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The impacts of Crassula helmsii on Northwest European freshwater macroinvertebrates

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The impacts of Crassula helmsii on Northwest European freshwater macroinvertebrates

Tasker, Samuel

Award date:
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THE IMPACTS OF *CRASSULA HELMSII* ON NORTHWEST EUROPEAN FRESHWATER MACROINVERTEBRATES

by

SAMUEL TASKER

A thesis submitted to the University of Plymouth

in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

School of Biological and Marine Sciences

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Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree has not formed part of any other degree either at the University of Plymouth or at another establishment.

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Publications

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Tasker, S J (2023). '*Bidessus unistriatus* rediscovered in East Sussex'. *Latissimus* 54: 1-2.

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The impacts of *Crassula helmsii* on Northwest European freshwater macroinvertebrates

Samuel Tasker

Abstract

Crassula helmsii (New Zealand pygmyweed) is an Australasian aquatic plant which has invaded European freshwaters since the 1950s, provoking widespread concern amongst conservationists. The ecological impacts of *C. helmsii* invasion remain unclear, however, particularly with respect to macroinvertebrates. The main aim of this thesis is therefore to determine how *Crassula helmsii* impacts recipient macroinvertebrate assemblages in small lentic waterbodies across its invasive range. To this end, I surveyed small lentic waterbodies across the UK, Belgium and the Netherlands, including invaded and ecologically similar uninvaded sites in each region, assessing the impacts of *C. helmsii* invasion on macroinvertebrates using a suite of taxonomic and functional diversity metrics. I found that *Crassula helmsii* invasion was associated with slightly elevated macroinvertebrate taxon richness, but that alien taxa were more abundant in *C. helmsii* sites. Additionally, assemblage composition apparently shifted following invasion, particularly with respect to taxa and traits associated with detritivory.

An additional aim of the thesis is to determine the mechanisms underpinning *C. helmsii*'s impacts on macroinvertebrate assemblages. To achieve this, I conducted mesocosm experiments investigating the preference of four herbivore and detritivore species for *C. helmsii* versus native macrophytes, as well as a field experiment to investigate the colonisation and breakdown of *C. helmsii* litter in nature. Macroinvertebrate consumers exhibited divergent preferences for *C. helmsii* vs. native macrophyte tissues, possibly

associated with interspecific differences in feeding mode and tolerance to phenolic defences. In the field, *Crassula helmsii* litter was colonised by a comparable macroinvertebrate assemblage to the native macrophyte *Callitriche stagnalis*, but decomposed at a slower rate, indicating resistance to mechanical and/or microbial breakdown. By the end of the trial, *C. helmsii* detritus hosted more abundant macroinvertebrates. The recalcitrance of *C. helmsii* detritus, along with its year-round abundance, is likely to drive impacts on macroinvertebrate detritivores that were observed in field surveys.

This thesis also aimed to contextualise *C. helmsii*'s impacts by summarising the global ecological consequences of alien aquatic macrophyte invasions. To achieve this, I conducted a meta-analysis, finding overall negative effects of alien macrophytes on the diversity of native biota, but a significant positive effect of submerged alien macrophytes on macroinvertebrates, congruent with the elevated macroinvertebrate taxon richness observed amongst *C. helmsii* in my field surveys.

In summary, *Crassula helmsii* appears to have limited, and not wholly negative, impacts on recipient NW European macroinvertebrate assemblages associated with dense vegetation in shallow waters. The palatability of *C. helmsii* varies between consumers but is not clearly different to that of native macrophytes. Impacts of *C. helmsii* on macroinvertebrates appear likely to be driven (in part) by the palatability, abundance and recalcitrance of *C. helmsii* detritus.

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Tables and figures

Chapter 1: General Introduction

1.1 Invasive species in freshwater

Surface freshwaters support 10% of multicellular life, including 1/3rd of vertebrate species, despite covering only 0.8% of the Earth (Balian *et al.*, 2008). Globally, freshwater biodiversity is declining faster than terrestrial or marine biodiversity (Tickner *et al.*, 2020). Freshwaters are threatened by most of the anthropogenic stressors affecting the terrestrial and marine realms, including climate change, habitat loss and pollution, which may be intensified by the position of freshwaters as recipients of runoff from their surrounding catchments. In addition, freshwaters are threatened by over-abstraction to supply growing human demands for water, and habitat fragmentation by dams constructed to provide water and/or electricity. In synergy with this array of stressors, freshwaters are increasingly impacted by the spread of invasive alien species (Seebens *et al.*, 2017; Reid *et al.*, 2019; Dudgeon, 2020; IPBES, 2023).

Alien invasions are not a modern phenomenon; almost since our appearance as a species, *Homo sapiens* has intentionally or unwittingly aided the dispersal of other organisms. Indeed, for much of our recent history we have been reliant on resources provided by non-native crops and domestic animals; some of the most widespread non-native plants were transported by early agriculturalists along with cereal grains (Elton, 1958; Preston, Pearman and Hall, 2004; Ricciardi, 2007). However, globalisation of trade and travel since the 19th century has rapidly accelerated the spread of alien taxa, resulting in the establishment of problematic invasive aliens across every continent and in almost all ecosystems (Dawson *et al.*, 2017; Seebens *et al.*, 2017). Biogeographic boundaries which have persisted since the Mesozoic have been widely breached, and recipient biotas exposed to invaders to which they are entirely evolutionarily naïve

(Torsvik and M. Cocks, 2016). These biological invasions have been linked to the degradation of ecosystem structure and function (Simberloff *et al.*, 2013; Gallardo *et al.*, 2016), loss of ecosystem services (Pejchar and Mooney, 2009; Diagne *et al.*, 2021) and, frequently, to species extinctions (Engbring and Fritts, 1988; Bellard, Cassey and Blackburn, 2016; Pyšek *et al.*, 2020). Globally, the spread of invasive alien species across biogeographic boundaries is leading to biotic homogenisation, in which geographically restricted species assemblages are replaced by a small number of globally successful invaders (McKinney and Lockwood, 1999; Olden, Comte and Giam, 2018). This process may be accelerated by 'invasional meltdown', wherein invasion by one alien species facilitates the invasion of other non-natives (Simberloff and Von Holle, 1999). The impacts of alien invasive species are highly context specific, and oftentimes invasive species act as passengers rather than drivers of change, invading ecosystems made less resilient and more invasible by prior anthropogenic degradation (Macdougall and Turkington, 2005; Britton *et al.*, 2023).

The insular, island-like nature of freshwaters - and particularly small lentic freshwaters – often with high endemism and high species turnover between basins, makes the biodiversity of many such systems particularly vulnerable to the impacts of invasive species, particularly where ecosystem resilience has been undermined by prior anthropogenic degradation (Moorhouse and Macdonald, 2015; Riley *et al.*, 2018; Dudgeon, 2020; Britton *et al.*, 2023). Freshwater invaders have been associated with drastic, and diverse, alterations to freshwater ecosystem structure. For instance, the invasive crayfish *Procambarus clarkii* has been demonstrated to shift densely vegetated, clearwater lakes into a turbid, phytoplankton-dominated phase via macrophyte fragmentation and consumption (Rodríguez, Bécares and Fernández-Aláez, 2003a; Gherardi and Acquistapace, 2007). Invasive zebra and quagga mussels (*Dreissena* spp.)

have been implicated in shifting lake ecosystems in the opposite direction, from turbid and phytoplankton-dominated to clear and macrophyte-dominated, through the regulation of phosphate cycling (Knapton and Petrie, 1999; Li *et al.*, 2021).

In response to the accelerating rate of biological invasions, non-native species management programmes have been implemented across the world by individual nations (UK Government, 2019; Ricciardi and MacIsaac, 2022; GB non-native species secretariat, 2023) and through intergovernmental agreements (European Parliament and Council of the European Union, 2014; International Maritime Organization, 2017). Efforts to manage the spread of invasive species consist primarily of:

a) **Prevention and containment.** Establishing biosecurity measures to close pathways of inter-/intra-country transport of exotic species. Creating barriers to the dispersal of invasive species once populations have been established.

b) **Eradication or control.** Destroying invasive populations or, where this is unfeasible, reducing invader abundance to minimise negative impacts on recipient native biota.

Informed by a rapidly growing body of applied invasion biology research (Simberloff, Parker and Windle, 2005; Booy *et al.*, 2017; Fournier *et al.*, 2019; Simberloff, 2020), invasive species control and eradication projects have achieved some success (Simberloff, 2009; Gherardi *et al.*, 2011; Rytwinski *et al.*, 2019; Coetzee *et al.*, 2021), but are typically resource intensive and, depending on methodology, may cause considerable collateral damage to native biota (Laufenberg *et al.*, 2005; Simmons *et al.*, 2007; Sethi *et al.*, 2017). Reliable quantitative assessments of the ecological impacts of invasive species are therefore vital to inform effective management and ensure appropriate allocation of the finite resources available to conservation.

1.2 Invasive alien macrophytes

Macrophytes are photosynthetic aquatic organisms visible to the naked eye, and include vascular plants, mosses and macroalgae (Pokorný and Kvet, 2003; Chambers *et al.*, 2007). Because of their foundational role as primary producers (Lodge, 1991; Newman, 1991; Bakker *et al.*, 2016) and ecosystem engineers (Carpenter and Lodge, 1986; Warfe and Barmuta, 2006; Thomaz and Cunha, 2010), invasion by alien macrophytes often drives marked shifts in recipient ecosystem structure and function.

The most conspicuous impacts of alien macrophytes on recipient ecosystems often relate to changes in physical structure and water chemistry, due to the tendency of many aliens to form dense, monotypic stands (Schultz and Dibble, 2012; Hussner *et al.*, 2021) in which native macrophyte competitors may be excluded (Hogsden, Sager and Hutchinson, 2007; Stiers *et al.*, 2011; Bassett *et al.*, 2012; Silveira, Alves and Thomaz, 2018; Lishawa *et al.*, 2019). These dense stands decrease light penetration, intensify vertical temperature gradients and retard water flow, which promotes the deposition of fine sediment. Oxygen fluxes are also intensified as macrophytes oxygenate surrounding water and sediment during the photoperiod, and deoxygenate their surroundings via night-time respiration (Carpenter and Lodge, 1986; Carter, Rybicki and Hammerschlag, 1991; Hogsden, Sager and Hutchinson, 2007). Where floating macrophytes form a surface mat, they may inhibit atmospheric oxygen exchange, drastically reducing dissolved oxygen in the water below (Caraco and Cole, 2002; Strayer *et al.*, 2003; Kornijów, Strayer and Caraco, 2010; Villamagna and Murphy, 2010). Dense alien macrophyte stands often increase habitat structural complexity, which can result in increased macroinvertebrate density (Kuehne, Olden and Rubenson, 2016). Altered physicochemistry and elevated structural complexity within alien macrophyte stands can compromise the foraging efficiency of larger predators (Troutman, Rutherford and Kelso, 2007; Theel and Dibble, 2008; Grutters *et al.*, 2015).

Alien macrophyte invasion may also alter the provision of food to consumers, both directly and indirectly. Changes to the quantity and nutritional quality of live macrophyte tissues available to herbivores may strongly impact recipient consumer assemblages since, contrary to historic views, herbivores typically remove over 40% of plant biomass in freshwaters, a greater proportion than is consumed by herbivores in terrestrial systems (Lodge, 1991; Bakker *et al.*, 2016). Remaining plant biomass enters the detrital pathway upon senescence, with consequent impacts on detritivores (Webster and Benfield, 1986; Cebrian and Lartigue, 2004). Macrophytes also provide a substrate for epiphytes, which often attain higher productivity than the macrophytes themselves (Sheldon and Boylen, 1975; Cattaneo and Kalff, 1980). Changes to the abundance and composition of epiphytes hosted by alien macrophytes can indirectly impact consumer assemblages (Strimaitis and Sheldon, 2011; Kovalenko and Dibble, 2014; Grutters, Gross, *et al.*, 2017). The production of allelopathic secondary metabolites by alien macrophytes may modulate impacts on consumers (Erhard, Pohnert and Gross, 2007; Grutters, Gross and Bakker, 2016; Grutters, Roijendijk, *et al.*, 2017).

As with all biological invasions, impacts are highly context-specific and may occur indirectly. For instance, invasion by submerged Eurasian water-milfoil (*Myriophyllum spicatum*) has been associated with both increases (Strimaitis and Sheldon 2011; Wilson and Ricciardi 2009) and declines (Cheruvelil et al. 2002; Keast 1984; Strimaitis and Sheldon 2011; Wilson and Ricciardi 2009) in macroinvertebrate abundance within North American lakes, varying between waterbodies and by season according, in part, to the epiphytic assemblage hosted by the plants (Strimaitis and Sheldon 2011; Wilson and

Ricciardi 2009). Impacts can also occur as a result of the facilitation of other alien species. Alien macrophytes have been demonstrated to facilitate the invasion of mussels (Michelan *et al.*, 2014; Wegner *et al.*, 2019), crayfish (Thouvenot *et al.*, 2017) and other macrophytes (Monks *et al.*, 2019). Impacts vary according to alien macrophyte traits, and particularly growth form (emergent, floating, submerged) (Schultz and Dibble, 2012; Hussner *et al.*, 2021). The ecological impacts of alien macrophytes are discussed further and systematically reviewed in Chapter 1.

1.3 Study species: *Crassula helmsii*

Crassula helmsii (T. Kirk) Cockayne (Crassulaceae: New Zealand pygmyweed, Australian swamp-stonecrop) is an invasive (semi-) aquatic plant, now widespread in Europe, which readily colonises small lentic waterbodies (Diaz, 2012; Smith and Buckley, 2020). In Northern Europe, small lentic waterbodies (e.g. ponds, ditches) contribute disproportionately to freshwater biodiversity (Williams *et al.*, 2004; Davies *et al.*, 2008), making the proliferation of *C. helmsii* particularly troubling to conservationists (see Section 1.4).

With a tendency to form dense marginal stands which may exclude other macrophytes (Ewald, 2014; van der Loop *et al.*, 2022), the plant is considered a major threat to European freshwater biodiversity, although insufficient quantitative research exists to validate this perspective (Dawson and Warman, 1987; Langdon *et al.*, 2004; Dean, 2012; Ewald, 2014; Smith, 2015; Smith and Buckley, 2015; van Kleef *et al.*, 2017), particularly in terms of impacts on animals.

1.3.1 Morphology and growth forms

Crassula helmsii is a succulent semi-aquatic perennial with creeping-terrestrial, emergent (Figure 1.1), submerged and free-floating growth forms, all of which may be exhibited by a single specimen. Lanceolate leaves are arranged in opposite pairs, with nodal spacing shortest in the emergent form and increasing with submergence.

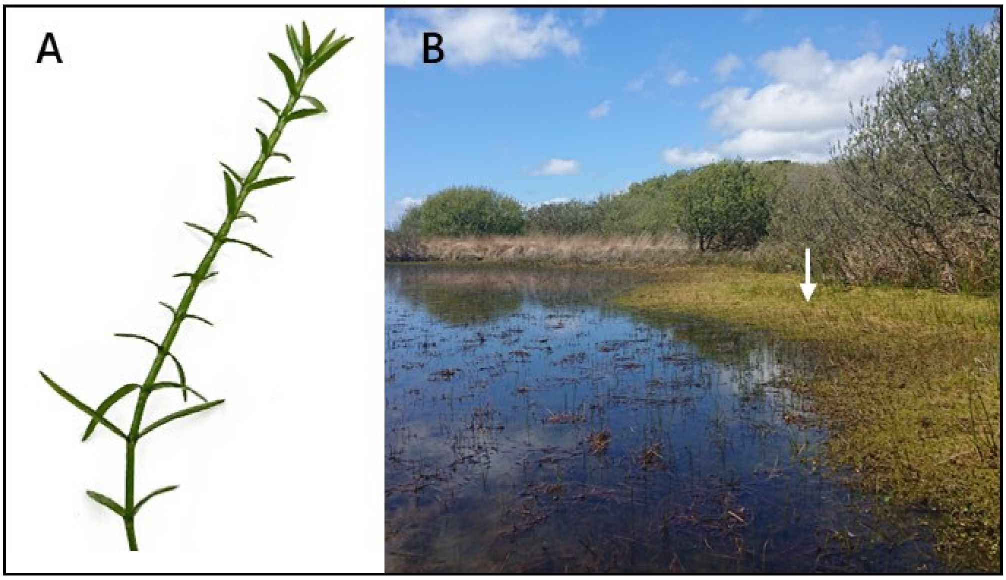

Figure 1.1. Typical architecture and growth form of marginal *Crassula helmsii*. **A**: emergent apical section of *Crassula helmsii*; **B**: dense emergent *C. helmsii* (arrowed) in marginal drawdown zone of waterbody, Lizard Peninsula, Cornwall, UK

In its naturalised European range, *C. helmsii* characteristically forms dense marginal stands of up to 1.5 kg/m^2 (Dawson and Warman, 1987), with nodal branching and adventitious nodal roots knitting plants into a loose turf (Figure 1.2) (Smith and Buckley, 2020).

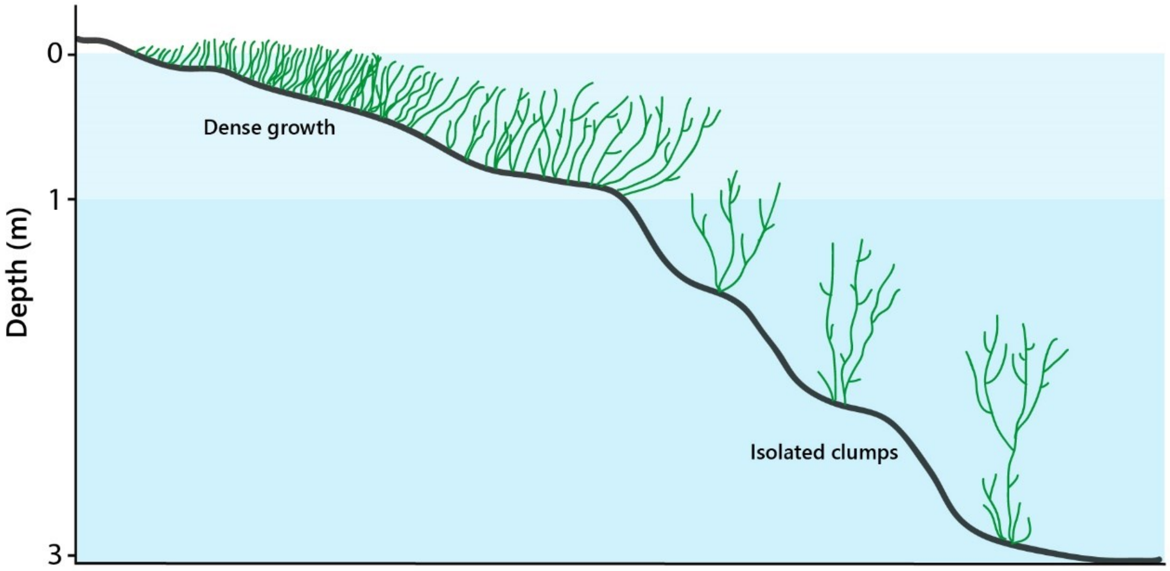

Figure 1.2*.* Growth forms of *Crassula helmsii*, varying with depth. Adapted from Dawson and Warman, 1987.

1.3.2 Australasian origins

C. helmsii is native to Australasia, where it is known by the vernacular name 'swamp stonecrop'. *C. helmsii* occurs in lentic and slow-flowing waterbodies across South Australia and Tasmania as well as the South Island of New Zealand. In Australia, *C. helmsii* occurs most frequently in small lentic freshwaters, whilst in New Zealand it appears largely restricted to the damp margins of waterbodies, including in brackish conditions (Dawson & Warman, 1987; Diaz, 2012; Kirby, 1964; Smith & Buckley, 2020). Several ecotypes are postulated to exist across Australasia, but this has not been investigated in any detail to date (Dawson & Warman, 1987; Diaz, 2012). In its native range, *C. helmsii* is restricted to areas with a mean diurnal temperature range of 0-15°C in winter/20-25°C in summer, and precipitation of 2-300 cm in winter/1-55 cm in summer (Leach and Dawson, 1999).

1.3.3 European invasion

Crassula helmsii is believed to have first arrived in the UK from Tasmania in 1911 (CEH, 2004). By 1927, the plant was available for sale as a pond oxygenator at Perry's Hardy Plant Nursery in Enfield, Greater London (CEH, 2004; Diaz, 2012). In 1956, a naturalised population of *C. helmsii* was discovered in nearby Essex (Laundon, 1961). In the following decades, *C. helmsii* spread rapidly across Britain and Northern Europe, arriving in Germany in 1981, Belgium in 1982 and Holland in 1995 (Smith and Buckley, 2020). Further anthropic introductions are considered to have been a key driver in this spread, as was local dispersal by water-users (e.g. via vegetative fragments on boots, amongst fishing gear) and likely water birds (Leach and Dawson, 1999; Figuerola and Green, 2002; Coughlan *et al.*, 2017; Lobato-de Magalhães *et al.*, 2023). Continued sale of *C. helmsii* as an ornamental likely facilitated these introductions, and remains a problem - whilst the plant has been prohibited from sale in England and Wales since 2014 (Plantlife, 2014), its trade remains legal in the European Union (European Commission, 2019). At present, *C. helmsii* occurs in France, Belgium, Holland, Germany, Spain, Denmark, Austria, Corsica, Ireland and Italy, but is most frequently recorded from Northwest Europe (EPPO, 2016; CABI, 2019; Van der Loop *et al.*, 2020). Isozyme analysis conducted by Dawson (1994) suggested that a single introduction is responsible for all populations of *C. helmsii* established in the British Isles.

1.3.4 Habitat

In its naturalised range, *C. helmsii* occurs on soft sediment in most lentic habitats, but is most frequently in ponds and ditches (Dawson & Warman, 1987; Smith & Buckley, 2020). *C. helmsii* is rarely recorded from flowing water in Europe, despite occurring in lotic habitats in Australasia (Diaz, 2012; Smith and Buckley, 2020). The relative scarcity of soft sediment in lotic systems has been posited as a possible explanation for this phenomenon (Dawson and Warman, 1987), but future riverine invasion remains possible. Indeed, *C. helmsii* has been demonstrated to grow faster in flow rates of up to 3 m/s (Dawson & Warman, 1987). *C. helmsii* has been recorded to a depth of 10 m, but typically colonises the marginal 'drawdown zone' of waterbodies (Hussner, 2007; Brouwer *et al.*, 2017). This may explain why *C. helmsii* is less common in large waterbodies, which typically have a narrower drawdown zone, although the vulnerability *of C. helmsii* to wave action has also been posited as a potential driver (Lockton, 2010). It is tolerant of a wide range of physicochemical conditions, but appears to favour circumneutral, nutrient-rich habitats (Dawson and Warman, 1987; Leach and Dawson, 1999; Hussner, 2009). *C. helmsii* is an adept coloniser of bare ground, particularly in eutrophic waterbodies (Dean *et al.*, 2015; Smith and Buckley, 2015; van der Loop *et al.*, 2020).

1.3.5 Reproduction and dispersal

Vegetative propagation appears to be the primary mechanism of reproduction for *C. helmsii* in Europe. The plant is capable of regeneration from readily dispersed single-node fragments (Hussner, 2009). This process is aided by the production of lateral turions in autumn that are easily separated from the plant (Dawson & Warman, 1987). In Europe, *C. helmsii* flowers August-September (Kirby, 1964). Historically it had been thought that seed from these *C. helmsii* populations was unviable, but recent germination experiments suggest that reproduction by seed may indeed be possible (Denys *et al.*, 2014; D'Hondt *et al.*, 2016). However, due to difficulties in handling individual seeds, authors used entire seed heads, possibly introducing nodes from which the apparent 'seedlings' in fact developed (Smith and Buckley, 2020). Where (apparent) germination has been observed, rates have typically been low, so sexual reproduction is likely to be rare in European populations by comparison to vegetative propagation (Nicol, Ganf and Pelton, 2003; D'Hondt *et al.*, 2016; Smith and Buckley, 2020; van der Loop, 2022).

Multiple mechanisms of dispersal have been implicated in the spread of *C. helmsii* across the region. As mentioned in Section 1.3.3, long-distance dispersal is likely to rely primarily on human vectors, including transfer of equipment between waterbodies and accidental or deliberate inclusion in the aquatics trade. Vegetative propagules of *Lemna* spp. consumed by wildfowl have recently been demonstrated to survive passage through the digestive tract (Paolacci *et al.*, 2023), indicating endozoochorous dispersal may similarly occur in *C. helmsii* (Denys *et al.*, 2014). Ectozoochorous dispersal by waterbirds – 'the duck's back' (Darwin, 1859) – is another possible vector (Figuerola and Green, 2002; Coughlan *et al.*, 2017). Dispersal within interconnected aquatic habitats occurs primarily via drift of vegetative fragments. Free-roaming vertebrates may disperse *C. helmsii* between proximate waterbodies, particularly where livestock (e.g. cattle and ponies) are used in conservation grazing of nature reserves (Dawson and Warman, 1987; Smith, 2015).

1.3.6 CAM metabolism and copper hyperaccumulation

As a member of the Crassulaceae family, *C. helmsii* is capable of Crassulacean Acid Metabolism (CAM photosynthesis), wherein inorganic carbon can be fixed as malic acid and stored in vacuoles for later use. In aquatic plants, this confers a competitive advantage by permitting carbon assimilation during the night, when other plants cannot photosynthesise and carbon is more abundant (Newman & Raven, 1995). CAM photosynthesis is thought to be linked to *C. helmsii*'s capacity for copper hyperaccumulation, with malate acting as a copper ligand and permitting the accumulation of copper to >9000 ppm in living tissues (Küpper *et al.*, 2009; Corzo Remigio *et al.*, 2021). Copper hyperaccumulation is thought to play a defensive and/or allelopathic role in some plants (Cappa and Pilon-Smits, 2014), but this seems unlikely to be relevant in C*. helmsii* invasions as accumulation does not occur until concentrations exceed 250 µg/L (Corzo Remigio *et al.*, 2021), whilst background concentrations of Cu in surface waters are generally below 5 µg/L (Flemming and Trevors, 1989).

1.3.7 Ecological impacts and interactions

Widely cited anecdotal reports on the extirpation of native macrophyte species following *C. helmsii* invasion (Dawson and Warman, 1987; Leach and Dawson, 1999) have led the plant to be viewed as highly destructive to European biodiversity, but to date there is limited quantitative research supporting this perspective.

1.3.7.1 Macrophytes

Ecological research has so far focused primarily on the impacts of *C. helmsii* on native macrophytes. In a study of ponds in the New Forest, UK, Ewald (2014) found that for every 10% increase in *C. helmsii* cover, native vegetation cover decreased by 5%, a correlation between expanding *C. helmsii* and declining native macrophyte abundance which was also observed by van Kleef *et al.* (2017) in North Brabant, Netherlands. In contrast, Dean (2012) found no significant relationship between *C. helmsii* abundance and the abundance of co-occurring vegetation in a study of fens in Cambridgeshire, UK. Germination suppression may be responsible for the trend observed by Ewald and van Kleef - in laboratory trials, Langdon *et al.* (2004) found that artificial mats of *C. helmsii* significantly suppressed the germination of 6 native macrophyte species (of 11 studied). To date, quantitative field studies have consistently failed to demonstrate a significant effect of *C. helmsii* invasion on native macrophyte species richness (Langdon *et al.*, 2004; Dean, 2012; Ewald, 2014; Smith and Buckley, 2015), although several anecdotal reports exist linking *C. helmsii* invasion to the loss of native macrophyte taxa (Cockerill, 1979; Swale and Belcher, 1982; Dawson and Warman, 1987; Leach and Dawson, 1999; van der Loop *et al.*, 2022). In a study of lentic habitats across Kent and East Sussex, UK, Smith and Buckley (2015) found that rare macrophyte species actually occurred more frequently in invaded waterbodies, perhaps due to the competitive release of rarer macrophyte species following displacement of dominant native plants by *C. helmsii*, although the authors noted that this effect could have been driven by environmental factors other than *C. helmsii* invasion, from which *C. helmsii* also benefitted.

1.3.7.2 Macroinvertebrates and other animals

Benthic macroinvertebrates are the dominant taxa of intermediate (and often top) trophic levels in most freshwaters (Covich, Palmer and Crowl, 1999). The impacts of *C. helmsii* invasion on wider ecosystem structure and function are therefore strongly conditional on the plant's interactions with macroinvertebrates. Two quantitative studies conducted in Southern England (neither published in peer-reviewed journals) demonstrated no significant impact of *C. helmsii* invasion on macroinvertebrate abundance (Smith, 2015) or species richness (Ewald, 2014; Smith, 2015). However, both studies were limited in spatial extent, and the impacts of *C. helmsii* invasion on macroinvertebrate community composition and functional diversity were unexplored.

Interactions with invertebrate herbivores are a possible mechanism for the putative community-level impacts of *C. helmsii* invasion on macroinvertebrates. *C. helmsii* was included in a large no-choice feeding trial (Grutters, Roijendijk, *et al.*, 2017) investigating the palatability of 40 macrophytes to generalist mollusc herbivores, which found that consumption of *C. helmsii* (1.26 mg g^{-1} day^{-1}) by the great pond snail (*Lymnaea stagnalis*) was lower than the mean consumption rate across all macrophytes (2.7 mg g^{-1} day^{-1}). This suggests that *C. helmsii* may be relatively unpalatable to herbivores, perhaps due to its low nutritional quality (high C:N ratio) and relatively high level of phenolic defence compounds (low N:phenolics ratio). The small leaves of *C. helmsii* are also likely to reduce feeding efficiency, further deterring grazers (Hanley *et al.*, 2007).

Interactions between *C. helmsii* and vertebrates have received patchy attention. Investigating the impact of *C. helmsii* on newts, Langdon *et al.* (2004) found that smooth newt (*Lissotriton vulgaris*) eggs hatched at a later developmental stage on *C. helmsii* than on native macrophytes, although there was no significant difference in developmental stage at hatching for great crested newts (*Triturus cristatus*). In a recent field experiment, van der Loop *et al*. (2023) reported negative impacts of *C. helmsii* on natterjack toad (*Epidalea calamita*) spawning and egg development but positive impacts on larval growth. There is some evidence that large vertebrates facilitate *C. helmsii* invasion success: in a field exclosure experiment, Dean *et al.* (2015) found that grazing vertebrates promote *C. helmsii* abundance by preferentially consuming native competitors. Eutrophication caused by direct large vertebrate defecation may also promote the establishment of *C. helmsii* (van der Loop et al., 2020).

1.3.8 Management of *Crassula helmsii* invasion

Whilst little quantitative evidence currently points to ecosystem degradation following *C. helmsii* invasion, it is widely perceived as destructive, and several methods for control or eradication of the plant are in widespread use. Small populations (<1 m^2) may be effectively removed by hand, but this is labour intensive and risks fragmentation and further dispersal of the plant (Hussner, 2009). Management of larger populations generally necessitates the use of more heavy-handed techniques, including the application of herbicides (e.g. glyphosate), waterbody burial and light exclusion (Dawson & Warman, 1987; Ewald, 2014; Sims & Sims, 2016). These techniques may impact severely upon non-target species, and are rarely successful in eradicating *C. helmsii* long-term (van der Loop et al., 2018). In most instances, minimising negative impacts and preventing the plant's further spread and establishment are likely to be more achievable management goals than total eradication (van der Loop et al., 2018, 2020). To this end, an 'ecosystem resilience approach' involving partial removal of *C. helmsii* followed by transplantation of native competitors has been developed, with promising initial results (van der Loop, van Kleef, *et al.*, 2023). Biocontrol may also represent a promising future avenue for the management of *C. helmsii* invasion: since 2018, ongoing UK field trials have sought to determine whether galls formed by a recently discovered host-specific Eriophyid mite (*Aculus crassulae*) can curtail *C. helmsii*'s competitive dominance (Knihinicki *et al.*, 2018; Varia *et al.*, 2022).

1.4 Study system: small lentic waterbodies

In Europe, *Crassula helmsii* occurs most frequently in small lentic waterbodies, including ponds and ditches (Smith and Buckley, 2020). Ponds can be defined as standing freshwaters with an area of less than 4 hectares. Ditches are linear freshwaters, which are usually man-made and often, bearing little relation to natural geographical features, behave as linear ponds (Biggs, von Fumetti and Kelly-Quinn, 2017; Riley *et al.*, 2018). Small waterbodies are the most abundant freshwater environments in the UK, although the number of ponds in England and Wales is thought to have fallen from around 800,000 in 1880 to 200,000 by 1980 - a decline of 75% - as a result of agricultural intensification (Wood, Greenwood and Agnew, 2003; Jeffries, 2012).

Small waterbodies are particularly important for freshwater biodiversity, due both to their abundance and their physical characteristics. Small waterbodies are strongly affected by their immediate surroundings, so provide a wider range of physicochemical conditions than larger lakes and rivers. Since small waterbodies typically have smaller catchments, they are also often less impacted by pollution than larger waterbodies (Biggs, von Fumetti and Kelly-Quinn, 2017; Riley *et al.*, 2018). Comparative studies conducted across NW Europe indicate that small waterbodies, and particularly ponds, support greater biodiversity at a landscape-scale than larger freshwaters. In addition, small waterbodies may support more rare and threatened species, including plants and animals which are unique to small waterbodies (Williams *et al.*, 2004; Davies *et al.*, 2008). Small waterbodies are also important in connecting meta-populations of aquatic and riparian organisms, acting as stepping stones between fragmented freshwater habitats (Sayer, 2014; Biggs, von Fumetti and Kelly-Quinn, 2017).

The high conservation value of small waterbodies makes invasion of these habitats by *C. helmsii* particularly concerning to land managers (van der Loop *et al.*, 2018). If *C. helmsii* undermines the biodiversity of invaded waterbodies, the plant’s ongoing spread could wreak significant harm to NW European freshwater biodiversity.

1.5 Thesis outline

The paucity of quantitative evidence on the ecological impacts of invasive *C. helmsii* undermines effective and proportionate management. To address this knowledge gap, this project sought to determine the impacts of *C. helmsii* on macroinvertebrates, the most abundant and functionally important freshwater macrofauna (Wallace and Webster, 1996; Covich, Palmer and Crowl, 1999).

In order to achieve this aim, the project was focused towards 3 objectives:

Assess changes in benthic macroinvertebrate assemblage composition associated with *Crassula helmsii* invasion. Whilst some attempts have been made in the past to quantify the impacts of *C. helmsii* on macroinvertebrates, these were limited in geographical scale and analytical scope, and results were inconclusive (Ewald, 2014; Smith, 2015). Through field surveys of multiple regions across NW Europe, a dataset sufficient to assess *C. helmsii*'s impacts on macroinvertebrates throughout its European range was generated. The results of field sampling are presented in **Chapter 2.**

Determine the mechanisms underpinning *C. helmsii*'s impacts on benthic macroinvertebrates. To date, there is no published information directly evaluating the mechanistic drivers of *C. helmsii*'s impacts on macroinvertebrate assemblages. Using laboratory experiments I investigated *C. helmsii*'s palatability to macroinvertebrate consumers, both live and as detritus. Supplementary laboratory experiments examined the long-term growth/mortality of juvenile macroinvertebrate consumers reared on *C. helmsii* (**Chapter 3**). In addition, a field experiment investigated the breakdown and colonisation of *C. helmsii* detritus by macroinvertebrates (**Chapter 4**).

Finally, a **meta-analysis of the ecological impacts of alien aquatic macrophytes** allowed findings on *C. helmsii* to be placed in a wider context. A significant body of primary literature exists on the ecological impacts of alien aquatic macrophytes, but this has never been quantitatively reviewed. I conducted a systematic review to reassess the ecological impacts of alien macrophyte invasion, and therefore contextualise the findings of this project with regards to the ecological impacts of *C. helmsii*. The results of this meta-analysis are presented in **Chapter 5**.

Chapter 2: Field study reveals nuanced impacts of *Crassula helmsii* on macroinvertebrate assemblages

2.1 Abstract

Invasive alien species are considered one of the greatest threats to global biodiversity, and are particularly problematic in aquatic systems. Given the foundational role of macrophytes in most freshwaters, alien aquatic plant invasions may drive strong bottom-up impacts on recipient biota. *Crassula helmsii* (New Zealand pygmyweed) is an Australasian macrophyte, now widespread in northwest Europe. *Crassula helmsii* rapidly invades small lentic waterbodies, where it is generally considered a serious threat to native biodiversity. The precise ecological impacts of this invasion remain poorly understood, however, particularly with respect to macroinvertebrates, which comprise the bulk of freshwater faunal biodiversity in the region. I conducted a field study of ponds, ditches and small lakes across the core of *C. helmsii*'s invasive range (United Kingdom, Belgium and the Netherlands), finding that invaded sites had higher macroinvertebrate taxon richness than uninvaded sites, and that many infrequent and rare macroinvertebrates co-occurred with *C. helmsii*. Alien macroinvertebrates were more abundant in *C. helmsii* sites, however, particularly the North American amphipod *Crangonyx pseudogracilis*. At the order level, water beetle (Coleoptera) richness and abundance were higher in *C. helmsii* sites, whereas true fly (Diptera) abundance was higher in uninvaded sites. Taxonomic and functional assemblage composition were both impacted by invasion, largely in relation to taxa and traits associated with detritivory, suggesting that the impacts of *C. helmsii* on macroinvertebrates are partly mediated by the availability and palatability of its detritus. The nuanced effects of *C. helmsii* on macroinvertebrates found here should encourage further quantitative research on the

impacts of this invasive plant, and perhaps prompt a more balanced re-evaluation of its effects on native aquatic macrofauna.

2.2 Introduction

Surface freshwaters cover 0.8% of the Earth, but support over 10% of extant multicellular life, including one third of known vertebrate species (Balian *et al.*, 2008). Coincident with this disproportionate biodiversity, freshwaters are impacted particularly strongly by interacting anthropogenic stressors, including alien invasive species (Dudgeon, 2020). Alien invasive species - defined here as non-native species which establish self-sustaining populations and spread widely from introduction sites (Pyšek *et al.*, 2004, 2020) - are proliferating globally (Keller *et al.*, 2011; Seebens *et al.*, 2017, 2021), with well-documented impacts on recipient biota (Clavero and García-Berthou, 2005; Bellard, Genovesi and Jeschke, 2016). Furthermore, alien invasive species have been associated with shifts in ecosystem functions (Scott *et al.*, 2012; Linders *et al.*, 2019) and services (Pejchar and Mooney, 2009; Diagne *et al.*, 2021; IPBES, 2023). Increasingly, however, positive impacts of invasive species are also being recognised (Davis *et al.*, 2011; Schlaepfer, Sax and Olden, 2011; Boltovskoy *et al.*, 2022; Sax, Schlaepfer and Olden, 2022). For instance, the alien invasive quagga and zebra mussels (*Dreissena* spp.), notorious in North America for biofouling of water infrastructure and native unionid bivalves, are now recognised for their role in the mitigation of anthropogenic phosphate inputs and associated phytoplankton blooms, reducing the costs of influent water treatment (Higgins and Vander Zanden, 2010; Li *et al.*, 2021; Boltovskoy *et al.*, 2022). In general, the impacts of alien invasive species are context and taxon-specific, and may involve both positive and negative effects on recipient ecosystems (Vilà *et al.*, 2011; Gallardo *et al.*, 2016; Boltovskoy *et al.*, 2022; Tasker, Foggo and Bilton, 2022). To use the same example, invasion by quagga and zebra mussels may push pelagic ecosystems closer to ‘natural’ pre-existing oligotrophic conditions through regulation of phosphate cycling, whilst simultaneously driving benthic ecosystems away from pre-existing natural conditions by transforming habitat structure and channelling nutrients and energy into the benthos (Ward and Ricciardi, 2007; Higgins and Vander Zanden, 2010; Boltovskoy *et al.*, 2022).

Given their foundational role in freshwater ecosystems, invasion by alien macrophytes might be expected to result in similarly transformative bottom-up impacts, both negative and positive (Schultz and Dibble, 2012; Gallardo *et al.*, 2016; Tasker, Foggo and Bilton, 2022). Aquatic macrophytes are key primary producers (Lodge, 1991; Newman, 1991) and ecosystem engineers (Carpenter and Lodge, 1986; Warfe and Barmuta, 2006; Thomaz and Cunha, 2010) in freshwaters. Within macrophyte beds, physicochemistry (light, temperature, DO, nutrients) and habitat structural complexity differ from adjacent unvegetated habitats (Ondok, Pokorný and Květ, 1984; Carpenter and Lodge, 1986; Carter, Rybicki and Hammerschlag, 1991; Miranda, Driscoll and Allen, 2000), with significant impacts on the composition and structure of associated faunal assemblages. Live macrophytes are consumed directly by both macroinvertebrate and vertebrate heterotrophs, and are also colonised by epiphyton (Allen, 1971; Sheldon and Boylen, 1975; Cattaneo and Kalff, 1980), which provides an additional source of forage for heterotrophic freshwater fauna. Upon senescence and death, macrophytes contribute to detritus production, and leach dissolved organic carbon, phosphorus, and nitrogen into the water column (Carpenter and Lodge, 1986). Decomposition of macrophyte detritus may also on occasion lead to localised oxygen depletion (Landers, 1982; Carpenter and Lodge, 1986; Carter, Rybicki and Hammerschlag, 1991). The effect of macrophytes on other components of the freshwater ecosystem varies with macrophyte identity due to variation in habitat preference, growth form, architecture, growth rate, phenology and palatability between (and within) species (Schultz and Dibble, 2012;

Grutters, Roijendijk, *et al.*, 2017; Hussner *et al.*, 2021; Tasker, Foggo and Bilton, 2022). Since alien macrophyte invasion may result in the replacement of pre-existing unvegetated habitat or displace pre-existing macrophytes, invasive macrophytes may alter all of the above abiotic and biotic interactions, with far-reaching impacts on recipient ecosystems (Schultz and Dibble, 2012; Gallardo *et al.*, 2016; Tasker, Foggo and Bilton, 2022).

Crassula helmsii (Kirk) Cockayne (New Zealand pygmyweed) is a semiaquatic succulent plant, originating in Australasia, which has spread widely across northwest Europe since its introduction to the region in the mid-20th century (Smith and Buckley, 2020). Typically colonising small, shallow lentic waterbodies (ponds, ditches, small lakes), *C. helmsii* is notable for its tendency to establish dense stands in marginal 'drawdown' zones, often apparently excluding native macrophytes (Dawson and Warman, 1987; Leach and Dawson, 1999; van der Loop *et al.*, 2022). These same small waterbodies are increasingly recognised as major repositories of NW European freshwater biodiversity (Williams *et al.*, 2004; Davies *et al.*, 2008; Biggs, von Fumetti and Kelly-Quinn, 2017), prompting widespread concern from conservationists and land managers regarding the impacts of *C. helmsii*. Consequently, programmes of control and eradication have been widely implemented, with mixed success and with inevitable knock-on impacts upon non-target biota (Dean, 2012; Ewald, 2014; Sims and Sims, 2016; van der Loop *et al.*, 2018; Varia *et al.*, 2022). Management has largely been justified by early (qualitative) accounts of the loss of native aquatic plants following colonisation by *C. helmsii* (Dawson and Warman, 1987; Leach and Dawson, 1999), but quantitative evidence for declines in native biota, particularly invertebrates, is often lacking (but see Ewald 2014; Langdon et al. 2004; Smith and Buckley 2015).

Macroinvertebrates – those invertebrates large enough to be seen with the naked eye - are the dominant taxa of intermediate (and often top) trophic levels in freshwaters, making up the bulk of macroscopic freshwater biodiversity (Covich, Palmer and Crowl, 1999). Aquatic macroinvertebrates constitute a major component of fish, amphibian and waterbird diets (e.g. Anderson et al. 1999; Skagen and Oman 1996; Wallace and Webster 1996), and, since many have a terrestrial adult phase (e.g. Ephemeroptera, Trichoptera, Diptera), are also responsible for aquatic-terrestrial subsidies (Lewis-Phillips *et al.*, 2020). As herbivores, detritivores and predators, macroinvertebrates also drive nutrient cycling and energy flows in aquatic systems (Wallace and Webster, 1996). Freshwater ecosystem function is thus contingent on macroinvertebrate functional composition, and sensitive to macroinvertebrate abundance and diversity (Wallace and Webster, 1996; Gessner *et al.*, 2010). Macroinvertebrate assemblages are significantly impacted by the modulated physicochemical conditions, physical structure and food provided by aquatic macrophytes (Schramm and Jirka, 1989; Posey, Wigand and Stevenson, 1993; McAbendroth *et al.*, 2005; Warfe and Barmuta, 2006; Schultz and Dibble, 2012). Given the central role of macroinvertebrates in freshwaters, the impacts of *C. helmsii* invasion on recipient ecosystem structure and function are likely to be strongly conditional on the plant's interactions with this component of the aquatic community. Despite this, no study to date has explored these impacts systematically, from a taxonomic and functional perspective.

Taxonomic diversity - species composition and abundance - is the dimension of biodiversity most frequently investigated when assessing the impacts of invasive species on recipient biota, and includes the use of metrics of taxon richness, Shannon/Simpson indices and Hill numbers (Magurran, 1988). Taxonomic diversity metrics are relatively intuitive and easy to calculate (Stevens and Tello, 2014), but their exclusive use risks overlooking the component of biodiversity most mechanistically impacted by changes to the environment, and most explicitly linked to ecosystem function: species traits, and functional diversity (Petchey and Gaston, 2006; Verberk, Van Noordwijk and Hildrew, 2013). Functional diversity is defined as 'the value and range of those species and organismal traits that influence ecosystem functioning' (Tilman *et al.*, 2001), and can be measured through the analysis of functional traits (e.g. mode of feeding, locomotion) present in an assemblage, rather than the species which possess those traits (Petchey and Gaston, 2006). Because traits-based functional diversity approaches transcend species identity, they have the potential to greatly enhance the generalisability of community ecology research across different species pools and ecoregions. Therefore, traits-based approaches could provide greater predictive power to (hitherto often largely descriptive) community ecology research (Petchey and Gaston, 2006; Verberk, Van Noordwijk and Hildrew, 2013). In invasion biology, traits-based approaches offer an opportunity to predict the impacts of invasive species on recipient biota in new ecoregions, and identify those invaders which are most likely to undermine ecosystem functioning (Renault *et al.*, 2022).

The taxonomic and/or functional diversity *within* an individual sample is known as alpha diversity, whilst diversity with respect to differentiation *between* samples is known as beta diversity (Magurran, 1988). This beta diversity can be further partitioned into two components: turnover and nestedness. Turnover reflects replacement of taxa between samples, whereas nestedness occurs where species poor samples contain a subset of taxa found in richer samples (Baselga and Leprieur, 2015). Beta-diversity is of particular interest in relation to the impacts of invasive species due to a process known as biotic homogenisation, wherein anthropogenic degradation (and mixing of biota between bioregions) causes compositionally distinct, geographically separated assemblages to become increasingly similar as rarer stenotopic species are replaced by widespread, eurytopic taxa, including non-natives (Olden, Comte and Giam, 2018). In this context, biotic homogenisation would be expected to result in reduced turnover and elevated nestedness (Baeten *et al.*, 2012).

