer a loss of fitness, not suffered during hydrochoric dispersal. According to the hypothesis of propagule pressure (Thomaz *et al.*, 2015), the likelihood of alien plant spread into new waterbodies increases with increasing movement of people, boats and equipment from invaded habitats and with increasing numbers of viable propagules.

Waterfowl and mammal mediated dispersal is also cited as another possible means of overland transfer of aquatic plant propagules from one isolated waterbody to another (Coughlan *et al.*, 2017a). Darwin even suggested that the wide ranges of freshwater plants globally may have been achieved by the carriage of seeds internally and vegetative fragments, externally (epizoochory), on the plumage and feet of waterfowl (Barrat-Segretain, 1996). Though epizoochoric dispersal may be a natural form of dispersal for native plants, some animals may also facilitate the dispersal of invasive alien macrophytes in their new range (Coughlan *et al.*, 2017b). Like anthropogenically dispersed fragments, epizoochoric fragments may also experience desiccation stress. Additionally, the size of the fragment is likely to affect the capability of attachment to the plumage and pelts of animals.

The extent to which native and alien aquatic plants differ in fragmentation rate and fragment size is not widely documented (Heidbüchel *et al.*, 2016). Though some species have the capacity to regenerate from a single node, others require larger fragments to regenerate (Kuntz *et al.*, 2014). Some debate exists in the literature as to whether large fragments of alien plants that possess multiple smaller regeneration units could increase propagule pressure as smaller fragments break off and remain viable (Heidbüchel *et al.*, 2016).

Li (2014) speculated that because aquatic plants do not typically suffer water stress all vegetative parts of an aquatic plant have the possibility to become vegetative propagules. When being dispersed overland this statement is no longer true. The ability of aquatic plants to tolerate water loss limits their ability to be dispersed overland. Plant fragments exposed to desiccation at the limits of their tolerance may exhibit reduced fitness and viability. It has thus been hypothesised that the success of a vegetative fragment as a propagule for reproduction and dispersal is limited by the plant's ability to survive fragmentation and, if dispersed over land, its ability to tolerate desiccation stress (Barnes *et al.*, 2013). The present study aimed to elucidate the extent to which aquatic invasive plants can tolerate fragmentation and desiccation stress. We tested a number of hypotheses with that aim in mind;

H₁. That fragment size influences propagule fitness and that a minimum fragment size threshold exists below which fragments are not viable.

H₂. That desiccation time and fragment size influence the rate at which water is lost from an aquatic plant fragment upon aerial exposure.

H₃. That desiccation time and fragment size influence propagule fitness and that there is a desiccation threshold beyond which fragments are not viable.

Methods

Asexual reproduction success through vegetative fragmentation and its limitation by desiccation was investigated in six invasive aquatic plant species. Four of these species are known to be invasive in Ireland (*Elodea canadensis*, *E. nuttallii*, *Lagarosiphon major* and *M. aquaticum*), and two species, native to Ireland are known to be invasive elsewhere (*M. spicatum* and *Ceratophyllum demersum* L.) (Hyldgaard & Brix, 2012; Moody & Les, 2010; Reynolds, 2002). All species are of the elodeid growth form, though *M. aquaticum* possesses emergent apical shoots and *C. demersum* is an unrooted species (Parnell *et al.*, 2012). All plants were field-collected from standing water habitats in east Co. Cork, Ireland. Collected plant material was washed in flowing tap water in order to remove sediment, invertebrates and epiphytic algae. In a glasshouse, plants were propagated in aerated troughs containing tap water and field collected sediments from the River Lee flood plain.

Healthy plants were screened for signs of damage, decay or discolouration. Unbranched, apical to midstem shoots (Mcalarnen *et al.*, 2012) were then selected as experimental material. The top 5cm of apical shoots were removed as per Evans *et al.*, (2011) in order to eliminate apical meristems and tightly clustered whorls of leaves. Fresh river water was collected from the River Lee at W608717 (Irish Grid) for use during experiments. Water was stored in aerated water butts and more collected as required. The monthly average trophic status of the river water was 0.006mg/l orthophosphate and 1.4mg/l total organic nitrogen (Beechinor, 2017). The River Lee and associated flood plain wetlands support populations of five of the six species investigated in this experiment; it was thus deemed an appropriate source of water and sediments for this experiment.

Fragment Size and Propagule Success; Fragment Viability & Growth.

A propagule viability experiment was designed in order to assess what the minimal viable fragment size for each species was. A range of propagule sizes were cut to include fragments with one, two, three, five, eight and ten nodes. In the *Myriophyllum* and *Ceratophyllum* genus nodes occur with leaves in whorls of four, while the *Elodea* species possess nodes with leaves in whorls of three (Parnell *et al.*, 2012). In the case of *L. major* which has spirally arranged leaves it was assumed that a spiral of three leaves was the equivalent of a whorled node (Fig. 1). Fragments were cut midway along the internodes and immediately placed in beakers of tap water so as to prevent desiccation damage. A random sample of ten fragments from each size treatment was selected. These fragments were gently blotted dry and starting biomass recorded.

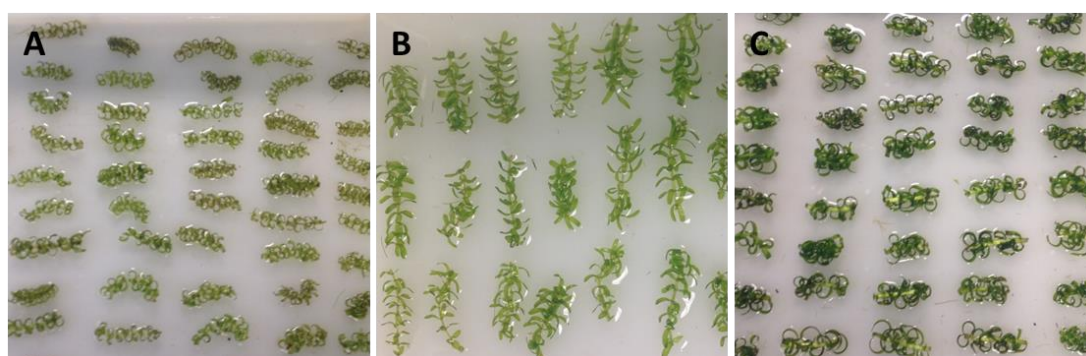


Figure 1. Fragments of species from the Hydrocharitaceae family in preparation. Leaves are in whorls of three in (A) *E. nuttallii* and (B) *E. canadensis* and are spirally arranged in (C). *L. major*.

Experimental plant propagules were transferred into unsealed magenta vessels with 300ml of river water. Magentas were then randomly placed in a temperature controlled (20°C) growth room under a 16hr photoperiod, with an average light intensity of $40\mu\text{mol.m}^{-2}.\text{s}^{-1}$. Plant fragments were allowed to grow for 31 days and topped up with river water as evaporation required. Plants were harvested after 31 days.

Harvested plants were assessed for viability. Plants were determined to have viably regenerated if new shoot or root production was observed (Redekop *et al.*, 2016). Newly produced shoots and roots were counted and lengths measured. Plant material was gently blotted dry and fresh biomass of roots, new shoots and original fragment were weighed. Percentage growth was calculated as the total biomass new shoots and new roots as a percentage of biomass of the initial fragment at the beginning of the experiment:

$$\text{Percentage Growth} = (\text{BM}_{\text{new}} / \text{BM}_{\text{start}}) * 100$$

Where BM_{new} represents the total biomass of any newly produced shoots and roots which grew over the course of the experiment, and BM_{start} represents the initial biomass of the plant fragment at the start of the experiment.

The hypothesis that there is a relationship between fragment size and fragment viability was tested using a binomial logistic regression. Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell procedure (Box & Tidwell, 1962). A Wald test was used to determine statistical significance of the independent predictor variable. In order to determine whether the relationships between fragment size and viability were different between species a Spearman's rank-order correlation, Fisher's Z-transformation and z-test were employed (Myers & Sirois, 2006).

The hypothesis that there is a relationship between fragment size and percentage growth was tested using a Spearman's rank-order correlation. The strength of the relationship was determined as per Cohen (1988). As per Myers and Sirois (2006) Spearman's correlation coefficients were transformed using Fisher's Z-Transformation, and z-tests were then performed to assess the equality of correlations for each possible combination of species.

Desiccation time, fragment size & water loss.

A desiccation experiment was implemented in order to investigate the rate at which water is lost from vegetative fragments that are removed from the aquatic environment and exposed to ambient air. Ten fragments of five or ten nodes were cut from healthy, unbranched stems of all six species. Excess water was gently blotted away and fragments

were weighed to establish baseline data. They were then arranged on plastic trays and placed in a humidity and temperature controlled room. Average ambient conditions in this room were 62.8% (SE 0.14) relative humidity and 21.1°C (SE 0.02). The biomass of each individual fragment was recorded after 0, 15, 30, 60, 120, 180, and 360 minutes of desiccation. Plant fragments were then oven dried until achieving a constant weight and average dry weight calculated. The relative water content of each individual was calculated using the following equation:

$$\text{Relative Water Content} = (BM_t - BM_d)/BM_{\text{start}}$$

Where BM_t is the fresh biomass of a fragment at a given time point, BM_d is the average dry biomass for fragments of that species and size and BM_{start} is the initial fresh biomass of the fragment at the start of the experiment.

Regression analysis was employed to test the hypothesis that there is a relationship between dessication time, fragment size and water content. To assess linearity a scatterplot with a superimposed regression line was plotted using R Studio. Visual inspection of these plots indicated a curvilinear relationship between the variables. There was homoscedasticity and normality of the residuals. No significant outliers were identified.

A quadratic polynomial regression equation that defines predicted responses (Y) in terms of the independent variables (X and Z) was fitted to the data using R Studio. The rate of change in water content (i.e. rate of water loss) are represented by a second order polynomial equation:

$$Y = b_0 + b_1X + b_2X^2 + b_3Z + b_4ZX + b_5ZX^2$$

Where Y is the predicted response, b_i are the coefficients, X is Time (min) and Z is Fragment Size. Combination of factors (such as ZX) represents an interaction between the individual factors in the respective term. The response (water content) is a function of the level of factors. The significance of the second-order model was evaluated by analysis of variance (ANOVA).

Desiccation Time, Fragment Size and Plant Propagule Success

Another desiccation experiment was run in parallel to the desiccation rate experiment above, using the same fragment size classes and desiccation periods. A total of 80

fragments of both size classes were arranged on plastic trays under the same ambient desiccation conditions as previously described for 0, 15, 30, 60, 120, 180 and 360 minutes. After exposure to the prescribed desiccation period ten fragments were randomly selected and transferred immediately to magentas containing 300ml of river water. Individuals were randomly placed in a growth room under previously described conditions for 31 days after which they were harvested. Plants were assessed for viability, measured and weighed as previously described.

To test the hypothesis that there is a relationship between the amount of time a fragment can be exposed to desiccating conditions, the size of the fragment and the viability of a fragment a binomial logistic regression was employed. Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell (1962) procedure. A Wald test was used to determine statistical significance for each of the independent predictor variables in the regression.

A multiple regression was run to test the hypothesis that there is a relationship between desiccation time, fragment size and percentage growth. Time and percentage growth data was LG10 transformed in order to meet the assumptions of linearity. There was linearity as assessed by partial regression plots and a plot of studentized residuals against the predicted values.

Results

Fragment Size and Propagule Success

Fragment Viability

The relationship between fragment size and fragment viability was investigated in a controlled experiment which measured viability of plant fragments of different sizes (as determined by no. of nodes) grown in optimum conditions over a 31-day period.

The relationship between fragment size and fragment viability is depicted for all six species in Fig. 2. *M. aquaticum* maintained 100% viability for all fragments in all size classes of this experiment; therefore, no correlation between fragment size and viability could be determined for this species. *M. spicatum* maintained similarly high viability rates throughout all treatments, with 100% viability in all size classes except the smallest which exhibited 70% viability. *E. nuttallii*, *E. canadensis* and *L. major* did not exhibit any viability at the smallest size classes as a result of high levels of mortality observed in smaller fragments. Viable fragments of *E. nuttallii* and *E. canadensis* had a minimum of three

nodes. The maximum viability observed for either of the *Elodea* species was 80%. Fragments of *L. major* required a minimum of 2 nodes to exhibit viability and achieved 100% viability in fragments of 8 nodes and larger. *C. demersum* only achieved 100% viability at 10 nodes but exhibited some viability in even the smallest of fragments.

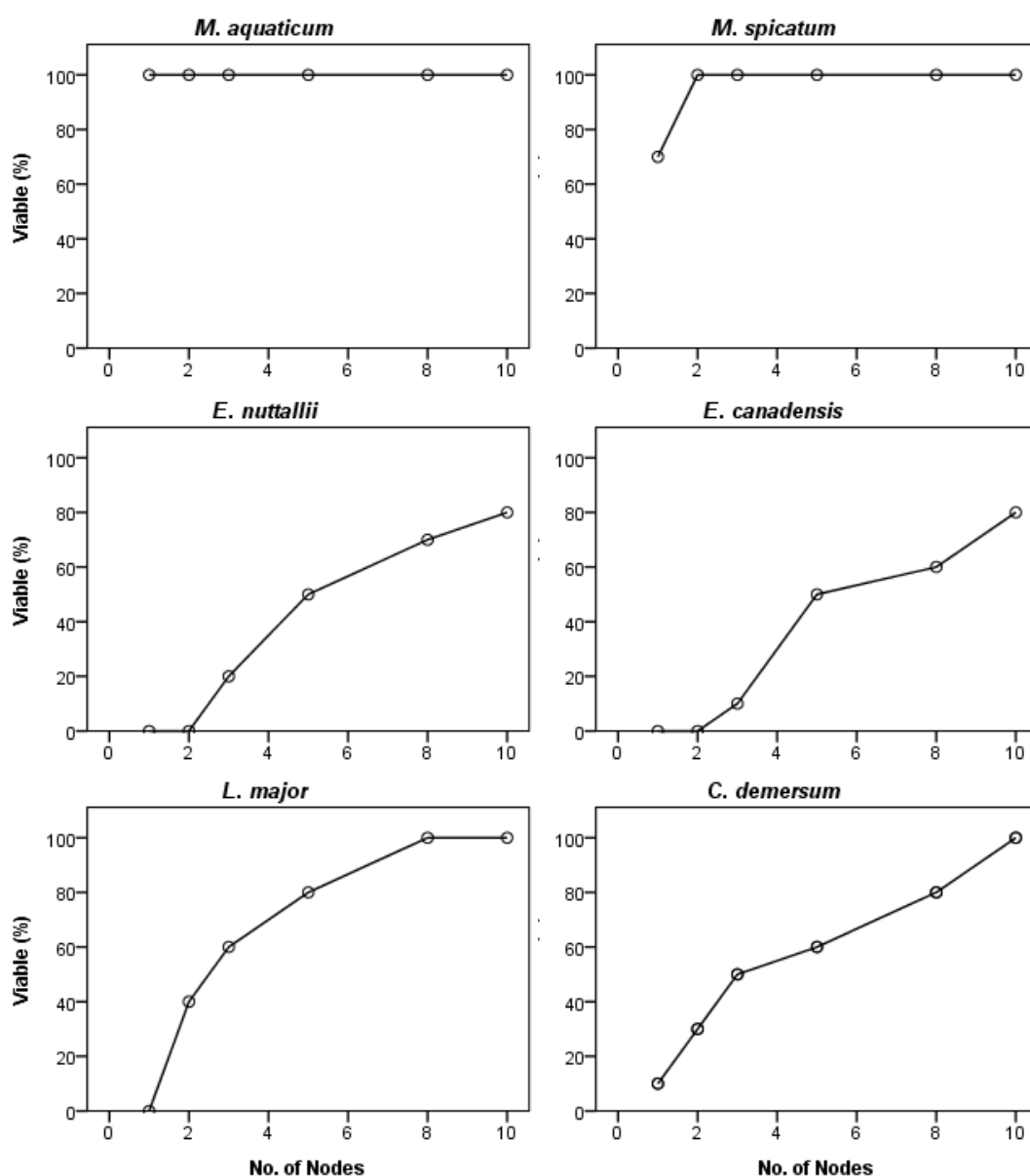


Figure2: The relationship between fragment size (as determined by number of nodes) and % of viable fragments (as determined by the production of new plant material).

A binomial logistic regression was performed to determine the effects of node number on fragment viability. *M. aquaticum* was excluded from this analysis as 100% of fragments were found to be viable for all treatments. Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell (1962) procedure. Based on this assessment, all continuous independent variables were found to be linearly related to the logit of the dependent variable. There were no outliers in the data. The logistic regression model was statistically significant for all five species, $\chi^2(1)$ ranged from 11.605–37.524, $p < 0.0005$ (Table 1). The model explained between 43.8% and 63.6% (Nagelkerke R^2) of the variance in fragment viability depending on the species (Table 1). The model correctly classified 75.0% to 95% of cases. Sensitivity ranged from 68.2–100% and specificity ranged from 0–86.8% across the study species (Table 1).

Table 1: Model fit for binomial logistic regression describing relationship between fragment size and viability.

<i>Species</i>	χ^2	<i>df</i>	<i>p</i>	Nagelkerke R^2	Correctly Classified (%)	Sensitivity (%)	Specificity (%)
<i>M. spicatum</i>	11.605	1	.001*	.537	95	100	0
<i>E. nuttallii</i>	27.813	1	.000*	.507	80	68.2	86.8
<i>E. canadensis</i>	27.644	1	.000*	.513	80	70	85
<i>L. major</i>	37.524	1	.000*	.636	83.3	89.5	72.7
<i>C. demersum</i>	23.812	1	.000*	.438	75	72.7	77.8

Table 2: Binomial logistic regression describing relationship fragment size & viability.

<i>Species</i>	β	<i>S.E.</i>	<i>Wald</i>	<i>df</i>	<i>p</i>	<i>Exp(β)</i>	95% C.I. for <i>EXP(β)</i>	
							Lower	Upper
<i>M. spicatum</i>	16.2	1576.43	.000	1	.992	10650521.44	.000	.
<i>E. nuttallii</i>	.530	.125	18.041	1	.000*	1.698	1.330	2.168
<i>E. canadensis</i>	.539	.128	17.742	1	.000*	1.714	1.334	2.203
<i>L. major</i>	.996	.289	11.921	1	.001*	2.708	1.538	4.767
<i>C. demersum</i>	.501	.131	14.639	1	.000*	1.650	1.277	2.132

A Wald test was used to determine statistical significance for each of the independent variables (Table 2). Fragment size (No. of nodes) was statistically significant ($p < 0.001$) for *E. nuttallii*, *E. canadensis*, *L. major* and *C. demersum*. Fragment size was not a significant predictor of viability of fragments of *M. spicatum*. For those species that fragment size was

a statistically significant predictor variable the odds ratio (Exp β) ranged from 1.65 in *C. demersum*, to 2.708 in *L. major* (Table 2). Odds ratios greater than 1 indicate a positive change in the odds for each increase number of nodes by one unit. Increasing the number of nodes on a fragment was thus associated with an increased likelihood of fragment viability for *E. nuttallii*, *E. canadensis*, *L. major* and *C. demersum*.

A Spearman's rank-order correlation was run to assess the relationship between fragment viability and fragment size as indicated by number of nodes on a fragment. The relationship was monotonic in five of the six study species, as assessed by visual inspection of a scatterplot. *M. aquaticum* exhibited 100% viability in all size treatments. A monotonic relationship could, therefore, not be determined and the species was excluded from the analysis.

The strength of the relationship was determined as per Cohen (1988). A strong positive correlation between fragment viability and number of nodes was identified for *E. nuttallii* ($r_s(58)=0.648$, $p<0.005$), *E. canadensis* ($r_s(58) = 0.642$, $p<0.005$), *L. major* ($r_s(58)=0.709$, $p<0.005$), and *C. demersum* ($r_s(58)=0.598$, $p<0.005$). A moderate correlation was determined for *M. spicatum* ($r_s(58)=0.336$, $p<0.05$). The results of the Spearman's rank-order correlation are summarised in table 3.

Table 3: Spearman's rank-order correlation between fragment size & viability.

<i>Species</i>	<i>Spearman's rho</i>
<i>M. aquaticum</i>	Correlation Coefficient .
	Sig. (2-tailed) .
	N 60
<i>M. spicatum</i>^{a,b,c}	Correlation Coefficient .336**
	Sig. (2-tailed) .009
	N 60
<i>E. nuttallii</i>^a	Correlation Coefficient .648**
	Sig. (2-tailed) .000
	N 60
<i>E. canadensis</i>^b	Correlation Coefficient .642**
	Sig. (2-tailed) .000
	N 60
<i>L. major</i>^c	Correlation Coefficient .709**
	Sig. (2-tailed) .000
	N 60
<i>C. demersum</i>	Correlation Coefficient .598**
	Sig. (2-tailed) .000
	N 60

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

^{a,b,c} . Species which share the same letter have statistically different correlation coefficients.

As per Myers & Sirois (2006) Spearman's correlation coefficients were transformed using Fisher's Z-Transformation, and z-tests were then performed to assess the equality of correlations for each possible combination of species. *M. spicatum* was determined to have a significantly different correlation coefficient from *E. nuttallii* (Z -2.254, $p < 0.05$), *E. canadensis* (Z -2.199, $p < 0.05$) and *L. major* (Z -2.859, $p < 0.005$). There were no statistical differences in any of the other pairwise comparisons of correlation coefficients between species.

Fragment Growth

The relationship between fragment size and growth of viable fragments was investigated in a controlled experiment which measured percentage growth of plant fragments of different sizes grown over a 31-day period. When analysing growth parameters, the binomial distribution of viable and non-viable fragments made it necessary to exclude non-viable fragments from the data analysis.

The relationship between fragment size and growth is depicted in figure 3. Percentage growth was within similar ranges for most species except *C. demersum*, which, compared to all other species, had lower percentage growth throughout its fragment size range (Note the different scale for *C. demersum* in figure 3).

Data did not meet the assumptions of linearity or homoscedasticity required to perform either a Pearson correlation or a linear regression. A Spearman's rank-order correlation was run to assess the relationship between number of nodes (fragment size) and percentage growth. Preliminary analysis showed the relationship to be monotonic in all six study species, as assessed by visual inspection of a scatterplot. The strength of the relationship was determined as per Cohen (1988). Only *E. canadensis* was found to have a significant correlation between fragment size and percentage growth ($r^2(20) = 0.808$, $p < 0.005$), this was a strong positive correlation (Table 4). As per Myers & Sirois (2006) Spearman's correlation coefficients were transformed using Fisher's Z-Transformation, and z-tests were then performed to assess the equality of correlations for each possible combination of species. The correlation coefficient of *E. canadensis* was found to be significantly different from that of all other species ($p < 0.005$). The correlation coefficient of *M. aquaticum* was also found to be significantly different from that of *C. demersum* ($p < 0.05$).

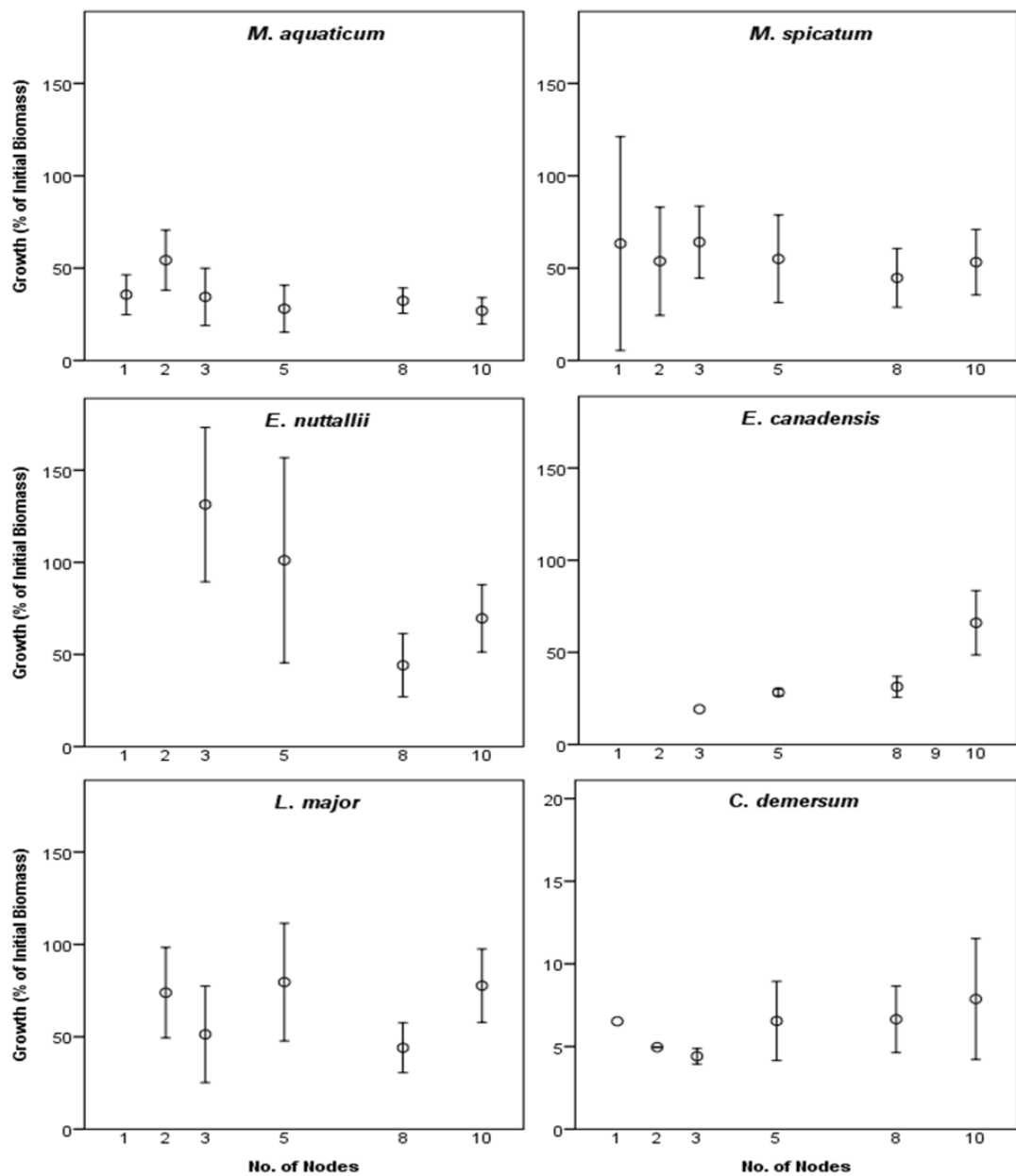


Figure 3: The relationship between fragment size (as determined by number of nodes) and fragment growth (measured as the combined biomass of freshly grown roots and shoots as a percentage of the initial biomass of plant fragments at the start of the experiment). Non-viable plants were excluded from the analysis.

Table 4: Spearman's rank-order correlation between fragment size & percentage growth.

<i>Species</i>	<i>Spearman's rho</i>	
<i>M. aquaticum</i> ^{a,f}	Correlation Coefficient	-.221
	Sig. (2-tailed)	.090
	N	60
<i>M. spicatum</i> ^b	Correlation Coefficient	.019
	Sig. (2-tailed)	.891
	N	57
<i>E. nuttallii</i> ^c	Correlation Coefficient	-.320
	Sig. (2-tailed)	.147
	N	22
<i>E. canadensis</i> ^{a,b,c,d,e,}	Correlation Coefficient	.808**
	Sig. (2-tailed)	.000
	N	20
<i>L. major</i> ^d	Correlation Coefficient	.101
	Sig. (2-tailed)	.547
	N	38
<i>C. demersum</i> ^{e,f}	Correlation Coefficient	.223
	Sig. (2-tailed)	.212
	N	33

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

^{a,b,c,d,e,f} . Species which share a letter have statistically different correlation coefficients.

Desiccation Time, Fragment Size and Water Loss

The rate at which water is lost from vegetative fragments that are removed from the aquatic environment was investigated in an experiment in which plant fragments of two sizes were exposed to ambient air. Average ambient conditions were 62.8% (SE 0.14) relative humidity and 21.1°C (SE 0.02). The data were fitted into a second order polynomial regression model to explain the relationship between independent variables (time and fragment size) and the predicted response (water content). The results for which are depicted in figure 4.

The relationship between desiccation time and water content is negative and curvilinear for all species, indicating that the rate of water loss changes over time. Large and small fragments of *M. aquaticum* & *M. spicatum* show similar regression lines. Divergence between fragments of different sizes is evident for all other species, indicating that desiccation rates may be influenced by fragment size.

The quality of the model fit was evaluated by the coefficient R^2 which represents the proportion of variation in water content. High R^2 was considered as an evidence for the applicability of the model in the range of variables included. Table 5 describes the model fit. The model was found to fit the range of variables for all six species, with adjusted R^2 values ranging from 0.88-0.96 ($p < 0.001$ in all cases). Coefficients for the fitted model are shown for each species in Table 6.

Table 5: Model fit for polynomial regression model describing the relationship between desiccation time and water content of fragments of five and ten nodes in size.

