The Enemy Release Hypothesis and beyond: *Lagarosiphon major* invasion dynamics and management options for New Zealand using native natural enemies from South Africa.

THESIS

Submitted in Fulfilment of the Requirement for the Degree

Doctor of Philosophy

at

Rhodes University

By

Nompumelelo Catherine Baso

G14b4197

Department of Botany

Rhodes University

P.O. Box 94

Grahamstown

6140

November 2023

Declaration: I have read and adhered to the Rhodes University plagiarism policy.

Abstract

Numerous scientific investigations have demonstrated the destructive impact that exotic species can have on ecosystem services beyond a specific threshold. There are many explanations for why introduced plants are likely to be more successful outside their native range. One such explanation is offered by the Enemy Release Hypothesis (ERH), which states that plants automatically become superior competitors outside of their natural range due to release from top-down stressors (herbivory, parasites, and diseases) that is evident in the absence of their natural enemies. The underlying assumption of the ERH is that natural enemies are important regulators of plant species populations, and that the pressures from these natural enemies are felt more readily by native species compared to alien plants. Consequently, in the absence of such pressures, the ERH assumes that exotic plants can allocate more resources towards growth and reproduction, while effectively maintaining accumulated biomass.

Classical biological control has previously been cited as evidence for the enemy release hypothesis. Therefore, the overarching aim and theme of this thesis was to investigate the role of ERH on the invasiveness of *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae) in New Zealand. Firstly, a literature search and a meta-analysis was used to synthesize existing studies in order to test for general applicability of this hypothesis to aquatic plant invasions. Furthermore, an empirical investigation was conducted in order to directly quantify enemy release in *L. major* populations invaded areas of New Zealand. To achieve this, various plant parameters of this plant, overall macrophyte and invertebrate diversity were measured and compared between sites in the native range in South Africa and the invaded areas in New Zealand.

Although the meta-analysis showed variable evidence for this hypothesis depending on various modulating factors such as study type, plant growth form and measured parameters, for *L.*

major, there was strong evidence of enemy release. The biogeographical comparisons showed that *L. major* exhibited increased fitness in most of the invaded sites, marked by elevated biomass accumulation, significantly higher shoot production, and the displacement of native plant species. The observed fitness advantages were directly correlated to a decrease in herbivory diversity and pressure upon the plant's introduction to New Zealand. Unlike the native populations, which contend with the presence of at least four co-occurring herbivores, including specialist herbivores, the invaded range had a substantially lower herbivore diversity, with only *Hygraula nitens* Butler (Lepidoptera: Crambidae) syn. *Nymphula nitens*, significantly damaging *L. major*.

These findings emphasize the importance of understanding invasion ecology and theories such as ERH in order to advance aquatic plant management and also present valuable insights for developing effective strategies to mitigate the impact of invasive alien species on aquatic ecosystems. Specifically, results from the empirical investigation provide evidence in support of the ERH and highlight the suitability of implementing biological control strategies to manage the *L. major* invasion in New Zealand. Previous studies have shown the suitability of two specialist herbivores, *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), and *Polypedilum tuburcinatum* Andersen (Diptera: Chironomidae), as potential biological control agents. This control strategy presents a sustainable and ecologically responsible approach, promoting coexistence between exotic plants and native species rather than displacement through competitive exclusion.

With the apparent dominance of *L. major* at various New Zealand localities, the subsequent objective of this thesis was to investigate the competitive interactions between *L. major* and another invasive Hydrocharitaceae, *Egeria densa* Planchon, as driven by herbivory. Combinations of two host specific Ephydrid flies, *H. lagarosiphon* and *H. egeriae*, were used at eight different factorial combination of planting densities. The analysis of plant parameters and the application of inverse linear models revealed that *L. major* often exhibits relatively higher fitness, especially in low monoculture treatments when the two insects were isolated.

However, multiple inverse linear models revealed that actual competitive outcomes are dependent on factors such as initial plant density and herbivory regime, with competitive interactions generally being mild. Nevertheless, the presence of *H. lagarosiphon* resulted in facilitation of *E. densa* growth. Thus, even at lower densities, these insects still had an impact on the observed interactions, further emphasizing suitability as damaging biological control agents.

Lastly, focusing on the abiotic component of *L. major* invasion, Species Distribution Models (SDMs) were employed to map potential suitable habitat for this species, as well as predict the consequences of climate change on this. Correlative and mechanistic modelling was also used to simulate suitable habitat for potential biological control agents, thus addressing the potential for mismatches between host plant distribution and insect suitable range. The Maximum Entropy Species Distribution Modelling (MaxEnt) algorithm revealed that more than 90% of all freshwater ecosystems in New Zealand are susceptible to *L. major* invasion, with suitability projected to expand further under future climate scenarios. Moreover, correlative modelling using this method suggests limited suitable habitat for both herbivores. However, degree-day modelling, which also takes into account the physiological requirements, showed that *H. lagarosiphon* has the potential to produce viable populations in several parts of New Zealand.

Overall, this thesis explored the intricate web of biotic and abiotic factors influencing the success of *L. major* outside its native range. The results emphasize the potential impacts of climate change on the invasion potential and management strategies for *L. major*. The findings also advocate for the implementation of sustainable and ecologically sound management solutions, such as biological control, to manage this species.

Acknowledgments

I would like to express my deepest gratitude to the following individuals and institutions who have played a pivotal role in my Ph.D. journey:

First and foremost, my academic mother, mentor and supervisor, Professor Julie Coetzee. When I would often suffer from the imposter syndrome, your passion for academic excellence was so radiant, it pulled me through some of the most difficult challenges during this journey. Thank you for your valuable insights and dedication to giving constructive feedback.

To the director of the Center for Biological Control, Professor Martin Hill, I am honored to have been part of your wonderful team. It is true that an organization is a reflection of its leadership, and often the most skilled leaders are the ones that lead with empathy and understanding. I admire your way of interacting with even the most unseen people, making every role as significant. Thank you specifically for providing prompt feedback to the many drafts I sent your way. Your guidance, mentorship, and expertise have been instrumental in shaping this research.

A special thanks to Dr Angela Bownes and Manaaki Whenua-Landcare Research of New Zealand for the financial support which made this research possible. A large piece of this thesis would have been missing if you and your team did not take it upon yourselves to go sampling on my behalf during the Covid-19 travel restrictions. Our meetings were also very instrumental in my progress.

Thank you to the rest of my colleagues, office mates and the rest of the Department of Zoology and Entomology, the Department of Botany, and Rhodes University for the community spirit and facilities. Specifical thanks to Mrs Jeanne van der Merwe for running the financials so efficiently. This thesis would have been harder without you.

To my soul twin, my rock and my husband, Wandisile Mdiza; I could not have asked for a better person to do this life thing with. I am profoundly grateful for your unwavering support, encouragement, and understanding during the demanding phases of this journey. Your belief in me has been a constant source of motivation. I owe this to you.

This journey also saw me going through many changes in my life, such as the death of umama wam, uMamngweve (my grandmother), and the birth of my first child, u-Esiphayo Mdiza. It’s as if the universe knew exactly what I needed, when I didn’t even know. Thank you to u-Makazi wam, u-Nombuyiselo for stepping up to the impossible role of filling my mothers’ shoes and being my go-to person for child minding when I had to be on the road for conferences and research.

I cannot end this without mentioning the most influential person to my love for education, my late grandfather, u-Mava Baso. I wish you were there to witness this huge milestone because I know you would proud. Your love of education lives on through me.

Enkosi.

I dedicate this thesis to umama wam u-Nohombile Lote (u-Mamgwevu: 1946-2020). Continue to rest in beautiful peace mama. You have been my rock even in your absence.

Table of contents

List of figures

List of tables

Chapter 1: General introduction

Globalisation has played a very important role in connecting people and therefore ecosystems. One of the negative consequences of this development is the spread of invasive species outside of their natural range. Furthermore, climate change and habitat destruction put native communities under even more stress while promoting further spread of the alien plant invaders. According to Rahel & Olden (2008), global climate change and biological invasion are two of the most prevalent features of the Anthropocene, and literature has shown how devastating the effects of alien invasive species can be on both the environment and the economy (McGregor & Gourlay, 2002; Van Ginkel, 2011; Hill & Coetzee, 2017). This problem of alien species invasion is prevalent in both terrestrial and aquatic ecosystem (Zimmermann et al., 2004; Hill & Coetzee, 2017). In both cases, native biodiversity tends to be under threat as the introduced species are usually better adapted to take advantage of various environmental and land use change (Vilà et al., 2007). Thus, native species are usually outcompeted, leaving their associated species vulnerable to predation and with no food sources (Sheldon & Creed, 1995).

1.1. Alien aquatic plant invasions

Aquatic pant invasions by exotic species have negative implications for both economic and ecological systems (Clayton, 1996; Zimmermann et al., 2004; Cuthbert et al., 2021). Effects may vary by growth form, or may be share between floating, emergent as well as submerged species. For example, many Invasive Alien Aquatic Plants (IAAPs) alter the aquatic environment by producing densa floating mats which block sunlight from penetrating the water, reduce dissolved oxygen concentration in water through reductions in photosynthesis, resulting in faunal die-off (Vilà et al., 2011; Hill & Coetzee, 2017; Zhang et al., 2019). Sensitive species such as mayflies are also unable to withstand the changes in water quality and chemistry as a result of these monospecific stands (Farooq et al., 2021; Xu et al., 2014). Similarly, high densities of submerged macrophytes, such as those found in the family Hydrocharitaceae, also form ideal habitats (slow-moving or still waters) for disease vectors like mosquitoes and blackflies (Mereta et al., 2013). In addition, the different growth forms may block waterways, clog irrigation systems, damage hydroelectric power infrastructures and reduce recreational activities when occurring in high enough densities (Clayton, 1996; Van Ginkel, 2011; Zhang et al., 2019). The negative effects of these plants increase as the plants become more abundant in the invaded environment (Vilà et al., 2011).

A notable example of how IAPs impact aquatic ecosystems is that of Lake Victoria in Uganda, given by Wilson et al. (2007). *Pontederia crassipes* (formerly *Eichhornia crassipes*) Mart. (Pontederiaceae), commonly known as water hyacinth, was first reported there in 1989. This is the world's second-largest freshwater lake, which provides people with hydropower and other ecosystem services. By the mid-1990s, water hyacinth had covered up to 20 000 ha of the lake. These densa floating mats had huge negative economic and ecological impacts in the surrounding areas as they infested some 80% of the Ugandan shorelines. This weed was a huge hindrance to socio-economic activities such as agriculture, lake transport, hydroelectric power generation, and general water supply. Ultimately, this also affected trade routes in and out of the ports, resulting in GDP losses for Kenya, Tanzania and Uganda which surround the lake.

Despite the previously noted negative impacts, it is important to note that in many other situations, macrophytes form an important part of the aquatic environment as they provide many ecosystem services, including improving water quality, facilitating nutrient cycling, and providing habitat for other species (Madsen et al., 2000; Schultz et al. 2003). These benefits are however mostly conferred when there is a diverse group of macrophytes occurring in moderation in the system. The advantages are significantly reduced in mono-specific stands typical of IAPs (Brown et al*.,* 1988; Wells et al., 1997), and therefore resource managers need to be able to weigh the cost against the benefits of these plants.

Although it is often difficult to quantify in economic terms the full cost of these invasive alien species (Pimentel et al., 2005), meaningful extrapolations can be made through quantifying the cost of managing these plants (Diagne et al., 2020), as well as the cost of losing ecosystem services that are usually offered by the affected aquatic ecosystems (Charles & Dukes, 2007; Cutthbert et al., 2021; Maluleke et al., 2021). For example, an old estimation by Pimentel et al. (2005) showed that the United States of America (USA) losses US$34 billion annually to invasive alien plants, while around US$2.81 billion is lost in the British economy (Kelly et al., 2013). When looking specifically at IAPs for example, the USA state of Florida spent an estimated US$ 43 million between 1980 and 1991 on programmes aimed at controlling aquatic weeds (Van Ginkel., 2011).

A more recent global cost analysis by Cuthbert et al. (2021) showed that the USA had the highest cost related to managing IAPs, followed by Brazil, India and France. According to Cuthbert et al. (2021), the InvaCost (Diagne et al., 2020) database used to conduct this analysis had limited data for the African continent, and even for South Africa, despite the vast amount of research into biological invasions in the country (EPPO, 2009; McConnachie et al., 2016; Coetzee et al., 2021). For example, Van Ginkel (2011) estimated that US$ 1.65 million is spent annually in the efforts of controlling the most noxious aquatic weed, water hyacinth in South Africa.

In New Zealand, alien invasive weeds threaten about 7% of the region’s vulnerable flora, corresponding to biodiversity loss of US$ 1.3 billion over a 10-year period (Williams & Timmins, 2002). This region has seen more than 1500 exotic plant species become naturalised over a period of about 150 years (Clayton, 1996). Approximately 10% of these are recognised by the Department of Conservation as weeds or potentially weedy (Williams & Timmins, 2002). According to Williams and Timmins (2002), Department of Conservation spends about US$ 60 million annually on managing alien invasive weeds, while also losing a further US$ 40 million per annum to invasions that affect the nation's productivity.

With regards to IAPs specifically, there is currently no available overall cost estimation for macrophyte control in New Zealand. However, according to Hudson & Harding (2004), expenses are projected to reach tens of millions annually. As an illustration, the annual expenses associated with controlling *Ceratophyllum demersum* L. (Ceratophyllaceae) in New Zealand amounts to roughly US$ 3.3 million per year. Thus, although the figure reported in this section may not look like big figures, the overall cost of not managing these invasions would be even greater in the long run (Hussner et al., 2017; Cuthbert et al., 2021).

1.2. Management options for biological plant invasions

Many countries have laws and regulation in place that mandate the control of invasive alien species (Williams & Timmins, 2002; Genovesi et al., 2015). In South Africa these are the NEMBA (2004) regulations, which classify exotic species into three categories according to their invasive vigour and anticipated impact. The Department of Conservation in New Zealand is actively involved in the developing and implementation of weed management legislation such as the Biosecurity Act of 1993 (Williams & Timmins, 2002). Other key stakeholders responsible for research and management of alien invasive weeds in New Zealand are university scientists, and Crown Research Institutes (Landcare Research, Atmospheric Research and National Institute of Water) (Williams & Timmins, 2002).

The main goal in managing alien species is to prevent their introduction in susceptible ranges. However, when this fails, regions that have systems for early detection, rapid response and prevention of further invasion are more likely to succeed in their quest for eradication (Genovesi et al., 2010; Hussner et al., 2017). There are three main options that may be employed in managing AIPs, and these are mechanical/manual, chemical and biological control (reviewed by Hussner et al., 2017). These management options may be used in isolation or in combination though an integrated management plan, offering varying degrees of success based on the invaded system and management goals (eradication, reduction, or containment). The available choices may sometimes be limited due to legislative restrictions, e.g., biological control in Europe (Sheppard et al., 2006), and the ban of various herbicides (Zehnsdorf et al., 2015).

1.2.1. Mechanical and Chemical control

Considerable research has been conducted on the management of aquatic weeds across the world (Hussner et al., 2017). Control measures were previously dominated by chemical and mechanical means of removal (Room & Thomas, 1985; Cilliers, 1991). Most European countries are still dependent on these two methods, and the most popular method being the use of cutter boats for submerged macrophytes (Zehnsdorf et al., 2015). This is because the cut and leave method (for biomass reduction) is considered cheaper than other control options (Zehnsdorf et al., 2015). However, mechanical control of AIPs is usually labour intensive, requires expensive heavy machinery, and is sometimes highly destructive. Inadequate cleaning of harvest material may also result in the spread of these AIPs to other non-invaded systems.

In many cases, these methods do not offer a long-term solution as some roots, and seeds and other reproductive propagules may remain in the system, allowing for regrowth (Howard-Williams et al., 1996). The destructive nature of mechanical control is also a concern as even small fragments left in the water may result in redistribution and recolonization (Chisholm, 2006). One way to mitigate against this is to install drift barriers in case of moving waters. Outcomes of physical management programmes are highly variable, depending on the machinery used, plant growth form, habitat and the general condition of the target system (Hussner et al., 2017).

The use of herbicides such as terbutryn, diquat, glyphosate and paraquat usually requires extensive research, repeated applications and assistance from manual clearing (Cilliers et al., 2003, Netherland, 2014; Hussner et al., 2017). The results of these programmes are usually short lived, while the cost is typically high in terms of both the environment and the economy (Chisholm, 2006; Malileke et al., 2021). Furthermore, chemical control creates an uninhabited niche which is highly susceptible to even more invasions. The chemicals may be unable to travel through the water column and reach the stems that are rooted in the soil, and this is even more challenging when plants have formed thick intertwined mats (Mafokoane, 2007; Chisholm, 2006).

A 2011 estimate showed that the annual cost of controlling *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae) in New Zealand using chemical and mechanical control is approximately US$ 1.5 million (Deloitte, 2011), while managing water hyacinth using harvesters in Europe can cost up to US$ 5.56 million per infested site per year for 50,000 tons of plant material (EPPO, 2009). It is important to note however that traditional methods do play an important role for eradication during the earlier phase of invasion, in smaller systems, and for reducing further spread, especially in cases where biological control is not suitable (Hussner et al., 2017). For example, frequent manual removal of water hyacinth or similar free floating growth forms using excavators may be enough for seasonal control, to reduce spread, or even eradication in smaller systems (Laranjeira & Nadais, 2008). However, this method may not be suitable for species that may be easily fragmented during removal, as this might facilitate further spread.

Herbicide applications may sometimes be cost effective and have less negative environmental impact than mechanical control if product labels are followed (Netherland, 2014). For example, in New Zealand, mechanical control programmes end up being five time more expensive than chemical applications (Hussner et al., 2017). Repeated herbicide application increases the prospect of eradication in some instances, but caution should be taken to avoid weed resistance (Netherland, 2014). However, despite its proven effectiveness, chemical control in freshwater systems faces heavy legislative restrictions due to the sensitivity of such systems to change. For example, in Europe, only glyphosate is authorized for regular use on emergent aquatic species, while there are no approved herbicides for submerged macrophytes (Hussner et al., 2017).

1.2.2. Biological control

Classical biological control is the use of introduced natural enemies of the problematic plant for its control in order to re-establish the natural top-down control of the alien invasive weed. This is based on the theory that herbivory plays an important role in controlling plant biomass in its natural environment (Williamson & Griffiths, 1996; Keane & Crawley, 2002). Indeed, Nachtrieb et al. (2011) found that direct herbivory is an important determinant of plant biomass in the aquatic environment.

These enemies are referred to as biological control agents, consisting of a variety of macro-invertebrates, mostly insects, as well as fungi and other pathogens (Cilliers, 1991). The selection process involves rigorous specificity tests, and as a result, biological control agents are usually highly specialised, in many cases only attacking one plant species (McClay & Balciunas, 2005). In general, the agents work by reducing plant fitness through damaging plant tissue, killing roots and reproductive parts such as seeds and flowers, and by reducing resource allocation to growth and reproduction by putting pressure on plant defences (Cilliers, 1991). As such, biological control is deemed one of the most effective and sustainable ways of controlling invasive alien plants, with minimal to no risk of non-target effects.

This type of invasive alien plant management strategy has been used successfully in 130 countries, with over 550 biological control agents having been released (Winston et al., 2014). For example, McConnachie et al. (2003) found that the use of biological control agents in the management of *Azolla filiculoides* L. (Salviniaceae) saves the country some US$ 589 per ha/yr. Furthermore, a significant number of invasive alien floating macrophytes which are considered among the worlds' worst aquatic weeds have been successfully controlled in certain parts of the world using classical biological control (Hill, 2003; McConnachie et al., 2014). For example, a recent economic analysis by Maluleke et al. (2021) showed that the most problematic aquatic weeds in South Africa were successfully managed using biological control at a fraction of the price compared to chemical control.

Regardless of the successes highlighted above, biological control programmes still have some risks associated with them, as with other alien invasive species management options. Examples of these include non-target effects, such as host shift or expansion, and indirect effects from changes in the food web (Hussner et al., 2017). As such, classical biological control has received significant scrutiny over the years (Callaway & Maron, 2006). This close examination of such programmes led to re-evaluation of safety measures during the assessment stages to ensure that these risks are reduced (Callaway & Maron, 2006).

In its early stages, safety standards for biological control were mainly focused on minimizing non-target effects on economic crops (Louda et al., 2003; Messing & Wright, 2006). However, this has since shifted to also include a wide variety of native plants, regardless of perceived value (Callaway & Maron, 2006; Messing & Wright, 2006). Thus, in the current landscape, no unanticipated effects have been documented, as screening procedures for potential biological control agents are optimised to predict and limit non-target effects for the whole ecosystem (Willis et al., 2003; Hill & Coetzee, 2017).

Besides host specificity testing, biological control programmes also have to show that the release of a specific biological control agent is warranted, and such measures will contribute to the control of the problematic weed. Thus, predicting the efficacy of an agent in the early stages is another challenge that biological control practitioners have to contend with (Callaway & Maron, 2006). Continuous assessment of the experimental design is also necessary to build on the existing techniques, and thus contribute to the continued improvement of biological control programmes and research.

Biological control programmes are not without limitations, as success of these programmes is highly dependent on a variety of environmental factors. Hill & Olckers (2001) highlight some of these factors for aquatic weed biological control. Firstly, they found that cold temperatures increase the time taken to control the weed. This is mainly observed in winter when insects become dormant and inactive (Coetzee et al., 2011). According to Hill & Olckers (2001) highly eutrophic water-bodies may enable aquatic weeds to overcompensate for herbivory.

Biological control activity may also be interrupted by periodic removal of the weed, natural enemy population fluctuations due to flooding and drought, interference from herbicide applications, and mechanical removal of the weed (Cilliers, 1991; Center et al., 1999; Coetzee et al., 2011). Furthermore, the biological control agents may require additional disturbance of the mats by wind and wave action in order to increase mortality caused by feeding damage, hence there may be less success in still waters (Hill & Olckers, 2001). In these instances, successful control of aquatic weeds requires an integrated approach that is also sensitive to the needs of the biological control agents (Van Ginkel, 2011).

1.2.3. Biological control of submerged invasive alien plants

Given the relative success biological control programmes have had in controlling floating weeds (Maluleke et al., 2021), there has since been a different challenge of submerged alien invasive weeds within this field of study (Coetzee et al., 2011). A study by Strange et al. (2019) found evidence for alternative stable states triggered by sudden increases in nutrient availability due to plant decomposition as a result of biological control. These have previously received less attention compared to other terrestrial and free-floating weeds, thus making biological control of these submerged macrophytes a novel idea for the most part.

Submerged alien invasive macrophytes, such as those in the family Hydrocharitaceae (*Egeria densa* Planchon, *Elodea canadensis* Michx, *Hydrilla verticillata* (L.f.) Royle and *L. major*) present a significant threat to aquatic ecosystems, thus demanding more attention. These species and many others have had biological control programmes initiated against them at various stages (Schooler et al., 2006; Mangan & Baars, 2023). For example, *H. verticillata* is a vigorous invader in the U.S.A. and so a biological control programme was deemed mandatory (Buckingham & Bennett 1998). This species has received significantly more attention compared to other alien invasive submerged macrophytes.

Two leaf-mining flies, *Hydrellia pakistanae* Deonier and *Hyrellia balciunasi* Bock (Diptera: Ephydridae) and two weevils, *Bagous affinis* Hustache and *Bagous hydrillae* O'Brien (Coleoptera: Curculionidae) have been released again *H. verticillata* in the U.S.A. As a result, parts of that region have seen considerable control achieved by *H. pakistanae* (Grodowitz et al., 2003). However, limitations such as extreme temperatures, parasitism and nitrogen content of the host plants all restrict insect population and damage. Therefore, management of *H. verticillata* is still largely dependent on chemical control in the U.S.A and elsewhere. In South Africa, *H. pakistanae* together with *B. hydrillae* were also selected for evaluation in South Africa on the basis of their establishment success and damage potential (Coetzee et al., 2011), but were not released. Instead, *Hydrellia purcelli* Deeming was considered sufficiently host specific for release in South Africa in 2015 (Coetzee et al., 2021).

Another highly problematic submerged AIP that has an advanced biological control programme in the USA is *Myriophyllum spicatum* L. (Haloragaceae). This is a more complex case study, involving accidental introductions of control agents, the use of a native North American weevil, *Euhrychiopsis lecontei* Dietz (Coleoptera: Curculionidae) as a biological control agent, and classical biological control with an aquatic midge, *Cricotopus myriophylli* Oliver (Diptera: Chironomidae) (Coetzee et al., 2011). Smith et al. (2019) also successfully developed and released *Hydrellia egeriae* Rodrigues (Diptera: Ephydridae), a biological control agent for the submerged *E. densa* in South Africa. This was the first successful implementation of a biological control agent for submerged weeds in the country.

The focal species of this study, *L. major* (Figure 1.1); commonly known as the African curly leaved waterweed, African elodea or oxygen weed; is another example of a highly problematic weed from the Hydrocharitaceae. This submerged perennial aquatic plant which originates from southern Africa (Obermeyer, 1964) has become a problematic weed in New Zealand, Ireland, UK, Australia and other European countries, probably introduced via aquarium trade (Wells et al., 1997; Champion & Tanner, 2000; McGregor & Gourlay, 2002). The common name of *L. major*, oxygen weed, has facilitated its spread because the aquarium industry often recommended it due to its fast photosynthetic ability which results in the production of oxygen (James et al., 1999). This plant is a vigorous invader in lentic freshwater systems and can produce densa floating mats that readily colonise infested habitats up to 6 meters in clear waters (Caffrey & Acevedo, 2007).

This species and a few other species in the family Hydrocharitaceae such as *E. densa, E. canadensis* and *H. verticillata* are highly invasive exotic weeds in various part of the world (McGregor & Gourlay, 2002; Schooler et al., 2006; Coetzee et al., 2011; Smith et al 2019). *Lagarosiphon major* is very similar to *Elodea sp.* (Figure 1.1) and has even been confused with *Elodea crispa* Hort. Ex Henkel in some instances (Caffrey & Acevedo, 2007). The main distinguishing feature of *L. major* from other *Elodea* species is its spiral alternate leaves (Figure 1.1). The existence of the *Lagarosiphon* genus as a whole has also been contested in the past and was thought to be a species of *Hydrilla*. However, this was later verified to be a distinct genus, and subsequently a number of *Lagarosiphon* species were described (Symoens & Triest, 1983).

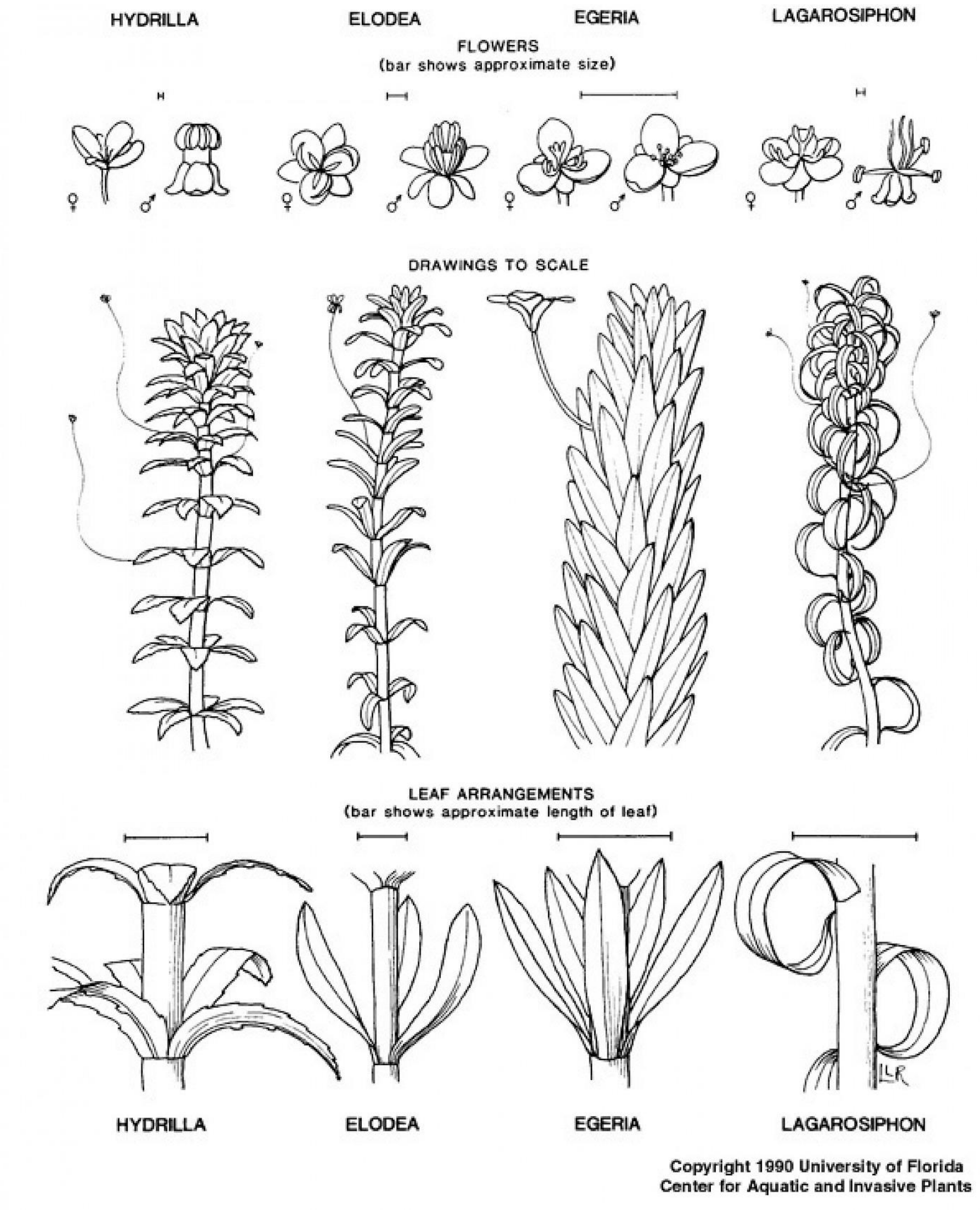


Figure 1.1: The structure of various Hydrocharitaceaes. Drawing from University of Florida, Center for Aquatic Plants (1990).

The leaves of *L. major* alternate and have tapered tips with small blunt unicellular spines on the leaf margins (Bowmer *et al*., 1995). These leaves are curled downwards towards the stem, with the top end of the crown having a densar cluster (Figure 1.1). The thick-walled epidermis gives the leaves a firm structure (Symoens & Triest., 1983). Stem diameter ranges from 3-5 mm, and branches continually beneath the water surfaces, with more repeated branching at the top resulting in the formation of densa mats of *L. major* (Caffrey & Acevedo, 2007). These stems are firm but fragile, and as a result they break off easily when disturbed to aid in dispersal Caffrey & Acevedo, 2007). Adventitious roots are produced at the nodes and grow laterally in the water, taking up nutrients, while longer (up to 50 cm) and tougher roots anchor the plant to the soil below (Bowmer *et al*., 1995). The plant produces inconspicuous male and female flowers in its native range (Obermeyer, 1964).

In its native, *L. major* is mainly found in shallow, muddy, alkaline waters but is also able to establish under most aquatic conditions in its invaded range (Baars et al., 2010; Martin et al., 2013). For example, in New Zealand, this plant grows in deeper, oligotrophic and clear water bodies (Wells et al., 1997; Caffrey & Acevedo, 2007). As a result, *L. major* displaces indigenous macrophyte communities through light starvation, leading to the formation of densa mono-specific stands of this invader (Clayton, 1996; James et al., 2006; Van Ginkel, 2011). Although this plant is known to support high densities of macroinvertebrates (some 300% more than other simple macrophytes) due to its structure (Kelly & Hawes, 2005), *L. major* has been banned in many of the countries listed above due to its invasive vigour (Champion & Tanner, 2000; CHAH, 2020).

Lagarosiphon major has been studied extensively by Caffrey and Acevedo (2007) in Ireland. The plant reached nuisance levels in Lough Corrib (Ireland's second largest lake) in 2005, and continued to grow, covering about 20% of the lake by 2007. This lake is of major ecological importance in this region as it supports 14 habitats and also houses six species of importance.

The local tourism economy also depends on the lake, as it is a popular angling destination for tourists and local communities. Densa stands of *L. major* alter the invaded area by accumulating sediment at the base of the plant, thus ensuring its survival even in oligotrophic conditions as the trapped sediments become a good source of nutrients for *L. major*. These densa stands also block light from penetrating the canopy, thereby displacing many of the native species (Rattray et al., 1994; Champion & Tanner, 2000; Caffrey et al. 2010). Because of these impacts, this species is part of the IAAPs of European Union Concern (EU Regulation, 2016; Genovesi et al., 2015; Morrissey et al., 2020).

Inland Fisheries Ireland, together with other partner agencies have made several attempts to control the spread of *L. major* in Lough Corrib, including chemical and mechanical measures such as benthic barriers (Caffrey & Acevedo, 2007; Morrissey et al., 2020). Results from Caffrey et al. (2010) show that biodegradable jute mats are able to kill *L. major*, while also promoting recolonization by other submerged native species. A more recent report from Morrissey et al. (2020) showed that management efforts from these agencies have been successful at limiting further increases in infestations of this species within the lake, as well as protecting the lower parts of the lake from invasion. Nevertheless, even though infestations have reduced compared to levels from 2013, there has been an overall increase in distribution of *L. major* on the lake (Morrissey et al., 2020).

New Zealand has also seen a similar trend in infestations of *L. major* in its water bodies both in the North Island (Whakapipi stream and Lake Tawera) and South Island (Lake Wanaka and Lake Dustan) (Wells et al., 1997; Champion & Tanner, 2000; McGregor & Gourlay, 2002). According to Clayton (1996), *L. major* is one of the most problematic submerged aquatic weeds in this region. Like Ireland, resource managers in New Zealand employ manual removal, as well as herbicide application in the form of Diquat gel to control *L. major* (Chisholm, 2006).

However, in both instances, these traditional methods of control have proved to be ineffective and highly costly in the long run (McGregor & Gourlay, 2002; Hussner et al., 2017).

1.2.4. Natural enemies of *Lagarosiphon major*

Prompted by the challenges posed by *L. major* in Ireland and European waterbodies, and the lack of information on natural enemies associated with this plant, Baars et al. (2010) conducted a survey of natural enemies of *L. major* in South Africa in order to initiate a biological control programme against this alien invasive weed. A short-term survey had previously been conducted at two sites in the country and showed the presence of natural enemies (Schutz, 2008). The 2010 survey by Baars et al. was more detailed, and as such led to the discovery of several phytophagous insects that were associated with this plant, including a stem mining fly, a number of leaf-feeding lepidopterans (Nymphulinae), and two shoot-boring, leaf-feeding *Bagous* sp. weevils (Curculionidae). One of the weevils had promising prospects but was shelved due to unforeseen taxonomic challenges (Baars et al., 2010). Most of the discovered insects were polyphagous, and so could not be studied any further. There were however two species, a chironomid midge *Polypedilum tuburcinatum* Andersen (Diptera: Chironomidae), and a leaf-mining fly, *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), that were prioritized for further study as possible biological control agents as they had promising monophagous traits (Baars et al., 2010; Deeming, 2012).

Chironomid larvae such as those in the *Polypedilum*/*Pentapedilum* genus form an important part of freshwater ecosystems (Armitage et al., 2012). The following section summarises the biology of *P. tuburcinatum* (Figure 1.2) as given by Earle et al. (2013) and Anderson et al. (2015). Mature females are usually able to lay one or two egg masses just 2-3 days after eclosion. These egg masses are typically laid on top of the water column, and once emerged, the hatchlings disperse in search of suitable plant material. The early instars enter the meristem of the shoots by feeding on the tip of actively growing shoots of *L. major*. This type of feeding usually damages the infested shoot and stops its growth (Figure 1.2), thus inducing the production of more side shoots that are also susceptible to further invasion by *P. tuburcinatum* larvae (Earle et al., 2013). The larvae may make use of multiple shoots during their development. Later instars tunnel down larger stems in preparation for the upcoming pupal stages. The complete life cycle from egg to adult takes about 30-50 days in warmer temperatures (~21ºC) (Earle et al., 2013).

This is a very promising biological control agent for *L. major* as its mode of damage attacks the shoots which are responsible for the plant's nuisance status. Indeed, *L. major* is completely dependent on the production of these shoots for growth and dispersal outside its native range (Caffrey & Acevedo, 2007). As such, this chironomid has the potential to reduce these structures by feeding on new growth, thus weakening the competitive edge of *L. major* over other native plants (Mangan & Baars, 2016).

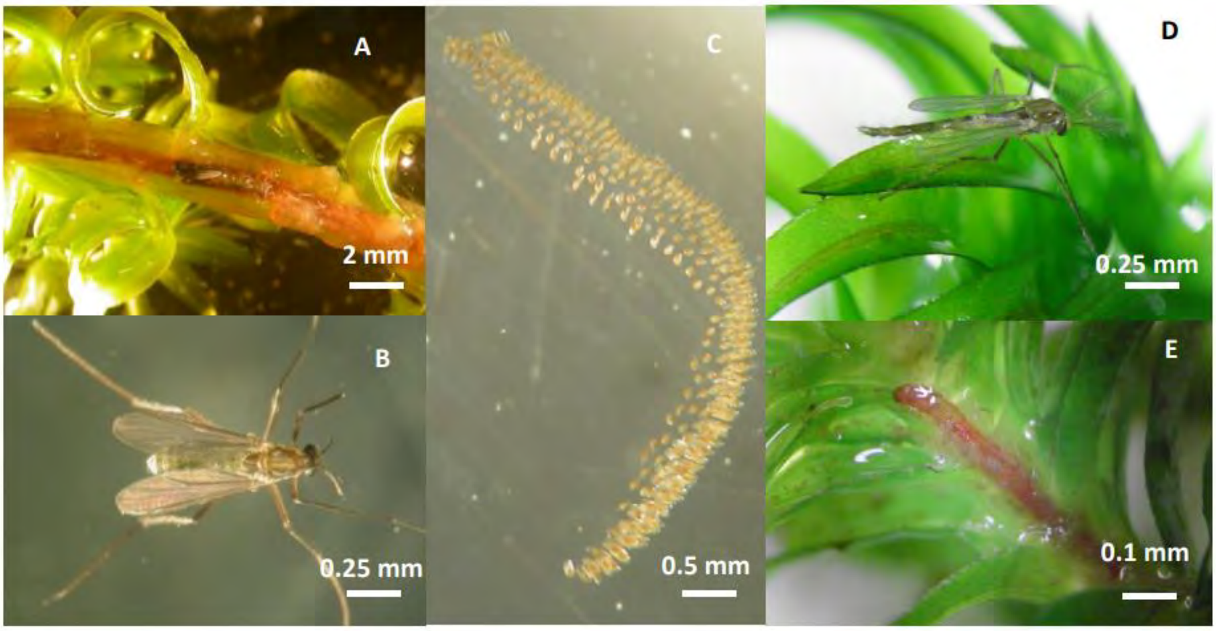


Figure 1.2: Various stages of *Polypedilum tuburcinatum* and tunnelling damage inflicted on *Lagarosiphon major*. Extracted from Earle (2013).

Hydrellia lagarosiphon (Figure 1.3) was shortlisted as a suitable biological control candidate due to its wide distribution in its native range and the amount of damage it causes to *L. major* (Baars et al., 2010). This species was also favoured because *Hydrellia* flies have previously been used in biological control programmes against species in the Hydrocharitaceae in several countries worldwide (Balciunas & Burrows, 1996; Wheeler & Center, 1996, Smith., 2019; see also session 1.2.3 above). According to studies conducted by Martin et al. (2013) and more recently, Mangan & Baars (2023), *H. lagarosiphon* seem to be a very promising biological control agent for *L. major*.

The following section gives a summary of the biology of *H. lagarosiphon* as given by Martin et al. (2013) and Baars et al. (2010). According to Martin et al. (2013), the biology of this fly is typical of other species in this genus that feed on Hydrocharitaceae plant species. Like other species in the *Hydrellia* genus, *H. lagarosiphon* lays its eggs singly or in small clusters on protruding vegetation of *L. major* and the hatchlings commence feeding on the soft tissue on the tip of the plant. The leaf-mining larvae then moves down the shoot to find slightly older undamaged leaves.

The larvae of *H. lagarosiphon* are responsible for causing damage to *L. major*. This potential biological control agent acts by feeding in-between the two epidermal layers which contain photosynthetic tissue. This action leaves behind transparent leaves that are unable to photosynthesize (Bownes, 2014; Weeks & Cuda, 2014). Each larva can feed on up to 50 leaves per shoot, and damaged leaves are left vulnerable to infections. Pupation also occurs within the leaf, taking around 14 days to complete. The emerging adults live just above the water surface, making use of the emerging vegetation for their habitat. These insects do not fly long distances, rather, they walk and fly-hop short distances on the water surface.

According to Martin et al. (2013) and Baars et al. (2010), the average infestation of *L. major* shoots by *H. lagarosiphon* was around 58% in the native range, but 100% infestations were also recorded. *Hydrellia lagarosiphon* has been shown to reduce branching of *L. major*, thus reducing its competitive edge over other submerged macrophytes (Martin et al., 2013). This implies that this agent could reduce weed infestations in invaded areas if sufficient starting densities of approximately 8 larvae per shoot are provided (Martin et al., 2013; Mangan & Baars, 2013), and further augmentative releases are done (Smith et al., 2022).

It is however possible that even at lower densities, *H. lagarosiphon* could still benefit local vegetation, by reducing *L. major* cover and thereby allowing native vegetation to regrow (Martin et al., 2013). This shows that despite being parasitized by various braconid wasps (Martin et al., 2018), *H. lagarosiphon* has the potential to be a valuable biological control agent of *L. major* in invaded areas such as Ireland, Europe, and New Zealand (Baars et al., 2010). *Hydrellia lagarosiphon* should be able to survive the cold temperatures in these temperate regions as it was found in areas where winter temperatures often reached between -2 and +4 °C in its native range (Martin et al., 2013). This is the most common insect herbivore found on *L. major* in the native range (South Africa), and previous studies show the ease with which the agent could be mass reared for released (Martin et al., 2013; Mangan & Baars, 2013). Its biology also makes it a good candidate for biological control because of its high reproductive rate (Mangan et al., 2023).

Figure 1.3: Various stages of *Hydrellia lagarosiphon* and damage inflicted on *Lagarosiphon major*. Extracted from Baars et al. (2010).

1.3. Aims and specific objectives

Biological control success has previously been cited as evidence for the enemy release hypothesis (Sheldon & Creed, 1995; Williamson & Griffiths, 1996). Thus, the overarching aim of this thesis was to investigate the Enemy Release Hypothesis (ERH) and its role on the invasiveness of *L. major* in New Zealand. It is unknown whether the biomass of this exotic weed in New Zealand exceeds typical values in the native range. Therefore, in order to achieve this aim, this thesis will present chapters dealing with specific objectives. The current chapter (Chapter 1) was used to present the rationale for the project by looking at existing literature and identifying opportunities that need further study within the realm of the ERH. Having achieved this, the following chapters deal with the gaps that have been identified, driven by specific objective.