By employing multiple taxonomic and functional indices of alpha and beta diversity, I aimed to make the first comprehensive assessment of *Crassula helmsii*'s impacts on European freshwater macroinvertebrates, capturing effects on the structure and functioning of macroinvertebrate assemblages within invaded systems, including effects on taxa of conservation concern, and determining whether *C. helmsii* invasion threatens to homogenise regional macroinvertebrate faunas. To this end, I conducted a field study across the United Kingdom, Belgium and the Netherlands, the most heavily invaded countries in *C. helmsii*'s European range, comparing the composition of macroinvertebrate assemblages between *C. helmsii* stands and uninvaded native vegetation. Specifically, I sought to address the following questions.

1. Do macroinvertebrate assemblages in sites invaded by *C. helmsii* differ from those in uninvaded sites? How do these trends vary:
    a. Between higher macroinvertebrate taxa (e.g. Orders)?
    b. Between alien and native macroinvertebrate taxa?
    c. Between rare and eurytopic macroinvertebrates?
2. Do macroinvertebrate assemblages associated with *C. helmsii* differ functionally from those occurring amongst native macrophytes in similar habitats (functional α-diversity)?
3. Does macroinvertebrate assemblage turnover or nestedness differ between invaded and uninvaded sites (taxonomic and functional β-diversity)?

2.3 Methodology

2.3.1 Site selection

Four regions across the United Kingdom, Belgium and the Netherlands – the most heavily invaded countries in *C. helmsii*'s European range (Smith and Buckley, 2020) - were selected for inclusion in the field study, on the basis of hydrogeomorphological diversity and the distribution of *C. helmsii* records.

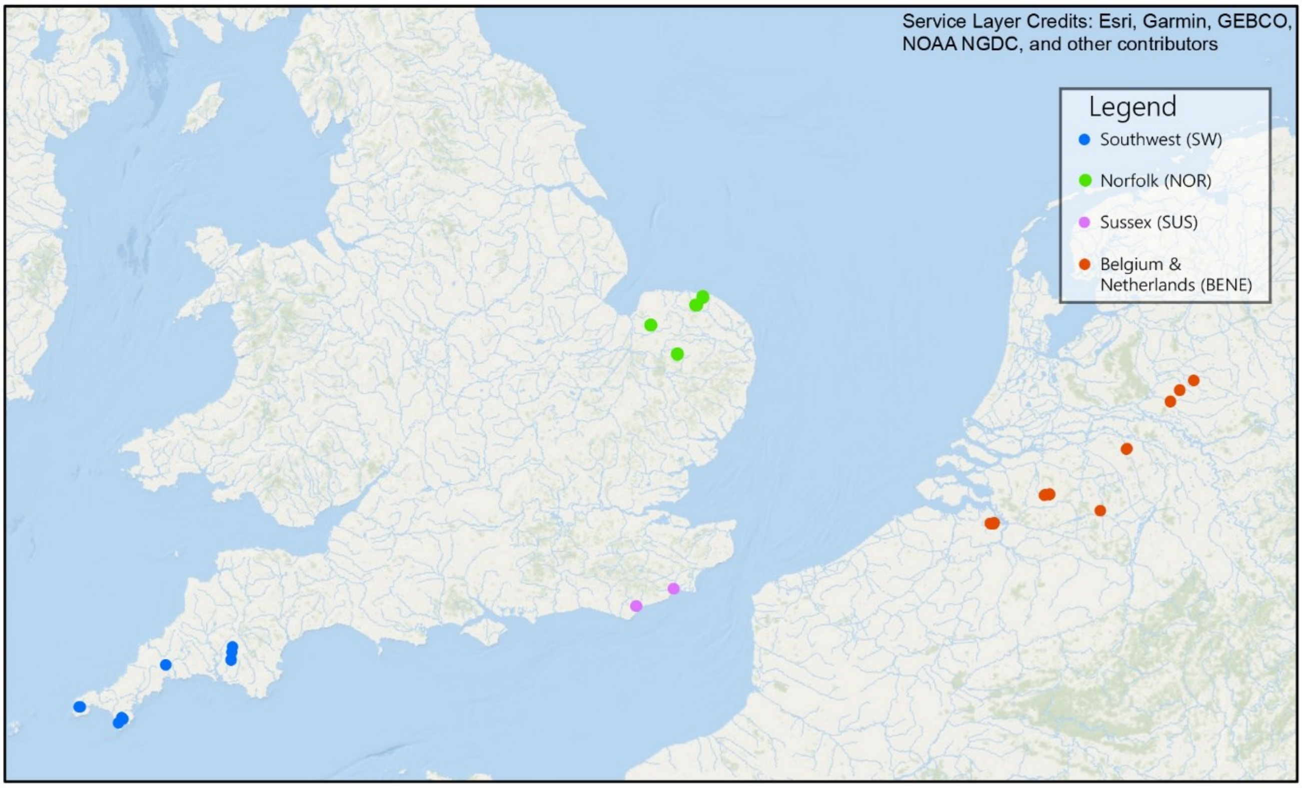

Figure 2.1. Map of northwest Europe, with points indicating sampling sites

Within these regions, sites were located using a mixture of biological records repositories, correspondence with researchers and conservation organisations, and site visits. Where possible, *C. helmsii*–invaded sites in proximity to comparable uninvaded sites were selected, to minimise confounding extraneous variation. A site list was compiled on the basis of accessibility (wrt. landowner permissions), and sites within designated nature reserves were selected where possible as a proxy for ecological quality. Ultimately, the majority of sampled sites (n = 41) were situated in semi-natural habitats, mostly within designated nature reserves, whilst the rest were in an agricultural setting (n = 15) with the exception of some sites in Belgium (A-F), which were mitigation ponds within predominantly industrial land usage.

Sampling was conducted in May 2021 (Cornwall, Devon, Norfolk (United Kingdom)) and 2022 (East Sussex, Norfolk (United Kingdom); Belgium; The Netherlands). Field sites were aggregated into 4 regions based on their spatial proximity: Cornwall and Devon UK (SW, n = 12); Norfolk UK (NOR, n = 18); Sussex UK (SUS, n = 12); Belgium & Netherlands (BENE, n = 20) (Figure 2.1, Appendix 1).

2.3.2 Study regions

2.3.2.1 Cornwall and Devon, UK (SW)

Cornwall and Devon are situated on the South West Peninsula of England. Pastoral agriculture is the predominant land use, and the region is dotted with areas of heathland and moorland, including Dartmoor and Bodmin Moor as well as smaller areas such as Penwith Moors SSSI. Surveyed field sites were situated amongst this semi-natural heathland (Figure 2.2). Waterbodies in Cornwall and Devon are typically acid due to the granitic Cornubian batholith which underlies much of the region. In contrast, the Lizard peninsula (field sites LP, CP, HK) is underlain by a complex massif of basic igneous rocks, gabbro, granite and ancient gneisses (Dudley Stamp, 1946), often producing highly base-rich waterbodies (inc. HK).

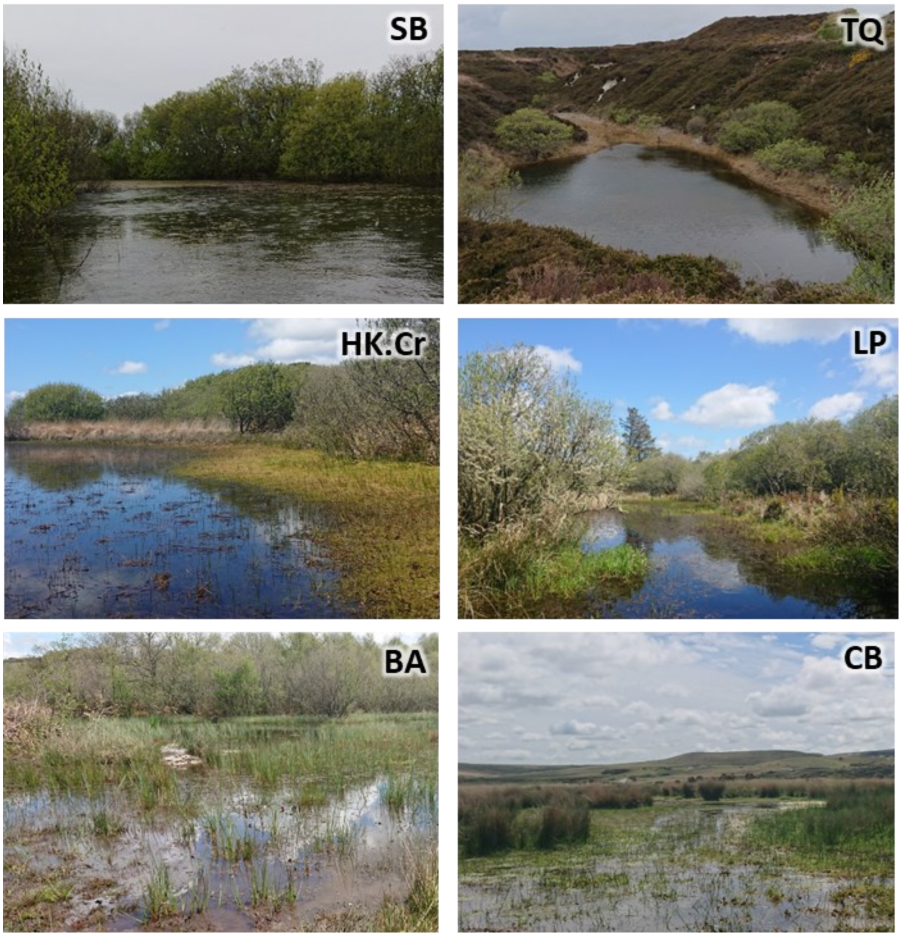

Figure 2.2. Characteristic field sites of Cornwall and Devon, UK (SW)

2.3.2.2 East Sussex, UK (SUS)

East Sussex is a county in south east England, encompassing much of the Weald, an eroded anticline of sandstone and clay, rimmed by the chalk of the North and South Downs (Dudley Stamp, 1946). Field sites consisted of ditches on grazing marsh within the Pevensey Levels SSSI (PA-PH), and the vegetated margins of Castle Water within Rye Harbour SSSI (RA-RD) (Figure 2.3).

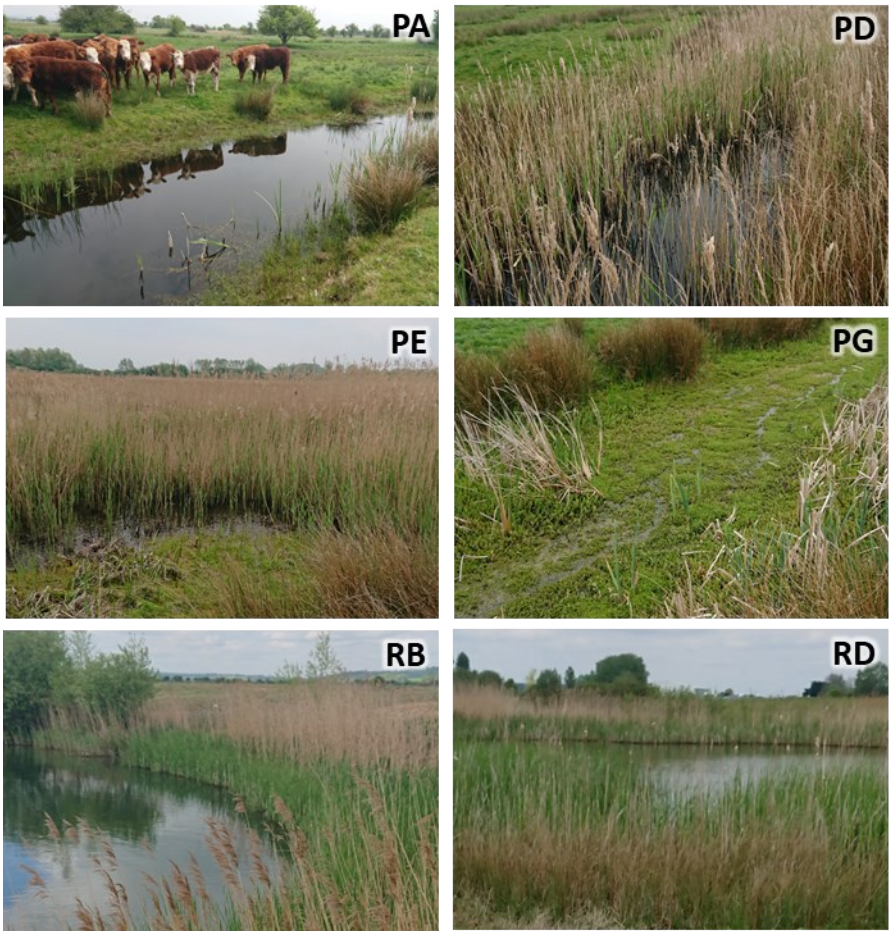

Figure 2.3. Characteristic field sites of East Sussex, UK (SUS).

2.3.2.3 Norfolk, UK (NOR)

Norfolk is a county in eastern England, predominantly covered by arable land. The county is primarily underlain by chalk bedrock, with superficial glacial sand and gravel deposits (Dudley Stamp, 1946). Field sites were a mixture of farm ponds (FA-FE, BP, MP) and periglacial pingo pools (TA-TC, GA-GH), which were typically circumneutral to base-rich and densely vegetated (Figure 2.4).

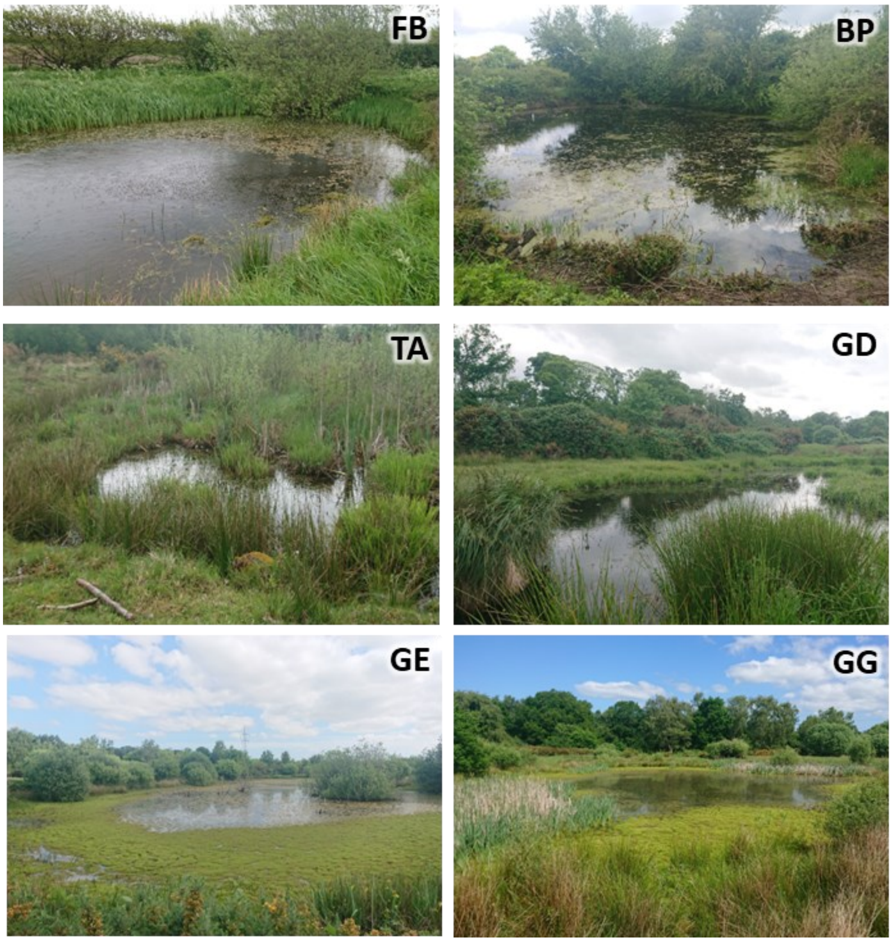

Figure 2.4. Characteristic field sites of Norfolk, UK (NOR)

2.3.2.4 Belgium and the Netherlands (BENE)

Belgium and the Netherlands, the 'Low Countries', occupy the Rhine-Meuse Scheldt delta of northwest continental Europe, and support the highest human population density in Europe (World Bank, 2023). The region is almost entirely underlain by deep (often >100m) deltaic sand and clay deposits (Pirson *et al.*, 2008). Field sites were mostly within designated nature areas apart from 6 mitigation ponds (BeA-BeF) around the

Linkeroever port terminal, Antwerp (Figure 2.5). Field sites were mostly fairly unproductive, with short macrophyte swards on sandy substrata.

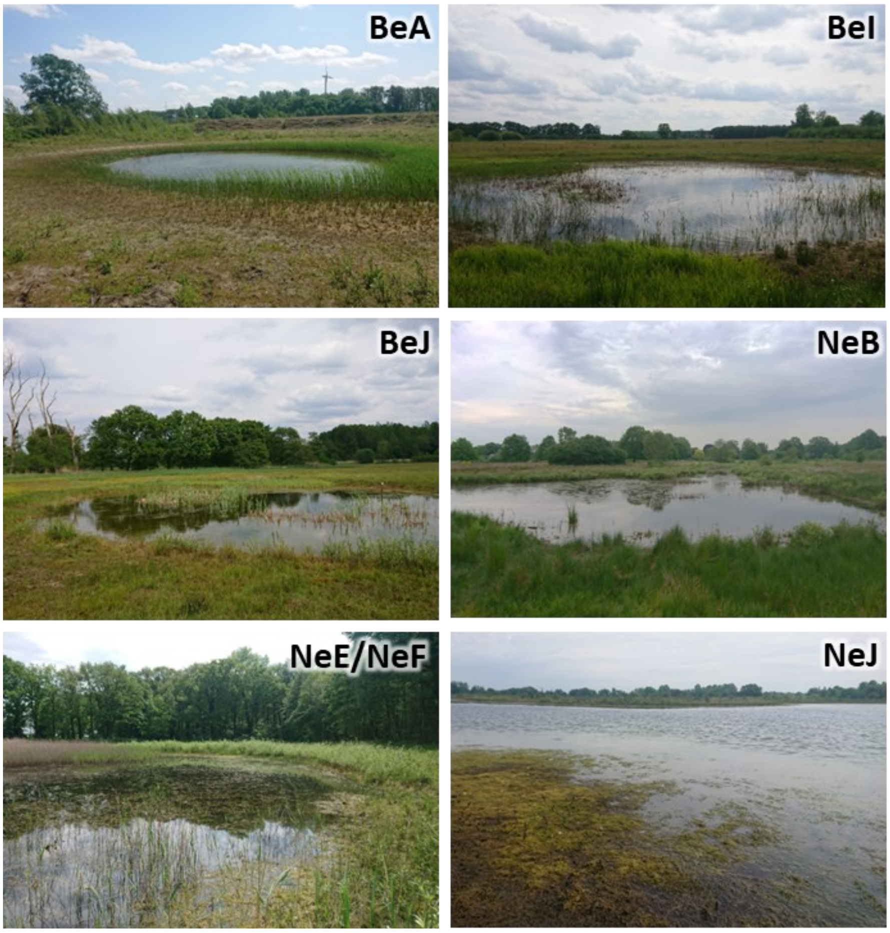

Figure 2.5. Characteristic field sites of Belgium and the Netherlands (BENE)

2.3.3 Sampling protocol

At each site, 4 macroinvertebrate samples were taken using a D-framed pond net (20 x 25 cm, 1 mm mesh), each sample consisting of 8 repeated sweeps over the same area (approx. 1 m^2). Samples were taken haphazardly within dense (> 50% cover) *C. helmsii* stands in invaded waterbodies, and haphazardly from the most densely vegetated marginal habitat in uninvaded 'control' waterbodies. Samples were transferred to 1 L pots and immediately fixed in either 70% industrial denatured alcohol (IDA) or 70% propylene glycol. Upon returning to the laboratory, all samples were drained and preserved in fresh IDA.

In the laboratory, samples were rinsed over a 500 µm sieve, and the remaining material placed in a water-filled tray marked out with a 4 x 4 cm grid, so that macroinvertebrates could be separated from vegetation and detritus. Macroinvertebrates were then identified and enumerated, using a binocular microscope (10-40x magnification) and a range of relevant literature (e.g. Brochard et al. 2016; Dobson et al. 2012; Edington and Hildrew 1995; Elliott et al. 1988; Foster et al. 2014; Foster and Friday 2011; Hammond et al. 1985; Nilsson 1996; Rowson et al. 2021; Savage 1989; Smallshire and Swash 2018; Wallace et al. 1990). Identification was to species level with the exception of Bivalvia (genus), Diptera ((sub)family), Annelida (subclass) and Platyhelminthes (class).

2.3.4 Data analysis

Data from the 4 samples from each site were pooled, and analysed at the site level (Coccia *et al.*, 2021). All analyses were conducted in the R programming environment (R Core Team, 2023).

2.3.4.1 Taxonomic diversity, abundance and assemblage composition

Differences in abundance, taxon richness and Shannon diversity between *C. helmsii* and uninvaded sites were assessed using generalised linear models, with region incorporated as a fixed effect. Model assumptions were checked graphically, and generalised least squares fits (package *nlme* (Pinheiro, Bates and R Core Team, 2023)) were used where issues of homogeneity of variance were evident. I applied these analyses to the whole dataset, to alien taxa alone, and individually to seven macroinvertebrate orders (Coleoptera, Diptera, Ephemeroptera, Gastropoda, Hemiptera, Odonata, Trichoptera,) and the subphylum Crustacea. For Diptera, Ephemeroptera and Crustacea I analysed abundance alone, excluding these groups from taxon richness analyses, since only 3 crustacean and 3 ephemeropteran species were present in samples, and dipterans were identified to (sub)family-level only.

Differences in assemblage composition between *C. helmsii* and uninvaded samples were assessed using permutational multivariate analysis of variance (PERMANOVA) on a Bray-Curtis dissimilarity matrix using *adonis2* in the package *vegan* (Oksanen *et al.*, 2022). For this purpose, abundance data were 4th root transformed to down-weight the influence of dominant taxa. Taxa contributing to differences in assemblage composition were then assessed using SIMPER analysis.

In order to investigate taxonomic beta-diversity, I transformed abundance data into a presence/absence database, from which I computed a distance matrix using Sorensen's Index of Dissimilarity, partitioned into its turnover and nestedness components for each sample in package *betapart* (Baselga *et al.*, 2023). I then applied a permutation test of multivariate homogeneity of group dispersions (PERMDISP) to this distance matrix

(Anderson, Ellingsen and McArdle, 2006) to assess differences in beta-diversity between *C. helmsii* and uninvaded samples.

To assess the prevalence of infrequent taxa amongst *C. helmsii*, I used macroinvertebrate presence/absence data to plot species accumulation curves with the Chao2 estimator, using *specpool* in the package *vegan* (Oksanen *et al.*, 2022). Chao2 effectively estimates the likelihood that taxa remain unsampled based on the relative frequency of taxa observed only once (‘singletons’) or twice (‘doubletons’) within sites and adds an expected number of as yet unencountered species to the number actually observed based on this likelihood. An elevated number of singletons produces a higher Chao estimate (Chao, 1984). I also checked species lists against conservation status assessments (Appendix 2) for the UK, Belgium and the Netherlands to explore whether threatened or near-threatened species (IUCN, 2023) were affected by *C. helmsii* invasion.

2.3.4.2 Functional trait database collation

I constructed a trait database using fuzzy coded trait data from Tachet *et al*. (2010 - (Schmidt-Kloiber and Hering, 2015). I selected food, feeding habits, locomotion, respiration, reproduction, dispersal and maximal body size for inclusion in the database (Table 2.1). These traits were selected both due to their roles in ecosystem functioning and on the basis of predicted impacts of *C. helmsii.* For instance, food, feeding habit and maximal body size determine rates of nutrient cycling and energy flows (Woodward *et al.*, 2005), whilst dispersal mode impacts aquatic-terrestrial subsidy (Lewis-Phillips *et al.*, 2020) and may be affected by the long-term seasonal permanence of perennial *C. helmsii* stands (Hargeby, 1990). Modes of locomotion and maximal size may be affected by *C. helmsii* stand density (Dawson and Warman, 1987; Warfe and Barmuta, 2004; McAbendroth *et al.*, 2005; Grutters *et al.*, 2015) and certain modes of respiration may be promoted or impaired by variation in dissolved oxygen concentrations within dense *C. helmsii* stands (Carter, Rybicki and Hammerschlag, 1991; Caraco and Cole, 2002; Ceschin *et al.*, 2020). Finally, modes of reproduction may be promoted or impaired by the altered availability and nature of oviposition sites within *C. helmsii* stands (Langdon *et al.*, 2004; Burkle, Mihaljevic and Smith, 2012). In order to weight all traits equally, fuzzy coded trait values were standardised across all traits (package *ade4*: (Thioulouse *et al.*, 2018)), so that the sum of all modalities equalled one.

As trait information in Tachet et al. (2010) is mostly encoded at the genus level, I pooled species within the dataset into their respective genera. Where organisms in the dataset were identified to family, trait values were estimated as the average of trait values for species/genera within that family. Similarly, where trait values were missing for genera in the dataset (*Cercyon*, *Coelostoma*, *Cryptopleurum*), but values were available for other genera within the same family, I used average values for the family to which these genera belonged (Sarremejane *et al.*, 2017; Coccia *et al.*, 2021). Where Tachet et al. (2010) contained no trait data pertaining to a family in the dataset, the family was removed from functional analyses (Johnson *et al.*, 2021). Hebridae, Rhagionidae, Trichoceridae, Curculionidae and Arachnida (0.16% of total individuals) were excluded from functional analyses on this basis.

Table 2.1. Macroinvertebrate functional traits included in the trait database (taken from Tachet *et al*. 2010).

Trait	**Modality**	**Trait**	**Modality**
Food	micro-organisms	Maximal size	< 0.25 cm
	detritus <1mm		> 0.25-0.5 cm
	dead plants >1mm		> 0.5-1 cm
	live microphytes		> 1-2 cm
	live macrophytes		> 2-4 cm
	dead animals >1mm		> 4-8 cm
	live microinvertebrates		> 8 cm
	live macroinvertebrates	Respiration	tegument
	vertebrates		gill
Feeding habits	absorber		plastron
	deposit feeder		spiracle
	shredder		hydrostatic vesicle
	scraper	Reproduction	ovoviviparity
	filter feeder		isolated eggs, free
	piercer		isolated eggs, cemented
	predator		clutches, cemented or fixed
	parasite		clutches, free
Locomotion and substrate relation	flier		clutches, in vegetation
	surface swimmer		clutches terrestrial
	pelagic swimmer		clutches terrestrial
	crawler		asexual
	burrower	Dispersal	aquatic passive
	interstitial		aquatic active
	temporarily attached		aerial passive
	permanently attached		aerial active

2.3.4.3 Functional diversity and assemblage composition

To calculate functional diversity indices, I created a trait-based distance matrix using Gower's distance. From this distance matrix, I produced a 4-dimensional functional space using Principal Coordinates Analysis (PCoA) (Maire *et al.*, 2015). Functional space computation was conducted using the *mFD* package (Magneville *et al.*, 2022). I used this functional space to compute Functional Richness (FRic), Functional Evenness (FEve) and Functional Divergence (FDiv) for each site. Functional richness represents the volume of the trait space occupied by each assemblage, bounded by taxa with the most extreme trait values in the assemblage. Functional evenness reflects how regularly taxa in an assemblage are distributed within the trait space, using the minimum spanning tree connecting all taxa in the assemblage. Functional divergence represents the distribution of taxa in relation to the functional centroid of the assemblage, reflecting the dissimilarity of taxa in each assemblage and indicating the degree of niche differentiation (Mason *et al.*, 2005; Mathers *et al.*, 2020; Coccia *et al.*, 2021). As with taxonomic analyses, I assessed the impacts of *C. helmsii* on these indices using generalised linear models, incorporating region as a fixed factor (Bates *et al.*, 2015).

To investigate the relative contribution of traits to the functional composition of macroinvertebrate assemblages in *C. helmsii* and uninvaded samples, I constructed a community weighted means (CWM) matrix by crossing the functional trait and taxon abundance databases, with which I produced a fuzzy principal components analysis (FPCA) ordination using *ade4* (Thioulouse *et al.*, 2018). Here, a PCA ordination (as opposed to the PCoA used to compute functional diversity metrics) has the advantage of permitting shifts in the trait space occupied by an assemblage to be correlated with particular trait modalities, enabling inferences to be made about the trait modalities promoted or impaired within *C. helmsii* sites (Bruno *et al.*, 2019; Guareschi *et al.*, 2021).

In order to assess functional beta-diversity, I transformed the taxon abundance database into presence/absence data and crossed this with the trait matrix to calculate Sorensen's Index of Dissimilarity, partitioned into its turnover and nestedness components (Baselga and Leprieur, 2015), with the *betapart* package (Baselga *et al.*, 2023). As with taxonomic beta-diversity, differences in functional beta-diversity between *C. helmsii* and uninvaded samples were assessed using a permutation test of multivariate homogeneity of group dispersions (Anderson, Ellingsen and McArdle, 2006).

2.4 Results

2.4.1 Taxonomic diversity, abundance and assemblage composition

Macroinvertebrate taxon richness was significantly higher in sites with *C. helmsii* than in uninvaded sites. Neither abundance nor Shannon diversity differed significantly between *C. helmsii* sites and uninvaded sites (Figure 2.6, Table 2.3). Measures of taxonomic β-diversity, including its two components, did not differ significantly between *C. helmsii* and uninvaded sites (Table 2.2).

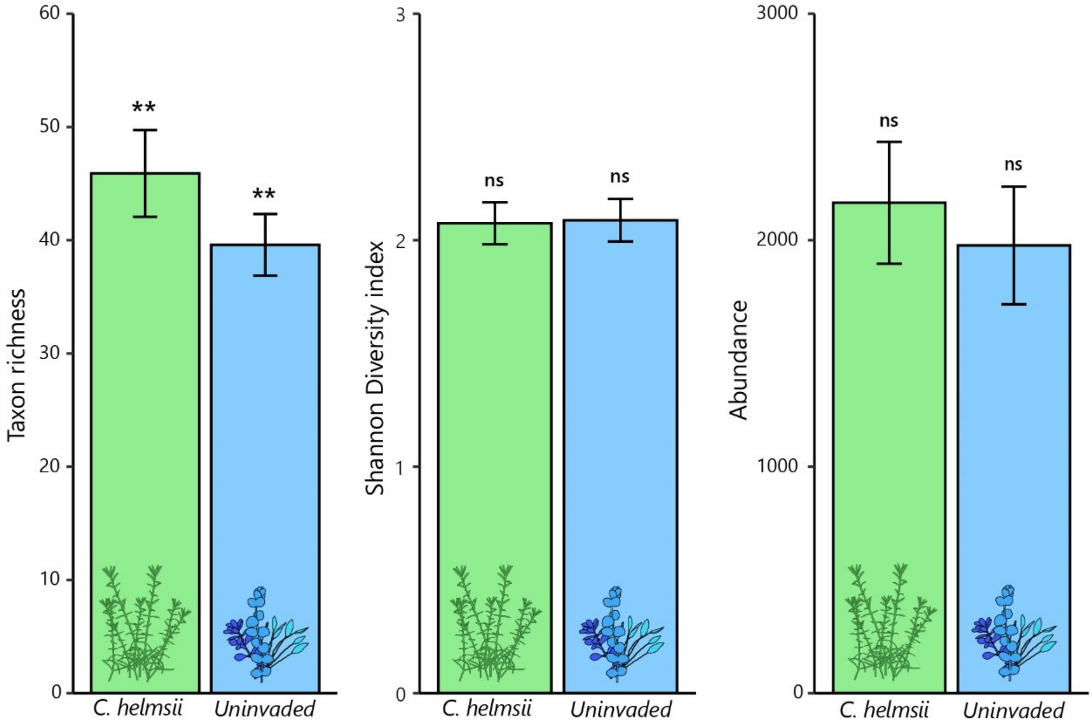

Figure 2.6. Indices of alpha taxonomic diversity (taxon richness, Shannon diversity and abundance), (mean ± SE, ** = p<0.01, ^{ns} = p>0.05).

Table 2.2. Taxonomic Sorensens beta-diversity, partitioned into turnover and nestedness. Average distances to median for *C. helmsii* and uninvaded distance matrices computed with PERMDISP, with F statistics and P values of permutation tests (999 permutations).

	C. helmsii	Uninvaded	F	P	
Total Beta	0.4493	0.4261	1.4813	0.21	ns
Turnover	0.3396	0.3437	0.0353	0.861	ns
Nestedness	0.10617	0.08615	1.099	0.329	ns

Table 2.3. Results of LM, GLM and GLS models testing the effects of *C. helmsii* invasion on taxonomic (taxon richness, Shannon diversity, abundance) alpha diversity indices, with region incorporated as a fixed effect. Type III LM fits tested by F tests, GLM fits by likelihood ratio tests, GLS fits by Wald chi-sq.

			df	F	X^2	P
Taxonomic	Taxon richness (Poisson GLM)	invasion	1	-	9.237	<0.01
		region	3	-	222.438	<0.001
		inv : reg	3	-	4.348	0.226
	Shannon diversity (LM)	invasion	1, 55	0.184	0.047	0.669
		region	3	1.733	1.319	0.171
		inv : reg	3	1.957	1.490	0.131
	Abundance (quasi-Poisson GLM)	invasion	1	-	0.382	0.536
		region	3	-	29.923	<0.001
		inv : reg	3	-	0.976	0.807

Alien macroinvertebrates were significantly more abundant in *C. helmsii* sites than in uninvaded sites, although this effect varied by region, being driven primarily by sites in Sussex, UK (SUS) and Southwest UK (SW) (Figure 2.7, Table 2.4). There was no significant difference in alien taxon richness between *C. helmsii* and uninvaded sites (Table 2.4).

Table 2.4. Results of LM and GLS models assessing impacts of *C. helmsii* invasion on taxon richness and abundance of alien macroinvertebrates, with region incorporated as a fixed effect. Type III LM fit tested by F test, GLS by Wald chi-sq.

			df	F	X^2	P
Alien diversity	Taxon richness (LM)	invasion	1	2.933	-	0.092
		region	3	13.610	-	<0.001
		inv : reg	3	2.095	-	0.111
	Abundance (GLS)	invasion	1	-	19.011	<0.001
		region	3	-	118.385	<0.001
		inv : reg	3	-	25.559	<0.001

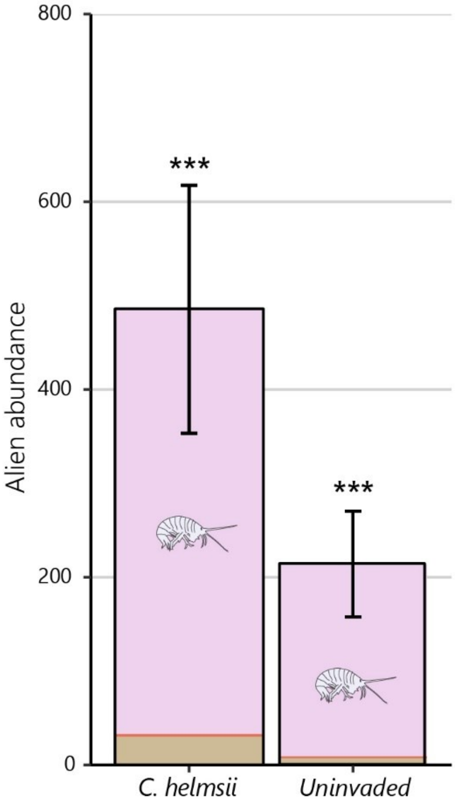

Figure 2.7. Alien macroinvertebrate abundance in *C. helmsii* and uninvaded sites, (mean ± SE, *** = p<0.001). Colours correspond to the following alien macroinvertebrate species: *Crangonyx pseudogracilis* 🟪 *; Ferrissia wautieri* 🟥 *; Physella acuta* 🟫

Removal of alien taxa from the dataset did not change overall taxonomic abundance and diversity trends (Table 2.5).

Table 2.5. Results of GLMs testing the effects of *C. helmsii* invasion on taxonomic (taxa richness, Shannon diversity, abundance) alpha diversity indices, with alien species removed from the dataset. Region incorporated as a fixed effect. Type III GLM fits tested by likelihood ratio tests.

			df	X^2	p
Native diversity	Taxa richness (GLM w. Poisson error distribution)	invasion	1	7.664	<0.01
		region	3	131.372	<0.001
		inv : reg	3	5.501	0.139
	Abundance (GLM w. Poisson error distribution)	invasion	1	0.267	0.605
		region	3	8.980	<0.05
		inv : reg	3	5.189	0.158

Coleopteran taxon richness was significantly higher in *C. helmsii* sites than in uninvaded sites. Coleopteran and crustacean abundance was also significantly higher in *C. helmsii* sites, varying by region (driven largely by Norfolk, UK (NOR) and Sussex, UK (SUS) sites). Dipteran abundance was significantly higher in uninvaded sites, an effect which did not differ significantly between regions (Figure 2.8).

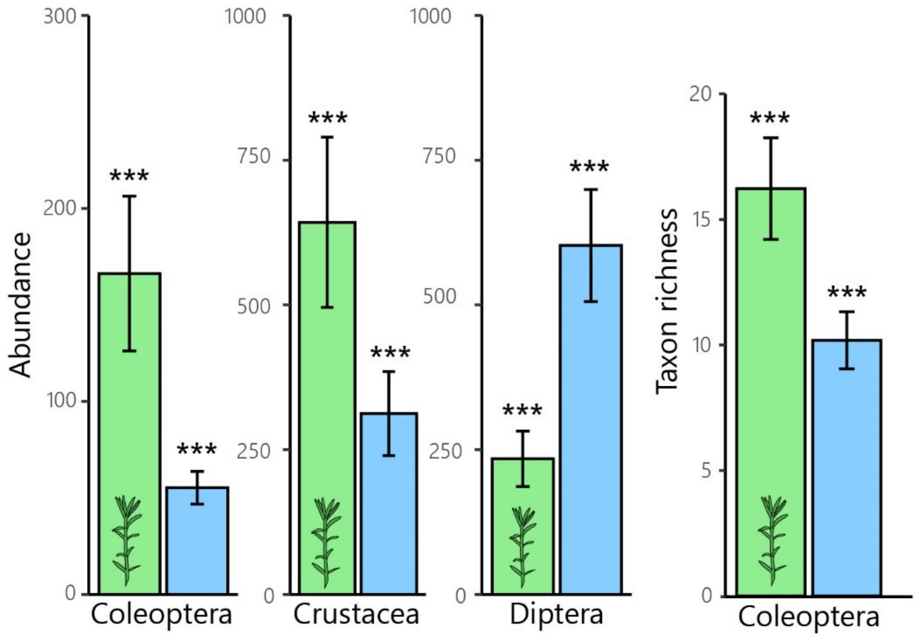

Figure 2.8. Abundance of Coleoptera, Crustacea and Diptera; taxon richness of Coleoptera in *C. helmsii* (🟩) and uninvaded (🟦) sites (mean ± SE, *** = $p<0.001$).

There were no significant differences in the taxon richness of Gastropoda, Hemiptera, Odonata nor Trichoptera between *C. helmsii*- and uninvaded sites, nor significant differences in the abundance of Ephemeroptera, Gastropoda, Hemiptera, Odonata or Trichoptera between *C. helmsii*- and uninvaded sites (Table 2.6).

Table 2.6. Results of GLM and GLS models testing the effects of *C. helmsii* invasion on abundance and taxa richness of Coleoptera, Crustacea, Diptera, Gastropoda, Hemiptera, Odonata, and Trichoptera, with region incorporated as a fixed effect. Type III GLM fits tested by likelihood ratio tests, GLS fits by Wald chi-sq.

			d.f	X^2	p
Abundance	Coleoptera (GLS)	invasion	1	14.222	<0.001
		region	3	28.186	<0.001
		inv : reg	3	15.731	<0.01
	Crustacea (GLS)	invasion	1	13.416	<0.001
		region	3	76.677	<0.001
		inv : reg	3	12.137	<0.01
	Diptera (GLS)	invasion	1	11.131	<0.001
		region	3	1.950	0.583
		inv : reg	3	3.973	0.264
	Ephemeroptera (GLS)	invasion	1	2.538	0.111
		region	3	2.685	0.443
		inv : reg	3	0.845	0.839
	Gastropoda (GLS)	invasion	1	0.417	0.518
		region	3	10.014	0.018
		inv : reg	3	7.644	0.053
	Hemiptera (GLS)	invasion	1	0.271	0.603
		region	3	4.254	0.235
		inv : reg	3	5.738	0.125
	Odonata (GLS)	invasion	1	0.003	0.959
		region	3	22.513	<0.001
		inv : reg	3	2.259	0.520
	Trichoptera (GLS)	invasion	1	0.461	0.497
		region	3	7.322	0.062
		inv : reg	3	1.515	0.679
Taxon richness	Coleoptera (Poisson GLM)	invasion	1	33.909	<0.001
		region	3	102.749	<0.001
		inv : reg	3	4.099	0.251
	Gastropoda (GLS)	invasion	1	0.435	0.510
		region	3	249.895	<0.001
		inv : reg	3	0.080	0.994
	Hemiptera (Poisson GLM)	invasion	1	0.958	0.328
		region	3	5.860	0.119
		inv : reg	3	6.598	0.086
	Odonata (Poisson GLM)	invasion	1	2.076	0.150
		region	3	31.831	<0.001
		inv : reg	3	1.435	0.697
	Trichoptera (Poisson GLM)	invasion	1	0.033	0.855
		region	3	16.034	<0.01
		inv : reg	3	0.635	0.888

Macroinvertebrate assemblages differed subtly but significantly between *C. helmsii* and uninvaded sites, with invasion status explaining only 2.9% of variation in assemblage composition (PERMANOVA: $F_{1,62} = 2.427$, $p < 0.01$; Appendix 4). Region explained 26.9% of variation, once invasion status had been taken into account. Overall differences in assemblage composition associated with invasion status were driven most strongly by detritivores including *Crangonyx pseudogracilis, Asellus aquaticus* and *Ampullaceana balthica*, which occurred more frequently within *C. helmsii* sites. *Cloeon dipterum* and non-biting midges (Chironomidae) occurred more frequently in uninvaded sites (Table 2.7).

Table 2.7. Taxa driving assemblage dissimilarity between uninvaded and *C. helmsii* samples, and their contribution to dissimilarity, up to 20% cumulative contribution. Abundances are back-transformed for easier interpretation here.

Taxon	Mean abundance: Uninvaded	Mean abundance: *C. helmsii*	Contribution (%)	Cumulative contribution (%)	
Crangonyx pseudogracilis	15.7	51.4	2.5	2.5	ns
Chironominae	161.3	15.7	2.2	4.7	***
Asellus aquaticus	6.5	22.2	2.1	6.8	ns
Ampullaceana balthica	6.2	25.4	2	8.8	ns
Cloeon dipterum	22.1	11	1.9	10.7	ns
Pisidium spp.	14	8.6	1.8	12.5	ns
Corixidae (nymph)	25.9	14.6	1.5	14	ns
Plea leachi	3.6	10.1	1.5	15.5	ns
Gyraulus crista	0.5	2.3	1.5	17	ns
Ischnura elegans	2.8	1.8	1.4	18.4	ns
Orthocladiinae	24.9	28.1	1.4	19.8	ns

ns $p > 0.05$ * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Chao species accumulation curves indicated the presence of more singletons and doubletons (taxa found in ≤2 sites) within *C. helmsii* sites than in uninvaded sites (Figure 2.9). Of taxa occurring in ≤4 sites, 8% were found exclusively within *C. helmsii* sites, compared with 2.7% exclusively from uninvaded sites. The remainder occurred across invaded and uninvaded sites. Fifty three taxa of conservation concern were found within samples, 20 of these exclusively in *C. helmsii* sites, compared with 13 exclusively in uninvaded sites and 20 in both invaded and uninvaded samples. Across regions, taxa of conservation concern were found on 73 occasions in *C. helmsii* sites, and 62 occasions in uninvaded sites (Appendix 2).

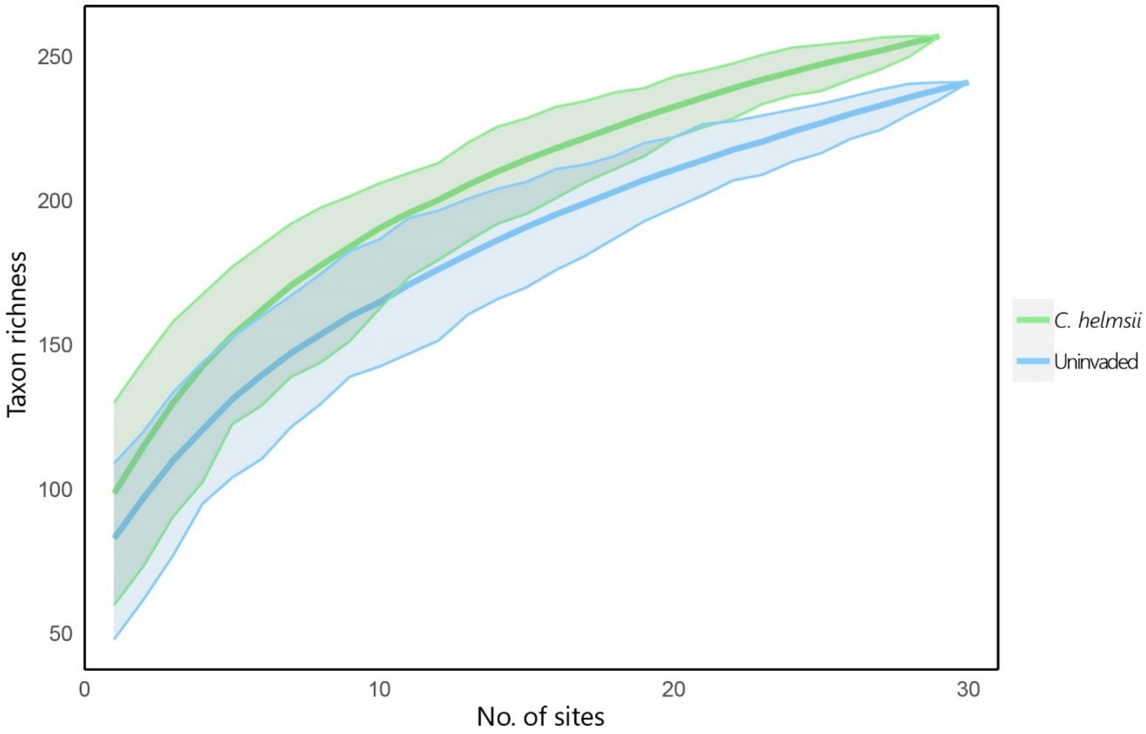

Figure 2.9. Species accumulation curves based on Chao2 estimator, using occurrence data. Envelope around lines represent upper/lower 95% confidence intervals.

2.4.2 Functional diversity and assemblage composition

Neither functional richness, functional evenness nor functional divergence were significantly different between *C. helmsii* and uninvaded sites (Figure 2.10, Table 2.8), nor did functional β-diversity (Table 2.9).