<i>Species</i>	<i>Residual SE</i>	<i>Df</i>	<i>Multiple R^2</i>	<i>Adjusted R^2</i>	<i>F-statistic</i>	<i>p-value</i>
<i>M. aquaticum</i>	0.04908	134	0.917	0.9139	296.2	< 2.2e-16***
<i>M. spicatum</i>	0.09589	134	0.8861	0.8818	208.4	< 2.2e-16***
<i>E. nuttallii</i>	0.08833	134	0.9199	0.9371	318.3	< 2.2e-16***
<i>E. canadensis</i>	0.08131	134	0.922	0.919	316.6	< 2.2e-16***
<i>L. major</i>	0.07196	134	0.9194	0.9164	305.6	< 2.2e-16***
<i>C. demersum</i>	0.07249	134	0.9484	0.9465	493	< 2.2e-16***

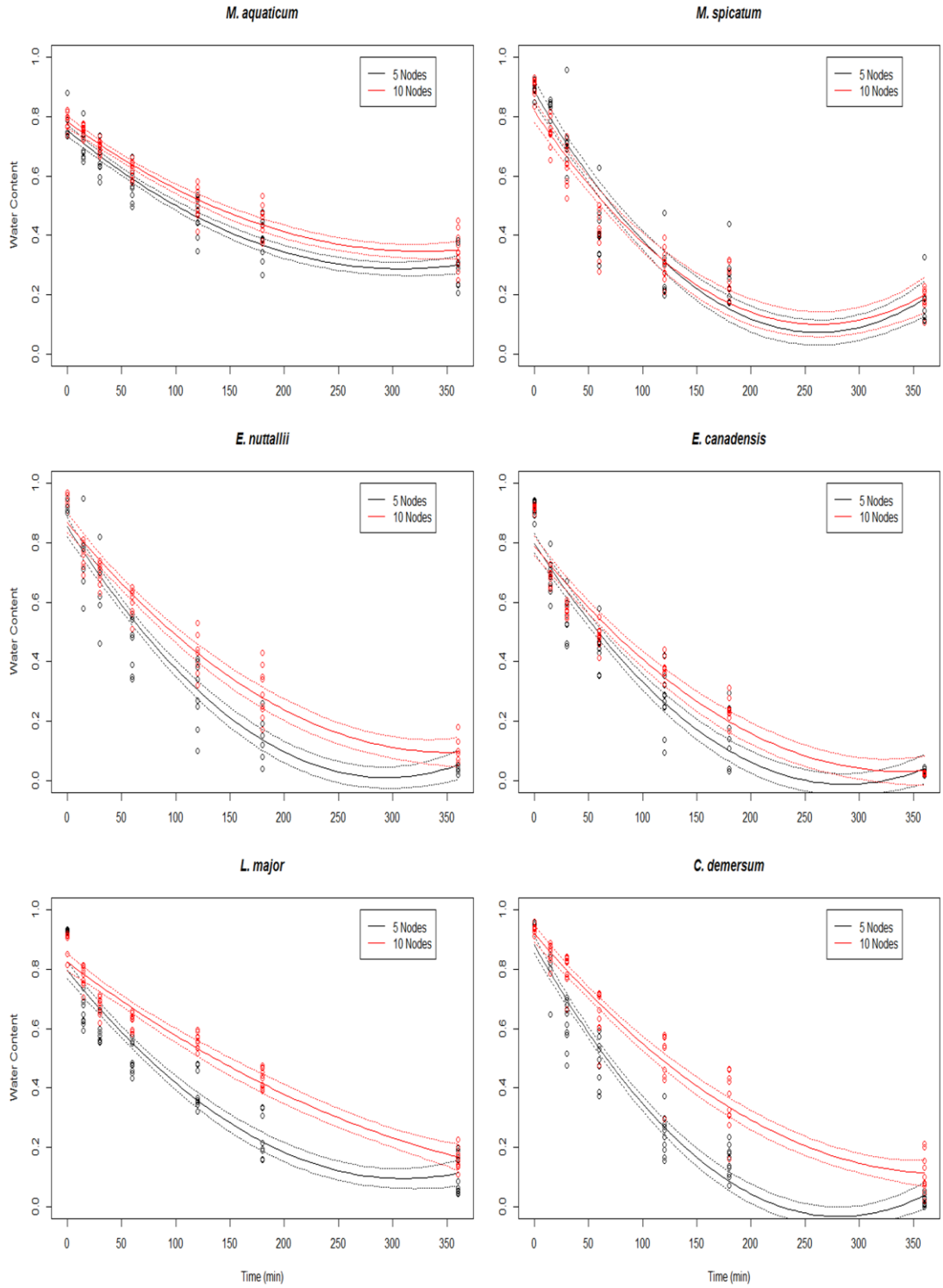


Figure 4: Water content of fragments of five or ten nodes over 360 minutes of exposure to ambient air conditions of 62.8% relative humidity and 21.1°C with fitted quadratic regression lines and 95% confidence intervals.

Table 6: Quadratic regression of water loss over time. Fitted model: $Y = b_0 + b_1X + b_2X^2 + b_3Z +$

<i>Species</i>	<i>Coefficient</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>t-value</i>	<i>Pr(> t)</i>
<i>M. aquaticum</i>	b_0 (Intercept)	7.54E-01	1.01E-02	74.566	<2e-16 ***
	b_1 (X)	-3.04E-03	1.77E-04	-17.178	<2e-16 ***
	b_2 (X^2)	4.94E-06	4.78E-07	10.344	<2e-16 ***
	b_3 (Z)	2.88E-02	1.43E-02	2.012	0.0463 *
	b_4 (ZX)	3.67E-04	2.50E-04	1.468	0.1444
	b_5 (ZX^2)	-8.61E-07	6.75E-07	-1.275	0.2046
<i>M. spicatum</i>	b_0 (Intercept)	8.84E-01	1.98E-02	44.756	<2e-16 ***
	b_1 (X)	-6.20E-03	3.46E-04	-17.941	<2e-16 ***
	b_2 (X^2)	1.18E-05	9.33E-07	12.682	<2e-16 ***
	b_3 (Z)	-6.55E-02	2.80E-02	-2.343	0.0206 *
	b_4 (ZX)	7.33E-04	4.89E-04	1.499	0.1363
	b_5 (ZX^2)	-1.44E-06	1.32E-06	-1.09	0.2775
<i>E. nuttallii</i>	b_0 (Intercept)	8.53E-01	1.65E-02	51.637	< 2e-16 ***
	b_1 (X)	-5.74E-03	2.89E-04	-19.86	< 2e-16 ***
	b_2 (X^2)	9.76E-06	7.80E-07	12.511	< 2e-16 ***
	b_3 (Z)	1.43E-02	2.34E-02	0.61	0.54269
	b_4 (ZX)	1.34E-03	4.09E-04	3.267	0.00138 **
	b_5 (ZX^2)	-3.49E-06	1.10E-06	-3.159	0.00196 **
<i>E. canadensis</i>	b_0 (Intercept)	7.98E-01	1.68E-02	47.61	< 2e-16 ***
	b_1 (X)	-5.65E-03	2.93E-04	-19.262	< 2e-16 ***
	b_2 (X^2)	9.82E-06	7.91E-07	12.418	< 2e-16 ***
	b_3 (Z)	-8.34E-03	2.37E-02	-0.352	0.72539
	b_4 (ZX)	1.17E-03	4.14E-04	2.826	0.00544 **
	b_5 (ZX^2)	-3.23E-06	1.12E-06	-2.888	0.00452 **
<i>L. major</i>	b_0 (Intercept)	7.97E-01	1.48E-02	53.725	< 2e-16 ***
	b_1 (X)	-4.53E-03	2.59E-04	-17.463	< 2e-16 ***
	b_2 (X^2)	7.30E-06	7.00E-07	10.429	< 2e-16 ***
	b_3 (Z)	2.73E-02	2.10E-02	1.302	0.195
	b_4 (ZX)	1.80E-03	3.67E-04	4.912	2.58e-06 ***
	b_5 (ZX^2)	-4.80E-06	9.90E-07	-4.842	3.48e-06 ***
<i>C. demersum</i>	b_0 (Intercept)	8.82E-01	1.49E-02	59.041	< 2e-16 ***
	b_1 (X)	-6.51E-03	2.61E-04	-24.909	< 2e-16 ***
	b_2 (X^2)	1.16E-05	7.05E-07	16.394	< 2e-16 ***
	b_3 (Z)	3.81E-02	2.11E-02	1.805	0.0734.
	b_4 (ZX)	2.24E-03	3.70E-04	6.065	1.27e-08 ***
	b_5 (ZX^2)	-5.94E-06	9.97E-07	-5.956	2.15e-08 ***

b = Coefficient, X = Time (min), Z = Fragment size (no. of nodes), ZX = Interaction.

Table 7: Analysis of variance of quadratic regression of water loss over time in aquatic plants.

<i>Species</i>	<i>ANOVA</i>	<i>Df</i>	<i>Sum Sq</i>	<i>Mean Sq</i>	<i>F value</i>	<i>P (>F)</i>
<i>M. aquaticum</i>	Time	1	3.05706	3.05706	1268.8776	< 2.2e-16 ***
	Time ²	1	0.42966	0.42966	178.3374	< 2.2e-16 ***
	Size	1	0.07615	0.07615	31.6085	1.052e-07 ***
	Time:Size	1	0.00183	0.00183	0.7600	0.3849
	(Time ²):Size	1	0.00391	0.00391	1.6248	0.2046
	Residuals	134	0.32284	0.00241		
<i>M. spicatum</i>	Time	1	6.9204	6.9204	752.6559	< 2.2e-16 ***
	Time ²	1	2.6088	2.6088	283.7294	< 2.2e-16 ***
	Size	1	0.0180	0.0180	1.9556	0.1643
	Time:Size	1	0.0238	0.0238	2.5909	0.1098
	(Time ²):Size	1	0.0109	0.0109	1.1891	0.2775
	Residuals	134	1.2321	0.0092		
<i>E. nuttallii</i>	Time	1	9.6268	9.6268	1496.7326	< 2.2e-16 ***
	Time ²	1	1.3588	1.3588	211.2575	< 2.2e-16 ***
	Size	1	0.1715	0.1715	26.6640	8.556e-07 ***
	Time:Size	1	0.0045	0.0045	0.7006	0.404071
	(Time ²):Size	1	0.0642	0.0642	9.9779	0.001958**
	Residuals	134	0.8619	0.0064		
<i>E. canadensis</i>	Time	1	8.9418	8.9418	1352.4449	< 2.2e-16 ***
	Time ²	1	1.4235	1.4235	215.3046	< 2.2e-16 ***
	Size	1	0.0455	0.0455	6.8796	0.009728 **
	Time:Size	1	0.0002	0.0002	0.0376	0.846488
	(Time ²):Size	1	0.0551	0.0551	8.3395	0.004525 **
	Residuals	134	0.8860	0.0066		
<i>L. major</i>	Time	1	6.9280	6.9280	1337.9705	< 2.2e-16 ***
	Time ²	1	0.5081	0.5081	98.1287	< 2.2e-16 ***
	Size	1	0.3509	0.3509	67.7653	1.444e-13 ***
	Time:Size	1	0.0046	0.0046	0.8892	0.3474
	(Time ²):Size	1	0.1214	0.1214	23.4484	3.485e-06 ***
	Residuals	134	0.6938	0.0052		
<i>C. demersum</i>	Time	1	10.6141	10.6141	2019.9603	< 2.2e-16 ***
	Time ²	1	1.5596	1.5596	296.8062	< 2.2e-16 ***
	Size	1	0.5850	0.5850	111.3269	< 2.2e-16 ***
	Time:Size	1	0.0081	0.0081	1.5379	0.2171
	(Time ²):Size	1	0.1864	0.1864	35.4764	2.146e-08 ***
	Residuals	134	0.7041	0.0053		

The statistical significance of the second order polynomial model was verified by ANOVA (Table 7). Analysis of variance indicated that the first-order effects of time ($p < 0.001$) and fragment size ($p < 0.01$) on water content were significant in all species. A significant second-order interaction between time and fragment size ($p < 0.01$) was detected for *E. canadensis*, *E. nuttallii*, *L. major* and *C. demersum* (Table 7). This interaction indicates that the rate of water loss over time differs with fragment size for these species. No significant interaction was identified between time and fragment size in *M. aquaticum* or *M. spicatum* (Table 7), indicating that fragment size does not cause a difference in the decline in water content in these two species.

The effects of time and fragment size on the water content of plant fragments are visualised in Figure 4. The decrease in water content over time is evident in all species and fragment sizes. The curvilinear trends identified by the fitted quadratic models indicate that the initial rate of water loss is great, and that the rate slows over time. The interactions between time and fragment size identified by the ANOVA are also visible in Figure 2, where larger fragments of *E. nuttallii*, *E. canadensis*, *L. major* and *C. demersum* lose water content at a slower rate, as represented by a gentler slope of the line.

Desiccation Time, Fragment Size and Propagule Success

Fragment Viability

The relationship between desiccation time, fragment size and fragment viability was investigated in a controlled experiment which measured viability of plant fragments after exposure to desiccating conditions for 0, 15, 30, 60, 120, 180 or 360 minutes. Viability was quantified after 31 days of growth post-desiccation exposure. Experiments were run with plant fragments of five and ten nodes

The resulting relationship between desiccation time, fragment size and fragment viability is depicted in figure 5. In general, larger fragments maintained a greater level of viability throughout the experiment, when compared to smaller fragments. A negative relationship between desiccation time and viability is visible for large and small fragments of all species, except *M. aquaticum*. Larger fragments of *M. aquaticum* maintained 100% viability even after exposure to 360min of desiccating conditions. Smaller fragments of *M. aquaticum* maintained 100% viability up to 180min after which a decline in viability was observed. No viability was observed for smaller fragments of *M. spicatum*, *L. major*, and *C. demersum*

after 360min desiccation exposure, but larger fragments of these species maintained some low levels of viability after 360min desiccation time. *E. canadensis* was the least tolerant of desiccation as small and large fragments exhibited no viability after 60min and 120min respectively. Small fragments of *E. nuttallii* were not viable after 180min of desiccation while larger fragments maintained low levels of viability up to 360min.

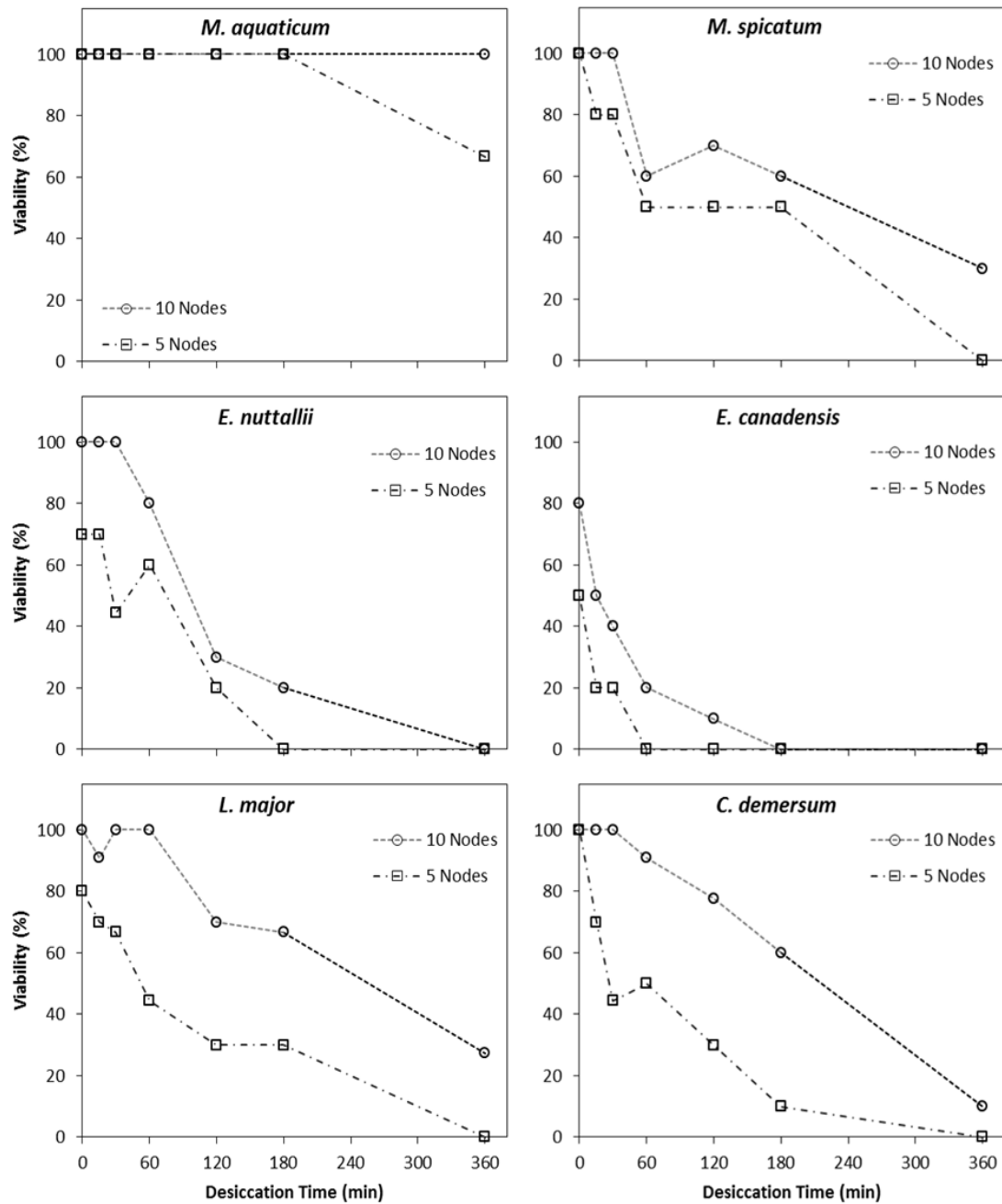


Figure 5: Viability of plant fragments of either five nodes or ten nodes post exposure to desiccating conditions for up to 360 minutes.

A binomial logistic regression was performed to determine the effect of desiccation time and fragment size on the viability of plant fragments from each species. Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell (1962) procedure. Based on this assessment, the continuous independent variable (desiccation time) was found to be linearly related to the logit of the dependent variable (viability). There were a number of studentized residuals which were kept in the analysis, standard deviations ranged from -4.669 to 6.047.

Table 8 describes the model fit for a binomial logistic regression describing the relationship between desiccation time, fragment size and viability. The model was statistically significant for all six species, $\chi^2(2)$ ranged from 16.776 in *M. aquaticum* to 85.128 in *E. nuttallii*, $p < .005$ in all cases. The model explained between 39.5% and 61.5% (Nagelkerke R^2) of the variance in fragment viability depending on the species. The model correctly classified 76.4% to 97.9% of cases. Sensitivity ranged from 44.8-100% and specificity ranged from 0-93.7% across the study species.

Table 8: Model fit for binomial logistic regression describing relationship between dessication time, fragment size and viability.

<i>Species</i>	χ^2	<i>df</i>	<i>p</i>	<i>Nagelkerke</i> R^2	<i>Correctly</i> <i>Classified</i> (%)	<i>Sensitivity</i> (%)	<i>Specificity</i> (%)
<i>M. aquaticum</i>	16.776	2	.000	.604	97.9	100	0
<i>M. spicatum</i>	46.919	2	.000	.395	76.4	91.4	46.8
<i>E. nuttallii</i>	85.128	2	.000	.608	80.7	81.2	80.3
<i>E. canadensis</i>	50.801	2	.000	.476	83.6	44.8	93.7
<i>L. major</i>	64.771	2	.000	.493	79.3	90.2	60.4
<i>C. demersum</i>	84.745	2	.000	.615	82.1	94.1	63.6

A Wald test was used to determine statistical significance for each of the independent predictor variables (Table 9). Both predictor variables, desiccation time and fragment size had statistically significant influences on viability in all species except *M. aquaticum* (Table 9). Increasing desiccation time was associated with reduced likelihood of exhibiting viability as evident by odds ratios (Exp β) less than 1 (0.944-0.984 reduction in odds for every minute increase in desiccation time). After controlling for desiccation time, larger fragments of ten nodes had up to 15.2 times higher odds (*C. demersum*) to exhibit viability than smaller fragments of five nodes (Table 9).

Table 9: Binomial logistic regression describing relationship between desiccation time, fragment size & viability.

<i>Species</i>	<i>Variables</i>	<i>B</i>	<i>S.E.</i>	<i>Wald</i>	<i>df</i>	<i>Sig.</i>	<i>Exp(B)</i>
<i>M. aquaticum</i>	<i>Minutes</i>	-.094	12.761	.000	1	.994	.910
	<i>Size(1)</i>	17.966	3847.695	.000	1	.996	-
	<i>Constant</i>	34.728	4594.003	.000	1	.994	-
<i>M. spicatum</i>	<i>Minutes</i>	-.012	.002	28.448	1	.000	.988
	<i>Size(1)</i>	1.033	.452	5.231	1	.022	2.810
	<i>Constant</i>	1.572	.350	20.174	1	.000	4.818
<i>E. nuttallii</i>	<i>Minutes</i>	-.029	.005	31.030	1	.000	.971
	<i>Size(1)</i>	1.906	.576	10.940	1	.001	6.727
	<i>Constant</i>	1.478	.401	13.610	1	.000	4.383
<i>E. canadensis</i>	<i>Minutes</i>	-.038	.010	13.527	1	.000	.963
	<i>Size(1)</i>	1.379	.528	6.833	1	.009	3.973
	<i>Constant</i>	-.441	.437	1.023	1	.312	.643
<i>L. major</i>	<i>Minutes</i>	-.013	.003	27.621	1	.000	.987
	<i>Size(1)</i>	2.705	.601	20.262	1	.000	14.957
	<i>Constant</i>	1.033	.335	9.495	1	.002	2.810
<i>C. demersum</i>	<i>Minutes</i>	-.019	.004	28.520	1	.000	.981
	<i>Nodes(1)</i>	2.720	.617	19.454	1	.000	15.174
	<i>Constant</i>	1.268	.367	11.934	1	.001	3.553

Fragment Growth

The relationship between desiccation time, fragment size and growth of viable fragments was investigated in a controlled experiment which quantified the growth of plant fragments after exposure to desiccating conditions for 0, 15, 30, 60, 120, 180 or 360 minutes. At 31 days post desiccation exposure growth was measured as the biomass of freshly grown roots and shoots as a percentage of the initial biomass of plant fragments. Experiments were run with plant fragments of five and ten nodes. When analysing growth parameters, the binomial distribution of viable and non-viable fragments made it necessary to exclude non-viable fragments from the data analysis.

The resulting relationship between desiccation time, fragment size and fragment growth is depicted in figure 6. A negative relationship between desiccation time and growth is visible for large and small fragments of all species. Percentage growth range was approximately the same for all species except *C. demersum*, which achieved a smaller range of growth than the other five species, as is evident by the difference in the scale of its y-axis in figure 6. In general, with the exception of *M. aquaticum*, larger fragments achieved slightly more growth throughout the experiment, when compared to smaller fragments of the same species, though the difference between percentage growth of large and small fragments is not statistically different for any species.

A multiple regression was run to predict the effect of desiccation time (Minutes) and fragment size (No. of Nodes) on the percentage growth. Time and percentage growth data was LG10 transformed. There was linearity as assessed by partial regression plots and a plot of studentized residuals against the predicted values. There was independence of residuals, as assessed by a Durbin-Watson statistic which ranged from 1.115-2.728. There was homoscedasticity, as assessed by visual inspection of a plot of studentized residuals versus unstandardized predicted values. There was no evidence of multicollinearity, as assessed by tolerance values greater than 0.1. *M. aquaticum*, *M. spicatum* and *E. nuttallii* each had one studentized deleted residuals greater than ± 3 standard deviations. There were no leverage values greater than 0.2, and values for Cook's distance above 1. The assumption of normality was met, as assessed by Q-Q Plot.

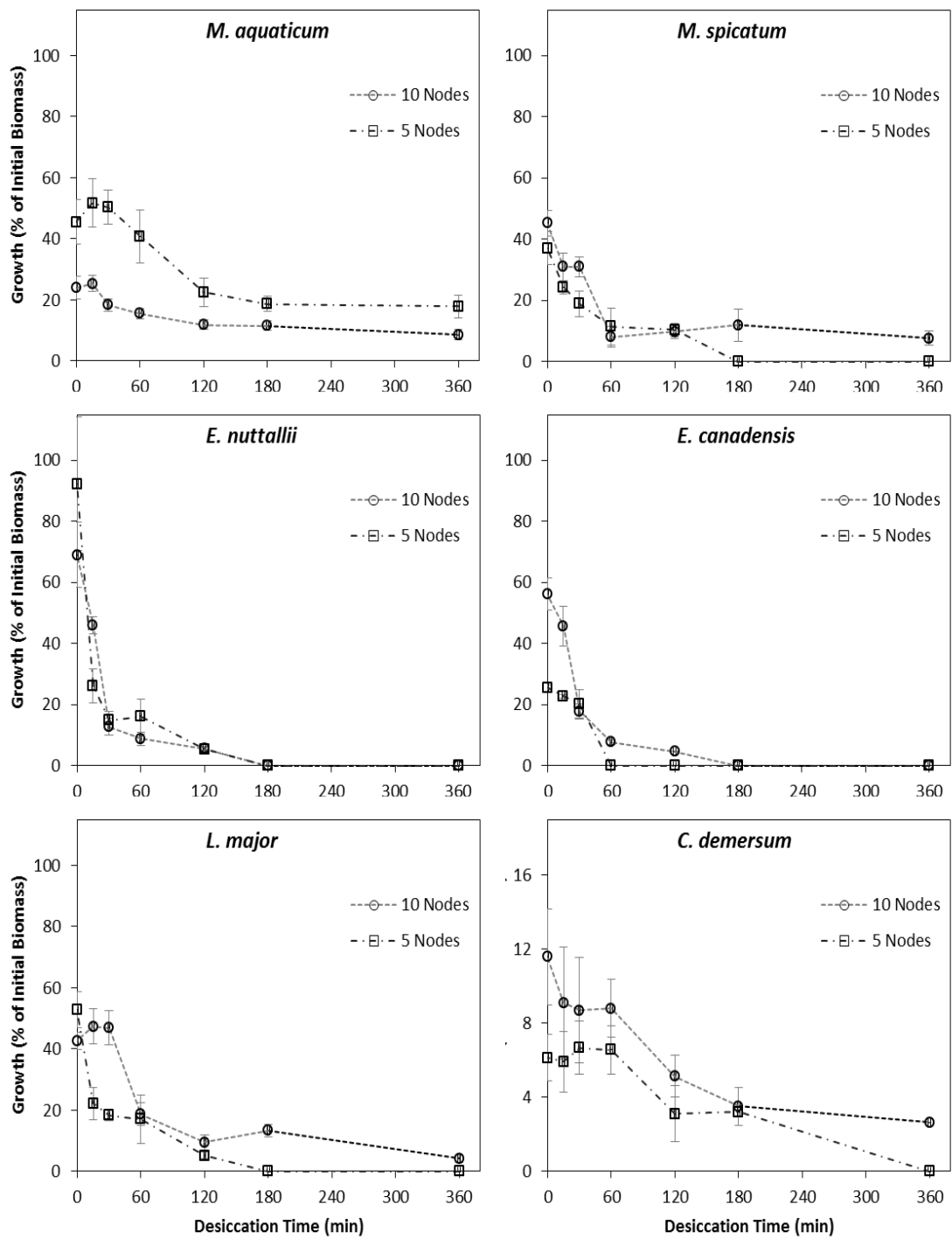


Figure 6: The relationship between desiccation time (min) and fragment growth (measured as the combined biomass of freshly grown roots and shoots as a percentage of the initial biomass of plant fragments at the start of the experiment) in fragments of either 5 or 10 nodes. Non-viable plants were excluded from the analysis.

The multiple regression model significantly ($p < 0.005$) predicted percentage growth for most species except *C. demersum* (Table 10). Desiccation time was significantly negatively associated with growth in all other species ($p < 0.05$). Increase in desiccation time was associated with a significant decrease in growth for all species. Reduction in p-growth for each additional minute of desiccation time ranged from -0.190 in *M. aquaticum* to -0.476 in *E. nuttallii*. Fragment size added significantly to the model for *M. aquaticum*, *E. canadensis* and *L. major* ($p < 0.05$). As fragment size variable was dichotomous the value of the slope coefficient represents the difference in growth between larger fragments of ten nodes and smaller fragments of five nodes. The relationship between fragment size and growth was negative for *M. aquaticum*, but positive for *E. canadensis* and *L. major*. This indicates that larger fragments of *M. aquaticum* exhibited less growth than smaller fragments of the same species throughout the desiccation experiment. The opposite was true for *E. canadensis* and *L. major* where large fragments exhibited statistically more growth than smaller fragments throughout all desiccation treatments. Multiple regression coefficients are shown in table 11.

Table 10: Analysis of Variance on Multiple Regression describing relationship between desiccation time^b, fragment size^b and growth^a.