Specifically, Chapter 2 used existing literature to investigate the general applicability of the ERH in aquatic plants by means of a meta-analysis and systematic review. The key questions that were answered in this section are as follows: (1) What is the overall strength of the evidence of the ERH in aquatic plants? (2) What factors modulate the observed results? Building on from the meta-analysis, Chapter 3 was used to conduct an empirical biogeographical investigation of the ERH and aimed to answer the following questions: (1) What typifies *L. major* sites in South Africa, and how does this relate to New Zealand? (2) How does the presence of this plant affect other macrophyte and invertebrate communities in each country? (3) Are there differences in reproductive output and biomass accumulation between native and invaded range populations? And how does this relate to herbivore abundance in each biogeographical region? This was done by measuring and comparing several plant parameters (biomass, shoot production, and cover) between South Africa (native range) where natural enemies of *L. major* are assumed to play a significant role in controlling the abundance of this plant species, and New Zealand (invaded area), to establish if abundance of *L. major* differs between the two regions.

It is also often assumed that alien invasive weeds are more abundant in their introduced range (Strong, 1977; Williamson & Griffiths, 1996; Brändle et al., 2008), but this is rarely quantified. Such explicit comparisons are crucial for testing the hypothesis that an absence of natural enemies facilitates weed invasion and yet quantitative studies are limited (Keane & Crawley, 2002; Hierro et al., 2005; Weyl & Coetzee, 2013). These data from the native range will also be instrumental in predicting the levels of control that may be expected from the release of biological control agents in New Zealand. This is because relative abundance of plant species in native and exotic habitats is a key predictor of biological control impact (Paynter et al., 2012).

According to Hierro et al. (2005), only a fraction of introduced plant species actually benefit from enemy release and become invasive. Therefore, the association of invertebrate with *L. major* between native and invaded range is important to quantify, in order to make direct correlation between plant damage and herbivore abundance. Thus, based on the ERH, because *L. major* does not have any natural enemies in New Zealand and therefore does not have added pressure to invest in plant defences, it should have significantly higher biomass, cover and rate of reproduction compared to South Africa.

Given the complex nature of freshwater ecosystems, it is not surprising therefore that direct benefit from enemy release does not universally explain the invasion ecology of many macrophytes (Keane & Crawley, 2002; Coulatti et al., 2004; Heger & Jeschke, 2014). Other biotic factors which determine species success include competitive vigour, mutualism, novel weapons, allelopathy, phenotypic plasticity and propagule pressure (Heger & Jeschke, 2014; Fleming & Dibble, 2015; Bolpagni, 2021). Therefore, after getting an overall understanding of what factors drive biological invasions with regards to the ERH, Chapter 4 used Spitters (1983) reciprocal-yield models to investigate the competitive ability of *L. major* against *E. densa* in the presence and absence of biological control by two host-specific leaf mining flies. My hypothesis for this experiment was that *L. major* will be a superior competitor to *E. densa* in the absence of biological control due to its previously documented competitive dominance against other similar macrophytes (Hofstra et al, 1999; Caffrey et al., 2010; Martin et al., 2014). Introduction of biological control agents is expected to further reduce the competitive vigour of *E. densa* compared to *L. major* due to the proven efficacy of *H. egeriae* as a biological control agent of *E. densa* (Smith, 2021).

Having studied the biotic mechanisms of plant invasion, Chapter 5 then explored the abiotic component of climate change on the future of *L. major* invasion in New Zealand by making use of correlative climate modelling techniques. The chapter also combined this method with degree day modelling to predict potential biocontrol agent suitability. The study focussed on desktop climate modelling to allow resource managers to predict changes in distribution of this species, and its control agents, under future climate scenarios projected by the IPCC (2001). Lastly, Chapter 6 provides a general discussion to link all the different concepts together, looking at how the obtained result impact our understanding of the ERH and the management implications thereof, specifically for biological control as a whole. The chapter also presents avenues for future research by identifying limitations to the current study.

Overall, because two species of potential host-specific agents have been identified for the control of *L. major*, the possibility of establishing a biological control programme for *L. major* in New Zealand using *H. lagarosiphon* and *P. tuburcinatum* could reduce the impact of this exotic weed. Due to their previous consideration as a biological control candidate for *L. major* in Ireland (Baars et al., 2010; Deeming, 2012; Mangan & Baars, 2013), there is a lot of information already available on the biology of these insects. And since there are no native species in the Hydrocharitaceae in New Zealand (De Lange & Rolfe, 2010), risk of non-target effects of introduced biological control agents is significantly reduced in this region. Ultimately, the release of *H. lagarosiphon* and *P. tuburcinatum* if approved, will allow for a more holistic and integrated management strategy in New Zealand, while also adding to the body of knowledge on the ERH. And since *L. major* is one of the most costly submerged invasive alien macrophytes to control in New Zealand (Clayton, 1996; McGregor & Gourlay, 2002; Deloitte, 2011) and elsewhere (Caffrey & Acevedo, 2007; Caffrey et al., 2010; Morrissey et al., 2020), biological control has the potential to significantly reduce these costs in the long run.

Chapter 2: Meta-analysis and systematic review of the Enemy Release Hypothesis as applied to aquatic plants.

2.1. Introduction

Understanding the factors that influence the success and distribution of species is an important part of ecology. According to the Enemy Release Hypothesis (ERH), alien species may experience a decrease in regulation by herbivores and other natural enemies when they are introduced to a new region, mainly due to the absence of their highly specialised monophagous natural enemies, which may allow a rapid increase in distribution and abundance (Williamson & Griffiths, 1996; Keane & Crawley, 2002). This is because the alien species have not co-evolve with the natural enemies in the new region, and as a result, those polyphagous natural enemies may not be as effective at suppressing the exotic species' population growth (Strong, 1977; Brändle et al., 2008). Therefore, the introduced species may have a competitive advantage over native plants due to their reduced allocation of resources to defence and increased allocation to growth and reproduction (Williamson & Griffiths, 1996; Zangerl & Rutledge, 1996).

The ERH has been tested on a variety of systems and taxa (Roy et al., 2011; Prior & Hellmann, 2013; also see Chapter 3), including aquatic plants which are an important component of freshwater ecosystems (Kwong et al., 2019; Petruzzella et al., 2020). However, there is still some debate about the strength of the evidence for the ERH in aquatic plants. This hypothesis is not universally supported by empirical evidence and may vary depending on the plant species, the enemy type, the environmental context, and the spatial and temporal scale of the study (Prior & Hellmann, 2013; Fleming & Dibble, 2015).

While some studies have found support for the ERH (Knevel et al., 2004; Vilà et al., 2005; Yan et al, 2022), there are many others which have not (Serra et al., 2013; Steele et al., 2018; Zhang et al, 2021). Therefore, there is a continuing need for a comprehensive and critical synthesis of the existing literature on the ERH, especially in aquatic plants, to evaluate its validity, generality, and applicability. This chapter aims to provide such a synthesis by conducting a systematic review and meta-analysis of the various studies that have tested the ERH in aquatic plants.

Meta-analysis involves the statistical synthesis of data from multiple independent studies, allowing for quantitative assessment and generalization of patterns and trends (Gurevitch & Hedges, 2001). These types of studies are abundant for terrestrial ecosystems, investigating a variety of response variables, including growth rates, reproductive success, biomass, and community-level metrics (Knevel et al., 2004; Vilà et al., 2005; Fleming & Dibble 2015). Invasive macrophytes tend to be included as an afterthought in these meta-analyses and as such, there are not many recent stand-alone syntheses which exist in this regard.

This meta-analysis pooled the results of published studies to assess the overall strength of the evidence for the ERH in aquatic plants with the following key objectives: [1] To quantify the effect size of enemy release on various plant traits (e.g., biomass, growth rate, reproduction) and compare it among different plant groups (e.g., submerged vs. emergent), and regions (e.g., native vs. invaded); [2] To identify the sources of heterogeneity and potential moderators of the enemy release effect (e.g., plant functional traits, enemy diversity and specificity, environmental factors).

This investigation will help to clarify the role of the ERH in the invasiveness of aquatic plants, and this has significant implications for conservation and management efforts of these species. For example, if introduced aquatic plants indeed experience enemy release, they may exhibit invasive behaviour and have detrimental impacts on native communities, potentially leading to biodiversity loss and ecological disruption (Clayton, 1996; Mooney & Cleland, 2001; Van Ginkel, 2011). Thus, reintroduction of specialised natural enemies, also known as biological control, may form part of management avenues to be considered. Conversely, if the ERH is not supported, this knowledge can inform strategies to control or mitigate the spread of alien invasive aquatic plants, by helping to determine the relevance of biological control for example. Additionally, the results of this study will contribute to our understanding of the mechanisms that drive the invasion success of exotic aquatic plants.

2.2. Methods

2.2.1. Systematic literature search

In order to synthesize existing data in relation to the ERH in macrophyte communities, the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) system was followed (Figure 2.1), in conjunction with the PICO framework (<u>Population</u>: Aquatic macrophytes <u>Intervention</u>: Invaded systems <u>Comparison/Control group</u>: Native species <u>Outcome</u>: Plant parameters, e.g., abundance, coverer and biomass, abundance) (Page et al., 2020). Using the framework, the following primary question was formulated: Do exotic plant species in freshwater ecosystems have increased fitness compared to native species?

The following inclusion and exclusion criteria for the studies were established prior to conducting the search: **Inclusion criteria**= [1] peer reviewed article; [2] Comparing enemy pressure and/or aquatic plant traits between native and invaded regions or between invasive and native plants within the same region; [3] Report quantitative data on enemy pressure (e.g., enemy abundance, damage, infection) and/or plant traits (e.g., biomass, growth rate, reproduction, defence) that is easily extractable. **Exclusion criteria**= [1] Not terrestrial organisms or large herbivores, or vertebrates, or crops, or fish or agricultural. Studies were excluded if they were not available in English or if they were review articles, book chapters, or conference abstracts without sufficient original data. Duplicate studies and articles were also excluded.

A literature search was initiated (17-21 June 2023) using Web of Science to find empirical studies relating to ERH published between 2012-2023 to capture the most recent literature without overwhelming the reviewer. The following key word combinations based on the inclusion/exclusion criteria were used to filter through the available literature and ensure that we capture all the possible synonyms: (invasi* OR invader OR alien OR exotic OR weed OR non-native OR introduced OR naturaliz) AND (native OR indigenous OR endemic) AND (submerged OR freshwater OR aquatic OR macrophyte OR “submerged plant” OR “aquatic plant” OR “freshwater plant” OR floating OR “floating plant” OR emergent OR “emergent plant”) AND ("reproduc*" OR “leaf area” OR length OR hight OR shoots OR cover OR growth OR abundance OR biomass OR competition) AND (“Enemy release" OR “native range” OR biogeographic OR intercontinental OR invertebrate OR insect OR herbivore) NOT (crop AND agric* AND terrestrial AND land AND vertebrates AND fish AND marine)

This yielded 244 peer reviewed scientific papers available at the following website: https://www.webofscience.com/wos/woscc/summary/68528c39-11d7-486a-a8c7-759621edf51e-93f2b365/relevance/1 [last accessed in August 2023]. A quick read of the title and abstract using the TclTk GUI (version 8.6) in R (version 4.3.1) (R Core Team, 2020) helped to determine which articles were most relevant and appropriate based on highlighted keywords from the previously stated inclusion and exclusion criteria (Figure 2.1). Inclusion was also determined by availability and type of data reported in those studies. No reviews or meta-analyses were included in the data, but empirical studies cited in those articles were incorporated when relevant. The full texts of 34 remaining articles were downloaded from Google Scholar after the initial screening process and assessed for eligibility using the same criteria before extracting the data. Furthermore, the snowballing method by described by Lajeunesse (2013) was also used to retrieve additional studies that may have been missed, and this was done by checking the reference lists of the screened articles for related material (Figure 2.1).

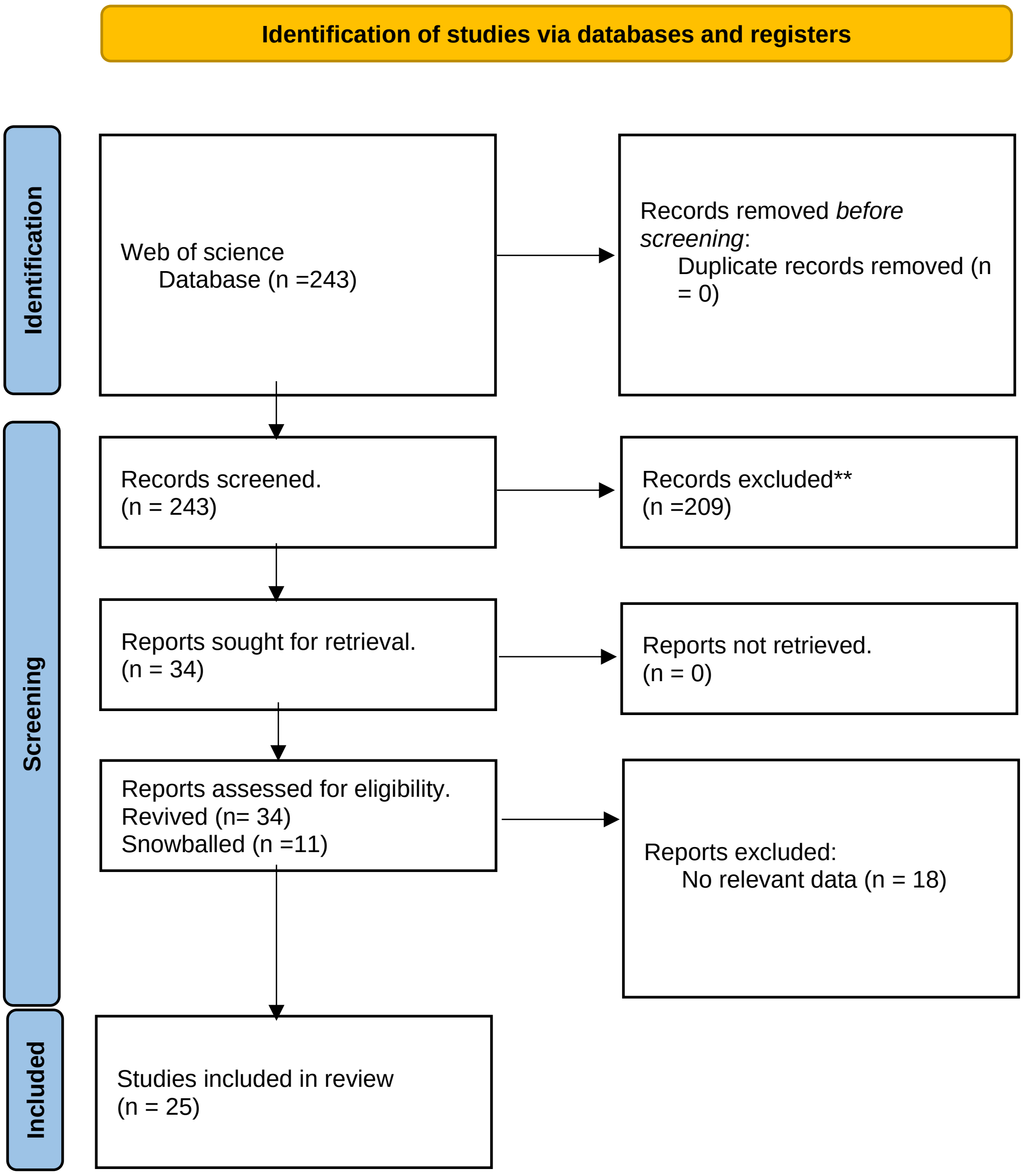


Figure 2.1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Page et al., 2020).

2.2.2. Data extraction and Meta-analysis

The following data were extracted from each eligible article: (1) study characteristics (study system, plant species, plant growth form, stress type, region, climate); (2) methodological details (study type, sample size, sampling period); (3) outcome data (mean and standard deviation of enemy pressure and/or plant trait of invasive and native species in each treatment group). Outcome data not reported in text or table format were extracted from plots using WebPlotDigitizer (https://apps.automeris.io/wpd/). The full data set is available in Appendix 3.1.

The Standardized Mean Difference (SMD) was used as the effect size of enemy release on plant traits. The SMD is defined as the difference in means between two groups divided by the pooled standard deviation. A positive SMD indicates that invasive plants have higher trait values or lower enemy pressure than native plants or that trait values are negatively correlated with enemy pressure (support for ERH). A negative SMD indicates the opposite. Hedges' d was used as an unbiased estimator of the SMD as well as calculated the variance of the SMD. Effect sizes were calculated based on the following operational definition and equation adopted from Hedges' *d* (Hedges 1981):

$$d= ((X_1-X_2/ sp)\ \text{x}\ ((N-3)/N-2.25))$$

Where d represents the effect size, X_1 and X_2 are the means of invasive and native species, respectively, sp is the pooled standard deviation of both groups and N is the total sample size of both groups. Each effect size was then multiplied by the following distribution bias correction formula to weight the quality and reliability of the studies according to sample size:

$$J_{(bias)}= 1 – (3 / (48 (n_{invasive} + n_{native}) -1)$$

Where n represents the sample size of each population.

Multivariate meta-analysis in the metafor package (version 4.2.0) (Viechtbauer, 2010) was used to perform random-effects meta-analysis in R (version 4.3.1) (R Core Team, 2020) to account for potential heterogeneity among studies. First, an estimation of the overall effect size of enemy release on plant traits across all studies was modelled. This is referred to as the grand-mean in the results. Study ID was included as a random effect to account for multiple effect sizes from the same study. The restricted maximum likelihood estimator was used to estimate the between-study variance and assessed the heterogeneity of effect sizes using the Q statistic. After running the model, the robust function was used to check and account for effect size dependencies.

Egger's test and funnel plot was used to test for publication bias, and it was found that there was a significant deviation from a symmetrical data set ($z = -3.820$, $P < 0.001$). This means that negative results were less likely to be published. The trim-and-fill method from metafor package was used to correct the observed asymmetry (Whiting et al., 2016). A sensitivity analysis was then conducted by re-running the meta-analysis with the trim-and-fill data and comparing the results with the original data set. Furthermore, the Vevea & Hedges (1995) weight-function model (weightr package) for publication bias was used to confirm results of the Egger's test. Upon model validation, forest plots were generated to visualize the effect sizes and their confidence intervals. Subgroup analyses were conducted based on the following factors or modulators to explore potential sources of heterogeneity: (1) growth form (submerged/ emergent), (2) study type (observational/biogeographical/ experimental) and (3) type of measurement (Defences, Consumption, Population, and Nutrient uptake). Differences in all the collected plant parameters between these modulators were used to test the ERH.

2.3. Results

2.3.1. Composition of studies synthesised.

A total of 284 (k) effects were extracted from 25 (n) studies, and the robust test showed that the effects were sufficiently independent as the results were similar to the original model (see appendix 3.2- 3.4 for which studies contributed most). Three influential effects from Serra et al., (2013), Zhang et al., (2021), and Yan et al., (2022) were identified as outliers by the metafor influence function (Figure 2.2). However, these were not removed from the study as there was no real reason found within the raw data to exclude them (e.g, significantly larger sample size relative to the rest of the studies).

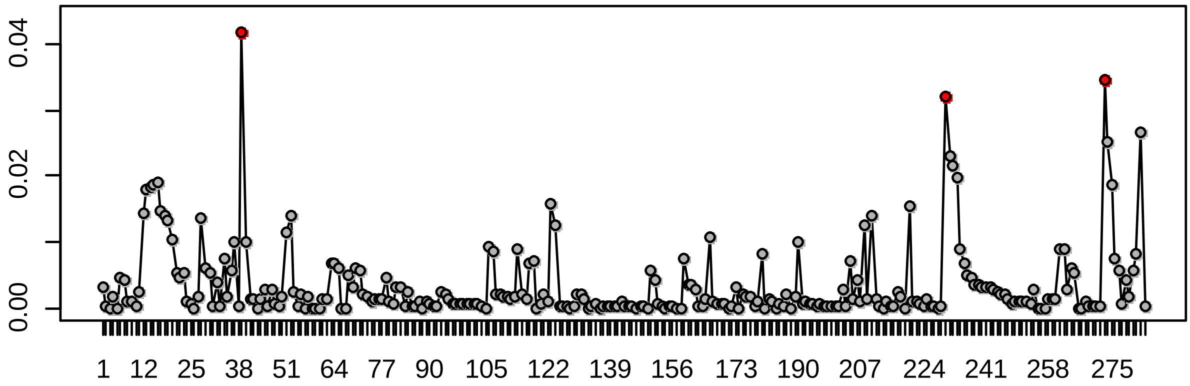


Figure 1.2: Cook's distance showing influence of individual points on the overall model. Outliers are shown in red.

Although there was significant evidence of funnel asymmetry (z= -3.820, *P* = 0.0001) according to Egger's test (Figure 2.3), comparison between the original grand-mean model and trim-fill result showed that the original effect size estimate is relatively robust to potential publication bias as adjusted estimate remained statistically insignificant, with confidence intervals comparable to the original analysis. This suggests that the observed effect is likely to be real and not solely driven by publication bias. This was further validated by the Vevea and

Hedges (1995) weight-function model for publication bias which also showed no real concern of publication bias (X^2= 0.447, *P*= 0.504).

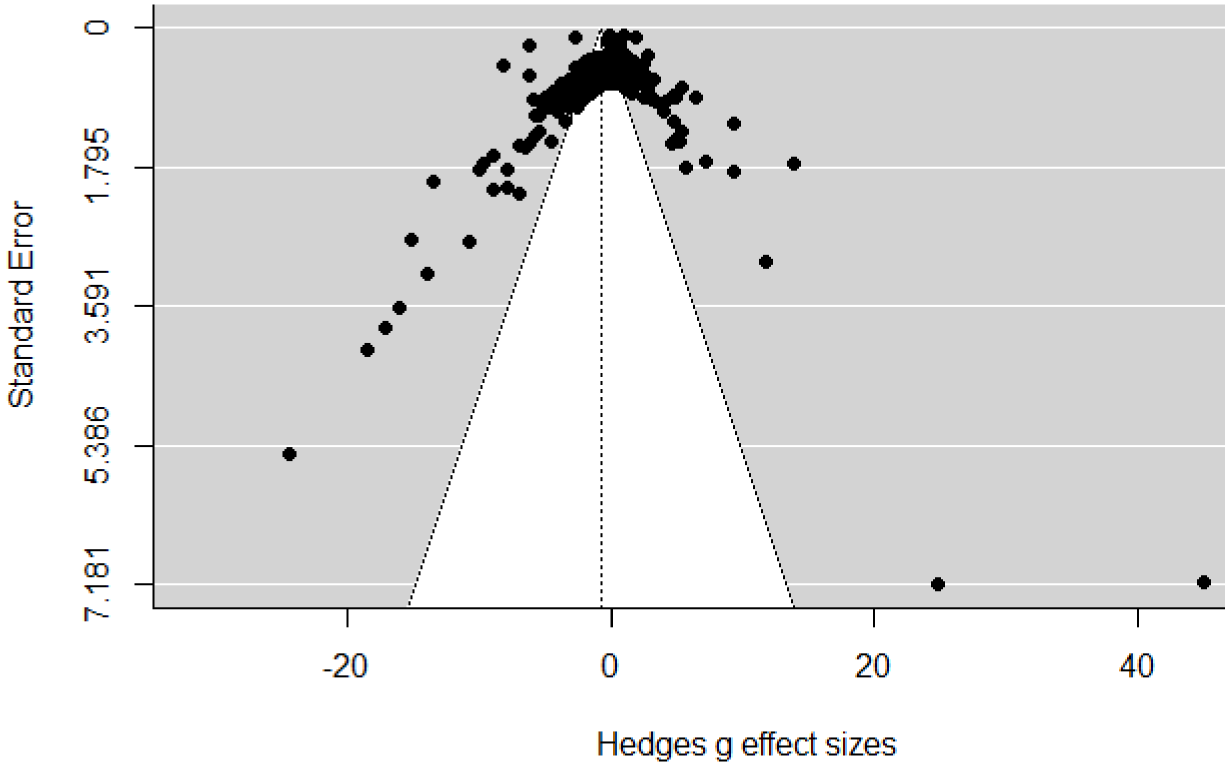


Figure 2.3: Egger's test of plot asymmetry testing for publication bias towards studies with significant *P* values.

2.3.2. Overall effect

The observed outcomes ranged from -24.755 to 45. 024, with the majority of estimates being negative (56%). This means that more than half of the extracted effects did not find evidence for the ERH. The estimated grand-mean outcome û=-0.530±0.645 (95% CI= -1.794 to 0.733). However, there was no difference between native and invasive plants (z = -0.823, *P* = 0.411). This means that overall, there was no evidence for or against the ERH. Nevertheless, according to the Q-test, the true outcomes appear to be highly heterogeneous ($Q_{(283)} = 4909.885$, $P < 0.001$). Therefore, although the grand mean was estimated to be negative, in some studies the true outcome may in fact be positive, as the 95% CI overlap with zero (see figures below). The following sections investigated this heterogeneity further by looking at the various modulating factors such as growth form, study type, etc.

2.3.3. Single Moderators

Overall, the analysis suggests that there were significant differences between authors in terms of their findings on ERH ($Q_{(25)} = 168.611$, $P < 0.001$). For example, Yan et al. (2022) found significant evidence for this hypothesis ($z= 5.602$, $P < 0.001$), while 11 other studies also found evidence, but these were not statistically significant (Figure 2.4). Furthermore, Serra et al. (2013), Steele et al. (2018) and others found significant evidence against the hypothesis (Figure 2.4). For these studies, native plant fitness was higher relative to invasive plants. Nevertheless, there was significant residual heterogeneity among authors ($Q_{(259)} = 4102$, $P < 0.001$). The following section investigated this heterogeneity further by looking at other possible modulating factors for the observed results.

Figure 2.4: Forest plot of outcomes grouped by author and coloured by type of study conducted. Hedges g estimates ±95% CI. Numbers represent k=number of outcomes per category.

With regards to the different species that were included in the selected ERH studies, although there was a lot of variation between species, ($Q_{(18)} = 318.3961$, $P < 0.001$), there was no evidence of this hypothesis either. This is with the exception of *Alternanthera philoxeroides* (Mart.) Griseb (Amaranthaceae), ($z= 2.193$, $P = 0.028$), and possibly *Pontederia crassipes* (formerly *Eichhornia crassipes*) Mart. (Pontederiaceae), ($z= 1.902$, $P = 0.057$), which showed significantly increased fitness outside their native range, or when compared to their native counterparts. Most of the species had confidence intervals that overlap with 0, which is an indication of no significant finding. On the other hand, *Ceratophyllum demersum* L. (Ceratophyllaceae), *Myriophyllum spicatum* L. (Haloragaceae) and *Salix fragilis* L. (Salicaceae) showed the opposite of what one would expect based on the hypothesis (Figure

2.5), suggesting better fitness in the native range or against other invasive plants. The presence of residual heterogeneity suggests that there is unexplained variability among the studies beyond what the model accounts for ($Q_{(265)} = 4455.9237$, $P < 0.001$), and this was explored further in the following sections.

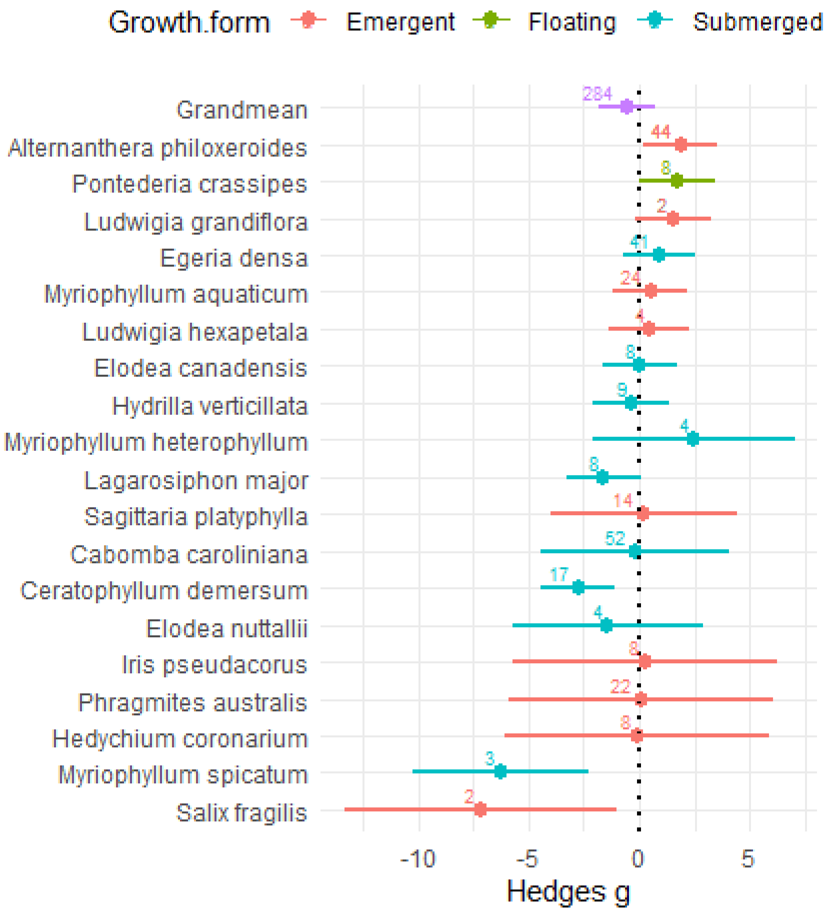


Figure 2.5: Forest plot of outcomes by species and growth form. Hedges g estimates ±95%CI. Numbers represents k=number of outcomes per category.

There was a significant difference in outcomes with regards to the ERH between plant response to treatment manipulation and no manipulation (control) ($Q=31.790$, $P <0.001$). In the absence of a stress factor or no manipulation, there was no significant difference between native and invasive plants as the Hedges g estimate confidence intervals overlap with zero (Figure 2.6). However, the introduction of a stress factor (e.g., temperature increase, competitions, herbivory, etc.) during manipulative experimentation leads to better performance of invasive macrophytes relative to their native counterparts (z= 5.638, *P* <0.001). Another model investigating the potential interaction between manipulation type and growth form showed that study outcomes varied according to growth form ($Q_{(6)} = 41.4369$, $P < 0.001$), however, the individual estimates from this model were not statisticaly significant.

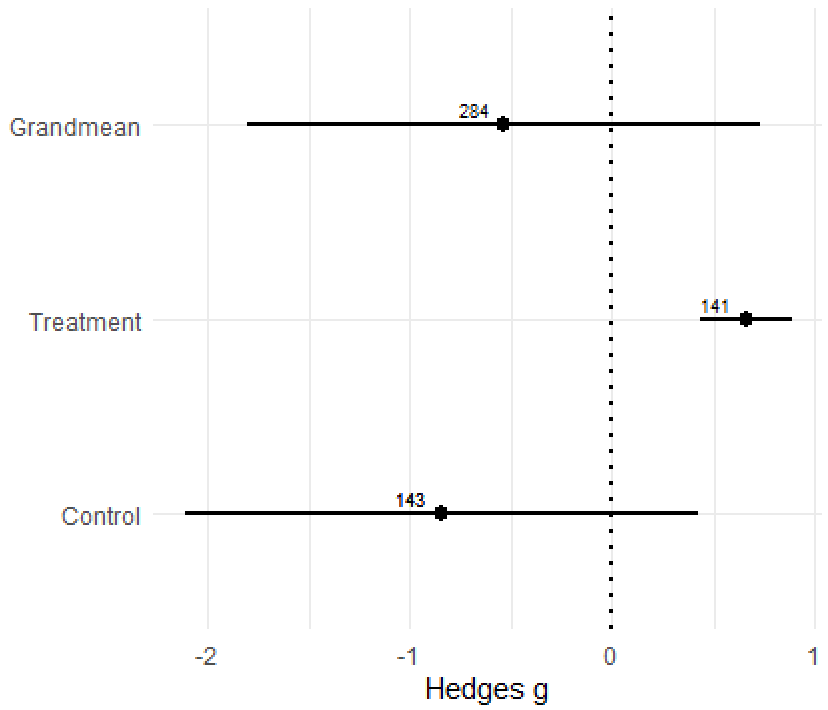


Figure 2.6: Forest plot clustered by type of manipulation. Hedges g estimates ±95% CI. Numbers represents k=number of outcomes per category.

When looking at growth forms, there was no significant difference in outcomes observed between submerged, floating and emergent macrophytes ($Q_{(2)} = 2.494$, $P = 0.287$). However, study outcomes varied according to the type of study conducted ($Q_{(3)} = 10{,}930$, $P < 0.001$). For biogiographical studies, there was very weak evidence of increased fitness outside of native range, and the same was true for manipulative experiment designs (no real difference between native and invasive plants) (Figure 2.7). Nevertheless, contrary to the ERH, observational studies within the invaded range showed that native species had significantly higher fitness relative to their invader counterparts (z= -3.974, *P* ~ 0.001). Again, there was considerable variation even within study types ($Q_{(281)}$ =4848.183, $P < 0.001$). A different model showed that the interaction between plant growth form and study type did not provide enough explanation for the observed variation either ($Q_{(8)}$ =12.870, *P* =0.1164).

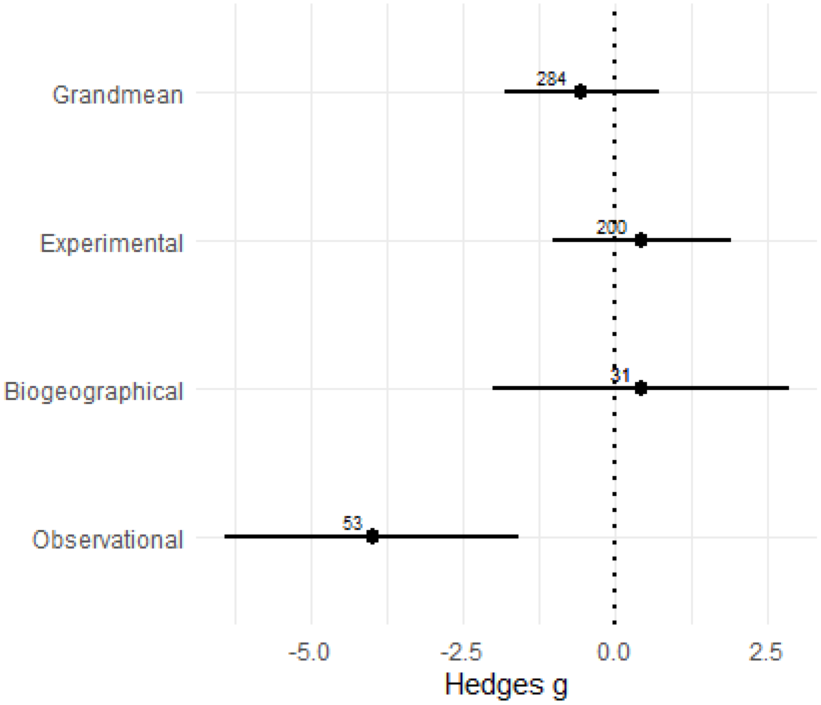


Figure 2.7: Forest plot clustered by study type. Hedges g estimates ±95% CI. Numbers represents k=number of outcomes per category.

Evidence for or against the ERH also varied according to plant fitness parameter reported ($Q_{(5)}$ =433.244, $P < 0.001$), with measures of consumption being the only variables to provide sufficient evidence for this hypothesis (z=2.480, *P* =0.013). This suggests that invasive species were less vulnerable to herbivory and diseases compared to native species (Figure 2.8). Nutrient competition measures (e.g. rate of nutrient uptake), growth parameters (RGR, photosynthetic rate, plant height, etc.), as well as plant defence measures (C: N, lignin, phenolics, etc.) provided neutral outcomes (Figure 2.8). On the contrary, plant population level measures (e.g. species richness, density, and abundance) showed that the presence of invasive species is also linked to overall population diversity (z= -3. 085, p=0.002).

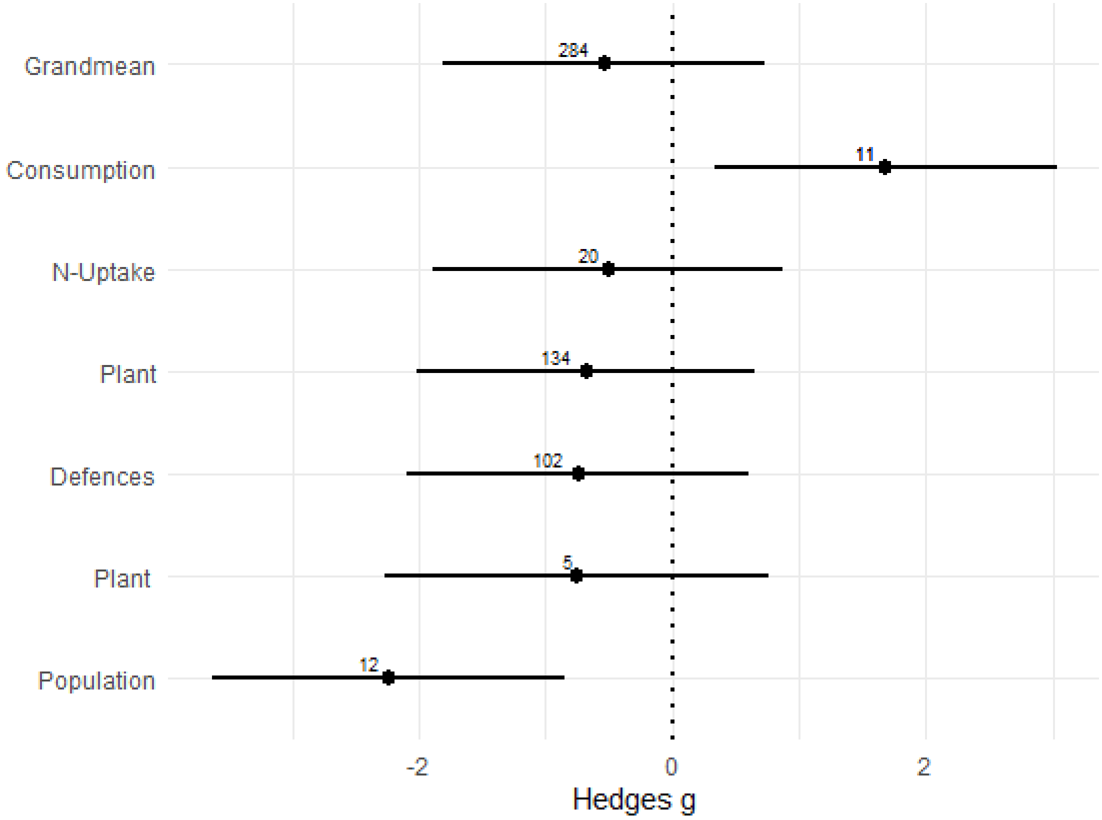


Figure 2.8: Forest plot of outcomes according to plant fitness measure reported. Hedges g estimates ±95% CI. Numbers represents k=number of outcomes per category.

2.3.4. Multiple moderators

To account for the high variability within the above model for reporting parameters (Q (282) =4903.561, p<0.001), a two-way interaction model was fitted. This model explored the relationship between study type and reporting parameter (Figure 2.9) and found that there was significant variation in reported plant parameter outcomes of studies in terms of the ERH based on study type (Q (10) =511, 313, p<0.001). For example, biogeographical studies have no real support for any of the parameters reported here. On the other hand, defence parameters for both observational and experimental studies tend to go against the hypothesis, showing that native rather than invasive macrophytes have higher fitness in the natural environment. This is shown by the negative estimate with 95% CIs that do not overlap with zero (Figure 2.9).

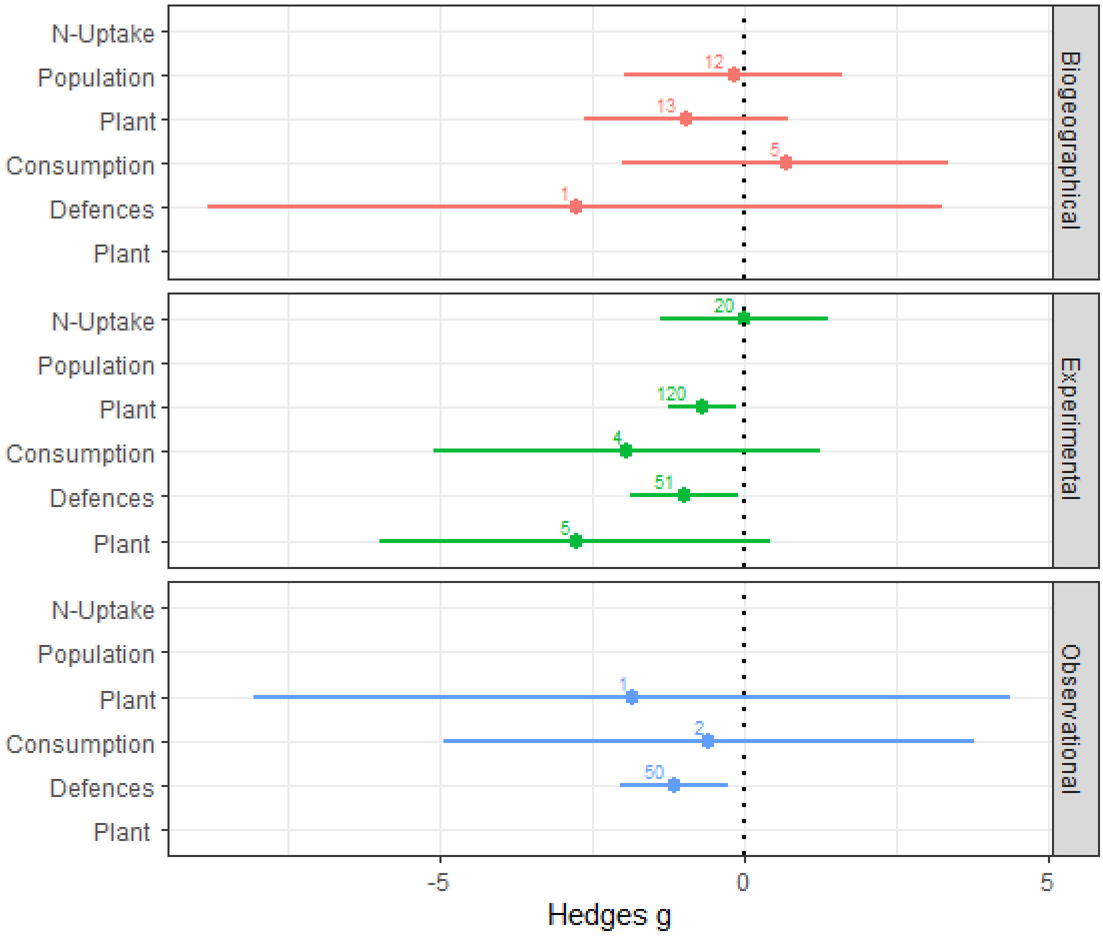


Figure 2.9: Forest plot of plant fitness measure outcomes according to study type. Hedges g estimates ±95% CI. Numbers represents k=number of outcomes per category.