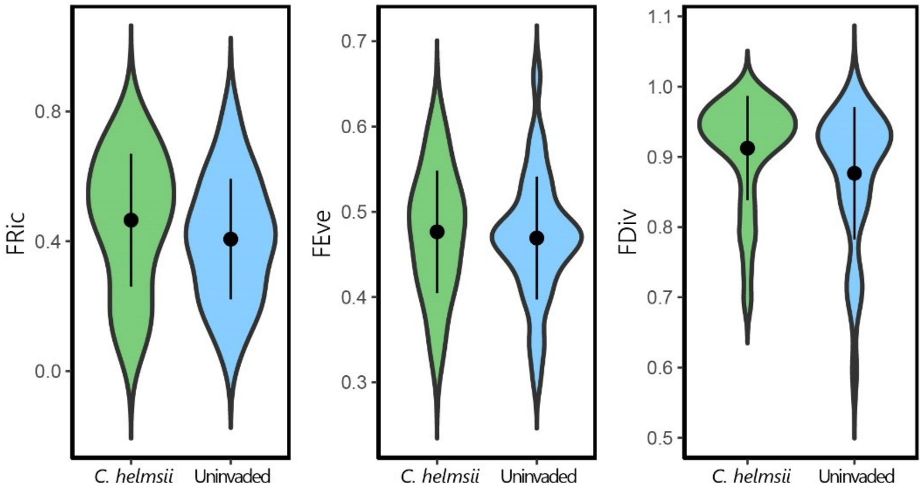

Figure 2.10. Violin plots showing the kernel probability density (mean ± 1 standard deviation) of functional alpha-diversity indices (functional richness (FRic), functional evenness (FEve) and functional divergence (FDiv)) of *C. helmsii* and uninvaded sites

Table 2.8. Results of LM and GLM models testing the effects of *C. helmsii* invasion on functional (functional richness, functional evenness, functional divergence) alpha diversity indices, with region incorporated as a fixed effect. Type III LM fits tested by F tests, GLM fits by likelihood ratio tests.

			df	F	X^2	p
	Functional richness (LM)	invasion	1.55	1.482	-	0.229
		region	3	8.514	-	<0.001
		inv : reg	3	0.172	-	0.915
	Functional evenness (LM)	invasion	1,55	0.241	-	0.626
		region	3	0.596	-	0.620
		inv : reg	3	0.830	-	0.483
	Functional divergence (quasi-Poisson GLM)	invasion	1	-	1.706	0.192
		region	3	-	0.269	0.966
		inv : reg	3	-	1.005	0.800

Shifts in functional assemblage composition on PCA axis 1 were associated with feeding type: shredder, food: dead plants >1 mm, reproduction: ovoviviparity and respiration: gill; trait modalities which are well represented amongst the detritivores which drove differences in taxonomic assemblage composition between *C. helmsii* and uninvaded sites. Differences on axis 2 were most strongly associated with respiration: tegument and locomotion: pelagic swimmer (Figure 2.11). The convex hull enclosing *C. helmsii* functional assemblage values occupied a greater volume of trait space than the convex hull enclosing uninvaded functional assemblage values.

Table 2.9. Functional Sorensens beta-diversity, partitioned into turnover and nestedness. Average distances to median for *C. helmsii* and uninvaded distance matrices computed with PERMDISP, with F statistics and P values of permutation tests (999 permutations).

	C. helmsii	Uninvaded	F	P	
Total Beta	0.2586	0.2679	0.0534	0.801	ns
Turnover	0.09174	0.10197	0.4548	0.485	ns
Nestedness	0.1828	0.1736	0.0444	0.83	ns

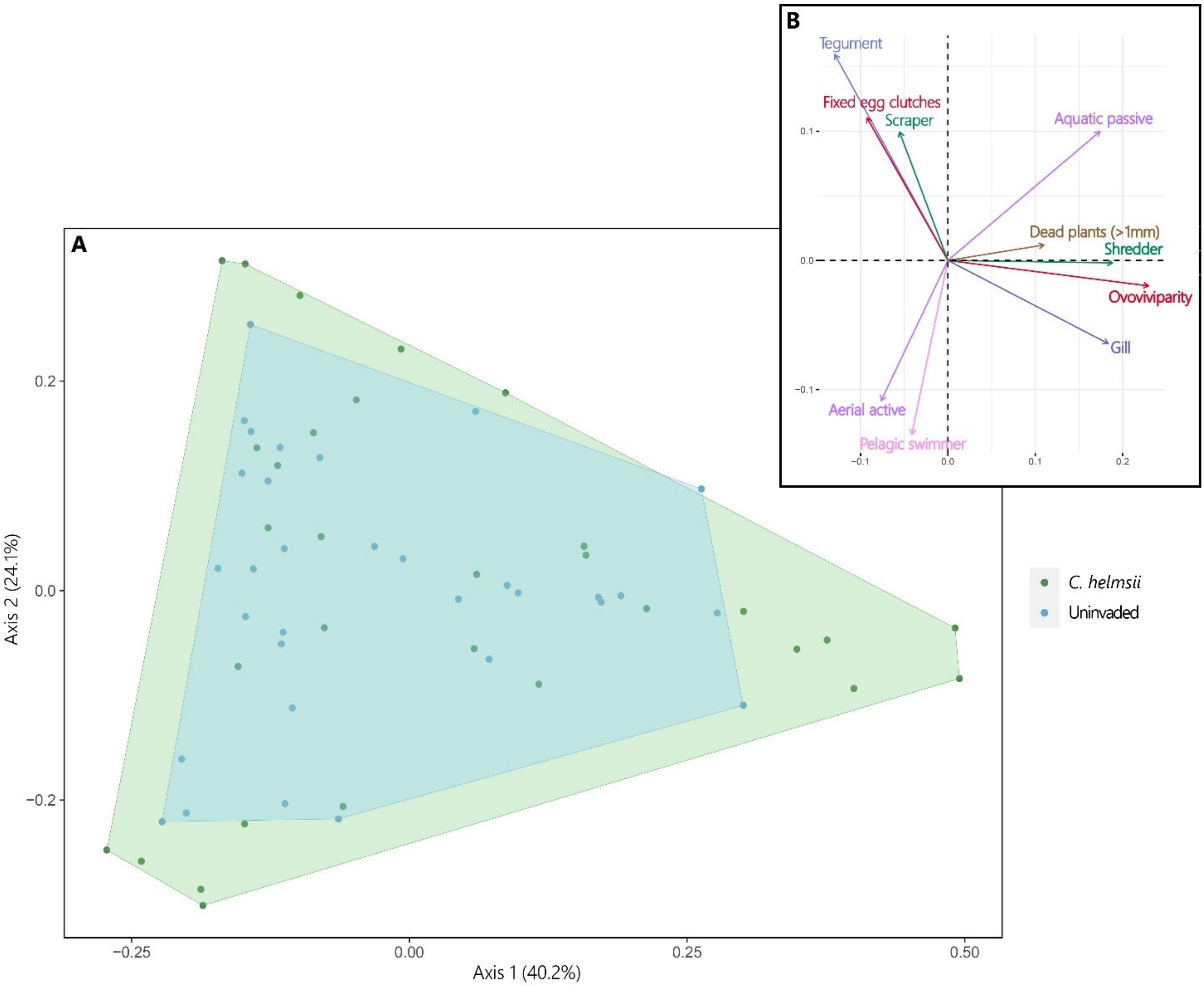

Figure 2.11. Fuzzy principal components analysis ordination, produced using a community weighted means matrix. First two axes account for 64.3% of the variation. Convex hulls enclose points representing the location in functional trait space of macroinvertebrate assemblages from *C. helmsii* (green convex hull) and uninvaded (blue convex hull) sites. B: Trait modalities most strongly driving assemblage functional composition. Green arrows: feeding mode; brown: food; red: reproduction; blue: respiration; purple: dispersal; pink: locomotion.

2.5 Discussion

Crassula helmsii is widely considered a pernicious invasive plant, despite limited quantitative evidence for negative effects on much of the recipient biota. In this study of 63 ponds across the most heavily invaded regions of NW Europe, I observed nuanced impacts of *C. helmsii* on macroinvertebrate assemblages. Contrary to expectations, *Crassula helmsii* sites had significantly higher macroinvertebrate taxon richness than uninvaded sites, whilst abundance, functional alpha-diversity and taxonomic & functional beta-diversity did not differ significantly between *C. helmsii* and uninvaded sites. Coleopteran taxa richness and abundance was higher within *C. helmsii* sites, whilst in contrast dipteran larval abundance was lower. I also found significantly higher alien macroinvertebrate abundance within *C. helmsii* sites, driven primarily by the North American amphipod *Crangonyx pseudogracilis*. Taxa occurring rarely in samples were equally likely to be found in *C. helmsii* and uninvaded sites; many of these species being habitat specialists (Appendix 3), meaning that *C. helmsii* invasion is not simply associated with an expansion of eurytopic taxa. Species of conservation concern in the study regions were also found across invaded and uninvaded sites relatively equally. Whilst finer taxonomic resolution (order-by-order) diversity results often varied by region, most likely due to underlying differences in macroinvertebrate species pools, overarching taxonomic and functional diversity analyses were consistent across regions. Macroinvertebrate assemblage composition was subtly different within *C. helmsii* compared with native vegetation, driven primarily by detritivores including *C. pseudogracilis*, *Asellus aquaticus* and *Ampullaceana balthica,* more common in *C. helmsii* sites, and *Cloeon dipterum,* more common in uninvaded sites. These shifts were reflected in functional composition analyses, which indicated that shredder and scraper detritivores were more prevalent within *C. helmsii* sites.

Crassula helmsii forms dense stands of up to 1.5 kg m^{-2} (Dawson and Warman, 1987), which are likely to impact generalist herbivores and detritivorous macroinvertebrate taxa by providing abundant food both directly (live macrophyte tissue, detritus upon senescence) and indirectly (epiphyton) (Sheldon and Boylen, 1975; Cattaneo and Kalff, 1980; Newman, 1991). This food supply is likely to remain available in fluctuating quantities throughout the year, since *C. helmsii* is perennial (Smith and Buckley, 2020). Taxonomic and functional composition results suggest that such shifts to food supply are a key mechanism by which *C. helmsii* impacts macroinvertebrate assemblages. The shredders *C. pseudogracilis* and *A. aquaticus* and the scraper *A. balthica* were more abundant within *C. helmsii* samples and amongst the taxa most responsible for assemblage dissimilarity, whilst the trait modalities food: dead plants, feeding type: shredder and feeding type: scraper occurred more within *C. helmsii* samples. Interestingly, larval pond olives (*C. dipterum*) and true flies (Diptera) were less abundant within *C. helmsii* sites. Many of these larvae feed upon fine particulate detritus (Tachet *et al.*, 2010), which may be less readily available amongst *C. helmsii* stands due to the recalcitrance (slow breakdown) of *C. helmsii* detritus (Tasker *et al.*, in prep.) and the replacement of bare substrata with dense vegetation.

Irrespective of macrophyte species identity, macroinvertebrate abundance and diversity tends to be higher in more architecturally complex macrophyte stands as a result of increased availability of microhabitats, including predator-free refugia - a trend which has been observed in both native stands and in invasions by multiple submerged alien macrophyte species (Kelly and Hawes, 2005; McAbendroth *et al.*, 2005; Hogsden, Sager and Hutchinson, 2007; Tasker, Foggo and Bilton, 2022). Alongside food provisioning (discussed above), altered structural complexity is likely to contribute to the higher taxon richness I observed within *C. helmsii* sites (Dawson and Warman, 1987; Smith and Buckley, 2020). As well as stem density, habitat structural complexity is contingent on plant growth form and architecture, with more complex structures (e.g. finely branched stems, finely dissected leaves) tending to increase the availability of microhabitats and refugia (Schramm and Jirka, 1989; Warfe and Barmuta, 2004; McAbendroth *et al.*, 2005; Thomaz and Cunha, 2010; Katayama, 2014). This effect is not consistent across taxa and is strongly modulated by the body size of predators and prey relative to interstitial spaces. For instance, macrophyte stands may exclude vertebrate predators, whilst permitting access for macroinvertebrate predators such as dragonfly larvae (Odonata) and diving beetles (Coleoptera: Dytiscidae) (Bartholomew, Diaz and Cicchetti, 2000; Grutters *et al.*, 2015). The functional composition PCA indicated that the locomotion: pelagic swimmer trait modality occurred more frequently within *C. helmsii* samples, suggesting that the interstitial space within these stands is sufficiently open to permit passage for many free-swimming macroinvertebrate taxa. In contrast, *C. helmsii'*s simple, narrowly lanceolate leaves may result in stands containing fewer refugia from macroinvertebrate predators for some taxa than those formed by finely dissected native macrophytes such as *Myriophyllum spicatum* L. (Diehl, 1988; Warfe and Barmuta, 2004; Smith and Buckley, 2020). The relatively lower abundance of non-biting midges (Chironomidae) within *C. helmsii* samples may in part also reflect this effect (Diehl, 1988).

The cross-seasonal permanence of dense *C. helmsii* stands may drive additional impacts on recipient macroinvertebrate assemblages. As mentioned above, *C. helmsii* is a perennial, and its stands typically remain in place throughout the year, perhaps facilitating its colonisation by relatively immobile taxa with weak powers of active dispersal (Hussner, 2009; Smith, 2015). This effect is likely to prove particularly strong in temporary waterbodies, with emersed *C. helmsii* stands providing moister conditions for aquatic taxa during the dry phase (Collinson *et al.*, 1995; Williams, 1997). Such habitat permanence may partly explain the elevated abundance of the crustaceans *C. pseudogracilis* and *A. aquaticus* – both relatively weak active dispersers – in *C. helmsii* sites (Hargeby, 1990; Verberk, Siepel and Esselink, 2008).

Increased abundance of alien taxa in *C. helmsii* sites is largely driven by *Crangonyx pseudogracilis*, a North American amphipod, with the North American gastropod *Physella acuta* also present in greater numbers amongst *C. helmsii*. The elevated abundance of alien macroinvertebrates observed within *C. helmsii* sites suggests facilitation (positive interactions between species) between *C. helmsii* and alien macroinvertebrates, raising the possibility of 'invasional meltdown', wherein invasion by one alien species facilitates the proliferation of other non-natives (Simberloff and Von Holle, 1999; Bruno, Stachowicz and Bertness, 2003). Macroinvertebrate functional composition differed in *C. helmsii* sites, linked to traits possessed by *C. pseudogracilis* (e.g. shredding mouthparts, ovoviviparity), suggesting *C. helmsii* may impact recipient ecosystems indirectly via facilitation of *C. pseudogracilis*. Increased abundance of alien macroinvertebrates in sites containing *C. helmsii* may also result from the broader invasibility of the site (e.g. elevated disturbance, proximity to alien propagule sources), which increases the likelihood of successful invasion by alien species, including *C. helmsii*. This seems unlikely to wholly explain the differences in alien abundance observed in this study, however, since alien abundance was significantly higher amongst *C. helmsii* even when compared with uninvaded sites which were in close proximity, interconnected and surrounded by closely comparable land use (e.g. Sussex, UK).

Although biological invasions have often been associated with declines in scarce, stenotopic species, taxa of conservation concern actually occurred more frequently in *C. helmsii* sites than in uninvaded sites in this study. In the UK, such taxa found exclusively in *C. helmsii* samples included the water beetles *Hydaticus seminiger* (De Geer, 1774) and *Limnoxenus niger* (Gmelin, 1790) (Foster, Bilton and Nelson, 2016; Foster *et al.*, 2020), as well as the diving beetle *Bidessus unistriatus* (Goeze, 1777)*,* found amongst dense *C. helmsii* in Sussex - the first record anywhere in the UK since 2008, where it is considered critically endangered (Foster, 2010; Tasker, 2023). In contrast, the Nationally Scarce caddisfly *Limnephilus binotatus* Curtis, 1834 (Wallace, 2016) was found only in uninvaded sites (see Appendix 2 for a complete list of scarce and threatened taxa found in surveys). Biological invasions have also been linked to biotic homogenisation (Olden, Comte and Giam, 2018; Muthukrishnan and Larkin, 2020), but I found no significant differences in taxonomic or functional beta-diversity (neither turnover nor nestedness) between *C. helmsii* and uninvaded samples, suggesting that *C. helmsii* invasions do not homogenise macroinvertebrate assemblages, at least in the regions surveyed here.

These results align broadly with past attempts to assess the ecological impacts of *C. helmsii*, which have also found nuanced impacts of this macrophyte. Unpublished studies from two regions of Southern England (Ewald, 2014; Smith, 2015) found no significant impact of C*. helmsii* on macroinvertebrate abundance or species richness. Early accounts of the extirpation of native flora (Dawson and Warman, 1987; Leach and Dawson, 1999) have proven similarly difficult to confirm quantitatively. A number of studies have reported a correlation between rising *C. helmsii* cover and declining cover of native macrophytes (Ewald, 2014; van Kleef *et al.*, 2017), although in some instances rising *C. helmsii* abundance may result from 'space filling' of bare ground unoccupied by fully aquatic native taxa (Dean, 2012). Generally, field studies have reported no significant decline in native plant species richness in *C. helmsii*- invaded waterbodies (Langdon *et al.*, 2004; Ewald, 2014; Smith and Buckley, 2015). In fact, Smith and Buckley (2015) found that waterbodies invaded by *C. helmsii* contained significantly more rare plant taxa than uninvaded waterbodies (although the authors noted that this effect could have been driven by factors other than *C. helmsii* invasion, from which *C. helmsii* also benefitted). Impacts on amphibians also appear mixed, with reports of negative impacts of *C. helmsii* on natterjack toad (*Epidalea calamita* (Laurenti, 1768)) spawning and egg development but positive impacts on larval growth (van der Loop, van Kleef, *et al.*, 2023), and negative impacts on smooth newt (*Lissotriton vulgaris* (L.)) egg development but no significant impact on great crested newt (*Triturus cristatus* (Laurenti, 1768)) egg development (Langdon *et al.*, 2004). In short, the ecological impacts of *C. helmsii* are perhaps less overwhelmingly negative than initial accounts suggested, particularly with respect to faunal assemblages. Nonetheless, ongoing reports of the disappearance of native plant taxa (e.g. van der Loop et al. 2022) warrant further quantitative investigation, with lag times between *C. helmsii* establishment and impact (Crooks, 2005) possibly masking negative effects on native macrophyte diversity. It is also worth noting that my study did not assess the impacts of *C. helmsii* on macroinvertebrate taxa associated with bare substrates, since I sampled from dense native vegetation in uninvaded sites. Future research on these impacts would clearly be instructive. Tipping points may exist in waterbodies where *C. helmsii* establishes near-complete coverage, beyond which native biota suffer greater negative impacts. Fostering biotic resistance by native floral assemblages could help to prevent complete *C. helmsii* domination and thus guard against the worst of these effects (Bakker and Wilson, 2004; Funk *et al.*, 2008; van der Loop, van Kleef, *et al.*, 2023).

2.5.1 Conclusion

Crassula helmsii, though considered one of the most pernicious invasive plants in NW Europe, appears to support a broadly comparable macroinvertebrate assemblage to native vegetation, albeit with somewhat elevated taxonomic diversity and subtly distinct assemblage composition, particularly with respect to detritivores. *C. helmsii* sites also contained more abundant alien macroinvertebrates, suggesting *C. helmsii* may facilitate invasion by at least some alien macroinvertebrates. Differences in taxonomic and functional assemblage composition between *C. helmsii* and uninvaded sites suggest that *C. helmsii* impacts macroinvertebrate assemblages via altered availability and quality of plant material, particularly detritus, and altered habitat structural complexity and stand permanence.

Chapter 3: Mesocosm trials indicate divergent and unstable preferences amongst consumers for *Crassula helmsii* vs. native macrophytes

3.1 Abstract

Herbivory may either help (enemy release) or hinder (biotic resistance) alien plant invasions. Impacts of alien plants on primary consumers (herbivores and detritivores) also mediate bottom-up ecosystem impacts of invasion. These interactions are largely determined by trait matching of consumers and alien plants. I conducted a series of mesocosm experiments to elucidate interactions between *Crassula helmsii* and consumers, relative to the co-occurring native macrophytes *Callitriche stagnalis*, *Lythrum portula*, *Hypericum elodes* and *Potamogeton polygonifolius*. I also conducted trials with decomposed plant material to extend results to detritivores. In choice feeding trials, the great pond snail *Lymnaea stagnalis* exhibited an unstable preference for native macrophytes, whereas the wandering pond snail *Ampullaceana balthica* exhibited no clear preference, and the crustaceans *Asellus aquaticus* and *Crangonyx pseudogracilis* tended to prefer *C. helmsii* over native macrophytes. In growth trials, juvenile *L. stagnalis* growth did not differ significantly between *C. helmsii* and native macrophytes. Stoichiometric and phenolic defence analyses suggested that herbivore preference for *C. helmsii* or native macrophytes may relate to the varying abilities of different herbivore taxa to process phenolic defences. The results of these trials indicate that the impacts of *C. helmsii* on herbivores are likely to vary somewhat according to herbivore species identity and traits, and that depending on the herbivore species pool in the recipient ecosystem, herbivory may either help or hinder *C. helmsii* invasion.

3.2 Introduction

Invasive species (Colautti and MacIsaac, 2004) are considered one of the greatest threats to global freshwater biodiversity (Dudgeon, 2020; IPBES, 2023). Invasive alien macrophytes may cause particularly drastic impacts to recipient ecosystems due to the foundational role of macrophytes as ecosystem engineers and primary producers in freshwaters (Carpenter and Lodge, 1986; Lodge, 1991; Newman, 1991; Thomaz and Cunha, 2010). There is therefore much interest in determining the factors which dictate macrophyte invasion success, and the impacts of a successful invasion (Fleming and Dibble, 2015).

One factor dictating both macrophyte invasion success and recipient ecosystem impacts is herbivory. The alternative ways in which herbivores in recipient ecosystems can respond to invasion have been encapsulated by two countervailing hypotheses: enemy release and biotic resistance (Elton, 1958; Jeschke, 2014). The enemy release hypothesis postulates that the success of invasive species is promoted by escape from natural enemies such as pathogens, predators and herbivores (Keane and Crawley, 2002). Under the predictions of the enemy release hypothesis, an invading macrophyte would be freed from the pressures imposed by coevolved herbivores, and therefore thrive in its new environment (Xiong *et al.*, 2008). According to the biotic resistance hypothesis, invasions may be suppressed by interactions with native biota, particularly within species-rich recipient systems (Maron and Vilà, 2001). Under the predictions of the biotic resistance hypothesis, an invading macrophyte might be preferentially consumed by herbivores in the recipient ecosystem, thereby hindering successful invasion (Parker and Hay, 2005; Morrison and Hay, 2011a). These two hypotheses are not necessarily mutually exclusive: for instance, macrophyte invaders may escape specialist herbivores from their native range whilst encountering new generalist herbivores against which they have evolved no defences (Morrison and Hay, 2011a). Evidence for both theories is equivocal (Jeschke *et al.*, 2012), and either may apply to a given invasion, depending on environmental context and the species identity of the invader (Maron and Vilà, 2001). It seems likely that interactions between plant invaders and herbivores in the recipient ecosystem are determined more by the traits of the invader than by evolutionary novelty (Maron and Vilà, 2001; Grutters, Roijendijk, *et al.*, 2017). Herbivore preference may therefore act as a driver of plant invasion success, a barrier or, depending on context, both.

Alongside top-down impacts of herbivores on invasive macrophytes, invasive macrophytes may impact recipient ecosystems via their bottom-up effects on consumers (Erhard, Pohnert and Gross, 2007). They may displace more palatable native plants, or conversely provide an abundance of palatable tissue for recipient herbivore populations (Xiong *et al.*, 2008). Further, as invasive macrophytes senesce and die, their tissues enter the detrital food web. As with live macrophyte tissue, the detritus of invasive macrophytes may differ in its palatability to that of native macrophytes (Suren and Lake, 1989; Kornijów, Gulati and Ozimek, 1995). Abundant invasive macrophytes are likely to produce copious detritus upon senescence (Carter, Rybicki and Hammerschlag, 1991). Where the phenology of an invading macrophyte differs from that of the pre-existing flora, seasonal availability of macrophyte tissue to herbivores and detritivores may also be altered (Wolkovich and Cleland, 2011). Since herbivore and detritivore traits vary widely, consumption of invasive macrophytes is likely to differ according to consumer species identity and traits (Morrison and Hay, 2011a). If an alien consumer readily eats the tissues of the invasive macrophyte, the consumer's invasion may be facilitated (Engelkes and Mills, 2013; Thouvenot *et al.*, 2017).

Alien macrophyte invasion success, and the impacts of a successful invasion, are predominantly determined by the traits of the alien macrophyte (Larkin *et al.*, 2012; Carniatto *et al.*, 2013; Fleming and Dibble, 2015; Brouwer *et al.*, 2017). Fast growth rate, clonal reproduction, phenotypic plasticity and allelopathy may enable the invader to outcompete native macrophytes (Galatowitsch, Anderson and Ascher, 1999; Zedler and Kercher, 2004; Michelan *et al.*, 2018; Hussner *et al.*, 2021), whilst low nutritional quality or mechanical (e.g. spines, sclerophylly) and chemical (e.g. phenols, terpenoids) defences may mitigate the pressure of herbivory, and impact herbivore assemblage composition within the recipient ecosystem (Lindén and Lehtiniemi, 2005; Erhard, Pohnert and Gross, 2007; Morrison and Hay, 2011b; Grutters, Roijendijk, *et al.*, 2017). Upon senescence and death, the rate and direction of changes to nutritional quality and plant defences will determine the impacts of the alien macrophyte on detritivore assemblage composition (Suren and Lake, 1989; Newman, Kerfoot and Hanscom, 1990; Carvalho *et al.*, 2015).

Crassula helmsii is a semiaquatic succulent plant, originally from Australasia, which has become widespread in NW Europe since its naturalisation in the mid-20th Century (Smith and Buckley, 2020). *Crassula helmsii* typically invades small lentic waterbodies, which in NW Europe represent arguably the most significant repository of freshwater biodiversity (Williams *et al.*, 2004; Davies *et al.*, 2008). Its rapid spread across the region has therefore provoked widespread concern amongst conservationists and land managers (Dawson and Warman, 1987; van der Loop *et al.*, 2018). *Crassula helmsii* often forms dense stands in the margins of invaded waterbodies, which increase macrophyte standing crop (Dawson and Warman, 1987) and may displace native macrophyte species (Ewald, 2014). Alongside indirect effects on recipient biota, driven by alterations to physicochemistry and habitat structural complexity (Dawson and Warman, 1987; Diaz, 2012), alterations to macrophyte species composition and abundance following *C. helmsii* invasion could have considerable direct impacts on herbivores and detritivores (Hussner, 2009). Herbivore preference may also mediate interspecies competition between *C. helmsii* and native macrophytes, influencing the likelihood of *C. helmsii*'s successful establishment in a recipient system (Thouvenot *et al.*, 2017).

Research conducted to date suggests that *C. helmsii* may be relatively unpalatable to native herbivores. In a series of no-choice feeding trials investigating the palatability of macrophytes to the generalist molluscan herbivores *Lymnaea stagnalis* and *Pomacea canaliculata* (Grutters et al., 2017), consumption of *C. helmsii* was lower for both species than the mean consumption rate across 40 macrophytes tested. Chemical analyses conducted alongside these feeding trials suggested that *C. helmsii* may be unpalatable to herbivores due to its low nutritional quality (high C:N ratio) and relatively high concentration of phenolic defence compounds (low N:phenolics ratio) (Mithöfer and Boland, 2012).

Here, I expand upon this work using a combination of choice feeding trials, performance trials and plant trait analysis to investigate interactions between *C. helmsii* and co-occurring invertebrate consumers. For these investigations, four macroinvertebrate herbivores and detritivores were selected: the molluscan scrapers *Lymnaea stagnalis* and *Ampullaceana balthica* and the crustacean shredders *Asellus aquaticus* and *Crangonyx pseudogracilis*. *L. stagnalis*, *A. balthica* and *A. aquaticus* are native to NW Europe, whilst *C. pseudogracilis* originates in North America (Tattersall, 1937). All four co-occur frequently in lentic waterbodies across NW Europe, and are known to consume both live tissue and detritus from macrophytes (Tachet *et al.*, 2010). Using these trials, I aim to assess:

1. The relative preference of macroinvertebrate consumers for *Crassula helmsii* vs. the native macrophytes *Callitriche stagnalis, Lythrum portula* and *Hypericum elodes*, both live and as detritus.

2. The consistency of observed preferences across seasons.

3. The mechanistic bases for observed preferences, and factors responsible for any observed shifts in preference.

3.3 Methodology

3.3.1 Invertebrate culturing and plant collection

Cultures commenced with wild *Lymnaea stagnalis* and *Ampullaceana balthica* collected from Chilton Polden, Somerset (51°10′50″N , 002°52′50″W) and *Asellus aquaticus* and *Crangonyx pseudogracilis* collected from Forder Valley, Plymouth (50°24′02″N , 004°05′58″W). Cultures were maintained in a 20 (±1)°C temperature controlled wet laboratory under a 12:12 hr photoperiod, in aquaria filled with artificial pond water (OECD, 2004: pH 7.7±1, O_2 (%) 80±6). Animals were fed washed lettuce (*Lactuca sativa*) and carrot (*Daucus carotta*) *ad libitum*. Prior to use in feeding trials, animals were acclimated to laboratory conditions for a minimum of 30 days.

Plant material for feeding trials was collected as required from Cadover Bridge, Dartmoor (50°27'55"N 4°02'09"W). Plants were rinsed thoroughly to remove epiphytes prior to use in trials. Where material was required for detritus trials, collected plants were placed in fine-mesh litter bags (20x30cm, 700µm mesh, each bag with approx. 150cm^3 plant material) and immersed in the waterbody from which the macrophytes were collected for either 7 or 30 days.

3.3.2 Choice feeding trials

I conducted choice feeding trials to assess the relative palatability of *C. helmsii* compared with native macrophytes, using both live (intact) plants and reconstituted gel diets.

3.3.2.1 Live plant trials

In live plant trials, I used the native macrophytes *Callitriche stagnalis*, *Lythrum portula* and *Hypericum elodes* alongside *Crassula helmsii* (n=15 for each plant). Each replicate consisted of a trial tank containing a single *Lymnaea stagnalis* adult (mean shell height

27.05 mm, wet mass 1.45 g), and a no-snail control tank, used to account for potential autogenic change in plant mass during the trial. Snails were selected from culture aquaria, and their shell height and wet mass (WM) recorded. Snails were then placed into trial aquaria filled with fresh artificial pond water, and starved for 24 hrs. Apical fragments of two macrophyte species (one *C. helmsii,* one native) were blotted, weighed (approx. 0.25 g), tethered with tungsten putty and placed at either end of both trial and control aquaria. After 72 hrs, plant fragments and snails were removed from aquaria and freeze-dried to obtain dry plant (DM) and snail masses. Tissue was subsequently removed from freeze-dried snail shells using concentrated bleach so that snail tissue mass (ST) could be calculated. Relative consumption rate (RCR) was calculated as: Trial DM_0 – Trial DM_1/ST/Time(days), where Trial DM_0 was calculated as: Trial WM_0 x Control DM_1/Control WM_0 (Elger and Barrat-Segretain, 2002; Burlakova *et al.*, 2009; Grutters, Roijendijk, *et al.*, 2017). *Ampullaceana balthica, Asellus aquaticus* and *Crangonyx pseudogracilis* were not used in live plant trials, since pilot studies demonstrated that the relatively small amount of plant tissue consumed by these smaller consumers was obscured by autogenic changes in plant mass.

3.3.2.2 Gel diet trials

In reconstituted gel diet trials, diets were created from the native macrophytes *C. stagnalis* and *L. portula* alongside *C. helmsii* (Hay, Kappel and Fenical, 1994; Amsler *et al.*, 2005; Hargrave *et al.*, 2017; Pessarrodona, Foggo and Smale, 2019). Trials were conducted with single adults of *Lymnaea stagnalis* (mean shell height 26.53 mm; WM 1.85 g), *Ampullaceana balthica* (12.8 mm; WM 0.43 g), *Asellus aquaticus* (mean body length 8.15 mm) and *Crangonyx pseudogracilis* (mean body length 4.64 mm). Macrophytes were freeze-dried, pulverised using an electric coffee grinder (Silvercrest SKME 180 B1) and passed through a 250 µm sieve. Then, 0.9 g of the resulting fine powder was mixed with 0.9 g pulverised *Lactuca sativa* (gem lettuce, added to induce feeding) and 15 ml of distilled water. A separate solution of 0.8 g agar in 20 ml of water was prepared, heated to liquify the agar, and mixed with the macrophyte solution. This mixture was set over gridded tiles, consisting of 1 x 1 mm fiberglass mesh glued to microscope slides. Tiles were sandwiched between two glass panes separated by spacers to produce a uniform gel thickness across all tiles. Different spacers were used according to the typical consumption of the trial consumer species: 0.25 mm for *Asellus aquaticus*, *Crangonyx pseudogracilis* and *Ampullaceana balthica*, and 0.5 mm for *Lymnaea stagnalis*. To the same end, quarter microscope slides were used for crustacean trials, whilst for snail trials I used half tiles. All herbivores were therefore supplied with sufficient gel to permit *ad libitum* feeding on either diet during the trial.

In snail trials, animals were selected from culture aquaria, and their shell height and wet mass recorded. Animals were then placed singly into 1 l trial aquaria filled with fresh artificial pond water, and starved for 24 hrs. After 24 hrs, gel tiles from both *C. helmsii* and a native macrophyte were placed at opposite ends of trial aquaria, and left in situ for 48 hrs. Afterwards, gel tiles were removed from aquaria and illuminated from below under a Meiji EMZ-5 stereomicroscope at 10x magnification. Empty mesh squares were counted to quantify gel consumption. Where fewer than 10 squares had been consumed across both tiles (e.g. due to disease or mortality in the consumer), the replicate was discarded. In crustacean trials, animals were selected from culture aquaria, and their body length recorded, before being placed singly in petri dishes filled with 50 ml of fresh artificial pond water. Due to their relatively smaller size, crustacean consumption of gel diets was too low to be effectively quantified by counting empty mesh squares. Orientation was therefore used as a proxy for consumption in crustacean trials, recording the location of each animal at 2, 4, 6, 24, 26, 28, 30 and 48 hours. Orientation was classified as A: on trial tile A, B: on trial tile B, or C: on neither tile (Figure 3.1).

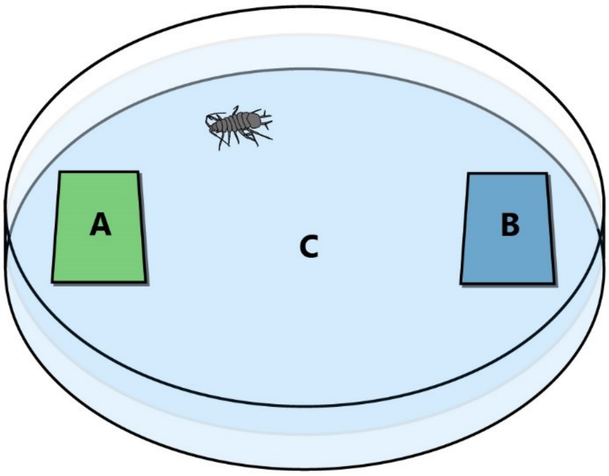

Figure 3.1. Design of orientation trials for the crustacean consumers *Asellus aquaticus* and *Crangonyx pseudogracilis*. **A**: on trial tile A, **B**: on trial tile B, or **C**: on neither tile

3.3.3 No-choice juvenile performance trials

A no-choice juvenile dietary performance trial was conducted to investigate the nutritional quality of *C. helmsii* to generalist aquatic herbivores. F1 juvenile *L. stagnalis* (2.6 ± 0.9 mm) were measured (shell height) using an ocular micrometer and placed individually in 1 l aquaria. The following day, apical fragments (0.2 ± 0.01 g) of freshly collected *Crassula helmsii*, *Callitriche stagnalis* or *Potamogeton polygonifolius* (n = 30: 10 for each macrophyte-snail combination) were placed into the trial aquaria. Every week subsequently, I replaced remnant macrophyte material with freshly collected apical fragments, and topped up water to account for evaporative loss. At 30 and 70 days, snails' shell height was again measured. Five snails were removed from the trial due variously to mortality and tank contamination. These snails were not included in subsequent analyses.

3.3.4 Macrophyte carbon: nitrogen ratios and phenolic defences

To assess carbon:nitrogen (C:N) ratios and phenolic defences, plants were freeze-dried, ground and passed through a 180 µm sieve. Resulting powders were analysed using Fourier-transform infrared spectroscopy (FTIR, Bruker, Massachusetts, USA, see Epstein, Foggo and Smale, 2019) to infer the relative concentration of phenolics within plant tissues, whilst C:N ratios were obtained using an elemental analyser (Elementar, Langensolbold, Germany).

3.3.5 Data analysis

Differences in consumption of fresh and reconstituted *C. helmsii* and native macrophytes in choice feeding trials were assessed using one sample t-tests. Preceding analysis, Shapiro-Wilks tests were used to ensure that differences were normally distributed, and where necessary, non-parametric Wilcoxon tests were substituted for t-tests. Orientation data from crustacean gel diet trials were converted to counts, wherein the total number of observations of animals on tile A or tile B throughout each trial were pooled, and 'C' observations (wherein animals were on neither tile) disregarded. Counts were then analysed using chi-squared tests. Significance thresholds were adjusted using the Šidák correction for multiple comparisons (Šidák, 1967).

To construct overall models assessing varying preference for *C. helmsii* amongst *L. stagnalis* (gel consumption), *A. balthica* (gel consumption), *A. aquaticus* and *C. pseudogracilis* (gel orientation), data were standardised as fractions of the total value for each trial replicate. Generalised linear mixed models with binomial error distribution were fitted to these transformed data, incorporating native macrophyte comparator and consumer species as fixed factors and plant decomposition state as a random effect. The effect of diet on the growth of juvenile *L. stagnalis* was assessed using linear models with diet (plant species) and timepoint as predictors. Model assumptions were checked graphically.

All analyses were conducted in the R computing environment (R Core Team, 2023). Linear models were fitted using lme4 (Bates *et al.*, 2015) and MASS (Venables and Ripley, 2002).

3.4 Results

3.4.1 Choice feeding trials

3.4.1.1 Live plants

In live plant choice feeding trials with *Lymnaea stagnalis* there was no significant difference in relative consumption rate (RCR) between *Crassula helmsii* and *Callitriche stagnalis* (one sample t-test, t(14)= 1.499, $p>0.05$), *Lythrum portula* (one sample t-test, t(14)=0.424, $p>0.05$) or *Hypericum elodes* (one sample t-test, t(14)=0.803, $p>0.05$), but in all three trials the native macrophyte was consumed more than *C. helmsii* (Figure 3.2). The mean RCR observed in these choice trials (0.83mg g^{-1} day^{-1} excl. negative values) was comparable to that reported in no-choice *L. stagnalis* feeding trials by Grutters *et al*. (2017) (3.3 mg g^{-1} day^{-1}), although they reported a mean RCR of 1.26 mg g^{-1} day^{-1} for *C. helmsii*, whilst in the trials reported here I observed a mean *C. helmsii* RCR of 0.08 mg g^{-1} day^{-1}, perhaps as a result of elevated growth of apical *C. helmsii* fragments in trial tanks. During trials, snails consumed between 0 and 34% of the initial mass provided of each macrophyte.

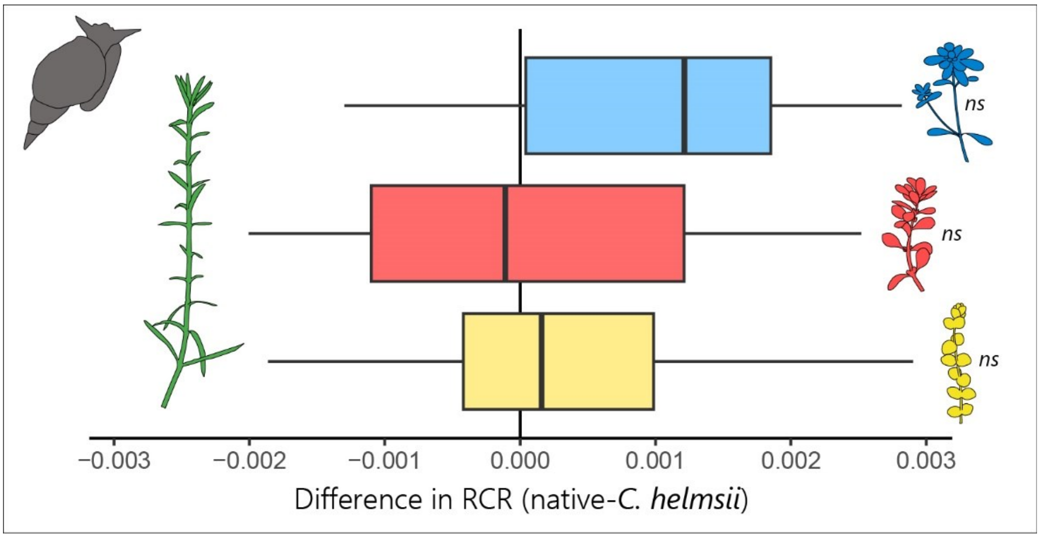

Figure 3.2. Results of live plant choice feeding trials with Lymnaea stagnalis, assessing relative consumption rate of *Crassula helmsii* ( ) vs. *Callitriche stagnalis* ( ), *Lythrum portula* ( ) and *Hypericum elodes* ( ). ns = $p > 0.05$.

3.4.1.2 Reconstituted gel diets

In gel diet choice trials (consumption or orientation), *C. helmsii* preference varied significantly by native macrophyte comparator and consumer species (Table 3.1).

Table 3.1. Generalised linear mixed model with binomial error distribution assessing preference of the macroinvertebrate consumers *Lymnaea stagnalis*, *Ampullaceana balthica*, *Asellus aquaticus* and *Crangonyx pseudogracilis* for *Crassula helmsii* versus native macrophytes. Native macrophyte and consumer species were incorporated as fixed factors, with plant decomposition state as a random effect. Type III GLM fits tested by Wald chi-sq.

	df	X^2	p
Native macrophyte (NM)	1	13.049	<0.001
Consumer	3	9.249	<0.05
NM : consumer	3	0.574	0.574

In gel diet consumption trials, there was no significant difference in consumption of *C. helmsii* vs. *C. stagnalis*, at any stage of conditioning, by either *Lymnaea stagnalis or Ampullaceana balthica*. Both *L. stagnalis* and *A. balthica* consumed significantly more *L. portula* than *C. helmsii* where diets were made from fresh plants. There was no significant difference in consumption of d7 *C. helmsii* vs. *L. portula* nor d30 *C. helmsii* vs. *L. portula* by *L. stagnalis* or *A. balthica*. *L. stagnalis* consumed more of native than *C. helmsii* in four of the six inter-plant gel trials, whilst *A. balthica* consumed more of the native in three trials, and more *C. helmsii* in three trials (Figure 3.3, Table 3.2).

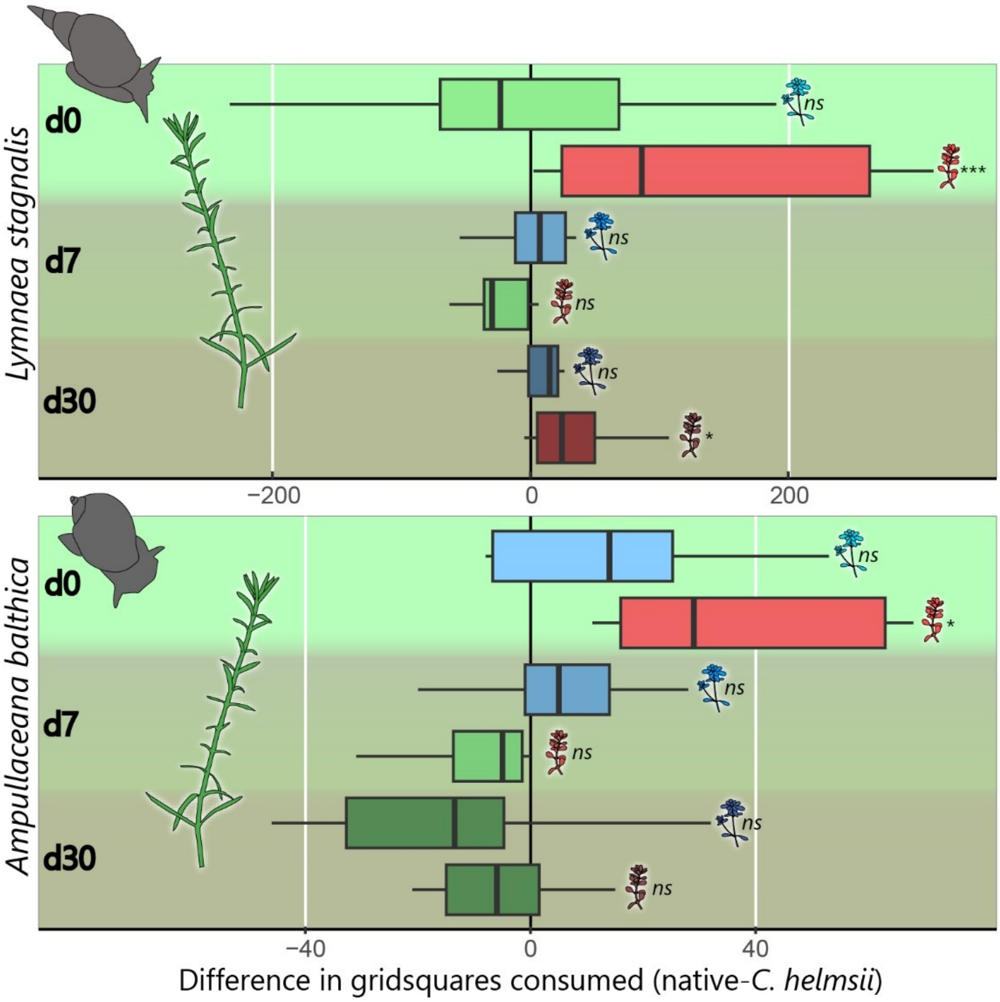

Figure 3.3. Results of choice feeding trials assessing consumption by *Lymnaea stagnalis* and *Ampullaceana balthica* of *Crassula helmsii* (🌿) vs. *Callitriche stagnalis* (✿) and *Lythrum portula* (✿) gel diets, made with fresh plants (d0) and d7/d30-conditioned detritus

Table 3.2. Consumption of reconstituted gel diets made from *Crassula helmsii* vs. native macrophytes by molluscan consumers *Lymnaea stagnalis* and *Ampullaceana balthica*. Šidák correction applied to p-value significance thresholds.