<i>Species</i>	<i>Model</i>	<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>M. aquaticum</i>	Regression	6.634	2	3.317	61.583	.000 ^b
	Residual	7.218	134	.054		
	Total	13.852	136			
<i>M. spicatum</i>	Regression	6.390	2	3.195	22.757	.000 ^b
	Residual	12.636	90	.140		
	Total	19.026	92			
<i>E. nuttallii</i>	Regression	8.323	2	4.162	40.737	.000 ^b
	Residual	6.844	67	.102		
	Total	15.168	69			
<i>E. canadensis</i>	Regression	1.328	2	.664	14.133	.000 ^b
	Residual	1.222	26	.047		
	Total	2.550	28			
<i>L. major</i>	Regression	7.440	2	3.720	38.954	.000 ^b
	Residual	8.022	84	.095		
	Total	15.462	86			
<i>C. demersum</i>	Regression	.567	2	.284	2.227	.114 ^b
	Residual	10.440	82	.127		
	Total	11.007	84			

^a. Dependent Variable: LG10(p-growth)

^b. Predictors: (Constant), Size (5 or 10 Nodes) , Time (LG10(Minutes))

Table 11: Coefficients^a of Multiple Regression Model describing relationship between desiccation time^b, fragment size^b and growth^a.

Species	Variable	Unstandardized Coefficients		Standardized Coefficients	Sig
		B	SE _B	β	
<i>M. aquaticum</i>	(Constant)	2.089	.074		.000
	Time ^b	-.190	.025	-.468	.000
	Size ^b	-.063	.008	-.497	.000
<i>M. spicatum</i>	(Constant)	1.565	.140		.000
	Time ^b	-.330	.049	-.582	.000
	Size ^b	.012	.016	.066	.449
<i>E. nuttallii</i>	(Constant)	1.898	.144		.000
	Time ^b	-.476	.053	-.739	.000
	Size ^b	-.004	.016	-.023	.777
<i>E. canadensis</i>	(Constant)	1.357	.153		.000
	Time ^b	-.286	.055	-.714	.000
	Size ^b	.037	.018	.290	.045
<i>L. major</i>	(Constant)	1.479	.125		.000
	Time ^b	-.368	.042	-.692	.000
	Size ^b	.039	.014	.219	.007
<i>C. demersum</i>	(Constant)	.717	.144		.000
	Time ^b	-.101	.050	-.222	.046
	Size ^b	.016	.016	.104	.346

^a Dependent Variable: Growth (LG10(p-growth))

^b Predictors: (Constant), Size (5 or 10 Nodes), Time (LG10(Minutes))

Discussion

It is well documented that vegetative fragments are important in the reproduction and dispersal of aquatic plants. Some studies have investigated the regenerative capacity of aquatic plant fragments in differing receptive environments, while others have studied the potential for hydrochoric and anthropogenic dispersal of plant fragments. It has been suggested that the success of a vegetative fragment as a propagule for reproduction and dispersal is limited by the plant's ability to survive fragmentation and, if dispersed over land, its ability to tolerate desiccation stress (Barnes *et al.*, 2013). In order to test this hypothesis this study first investigated the relationship between fragment size and propagule fitness, in terms of regenerative viability and growth. It then investigated the

rate at which plant fragments became desiccated and the relationship between desiccation time, fragment size and fragment viability and growth.

The Relationship between Fragment Size and Propagule Success

Allofragments can be generated in a range of sizes (Heidbüchel *et al.*, 2016). The present study hypothesised that the size of a vegetative fragment from an aquatic plant influences its regenerative viability and growth potential. There was a strong positive relationship between fragment size and propagule viability found for *E. nuttallii*, *E. canadensis*, *L. major* and *C. demersum*. A decrease in fragment size therefore is associated with reduced likelihood of propagule viability in these species.

No viability was recorded in fragments of less than three nodes for either *Elodea* species, making them the least tolerant of fragmentation into small pieces. *C. demersum* maintained some low levels (10%) of viability in even the smallest of fragments. *M. spicatum* exhibited high levels of viability (70-100%) throughout all treatments. *M. aquaticum* displayed equally high viability (100%) in every size treatment. Both *Myriophyllum* species are therefore highly tolerant of fragmentation (Fig. 7). In fact, this study showed that *M. aquaticum*, *M. spicatum* and *C. demersum* were capable of regenerating from a single node. *L. major* remains viable at two nodes while both *Elodea* species require a minimum of three nodes to remain viable. The observed minimal viable sizes are supported by data indirectly gleaned from other studies on *M. aquaticum*, *M. spicatum*, *E. nuttallii*, *E. canadensis* and *C. demersum* (Hoffmann *et al.*, 2014; Hussner, 2009; Kuntz *et al.*, 2014; Redekop *et al.*, 2016; Riis *et al.*, 2009). This research offers new insights into the fragmentation tolerance of the highly invasive species *L. major*.



Figure 7: Regeneration of roots and shoots in viable 3 node fragments of *M. aquaticum*.

As shown in this study, propagule viability increases with fragment size. Previous work conducted using different aquatic plant species (*Limnophila sessiflora* (Vahl) Blume, *Hygrophila polysperma* (Roxb.) T. Anderson, *Ipomoea aquatica* Forsk., *A. philoxeroides*) found similar relationships between fragment size/node number and regeneration success (Dong *et al.*, 2010; Lin *et al.*, 2012; Spencer and Bowes, 1985), but this is the first time that this has been demonstrated for the present suite of species.

A positive correlation between fragment size/number of nodes, and regenerative viability may be explained by the presence of meristematic tissue which allows for the development of new tissue that is usually located in the nodes or buds of aquatic plants (Barrat-Segretain *et al.*, 1998). Also abundance of nodes controls the number of leaves a fragment possesses; a greater number of photosynthesising leaves may enhance survival rates and viability (Hussner, 2009) by providing additional photosynthetic capacity. Alternatively, carbohydrate and nutrient reserves in larger fragments may aid the survival and regeneration of plant propagules. In shoot or stoloniferous fragments internodes have been shown to play a role in propagule survival because they are a source of soluble proteins and non-structural carbohydrates that can be reallocated to the regenerating node (Dong *et al.*, 2010). This may be especially important for unrooted vegetative propagules that lack capacity to acquire nutrients and water from the surrounding environment. Fitness and survival of vegetative fragments may thus be correlated with fragment length for a number of reasons.

Fragment size was not found to be significantly correlated with percentage growth, except in the case of *E. canadensis*. This was a strong positive correlation indicating that larger fragments of *E. canadensis* are not only more likely to be viable but will also grow at a greater rate. For the other species, the fact that there is no relationship between fragment size and percentage growth indicates viable fragments of all sizes will grow at similar rates. Similarly, Li *et al.*, (2016) showed that fragment size did not have an effect on the relative growth rate of viable *M. spicatum* fragments. Thus, if viable, propagules may become equally problematic in receiving ecosystems regardless of their size.

Aquatic plant fragments are regularly found in the wild as a result of auto fragmentation or allofragmentation caused by natural or anthropogenic disturbances (Heidbüchel *et al.*, 2016). Different species show differences in size class distribution of fragments, e.g. *E. canadensis* produces, on average, significantly longer fragments than *L. major* as a result of disturbance by water velocity (Redekop *et al.*, 2016). It has been documented that smaller

plant fragments are more easily dispersed by water (Riis and Sand-Jensen, 2006) and more likely to be dispersed, anthropogenically, overland (Rothlisberger *et al.*, 2010). It is evident that all species studied in the present study are capable of regenerating from relatively small fragments and maintaining growth. This has important implications for the management and dispersal of such species.

Desiccation Time, Fragment Size and Water Loss

The rate at which water is lost from a plant fragment upon aerial exposure may influence the duration a propagule can tolerate being exposed to desiccating conditions. It was hypothesised that both the duration of aerial exposure and the fragment size influence the rate of water loss, thus impacting on fragment fitness.

The relationship between desiccation time and water content was found to be negative and curvilinear for all studied species. A negative curvilinear trend indicates an exponential decay in water content over time. The curvilinear trends identified by the fitted quadratic models indicate that the initial rate of water loss is great, and that the rate slows over time as the fragment approaches its final dry mass. This pattern of water loss has previously been observed in a suite of aquatic plant species (Barnes *et al.*, 2013; Basiouny *et al.*, 1978; Evans *et al.*, 2011; Jerde *et al.*, 2012).

M. aquaticum fragments lost water at a slower rate than all other species. This is, most likely, because fragments of emergent (aerial/above water) shoots of this species were used in this experiment. Emergent tissue of *M. aquaticum* is more tolerant of desiccation than its submerged counterpart because it possesses a thicker cuticle than submerged parts which have strongly reduced cuticle (Ebke *et al.*, 2013). The cuticle of emergent tissue of *M. aquaticum* is also thicker than that of other aquatic plant species, though compared to land plants this cuticle is modest (Do Amaral *et al.*, 1990).

Importantly, the present study detected an interaction between desiccation time and fragment size on the resulting water content of fragments of *E. canadensis*, *E. nuttallii*, *L. major* and *C. demersum*. In this study, larger fragments of these species lost water at a slower rate than smaller fragments of the same species. Such an interaction has not previously been observed in these species. No interaction between fragment size and desiccation rate was found for fragments of *M. aquaticum* or *M. spicatum* in this study. Likewise, Jerde *et al.*, (2012), did not identify any difference in desiccation rate between *M. spicatum* fragments of different lengths. However, another study has previously shown that smaller fragments of *M. spicatum* dry out more quickly than larger fragments

(Mcalarnen *et al.*, 2012). Differentiation in desiccation rate by fragment size may play a role in fragment survival and viability post desiccation.

Desiccation Time, Fragment Size and Propagule Success

The rate of water loss and impact of desiccation on fitness has previously been investigated in a number of aquatic plant species (Barnes *et al.*, 2013; Basiouny *et al.*, 1978; Bickel, 2015; Bruckerhoff *et al.*, 2015; Evans *et al.*, 2011; Jerde *et al.*, 2012; Michelin *et al.*, 2010). However, studies that investigate the relationship between fragment size, desiccation and propagule fitness are limited and restricted to studies on *M. spicatum* and *Hydrilla verticillata* (L.f.) Royle (Baniszewski *et al.*, 2016; Jerde *et al.*, 2012; Mcalarnen *et al.*, 2012). This study investigates the relationship between fragment size, desiccation and propagule fitness of six aquatic plant species.

A negative relationship between desiccation time and fragment viability was observed in both large and small fragments of all study species, with the exception of *M. aquaticum* which maintained high viability throughout. Results were consistent with previous studies that also showed a decline in viability as desiccation time was increased (Barnes *et al.*, 2013; Basiouny *et al.*, 1978; Bickel, 2015; Bruckerhoff *et al.*, 2015; Evans *et al.*, 2011; Jerde *et al.*, 2012; Mcalarnen *et al.*, 2012). Viability of *M. aquaticum* had previously been shown to be unaffected by desiccation for up to three hours in low humidity (Barnes *et al.*, 2013).

In general, larger fragments maintained a greater level of post-desiccation viability throughout the experiment, when compared to smaller fragments. The influence of desiccation time and fragment size on the post-desiccation viability of propagules was statistically significant for all species except *M. aquaticum*. After controlling for desiccation time, larger fragments had up to 15 times higher odds to exhibit viability than smaller fragments. This loss of viability in smaller fragments is likely to be related to the increased loss of water from such fragments. Small fragments of *E. canadensis* were least tolerant of desiccation and were not viable after an hour of desiccation. Large fragments of *M. aquaticum*, *M. spicatum*, *L. major* and *C. demersum* still exhibited viability after six hours of desiccation, while *E. nuttalli* was viable up to six hours and *E. canadensis* up to three hours.

Similarly, as increase in desiccation time was associated with a significant decrease in percentage growth of both large and small viable fragments, with a reduction in growth for each additional minute of desiccation time predicted. Of the six species, percentage growth of viable fragments of *M. aquaticum* were affected the least by desiccation time, while desiccation time had the greatest effect on growth of viable fragments of *E. nuttalli*. After

accounting for the influence of desiccation time, fragment size was found to significantly influence post-desiccation growth in *M. aquaticum*, *E. canadensis*, and *L. major*. In the case of *E. canadensis* and *L. major* larger fragments exhibited more growth than smaller fragments post desiccation.

In the case of *M. spicatum*, Jerde *et al.*, (2012) had previously found that fragments up to 15cm in length did not survive more than three hours of desiccation at 40% humidity, however much longer fragments, up to 65cm in length, were shown to survive up to five hours if coiled around themselves and a piece of boating equipment. The enhanced survival and viability of clumped and coiled plants is likely a result of insulation against desiccation stress, as the outer layers protect inner material and reduce water loss, by reducing the surface area exposed to the air (Bruckerhoff *et al.*, 2015; Jerde *et al.*, 2012), despite remaining viable for long periods of desiccation, coiled fragments still experience a reduction in growth as desiccation time increased (Bruckerhoff *et al.*, 2015).

The physiological mechanisms involved in reducing aquatic plant fitness after desiccation have been described in detail in the case of *H. verticillata*, a member of the Hydrocharitaceae family of which *Elodea* and *Lagarosiphon* are also part (Basiouny *et al.*, 1978; Kar and Choudhuri, 1982; Kar, 1988). At the biochemical level the plant experiences loss in chlorophyll, protein and carbohydrates as the plant respiration rate increases (Basiouny *et al.*, 1978; Kar, 1988). This is accompanied by deteriorative changes such as general cellular disruption, including an increase in tissue permeability (the leakage of ions from leaf tissue) and a decline in RNA concentration. Increase in tissue permeability indicates that membrane systems are rapidly affected by desiccation (Kar and Choudhuri, 1982; Kar, 1988). Studies on other species of aquatic plant have shown that photosynthetic activity also declines as a result of desiccation stress (Adams and Bate, 1994). Recovery from physiological damage is possible after short periods of desiccation stress (Adams and Bate, 1994; Kar and Choudhuri, 1982); however, prolonged exposure will result in overall cellular death and tissue degradation.

Knowledge Applications

Current management practises are often restricted to mechanical removal of aquatic invasive plant species. Such practises involve cutting and/or dredging of large volumes of plant biomass out of the water (Hussner *et al.*, 2017). Such methods frequently result in the production of large numbers of vegetative fragments that break away and remain in the aquatic environment (Bowmer *et al.*, 1979; Caffrey *et al.*, 2011; Hussner, 2009; Zehnsdorf

et al., 2015). This study and others show that even the smallest of fragments that remain in the water have the potential to regenerate and recolonize the managed habitat. Additionally, such small fragments are capable of being dispersed long distances by moving water (Riis and Sand-Jensen, 2006). This study shows that viability and growth potential are enhanced when fragments are larger and possess more nodes. This study only considered fragments without apical tips; however, it has been shown that fragments with apical tips have even higher survival rates and greater regeneration capacity (Riis *et al.*, 2009). Further, it is known that season and the abiotic conditions of the receiving environment also influence fragment success; abiotic factors influencing fragment success include light, nutrient availability, temperature and substrate (Barrat-Segretain and Bornette, 2000; Hoffmann *et al.*, 2014; Kuntz *et al.*, 2014; Li *et al.*, 2016; Li, 2014; Mielecki and Pieczyńska, 2005; Vári, 2013).

The size distribution of fragments generated by mechanical removal of aquatic plants has not been investigated, though it may be assumed that both large and small fragments are accidentally produced. The present study cannot exactly predict the likelihood of fragment regeneration and establishment after mechanical removal of invasive vegetation. However, the study, coupled with others can inform managers of potential risks associated with anthropogenic allofragmentation of invasive plants. Care must be taken to minimize plant fragmentation during the removal process, and due caution practised with respect to removal of vegetative debris. This study suggests that mechanical management may not always be an effective and appropriate means of aquatic invasive plant management. This may especially be the case for species such as *M. aquaticum*, which has extremely high regenerative success rate at all fragment sizes. In the Irish context *M. aquaticum* is at the early stages of its invasion (Kelly *et al.*, 2014). Its distribution in the wild is limited and remains manageable compared to the ubiquitous distribution of other species such as *E. canadensis*. Alternative means of control are thus recommended for *M. aquaticum*.

Because of the limitations of various methods of invasive species control focus should shift toward the prevention of dispersal and establishment of invasive species (Puth and Post, 2005). The spread of aquatic invasive species is repeatedly attributed to the overland movement of equipment by waterway users such as boaters, canoeists and anglers. In Europe some 36% of non-native species are thought to have been introduced by such activities (Anderson *et al.*, 2014). In North America, most boat users report removing aquatic plants that they notice attached to their boats. However, small propagules are

more likely to go unnoticed by visual inspection and less than a quarter of boaters were likely to clean and dry their boats effectively when moving between waterways (Rothlisberger *et al.*, 2010). Interestingly, 27% of boaters who removed their boats from the water intended to refloat their boats within 24 hours (Johnson *et al.*, 2001). Similarly, canoeists and anglers in the UK move frequently between waterbodies. Only 6% of canoeists and 21% of anglers clean and dry their kit after every use (Anderson *et al.*, 2014). The present study suggests that hitchhiking fragments are likely to regenerate if not completely desiccated before being reintroduced to the aquatic environment.

Rothlisberger *et al.*, (2010) found that smaller plant fragments are more frequently attached to boats. Some 45% of inspected boats and 36% of trailers carried plant fragments externally while moving overland (Johnson *et al.*, 2001; Rothlisberger *et al.*, 2010). The present study provides valuable data on plant fragment survival and regeneration potential post desiccation. Prolonged desiccation by aerial exposure reduces plant fragment viability. Plant fragments that remain viable post desiccation experience reduced growth and may be less likely to survive long term in a new receiving environment. It is evident from our study that not every species tolerates desiccation stress to the same extent, implying that fragments of some species may be more successful at surviving and regenerating after overland dispersal than others. *M. aquaticum* is most suited for such overland dispersal due to its high capacity to remain viable for lengthy periods. While other species may be less tolerant of desiccation under lab conditions the results of this study are conservative and aquatic plant fragments may be capable of remaining viable for longer periods than reported when exposed to conditions outside of the lab. This is particularly true for plant fragments that will dry out more slowly in cooler temperatures, higher humidity or exposed to precipitation (Bruckerhoff *et al.*, 2015). Further, larger plant propagules that occur in clumps of vegetation, or are coiled around themselves are better insulated from desiccation stress (Bruckerhoff *et al.*, 2015). Indeed, there are ample opportunities for plant fragments to remain insulated and moist while being transported overland, including fragments sitting in unintentional reservoirs of water such as boat interiors, bilge water, bait buckets, wet netting and carpet etc. (Jerde *et al.*, 2012; Rothlisberger *et al.*, 2010). Thorough drying of equipment must thus be employed to enhance biosecurity in conjunction with washing of equipment and education of waterway users on biosecurity issues.

Conclusions

It is evident that aquatic plants have a high capacity to tolerate fragmentation and regenerate from the smallest of vegetative fragments in most cases. *M. aquaticum*, followed by *M. spicatum*, *C. demersum* and *L. major* can remain viable at the smallest of size classes. This has implications for mechanical management of aquatic invasive species and anthropogenic fragmentation and dispersal by waterway users. Viability and fitness of propagules that are dispersed over land are not only limited by fragmentation tolerance but are also limited by their ability to tolerate desiccation. Desiccation tolerance is species specific and influenced by fragment size. The rate of water loss in aquatic plant fragments is curvilinear and slows with time. Desiccation of plant fragments reduces viability with time. Larger fragments are more likely to remain viable post desiccation but the subsequent regrowth upon rehydration is reduced by prolonged aerial exposure. It is thus recommended that thorough inspection and drying of equipment is used by waterway users as a means of biosecurity in combination with thorough inspection and cleaning of equipment and effective education. Provision should thus be made by water way managers for access to the required biosecurity washing facilities and every effort made to effectively educate waterway users in biosecurity techniques and measures.

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Chapter 5

Does the Freshwater Alien Species
Myriophyllum aquaticum have
Potential to Invade Brackish Waters
in Ireland?

Introduction

Estuaries, lagoons, saltmarshes, tidal rivers and other coastal wetlands are important ecosystems which occupy the transitional area between marine, freshwater and terrestrial environments. Known collectively as transitional wetlands (McLusky & Elliott, 2007) these ecosystems are diverse in nature and highly productive (Zaldivar *et al.*, 2008). Further they provide many essential ecosystem services globally (Barbier *et al.*, 2011; Mouillot *et al.*, 2006; Levin *et al.*, 2001; Davidson, 1991) and are thus of high socioeconomic value (Newton *et al.*, 2014). Neither freshwater nor marine, these transitional waters are most often brackish in nature (Basset *et al.*, 2013). The physicochemical conditions of transitional waters are under the influence of both the marine and freshwater environment (McLusky & Elliott, 2007), thus abiotic factors such as salinity of the water and substrate are known to vary according to the tides, freshwater influx, weather and climatic events (Lettice, 2014; Telesh & Khlebovich, 2010; Tagliapietra *et al.*, 2009).

Typically, species richness is lower in brackish environments compared to adjacent freshwater and marine habitats (Paavola *et al.*, 2005; Palmer *et al.*, 2011). This is because those environments where salinity gradients or fluctuations occur impose physiological constraints on the biota, and only a few species have developed mechanisms to either avoid or tolerate stress due to fluctuations in salinity (Saiz-Salinas & Gonzalez-Oreja, 2000). Stress has been defined as any environmentally induced “constraint which limits the potential productivity of biota” (Freedman 1995; Saiz-Salinas & Gonzalez-Oreja, 2000). Specific constraints are sometimes also referred to as stressors, i.e. organisms of transitional waters are subjected to frequent and repeated osmotic and ionic stressors, which may cause stress in these organisms (Lasserre, 1976).

The distributions of plants in transitional wetlands are controlled by a complex suite of biotic and abiotic factors which interact to define the ecological niche of a given species (Crain *et al.*, 2004; Cott *et al.*, 2011; Cott *et al.*, 2013; Ungar, 1998). Many studies have investigated the role salinity plays in controlling the distribution of macrophytes in coastal wetlands such as salt marshes, tidal rivers, estuaries and lagoons (e.g. Crain *et al.*, 2014; Silvestri *et al.*, 2005; Watson & Byrne, 2009). Halophytic plants are those which have evolved to tolerate and, in some cases, thrive in saline conditions (Flowers and Colmer, 2008). Other studies have investigated the impact of salinization on freshwater species which are not typically capable of responding to salt stress in the same way halophytes do (James *et al.*, 2003; Brock *et al.*, 2005; Nielson *et al.*, 2003).

Like most aquatic habitats, transitional wetlands are at risk from a variety of anthropogenic pressures including eutrophication, hydromorphological modification and invasion by alien species (Newton *et al.*, 2014; Williams & Grosholz, 2008). However, in Ireland and Britain most biological invasions of brackish waters appear to be by invertebrates and algae (Minchin, 2007; Minchin, 2013). In inventories of alien species established in coastal waters of Britain and Ireland the only plant species listed as invasive in brackish waters are from the halophytic *Spartina* genus (Minchin, 2007; Minchin 2013). However, a similar inventory of alien species in San Francisco Bay recorded a total of 12 alien plant species established in brackish waters, five of which also occurred in freshwater habitats (Cohen & Carlton, 1998).

The role environmental stressors play in invasion ecology is not comprehensively understood. It has been suggested that fast growing, competitive, non-native species may be less well adapted toward the constraints in a given habitat compared to species native to that location (Alpert *et al.*, 2000) and in that sense stressful environments may represent a barrier to invasion (Pauchard *et al.*, 2009). However, it appears that this is not always the case and that the success of an invasion by an alien species in a stressful environment depends on the biological traits of the taxa, the composition of the native community, resource availability and the nature of the stressor in question (Paavola *et al.*, 2005; Davis *et al.*, 2000; MacDougall *et al.*, 2006; Pan *et al.*, 2006).

Myriophyllum aquaticum (Vell.) Verdc. is a heterophyllous aquatic plant species characterised by both a submerged and emergent growth form (Sytsma & Anderson, 1993; Wersal & Madsen, 2011). The species is native to freshwater habitats of South America (Orchard, 1981) but as a result of its popularity in the aquarium and horticultural trade has become naturalised in North America, Southern Africa, Australasia and Europe (Orchard, 1981; Hussner, 2012; Tsiamis *et al.*, 2017; Gillard *et al.*, 2017). An invader of slow flowing and standing waters, *M. aquaticum* is considered to be ecologically and economically destructive throughout its invasive range (Oreska & Aldridge, 2011; Rumlerová *et al.*, 2016). It has been known to alter the physicochemical environment and impact on floral and faunal communities, and in some locations it poses a significant threat to human health and socioeconomic activity associated with wetlands (Stiers *et al.*, 2011; Schultz & Dibble, 2012; Orr & Resh, 1992; Douglas, 2009; Stone *et al.*, 2009; Desa & Lee, 2018). As a result of its economic and ecological impacts the species has been classified as an 'Invasive Alien Species of Union Concern' throughout the EU (Tsiamis *et al.*, 2017). As such EU member states are required to put in place measures for early detection and rapid eradication of the

species where it occurs and to manage populations which have already spread throughout the EU (Tsiamis *et al.*, 2017).

M. aquaticum was first recorded in Ireland in Co. Down in 1988 (Reynolds, 2002) and its distribution has expanded in recent years. Based on its current distribution, which is primarily coastal, Kelly *et al.*, (2014) constructed environmental niche models which showed that at present *M. aquaticum* occupies only 2% of its potential freshwater range in Ireland and over the coming decades it is likely to spread. In Ireland the genus *Myriophyllum* consists of three native species, *M. spicatum* L., *M. verticillatum* L. and *M. alterniflorum* DC. (Parnell *et al.*, 2012). *M. spicatum* is a submerged species native throughout Eurasia, and parts of Africa (Aiken, 1979; Weyl *et al.*, 2016). It is known to be an invasive species outside of its native range, and has successfully invaded North America, representing a serious ecological and economic threat to some regions (Moody *et al.*, 2016; Pimentel, 2009; Eischer *et al.*, 2000).

Both *M. aquaticum* and *M. spicatum* are considered to be predominantly freshwater species. For instance, *M. spicatum* has been assigned an Ellenberg score of 0 for salinity implying that it has no salt tolerance (Hill *et al.*, 1999). However, *M. spicatum* is also considered a characteristic species of lagoons in its native range (Fossit, 2000; Verhoeven & Van Vierssen, 1978) and though predominantly an invader of freshwater habitats, is also a well-documented invader of brackish waters of North America (Menzie, 1979; Anderson *et al.*, 1966, Orth, 1994) where it has been shown to have altered native communities (Valenoti *et al.*, 2011; Chaplin & Valentine, 2008; Martin & Valentine, 2011; Duffy & Baltz, 1998). A number of reports also exist of *M. aquaticum* occurring in, but not dominating, brackish waters at the limits of its native range (Ramirez *et al.*, 1989; Calliari *et al.*, 1997). It has been speculated that that *M. aquaticum* is a potential invader of brackish waters. Given its freshwater, but primarily coastal distribution in Ireland it may pose a serious risk to transitional wetlands.

In order to determine the invasion potential of *M. aquaticum* in brackish waters this study takes a comparative approach, identifying differences between *M. spicatum* and *M. aquaticum*. Such a comparative approach is useful in identifying the traits responsible for the competitive success of an invasive species. Recognising that different barriers to invasion success occur at the various life stages of a species (Sakai *et al.*, 2001), the present study investigates the response of both *Myriophyllum* species to salinity at the propagule and established plant stage. The hypotheses tested in this study were:

H₁. That, under *ex situ* conditions, the viability and success of *Myriophyllum* spp. propagules are influenced by the salinity of the receiving environment.

H₂. That there is a relationship between waterbody salinity and the growth and morphology of *Myriophyllum* spp. under field conditions.

H₃. That *M. aquaticum* has the potential to colonize and become established as an invasive species of brackish waters and coastal wetlands in Ireland.

Methods

This study utilises a factorial design to establish the effect of salinity on *Myriophyllum* spp. propagule viability and growth under laboratory conditions and the effect of salinity on the growth and morphology of established plants under field conditions. As *M. aquaticum* is a restricted invasive species within the EU all plant material used in this study was collected, transported and propagated under license granted by the National Parks and Wildlife Service, Ireland (License No: IAS 7/2014).

The Effect of Receptive Environment Salinity on *Myriophyllum* Propagules

The effect of salinity on the viability and success of propagules of *M. aquaticum* and *M. spicatum* was investigated under *ex situ* conditions. In the context of this experiment plant propagules were determined to have viably regenerated if new shoot or root production was observed (Redekop *et al.*, 2016) and the quantitative amount of propagule biomass production was used as an indicator of success (Chen *et al.*, 2011). Material of *M. spicatum* (Latitude: 51.911291, Longitude: -8.269594) and *M. aquaticum* (Latitude: 51.901892, Longitude: -8.298476) was collected from freshwater ponds in East Cork in September and October 2015 respectively. Collected plant material was gently washed in flowing tap water in order to remove sediment, invertebrates and epiphytic algae. The plants were propagated in aerated troughs containing tap water and sediments collected from the flood plain of the River Lee, Co. Cork.