The potential relationship between reporting parameter and growth form also plays an important role in modulating study outcomes in terms of the ERH ($Q_{(11)} = 523.202$, $P < 0.001$). Here, it is evident that only submerged macrophytes are less vulnerable to consumption relative to native plants ($z=2.795$, $P = 0.005$), providing evidence for the hypothesis. In terms of floating plants, there is no real indication that invasive macrophytes in this growth form perform better or worse than their native counterparts or outside of their native range. This is because the available estimates within this group all overlap with zero in terms of the 95% CI (Figure 2.10). Nevertheless, the negative estimates for defence parameters in submerged plants (z= -2.142, *P* =0.032), and population level estimates for emergent macrophytes (z= -3.228, *P* =0.001) indicated that for these groups, the ERH does not hold true (Figure 2.10). Rather, it is the native submerged species that have fewer defences than invasive conspecifics, as well as increased species richness in invaded areas.

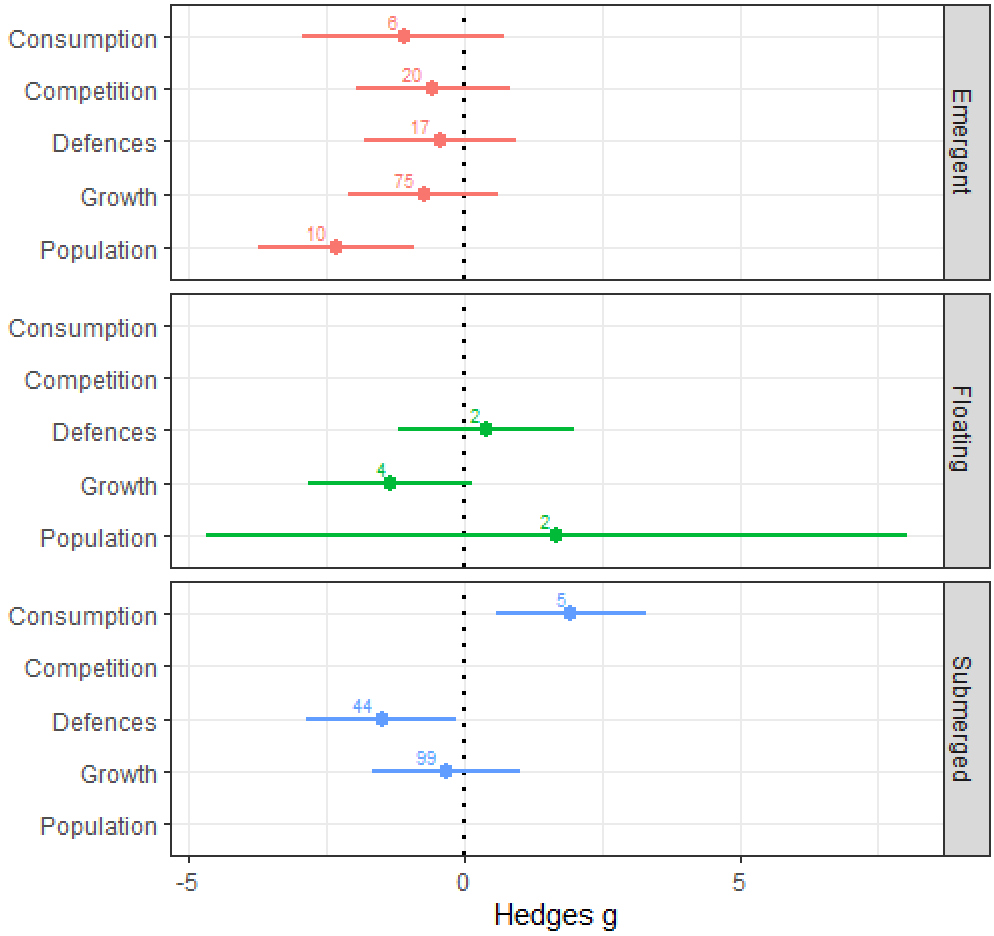


Figure 2.10: Forest plot of plant fitness measure outcomes according to plant growth form. Hedges g estimates ±95% CI. Numbers represents k=number of outcomes per category.

2.4. Discussion

The aim of this study was to use existing literature to investigate the applicability of the ERH within invaded aquatic plant communities. The results reported here attempted to identify and dissect the intricate interplay of variables that modulate observed study outcomes in relation to this hypothesis. By examining diverse growth forms, assessing multiple fitness parameters, considering the influence of stressors, and scrutinizing different study methodologies, this study provided a comprehensive overview of the contextual complexities for the evidence supporting the ERH.

Combining the outcomes from all the studies included here provided no meaningful information with regards to the ERH. This is because even though the overall outcome was negative [no evidence for the hypothesis], there was very high variability between the captured outcomes. This variation can be attributed to diverse factors such as differences in experimental design, ecological context, and inherent variation in ecological systems. This aligns with previous studies that have shown the complexity of applicability and evidence for this hypothesis (Keane and Crawley, 2002; Coulatti et al., 2004; Fleming & Dibble, 2015).

Further investigation of the many possible modulating factors provided valuable information and interesting trends. Firstly, as expected, there was much variation between studies and between the different species, with most systems exhibiting neutral outcomes. This was further broken down into manipulative vs non-manipulative studies. Here, there was significant evidence of the ERH in manipulative experiments. This change in ecological dynamics under specific stressors highlights the dynamic nature of ecological interactions and the critical role of stressors in shaping these interactions (Vitousek, 1990). Manipulation of stressors like temperature increases, competition, and herbivory can alter species interactions, favouring invasive species under specific conditions (Ricciardi et al., 2013; Denley et al., 2019).

Differentiating between study types was an important step in this investigation as different studies provide distinct insights into ecological interactions (Colautti et al., 2004). Biogeographical studies, for instance, consider historical factors and evolutionary dynamics (Keane and Crawley, 2002, Silveira et al., 2018; Kwong et al., 2019), whereas manipulative experiments allow controlled exploration of mechanisms (Mozdzer et al., 2010; Gufu et al., 2018). Observational studies, on the other hand, capture the complexity of natural interactions in situ (Serra et al., 2013; Fleming & Dibble, 2015; Redekop et al., 2018).

Results from this study showed that experimental designs and biogeographical studies provided only marginal support for the ERH, whereas outcomes from observational studies significantly deviated from assumptions of this hypothesis. Here, native species appear to be doing better than invasive conspecifics. One explanation for this could be that native species which are better adapted to the local resource competition and defence dynamics, may perform better in the presence of invasive species, which tend to downregulate defence mechanisms in new environment in support of growth (Callaway & Ridenour, 2004; Hejda et al., 2009). This is especially true when there is a dominance of generalist herbivores within the invaded landscape.

The consideration of varied outcomes according to plant parameter reported revealed consumption parameter outcomes that were in agreement with this hypothesis, while population level parameters went against the assumptions of this framework. The observed trend of increased community fitness in invaded areas challenges the assumptions of reduced population diversity due to competition from these supposedly superior invaders. These findings resonate with studies that show that invasions can create novel niches, benefiting both native and invasive species (Denley et al., 2019; Zhang et a., 2021).

The complexity of ecological factors in real world scenarios such as competition, resource availability, and biotic interactions as seen here can lead to outcomes that diverge from theoretical expectations such as those of the ERH. Therefore, there is a need to explore other mechanisms that may interact with or override the ERH in aquatic plants, such as competition, mutualism, evolution of increased competitive ability, novel weapons, allelopathy, phenotypic plasticity, propagule pressure, and climate suitability (Fleming & Dibble, 2015; Bolpagni, 2021). Although the ERH is still a useful way to think about invasive species, it is a very broad and imprecise hypothesis (Colatutti et al., 2004). As a result, Heger & Jeschke (2014) argue that this should rather be looked at as a hierarchy of hypotheses consisting of more specific and testable sub-hypotheses.

The initial search was only conducted in one database and did not include grey literature. It is possible that the information contained in grey literature might provide meaningful results, but this was outside of the scope of this investigation. Secondly, article screening and data extraction for the meta-analysis was conducted by a single person. Usually, data collection for these types of studies are conducted by at least two or three people to reduce human error (Lajeunesse, 2013). To account for this, the screening process occurred twice to ensure that all relevant articles were captured.

There were also limitations with regards to data availability and comparability between studies. Thus, to convert to a common currency, means and SD of 6 out of 7 outcomes extracted from Silveira et al. (2018) had to be estimated or computed from median and interquartile range with the assumption of normality (multiplied by 0.741). For Kwong et al. (2017) and Kwong et al. (2019), SD was computed from Standard Error of Difference (SED) with the assumption that the two groups being compared have equal variances. For Gillard et al. (2021), the SD was computed from the 95% CI with an assumed critical value of 1.96 which corresponds to the standard normal distribution's value at the 97.5th percentile (Lajeunesse, 2013). And lastly, for

Mozdzer et al. (2010), two outcomes were computed by converting mean ± 1 SE to mean ± SD by assuming a symmetric distribution of the confidence interval.

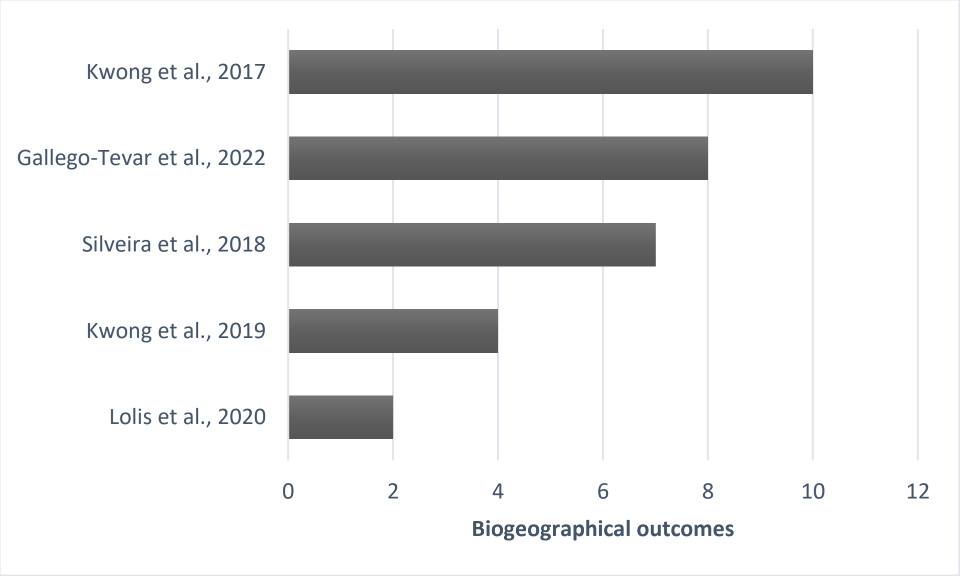
Another possible shortfall of this meta-analysis is that leaf nitrogen (N) content was categorised as a growth and not defence indicator. Although nitrogen plays a crucial role in both the growth and defence of plants (Callaway & Ridenour, 2004), this was an important distinction to make for this study in order to successfully investigate the ERH. Thus, increased N in invasive macrophytes was interpreted as evidence for this hypothesis. However, this might not hold true in all situations as N is also an important contributor to nitrogen-based defences (Mur et al., 2017), which would then mean a different outcome in terms of the ERH which assumes reduced defences in exotic ecosystems (Williamson & Griffiths, 1996; Zangerl & Rutledge, 1996).

Lastly, various scholars have suggested that the enemy release should primarily be studied at a biogeographical scale (Keane & Crawley, 2002; Colautti et al., 2004; Fleming & Dibble, 2015). Nevertheless, these types of empirical studies making direct comparisons of specific species in native and invaded range which meet the criteria of this study were limited (n=5). As a result, observational (n=5) as well as experimental studies (n=15) were included if they made comparisons between specific native and invasive species with similar life histories. Due to this biogeographical data limitation, statistical power to make concrete conclusions about the available evidence for ERH in aquatic plant systems is limited. Thus, more empirical studies investigating ERH at this level in these types of ecosystems is needed. The following chapter will therefore conduct such a comparison for *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae), which is the focal species of this thesis.

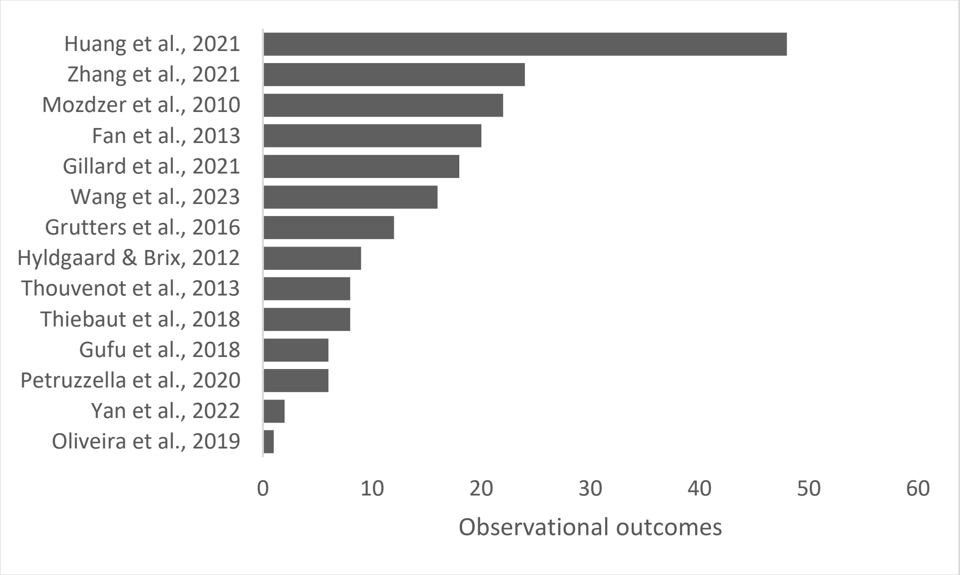
Overall, there was no real evidence that escape from natural enemies confers an advantage to aquatic invasive species as studies were highly variable, with different study designs, plant growth forms and plant parameter measurements. However, further investigation of modulators revealed a complex patten of interactions that cannot be solely explained by the ERH. Although there was empirical support under specific conditions, it is not a universal or consistent mechanism to explain the success of invasive aquatic plants. Nevertheless, managers can still use the results from this synthesis to inform their decisions on the prevention, detection, and control of invasive aquatic plants, taking into account the context-specificity and uncertainty of the ERH and its implications for ecosystem functioning. For example, plants in disturbed ecosystems such as those with high rates of eutrophication were shown to benefit more from enemy release, and therefore managers can use this evidence to argue for biological control in such scenarios. Furthermore, the encountered limitations outlined above highlight a still limited database, requiring more empirical studies in these systems.

2.5. Appendices

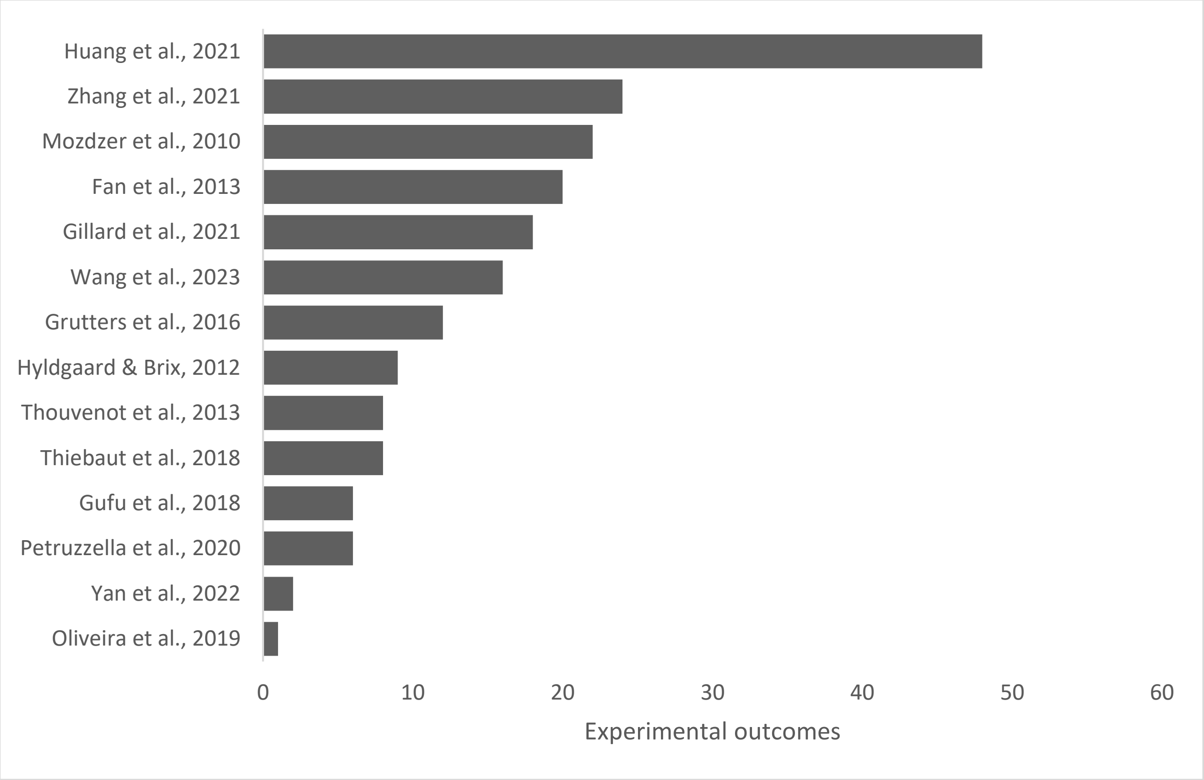
Appendix 2.1: QR code to complete data set of effect sizes extracted.



Appendix 2.2: Biogeographical outcomes extracted from each author.



Appendix 2.3: Observational outcomes extracted from each author.



Appendix 2.4: Experimental outcomes extracted from each author.

Chapter 3: Biogeographical evidence of Enemy Release in *Lagarosiphon major*

3.1. Introduction

Increased international travel has led to an increase in biological invasions (Long, 2003; Rahel & Olden, 2008). However, during the initial phase, there is no definite way to tell whether an introduced species will become invasive or not. This is with the exception of known exotic species that have a track record of being successful invaders elsewhere, or in the case of well-studied species with easily predictable growth models (Hussner et al., 2017). Indeed, the majority of exotic species that do survive outside of their native range end up with marginal populations within the new habitats due to a variety of environmental constraints and biotic interactions that limit their expansion (Alpert et al. 2000; Hierro et al., 2005). However, there are a few that are able to overcome these constraints and become highly invasive and problematic, both ecologically and financially (McGregor & Gourlay, 2002; Zimmermann et al., 2004; Hill & Coetzee, 2017).

For example, around 10% of exotic species are considered potential invaders or actual weeds in New Zealand (Williams & Timmins, 2002). Furthermore, exotic species in North America make up about 21% of the flora, with only 2% successful invaders (Rejmánek, 2000). Although these percentages may seem low, those that are successful invaders often cause a lot of damage (Clayton, 1996; Van Ginkel, 2011). *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae) in New Zealand is a highly successful and problematic invader, costing the country close to US$ 1.5 million yearly (Deloitte, 2011 via Soliman & Inglis, 2018). Similarly, the limited number of alien aquatic plants such as water hyacinth [*Pontederia crassipes* (formerly *Eichhornia crassipes*) Mart. (Pontederiaceae] and giant salvinia [*Salvinia molesta* D.

Mitch. (Salviniaceae)] that invade the South African waterbodies are responsible for the loss of many ecosystem services, and millions of Dollars lost in trying to control them (McConnachie et al., 2016; Hill & Coetzee, 2017).

Ecologists put forward a number of theories and hypotheses to explain why some species become invasive, while others do not. According to Alpert et al. (2000), the invasiveness of an exotic species is determined by a combination of its traits and the local abiotic factors of the new environment, which interact to successfully regulate or not regulate excessive growth. In addition to habitat quality, there are many other explanations for why alien invasive plants are likely to be more successful outside their native range (Fleming & Dibble, 2015). One such explanation is offered by the Enemy Release Hypothesis (ERH) which states that plants tend to become superior competitors outside of their natural range due to release from top-down stressors (herbivory and disease) that is evident in the absence of their natural enemies (Williamson & Griffiths, 1996). This is because unlike their native counterparts, the exotic plant invaders experience reduced regulatory pressure from their natural enemies and are therefore able to spread and reproduce more rapidly (Keane & Crawley, 2002) (Figure 3.1).

The underlying assumption of the ERH is that natural enemies are important regulators of plant species populations, and that the pressures from these natural enemies are felt more readily by the native species compared to alien plants (Keane & Crawley, 2002). In the absence of such pressures, the exotic plants are able to redirect more resources towards growth and reproduction while also successfully keeping the accumulated biomass (Fleming & Dibble, 2015). This ultimately leads to increased competitive ability (Figure 3.1).

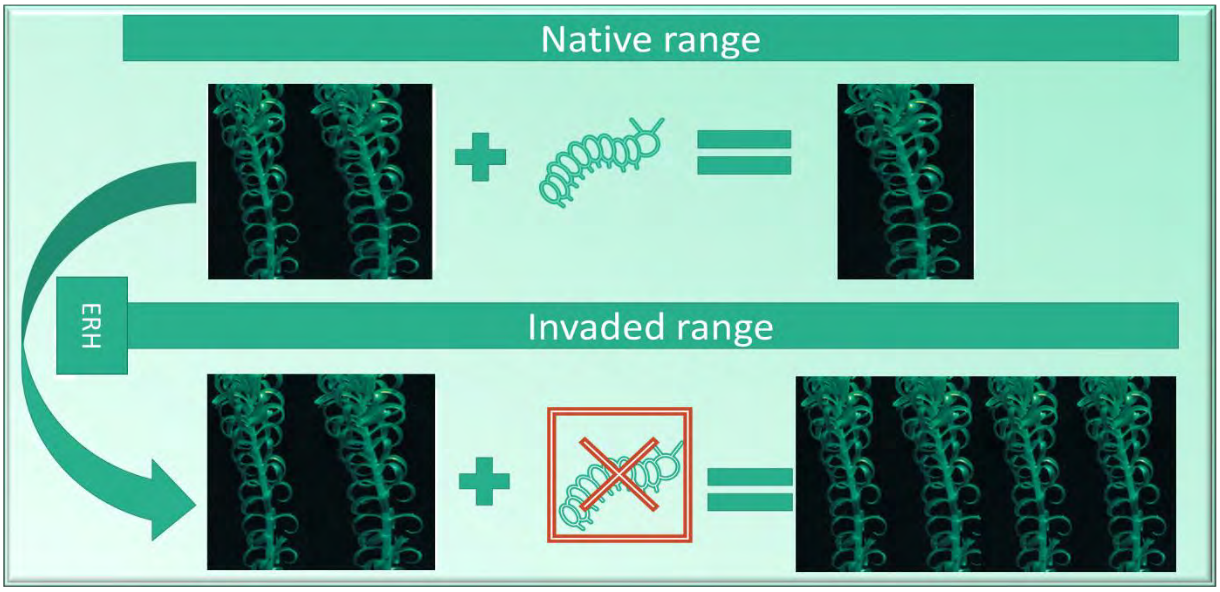


Figure 3.1: Simplified visual representation of the Enemy Release Hypothesis.

Classical biological control practitioners use this hypothesis as a basis for their approach in controlling invasive species (Keane & Crawley, 2002). Specifically, this is where host specific natural enemies such as insects and fungi are re-introduced to the target plant which is usually causing damage outside of its native range (Cilliers, 1991). This method of controlling biological invasion has proven to be effective in many instances (Hill 2003; McConnachie et al., 2014; Winston et al., 2014; Maluleke et al., 2021), and those successes are often cited as further evidence for the ERH (Sheldon & Creed, 1995; Williamson & Griffiths, 1996; Colautti et al., 2004; Liu & Stiling, 2006).

This hypothesis has also been rigorously tested in a variety of ecosystems and on several taxonomic groups (Roy et al., 2011; Prior & Hellmann, 2013), with varied levels of support depending on the environmental context, plant species, enemy type and spatial and temporal scale of the study (Fleming & Dibble, 2015, Chapter 2). For example, Kwong et al. (2017) conducted a biogeographical comparison of *Sagittaria platyphylla* (Engelm.) J.G. Sm (Alismataceae) in the native United States of America, and two introduced ranges in Australia and South Africa. Kwong et al. (2017; 2019) found that introduced populations were 40% and

50% more successful at reproduction, and experience reduced levels of herbivory compared to native populations, which they concluded was support of the ERH. However, all other plant parameters measured in that study where not consistent with the assumptions of this hypothesis.

Observational studies making such comparisons with native and invasive species at a landscape scale or at an ecosystem level such as those reported by Canavan et al. (2019) found that herbivore pressure was not different between two native Poaceae, *Phragmites australis* Trin. ex Steud and *Phragmites mauritianus* Kunth compared to the closely related alien invasive *Arundo donax* L. (Poaceae). Thus, there was no real evidence of enemy release in this case study. Lastly, with regards to manipulative techniques, Grutters et al., (2016) showed that the generalist moth *Parapoynx stratiotata* L. (Lepidoptera: Crambidae) does not discriminate between various native and invasive macrophytes in controlled mesocosms. Therefore, the success of these invasive plants may not be explained by the ERH. However, in the same breath, it is highly probable that under natural conditions, the native plants are also subject to specific herbivore pressures, aligning with the principles of the enemy release hypothesis.

Having seen the variability in applicability, Chapter 2 provided a meta-analysis in order to test for overall strength of the evidence for the ERH in macrophyte communities. That chapter also discussed the advantages of studying this hypothesis using both mesocosm level comparisons, as well as biogeographical evaluations. From the meta-analysis, it was evident that experimental studies are more common, while biogeographical comparisons are lacking (Chapter 2). This is probably due to the logistical challenges involved in conducting a study at this scale. However, according to Keane and Crawley (2002), investigations at these levels hold more weight in terms of providing evidence for the ERH as they provide a direct comparison of the same species in its native as well as invaded range. Therefore, the current chapter aims to contribute to this body of knowledge, in order to ensure that biogeographical studies feature more equally in future meta-analyses of this very important ecological hypothesis.

3.1.1 Enemy release hypothesis case study: *Lagarosiphon major* in New Zealand

Lagarosiphon major has a suite of natural enemies in its native range (Schutz, 2008; Baars et al., 2010); also see review in Chapter 1). However, this is presumed to not to be the case in areas such as New Zealand and Ireland where this plant has been introduced (Caffrey & Acevedo, 2007). Indeed, phytophagous insect diversity of any plant is determined by the evolutionary history of the plant in any specific region (Strong, 1977; Brändle et al., 2008; Weyl & Coetzee, 2013). Thus, *L. major* is a major nuisance in these regions of the world, and this is possibly due to the absence of its natural enemies. According to the 'Time-spent Hypothesis' the longer a plant species is in a region, the more natural enemies it is likely to accumulate, and in the case of a range expansion, existing phytophagous insects may attempt to transfer to the new species (Strong, 1977; Brändle et al., 2008; Weyl & Coetzee, 2013). This is usually a very slow evolutionary process, but given enough time, the new plant will eventually attract an asymptomatic number of natural enemies (Thompson, 1999).

Due to the slow nature of this process, natural resource managers are usually tasked with controlling invasive weeds such as *L. major* to avoid the many implications of having mono-specific stands of this plant. Using traditional methods of control such as chemical and mechanical control has proven to be challenging due to the life history of this plant (Deloitte, 2011). *Lagarosiphon major* is a submerged aquatic plant that is able to reproduce via vegetative propagation from even small fragments (Caffrey & Acevedo, 2007). Therefore, it is a poor candidate for herbicide application, and biological control might be more appropriate. This is a process which attempts to accelerate the 'time-spent hypothesis' by reintroducing specialist natural enemies to the plant (Cilliers, 1991).

The need to find alternative ways to control *L. major* also presents an opportunity to actively study the ERH in the process of developing a biological control programme for New Zealand. This was done by comparing various biological parameters of this plant between South Africa (native range) and New Zealand, where *L. major* is assumed to have escaped the top-down control from its natural enemies that are present in the native range. My hypothesis is that *L. major* has fewer natural enemies attacking it in the invaded range (New Zealand) compared to its native range (SA). Therefore, we also expect to find more *L. major* biomass, overall cover and therefore less plant biodiversity in the invaded areas due to competition.

3.2. Materials and methods

3.2.1. Study sites

The climate in New Zealand is typical of a temperate region, with warm temperatures and moderate rainfall. Due to its biogeographical isolation, most of the flora in New Zealand is endemic to the region (Williams & Timmins, 2002). However, due to the rise in globalisation, there is now also as many exotic species as there is native flora (Clayton, 1996; Williams & Timmins, 2002). There is a diversity of aquatic ecosystems, ranging from rivers, pristine lakes and dams, from relatively small to very large. Surveys were conducted in water bodies throughout the introduced range (North and South Island), ensuring that the full range of climates, water types and quality that *L. major* has colonised in New Zealand are represented (Figure 3.2; Plate 3.1).

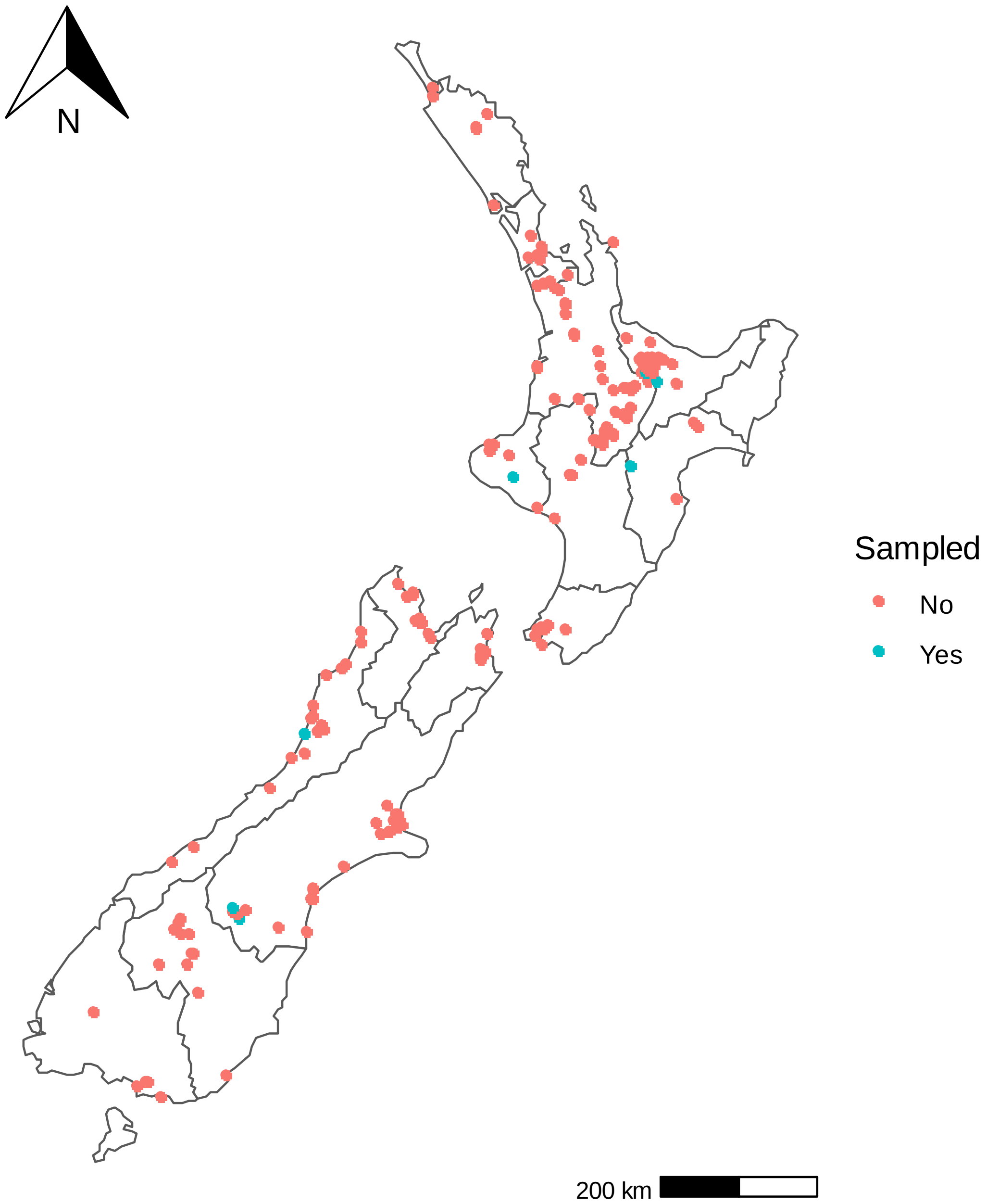


Figure 3.2: Distribution of *Lagarosiphon major* in New Zealand. Data extracted from GBIF. Sites sampled for this study are highlighted in blue (7 localities).

Plate 3.1: Various aquatic ecosystems with *Lagarosiphon major* in New Zealand representing a typical site in the invaded range.

In South Africa, study sites were primarily located in the temperate regions of the Eastern Cape Province and high-altitude sites in Kwa-Zulu Natal and Mpumalanga (Figure 3.3; Plate 3.2), which are climatically matched to New Zealand. Surveys were conducted in summer of 2020-2023 in both countries to account for potential temporal variation in herbivore activity and plant growth. Data were collected from a minimum of three infested sites in each of the South African regions mentioned above to ensure that all key associations and trends were detected across a climatic range as well as temporal range (Figure 3.3; Plate 3.2). Thus, nine localities were sampled in the native range and seven sites were visited in New Zealand.

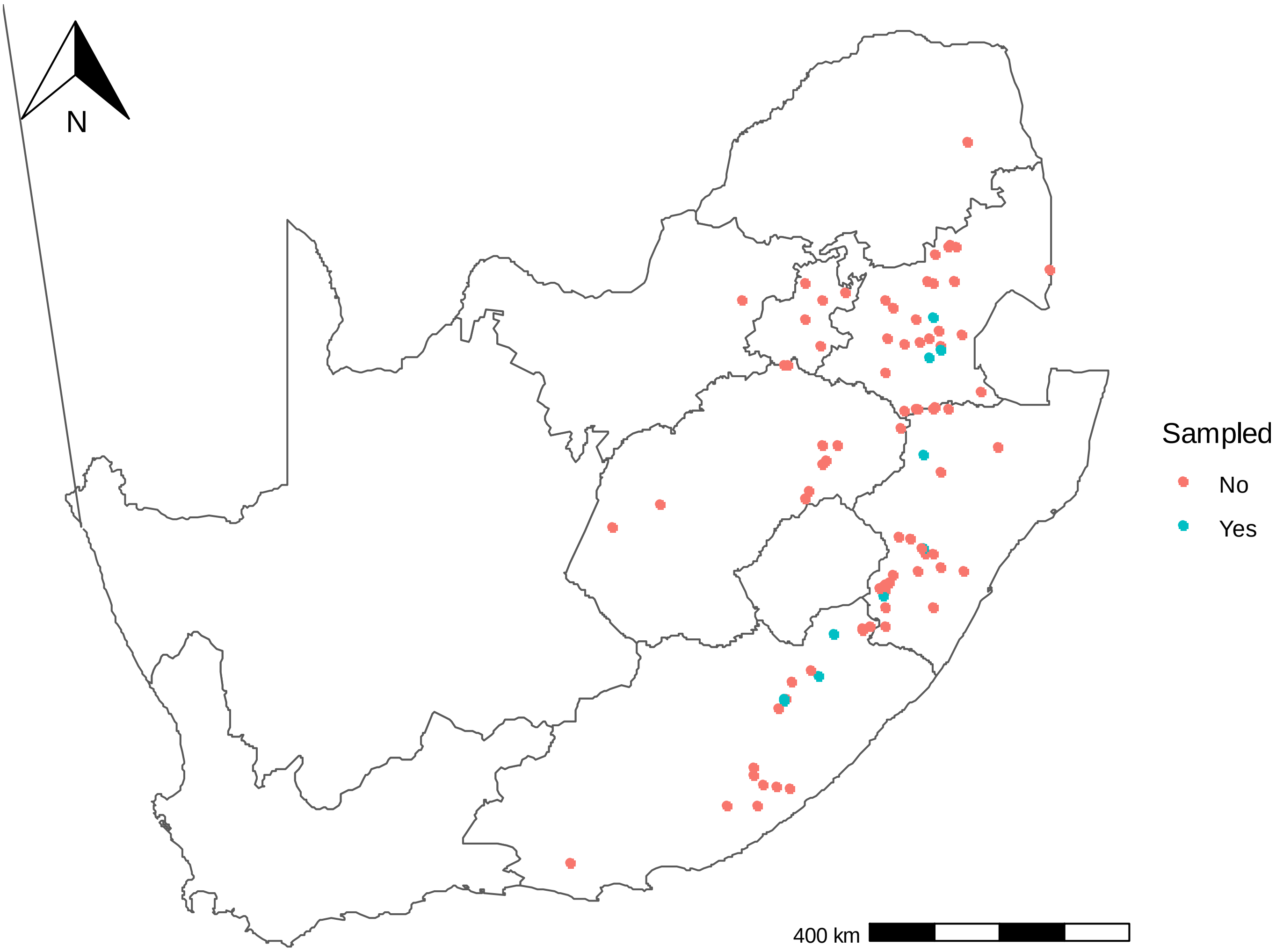


Figure 3.3: Distribution of *Lagarosiphon major* in South Africa. Data extracted from GBIF. Sites sampled for this study are highlighted in blue (9 localities across three provinces).

Plate 3.2: Various farm dams with *Lagarosiphon major* in South Africa representing a typical site in the native range.

3.2.2. Sampling protocol

Upon arrival at any site, physicochemical parameters such as water temperature (℃), pH, and total dissolved solids (TDS) (ppm) were taken using a hand-held multi-meter probe (Hanna HI 98130). Dissolved Oxygen (mg/l) was also recorded using a Sper Scientific Pen Meter. Depth at site (cm), and turbidity (using a Secchi disc) (%), coordinates, and fixed-point photographs were also taken. Furthermore, three water (250 ml) samples were collected for Phosphorus (mg/l), Ammonia (mg/l), and Nitrate (mg/l) analysis in the laboratory. These were preserved in a frozen state to minimize nutrient breakdown until they underwent analysis. The analysis involved the use of ion-selective electrodes designed for ammonia (NH_4+) (NH_4-BTA), nitrates (NO_3-) (NO_3-BTA) and phosphate (PO_4^{3-}) (PO_4^{3-}-BTA) which were connected to a LabQuest®2 digital interface (also from Vernier, order code LABQ2).

An estimation of the overall percentage cover of aquatic vegetation by species was recorded at each site using the following criterion: 1 = sparse (1-10% cover); 2 = low (11-25%); 3 = moderate (26 – 50%); 4 = high (51- 75%); 5 = very high (76 - 100%). A closer assessment of plant cover was made using five quadrats (1 m^2) within a 5 m^2 plot to estimate cover and shoot production of *L. major* and any other submerged macrophytes within the defined area. This was done by wading in depths of up to 1.5 m for closer inspection, with exact depth recorded for each quadrat. These data were also used to determine species richness/diversity in the defined area.

Field sampling to establish ecological networks was conducted using a sampling approach developed by Weyl and Coetzee (2013). This method has been proven to be highly effective in determining plant biomass and insect associations for submerged aquatic plants. These ecological networks will help predict the level of biotic resistance that can be expected and any other impacts on the ecosystem that will result from the release of biological control agents. The Howard-Williams and Longman sampler (hereafter the ‘crank’ sampler) was used specifically to quantify plant biomass (kg) within a 25 X 25 cm quadrat (as described by Weyl & Coetzee, 2013), replicated three times at each site. One full rotation (360°) sufficient for the blades to cut the plants from the base and wrap around the sampler was used.

The collected material was placed immediately in a 20 L bucket with water enough to submerge the plants and shaken forcefully to dislodge any mud as well as other macrophytes present. The resulting wet biomass was weighed immediately, and from each of the replicates of the crank sampler, up to 1 kg plant material was collected and brought back to the laboratory for processing through a Berlese funnel to collect and identify invertebrates that were possibly associated with *L. major*. In this setup, plants were placed in the funnels and subjected to a gradual drying process using the heat generated by light bulbs. This process encouraged insects to move into a collection cup at the bottom, which underwent daily inspections. The emerging functional feeding groups were identified using a dissecting microscope and classified into the following categories: Dominant >10, Frequent > 5, Occasional >3, Rare < 3.

Furthermore, 20 shoot tips, 20 cm long, were randomly collected from the weed mat. These were examined for larvae, pupae, and insect damage in the laboratory using a dissecting microscope. Insect damage was scored according to the following criteria: (1 = <5%, 2 = 5-25%, 3 = 25-50%, 4 = 50-75%, 5=75%-100%) relative to undamaged shoots in those selected sites. All collected plant samples were kept in the dark, below 5 ^{O}C using ice tubes for no more than three days before processing.

3.2.3. Data analysis

Abiotic parameters were analysed using the princomp function in R v 4.3.1 (R Core Team, 2023) to run a Principal Component Analysis (PCA) using the correlation matrix. This helped reduce the dimensionality of the data in order to identify the main sources of variation in *L. major* sites for both the native as well as the invaded region. This function also scales the data to ensure that the PCA results are not biased by variables with large values. The Autoplot function in R was then used to visualize the PC scores and loadings for the different variables. Furthermore, a Mann-Whitney U-test was used for the comparison of phosphorus, ammonia, and nitrate between native and invaded range.

To statistically compare wet biomass production of *L. major* between New Zealand and South Africa, results were analyzed using Generalized Mixed Models in RStudio interface (v2022.07.1+554) with site name and year of sampling as random effects, and region as fixed effects. The Gamma family distribution was used for this analysis as the data were skewed to the right. For plant cover, a Mixed-Effects Ordinal Logistic Regression model from 'ordinal' package in R was used, where the different plant covers were treated as factor levels. The setup for this was similar to the model described above for biomass comparisons but used a different package due to the different data structures.

An independent two-sample *t*-test was used to compare shoot production between the two regions. This was chosen over mixed models as year of sampling and different sites did not provide additional information for that model. An alpha level of 0.05 was chosen as the indicator for statistical difference between the two regions (Native vs Invaded) for all analysis. Lastly, to examine the effect of *L. major* on ecosystem health in terms of biodiversity, species Evenness (J0), and Shannon-Wiener α-diversity indices (H0) were calculated for both plant species and invertebrate communities in invaded and native region using the 'vegan: ecological diversity' package in the R-software. These data were then analysed using a Mann-Whitney U-test.

3.3. Results

3.3.1. Site characteristics

As expected, there was a lot of variation in physiochemical parameters measured, both in terms of site and region (Table 3.1). However, invaded sites in New Zealand had overall better water quality with relatively higher dissolved O_2 (mg/L), lower TDS (ppm) and overall higher water clarity (%), while values for South Africa (native range) were indicative of relatively poor water quality (Table 3.1). The mean pH was slightly higher in the native range (7.77±1.49, n=70) compared to New Zealand (7.38±0.84, n=39), indicative of more alkaline waters.

Lagarosiphon major also occupied relatively deeper and cooler waters in the invaded range relative to native sites (Table 3.1). Furthermore, phosphorus, ammonia and nitrates were significantly higher in the native range relative to the invaded range (W= 3, $P < 0.001$; W= 9, $P <0.001$: and W=55, $P <0.001$ respectively), with limited variability between invaded sites (Figure 3.4).