Consumer	**Plant**	**Mean consumption**	**SE**	**Mean difference**	**Test**
Lymnaea stagnalis	*C. helmsii*	73.2	18.7	+ 15.3	*t*-test, t(19) = -0.536^{ns}
	C. stagnalis	57.9	18.1		
	C. helmsii	31	5.8	- 139.3	Wilcoxon test, v = 190, n = 20***
	L. portula	170.3	31.9		
	d7 *C. helmsii*	27.1	10.4	- 0.7	*t*-test, t(9) = 0.073^{ns}
	d7 *C. stagnalis*	27.8	6.6		
	d7 *C. helmsii*	44.4	9.8	+ 25	*t*-test, t(9) = 0.073^{ns}
	d7 *L. portula*	19.4	3.7		
	d30 *C. helmsii*	40.5	7.0	- 11.5	*t*-test, t(9) = 1.380^{ns}
	d30 *C. stagnalis*	52	9.5		
	d30 *C. helmsii*	34.3	6.7	- 36.6	*t*-test, t(9) = 2.791^{ns}
	d30 *L. portula*	70.9	14.7		
Ampullaceana balthica	*C. helmsii*	26	12.1	- 1.9	*t*-test, t(9) = 0.143^{ns}
	C. stagnalis	27.9	4.6		
	C. helmsii	5.2	1.21	- 37.9	*t*-test, t(9) = 4.763*
	L. portula	43.1	7.8		
	d7 *C. helmsii*	13.9	3.5	- 6.4	*t*-test, t(8) = 1.277^{ns}
	d7 *C. stagnalis*	20.3	5.2		
	d7 *C. helmsii*	23	14.3	+ 10.3	*t*-test, t(3) = -1.439^{ns}
	d7 *L. portula*	12.8	7.5		
	d30 *C. helmsii*	34.1	6.8	+ 12.9	*t*-test, t(9) = -1.599^{ns}
	d30 *C. stagnalis*	21.2	5.5		
	d30 *C. helmsii*	15.3	3.3	+ 5.6	*t*-test, t(6) = -1.173^{ns}
	d30 *L. portula*	9.7	3.2		

ns p >0.05   * p < 0.05   ** p < 0.01   *** p < 0.001

In gel diet orientation trials, *Asellus aquaticus* and *C. pseudogracilis* were observed significantly more on tiles made from fresh and 30-day conditioned *C. helmsii* than those made from d0/d30 *C. stagnalis*, but exhibited no significant preference for either plant after 7 days of conditioning. *Asellus aquaticus* was observed significantly more on fresh and 7-day conditioned *C. helmsii* than d0/d7 *L. portula*, but exhibited no significant preference for either plant after 30 days of conditioning. There was no significant difference in the orientation of *C. pseudogracilis* on *C. helmsii* vs. *L. portula* at any stage of conditioning. *C. helmsii* was preferred in five of six orientation trials by *A. aquaticus*, and four of six orientation trials by *C. pseudogracilis* (Figure 3.4, Table 3.3).

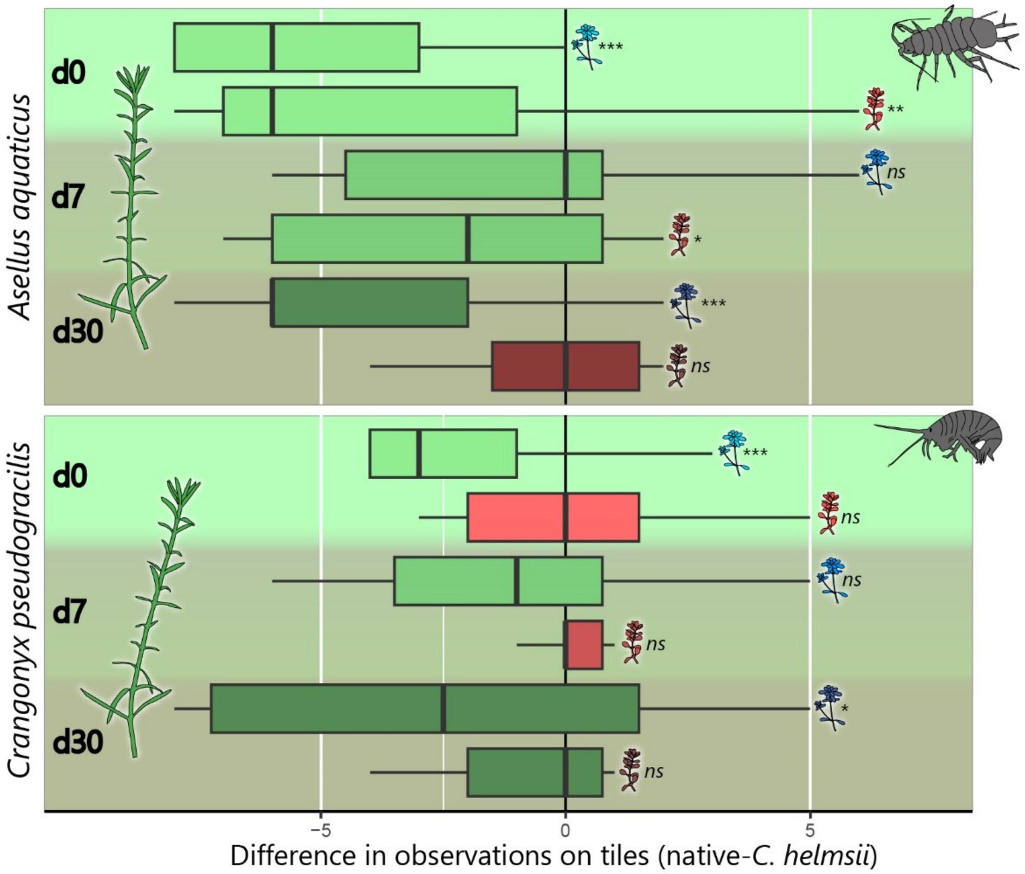

Figure 3.4. Results of choice feeding trials assessing orientation of *Asellus aquaticus* and *Crangonyx pseudogracilis* on *Crassula helmsii* (🌿) vs. *Callitriche stagnalis* (✿) and *Lythrum portula* (✿) gel diets, made with fresh plants (d0) and d7/d30-conditioned detritus

Table 3.3. Orientation in relation to reconstituted gel diets made from *Crassula helmsii* vs. *native macrophytes* by crustacean consumers *Asellus aquaticus* and *Crangonyx pseudogracilis*. Šidák correction applied to p-value significance thresholds.

Consumer	**Plant**	**Mean observations (consumer on tile)**	**SE**	**Mean difference**	**Test**
A. aquaticus	*C. helmsii*	5.7	0.55	+ 4.5	X^2 (1, n=10) = 28.7 ***
	C. stagnalis	1.2	0..55		
	C. helmsii	6.1	0.83	+ 3.7	X^2 (1, n=10) = 12.8 **
	L. portula	2.4	0.86		
	d7 *C. helmsii*	3.4	0.64	+ 0.9	X^2 (1, n=10) = 1.37 ^{ns}
	d7 *C. stagnalis*	2.5	0.62		
	d7 *C. helmsii*	4.1	0.75	+ 2.4	X^2 (1, n=10) = 9.93 *
	d7 *L. portula*	1.7	0.56		
	d30 *C. helmsii*	6.1	0.55	+ 4.4	X^2 (1, n=10) = 24.8 ***
	d30 *C. stagnalis*	1.7	0.5		
	d30 *C. helmsii*	2.8	0.7	- 1.0	X^2 (1, n=10) = 1.52 ^{ns}
	d30 *L. portula*	3.8	0.83		
C. pseudogracilis	*C. helmsii*	2.9	0.52	+ 2.7	X^2 (1, n=10) = 14.23 ***
	C. stagnalis	0.5	0.34		
	C. helmsii	1.3	0.47	- 0.4	X^2 (1, n=10) = 0.53 ^{ns}
	L. portula	1.7	0.7		
	d7 *C. helmsii*	2.8	0.59	+ 1.2	X^2 (1, n=10) = 3.27 ^{ns}
	d7 *C. stagnalis*	1.6	0.52		
	d7 *C. helmsii*	0.5	0.17	- 0.2	X^2 (1, n=10) = 0.33 ^{ns}
	d7 *L. portula*	0.7	0.15		
	d30 *C. helmsii*	4.7	0.90	+ 2.4	X^2 (1, n=10) = 8.23 *
	d30 *C. stagnalis*	2.3	0.73		
	d30 *C. helmsii*	1.3	0.52	+ 0.5	X^2 (1, n=10) = 1.19 ^{ns}
	d30 *L. portula*	0.8	0.49		

I observed differences in the direction of consumption by *L. stagnalis* of gel diets made from plants collected in winter (January-March 2022) and summer (June-October 2022). *L. stagnalis* consumed significantly more winter-collected *C. stagnalis* than *C. helmsii*, but significantly more summer-collected *C. helmsii* than *C. stagnalis*. *Lymnaea stagnalis* consumed significantly more *L. portula* than *C. helmsii* in summer but exhibited no significant preference between winter-collected plants (Figure 3.5, Table 3.4).

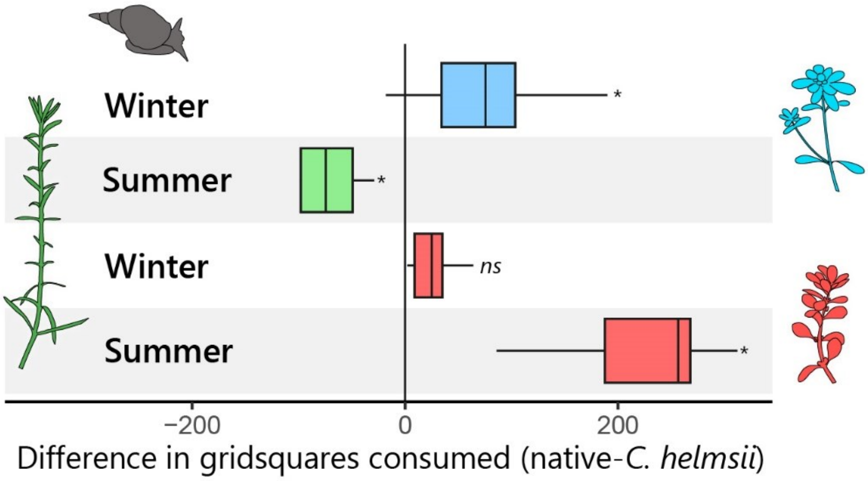

Figure 3.5. Results of choice feeding trials assessing consumption by *Lymnaea stagnalis* of *Crassula helmsii* () vs. *Callitriche stagnalis* () and *Lythrum portula* () gel diets, made with plants collected in winter and summer.

Table 3.4. Variation in consumption of *Crassula helmsii* vs. native macrophytes collected in summer and winter by *Lymnaea stagnalis*. Šidák correction applied to p-value significance thresholds.

Season	**Plant**	**Mean consumption**	**SE**	**Mean difference**	**Test**
Winter	*C. helmsii*	32.2	9.0	- 78.4	*t*-test, t(9) = 3.609*
	C. stagnalis	110.6	27.8		
Summer	*C. helmsii*	114.2	32.1	+ 109	Wilcoxon test, v = 0, n = 10*
	C. stagnalis	5.2	1.6		
Winter	*C. helmsii*	19.0	7.1	- 55.9	Wilcoxon test, v = 45, n = 10^{ns}
	L. portula	74.9	33.6		
Summer	*C. helmsii*	41.8	7.8	- 214.3	Wilcoxon test, v = 55, n = 10*
	L. portula	256.1	35.0		

In intra-plant comparisons, gel diets made from fresh *C. helmsii* were consumed significantly more by *L. stagnalis* than diets made from *C. helmsii* conditioned for 7 days, but there was no significant difference in consumption of fresh vs. 30-day conditioned *C. helmsii* diets. (Figure 3.6, Table 3.5). There were no significant differences in consumption between fresh and conditioned *C. stagnalis* gels.

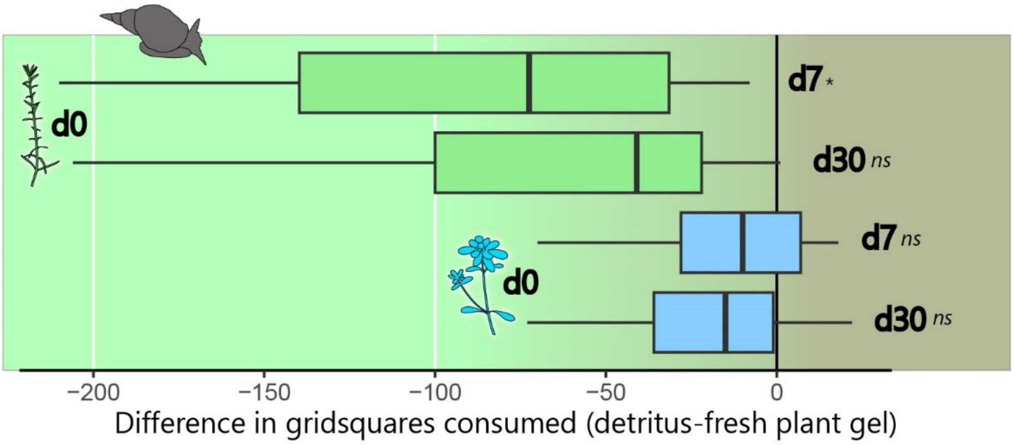

Figure 3.6. Results of choice feeding trials assessing preference of *Lymnaea stagnalis* for gel diets made with freshly collected *Crassula helmsii* (🌿) and *Callitriche stagnalis* (✿) vs. gels made with d7/d30-conditioned detritus.

Table 3.5. Consumption of fresh vs. 7/30-day conditioned *C. helmsii* and *C. stagnalis* by *Lymnaea stagnalis*. Šidák correction applied to p-value significance thresholds.

Plant	**Conditioning stage**	**Mean consumption**	**SE**	**Mean difference**	**Test**
C. helmsii	Fresh	164.7	24.3	+ 93.7	*t*-test, t(9) = -4.076*
	7 days	71.0	71.0		
	Fresh	96.4	29.3	+ 69.0	*t*-test, t(8) = -3.147^{ns}
	30 days	27.4	9.7		
C. stagnalis	Fresh	62.4	21.1	+ 26.0	Wilcoxon test, v = 14, n = 9^{ns}
	7 days	36.4	5.9		
	Fresh	50.8	16.8	+ 21.2	*t*-test, t(8) = -2.025^{ns}
	30 days	29.6	8.1		

3.4.2 No-choice juvenile performance trials

I observed no significant differences in growth between *L. stagnalis* juveniles reared on *C. helmsii*, *C. stagnalis* and *P. polygonifolius* (LM, $F_{2,69} = 0.22$, $p > 0.05$, Figure 3.7).

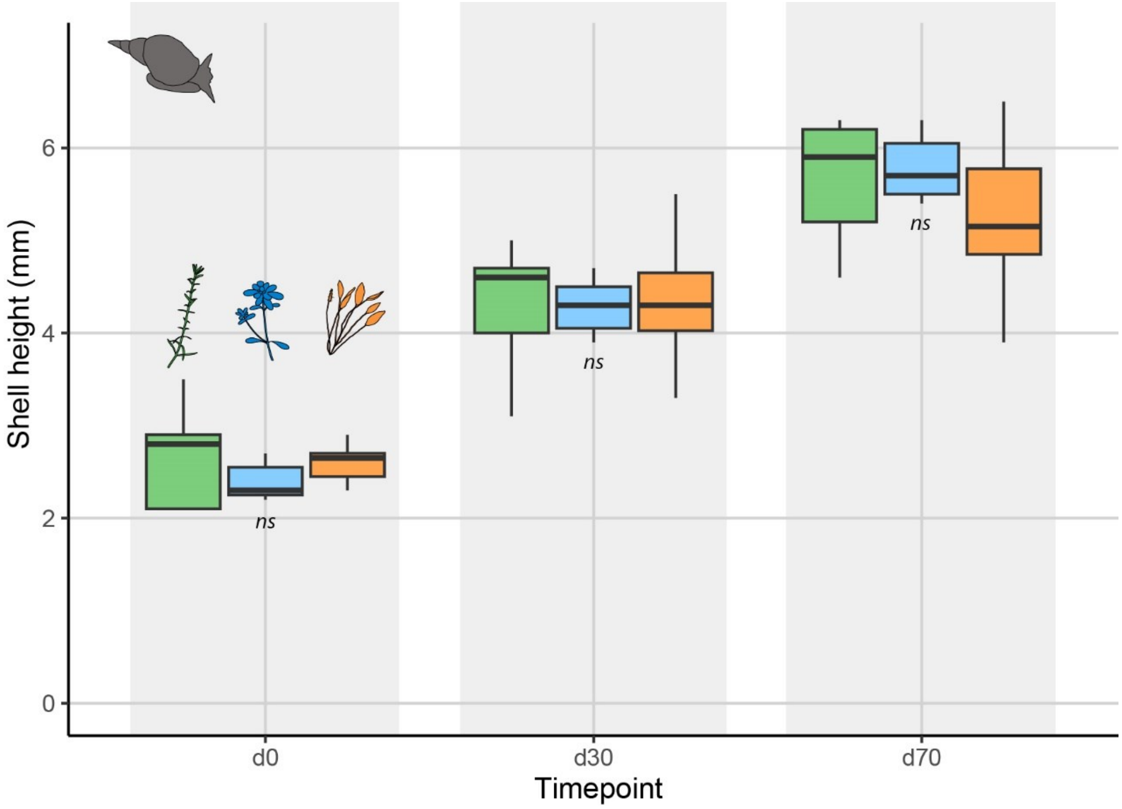

Figure 3.7. Results of performance trial assessing growth (shell height) of juvenile *Lymnaea stagnalis* reared on apical fragments of *Crassula helmsii* (🌿), *Callitriche stagnalis* (✿) or *Potamogeton polygonifolius* (🌾), measured at 0, 30 and 70 days.

3.4.3 Macrophyte carbon: nitrogen ratios and phenolic defences

FTIR spectra for *C. helmsii*, *C. stagnalis* and *L. portula* exhibited peaks at 3400-3200cm^{-1}, indicating the presence of phenolic compounds (Coates, 2006). Phenolics concentrations appeared to decline during decomposition of *C. helmsii, C. stagnalis* and *L. portula*. Spectra suggested that *C. helmsii* samples had consistently lower phenolic concentrations than *L. portula* and *C. stagnalis* (Figure 3.8).

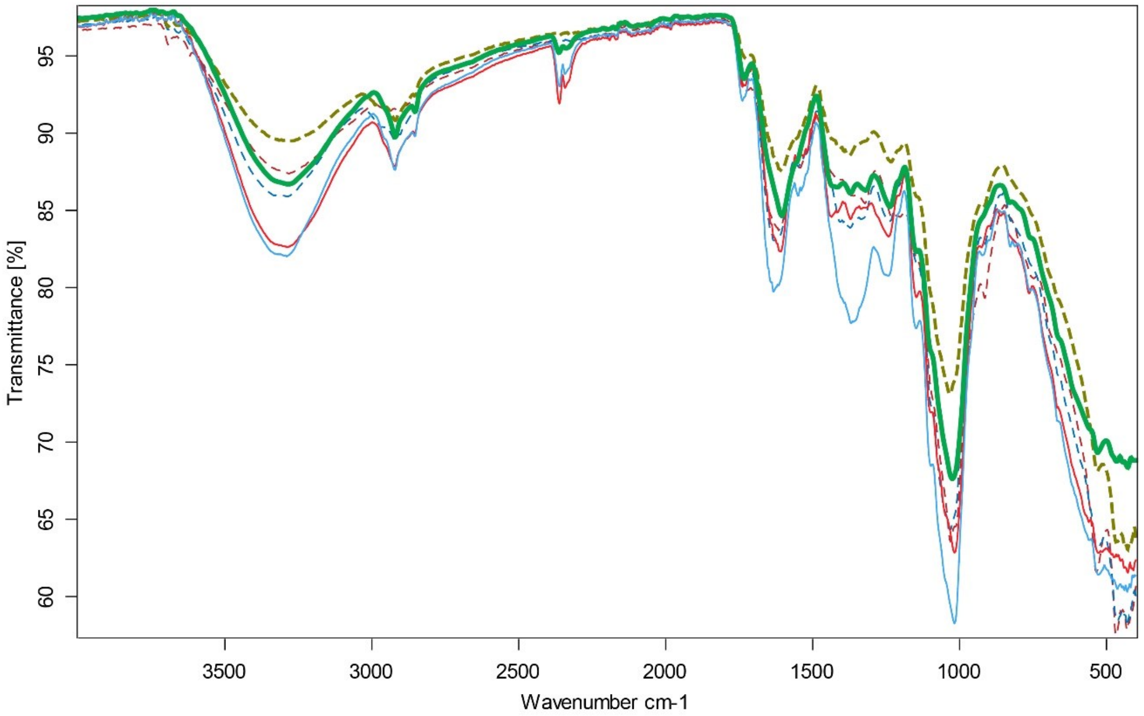

Figure 3.8. Fourier-transform infrared spectroscopy (FTIR) spectra of fresh (d0: solid line) and conditioned (d30: dashed line) *Crassula helmsii* (▬), *Callitriche stagnalis* (—) and *Lythrum portula* (—).

Carbon:nitrogen ratios declined during conditioning of plant material used in trials (d0>d7>d30). *C. helmsii* material had a higher C:N ratio than native macrophytes *C. stagnalis* and *L. portula* at each stage of conditioning (Figure 3.9).

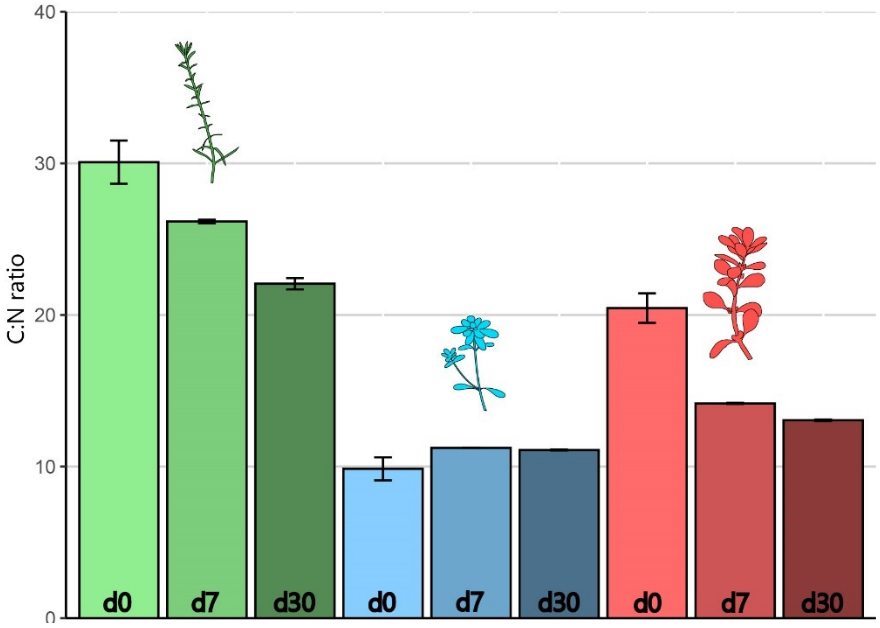

Figure 3.9. Carbon : nitrogen (C:N) ratio of fresh (d0) and conditioned (d7/d30) *Crassula helmsii* (🌿), *Callitriche stagnalis* (🌿) and *Lythrum portula* (🌿).

3.5 Discussion

Feeding trials revealed divergent preferences for *Crassula helmsii* vs. native macrophytes between macroinvertebrate consumer species, somewhat modulated by season and macrophyte decomposition. In choice feeding trials with live plants and gel diets, *Lymnaea stagnalis* exhibited an unstable preference for the native macrophytes *Callitriche stagnalis* and *Lythrum portula* over *C. helmsii*. In gel diet trials, *Ampullaceana balthica* did not demonstrate an overall preference for *C. helmsii* or either native macrophyte, although fresh *L. portula* gels were preferred over gels made from *C. helmsii*. Both *Asellus aquaticus* and *Crangonyx pseudogracilis* exhibited a general preference for *C. helmsii* over native macrophytes. In intra-plant trials, *L. stagnalis* exhibited a preference for fresh- over decomposed *C. helmsii*. In seasonality trials, preference of *L. stagnalis* for *C. helmsii* vs. *C. stagnalis* varied according to the season in which plant material was collected. In juvenile performance trials, growth rates did not differ significantly between *L. stagnalis* reared on *C. helmsii*, *C. stagnalis* or *Potamogeton polygonifolius*.

Fresh *Crassula helmsii* had a higher carbon:nitrogen (C:N) ratio than both native macrophytes included in assays and *L. portula* had a higher C:N ratio than *C. stagnalis*. Since nitrogen is generally a limiting nutrient for herbivores (Mattson, 1980; Bakker and Nolet, 2014; Bakker *et al.*, 2016; Grutters, Gross and Bakker, 2016), these results indicate that live *C. helmsii* is of low nutritional quality by comparison to *C. stagnalis* and *L. portula*. FTIR spectra indicated that phenolic concentrations were highest in *C. stagnalis*, followed by *L. portula* and then *C. helmsii*. Phenolics are ubiquitous allelochemicals in plants, widely associated with defence against herbivory (Lodge, 1991; Mithöfer and Boland, 2012). These results therefore suggest that *C. helmsii* is poorly defended compared with native macrophytes. However, other plant secondary metabolites were unassessed, which may compensate for lower phenolic concentrations in *C. helmsii* tissues (Grutters, Saccomanno, *et al.*, 2017). Together, assays indicate that live *C. helmsii* is of poor nutritional quality, but perhaps poorly defended, at least in comparison to the native macrophytes tested. Plant defences typically arise at the expense of growth rate, and *C. helmsii*'s relatively weak phenolic defences likely reflect a trade-off which permits the invasive plant's rapid growth (Coley, Bryant and Chapin, 1985; Dawson and Warman, 1987; Hussner, 2009; Smith and Buckley, 2020).

Carbon: nitrogen ratios declined as *C. helmsii* and *L. portula* tissues senesced, but remained comparatively stable in *C. stagnalis.* As a result, differences in nutritional quality between *C. helmsii* and *C. stagnalis* were likely reduced during decomposition. Phenolic concentrations appeared to decline evenly across the three macrophyte species during decomposition. Litter nutrient flux during decomposition frequently varies according to plant species identity (Longhi, Bartoli and Viaroli, 2008; Tiegs *et al.*, 2013; Carvalho *et al.*, 2015). The nutrient profile of macrophyte detritus in my assays is likely to have been influenced by colonisation by microbial communities, which may uptake nutrients including nitrogen and immobilise them within the litter. As a rule of thumb, nitrogen is released from detritus where C:N < 20, and immobilised where C:N > 20, as observed in these assays (Longhi, Bartoli and Viaroli, 2008).

Macrophyte-herbivore interactions are likely to be determined by matching between macrophyte traits and herbivore habits, physiology and feeding mechanisms (Carmona, Lajeunesse and Johnson, 2011; Pearse *et al.*, 2013). In the trials reported here, differences in preference for *C. helmsii* vs. *C. stagnalis* and *L. portula* may have resulted from differences in tolerance to chemical (phenolic) and mechanical defences between consumers. The digestive physiology of the crustaceans *A. aquaticus* and *C. pseudogracilis* may be maladapted to process defensive phenolics, resulting in the preference I observed for *C. helmsii* over native macrophytes in these species, despite *C. helmsii*'s relatively low nutritional quality. By contrast, *L. stagnalis* may be more tolerant of chemical defences and therefore prefers *C. stagnalis* and *L. portula* due to their higher nutritional quality, despite their relatively strong phenolic defences. In intra-species trials, however, *L. stagnalis* preferred fresh *C. helmsii* to decomposed *C. helmsii*, despite the higher nutritional quality and lower phenolic concentration of decayed material. Mechanical defences such as trichomes, mineral secretion and schlerophylly may also play a role in determining herbivore interactions with *C. helmsii* and native macrophytes (Hanley *et al.*, 2007). If pulverisation into gel diets left some mechanical defences (e.g. silicates) intact, reversal of preferences for *C. helmsii* gels vs. native macrophyte gels between *L. stagnalis* and *A. aquaticus/C. pseudogracilis* might be explained by differences in feeding mode – and therefore ease of processing structural defences - between the scraper *L. stagnalis* and the shredders *A. aquaticus/C. pseudogracilis*.

Herbivore preference can be unstable. For instance, preference may vary according to seasonal or ontogenetic variation in macrophyte palatability (Hanley *et al.*, 2007). In my trials, *Lymnaea stagnalis* demonstrated a stable preference for *L. portula* over *C. helmsii* in both summer and winter, but preferred *C. stagnalis* over *C. helmsii* where plants were collected in winter, and *C. helmsii* over *C. stagnalis* in summer-collected plants. The reversal in *L. stagnalis*' preference for *C. helmsii* vs. *C. stagnalis* may relate to ontogenetic changes in plant nutritional quality and defences. In the waterbody from which trial plants were collected (Devon, UK), *Crassula helmsii* grows most profusely in spring/summer, whereas *C. stagnalis* tends to produce stands in autumn and winter. *Lymnaea stagnalis'* preference therefore correlates with the production of new growth, which appears to be more palatable than mature tissue (Elger, Barrat-Segretain and Willby, 2006). Seasonal variation in preference was not tested for *Asellus aquaticus* or *Crangonyx pseudogracilis*, but trials for both species were conducted with summer-collected plants, so their general preference for *C. helmsii* may similarly be (partially) explained by macrophyte phenology.

Several plant traits which might exert an influence on herbivore preference were not directly examined in these trials, including olfactory cues and the alignment of a plant's growing cycle with herbivore phenology (Pearse *et al.*, 2013). In freshwaters, epiphytes often make a contribution to overall primary productivity comparable to that of the macrophytes upon which they grow (Sheldon and Boylen, 1975; Cattaneo and Kalff, 1980). Macrophytes can therefore indirectly effect herbivores via the abundance and composition of the epiflora which they support. This varies according to plant traits such as architecture, growth rate and stand density (Carpenter and Lodge, 1986; Grutters, Gross, *et al.*, 2017). In the trials reported here, plant material was washed to remove epiphytes, but any remaining epiphyton is likely to have influenced apparent herbivore preference for *C. helmsii* vs. native plants, as well as CHN/FTIR results. In addition to traits related to palatability, herbivore preference may also be altered indirectly by the volume of predator-free refugia available within macrophyte stands, as dictated by macrophyte growth form and architecture (Pearse *et al.*, 2013; Grutters *et al.*, 2015; Tasker, Foggo and Bilton, 2022).

Although growth rates of juvenile *L. stagnalis* were comparable between those reared on *C. helmsii* and those reared on native macrophytes, choice feeding trials with adults suggest that native macrophytes are preferred by this snail. By contrast, *A. aquaticus* and *C. pseudogracilis* exhibited a general preference for *C. helmsii* over native macrophytes.

These results suggest that *C. helmsii* invasion is likely to have differential impacts on different herbivorous and detritivorous taxa, perhaps varying according to feeding mode or tolerance to allelochemicals. The preference of the alien amphipod *C. pseudogracilis* for *C. helmsii* suggests that *C. helmsii* invasion may facilitate invasion by this species. Note that all 4 consumers used in these trials are considered generalists, and impacts of *C. helmsii* may differ considerably for specialists (e.g. aquatic Lepidoptera, phytophagous aquatic Coleoptera) (Morrison and Hay, 2011a; Grutters, Gross and Bakker, 2016). Irrespective of consumer species identity and tissue palatability, the rapid growth rates often attained by *C. helmsii* may result in elevated consumer abundance due simply to elevated primary production, with absolute consumption increasing despite the percentage of primary production consumed by herbivores and detritivores remaining the same or declining (Cebrian and Lartigue, 2004).

Historically, the role of herbivory has been somewhat overlooked within freshwaters, but recent research suggests that rates of herbivory in freshwaters often exceed those in terrestrial systems (Bakker *et al.*, 2016; Wood *et al.*, 2016). Herbivores may structure macrophyte assemblages both directly (macrophyte consumption) and indirectly (peri-/epiphyte consumption, fragmentation and propagule transport, bioturbation) (Bakker *et al.*, 2016). Concurrent with bottom-up impacts on the macroinvertebrate assemblage by *C. helmsii*, my results suggest that the macroinvertebrate assemblage may exert a direct top-down influence on *C. helmsii*, varying according to the identity of the herbivores present in the recipient ecosystem. Herbivores may either suppress *C. helmsii* by consuming its tissues (biotic resistance) or promote its competitive success by preferentially consuming native macrophytes (enemy release) (Parker and Hay, 2005; Xiong *et al.*, 2008; Morrison and Hay, 2011a). Counterintuitively, the activity of macroinvertebrate shredders such as caddisfly larvae may at times indirectly *promote C.*

helmsii invasion, by fragmenting plants and creating vegetative propagules (Crane *et al.*, 2021; Tasker and Bilton, 2023). Grazing on epiphyton represents another significant indirect interaction between herbivores and macrophytes, overlooked by this study (Cattaneo and Kalff, 1980). Epiphyton herbivory is thought to often exceed direct grazing on live macrophytes (Brönmark, 1989), and could promote *C. helmsii* invasion by permitting greater plant growth if *C. helmsii* were to host a more palatable epiphyte assemblage (Jones and Sayer, 2003; Strimaitis and Sheldon, 2011).

3.5.1 Conclusion

The results of these feeding trials provide no conclusive evidence for the occurrence of enemy release or biotic resistance in herbivore interactions with *Crassula helmsii*. The unstable - and sometimes opposing - preferences exhibited by snail and crustacean consumers in trials suggest that plant traits are not a consistent predictor of herbivore preference in this case (Grutters, Roijendijk, *et al.*, 2017), but rather that trait matching dictates herbivore preference (Pearse *et al.*, 2013). Depending on the herbivore species pool within the recipient ecosystem, herbivory may hinder or promote *C. helmsii* invasion success. By the same token, *C. helmsii* invasion may alter herbivore assemblage composition in recipient ecosystems via differential provision of food.

Chapter 4: Litter decomposition field experiment suggests detritus may mediate impacts of *Crassula helmsii* on macroinvertebrates

4.1 Abstract

Aquatic plants are generally consumed more as detritus than when alive, and their decomposition influences nutrient cycling and energy flows in many freshwater environments. Because of the high growth rates often achieved by invasive alien macrophytes, their establishment in recipient ecosystems is likely to strongly alter the abundance and composition of litter entering detrital pathways, representing a significant - but largely overlooked – facet of the ecological impacts of alien macrophyte invasion. I conducted a litterbag experiment to investigate decomposition of litter from the invasive alien macrophyte *Crassula helmsii* (New Zealand pygmyweed) against native plant *Callitriche stagnalis* (pond water-starwort), and assess interactions of recipient macroinvertebrate detritivores with *C. helmsii* litter. Alien *C. helmsii* litter decomposed more slowly, and was ultimately colonised by more abundant macroinvertebrates. Macroinvertebrate assemblage composition was comparable between macrophyte species, but shifted during the experiment from taxa and functional traits associated with coarse particulate organic matter (non-native shredder *Crangonyx pseudogracilis*) towards those associated with finer detritus (suspension feeding *Pisidium casertanum*). The results of this trial indicate that *C. helmsii* invasion may impact macroinvertebrate assemblages via production of long-lasting and comparably palatable detritus, which, given the density often attained by invading *C. helmsii*, may be available in great abundance.

4.2 Introduction

Invasive species are proliferating worldwide, aided by human vectors of dispersal and anthropogenic change to recipient ecosystems (Seebens *et al.*, 2017). Establishing the factors which govern the ecological impacts of invasion is a key research goal in invasion biology and could enable problematic invasions to be pre-empted and acted against (Simberloff, Parker and Windle, 2005; Simberloff *et al.*, 2013). Self-evidently, interactions between invasive species and recipient biota are key determinants of both ecosystem resilience to invasions, and the impacts of successful invasion (Maron and Vilà, 2001; Simberloff *et al.*, 2013). Because of the foundational role of macrophytes (macroscopic green plants and macroalgae) in terrestrial, freshwater and marine ecosystems, macrophyte invasions may cause far-reaching impacts on recipient ecosystems (Vilà *et al.*, 2011; Maggi *et al.*, 2015; Tasker, Foggo and Bilton, 2022). In freshwaters, much attention has been paid to determining the rules governing interactions between alien macrophytes and recipient herbivores, mostly due to herbivory's potential role in fostering ecosystem resilience through biotic resistance, wherein invasions may be suppressed by interactions with native biota (Parker and Hay, 2005; Morrison and Hay, 2011a; Grutters, Roijendijk, *et al.*, 2017; Oliveira *et al.*, 2019). By comparison, very little research focuses on interactions between alien macrophytes and detritivores (but see Cuassolo et al. 2020; Dekanová et al. 2021). Whilst detritivory clearly cannot play a direct role in biotic resistance, it may be instrumental to the wider ecosystem impacts of invasive macrophytes. Aquatic macrophytes are generally more frequently consumed as detritus than whilst alive, and macrophyte litter decomposition (conducted in part by detritivores) strongly influences freshwater nutrient cycling and energy flows (Newman, 1991; Shilla *et al.*, 2006; Bakker *et al.*, 2016; Dekanová *et al.*, 2021; Thornhill *et al.*, 2021).

Impacts of a macrophyte invader on detritivores are therefore likely to be more significant than impacts on herbivores in determining the invasion's impacts on the recipient ecosystem.

The freshwater decomposition of vascular plant detritus can be thought of as a 3-part process, consisting of 1) leaching; 2) microbial decomposition and 3) mechanical/invertebrate fragmentation (Webster and Benfield, 1986). Leaching of water-soluble compounds leads to considerable early mass loss (Gessner, Chauvet and Dobson, 1999; Pope, Gordon and Kaushik, 1999; Carvalho *et al.*, 2015), concurrent with the beginnings of colonisation by microbes (e.g. bacteria, hyphomycete fungi) and fragmentation of detritus by invertebrate detritivores and/or mechanical action (Webster and Benfield, 1986; Santonja, Pellan and Piscart, 2018). The latter two processes act in a positive feedback loop: microorganisms 'condition' the detritus, making it softer and more palatable for detritivores. In addition, microbial colonisation can increase the nutritional quality of material ingested by detritivores. Resulting detritivore fragmentation increases the surface area of the detritus, promoting further microbial colonisation and decomposition (Newman, 1991; Longhi, Bartoli and Viaroli, 2008; Anderson, Pond and Mayor, 2016). The rate of decomposition is influenced by detritivore assemblage composition (Jonsson and Malmqvist, 2000; Gessner *et al.*, 2010) as well as physicochemical factors including temperature, pH, oxygen concentration and waterbody trophic state (Webster and Benfield, 1986).

Although freshwater litter decomposition experiments have played an important role in the development of biodiversity-ecosystem function paradigms, the majority of research has focused on allochthonous sources of litter, and especially on woody plant leaves in lotic systems (Petersen and Cummins, 1974; Wallace and Webster, 1996; Jonsson and

Malmqvist, 2000; Gessner *et al.*, 2010; Handa *et al.*, 2014). Given the markedly different properties of aquatic plant tissues, aquatic plant decomposition is likely to follow a different trajectory to decomposition of allochthonous woody litter, perhaps also driven in part by differences in the composition of associated detritivores (Cebrian and Lartigue, 2004; Carvalho *et al.*, 2015; Bakker *et al.*, 2016). Divergence in decomposition processes is likely to be particularly strong in small lentic waterbodies, where wave action and flow are insignificant and the detritivore species pool differs markedly from that in lotic systems (Webster and Benfield, 1986; Pope, Gordon and Kaushik, 1999; Santonja, Pellan and Piscart, 2018). Autochthonous aquatic plant litter production is also likely to significantly exceed allochthonous terrestrial litter supply in most small lentic waterbodies, and so make a greater contribution to nutrient and energy flows (Wetzel, 1992).

Crassula helmsii (New Zealand pygmyweed) is an alien aquatic plant, originally from Australasia, which has spread widely across small lentic waterbodies throughout NW Europe since its introduction in the mid-20th century (Smith and Buckley, 2020). *Crassula helmsii* is notorious for profuse biomass production in the margins of these waterbodies, up to 1.5 kg m^{-2} (Dawson and Warman, 1987). As plants within these dense stands senesce, they can be expected to produce considerable volumes of litter (Carpenter and Lodge, 1986; Newman, 1991). Because *C. helmsii* is perennial, and retains aboveground biomass through the winter in many areas (Hussner, 2009; Smith and Buckley, 2020), this material is likely to be available in varying quantities throughout much of the year.

Whilst the aquatic decomposition of invasive riparian, emergent and floating plant litter has been subject to some investigation (Chimney and Pietro, 2006; Saulino, Thompson and Trivinho-Strxino, 2018; Cuassolo, Díaz Villanueva and Modenutti, 2020; Dekanová *et* *al.*, 2021), to my knowledge the decomposition of alien submerged plant litter has rarely been studied to date (Carpenter and Adams, 1979; Shilla *et al.*, 2006). Given the importance of detritus to freshwater nutrient and energy flows, the availability and palatability of invasive macrophyte detritus is likely to strongly influence impacts on recipient ecosystems (Cebrian and Lartigue, 2004; Saulino, Thompson and Trivinho-Strxino, 2018). Availability may vary according to macrophyte phenology and biomass production, and the rate of microbial decomposition. Palatability may vary according to nutrient concentrations, particularly nitrogen and phosphorus, and the retention of defensive chemicals, such as phenolic compounds, or structures, such as trichomes and sclerophylly (Webster and Benfield, 1986; Newman, 1991; Chimney and Pietro, 2006; Hanley *et al.*, 2007). Impacts on the invaded ecosystem will be mediated by match/mismatch between these plant traits and the traits present in the recipient detritivore assemblage (Tiegs *et al.*, 2013; Carvalho *et al.*, 2015).

Prior field surveys (Chapter 2) revealed marked shifts in the taxonomic and functional composition of macroinvertebrate detritivores within *C. helmsii*-invaded sites compared to uninvaded waterbodies, suggesting that the impacts of *C. helmsii* invasion on detritivores are indeed a major determinant of the plant's impacts on ecosystem structure and function (Petchey & Gaston, 2006; Schmera et al., 2016). In order to investigate the mechanisms underpinning the impacts of *C. helmsii* on macroinvertebrate detritivores, I designed a field experiment to compare *C. helmsii* breakdown with that of an architecturally similar co-occurring native macrophyte. Litterbags were deployed in a *C. helmsii*- invaded pond in West Cornwall, UK, containing either *C. helmsii* or the widespread co-occurring native macrophyte, *Callitriche stagnalis* (water starwort). I recovered litterbags periodically, weighed remaining plant material and recorded associated macroinvertebrates. Through this experiment, I aimed to determine:

1. The rate of breakdown of *Crassula helmsii* vs. *Callitriche stagnalis* detritus
2. The abundance, taxonomic and functional trait composition of macroinvertebrates colonising alien *C. helmsii* vs. native *C. stagnalis*

4.3 Methodology

4.3.1 Field experiment: detritus colonisation and breakdown

4.3.1.1 Experiment site

The field experiment was conducted in a circumneutral permanent pond with an area of 0.11 ha (pH 5.85, conductivity 141 µS/cm (May 2021)), surrounded by grassland and heathland in Sancreed, west Cornwall, United Kingdom (50°06’18”N , 005°38’03”W). The waterbody has a mean depth of 1 m, and is well vegetated with submerged macrophytes throughout, including abundant *Crassula helmsii* amongst a mosaic of other macrophytes (Figure 4.1). I did not record *Callitriche stagnalis* during the trial, although it occurs widely in similar habitats across the region (NBN Trust, 2023). Marginal areas are mostly shaded by riparian *Salix* spp. The study was conducted between April and June 2023, during which time local monthly air temperatures averaged 13.1°C (mean daily max. 16.1°C, min. 10.2°C) (Met Office, 2023).

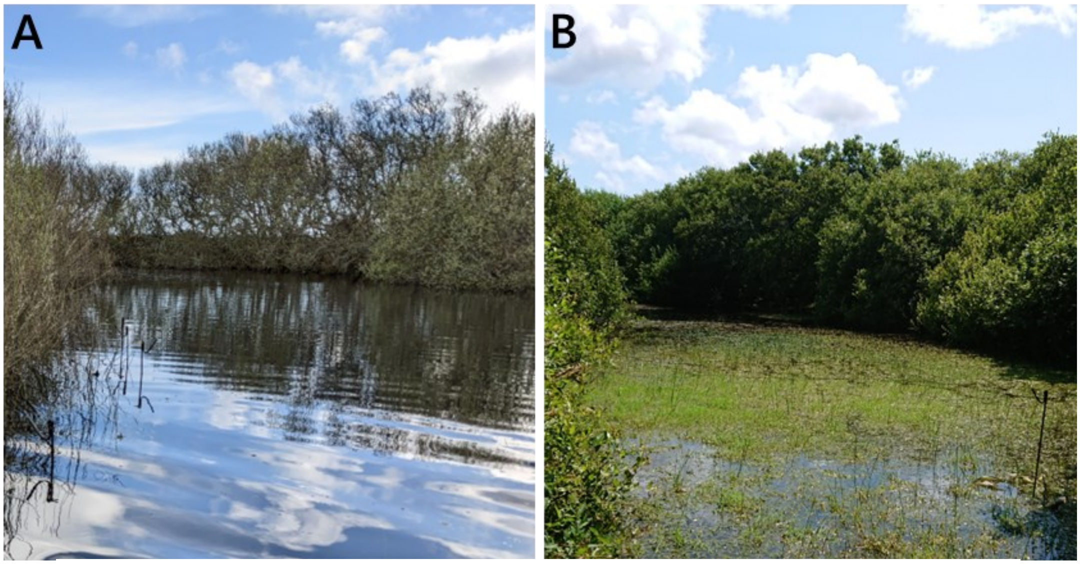

Figure 4.1. Field experiment site in Sancreed, Cornwall, UK. **A**: d0 (April 7 2023); **B**: d83 (29 June 2023)

4.3.1.2 Experimental procedure

Crassula helmsii and *C. stagnalis* were collected from Cadover Bridge, Dartmoor (50°27'55"N 4°02'09"W), thoroughly rinsed to remove epiphytes and air-dried at a temperature of 26±3°C for two weeks. Once plants had attained constant mass, they were split into 5 ± 0.1 g portions and placed into 20 x 30 cm mesh litter bags (N = 42). Of these, 24 coarse mesh bags had a 700 µm mesh base and 7 mm mesh on the upper side (adapted from Bedford, 2004), permitting access for macroinvertebrate detritivores. The remaining 18 fine mesh bags were composed entirely of 700 µm mesh, for the quantification of microbial and meiofaunal decomposition in the absence of macroinvertebrates. Upon arrival at the experimental site, bags were weighted down with cleaned glass marbles, shut with cable ties (coloured to indicate the plant species within) and secured in groups to randomly distributed stakes in the margins of the waterbody (depth < 1 m). Fine and coarse mesh bags containing *C. helmsii* and *C. stagnalis* were distributed evenly across these stakes, so that for each retrieval date, an even number of bags of both macrophyte species were retrieved from each stake, negating potentially confounding variation in abiotic conditions across the waterbody. 14 bags were extracted from the waterbody on each of 3 retrieval dates, after 10 (d10), 35 (d35) and 83 (d83) days. Retrieval dates were selected to encompass all stages of litter decay (rapid early mass loss through to slow breakdown of recalcitrant litter components), and collect macroinvertebrates associated with each stage of decomposition (Carvalho *et al.*, 2015). Individual bags were retrieved using a large 500 µm mesh bag to prevent loss of plant material or invertebrates. Upon retrieval, litterbags were placed singly in 1 l pots containing 70% IDA (for invertebrate fixation) and transferred to the laboratory for processing.

In the laboratory, macroinvertebrates were separated from plant material, which was then air dried to constant mass and weighed. Macroinvertebrates were then identified and enumerated. Where possible, specimens were identified to species level using a range of resources (Hammond, Merritt and Gardner, 1985; Elliott, Humpesch and Macan, 1988; Savage, 1989; Wallace, Wallace and Philipson, 1990; Edington and Hildrew, 1995; Nilsson, 1996; Foster and Friday, 2011; Dobson *et al.*, 2012; Foster, Bilton and Friday, 2014; Brochard *et al.*, 2016; Smallshire and Swash, 2018; Rowson *et al.*, 2021), with the exception of Bivalvia (species/genus), Diptera (subfamily) and Annelida (subclass).