Plants were screened for signs of damage, decay or discolouration. Unbranched apical to mid-stem shoots (Mcalarnen *et al.*, 2012) were selected as experimental material, thus excluding the submerged lower stem, tissue of *M. aquaticum*. The top 5cm of apical shoots were removed as per Evans *et al.*, (2011) in order to eliminate apical meristems and tightly

clustered whorls of leaves. Shoots were cut into fragments of 3 nodes by cutting through the midpoint of the internode. Propagules were immediately stored in tap water to prevent desiccation damage and acclimatised to growth room conditions for 48hrs. A random set of ten propagules of each species were selected, gently blotted dry, biomass recorded and mean starting biomass obtained.

In order to assess the relationship between salinity and propagule viability an experiment with a range of salinities from 0ppt (freshwater) to 18ppt (approximately half strength sea water) was designed. Salinity treatments in the experiment were 0, 1, 2, 3, 4, 8, 11, 14 and 18ppt. Full strength sea water was UV sterilised and diluted with distilled water until the desired salinity was achieved. Nutrients were supplied by adding Hoagland's nutrient solution (Roshon *et al.*, 1996) to the media to a concentration of 2.5%. Experimental plant propagules were transferred into unsealed magentas containing 300ml of media. There were five replicates per salinity treatment for each species, resulting in a total of 90 plant propagules in the experiment. Magentas were then randomly placed in a temperature controlled (20°C) growth room under a 16hr photoperiod, with an average light intensity of $40\mu\text{mol.m}^{-2}\text{s}^{-1}$. Plant fragments were allowed to grow for 42 days and topped up with distilled water as evaporation required. The media was replaced every two weeks to prevent algal growth and to replenish nutrients.

Harvested plants were assessed for viability. Plants were determined to have viably regenerated if new shoot or root production was observed (Redekop *et al.*, 2016). Newly produced shoots and roots were counted and lengths measured. Excess water was gently blotted from plant material and fresh biomass of roots, new shoots and original fragment were weighed. Percentage growth was calculated as the total biomass of newly produced shoots and roots as a percentage of biomass of the initial fragment at the beginning of the experiment:

$$\text{Percentage Growth} = (\text{BM}_{\text{new}} / \text{BM}_{\text{start}}) * 100$$

BM_{new} represents the total biomass of any newly produced shoots and roots which grew over the course of the experiment. BM_{start} represents the initial biomass of the plant fragment at the start of the experiment.

The hypothesis that there is a relationship between salinity and propagule viability was tested for each species using a binomial logistic regression. Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-

Tidwell procedure (Box & Tidwell, 1962). A Wald test was used to determine statistical significance for the independent predictor variables, salinity and species. Because of low viability rates at higher salinities any further analysis was conducted on data obtained from viable propagules grown in treatments of 0-11ppt only.

In order to determine if salinity had an effect on propagule growth and whether there was a difference in response between species a two-way ANOVA was employed. Dependent variables tested by two-way ANOVA included overall biomass growth, shoot biomass growth and shoot length. Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by inspection of a boxplot, normality was assessed using Shapiro-Wilk's normality test for each cell of the design and homogeneity of variances was assessed by visual inspection of a scatterplot of the residuals. Where necessary data was square root transformed to obtain normality. Two plants generated outliers in the data which were kept in the analysis. Residuals were normally distributed except for a single cell in the experimental design which approximated normality. The ratio of the largest group variance to the smallest group variance was always less than 3. In cases where a significant interaction between variables was identified a simple main effects model was performed with statistical significance receiving a Bonferroni adjustment.

A Spearman's rank-order correlation was run to assess the relationship between salinity and root production in viable propagules. The relationship was monotonic in both species. The strength of the relationship was determined as per Cohen (1988). A large proportion of the viable propagules failed to produce roots, thus the assumptions of a two-way ANOVA were violated. It was therefore not possible to analyse the interaction between species and salinity on parameters such as root number, root biomass or root length. Alternatively, in cases where there was a monotonic relationship between variables a Spearman's rank-order correlation was conducted. In cases where the relationship was determined to be non-monotonic a Kruskal Wallis H-Test was conducted; In which case the distribution of the dependent variable was determined by the visual inspection of boxplots. Post-hoc pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. Adjusted p-values are presented. These tests were conducted independently for each species using only data collected from propagules that successfully produced roots.

The Effect of Waterbody Salinity on *Myriophyllum* Plants in the Field

In order to determine if there is a relationship between salinity and the growth and morphology of established *M. aquaticum* or *M. spicatum* plants in the wild a field based experiment was established. This experiment measured the growth responses of plants grown in semi-natural waterbodies of varying salinity.

Field Site & Environmental Variables

M. aquaticum and *M. spicatum* co-occur in semi-natural ponds at the Fota Island Golf Club, Co. Cork. This is a coastal site on an island in Cork Harbour, surrounded by estuarine habitats. Personal communication with Simon O'Hara, the head greenkeeper at the golf club revealed that the ponds on site were influenced by saline intrusions from the estuary which rendered some of the ponds unusable for irrigation of the golf course. A total of nine waterbodies on the island were inspected for the presence of *M. aquaticum* and *M. spicatum* and preliminary salinity measurements were taken. A complex of ponds and channels on the golf course (Fig. 1) was found to contain both *Myriophyllum* species in varying levels of abundance. These waterbodies are closely clustered together and vary in salinity. They are separated from the estuary by a sea wall and unidirectional sluice which prevents tidal inundation of the golf course, a portion of which lies below the high water mark. At high tide, when ground water levels rise, saline water intrudes the wetland complex via a bore hole (O'Hara, pers comm). At low tide surface waters exit through the unidirectional sluice to the estuary.

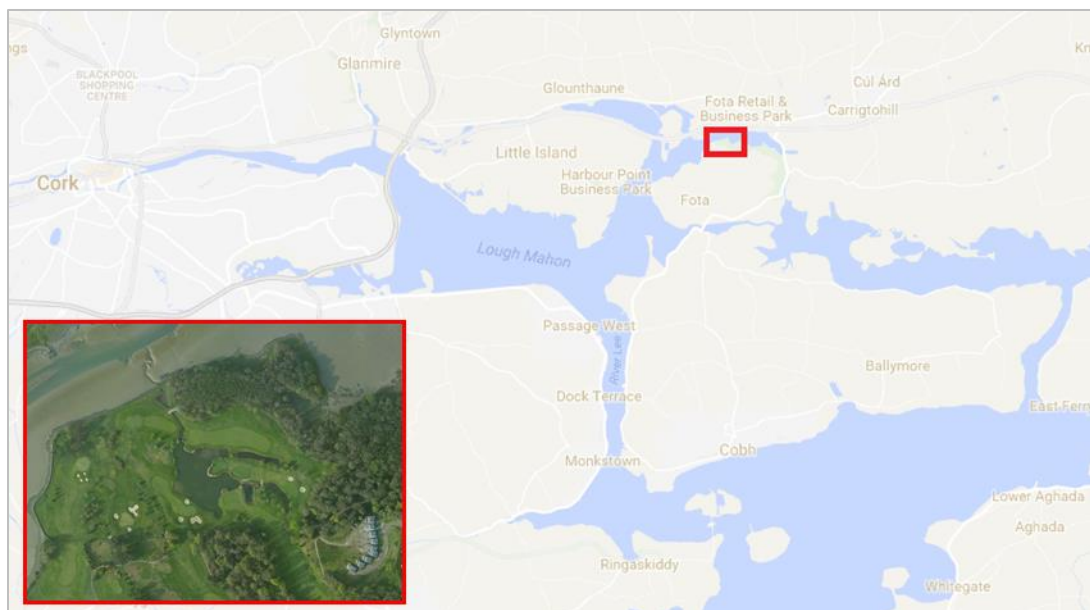


Figure 1: The location of the selected field site at the Fota Island Golf Club, located on the the North of Fota Island, an Island in Cork Harbour.

The Fota Island site (Fig. 1) was selected as an experimental field site as it offered a number of waterbodies under the same management conditions in close proximity to each other. These waterbodies naturally varied in salinity. *M. aquaticum* and *M. spicatum* were both abundant at the location indicating that conditions were suitable for the growth of both species, and because *M. aquaticum* was already present no ethical issues associated with experimentally growing an invasive species in the wild would arise. Permission to access and conduct field work at the location was granted by the Golf Club.

A site walkover was conducted and preliminary measurements of physicochemical parameters such as salinity, pH and conductivity were recorded. An inventory of macrophytes species present was constructed and their abundance was estimated. Wetland habitats were classified according to Fossit (2000) based on physicochemical and macrophyte data. Based on the preliminary data, four ponds that encompassed the greatest range in salinity, including a freshwater pond, were selected for experimental purposes. The ponds were approximately the same area (0.06-0.1ha) in size and no more than 232m apart. All four ponds were adjacent to playable golf course fairways, tee boxes and/or greens. In order to establish the average pH of each pond a portable pH meter was used to measure the pH *in situ* at four equidistant locations around the perimeter.

In order to establish the salinity range of each pond the salinity was measured *in situ* on six occasions. This included three low tide measurements and three high tide measurements. An average salinity was established for each pond on each sampling occasion by measuring the salinity at four equidistant sampling locations around the perimeter of the pond. Salinity measurements were taken from 10cm below the surface (Lettice, 2014) using a Wissenschaftlich Technische Werkstätten Meter, Model 330i. On one occasion, in order to estimate a maximum value in the salinity range of the ponds, high tide and low tide measurements were recorded following a period of four days without precipitation. Based on the recorded salinity range ponds were classified as being of freshwater, low, moderate or high salinity.

A two-way ANOVA was conducted to examine whether physicochemical variables (pH and salinity) differed between ponds at high and low tide. Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by inspection of a boxplot, normality was assessed using Shapiro-Wilk's normality test for each cell of the design and homogeneity of variances was assessed by visual inspection of a scatterplot of the residuals. Salinity was square root transformed to obtain normality and homogeneity of

variance. There were no outliers. Residuals were normally distributed and the ratio of the largest group variance to the smallest group variance was less than 3, except in the case of salinity in Pond No. 1 which is a freshwater pond and therefore salinity was 0ppt on the majority of sampling occasions. This pond was retained in the analysis as it did not alter the overall outcome of the test. In cases where a significant interaction between variables was identified a simple main effects model was performed with statistical significance receiving a Bonferroni adjustment.

Established Plant Growth & Morphology.

Plant material of both *M. aquaticum* and *M. spicatum* was collected from a freshwater pond where they co-occurred at Fota Island Golf Club (Latitude: 51.901892, Longitude: -8.298476) on August 12th 2016. Plant material was gently washed under flowing tap water in order to remove sediment, invertebrates and epiphytic algae. Unbranched apical shoots, 15cm in length were cut at the nearest node. Shoots were planted in polypropylene plastic, 0.36L, plant pots which contained Westland Aquatic Compost. Shoots were planted 4-5cm into the compost so that there was approximately 10cm of shoot aboveground. Plants were grown outdoors in 12L buckets of water, four plants per bucket. Plants were checked weekly until a healthy root ball had established. After three weeks ten random individuals of *M. aquaticum* and 14 individuals of *M. spicatum* were harvested, measured and weighed in order to establish the average size and morphology of plants at the beginning of the experiment. Parameters measured included total biomass, shoot biomass, root biomass, apical shoot length, number of branches and branch length.

On September 2nd 2016 experimental plants were transported to the field site in the buckets in which they were grown. Still in their pots, ten replicate plants of each species were randomly set into a 49x49cm weighted frame which held them in place. After wading into the pond to a depth of ~50cm the frames were set 6cm into the sediment, so that the sediment was level with the rim of the pots. In all four ponds an area void of vegetation was selected in order to minimise competition with the natural plant community. Plants were left in place for six weeks and harvested October 14th 2016. Harvested plants were transported and stored in labelled zip lock bags containing pond water and processed immediately upon arrival in the laboratory. Plants were gently washed under flowing tap water, blotted dry and mortality, final biomass, root and shoot biomass, apical shoot length, number of branches and branch length were measured.

Plant survival rates were determined by assessing the proportion of plants which remained alive upon completion of the experiment. Relative growth rate (RGR) was calculated for biomass data using the following formula:

$$\text{RGR} = (\ln W_2 - \ln W_1)/T$$

Where $\ln$ is the natural log, W_1 is the biomass at the start of the experiment, W_2 is the final biomass at the end of the experiment and T is time in days. In the case of *M. aquaticum* the ratio of emergent shoot biomass to submerged shoot biomass was also calculated.

A Spearman's rank-order correlation was run to assess the relationship between salinity and plant survival. The relationship was monotonic in both species. The strength of the relationship was determined as per Cohen (1988).

Apical shoots were broken from a number of plants over the course of the experiment and were thus excluded from further analysis. The assumptions of a two-way ANOVA were violated as a result of the 100% mortality of *M. spicatum* under highly saline conditions. Thus, in order to assess the relationship between salinity and plant growth responses a one-way ANOVA was conducted separately for each species using only data collected from living and intact plants. Outliers were assessed by inspection of a boxplot, normality was assessed using Shapiro-Wilk's normality test for each cell of the design. Data is square root transformed to achieve normality where appropriate. Homogeneity of variances was assessed by Levene's test for equality of variances. Where the assumption of homogeneity of variance was met a Tukey post hoc test was conducted. Where the assumption of homogeneity of variance was violated the Welch ANOVA was interpreted instead, followed by a Games-Howell post hoc test.

Alternatively, where the assumption of normality was not met a Kruskal-Wallis H test was employed. Distribution of the dependent variable was assessed by visual inspection of a boxplot. Post hoc pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons.

Results

The Effect of Receptive Environment Salinity on *Myriophyllum* Propagules

The effect of the salinity of the receptive environment on the viability and fitness on propagules of *M. aquaticum* and *M. spicatum* was investigated under laboratory conditions. In the context of this experiment plant propagules were determined to have viably regenerated if new shoot or root production was observed (Redekop *et al.*, 2016) and the quantitative amount of propagule biomass production and shoot growth was used as an indicator of propagule performance (Chen *et al.*, 2011).

Propagule Viability, *Ex-Situ*

The relationship between salinity and propagule viability is depicted for *M. spicatum* & *M. aquaticum* in figure 2. *M. spicatum* maintained 100% viability up to 8ppt but viability became reduced at salinity concentrations greater than 8ppt. Only 20% of *M. spicatum* propagules remained viable at 14ppt salinity and no fragments remained viable at a concentration of 18ppt. *M. aquaticum* also maintained high viability (80-100%) up to salinities of 8ppt. Viability of *M. aquaticum* propagules declined more rapidly than *M. spicatum* at higher salinities and no fragments were determined to be viable at salinities of 14ppt or greater.

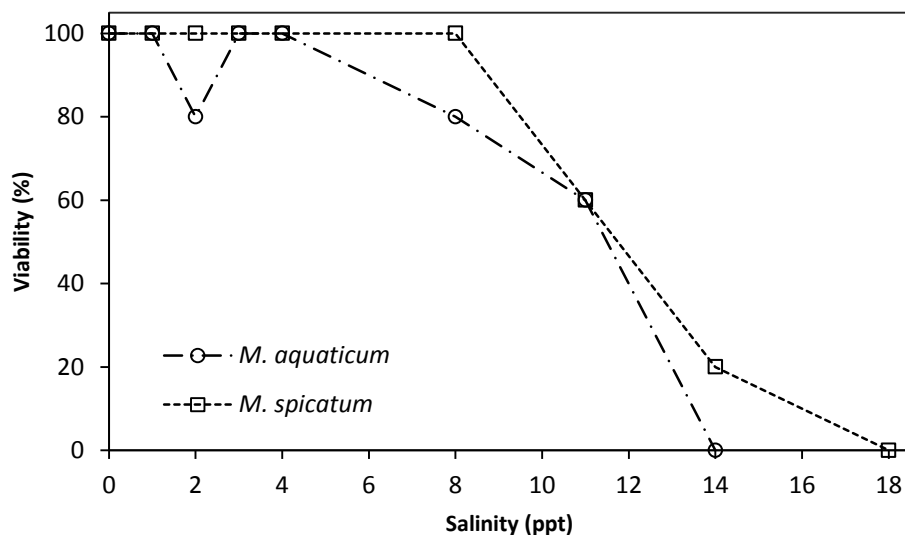


Figure 2: The relationship between salinity and *Myriophyllum* spp. propagule viability in under *ex situ* conditions.

A binomial logistic regression was performed to determine the effects of salinity on propagule viability. Salinity was found to be linearly related to the logit of viability. There was one outlier in the data which was kept in the analysis (Studentized residual value of -11.35 standard deviations). The logistic regression model was statistically significant ($p < 0.005$, $\chi^2 = 79.725$). The model explained 75% of the variance in fragment viability (Nagelkerke R^2) and correctly classified 92% of cases. A Wald test was used to determine statistical significance for the independent variables. Salinity was found to be a statistically significant ($p < 0.005$) predictor of viability. Increasing salinity was associated with a reduced likelihood of propagule viability as was evident by an odds ratio less than one (0.534 reductions in odds for every unit increase in salinity). Species was also found to be a statistically significant ($p = 0.022$) predictor of viability. After controlling for salinity *M. spicatum* propagules had 6.6 times higher odds to exhibit viability than those of *M. aquaticum*, meaning that *M. spicatum* was more likely to remain viable than *M. aquaticum* under saline conditions.

Propagule Growth, *Ex-Situ*

Percentage growth in terms of newly produced biomass as a percentage of the initial biomass of the propagule was calculated for total new biomass, shoot biomass and root biomass (Fig. 3). *M. spicatum* exhibited greater percentage overall, shoot and root growth than *M. aquaticum* across all salinity treatments.

Under control conditions mean overall growth was $19\% \pm 2.8\%$ for *M. spicatum* and $6.6\% \pm 1.7\%$ for *M. aquaticum*. *M. spicatum* exhibited a steady increase in growth at low salinities while *M. aquaticum* maintained relatively stable growth at low salinities. Both species achieved maximum growth at 4ppt where mean percentage growth was $71\% \pm 12\%$ for *M. spicatum* and $9.7\% \pm 3.1\%$ for *M. aquaticum*. Both species exhibited an observable decline from maximum in percentage growth at salinities greater than 4ppt. The maximum salinity at which *M. spicatum* and *M. aquaticum* exhibited growth was 14ppt and 11ppt respectively, beyond which no propagules remained viable.

A two-way ANOVA was conducted to examine the effects of salinity on the overall growth of *M. aquaticum* & *M. spicatum*. There was a statistically significant interaction between species and salinity on propagule growth, $F(6, 50) = 4.813$, $p = 0.001$, partial $\eta^2 = 0.366$. Therefore, an analysis of simple main effects for salinity and species was performed with statistical significance receiving a Bonferroni adjustment. When compared to *M. aquaticum*, propagules of *M. spicatum* exhibited significantly greater growth at all salinity

levels, but the magnitude of the difference was greater as salinity levels were increased. Significance ranged from $p=0.026$ at salinities of 0ppt to $p<0.0005$ at 11ppt. The simple main effect of salinity was significant for *M. spicatum* ($F(6, 50)=7.639$, $p<0.0005$, partial $\eta^2=0.478$) but not for *M. aquaticum* ($F(6, 50)=1.077$, $p=0.389$, partial $\eta^2=0.114$). Pairwise comparisons of salinity treatments in the simple main effects model revealed that growth at salinities of 3, 4 & 8ppt was significantly greater than that of the control for *M. spicatum* (Fig. 3.A).

Maximum growth was observed at 4ppt, which was significantly greater than that at lower concentrations but not significantly different from growth at higher salt concentrations. No significant differences between salinity treatments were observed for *M. aquaticum* indicating that there was no difference in the growth response of *M. aquaticum* to salinity treatments.

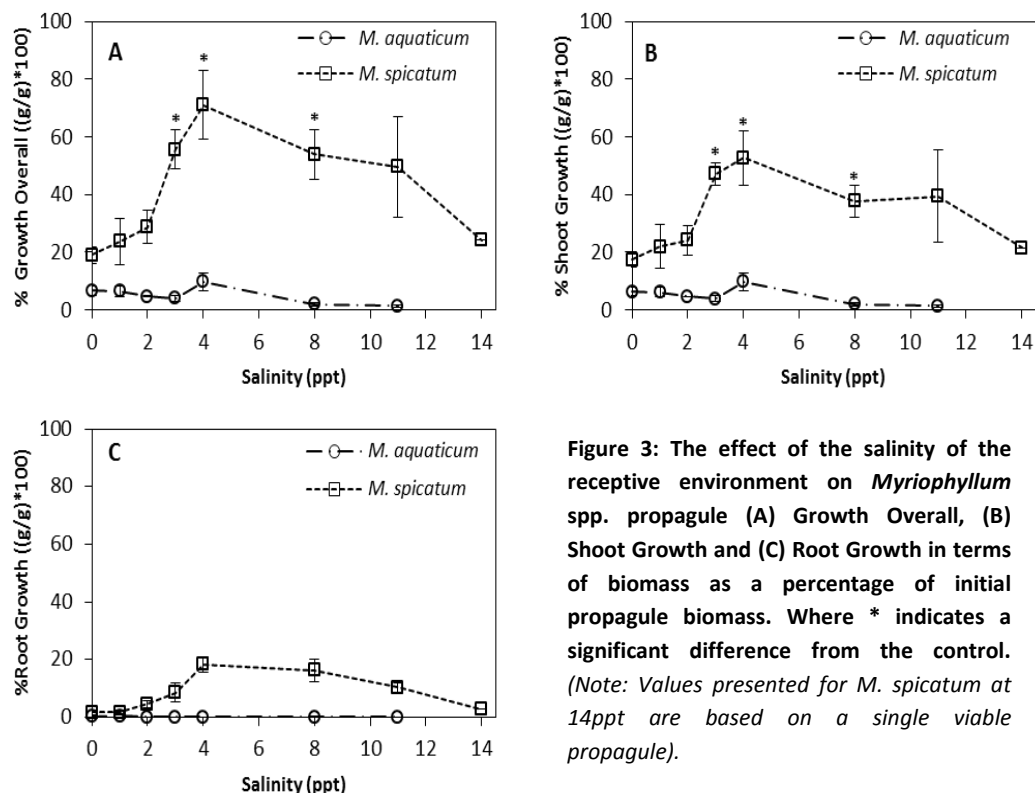


Figure 3: The effect of the salinity of the receptive environment on *Myriophyllum* spp. propagule (A) Growth Overall, (B) Shoot Growth and (C) Root Growth in terms of biomass as a percentage of initial propagule biomass. Where * indicates a significant difference from the control. (Note: Values presented for *M. spicatum* at 14ppt are based on a single viable propagule).

In both species the percentage growth of shoot biomass followed the same pattern as overall plant growth (Fig. 3.B). Average shoot growth at 0ppt was $6.3\% \pm 1.5\%$ in *M. aquaticum* and $17.4\% \pm 2.8\%$ in *M. spicatum*. Optimum shoot growth occurred at 4ppt for both species. Maximum shoot growth achieved was $9.7\% \pm 3.1\%$ and $52.7\% \pm 9.4\%$ in *M. aquaticum* and *M. spicatum* respectively. A statistically significant interaction between species and salinity on shoot growth ($F(6, 50)=3.391$, $p=.007$, partial $\eta^2=0.28$) was identified by a two-way ANOVA. Analysis of the simple main effects showed that *M. spicatum* propagules exhibited significantly greater shoot growth than *M. aquaticum* at all salinity levels ($p = 0.029 - p < 0.005$). The simple main effect of salinity on shoot growth was significant for *M. spicatum* ($F(6, 50)=7.156$, $p<0.0005$, partial $\eta^2=0.383$) but not for *M. aquaticum* ($F(6, 50)=1.174$, $p=0.335$, partial $\eta^2=0.123$). Pairwise comparisons of salinity treatments in the simple main effects model revealed that growth at salinities of 3, 4 & 8ppt was significantly greater than that of the control for *M. spicatum*. Maximum growth was observed at 4ppt, which was significantly greater than that at lower concentrations but not significantly different from growth at higher salt concentrations. No significant differences in percentage shoot growth between salinity treatments were observed for *M. aquaticum*.

The relationship between salinity and root growth in terms of biomass was assessed using a Kruskal Wallis H-Test. Distributions of root growth scores were not similar for all groups. No statistically significant differences in distribution were identified for *M. aquaticum*. In *M. spicatum* the distributions were statistically significantly different between groups ($H(7)=18.716$, $p=0.005$). Post hoc analysis revealed statistically significant differences in root growth between propagules grown at 4ppt and 1ppt salinity ($p=0.02$). No significant differences were identified between any other pairwise combinations.

Propagule Morphology, *Ex-Situ*

All viable propagules produced shoots in this experiment. Shoot number ranged from 1-5 in *M. aquaticum* and 1-4 in *M. spicatum* (Fig. 4.A). A Kruskal Wallis H-Test was used to assess the relationship between salinity and number of shoots produced by viable propagules. No statistically significant differences in shoot number were identified at any salinity in either species.

Under control conditions of 0ppt salinity the total length of shoots produced by viable propagules of *M. aquaticum* and *M. spicatum* were $17.8\text{mm} \pm 1.8\text{mm}$ and $13.6\text{mm} \pm 2\text{mm}$, respectively. Reflecting the trends observed for plant biomass the shoot length increased

to a maximum of $47\text{mm} \pm 12.2\text{mm}$ and $39.2\text{mm} \pm 4.5\text{mm}$ at 4ppt. At salinities greater than 4ppt a decline from the maximum shoot length was observed. A two-way ANOVA revealed a statistically significant interaction between species and salinity on shoot length ($F(6, 50)=4.104$, $p<0.005$, partial $\eta^2=0.33$). This interaction is visualised in Figure 4.B where the difference in shoot length between species is greater at higher salinities. Analysis of the simple main effects showed that *M. spicatum* shoots were significantly longer than those of *M. aquaticum* at salinities of 8ppt ($F(1, 50) = 14.483$, $p < 0.005$) and 11ppt ($F(1,50)= 8.43$, $p = 0.005$). Salinity, as a simple main effect on shoot length was significant for *M. aquaticum* ($F(6, 50) = 5.311$, $p < 0.0005$, partial $\eta^2 = 0.389$) and *M. spicatum* ($F(6, 50) = 5.454$, $p < 0.005$, partial $\eta^2 = 0.3.96$). Pairwise comparisons of salinity treatments in the simple main effects model revealed that shoot length of propagules grown at 4ppt salt were significantly greater than those grown at 0 ($p=0.023$), 1 ($p=, 0.007$) 8 ($p = 0.003$) and 11ppt ($p = 0.001$) for *M. aquaticum*. Propagules of *M. spicatum* grown at 4ppt and 8ppt salinity produced significantly longer shoots than those grown in the control ($p = 0.007$ and $p = 0.004$) and at 1ppt ($p=0.018$ and $p=0.011$), but did not differ significantly from those grown at 11ppt.

Unlike shoot production, not all viable plant propagules produced roots. The percentage of viable propagules that produced roots is illustrated in figure 4.C. A high proportion (80-100%) of viable *M. aquaticum* propagules produced roots at low salinities of 0-2ppt, however at concentrations greater than 2ppt a decline in successful root production was observed. Viable propagules of *M. aquaticum* failed to produce any roots at salinities greater than 8ppt. Contrastingly, only 40% of viable *M. spicatum* propagules produced roots at low salinities, but the rate of successful root production increased with salinity, until all viable propagules produced roots at salinities of 3ppt and greater. Viable *M. spicatum* propagules successfully produced roots up to a maximum of 14ppt. A Spearman's rank-order correlation was run to assess the relationship between salinity and root production in viable propagules. The relationship was monotonic in both species. The species responded differently to salinity. A moderate positive correlation between salinity and root production was identified in *M. spicatum* ($rs(34)=0.4931$, $p=0.003$). Contrastingly, a strong negative correlation was observed for *M. aquaticum* ($rs(31)=-0.538$, $p=0.002$).