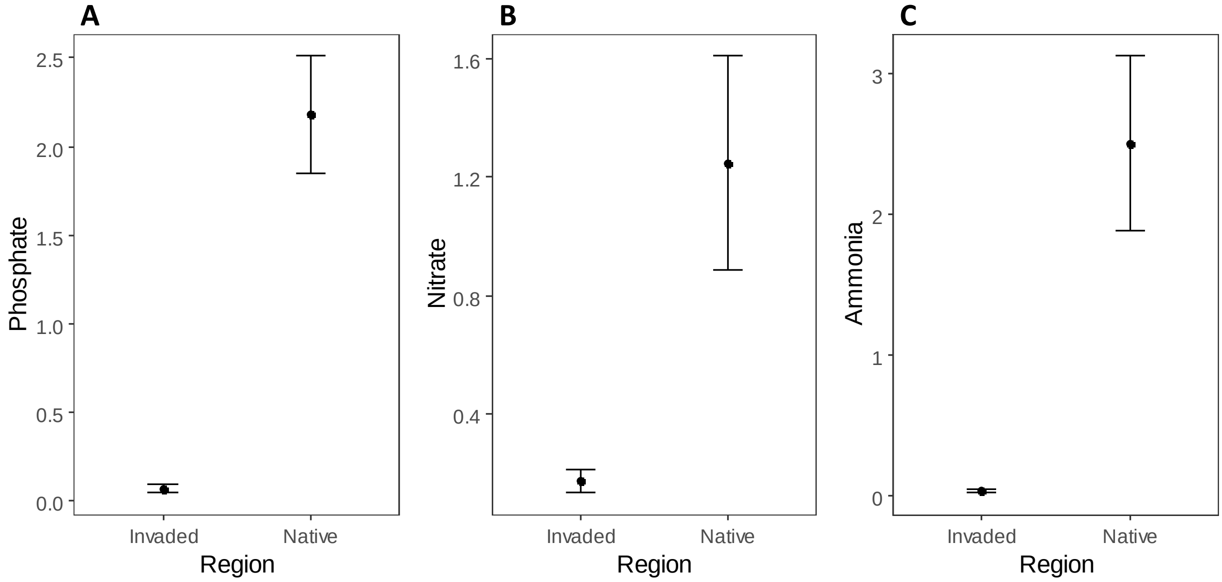


Figure 3.4: Amount of (A) Phosphate (mg/l), (B) Nitrate (mg/l), and (C) Ammonia (mg/l) measured in water with *Lagarosiphon major* in the native and invaded range. Mean ± se.

Table 3.1: Mean ± SD physicochemical parameters at *Lagarosiphon major* site (NZ = New Zealand; EC1 = Eastern Cape; Kwa-Zulu Natal; MPL = Mpumalanga) in native and Invaded range.

Region	Site	Range	N	Ph	Depth (cm)	Clarity (%)	Temperature (℃)	O_2 (mg/l)	TDS (ppm)
South Africa	EC1	Native	5	7.61±1.87	68.2±25.38	64±25.53	16.2±4.91	7.85±3.13	81.78±36.52
	EC2	Native	3	5.9±0.38	63.7±1.53	92±2.52	14.5±0.06	8.97±0.64	45.13±2.63
	EC3	Native	2	5.69±0.13	80.4±17.63	36±46.19	16.9±0	6.9±0	30.65±0.35
	KZN1	Native	7	8±0.1	33.4±9.26	100±0	22.7±1.02	-	43.01±15.33
	KZN2	Native	6	7.2±1.23	73.1±10.14	71±18.7	23.5±0.43	4.57±0.12	198.5±28.91
	KZN3	Native	4	6.7±0.69	39.3±6.45	94±11.7	18.3±2.38	3.23±0.42	64.97±1.7
	MPL1	Native	9	8.21±1.4	58.9±12.11	48±10.77	24.8±2.5	6.5±3.52	321.7±195.41
	MPL2	Native	3	6.47±1.06	48±4.36	4±0.44	25.1±0.35	5±1.73	793±39.89
	MPL3	Native	6	9.07±1.93	43.1±12.51	36±34.58	27±0.49	5.68±1.88	240.5±109.34
New Zealand	NZ1	Invasive	6	6.9±0.11	147.5±2.74	68±1.21	25±1.48	6.89±0.44	130.42±1.75
	NZ2	Invasive	6	6.36±0.09	70±15.23	100±0	15±1.63	11.16±0.75	81.4±0.55
	NZ3	Invasive	3	8.86±0.16	57.7±9.07	100±0	15.3±0.84	12.74±1.61	83.13±2.21
	NZ4	Invasive	6	8.44±0.27	124.8±8.01	80±5.52	23.1±0.79	9.43±0.44	506.33±6.71
	NZ5	Invasive	6	7.5±0.72	62.3±12	100±0	18.1±2.19	9.6±1.02	69.37±2.14
	NZ6	Invasive	6	7.52±0.16	66.3±10.69	100±0	17.2±2.34	9.5±0.09	66.98±3.01
	NZ7	Invasive	6	6.82±0.33	71.8±18.98	100±0	22.2±0.58	7.55±2.11	86.92±3.5
	Mean	*Invasive*	*39*	*7.38±0.84*	*87.94±35.25*	*92.03±12.79*	*19.72±3.96*	*9.31±1.97*	*151.23±50.17*
	Mean	*Native*	*70*	*7.77±1.49*	*59.54±19.94*	*63.45±31.6*	*21.13±4.4*	*6.29±2.55*	*160.35±44.24*

The PCA showed a separation in water quality parameters for populations of *L. major* in the native and invade range, suggesting different abiotic conditions in these ecosystems (Figure 3.5). The first two components explained 52% of the variation observed, with the first component contributing 35% and being mainly positively correlated with phosphate, turbidity, and ammonia, while being negatively correlated with dissolved Oxygen (Figure 3.5). This suggests that these variables are the most important in discriminating between the native and invaded sites, as native ecosystems tend to have higher phosphate, turbidity, and ammonia readings, and lower dissolved oxygen than invaded ecosystems (Table 3.1; Figure 3.5).

The second component explained 17% of the observed variation, mainly being associated conductivity, pH, depth and temperature, all showing negative correlation with that component (Figure 3.4). These were also more closely associated with invaded sites. Other variables such as nitrates, TDS and clarity did not appear to have a strong effect on the differentiation of these ecosystems. Furthermore, there was a slight overlap between the invaded and native ecosystems based on the second PC, which accounts for less than one-fifth of the variation in the data. Thus, this PC does not discriminate well between the two groups, as some sites have similar values for PC2 in both regions (Figure 3.5).

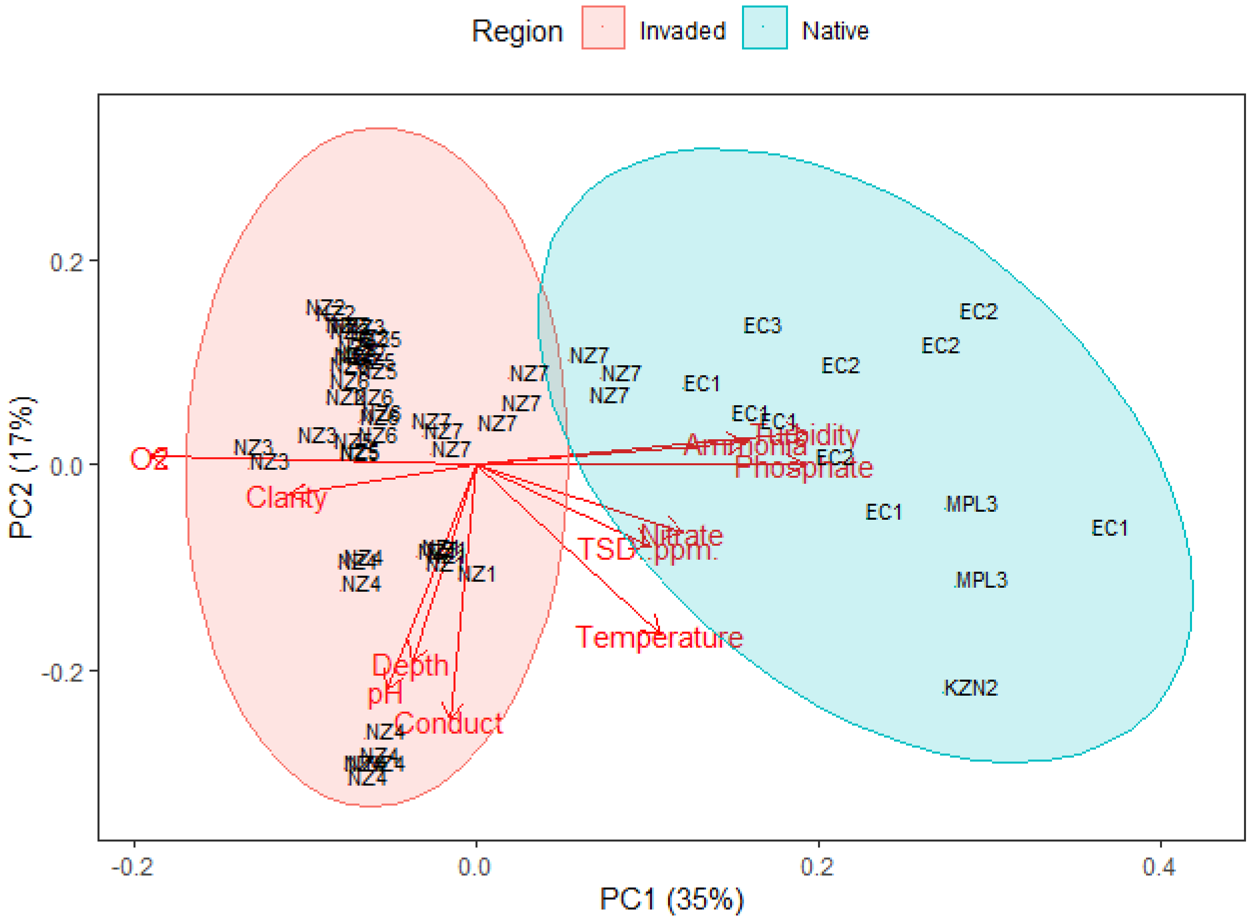


Figure 3.5: Principal Component Analysis scores showing physicochemical parameters for *Lagarosiphon major* sites in native and invaded range compressed into 2 dimensions. Ellipses indicate the 95% confidence intervals. The data points are labelled with codes indicating the location (NZ = New Zealand; EC1 = Eastern Cape; Kwa-Zulu Natal; MPL = Mpumalanga). The vectors show the direction and magnitude of the correlation of different environmental variables (e.g., Temperature, Depth, Conductivity) with the principal components.

3.3.2. Plant parameters

Biomass production of *L. major* was highly variable both in terms of sites, as well as regions (Figure 3.6). Nevertheless, after controlling for the year and site of sampling, there was overall significantly higher *L. major* biomass in the invaded range (β=2.166, SE=0.007, *P*= 0.030), with a mean of 55.2±6.612 (n=60), compared to the native range which had a mean of

27.09±2.25 (n=88), almost half of what is observed in the invaded range. A similar trend was observed with *L. major* shoot production, where the invaded range had a mean of 147±16.43 (n=9) and native plants in South Africa had a mean of 77±8.42 (n=27) shoots per m^2 ($t_{(12)}$ = 3.79, *P* =0.002).

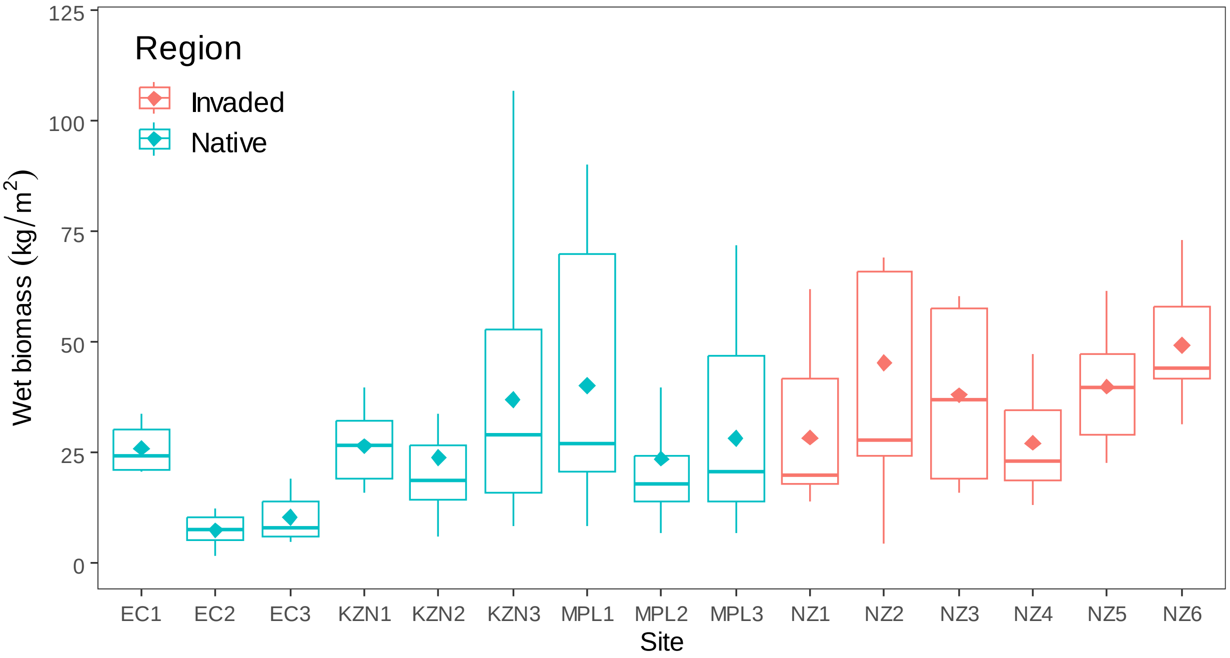


Figure 3.6: Wet *Lagarosiphon major* biomass (kg/m^2) per site (NZ = New Zealand; EC = Eastern Cape; KZN= Kwa-Zulu Natal; MPL= Mpumalanga), in both native and invaded range.

The cover of *L. major* at the site scale (>5 m^2) did not differ between native and invaded range (z=0.789, *P*=0.43), after controlling for site and year of sampling. However, at a finer scale (~1 m^2), the cover of *L. major* at most of the quadrats in the invaded site were in the high range (75-100%), indicative of higher percentage cover at those sites (Figure 3.7). Quadrats in the native range had a more even split between the five groups of percentages (Figure 3.7). Therefore, at this scale, the analysis showed that the cover of *L. major* was overall higher in the invaded range compared to the native range (z= -3.137, *P*=0.002).

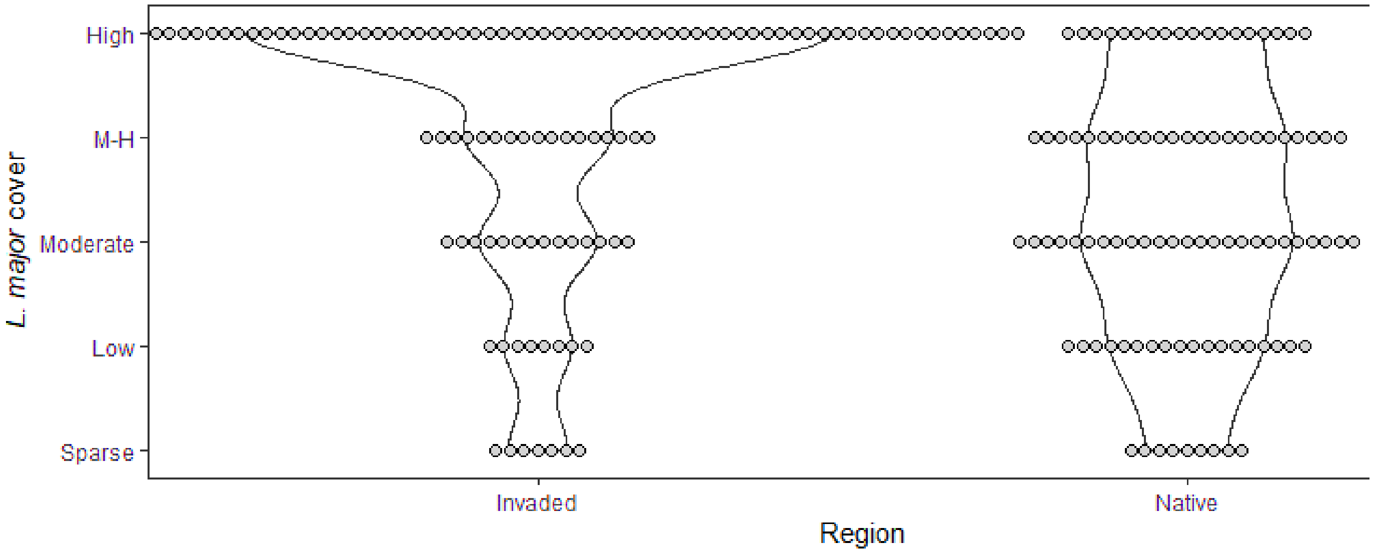


Figure 3.7. The range of *Lagarosiphon major* cover per quadrat (1 m^2) for native populations in South Africa and invasive populations in New Zealand. Sparse=1-10%; Low =11-25%; Moderate =26 – 50%; High=51- 75%; Very high (76 - 100%).

Lagarosiphon major was the most dominant macrophyte species in both the native as well as the invaded range, with relatively more *L. major* in the invaded range at the quadrat scale (Figure 3.8). Furthermore, there was a higher number of other plant species co-occurring with *L. major* in the native range compared to the invaded range, both at a large scale (> 5m^2 site) and at a finer scale (1 m^2) (Figure 3.8). With regards to plant diversity indices, there was a significant negative effect of *L. major* cover on overall plant species density ($t_{(13)} = -6$, richness ($t_{(15)} = -5$, $P<0.001$) and diversity (W = 3, $P = 0.002$) in the invaded range compared to the native range (Figure 3.9). However, although there was also a negative association of species evenness and *L. major* cover, this relationship was not significant (W = 16, $P = 0.6$). Nevertheless, at the finer scale, species richness (W = 8, $P = 0.008$), density ($t_{(12)} = -2$, $P = 0.03$), evenness (W = 12, $P = 0.03$) and diversity (W = 8, $P = 0.008$) were all shown to be significantly reduced in the invaded range.

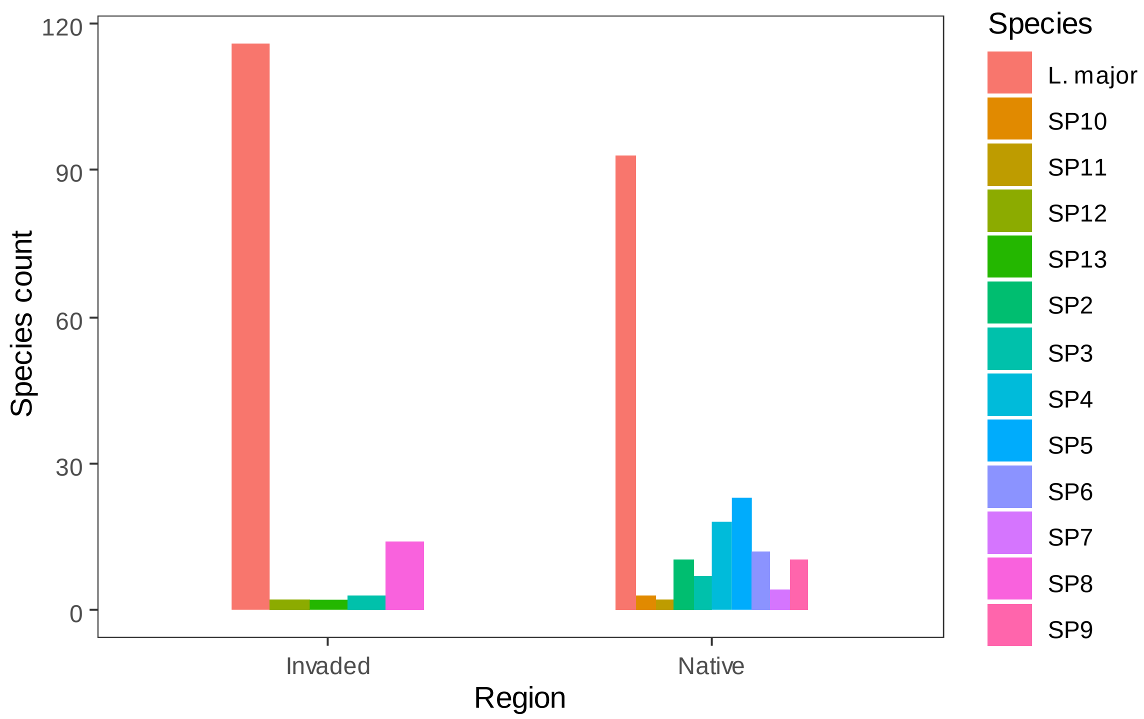
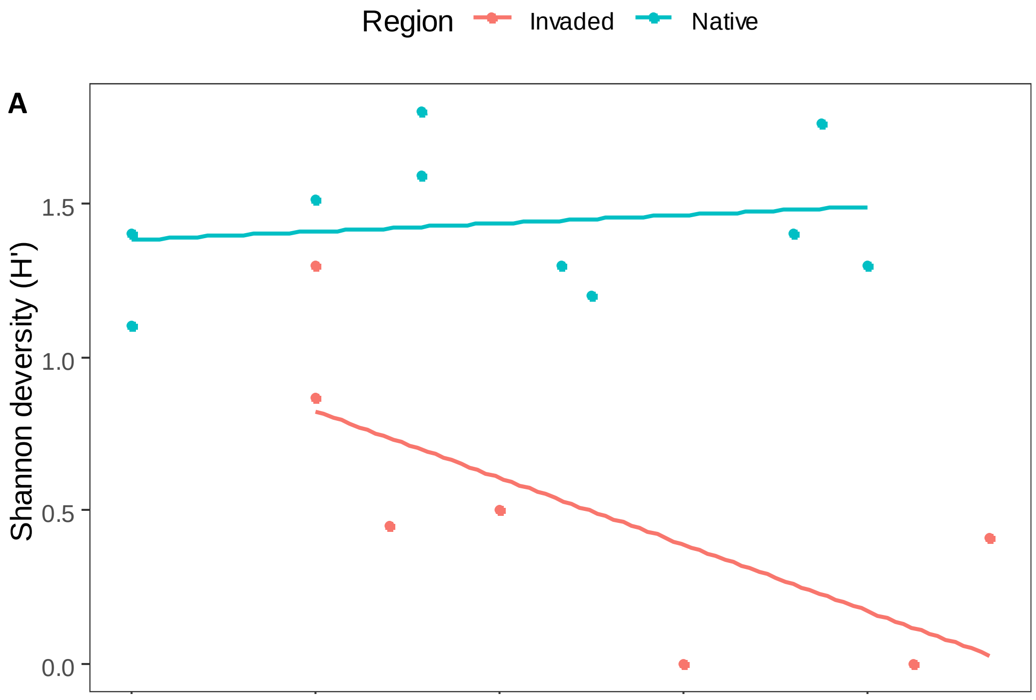


Figure 2.8: Species frequency/m^2 according to region (native and invasive). Different colours indicate different plant species (SP2 to SP10). *Lagarosiphon major* is represented by L. major.



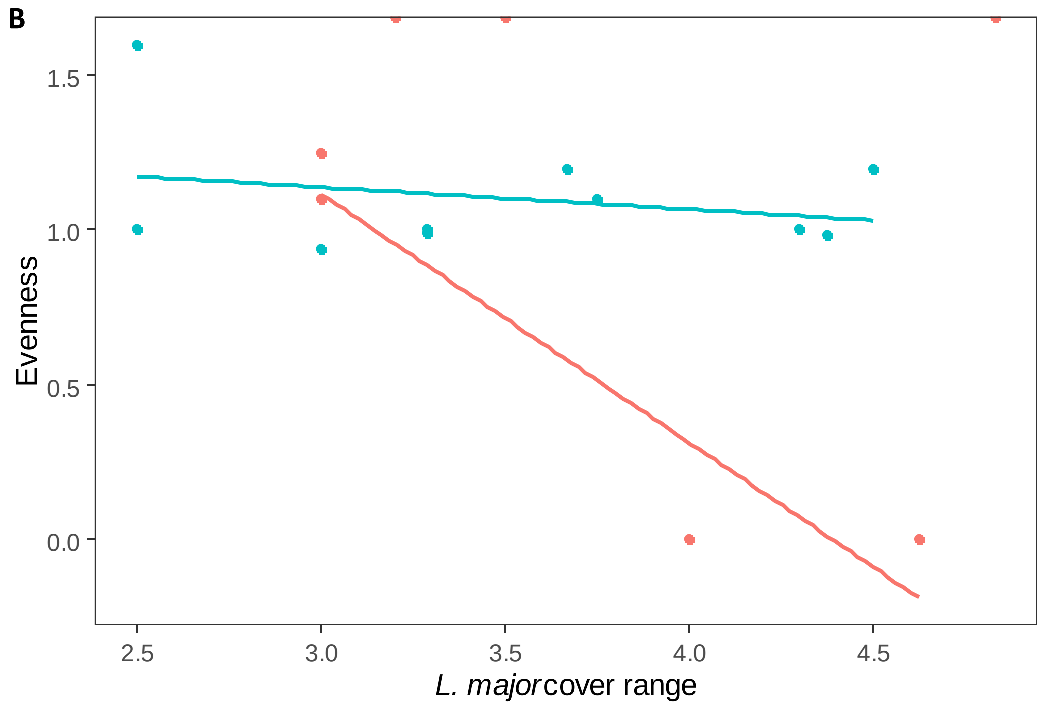


Figure 3.9: (A) Shannon diversity and (B) Evenness of aquatic plant species per site, as affected by *Lagarosiphon major* cover in native and invaded range.

3.3.3. Invertebrate community

The most dominant invertebrate groups in native populations of *L. major* were Ceratopogonidae (Diptera) (detritivores), Hydrachnidiae (Trombidiformes) (predators), as well as the specialist herbivore *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae) (Table 3.2). Other groups were either rare or occasional, with the exception of another specialist *L. major* herbivore, *Polypedilum tuburcinatum* Andersen (Diptera: Chironomidae) which occurred more frequently (Table 3.2).

In the invaded range of *L. major*, the most common invertebrate groups were Aphids (Hemiptera) (herbivore), *Hygraula nitens* Butler (Lepidoptera: Crambidae), syn. *Nymphula nitens* (*L. major* associated herbivore), *Microvelia macgregori* Kirkaldy (Hemiptera: Veliidae) (predator), and *Paratya curvirostris* Heller (Decapoda: Atyidae) (omnivore) (Table 3.2).

Again, other groups were either rare or frequent, with the exception of Caddisfly (herbivore) and Hydrachnidia (predator), which were both frequent (Table 3.2).

Overall, the native range had more herbivores directly associated with *L. major,* compared to the invaded range (Table 3.2). There were also no parasitoids of *L. major* herbivores recorded in the invaded range, while they were present in native populations. With regards to overall invertebrate species richness ($t_{(6)} = 3$, $P = 0.03$), density ($t_{(8)} = 6$, $P<0.001$), evenness ($W = 4$, $P = 0.05$) and diversity ($W = 22$, $P = 0.2$), there was a significant difference in the number of invertebrates associated with *L. major* in the native range compared to the invaded range (Table 3.2, Figure 3.10).

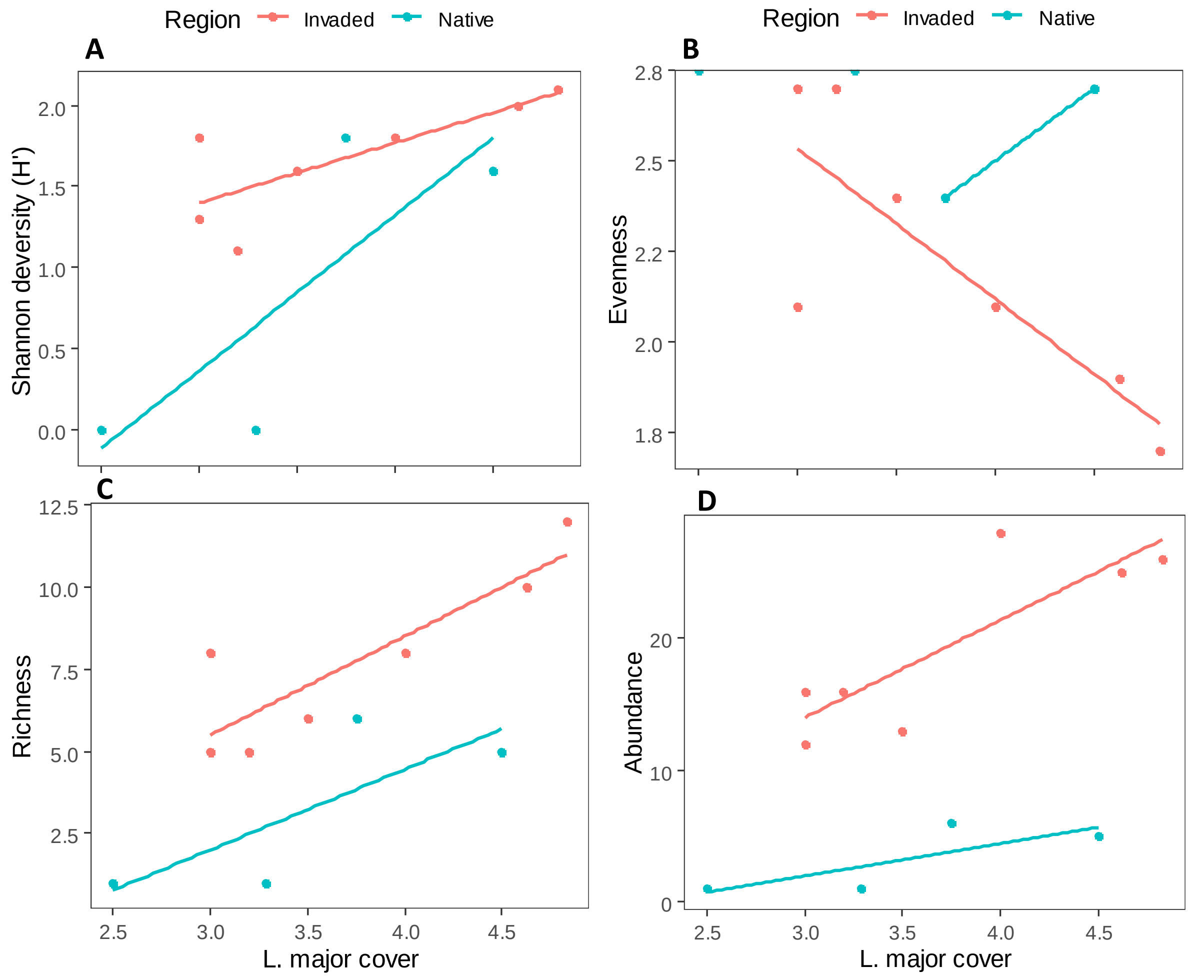


Figure 3.10: Insect diversity and richness per kg of *Lagarosiphon major* as affected by *L. major* cover in native and invasive populations.

Table 3.2: Invertebrates associated with *Lagarosiphon major* at different sites in native and invaded range. Insects known to actively feed on or damage *L. major* are underlined.

Country	Region	Group ID	Sites	Count/kg	Sd	FFG	Frequency
South Africa	Native	*Bagous* spp,	4	1.75	0.96	Herbivore	Rare
	Native	Braconidae	3	3	1.5	Parasitoid	Occasional
	Native	Ceratopogonidae	3	14.67	15.14	Detritivore	Dominant
	Native	Glossiphoniidae	3	2	0	Predator	Rare
	Native	*H. lagarosiphon*	8	15.5	12.40	Herbivore	Dominant
	Native	Hydrachnidia	3	15.67	11.02	Predator	Dominant
	Native	Hydroids	1	1		Collector	Rare
	Native	Ephemeroptera	1	1		Collector	Rare
	Native	*Nymphulinae.*	2	3	2.83	Herbivore	Occasional
	Native	Odonata	2	3.5	2.12	Predator	Occasional
	Native	Oligochaeta	2	4.5	2.12	Detritivore	Occasional
	Native	*P. tuburcinatum*	2	6.5	7.78	Herbivore	Frequent
	Native	Thrips	1	1		Herbivore	Rare
New Zealand	Invaded	Aphid	3	15.67	12.74	Herbivore	Dominant
	Invaded	Bivalve	1	3		Filter feeder	Occasional
	Invaded	Caddisfly	12	7	4.67	Herbivore	Frequent
	Invaded	Cecidomyiidae	2	7.5	7.78	Predator	Frequent
	Invaded	Chironomid	3	3.67	2.31	Diverse	Occasional
	Invaded	Tipulidae	1	5		Collector	Frequent
	Invaded	Glossiphoniidae	3	1.33	0.58	Predator	Rare
	Invaded	Hydrachnidia	11	8.36	6.73	Predator	Frequent
	Invaded	*Hygraula nitens*	42	13.05	13.05	Herbivore	Dominant
	Invaded	Limpet	1	5		Grazer	Frequent
	Invaded	Microvelia sp.	4	12.33	9.29	Predator	Dominant
	Invaded	Nematoda	3	1.33	0.58	Predator	Rare
	Invaded	Oligochaeta	1	1		Detritivore	Rare
	Invaded	Paratya shrimp	5	12.6	13.22	Omnivore	Dominant
	Invaded	Stratiomyidae	1	4		Omnivore	Occasional
	Invaded	Zygoptera	1	1		Predator	Rare

In the native range, the level of damage experienced by *L. major* was highly variable, with sites EC1, KZN2 and MPL1 showing higher damage compared to sites like EC2 and MPL4 (Figure 3.11). With regards to the invaded range, most sites had a lower level of damage compared to the native populations, with the exception of sites NZ1 and NZ7, which also showed higher levels of damage relative to the rest of that regions (Figure 3.11). However, although varied between sites, the overall levels of damage experienced by *L. major* in the native range were significantly higher compared to invaded range (z value= 2.25, *P*= 0.024), with means of 3.057±1.16 (n=104) and 2.495±1.5 (n=234), respectively.

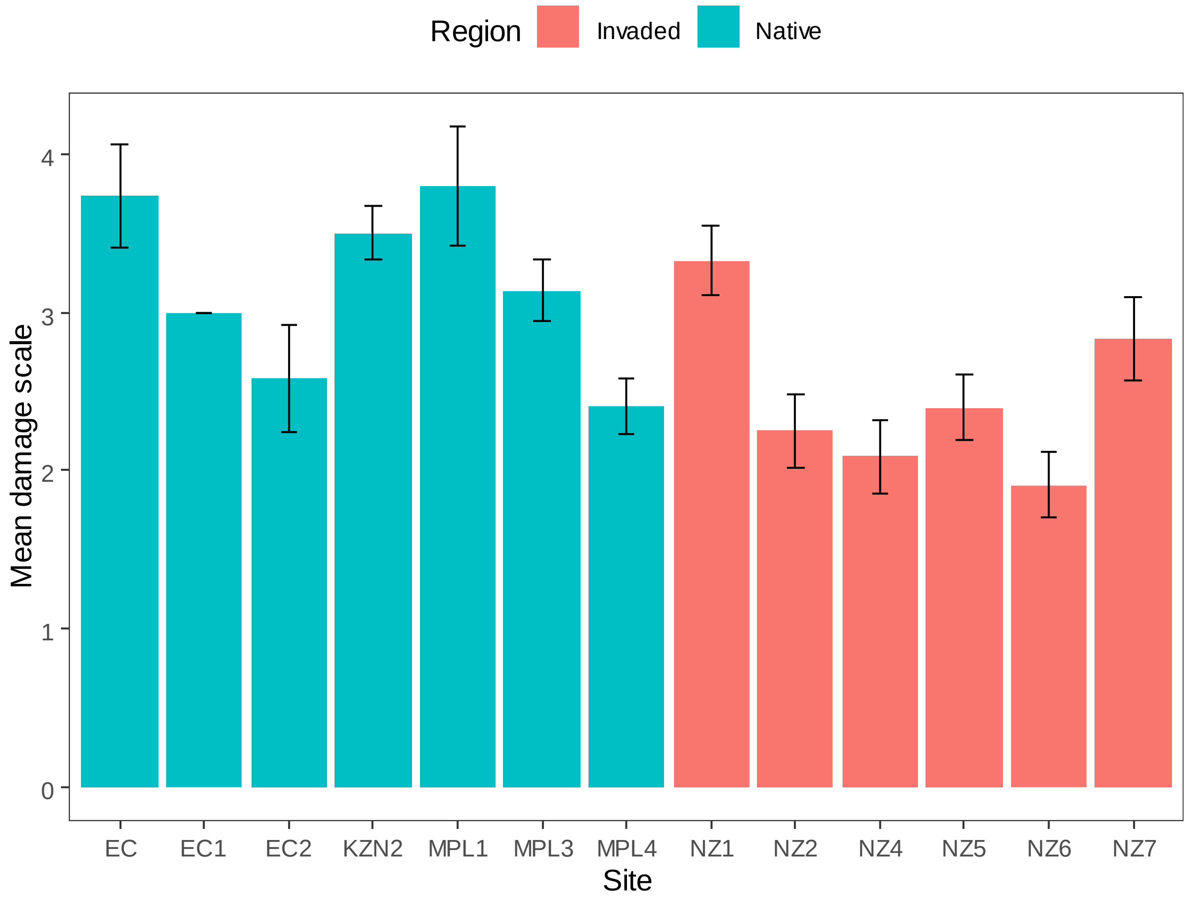


Figure 3 .11: *Lagarosiphon major* damage scale per site (NZ = New Zealand; EC = Eastern Cape; KZN= Kwa Zulu Natal; MPL= Mpumalanga), in both native and invaded range. Mean ±SE.

3.4. Discussion

The aim of this study was to provide a biogeographical comparison of *L. major* between South Africa and New Zealand. Firstly, the tolerance of *L. major* to different environmental conditions was evident in both the native, as well as the invaded range (Champion & Tanner, 2000; Caffrey & Acevedo, 2007). However, there were some notable differences in the water quality parameters between the two regions. One of the most striking differences was the higher level of nutrients, especially phosphorus, ammonia and nitrates, in the native range compared to the invaded range. This is indicative of eutrophication and is not surprising, as freshwater systems in South Africa are some of the most polluted in the world (Van Ginkel et al. 2000; Oberholster & Ashton 2008). New Zealand on the other hand is known for its near-pristine waters in comparison (McGregor & Gourlay, 2002; Larned et al, 2016). This means that success of *L. major* in this region is possibly not determined by nutrient availability, but by lack of herbivory.

Another related key difference between these two regions was greater water clarity and higher dissolved oxygen concentrations in the invaded range compared to the native range. This also translated to *L. major* occupying deeper and cooler waters in the invaded range than in the native range due to increased light penetration (Caffrey & Acevedo, 2007; Morrissey et al., 2020). Furthermore, higher dissolved oxygen and water clarity in the invaded range suggest that *L. major* may have a positive effect on these parameters by increasing oxygen production and reducing turbidity through sediment stabilization (James et al., 2006; Hussner et al., 2017). However, these effects are directly dependent on the density and biomass of *L. major*, as high levels of plant growth can have vast negative impacts, including reduces dissolved oxygen and water clarity through shading and decomposition (Clayton, 1996; James et al., 2006; Van Ginkel, 2011). For future studies, it would be interesting to see how these physicochemical properties compare to sites where *L. major* is not present in both biogeographic regions.

With regards to plant parameters, *L. major* was the most dominant species overall in both regions. Nevertheless, *L. major* cover, biomass, and shoot production was higher in the invaded range, and was also associated with reduced species richness, evenness, and diversity. Similar results were reported by Gallego-Tévar et al. (2022) after conducting a similar biogeographical comparison of *Iris pseudacorus* L. (Iridaceae) in native Spain vs invaded USA. They specifically showed that in the native range, *I. pseudacorus* promoted plant diversity by increasing evenness and species richness and thus coexisted successfully with many other native species. However, in the invaded range, this species was associated with reduced plant diversity of other native species.

Both the current study and the study conducted by Gallego-Tévar et al. (2022) show the competitive dominance of species outside their native range, possibly due to limited top-down pressure (Williamson & Griffiths, 1996; Keane & Crawley, 2002). Furthermore, the differences in cover based on scale of measurement implies that although a site may not be completely covered with *L. major*, the patches of this species in those sites tend to be more clumped in the invaded range compared to native. This could be interpreted in terms of the ERH as support for the hypothesis that escape from natural enemies allows for this plant to successfully accumulate more of its biomass outside of its native range, while top-down pressures in the native range regulate growth more successfully (Zangerl & Rutledge, 1996).

Lagarosiphon major has been shown to be a superior competitor against a variety of closely related macrophytes (Martin et al., 2013; Silveira & Thiébaut, 2020), with the ability to form densa mats that shade out and displace native species (Rattray et al., 1994; Champion & Tanner, 2000; Caffrey et al. 2010). These monoculture stands have marked negative impacts on the biodiversity and ecosystem functioning of affected aquatic ecosystems (Clayton, 1996; James et al., 2006; Van Ginkel, 2011). Indeed, the current study showed that *L. major* reduces the density, richness, diversity, and evenness of other plant species in the invaded range, which may affect the food web structure and trophic interactions of aquatic organisms (Rattray et al. 1994; Champion & Tanner, 2000; Caffrey et al. 2010). Moreover, the dominance of *L. major* may reduce ecosystem services derived from invaded landscapes (Van Ginkel, 2011; Kelly et al. 2013).

The invertebrate community associated with *L. major* differed between the native and invaded range, both in terms of composition and abundance. Specifically, the native range had a higher species richness, density, evenness, and diversity of invertebrates associated with *L. major* than the invaded range. This reflects the higher complexity and heterogeneity of the native habitat as seen with plant species diversity, which were shown to support more diverse and abundant invertebrate assemblages. These results are also in line with the 'time-spent hypothesis' which argues that the number of invertebrate associations is directly linked evolutionary history (Strong, 1977; Brändle et al., 2008; Weyl & Coetzee, 2013). The native range of South Africa had more invertebrate groups which fed on *L. major*, such as generalist moths *Synclita* sp. and *Parapoynx* sp. (Lepidoptera: Crambidae), *Bagous* sp. weevils (Coleoptera: Curculionidae), as well as two specialist herbivores, *H. lagarosiphon* and *P. tuburcinatum* which were encountered frequently at different sites (Schutz, 2008; Baars et al., 2010).

These groups may exert top-down control on the population dynamics of this plant in its native habitat, reducing its biomass and cover, as reported here. The level of damage experienced by *L. major* was overall higher in the native range than in the invaded range. This may be an indication that *L. major* is exposed to more biotic stressors, such as herbivory and competition in its native habitat than in its invaded habitat. The two specialist insects mentioned above have previously been shown to significantly damage *L. major* (Baars et al., 2010; Martin et al., 2013; Mangan & Baars, 2016) and are being considered for biological control of this weed in New Zealand and Ireland (Earle et al., 2013; Paynter et al., 2015; Olckers et al., 2021; Mangan & Baars, 2023).