4.3.2 Carbon: nitrogen analysis

To assess carbon:nitrogen (C:N) ratios, litter was freeze-dried, ground, and passed through a 180 µm sieve. I weighed out c. 5mg of resulting powders into tin cups for C:N analysis in an elemental analyser (Elementar, Langensolbold, Germany). To minimise contamination by invertebrates and extraneous detritus, I included only litter from fine mesh bags in this analysis.

4.3.3 Data analysis

Decomposition rate was calculated based on the exponential decay model (Petersen and Cummins, 1974; Bärlocher, 2005; Thornhill *et al.*, 2021), using the formula:

$$-k = \frac{ln(DM_1/DM_0)}{d}$$

where DM_0 is initial dry mass, DM_1 is dry mass upon recovery and d is the number of days submersed. For convenience, $-k$ is expressed positively hereafter.

Differences in mass loss and C:N ratio between *C. helmsii* and *C. stagnalis* litter were assessed using linear models, with mesh size as a fixed factor. Differences in the taxon richness and abundance of macroinvertebrates associated with coarse litter bags were assessed using generalised linear models (packages *lme4* (Bates *et al.*, 2015) and *MASS* (Venables and Ripley, 2002)). Model assumptions were checked graphically, and generalised least squares fits (package *nlme* (Pinheiro, Bates and R Core Team, 2023)) used where issues with homogeneity of variance were evident. For all models, homogeneity of response (equivalence of breakdown slopes) was tested using Type III (simultaneous) ANCOVA (package *car* (Fox and Weisberg, 2018)). If no significant interaction between independent variables was observed, Type I (sequential) ANCOVA was used for significance testing of main effects, whereas results of Type III ANCOVA were reported where interactions were significant.

Differences in taxonomic assemblage composition of macroinvertebrates associated with *C. helmsii* and *C. stagnalis* litter bags during breakdown were assessed using permutational multivariate analysis of variance (PERMANOVA) on a Bray-Curtis dissimilarity matrix (package *vegan* (Oksanen *et al.*, 2022)). For this purpose, abundance data were square-root transformed to down-weight the influence of dominant taxa.

To assess differences in functional assemblage composition, I constructed a functional trait database using fuzzy-coded data (Tachet *et al.*, 2010) encompassing 3 biological traits: food, feeding type and body size (Table 2.1). I then constructed a community weighted means (CWM) matrix by crossing this functional trait database with my taxon abundance database (package *ade4* (Thioulouse *et al.*, 2018)), and used the matrix to compute an ordination using fuzzy principal components analysis (FPCA) (Guareschi *et al.*, 2021).

All analyses were conducted in the R computing environment (R Core Team, 2023).

4.4 Results

4.4.1 Litter decomposition

4.4.1.1 Mass loss

In the field trial, *C. stagnalis* litter lost mass at a significantly faster rate than that of *C. helmsii.* Significantly more mass was lost from coarse-mesh bags than from fine-mesh bags, but there was no significant interaction between mesh type and macrophyte species (Table 4.1).

Table 4.1. Results of linear models assessing decomposition rate and carbon: nitrogen (C:N) ratio of *Crassula helmsii* or *Callitriche stagnalis* litter. Mesh size incorporated as a fixed factor in decomposition rate model. Type I LM fits assessed with F test.

		df	F	p
Decomposition rate (Type I LM)	retrieval date	1, 37	90.735	<0.001
	plant	1	43.168	<0.001
	mesh	1	25.917	<0.001
	plant : mesh	1	3.661	0.063
C:N ratio (Type I LM)	retrieval date	1, 21	1.541	0.228
	plant	1	70.860	<0.001

Averaged across mesh sizes and retrieval date, decomposition rate *k* equalled 0.018±0.002 d^{-1} for *Crassula helmsii* and 0.029±0.002 d^{-1} for *Callitriche stagnalis* (Table 4.2). After 83 days of decomposition, only 21.1% of *C. stagnalis*' mass remained across coarse and fine-mesh litter bags, whilst *C. helmsii* retained 49.1% of its original mass. (Figure 4.2).

Table 4.2. Leaf decomposition rates (k d^{-1}) of *Crassula helmsii* and *Callitriche stagnalis* in coarse and fine mesh litterbags over 83 days.

	Leaf processing rate ($k\ d^{-1}$) ± SE		
	Coarse mesh	Fine mesh	Overall
Crassula helmsii	0.0205 ± 0.0033	0.0155 ± 0.0028	0.0183 ± 0.0022
Callitriche stagnalis	0.0333 ± 0.0028	0.0223 ± 0.0018	0.0286 ± 0.0021

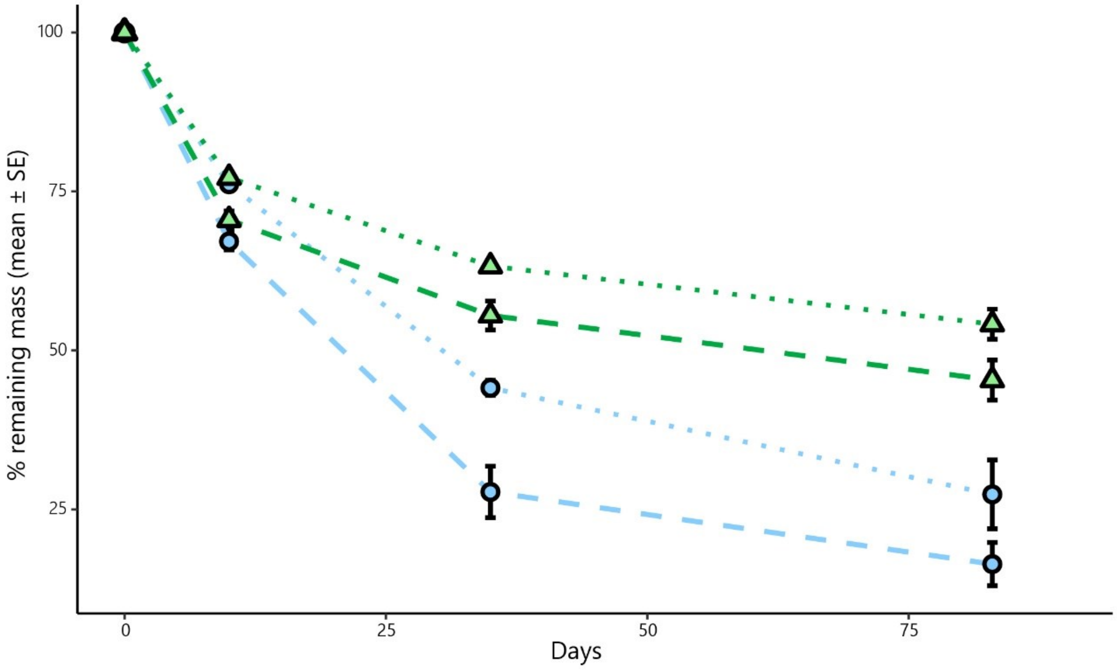

Figure 4.2. Mass loss (mean ± SE) of *Crassula helmsii* (△) *and Callitriche stagnalis* (●) over 83 days. Dotted bars = coarse mesh; dashed bars = fine mesh.

4.4.1.2 Carbon: nitrogen ratio

C. helmsii litter had a significantly higher carbon: nitrogen ratio than *C. stagnalis* litter, but litter carbon: nitrogen ratios did not change significantly throughout the experiment (Figure 4.3, Table 4.1).

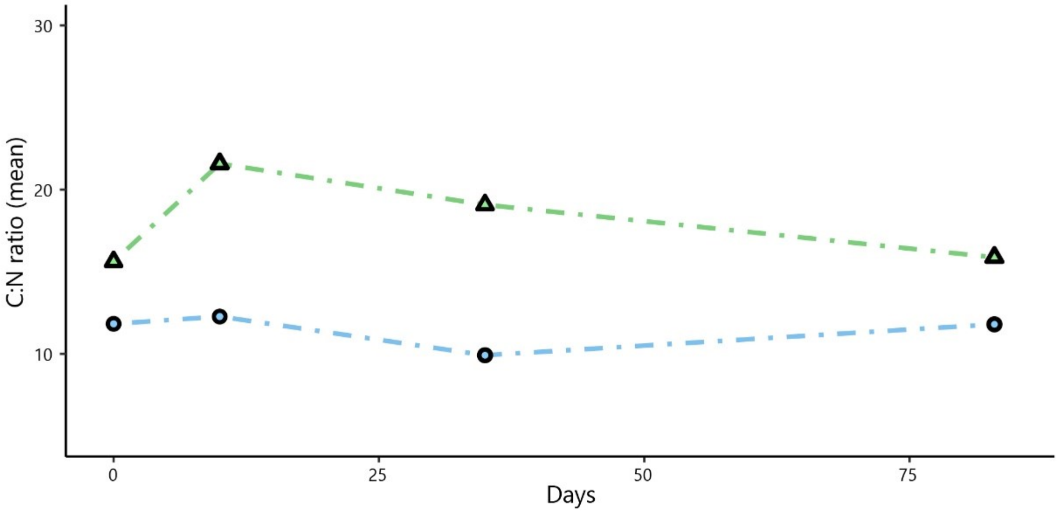

Figure 4.3. Mean changes to carbon: nitrogen ratio of *Crassula helmsii* (△) and *Callitriche stagnalis* (●) litter over 83 days in fine mesh bags. Error bars (SE) too small to be visible, so not included.

4.4.2 Macroinvertebrate colonisation

4.4.2.1 Taxonomic diversity and abundance

I observed a significant interaction effect of plant species and litterbag retrieval date on macroinvertebrate abundance, with *C. helmsii* litterbags containing fewer macroinvertebrates than *C. stagnalis* bags after 10 days, but more macroinvertebrates after 83 days (Figure 4.4, Table 4.3).

Table 4.3. Results of GLM and GLS models assessing taxon richness, total abundance and abundance of *Crangonyx pseudogracilis* and *Pisidium casertanum* associated with *Crassula helmsii* or *Callitriche stagnalis* litter, with litterbag retrieval date incorporated as a fixed factor. Type I GLS fits assessed with F test, Type III with Wald chi-sq. Type I GLM fits assessed with Wald chi-sq, Type III with likelihood ratio test.

		df	F	X^2	p
Taxon richness (Type I Poisson GLM)	Retrieval date	1, 21	2.944		0.101
	Plant	1	0.120		0.732
Total abundance (Type III GLS)	Retrieval date	1		2.921	0.087
	Plant	1		1.083	0.298
	Plant : retrieval date	1		5.523	<0.05
C. pseudogracilis abundance (Type III negative binomial GLM)	Plant	1		3.825	0.051
	Retrieval date	1		4.950	<0.05
	Plant : retrieval date	1		5.270	<0.05
P. casertanum abundance (Type I negative binomial GLM)	Retrieval date	1		22.407	<0.001
	Plant	1		1.714	0.191

Macroinvertebrate taxon richness did not differ significantly between *C. helmsii* and *C. stagnalis*, nor between litterbag retrieval dates. I separately analysed the abundance of *Crangonyx pseudogracilis* and *Pisidium casertanum,* the two most abundant macroinvertebrate taxa in my litterbags, finding a significant interaction effect between plant and retrieval date on *C. pseudogracilis* abundance, with *C. helmsii* litterbags containing fewer *C. pseudogracilis* than *C. stagnalis* bags after 10 days, but more *C. pseudogracilis* after 83 days. *Pisidium casertanum* abundance was significantly higher in litter of both macrophyte species at later retrieval dates (Figure 4.4, Table 4.3).

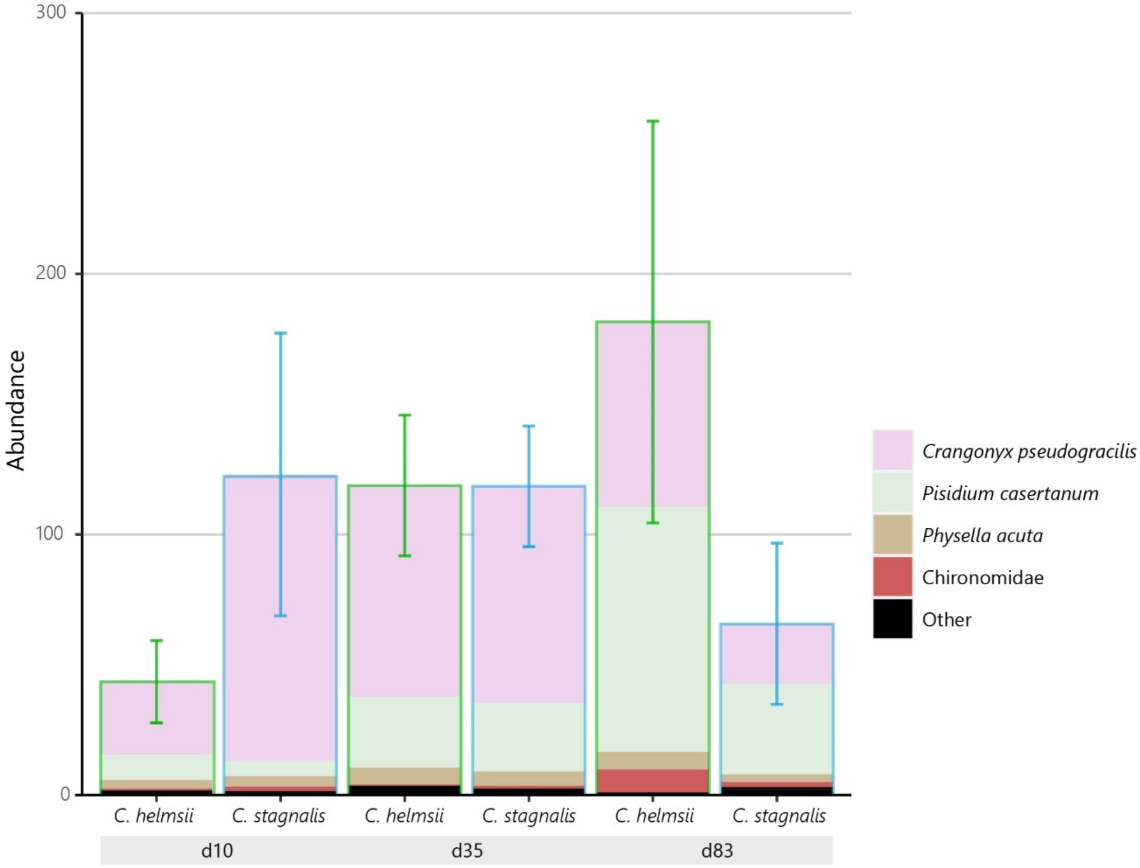

Figure 4.4. Abundance of macroinvertebrates associated with *Crassula helmsii* and *Callitriche stagnalis* litter bags after 10, 35 and 83 days (mean ± SE).

4.4.2.2 Taxonomic and functional assemblage composition

Macroinvertebrate taxonomic composition differed significantly according to retrieval date (PERMANOVA: $F_{1,18}$ = 4.750, $p < 0.01$), but not according to macrophyte species (PERMANOVA: $F_{1,18}$ = 0.568, $p > 0.05$). Consequently, functional assemblage composition shifted during litter decomposition from trait space within the FPCA ordination associated with trait modalities food: dead plants (>1 mm) and feeding mode: shredder towards space associated with the trait modalities food: detritus (<1 mm) and feeding mode: filter feeder (Figure 4.5).

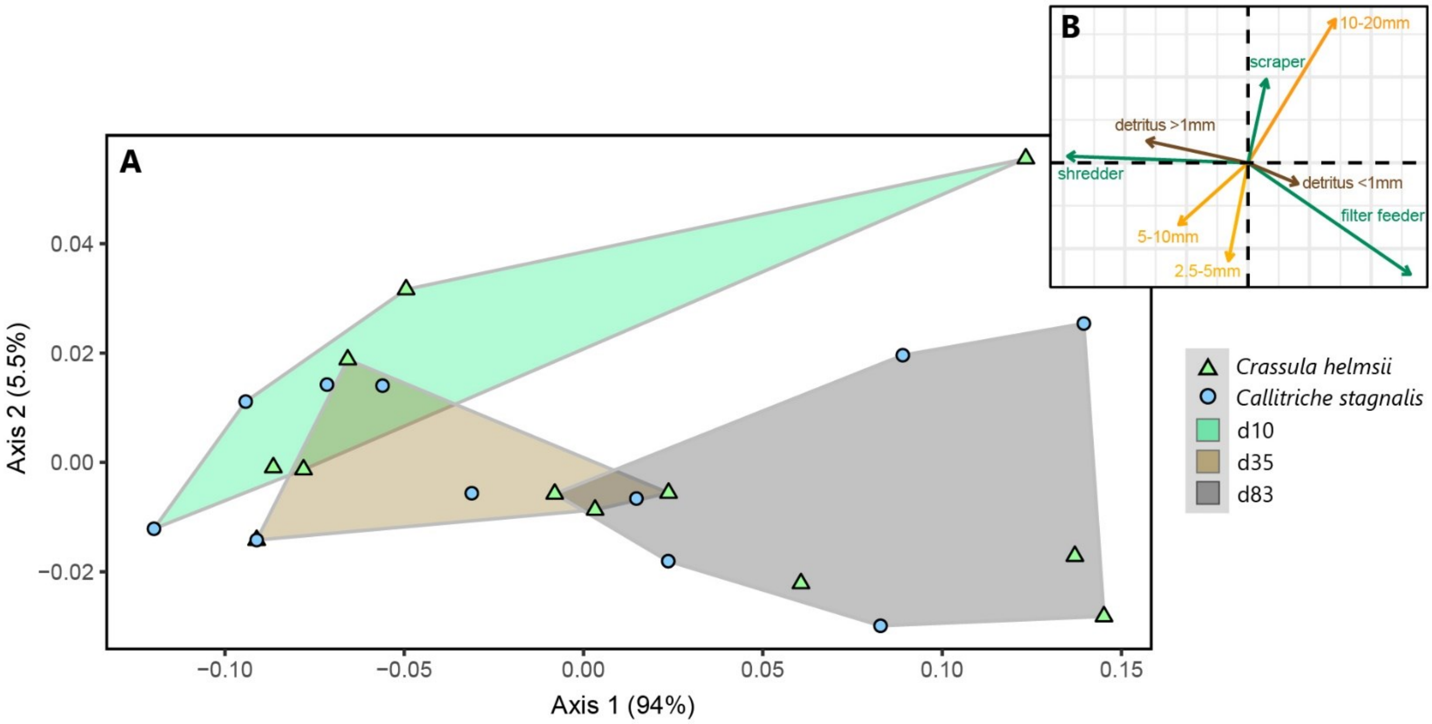

Figure 4.5. **A**: fuzzy principal components analysis ordination, produced using a community weighted means matrix. First two axes account for 99.5% of the variation. Convex hulls represent the location in functional trait space of macroinvertebrate assemblages from d10, d35 and d83 litterbags. **B**: Trait modalities most strongly driving assemblage functional composition. Green arrows: feeding mode; brown: food; orange: body size.

4.5 Discussion

Given the major contribution made by detritus to aquatic energy flows and nutrient cycling (Webster and Benfield, 1986; Cebrian and Lartigue, 2004; Shurin, Gruner and Hillebrand, 2005), changes to the quantity and quality of detritus are likely to be a key factor determining the impacts of alien macrophyte invasion. In my field experiment, alien *Crassula helmsii* litter decomposed at a significantly slower rate than native *Callitriche stagnalis* litter. Initially, *C. stagnalis* litter supported more abundant macroinvertebrates, but after 83 days, *C. helmsii* litterbags supported higher macroinvertebrate abundance. Litter mass loss was highest during the first 10 days of litter decomposition, likely due to leaching of water-soluble compounds. During this phase, the colonising invertebrate assemblage was dominated by *Crangonyx pseudogracilis*, an abundant non-native amphipod shredder (Tattersall, 1937). Fragmentation by *C. pseudogracilis* is likely to have contributed significantly to the elevated mass loss in coarse litter bags from 10-35 days. In the last 6 weeks of the trial (d35-d83), *C. pseudogracilis* was replaced by *Pisidium casertanum*, a suspension feeding bivalve (Lopez and Holopainen, 1987), as the dominant member of the colonising macroinvertebrate assemblage.

These results suggest that *C. helmsii* detritus is processed by a similar macroinvertebrate assemblage to native macrophytes, but that *C. helmsii* detritus persists for longer, and ultimately hosts more abundant detritivores per unit of litter. I observed no significant interaction between litterbag mesh size and macrophyte species, indicating that the percentage of detritus consumed by detritivores is comparable between *C. helmsii* and *C. stagnalis*, and that differences in mass loss between *C. helmsii* and *C. stagnalis* litter are likely driven by resistance of *C. helmsii* litter to mechanical breakdown and/or microbial decomposition (Webster and Benfield, 1986; Santonja, Pellan and Piscart, 2018). Resistance of *C. helmsii* litter to mechanical breakdown could be explained by the relative sclerophylly of its tissues (by comparison to other submerged macrophytes; pers.obs.), whilst microbial decomposition could be retarded by *C. helmsii*'s low nutritional quality, as revealed by its comparatively high carbon: nitrogen ratio (Li *et al.*, 2012).

Decomposition rates vary strongly across studies according to detritivore assemblage composition, waterbody physicochemistry and experimental design (e.g. litter bag mesh aperture), so studies are rarely directly comparable. Nonetheless, the breakdown rate I observed for *C. helmsii* (0.018 $k\ d^{-1}$) is less than half the mean rate of 0.047 $k\ d^{-1}$ calculated by Chimney and Pietro (2006) for submerged freshwater macrophytes, suggesting *C. helmsii* does produce unusually recalcitrant detritus. This is more likely to be a product of *C. helmsii*'s traits than its evolutionary novelty to detritivores (Elton, 1958; Keane and Crawley, 2002; Jeschke, 2014; Grutters, Roijendijk, *et al.*, 2017), and is unlikely to represent a generalisable pattern for non-native submerged macrophytes in general. For example, in a field experiment in Myall Lake, Australia, Shilla *et al*. (2006) observed faster breakdown of litter from alien *Vallisneria gigantea* than from the native macrophytes *Najas marina* and *Myriophyllum sulsagineum.* I observed faster rates of decomposition of both *C. helmsii* and *C. stagnalis* (0.029 $k\ d^{-1}$) than those reported for beech (*Fagus sylvatica*: 0.0015 $k\ d^{-1}$) (Thornhill *et al.*, 2021), *Phragmites australis* (0.0057 $k\ d^{-1}$) and *Typha angustifolia* (0.0058 $k\ d^{-1}$) (Dekanová *et al.*, 2021) in field experiments conducted in comparable small temperate lentic waterbodies, fitting the general pattern that submerged macrophytes break down more quickly than emergent or riparian plants (Chimney and Pietro, 2006; Shilla *et al.*, 2006).

Because of the high biomass production often attained by *C. helmsii* (and consequently high detritus production), absolute consumption by detritivores will be higher than that of slower growing native macrophytes such as *C. stagnalis* if the percentage of detritus consumed is similar (Dawson and Warman, 1987; Cebrian and Lartigue, 2004). In addition, *C. helmsii* is a perennial, and tends to retain aboveground biomass in winter, so will yield varying quantities of detritus throughout much of the year, as opposed to the seasonal glut typical of most native macrophytes characteristic of the shallow fluctuating waters colonised by *C. helmsii* (Carpenter and Lodge, 1986; Hussner, 2009; Smith and Buckley, 2020). This reliable supply of abundant detritus is likely to promote the expansion of detritivore populations within recipient ecosystems, and *C. helmsii* may facilitate further alien invasions where such detritivores are non-native. The alien amphipod *C. pseudogracilis* was highly abundant amongst *C. helmsii* detritus, particularly in d83 litterbags. The non-native bladder snail *Physella acuta* (Rowson *et al.*, 2021) was also present in *C. helmsii* litterbags across all removal dates, so may similarly be facilitated by *C. helmsii* invasion.

The decomposition of aquatic vascular plant litter is understudied in comparison to the aquatic decomposition of allochthonous terrestrial plant material, particularly in lentic systems (Cummins *et al.*, 1973; Gessner, Chauvet and Dobson, 1999; Gessner *et al.*, 2010). Decomposition pathways of aquatic plant litter in lentic systems differ from better-studied processes of woody litter decomposition in lotic systems for several reasons. Firstly, aquatic vascular plants typically have higher available nutrient concentrations than terrestrial plants, due largely to the absence of unpalatable structural components such as lignin, and detritus nutritional quality is strongly correlated with the percentage of detrital production which is consumed in freshwaters (Cebrian and Lartigue, 2004; Shilla *et al.*, 2006; Bakker *et al.*, 2016). Secondly, differing litter properties and detritivore species pools will mean that colonising detritivore assemblages will differ between habitats (Pope, Gordon and Kaushik, 1999; Cebrian and Lartigue, 2004; Carvalho *et al.*, 2015; Bakker *et al.*, 2016). In addition, litter decomposition in small lentic waterbodies will proceed differently to decomposition in lotic systems (or larger lakes) due to the relative insignificance of mechanical breakdown by flow or wave action (Webster and Benfield, 1986; Santonja, Pellan and Piscart, 2018). The unanticipated colonisation of litterbags by abundant *Pisidium casertanum* might represent one such divergence from better-studied processes of lotic woody litter decomposition (Cummins *et al.*, 1973; Petersen and Cummins, 1974; Gessner *et al.*, 2010). The fragmentation of microbially conditioned coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM) by macroinvertebrate shredders is a well-understood and near-ubiquitous component of litter decomposition in freshwaters (Cummins *et al.*, 1973; Webster and Benfield, 1986; Pope, Gordon and Kaushik, 1999; Santonja, Pellan and Piscart, 2018; Thornhill *et al.*, 2021), but the role of macroinvertebrate collector-gatherers and suspension feeders in detritus processing has been less well studied, and the mass colonisation of litter by *Pisidium* spp. has, to my knowledge, not been reported from litter experiments to date (Cummins *et al.*, 1973; Wallace and Webster, 1996; Pope, Gordon and Kaushik, 1999; Carvalho *et al.*, 2015; Dekanová *et al.*, 2021). *P. casertanum* is a small bivalve mollusc which is thought to primarily feed in the interstices of sediment, filtering dense suspended FPOM agitated into suspension by pumping water through the pedal aperture (Lopez and Holopainen, 1987). Colonisation of litterbags by abundant *P. casertanum* in the last 6 weeks of the trial suggests that *Pisidium* spp. may play an underappreciated role in assimilation of detrital carbon and nutrients into macrofaunal food webs within small lentic waterbodies. Alongside direct assimilation, suspension feeding by *Pisidium* spp. may increase detritus particle size via egestion of faecal pellets, enabling further uptake of detrital carbon and nutrients by collector-gatherers (Wallace and Webster, 1996). Without further study, it is difficult to determine the generalisability of these findings, however.

In general, additional research on aquatic plant decomposition is much needed, given the key role of autochthonous detrital pathways in energy and nutrient flow through many freshwater ecosystems (Cebrian and Lartigue, 2004; Bakker *et al.*, 2016), and possible contributions to carbon burial (Taylor *et al.*, 2019). To my knowledge, whilst the role of emergent aquatic plant invasions in altering litter supply has been highlighted in previous studies (Cuassolo, Díaz Villanueva and Modenutti, 2020; Dekanová *et al.*, 2021), this field experiment represents a first attempt to assess the impacts of submerged plant invasion on detritivorous macroinvertebrates. Impacts are inevitably context- and taxon-specific, so it is difficult to draw any generalisations from these results. In future, general trends (and a predictive framework) might be elucidated via similar litter experiments using multiple alien macrophytes alongside a suite of native comparators, with trait information incorporated into analyses (Grutters, Roijendijk, *et al.*, 2017).

4.5.1 Conclusion

This field experiment indicates that *Crassula helmsii* detritus is colonised by a comparable macroinvertebrate assemblage to native macrophyte detritus, but decomposes slower, and ultimately may support more abundant detritivores. Given the dense stands typically formed by *C. helmsii* (Dawson and Warman, 1987), its perennial growth (Smith and Buckley, 2020) and the recalcitrance of its litter demonstrated here, *C. helmsii* is likely to produce copious, long-lasting detritus throughout the year, driving considerable impacts on the detritivore assemblage of invaded waterbodies, and consequently upon wider ecosystem structure and functioning. Incidentally, the colonisation of litter bags by abundant *Pisidium casertanum* may indicate an underappreciated contribution of *Pisidium* spp. to detritus processing within small lentic waterbodies.

Chapter 5: Quantifying the ecological impacts of alien aquatic macrophytes: a global meta-analysis of effects on fish, macroinvertebrate and macrophyte assemblages

5.1 Abstract

Biological invasions constitute a pervasive and growing threat to the biodiversity and functioning of freshwater ecosystems. Macrophytes are key primary producers and ecosystem engineers in freshwaters, meaning alien macrophyte invasions have the capacity to alter the structure and function of recipient aquatic ecosystems profoundly. Although prevailing wisdom holds that alien macrophyte invasions tend to compromise freshwater ecosystem structure and function, the ecological impacts of alien macrophyte invasion have not been quantitatively reviewed to date.

Here I present a global meta-analysis of 202 cases from 53 research articles, exploring the impacts of alien macrophyte invasion on the abundance and diversity of three ubiquitous and ecologically important focal groups, which together comprise the bulk of non-microbial freshwater biodiversity: resident macrophytes, macroinvertebrates and fish. This synthesis includes data from all continents except Antarctica and Asia, covering 25 alien macrophyte species, but reveals considerable taxonomic and geographical biases in knowledge.

Meta-analysis results reveal that invasion by alien macrophytes has an overall negative impact on taxonomic diversity of the three focal groups, but no consistent effect on abundance. At a finer resolution, I detect a strong negative effect of alien macrophyte invasion on resident macrophyte abundance and diversity, and a significant but smaller

positive effect of submerged alien macrophyte invasion on macroinvertebrates. Effects on fish appear inconsistent.

These findings emphasise the importance of context- and taxon-specific ecological research in informing appropriate and proportionate management of alien macrophyte invasions, since alien macrophyte impacts are not consistently negative. I also identify significant geographical and taxonomic limitations in coverage of existing studies, quantitative data being lacking for many alien taxa.

5.2 Introduction

In the Anthropocene, alien species have become near-ubiquitous components of biological assemblages across the world (Keller *et al.*, 2011; Lewis and Maslin, 2015). Invasion by alien species can disrupt ecosystem composition and function, with knock-on effects for the provision of ecosystem services and the resilience of the system to subsequent environmental change (Strayer *et al.*, 2006; Hershner and Havens, 2008; Pejchar and Mooney, 2009). Losses and expenditures associated with biological invasions are estimated to have cost the global economy US$1.288 trillion since 1970, and continue to mount (Diagne *et al.*, 2021). Biological invasions often act synergistically with other anthropogenic stressors, replacing geographically restricted species with a small number of globally successful invaders and homogenising regional biotas (McKinney and Lockwood, 1999; Olden, Comte and Giam, 2018; Petsch *et al.*, 2022). Such biotic homogenisation may be accelerated by 'invasional meltdown', wherein invasion by one alien species facilitates the invasion of other non-natives (Simberloff and Von Holle, 1999). Alien invasions are the driver most frequently associated with amphibian, reptile and mammal extinctions on the IUCN Red List, and the second- and fourth-most frequent driver of bird and plant extinctions respectively (Bellard, Cassey and Blackburn, 2016).

The deleterious impacts of a handful of particularly problematic invaders may, however, overshadow the relatively benign nature of most alien species, skewing the perspective of conservation biologists and land managers (Davis *et al.*, 2011; Schlaepfer, Sax and Olden, 2011). In some instances, aliens may not themselves be agents of degradation, but rather ride on the coat-tails of more insidious stressors (e.g. nutrient enrichment, habitat destruction) (Didham *et al.*, 2005; Macdougall and Turkington, 2005). Therefore, whilst complete protection of near-pristine areas should clearly remain a priority, it is unfeasible, and perhaps even counter-productive, to apply this approach to the heavily modified ecosystems now covering much of the Earth (Dudgeon, 2020). Conservationists and land managers must instead develop proportionate and cost-effective strategies for the conditional management of alien species, accounting objectively for the risks posed by a given invader. In this respect, quantitative meta-analysis of primary ecological research represents a powerful tool, reaching beyond potential bias to assess the typical impacts of alien species, as well as gaps in our current knowledge (Vilà *et al.*, 2011; Gurevitch *et al.*, 2018).

Freshwaters are arguably more invasible than most terrestrial ecosystems, owing in part to high propagule pressure from uniquely aquatic vectors (e.g. the ornamental aquatics trade; the release of ballast water etc.), the comparative ease of dispersal through interconnected drainage systems (Moorhouse and Macdonald, 2015) and the anthropogenic depletion of pre-existing biota, all of which facilitate the spread of invaders (Tilman, 2004; Strayer, 2010; Dudgeon, 2020). Freshwaters are also disproportionately diverse, hosting almost 10% of all described non-microbial species despite covering less than 1% of the earth's surface (Dudgeon, 2020). The insular, island-like nature of freshwater systems, with high endemism and high species turnover between basins, means that many freshwater taxa are disproportionately vulnerable to extinction. The impact of invasive species is considered a key driver in many such extinctions (Strayer and Dudgeon, 2010; Moorhouse and Macdonald, 2015; Dudgeon, 2020).

Alien macrophyte invasion may drive particularly drastic shifts in freshwater ecosystem composition and function, since macrophytes - photosynthetic aquatic organisms visible with the naked eye (Chambers *et al.*, 2007) - are key primary producers (Lodge, 1991; Newman, 1991) and ecosystem engineers (Carpenter and Lodge, 1986; Warfe and Barmuta, 2006; Thomaz and Cunha, 2010). Physicochemical microhabitats with distinct light, temperature, dissolved oxygen and nutrient concentrations are maintained within macrophyte beds, and attenuated water movement promotes the deposition of fine sediment and retention of detritus (Ondok, Pokorný and Květ, 1984; Carpenter and Lodge, 1986; Carter, Rybicki and Hammerschlag, 1991; Miranda, Driscoll and Allen, 2000). Decaying macrophytes leach dissolved organic carbon, phosphorus and nitrogen into the water column, whilst microbial decomposition of macrophyte detritus may lead to localised oxygen depletion (Landers, 1982; Ondok, Pokorný and Květ, 1984; Carpenter and Lodge, 1986; Carter, Rybicki and Hammerschlag, 1991; Miranda, Driscoll and Allen, 2000). Alongside these physicochemical effects, macrophytes are key to an array of biotic interactions. They are colonised by diverse epiphytic assemblages which often make a contribution to productivity and nutrient exchange comparable to that of the macrophyte itself (Allen, 1971; Sheldon and Boylen, 1975; Cattaneo and Kalff, 1980). In addition, protection from predators and abundant food (e.g. epiphyton, live macrophyte tissue, detritus, animal prey) within macrophyte beds attracts macroinvertebrates and fish in higher densities than are found in adjacent unvegetated habitats (Killgore, Morgan and Rybicki, 1989; Schramm and Jirka, 1989; Thorp, Jones and Kelso, 1997; Hatzenbeler *et al.*, 2000; Strayer *et al.*, 2003).

Schultz and Dibble (2012) qualitatively reviewed alien macrophyte impacts, identifying changes to habitat structure, oxygen depletion, the release of allelopathic compounds and facilitation of other alien species as major drivers of ecosystem change following invasion. Habitat structure may be altered dramatically by alien macrophyte invasion due to the tendency of many alien species to form dense, monotypic stands. Where such stands increase plant biomass and structural complexity, macroinvertebrate density may increase (Kuehne, Olden and Rubenson, 2016), whilst the foraging efficiency of larger predators is compromised (Theel and Dibble, 2008). Dense alien macrophyte canopies may also decrease atmospheric exchange with water, reducing dissolved oxygen concentrations and further impairing predator foraging efficiency (Caraco and Cole, 2002; Troutman, Rutherford and Kelso, 2007). Allelochemicals exuded by alien macrophytes have been demonstrated to reduce lepidopteran larval growth and feeding (*Elodea nuttallii*: Erhard, Pohnert and Gross, 2007), stickleback larval foraging (*Myriophyllum spicatum*: Lindén and Lehtiniemi, 2005) and the germination and growth of native macrophyte competitors (*Ludwigia* spp.: Dandelot *et al.*, 2008; Thiébaut, Thouvenot and Rodríguez-Pérez, 2018). Alien macrophytes have also been demonstrated to facilitate the invasion of non-native species, including mussels (Michelan *et al.*, 2014; Wegner *et al.*, 2019), crayfish (Thouvenot *et al.*, 2017) and other macrophytes (Monks *et al.*, 2019). Many of these impacts are contingent on the growth form of the alien macrophyte in question. For instance, floating-leaved and free-floating macrophytes are most likely to form hypoxia-inducing closed canopies (Caraco and Cole, 2002), whereas, due to a relatively higher proportion of biomass suspended in the water column, submerged macrophytes might be expected to have the greatest effect on aquatic habitat complexity post-invasion (Kuehne, Olden and Rubenson, 2016). The growth form of an alien macrophyte also determines to a great extent which habitats it is able to invade, although many invasive aliens exhibit growth form plasticity, enabling successful invasion of suboptimal habitats (Hussner *et al.*, 2021).

Although prevailing wisdom holds that alien macrophyte invasions tend to compromise freshwater ecosystem structure and function (Brundu, 2014; Fleming and Dibble, 2015; Dudgeon, 2020), there is considerable variability in the impacts of alien macrophyte invasion, with some invasions having a negligible or even apparently beneficial effect on recipient native taxa. Despite selection bias arising from disproportionate focus on the most problematic non-native taxa (Evangelista, Magela Thomaz and Umetsu, 2014), alien macrophyte invasions have been associated variously with elevated native macrophyte diversity (Kuehne, Olden and Rubenson, 2016) and the promotion of rare native plant taxa (Smith and Buckley, 2015); elevated invertebrate density (Toft *et al.*, 2003; Hogsden, Sager and Hutchinson, 2007) and diversity (Kuehne, Olden and Rubenson, 2016) and increased fish biomass (Barrientos and Allen, 2008; Bickel and Closs, 2008). Clearly, the impacts of alien macrophyte invasion are not consistently negative, and warrant thorough quantitative review.

A recent meta-analysis by Gallardo *et al*. (2016) reviewing the impacts of aquatic biological invasions found that invasion by alien primary producers significantly reduced macrophyte diversity and fish and macroinvertebrate abundance. Due to the inclusion of non-macrophyte taxa (e.g. microalgae) and brackish-water systems in their analyses, the typical impacts of alien macrophyte invasion in freshwaters remain unclear, however. In addition, these authors did not explore the differential effect of alien growth form on native assemblages. Here, I present a focused meta-analysis of primary research investigating the effects of alien aquatic macrophyte invasion on three well-studied freshwater focal taxa: resident macrophytes, macroinvertebrates and fish. Analyses also investigate the influence of growth form (submerged, emergent or floating) on the ecological impacts of alien macrophytes, and the specific impacts of the best-studied alien macrophyte taxa. I explore the taxonomic and geographical coverage of studies in the database to contextualise results and investigate the biogeography of alien macrophyte invasions and the generalisability (and potential limitations) of work conducted to date.

5.3 Methods

5.3.1 Literature search

5.3.1.1 Identification of relevant literature

I conducted a literature search for research investigating the effects of alien macrophyte invasion on macrophyte, macroinvertebrate and fish assemblages. A search conducted on Scopus (Elsevier Co., USA) for titles, abstracts or keywords containing the terms 'inva* OR alien OR "non native" OR exotic OR introduc* PRE/2 macrophyte OR plant OR weed AND freshwater OR aquatic OR stream OR river OR wetland OR pond OR lake OR reservoir AND abundance OR cover OR density OR biomass OR richness OR diversity' yielded 1672 results. I included all published records up to 31/12/2020 within the subject areas of environmental/agricultural and biological sciences. After screening of search results (titles/abstracts) and supplementary searching of the bibliographies of retrieved articles, 192 articles (published 1982-2020) were individually assessed against criteria for inclusion.

5.3.1.2 Criteria for inclusion

To meet the criteria for inclusion, articles were required to report the impact of alien aquatic macrophyte invasion on the abundance and/or taxonomic diversity of one or more focal taxa (resident macrophytes, macroinvertebrates, fish) in freshwaters. Invasive native species were excluded, except where a non-native lineage had been implicated in the invasion, e.g. *Typha x glauca* and *Phragmites australis* in North America (Saltonstall, 2002; Travis *et al.*, 2010). Articles were also required to report the mean and standard error/standard deviation of effect size and number of invaded and control sites (>1), in tabular or graphical form. Where summary statistics were only available in graphical form (>50% of articles), I used WebPlotDigitizer v4.4 (Rohatgi, 2020) to extract the necessary data.

5.3.1.3 Database collation

The resulting database collates results from 53 articles representing 202 cases of ecological impact (Appendix 6). Studies span 25 species of alien macrophyte (Table 5.1), encompassing 116 effects on macroinvertebrates, 56 effects on macrophytes and 30 effects on fish. Most studies in the database were conducted in North America (141 cases).

Table 5.1. Alien macrophyte species of each growth form included in the complete dataset. Values a, b correspond to: a) the number of cases of ecological impact recorded for each macrophyte species; b) the number of articles from which these cases were sourced.

SUBMERGED		FLOATING		EMERGENT	
Myriophyllum spicatum	*40, 7*	*Eichhornia crassipes*	14, 3	*Typha* spp.	32, 14
Hydrilla verticillata	*39, 10*	*Hydrocharis morsus-ranae*	6, 1	*Phragmites australis*	13, 5
Lagarosiphon major	*7, 2*	*Trapa natans*	4, 2	*Hymenachne amplexicaulis*	9, 1
Cabomba caroliniana	*4, 1*	*Pistia stratiotes*	3, 1	*Urochloa mutica*	8, 1
Elodea canadensis	3, 1	*Azolla filiculoides*	2, 1	*Myriophyllum aquaticum*	6, 3
Ranunculus fluitans	3, 1	*Ceratopteris thalictroides*	1, 1	*Lythrum salicaria*	2, 2
Egeria densa	1, 1	*Hydrocotyle ranunculoides*	1, 1	*Ludwigia grandiflora*	1, 1
		Lemna minuta	1, 1		
Total	**99, 23**	Total	**32, 11**	Total	**71, 27**

In addition to recording summary statistics, I classified studies according to:

Alien macrophyte growth form: submerged, floating (sediment-rooted with floating leaves and/or free-floating), emergent

Habitat: lotic (rivers, streams); lentic (ponds, lakes, backwaters, reservoirs); wetland (defined as the boundary area between open water and dry land)

Climate: subtropical, temperate, tropical

Study type: observational (field studies); manipulative (field experiments); mesocosm (experiments in aquaria/outdoor tubs)

Control type: analogous uninvaded site; before-after invasion; treated plot (wherein alien is subjected to control or eradication technique); native vegetation; no vegetation

Following Gallardo *et al.* (2016), I considered each treatment: control comparison as a separate case in the database where data from multiple control groups (e.g. both native vegetation and no vegetation) were reported in an article. Where multiple treatment groups of varying alien density were reported, I used only data from the highest alien-density treatment, and where multiple sampling dates were reported, I used only data from the last available date.

5.3.2 Data analysis

5.3.2.1 Coverage

I explored the coverage of the database by study region, climate, habitat type, study type and alien species identity to assess the generalisability of findings across taxa, habitats and regions and to investigate gaps in knowledge of alien macrophyte invasions.

5.3.2.2 Effect size calculation and preliminary analyses

For each case of alien macrophyte impact, I calculated effect sizes using Hedge's g, a measure of standardised mean difference (SMD) that is not biased by small sample sizes (Hedges, 1981; Lüdecke, 2019; R Core Team, 2023):

$$Hedge's\ g = \frac{treatment\ mean - control\ mean}{pooled\ standard\ error}\ x\ weighting\ factor\ (based\ on\ no. of\ replicates)$$

Hedge's g is unitless and ranges from −∞ to +∞, with the value's magnitude and sign corresponding respectively to the size and direction of the effect.

In the literature, the effects of alien macrophyte invasion were often reported using different measures of abundance (density, biomass, cover, catch-per-unit-effort) and diversity (taxa richness, Simpson's index, Shannon's index). Since resulting effect sizes were not significantly different (Table 5.2), metrics were pooled under abundance and diversity, respectively.

Table 5.2. Results of ANOVAs testing differences between metrics of abundance and diversity.

Variable	Output
Total abundance	$F_{3,110} = 0.751$, $P = 0.524$
Total diversity	$F_{3,84} = 0.416$, $P = 0.742$
Macrophyte abundance	$F_{2,14} = 1.44$, $P = 0.27$
Macrophyte diversity	$F_{1,37} = 1.11$, $P = 0.299$
Macroinvertebrate abundance	$F_{1,79} = 0.012$, $P = 0.913$
Macroinvertebrate diversity	$F_{2,32} = 0.024$, $P = 0.976$
Fish abundance	$F_{2,13} = 0.102$, $P = 0.904$
Fish diversity	$F_{2,11} = 0.388$, $P = 0.687$

5.3.2.3 Meta-analysis

Using restricted maximum likelihood estimation, I ran multi-level random-effects (MLRE) models in R-package 'metafor' (Viechtbauer, 2020) to assess the impact of alien macrophyte invasion on 3 focal taxa: resident macrophytes, macroinvertebrates and fish. A multi-level structure (with cases nested within articles) was employed in these models to account for non-independence arising where multiple effect sizes were extracted from the same study (Habeck and Schultz, 2015; Cheung, 2019; Harrer *et al.*, 2019).

Preliminary analyses revealed contrasting effects of alien macrophyte invasion on resident macrophytes, macroinvertebrates and fish. Therefore, I proceeded to analyse each focal taxon independently, alongside all-taxon 'grand mean' analysis.

I ran an additional set of MLRE models to test the effects of growth form on the ecological impacts of alien macrophytes, and another set to examine the ecological impacts of the specific alien macrophyte taxa most highly represented in the database: *Typha* spp. (*Typha angustifolia*, *Typha x glauca* (Typhaceae)), *Phragmites australis* (Poaceae), *Myriophyllum spicatum* (Haloragaceae) and *Hydrilla verticillata* (Hydrocharitaceae) and submerged macrophytes excl. *M. spicatum/H. verticillata*. In order to maintain statistical power for these subgroup analyses, I aggregated abundance and diversity results for each focal taxon/growth form (Coetzee, Gaston and Chown, 2014; Gallardo *et al.*, 2016). Furthermore, I included only those subgroups with ≥10 effect sizes from ≥3 articles (Habeck and Schultz, 2015).

Between-study heterogeneity was assessed for each dataset using Q and I^2 statistics (Harrer *et al.*, 2019). A significant Q value indicates the presence of significant heterogeneity in the dataset, unaccounted for by the model. I^2 represents the percentage of variability in effect sizes not caused by sampling error. An I^2 value exceeding 75% indicates substantial heterogeneity (Higgins *et al.*, 2003), but it is worth noting that I^2 values are typically higher than 75% in most ecological and evolutionary meta-analyses due to the intrinsic variability of the study systems (Senior *et al.*, 2016). In an attempt to explain residual heterogeneity, I ran duplicate MLRE models separately incorporating the following moderators: control type nested within study type; habitat; climate; alien species identity. The addition of these covariates did not consistently increase the explanatory power of models (Likelihood Ratio Test, p>0.05; Table 5.3), however, so I proceeded using the original (reduced) multi-level random effects models wherein cases were nested within articles, with no additional covariates.