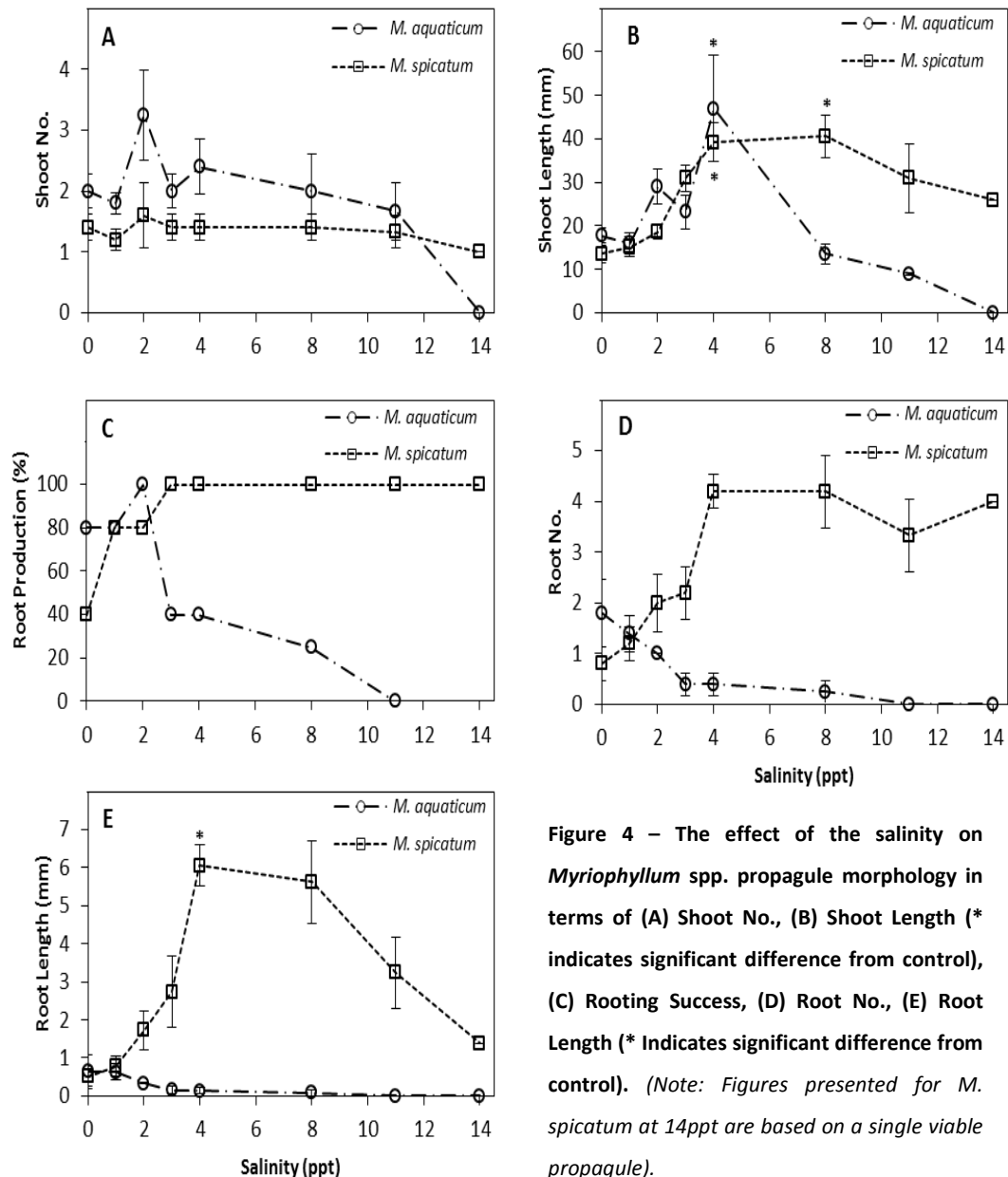


Figure 4 – The effect of the salinity on *Myriophyllum* spp. propagule morphology in terms of (A) Shoot No., (B) Shoot Length (* indicates significant difference from control), (C) Rooting Success, (D) Root No., (E) Root Length (* Indicates significant difference from control). (Note: Figures presented for *M. spicatum* at 14ppt are based on a single viable propagule).

The relationship between salinity and number of roots produced was also assessed using a Spearman’s rank-order correlation. The relationship was monotonic in both species. A strong positive correlation between salinity and root number was identified in *M. spicatum* ($rs(30)=0.651$, $p<0.01$). Contrastingly, a strong negative correlation was observed for *M. aquaticum* ($rs(17)= -0.538$, $p=0.015$).

A Kruskal Wallis H-Test was used to assess the relationship between salinity and root length. No statistically significant differences in root length were identified for *M. aquaticum*. For *M. spicatum* the distributions were statistically significantly different between groups, $H(7)=19.062$, $p=0.008$. Post hoc analysis showed that combined root

length of propagules grown at 4ppt (mean rank=26) was significantly different from those grown under control conditions (mean rank=5.67, $p=0.044$) and 1ppt (mean rank=6.75, $p=0.031$).

The Effect of Waterbody Salinity on *Myriophyllum* Plants in the Field

Established plants of *M. aquaticum* and *M. spicatum* were grown under field conditions in semi-natural wetlands which varied in salinity. This field experiment was designed to determine the effects of wetland salinity on the growth and morphology of *Myriophyllum* spp. plants.

Habitat Characteristics

All wetland habitats within the selected study site at Fota Island, Co. Cork were classified according to Fossit (2000). Figure 5 depicts the assigned habitat classification of wetland habitats within the study site. All but one of the waterbodies were classified as “CW1 – Lagoons and Saline Lakes” according to Fossit (2000). This classification was based on detectable salinity (>1ppt) in the water, tidal induced water fluctuations and the presence of characteristic lagoon flora such as *Ruppia* spp., *Potamogeton pectinatus* L., *Chara* spp., and *M. spicatum*. The presence of brackish water fauna such as shrimp and crab was observed in some of the saline waterbodies (Ponds No. 4, 5 and 6 in Fig. 5). One pond did not have detectable salinity in the water and was therefore considered to be a freshwater habitat and classified as “FL5 – Eutrophic Lake”. The key indicating characteristics of FL5 habitats were basic pH, abundant algae, and the presence of macrophytes such as *Lemna* spp., *P. pectinatus* and *M. spicatum*. Other wetland habitats such as “FS1 - Reed and Large Sedge Swamps” occurred on the fringes of the brackish waters. A notable macrophyte frequent in the wetland complex was *Potamogeton natans* L., which is not listed as an indicator species of either FL5 or CW1 by Fossit. In addition to *M. aquaticum* a second invasive species, *Nymphoides peltata* (S.G. Gmel.) Kuntze was also present. *M. spicatum*, *M. aquaticum* and *N. peltata* ranged from being dominant in some ponds to rare and absent in others.



Figure 5: Wetland habitats at Fota Island Golf Club, Co. Cork, classified according to Fossit (2000). Pond No. 1 = FL5-Eutrophic Lake, Ponds No. 2-6 = CW1-Lagoons & Saline Lakes. Where Ponds No. 1-4 are those used in the present field experiment. No. 1 = Freshwater (0-0.05ppt), No. 2 = Low Salinity (0.3-1.1ppt), No. 3 = Moderate Salinity (1.7-7.2ppt) and No. 4 = High Salinity (2.3-13.4ppt).

The freshwater pond (Pond No. 1) and three of the lagoon ponds (Ponds No. 2, 3 and 4) were selected for detailed monitoring of salinity and pH because they represented the greatest range in salinity in preliminary observations. Figure 5 illustrates the location of the selected ponds at the study site. Pond No. 1 was determined to be a freshwater pond with salinity ranging from a minimum of 0ppt at low tide to a maximum 0.05ppt at high tide. Pond No. 2 maintained low salinity throughout the experiment, ranging from a minimum of 0.3ppt at low tide to a maximum of 1.1ppt at high tide. Pond No. 3 was moderately salty ranging from a minimum of 1.7ppt at low tide to a maximum of 7.2ppt at high tide. The highest salinity and greatest range was observed in Pond No. 4. Here salinity ranged from a minimum of 2.3ppt at low tide to a maximum of 13.4ppt at high tide. The salinity range for each pond at high and low tide is depicted in Figure 6.A.

A two-way ANOVA was conducted to examine whether salinity levels differed between ponds at high and low tide. There was a statistically significant interaction between ponds and tides on salinity, $F(3, 16)=17.353$, $p<0.005$, partial $\eta^2=0.765$. Therefore, an analysis of simple main effects for pond and tide status was performed with statistical significance receiving a Bonferroni adjustment. The salinity of Pond No. 3 and No. 4 were significantly

greater ($P < 0.005$) at high tide when compared to low tide, indicating that salinity levels in these ponds fluctuate throughout the tidal cycle. There was no significant difference in the salinity at high and low tide in Pond No. 1 and No. 2 indicating that the salinity of these ponds remains more stable throughout the tidal cycle. Pairwise comparisons of ponds in the simple main effects model revealed that at low tide salinity levels of Ponds No. 2, No. 3 and No. 4 was significantly greater than that of the freshwater Pond No. 1. The third and fourth ponds were also significantly saltier than the second pond at low tide. At low tide there was no significant difference between salt levels of Pond No. 3 and No. 4. At high tide salinity was significantly different in all pairwise comparisons of ponds.

The pH of the water was slightly basic in all four study ponds, ranging from a minimum average pH of 7.65 at low tide in Pond No. 1 to a maximum average pH of 8.03 at high tide in Pond No. 2. (Fig. 6.B) A two-way ANOVA was conducted to determine if pH differed between ponds or according to the tide. No significant interaction was identified between ponds and tides. Neither ponds nor tide had a significant main effect on pH, indicating that there was no statistical difference in pH between ponds.

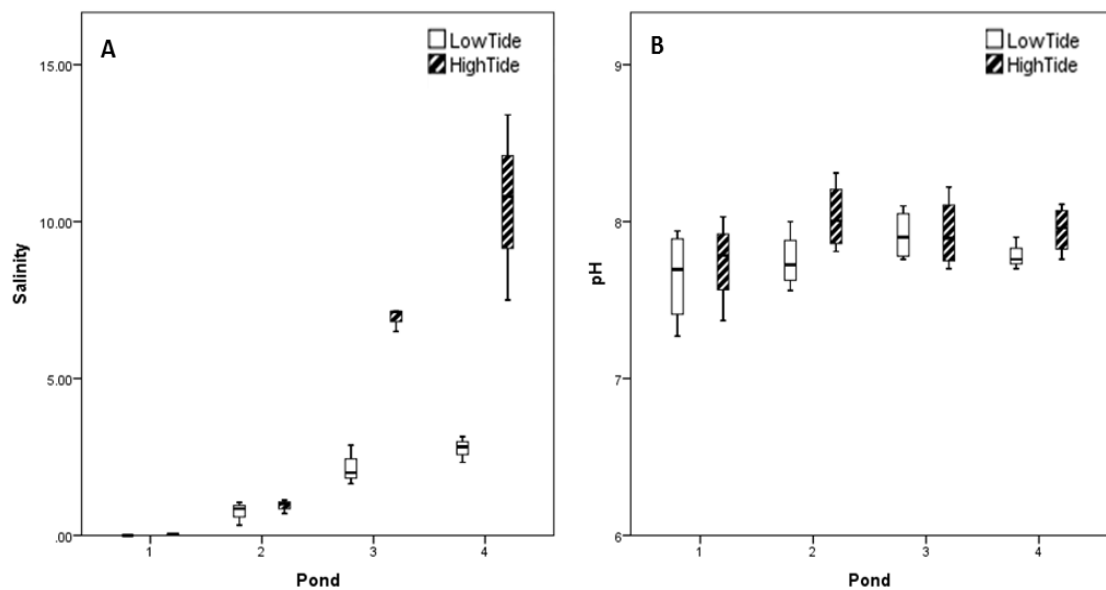


Figure 6: (A) The salinity range of experimental ponds sampled at high tide and low tide, (B) The pH range of experimental ponds sampled at high tide and low tide.

Plant Survival in the Field

Plant survival rates were determined by assessing the proportion of plants which remained alive upon completion of the field experiment (Fig. 7). *M. aquaticum* maintained high survival rates (80-100%) throughout the experiment. *M. spicatum* maintained 100% survival at 0ppt and low salinities, however survival declined to 80% at moderate salinities and at the highest salinity no plants were alive by the end of the experiment. A Spearman's rank-order correlation was run to assess the relationship between salinity and plant survival. A small negative correlation between salinity and plant survival was identified in *M. aquaticum*, though this relationship was not statistically significant ($r_s(40)=-0.308$, $p=0.053$). A statistically significant, strong negative correlation between salinity and plant survival was observed for *M. spicatum* ($r_s(41)=-0.777$, $p<0.005$). As no *M. spicatum* plants remained alive at high salinity all further analysis of *M. spicatum* growth responses was conducted on data collected from freshwater, low and moderate treatments only.

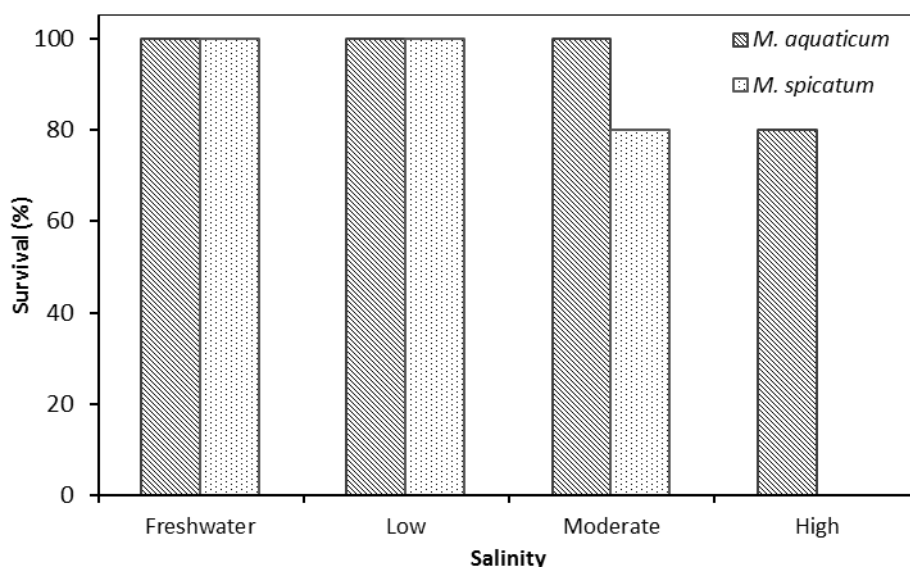


Figure 7: The effect of waterbody salinity on the survival of established plants of *Myriophyllum* spp. grown under semi-natural field conditions. Where 'Freshwater' = 0-0.05ppt, 'Low' = 0.3-1.1ppt, 'Moderate' = 1.7-7.2ppt and 'High' = 2.3-13.4ppt.

Plant Growth in the Field

The relative growth rate of living plants grown in semi-natural waterbodies of different salinities was measured (Fig. 8). RGR was measured in terms of the change in biomass of the average plant over the course of the experiment. Whole plant RGR increased in both species from those grown in freshwater to those grown at low and moderate salinities (Fig. 8.A). *M. aquaticum* plants grown under the most saline conditions exhibited loss of biomass which resulted in a negative RGR. A one-way ANOVA was employed to analyse the relationship between waterbody salinity and whole plant RGR. There were no outliers in the data and RGR was normally distributed, as assessed by Shapiro-Wilk's test ($p > 0.05$). There was homogeneity of variances, as assessed by Levene's test for equality of variances for *M. spicatum* ($p = 0.071$) but not for *M. aquaticum*.

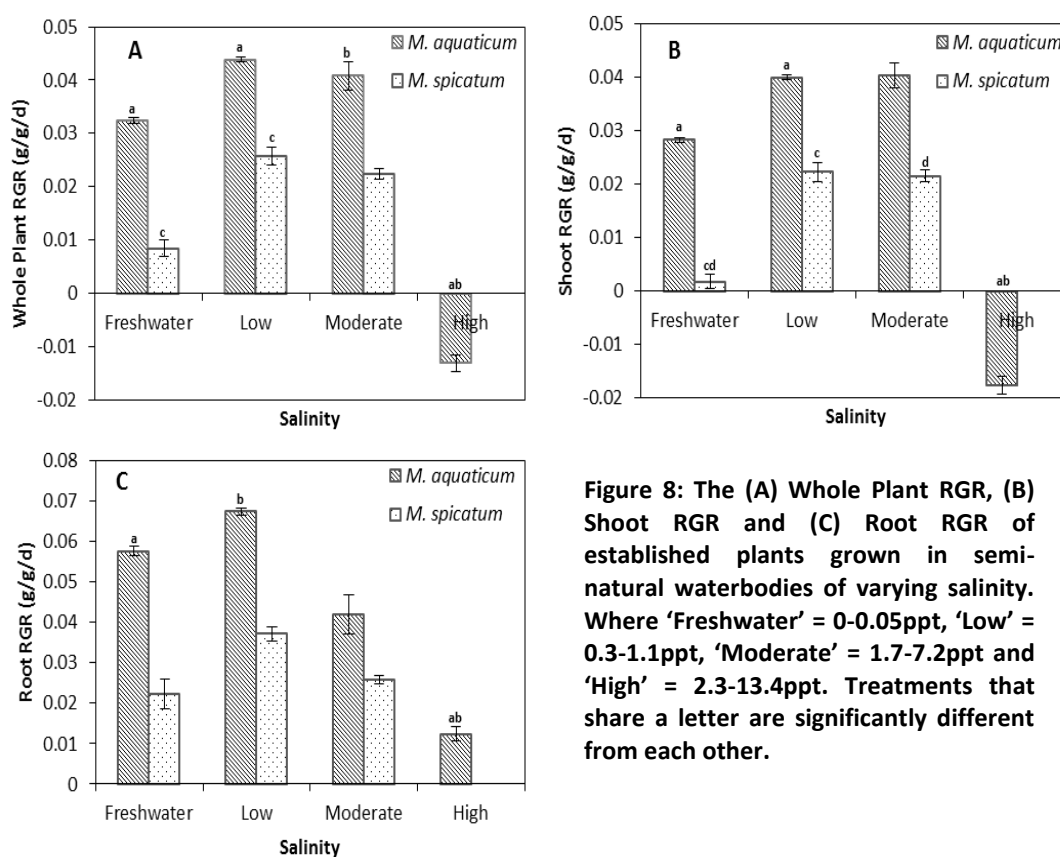


Figure 8: The (A) Whole Plant RGR, (B) Shoot RGR and (C) Root RGR of established plants grown in semi-natural waterbodies of varying salinity. Where 'Freshwater' = 0-0.05ppt, 'Low' = 0.3-1.1ppt, 'Moderate' = 1.7-7.2ppt and 'High' = 2.3-13.4ppt. Treatments that share a letter are significantly different from each other.

Whole plant RGR was statistically significantly different between salinity treatments for *M. spicatum* ($F(2, 26)=3.772$, $p=0.036$) and *M. aquaticum* ($F(3, 12.4)=55.6$, $p<0.005$). Post hoc tests showed that *M. spicatum* plants grown at low salinities had a significantly greater RGR than those grown in the freshwater pond ($p=0.036$). Similarly *M. aquaticum* plants grown at low salinities also had a significantly greater RGR than those grown in freshwater conditions ($p<0.005$). The negative RGR exhibited by *M. aquaticum* grown in the most saline pond was significantly different from plants grown under all other conditions ($p<0.005$).

In terms of biomass accumulation, shoot RGR followed the same trend as whole plant RGR in both species (Fig. 8.B). There were no outliers in the data, as assessed by inspection of a boxplot. RGR was normally distributed, as assessed by Shapiro-Wilk's test ($p>0.05$). There was homogeneity of variances, as assessed by Levene's test for equality of variances for *M. spicatum* ($p=0.102$) but not for *M. aquaticum* ($p=0.003$). Shoot RGR was statistically significantly different between salinity treatments for *M. spicatum* ($F(2, 26)=5.864$, $p=0.008$) and *M. aquaticum* ($F(3, 12.5)=51.4$, $p<0.005$). Post hoc tests showed that *M. spicatum* plants grown at low and moderate salinities had a significantly greater shoot RGR than those grown in the freshwater pond ($p=0.012$ and $p=0.027$, respectively). *M. aquaticum* plants grown at low salinities also had a significantly greater shoot RGR than those grown in freshwater conditions ($p<0.005$). The negative shoot RGR exhibited by *M. aquaticum* grown in the most saline pond was significantly different from plants grown under all other conditions ($p<0.005$).

Root RGR (Fig 8.C) did not follow the same trend as that of shoot RGR in the case of *M. aquaticum*. Unlike its shoots, the roots of *M. aquaticum* continued to grow under the most saline conditions. Root RGR data for *M. spicatum* was square root transformed to obtain normality). Root RGR was normally distributed, as assessed by Shapiro-Wilk's test ($p>0.05$). Both species failed the test for homogeneity of variances, as assessed by Levene's test for equality of variance, $p=0.04$ for *M. spicatum* and $p=0.002$ for *M. aquaticum*. Root RGR was statistically significantly different between salinity treatments for *M. aquaticum* ($F(3, 12.9)=29.99$, $p<0.005$) but not for *M. spicatum* ($F(2, 15.8)=2.227$, $p<0.141$). Post hoc tests showed that *M. aquaticum* plants grown at high salinities had a significantly lower root RGR than those grown in freshwater and low salinities ($p<0.005$) but did not differ significantly from those grown at moderate salinity.

Plant Morphology in the Field

The morphology of *Myriophyllum* spp. plants grown at different salinities under semi-natural field conditions was measured in terms of apical shoot length, branch number, branch length and, in the case of *M. aquaticum*, the ratio of emergent to submerged biomass (Fig. 9).

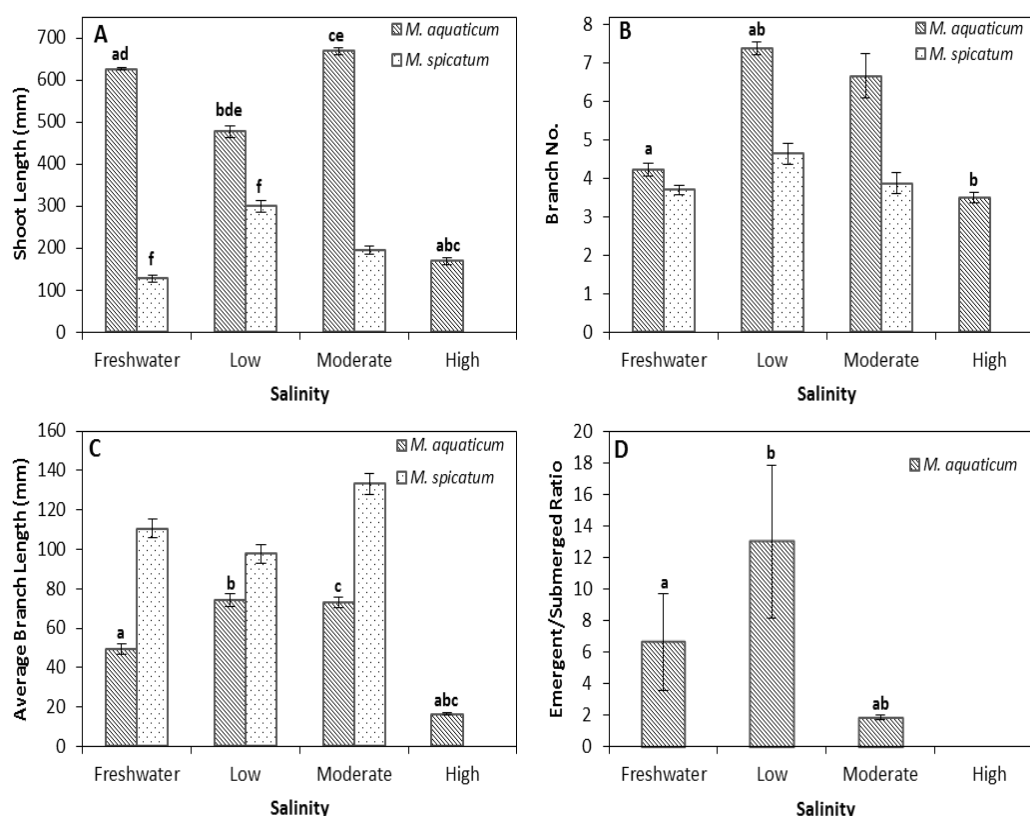


Figure 9: Morphological characteristics of plants grown in semi-natural waterbodies of varying salinity. (A) Apical shoot length, (B) No. of branches (C) Average branch length and (D) The ratio of emergent:submerged shoot biomass. Where 'Freshwater' = 0-0.05ppt, 'Low' = 0.3-1.1ppt, 'Moderate' = 1.7-7.2ppt and 'High' = 2.3-13.4ppt. Treatments that share a letter are significantly different from each other.

On average the apical shoots of *M. aquaticum* plants were longer than those of *M. spicatum* plants grown at all salinity levels (Fig 9.A). The shortest *M. aquaticum* plants (169 ± 8 mm) occurred in the most saline pond while on average the tallest *M. aquaticum* plants were grown at moderate salinity. In the case of *M. spicatum* the shortest plants (128 ± 8 mm) were those grown under freshwater conditions while the tallest plants (300 ± 15 mm) were those grown in low salinities. Apical shoot length was normally distributed in both species throughout the experiment and there were no significant

outliers in the data. Neither *M. aquaticum* ($p=0.002$) nor *M. spicatum* ($p=0.021$) met the assumption of homogeneity of variance.

Apical shoot length was statistically significantly different between salinity treatments for *M. aquaticum* ($F(3, 12.3)=109.249$, $p<0.005$) and for *M. spicatum* ($F(2, 16.9)=5.367$, $p=0.016$). Post hoc tests showed that *M. aquaticum* plants grown at high salinities were significantly shorter than those grown in freshwater, low and moderate salinities ($p<0.005$). *M. aquaticum* plants grown in low salinities were found to be significantly shorter than those grown in freshwater ($p=0.034$) and moderately saline ($p=0.001$) conditions. Contrastingly, *M. spicatum* plants grown under low salinities were found to be significantly longer than those grown in freshwater ($p=0.015$), but not significantly different from those grown in moderately saline conditions.

The average number of branches (to the nearest whole number) produced by *M. aquaticum* and *M. spicatum* plants in freshwater conditions was 4. At low salinities the number of branches increased to a maximum of 7 and 5 in *M. aquaticum* and *M. spicatum*, respectively. In *M. aquaticum* the minimum number of branches was observed at high salinities. Data was not normally distributed in either species and there was one significant outlier. A Kruskal-Wallis H Test was thus employed. Distribution of branch number was not evenly distributed in either species as assessed by visual inspection of a box plot. No statistically significant difference in branches produced by *M. spicatum* was observed in this experiment. There was a statistically significant difference in the number of branches produced by *M. aquaticum* under different salinity treatments, $H(3)=15.547$, $p=0.001$. Post hoc pairwise comparisons revealed statistically significant differences in branch number between freshwater and low salinity treatments ($p=0.024$), and low and high salinity treatments ($p=0.001$) but not between any other pairwise comparison.

The average branch length of *M. aquaticum* plants was typically shorter than that of *M. spicatum* plants throughout the experiment. Average branch length of *M. aquaticum* increased from $49\pm3\text{mm}$ when grown under freshwater conditions to $74\pm3\text{mm}$ and $73\pm3\text{mm}$ in low and moderate salinities. The shortest *M. aquaticum* branches were $16\pm1\text{mm}$ on average and occurred on plants grown at high salinities. Average branch length of *M. spicatum* ranged from $98\pm5\text{mm}$ when grown in the low salinity treatment to a maximum of $113\pm5\text{mm}$ when grown in a moderately saline treatment. Average branch length data was square root transformed to achieve normality. There were no significant outliers in the data. Both species met the assumption of homogeneity of variance. Average

branch length was statistically significantly different between salinity treatments in *M. aquaticum* ($F(3, 27)=20.763$, $p<0.005$). Post hoc tests showed that *M. aquaticum* plants grown at high salinities had a significantly shorter average branch length than those grown in all other treatments ($p<0.005$ in all cases). There was no statistically significant difference in average branch length between salinity treatments in *M. spicatum* ($F(2, 26)=1.382$, $p=0.269$).

The ratio of emergent shoot biomass to submerged shoot biomass was calculated for *M. aquaticum* plants. The ratio of emergent to submerged tissue was at its greatest in low salinities and at its lowest in moderate salinities. No emergent biomass was recorded in plants grown in the most saline conditions. Pond No. 4 was thus excluded from further analysis of emergent to submerged biomass ratios. Data from freshwater, low and moderate salinity treatments was not normally distributed and there were two significant outliers which were kept in the analysis. A Kruskal-Wallis H Test was thus employed. Distribution of ratios was not evenly distributed as assessed by visual inspection of a box plot. There was a statistically significant difference in ratio distribution between different salinity treatments, $H(2)=12.632$, $p=0.002$. Post hoc pairwise comparisons showed statistically significant differences in the ratio of emergent:submerged tissue between plants grown in moderate salinities and those grown in freshwater ($p=0.012$) and low ($p=0.002$) salinities.

Discussion

In coastal wetlands the salinity of sediment and water is known to play a key role in controlling the distribution of many aquatic or semi-aquatic organisms (Palmer *et al.*, 2011, Saiz-Salinas & Gonzalez-Oreja, 2000). Plant communities in habitats such as salt marshes, tidal rivers, estuaries and lagoons are usually dominated by halophytic species which have evolved a variety of processes to avoid and/or tolerate salt stress (Flowers and Colmer, 2008; Crain *et al.*, 2014; Silvestri *et al.*, 2005; Watson & Byrne, 2009; Ungar, 1998). Freshwater macrophytes are not typically capable of tolerating salt stress in the same way halophytes do (James *et al.*, 2003; Brock *et al.*, 2005; Nielson *et al.*, 2003). It has been hypothesised that stressful environments may represent a barrier to invasion by non-native species (Alpert *et al.*, 2000; Pauchard *et al.*, 2009).