However, *L. major* and *H. lagarosiphon* are also associated with various parasitoids, such as *Chaenusa* spp. (Hymenoptera: Braconidae), which affects the survival and reproduction of this insect herbivore in the native range (Baars et al., 2010; Martin et al., 2018). Similar groups have also been shown to reduce biological control of the invasive *Egeria densa* Planch. (Hydrocharitaceae) in South Africa (Coetzee et al., 2021; Smith 2021; Van Noort et al. 2021). Furthermore, Paynter et al. (2010) found that parasitism was positively correlated with failure of biological control programmes in New Zealand. However, the Brachonid parasitoids are thought to be absent in New Zealand, which could improve the efficacy of *H. lagarosiphon* as biological control agents in that region.

There were two sites in New Zealand where *L. major* was found to have relatively higher levels of damage than some sites in the native range. This implies that there may be some local factors that increase the biotic stress experienced by *L. major* in these sites, such as the presence of native herbivores such as *H. nitens* which has been reported to attack *L. major* (Redekop et al., 2018). These were found in high abundance at the two sites. This endemic New Zealand moth is a herbivore that can switch between different macrophyte hosts, and has shown to have preferential feeding on exotic Hydrocharitaceae such as *Hydrilla verticillata* (L.f.) Royle, *Elodea canadensis* Michx, as well as *L. major* (Habeck & Balciunas, 2005; Wells, 2015; Redekop et al., 2018).

This insect may be used as a native biological control agent, an alternative to classical biological control which does not use re-introduction of natural enemies but involves the use of native herbivores to control invasive species (Madsen et al., 2000; Wells, 2015). Thus, *H. nitens* would be mass reared and released in sites where *L. major* is causing ecological harm. According to Sheldon & Creed (1995), this strategy poses less risk to native populations as it is more economical in terms of logistics involved in the development of a new biological control programme. For example, Sheldon & Creed (2003), showed that a native North

American weevil (*Euhrychiopsis lecontei* Dietz [Coleoptera: Curculionidae]) may be a suitable alternative to classical biological control for managing various exotic *Myriophyllum* species.

Overall, the invaded range had fewer invertebrate groups that are directly associated with *L. major*, and more generalist or opportunistic groups. For example, Aphids are herbivores that can feed on a wide range of aquatic plants, *P. curvirostris* is an omnivore that can consume both plant and animal matter, and *M. macgregori* is a predator of various aquatic insects. These groups may benefit from the high biomass and cover of *L. major* in the invaded habitat, but they may not have a significant impact on its growth and spread.

In conclusion, the results of this study demonstrate that *L. major* is a highly invasive aquatic plant that has enhanced growth in New Zealand compared to South Africa, and possibly benefits from having fewer natural enemies in the invaded range. Therefore *L. major* invasion poses a serious threat to the native biodiversity and ecosystem services of freshwater habitats in New Zealand. Consequently, effective management strategies are needed to prevent its further spread and reduce its impacts. As such, based on the assumptions of the ERH, I would argue that the *L. major* invasion in New Zealand could be successfully managed using biological control. The stem-mining midge, *P. tuburcinatum* and the leaf-mining fly *H. lagarosiphon* are strong candidates, as they have been shown to be damaging and host specific enough (Baars et al., 2010; Earle et al., 2013; Martin 2013; Mangan & Baars, 2023), are actively being pursued for introduction to both New Zealand and Ireland (Earle et al., 2013; Paynter et al., 2015; Olckers et al., 2021). Specifically, for the *H. lagarosiphon*, there are plenty of opportunities to learn and develop mass rearing techniques from previous studies using insects in this genus (Smith, 2021; Mangan et al., 2015). All these recourses will be advantageous in developing and implementing this biological control programme.

Chapter 4: Competition between *Lagarosiphon major* and *Egeria densa* as mediated by biological control.

4.1. Introduction

Invasive aquatic plant species pose a significant threat to freshwater ecosystems, causing environmental and economic harm globally McGregor & Gourlay, 2002; Van Ginkel, 2011; Hill & Coetzee, 2017). *Lagarosiphon major* (Ridl.) and *Egeria densa* (Planch) are two such invasive species from the family Hydrocharitaceae that have rapidly spread in freshwater ecosystems worldwide (Champion & Tanner, 2000; McGregor & Gourlay, 2002; Caffrey & Acevedo, 2007). *Egeria densa* originates from South America, while *L. major* is native to southern Africa (Obermeyer, 1964). Their spread across the world was facilitated by their use in the aquarium trade (James et al., 1999), and both species are considered invasive in New Zealand (Caffrey et al., 2010).

Lagarosiphon major is commonly referred to as oxygen weed, curly waterweed or African elodea, while *E. densa* is commonly referred to as Brazilian waterweed. Both species have a similar growth form, occurring as submerged macrophytes which can form densa monoculture stands in slow moving water bodies (see Chapter 1 for a detailed description of *L. major*). The main distinguishing factor between these two species is the shape and colour of their leaves. The leaves of *L. major* tend to alternate and are generally more curly and dark green due to the relatively thick-walled epidermis (Obermeyer, 1964; Bowmer *et al*., 1995), while *E. densa* leaves occur in whorls of four, are less curly and are usually a brighter green colour in comparison (Yarrow et al., 2009). The former also has a relatively firm structure with small blunt spines on the leaf margins (Symoens & Triest., 1983; Bowmer *et al*., 1995), compared to the more fragile *E. densa*, which facilitates dispersal (Yarrow et al., 2009; Redekop et al., 2016).

Lagarosiphon major may be considered an ecosystem engineer as it changes sediment structure of the invaded system, while both species can tolerate a wide range of environmental conditions and exhibit aggressive growth patterns, forming densa stands that often outcompete native aquatic plants (McGregor & Gourlay, 2002; Caffrey & Acevedo, 2007). As a result, native macrophytes are displaced, altering entire ecosystems. For example, *L. majo*r has successfully displaced many native *Myriophyllum* spp. in New Zealand (Rattray et al. 1994), and various charophyte species in Ireland (Caffrey et al. 2010). The resulting impact of invasion by these species is therefore loss of biodiversity, reduced water quality, impaired hydroelectric power generation and disruption to recreational activities (Clayton, 1996; Wilson et al., 2007; Van Ginkel, 2011).

Chapter 3 investigated the importance of top-down regulation in plant communities through natural enemies. It suggested that the absence of natural enemies of *L. major* in New Zealand (exotic range) may be one of the explanations why this plant has become such a successful invader. Nevertheless, the meta-analysis in Chapter 2 revealed the real complexity of natural ecosystems, showing that release from natural enemies is only part of the biological invasion process, and may not always be an applicable explanation. Therefore, other mechanisms of invasion such as competition, mutualism, novel weapons, allelopathy, phenotypic plasticity and propagule pressure need to be explored (Fleming & Dibble, 2015; Bolpagni, 2021). This chapter specifically investigates the role of competition for limited resources in biological invasions. This is because understanding the dynamics of competitive interactions within plant communities is crucial for effective management and conservation of freshwater ecosystems (Van et al. 1999).

There are two types of competition that govern plant community interactions, intra-specific and inter-specific competition. Intra-specific competition occurs within populations of the same species, and inter-specific competition occurs across different species within a community (Lacoul & Freedman, 2006). The former is a stronger form of competition as requirements for resources are very similar within species (Coetzee et al. 2005; Monacelli & Wilcox, 2021), with resource acquisition taking place in the same manner (Van et al. 1999). For example, Martin et al. (2014) showed that the influence of *L. major* on co-occurring conspecifics is much greater than competition with *Myriophyllum spicatum* L. (Haloragaceae). Nevertheless, this can also be true for different species if they have overlapping life-history strategies (Stiers et al. 2011), as is the case with *L. major* and *E. densa* (Caffrey & Acevedo, 2007).

Interspecific competition is usually mediated by other limiting factors such as nutrient availability, dissolved oxygen, light intensity, seasonal variability, as well as herbivore pressure (Titus & Adams 1979; Coetzee et al. 2005; Caffrey et al. 2010). For example, Caffrey et al. (2010) showed that even under low CO_2 and high pH, *L. major* remains a superior competitor in terms of photosynthetic rate relative to other Hydrocharitaceae such as *Elodea canadensis* Michx and *Elodea nuttallii* Planch.

Various studies have investigated the competitive vigour of *L. major* against other macrophytes at different environmental conditions (Hofstra et al, 1999; Martin et al., 2014), as well as *L. major* against *E. densa* (Strange, 2017; Silveira & Thiébaut, 2020). These community level studies show that competition is an important factor that influences the success of invasive plant species, especially in new environments. The ability of an invasive species to compete with native species can determine its impact on the ecosystem (Van et al. 1999; Monacelli & Wilcox, 2021).

Herbivory plays an important role in mediating these competitive interactions (Coetzee et al. 2005; Caffrey et al. 2010). For example, Fan et al (2013) showed that a generalist grasshopper (*Atractomorpha sinensis* Bolivar [Orthoptera: Pyrgomorphidae]) is able to shift the competitive advantage between a native and an invasive species of Alternanthera. The invasive alligator weed (*Alternanthera philoxeroides* (Mart.) Griseb.: Amaranthaceae), exhibited higher biomass, photosynthetic rate as well as higher leaf nitrogen relative to the native *Alternanthera sessilis* (L.) DC. (Amaranthaceae), also known as the Brazilian spinach, in the absence of herbivory. The introduction of the grasshopper changed this dynamic, so that native plant performance was higher.

Based on this, one would expect that biological control would also have a similar impact in terms of mediating competitive dominance between native and invasive plants. This is one of the approaches used to manage invasive plant species, which involves using highly specific natural enemies, such as herbivorous insects, to control the spread of the invader (Cilliers, 1991; Winston et al., 2014). *Hydrellia* flies are a group of insects from the Ephydridae that are used in the biological control of various species within the submerged Hydrocharitaceae (Balciunas & Burrows, 1996; Wheeler & Center, 1996, Coetzee et al., 2011; Smith, 2017). These are aquatic flies whose larvae mine the leaves of their host plant species and tend to be monophagous. They act by reducing the photosynthetic tissue of the plant, resulting in decline in growth rate, and therefore, biomass (Bownes, 2014; Weeks & Cuda, 2014).

The use of *Hydrellia pakistanae* (Deonier) (Ephydridae) against *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) in the USA is the flagship of biological control of submerged macrophytes, although with varying degrees of success (Wheeler & Center, 1996; Center et al., 1997). More recently, *H. egeriae* has been released to control *E. densa* in South Africa (Smith et al., 2019), while *Hydrellia lagarosiphon* (Deeming) [Diptera: Ephydridae], is a potential suitable biological control agent of *L. major* (Baars et al., 2010; Martin et al., 2013).

This study used reciprocal yield models in order to understand the potential outcomes of introducing either *H. lagarosiphon* or *H. egeriae* on the competitive interactions between *L. major* and *E. densa*, should either or both species be considered for release in New Zealand. These models describe the yield of two competing species as a function of their densities and can be used to estimate the competitive ability of each species under different environmental conditions, and in this case, herbivory (e.g., Van, 1999; Coetzee et al., 2005 and Martin et al., 2018).

Thus, the aim of this study was to use Spitters' (1983) reciprocal-yield model to evaluate the competitive ability of these two invasive species in the presence and absence of their respective biological control agent. Specifically, the objective of this chapter was to provide insights into the role of biological control in mediating competitive interactions between these two macrophytes. I hypothesised that *L. major* would emerge as the superior competitor to *E. densa* due to its more rigid structure and high phenotypic plasticity. When both species are exposed to biological control, this competitive edge should be exacerbated by the addition of herbivory, due to the destructive nature of *H. egeriae* relative to *H. lagarosiphon*.

The results of the study will provide valuable insights into the competitive ability of *L. major* against *E. densa* and the role of biological control in mediating this competition and therefore should be taken into consideration when managing these plants. This is because resource managers are usually tasked with controlling multiple invasive species in large areas on a limited budget. Therefore, studies like this could provide a good starting point for prioritisation efforts when it comes to choosing which biological control programme to fund. This is an important contribution that will help resource managers with allocation of funds to the most impactful project (Paynter et al., 2015).

In the context of New Zealand and elsewhere where both species are a nuisance, this study will help predict what the interaction might be in cases where *L. major* and *E. densa* co-occur, and how introducing biological control of one species or the other might affect the observed interactions at the community level. This study is also crucial for the native range of *L. major* in South Africa where *E. densa* is spreading, because although no co-occurrences have been reported thus far, this is still an important consideration for continued effective management of *E. densa*.

4.2. Materials and Methods

4.2.1. Experimental design

Spitters (1983) reciprocal-yield models were used to investigate the competitive ability of *L. major* against *E. densa* in the presence and absence of biological control by *H. lagarosiphon* and *H. egeriae*, respectively. The experiment was conducted inside a greenhouse tunnel at the Waainek Research Facility at Rhodes University, South Africa from October until early December 2022. The setup consisted of eight different factorial combination of planting densities of *L. major*: *E. densa* as follows: 0:3, 0:9, 3:3, 3:9, 9:3, 3:0, 9:0; 9:9, with and without biological control. This resulted in four herbivory treatment combinations (1. Control=No herbivory; 2. Only *H. lagarosiphon*; 3. Only *H. egeriae*; 4. Both *H. lagarosiphon* and *H. egeriae*) according to the different planting densities, with each treatment replicated four times.

The experiment was conducted in four flow-through systems of five connected tanks (120cm x 100cm x 70cm each, 800 L), filled with water which was continually circulated by means of a submersible pump. The fifth tank was used to house the filtration system. Apical shoots (~25 cm in length with a mean dry biomass of 0.16 ±SE (n=20 per species), and no side shoots) were planted according to their experimental planting densities, in 7L plastic tubs (25 cm diam. × 14 cm deep) containing pond sediment and no added fertiliser. A thin layer of silica sand on top of the sediment minimised the growth of algae and maintained water clarity. Each tank contained eight randomly placed tubs, representative of the different planning densities, and with enough distance between tubs to minimise the interaction of plants from different tubs (Figure 4.1).

Herbivore inoculation using two newly hatched larvae per respective host plant shoot was initiated four weeks after the experiment setup, on the emergent part of each plant. All treatments were enclosed with a single layer of 50% shade cloth to provide optimum conditions for submerged plant growth (Yarrow et al. 2009), and to avoid escape and cross-contamination of insects. Each treatment was replicated four times and spread out across 16 tanks (each tank representing a replicate of the four herbivory treatments), equating to three flowthrough systems. The experiment was run for a total of 10 weeks, with an acclimation period of four weeks before the commencement of the herbivory treatment. At the end of the experiment period, length of the longest shoot per tub, and the number of side shoots for the longest shoot were determined for each species before harvesting. The harvested material was washed and separated if planted in mixed cultures, then oven dried for 76 hours at 60 ^{o}C in order to obtain dry biomass of roots and shoots for each species.

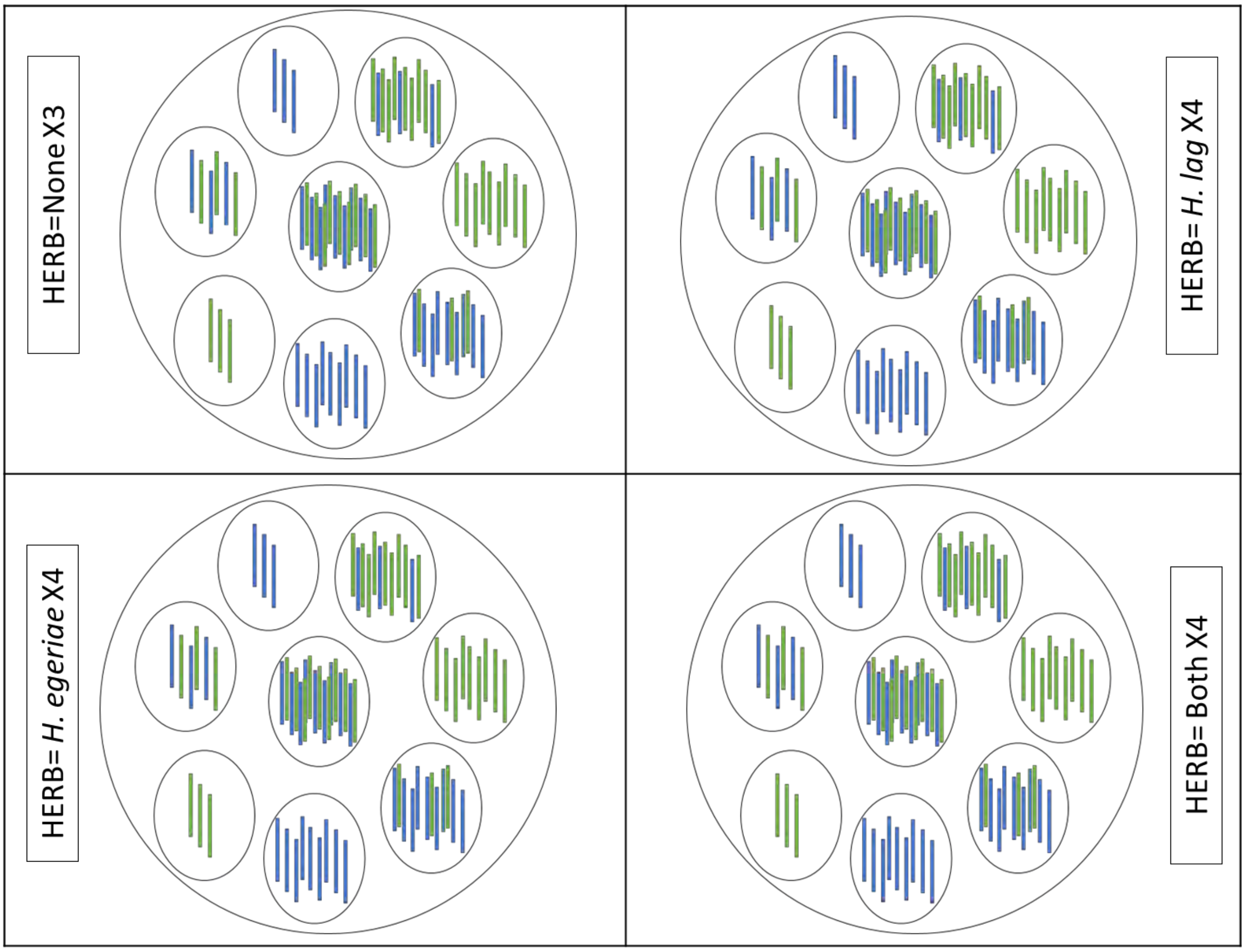


Figure 4.1: Schematic representation of the competition experiment layout for each species combination for the herbivory treatments (HERB) as housed in the flowthrough system tanks. *H. lag* represents *Hydrellia lagarosiphon*, and *H. egeriae* represents *Hydrellia egeria*. Different colours indicate different plant species (i.e. *Lagarosiphon major* and *Egeria densa*). Placement of tubs was randomised in the actual experiment.

4.2.2. Data analysis

To investigate the relationship between herbivory and plant density (and therefore competition), results from all plant parameters (shoot length, number of shoots, and dry biomass) were analysed using Generalised Linear Models (GLM-ANOVA) with specified family distribution (Gaussian, Gamma or Poisson regression) and link function based on data features. GLM were chosen over Generalised Mixed Models because upon inspection, the amount of variance explained by the tank effect (random effect with four replicates or categories) was not distinguishable from zero for all the response variables. This means that there was not much variation when comparing between tanks where the different densities were housed.

The GLM had three factors for each response variable (Density, Plant and Herbivory), and the car package Anova function was used to perform type-II analysis of deviance. This allowed the significance of individual terms and groups of terms in the GLM to be tested. Parameters that showed a significant result were further analysed using multiple post hoc comparisons. In order to reduce Type II error, the emmeans function with the Bonferroni adjustment was implemented within the lme4 package in RStudio. The rate of biomass accumulation under the different herbivory treatments and planting densities was determined by calculating the relative growth rate (RGR) of dry above ground biomass for each species. This was done in R studio using the standard formula described by Evans (1972) (equation 1).

$$\text{Equation 1:} \quad RGR = (lnW_2 - lnW_1)/ (t_2 - t_1)$$

Here W_1 and W_2 represent the dry biomass at the start and end of the experiment, respectively at times t_1 and t_2 (the start and end time in weeks). This was again analysed using GLM and followed similar steps to the analysis of other plant parameters measured above. The results of this analysis were used to determine whether the mean end-yields of *L. major* and *E. densa* were significantly different between the treatments and whether the mean RGR of these two plants species were significantly different between the treatments.

Inverse linear models were used to directly quantify the competitive effect of *L. major* and *E. densa* as driven by different herbivory regimes. This was done by running multiple regressions on the above ground dry biomass according to Spitters's (1983) reciprocal yield. Equation 2 and 3 below show the structure of the models for *L. major* and *E. densa*, respectively.

$$\text{Equation 2:} \quad 1/W_L = a_{L0} + a_{LL}d_L + a_{LE}d_E$$

$$\text{Equation 3:} \quad 1/W_E = a_{E0} + a_{EE}d_E + a_{EL}d_L$$

Here, 1/W represents the inverse dry biomass yield, while the intercepts a_{L0} and a_{E0} represent the respective reciprocal of the maximum mass of isolated plants. The planting densities are shown by d_L and d_E. Therefore, the partial regression coefficients $a_{LL}d_L$ and $a_{EE}d_E$ are representative of intraspecific competition, while the coefficients $a_{LE}d_E$ and $a_{EL}d_L$ characterise interspecific competition in terms of their effects on the reciprocal yield of *L. major* and *E. densa*, respectively.

Taking the ratio of intra and interspecific competition (a_{LL}/a_{LE} or a_{EE}/a_{EL}) gives the magnitude of competitive advantage that each species has against the other in terms of mean dry biomass. The effect on *L. major* by *E. densa* is measured by coefficient a_{LE} and the opposite is true for a_{EL}. Similarly, a_{LL} and a_{EE} are defined as the effect of each plant species on itself. Thus, a competition ratio =1 shows a neutral interaction, while values > 1 illustrate the dominance of intraspecific competition, and a value of < 1 indicates greater interspecific competition (Monacelli & Wilcox, 2021). Here, negative values indicate a facilitative effect rather than a competitive interaction (Thiébaut et al., 2019; Silveira & Thiébaut, 2020; Monacelli & Wilcox, 2021).

An alpha level of 0.05 was chosen for all statistical tests, and all graphs and analyses were conducted in R Studio v 4.2.2 (R Core Team, 2023). The mean ± SE and post hoc groupings for plant parameters (including RGR) were graphed using the ggplot2 package. The inverse yield and therefore plant competition was visualised using three dimensional figures with multiple linear regressions captured by the regression plane plotted using the scatterplot3d package in Rstudio.

4.3. Results

4.3.1. Plant parameters

The present study investigated the effects of herbivory and plant density on the fitness and reproduction of two aquatic plant species, *L. major* and *E. densa*. The experiment examined shoot length, shoot number, biomass production, and relative growth rate as indicators of plant performance in response to herbivory by their respective biological control agents, *H. lagarosiphon* and *H. egeriae* across different plant densities. The findings reveal intriguing patterns that highlight the complex interactions between planting densities and herbivory across the two plant species.

Shoot length was significantly affected by all terms and main interactions (Table 4.1). The post-hoc test however showed varied responses depending on herbivore combination and starting densities. Firstly, both plant species were surprisingly not affected by herbivory treatments nor density for monocultures (Figure 4.2). Nevertheless, for the mixed cultures, herbivory treatment was an important determinant of shoot length (Table 4.1; Figure 4.2). For example, although *L. major* did not have a significant response to herbivory in the low-density mixed cultures (3:3), *E. densa* with no herbivory had significantly longer shoots than those that were exposed to herbivory by either *H. egeriae* or *H. lagarosiphon*, for this density treatment (Figure 4.2). However, when exposed to both insects at the same time, this herbivory effect was not evident.

The importance of herbivory as a determinant of plant shoot length was more evident in the high-density mixed cultures (9:9). Here, the length of *L. major* was significantly reduced by herbivory by both agents, regardless of whether they were isolated or combined (Table 4.1; Figure 4.2). This is in contrast to the response of *E. densa* reported above, where agents were only effective in isolation. Surprisingly though, *H. egeriae* herbivory had a greater impact on *L. major* than *H. lagarosiphon* which is its candidate biological agent. With regards to *E. densa*, herbivory was also an important determinant of shoot length within the high-density mixed cultures (9:9), except for those treatments with only *H. lagarosiphon*, which was not significantly different from the control (Figure 4.2).

When the density of plants was varied, only *L. major* plants that were exposed to both agents simultaneously had significantly reduced shoot length in the treatment with lower *L major* densities (3:9). In this case, the combined effect of the two insects appears to foster biological control of *L. major*, rather than restrain it as seen with *E. densa*. Higher densities of *E. densa* within this treatment saw *H. egeriae* being the only herbivory regime that successfully reduced shoot growth (Figure 4.2). This pattern changed when looking at the converse results, where *L. major* was the dominant plant (9:3). Here, herbivory did not affect *L. major* shoot length. *Egeria densa* on the other hand was significantly affected by *H. lagarosiphon* relative to the control, and its biological control agent, *H. egeriae* did not perform better than the control. In summary, the biological control agents were more successful at reducing shoot lengths in mixed cultures (Table 4.2).

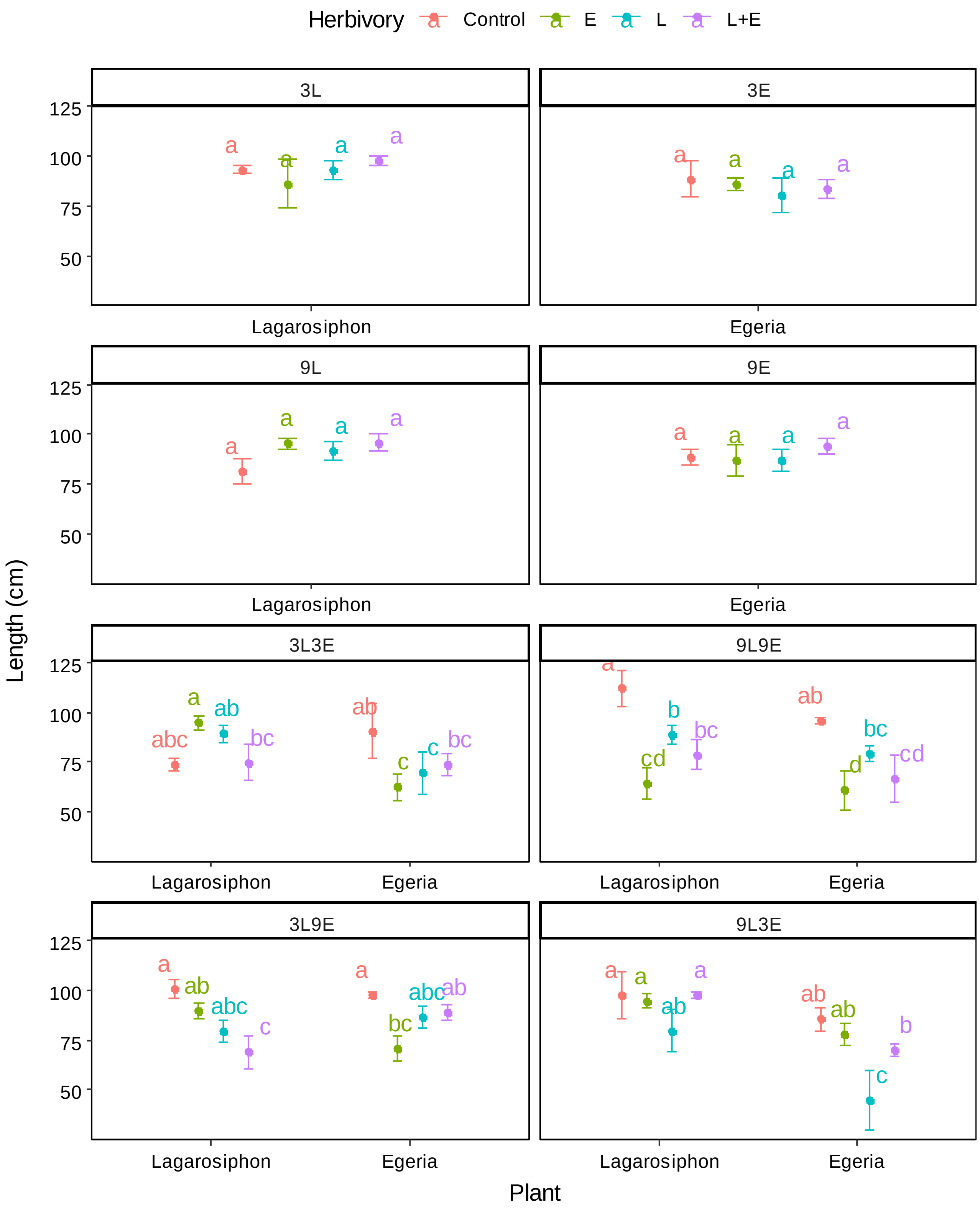


Figure 4.2: Length (mean ± S.E, cm) of *Lagarosiphon major* and *Egeria densa* as affected by planting densities and herbivory treatment. Different colours show herbivory treatments, where Control = No herbivory, E = *Hydrellia egeriae*, L= *Hydrellia lagarosiphon* and L+E = both insects combined. Panels represent starting densities (e.g., 3L= 3 *L. major*, 3E= 3. *E. densa*). Different letters denote significant differences between treatments.

The results of the mean number of shoots showed a similar pattern to mean shoot length findings (Table 4.2), indicating no significant effect of herbivory on the monocultures of both species (Table 4.1). This is with the exception of the low-density stands of *L. major*, where herbivory by *H. egeriae* had a significant reduction on the number of shoots produced relative to the control (Figure 4.3). Note however that although all main terms were significant, the interaction between plant species and herbivory, as well as the three-way interaction between the main effects, were not significant (Table 4.1).

For the mixed cultures, herbivory was also not an important determinant of the number of shoots produced by both plant species in the low-density treatment (3:3) (Figure 4.3). However, mean shoot number was overall lower for *E. densa* compared to *L. major*, and a similar trend was evident across the different treatments (Table 4.1). In the high-density mixed cultures (9:9), again we see the effect of *H. egeriae* being more readily felt by *L. major* in terms of vegetative reproduction, while *E. densa* did not appear to be affected by herbivory from either insect (Figure 4.3). When the density of plants was varied, the same pattern of *H. egeriae* dominance in *L. major* stands and overall low number of shoots in *E. densa* was observed.

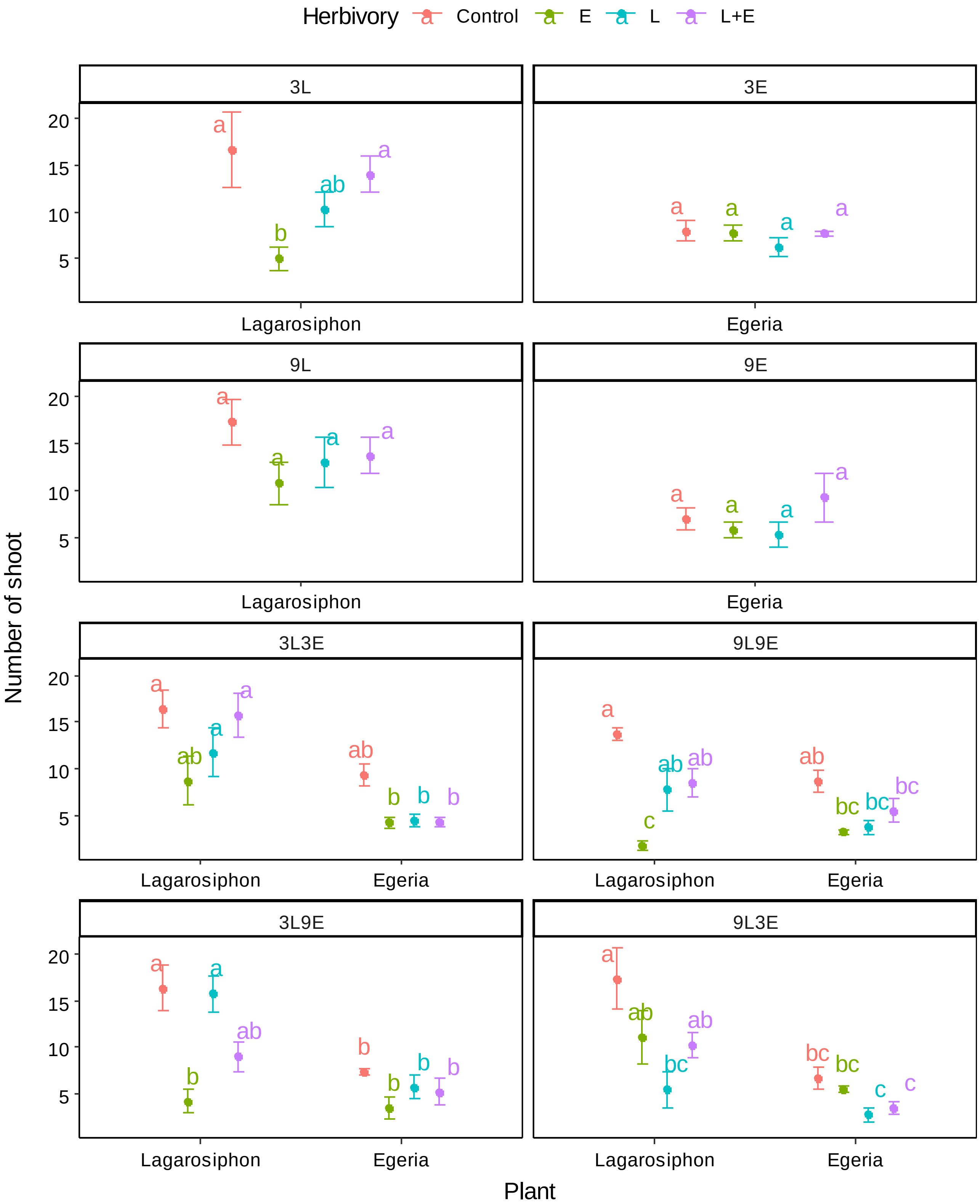


Figure 4.3: Number of side shoots (mean ± S.E) of *Lagarosiphon major* and *Egeria densa* as affected by planting densities and herbivory treatment. Different colours show herbivory treatments, where Control = No herbivory, E = *Hydrellia egeriae*, L= *Hydrellia lagarosiphon* and L+E = both insects combined. Panels represent starting densities (e.g., 3L= 3 *L. major*, 3E= 3. *E. densa*). Different letters denote significant differences between treatments.

Overall, post hoc test results showed that the influence of herbivory on plant biomass production was directly influenced by density, although three-way interactions were again not significant (Table 4.1). Specifically, biomass production of *L. major* within the low-density monoculture stands was significantly reduced by both insects in isolation, but not when combined (Figure 4.4). In the high-density monocultures, the effect of herbivory was not an important determinant of *L. major* biomass (Figure 4.4).

The reverse was true for *E. densa*. Here, low-density stands were not affected by herbivory while the high-density treatment responded negatively to herbivory by *H. lagarosiphon* (Table 4.1; Figure 4.4). For the mixed cultures, herbivory treatment did not affect biomass production of *L. major* regardless of density, while *E. densa* was negatively affected by both agents in isolation at low densities (Figure 4.4). However, at high density, this effect was not observed (Table 4.1; Figure 4.4).

When the density of *L. major* was lower than *E. densa* (3:9), biomass production of *L. major* was reduced successfully only when both insects were present, while *E. densa* was not affected by herbivory. However, switching the densities around so that *L. major* was more dominant (9:3) resulted in both plants having reduced biomass in the presence of herbivory (Figure 4.4). This is consistent with the other plant parameters reported above (Table 4.2).

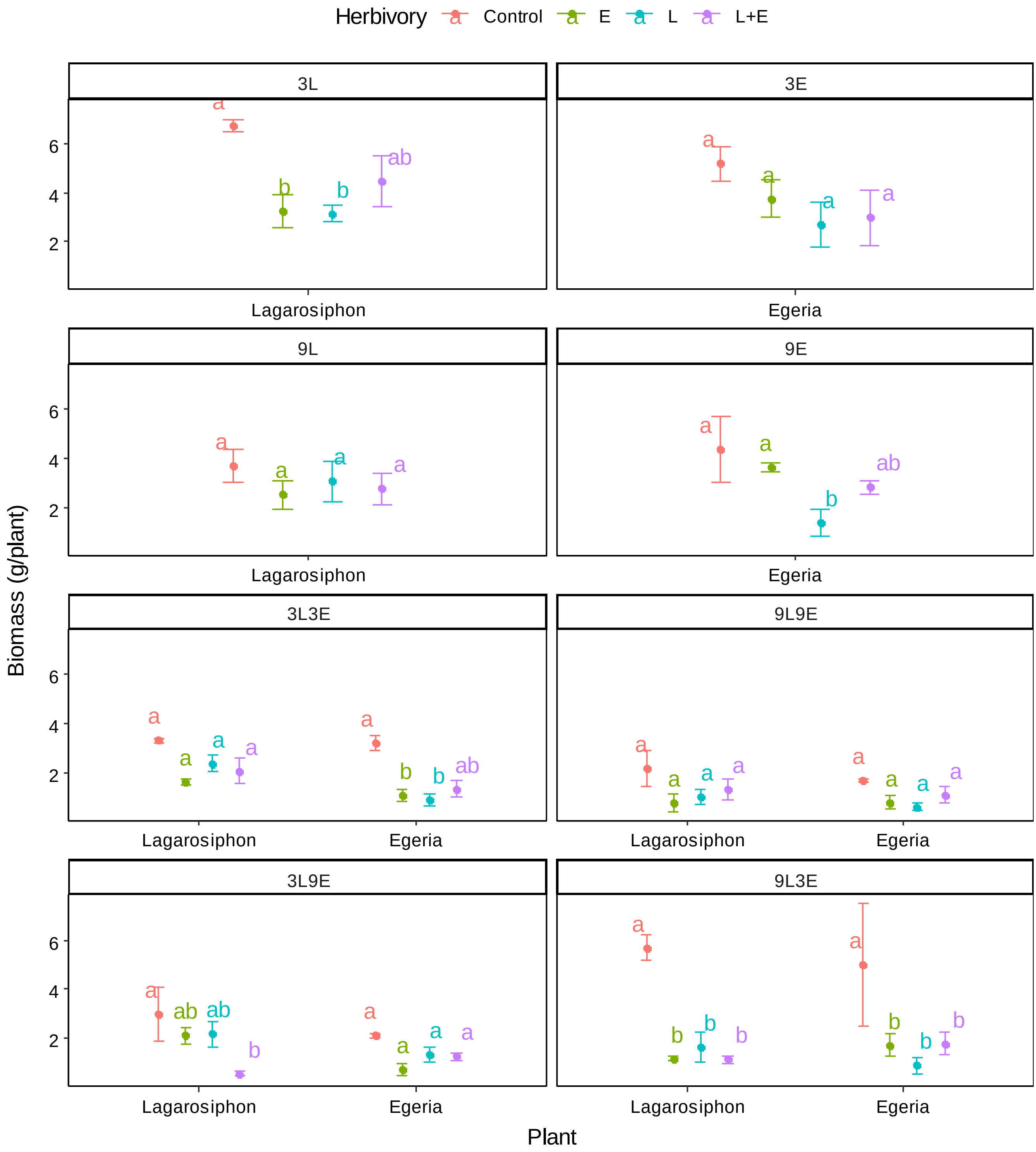


Figure 4.4: Dry aboveground biomass (mean ± S.E, g) of *Lagarosiphon major* and *Egeria densa* as affected by planting densities and herbivory treatment. Different colours show herbivory treatments, where Control = No herbivory, E = *Hydrellia egeriae*, L= *Hydrellia lagarosiphon* and L+E = both insects combined. Panels represent starting densities (e.g., 3L= 3 *L. major*, 3E= 3. *E. densa*). Different letters denote significant differences between treatments.

The relative growth rate of *L. major* monocultures was not affected by herbivory, regardless of density (Table 4.1; Figure 4.5). This finding aligns with the results obtained for shoot length (Figure 4.1) and vegetative reproduction (Figure 4.3). The same was true for *E. densa* at low densities (Table 4.2). However, at high density monocultures, herbivory by *H. lagarosiphon* had a negative effect on relative growth rate of this plant but did not affect *L. major* relative growth rate (Table 4.1; Figure 4.5).

For the mixed cultures, herbivory treatment did not affect the growth rate of *L. major* at low densities, while *E. densa* was negatively affected by both agents in isolation for this density treatment (Figure 4.5). However, at high mixed densities (9:9), no herbivory effect was observed for *E. densa*, while *L. major* growth rate was significantly reduced by *H. egeriae* (Table 4.1; Figure 4.5).

When plant densities were varied, specifically when the density of *L. major* was lower than *E. densa* (3:9), the growth rate of *L. major* was reduced significantly only when both insects were present, while *E. densa* was only negatively affected by its biological control agent (Figure 4.5). However, when *L. major* was more dominant (9:3), all herbivory combinations significantly reduced the relative growth rate of this plant species, while *E. densa* was again only responsive to *H. lagarosiphon* at these densities (Table 4.1; Figure 4.5).