Table 5.3. Results of likelihood ratio tests to assess relative fit of models incorporating additional covariates.

Covariate	Focal taxon	Likelihood ratio test statistics
Control type nested within study type	All-taxon	**Abundance: $\chi^2_1 = 8.5$, $p < 0.05$ (↓AIC >2).** Diversity: $\chi^2_1 = 2.5$, $p > 0.05$.
	Macrophytes	Abundance: $\chi^2_1 = 0.7$, $p > 0.05$. Diversity: $\chi^2_1 = 1.1$, $p > 0.05$.
	Macroinvertebrates	Abundance: $\chi^2_1 = 1.2$, $p > 0.05$. Diversity: $\chi^2_1 = 0.4$, $p > 0.05$.
	Fish	Abundance: $\chi^2_1 = 0.0$, $p > 0.05$. Diversity: $\chi^2_1 = 0.0$, $p > 0.05$.
Habitat	All-taxon	**Abundance: $\chi^2_1 = 4.4$, $p < 0.05$ (↓AIC >2).** Diversity: $\chi^2_1 = 3.4$, $p > 0.05$
	Macrophytes	Abundance: $\chi^2_1 = 1.4$, $p > 0.05$. Diversity: $\chi^2_1 = 0.15$, $p > 0.05$.
	Macroinvertebrates	Abundance: $\chi^2_1 = 0.0$, $p > 0.05$. Diversity: $\chi^2_1 = 0.0$, $p > 0.05$.
	Fish	Abundance: $\chi^2_1 = 3.4$, $p > 0.05$. Diversity: $\chi^2_1 = 0.1$, $p > 0.05$.
Climate	All-taxon	Abundance: $\chi^2_1 = 0.5$, $p > 0.05$. Diversity: $\chi^2_1 = 0.3$, $p > 0.05$.
	Macrophytes	Abundance: $\chi^2_1 = 3.6$, $p > 0.05$. Diversity: $\chi^2_1 = 1.2$, $p > 0.05$.
	Macroinvertebrates	Abundance: $\chi^2_1 = 0.6$, $p > 0.05$. Diversity: $\chi^2_1 = 0.5$, $p > 0.05$.
	Fish	Abundance: $\chi^2_1 = 0.0$, $p > 0.05$. Diversity: $\chi^2_1 = 0.2$, $p > 0.05$.
Alien species identity	All-taxon	Abundance: $\chi^2_1 = 1.6$, $p > 0.05$. **Diversity: $\chi^2_1 = 6.7$, $p < 0.01$ (↓AIC >2).**
	Macrophytes	Abundance: $\chi^2_1 = 0.1$, $p > 0.05$. Diversity: $\chi^2_1 = 2.0$, $p > 0.05$.
	Macroinvertebrates	Abundance: $\chi^2_1 = 0.9$, $p > 0.05$. Diversity: $\chi^2_1 = 0.2$, $p > 0.05$.
	Fish	Abundance: $\chi^2_1 = 0.0$, $p > 0.05$. Diversity: $\chi^2_1 = 2.4$, $p > 0.05$.

Meta-analyses may be distorted by the ‘file-drawer problem’, a form of publication bias wherein non-significant results (particularly those resulting from studies with small sample sizes) are less likely to reach publication. To assess whether the file drawer problem affected my meta-analyses, I evaluated plots using Egger’s test (Egger *et al.*, 1997) by modifying models to include the variance of the effect sizes as a moderator. Analyses were considered biased where the intercept of this model differed significantly from zero ($p<0.1$). Meta-analyses may also be distorted by a handful of highly influential cases. To evaluate whether pooled results were skewed by the presence of influential cases, I conducted leave-one-out analyses, iteratively removing one case at a time and recalculating the pooled effect size in its absence. I defined influential cases as those with DFBETAs (differences in beta values) above 1 (Viechtbauer and Cheung, 2010). Where a case exceeded this cut-off, I conducted a sensitivity analysis, running the relevant MLRE model again with the case in question removed.

5.4 Results

5.4.1 Geographical, taxonomic and methodological coverage

The invasions included in meta-analyses occur largely in North America (70% of cases; Figure 5.1), although Central and South America are also well represented. Palaeotropical coverage is poor, reflecting the comparative dearth of published quantitative ecological research on alien macrophyte invasions in these regions. Studies focus mostly on shallow wetlands and lentic ecosystems such as lakes and river backwaters, leaving lotic systems underrepresented (12% of cases). Only five cases in the database are drawn from invasions of small waterbodies such as ponds and streams (Appendix 5). Manipulative field experiments and mesocosm trials make up only 24% of cases in the database, with the remaining three-quarters drawn from observational studies.

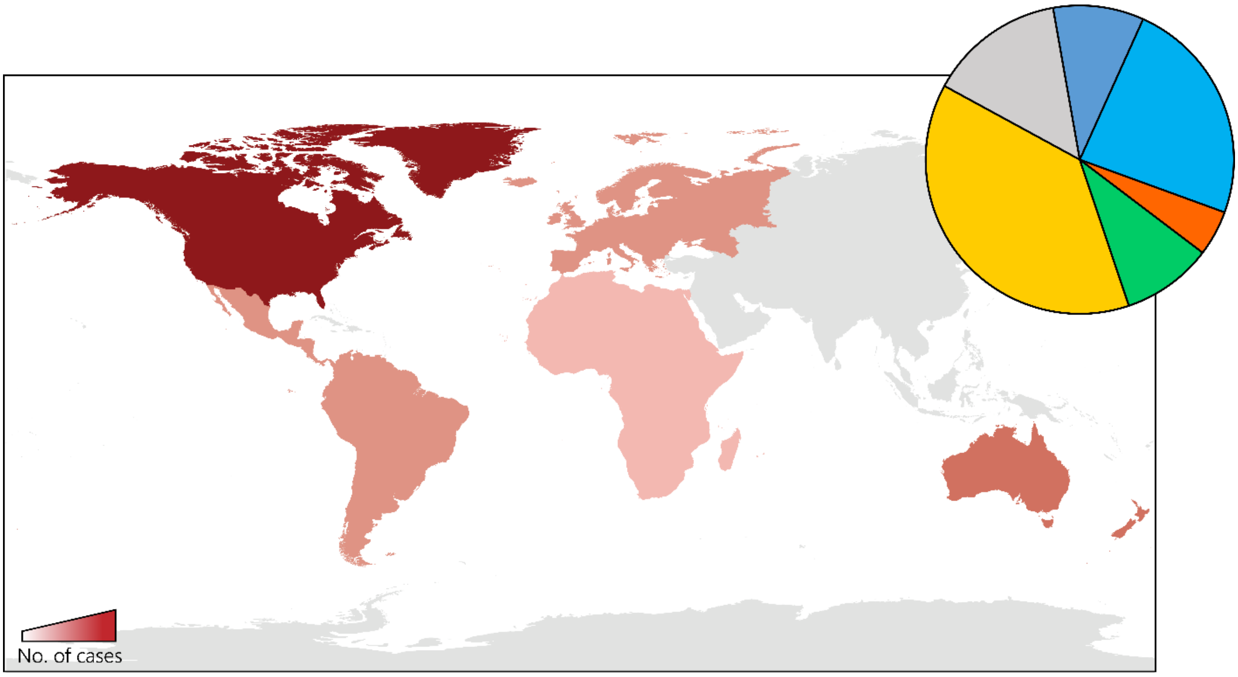

Figure 5.1. Number of cases from each continent included in the meta-analysis, and the biogeographic origins of the alien macrophytes upon which these studies focus: Nearctic (🟦); Palearctic (🟦); Oriental (🟧); Ethiopian (🟩); Neotropical (🟨); Cosmopolitan (⬜).

Although the database includes 25 species of alien macrophyte, *Typha* spp., *Hydrilla verticillata* and *Myriophyllum spicatum* together contribute more than half of the cases included (Table 5.1). Other well-studied aliens include *Phragmites australis* and *Eichhornia crassipes* (Pontederiaceae). Alien macrophyte taxa in the database mostly originate in the tropics, particularly the Neotropical region (8 species). Only eight species in the database originate from the Holarctic.

5.4.2 Meta-analyses

I found a significant negative overall effect of alien macrophyte invasion on all-taxon diversity, whilst the effect on all-taxon abundance was non-significant. Alien macrophytes caused significant reductions to resident macrophyte abundance and diversity, but had no significant pooled effect on macroinvertebrate abundance, macroinvertebrate diversity, fish abundance or fish diversity (Figure 5.2, Table 5.4).

Table 5.4. Multilevel random effects models assessing the impacts of alien macrophyte invasion on abundance and diversity of macrophyte, macroinvertebrate and fish assemblages. Q (and its associated p-value) and I^2 provide estimates of residual heterogeneity. An I^2 value exceeding 75% indicates substantial residual heterogeneity.

Focal taxon	**Response variable**	**Mean effect**	**95% CI**	***P***	**Heterogeneity statistics**
All-taxon	Abundance	-0.15	-0.50, 0.21	ns	Q = 873.03, df = 113, $p < 0.0001$; I^2 = 90.31%
	Diversity	-0.65	-1.16, -0.14	*	Q = 710.34, df = 87, $p < 0.0001$; I^2 = 92.85%
Macrophytes	Abundance	-1.27	-2.07, -0.46	**	Q = 91.48, df = 16, $p < 0.0001$; I^2 = 88.55%
	Diversity	-1.38	-1.99, -0.77	***	Q = 322.26, df = 38, $p < 0.0001$; I^2 = 91.02%
Macroinvertebrates	Abundance	0.16	-0.32, 0.64	ns	Q = 628.25, df = 80, $p < 0.0001$; I^2 = 89.56%
	Diversity	0.12	-0.57, 0.82	ns	Q = 124.35, df = 34, $p < 0.0001$; I^2 = 84.57%
Fish	Abundance	-0.23	-0.76, 0.30	ns	Q = 56.41, df = 15, $p < 0.0001$; I^2 = 85.84%
	Diversity	0.32	-0.23, 0.87	ns	Q = 73.78, df = 13, $p < 0.0001$; I^2 = 84.93%

Influence analyses indicated the presence of influential outlying cases in macroinvertebrate diversity and fish abundance meta-regressions. Following the removal of these cases (1 from each dataset), models were recalculated to assess the robustness of initial findings. Whilst the effect of alien macrophyte invasion on macroinvertebrate diversity remained non-significant (mean estimate = 0.37; 95% CI = -0.1, 0.83; P>0.05), fish abundance became negatively correlated with alien macrophyte invasion (mean estimate = -0.4; 95% CI = -0.73, -0.08; P<0.05). I detected evidence of publication bias ('file-drawer problem') in the datasets evaluating overall diversity (P = 0.009) and macroinvertebrate diversity (P = 0.06). A significant amount of residual heterogeneity remained unexplained for all models (Table 5.4).

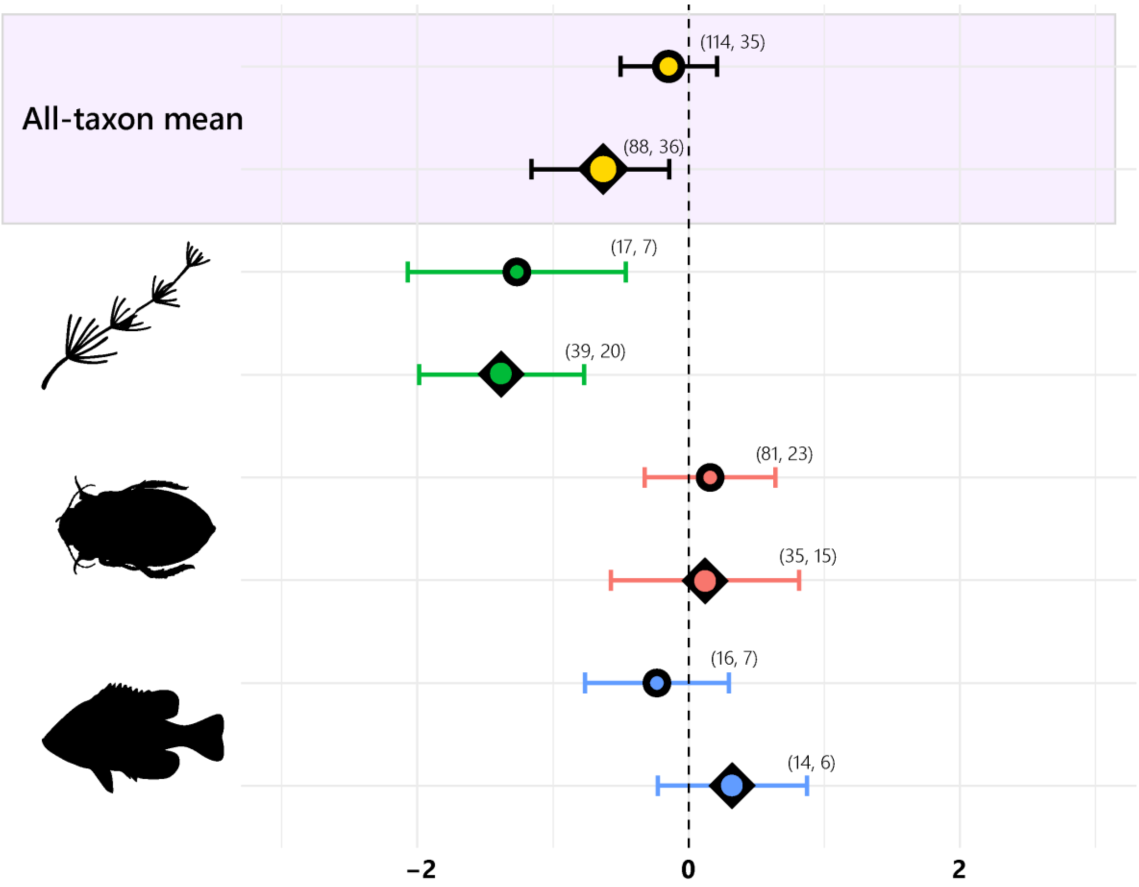

Figure 5.2. Effects of invasive alien macrophytes on the abundance (●) and diversity (◆) of macrophyte, macroinvertebrate and fish assemblages. Means are taken from standardised mean difference (Hedge's g) of treatment and control groups. Error bars represent 95% confidence intervals. Values in parentheses (a, b) represent: a) the number of effect sizes used in the model; b) the number of articles from which the effect sizes were sourced. Credit to Maxime Dahirel (https://creativecommons.org/licenses/by/3.0/) and Sergio A. Muñoz-Gómez (https://creativecommons.org/licenses/by-nc-sa/3.0/) for taxa illustrations.

When split by growth form, emergent alien macrophyte invasion had a strong negative impact on resident macrophytes (aggregated abundance and diversity), whilst submerged alien macrophyte invasion had a smaller positive effect on macroinvertebrates (Figure 5.3, Table 5.5).

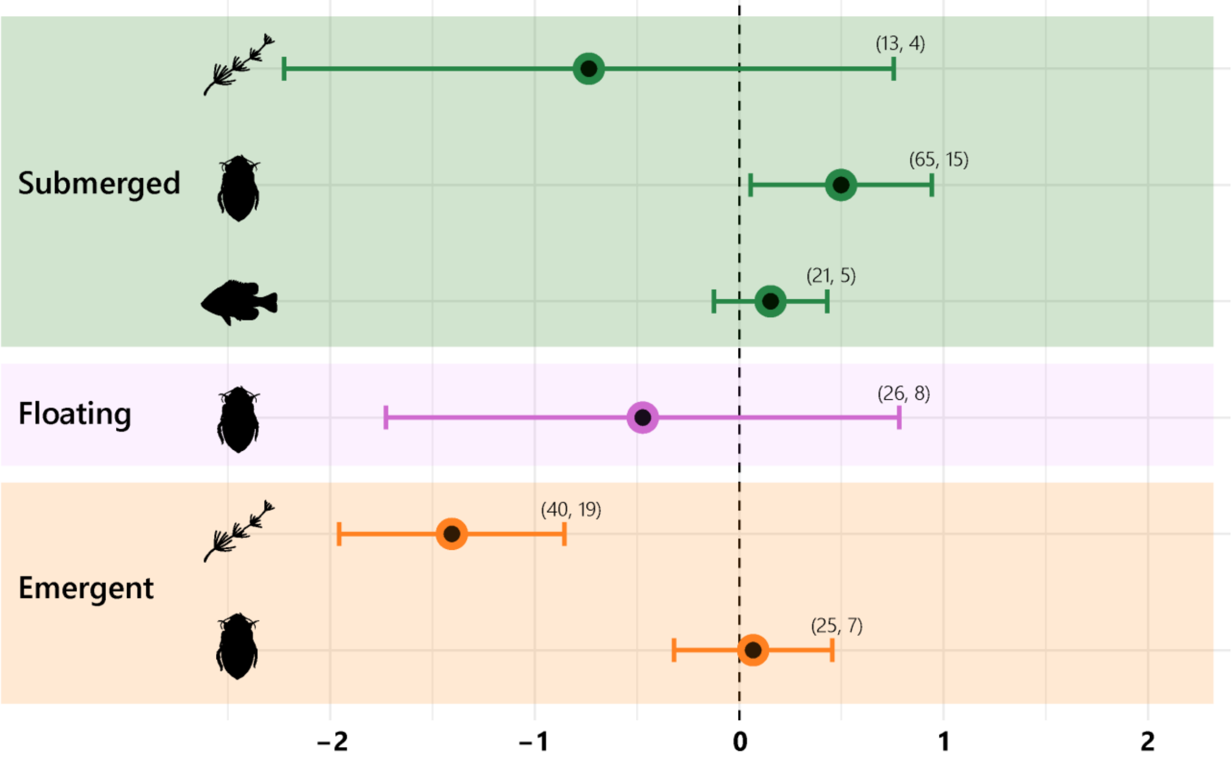

Figure 5.3. Response of resident macrophyte, macroinvertebrate and fish assemblages to submerged, floating and emergent alien macrophytes. Error bars represent 95% confidence intervals. Values in parentheses (a, b) represent: a) the number of effect sizes used in the model; b) the number of articles from which the effect sizes were sourced. Abundance and diversity results are pooled for each subgroup.

In order to determine whether this positive effect was an artefact of comparison with unvegetated control sites, I repeated the model including only those results wherein the macroinvertebrates amongst alien vegetation were compared to those amongst native vegetation (55 of 65 cases), finding a similarly positive effect (mean estimate = 0.46; 95% CI 0.09, 0.89, $P<0.05$). Submerged alien macrophyte invasion had no significant effect on resident macrophytes nor fish. Floating and emergent alien macrophytes had no significant effect on macroinvertebrates ($P>0.05$). I excluded effects of emergent alien macrophytes on fish, effects of floating alien macrophytes on fish and effects of floating alien macrophytes on macrophytes from these subgroup analyses due to insufficient data (see Section 5.3.2.3). I detected no evidence of influential outlying cases nor publication bias in these analyses. A significant amount of residual heterogeneity remained unexplained for all models, however (Table 5.5).

Table 5.5. Multilevel random effects models assessing the influence of growth form on the ecological impacts of alien macrophyte invasion. Q and I^2 provide estimates of residual heterogeneity. An I^2 value exceeding 75% indicates substantial residual heterogeneity. Abundance and diversity results are pooled for each subgroup.

Focal taxon	**Alien growth form**	**Mean effect**	**95% CI**	***P***	**Heterogeneity statistics**
Macrophytes	Submerged	-0.74	-2.23, 0.76	ns	Q = 142.84, df = 12, p <0.0001; I^2 = 94.31%
	Emergent	-1.41	-1.96, -0.85	***	Q = 258.24, df = 39, p < 0.0001; I^2 = 89.24%
Macroinvertebrates	Submerged	0.5	0.06, 0.94	*	Q = 430.51, df = 64, p < 0.0001; I^2 = 87%
	Floating	-0.47	-1.73, 0.78	ns	Q = 226.50, df = 25, p < 0.0001; I^2 = 91.57%
	Emergent	0.07	-0.32, 0.46	ns	Q = 62.09, df = 24, p < 0.0001; I^2 = 57.43%
Fish	Submerged	0.15	-0.12, 0.43	ns	Q = 53.87, df = 20, p < 0.0001; I^2 = 62.54%

When split by alien species identity, invasion by emergent *Typha* spp. caused strong resident macrophyte declines, whilst *Phragmites australis* caused weaker but still significant resident macrophyte declines (aggregated abundance and diversity). Invasion by submergent *Myriophyllum spicatum* and *Hydrilla verticillata* had significant (but somewhat weaker) positive effects on macroinvertebrates. Invasion by *Hydrilla verticillata* had a non-significant impact on fish. With *M. spicatum* and *H. verticillata* removed from the subgroup, remaining submerged macrophytes had no significant impact on macroinvertebrates (Figure 5.4, Table 5.6).

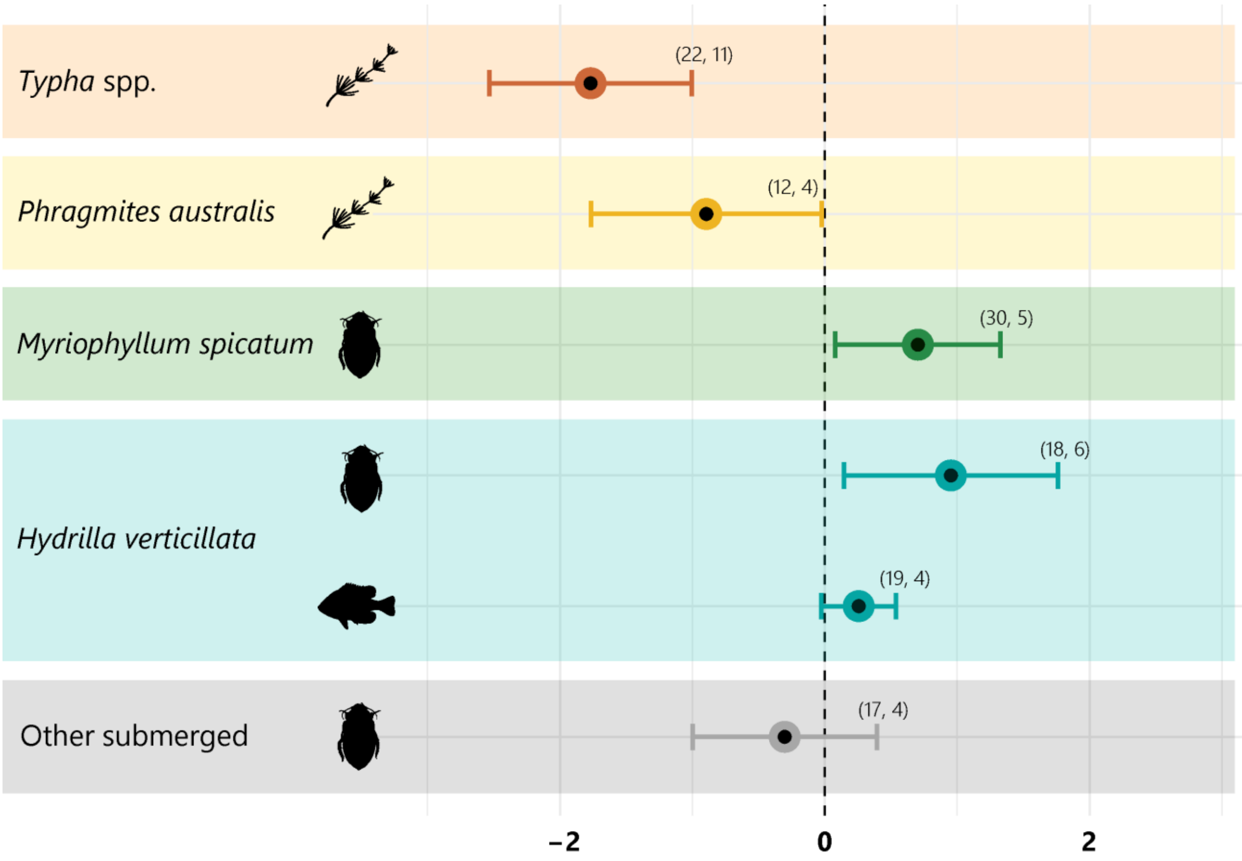

Figure 5.4. Effects of the alien macrophytes *Typha* spp., *Phragmites australis*, *Myriophyllum spicatum*, *Hydrilla verticillata* and submerged alien macrophytes excl. *M. spicatum*/*H. verticillata* on macrophyte, macroinvertebrate and fish assemblages. Error bars represent 95% confidence intervals. Values in parentheses (a, b) represent: a) the number of effect sizes used in the model; b) the number of articles from which the effect sizes were sourced. Abundance and diversity results are pooled for each subgroup.

I could not analyse the effects of *Typha* spp. or *Phragmites australis* on fish and macroinvertebrates, the effects of *Myriophyllum spicatum* on fish and macrophytes or the effects of *Hydrilla verticillata* on macrophytes in these subgroup analyses due to insufficient data. I found evidence of publication bias in the other submerged aliens: macroinvertebrates dataset. I also detected two influential outlying cases in the *H. verticillata*: macroinvertebrates dataset. Following removal of these influential cases, the impact of *H. verticillata* invasion on macroinvertebrates remained significantly positive (mean estimate = 0.43; 95% CI = 0.03, 0.83; P<0.05). A significant amount of residual heterogeneity remained unexplained for all models (Table 5.6).

Table 5.6. Multilevel random effects models assessing the effects of the alien macrophytes *Typha* spp., *Myriophyllum spicatum*, *Hydrilla verticillata* (and other submerged alien macrophytes, excl. *M. spicatum*/*H. verticillata*) on macrophyte, macroinvertebrate and fish assemblages. Q and I^2 provide estimates of residual heterogeneity. An I^2 value exceeding 75% indicates substantial residual heterogeneity. Abundance and diversity results are pooled for each subgroup.

Alien macrophyte	Focal taxon	Mean effect	95% CI	*P*	Heterogeneity statistics
Typha spp.	Macrophytes	-1.77	-2.53, -1.01	***	*Q* = 165.30, df = 21, *p* <0.0001; I^2 = 89.98%
Phragmites australis	Macrophytes	-0.89	-1.77, -0.02	*	Q = 62.31, df = 11, *p* <0.0001 I^2 = 84.18%
Myriophyllum spicatum	Macroinvertebrates	0.70	0.08, 1.33	*	*Q* = 117.69, df = 29, *p* <0.0001; I^2 = 78.6%
Hydrilla verticillata	Macroinvertebrates	0.95	0.14, 1.76	*	*Q* = 71.60, df = 17, *p* <0.0001; I^2 = 86.92%
	Fish	0.26	-0.03, 0.54	ns	*Q* = 42.11, df = 18, *p* = 0.0011; I^2 = 54.58%
Other submerged alien macrophytes	Macroinvertebrates	-0.30	-1, 0.39	ns	Q = 156.66, df = 16, *p* <0.0001 I^2 = 89.43

5.5 Discussion

Alien macrophyte invasions are often perceived as wholly negative for native freshwater assemblages (Brundu, 2014; Fleming and Dibble, 2015; Hussner *et al.*, 2017), and indeed, the meta-analyses reported here demonstrate that, in the literature I review, alien macrophytes have an overall negative impact on the taxonomic diversity of invaded assemblages (although impacts on abundance are inconsistent). At a finer resolution, however, these meta-analyses reveal a more nuanced picture. I detected a significant negative relationship between alien macrophyte invasion and resident macrophyte abundance and diversity, but found no significant, consistent effect on fish or macroinvertebrate abundance or diversity. Split by alien macrophyte growth form, I found a strong negative effect of emergent alien macrophytes on resident macrophytes, but a significant positive effect of submerged alien macrophytes on macroinvertebrates. Split by alien species identity, *Typha* spp. and *Phragmites australis* had significant negative effects on macrophytes, whilst *Myriophyllum spicatum* and *Hydrilla verticillata* had significant positive impacts on macroinvertebrates. The consistently high residual heterogeneity of my meta-regression models illustrates the prevalence of context-dependent variation in the responses of freshwater biota to alien macrophyte invasion. Some of this heterogeneity may also arise due to lags between alien macrophyte arrival and the onset of invasive proliferation, with consequent delayed impacts on resident biota (Crooks, 2005). These findings challenge the notion that alien macrophyte impacts are consistently negative (Goodenough, 2010), and reiterate the need to consider environmental context, growth form and species identity in assessments of alien macrophyte threat.

Most of the invasions included here occurred in North America. This is chiefly a reflection of the comparative dearth of quantitative studies meeting inclusion criteria which have been conducted elsewhere. Most of the alien macrophyte species included in these meta-analyses originate in the tropics, particularly the Neotropical realm – a macrophyte diversity hotspot (Murphy *et al.*, 2019).

5.5.1 Macrophytes

Despite considerable heterogeneity in the response of resident macrophytes to aliens, I detected a strong association between alien macrophyte invasion and the degradation of macrophyte assemblages. This effect, however, is chiefly driven by emergent macrophytes, notably *Phragmites australis* and the North American *Typha* species complex. Emergent invaders (e.g. *Typha x glauca*, *Phalaris arundinacea*) are typically equipped with a suite of characters (tall, fast-growing, capable of clonal integration) which readily facilitate the competitive exclusion of native macrophytes via superior resource acquisition (Galatowitsch, Anderson and Ascher, 1999; Zedler and Kercher, 2004; Michelan *et al.*, 2018; Hussner *et al.*, 2021). In addition, the fibrous litter produced by many invasive emergent taxa indirectly displaces native macrophytes through nutrient enrichment and light reduction (Farrer and Goldberg, 2009; Vaccaro, Bedford and Johnston, 2009; Holdredge and Bertness, 2011; Larkin *et al.*, 2012). Floating and submerged invasive macrophytes may also displace native vegetation (Boylen, Eichler and Madsen, 1999; Houston and Duivenvoorden, 2002; Silveira, Alves and Thomaz, 2018; Pinero-Rodríguez *et al.*, 2021), most likely via superior resource acquisition (Madsen, 1998) and/or phenotypic plasticity (Riis *et al.*, 2012; Fleming and Dibble, 2015). Displacement of native macrophytes by aliens may also be promoted by the production of plant secondary metabolites which defend the invader from herbivores

(Erhard, Pohnert and Gross, 2007; Grutters, Roijendijk, *et al.*, 2017) or inhibit the growth of native macrophyte competitors (Dandelot *et al.*, 2008; Thiébaut, Thouvenot and Rodríguez-Pérez, 2018). Although responsibility for the replacement of native vegetation is often ascribed to the alien invader itself, the competitive dominance of invasive alien macrophytes does not arise in a vacuum. Rather, competitive interactions are often mediated by extraneous factors such as climate change (Hussner *et al.*, 2014; You *et al.*, 2014; Calvo *et al.*, 2019) and anthropogenic nutrient/contaminant loading (Galatowitsch, Anderson and Ascher, 1999; Richburg, Patterson and Lowenstein, 2001; Zedler and Kercher, 2004; Chase and Knight, 2006; You *et al.*, 2014; van der Loop *et al.*, 2020). For example, eutrophication has been demonstrated to facilitate the replacement of native macrophytes by *Myriophyllum spicatum* (Chase and Knight, 2006) and promote colonisation of bare soil by *Crassula helmsii* (van der Loop *et al.*, 2020), whilst eutrophication and warming interactively promote the growth and clonal propagation of *Eichhornia crassipes* (You *et al.*, 2014). In such instances, invaders might be better considered as passengers, rather than drivers, of environmental change.

5.5.2 Macroinvertebrates

The considerable residual heterogeneity in macroinvertebrate abundance and diversity meta-regressions indicates strong context-dependent variation in the effects of alien macrophyte invasion on macroinvertebrates, and whilst the pooled effects of invasion on macroinvertebrate abundance and diversity were non-significant, invasion by submerged alien macrophytes had a significant positive effect on macroinvertebrates. In this context, it is important to remember that the physical structure provided by vegetation may be of greater importance to most macroinvertebrates than macrophyte species identity (McAbendroth *et al.*, 2005; Thomaz *et al.*, 2008). Due to the strong role played by physical structure in governing the response of macroinvertebrates to alien macrophyte invasion, macroinvertebrate abundance and diversity can increase even where a diverse native macrophyte mosaic is replaced by monotypic alien macrophyte stands, provided that structural complexity is increased (Kelly and Hawes, 2005; Hogsden, Sager and Hutchinson, 2007). The positive impacts of invasion by architecturally complex *Hydrilla verticillata* and *Myriophyllum spicatum* drive the positive effect of submerged alien macrophytes on macroinvertebrate abundance/diversity I observe in this meta-analysis, with the effect disappearing once *H. verticillata/M. spicatum* are removed from the subgroup. Whilst structural change is likely the most common determinant of the impacts of alien macrophyte invasion on macroinvertebrates, alteration of hydrochemistry might in some instances drive equally strong shifts in macroinvertebrate assemblage structure. Dense mats formed by floating alien macrophytes can reduce atmospheric exchange of oxygen, leading to declines in the abundance of hypoxia-sensitive macroinvertebrate taxa (Ceschin *et al.*, 2020, but see Kornijów, Strayer and Caraco, 2010), whilst the allelopathic exudates of alien macrophytes may alter community composition and diversity via the mortality of sensitive taxa or the deterrence of herbivores (Lindén and Lehtiniemi, 2005; Erhard, Pohnert and Gross, 2007). In general, the effects of alien macrophyte invasion may differ for oligophagous herbivorous macroinvertebrates, since shifts in macrophyte assemblage composition impact directly on their nutrition (Erhard, Pohnert and Gross, 2007; Grutters, Roijendijk, *et al.*, 2017). Similarly, alien macrophyte invasion may alter autochthonous detritus production and retention, impacting on the nutrition of detritivorous macroinvertebrates (Cuassolo, Díaz Villanueva and Modenutti, 2020). As with resident macrophytes, alien taxon identity and context are important determinants of the outcomes of alien macrophyte invasion for macroinvertebrates.

5.5.3 Fishes

Due to the comparatively large spatial scale of habitat use by many fishes, the extent to which alien macrophyte invasion affects a fish assemblage may often be dictated by the invasion's scale (Keast, 1984). As well as potentially impacting individual movement and refuge, the impacts of alien macrophyte invasion on fishes are expected to depend strongly on the effects of that invasion on prey taxa abundance and availability (Dibble and Harrel, 1997; Bickel and Closs, 2008; Carniatto *et al.*, 2020). Alien macrophyte invasion may also affect fish assemblage structure by altering interspecific interactions between fishes (Schultz and Dibble, 2012). For instance, increased structural complexity following alien macrophyte invasion may impair foraging by piscivorous fishes by enhancing availability of refugia for prey taxa (Collingsworth and Kohler, 2010), whilst secondary metabolites produced by alien macrophytes may alter the behaviour of predator or prey (Lindén and Lehtiniemi, 2005). A number of studies have detected elevated abundance of non-native fishes in alien macrophyte beds (Houston and Duivenvoorden, 2002; Kuehne, Olden and Rubenson, 2016), suggesting that alien macrophytes may facilitate alien fishes. Whilst I found no consistent effect of alien macrophyte invasion on fish abundance or diversity, it should be noted that a general trend in the impacts of alien macrophyte invasion on fish might remain undetected by this meta-analysis, given the relatively small number of fish articles which met the criteria for inclusion.

5.5.4 Conclusion

I found a significant negative impact of alien macrophyte invasion on all-taxon diversity, but considerable variation in impacts at a finer resolution. These meta-analyses should therefore serve to emphasise that, whilst growth form appears to be a major determinant of alien macrophyte impact, the effects of alien aquatic plant invasions depend strongly on species identity and ecological context. Context- and taxon-specific ecological research is therefore an irreplaceable prerequisite to the development of proportionate and cost-effective alien macrophyte management, and should be pursued wherever possible. Currently there is a notable bias (Evangelista, Magela Thomaz and Umetsu, 2014) towards the study of a handful of widely distributed, well-established invasive plants (e.g. *Myriophyllum spicatu*m, *Typha x glauca*, *Hydrilla verticillata*), reflected in the articles included in these meta-analyses. Past work has often relied on qualitative assessment of ecological impacts, and published quantitative data (suitable for meta-analysis) is lacking for a number of widespread and problematic invasive macrophytes (e.g. *Crassula helmsii, Alternanthera philoxeroides*). The publication of quantitative ecological research on such plants will better serve the evidence-based management of alien macrophytes.

Beyond abundance and diversity, the potential impacts of alien macrophyte invasion on recipient communities are, of course, unexamined by this meta-analysis. Changing community composition following alien macrophyte invasion is a pressing concern, given the threat of biotic homogenisation posed by widespread alien invaders (Olden *et al.*, 2004). Whilst the metrics included in this meta-analysis tend to correlate positively with other measures of diversity (e.g. functional diversity, β-diversity) (Strecker *et al.*, 2011; Pool, Grenouillet and Villéger, 2014; Stevens and Tello, 2014), positive effects of alien macrophyte invasion on the site-scale abundance and taxonomic α-diversity of pre-existing assemblages may mask degradation of other dimensions of biodiversity (Devictor *et al.*, 2010; Strecker *et al.*, 2011), or the degradation of biodiversity at a landscape or global scale. In biotic homogenisation, for instance, macroinvertebrate diversity could potentially increase at the site-scale due to the facilitation of widespread eurytopic species, whilst global diversity is compromised by the loss of geographically restricted endemic taxa (McKinney and Lockwood, 1999; Olden *et al.*, 2004).

As noted above, the impacts of alien macrophytes on fish remain particularly poorly studied (Evangelista, Magela Thomaz and Umetsu, 2014). Lotic systems are also understudied, despite their significance as invasion corridors (Johansson, Nilsson and Nilsson, 1996; Čuda *et al.*, 2017). Additionally, ponds and other small waterbodies are underrepresented in the primary literature reviewed here, despite their disproportionate contribution to freshwater biodiversity and their intrinsic vulnerability to invasion (Williams *et al.*, 2004; Davies *et al.*, 2008; Stiers *et al.*, 2011). Clearly, these subjects warrant further attention.

Chapter 6: General discussion

6.1 Invasive species as a threat to global biodiversity

Invasive species are proliferating worldwide, displacing geographically restricted native species and homogenising regional biotic assemblages (McKinney and Lockwood, 1999; Ricciardi, 2007; Olden, Comte and Giam, 2018; Dudgeon, 2020; IPBES, 2023). As warned by Charles Elton in his seminal book 'The Ecology of Invasions by Animals and Plants' (1958), biogeographic boundaries are beginning to blur as we witness a 'great dislocation in nature'. Invasive species are considered a leading cause of extinctions (Engbring and Fritts, 1988; Clavero and García-Berthou, 2005; Bellard, Cassey and Blackburn, 2016), and innumerable examples exist of biological invasions resulting in drastic alterations to recipient ecosystems (Ogutu-Ohwayo, 1990; Rodríguez, Bécares and Fernández-Aláez, 2003b; Li *et al.*, 2021). Oftentimes, invasive species capitalise on prior anthropogenic degradation. In these instances, disentangling the effects of invasive species from those of pre-existing stressors can be difficult, meaning invasive species may be labelled as drivers of anthropogenic degradation where, more accurately, they might be considered as passengers (Didham *et al.*, 2005; Macdougall and Turkington, 2005; Britton *et al.*, 2023). The field of invasion biology has burgeoned immensely in response to the unremitting spread of invasive species (Campbell and Simberloff, 2022), informing the prediction and management of biological invasions (Crooks, 2005; Booy *et al.*, 2017; Fournier *et al.*, 2019) and yielding insights into fundamental ecological and evolutionary processes such as speciation (Bush, 1969) and diversity-stability relationships (Lodge, 1993; McCann, 2000; Sax *et al.*, 2007). More recently, researchers have warned of persistent biases in invasion biology research, which risk overstating negative impacts and overlooking possible positive effects of invasive species (Davis *et al.*, 2011;

Schlaepfer, Sax and Olden, 2011; Boltovskoy *et al.*, 2022; Sax, Schlaepfer and Olden, 2022)

6.2 Perceptions of Crassula helmsii

Since its arrival in NW European freshwaters in the mid-20th century, *Crassula helmsii* has acquired a reputation as destructive, largely as a result of initial qualitative accounts of the disappearance of native aquatic plants following *C. helmsii* invasion (Dawson and Warman 1987; Leach and Dawson 1999). Most quantitative ecological research has focused on the impacts of *C. helmsii* on native macrophytes. Increasing *C. helmsii* cover has been correlated with reduced cover of native macrophytes (Ewald, 2014; van Kleef *et al.*, 2017), but to date no significant impacts of *C. helmsii* on plant species richness have been reported (Ewald 2014; Langdon et al. 2004; Smith and Buckley 2015). Prior to this project, the impacts of *C. helmsii* on macroinvertebrates had been little studied. Two non-peer-reviewed reports, based on research conducted in Kent and the New Forest, found no significant impact of *C. helmsii* invasion on macroinvertebrate abundance (Smith 2015) or species richness (Ewald 2014; Smith 2015). Interactions between *C. helmsii* and vertebrates have received similarly patchy attention. In laboratory trials, smooth newt (*Lissotriton vulgaris*) eggs have been shown to hatch at a later developmental stage on *C. helmsii* than on native macrophytes, although the authors found no significant difference in developmental stage at hatching for great crested newts (*Triturus cristatus*) (Langdon *et al.* 2004). A field experiment conducted in Noord-Brabant, the Netherlands, demonstrated negative impacts of *C. helmsii* on natterjack toad (*Epidalea calamita*) spawning and egg development but positive impacts on larval growth (van der Loop *et al.* 2023a).

6.3 Project findings

I set out to address this dearth of evidence with a mixture of field survey and experimentation. I conducted field surveys in multiple regions across *C. helmsii*'s invasive range to determine the typical impacts of *C. helmsii* invasion on macroinvertebrate assemblages. Concurrently, I conducted laboratory mesocosm experiments to disentangle the drivers of (putative) impacts of *C. helmsii* on macroinvertebrate consumers. Finally, I conducted a litter decomposition field experiment to elucidate the mechanistic bases for the impacts on detritivorous macroinvertebrates that were observed in field surveys. Alongside investigations of the impacts of *C. helmsii* on macroinvertebrates, I conducted a meta-analysis to contextualise findings amongst the ecological impacts of alien aquatic macrophyte taxa worldwide.

Across Belgium, the Netherlands and the UK, field surveys suggested nuanced, and not wholly negative, impacts of *C. helmsii* on macroinvertebrates. Overall, macroinvertebrate taxon richness was significantly higher within *C. helmsii* sites. This was not accompanied by elevated nestedness within *C. helmsii* sites, and habitat specialists occurring infrequently within samples (e.g. *Agabus labiatus*, *Stagnicola fuscus*) were equally likely to be found in *C. helmsii* as native sites. Clearly, it seems, the elevated taxon richness observed within *C. helmsii* sites could not be explained simply by the promotion of eurytopic taxa. Alien macroinvertebrates were more abundant amongst *C. helmsii*, in particular the detritivorous amphipod *Crangonyx pseudogracilis*, raising the possibility of facilitation. Macroinvertebrate taxonomic and functional assemblage composition shifted in *C. helmsii* sites, most markedly with respect to detritivores: *Ampullaceana balthica*, *Asellus aquaticus* and *C. pseudogracilis* were more abundant amongst *C. helmsii*, whilst *Cloeon dipterum* was more abundant in uninvaded sites. Functional composition shifted in *C. helmsii* sites towards the shredder feeding modality and dead plant food modality.

Mesocosm trials revealed unstable preferences for *C. helmsii* versus native macrophytes, which appeared to bifurcate according to the taxonomy (and traits) of the consumer. The great pond snail, *Lymnaea stagnalis*, exhibited an unstable preference for the native macrophytes *Callitriche stagnalis* and *Lythrum portula*, whereas the crustaceans *Asellus aquaticus* and *Crangonyx pseudogracilis* demonstrated a preference for *C. helmsii*. The preferences of *L. stagnalis* were modulated according to the season in which the plants were collected, suggesting that in some instances phenology, rather than macrophyte species identity, may dictate consumer preferences (Elger et al. 2006). In all consumers, preferences varied according to the degradation state of the macrophyte tissue used in trials. Stoichiometric (carbon: nitrogen) and plant defence (phenolic) analyses suggested that *C. helmsii* is nutritionally poor, but relatively poorly defended. Differences between consumers in preference for *C. helmsii* vs native macrophytes may therefore relate to trait differences, with snails perhaps better equipped than crustaceans to process phenolic defences and therefore able to consume better defended - but more nutritious - native macrophytes.

The litter decomposition field experiment revealed that *C. helmsii* was colonised by a comparable macroinvertebrate assemblage to the native macrophyte *Callitriche stagnalis*, but decomposed at a much slower rate, perhaps due to retarded microbial decomposition. The impacts of *C. helmsii* on macroinvertebrate detritivores observed in field surveys therefore appear likely to be driven by the elevated year-round availability of recalcitrant detritus from dense, perennial *C. helmsii* stands.

Meta-analyses revealed that submerged alien aquatic macrophytes typically impacted recipient macroinvertebrates positively. The mechanisms most likely to underpin this trend are elevated primary production and elevated habitat structural complexity following macrophyte invasion. These appear the most likely drivers of effects on macroinvertebrates I observed in *C. helmsii* field surveys, with detritivore composition altering due to increased availability of macrophyte detritus, and taxon richness increasing due to elevated structural complexity (Hogsden et al. 2007; Kelly and Hawes 2005).

6.3.1 Limitations of project methodology

Although, to date, the field study presented here represents the most comprehensive assessment of the impacts of *Crassula helmsii* on European macroinvertebrates, there are a handful of methodological constraints which militate against incautious generalisation of its findings to *all* situations where *C. helmsii* invades. Firstly, since the study functions as a space-for-time substitution, and successful invasion by *C. helmsii* is a non-random process determined in part by recipient biota and physicochemical conditions, one cannot say with *absolute* certainty that the differences observed between uninvaded and *C. helmsii*-invaded waterbodies are analogous to those which would occur in a single waterbody prior to- and after invasion by *C. helmsii*. This constraint was addressed insofar as possible by pairing *C. helmsii* sites with nearby (and physicochemically analogous) uninvaded sites, however. Secondly, since I sampled from dense native vegetation in uninvaded sites, the field study cannot encompass the impacts of *C. helmsii* on those macroinvertebrate taxa which are found in association with bare or relatively open substrates. This relates to the first constraint, since in *C. helmsii*-invaded sites, *C. helmsii* may have replaced bare ground rather than dense native vegetation (Ewald, 2014; Smith, 2015). Adopting this sampling protocol was necessary to avoid skewing findings, given that vegetated habitats support more diverse and abundant macroinvertebrate assemblages irrespective of macrophyte species identity. Nonetheless, it is important to note that bare-ground specialists (e.g. *Limnephilus vittatus, Caenis* spp.) may decline following *C. helmsii* invasion. More generally, *C. helmsii* invasion represents a probable risk, unencompassed by my study, to specialist floral and faunal assemblages associated with bare sediments. These include alliances of 'isoetids' such as *Litorella uniflora*, and the rare aquatic fern *Pilularia globulifera* (Ewald, 2014), through macroinvertebrate faunas and on to vertebrates including amphibians (e.g. *Epidalea calamita*: van der Loop, van Veenhuisen, *et al.*, 2023) and wading birds (Everard and Noble, 2008).