This study compared *M. spicatum* (a native species in Ireland) and *M. aquaticum* (an invasive alien species in the freshwaters of Europe, North America, Southern Africa and

Australasia) in order to determine if *M. aquaticum* has the potential to become a successful invader of brackish waters and transitional wetland habitats in Ireland. Recognising that different barriers to invasion success occur at the various life stages of a species (Sakai *et al.*, 2001), the present study investigates the response of both *Myriophyllum* species to salinity at the asexual propagule and established plant stage.

The Effect of Receptive Environment Salinity on *Myriophyllum* Propagules

Amongst other factors, plant fitness and invasion success depend on the ability to reproduce and persist in an environment. In many species salinity has a negative impact on reproduction. For example, a reduction in flowering in response to salinity has been reported for *M. spicatum* (Twilley and Barko, 1990; Martin & Valentine, 2014). Further, Nowak *et al.*, (2017) demonstrated that even when *M. spicatum* was abundant in coastal waters its seeds were absent from the sediment. Capers (2003) showed that the colonisation of a tidal marsh by freshwater submerged macrophytes was primarily by means of vegetative propagules. Thus, the capacity of *Myriophyllum* spp. to reproduce asexually from allofragments under saline conditions appears to be vital to allow the species to colonize brackish water environments.

The reproductive success of a propagule is partially dependent on the suitability of the receptive environment (Kuntz *et al.*, 2014; Li *et al.*, 2016). Previous lab based investigations of the response of *Myriophyllum* spp. to salinity have focused on large apical shoots or established plants rather than small, easily dispersed, allofragments as reproductive propagules. The present study hypothesised that the regenerative success of *Myriophyllum* spp. propagules are influenced by the salinity of the receptive environment into which it has been dispersed.

Propagule Success, *Ex-Situ*

Plant propagules were determined to have viably regenerated if new shoot or root production was observed. Examining the relationship between salinity and propagule viability revealed that for every unit that salinity was increased propagules of *M. aquaticum* and *M. spicatum* were less likely to regenerate. An increase in salinity is therefore associated with reduced likelihood of propagule viability in *Myriophyllum* spp., though the observed negative relationship had shifted to lower salinities for *M. aquaticum* compared to *M. spicatum*. No viability was recorded at salinities greater than 11ppt in *M. aquaticum* making the species incapable of successful regeneration in highly saline conditions.

For *M. aquaticum*, an increase in shoot length was promoted by moderate salinity of 4ppt, but shoot biomass was not promoted in the same way. Viable propagules exposed to salt concentrations up to 11ppt maintained a level of biomass production that was not significantly different from that of propagules grown in freshwater. The findings of the present study are corroborated by previous works which found that, under lab conditions, the biomass growth of apical shoots of emergent *M. aquaticum* were unaffected by salinities of up to 5ppt (Haller *et al*, 1974; Thouvenot *et al.*, 2012). Previously, recorded salt concentrations with negative impacts on apical shoots of *M. aquaticum* were reported to be between 6ppt and 13ppt (Haller *et al*, 1974; Thouvenot *et al.*, 2012). The present study showed that moderate salinity reduced the likelihood of *M. aquaticum* propagules producing roots and reduced rooting success in terms of root number, biomass and length. Root production was completely inhibited at salinities greater than 8ppt. This is supported by Thouvenot *et al* (2012) who reported a similar negative relationship between salinity and root production in apical shoot fragments of *M. aquaticum*. Thus, the success of viable *M. aquaticum* propagules colonising brackish environments could be limited by impaired root production and the ability to successfully anchor to the substrate (Barrat-Segretain *et al.*, 2003). The present study also shows that if a viable propagule successfully produces roots in brackish waters shoot production and overall growth will not be impaired by saline conditions, indicating that successful establishment is likely upon rooting success.

In contrast to *M. aquaticum*, propagules of *M. spicatum* were more likely to root as salinity is increased. As with shoot production, optimum conditions for root success in terms of root number, length and biomass were at salinities of 4-8ppt. The ability of viable *M. spicatum* propagules to colonize a brackish habitat is thus less likely to be limited by the ability to successfully root. Propagule success may even be enhanced by low to moderate salinities as growth in terms of shoot and root biomass and shoot length was promoted by salt concentrations of 3-8ppt.

The Effect of Waterbody Salinity on *Myriophyllum* Plants in the Field.

The Context of the Transitional Wetland

The physicochemical conditions of transitional waters are under the influence of both the marine and freshwater environment (McLusky & Elliott, 2007). Thus the salinity of the water and substrate of coastal wetlands are known to fluctuate and change according to the tides, freshwater influx, weather and climatic events (Lettice, 2014; Telesh &

Khlebovich, 2010; Tagliapietra *et al.*, 2009). A freshwater pond and three lagoon ponds (Fossit, 2000) located in close proximity to each other were used in an experimental study of the effect of salinity on *M. aquaticum* and *M. spicatum* grown under semi-natural conditions. The influence of the tide on the salinity and pH of these four coastal wetlands was monitored on six sampling occasions in order to establish their salinity range and pH range.

The pH of the individual study ponds was not altered by the tides and there was no significant difference in pH between ponds. The salinity of the four study ponds ranged from freshwater with undetectable salinity to a maximum of 13.4ppt at high tide. The three ponds with detectable salinity were classified as lagoons according to Fossit (2000). Irish lagoons vary from oligohaline (0.5-5ppt) through to euhaline (30-40ppt) (Healy, 2003) and the salinity range of lagoons in the present study fell well within the salinity range of other nearby lagoons on the Cork coast (Lettice, 2014). Therefore, it is plausible that the actual salinity range of the study ponds may be greater than the recorded range. The freshwater pond and the low salinity lagoon were found to maintain a stable salinity which did not fluctuate with the tide. By contrast, tidal intrusion significantly increased the salinity of both the moderate and high salinity lagoon ponds. Over all there was a difference in salinity between each pairwise comparison of ponds, but the magnitude of this difference was greatest at high tide.

The close proximity, stable pH and differences in salinity made this field site an appropriate study site to investigate the influence of salinity on the growth and success of *Myriophyllum* species in transitional waters. In this field based experiment well established plants were grown in ponds of different salinities for six weeks.

Waterbody Salinity & Established Plant Performance.

M. aquaticum and *M. spicatum* growth responses to saline conditions have previously been investigated under laboratory and mesocosm conditions. In comparison, this experiment investigated performance of both species under field conditions in semi-natural waterbodies. In the case of *M. aquaticum*, previous studies (Haller *et al.*, 1974, Thouvenot *et al.*, 2012; Thouvenot *et al.*, 2015) found that overall growth was unaffected by low salinities (0-4ppt), slowed by moderate concentrations (4-6ppt) and ceased at high concentrations (10-13ppt). In the literature the response of *M. spicatum* to salinity exposure appears to be less consistent, with one study reporting that high salinity concentrations of 12ppt did not affect the species' growth (Twilley & Barko, 1990), while

another study reported almost complete die off at moderate salinity concentrations of 6ppt (van Wijck *et al.*, 1994). In the present field experiment deleterious effects of salt were observed for both species at high salinities in terms of plant survival (*M. spicatum* suffered 100% mortality at highest salinity treatments), overall RGR, shoot biomass, root biomass, apical shoot length and branch length.

The present study experimentally showed that low to moderate salinity promoted growth of both species in terms of overall RGR, shoot biomass and number of branches. This suggests that optimum conditions for *M. spicatum* and *M. aquaticum* growth are in low salinity environments and that plants grown at moderate salinity perform as well as plants grown in freshwater. The observed growth promotion of freshwater plants by low to moderate salt concentrations is not unique to this study. Previous experimental works have shown that low to moderate salinities (1-5ppt) can enhance the growth of a variety of typically freshwater macrophytes including *Myriophyllum simulans* Orch., *Lemna minor* L. and *Phragmites australis* (Cav.) Trin. ex Steud. (Haller *et al.*, 1974; Goodman *et al.*, 2010; Lissner & Schierup, 1997; van Wijck *et al.*, 1994). Haller *et al.*, (1974) speculated that “comparatively high sodium or chloride, or both, are required for maximum production” in some species. Sodium for example is an essential element for growth in some halophytic plants (Evans & Sorger, 1966).

Both *M. aquaticum* and *M. spicatum* occur abundantly in semi-natural brackish water lagoon habitats at Fota. This finding is consistent with reports relating to the distribution of both species in the wild. *M. aquaticum* has been reported as occurring in brackish waters of lagoons, estuaries and oligohaline tidal rivers in its native range (Ramirez *et al.*, 1989; Calliari *et al.*, 1997, Aston, 1967; Estevez *et al.*, 2000), though the salinity range of the habitat or the abundance of the species in the habitat is not reported in either case. Similarly, *M. spicatum* is a characteristic species of lagoons in Ireland (Hatch, 1996; Roden, 1998; Fossit, 2000) and is a successful invader of brackish waters in its invasive range (Menzie, 1979; Anderson *et al.*, 1966; Orth, 1994). The limits of *M. spicatum* distribution in its invasive range have been investigated (Anderson *et al.*, 1966, Orth, 1994) and the species has been found to occur in brackish waters with a greater salinity range (0.5-18ppt) than that measured in the present experiment. The 100% mortality rate of *M. spicatum* in the high salinity treatment in the present study may be inconsistent with its distribution in its invasive range because of confounding ecological factors. An example of such may be

the herbivory of *M. spicatum* by marine invertebrates in its native range but the release from herbivorous enemies in its invasive range (Valinoti *et al.*, 2011; Hansen *et al.*, 2011).

Morphological Mechanisms of Salt Avoidance & Tolerance

Salt stress in plants is caused by a combination of osmotic stress which occurs when water is lost from plant cells to the surrounding saline environment and ionic stress which occurs due to the accumulation salts inside cells (Munns, 2002). The ability of plants to tolerate salt stress is partially determined by their ability to retain water and maintain ion homeostasis (Parida & Das, 2005). When evapotranspiration occurs plants not only lose water but also accumulate salts which have been carried in the transpiration stream and have become deposited in the leaves as water evaporates (Munns & Tester, 2008). If the plant is unable to compartmentalize the salt in the vacuole, concentrations will eventually accumulate until toxic levels are reached causing the cells and leaves to die (Munns, 2002). Submerged aquatic plants, because they are not exposed to the air, are not subject to transpiration. In this way *M. aquaticum* differs from *M. spicatum* as it is heterophyllous and possesses a large proportion of emergent tissue. *M. aquaticum* is thus subject to transpiration and the accumulation of salts in the emergent leaf tissue.

It has been proposed that phenotypic plasticity contributes to the invasive success of plants (Baker, 1965; Davidson *et al.*, 2011), thus the morphological variation of *M. aquaticum* in relation to salinity treatments was investigated. Specifically, with the role of transpiration in salt tolerance in mind, the ratio of emergent to submerged *M. aquaticum* biomass was investigated under field conditions. Emergent biomass was entirely absent at high salinities and the ratio of emergent biomass to submerged biomass was significantly reduced at moderate salinities compared to plants grown in freshwater and low salinity (Fig. 10). This would most likely result in a reduction of transpiration for the plant, and subsequently the slower accumulation of salts, allowing the species to maintain normal growth rates. In the case of plants grown at high salinity the complete lack of emergent tissue means that those plants are not subject to transpiration by aerial exposure. The observation of Ramirez *et al.*, (1989) that *M. aquaticum* occurred only in its submerged form in estuarine environments in its native range is consistent with the finding of the present study.

As the root is the primary location of ion uptake a reduction in root growth is thought to be a response to root cell damage and to reduce ion uptake in stressful environments (Panda & Upadhyay, 2003). The deleterious effects of salinity on the roots of freshwater plants is a common occurrence in freshwater macrophytes with floating or emergent leaves and has

been recorded in a variety of species such as *Salvinia natans* L., *Ludwigia grandiflora* (Michx.) Greuter & Burdet, *L. minor*, *Lemna gibba* L., *Spirodela polyrhiza* (L.) Schleid. and *Triglochin procerum* R. Br. (Yilmaz, 2007; Goodman, *et al.*, 2010; Cheng, 2011; Jampeetong & Brix, 2009; Thouvenot *et al.*, 2012, Thouvenot *et al.*, 2015; Panda & Upadhyay, 2004). In the present study increasing salinity had a negative effect on the roots of established *M. aquaticum* plants slowing their growth rate in the field. No such negative affect was observed for *M. spicatum* in the present study, perhaps because the absence of emergent leaves means that there is no transpiration stream to drive ionic uptake.

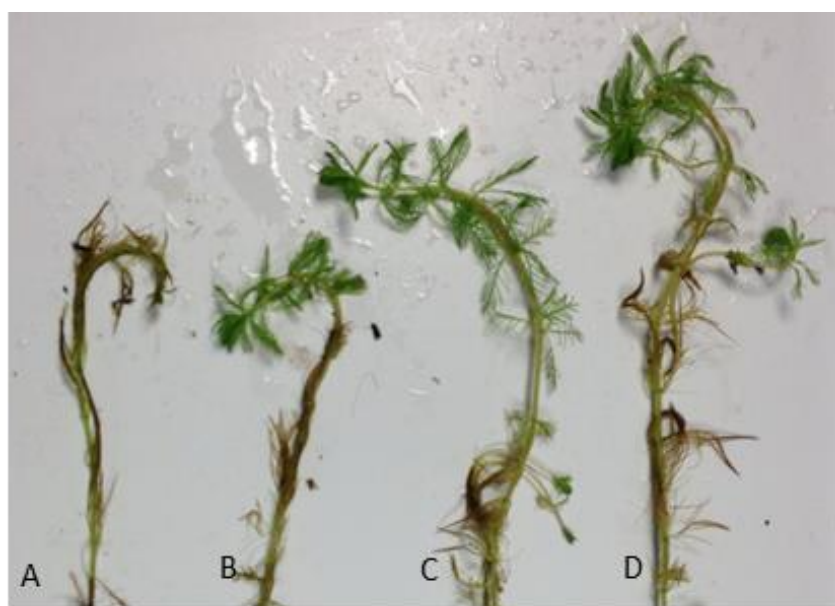


Figure 10. Emergent biomass was entirely absent in *M. aquaticum* plants grown at (A) high salinities and the ratio of emergent biomass to submerged biomass was significantly reduced at (B) moderate salinities compared to plants grown in (C) freshwater and (D) low salinity

Study Limitations

It is often noted that lab-based experiments represent a simplification of the processes underway in natural ecosystems. With this in mind, the present, lab-based, propagule experiment is best interpreted in the context of the subsequent field experiment. The lab-based experiments maintained a constant salinity that did not fluctuate throughout the experiment, thus plant propagules were exposed to persistent salinity treatments over a six-week period. Contrastingly, in the field based experiment salinity fluctuated on a twice daily basis with the tides and was also likely to vary with freshwater input, weather and

climatic events (Lettice, 2014; Telesh & Khlebovich, 2010; Tagliapietra *et al.*, 2009). The fluctuation in salinity was particularly pronounced in the moderate and high salinity treatments in the present field experiment as is evident by the salinity range recorded. The fluctuating salinity of semi-natural waterbodies may offer respite from persistent osmotic stress, thus allowing the plants to successfully grow, even in environments where the range in salinity is high. The importance of the duration of exposure to saline environments has previously been demonstrated for freshwater macrophytes and halophytes such as *Myriophyllum crispatum* Orch. and *Spartina alterniflora* Loisel (James and Hart, 1993; Brown *et al.*, 2006). Indeed, Brock (1986) argued that rather than the capacity to tolerate extreme levels of salinity, the ability to tolerate fluctuating salinity in combination with fluctuations of other environmental factors will determine the success of a plant species in an aquatic habitat.

The present propagule study used small, midstem, allofragments, only three nodes in size but if the fragment possesses an apical tip its viability may be enhanced (Riis *et al.*, 2009). It has also been shown that for some aquatic plant species the likelihood of remaining viable increases with fragment size. Further, larger fragments of both *M. aquaticum* and *M. spicatum* were better able to tolerate desiccation stress (Reidy, 2018; See Chapter 4) and it is known that, in plants, the stress response induced by desiccation is similar to that induced by salt (Mahajan & Tuteja, 2005). The results of the present study must, thus, be interpreted conservatively and it should be emphasised that larger fragments may be considerably more successful in establishing in brackish environments than the data in this study indicate.

Knowledge Application

The use of seawater inundation as a control method for invasive macrophytes of coastal wetlands has been advocated by some authorities (Dean *et al.*, 2013; Grillas, 2004; Charlton *et al.*, 2011). However, Kettering and Adams (2011) cautioned about the unintended negative effects invasive plant control can have on native species. The present study indicates that established populations of the alien *M. aquaticum* are more resistant to high salinities than those of its native counterpart, *M. spicatum*, in Ireland. The use of seawater inundation as a control method may potentially be ineffective against *M. aquaticum* but have unintentional negative effects on native species such as *M. spicatum* and others. However, Charlton *et al.* (2011) argue that, depending on the degree of invasion and native community composition, the benefit of invasive species eradication may be deemed greater than negative impacts on native co-occurring species.

Conclusions

There are multiple barriers to plant invasion success in brackish waters. Propagules must first be dispersed into the brackish environment, remain viable and successfully colonize the habitat. Upon successful colonisation of a habitat established plants must then tolerate fluctuating levels of salinity that persists for both short and prolonged periods in order to grow and spread. Alpert *et al.*, (2000) hypothesised that the presence of environmental stressors may allow native species, which are assumed to be adapted to local conditions a competitive advantage over alien species. The present study appears to support this hypothesis at the propagule stage of the asexual *Myriophyllum* life cycle. However, as established plants the alien species, *M. aquaticum*, appears to have the competitive advantage in semi-natural brackish environments. As suggested by Nielson *et al.*, (2003) salt sensitivity may differ among various life stages of a species.

This study showed that propagules of *M. aquaticum* and *M. spicatum* are capable of overcoming the barrier of salt stress with varying success in low, moderate and high salinities. In this study *M. aquaticum* propagules remained viable, maintained normal growth rates and successfully produced roots in salinities of up to 8ppt. When *M. spicatum* is compared to *M. aquaticum* at the propagule stage, *M. spicatum* is more tolerant of higher salinities of up to 11ppt, but also exhibits enhanced growth at low to moderate salinities. Propagules of both species are thus capable of colonizing and establishing populations in transitional wetland habitats.

The enhanced performance of established *M. aquaticum* plants, observed at low to moderate salinities under field conditions, indicates that the species, like *M. spicatum*, is capable of maintaining growth and invading brackish waters outside of its native range. In particular, *M. aquaticum*, exhibits morphological plasticity, adjusting its emergent to submerged biomass ratio in response to salt, allowing it to maintain normal growth at moderate salinities. This study recorded the presence of *M. aquaticum* in coastal wetlands with the ecological characteristics of lagoons in Ireland. These habitats are within the salinity ranges of other transitional waters recorded around the Irish coast. Combined with *M. aquaticum*'s capacity to tolerate other related stressors such as drought and fluctuating water levels, *M. aquaticum* is thus potentially a successful invader of transitional waters in Ireland.

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Chapter 6

A Case of Mistaken Identity: Cryptic Identity of Alien *Myriophllym* Spp. in Ireland has Implications for Invasive Alien Species of E.U. Concern

Introduction

Cryptic invasions have been defined as “the occurrence of a species or genotype that was not previously recognised as alien in origin or not distinguished from other aliens” (Novak, 2011). Cryptic invasions arise when there are morphological similarities between invasive alien species and/or between alien and native species. Such morphological similarity can result in the misidentification of a non-native species as a native species or another closely related alien species (Morais & Reichard, 2018). As a result of cryptic identity, invasion by some alien species can go unnoticed or result in the mismanagement of alien taxa in their non-native range. Freshwater environments are particularly vulnerable to cryptic invasions and have been recorded for taxa of aquatic plants, invertebrates, fish and amphibian (Morais & Reichard, 2018; Kettering & Mock, 2012; Tavalire *et al.*, 2012; van Bocxlaer *et al.*, 2015; Lucek, 2016; O'Donnell *et al.*, 2017). Cryptic invasions of the freshwater environment have been associated with negative impacts such as hybridization with native species, replacement of similar native species and loss of biodiversity. Aquatic plant species such as those in the *Myriophyllum* genus are particularly difficult to identify correctly because they often possess “reduced floral characters and convergent vegetative morphology” (Moody *et al.*, 2008). Two *Myriophyllum* spp. in particular, *Myriophyllum aquaticum* (Vell.) Verdc. and *Myriophyllum heterophyllum* Michx. are considered to be freshwater invasive alien plant species of international concern in the European Union (Regulation (EU) 1143/2014; European Union, 2017).

M. aquaticum is native to freshwater habitats in the lowlands of central eastern and western South America (Orchard, 1981) and *M. heterophyllum* is native to the eastern regions of North America (Aiken, 1981; Thum *et al.*, 2011). Globally, *M. aquaticum* and *M. heterophyllum* are popular species in the aquatic horticulture industry and the aquarium trade due to their ease of cultivation and attractive aesthetic (Kay & Hoyle, 2001; Thum *et al.*, 2012; Ghahramanzadeh *et al.*, 2013). Both species have subsequently escaped captivity and established invasive populations in their introduced range (Orchard, 1981; Thum *et al.*, 2011; Hussner, 2012; Yu *et al.*, 2012; Tsiamis *et al.*, 2017; Gillard *et al.*, 2017). They are invaders of slow flowing or standing aquatic habitats, such as shallow wetlands, lakes, canals, ponds and backwaters (Thum & Lennon, 2010; Wersal & Madsen, 2011). Both species are considered to be ecologically and economically destructive throughout their non-native range. Like many aquatic nuisance species, they are known to have high

biomass production, which commonly forms dense monospecific stands and surface mats (Hussner, 2009). Such extensive stands of biomass can alter hydrology, modify the physiochemical properties of aquatic habitats and impact biodiversity by displacing native flora, hybridising with native congeneric species and altering faunal community composition (Desa & Lee, 2018; Hussner, 2009; Thum & Lennon, 2006; Stiers *et al.*, 2011; Schultz & Dibble, 2012; Orr & Resh, 1992; Lastrucci *et al.*, 2017). Invasive populations of these species also pose a threat to socioeconomic activity and human health (Douglas, 2009; Stone *et al.*, 2009; Halstead *et al.*, 2003; Hussner *et al.*, 2010). In their invasive range, high-cost intensive mechanical and chemical control programmes have been conducted on *M. aquaticum* and *M. heterophyllum* to varying levels of success (Bailey & Calhoun, 2008; Oreska & Aldridge, 2011; Hussner *et al.*, 2017). Restrictions have recently been imposed on the importation and sale of both species throughout the E.U. and member states are required to take measures for their early detection, rapid eradication and/or management (European Commission, 2014: Regulation (EU) 1143/2014).

The reproduction and secondary spread of *M. aquaticum* in its introduced range is thought to be entirely asexual as it is a dioecious species and only pistillate plants have been recorded throughout its introduced range (Orchard, 1981; Wersal & Madsen, 2011; CABI, 2007). By contrast *M. heterophyllum* is capable of both asexual and sexual reproduction in its invasive range and it hybridises readily with other *Myriophyllum* spp (Barnes *et al.*, 2013; Moody & Les, 2002; Thum & Lennon, 2006; Thum *et al.*, 2011). *M. heterophyllum* has not yet been recorded on the island of Ireland (National Biodiversity Data Centre, 2018; Botanical Society of Britain and Ireland, 2018), while *M. aquaticum* is a relatively recent addition to the Irish flora having been first recorded in Co. Down, in 1988 (Reynolds, 2002). Using environmental niche models under current environmental data sets and predicted climate scenarios, Kelly *et al.*, (2014) showed that at present *M. aquaticum* occupies only 2% of its potential range in Ireland and over the coming decades it is likely to spread. By the commencement of this study in October 2016 *M. aquaticum* had been recorded in 27 of the 10km atlas squares in Ireland (National Biodiversity Data Centre, 2016; Botanical Society of Britain & Ireland, 2016). However, the current distribution of the species seems to be disjointed with four main clusters occurring in the North, East, South and South West of the island, and a single population in the midlands (Fig. 1).

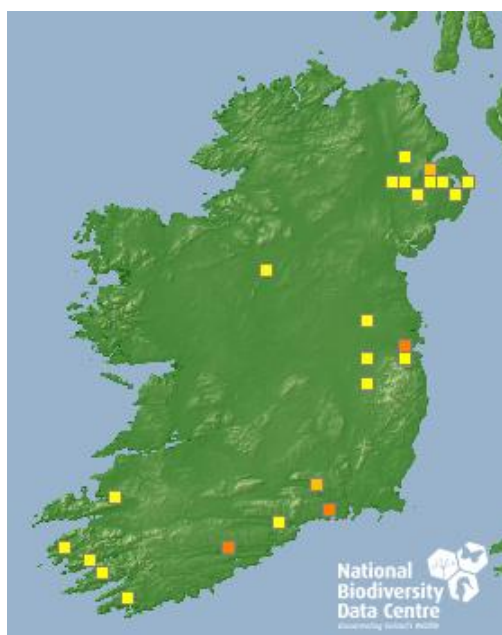


Figure 1: Known distribution of *M. aquaticum* in Ireland prior to the commencement of this study. (Colour intensity represents frequency of records within a 10km atlas square). Map adapted from National Biodiversity Data Centre (2016)

Myriophyllum species are renowned for being difficult to identify morphologically (Aiken, 1981; Moody & Les, 2010; Thum *et al.*, 2012; Moody & Les, 2007; Sturtevant *et al.*, 2009) and they often exhibit morphological plasticity depending on abiotic conditions (Aiken, 1981; Strand *et al.*, 2001; Wersal & Madsen, 2011; Tóth *et al.*, 2017; Weyl & Coetzee, 2016). Members of the genus are repeatedly mistakenly identified, leading to sometimes unrecognised cryptic invasions and causing difficulties with the regulation, management and eradication of nuisance species. *M. aquaticum* is a heterophyllus species characterised by both a submerged and emergent growth form (Parnell *et al.*, 2012; Orchard, 1981). This is dissimilar from the three native *Myriophyllum* spp. in Ireland (*M. alterniflorum* DC., *M. spicatum* L. and *M. verticillatum* L.) which are almost entirely submerged but for emergent flowering pedicels. Thus, the densely glandular aerial leaves of emergent *M. aquaticum* shoots are an important characteristic in differentiating the species from its native congeneric species in Ireland (Parnell *et al.*, 2012; Preston, 1998). However, in its submerged form, there can be considerable morphological overlap with congeneric species, including *M. heterophyllum* (Parnell *et al.*, 2012; Preston, 1998, Faegri, 1982; Orchard, 1985; Torres Robles, 2011). Indeed, throughout the genus submerged plants frequently share many vegetative traits with other congeneric species (Orchard, 1985; Orchard, 1981; Aiken, 1981).

Molecular techniques are becoming increasingly important for the early detection of aquatic invasive species (Newton *et al.*, 2016; Darling & Mahon, 2011) and for the accurate identification of cryptic aquatic plants such as those in the *Myriophyllum* genus (e.g. Ghahramanzadeh *et al.*, 2013; Thum *et al.*, 2012; Thum *et al.*, 2010; Sturtevant *et al.*, 2009; Moody & Les, 2010; Moody *et al.*, 2008). Evidence suggests that genetic variation is an important factor which can influence the invasiveness of an alien species and contribute to its resistance to eradication efforts (Roman & Darling, 2007; Ward *et al.*, 2008; Thum *et al.*, 2011). Further, the advancement of molecular techniques provides a platform with which to elucidate the provenance of an alien species, trace the introduction history and infer subsequent spread (Weyl, *et al.*, 2016; Moody *et al.*, 2016; Zhang *et al.*, 2010). Given Ireland's obligation toward early detection, rapid eradication and effective management of *M. aquaticum* and *M. heterophyllum* under international law, it is important to accurately discern the identity of non-native *Myriophyllum* species in Ireland and to determine how genetically diverse established populations are. This study hypothesised that:

1. Previously recorded populations of *M. aquaticum* in Ireland are not genetically identical.
2. The accurate identification of non-native *Myriophyllum* taxa in Ireland is confounded.
3. The use of molecular tools can be used to elucidate the genetic diversity of introduced *Myriophyllum* taxa and discern putative cryptic invasions of freshwater environments in Ireland.

This study employed the established amplified fragment length polymorphism (AFLP) molecular marker technique (LaRue *et al.*, 2013; Thum *et al.*, 2011; Vos *et al.*, 1995) to investigate the genetic variations among recorded *M. aquaticum* populations in Ireland. Additionally, given the increasing reliance on molecular methods in the identification of cryptic populations of *Myriophyllum*, DNA sequence techniques were used for taxonomic identification of selected accessions (Moody & Les, 2010). It is important to elucidate the taxonomic identity and genetic variation among recorded populations of *M. aquaticum* in Ireland as it will aid in understanding the frequency of introduction events and how the species has spread throughout the island. Additionally, it may facilitate the early detection of other non-native cryptic *Myriophyllum* species in Ireland. Further, it will also inform future legislation, management and eradication of invasive alien species of international concern at the early stages of invasion in Ireland.