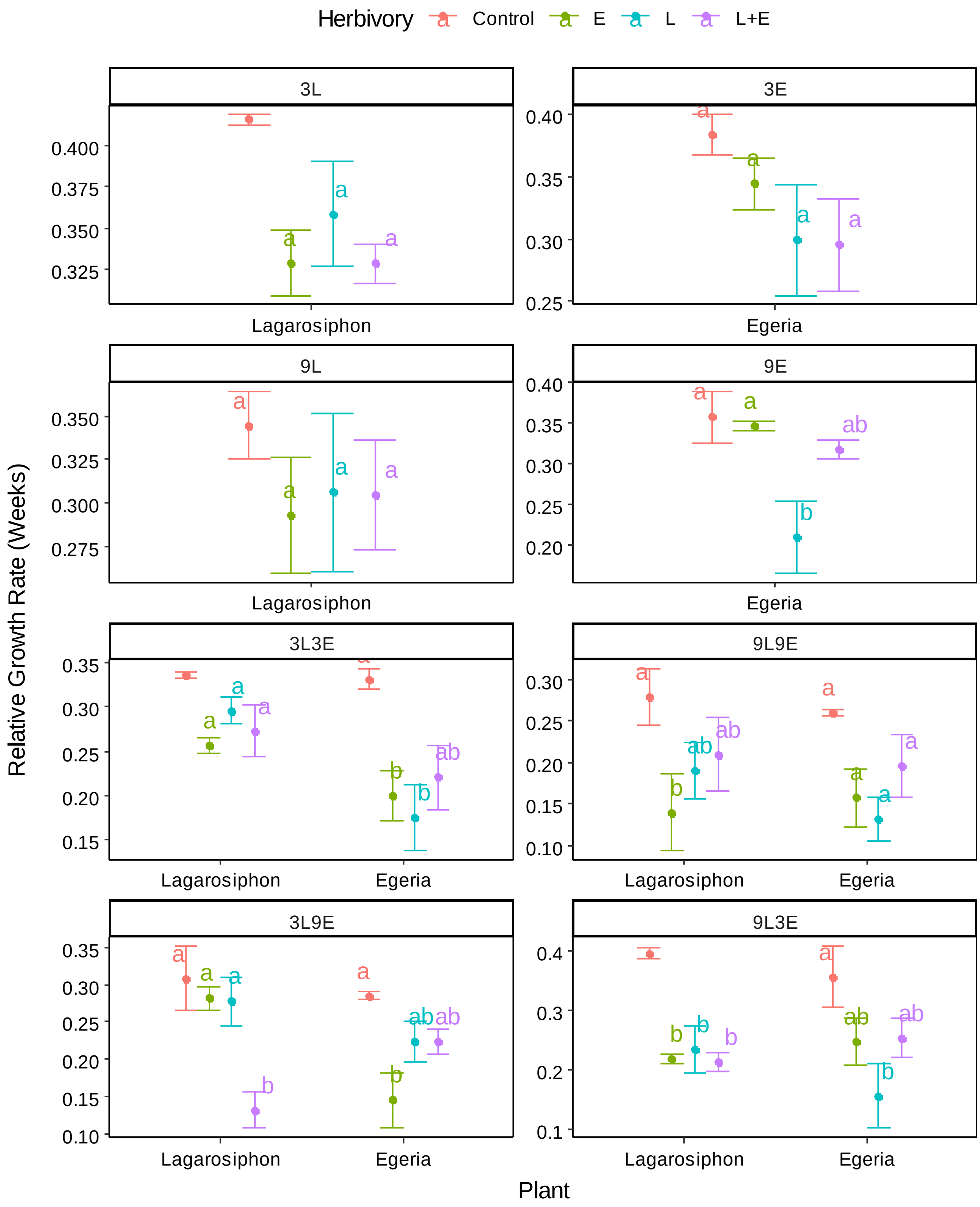
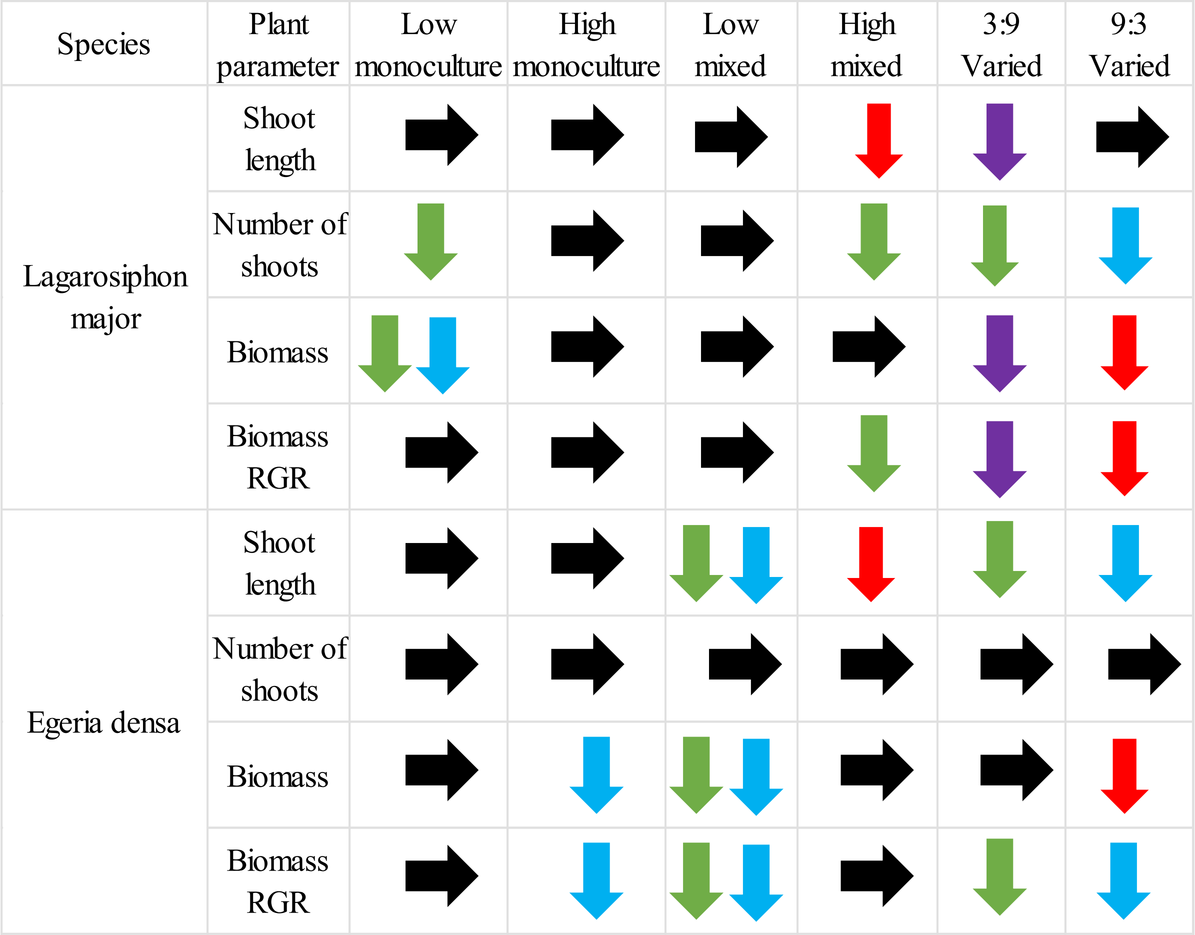


Figure 4.5: Biomass relative growth rate (mean ± S.E, weeks) of *Lagarosiphon major* and *Egeria densa* as affected by planting densities and herbivory treatment. Different colours show herbivory treatments, where Control = No herbivory, E = *Hydrellia egeriae*, L= *Hydrellia lagarosiphon* and L+E = both insects combined. Panels represent starting densities (e.g., 3L= 3 *L. major*, 3E= 3. *E. densa*). Different letters denote significant differences between treatments.

Table 4.1: Generalised linear model-ANOVA summary results for each plant parameter.

Plant parameter	Coefficients	X^2	Df	*P*
Shoot length (cm)	*Density*	51.987	7	**<0.001**
	Plant	42.415	1	**<0.001**
	Herbivory	39.893	3	**<0.001**
	Density:Plant	30.601	3	**<0.001**
	Density:Herbivory	124.555	21	**<0.001**
	Plant:Herbivory	16.220	3	**0.0012**
	Density:Plant:Herb	50.718	9	**<0.001**
Shoot number	*Density*	40.035	7	**<0.001**
	Plant	117.679	1	**<0.001**
	Herbivory	92.709	3	**<0.001**
	Density:Plant	9.036	3	**0.028**
	Density:Herbivory	70.169	21	**<0.001**
	Plant:Herbivory	7.199	3	0.065
	Density:Plant:Herb	9.084	9	0.429
Plant biomass (g)	*Density*	132.125	7	**<0.001**
	Plant	6.041	1	**0.014**
	Herbivory	86.447	3	**<0.001**
	Density:Plant	2.494	3	0.476
	Density:Herbivory	35.846	21	**0.023**
	Plant:Herbivory	5.548	3	0.136
	Density:Plant:Herb	9.509	9	0.392
Relative growth rate (weeks)	*Density*	113.541	7	**<0.001**
	Plant	8.015	1	**0.005**
	Herbivory	62.902	3	**<0.001**
	Density:Plant	1.849	3	0.604
	Density:Herbivory	34.181	21	**0.023**
	Plant:Herbivory	9.443	3	**0.024**
	Density:Plant:Herb	15.671	9	0.074

Table 4.2: Summary of the response of *Lagarosiphon major* and *Egeria densa* to herbivory as driven by planting densities. **Black**= no herbivory effect, Red= all herbivory treatments have an impact, Purple= effect only when both agents are present simultaneously, Green= only *Hydrellia egeriae* has an impact, Blue= only *Hydrellia lagarosiphon* has an impact, Green + Blue= both agents have an impact on that treatment only when isolated.



Species	Plant parameter	Low monoculture	High monoculture	Low mixed	High mixed	3:9 Varied	9:3 Varied
Lagarosiphon major	Shoot length	→	→	→	↓	↓	→
	Number of shoots	↓	→	→	↓	↓	↓
	Biomass	↓ ↓	→	→	→	↓	↓
	Biomass RGR	→	→	→	↓	↓	↓
Egeria densa	Shoot length	→	→	↓ ↓	↓	↓	↓
	Number of shoots	→	→	→	→	→	→
	Biomass	→	↓	↓ ↓	→	→	↓
	Biomass RGR	→	↓	↓ ↓	→	↓	↓

4.3.2. Reciprocal yield models

The results obtained from reciprocal yield models provide further insight into the influence of intraspecific and interspecific competition on the growth of two plant species studied here, as well as the impact of herbivory from their biological control agents on these interactions. Overall, competitive interactions were varied depending on the herbivory context. The following figures illustrate the changes in competitive relationships based on three-dimensional reciprocal yield planes corresponding to the competition coefficients for the *L. major* and *E. densa* plant species under investigation here. The higher the values on the figure, the lower the actual yield, and the greater the slope, the larger the competitive coefficient. The slope in one direction represents the effect of the density of one plant species on its own yield (intraspecific competition), while a slope in the other direction represents the effects of density across the two species (interspecific competition).

Multiple linear regressions of reciprocal yield showed that intraspecific competition within *L. major* had no significant influence on the growth of this plant for the control treatment and when exposed to both insects simultaneously (Table 4.3; Figure 4.6A and D, respectively). However, when the insects were isolated, both had a significant effect on *L. major* growth (coefficient = 0,118; *P*=0.021 for *H. egeriae;* and coefficient =0.078, *P*= 0.014 for *H. lagarosiphon*), which intensified competition between individual plants (Table 4.3), evident by the steep slope of the regression planes towards the x-axis where *L. major* density is represented (Figure 4.6B-C). Here, intraspecific competition within *L. major* plants was greater in the presence of *H. egeriae* compared to *H. lagarosiphon*.

Interspecific competition on the other hand was affected by all herbivory treatments for *L. major* plants (Table 4.3), evident by the direction of the slopes, where exposure to both insects simultaneously led to the greatest competitive interaction between the two plants at 0.141 relative to 0.033 for the control (Figure 4.6A-D). These findings further support the earlier observations on plant parameters, which indicated that herbivory had significant effects on shoot length, shoot number, biomass production, and relative growth rate of *L. major* depending on plant densities.

The highest competitive ratio for *L. major* was found when the two plants were competing in the presence of *H. lagarosiphon*, with 1 *L. major* being equivalent to 1.7 *E. densa* plants (Table 4.3). This indicates that *L. major* had a slight competitive advantage over *E. densa* when competing in the presence of this insect (Figure 4.6B). The lowest ratio of competition was observed when plants were competing in the presence of both insects simultaneously, as well as in the absence of both insects (Table 4.3) (Figure 4.6D). Generally, the competitive interactions between *L. major* and *E. densa* were highly dependent on the herbivory context but were overall mild as no values were greater than 2 to signify real competitive advantage (Table 4.1, Figure 4).

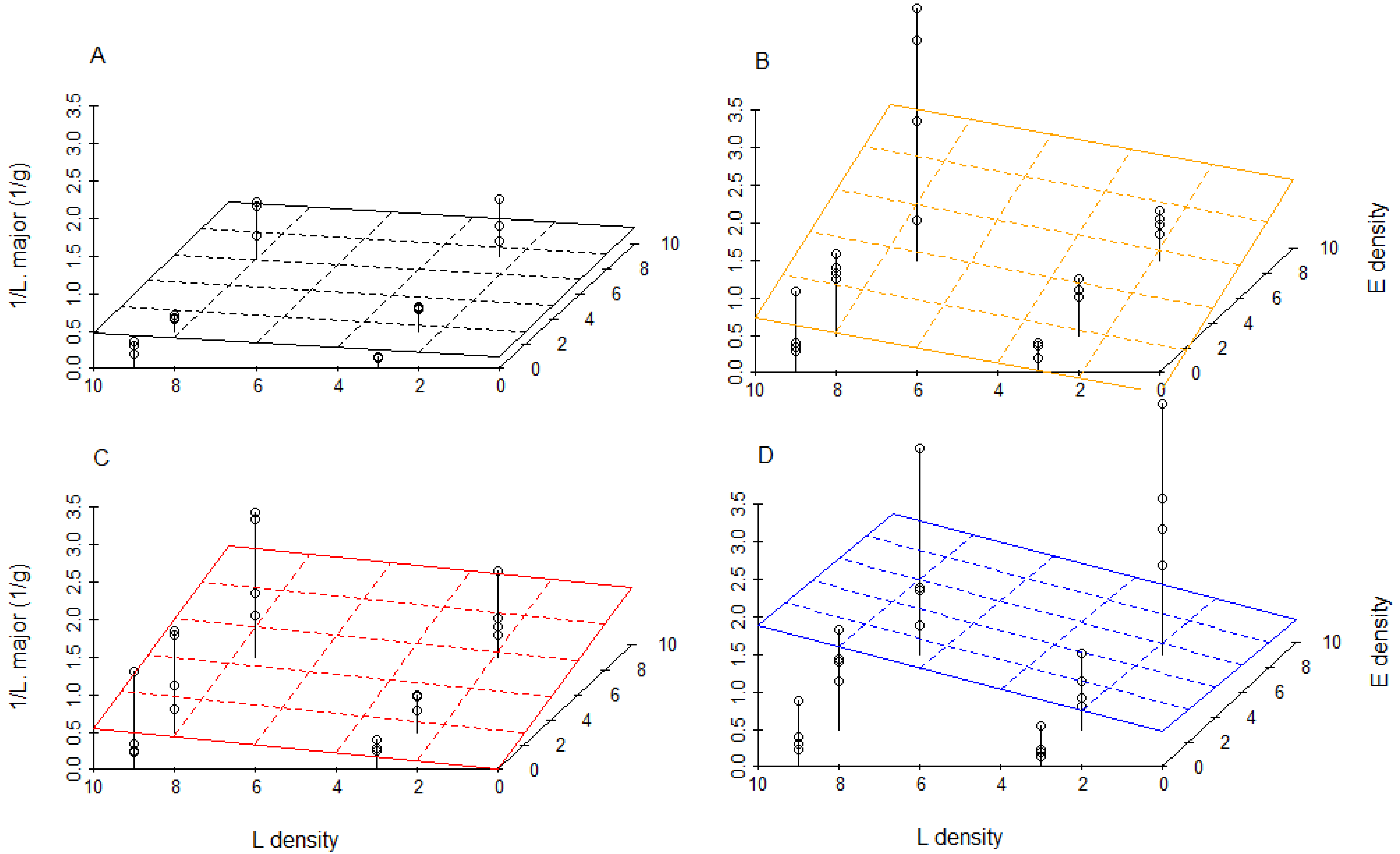


Figure 4.6: Multiple regression planes demonstrating the combined effect of *Lagarosiphon major* (L density) and *Egeria densa* (E density) planting densities on the inverse mean dry aboveground biomass (1/g) of one *L. major* plant, according to herbivory treatment (A= Control, B= *Hydrellia egeriae*, C= *Hydrellia*

lagarosiphon and D= Both *H. egeriae* and *H. lagarosiphon*). Points indicate observations (n=4 for treatment and n=3 for control) and the vertical lines between data points represent the residuals. Values on X and Z axes represent planting densities at the beginning of the experiment.

Table 4.3 Multiple regression analysis of above ground competition, in terms of dry biomass, between *Lagarosiphon major* and *Egeria densa* grown under different densities and herbivory regimes.

1/g/Plant	Herbivory	Intercept	Intraspecific competition	Interspecific competition	Ratio of competition coefficients	R^2	F	df
Lagarosiphon major	Control	0.151	0.006; *P*=0.614	0.033; ***P*=0.004**	*0.190*	0.438	5.838	2;15
	H. egeriae	-0.227	0.118; ***P*=0.021**	0.102; ***P*=0.006**	*1.169*	0.459	8.471	2; 20
	H. lag	0.009	0.078; ***P*=0.014**	0.057; ***P*=0.024**	*1.368*	0.384	6.544	2; 21
	Both	0.508	-0.024; *P*=0.623	0.141; ***P*=0.001**	*-0.171*	0.425	7.018	2;19
Egeria densa	Control	0.092	0.03; ***P*=0.005**	0.021; ***P*=0.011**	*0.700*	0.5	9.642	2; 15
	H. egeriae	0.128	0.094; *P*=0.106	0.088; *P*=0.061	*0.936*	0.244	3.393	2; 21
	H. lag	0.904	-0.0125; *P*=0.879	0.149; ***P*=0.033**	*-11.920*	0.199	2.619	2; 21
	Both	0.502	0.014; *P*=0.74	0.057; *P*=0.11	*4.071*	0.127	1.378	2; 19

The results for *E. densa* reveal some contrasting patterns compared to what was observed above for *L. major*. In the case of intraspecific competition within *E. densa*, the reciprocal yield models indicated that only in the absence of herbivory was there a small but statistically significant intraspecific competition within *E. densa* plants (coefficient = 0.03, *P*= 0.005), and the introduction of herbivory negated this interaction (Table 4.3; Figure 4.7A-D).

On the other hand, both the control treatment and *E. densa* plants exposed to *H. lagarosiphon* showed a significant effect of interspecific competition on biomass yield, with the greatest competition coefficient being 0.149 for *H. lagarosiphon* and 0.021 in the absence of herbivory (Table 4.3; Figure 4.7A and C, respectively). Surprisingly, the *E. densa* biological control agent, *H. egeriae*, was not a significant determinant of both interspecific as well as intraspecific competition for this species regardless of the marked slope (Table 4.3; Figure 4.7B).

In terms of the magnitude of competition between the two plant species, the lowest competitive ratio for *E. densa* was found when the plants were not exposed to herbivory (Table 4.3; Figure 4.7D). For this treatment, one *E. densa* plant was equivalent to 0.70 *L. major* plants, thus showing the importance of mild interspecific competition rather than intraspecific competition under these conditions. Furthermore, despite the apparent relative dominance of *E. densa* when plants were exposed to both insects at the same time, evidenced by the 4.07 competitive ratio, this herbivory regime was not statistically significant in terms of determining competitive interactions (Table 4.3). The low R^2 value further shows that other factors that were not recorded for this study (e.g., temperatures and light availability) may be responsible for the observed competitive interactions (Table 4.3).

Lastly, the greatest ratio of competition was observed when plants were exposed to *H. lagarosiphon* herbivory only, at -11.92 *E. densa* plants for every *L. major* plant (Table 4.3; Figure 4.7D). The negative value here shows that one *L. major* plant was facilitating an equivalent of approximately 12 *E. densa* individuals under these conditions. In other words, *E. densa* becomes a superior competitor here. However, again, even though interspecific competition was found to be significant, herbivory only explains around 20 % of the variation observed in the data (Table 4.3). In summary, the reciprocal yield models show that *L. major* was overall more responsive to herbivory treatments in terms of competitive interactions compared to *E. densa*, while for the latter, interactions were mainly determined by plants exposed to *H. lagarosiphon* only (Table 4.3).

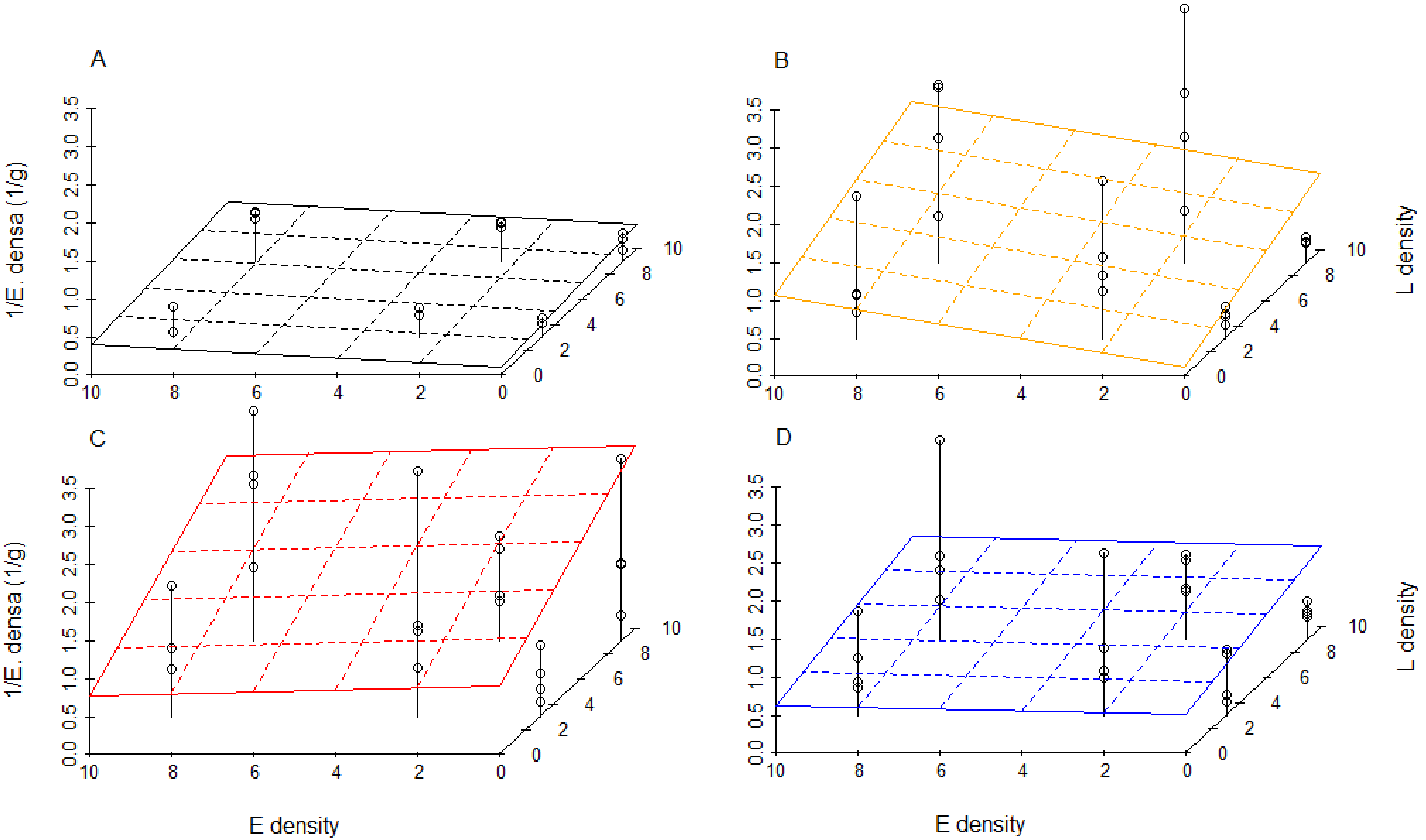


Figure 4.7: Multiple regression planes demonstrating the combined effect of *Lagarosiphon major* and *Egeria densa* planting densities on the inverse mean dry aboveground biomass (1/g) of one *Egeria densa* plant, according to herbivory treatment (A= Control, B= *Hydrellia egeriae*, C= *Hydrellia lagarosiphon* and D=

Both insects). Points indicate observations (n=4 per treatment) and the vertical lines between data points represent the residuals. Values on X and Z axes represent planting densities at the beginning of the experiment.

4.4. Discussion

The analysis of plant parameters and reciprocal yield models presented here provides valuable insights into the interactions between herbivory, competition, and plant fitness in the context of *L. major* and *E. densa* plant communities as determined by planting densities. These types of models have previously been used to study plant competition in the presence of herbivory (Van, 1999; Coetzee et al., 2005 and Martin et al., 2014; 2018). They are also a useful tool for investigating the competitive ability of invasive species against native species.

In contrast to findings by Strange (2017), there was no strong competitive interaction between *L. major* and *E. densa* for the current study with regards to competitive ratios, although plant parameter comparisons showed the former to be overall more successful. This was especially true in low density cultures. According to Strange (2017), *E. densa* was a far more superior competitor relative to *L. major*. However, those studies did not include a herbivory effect, and there were high mortalities of *L. major* recorded, seemingly due to high temperatures. On the other hand, various studies have found that *L. major* tends to be a superior competitor outside its native range, with the ability to actively outcompete other submerged macrophytes, such as *M. spicatum*, *M. triphyllum* (Haloragaceae), *E. canadensis* and *E. densa* (Rattray et al. 1994; James et al. 1999; Hofstra et al. 1999; Caffrey et al. 2010).

In general, *L. major* was able to tolerate herbivory when planted in isolation, regardless of starting density. This highlights the potential limitations of the candidate biological control agent, *H. lagarosiphon*, in effectively controlling *L. major*, as mostly it was *H. egeriae* or at least both species in combination driving the observed changes in *L. major* growth. This is contrary to expectations based on previous studies which showed that *H. lagarosiphon* affects plant growth and is damaging and specific enough to be considered for biological control of *L. major* (Baars et al., 2010; Martin et al., 2013).

Alternatively, it is also possible that the observed tolerance is due to limited insect numbers, which were probably not high enough to make a significant reduction in plant growth under these conditions. According to Mangan & Baars (2016), densities of three to four *H. lagarosiphon* larvae per shoot tip are required for effectively reducing the growth of *L. major*. However, herbivory inoculation for this study only used two larvae per plant shoot due to the size of the experiment, and the unavailability of large enough insect numbers.

In most instances, co-occurrence of biological control agents reduced efficacy of *E. densa* control, relative to when plants were only exposed to *H. egeriae*. Although these insects have been shown to have preferences for their host plant (Baars et al., 2010 and Martin et al., 2013 for *H. lagarosiphon* on *L. major* and Cabrera Walsh et al., 2013 for *E. densa*), they are also able to feed and reproduce on either species, although not over multiple generations (Smith et al., 2019). Therefore, the reduced efficacy might be due to competition between the two insects for this plant species. This is because *H. egeriae* and *H. lagarosiphon* have very similar life histories, which means they can compete for resources such as oviposition sites and food.

A systematic review by Stephens et al. (2013) showed compelling evidence of this competition phenomenon. They investigated the effect of releasing multiple natural enemies on plant performance of various species and found that insects feeding on the same part of the plant had antagonistic effects. An earlier similar review showed that insects that are closely related, as is the case here, are likely to compete more aggressively for resources (Denno et al., 1995). On the other hand, while co-occurrence of the two species of insects reduced efficacy for *E. densa* control, combining *H. lagarosiphon* and *H. egeriae* was overall more efficient in reducing *L. major* fitness. In this case, the presence of *H. egeriae* appeared to complement *H. lagarosiphon* efforts.

Studies by Smith et al. (2019) have shown that *L. major* is susceptible to herbivory by *H. egeriae*. However, the release of this insect was permitted because the risk of non-target effects on *L. major* was shown to be minimal compared to *E. densa* for that investigation. However, for the current study, the effect by the candidate biological control *H. lagarosiphon* was mostly negligible, while non-target effects by *H. egeriae* were significant. Thus, *H. egeriae* appeared to be more non-discriminant than *H. lagarosiphon* in terms of host selection and overall damage. However, according to Smith et al. (2019), the native *L. major* is unable to sustain *H. egeriae* beyond three generations. Thus, despite the results obtained here, this insect is not a threat to native populations of *L. major* in South Africa.

Nevertheless, this is still in contrary to the expectation that each insect would affect its target weed more readily than the non-target. Therefore, this might possibly be a density effect more than a herbivory effect. Indeed, *L. major* was generally more successful at competing for resources than *E. densa* in terms of vegetative growth, provided that there was no biological control effect. Furthermore, overall insect performance was better in mixed cultures, presumably due to reduced plant fitness under interspecific competition (Aerts., 1999). This is similar to findings by Martin et al. (2018) on the same species. These findings highlight the importance of plant density in mediating the impact of herbivory.

Reciprocal yield models revealed that in the absence of herbivory, as well as in cases where *H. lagarosiphon* and *H. egeriae* co-existed, intraspecific competition did not play an important role in determining *L. major* growth. Rather, interspecific competition was the main determinant of *L. major* growth under these conditions. However, the ratio of competition between the two plant species showed only a mild interaction, with *L. major* being only 19% as competitive as *E. densa* under natural conditions. This reduced competitive edge has also been documented by Strange (2017) between these two species, but the strength of the interaction was far greater than what was observed here.

Competition between these two plants was directly influenced by all herbivory regimes, emphasizing the dominance of herbivory effects in determining overall competitive interactions and performance in mixed cultures (Martin et al., 2018). Various other studies have shown the pivotal role that herbivores play in shaping competitive interactions, and therefore overall plant community structures (Coetzee et al. 2005; Caffrey et al. 2010; Fan et al., 2013). In this case, herbivory by *H. lagarosiphon* resulted in *L. major* having a slight competitive advantage over *E. densa*, presumably due to compensatory growth or tolerance to herbivory which was also evident in the plant parameters. Alternatively, this could be an artifact of low insect numbers (Mangan, 2016), as stated in the previous section.

As expected, *H. egeriae* also promoted slight competitive dominance of *L. major* by feeding on, and therefore reducing, the competitive ability of *E. densa* (Smith et al., 2019). However, overall, competitive ratios showed that *L. major* did not have a pronounced competitive advantage to *E. densa*, regardless of herbivory regime. This is in contrast to the hypothesis that biological control will promote or reduce competitive interactions as seen in previous studies (Coetzee et al. 2005; Martin et al., 2018). Furthermore, numerous studies have consistently found *L. major* to more competitive relative to various other species (Rattray et al. 1994; James et al. 1999; Hofstra et al. 1999; Caffrey & Acevedo 2007; Caffrey et al., 2010). The lack of great competitive dominance in this case may be due to abundance of resources and factors beyond the scope of this study such as light and oxygen.

With regards to *E. densa,* intraspecific competition was in fact evident in the absence of herbivory, highlighting the importance of competition for resources within this species under natural conditions, although the effect was only marginal. This type of competition is usually more dominant due to similarities in resource acquisition (Van et al. 1999; Coetzee et al. 2005; Martin et al. 2013; Monacelli & Wilcox, 2021). Nevertheless, the introduction of herbivory disrupted this interaction, thereby further outlining the influence of herbivory in altering the competitive dynamics in plant communities (Coetzee et al. 2005; Fan et al., 2013; Martin et al., 2018).

Interspecific competition was an important determinant of plant yield only when plants were not exposed to herbivory or only exposed to *H. lagarosiphon*. The low competitive ratio in the absence of herbivory might be an indication that *E. densa* and *L. major* are able to co-exist in natural conditions, with the latter even being slightly more competitive. There are no currently reported co-occurrences of these two plant species in the South African native range of *L. major*, but there is evidence of co-occurrence in New Zealand, with *L. major* being the dominant of the two species (Chapter 2, pers. obs).

According to the results of reciprocal yield, the introduction of *H. lagarosiphon* had a facilitative effect on the yield of *E. densa*, with 1 *L. major* facilitating the growth of close to 12 *E. densa* plants. This is a surprising, but not an unexpected finding as *H. lagarosiphon* is said to have a preference for *L. major* (Martin et al., 2013; Mangan & Baars, 2023), which would naturally reduce its competitive ability (Martin et al., 2018). This may even translate to a facilitative effect if the wounded plants change the water/soil chemistry in the surrounding area to favour *E. densa* growth. Facilitative interactions are not uncommon in plant communities (Thiébaut et al., 2019; Silveira & Thiébaut, 2020). For example, Monacelli & Wilcox (2021) reported native species having a facilitative effect on invasive species seed production.

In general, this study highlights the intricate interplay between herbivory and competition in shaping plant community dynamics. Interspecific competition between the plants appears to have played a significant role in increasing the overall efficacy of biological control agents, with specific reference to *L. major*. This is because the presence of other plant species tends to reduce plant fitness, as resources need to be allocated to growth for successful competition for resources, as well as defences to overcome the effects of herbivory (Aerts., 1999). On the other hand, herbivory success was also affected by competition between the two insects. In many cases, when both insects were present, they competed for resources, which reduced their overall efficacy in controlling *E. densa,* whereas, for *L. major*, the insects were generally more successful at reducing plant fitness when co-occurring. This is something that needs to be considered when selecting insects for biological control programmes.

Furthermore, according to this study, *L. major* may be more difficult to control than *E. densa*, as it was shown to have higher overall fitness (in terms of plant parameters) and also lower intraspecific competition, which may allow it to dominate the aquatic vegetation in some habitats, as is the case in many aquatic ecosystems in New Zealand (Rattray et al. 1994). In this regard, it may be better to rather use *E. densa* and *H. egeriae* as a flagship of submerged aquatic plant biological control in New Zealand as this system already has a proven track record. This insect been shown to significantly affect the leaves of *E. densa* in both South America and South Africa (Cabrera Walsh et al., 2013; Smith et al., 2019; Smith, 2021). The successful introduction and implementations of this project could then be used as a motivation to funding agencies for continued work with other macrophytes such as *L. major*, and control agents such as *P. turbucinatum*. The same management strategy could be adopted in Ireland and the United Kingdom, where these species are also causing significant ecological harm.

Overall, the effect of herbivory on plant growth is complex and depends on several factors, including the density of the plants, the type of insect, and the presence of competition between the insects and between plant species. Nevertheless, the results of this study could be instrumental in the development of more effective strategies for managing aquatic invasive plant species by guiding prioritisation efforts for biological control. However, a more holistic approach to invasive plant management is also recommended, which involves prevention, early detection, rapid response, integrated control, monitoring, and evaluation.

Chapter 5: Modelling the distribution of *Lagarosiphon major* and its potential biological control agents in invaded ranges.

5.1. Introduction

Loss of biodiversity due to climate change has become one of the key features of the Anthropocene (Rahel & Olden, 2008). In the 2007 report, the Intergovernmental Panel on Climate Change (IPCC, 2007) projected a 0.2 °C increase in temperature per decade. Although this might seem like a minor change, it has far-reaching implications for plant communities (McGregor & Gourlay, 2002; Van Ginkel, 2011; Hill & Coetzee, 2017), especially those with limited habitats and endangered species (Khanum et al., 2013). The spread of invasive species undermines the efforts of biodiversity conservation, which is crucial to the continued functioning of our ecosystems (Sheldon & Creed, 1995; Vilà et al., 2007). Furthermore, the projected changes in future climates could help facilitate further spread of these invasive alien species through the expansion of suitable areas (Coetzee et al., 2007). In addition, non-invasive species may also become problematic in future, due to climate change.

The previous chapters investigated the role of biotic factors such as enemy release (Chapter 2 and 3) and competition (Chapter 4) in facilitating aquatic plants invasions. The current chapter explores the role of abiotic factors, specifically climatic factors. Making use of climate models and machine learning to predict how changes in temperature, humidity, and rainfall will influence the spatial distribution of invasive species over time should form the basis of any management or conservation plan. This is because these types of studies may be useful in helping to prioritise conservation efforts in areas that are identified as the most vulnerable to invasive species expansion (Kumar et al., 2014). Furthermore, biological control programmes also benefit immensely from these types of studies, as we can predict the ideal areas for use of a specific agent through climate matching (Sutton, 2019; Minuti et al., 2022).

One of the requirements for an effective biological control programme is ensuring climate suitability for the introduced agent. This is because the development times, survival, and reproductive rates of many insects are directly influenced by a variety of climatic factors, including temperature, humidity as well as precipitation (Smith et al., 2022). Therefore, biological control agent establishment, distribution and overall abundance will ultimately be determined by these climatic factors in the introduced range. Indeed, studies have shown that climate incompatibility between native and invaded range may lead to the failure of an otherwise good biological programme (Fisher et al., 2011; Harms et al., 2021).

Correlating known localities to a variety of bioclimatic data through machine learning allows researchers to predict areas with the suitable climatic envelope for the species at hand before even going out into the field (Sutton, 2019). Desktop studies such as species distribution modelling and ecological niche modelling provide a cost-effective way of assessing the suitability of a biological control agent while the project is still in the early stages. This is because the development of a valuable biological control programme can cost close to $ 5 000 000 (Paynter et al., 2015) due to the amount of fieldwork and manpower required in the early stages. Thus, incorporating species distribution models in such projects can help prioritise biological control agents which have a better potential of establishment in the invaded range.

There are many techniques to Species Distribution Modelling (SDM) and Ecological Niche Modelling (ENM). One such technique is the Maximum Entropy Species Distribution Modelling (MaxEnt) algorithm which has been used extensively to predict species distribution for presence-only data (Phillips et al., 2017). This modelling approach is usually preferred over others due to its ability to use both presence data as well as categorical variables (Çoban et al., 2020). MaxEnt modelling is also reliable for use in studies with a small sample size (limited presence points) (Phillips et al., 2017). For example, Hernandez et al. (2006) found that MaxEnt was the most capable method of modelling rare species distribution out of the four methods they tested, producing useful results even with sample sizes as small as 5 occurrences.

Freshwater plants, such as *Lagarosiphon major*, are highly sensitive to temperature change (Coetzee et al., 2007; Heino & Toivonen, 2009; Riis et al., 2012). This submerged aquatic plant is native to southern Africa and is invasive in many parts of the world, including Europe, New Zealand, the United Kingdom, and Ireland (Wells et al., 1997; Champion & Tanner, 2000; McGregor & Gourlay, 2002). *Lagarosiphon major* exhibits characteristics of an ecosystem engineer as it can modifying its immediate environment, thereby promoting its own dominance over other co-occurring macrophytes. This modification is primarily achieved through sediment trapping which results in broader changes in the biogeochemical conditions of the invaded environment (Caffrey & Acevedo in 2007). Consequently, within its invaded range, this species has the potential to diminish overall biodiversity and therefore ecosystem services, leaving behind monophyletic stands which further exacerbate sediment accumulation (Clayton, 1996).

Resource managers are therefore required to implement control measures to reduce these impacts. However, management of *L. major* using traditional means such as chemical and mechanical removal has proven to be challenging, as this plant can germinate from even small fragments leftover in the system (McGregor & Gourlay, 2002; Caffrey & Acevedo, 2007; Hussner et al., 2017). Because of this, biological control options have been explored. Previous studies identified two potential biological control agents, *Polypedilum tuburcinatum* and *Hydrellia lagarosiphon*, which cause considerable damage to *L. major* in South Africa (Baars et al., 2010; Deeming, 2012).

Plans are underway to get these two agents released in New Zealand and Ireland, as preliminary host specificity tests and risk assessment show limited risk of nontarget effects on the native flora of both regions (Baars et al., 2010; Earle et al. 2013; Deeming, 2012; Martin et al., 2013; Mangan & Baars, 2023). Nevertheless, novel climate in the introduced range will also play an important role in the establishment and efficacy of these biological control agents should permission for release be granted. This is an important consideration as climate mismatches are especially common in cases where the source population is being transferred from tropical to more temperate or subtropical regions (see review by Harms et al., 2021). In the case of *L. major* and its candidate agents however, both the plant and insects are restricted to high-lying areas of South Africa which tend to be more temperate (Baars et al., 2010; Martin et al., 2013), similar to New Zealand climate. Nevertheless, exploring how global warming in future climates might influence the suitable range for both *L. major* and its potential biological control agents is of major importance to the overall management of this species.

Besides the correlative modelling described above, another way to predict climate suitability is the use of degree day modelling. This provides a mechanistic approach to predicting climate suitability by making use of known biological data of the organism in question together with local climate data (Coetzee et al., 2007; Kearney et al., 2010). The end product is cumulative effect of temperature on reproduction above a specified threshold which is typically informed by the organism's life tables which arise from empirical observations and experimentation. For example, Smith et al. (2022) used this modelling approach to show that *Hydrellia egeriae* would be able to persist in all sites invaded by *Egeria densa* in South Africa.

Although a useful tool, degree-day modelling should be accompanied by estimates of reproductive success, because population persistence is not only linked to generation turnover, but also is directly dependent on net reproductive rate (Ro). Here, Ro with values less than 1 is an indication that populations might not be sustainable, while values greater than 1 are indicative of successful population persistence (Deevey, 1947). For example, using both methods, Mangan and Baars (2013) predicted that *H. lagarosiphon* was climatically suitable for release in most parts of Europe and Ireland invaded by *L. major*, as most investigated sites showed that populations of this agent would successfully persist in these regions.

Species distribution is expected to change under future climate scenarios. Thus, the aim of this chapter is to predict how changes in temperature, humidity, rainfall, and other bioclimatic variables will affect the distribution of *L. major* in New Zealand, Australia and Europe (invaded range) through the use of MaxEnt climate modelling. I also examined whether there are any projected mismatches between *L. major* and two of its natural enemies and potential biological control agents, *P. tuburcinatum* and *H. lagarosiphon* in New Zealand using correlative as well as mechanistic modelling. Lastly, this study will determine the bioclimatic envelope of *L. major* and its potential agents in both regions and reveal which climatic data best determines the observed distribution. These results will be instrumental in the management of *L. major* in the invaded range under future climates as understanding possible changes in the distribution of this species will be vital in the successful implementation of regulations and management plans.

5.2. Materials and methods

5.2.1. Species occurrence records

The accuracy of any species distribution model is directly tied to the quality of occurrence data used, and this is determined primarily by site accessibility as well as sampling effort (Minuti et al., 2022). Online databases are also reliant on effective data sharing, and as such they are susceptible to sampling bias reporting, leading to over or underrepresentation. This can be offset by effective data cleaning and selecting the correct parameters during modelling selection.

For the current study, worldwide occurrence records of *L. major* were downloaded from the Global Biodiversity Information Facility (GBIF) portal (https://www.gbif.org/) and supplemented with data obtained from field surveys carried out by the authors. After extensive data cleaning, removal of duplicate records, cell filtering (one record per 2.5 arc min resolution) and spatial thinning, the final dataset had 803 global occurrence records (Figure 5. 1). The global species distribution of *L. major* was modelled using the cleaned total occurrence data set and then cropped to the regions of interest for further analysis and evaluation.