In interpreting the results of the litter decomposition field experiment, readers should bear in mind that, due to logistical constraints, the trial included only one native macrophyte comparator. As a result, I cannot be confident that the observed trends (comparable colonisation by macroinvertebrates, slower breakdown) would be borne out in comparison with *all* relevant native macrophytes, although the decomposition rate (*k*) recorded for *C. helmsii* was indeed markedly lower than those typically reported for submerged macrophytes in past studies (Chimney and Pietro, 2006). In interpreting laboratory feeding trials, readers should similarly note the number of macrophyte species against which *C. helmsii* was tested, as well as the diversity of consumers employed in trials.

6.3.2 Future research

Clearly, the field and laboratory experiments presented here could be augmented with additional native macrophyte species, and laboratory trials could be performed with additional consumers. These extensions would increase the generalisability of findings, and permit more robust inferences to be made on the mechanisms of *C. helmsii*'s impacts on macroinvertebrates. Field surveys could be extended by the addition of samples taken from unvegetated substrata, as a second control against which *C. helmsii* samples could be compared. In this way, the impacts of *C. helmsii* on macroinvertebrates associated with bare substrata could be elucidated.

Further research could also investigate other putative mechanisms of *C. helmsii* impact. Most pressingly, *C. helmsii* and native macrophyte stand fractal complexity could be quantified (McAbendroth *et al.*, 2005) and correlated with indices of macroinvertebrate diversity to determine whether, as hypothesised here, habitat structural complexity is (partially) responsible for the impacts of *C. helmsii* on macroinvertebrates. Epiphyte composition on *C. helmsii* and co-occurring native macrophytes could also be assessed, to determine whether impacts on macroinvertebrate consumers (herbivores and detritivores) are mediated by epiphyte composition (Cattaneo and Kalff, 1980; Newman, 1991; Grutters, Gross, *et al.*, 2017). Ongoing reports of the extirpation of native flora following *C. helmsii* invasion (e.g. van der Loop *et al.*, 2022) demonstrate that further research to quantify these effects is still vitally needed.

6.3.3 A perspective on management

Surprisingly, I observed limited - and not overwhelmingly negative - impacts of *Crassula helmsii* on macroinvertebrates in both field and laboratory studies. These findings should inform future approaches to management. Concern regarding invasive species in freshwaters is undeniably justified. In freshwaters, invasive species have been associated variously with declines in plant (Lodge *et al.*, 1994; Hogsden, Sager and Hutchinson, 2007; Stiers *et al.*, 2011), macroinvertebrate (Lodge *et al.*, 1994; Coetzee, Jones and Hill, 2014; Lawrence *et al.*, 2016), fish (Ogutu-Ohwayo, 1990; Schrank and Lishawa, 2019), amphibian (Nystrom *et al.*, 2001; Weldon *et al.*, 2004), mammal (Rushton *et al.*, 2000) and bird (Brzeziński *et al.*, 2020) assemblages. However, less attention has been paid to documenting those non-native species which have few negative, or indeed positive, impacts on recipient biota (Davis *et al.*, 2011; Schlaepfer, Sax and Olden, 2011; Sax, Schlaepfer and Olden, 2022). Arguably, a guilty until proven innocent mindset has arisen within invasion biology and the wider conservation movement, so that where declines in native biota occur in the presence of invasive species, the invasive(s) are implicated by default until they can be conclusively exonerated. Whilst this perspective is useful in provoking land managers to take rapid action where fragile assemblages are under threat, it is important that researchers work to quickly determine the necessity of such action, particularly where costs and/or risks of collateral damage to native biota are high. In the case of *C. helmsii*, where this field study adds to past evidence suggesting nuanced impacts on plants, macroinvertebrates and amphibians, I would suggest that in most instances, control techniques which minimise impacts to native biota may be most appropriate. The ecosystem resilience approach (van der Loop, van Kleef, *et al.*, 2023) is particularly promising in this respect, as is the development of the mite biocontrol agent *Aculus crassulae* (Knihinicki *et al.*, 2018; Varia *et al.*, 2022).

6.4 Conclusion

In the decades since its European naturalisation, *Crassula helmsii* has generally been portrayed as an aggressive menace, and a major threat to the region's freshwater biodiversity. Increasingly, such a view seems at variance with currently available evidence, at least with respect to impacts on native macrofauna. Past quantitative research has indicated that *C. helmsii* has nuanced, often subtle impacts on native plants

(Langdon *et al.*, 2004; Ewald, 2014; Smith and Buckley, 2015) and amphibians (Langdon *et al.*, 2004; van der Loop, van Veenhuisen, *et al.*, 2023), although the many non-quantitative reports of the loss of native plants from heavily invaded waterbodies should not be disregarded (Dawson and Warman, 1987; Leach and Dawson, 1999; van der Loop *et al.*, 2022). In the most comprehensive field study conducted to date, I observed only limited impacts of *C. helmsii* on macroinvertebrates, the most numerous and functionally important freshwater macrofauna, whilst laboratory and field experimentation demonstrated that the plant is readily processed by a range of macroinvertebrate consumers. Whilst every care should be taken to prevent the further spread of *C. helmsii*, and targeted control (e.g. van der Loop et al. 2023a; Varia et al. 2022) pursued where practicable, the indiscriminately destructive control techniques which have been employed in the past (Charlton, Gurney and Lyons, 2010; van der Loop *et al.*, 2018) should be viewed as a last resort, and implemented only in situations where there is a demonstrable risk to native taxa or ecosystem services.

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Appendix 1. Field study sites

Table S1. Field sampling site details. Dashes indicate absence of data.

Site	**Code**	**Region**	**Lat./long.**	**Type**	**Location**	**Land use**	**DO (%)**	**Conductivity (µS/cm)**	**Temperature**	**pH**	***C. helmsii* category**
Tredinney	TQ	SW	50.1017, -5.6456	Pond	Penwith Moors SSSI	Semi-natural	98	142	13.1	5.49	Uninvaded
Sancreed A	SA	SW	50.1049, -5.6341	Pond	Penwith Moors SSSI	Semi-natural	61	141	12.8	5.85	Abundant
Sancreed B	SB	SW	50.1055, -5.6360	Pond	Penwith Moors SSSI	Semi-natural	70	125.7	13.5	5.72	Dominant
Leech Pool	LP	SW	50.0451, -5.1874	Pond	Goonhilly Downs SSSI	Semi-natural	52	186.2	11	5.25	Uninvaded
Croft Pascoe	CP	SW	50.0348, -5.1692	Pond	Goonhilly Downs SSSI	Semi-natural	122	255	19.1	6.81	Uninvaded
Hayle Kimbro	HK	SW	50.0085, -5.2183	Pond	Goonhilly Downs SSSI	Semi-natural	82	358	20	9.6	Abundant
Breney A	BA	SW	50.4172, -4.7374	Pond	Breney Common SSSI	Semi-natural	58	71.8	17	5.5	Uninvaded
Breney B	BB	SW	50.4167, -4.7384	Pond	Breney Common SSSI	Semi-natural	34	71.9	12	6	Abundant
Yelverton	YR	SW	50.5179, -4.0369	Pond	Dartmoor NP	Semi-natural	70	61.8	14.8	4.9	Uninvaded
Yellowmeade	YQ	SW	50.5539, -4.0270	Pond	Dartmoor NP	Semi-natural	79	36.3	15.4	5.4	Uninvaded
Cadover A	CA	SW	50.4650, -4.0366	Pond	Dartmoor NP	Semi-natural	81	162	18.7	7	Dominant
Cadover B	CB	SW	50.4655, -4.0359	Pond	Dartmoor NP	Semi-natural	94	70	20	5.9	Abundant
Manor Farm A	FA	NOR	52.8546, 1.0832	Pond	Manor Farm	Agricultural	145	374	13.2	8.2	Uninvaded
Manor Farm B	FB	NOR	52.8554, 1.0879	Pond	Manor Farm	Agricultural	33	271	12	7	Uninvaded
Manor Farm C	FC	NOR	52.8532, 1.0766	Pond	Manor Farm	Agricultural	51	1175	13.5	9.4	Dominant
Manor Farm D	FD	NOR	52.8529, 1.0817	Pond	Manor Farm	Agricultural	120	326	13.5	6.8	Abundant

Manor Farm E	FE	NOR	52.8535, 1.0858	Pond	Manor Farm	Agricultural	104	259	13	8.4	Abundant
Thompson A	TA	NOR	52.5248, 0.8523	Pond	Thompson Common SSSI	Semi-natural	55	267	13.2	6.82	Uninvaded
Thompson B	TB	NOR	52.5236, 0.8480	Pond	Thompson Common SSSI	Semi-natural	41	87.5	15.4	5.75	Uninvaded
Thompson C	TC	NOR	52.5248, 0.8518	Pond	Thompson Common SSSI	Semi-natural	43	345	14.7	7.1	Patchy
Sayer's Pit	BP	NOR	52.9116, 1.1608	Pond	Bodham	Agricultural	66	536	13	6.8	Uninvaded
Marl Pit	MP	NOR	52.9036, 1.1613	Pond	Bodham	Agricultural	58	418	12.6	7.2	Patchy
Gayton A	GA	NOR	52.7301, 0.5612	Pond	West Acre Estate	Semi-natural	34	212	16.7	7.1	Uninvaded
Gayton B	GB	NOR	52.7304, 0.5628	Pond	West Acre Estate	Semi-natural	29	249.6	18.4	8.1	Uninvaded
Gayton C	GC	NOR	52.7297, 0.5604	Pond	West Acre Estate	Semi-natural	87	365.1	18.6	7.2	Uninvaded
Gayton D	GD	NOR	52.7287, 0.5635	Pond	West Acre Estate	Semi-natural	45	198	18	6.5	Uninvaded
Gayton E	GE	NOR	52.7287, 0.5613	Pond	West Acre Estate	Semi-natural	61	151.1	15.2	7.0	Dominant
Gayton F	GF	NOR	52.7280, 0.5605	Pond	West Acre Estate	Semi-natural	91	246.7	15.2	6.8	Dominant
Gayton G	GG	NOR	52.7287, 0.5604	Pond	West Acre Estate	Semi-natural	85	88.9	14.7	6.4	Dominant
Gayton H	GH	NOR	52.7291, 0.5609	Pond	West Acre Estate	Semi-natural	66	264	16.3	7.1	Dominant
Pevensey A	PA	SUS	50.8263, 0.3292	Ditch	Montague Farm	Agricultural	-	-	-	-	Uninvaded
Pevensey B	PB	SUS	50.8263, 0.3280	Ditch	Montague Farm	Agricultural	-	-	-	-	Uninvaded
Pevensey C	PC	SUS	50.8268, 0.3254	Ditch	Montague Farm	Agricultural	-	-	-	-	Uninvaded
Pevensey D	PD	SUS	50.8270, 0.3255	Ditch	Montague Farm	Agricultural	-	-	-	-	Uninvaded
Pevensey E	PE	SUS	50.8262, 0.3261	Ditch	Montague Farm	Agricultural	-	-	-	-	Dominant
Pevensey F	PF	SUS	50.8263, 0.3260	Ditch	Montague Farm	Agricultural	-	-	-	-	Abundant

Pevensey G	PG	SUS	50.8275, 0.3224	Ditch	Montague Farm	Agricultural	-	-	-	-	Dominant
Pevensey H	PH	SUS	50.8277, 0.3223	Ditch	Montague Farm	Agricultural	-	-	-	-	Dominant
Rye A	RA	SUS	50.9387, 0.7357	Lake	Rye Harbour SSSI	Semi-natural	-	-	-	-	Uninvaded
Rye B	RB	SUS	50.9386, 0.7362	Lake	Rye Harbour SSSI	Semi-natural	-	-	-	-	Uninvaded
Rye C	RC	SUS	50.9400, 0.7404	Lake	Rye Harbour SSSI	Semi-natural	-	-	-	-	Patchy
Rye D	RD	SUS	50.9393, 0.7395	Lake	Rye Harbour SSSI	Semi-natural	-	-	-	-	Patchy
Belgium A	BeA	BENE	51.2459, 4.2540	Pond	Linkeroever	Brownfield	109.4	499	22.5	8.7	Uninvaded
Belgium B	BeB	BENE	51.2548, 4.2592	Pond	Linkeroever	Brownfield	138.4	664	21.8	9.0	Abundant
Belgium C	BeC	BENE	51.2529, 4.2136	Pond	Linkeroever	Brownfield	154.4	1766	25.3	8.0	Uninvaded
Belgium D	BeD	BENE	51.2464, 4.2267	Pond	Linkeroever	Brownfield	144	264	25.9	9.4	Abundant
Belgium E	BeE	BENE	51.2456, 4.2273	Pond	Linkeroever	Brownfield	122.5	292	21.7	8.2	Dominant
Belgium F	BeF	BENE	51.2521, 4.2152	Pond	Linkeroever	Brownfield	91.5	615	25.4	7.8	Uninvaded
Belgium G	BeG	BENE	51.4133, 4.8377	Pond	Wortel-Kolonie	Semi-natural	121.9	53.3	21.1	4.7	Uninvaded
Belgium H	BeH	BENE	51.4102, 4.8338	Pond	Wortel-Kolonie	Semi-natural	114	51.1	23.6	7.0	Abundant
Belgium I	BeI	BENE	51.4145, 4.8910	Pond	Vallei van het Merkske	Semi-natural	115.6	127.9	23.8	8.1	Uninvaded
Belgium J	BeJ	BENE	51.4128, 4.8932	Pond	Vallei van het Merkske	Semi-natural	135.4	89.4	22.9	9.2	Uninvaded
Netherlands A	NeA	BENE	51.9574, 6.3145	Pond	De Zumpe	Semi-natural	-	84.2	-	9.4	Patchy
Netherlands B	NeB	BENE	51.9582, 6.3132	Pond	De Zumpe	Semi-natural	-	527	-	7.0	Uninvaded
Netherlands C	NeC	BENE	52.0265, 6.4347	Pond	Ruurlo	Semi-natural	-	51.1	-	8.2	Uninvaded
Netherlands D	NeD	BENE	52.0284, 6.4316	Pond	Ruurlo	Semi-natural	-	43.7	-	8.8	Abundant

Netherlands E	NeE	BENE	52.0811, 6.5977	Pond	Eibergen	Semi-natural	-	301	-	7.7	Patchy
Netherlands F	NeF	BENE	52.0813, 6.5975	Pond	Eibergen	Semi-natural	-	301	-	7.7	Uninvaded
Netherlands G	NeG	BENE	51.6683, 5.7788	Pond	Molenheide	Semi-natural	-	57.1	-	6.8	Abundant
Netherlands H	NeH	BENE	51.6701, 5.7808	Pond	Molenheide	Semi-natural	-	48.3	-	5.8	Uninvaded
Netherlands I	NeI	BENE	51.2693, 5.4244	Pond	De Plateaux-Hagevean	Semi-natural	-	55.1	-	6.8	Uninvaded
Netherlands J	NeJ	BENE	51.2671, 5.4201	Pond	De Plateaux-Hagevean	Semi-natural	-	208	-	7.8	Dominant

Appendix 2. Taxa of conservation concern collected during field surveys.

Scarce and/or threatened species found exclusively in *C. helmsii* sites or uninvaded sites. Species included if assigned to any rarity category in source document/expert opinion (e.g.nationally scarce/near-threatened – critically endangered).

UK		**Belgium**		**Netherlands**	
C. helmsii	**Uninvaded**	***C. helmsii***	**Uninvaded**	***C. helmsii***	**Uninvaded**
Limnoxenus niger (3 sites)	*Limnephilus binotatus (3)*	*Helophorus nubilus (1)*	*Dryops auriculatus (1)*	*Leptocerus tineiformis (2)*	*Omphiscola glabra (1)*
Hydaticus seminiger (2)	*Hydrophilus piceus (1)*	*Sigara fossarum (1)*	*Corixa panzeri (1)*	*Ilybius montanus (1)*	
Agabus labiatus (1)	*Graptodtyes flavipes (1)*	*Holocentropus stagnalis (1)*	*Glaenocorisa propinqua (1)*	*Hydroporus incognitus (1)*	
Agabus uliginosus (1)	*Hydrovatus clypealis (1)*		*Sigara stagnalis (1)*	*Enochrus nigritus (1)*	
Agabus undulatus (1)	*Segmentina nitida (1)*		*Leucorrhinia rubicunda (1)*	*Haliplus fulvus (1)*	
Dryops anglicanus (1)	*Erotesis balthica (1)*		*Sympetrum flaveolum (1)*		
D. griseus (1)					
D. similaris (1)					
Haliplus variegatus (1)					
Enochrus quadripunctatus (1)					
Bidessus unistriatus (1)					
Laccornis oblongus (1)					
Observations of scarce and/or threatened taxa (≥1 individual) in *C. helmsii* or uninvaded sites					
58	**49**	**5**	**8**	**10**	**5**

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Expert opinion – Kevin Scheers, Team Zoetwaterhabitats/ Freshwaterhabitats, Instituut voor Natuur- en Bosonderzoek (INBO)

Netherlands

Ministerie van Landbouw, Natuur en Voedselkwaliteit – red lists. Government Gazette 2004, 218 (https://minez.nederlandsesoorten.nl/)
Rarity of the macrofauna of Dutch inland waters (https://iplo.nl/thema/water/monitoring-water/kennisgroepen-monitoring/macrofaunanieuws/literatuur/literatuur/zeldzaamheid-macrofauna-nederlandse-binnenwateren/)

Appendix 3. Taxa occurring rarely in samples (≤3 sites)

United Kingdom			
Taxon	**Notes**	***C. helmsii***	**Uninvaded**
Acentria ephemerella	Phytophagous	3	0
Acilius sulcatus	Fish-free waters	0	1
Aeshna cyanea		0	1
Aeshna mixta		0	1
Agabus labiatus	Fish-free, usually ancient, temporary waters	1	0
Agabus nebulosus	Exposed fine substrate	1	0
Agabus sturmii	Meso-eutrophic lentic waters	1	1
Agabus uliginosus	Shallow fluctuating pools, typically in grassland	1	0
Agabus undulatus	Well-vegetated permanent waterbodies within fenland	1	0
Agabus unguicularis	Nutrient-rich and mesotrophic fens, with mosses or fine grasses	2	1
Agrypnia varia		0	2
Anacaena globulus	Eurytopic	1	1
Anax imperator		3	0
Bagous collignensis	Well vegetated margins	1	1
Bagous glabrirostris	Host plants *Ceratophyllum submersum, Stratiotes aloides*	2	1
Bagous limosus	Host plants *Potamogeton* spp., *Hydrocharis morsus-ranae*	1	1
Bagous puncticollis	Host plants *Stratiotes aloides, Elodea canadensis, H. morsus-ranae*	2	1
Bathyomphalus contortus	Richly vegetated permanent waters	0	2
Berosus affinis	Vegetated waters, usually permanent and eutrophic	0	2
Berosus signaticollis	Vegetated waters, often temporary	0	1
Bidessus unistriatus	Soft substrata (clay and peat), ponds and ditches w. fluctuating margins. RARE	1	0
Caenis luctuosa	Interstitial silt between gravel and stones	2	1
Callicorixa praeusta		0	1
Cercyon tristis	Vegetation and detritus in margins	2	1
Chalcolestes viridis	Tree-lined standing and slow-flowing waters	1	0
Circulionidae (larva)		0	1
Coenagrion pulchellum		1	1
Colymbetes fuscus	Mostly temporary waters	2	1
Corixa affinis		0	1
Corixa dentipes		0	1
Corixa panzeri		0	2
Corixa punctata		0	1
Cryptopleurum minutum	Vegetable detritus	1	0

Cylindrotomidae		1	1
Dolomedes sp.		2	0
Donacia clavipes	*Phragmites australis* specialist herbivore	0	1
Dryops anglicanus	Primary fen habitats	1	0
Dryops griseus	Primary fen habitats	1	0
Dryops similaris	Mainly primary fen habitats	1	0
Dytiscus marginalis	Eurytopic	0	1
Elophila nymphaeata	Phytophagous	1	0
Empididae		0	1
Enochrus fuscipennis	Acid water, often on peat and amongst *Sphagnum*	0	1
Enochrus melanocephalus	Lowland, sparesly vegetated waters, often polluted/disturbed	1	2
Enochrus quadripunctatus	Lowland, base-rich waters with some exposed mineral substrata/mesotrophic fens	1	0
Erotesis baltica	Dykes and lake-fens, amongst roots	0	1
Graptodytes flavipes	Sparsely vegetated, hard-bottom acid heathland pools, sometimes also on clay	0	1
Graptodytes granularis	Well-vegetated, permanent (fluctuating) ponds	1	0
Gyrinus marinus	Larger typically eutrophic waters	0	1
Gyrinus substriatus	Eurytopic	1	0
Haliplus flavicollis	Exposed substrata with sparse vegetation	1	1
Haliplus fulvus	Exposed substrata with sparse vegetation	2	1
Haliplus lineatocollis	Eurytopic	1	1
Haliplus obliquus	Feeds on charophytes, typically associated with base-rich waters	0	1
Haliplus variegatus	Feeds on charophytes in stagnant fens on soft peat or clay. Clean water.	1	0
Hebrus ruficeps		3	0
Helophorus alternans	Sun-exposed heathland pools (req. warmth)	1	0
Helophorus flavipes	Eurytopic in acid-neutral waters	1	0
Helophorus grandis	Eurytopic, breeds in temporary waters	2	0
Helophorus griseus	Usually in relatively base-rich waters	1	0
Helophorus obscurus	Muddy neutral - alkaline waters	1	2
Hesperocorixa moesta		2	1
Holocentropus dubius	Vegetated acid waters	1	1
Hydaticus seminiger	Dense vegetation	2	0
Hydraena testacea	Margins of rivers and ponds, rare	1	0
Hydrobius subrotundus	Acid waters, often amongst *Sphagnum*	1	1
Hydrochus elongatus	Base-rich fens, often reedbeds	2	1
Hydrophilus piceus	Permanent, richly vegetated ponds, dykes in grazing fen	0	1
Hydroporus gylenhalii	Dystrophic or mesotrophic pools	1	1
Hydroporus obscurus	Typically associated with *Sphagnum*	0	2
Hydroporus striola	Fen habitats, both recent and old	1	0

Hydrovatus clypealis	Edges of ponds on soft sediment, floating rafts of vegetation	0	1
Ilybius (larva)		1	2
Ilybius ater	Marginal, vegetated mud or peat	2	0
Ilybius chalconatus	Temporary pools, often wooded	1	0
Ilybius fenestratus	Sparsely vegetated permanent waters	1	0
Ilybius fuliginosus	Eurytopic	1	1
Ilybius quadriguttatus	Densely vegetated ponds, ditches, canals	1	0
Laccobius bipunctatus	Muddy shallows in lowland waters	0	1
Laccobius minutus	Eurytopic	0	2
Laccophilus hyalinus	Usually base-rich sites	1	0
Laccornis oblongus	Relict fenland habitat	1	0
Leptoceridae (early instar)		1	0
Lestes dryas	Densely vegetated ponds and ditches	1	2
Lestes sponsa	Small standing waters with abundant emergent plants	1	2
Libellula depressa		1	2
Libellulidae (early instar)		0	1
Limnebius truncatellus		0	1
Limnephilus auricula	Temporary grassy pools and ditches	1	0
Limnephilus binotatus	Emergent vegetation in fens, lake margins	0	3
Limnephilus decipiens		1	1
Limnephilus rhombicus		0	1
Limnephilus vittatus	Sandy or silty substrata	0	3
Limnius volckmari	Usually running water but will fly	1	0
Limnoxenus niger	Well vegetated ponds and ditches	3	0
Mesovelia furcata		1	0
Microvelia pygmaea		0	1
Microvelia reticulata		2	0
Mystacides longicornis		0	1
Nepa cinerea		1	1
Noterus crassicornis	Permanent, base-rich waters	1	1
Ochthebius dilatatus	Muddy sites	0	1
Ochthebius minimus	In vegetation	3	0
Paracymus scutellaris	Typically amongst mosses in seepage over peat	1	0
Pediciidae		1	0
Phytobius leucogaster	Host plants *Myriophyllum* spp.	2	1
Porhydrus lineatus	Patchily vegetated base-rich waters, esp. grazing fen ditches	2	0
Potamopyrgus antipodarum		1	1
Prasocuris phellandrii	Primary host plant is *Caltha palustris*	1	1
Ptychopteridae		0	1
Radix auricularia		1	0
Ranatra linearis		0	3
Rhagionidae		1	0

Rhantus frontalis	Lowland pools, amonst vegetation. Often some exposed substrata e.g. sand	0	1
Rhantus suturalis	Widespread, but mainly open base-rich sites	1	0
Segmentina nitida	Marshes and shallow drainage ditches with dense emergent vegetation	0	1
Sialis lutaria		0	2
Sigara concinna		0	1
Sigara distincta	Typically amongst emergent vegetation	0	1
Sigara dorsalis		2	0
Sigara fossarum		0	1
Sigara lateralis		0	1
Sigara nigrolineata		0	1
Sigara scotti		1	1
Stagnicola fuscus	Richly vegetated waters	1	2
Stenopelmus rufinasus		1	0
Stictonectes lepidus	Over hard peat or rock	0	1
Suphrodytes (larva)		2	0
Suphrodytes figuratus	Fen conditions in vegetation	2	0
Sympetrum flaveolum	Shallow acidic waters w. emergent vegetation	1	2
Sympetrum striolatum		1	1
Tanysphyrus lemnae	Host plant *Lemna* spp.	1	1
Thryogenes sp.		1	0
Tipulidae		2	1
Trichoceridae		1	1
Trichostegia minor	Temporary/fluctuating ponds and ditches w. abundant leaf litter	1	2
Belgium			
		C. helmsii	**Uninvaded**
Aeshna cyanea		0	1
Agabus bipustulatus		2	0
Agabus labiatus		0	1
Agabus nebulosus		2	0
Agrypnia varia		0	2
Anax parthenope		1	0
Berosus (larva)		1	1
Berosus luridus		1	1
Berosus signaticollis		1	1
Bidessus unistriatus		2	1
Coenagrion mercuriale		0	1
Coenagrion puella		1	2
Coenagrion pulchellum		0	1
Colymbetes fuscus		1	0
Corixa panzeri		0	1

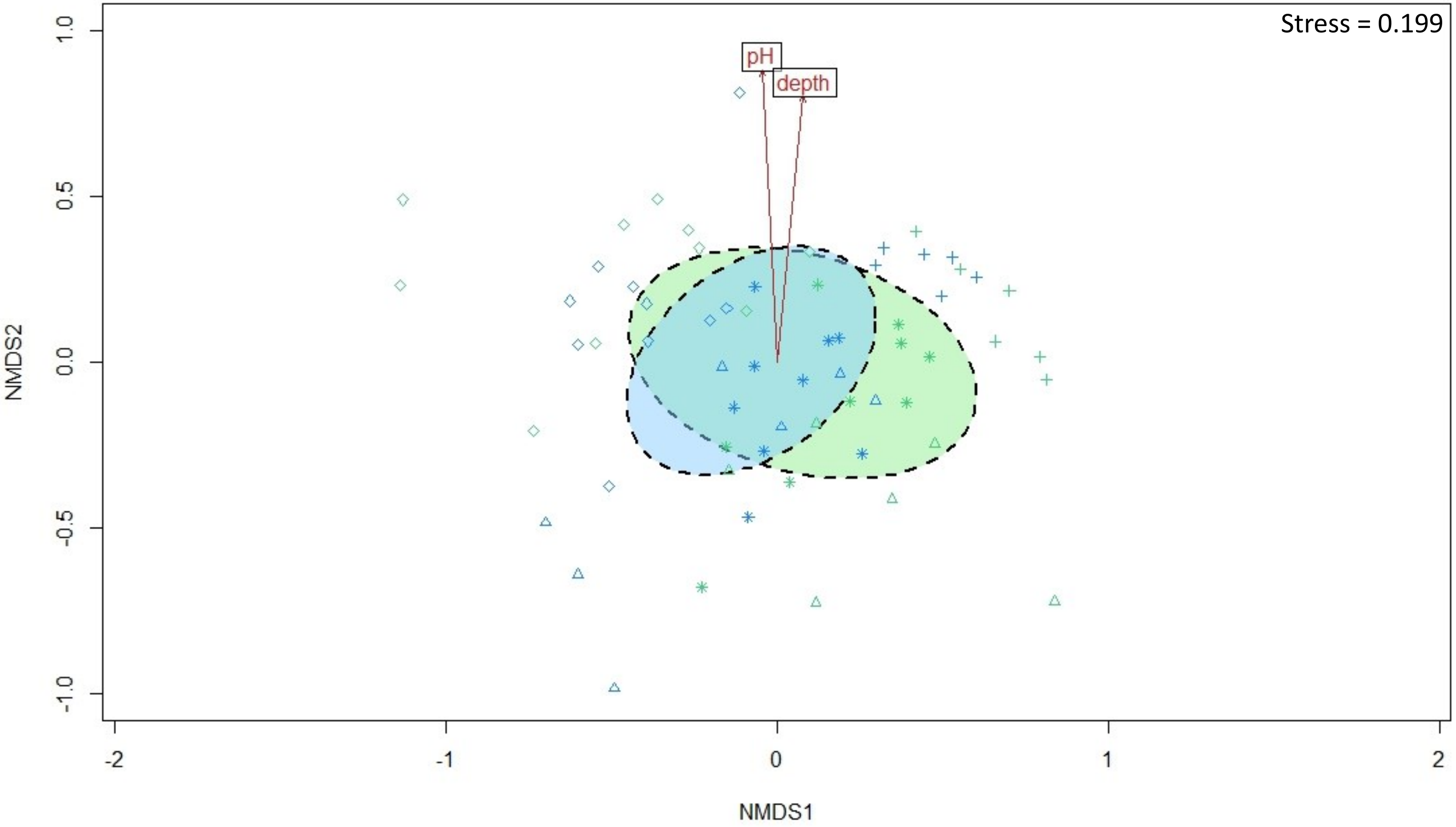
Crangonyx pseudogracilis		1	0
Crocothemis erythraea		1	2
Cymatia coleoptrata		2	0
Cymbiodyta marginellus		1	0
Dixidae		0	1
Dolichopodidae		0	1
Dryops (larva)		1	1
Dryops auriculatus		0	1
Ecnomus tenellus		1	0
Enallagma cyathigerum		2	0
Enochrus melanocephalus		1	0
Enochrus quadripunctatus		1	0
Ephydridae		1	1
Gerridae		0	1
Glaeonocorisa propinqua		0	1
Graphoderus (larva)		1	2
Gyraulus albus		1	0
Gyraulus crista		1	0
Gyrinus (larva)		1	2
Haliplus (larva)		0	2
Haliplus (*ruficollis* group)		1	0
Haliplus fulvus		1	0
Haliplus lineatocollis		1	0
Helochares lividus		1	0
Helochares punctatus		0	1
Helophorus aequalis		2	0
Helophorus brevipalpis		2	0
Helophorus grandis		2	1
Helophorus minutus		1	0
Helophorus nubilus		1	0
Hippeutis complanatus		1	0
Holocentropus stagnalis		1	0
Hydrophiloidea (larva)		0	1
Hydroporinae (larva)		0	1
Hydroporus angustatus		0	1
Hydroporus erythrocephalus		0	1
Hydroporus obscurus		0	1
Hydroporus planus		2	0
Hydroporus pubescens		1	0
Hydrovatus cuspidatus		1	1
Hygrobia hermanni		2	0
Hygrotus decoratus		1	1
Hygrotus impressopunctatus		1	0
Hyphydrus ovatus		0	1
Ilybius chalconatus		1	0
Laccobius minutus		1	0

Laccophilus minutus		1	1
Leptocerus tineiformis		2	1
Lestes sp. (early instar)		1	1
Leucorrhinia rubicunda		0	1
Libellula depressa		0	1
Libellula quadrimaculata		1	1
Limnephilus vittatus		2	1
Limoniidae		0	1
Liopterus (larva)		0	1
Liopterus haemorrhoidalis		1	1
Noterus crassicornis		0	1
Notonecta glauca		0	1
Notonecta obliqua		0	1
Nymphula stagnata		1	0
Oecetis lacustris		1	0
Oecetis ochracea		1	0
Peltodytes (lv)		1	0
Pisidium spp.		1	2
Potamopyrgus antipodarum		1	0
Rhantus suturalis		1	0
Sialis lutaria		0	1
Sigara fossarum		1	0
Sigara lateralis		1	0
Sigara scotti		2	1
Sigara stagnalis		0	1
Sympetrum danae		1	1
Sympetrum flaveolum		0	1
Sympetrum fonscolombei		2	1
Sympetrum sp. (early inst.)		1	0
Syrphidae		0	1
Tabanidae		1	1
Tipulidae		1	0
Triaenodes bicolor		0	1
Trichoptera (pupa)		1	0
Turbellaria		1	0
Netherlands			
		C. helmsii	**Uninvaded**
Aeshnidae (early instars)		1	1
Agabus bipustulatus		1	0
Ampullaceana balthica		2	1
Anacaena limbata		1	0
Anacaena lutescens		1	0
Asellus aquaticus		1	2
Athripsodes aterrimus		1	1

Bidessus unistriatus		2	1
Caenis luctuosa		1	2
Coenagrion pulchellum		0	1
Cordulia aenea		1	0
Crangonyx pseudogracilis		0	2
Crocothemis erythraea		2	0
Culicidae		1	0
Cybister lateralimarginalis		1	0
Cymatia coleoptrata		1	1
Dixidae		1	0
Dryops (larva)		0	2
Dryops luridus		1	0
Dytiscus (larva)		2	0
Enallagma cyathigerum		0	1
Enochrus coarctatus		1	0
Enochrus nigritus		1	0
Enochrus ochropterus		0	1
Enochrus testaceus		1	0
Ephydridae		0	1
Ferrissia wautieri		2	1
Gerridae		1	0
Graphoderus zonatus		1	1
Gyraulus albus		0	1
Haliplus (larva)		1	0
Haliplus (*ruficollis* group)		1	0
Haliplus confinis		0	1
Haliplus flavicollis		1	1
Haliplus fluviatilis		1	0
Haliplus fulvus		1	0
Haliplus immaculatus		1	0
Helochares lividus		1	0
Helochares punctatus		1	0
Helophorus aequalis		1	0
Helophorus brevipalpis		1	0
Helophorus grandis		1	0
Hesperocorixa castanea		1	0
Hesperocorixa sahlbergi		0	1
Hippeutis complanatus		1	1
Hirudinea		1	2
Hydroglyphus geminus		2	0
Hydrophiloidea (larva)		1	1
Hydroporinae (larva)		1	1
Hydroporus erythrocephalus		2	0
Hydroporus incognitus		1	0
Hydroporus planus		1	0
Hydroporus pubescens		1	0

Hygrotus decoratus		1	0
Hygrotus impressopunctatus		2	0
Hygrotus inaequalis		1	1
Hyphydrus ovatus		1	1
Ilybius montanus		1	0
Leptocerus tineiformis		2	0
Lestes sp. (early instar)		1	1
Libellula depressa		1	1
Limnephilidae (early instar)		1	0
Limnephilus flavicornis		0	1
Limnephilus vittatus		1	2
Liopterus (larva)		0	1
Lymnaea stagnalis		1	1
Microvelia reticulata		1	0
Nartus (larva)		1	0
Nymphula stagnata		1	0
Oecetis lacustris		0	2
Omphiscola glabra		0	1
Peltodytes caesus		1	0
Planorbarius corneus		1	1
Planorbis planorbis		0	1
Psychodidae		2	1
Ranatra linearis		1	0
Rhantus exsoletus		1	1
Sigara limitata		1	0
Sigara scotti		2	1
Sphaerium		1	0
Sympetrum danae		2	1
Sympetrum flaveolum		0	1
Sympetrum fonscolombei		2	1
Sympetrum sanguineum		1	2
Trichoptera (pupa)		0	2
Turbellaria		3	0
Vavata cristata		1	1

Appendix 4. Taxonomic assemblage composition ordination



Non-metric multidimensional scaling (nMDS) plot derived from 4th root transformed abundances of macroinvertebrate taxa, illustrating macroinvertebrate community composition in *C. helmsii* (green) and uninvaded (blue) sites. Confidence ellipses show 1SD around centroid for *C. helmsii/*uninvaded sites. Symbols denote regions: Δ SW, + SUS, * NOR, ◊ BENE.

Appendix 5. Habitats, study types and control types in meta-analysis database.

Habitat	*Details*	*Study type*	*Control type*	*Species*	*Cases*
Lentic	**Backwater**	Observational	**Native vegetation**	*Hymenachne amplexicaulis*	9
	Canal	Observational	**Uninvaded**	*Myriophyllum aquaticum*	4
	Lake	Manipulative	**Uninvaded**	*Hydrocharis morsus-ranae*	6
		Observational	**Before-after**	*Myriophyllum spicatum*	4
			Native vegetation	*Cabomba caroliniana*	4
				Eichhornia crassipes	6
				Hydrilla verticillata	14
				Myriophyllum spicatum	28
			No vegetation	*Hydrilla verticillata*	2
			Uninvaded	*Hydrilla verticillata*	8
				Myriophyllum spicatum	6
	Lake/ backwater	Observational	**Native vegetation**	*Eichhornia crassipes*	3
				Elodea canadensis	3
				Hydrilla verticillata	3
				Lagarosiphon major	3
				Ranunculus fluitans	3
	Pond	Observational	**Uninvaded**	*Hydrocotyle ranunculoides*	1
				Ludwigia grandiflora	1
				Myriophyllum aquaticum	1
				Lemna minuta	1
	Reservoir	Manipulative	**Treated plot**	*Lagarosiphon major*	6
Lotic	**Freshwater estuary**	Observational	**Native vegetation**	*Trapa natans*	1
			No vegetation	*Trapa natans*	1
	River	Observational	**Native vegetation**	*Egeria densa*	1
				Hydrilla verticillata	7
				Myriophyllum aquaticum	1
				Myriophyllum spicatum	2
				Trapa natans	2
	Stream	Observational	**Native vegetation**	*Ceratopteris thalictroides*	1
	Tidal freshwater	Manipulative	**No vegetation**	*Hydrilla verticillata*	1
		Observational	**Native vegetation**	*Eichhornia crassipes*	5
			No vegetation	*Hydrilla verticillata*	2

Habitat	*Study type*	*Control type*	*Species*	*Cases*
Wetland	Manipulative	**Before-after**	*Phragmites australis*	9
			Typha spp.	2
		Treated plot	*Phragmites australis*	2
			Typha spp.	15
	Observational	**Native vegetation**	*Lythrum salicaria*	1
			Phragmites australis	1
			Typha spp.	9
			Urochloa mutica	8
		Uninvaded	*Lythrum salicaria*	1
			Phragmites australis	1
			Typha spp.	5
Mesocosm	Manipulative	**Native vegetation**	*Hydrilla verticillata*	1
		No vegetation	*Hydrilla verticillata*	1
		Uninvaded	*Azolla filiculoides*	2
			Pistia stratiotes	3
			Typha spp.	1

Appendix 6. Summary of primary research cases included in meta-analysis

Focal taxon	**Alien growth form**	**Alien species**	**Location**	**Climate**	**Habitat**	**Study type**	**Metric**	**Control**	**Effect size**	**SE**	**Lead author, Year**	**Title**
Macrophytes	Submerged	*Myriophyllum spicatum*	Horseshoe Lake, Midwest USA	Temperate	Lake	Observational	Biomass	Uninvaded	**-0.618**	**0.324**	Van Goethem, 2020	Effects of Invasive Watermilfoil on Primary Production in Littoral Zones of North-Temperate Lakes
Macrophytes	Submerged	*Myriophyllum spicatum*	Iron Lake, Midwest USA	Temperate	Lake	Observational	Biomass	Uninvaded	**-0.027**	**0.316**	Van Goethem, 2020	Effects of Invasive Watermilfoil on Primary Production in Littoral Zones of North-Temperate Lakes
Macrophytes	Submerged	*Myriophyllum spicatum*	Islington Bay, Midwest USA	Temperate	Lake	Observational	Biomass	Uninvaded	**-1.146**	**0.342**	Van Goethem, 2020	Effects of Invasive Watermilfoil on Primary Production in Littoral Zones of North-Temperate Lakes
Macrophytes	Submerged	*Myriophyllum spicatum*	Sturgeon Sloughs, Midwest USA	Temperate	Lake	Observational	Biomass	Uninvaded	**-0.116**	**0.317**	Van Goethem, 2020	Effects of Invasive Watermilfoil on Primary Production in Littoral Zones of North-Temperate Lakes
Macrophytes	Submerged	*Myriophyllum spicatum*	Lake St. Helen, Midwest USA	Temperate	Lake	Observational	Biomass	Uninvaded	**-0.650**	**0.325**	Van Goethem, 2020	Effects of Invasive Watermilfoil on Primary Production in Littoral Zones of North-Temperate Lakes
Macrophytes	Submerged	*Myriophyllum spicatum*	Torch Lake, Midwest USA	Temperate	Lake	Observational	Biomass	Uninvaded	**0.088**	**0.316**	Van Goethem, 2020	Effects of Invasive Watermilfoil on Primary Production in Littoral Zones of North-Temperate Lakes
Macrophytes	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Richness	Uninvaded	**0.743**	**0.405**	Hoyer, 2008	Lack of exotic hydrilla infestation effects on plant, fish and aquatic bird community measures
Macrophytes	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Richness	Uninvaded	**0.774**	**0.602**	Hoyer, 2008	Lack of exotic hydrilla infestation effects on plant, fish and aquatic bird community measures
Macrophytes	Submerged	*Cabomba caroliniana*	Ontario, Canada	Temperate	Lake	Observational	Shannon	Native vegetation	**-0.404**	**0.477**	Hogsden, 2007	The Impacts of the Non-native Macrophyte Cabomba caroliniana on Littoral Biota of Kasshabog Lake, Ontario

Macrophytes	Submerged	*Myriophyllum spicatum*	New York, USA	Temperate	Lake	Observational	Richness	Before-after	**-3.782**	**0.477**	Boylen, 1999	Loss of native aquatic plant species in a community dominated by Eurasian watermilfoil
Macrophytes	Submerged	*Myriophyllum spicatum*	New York, USA	Temperate	Lake	Observational	Richness	Before-after	**-2.603**	**0.387**	Boylen, 1999	Loss of native aquatic plant species in a community dominated by Eurasian watermilfoil
Macrophytes	Submerged	*Myriophyllum spicatum*	New York, USA	Temperate	Lake	Observational	Richness	Before-after	**-2.340**	**0.369**	Boylen, 1999	Loss of native aquatic plant species in a community dominated by Eurasian watermilfoil
Macrophytes	Submerged	*Myriophyllum spicatum*	New York, USA	Temperate	Lake	Observational	Richness	Before-after	**-2.720**	**0.395**	Boylen, 1999	Loss of native aquatic plant species in a community dominated by Eurasian watermilfoil
Macrophytes	Floating	*Azolla filiculoides*	SW Spain	Subtropical	n/a	Mesocosm	Biomass	Uninvaded	**-3.142**	**0.536**	Pinero-Rodriguez, 2020	The invasive aquatic fern Azolla filiculoides negatively impacts water quality, aquatic vegetation and amphibian larvae in Mediterranean environments
Macrophytes	Floating	*Azolla filiculoides*	SW Spain	Subtropical	n/a	Mesocosm	Richness	Uninvaded	**-1.377**	**0.395**	Pinero-Rodriguez, 2020	The invasive aquatic fern Azolla filiculoides negatively impacts water quality, aquatic vegetation and amphibian larvae in Mediterranean environments
Macrophytes	Floating	*Hydrocotyle ranunculoides*	Belgium	Temperate	Pond	Observational	Richness	Uninvaded	**-1.038**	**0.346**	Stiers, 2011	Impact of three aquatic invasive species on native plants and macroinvertebrates in temperate ponds
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Manipulative	Richness	Treated plot	**-1.883**	**0.388**	Lishawa, 2020	Wetland Waterbird Food Resources Increased by Harvesting Invasive Cattails
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Manipulative	Shannon	Treated plot	**-1.325**	**0.356**	Lishawa, 2020	Wetland Waterbird Food Resources Increased by Harvesting Invasive Cattails
Macrophytes	Emergent	*Phragmites australis*	Midwest USA	Temperate	Wetland	Manipulative	Richness	Treated plot	**-1.721**	**0.702**	Bonello, 2020	Plant community recovery after herbicide management to remove Phragmites australis in Great Lakes coastal wetlands

Macrophytes	Emergent	*Phragmites australis*	Midwest USA	Temperate	Wetland	Manipulative	Shannon	Treated plot	**-4.705**	**1.160**	Bonello, 2020	Plant community recovery after herbicide management to remove Phragmites australis in Great Lakes coastal wetlands
Macrophytes	Emergent	*Typha*	Midwest USA	Temperate	Wetland	Manipulative	Richness	Before-after	**-1.150**	**0.916**	Lishawa, 2019	Invasive species removal increases species and phylogenetic diversity of wetland plant communities
Macrophytes	Emergent	*Typha*	Midwest USA	Temperate	Wetland	Manipulative	Richness	Treated plot	**-0.702**	**0.855**	Lishawa, 2019	Invasive species removal increases species and phylogenetic diversity of wetland plant communities
Macrophytes	Emergent	*Typha*	Midwest USA	Temperate	Wetland	Observational	Richness	Native vegetation	**-0.274**	**0.410**	Schrank, 2019	Invasive cattail reduces fish diversity and abundance in the emergent marsh of a Great Lakes coastal wetland
Macrophytes	Emergent	*Typha*	Midwest USA	Temperate	Wetland	Observational	Shannon	Native vegetation	**-0.247**	**0.410**	Schrank, 2019	Invasive cattail reduces fish diversity and abundance in the emergent marsh of a Great Lakes coastal wetland
Macrophytes	Emergent	*Phragmites australis*	Point Mouillee, Midwest USA	Temperate	Wetland	Manipulative	Richness	Before-after	**-1.417**	**0.723**	Judd, 2019	Short-term impacts of Phragmites management on nutrient budgets and plant communities in Great Lakes coastal freshwater marshes
Macrophytes	Emergent	*Phragmites australis*	Point Mouillee, Midwest USA	Temperate	Wetland	Manipulative	Shannon	Before-after	**-1.286**	**0.708**	Judd, 2019	Short-term impacts of Phragmites management on nutrient budgets and plant communities in Great Lakes coastal freshwater marshes
Macrophytes	Emergent	*Phragmites australis*	Brancheau, Midwest USA	Temperate	Wetland	Manipulative	Richness	Before-after	**0.526**	**0.646**	Judd, 2019	Short-term impacts of Phragmites management on nutrient budgets and plant communities in Great Lakes coastal freshwater marshes