Methods

Sample Collection

Up to date records of the distribution of *M. aquaticum* on the island of Ireland were obtained from the National Biodiversity Data Centre (2016) and the Botanical Society of Britain & Ireland (2016) and combined with the author's personal records. 90% of confirmed locations in Ireland were visited in early October 2016 and permission to access the site and collect plant material obtained from land owners. In total 15 populations were visited in the Republic of Ireland and 12 in Northern Ireland, but plant material was successfully collected from just 14 populations in the Republic of Ireland. This comprised of 12 populations occurring in natural/seminatural waterbodies and two ornamental populations in domestic gardens. No specimens were recovered from any of the previously recorded Northern Irish populations. Specimens were identified as *M. aquaticum* based on the morphological characteristics using available dichotomous keys (Parnell *et al.*, 2012; Preston, 1998). The dominant growth form (emergent or submerged) exhibited by population was recorded. The emergent growth form primarily consisted of robust vegetation, possessing the characteristic, densely glandular, aerial leaves in whorls of four, laying in prostrate mats on the water surface or emerging erectly out of the water (Fig. 2). The submerged growth form was characterised by vertical growth with characteristic submerged, narrow-linearly segmented, pinnate leaves in whorls of four to six (Orchard, 1981; Sytsma & Anderson, 1993; Jeune & Lacroix, 2009; Parnell *et al.*, 2012) (Fig. 2). As *M. aquaticum* is a restricted species, the collection, transport and propagation of *M. aquaticum* for research purposes was permitted under a license granted by the National Parks and Wildlife Service, Ireland (License No: IAS 7/2014).

Ten healthy apical shoots of *M. aquaticum* were collected from each population. Material was collected either by hand or by rake toss. Material was collected from individuals that were approximately evenly distributed around the circumference of the waterbody, with care being taken not to collect material from the same individual plant twice where possible. Minimum distance between individuals was 1m in the smallest waterbody. Upon return to the lab plant material was washed under running tap water to remove sediment, periphyton and invertebrates. Gloves were changed between washing each individual in order to minimize possibility of cross contamination with genetic material. Washed plants were frozen at -80°C. Frozen meristematic shoots were then freeze dried to a constant weight.



Figure 2: Morphological variation in plant specimens collected from previously recorded populations of *M. aquaticum*. (A) plants emerging erectly above the water, (B) plants prostrate on the water surface, and (C) submerged plants.

Genetic Variation - AFLP

Amplified fragment length polymorphism (AFLP) analysis was employed to investigate if there was genetic variation between recorded populations of *M. aquaticum*. AFLP markers are considered suitable for studies of interspecific and intraspecific variation as it is possible to amplify many loci in order to identify those that are polymorphic (Nybom, 2004). AFLPs were conducted following protocol modified from Vos *et al.*, (1995) and primers were selected from Thum *et al.*, (2011) and La Rue *et al.*, (2013) as they have been successful for other species in the *Myriophyllum* genus. Genomic DNA was extracted from plant material using Qiagen DNEasy extraction kits following the manufacturer's instructions. Extractions were run in duplicate for three individuals from each population in order to assess reproducibility of the AFLP fingerprinting (Ley & Hardy, 2015). EcoRI and MseI restriction enzymes were chosen to reduce genome complexity in this project. PCR amplification was performed in two stages: Preselective reactions employed the EcoRI-A and MseI-C primers and selective reactions employed VIC EcoRI AGG - MseI CAC and NED EcoRI ACG – MseI CAG primers. Selective PCR product was then sequenced on an ABI 3730xl at the University of Illinois-Urbana-Champaign Sequencing Core.

Chromatograms of AFLP sequenced data was scored according to the absence/presence of peaks using GeneMapper version 5.2 (Applied Biosystems) software. Chromatogram quality was inspected; failed or poor quality accessions were excluded and 76 accessions were included. Analysis of fragments was limited to between 100 and 500 base pairs in length. In total 666 loci were included in the analysis when primer combinations were concatenated. GeneMapper generated a binary matrix and data was analysed using SpAgEDi 1.5 (Hardy & Vekemans, 2002) and GenAlEx 6.5 (Peakall & Smouse, 2012) software respectively. It is common that, due to error, AFLP fingerprints are not 100% identical for two samples from the same individual plant (Douhovnikoff & Dodd, 2003). Thus, distance between duplicates of individuals was calculated in order to estimate the error rate. PCoA analysis was employed to visualise genetically similar individuals. Genetically similar accessions were grouped together and visualised using PCoA analysis.

Genotypes were evaluated using Structure v2.3 (Pritchard *et al.*, 2000; Falush *et al.*, 2007). An admixture model was run with no predefined population information and an alpha value of 1. A burnin period of 10000 was employed with 10000 MCMC reps after the burnin. Analysis was run for possible K values ranging from 1-6 (based on the number of groups in the PCoA plot). and the number of distinct genetic clusters (K) was evaluated using the ΔK statistic (Evanno *et al.*, 2005) calculated by Structure Harvester.

Molecular Identification - ITS

Molecular identification of selected accession from each group were performed following established protocol for the *Myriophyllum* genus (Moody & Les, 2002; Moody & Les, 2010; Thum *et al.*, 2011; Zuellig & Thum, 2012; Thum *et al.*, 2012). Identification was based on molecular analysis of DNA sequences from the nuclear DNA internal transcribed spacer 1 and 2 and the intervening 5.8s ribosomal subunit (hereafter ITS). Methods for the amplification and sequencing of ITS follow those used by Thum *et al.*, (2011). Molecular identification was performed by comparing ITS sequences from the present study to those from Moody and Les' (2010) and Thum *et al.*'s (2012) accessions of the *Myriophyllum* genus which have been deposited on GenBank. Methods for alignment of accessions with GenBank accessions follow Thum *et al.* (2012). Sequences from the present study were aligned to GenBank accessions using the ClustalW algorithm as implemented in MEGA version 4 (Tamura *et al.*, 2007). Gaps were treated as missing. In total eight individuals were selected for ITS analysis these were individuals No. 7, 8, 10, 11, 22, 28, 40 and 41 (See table 1 for population of origin).

Results

Specimens morphologically identified as *M. aquaticum* were successfully collected from 14 of the recorded populations in the Republic of Ireland. Two growth forms, emergent and submerged, were observed in the field. The emergent growth form of *M. aquaticum* was the most frequently recorded growth form, occurring in eleven of the populations distributed throughout the country. Three populations, one in the east (Grand Canal, Co. Dublin), one in the mid-west (Lough Boder, Co. Roscommon), and one in the south-west (Sneem, Co. Kerry) were found to have entirely submerged populations (Tab. 1). The population at Fota, Co. Cork had a mixture of emergent and submerged individuals.

Genetic Variation - AFLP

AFLP primer combinations were concatenated and revealed a total of 666 polymorphic loci which were identified by AFLP analysis. Each polymorphic loci was scored as present or absent. 91 out of the 666 polymorphic loci were scored exactly the same in all duplicated individuals. This represented an average error rate of 8.3% per loci in duplicated individuals. No two duplicates of the same individual were scored exactly the same and overall variation between duplicates of the same individual ranged from 2.5% to 22%. A PCoA plot was used to visualise the variation between duplicates and individuals. The PCoA plots identified four distinct clusters, which represent different groups of genetically similar plants, in the studied populations recorded as *M. aquaticum*. These groups are visualised in Fig. 3 with the first two axes cumulatively explaining 46.5% of variation within the data. In Fig. 3 samples are visually assigned to four groups in which individuals cluster together.

Grouping of accessions in the Structure analysis corresponded with and confirmed the PCoA. All four clusters were clearly differentiated when K=4. Figure 4 illustrates the genetic similarity of all analysed samples. The majority of individuals were assigned to one cluster (G1, illustrated in blue). Based on the bar plot (Fig. 4) it is evident that there is further genetic variation within groups and the average distance between accessions calculated by structure (0.3138) was greatest for this group. A hierarchical approach is recommended to identify further genetic structuring within groups.

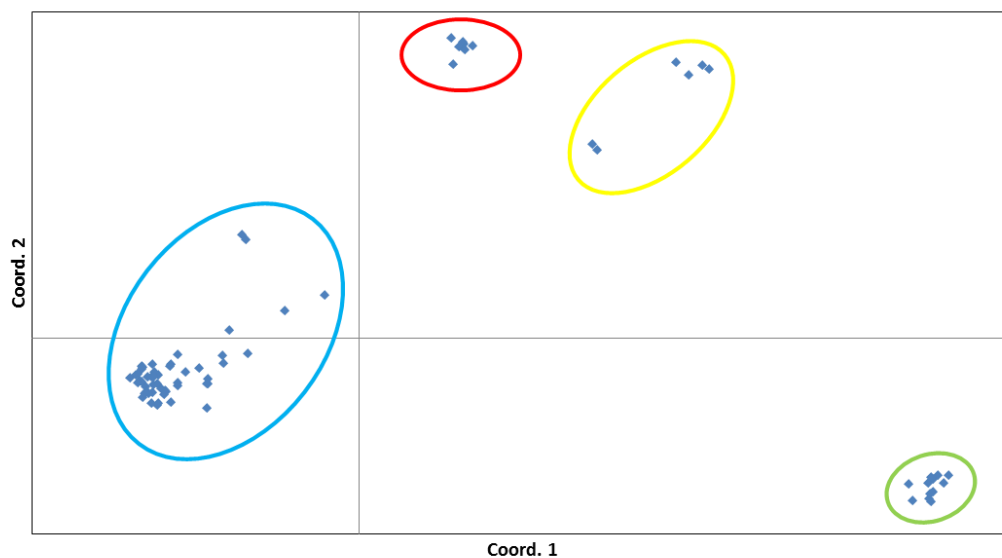


Figure 3: Principal coordinates analysis of AFLP data using 666 loci from concatenated primer combination 1 (VIC EcoR1 AGG – Mse1 CAC and NED EcoR1 ACG – Mse1 CAG). Genetically similar individuals are visually grouped. Blue = G1, Red = G2, Green = G3, Yellow = G4.

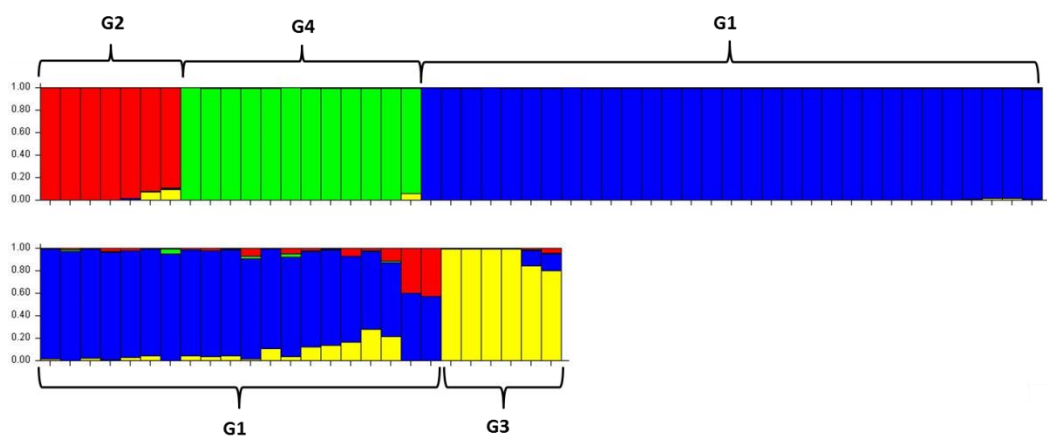


Figure 4: Structure analysis of genetic variation in AFLP accessions when K=4. Each bar represents an accession. Accessions are assigned to a group based on colour dominance. Bars dominated by Blue = G1, Red = G2, Yellow = G3 and Green = G4. Variation in colour within a bar indicates genetic variation from others in that group and similarity with other groups.

Table 1: Sampled *Myriophyllum* populations, with growth form as observed in the field and visually assigned genetic group based on principal coordinates analysis of 666 polymorphic loci identified by AFLP analysis.

Population	Accession	Geographic Location	Growth Form	Assigned
Thornbrook	1-3	52.21365, -7.24046	Emergent	G1
Schull	4-6	51.52407, -9.52745	Emergent	G1
Skibbereen	7-9	51.57389, -9.3046	Emergent	G2
Grand Canal	10-12	53.33557, -6.31779	Submerged	G4
Lough Boderg	13-15	53.86174, -7.99641	Submerged	G4
Usk	16-18	53.06238, -6.74099	Emergent	G1
Magaha	19-21	52.14775, -7.76956	Emergent	G1
Annestown	22-24	52.15509, -7.26517	Emergent	G1
Iniscarra	25-27	51.89497, -8.60426	Emergent	G1 & G2
Derreen	28-30	51.76803, -9.78261	Emergent	G1
Fota	31-33	51.90212, -8.29773	Emergent	G1
	40-42		Submerged	G3
Tralelee	34-36	52.26539, -9.71807	Emergent	G1
Valentia	37-39	51.9233, -10.31798	Emergent	G1
Sneem	43-45	51.79978, -9.95755	Submerged	G1

Most sampled populations contained individuals from just one group per population, with the exception of the Fota and Iniscarra populations which possessed individuals from two groups. The majority of populations (11 out of 14 populations) were assigned to the first group, G1. Most individuals assigned to G1 exhibited the emergent growth form with the exception of those from the Sneem population which were submerged. A second group (G2) contained the Skibbereen population and an individual from the Iniscarra population. A third group (G3) contained only the submerged individuals of the Fota population. The Grand Canal and Lough Boderg populations, both submerged, were assigned to the fourth group (G4). Table 1 summarises the observed growth form and visually allocated group based on PCoA and Structure analysis. The geographical distribution of identified groups is visualised in figure 5. No clear geographical structuring of groups was evident, with overlap in the geographic distribution of groups G1, G2 and G3. Group G4 occurs in the two most northerly populations analysed.

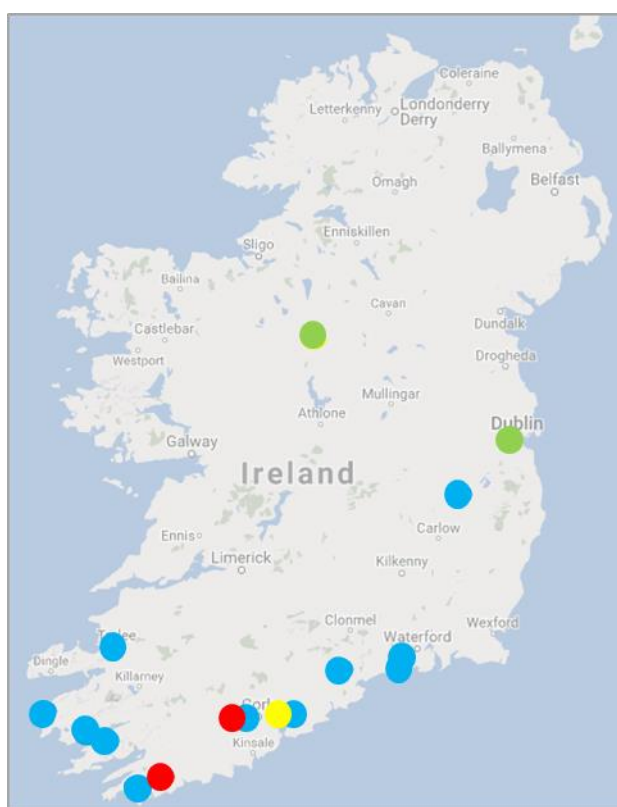


Figure 5: Geographic distribution of populations with assigned groups based on AFLP analysis. Blue=G1, Red=G2, Yellow=G3, Green=G4.

Molecular Identification - ITS

Two individual accessions from each of the AFLP determined groups were selected for taxonomic identification by comparing ITS sequences from the present study to those from Moody and Les' (2010) and Thum *et al.*'s (2012) molecular studies of the *Myriophyllum* genus which have been lodged with GenBank. This study identified four different ITS sequences (Tab. 2). Individuals No. 22 (Annestown, Co. Waterford) and No. 28 (Derreen Co. Kerry) were selected from G1 and were found to align with GenBank accessions of *M. aquaticum*. Individuals No. 7 and No. 8 (Skibbereen, Co. Cork) were selected from G2 and were found to most closely resemble *Myriophyllum* sp. 'red 1' Moody and Les. Individuals No. 40 and No. 41 (Fota, Co. Cork), were selected from G3 and were identified as *M. heterophyllum* when compared to GenBank accessions. Finally, individuals from G4, No. 10 and No. 11 (Grand Canal, Co. Dublin) matched with GenBank accessions of *M. verticillatum*. It was assumed that individuals within an assigned group share taxonomic identity. Table 2 summarises the assigned taxonomic identity of each group and figure 4 illustrates their sampled distribution in Ireland.

Table 2: The molecular identity of *Myriophyllum* spp. in this study, based on comparing ITS sequences to GenBank accessions.

Population	Accession No.	Assigned Group	Molecular ID (ITS)
Skibbereen	7	G2	<i>M. sp. 'red 1'</i>
Skibbereen	8	G2	<i>M. sp. 'red 1'</i>
Grand Canal	10	G4	<i>M. verticillatum</i>
Grand Canal	11	G4	<i>M. verticillatum</i>
Annestown	22	G1	<i>M. aquaticum</i>
Derreen	28	G1	<i>M. aquaticum</i>
Fota	40	G3	<i>M. heterophyllum</i>
Fota	41	G3	<i>M. heterophyllum</i>

Discussion

With the clonal nature of *M. aquaticum* in mind, this study employed the established AFLP molecular marker technique to investigate the genetic variation among recorded populations of the species in Ireland. Analysis revealed that previously recorded populations of *M. aquaticum* were highly variable genetically and that four distinct groups of individuals existed among recorded populations. Cognisant of the cryptic nature of the *Myriophyllum* genus and the high degree of overlap in vegetative morphology within the genus (Figure 6), further genetic analysis of the four groups identified in this study was conducted. ITS sequencing was employed to taxonomically identify selected individuals from each group. The present study thus elucidated the cryptic identities of non-native *Myriophyllum* populations in the Rep. of Ireland and identified four different taxa among populations previously morphologically identified as *M. aquaticum*. 71% of studied populations contained individuals of *M. aquaticum* which were correctly identified based on morphological characteristics. However, five of the studied populations contained plants which were incorrectly identified as *M. aquaticum* based on morphological traits. These populations were composed of one species native to Ireland, *M. verticillatum* and two taxa which are alien to Ireland and were previously unrecorded on the island of Ireland, namely *M. heterophyllum* and *M. sp 'red 1'*.

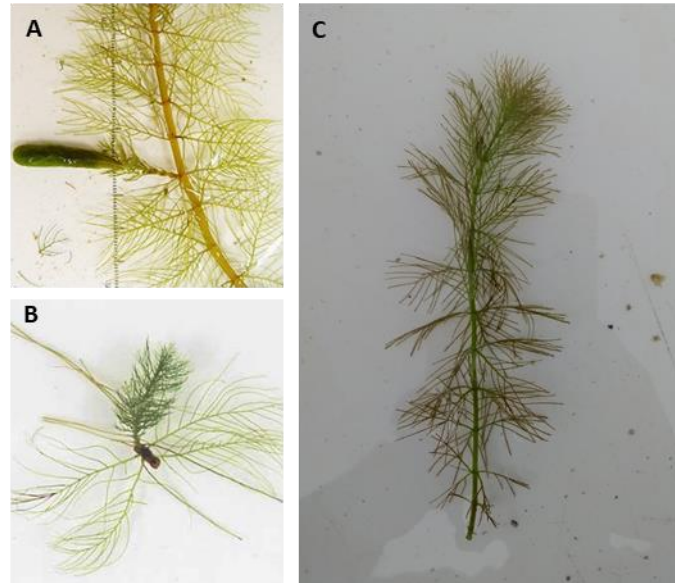


Figure 6: Examples of overlap in vegetative morphology within the *Myriophyllum* genus including submerged specimens of (A). *M. verticillatum*, (B) *M. heterophyllum* and (C) *M. aquaticum*.

(Images: Cameron, 2018; Hussner & Krause, 2007; Reidy, 2018).

Cryptic Identity of Non-Native *Myriophyllum* spp.

It has been stated that “aquatic plants are particularly difficult to identify properly due to their often reduced floral characters and convergent vegetative morphology” (Moody *et al.*, 2008) and it would appear that *M. aquaticum* is no exception. Recent investigations into the typification of *M. aquaticum* have suggested that there is ambiguity surrounding the lectotype for the species (Tur *et al.*, 2009). This taxon was known as *M. brasiliense* Cambess. until 1973 but was then combined with *Enydria aquatic* Vell. (1825) and renamed *M. aquaticum* (Vell.) Verdc. (Tur *et al.*, 2009). The species has also previously been known by the synonym *M. proserpinacoides* Gill. ex Hook. & Arn. (Torres Robles *et al.*, 2011). Adding further confusion to the taxonomic identity of *M. aquaticum*, Torres Robles *et al.*, (2011) have argued that the species is not strictly dioecious, as previously taxonomically described, having observed both dioecious and moneocious specimens in its native range. Indeed, closely related species in the *M. aquaticum* clade (*Myriophyllum* subgenus *Myriophyllum* section *Pectinatum*) have also been the subject of taxonomic debate (Moody & Les, 2010). Included in this clade are two undescribed, but molecularly distinct taxa (one of which was identified in the present study), *M. sp. “red 1”* and *M. sp. “red 2”* Moody and Les which are of unknown geographic origins but are morphologically very similar to *M. aquaticum* though more compact in habit (Moody & Les, 2010). The high degrees to which

morphological traits overlap between *M. aquaticum* and congeneric species have been repeatedly recognised. Orchard (1985) previously described the significant morphological overlap between *M. aquaticum*, *M. verticillatum* and *M. heterophyllum* and placed them together in a taxonomic alliance based on such traits.

Molecular techniques have become particularly useful in overcoming taxonomical identification problems associated with morphological convergence and character loss (Moody *et al.*, 2008). They are also becoming increasingly popular for the identification and early detection of invasive species. In its invasive range *M. heterophyllum* has repeatedly proven difficult to differentiate from *M. verticillatum*, other congeneric species and hybrids without the assistance of molecular tools (Thum *et al.*, 2006, Thum *et al.*, 2010; Thum *et al.*, 2011; Tavalire *et al.*, 2012). Additionally, recent genetic research conducted on *Myriophyllum* plants available in the aquarium trade revealed that *M. heterophyllum* and *M. aquaticum* are often misidentified by suppliers and are labelled and sold as incorrect taxa (Thum *et al.*, 2012; Moody *et al.*, 2008). The misidentification of individuals of *M. verticillatum*, *M. heterophyllum* and *M. sp. "red 1"* amongst Irish *Myriophyllum* populations previously recorded as *M. aquaticum* is therefore not surprising but the elucidation of their true identity using molecular techniques is an important advancement in the context of invasive species recording in Ireland.

Myriophyllum aquaticum

The majority of populations investigated in this study were grouped in G1 which was molecularly confirmed as *M. aquaticum*. All of these populations occur in naturalised states except for one which occurred as an ornamental plant in a domestic garden. The earliest known record of *M. aquaticum* in a naturalised setting in Ireland is from Co. Down, Northern Ireland in 1988 (Reynolds, 2002). It is not known if such early populations contained just a single clone or consisted of multiple clones. It is also not known how many introduction events have occurred since then, or the extent to which established populations have been secondarily dispersed throughout the island post introduction. This study failed to successfully collect plant material from any of the documented Northern Irish populations and was thus unable to answer questions about the earliest introductions of *M. aquaticum* to Ireland.

The study did however provide evidence that there is a degree of genetic variation among individuals confirmed to be *M. aquaticum* (G1), though further hierarchical Structure analysis of the AFLP data is necessary to establish within group distance thresholds in order

to determine exactly how much genetic variation occurs. Given the high tendency for clonal reproduction of *M. aquaticum*, even in its native range (Orchard, 1981), the variation found in this study indicates that material introduced to Ireland may have originated in multiple source populations. This study did not attempt to identify the source of these clones, but using AFLP, it would be possible to identify potential source populations from material collected from throughout the native and introduced range as has been done for other clonal aquatic and terrestrial invasive species (Thum *et al.*, 2010; Amsellem *et al.*, 2000; Zhang *et al.*, 2010). In this study, at least one population (Iniscarra, Co. Cork) of *M. aquaticum* originated in the ornamental plant trade, as confirmed by personal communication with the landowner.

Myriophyllum heterophyllum

Submerged plants from Fota, Co. Cork were molecularly identified as *M. heterophyllum* while emergent plants from the same location were molecularly identified as *M. aquaticum*. *M. heterophyllum* has been predicted to be among the most serious ecological and economic threats to Irish waters (Gallardo & Aldridge, 2013). The species is recognised by the European Union as an ‘invasive alien species of Union concern’ because of the significant ecological and socioeconomic threats it poses outside of its native range (European Union, 2017). Since 2017 restrictions have been imposed on the importation and sale of *M. heterophyllum* throughout the E.U. and member states are required to take measures for their early detection, rapid eradication and/or management (Regulation (EU) 1143/2014). The species is widely distributed throughout mainland Europe and is established in Austria, Belgium, Croatia, France, Germany, The Netherlands, Spain and Switzerland (Jasprica *et al.*, 2017; Brundu, 2015; Hussner, 2012), but *M. heterophyllum* has not previously been recorded in Ireland. The present study thus represents the first record of *M. heterophyllum* naturalised in a seminatural habitat in Ireland. Further, it is only the third record of *M. heterophyllum* on the islands of Britain and Ireland, the two previous records being from England in 1941 and 2016 (Smith *et al.*, 2017). The earlier record of *M. heterophyllum* from England has not been observed since its initial record and is thought to have gone extinct when the wetland was drained in 1947 (Smith *et al.*, 2017). The present study thus represents an important step in the early detection of a highly invasive species that poses a serious risk to European waters, while it still at the early stages of its invasion in Ireland.

The origin of *M. heterophyllum* in Ireland is unknown though it most likely originated from the aquatic horticulture or aquarium trade (Anderson *et al.*, 2015). Despite being listed as a

restricted species under Regulation (EU) 1143/2014, existing stocks of *M. heterophyllum* may continue to be sold under certain circumstances until February 2019 (European Commission, 2014). It is also well documented that in the aquatic horticulture industry *M. heterophyllum* is commonly incorrectly identified and distributed under false names (Thum *et al.*, 2012; Ghahramanzadeh *et al.*, 2013; Oele *et al.*, 2015; Van Valkenburg & Boer, 2015), representing continued introduction pathways if comprehensive monitoring of imported plants does not occur. Secondary dispersal of the *M. heterophyllum* by anthropogenic activity is also likely as it has been shown that propagules of the species are capable of tolerating overland dispersal between waterbodies (Barnes *et al.*, 2013; Rothlisberger *et al.*, 2010). Using predictive species distribution models for *M. heterophyllum* invasion, Gallardo & Aldridge (2013) predicted that the east and northern regions of the island were most favourable, while the southern coast was not deemed suitable. To the contrary, the present study found that the first known Irish population of *M. heterophyllum* occurred on the southern coast of Co. Cork, well outside of the previously predicted suitable range (Gallardo & Aldridge, 2013).

Myriophyllum sp. “red 1”

The *M. aquaticum* clade (*Myriophyllum* subgenus *Myriophyllum* section *Pectinatum*) contains the two morphologically undescribed taxa, *M. sp.* “red 1” and *M. sp.* “red 2”. These taxa are entirely uncharacterized outside the aquatic plant trade and have never been observed in flower. These taxa are most closely related to, but molecularly distinct from *M. aquaticum*. All three species are morphologically similar in their emergent form though *M. sp.* “red 1” and *M. sp.* “red 2” appear more compact in habit (Moody & les, 2010).

M. sp. “red 1” and *M. sp.* “red 2” taxa have not been observed in the wild and have previously only been detected through molecular studies of *Myriophyllum* spp. available through the aquatic plant trade in the USA and Australia (Thum *et al.*, 2012; Oele *et al.*, 2015; Moody & Les, 2010). It has previously been demonstrated that *M. sp.* “red 1” and *M. sp.* “red 2” plants sold in the aquatic plant trade are repeatedly mislabelled as *M. aquaticum* (or its synonyms), *M. heterophyllum* and other *Myriophyllum* spp. (Thum *et al.*, 2012; Oele *et al.*, 2015). In an extensive molecular screening of *Myriophyllum* spp. available through the aquatic plant trade in The Netherlands Ghahramanzadeh *et al.*, (2013) did not detect either *M. sp.* “red 1” or *M. sp.* “red 2”. Individuals of *M. sp.* “red 1” recorded in the present study thus represent the first confirmed European accessions of the taxa. In the present study both recorded populations of *M. sp.* “red 1” were found to occur in domestic

garden pond settings. Personal communication with property owners revealed that in both cases the plant material used to establish these garden populations was provided by horticultural suppliers or garden centres. This implies that the taxa was, and might still be available through commercial trade in Ireland.