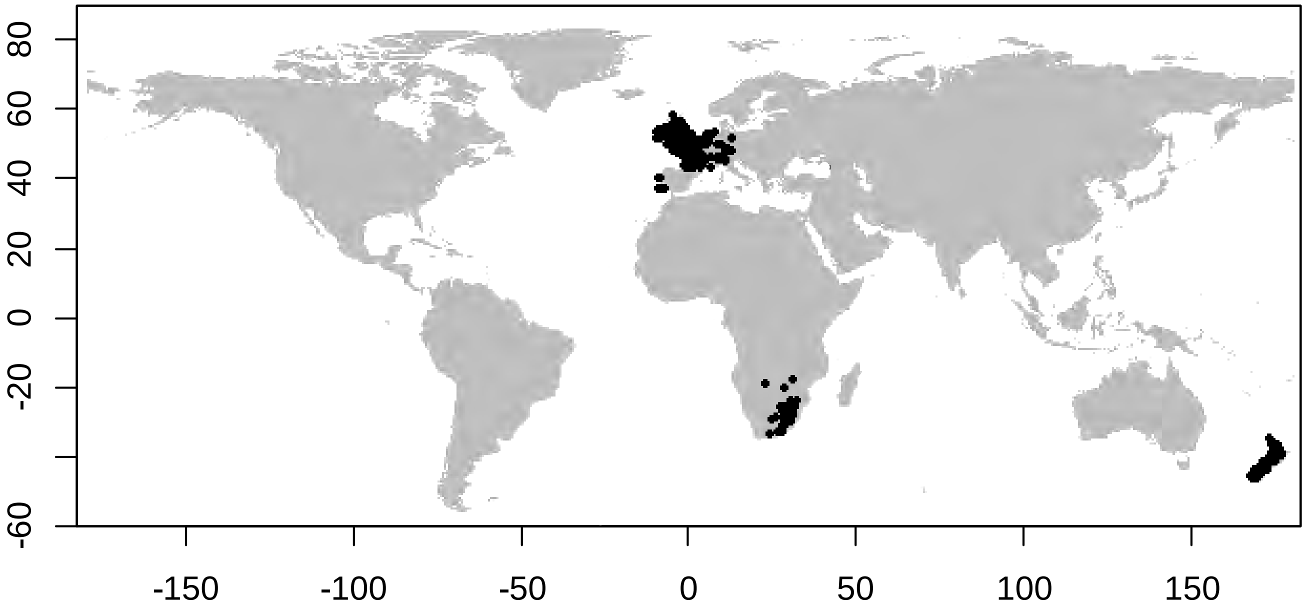


Figure 5.1: Worldwide distribution of *Lagarosiphon major*, with 4181 uncleaned occurrence records. Data cleaning removed 2529 occurrence localities that shared the same grid cell, 6 occurrence points with NA predictor variable values, and spatial thinning removed 1720 records, resulting in 803 remaining occurrence points for modelling.

5.2.2. Environmental variables

Predictive environmental variables for both current and future climate were sourced from the WorldClim database 2.1 (https://www.worldclim.org/data/worldclim21.html) at a special resolution of 2.5 arc minutes (~5 km). This provides a set of 19 bioclimatic variables which are derived from measures of rainfall, temperature, and altitude from weather stations all around the world, indicative of annual, quarterly, and monthly means, as well as extreme ranges (Fick & Hijmans,2017). The available data also gives future climate projections from the IPPC5 Global Climate Models (GCM).

In order to predict the future distribution of *L. major* and its potential two biological control agents, spatially downscaled bioclimatic variables were extracted from the WorldClim database using RStudio (v2022.07.1+554). Using the getData command from the raster package, I downloaded atmosphere-only GCM (ACCESS1.0) projections following the Coupled Model Intercomparison Projects 5 (CMIP5) protocol and using the 8.5 Representative Concentration Pathway (RCP) of greenhouse gas for the year 2080.

The WorldClim database has been used extensively in previous studies to model species distribution for a variety of organisms (Khanum et al., 2013; Khosa et al., 2019; Sutton, 2019; Minuti et al., 2022). Due to the unavailability of accurate water quality data relevant to species distribution modelling in freshwater systems, atmospheric variables such as those offered by this database are usually used as proxies (Khosa et al., 2019; Minuti et al., 2022). These variables are considered effective predictors of macrophyte niche distribution and have been used in similar studies (Coetzee et al., 2007; Hoveka et al., 2016).

The model outcome of species distribution modelling is determined by the predictive environmental layers used, and the most relevant layers depend on the species under investigation. Furthermore, including highly correlated predictor variables may lead to model overfitting and overall poor model optimisation (Kass et al., 2021). Therefore, only uncorrelated bioclimatic variables (correlation coefficient < 0.75) which were predetermined using vifcor (usdm package in RStudio) were used to train the model for current *L. major* distribution (Table 5.1).

Table 5.1: Bioclimatic envelope of *Lagarosiphon major* based on occurrence data. N= 574 for global and N=74 for SA

Variable	**Global mean**	**Native mean**
Mean diurnal range in temp (°C, Bio 2)	9.68±2.02	14.26±1.36
Mean temperature of wettest quarter (°C, Bio 8)	10.34±4.16	19.6±2.1
Mean temperature of driest quarter (°C, Bio 9)	14.12±4.58	10.33±2.46
Precipitation of driest month (mm, Bio 14)	71.23±44.89	10.51±5.48
Precipitation seasonality (CV) (mm, Bio 15)	23.27±18.15	66.05±12.84
Precipitation of warmest quarter (mm, Bio 18)	294.5±135.79	337.47±86.02

In order to determine and visualise niche overlap between the distribution of *L. major* in the native (SA) as well as invaded ranges, a Principal Component Analysis (PCA) was run in RStudio. To do this, the raster package was used to extract bioclimatic data for each occurrence point, and this was then used to produce a niche overlap summary using the first two dimensions of the PCA.

5.2.3. Species distribution modelling

Current climate suitability and projected changes in habitat suitability under future climates were modelled using the maximum entropy algorithm. This was implemented using the ENMeval package (v2.03) in RStudio using manxent.jar (v3.4.3) from the dismo package (v1.3.8). This package offers an automated way of selecting the best model settings to use when running MaxEnt (Kass et al., 2021). This is an important step in SDM as model complexity may lead to overfitting when the default settings are used. The automation of model tuning also allows for transferability and reproducibility of results, which is always an important consideration in any scientific study. The ENMeval package has gained popularity in ecological niche modelling due to its ease of use (see review by Kass et al.,2021).

Spatial partitioning and cross-validation using the block method also reduced the effect of sampling bias and ensured increased special extrapolation to novel conditions in the case of *L. major*. The jackknife partitioning method was more appropriate for the two biological control agents, due to the small sample size (*H. lagarosiphon* = 42, *P. tuburcinatum* = 27). Pseudo-absence points necessary for running the presence-only MaxEnt algorithm were randomly generated from the species-defined background within ENMevaluate during model evaluation and selection. The selection of these pseudo-absences has a significant influence on the resultant projections (Phillips et al., 2017; Kass et al., 2021), thus the random.seed command in RStudio was used in order to get the same random points each time and ensure duplication.

Model settings from the ENMeval output which best minimised the Akaike Information Criterion corrected for small samples sizes (AICc) were used to run the suitability models. The resultant models from this also gave the maximum Area Under the Curve (AUC) scores (Table 5.2), a metric used to evaluate model performance, where a value of 0.5 indicates a model that is not different from a randomly generated one, and 0.75 is indicative of an acceptable model, while values closer to 1 indicate high model performance.

The final products from these models were set to produce cloglog transformed MaxEnt output maps which predict the probability of species occurrences. These maps were also converted into binary maps of presence or absence using 10 Percentile Training Presence threshold, in order to make predictions about actual changes in km^2 and percentages. In order to determine climate compatibility for the two biological control agents in New Zealand, the resultant models which were trained with South African presence data was projected onto New Zealand for both current and future climates.

Table 5.2: Best MaxEnt model settings with maximum AUC from the ENMeval package.

Species and area	Feature class	RM	AUC	CBI	10p
Global *L. major*	LQH	1	0.962	0.927	0.277
H. lagarosiphon	LQ	2	0.868	0.763	0.158
P. tuburcinatum	LQ	2	0.871	0.867	0.192

5.2.4. Degree-day modelling and net reproduction

Hydrellia lagarosiphon development data from Mangan & Baars (2013) as well as daily minimum and maximum temperatures from local weather stations around invaded areas in New Zealand were used to predict population persistence of *H. lagarosiphon* in those regions. The weather data was sourced from the Cliflo database (https://cliflo.niwa.co.nz/) which provides access to a variety of climate data from more than 600 stations. Temperature data from the past 10 years (2012-2022) was requested from 18 weather stations across New Zealand and used in the following equation 5.1 to estimate the number of generations this potential agent would be able to complete at the specified sites based on mean accumulated degree-days:

Equation 5.1: $K=\sum(Tmax+Tmin)/2-t$ (where $Tmin < t$, t was used)

Here, *Tmax* and *Tmin* represent mean daily minimum and maximum temperatures at each site, and *t* represents the lower development threshold which was determined to be 7.5 °C for *H. lagarosiphon* by Mangan and Baars (2013). Furthermore, the study also determined Ro to be estimated by the following equation: $R_o = -10.5507 + 01.1995\chi + 0.0035\chi^2$, where χ represents the daily average temperature per site. Using the reduced major axis regression equation (Ikemoto & Takai, 2000), they estimated that *H. lagarosiphon* requires 571-degree days above their development threshold of 7.5 °C (Mangan & Baars., 2013). For the current chapter, χ was substituted by relevant temperatures for New Zealand, and the resultant values were averaged over the entire year to give net reproductive rate per year at each site. As noted previously, a value less than 1 was taken as an indication that population persistence would not be possible. All data analyses and visualizations were done in the R environment (R Core Team, 2023).

5.3. Results

The high AUC and CBI scores (0.962 and 0.927 respectively) revealed that the current *L. major* global distribution was predicted very well by the MaxEnt models settings selected from ENMeval compared to random (AUC= 0.5) (Table 5.2). The PCA showed a separation in the distribution of *L. major* in environmental space across the three different regions, with a slight overlap between the native range of South Africa and Europe, as well as a complete separation between South Africa and New Zealand (Figure 5.2).

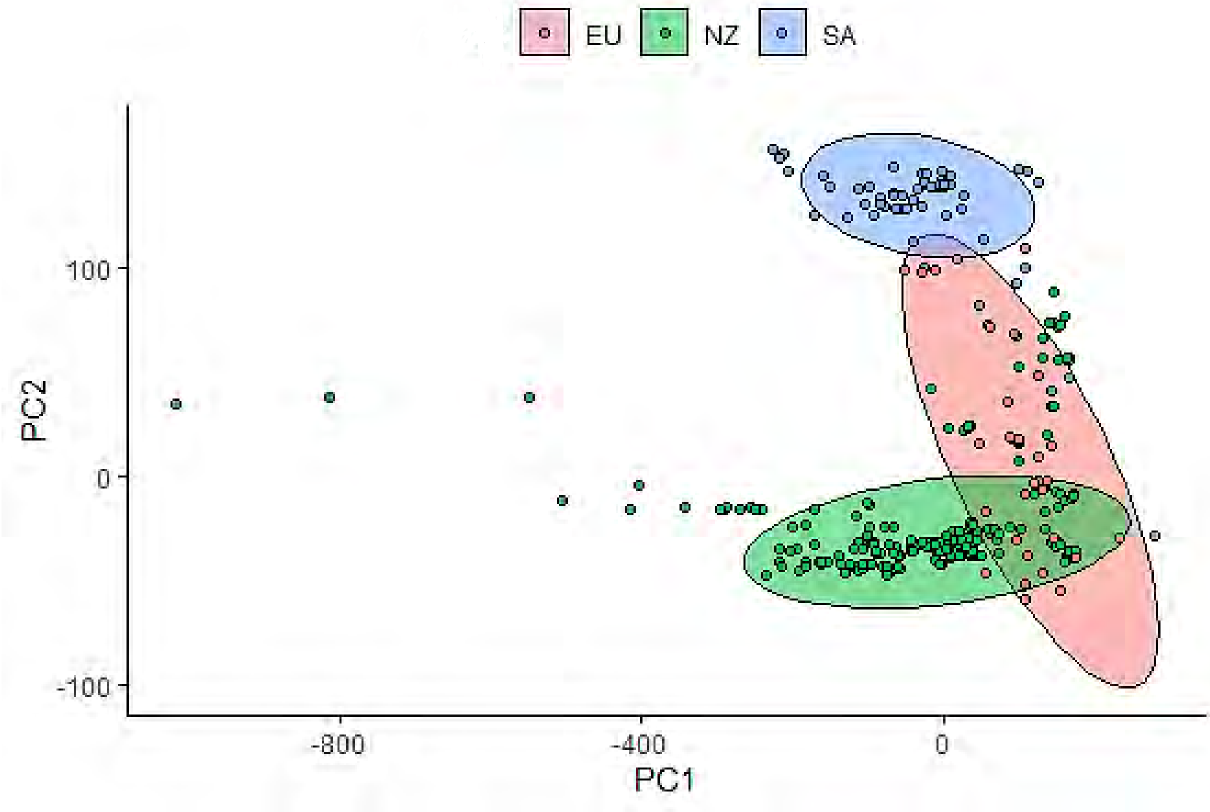
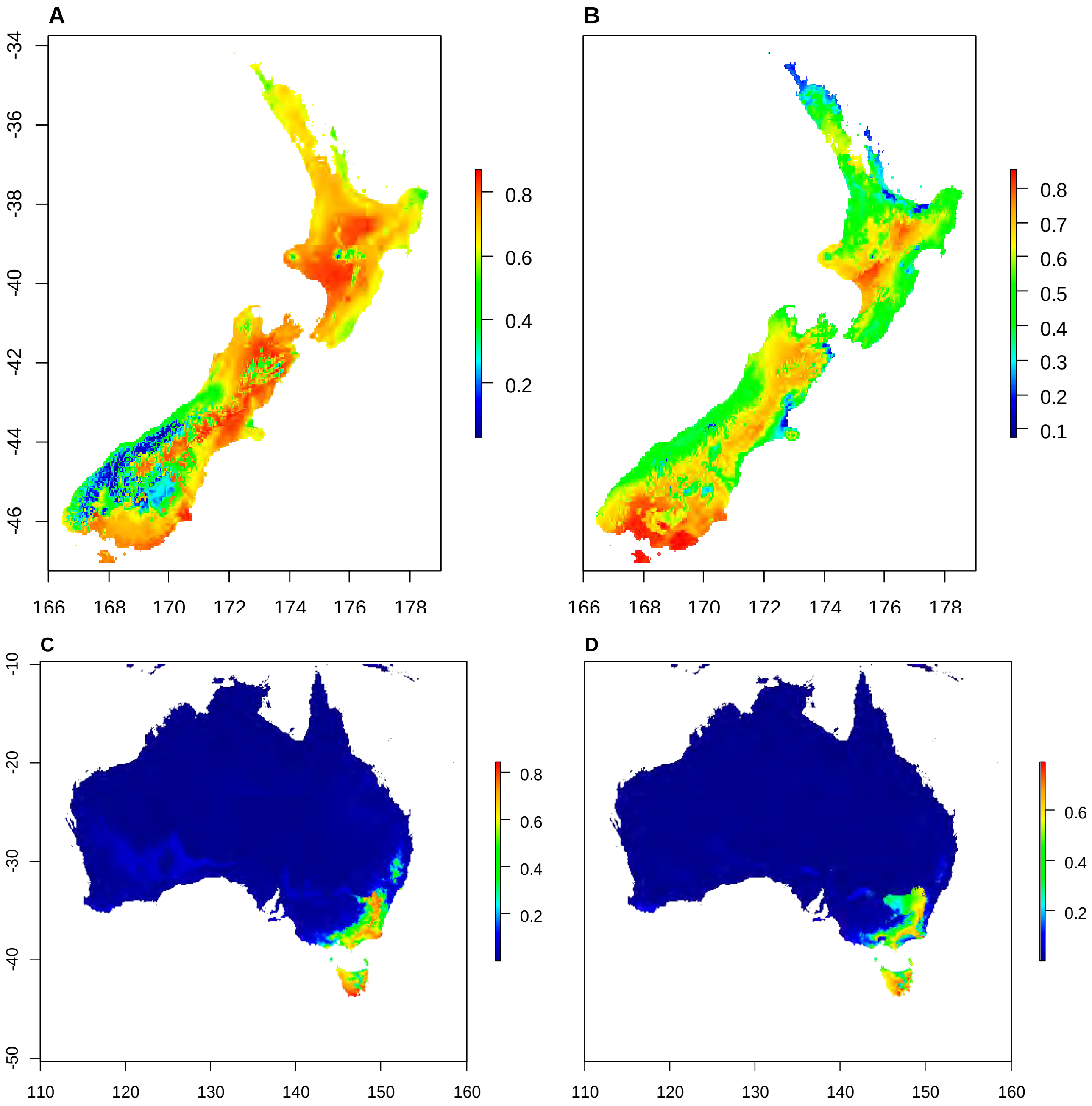


Figure 3.2: Principal Component Analysis for *Lagarosiphon major* occurrence records as separated by bioclimatic variables compressed into 2 dimensions (PC1= 78%, PC2= 2%. Ellipses indicate the 95% confidence interval.

5.3.1. Lagarosiphon major ecological niche modelling

Current and future suitability maps for *L. major* in the invaded range are presented in Figure 5.3, with warmer colours indicating areas of high predicted climate suitability (red=high probability of suitability, green= typical *L. major* climate envelop, blue=probably unsuitable). For New Zealand, the continuous probability maps show that the majority of the North Island is currently highly suitable for *L. major* growth, and future climates are only going to slightly reduce suitability. In the South Island, the maps show a range shift in highly suitable areas from a more even distribution in current climates to a more southern shift (Figure 5.3A-B). For Australia, *L. major* suitability is currently restricted to New South Wales, Victoria, and Tasmania. According to future climate projections, the suitability of *L. major* will not change much for this region (Figure 5.3C-D). Climate suitability for *L. major* in Europe is currently mainly restricted to the western region, with very high projected suitability in France, Ireland and the United Kingdom (Figure 5.3E). Results from future climate modelling indicate a shift toward Eastern Europe (Figure 5.3F).



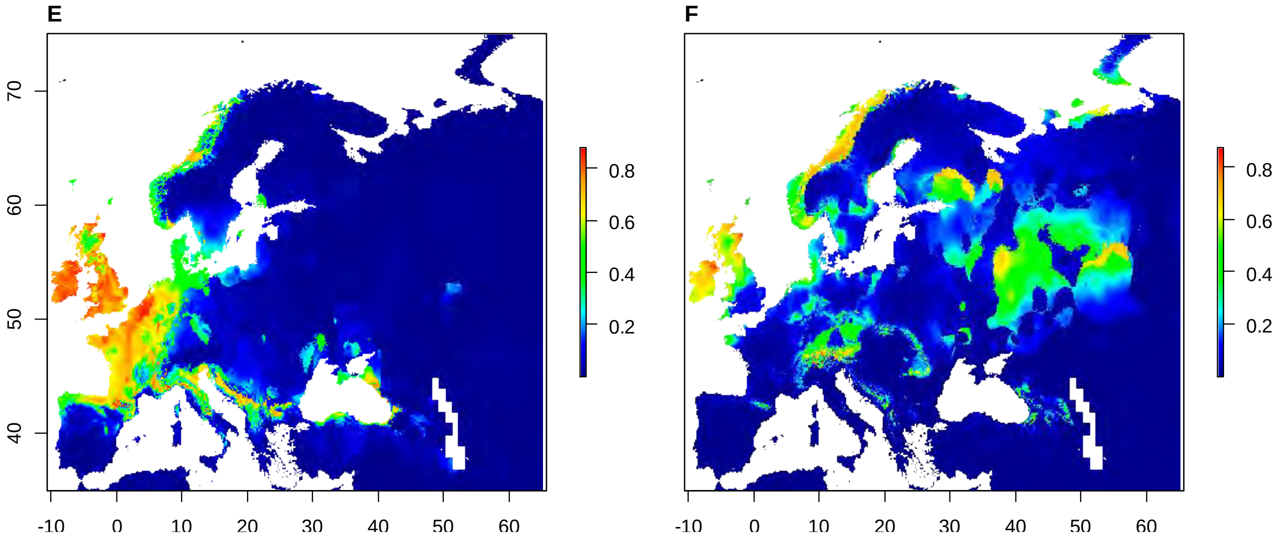


Figure 5.3: MaxEnt ecological niche modelling for *Lagarosiphon major*. Future predictions (2080) were modelled using climate scenario RCP 8.5. Warmer colours indicate higher predicted *L. major* suitability. (A) Currently suitable area in New Zealand, (B) Future climate suitability in New Zealand, (C) Current suitability in Australia, (D) Future climate suitability in Australia, (E) Current suitability in Europe and (F) Future climate suitability in Europe.

To make inferences about the actual spatial extent of *L. major* suitability in km^2, the continuous probability of suitability maps were converted to binary maps using the 10 Percentile Training Presence Threshold. According to these maps, the currently suitable area for *L. major* growth in New Zealand is 263119 km^2 (indicated by green), approximately 93% of the total available area (Figure 5.4A, Table 5.3). This is projected to increase slightly to about 272830 km^2 (3.7% increase indicated by red) under future climates (Figure 5.4A, Table 5.3).

In Australia, the current suitable niche for *L. major* invasion is predicted to be around 260533 km^2 (indicated by green in Figure 5.4B, Table 5.3), representing only about 3.3% of the country. While a 12% range reduction to 229623 km^2 is expected in the future (indicated by red, Figure 5.4B, Table 5.3). In Europe, the currently suitable area for *L. major* invasion is approximately 12% (1736495 km^2), and this is projected to decrease by around 4% to 1663846 km^2 under future climate scenarios (Figure 5.4, Table 5.3). There is also an overlap between current and future suitability, as well as a range expansion and shift towards the east.

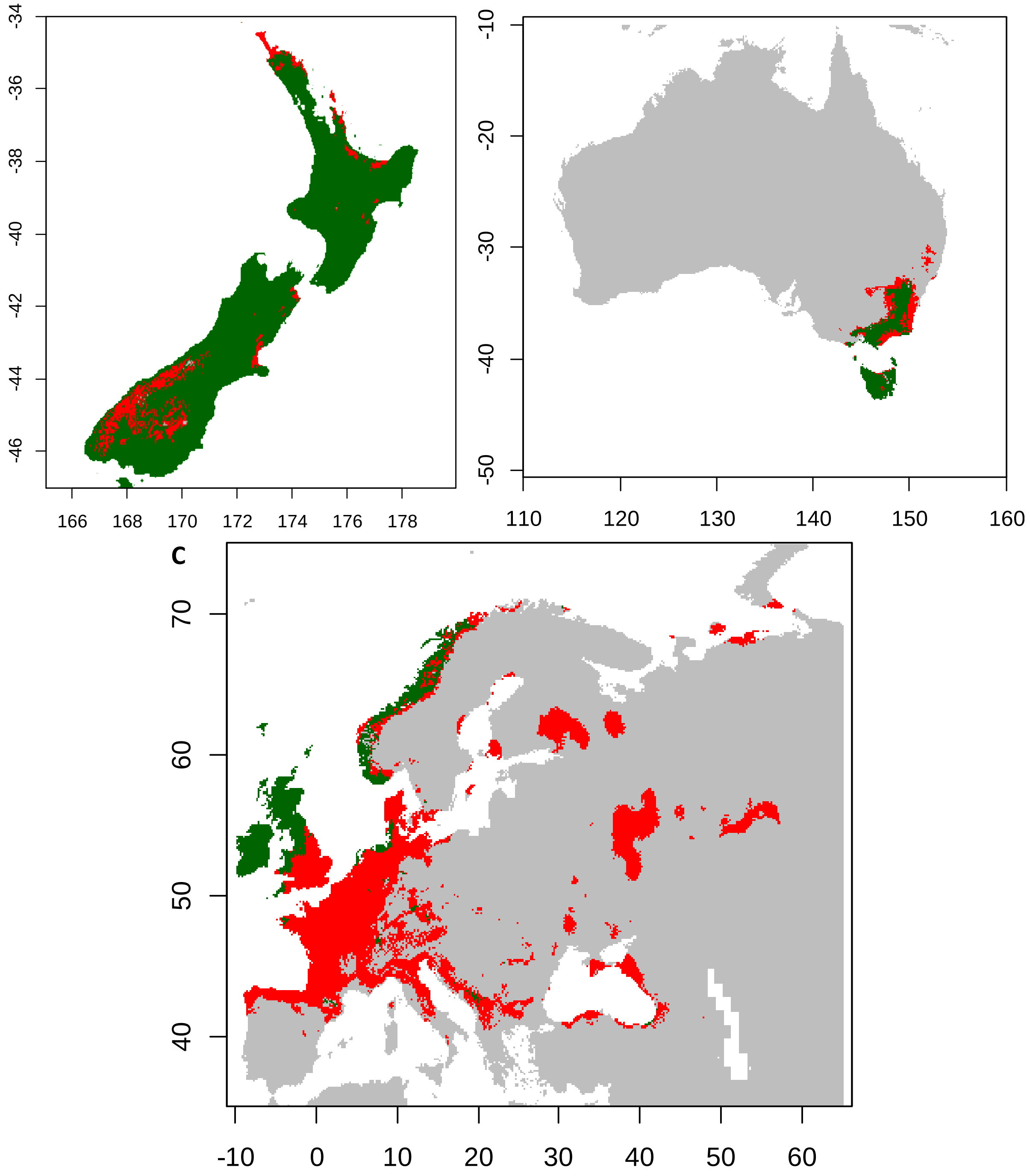


Figure 5.4: Binary maps of *Lagarosiphon major* climate suitability change for (A) New Zealand, (B) Australia and (C) Europe. Grey= not suitable, green= currently suitable, and red= future suitability.

Table 5. 1: Current and future raster area (km^2) suitability.

Species and area	Total (km^2)	Current (km^2)	Future (km^2)	Change
L. major New Zealand	282886.4	263119	272830	+3.7 %
L. major Australia	7786129	260533	229623	-12%
L. major Europe	14177694	1736495	1663846	-4%
H. lagarosiphon	282886.4	31009	25839	-17%
P. tuburcinatum	282886.4	26502	24377	-8%

The most important bioclimatic contributors to the predicted global suitability of *L. major* were Precipitation Seasonality (Bio 15), Precipitation of Driest Month (Bio 14), Mean Temperature of Driest Quarter (Bio 9) and Mean Temperature of Wettest Quarter (Bio 8), together contributing more than 85% of the model information (Figure 5.5, Table 5.1). Changes in Bio 15 led to a marked reduction in the projected *L. major* suitability, while Bio 14, Bio 9 and Bio 8 showed relationships that increase *L. major* suitability up to a certain threshold (Figure 5.6, Table 5.1).

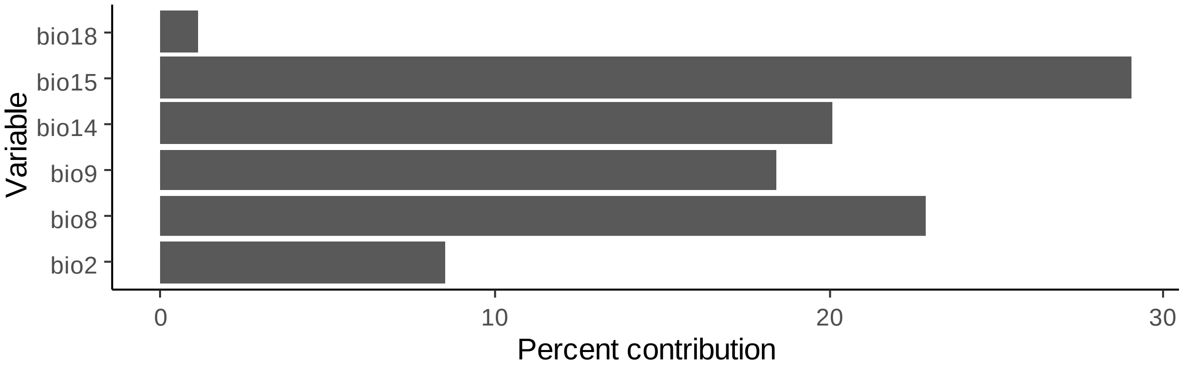


Figure 5.5: Percentage contribution of each variable to the current model prediction for global climate suitability of *Lagarosiphon major*.

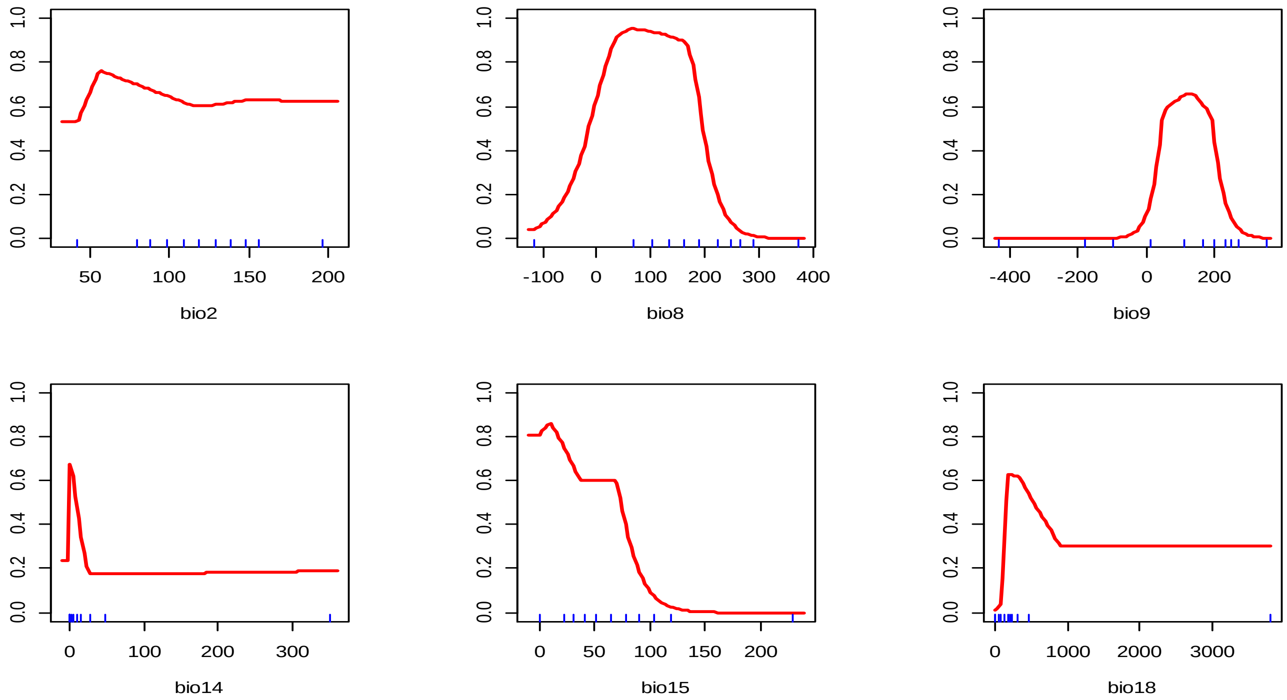


Figure 5.6: Bioclimatic variable response curves for current *Lagarosiphon major* global suitability model.

5.3.2. Ecological niche modelling for two potential biological control agents

In order to predict the area of suitability for two potential biological control agents in New Zealand, their current suitable range was modelled in South Africa and then projected onto New Zealand’s climatic layers. The resultant MaxEnt models from the ENMeval package tuning were considered trustworthy for extrapolation onto novel conditions as they had high AUC scores (0.868 for *H. lagarosiphon*, and 0.871 for *P. tuburcinatum*) (Table 5.2).

Current and future suitability maps for *H. lagarosiphon* and *P. tuburcinatum* in New Zealand (*L. major* invaded range) are presented below (Figure 5.7A-D respectively), with warmer colours indicating areas of high predicted climate suitability. Climate suitability for both *H. lagarosiphon,* and *P. tuburcinatum* is currently restricted to the South Island, and future climate projections also show the same results. However, there is also an apparent range suitability reduction for *H. lagarosiphon*, and a slight shift for *P. tuburcinatum* under future climates (Figure 5.7).

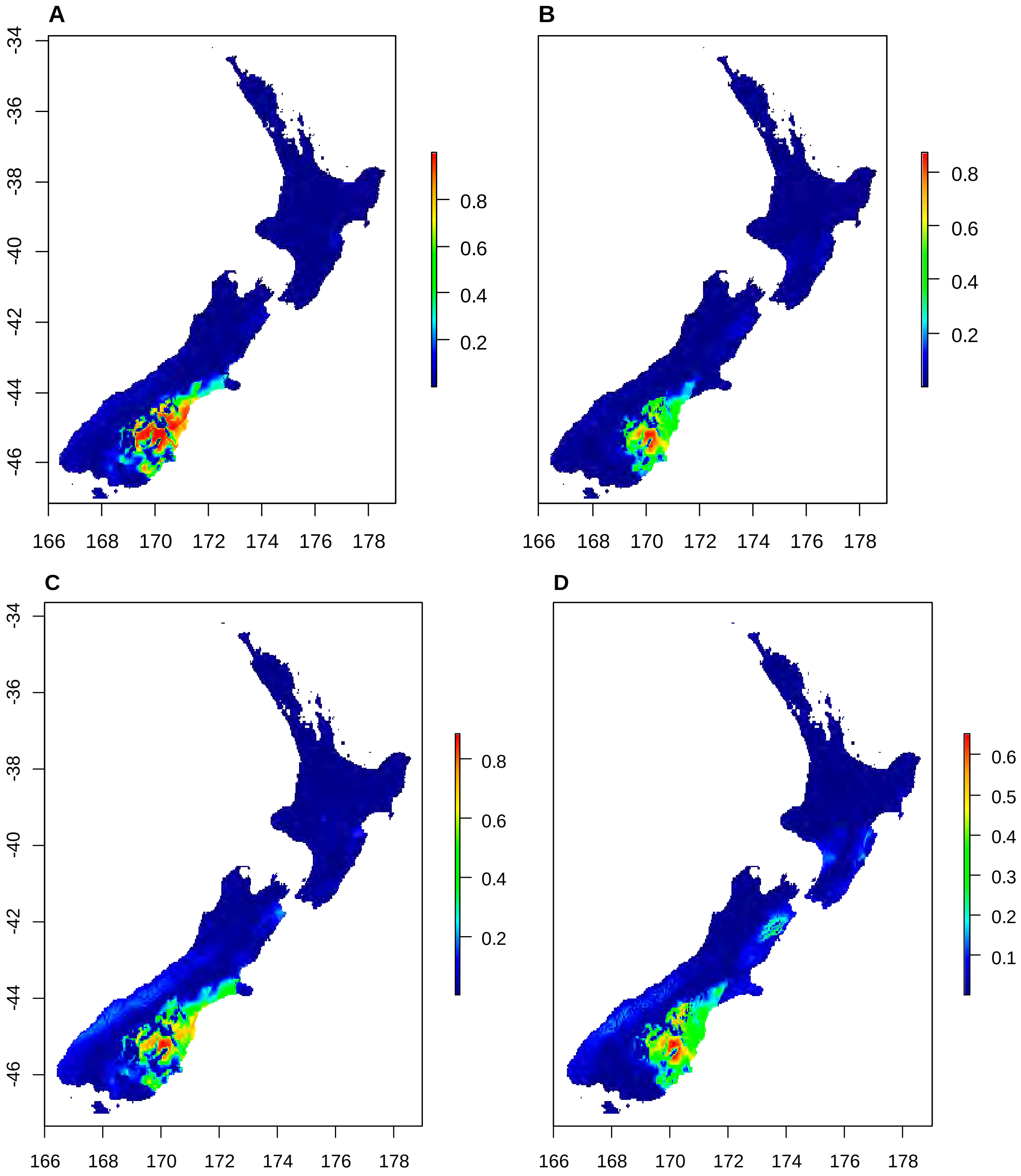


Figure 5.7: MaxEnt ecological niche modelling for *Hydrellia lagarosiphon,* and *Polypedillum tuburcinatum* in New Zealand (invaded range) for current and future climate conditions. Models were trained using South African occurrence data, where future predictions (2080) were modelled using climate scenario RCP 8.5. Warmer colours indicate higher predicted suitability. (A) Currently suitable area for *H. lagarosiphon;* (B) Future climate suitability for *H. lagarosiphon;* (C) Current *P. tuburcinatum* suitability; (D) Future climate suitability for *P. tuburcinatum*.

The binary maps for *H. lagarosiphon* in New Zealand show a suitable area of around 31009 km^2 (11%) under the current climate conditions (indicated by green in Figure 5.8A, Table 5.3), while future climates are projected to cause habitat suitability loss of around 17%, resulting in 25839 km^2 suitable range (indicated by red, Figure 5.8B, Table 5.3). For *P. tuburcinatum* under current conditions, the total area of suitability in New Zealand was modelled to be around 26502 km^2, approximately 9% of the total area (indicated by green, Figure 5.8B, Table 5.3). This species is also projected to experience a range reduction to about 24377 km^2 (~8% loss) under the RCP 8.5 climate scenario by 2080 (indicated by red, Figure 5.8B, Table 5.3).

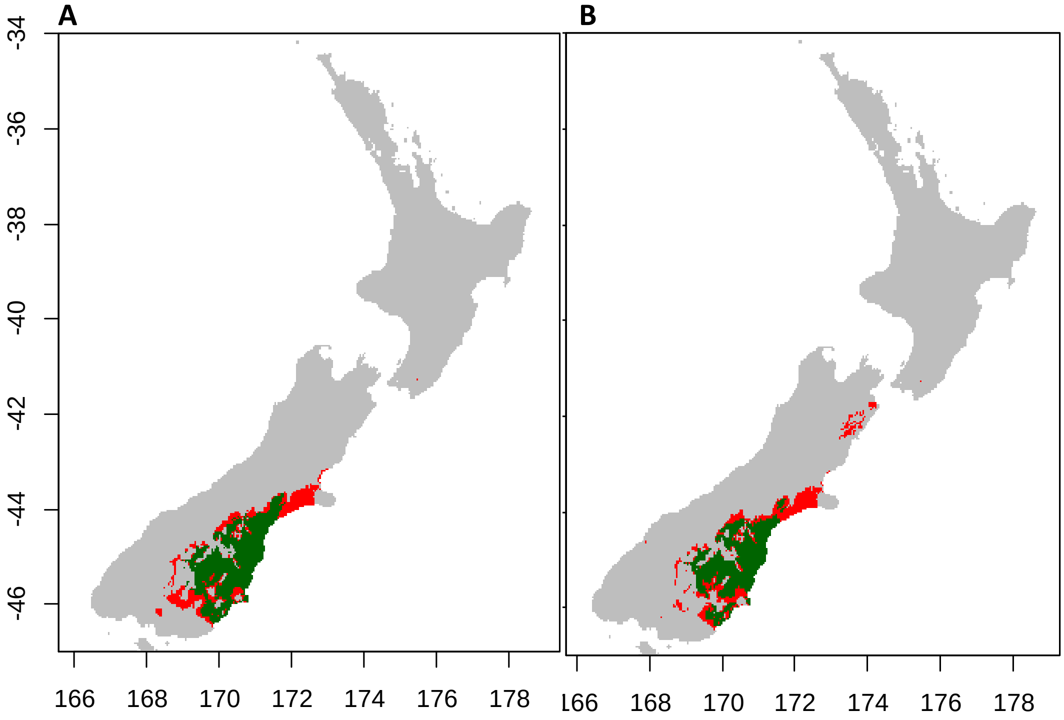


Figure 5.8: Binary map of climate suitability changes for (A) *Hydrellia lagarosiphon* and (B) *Polypedilum tuburcinatum*. Grey= not suitable, green= currently suitable, and red= future suitability.

Unlike *L. major*, modelled climate suitability for the two potential biological control agents was highly influenced by changes in Precipitation of Warmest Quarter (Bio 18), where agent suitability increased as this bioclimatic variable increased, contributing more than 40% to the *H. lagarosiphon* model predictions, and close to 60% for *P. tuburcinatum* (Figure 5.9A-B respectively). Other important variables were Mean Temperature of Driest Quarter (Bio 9) and Mean Temperature of Wettest Quarter (Bio 8), with a combined contribution of ~50% for *H. lagarosiphon*, and ~35% for *P. tuburcinatum* (Figure 5.9A-B).

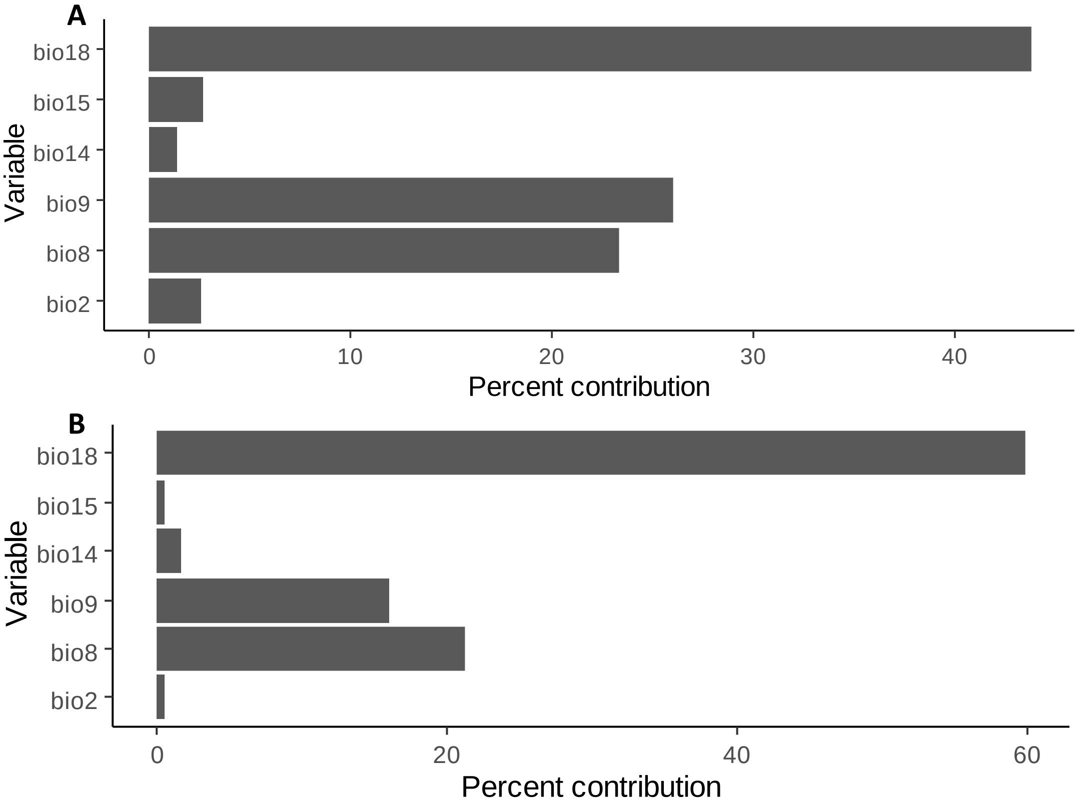


Figure 5.9: Percentage contribution of each variable to the current model prediction for climate suitability of (A) *Hydrellia lagarosiphon* and (B) *Polypedilum tuburcinatum* in South Africa.

5.3.3. Degree-day modelling of *Hydrellia lagarosiphon*

According to degree-day modelling, *H. lagarosiphon* was predicted to produce between 4.5 and 9.3 generations per year in New Zealand, depending on temperature variations between sites (Figure 5.10A). The lowest generation turnover was at the lower parts of the Southland region in the South Island, while the highest turnover was predicted in the Northland region of the North Island (Figure 5.10A). A similar pattern was observed with regards to net reproductive rate (Ro). Here, Ro ranged between 2.9 and 10.5, with no sites having a value less than 1 (Figure 5.10B).