Macrophytes	Emergent	*Phragmites australis*	Brancheau, Midwest USA	Temperate	Wetland	Manipulative	Shannon	Before-after	**0.389**	**0.640**	Judd, 2019	Short-term impacts of Phragmites management on nutrient budgets and plant communities in Great Lakes coastal freshwater marshes
Macrophytes	Emergent	*Phragmites australis*	NY, USA	Temperate	Wetland	Manipulative	Cover	Before-after	**1.951**	**0.551**	Zimmerman, 2018	Native Plant Recovery following Three Years of Common Reed (Phragmites australis) Control
Macrophytes	Emergent	*Phragmites australis*	NY, USA	Temperate	Wetland	Manipulative	Cover	Before-after	**-0.887**	**0.471**	Zimmerman, 2018	Native Plant Recovery following Three Years of Common Reed (Phragmites australis) Control
Macrophytes	Emergent	*Phragmites australis*	NY, USA	Temperate	Wetland	Manipulative	Cover	Before-after	**-1.787**	**0.536**	Zimmerman, 2018	Native Plant Recovery following Three Years of Common Reed (Phragmites australis) Control
Macrophytes	Emergent	*Phragmites australis*	NY, USA	Temperate	Wetland	Manipulative	Cover	Before-after	**-1.513**	**0.512**	Zimmerman, 2018	Native Plant Recovery following Three Years of Common Reed (Phragmites australis) Control
Macrophytes	Emergent	*Phragmites australis*	NY, USA	Temperate	Wetland	Manipulative	Cover	Before-after	**-1.993**	**0.555**	Zimmerman, 2018	Native Plant Recovery following Three Years of Common Reed (Phragmites australis) Control
Macrophytes	Emergent	*Myriophyllum aquaticum*	Central Italy	Subtropical	Canal	Observational	Richness	Uninvaded	**-1.816**	**0.538**	Lastrucci, 2018	Impacts of Myriophyllum aquaticum invasion in a Mediterranean wetland on plant and macroarthropod communities
Macrophytes	Emergent	*Myriophyllum aquaticum*	Central Italy	Subtropical	Canal	Observational	Shannon	Uninvaded	**-1.985**	**0.554**	Lastrucci, 2018	Impacts of Myriophyllum aquaticum invasion in a Mediterranean wetland on plant and macroarthropod communities
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Manipulative	Richness	Before-after	**-3.280**	**0.791**	Lishawa, 2015	Biomass harvest of invasive Typha promotes plant diversity in a Great Lakes coastal wetland
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Manipulative	Shannon	Treated plot	**-6.085**	**1.243**	Lishawa, 2015	Biomass harvest of invasive Typha promotes plant diversity in a Great Lakes coastal wetland
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	n/a	Mesocosm	Biomass	Uninvaded	**-1.856**	**0.782**	Lishawa, 2014	Denitrification in a Laurentian Great Lakes coastal wetland invaded by hybrid cattail (Typha x glauca)

Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Manipulative	Density	Treated plot	**-2.527**	**0.612**	Farrer, 2014	Mechanisms and reversibility of the effects of hybrid cattail on a Great Lakes marsh
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Manipulative	Density	Treated plot	**-2.920**	**0.961**	Farrer, 2014	Mechanisms and reversibility of the effects of hybrid cattail on a Great Lakes marsh
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Manipulative	Richness	Treated plot	**-5.927**	**1.598**	Farrer, 2014	Mechanisms and reversibility of the effects of hybrid cattail on a Great Lakes marsh
Macrophytes	Emergent	*Typha*	NW Costa Rica	Tropical	Wetland	Manipulative	Richness	Treated plot	**-2.260**	**0.472**	Osland, 2011	Restoring diversity after cattail expansion: disturbance, resilience, and seasonality in a tropical dry wetland
Macrophytes	Emergent	*Typha*	NW Costa Rica	Tropical	Wetland	Manipulative	Shannon	Treated plot	**-2.766**	**0.518**	Osland, 2011	Restoring diversity after cattail expansion: disturbance, resilience, and seasonality in a tropical dry wetland
Macrophytes	Emergent	*Ludwigia grandiflora*	Belgium	Temperate	Pond	Observational	Richness	Uninvaded	**-2.099**	**0.392**	Stiers, 2011	Impact of three aquatic invasive species on native plants and macroinvertebrates in temperate ponds
Macrophytes	Emergent	*Myriophyllum aquaticum*	Belgium	Temperate	Pond	Observational	Richness	Uninvaded	**-0.948**	**0.358**	Stiers, 2011	Impact of three aquatic invasive species on native plants and macroinvertebrates in temperate ponds
Macrophytes	Emergent	*Typha*	Midwest USA	Temperate	Wetland	Observational	Richness	Uninvaded	**-2.204**	**0.416**	Mitchell, 2011	Time-Dependent Impacts of Cattail Invasion in a Great Lakes Coastal Wetland Complex
Macrophytes	Emergent	*Typha*	Midwest USA	Temperate	Wetland	Observational	Shannon	Uninvaded	**-1.784**	**0.387**	Mitchell, 2011	Time-Dependent Impacts of Cattail Invasion in a Great Lakes Coastal Wetland Complex
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Observational	Shannon	Uninvaded	**-0.448**	**0.192**	Lishawa, 2010	Water Level Decline Promotes Typha X glauca Establishment and Vegetation Change in Great Lakes Coastal Wetlands

Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Observational	Richness	Uninvaded	-0.526	**0.193**	Lishawa, 2010	Water Level Decline Promotes Typha X glauca Establishment and Vegetation Change in Great Lakes Coastal Wetlands
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Observational	Richness	Uninvaded	0.827	**0.253**	Lishawa, 2010	Water Level Decline Promotes Typha X glauca Establishment and Vegetation Change in Great Lakes Coastal Wetlands
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Manipulative	Density	Treated plot	-1.099	**0.483**	Farrer, 2009	Litter drives ecosystem and plant community changes in cattail invasion
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Manipulative	Richness	Treated plot	-0.498	**0.455**	Farrer, 2009	Litter drives ecosystem and plant community changes in cattail invasion
Macrophytes	Emergent	*Lythrum salicaria*	New York, USA	Temperate	Wetland	Observational	Biomass	Native vegetation	-0.219	**0.317**	Mahaney, 2006	Impacts of Lythrum salicaria invasion on plant community and soil properties in two wetlands in central New York, USA
Macrophytes	Emergent	*Typha x glauca*	Midwest USA	Temperate	Wetland	Observational	Shannon	Native vegetation	-3.238	**0.737**	Angeloni, 2006	Effects of an invasive cattail species (Typha × glauca) on sediment nitrogen and microbial community composition in a freshwater wetland
Macrophytes	Emergent	*Phragmites australis*	Massachusetts, USA	Temperate	Wetland	Observational	Richness	Uninvaded	0.498	**0.547**	Richburg, 2001	Effects of road salt and Phragmites australis invasion on the vegetation of a Western Massachusetts calcareous lake-basin fen
Macrophytes	Emergent	*Lythrum salicaria*	Canada	Temperate	Wetland	Observational	Richness	Uninvaded	0.051	**0.312**	Treberg, 1999	Relationship between the abundance of Lythrum salicaria (purple loosestrife) and plant species richness along the Bar River, Canada
Macroinvertebrates	Submerged	*Hydrilla verticillata*	S Brazil	Subtropical	River	Observational	Density	Native vegetation	1.291	**0.644**	Carniatto, 2020	An invasive and a native macrophyte species provide similar feeding habitat for fish
Macroinvertebrates	Submerged	*Hydrilla verticillata*	S Brazil	Subtropical	River	Observational	Richness	Native vegetation	0.474	**0.587**	Carniatto, 2020	An invasive and a native macrophyte species provide similar feeding habitat for fish

Macroinvertebrates	Submerged	*Hydrilla verticillata*	S Brazil	Subtropical	River	Observational	Shannon	Native vegetation	**0.127**	**0.578**	Carniatto, 2020	An invasive and a native macrophyte species provide similar feeding habitat for fish
Macroinvertebrates	Submerged	*Egeria densa*	Washington State, USA	Temperate	River	Observational	Shannon	Native vegetation	**1.941**	**1.563**	Kuehne, 2016	Multi-trophic impacts of an invasive aquatic plant
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Pennsylvania	Temperate	Lake	Observational	Density	Native vegetation	**0.906**	**0.880**	Baron, 2010	The Effects of Macrophyte Tannins on the Epiphytic Macroinvertebrate Assemblages in Sandy Lake, Pennsylvania
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Pennsylvania	Temperate	Lake	Observational	Density	Native vegetation	**0.343**	**0.826**	Baron, 2010	The Effects of Macrophyte Tannins on the Epiphytic Macroinvertebrate Assemblages in Sandy Lake, Pennsylvania
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Pennsylvania	Temperate	Lake	Observational	Density	Native vegetation	**1.278**	**0.938**	Baron, 2010	The Effects of Macrophyte Tannins on the Epiphytic Macroinvertebrate Assemblages in Sandy Lake, Pennsylvania
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Pennsylvania	Temperate	Lake	Observational	Density	Native vegetation	**1.471**	**0.974**	Baron, 2010	The Effects of Macrophyte Tannins on the Epiphytic Macroinvertebrate Assemblages in Sandy Lake, Pennsylvania
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Eastern USA	Temperate	Lake	Observational	Biomass	Native vegetation	**-0.452**	**0.564**	Wilson, 2009	Epiphytic macroinvertebrate communities on Eurasian watermilfoil (Myriophyllum spicatum) and native milfoils Myriophyllum sibericum and Myriophyllum alterniflorum in eastern North America
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Eastern USA	Temperate	Lake	Observational	Biomass	Native vegetation	**-0.962**	**0.501**	Wilson, 2009	Epiphytic macroinvertebrate communities on Eurasian watermilfoil (Myriophyllum spicatum) and native milfoils Myriophyllum sibericum and Myriophyllum alterniflorum in eastern North America

Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Eastern USA	Temperate	River	Observational	Biomass	Native vegetation	**-1.269**	**0.593**	Wilson, 2009	Epiphytic macroinvertebrate communities on Eurasian watermilfoil (Myriophyllum spicatum) and native milfoils Myriophyllum sibericum and Myriophyllum alterniflorum in eastern North America
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Eastern USA	Temperate	Lake	Observational	Biomass	Native vegetation	**0.021**	**0.333**	Wilson, 2009	Epiphytic macroinvertebrate communities on Eurasian watermilfoil (Myriophyllum spicatum) and native milfoils Myriophyllum sibericum and Myriophyllum alterniflorum in eastern North America
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Eastern USA	Temperate	Lake	Observational	Density	Native vegetation	**-0.579**	**0.570**	Wilson, 2009	Epiphytic macroinvertebrate communities on Eurasian watermilfoil (Myriophyllum spicatum) and native milfoils Myriophyllum sibericum and Myriophyllum alterniflorum in eastern North America
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Eastern USA	Temperate	Lake	Observational	Density	Native vegetation	**1.124**	**0.511**	Wilson, 2009	Epiphytic macroinvertebrate communities on Eurasian watermilfoil (Myriophyllum spicatum) and native milfoils Myriophyllum sibericum and Myriophyllum alterniflorum in eastern North America
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Eastern USA	Temperate	River	Observational	Density	Native vegetation	**-1.190**	**0.586**	Wilson, 2009	Epiphytic macroinvertebrate communities on Eurasian watermilfoil (Myriophyllum spicatum) and native milfoils Myriophyllum sibericum and Myriophyllum alterniflorum in eastern North America
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Eastern USA	Temperate	Lake	Observational	Density	Native vegetation	**0.580**	**0.341**	Wilson, 2009	Epiphytic macroinvertebrate communities on Eurasian watermilfoil (Myriophyllum spicatum) and native milfoils Myriophyllum sibericum and Myriophyllum alterniflorum in eastern North America

Macroinvertebrates	Submerged	*Lagarosiphon major*	New Zealand	Temperate	Reservoir	Manipulative	Shannon	Treated plot	**0.000**	**0.200**	Bickel, 2009	Impact of Partial Removal of the Invasive Macrophyte Lagarosiphon Major (Hydrocharitaceae) on Invertebrates and Fish
Macroinvertebrates	Submerged	*Lagarosiphon major*	New Zealand	Temperate	Reservoir	Manipulative	Richness	Treated plot	**0.346**	**0.202**	Bickel, 2009	Impact of Partial Removal of the Invasive Macrophyte Lagarosiphon Major (Hydrocharitaceae) on Invertebrates and Fish
Macroinvertebrates	Submerged	*Lagarosiphon major*	New Zealand	Temperate	Reservoir	Manipulative	Biomass	Treated plot	**-1.329**	**0.221**	Bickel, 2009	Impact of Partial Removal of the Invasive Macrophyte Lagarosiphon Major (Hydrocharitaceae) on Invertebrates and Fish
Macroinvertebrates	Submerged	*Lagarosiphon major*	New Zealand	Temperate	Reservoir	Manipulative	Density	Treated plot	**-3.267**	**0.307**	Bickel, 2009	Impact of Partial Removal of the Invasive Macrophyte Lagarosiphon Major (Hydrocharitaceae) on Invertebrates and Fish
Macroinvertebrates	Submerged	*Hydrilla verticillata*	Mississippi, USA	Subtropical	n/a	Mesocosm	Richness	Native vegetation	**1.677**	**1.441**	Theel, 2008	Differential influence of a monotypic and diverse native aquatic plant bed on a macroinvertebrate assemblage; an experimental implication of exotic plant induced habitat
Macroinvertebrates	Submerged	*Hydrilla verticillata*	Mississippi, USA	Subtropical	n/a	Mesocosm	Richness	No vegetation	**4.312**	**2.849**	Theel, 2008	Differential influence of a monotypic and diverse native aquatic plant bed on a macroinvertebrate assemblage; an experimental implication of exotic plant induced habitat
Macroinvertebrates	Submerged	*Cabomba caroliniana*	Ontario, Canada	Temperate	Lake	Observational	Density	Native vegetation	**1.076**	**0.508**	Hogsden, 2007	The Impacts of the Non-native Macrophyte Cabomba caroliniana on Littoral Biota of Kasshabog Lake, Ontario
Macroinvertebrates	Submerged	*Cabomba caroliniana*	Ontario, Canada	Temperate	Lake	Observational	Biomass	Native vegetation	**-1.771**	**0.564**	Hogsden, 2007	The Impacts of the Non-native Macrophyte Cabomba caroliniana on Littoral Biota of Kasshabog Lake, Ontario

Macroinvertebrates	Submerged	*Cabomba caroliniana*	Ontario, Canada	Temperate	Lake	Observational	Shannon	Native vegetation	**-0.278**	**0.474**	Hogsden, 2007	The Impacts of the Non-native Macrophyte Cabomba caroliniana on Littoral Biota of Kasshabog Lake, Ontario
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	MI, USA	Temperate	Lake	Observational	Density	Native vegetation	**2.012**	**0.452**	Cheruvelil, 2000	Macroinvertebrates associated with submerged macrophytes: Sample size and power to detect effects
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	MI, USA	Temperate	Lake	Observational	Density	Native vegetation	**1.336**	**0.406**	Cheruvelil, 2000	Macroinvertebrates associated with submerged macrophytes: Sample size and power to detect effects
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	MI, USA	Temperate	Lake	Observational	Density	Native vegetation	**0.000**	**0.365**	Cheruvelil, 2000	Macroinvertebrates associated with submerged macrophytes: Sample size and power to detect effects
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	MI, USA	Temperate	Lake	Observational	Density	Native vegetation	**-0.175**	**0.366**	Cheruvelil, 2000	Macroinvertebrates associated with submerged macrophytes: Sample size and power to detect effects
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	MI, USA	Temperate	Lake	Observational	Biomass	Native vegetation	**1.234**	**0.400**	Cheruvelil, 2000	Macroinvertebrates associated with submerged macrophytes: Sample size and power to detect effects
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	MI, USA	Temperate	Lake	Observational	Biomass	Native vegetation	**1.007**	**0.389**	Cheruvelil, 2000	Macroinvertebrates associated with submerged macrophytes: Sample size and power to detect effects
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	MI, USA	Temperate	Lake	Observational	Biomass	Native vegetation	**0.209**	**0.366**	Cheruvelil, 2000	Macroinvertebrates associated with submerged macrophytes: Sample size and power to detect effects
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	MI, USA	Temperate	Lake	Observational	Biomass	Native vegetation	**0.158**	**0.366**	Cheruvelil, 2000	Macroinvertebrates associated with submerged macrophytes: Sample size and power to detect effects
Macroinvertebrates	Submerged	*Hydrilla verticillata*	E USA	Temperate	Tidal freshwater	Observational	Density	No vegetation	**3.892**	**1.153**	Thorp, 1997	A comparison of water-column macroinvertebrate communities in beds of differing submersed aquatic vegetation in the tidal freshwater Potomac River
Macroinvertebrates	Submerged	*Hydrilla verticillata*	E USA	Temperate	Tidal freshwater	Observational	Density	No vegetation	**0.521**	**0.645**	Posey, 1993	Effects of an introduced aquatic plant, hydrilla verticillata, on benthic communities in the upper chesapeake bay

Macroinvertebrates	Submerged	*Hydrilla verticillata*	E USA	Temperate	Tidal freshwater	Manipulative	Density	No vegetation	**4.039**	**1.183**	Posey, 1994	Effects of an introduced aquatic plant, hydrilla verticillata, on benthic communities in the upper chesapeake bay
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Wisconsin, USA	Temperate	Lake	Observational	Density	Native vegetation	**0.899**	**0.611**	Chilton, 1990	Macroinvertebrate communities associated with three aquatic macrophytes (Ceratophyllum demersum, Myriophyllum spicatum, and Vallisneria americana) in Lake Onalaska, Wisconsin
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Wisconsin, USA	Temperate	Lake	Observational	Density	Native vegetation	**1.902**	**0.714**	Chilton, 1990	Macroinvertebrate communities associated with three aquatic macrophytes (Ceratophyllum demersum, Myriophyllum spicatum, and Vallisneria americana) in Lake Onalaska, Wisconsin
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Wisconsin, USA	Temperate	Lake	Observational	Density	Native vegetation	**-5.990**	**1.445**	Chilton, 1990	Macroinvertebrate communities associated with three aquatic macrophytes (Ceratophyllum demersum, Myriophyllum spicatum, and Vallisneria americana) in Lake Onalaska, Wisconsin
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Wisconsin, USA	Temperate	Lake	Observational	Density	Native vegetation	**1.292**	**0.644**	Chilton, 1990	Macroinvertebrate communities associated with three aquatic macrophytes (Ceratophyllum demersum, Myriophyllum spicatum, and Vallisneria americana) in Lake Onalaska, Wisconsin
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Wisconsin, USA	Temperate	Lake	Observational	Shannon	Native vegetation	**3.138**	**0.903**	Chilton, 1990	Macroinvertebrate communities associated with three aquatic macrophytes (Ceratophyllum demersum, Myriophyllum spicatum, and Vallisneria americana) in Lake Onalaska, Wisconsin

Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Wisconsin, USA	Temperate	Lake	Observational	Shannon	Native vegetation	**1.684**	**0.687**	Chilton, 1990	Macroinvertebrate communities associated with three aquatic macrophytes (Ceratophyllum demersum, Myriophyllum spicatum, and Vallisneria americana) in Lake Onalaska, Wisconsin
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Wisconsin, USA	Temperate	Lake	Observational	Shannon	Native vegetation	**2.752**	**0.839**	Chilton, 1990	Macroinvertebrate communities associated with three aquatic macrophytes (Ceratophyllum demersum, Myriophyllum spicatum, and Vallisneria americana) in Lake Onalaska, Wisconsin
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Wisconsin, USA	Temperate	Lake	Observational	Shannon	Native vegetation	**0.300**	**0.581**	Chilton, 1990	Macroinvertebrate communities associated with three aquatic macrophytes (Ceratophyllum demersum, Myriophyllum spicatum, and Vallisneria americana) in Lake Onalaska, Wisconsin
Macroinvertebrates	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Density	Native vegetation	**-0.420**	**0.337**	Schramm, 1989	Effects of aquatic macrophytes on benthic macroinvertebrates in two Florida lakes
Macroinvertebrates	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Biomass	Native vegetation	**-0.211**	**0.334**	Schramm, 1989	Effects of aquatic macrophytes on benthic macroinvertebrates in two Florida lakes
Macroinvertebrates	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Density	No vegetation	**0.156**	**0.334**	Schramm, 1989	Effects of aquatic macrophytes on benthic macroinvertebrates in two Florida lakes
Macroinvertebrates	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Biomass	No vegetation	**0.369**	**0.336**	Schramm, 1989	Effects of aquatic macrophytes on benthic macroinvertebrates in two Florida lakes
Macroinvertebrates	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Density	Native vegetation	**1.235**	**0.262**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes
Macroinvertebrates	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Biomass	Native vegetation	**1.106**	**0.258**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes

Macroinvertebrates	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Density	Native vegetation	**0.879**	**0.249**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes
Macroinvertebrates	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Biomass	Native vegetation	**0.874**	**0.249**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes
Macroinvertebrates	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Density	Native vegetation	**-1.140**	**0.384**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes
Macroinvertebrates	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Biomass	Native vegetation	**0.222**	**0.367**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Ontario, Canada	Temperate	Lake	Observational	Density	Native vegetation	**1.977**	**0.554**	Keast, 1984	The introduced aquatic macrophyte, Myriophyllum spicatum, as habitat for fish and their invertebrate prey
Macroinvertebrates	Submerged	*Myriophyllum spicatum*	Ontario, Canada	Temperate	Lake	Observational	Density	Native vegetation	**0.948**	**0.474**	Keast, 1984	The introduced aquatic macrophyte, Myriophyllum spicatum, as habitat for fish and their invertebrate prey
Macroinvertebrates	Submerged	*Elodea canadensis*	New Zealand	Temperate	Lake/ backwater	Observational	Density	Native vegetation	**1.238**	**0.708**	Biggs, 1982	Macroinvertebrates associated with various aquatic macrophytes in the backwaters and lakes of the upper clutha valley, New zealand
Macroinvertebrates	Submerged	*Elodea canadensis*	New Zealand	Temperate	Lake/ backwater	Observational	Density	Native vegetation	**0.152**	**0.659**	Biggs, 1982	Macroinvertebrates associated with various aquatic macrophytes in the backwaters and lakes of the upper clutha valley, New zealand
Macroinvertebrates	Submerged	*Elodea canadensis*	New Zealand	Temperate	Lake/ backwater	Observational	Density	Native vegetation	**-1.732**	**0.753**	Biggs, 1982	Macroinvertebrates associated with various aquatic macrophytes in the backwaters and lakes of the upper clutha valley, New zealand
Macroinvertebrates	Submerged	*Lagarosiphon major*	New Zealand	Temperate	Lake/ backwater	Observational	Density	Native vegetation	**5.271**	**2.467**	Biggs, 1982	Macroinvertebrates associated with various aquatic macrophytes in the backwaters and lakes of the upper clutha valley, New zealand

Macroinvertebrates	Submerged	*Lagarosiphon major*	New Zealand	Temperate	Lake/ backwater	Observational	Density	Native vegetation	**0.535**	**0.942**	Biggs, 1982	Macroinvertebrates associated with various aquatic macrophytes in the backwaters and lakes of the upper clutha valley, New zealand
Macroinvertebrates	Submerged	*Lagarosiphon major*	New Zealand	Temperate	Lake/ backwater	Observational	Density	Native vegetation	**-0.667**	**0.958**	Biggs, 1982	Macroinvertebrates associated with various aquatic macrophytes in the backwaters and lakes of the upper clutha valley, New zealand
Macroinvertebrates	Submerged	*Ranunculus fluitans*	New Zealand	Temperate	Lake/ backwater	Observational	Density	Native vegetation	**1.120**	**0.911**	Biggs, 1982	Macroinvertebrates associated with various aquatic macrophytes in the backwaters and lakes of the upper clutha valley, New zealand
Macroinvertebrates	Submerged	*Ranunculus fluitans*	New Zealand	Temperate	Lake/ backwater	Observational	Density	Native vegetation	**-0.665**	**0.851**	Biggs, 1982	Macroinvertebrates associated with various aquatic macrophytes in the backwaters and lakes of the upper clutha valley, New zealand
Macroinvertebrates	Submerged	*Ranunculus fluitans*	New Zealand	Temperate	Lake/ backwater	Observational	Density	Native vegetation	**-1.234**	**0.930**	Biggs, 1982	Macroinvertebrates associated with various aquatic macrophytes in the backwaters and lakes of the upper clutha valley, New zealand
Macroinvertebrates	Floating	*Pistia stratiotes*	Eastern Cape, South Africa	Subtropical	n/a	Mesocosm	Density	Uninvaded	**-2.890**	**1.325**	Coetzee, 2020	Biological control of water lettuce, Pistia stratiotes L., facilitates macroinvertebrate biodiversity recovery: a mesocosm study
Macroinvertebrates	Floating	*Pistia stratiotes*	Eastern Cape, South Africa	Subtropical	n/a	Mesocosm	Shannon	Uninvaded	**-1.749**	**1.032**	Coetzee, 2020	Biological control of water lettuce, Pistia stratiotes L., facilitates macroinvertebrate biodiversity recovery: a mesocosm study
Macroinvertebrates	Floating	*Pistia stratiotes*	Eastern Cape, South Africa	Subtropical	n/a	Mesocosm	Richness	Uninvaded	**-5.495**	**2.144**	Coetzee, 2020	Biological control of water lettuce, Pistia stratiotes L., facilitates macroinvertebrate biodiversity recovery: a mesocosm study
Macroinvertebrates	Floating	*Lemna minuta*	Central Italy	Subtropical	Pond	Observational	Richness	Uninvaded	**-3.478**	**0.552**	Ceschin, 2020	Habitat change and alteration of plant and invertebrate communities in waterbodies dominated by the invasive alien macrophyte Lemna minuta Kunth

Macroinvertebrates	Floating	*Ceratopteris thalictroides*	Western Australia	Tropical	Stream	Observational	Richness	Native vegetation	-0.382	**0.412**	Carey, 2018	Impacts of Indian waterfern (Ceratopteris thalictroides (L.) Brongn.) infestation and removal on macroinvertebrate biodiversity and conservation in spring?fed streams in the Australian arid zone
Macroinvertebrates	Floating	*Hydrocharis morsus-ranae*	New York, USA	Temperate	Lake	Manipulative	Density	Uninvaded	-0.681	**0.853**	Zhu, 2015	Effects of invasive European frogbit and its two physical control methods on macroinvertebrates
Macroinvertebrates	Floating	*Hydrocharis morsus-ranae*	New York, USA	Temperate	Lake	Manipulative	Richness	Uninvaded	1.859	**1.057**	Zhu, 2015	Effects of invasive European frogbit and its two physical control methods on macroinvertebrates
Macroinvertebrates	Floating	*Hydrocharis morsus-ranae*	New York, USA	Temperate	Lake	Manipulative	Simpson	Uninvaded	1.457	**0.971**	Zhu, 2015	Effects of invasive European frogbit and its two physical control methods on macroinvertebrates
Macroinvertebrates	Floating	*Hydrocharis morsus-ranae*	New York, USA	Temperate	Lake	Manipulative	Density	Uninvaded	-2.077	**1.108**	Zhu, 2015	Effects of invasive European frogbit and its two physical control methods on macroinvertebrates
Macroinvertebrates	Floating	*Hydrocharis morsus-ranae*	New York, USA	Temperate	Lake	Manipulative	Richness	Uninvaded	-0.081	**0.817**	Zhu, 2015	Effects of invasive European frogbit and its two physical control methods on macroinvertebrates
Macroinvertebrates	Floating	*Hydrocharis morsus-ranae*	New York, USA	Temperate	Lake	Manipulative	Simpson	Uninvaded	1.064	**0.902**	Zhu, 2015	Effects of invasive European frogbit and its two physical control methods on macroinvertebrates
Macroinvertebrates	Floating	*Trapa natans*	New York, USA	Temperate	Freshwater estuary	Observational	Density	No vegetation	1.719	**0.841**	Strayer, 2003	Invertebrate communities associated with a native (Vallisneria americana) and an alien (Trapa natans) macrophyte in a large river
Macroinvertebrates	Floating	*Trapa natans*	New York, USA	Temperate	Freshwater estuary	Observational	Density	Native vegetation	1.222	**0.638**	Strayer, 2003	Invertebrate communities associated with a native (Vallisneria americana) and an alien (Trapa natans) macrophyte in a large river
Macroinvertebrates	Floating	*Eichhornia crassipes*	California, USA	Subtropical	Tidal freshwater	Observational	Density	Native vegetation	0.145	**0.633**	Toft, 2003	The effects of introduced water hyacinth on habitat structure, invertebrate assemblages, and fish diets

Macroinvertebrates	Floating	*Eichhornia crassipes*	California, USA	Subtropical	Tidal freshwater	Observational	Density	Native vegetation	**0.506**	**0.645**	Toft, 2003	The effects of introduced water hyacinth on habitat structure, invertebrate assemblages, and fish diets
Macroinvertebrates	Floating	*Eichhornia crassipes*	California, USA	Subtropical	Tidal freshwater	Observational	Density	Native vegetation	**-0.392**	**0.640**	Toft, 2003	The effects of introduced water hyacinth on habitat structure, invertebrate assemblages, and fish diets
Macroinvertebrates	Floating	*Eichhornia crassipes*	California, USA	Subtropical	Tidal freshwater	Observational	Density	Native vegetation	**-0.924**	**0.673**	Toft, 2003	The effects of introduced water hyacinth on habitat structure, invertebrate assemblages, and fish diets
Macroinvertebrates	Floating	*Eichhornia crassipes*	California, USA	Subtropical	Tidal freshwater	Observational	Density	Native vegetation	**0.125**	**0.633**	Toft, 2003	The effects of introduced water hyacinth on habitat structure, invertebrate assemblages, and fish diets
Macroinvertebrates	Floating	*Trapa natans*	New York, USA	Temperate	River	Observational	Density	Native vegetation	**-1.709**	**0.529**	Feldman, 2001	Taxonomic and size structures of phytophilous macroinvertebrate communities in Vallisneria and Trapa beds of the Hudson River, New York
Macroinvertebrates	Floating	*Trapa natans*	New York, USA	Temperate	River	Observational	Richness	Native vegetation	**-0.878**	**0.470**	Feldman, 2001	Taxonomic and size structures of phytophilous macroinvertebrate communities in Vallisneria and Trapa beds of the Hudson River, New York
Macroinvertebrates	Floating	*Eichhornia crassipes*	Florida, USA	Subtropical	Lake	Observational	Density	Native vegetation	**2.082**	**0.325**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes
Macroinvertebrates	Floating	*Eichhornia crassipes*	Florida, USA	Subtropical	Lake	Observational	Biomass	Native vegetation	**2.491**	**0.349**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes
Macroinvertebrates	Floating	*Eichhornia crassipes*	Florida, USA	Subtropical	Lake	Observational	Density	Native vegetation	**1.966**	**0.316**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes
Macroinvertebrates	Floating	*Eichhornia crassipes*	Florida, USA	Subtropical	Lake	Observational	Biomass	Native vegetation	**2.256**	**0.332**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes

Macroinvertebrates	Floating	*Eichhornia crassipes*	Florida, USA	Subtropical	Lake	Observational	Density	Native vegetation	**0.686**	**0.388**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes
Macroinvertebrates	Floating	*Eichhornia crassipes*	Florida, USA	Subtropical	Lake	Observational	Biomass	Native vegetation	**1.365**	**0.412**	Schramm, 1987	Epiphytic macroinvertebrates on dominant macrophytes in two central Florida Lakes
Macroinvertebrates	Emergent	*Myriophyllum aquaticum*	Central Italy	Subtropical	Canal	Observational	Richness	Uninvaded	**0.166**	**0.448**	Lastrucci, 2018	Impacts of Myriophyllum aquaticum invasion in a Mediterranean wetland on plant and macroarthropod communities
Macroinvertebrates	Emergent	*Myriophyllum aquaticum*	Central Italy	Subtropical	Canal	Observational	Shannon	Uninvaded	**-0.019**	**0.447**	Lastrucci, 2018	Impacts of Myriophyllum aquaticum invasion in a Mediterranean wetland on plant and macroarthropod communities
Macroinvertebrates	Emergent	*Myriophyllum aquaticum*	Washington State, USA	Temperate	River	Observational	Shannon	Native vegetation	**2.018**	**1.600**	Kuehne, 2016	Multi-trophic impacts of an invasive aquatic plant
Macroinvertebrates	Emergent	*Typha*	Midwest USA	Temperate	Wetland	Observational	Density	Native vegetation	**-0.603**	**0.278**	Lawrence, 2016	Typha invasion associated with reduced aquatic macroinvertebrate abundance in northern Lake Huron coastal wetlands
Macroinvertebrates	Emergent	*Typha*	Midwest USA	Temperate	Wetland	Observational	Biomass	Native vegetation	**-0.521**	**0.277**	Lawrence, 2016	Typha invasion associated with reduced aquatic macroinvertebrate abundance in northern Lake Huron coastal wetlands
Macroinvertebrates	Emergent	*Phragmites australis*	Ohio, USA	Temperate	Wetland	Observational	Density	Native vegetation	**0.153**	**0.216**	Holomuzki, 2009	Invasive reed effects on benthic community structure in Lake Erie coastal marshes
Macroinvertebrates	Emergent	*Typha angustifolia*	Ohio, USA	Temperate	Wetland	Observational	Density	Native vegetation	**-0.360**	**0.197**	Holomuzki, 2009	Invasive reed effects on benthic community structure in Lake Erie coastal marshes
Macroinvertebrates	Emergent	*Typha*	Kansas, USA	Subtropical	Wetland	Manipulative	Richness	Treated plot	**1.161**	**0.343**	Kostecke, 2005	Macroinvertebrate response to cattail management at Cheyenne Bottoms, Kansas, USA
Macroinvertebrates	Emergent	*Typha*	Kansas, USA	Subtropical	Wetland	Manipulative	Shannon	Treated plot	**-0.040**	**0.316**	Kostecke, 2005	Macroinvertebrate response to cattail management at Cheyenne Bottoms, Kansas, USA

Macroinvertebrates	Emergent	*Typha*	Kansas, USA	Subtropical	Wetland	Manipulative	Biomass	Treated plot	**0.317**	**0.318**	Kostecke, 2005	Macroinvertebrate response to cattail management at Cheyenne Bottoms, Kansas, USA
Macroinvertebrates	Emergent	*Typha*	Kansas, USA	Subtropical	Wetland	Manipulative	Biomass	Treated plot	**0.611**	**0.324**	Kostecke, 2005	Macroinvertebrate response to cattail management at Cheyenne Bottoms, Kansas, USA
Macroinvertebrates	Emergent	*Urochloa mutica*	N Australia	Tropical	Wetland	Observational	Richness	Native vegetation	**13.243**	**8.255**	Douglas, 2003	Effects of the exotic macrophyte, para grass (Urochloa mutica), on benthic and epiphytic macroinvertebrates of a tropical floodplain
Macroinvertebrates	Emergent	*Urochloa mutica*	N Australia	Tropical	Wetland	Observational	Richness	Native vegetation	**-0.766**	**1.107**	Douglas, 2003	Effects of the exotic macrophyte, para grass (Urochloa mutica), on benthic and epiphytic macroinvertebrates of a tropical floodplain
Macroinvertebrates	Emergent	*Urochloa mutica*	N Australia	Tropical	Wetland	Observational	Richness	Native vegetation	**1.955**	**1.569**	Douglas, 2003	Effects of the exotic macrophyte, para grass (Urochloa mutica), on benthic and epiphytic macroinvertebrates of a tropical floodplain
Macroinvertebrates	Emergent	*Urochloa mutica*	N Australia	Tropical	Wetland	Observational	Richness	Native vegetation	**-1.973**	**1.578**	Douglas, 2003	Effects of the exotic macrophyte, para grass (Urochloa mutica), on benthic and epiphytic macroinvertebrates of a tropical floodplain
Macroinvertebrates	Emergent	*Urochloa mutica*	N Australia	Tropical	Wetland	Observational	Richness	Native vegetation	**3.282**	**2.264**	Douglas, 2003	Effects of the exotic macrophyte, para grass (Urochloa mutica), on benthic and epiphytic macroinvertebrates of a tropical floodplain
Macroinvertebrates	Emergent	*Urochloa mutica*	N Australia	Tropical	Wetland	Observational	Richness	Native vegetation	**1.730**	**1.465**	Douglas, 2003	Effects of the exotic macrophyte, para grass (Urochloa mutica), on benthic and epiphytic macroinvertebrates of a tropical floodplain

Macroinvertebrates	Emergent	*Urochloa mutica*	N Australia	Tropical	Wetland	Observational	Density	Native vegetation	**1.923**	**1.554**	Douglas, 2003	Effects of the exotic macrophyte, para grass (Urochloa mutica), on benthic and epiphytic macroinvertebrates of a tropical floodplain
Macroinvertebrates	Emergent	*Urochloa mutica*	N Australia	Tropical	Wetland	Observational	Density	Native vegetation	**2.891**	**2.049**	Douglas, 2003	Effects of the exotic macrophyte, para grass (Urochloa mutica), on benthic and epiphytic macroinvertebrates of a tropical floodplain
Macroinvertebrates	Emergent	*Hymenachne amplexicaulis*	E Australia	Subtropical	Backwater	Observational	Density	Native vegetation	**-1.463**	**0.823**	Houston, 2002	Replacement of littoral native vegetation with the ponded pasture grass Hymenachne amplexicaulis: effects on plant, macroinvertebrate and fish biodiversity of backwaters in the Fitzroy River, Central Queensland, Australia
Macroinvertebrates	Emergent	*Hymenachne amplexicaulis*	E Australia	Subtropical	Backwater	Observational	Density	Native vegetation	**-2.045**	**0.920**	Houston, 2002	Replacement of littoral native vegetation with the ponded pasture grass Hymenachne amplexicaulis: effects on plant, macroinvertebrate and fish biodiversity of backwaters in the Fitzroy River, Central Queensland, Australia
Macroinvertebrates	Emergent	*Hymenachne amplexicaulis*	E Australia	Subtropical	Backwater	Observational	Density	Native vegetation	**-1.571**	**0.839**	Houston, 2002	Replacement of littoral native vegetation with the ponded pasture grass Hymenachne amplexicaulis: effects on plant, macroinvertebrate and fish biodiversity of backwaters in the Fitzroy River, Central Queensland, Australia

Macroinvertebrates	Emergent	*Hymenachne amplexicaulis*	E Australia	Subtropical	Backwater	Observational	Richness	Native vegetation	**2.539**	**1.016**	Houston, 2002	Replacement of littoral native vegetation with the ponded pasture grass Hymenachne amplexicaulis: effects on plant, macroinvertebrate and fish biodiversity of backwaters in the Fitzroy River, Central Queensland, Australia
Macroinvertebrates	Emergent	*Hymenachne amplexicaulis*	E Australia	Subtropical	Backwater	Observational	Richness	Native vegetation	**0.400**	**0.716**	Houston, 2002	Replacement of littoral native vegetation with the ponded pasture grass Hymenachne amplexicaulis: effects on plant, macroinvertebrate and fish biodiversity of backwaters in the Fitzroy River, Central Queensland, Australia
Macroinvertebrates	Emergent	*Hymenachne amplexicaulis*	E Australia	Subtropical	Backwater	Observational	Richness	Native vegetation	**0.698**	**0.735**	Houston, 2002	Replacement of littoral native vegetation with the ponded pasture grass Hymenachne amplexicaulis: effects on plant, macroinvertebrate and fish biodiversity of backwaters in the Fitzroy River, Central Queensland, Australia
Fish	Submerged	*Hydrilla verticillata*	S Brazil	Subtropical	River	Observational	Density	Native vegetation	**-1.074**	**0.624**	Cunha, 2011	Small-sized fish assemblages do not differ between a native and a recently established non-indigenous macrophyte in a neotropical ecosystem
Fish	Submerged	*Hydrilla verticillata*	S Brazil	Subtropical	River	Observational	Biomass	Native vegetation	**-1.157**	**0.632**	Cunha, 2011	Small-sized fish assemblages do not differ between a native and a recently established non-indigenous macrophyte in a neotropical ecosystem
Fish	Submerged	*Hydrilla verticillata*	S Brazil	Subtropical	River	Observational	Shannon	Native vegetation	**1.019**	**0.620**	Cunha, 2011	Small-sized fish assemblages do not differ between a native and a recently established non-indigenous macrophyte in a neotropical ecosystem

Fish	Submerged	*Hydrilla verticillata*	S Brazil	Subtropical	River	Observational	Richness	Native vegetation	**1.078**	**0.625**	Cunha, 2011	Small-sized fish assemblages do not differ between a native and a recently established non-indigenous macrophyte in a neotropical ecosystem
Fish	Submerged	*Lagarosiphon major*	New Zealand	Temperate	Reservoir	Manipulative	CPUE	Treated plot	**-0.584**	**0.264**	Bickel, 2009	IMPACT OF PARTIAL REMOVAL OF THE INVASIVE MACROPHYTE Lagarosiphon major (HYDROCHARITACEAE) ON INVERTEBRATES AND FISH
Fish	Submerged	*Lagarosiphon major*	New Zealand	Temperate	Reservoir	Manipulative	Biomass	Treated plot	**-0.327**	**0.260**	Bickel, 2009	IMPACT OF PARTIAL REMOVAL OF THE INVASIVE MACROPHYTE Lagarosiphon major (HYDROCHARITACEAE) ON INVERTEBRATES AND FISH
Fish	Submerged	*Hydrilla verticillata*	Guatemala	Tropical	Lake	Observational	Shannon	Native vegetation	**0.631**	**0.651**	Barrientos, 2008	Fish abundance and community composition in native and non-native plants following hydrilla colonisation at Lake Izabal, Guatemala
Fish	Submerged	*Hydrilla verticillata*	Guatemala	Tropical	Lake	Observational	Density	Native vegetation	**0.325**	**0.638**	Barrientos, 2008	Fish abundance and community composition in native and non-native plants following hydrilla colonisation at Lake Izabal, Guatemala
Fish	Submerged	*Hydrilla verticillata*	Guatemala	Tropical	Lake	Observational	Biomass	Native vegetation	**0.208**	**0.635**	Barrientos, 2008	Fish abundance and community composition in native and non-native plants following hydrilla colonisation at Lake Izabal, Guatemala
Fish	Submerged	*Hydrilla verticillata*	Guatemala	Tropical	Lake	Observational	Shannon	Native vegetation	**1.235**	**0.702**	Barrientos, 2008	Fish abundance and community composition in native and non-native plants following hydrilla colonisation at Lake Izabal, Guatemala
Fish	Submerged	*Hydrilla verticillata*	Guatemala	Tropical	Lake	Observational	Density	Native vegetation	**-0.522**	**0.646**	Barrientos, 2008	Fish abundance and community composition in native and non-native plants following hydrilla colonisation at Lake Izabal, Guatemala

Fish	Submerged	*Hydrilla verticillata*	Guatemala	Tropical	Lake	Observational	Biomass	Native vegetation	**0.952**	**0.675**	Barrientos, 2008	Fish abundance and community composition in native and non-native plants following hydrilla colonisation at Lake Izabal, Guatemala
Fish	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Density	Uninvaded	**-0.116**	**0.392**	Hoyer, 2008	Lack of exotic hydrilla infestation effects on plant, fish and aquatic bird community measures
Fish	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Richness	Uninvaded	**0.581**	**0.400**	Hoyer, 2008	Lack of exotic hydrilla infestation effects on plant, fish and aquatic bird community measures
Fish	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Simpson	Uninvaded	**0.751**	**0.406**	Hoyer, 2008	Lack of exotic hydrilla infestation effects on plant, fish and aquatic bird community measures
Fish	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	CPUE	Uninvaded	**-0.524**	**0.589**	Hoyer, 2008	Lack of exotic hydrilla infestation effects on plant, fish and aquatic bird community measures
Fish	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Richness	Uninvaded	**0.278**	**0.581**	Hoyer, 2008	Lack of exotic hydrilla infestation effects on plant, fish and aquatic bird community measures
Fish	Submerged	*Hydrilla verticillata*	Florida, USA	Subtropical	Lake	Observational	Simpson	Uninvaded	**0.893**	**0.610**	Hoyer, 2008	Lack of exotic hydrilla infestation effects on plant, fish and aquatic bird community measures
Fish	Submerged	*Hydrilla verticillata*	Louisiana, USA	Subtropical	Lake/ backwater	Observational	Biomass	Native vegetation	**-0.309**	**0.166**	Troutman, 2007	Patterns of Habitat Use among Vegetation-Dwelling Littoral Fishes in the Atchafalaya River Basin, Louisiana
Fish	Submerged	*Hydrilla verticillata*	Louisiana, USA	Subtropical	Lake/ backwater	Observational	Density	Native vegetation	**0.192**	**0.166**	Troutman, 2007	Patterns of Habitat Use among Vegetation-Dwelling Littoral Fishes in the Atchafalaya River Basin, Louisiana
Fish	Submerged	*Hydrilla verticillata*	Louisiana, USA	Subtropical	Lake/ backwater	Observational	Richness	Native vegetation	**0.711**	**0.171**	Troutman, 2007	Patterns of Habitat Use among Vegetation-Dwelling Littoral Fishes in the Atchafalaya River Basin, Louisiana

Fish	Floating	*Eichhornia crassipes*	Louisiana, USA	Subtropical	Lake/ backwater	Observational	Biomass	Native vegetation	**-0.154**	**0.168**	Troutman, 2007	Patterns of Habitat Use among Vegetation-Dwelling Littoral Fishes in the Atchafalaya River Basin, Louisiana
Fish	Floating	*Eichhornia crassipes*	Louisiana, USA	Subtropical	Lake/ backwater	Observational	Density	Native vegetation	**-0.726**	**0.173**	Troutman, 2007	Patterns of Habitat Use among Vegetation-Dwelling Littoral Fishes in the Atchafalaya River Basin, Louisiana
Fish	Floating	*Eichhornia crassipes*	Louisiana, USA	Subtropical	Lake/ backwater	Observational	Richness	Native vegetation	**0.296**	**0.168**	Troutman, 2007	Patterns of Habitat Use among Vegetation-Dwelling Littoral Fishes in the Atchafalaya River Basin, Louisiana
Fish	Emergent	*Typha*	Midwest USA	Temperate	Wetland	Observational	Richness	Native vegetation	**-0.872**	**0.247**	Schrank, 2019	Invasive cattail reduces fish diversity and abundance in the emergent marsh of a Great Lakes coastal wetland
Fish	Emergent	*Typha*	Midwest USA	Temperate	Wetland	Observational	Shannon	Native vegetation	**0.846**	**0.246**	Schrank, 2019	Invasive cattail reduces fish diversity and abundance in the emergent marsh of a Great Lakes coastal wetland
Fish	Emergent	*Typha*	Midwest USA	Temperate	Wetland	Observational	CPUE	Native vegetation	**-1.016**	**0.251**	Schrank, 2019	Invasive cattail reduces fish diversity and abundance in the emergent marsh of a Great Lakes coastal wetland
Fish	Emergent	*Hymenachne amplexicaulis*	E Australia	Subtropical	Backwater	Observational	CPUE	Native vegetation	**1.208**	**0.315**	Houston, 2002	Replacement of littoral native vegetation with the ponded pasture grass Hymenachne amplexicaulis: effects on plant, macroinvertebrate and fish biodiversity of backwaters in the Fitzroy River, Central Queensland, Australia
Fish	Emergent	*Hymenachne amplexicaulis*	E Australia	Subtropical	Backwater	Observational	Richness	Native vegetation	**-0.292**	**0.290**	Houston, 2002	Replacement of littoral native vegetation with the ponded pasture grass Hymenachne amplexicaulis: effects on plant, macroinvertebrate and fish biodiversity of backwaters in the Fitzroy River, Central Queensland, Australia

Fish	Emergent	*Hymenachne amplexicaulis*	E Australia	Subtropical	Backwater	Observational	Shannon	Native vegetation	**-1.459**	**0.326**	Houston, 2002	Replacement of littoral native vegetation with the ponded pasture grass Hymenachne amplexicaulis: effects on plant, macroinvertebrate and fish biodiversity of backwaters in the Fitzroy River, Central Queensland, Australia