The native distribution and ecology of *M. sp. "red 1"* is not known. However, given the close relationship between *M. aquaticum* and *M. sp. "red 1"* it has been speculated that both taxa may have the same invasive potential (Thum *et al.*, 2012). It is possible that the taxa occur more widely in horticultural settings throughout Ireland and has the potential to spread out of captivity. It is also possible that the taxa already occur in the wild as a cryptic invader, having been misidentified as *M. aquaticum* or another related species.

Myriophyllum verticillatum

In the present study, *Myriophyllum* specimens collected from two locations where *M. aquaticum* had previously been recorded (Grand Canal, Co. Dublin and Lough Boderg, Co. Roscommon) and in the present study were morphologically identified as *M. aquaticum* in its submerged form were later molecularly identified as the native species *M. verticillatum*. Reiche (1989) once alluded that *M. aquaticum* in its submerged form was identical to *M. verticillatum* (Torres Robles, 2011). Despite being native to Ireland, *M. verticillatum* is a highly obstructive macrophyte in Irish canals and has been the subject of both mechanical and chemical control (Caffrey & Monahan, 2006). The obstructive and seemingly invasive growth of *M. verticillatum* in Ireland may further confound the species identification process or mask other cryptic invaders of the genus.

No plant accessions from these populations were molecularly identified as *M. aquaticum*. This may represent a case of misidentification by both the initial recorders and the present author or alternatively a situation where in *M. aquaticum* populations were eradicated subsequent to their initial record and replaced by *M. verticillatum* post eradication. For example, the 2016-2020 Dublin City Invasive Alien Species Action Plan highlights the recorded Grand Canal population of *M. aquaticum* and prioritises it for eradication (Dublin City Council, 2016). Initial recorders and local authorities responsible for the management of the waterways in question have been consulted for further information on the nature of the vegetative material upon which the initial identification was based and any subsequent action undertaken on the basis of the record, but a response is awaited. In Ireland, the potential for future misidentification of *M. verticillatum* as *M. aquaticum*, or *vice versa*,

could result in incorrect allocation of invasive species management resources or the mismanagement of a cryptic invasive species thought to be a native species.

Legislative Implications

In 2014 the European Parliament adopted the EU Invasive Alien Species Regulation (Regulation (EU) 1143/2014), implementing Target 5 of the EU Biodiversity Strategy to 2020 (European Commission, 2011). The regulation provides for combatting invasive alien species through prevention, early detection, rapid eradication and management of alien species. Unlike EU Directives, EU Regulations become national law without having to be transposed and therefore was immediately enforceable by law in all Member States by January 2015 (Genovesi *et al.*, 2015). Central to this legislation is a “black list” of “Invasive Alien Species of Union Concern” which were identified through detailed risk assessments by member states. *M. aquaticum* and *M. heterophyllum* are currently two of 23 listed plant species of Union concern with an EU wide ban on importation, trade, possession, breeding, growing, transporting, use and release to the environment according to the regulation (European Union, 2017).

According to some commentators, enforcement of such restrictions on listed species is very challenging (Genovesi *et al.*, 2015). Species specific legislation or “black lists” of species are finite and have been open to criticism for not being effective in reducing trade of regulated plants (Oele *et al.*, 2015). In order to be effective such restrictions require high levels of industry compliance (Hulme *et al.*, 2017) and it has been well documented that plants sold in the aquarium trade are often misidentified, mislabelled or sold under incorrect names (Keller & Lodge, 2009; Maki & Galatowitsch, 2004; Martin & Coetzee, 2011). Indeed, this has been documented to be the case for *M. aquaticum*, *M. heterophyllum*, *M. sp. “red 1”* and other *Myriophyllum spp.* on multiple occasions (Thum *et al.*, 2012, Moody *et al.*, 2008; Oele *et al.* 2015; Ghahramanzadeh *et al.*, 2013). The cryptic nature of the *Myriophyllum* genus may provide regulatory loopholes through which restricted species such as *M. aquaticum* and *M. heterophyllum* could continue to be introduced to the EU. Additionally, existing populations of other invasive plants which may have been misidentified as *M. aquaticum* and *vice versa* are not subject to the legislative enforcement of eradication and control as deemed appropriate by international law.

Because of the slow pace at which the legislative process progresses it is difficult for species specific “black lists” to address such taxonomic loopholes as they arise. This has led

some authors to propose the “white list” approach which is implemented in Australia; such an approach prohibits the entry of any organism unless it is on an authorised list of species that pose little risk of invasion (Genovesi *et al.*, 2015; Garcia-de-Lomas & Vila, 2015).

Conclusions & Recommendations

This study demonstrates that molecular methods are a valuable resource for use in the rapid detection and monitoring of aquatic invasive plant species in Ireland. This study confirmed that the majority of previously recorded populations of *M. aquaticum* in seminatural habitats have been identified correctly. The study provided evidence that there is some genetic variation among and between *M. aquaticum* populations in Ireland, though further analysis of the AFLP data is necessary to determine exactly how much genetic variation occurs. Genetic variation and the occurrence of at least one ornamental garden population which originated in the aquatic plant trade suggest that *M. aquaticum* may have been introduced multiple times to Ireland.

This study also demonstrates the potential for the native *M. verticillatum* to be mistaken for submerged *M. aquaticum*, and *vice versa*, in the wild. The study detected the first known occurrence of *M. heterophyllum* in Ireland, where it co-occurred with *M. aquaticum* in a seminatural wetland. Furthermore, this study also detected and reported the first European occurrence of *M. sp. “red 1”* which originated in the aquatic plant trade and now occurs in domestic gardens in Ireland. It is possible that the distribution of *M. aquaticum*, *M. heterophyllum* and *M. sp. “red 1”* in Ireland are not limited to the populations investigated in this study but exist as cryptic populations elsewhere. A number of previously recorded populations of *M. aquaticum* on the island of Ireland were not included in this study, further investigation of these populations would be worthwhile in order to confirm their identity or uncover any other alien *Myriophyllum* taxa potentially hidden amongst them. Given the ease with which *M. aquaticum* and *M. heterophyllum* can be mistaken for and sold as native or unregulated taxa it is also highly recommended that importation of plants for the aquatic plant trade be screened for cases of mistaken identity in order to prevent further introductions of illicit species.

The presence of a previously undetected population of *M. heterophyllum* in Ireland is particularly alarming. Eradication of this population of *M. heterophyllum* must thus be prioritised. The early detection of an invasive species while they remain in low densities is

critical to rapid eradication and effective management of an invasive species once it has colonized and become established in a new range (Mehta *et al.*, 2007). Environmental DNA (eDNA) has been identified as a particularly useful approach for the early detection of aquatic invasive animal species which occur in low densities or in difficult to survey aquatic habitats (Darling & Mahon, 2011). eDNA techniques appropriate for the detection of aquatic invasive plants, including invasive *Myriophyllum* species are in development and have been successfully trialled (Scriver *et al.*, 2015; Newton *et al.*, 2016). The present study highlights the usefulness of molecular techniques in the early detection of cryptic aquatic invasive species in Ireland.

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Chapter 7

Seeing the Wetland through the Weeds: Synthesis & Conclusions

Summary of Research Results

The overarching aim of the research was to characterise the ecological traits, of the environment, biological communities and individuals, responsible for the successful dispersal, establishment and spread of alien macrophytes. To that end hypotheses relating to propagule pressure, habitat disturbance, resource availability, community invasibility, stress tolerance and cryptic invasions were tested.

Chapter 2

An *in situ* investigation of alien macrophyte species in the Co. Cork landscape found that the occurrence and distribution of invasive alien species in standing waterbodies in Ireland is substantially greater than previously recorded and that more waterbodies were invaded than were not. Invaded waterbodies were richer in nutrients and TP was positively related to the biomass of *Elodea canadensis* Michx.. The relationship with the abiotic environment was species specific and it was demonstrated that *E. canadensis* and *Elodea nuttallii* (Planch.) H. St. John occupied marginally different niches. Overall, the intensity of human amenity use was the best predictor of the occurrence of invasive alien species. Invasive alien species often co-occurred, with up to four alien species simultaneously occupying the same habitat. Here the theory of propagule pressure (Lockwood *et al.*, 2005) due to the intensity of amenity activity was integrated with the concepts of disturbance and resource availability (Catford *et al.*, 2012; Davis *et al.*, 2000) to best explain the occurrence, distribution and success of alien macrophytes in a landscape dominated by human activity.

Chapter 3

The same *in situ* investigation studied the association between invasive alien species and native macrophyte assemblages. The likelihood of occurrence of an alien species was not influenced by native species richness or abundance, and the measured characteristics of native communities of invaded habitats did not differ from those of uninvaded habitats. Rather, the influence of alien species was additive to the native community, increasing the complexity of native assemblages in terms of richness, biomass, diversity and function of invaded ecosystems. Neutral responses of the macrophyte community were scale dependent and were only evident at the coarser habitat scale, while density dependent negative associations were discernible at finer, sub-habitat scales. Like the relationship with the abiotic environment, alien macrophytes had species specific associations with the native community, and those native species excluded by alien species tended to be morphologically similar. The depletion of biodiversity in invaded ecosystems could not be

disentangled from habitat degradation as a result of disturbance by nutrient enrichment. Here the theories of disturbance, resource availability and vacant niches were combined to conclude that in a eutrophic landscape invasive species are both drivers and passengers of ecological change (Catford *et al.*, 2012; Davis *et al.*, 2000, Levine *et al.*, 2004; Richardson and Pysek, 2006; Elton. 1958).

Chapter 4

Ex situ experiments investigated the mechanisms by which invasive alien macrophyte species reproduce and overcome barriers to dispersal. It was evident that most invasive species studied had high capacity to tolerate fragmentation and to regenerate from the smallest of vegetative fragments. Viability and fitness of propagules that are dispersed over land are not only limited by fragmentation tolerance but are also limited by their ability to tolerate desiccation (Barnes *et al.*, 2013). Desiccation tolerance is species specific and influenced by fragment size. Desiccation of plant fragments reduces viability with time. Larger fragments are more likely to remain viable post desiccation but the subsequent regrowth upon rehydration is reduced by prolonged aerial exposure. Here it was concluded that aquatic invasive plant species have the capacity to tolerate stressful conditions to overcome barriers to dispersal and colonization. Small asexual propagules remain viable and reproduce effectively. The ability of invasive alien macrophytes to tolerate fragmentation and desiccation during overland dispersal makes them ideal candidates to be dispersed by the anthropogenic activity identified as vectors of dispersal in Chapter 2 (Johnson *et al.*, 2001)

Chapter 5

A comparative approach between an invasive alien macrophyte (*Myriophyllum aquaticum* (Vell.) Verdc.) and a native congeneric species with invasive tendencies (*Myriophyllum spicatum* L.) built on the concept that barriers to invasion success occur throughout the invasion cycle (Richardson *et al.*, 2000). Propagules of invasive plants must sometimes tolerate stressful conditions of the receptive environment in order to successfully establish themselves (Berg *et al.*, 2016). An *ex situ* experiment was conducted to analyse the response of *Myriophyllum* spp. propagules to saline receptive environments. In the propagule phase, native species adapted to local stressful conditions, appeared more tolerant and were more likely to become established. However, it was found that those propagules of *M. aquaticum* that did survive saline conditions had the capacity to become competitive plants capable of persisting, growing and spreading in saline environments.

An *in situ* factorial experiment was then conducted to identify whether the same inherent ability to tolerate salinity stress facilitated the persistence and spread of the invasive *M. aquaticum* in saline environments. Maximum salinities tolerated by *M. aquaticum* under semi-natural conditions were documented and phenotypic plasticity (Davidson *et al.*, 2011) was identified as a mechanism by which *M. aquaticum* could become a successful invader of brackish ecosystems. It was concluded that the species had the potential to spread into, and invade, ecologically important coastal wetlands such as lagoon, saltmarshes and tidal rivers in Ireland.

Chapter 6

The concept of cryptic invasions (Morais & Reichard, 2017) was introduced and molecular techniques were utilised to investigate the cryptic identities of alien *Myriophyllum* spp. in Ireland. Molecular analysis showed that a number of populations morphologically identified as *M. aquaticum* were in fact misidentified. This study demonstrated the potential for the native *M. verticillatum* L. to be mistaken for submerged *M. aquaticum*, and *vice versa*, in the wild. The study detected the first known occurrence of *M. heterophyllum* Michx. in Ireland, where it co-occurred with *M. aquaticum* in a semi-natural wetland. Furthermore, this study also detected and reported the first European occurrence of *M. sp. "red 1"* Moody and Les which originated in the aquatic plant trade and now occurs in domestic gardens in Ireland. The presence of a previously undetected population of *M. heterophyllum* in Ireland is particularly alarming and it is possible that *M. heterophyllum* and *M. sp. "red 1"* are not limited to the populations investigated in this study but also exist as cryptic populations elsewhere. Highlighting the potential of ongoing cryptic invasions is important, especially considering the degree to which aquatic invasions were found to be under recorded in Chapter 2. The discovery of multiple cryptic taxa originating in the horticulture trade and domestic gardens is also of concern given that gardening/landscaping were identified as vectors of invasive plant propagules in Chapter 2.

Synthesis of the Research

This research demonstrated that invasive freshwater plant species in Ireland are tolerant of stressful conditions throughout their life cycle. Propagules were shown to withstand stress during dispersal (Chapter 4) and colonisation (Chapter 5), and they are capable of persisting and growing in conditions that are suboptimal for some native species (Chapter 5) and communities (Chapter 3). They are widely distributed, colonising a wide variety of habitat types and environmental conditions (Chapter 2, 3 & 5). Despite frequently co-

occurring, their relationship with abiotic environment and the native community is species specific and influenced by the form and function of the particular species (Chapter 2 & 3). Phenotypic plasticity was identified as a mechanism through which some invasive species can successfully invade such a wide variety of habitat types (Chapter 5) and the morphology of some species can vary significantly between populations (Chapter 6).

European Union member states are required to take measures for the early detection, rapid eradication and/or management of invasive species (European Union, 2017). The finding of this research can assist the implementation of such measures in Ireland. The degree to which invasive alien macrophytes are currently under recorded (Chapter 2) begs concern and effective monitoring programmes should be put in place. The occurrence and impact of macrophyte invasions have been demonstrated to be both scale and density dependent (Chapter 3) which may render detection difficult for practitioners (Byers & Noonburg, 2003; Yokomizo *et al.*, 2009). Targeted molecular techniques such as AFLP and ITS profiling have also been demonstrated to be particularly useful in the identification and detection of cryptic taxa at the early stages of their invasion in Ireland (Chapter 6). Here the early detection of *M. heterophyllum* in Ireland (Chapter 6) will be crucial to the effective eradication of the species before it can become widespread (Mehta *et al.*, 2007).

It was found that *E. canadensis* and *E. nuttallii* had marginally different ecological niches

Waterbodies adjacent to horticultural amenities or landscaped areas were identified to be particularly vulnerable to invasion (Chapter 2). The sale of many invasive species is now banned but the occurrence of potentially invasive cryptic taxa in the horticultural trade in Ireland was identified by this study (Chapter 6). This leaves current legislation open to loopholes through which blacklisted plant species can continue to be introduced (Thum *et al.*, 2012). Thus, there is a need for the effective monitoring of importations before they reach the market and the need for regulation of the online plant trade. Ireland, being an island has the potential to effectively do so.

Boating and fishing activity were also deemed to be effective vectors of aquatic invasive alien plants (Chapter 2) and species of concern were capable of being dispersed within and between waterbodies (Chapter 4) (Johnson *et al.*, 2001; Anderson *et al.*, 2014). The varying degrees of tolerance that studied invasive macrophytes have toward fragmentation and desiccation (Chapter 4) highlights how essential effective biosecurity is to prevent introduction and secondary dispersal of invasive macrophytes (Bruckerhoff *et al.*, 2015).

A relationship between environmental degradation due to nutrient enrichment and the occurrence, richness and abundance of invasive species was detected (Chapter 2) (MacDougall & Turkington, 2005). The restoration of water quality to reference conditions is unlikely to eliminate the occurrence of invasive species entirely but it may be effective in limiting the excessive growth of species such as *E. canadensis* which had a positive relationship with TP enrichment (Chapter 2). The restoration of water quality however may also result in the recovery of depleted native macrophyte communities with preference for lower trophic states (Chapter 3). Here, by reducing disturbance impacts and resource availability, biotic resistance may possibly be enhanced, thus protecting uninvaded habitats against future invasions (Funk *et al.*, 2008).

This study also showed that freshwater habitats specifically designated for the conservation of biodiversity are not immune to invasion (Chapter 3). Coastal wetlands such as lagoons, saltmarshes and tidal rivers are particularly important for biodiversity and ecosystem services (Barbier *et al.*, 2011), but are also vulnerable to invasion (Chapter 5). Further, the structure and function of native communities can be altered by the invasion alien macrophytes and may represent a threat to their conservation status (Chapter 3). The management of wetlands for the conservation of biodiversity may benefit from the eradication of invasive species. However, the appropriate eradication tools must be selected. Mechanical control of invasive species is temporary at best, due to the high potential for regeneration from allofragments generated by the cutting process (Chapter 4). These fragments may be viable, resulting in further spread. Non-invasive techniques are preferential where appropriate.

Limitations and Scope for Future Research

The abiotic characteristics of lentic habitats are known to fluctuate in space and time. Within a waterbody, physiochemical properties such as pH, conductivity, nutrient availability and many others can fluctuate seasonally and vary depending on surrounding land use, geology, water retention time and climate/weather events (Brönmark & Hansson, 2017). This study investigated the relationship between invasive alien plant species and the abiotic characteristics of waterbodies at a representative moment in time. Further long term studies monitoring macrophyte responses to temporal and phenological changes in environmental conditions may provide further insight into the mechanism of invasion success and resistance (Nino *et al.*, 2007; Klein & Verlaque, 2009). There was insufficient data to explore relationships between waterbody characteristics and less frequent invasive

species. Species specific studies targeting habitats occupied by target species would be beneficial and provide a detailed description of the environmental envelopes occupied by other invasive macrophytes in Ireland.

It was not possible to empirically distinguish between cause and effect in the relationships between invasive macrophytes and the native communities they occupied (MacDougall, & Turkington, 2005). In order to definitively answer this question long term monitoring of native communities, before, during and after an invasion event is necessary. Long term invasion experiments conducted in mesocosms/ponds with well established macrophyte communities may offer a viable mechanism to answer such questions (Kercher *et al.*, 2007). There is also scope to investigate whether the restoration of water quality will assist in the restoration of native communities (Søndergaard *et al.*, 2007) capable of resisting invasion or outcompeting existing invasive species (Byers & Noonberg, 2003).

The present study investigated fragmentation and dessication tolerance under controlled conditions. Under field conditions plant fragments are exposed to varying levels of dessication, humidity and moisture as they are transported overland (Bruckerhoff *et al.*, 2015). Viability and fitness may also be enhanced if propagules possess an apical tip or colonize a particularly receptive environment (Riis *et al.*, 2009). The present results must thus be interpreted conservatively as propagules may remain viable for periods longer than described. At present mechanical, physical and chemical control of aquatic plants all generate allofragments to varying amounts. This represents a barrier to the successful eradication of invasive species populations if the generated fragments can remain viable. A comparative investigation quantifying the size, abundance and viability of fragments generated by various control techniques would be beneficial.

The survival of *M. aquaticum* propagules under ex-situ experimental conditions may have been limited by their constant exposure to saline conditions (Goodman *et al.*, 2006). Under field conditions, fluctuations in salinity and freshwater influxes may offer respite from osmotic stress and allow greater viability rates than those reported from the ex-situ salinity tolerance experiment.

Comparisons between congeneric native and invasive species can be particularly useful in the identification of mechanisms of invasive success (Paolacci *et al.*, 2018). *M. aquaticum* is an ideal candidate for further comparative studies as there are three species of *Mriophyllum* native to Ireland; *M. spicatum*, *M. verticillatum* and *M. alterniflorum* DC.

(Parnell *et al.*, 2012). *In-situ* comparisons where alien and native species of the genus co-occur might be particularly useful, offering insights into competitive traits and responses to ecological conditions. If they do not co-occur *ex-situ* experiments under a variety of conditions may elucidate ecophysiological mechanisms to invasion success.

The present genetic investigations of *Myriophyllum* spp. in Ireland were limited to a small sample size from recorded populations of *M. aquaticum* in the Rep. of Ireland. The study failed to successfully collect samples from any of the recorded populations of *M. aquaticum* in Northern Ireland. Given that the earliest records of *M. aquaticum* in Ireland are from Northern Ireland (Reynolds, 2002), this study was unable to answer any questions about the earliest populations of the species on the island. Despite the limited sample pool, some genetic diversity was detected amongst and between Irish populations of *M. aquaticum*, indicating that more than one introduction event has taken place. Further investigations employing the AFLP technique may be used to identify the populations of origin and number of introduction events (Moody *et al.*, 2016). The identification of cryptic populations of *M. heterophyllum* and *M. sp* “red 1” was also limited by the low sampling intensity of this study. Sampling of populations of other *Myriophyllum* spp. may render a greater frequency of previously undetected cryptic species. At present the use of molecular approaches to detect invasive macrophytes is limited to the use of species specific molecular tools. The future development of passive approaches to eDNA surveillance, where a community of species is identified from a single sample, would enable the detection of unexpected species (Simmons *et al.*, 2015)

The study did not directly investigate the prevalence of putative cryptic species in the horticultural trade, but infers its occurrence based on limited data. A deliberate investigation of the molecular identities of aquatic plants in the horticultural and aquarium trades would yield important information of the prevalence of restricted, cryptic and invasive species entering into Ireland (Thum *et al.*, 2012).

Lessons for Invasive Species Management

EU Regulation 1143/2014 on Invasive Alien Species requires all member states to put in place measures in relation to invasive alien species included on the list of “Invasive Alien Species of Union” concern. Three distinct types of measures are envisaged, which follow an internationally agreed hierarchical approach to combatting invasive alien species. E.U. member states must put in place a series of measures to prevent the introduction and

spread of invasive species, detect and eradicate invasive species at the early stages of the invasion process, and manage or control invasive species that are already well established (European Union, 2017).

Prevention

Restrictions on the importation, dispersal, propagation and sale of listed alien plant and animal species already exist in Ireland (S.I. No. 477/2011 & Regulation (EU) 1143/2014). These legal instruments are designed to prevent the introduction and spread of blacklisted invasive species. Species such as *A. filiculoides*, *E. canadensis*, *E. nuttalli*, *L. major*, *M. aquaticum*, *M. heterophyllum* and *N. peltata* are banned from importation and sale. However, the monitoring and enforcement of these laws is limited. The present research has demonstrated the need for stringent enforcement of the ban on sale and propagation of these species. Horticultural activity was identified as one of the primary modes of introduction of invasive species to waterbodies in Cork. Further the cryptic identity of many plants may create loopholes in existing blacklists through which invasive species may be imported and sold. The detection of *M. sp* “red 1” in the horticultural trade in Cork represents just one example the importance of closing such loopholes.

In addition to the prevention of initial introduction events, the prevention of secondary dispersal to new waterbodies is also essential. This research identified boating and fishing activity as some of the primary vectors in the spread of freshwater invasive plants in Cork. This research also demonstrated how even small fragments tolerate aerial exposure and can be readily dispersed overland, within and between freshwater habitats. This highlights the importance of educating waterway users in biosecurity and phytosanitation measures (Anderson *et al.*, 2014). The established ‘check, clean, dry’ protocol is advocated, with a reminder of the need to be thorough because tiny single node fragments of some invasive species may go unnoticed, while still being viable.

Ensuring that habitats are resistant to invasion (Capers *et al.*, 2007) is essential in the prevention of the spread of invasive species should barriers to introduction and dispersal fail. This research demonstrated that, in Ireland the occurrence and abundance of invasive macrophytes are associated with greater nutrient availability. Simultaneously, native macrophyte communities are depleted under such conditions. The restoration of good water quality and native macrophyte communities (Jeppesen *et al.*, 2005) may represent a novel approach to the prevention of the colonisation of freshwater habitats by invasive species in Ireland.

Early Detection

If the introduction and spread of an invasive species takes place, the earlier it is detected in its new environment the more likely it is to be successfully eradicated (Mehta *et al.*, 2007). Knowledge of current distribution of freshwater invasive species is thus a key tool in their management. This research highlighted the fact that many freshwater invasive species are under recorded in Ireland. The early detection of invasive species upon spreading to new waterbodies is thus unlikely unless systematic monitoring regimes are put in place. At present the majority of invasive species records occur through the voluntary efforts of citizens who contribute their records to the National Biodiversity Data Centre and the Botanical Society of Britain and Ireland. If resources for the detection of invasive species are limited, areas of high conservation value should be prioritised; this research showed that waterbodies designated for the conservation of biodiversity are vulnerable to invasion.

Because of the difficulty in accurate identification of some taxa of aquatic plants, invasions by cryptic taxa may go undetected. The potential for the development of molecular tools such as eDNA techniques to assist with early detection of occurrence is promising. eDNA techniques are considered to be particularly powerful when the invasion status of a waterbody is unknown or in the early stages of colonization when small populations may otherwise go undetected (Newton *et al.*, 2016; Scriver *et al.*, 2017). Extensive use of molecular techniques for the identification and detection of invasive species may be limited by greater cost (compared to traditional morphological identification techniques) and the need for specialist laboratory facilities. However, the accuracy provided by molecular techniques may provide long term reward, as it has been repeatedly demonstrated that even experienced practitioners have difficulty in the accurate identification of aquatic plants (Thum *et al.*, 2006).

The early detection of *M. heterophyllum* through this research represents the first step in the successful eradication of this species before it can be dispersed. In addition, the extensive field surveys detected a number of other waterbodies where the populations of other invasive taxa were not yet well established. Successful eradication of these populations may be achieved if the appropriate actions are put in place, and care is taken not to disperse or spread the species in the process.

Eradication and Control

Traditional approaches to the eradication and management of aquatic plants include a variety of mechanical, chemical, physical and biological methods (Hussner *et al.*, 2017).

Mechanical techniques are most commonly used in the control of submerged macrophytes. This research showed that freshwater invasive species are highly tolerant of fragmentation. Thus, the use of mechanical cutting or excavation techniques may not be an effective means of entirely eradicating a population of an invasive species. However, such techniques may be necessary on occasions where rapid but temporary reduction in invasive plant biomass is desired. Because of the risk of hydrochoric dispersal of vegetative propagules the use of drift barriers is recommended, as is the removal of all plant material from the water (Hussner *et al.*, 2017). Biosecurity measures must also be put in place in order to prevent the overland dispersal of plant fragments (Inland Fisheries Ireland, 2010).

The use of herbicides in the eradication and control of invasive macrophytes is common practice in some parts of the world (Getsinger *et al.*, 2008). Commonly used chemical agents include diquat, endothall, fluridone and 2,4-D, however their use in or adjacent to waterways throughout the E.U. is restricted (Hussner *et al.*, 2017). Concerns relating to the persistence and potential contamination of drinking water exist over the use of herbicides in aquatic environments (Brock *et al.*, 2006). Glyphosate is permitted for targeted use on emergent macrophyte species (Hussner *et al.*, 2017) and may present an opportunity for the control of emergent populations of *M. aquaticum* (Emerine *et al.*, 2010). However, *M. aquaticum* does not always exhibit emergent phenotypes.

The use of seawater inundation as a control method for invasive macrophytes of coastal wetlands has been advocated by some authorities (Dean *et al.*, 2013; Charlton *et al.*, 2011). This method may potentially be ineffective against *M. aquaticum* and have unintentional negative effects on native species such as *M. spicatum* and others. However, Charlton *et al.* (2011) argue that, depending on the degree of invasion and native community composition, the benefit of invasive species eradication may be deemed greater than negative impacts on native co-occurring species.

The innovative use of light exclusion in the eradication of aquatic invasive plants appears to be a promising alternative to herbicides and mechanical control. In this method geotextiles or biodegradable jute matting is used as an effective benthic barrier, covering submerged aquatic plants, occluding light, and causing subsequent dieback of vegetation (Caffrey *et al.*, 2010; Caffrey *et al.*, 2011; Hoffman *et al.*, 2013). The exclusion of light has effectively controlled populations of *L. major* in Ireland while simultaneously allowing the recovery of native plant communities which germinate from the seed bank and penetrate through the benthic barrier (Caffrey *et al.*, 2011). Though not yet quantified in the literature, the

method is less invasive than mechanical control techniques and no-doubt generates less fragments. Furthermore, the use of biodegradable materials does not have the same environmental concerns that associated with herbicides, and can be employed in freshwater and coastal wetlands alike.

Conclusions

This research has furthered knowledge relating to the ecology of invasive aquatic plants in Ireland and highlighted its implications for their management. It has characterised some of the relationships between invasion success and the environments and communities that these invasive species occupy. It has also demonstrated how species specific responses and the biological traits of the individual interact with dynamic ecosystems to influence the dispersal, distribution and performance of invasive macrophytes in freshwater and brackish habitats. These relationships are complex and dependent on ability to reproduce, be dispersed, tolerate abiotic conditions, and compete for resources with the native community. Thus the invasion success of aquatic invasive alien plants in Ireland is indeed the product of environment, community and the individual.

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Now that I am finished I've a lot of catching up to do. Hey everyone, let's grab coffee soon.