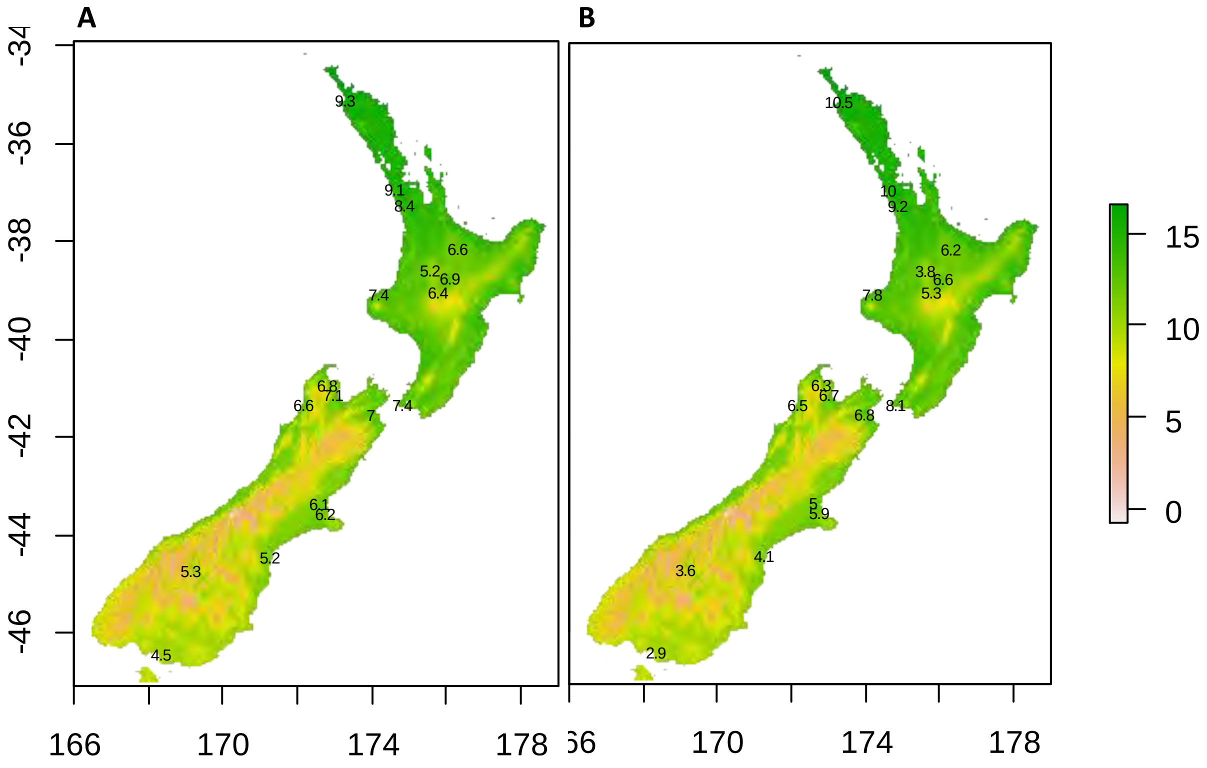


Figure 5. 10: (A) Number of *Hydrellia lagarosiphon* generations per year and (B) Net reproductive rate (Ro) per year estimated from Mangan & Baars (2013) reduced major axis regression model and New Zealand temperature data for the past 10 years (2012-2020).

5.4. Discussion

Climate compatibility is one of the primary requirements which determines the geographic distribution of most plants and animal species (Fisher et al., 2011; Khosa et al., 2019). Thus, species distribution models are an important part of natural resource management and conservation because they offer valuable information about the habitat suitability of both problematic invaders and endangered species (Coetzee et al., 2007; Khanum et al., 2013; Sutton, 2019). The current study modelled current and future *L. major* climate suitability. Model predictions were good, with high AUC values, and are transferable to areas outside the native range because model training was done using the global occurrence data.

The models revealed that the majority of New Zealand (93%) is suitable for *L. major* growth, and climate change will further increase suitability. This is an expected prediction because New Zealand generally has a more temperate climate profile, with clear oligotrophic freshwater systems which are ideal for *L. major* growth (Caffrey & Acevedo, 2007). This plant is known to prefer cooler regions in its native range (Clayton, 1996; Martin et al., 2013). As such, there are many localities in New Zealand where *L. major* forms large densa mats that negatively impact those ecosystems, thus reducing overall functioning and ecosystem services (Caffrey & Acevedo, 2007). The projected increase in suitability is concerning as it indicates that *L. major* is not currently at its invasion equilibrium and may continue to invade and cause problems in even more localities.

On the other hand, model predictions for *L. major* distribution in Australia only showed a 3% area of suitability, and future climate conditions are expected to reduce suitability even further. The most southern parts of New South Wales, Victoria and Tasmania are suitable for invasion by this plant. These are mostly temperate to cool temperate regions, with only mild seasonality. There are currently no naturalized populations of *L. major* in Australia, and previous infestations were successfully eradicated (CHAH, 2020). This absence of problematic infestations might be due to differences in climatic requirements, or other barriers of entry. Indeed, *L. major* is listed among prohibited species in Australia (CHAH, 2020).

In Europe, a relatively small area (~12%) was projected to be suitable for *L. major* invasion, and this was mainly in the western region, including Ireland and the United Kingdom. This is in line with the current known localities of *L. major* in this region. Future climates are projected to result in a range reduction of about 4% and a shift towards Eastern Europe, where warming is projected to increase the suitability of previously inhabitable areas.

The marked separation in climatic envelope between South Africa and New Zealand evident in this study could be interpreted as an adaptation to a wide variety of environmental conditions (Rodríguez-Merino et al., 2019), as this plant has successfully invaded many parts New Zealand despite this supposed climate mismatch. According to Gallagher et al. (2010) climatic niche separation between native and invaded range is not uncommon as the invader population usually has the added advantage of release from top-down constraints (Mooney & Cleland, 2001; also see Chapter 2).

Correlative model predictions for both current and future climate scenarios show agent suitability to be restricted only to a relatively small portion of the South Island in New Zealand, and climate change is projected to further reduce suitability for both *H. lagarosiphon* and *P. tuburcinatum*. This might be due to a lack of understanding of real distributions in the native range, as observations are mostly reported in areas that are easy to access (Sutton et al., 2019; Minuti et al.,2022). Therefore, these models should not be interpreted as indicative of the actual limits of these species. Rather, these results show areas where agent populations have a higher chance of establishment due to climate matching with the place of origin (Sutton et al., 2019; Minuti et al.,2022), and they could expand beyond the MaxEnt projected area.

Baars et al. (2010) showed that *H. lagarosiphon* is more widely distributed in the native range, compared to *P. tuburcinatum* which also occupies a relatively large climatic envelope. Climate suitability of the two potential biological control agents was mainly determined by Precipitation of warmest quarter (bio-18), whereas *L. major* suitability was mainly dependent on Precipitation seasonality (bio-15). This means that the climate suitability for *L. major* invasion is determined by a different set of climatic factors compared to those of its potential agents. Therefore, it is possible that a larger range of suitable climate in New Zealand could also translate into more sites which are suitable for biological control establishment as insect survival depend on host plant availability (Sutton et al., 2019).

Indeed, mechanistic modelling informed by insect biology from Mangan and Baars (2013), as well as this study, showed that *H. lagarosiphon* would be able to complete at least two generations in Ireland, and even more in New Zealand. However, due to various limitations, it is still not established whether *P. tuburcinatum* will follow a similar pattern to *H. lagarosiphon* in the invaded areas as it does in the native range. Implications of micro-climate also form an important determining factor of realised establishment and distribution success as macrophyte weed beds tend to have higher minimum temperatures relative to the surrounding open waters (Wheeler & Center, 2001; Mangan, 2012; Smith et al., 2019). Therefore, beyond these two desktop modelling approaches discussed here, ground-truthing studies are still a fundamental part of any successful management strategy as they can be used to help build adaptive strategies in order to maximise biological control efforts (Muskett et al. 2020; Smith et al., 2022).

This chapter used various tools to estimate the distribution of *L. major* and its potential biological control agents, with a particular focus on *H. lagarosiphon* and New Zealand invaded sites. MaxEnt modelling showed varied response to future climates according to region, with New Zealand increasing in suitability, while Australia and Europe are projected to see a further reduction in *L. major* suitable habitat. These results however should be analysed with caution as they are only based on atmospheric data, with no available comprehensive layers for freshwater systems (Khosa et al., 2019). Furthermore, the influence of background selection and model parameters should also be considered in this regard, as results are highly dependent on model tuning (Phillips et al., 2017; Kass et al., 2021). However, these climate models still offer valuable information about the current and future suitability of the different modelled regions for *L. major* invasion, and this can be used to simplify monitoring efforts by focusing resources on those areas that have predicted higher suitability. And although forecasted MaxEnt suitability is limited for both *H. lagarosiphon,* and *P. tuburcinatum*, mechanistic modelling showed that *H. lagarosiphon* would be able to sustain viable populations throughout New Zealand. Although there is still much work still needed, especially for *P. tuburcinatum*, it is generally expected that its realised distribution would be greater than the MaxEnt predictions. Therefore, if approved for release in New Zealand, these two agents could help reduce the impact of this weed in the invaded sites.

Chapter 6: General discussion

Biological invasions are driven by a variety of factors in the newly invaded ecosystem, and understanding these drivers is essential for successful management. The conceptual framework by Lacoul & Freedman (2006) explored different variables affecting the distribution and abundance of aquatic plants across different spatial scales (regional, catchment, and local) and environmental complexes (biogeography, climate, and geomorphology), and directly relates to the multi-faceted approach and scale considerations within the current study.

According to this framework, the scale of analysis can range from very broad regional factors such as continental geography, biome specific distributions (e.g., tropical and temperate regions) as well as environmental conditions such as overall regional water quality (e.g., acidity, clarity, and salinity). Furthermore, the medium scale may explore hydrological effects, such as watersheds or whole bodies of water within the catchment level, as well as the general physiological conditions including nutrient status, pH, and turbidity. And lastly, the smaller scale may delve into specific habitats and communities, considering factors like competition, herbivory, and sediment attributes (Lacoul & Freedman., 2006).

Biotic and abiotic drivers are directly linked across the different scales (Lacoul & Freedman., 2006), and understanding how these levels interact is a key step in implementing an effective management plan. Thus, following this framework, the overall objective of this thesis was to explore some of those mechanisms in the context of invasive macrophytes, and more specifically *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae). The second objective was to contribute to the growing body of literature which investigates the potential use of biological control agents *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae) and *Polypedilum tuburcinatum* (Diptera: Chironomidae) to control *L. major* invasions (Baars et al., 2010; Martin et al., 2013; Mangan & Baars 2013; 2023).

To achieve these objectives, the thesis adopted a multidisciplinary approach that combined biogeographical, meta-analytic, experimental, and modelling methods in order to reflect and capture the multifaceted nature of these ecosystems (Lacoul & Freedman., 2006). The following interprets the main findings and contributions of each chapter, as well as their implications for management of *L. major*. The last section discusses further potential for future research according to gaps identified but not fulfilled by the current study.

Chapter 1 provided a general introduction and literature review on the biological invasion dynamics of *L. major* and related aquatic plants, with a particular focus on the Enemy Release Hypothesis (ERH) as the main theme of this thesis. The chapter highlighted the history, causes, impacts, and management strategies of aquatic plant invasions, as a brief introduction to some of the key concepts and hypotheses in invasion ecology. The chapter also identified some of the knowledge gaps and research questions that motivated the subsequent chapters of the thesis.

6.1. Biotic drivers of plant invasion

Firstly, to help build on the theoretical understanding of macrophyte invasion dynamics, this thesis presented a meta-analysis and qualitative systematic review of ERH in aquatic plants. The chapter synthesized the evidence for ERH in aquatic plants based on 24 out of more than 200 studies screened as per predetermined criteria. This review included studies of various methodologies, including biogeographical comparisons, observational studies, as well as manipulative experimentation using mesocosms. This was a valuable exercise as it allowed for the exploration and incorporation of the multiple levels and factors identified by Lacoul & Freedman (2006).

The advantages and disadvantages of each method were also highlighted in Chapter 2 as discussed by Keane & Crawley (2002), as well as Fleming & Dibble, (2015). Similar to previous investigations (Zangerl & Rutledge, 1996; Colautti et al., 2004; Redekop et al., 2018), the results were highly variable depending on specific modulators, such as species under investigation, type of methodology employed, plant parameter measurements recorded, as well as plant growth form. Therefore, the overall synthesis provided no absolute conclusions when studies were clustered as there was no real evidence for, or against the ERH due to high variability.

Evidence was also limited even after the analyses were broken down according to the various modulating factors identified. This further shows complexity in patterns of interactions in biotic and abiotic factors, which cannot be solely explained by the ERH (Lacoul & Freedman., 2006; Fleming & Dibble, 2015; Bolpagni, 2021). However, despite the high variability observed, there was general applicability of the hypothesis in case of disturbance in manipulative experiments. It appears that disturbances such as temperature fluctuations, the presence of generalist herbivores, and shifts in nutrient availability, tend to promote the proliferation of invasive species, aligning with the findings of previous studies which found an enemy release advantage under these conditions (Vitousek, 1990; Ricciardi et al., 2013; Denley et al., 2019).

Overall, Chapter 2 provided a comprehensive and quantitative overview of ERH in aquatic plants, as well as a framework for future research on this topic, which include a need for more biogeographical studies, an incorporation of more floating growth forms, as well as the consideration of other factors outside of the ERH which may explain the observed results. This includes biotic resistance due to competition from related species, allelopathy, as well as other abiotic factors such as climate suitability and nutrient availability (Colatutti et al., 2004; Lacoul & Freedman., 2006; Fleming & Dibble, 2015; Bolpagni, 2021). The subsequent chapters thus addressed some of these factors.

To simplify some of the inherent complexities of working with entire freshwater ecosystems, this study used *L. major* as a case study to present an empirical biogeographical comparison of this species between South Africa and New Zealand. This was done in order to test whether this plant derives a benefit in the introduced range due to release from its associated natural enemies, as per the assumptions of the ERH (Williamson & Griffiths, 1996; Keane and Crawley, 2002; Fleming & Dibble, 2015).

The study used various plant parameters and diversity indices to assess the fitness of *L. major* and correlated this to the prevailing environmental conditions, herbivore diversity and therefore plant damage in the two regions. From these data, it was clear that *L. major* has increased fitness in most of the invaded sites as it had more biomass accumulation, produced significantly more shoots, and also displaced other native plants. This was possibly linked to natural enemy escape, as *L. major* experienced a significant reduction in herbivory pressure after its introduction to New Zealand (see Chapter 3).

Unlike native populations which were exposed to four or more frequently co-occurring herbivores (generalist moths, weevils and two specialist insects, *P. tuburcinatum*, and *H. lagarosiphon*), in the invaded range, only *Hygraula nitens* Butler (Lepidoptera: Crambidae), syn. *Nymphula nitens,* which was recorded in abundance at two sites, is known to significantly damage *L. major* in New Zealand (Redekop et al., 2018). Thus, these results supported the ERH and showed that *L. major* has a high invasive potential due to escape from its natural enemies. Chapter 3, therefore, made a direct contribution to existing literature and our understanding of the biogeographical patterns and mechanisms of biological invasions in aquatic plants, as per the gaps and limitations identified in Chapter 2 with regards to the ERH.

Furthermore, the drivers of *L. major* invasions identified here have meaningful implications for successful management of this weed. The challenges associated with controlling *L. major* using traditional methods make it a suitable candidate for biological control, which has the potential to promote co-existence in a more stable state, where this exotic plant can co-exist successfully with other native species instead of displacing them (Rattray et al. 1994; McGregor & Gourlay, 2002; Caffrey & Acevedo, 2007). For example, the reintroduction of natural enemies of various highly problematic invasive macrophytes in South Africa has been effective at controlling some of the worst invasions in the country (McConnachie et al., 2004; Coetzee et al., 2021).

The two specialist herbivores mentioned above have been proved to be good potential biological control candidates for *L. major* (Martin et al., 2013, Baars et al., 2023; Mangan & Baars, 2023), with limited prospect for *H. lagarosiphon* parasitism that is pervasive in the native range (Baars et al., 2010; Martin et al., 2018). The absence of these predators in the invaded range is likely to increase biological efficacy, as these parasitoids were previously shown to have a negative influence on the abundance of *H. lagarosiphon*, which then reduced herbivory pressure, thus making *L. major* a superior competitor (Martin et al., 2018).

Managing biological invasions within the aquatic environment using traditional means of control has proved to be challenging and unsustainable in the long run (Howard-Williams et al., 1996; Hussner et al., 2017; Maluleke et al., 2021). Additionally, submerged macrophytes like *L. major* which use asexual reproduction are highly invasive and therefore require alternative control measures. Hence, such plants become ideal candidates for biological control programmes, which are based on the premise that natural enemies are important regulators of plant populations in their native range (Cilliers, 1991; Keane & Crawley, 2002; Nachtrieb et al., 2011).

The use of *Hydrellia pakistanae* Deonier (Diptera; Ephydridae) in the United States of America and its contribution to controlling *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) provided the first evidence that the *Hydrellia* genus can be used to manage invasive plants in the Hydrocharitaceae (Buckingham & Bennett 1998; Grodowitz et al., 2003). Furthermore, successful establishment of *Hydrellia egeriae* in South Africa and elsewhere to control *Egeria densa* Planchon (Hydrocharitacea) (Smith, 2021) also provides confidence in the use of such insect to control submerged macrophytes which have otherwise been neglected in biological control programmes (Coetzee et al., 2011).

Having established the dominance of *L. major* in New Zealand sites at the regional scale as per the conceptual framework by Lacoul & Freedman (2006), the next objective of this thesis was to determine what competitive interactions could be expected between this species and another highly problematic Hydrocharitaceae, *E. densa* which is also invasive in that region (Caffrey et al., 2010). The overall competitive ability of a species is primarily determined by the degree of biomass accumulation and density, compared to other co-occurring congeners, with invasive species usually forming densa canopies that are vigorous competitors for limited resources (Clayton, 1996; James et al., 2006; Coetzee et al. 2005; Van Ginkel, 2011).

Chapter 4 examined how the competition between these two very similar invasive macrophytes, in the presence of herbivory by two host specific Ephydridae flies (*H. lagarosiphon* and *H. egeriae*) modulates the observed interactions at the finer scale. The chapter used a mesocosm approach to simulate different scenarios of plant density, herbivore presence and absence combinations on the growth and biomass of both plants. Various plant parameters, as well as multiple inverse linear models developed by Spitters (1983) were then used to quantify plant fitness and overall competitive dominance under these different treatments.

The results from plant parameters measures such as biomass production, shoot length and relative growth rate showed that *L. major* performed better in most of the treatments, although starting plant density as well as herbivory regime were important determinants of the observed interactions. Other studies have reported *L. major* as a superior competitor to various other species (Rattray et al. 1994; Caffrey et al. 2010; Martin et al., 2014). However, despite *L. major* showing higher growth parameters, this did not translate to this species being a better competitor than *E. densa*.

Competitive interactions were generally limited, except for the mesocosms which were inoculated with *H. lagarosiphon*. Here, according to the reciprocal yield models, the presence of this *L. major* herbivore appears to have facilitated the growth of *E. densa* by feeding on *L. major*. These facilitative interactions are not uncommon in plant communities (Thiébaut et al., 2019; Silveira & Thiébaut, 2020; Monacelli & Wilcox, 2021). In this case, *E. densa* was equivalent to 11 *L. major* plants, but this was not fully explained by the herbivory treatment, as it was also apparent in Chapter 4 that *L. major* was able to withstand herbivory and continue to grow successfully in the presence of *H. lagarosiphon*.

It was postulated that there were possibly insufficient insect numbers to trigger a significant reduction in *L. maj*or fitness in terms of growth. According to Martin et al. (2013) and Mangan & Baars (2016), at least four insects are required per shoot to cause meaningful damage. However, the results suggest that even at low numbers, this insect could affect *L. major* competitive vigour, although co-occurrence of insects and plants in mixed cultures was the most successful way to reduce growth parameters. Generally, *H. egeriae* appeared to be more successful at suppressing its host plants, *E. densa*, and this was particularly true in isolated insect cultures.

Overall, Chapter 4 presented the intricate interplay of herbivory and plant density in determining plant competitive interactions, further highlighting the complexities of ecological interactions as seen with the meta-analysis in Chapter 2. The chapter also showed that biological control may be a viable option for managing submerged macrophyte invasions, especially for *E. densa*. This same method of control could be successful for *L. major*, provided enough insect density numbers are achieved (Baars et al., 2013; Mangan & Baars, 2023). For example, Martin et al. (2013) showed that herbivory by the fly reduced the competitive ability of *L. major*, which has important consequences for succession and dominance in aquatic communities. Competition and herbivory both in isolation as well as in combination are an important driving source for overall species distributions and assemblages (Van et al. 1999; Coetzee et al. 2005).

Although controlled experiments such as the one presented in Chapter 4 cannot necessarily predict competitive outcomes under natural conditions, these types of studies are still a useful tool to at least anticipate overall interactions. These predictive powers are highly advantageous to the biological control field, as we can see how introducing natural enemies might change the dynamics of the ecosystem even before the actual introduction. Nevertheless, besides competitive interactions, other attributes may also play an important role in determining exotic species dominance, based on other environmental conditions (Colatutti et al., 2004; Lacoul & Freedman., 2006; Fleming & Dibble, 2015; Bolpagni, 2021).

6.2. Abiotic drivers of plant invasion

As seen from the previous chapters, there are many factors which may influence exotic species success. So far, this discussion has mainly focussed on the biotic components such as herbivory and competition. However, abiotic factors related to climate change are also likely to affect overall habitat suitability, and therefore the abundance and distribution of invasive species (Lacoul & Freedman., 2006). Consequently, Chapter 5 was aimed at investigating the abiotic components to *L. major* invasion. Specifically, Species Distribution Models (SDMs) were used to map potentially suitable habitat for *L. major* in the invaded range, and also predict how climate change will impact this. Furthermore, the chapter made use of both correlative and mechanistic modelling approaches to simulate suitable habitat for the two potential biological control agents under consideration for release in New Zealand. This was done to test for potential mismatches in host plant distribution and insect suitable range, as well as to identify optimum release sites should permission be granted.

The Maximum Entropy Species Distribution Modelling (MaxEnt) algorithm showed that almost all of New Zealand is susceptible to *L. major* invasion, and this is likely to further expand by 4% based on the 2080 climate scenario RCP 8.5 (IPCC, 2001). The meta-analysis in Chapter 2 showed that invasive species respond better to disturbances such as these. Furthermore, other studies have demonstrated that invasive plants will likely respond better to climate change (Cornelissen, 2011; Reeves, 2017), thus pushing the need for effective and sustainable management plans. According to Alahuhta et al. (2011), climate change is likely to affect freshwater species in colder regions more readily, favouring canopy forming species such as *L. major* and *E. densa*, while reducing overall species richness, especially for cold tolerant species. Furthermore, the projected temperature changes will have marked effects on catchment hydrology, and ultimately macrophyte assemblages (Lacoul & Freedman., 2006).

With regards to insect suitability, correlative methods revealed that the distribution of *H. lagarosiphon* and *P. tuburcinatum* would likely be limited to a small part of the South Islands. However, due to various limitations of this modelling approach discussed in Chapter 5 (Sutton, 2019l; Minuti et al., 2022), which do not take into account the physiology of the target organism, this provided only a starting point in terms of potential distribution on these *L. major* herbivores. Thus, degree day modelling was used to supplement results for *H. lagarosiphon*. This method makes use of climate data as well as organism development data to predict generation turnover at specific locations (Coetzee et al., 2007; Kearney et al., 2010).

Using life tables from Mangan & Baars (2013) and temperature data from various *L. major* infested localities, it was shown that this insect would be able to establish viable populations at various parts of New Zealand, in both the South and North Islands. A similar result would therefore be expected for *P. tuburcinatum* as these two herbivores occupy the same climatic envelope in the region of origin (Baars et al., 2013; Martin et al., 2013). Overall, the chapter indicated that climate change can have significant impacts on the invasion potential and management prospects of *L. major*.

6.3. Recommendations for future research

This study followed the theoretical model developed by Lacoul & Freedman. (2006) to determine the relative importance of various contributing factors within the larger framework in order to facilitate an understanding of the drivers associated with *L. major* invasion and management options thereof. Nevertheless, although extensive, this thesis does not cover all aspects of *L. major* invasion, due to the inherent complexities of studying such multifaceted phenomena. Therefore, future research could investigate some of the other important factors which could possibly affect invasion and management success.

Firstly, following the distribution of *L. major* in New Zealand using modern technologies such as remote sensing could aid with management decisions. For example, the low-cost sonar mapping used to map *L. major* invasions in Ireland by Morrissey et al. (2020) could be adopted in New Zealand. Alternatively, satellite image mapping similar to the tracking of water hyacinth in South Africa which does not require return visits for up-to date assessments could be highly beneficial (Coetzee et al., 2022).

With regards to the potential biological control agents, thermal limitations still need to be studied. This will further contribute to our understanding and predictions for suitable habitats of introduction. Mangan & Baars (2013) have already made good progress in this regard for *H. lagarosiphon*. However, similar investigations are still needed for *P. tuburcinatum*. Furthermore, continued host specificity testing of this insect for New Zealand is also needed. This would add to the test plant list already examined by Baars et al. (2010) for Ireland, which showed promising results.

The continued absence of this agent at various sites in the native range, as well as difficulty rearing the midge populations were the main limiting factors of this component for this thesis. Therefore, before further work is done in this regard, there first needs to be an investigation into the reason for this sporadic appearance and disappearance. Once this has been established, it would be interesting to conduct similar competition experiments as presented in Chapter 4, using *P. tuburcinatum* as part of the herbivory regimes.

Lagarosiphon major is known to have a highly sophisticated photosynthetic system, with ability to take up both CO_2 and HCO_3^- from the water (James et al*.,* 1999). According to Barko & Smart (1986), rooted macrophytes with surface forming canopies, such as *L. major,* are able to take up nutrients and gases from the water column, sediment as well as the atmosphere. This presents further scope for climate change related studies, as this plant may benefit from projected CO_2 increase (IPCC, 2002).

Hussner et al. (2019) simulated this CO_2 increase on various submerged macrophytes, including *E. densa* and *L. major*. The investigation showed that although there was no overall effect on photosynthesis, elevated dissolved inorganic carbon (DIC) and atmospheric CO_2 levels were generally associated with reduced leaf nitrogen content. Thus, this indicates that although submerged aquatic HCO_3^- users are less affected by rising atmospheric CO_2 when in DIC-rich environments compared to obligate CO_2 users in CO_2-limited conditions, increasing atmospheric CO_2 still has an impact on nitrogen absorption by these submerged plants, and this has the potential to affect ecosystem dynamics (Hussner et al., 2019).

Previous research by Baso et al. (2021) and others show that the efficacy of biological control is directly related to plant host quality, and this is partly determined by CO_2 and nutrient availability (Cornelissen, 2011; Reeves, 2017; Smith, 2021). Thus, this could also be an important factor to incorporate into further competition studies, to inform management expectations under future climates, which are directly linked to elevated atmospheric CO_2. According to Riis et al. (2010), CO_2 availability is one of the key elements controlling *L. major* size in New Zealand's freshwaters. Therefore, an investigation of increased CO_2 and its effects on biological control is needed.

6.4. Conclusion

The main conclusion from this undertaking is that ERH plays an important role in the biological invasion of *L. major*, but it is not the only factor that determines its success or failure. Other factors, such as environmental conditions, competition, biological control, and climate change, can also influence the invasion dynamics and management outcomes. Thus, although biological control may be a viable strategy, it may be better to develop a more holistic management programme that takes the needs of the ecosystem as well as the goals of resource managers into consideration, whether that includes other control options or not (Coetzee et al., 2011; Hussner et al., 2017). This is because none of the previous submerged biocontrol programmes have been as successful as those implemented against floating invasive macrophytes, and this is due to various challenges and uncertainties associated with biological control of submerged IAPs, such as parasitism of the Ephydridae flies, as well as other environmental variables such as temperature differences in open water vs weed stands and the microclimates which the control agents have to contend with (Grodowitz et al., 2003; Smith et al., 2022).

Biggs et al. (2012) suggested seven principles for building social ecological resilience, which include fostering complex adaptive systems thinking, maintaining diversity and redundancy. Therefore, an adaptable integrated approach consisting of diverse control methods, including biological control, as opposed to a singular approach might provide a better understanding and a more successful manage plan (Coetzee et al., 2011; Hussner et al., 2017), taking into account the multiple faceted nature of these systems. Furthermore, the dynamic nature of invasive species and their interactions with their new environments requires ongoing scientific inquiry as per Biggs's et al. (2012) principle of encouraging learning, to ultimately better inform conservation and active restoration efforts while addressing the challenges posed by invasive species like *L. major*. Overall, the work presented here contributes significantly to our understanding of ecological relationships and management strategies.

References

Aerts, R., 1999. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant-soil feedbacks. *Journal of Experimental Botany* 50 (330): 29-37.

Alahuhta, J., Heino, J. and Luoto, M., 2011. Climate change and the future distributions of aquatic macrophytes across boreal catchments. *Journal of Biogeography* 38 (2): 383-393.

Alpert, P., Bone, E. and Holzapfel, C., 2000. Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspectives in Plant Ecology, Evolution and Systematics* 3 (1): 52-66.

Andersen, T., Bello Gonzalez, O.C., Baars, J.R. and Earle, W., 2015. A new invasive weed-feeding species of *Polypedilum* (Pentapedilum) Kieffer from South Africa (Diptera: Chironomidae, Chironominae). *Zootaxa,* 4000 (5): 559-570.

Armitage, P.D., Pinder, L.C. and Cranston, P.S. eds., 2012. The Chironomidae: biology and ecology of non-biting midges. Springer Science & Business Media, Dordrecht.

Baars, J.R., Coetzee, J.A., Martin, G., Hill, M.P. and Caffrey, J.M. 2010. Natural enemies from South Africa for biological control of *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae) in Europe. *Hydrobiologia* 656:149-158.

Balciunas, J.K. and Burrows, D.W., 1996. Distribution, Abundance and Field Host-Range of *Hydrellia balciunasi* Bock (Diptera: Ephydridae) a Biological Control Agent for the Aquatic Weed *Hydrilla verticillata* (Lf) Royle. *Australian Journal of Entomology* 35(2): 125-130.

Baso, N.C., Coetzee, J.A., Ripley, B.S. and Hill, M.P., 2021. The effects of elevated atmospheric CO_2 concentration on the biological control of invasive aquatic weeds. *Aquatic Botany* 170: 103348.

Biggs, R., Schlüter, M., Biggs, D., Bohensky, E.L., BurnSilver, S., Cundill, G., Dakos, V., Daw, T.M., Evans, L.S., Kotschy, K. and Leitch, A.M., 2012. Toward principles for enhancing the resilience of ecosystem services. *Annual review of environment and resources* 37: 421-448.

Brändle, M., Kuhn, I., Klotz, S., Belle, C. & Brandl, R. 2008. Species richness of herbivores on exotic host plants increases with time since introduction of the host. *Diversity and Distributions* 14: 905-912.

Bolpagni R., 2021. Towards global dominance of invasive alien plants in freshwater ecosystems: the dawn of the Exocene? *Hydrobiologia* 848:2259-2279.

Bownes, A., 2014. Suitability of a leaf-mining fly, *Hydrellia* sp., for biological control of the invasive aquatic weed, *Hydrilla verticillata* in South Africa. *BioControl* 59 (6): 771-780.

Bowmer, K.H., Jacobs, S.W.L. and Sainty, G.R., 1995. Identification, biology and management of *Elodea canadensis*, Hydrocharitaceae. *Journal of Aquatic Plant Management* 33: 13-19.

Brown, C.A., Thomas, P., Poe, J., French, R.P. & Schloesser, D.W. 1988. Relationships of phytomacrofauna to surface area in naturally occurring macrophyte stands. *Journal of the North American Benthological Society* 7: 129-139.

Buckingham, G.R. and Bennett, C.A., 1998. Host range studies with *Bagous affinis* (Coleoptera: Curculionidae), an Indian weevil that feeds on hydrilla tubers. *Environmental Entomology* 27 (2): 469-479.

Cabrera Walsh, G., Magalí Dalto, Y., Mattioli, F.M., Carruthers, R.I. and Anderson, L.W., 2013. Biology and ecology of Brazilian elodea (*Egeria densa*) and its specific herbivore, *Hydrellia sp*., in Argentina. *Biocontrol* (58): 133-147.

Caffrey, J. and Acevedo, S., 2007. *Lagarosiphon major* –an aggressive invasive species in Lough Corrib. *Central Fisheries Board, Ireland*.

Caffrey, J., Millane, M., Evers, S.L., Moran, H. and Butler, M., 2010. A novel approach to aquatic weed control and habitat restoration using biodegradable jute matting. *Aquatic Invasions* 5 (2): 123-129.

Callaway, R. M., & Ridenour, W. M., 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2 (8): 436-443.

Callaway, R.M. and Maron, J.L., 2006. What have exotic plant invasions taught us over the past 20 years?. *Trends in Ecology & Evolution* 21 (7): 369-374.

Canavan, K., Paterson, I.D., Hill, M.P. and Dudley, T.L., 2019. Testing the Enemy Release Hypothesis on tall-statured grasses in South Africa, using *Arundo donax*, *Phragmites australis*, and *Phragmites mauritianus* as models. *Bulletin of Entomological Research* 109 (3): 287-299.

Center, T.D., Grodowitz, M.J., Cofrancesco, A.F., Jubinsky, G., Snoddy, E. and Freedman, J.E., 1997. Establishment of *Hydrellia pakistanae* (Diptera: Ephydridae) for the Biological Control of the Submersed Aquatic Plant *Hydrilla verticillata* (Hydrocharitaceae) in the Southeastern United States. *Biological Control* 8 (1): 65-73.

CHAH (COUNCIL OF HEADS OF AUSTRALASIAN HERBARIA) 2020. Online at: https://profiles.ala.org.au/opus/weeds-australia/profile/Lagarosiphon%20major Accessed 15 September 2020.

Champion, P.D. and Tanner, C.C., 2000. Seasonality of macrophytes and interaction with flow in a New Zealand lowland stream. *Hydrobiologia 441* (1): 1-12.

Charles, H. and Dukes, J.S., 2007. Impacts of invasive species on ecosystem services. *Biological Invasions*. 217-237.

Chisholm, W.P 2006. Review of aquatic weed control methods in New Zealand. In: Preston C, Watts JH, Crossman ND eds *Proceedings of the 15th Australian Weeds Conference*. 787–790.

Cilliers, C.J. 1991. Biological control of water hyacinth, *Eichhornia crassipes* (Pontederiaceae), in South Africa. *Agriculture, Ecosystems & Environment* 37 (1-3): 207-217.

Cilliers, C.J., Hill, M.P., Ogwang, J.A. and Ajuonu, O., 2003. Aquatic weeds in Africa and their control. *Biological control in IPM systems in Africa* 161-178.

Clayton, J.S., 1996. Aquatic weeds and their control in New Zealand lakes. *Lake and Reservoir Management* 12 (4): 477-486.

Çoban, H.O., Örücü, Ö.K. and Arslan, E.S., 2020. MaxEnt modelling for predicting the current and future potential geographical distribution of *Quercus libani* Olivier. *Sustainability* 12 (7): 2671.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A. and MacIsaac, H.J., 2004. Is invasion success explained by the enemy release hypothesis?. *Ecology Letters* 7 (8): 721-733.

Coetzee, J.A., Center, T.D., Byrne, M.J. and Hill, M.P., 2005. Impact of the biocontrol agent *Eccritotarsus catarinensis*, a sap-feeding mirid, on the competitive performance of water hyacinth, *Eichhornia crassipes*. *Biological Control* 32 (1): 90-96.

Coetzee, J.A., Bownes, A. and Martin, G.D., 2011. Prospects for the biological control of submerged macrophytes in South Africa. *African Entomology* 19 (2): 469-487.

Coetzee J.A., Byrne M.J. & Hill M.P. 2007. Predicting the distribution of *Eccritotarsus catarinensis*, a natural enemy released on water hyacinth in South Africa. *Entomologia Experimentalis et Applicata* 125: 237–247.

Coetzee, J.A., Bownes, A., Martin, G.D., Miller, B.E., Smith, R., Weyl, P.S.R. and Hill, M.P., 2021. A review of the biocontrol programmes against aquatic weeds in South Africa. *African Entomology* 29 (3): 935-964.

Coetzee, J.A., Miller, B.E., Kinsler, D., Sebola, K. and Hill, M.P., 2022. It's a numbers game: inundative biological control of water hyacinth (*Pontederia crassipes*), using *Megamelus scutellaris* (Hemiptera: Delphacidae) yields success at a high elevation, hypertrophic reservoir in South Africa. *Biocontrol Science and Technology* 32 (11): 1302-1311.

Cornelissen, T., 2011. Climate change and its effects on terrestrial insects and herbivory patterns. *Neotropical Entomology* 40*:* 155-163.

Cuthbert, R.N., Pattison, Z., Taylor, N.G., Verbrugge, L., Diagne, C., Ahmed, D.A., Leroy, B., Angulo, E., Briski, E., Capinha, C. and Catford, J.A., 2021. Global economic costs of aquatic invasive alien species. *Science of the Total Environment* 775: 145238.

Deeming, J.C. 2012. A new species of *Hydrellia* Rob. Desv. (Diptera: Ephydridae) developing in *Lagarosiphon major* (Hydrocharitaceae) in South Africa. *African Entomology* 20:217-221.

Deevey Jr, E.S., 1947. Life tables for natural populations of animals. *The Quarterly Review of Biology* 22 (4): 283-314.

De Lange PJ, and Rolfe JR 2010. New Zealand indigenous vascular plant list. *Wellington, New Zealand Plant Conservation Network.*

Deloitte., 2011. Styela clava: Economic impact assessment. *Report to the Ministry of Agriculture & Forestry, Wellington.*

Denley, D., Metaxas, A., & Fennel, K., 2019. Community composition influences the population growth and ecological impact of invasive species in response to climate change. *Oecologia* 189 (2): 537-548.

Denno, R.F., McClure, M.S. and Ott, J.R., 1995. Interspecific interactions in phytophagous insects: competition re-examined and resurrected. *Annual Review of Entomology* 40 (1): 297-331.

Diagne, C., Leroy, B., Gozlan, R.E., Vaissière, A.C., Assailly, C., Nuninger, L., Roiz, D., Jourdain, F., Jarić, I. and Courchamp, F., 2020. InvaCost, a public database of the economic costs of biological invasions worldwide. *Scientific Data* 7 (1): 277.

Earle, W., 2013. Field and laboratory-based evaluation of natural enemies screened as biological control agents of the aquatic weed *Lagarosiphon major*. PhD thesis, University College Dublin.

Earle, W., Mangan, R., O'Brien, M and Baars, J.R. 2013. Biology of *Polypedilum* n. sp. (Diptera: Chironomidae), a promising candidate agent for the biological control of the aquatic weed *Lagarosiphon major* (Hydrocharitaceae) in Ireland. *Biocontrol Science and Technology* 23 (11): 1267-1283.

EPPO, 2009. *Eichhornia crassipes*. *Bulletin OEPP/EPPO Bulletin* 39 (1)*:* 460-464.

Fan, S., Yu, D. and Liu, C., 2013. The invasive plant *Alternanthera philoxeroides* was suppressed more intensively than its native congener by a native generalist: implications for the biotic resistance hypothesis. *PLoS One* 8 (12): 83619.

Farooq, M., Li, X., Tan, L., Fornacca, D., Li, Y., Cili, N., Tian, Z., Yang, L., Deng, X., Liu, S. and Xiao, W., 2021. Ephemeroptera (Mayflies) Assemblages and Environmental Variation along Three Streams Located in the Dry-Hot Valleys of Baima Snow Mountain, Yunnan, Southwest China. *Insects* 12(9): 775.

Fick, S.E. and Hijmans, R.J., 2017. WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International journal of climatology* 37 (12): 4302-4315.

Fisher, A.J., Smith, L. and Woods, D.M., 2011. Climatic analysis to determine where to collect and release *Puccinia jaceae var. solstitialis* for biological control of yellow starthistle. *Biocontrol Science and Technology* 21 (3): 333-351.

Fleming, J.P. and Dibble, E.D., 2015. Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia* 746 (1): 23-37.

Gallagher, R.V., Beaumont, L.J., Hughes, L. and Leishman, M.R., 2010. Evidence for climatic niche and biome shifts between native and novel ranges in plant species introduced to Australia. *Journal of Ecology* 98 (4): 790-799.

GBIF, 2022. GBIF Occurrence. Online website: https://doi.org/10.15468/dl.yudke3. Accessed 25 August.

Genovesi, P., Carboneras, C., Vilà, M. and Walton, P., 2015. EU adopts innovative legislation on invasive species: a step towards a global response to biological invasions?. *Biological Invasions* 17*:* 1307-1311.

Genovesi, P., Scalera, R., Brunel, S., Roy, D., Solarz, W., 2010. Towards an early warning and information system for invasive alien species (IAS) threatening biodiversity in Europe. *EEA Technical report*, Copenhagen 5(2010): 47

Gallego-Tévar, B., Grewell, B.J., Whitcraft, C.R., Futrell, J.C., Bárcenas-Moreno, G. and Castillo, J.M., 2022. Contrasted impacts of Yellow Flag Iris (*Iris pseudacorus*) on plant diversity in tidal wetlands within Its native and invaded distribution ranges. *Diversity* 14 (5): 326.

Gillard, M.B., Caudal, J.P., Deleu, C. and Thiébaut, G., 2021. Heterogeneous Impact of Water Warming on Exotic and Native Submerged and Emergent Plants in Outdoor Mesocosms. *Plants 10* (7): 1324.

Grodowitz, M.J., Cofrancesco, A.F., Stewart, R.M., Madsen, J. & Morgan, D., 2003. Possible impact of Lake *Seminole hydrilla* by the introduced leaf-mining fly *Hydrellia pakistanae*. U.S. Army Engineer Research and Development Center, Vicksburg, Mississippi, U.S.A. 3*: 18*-35.

Grutters, B.M., Gross, E.M. and Bakker, E.S., 2016. Insect herbivory on native and exotic aquatic plants: phosphorus and nitrogen drive insect growth and nutrient release. *Hydrobiologia* 778*:* 209-220.

Gufu, G.D., Manea, A., Vorreiter, L. and Leishman, M.R., 2018. Do invasive exotic and native freshwater plant species respond similarly to low additional nitrate doses?. *Aquatic Botany* 151*:* 1-8.

Gurevitch, J. & Hedges, L.V., 2001. Meta-analysis: combining the results of independent experiments. *Design and Analysis of Ecological Experiments* (eds S.M. Scheiner & J. Gurevitch) 347–369. Oxford University Press, New York.

Habeck, D.H. and Balciunas, J.K., 2005. Larvae of Nymphulinae (Lepidoptera: Pyralidae) associated with *Hydrilla verticillata* (Hydrocharitaceae) in North Queensland. *Australian Journal of Entomology* 44 (4): 354-363.

Harms, N.E., Knight, I.A., Pratt, P.D., Reddy, A.M., Mukherjee, A., Gong, P., Coetzee, J., Raghu, S. and Diaz, R., 2021. Climate mismatch between introduced biological control agents and their invasive host plants: improving biological control of tropical weeds in temperate regions. *Insects* 12 (6): 549.

Hedges LV., 1981. Distribution theory for Glass's estimator of effect size and related estimators. *Journal of Educational Statistics* 6:107–128.

Heger, T. and Jeschke, J.M., 2014. The enemy release hypothesis as a hierarchy of hypotheses. *Oikos* 123 (6): 741-750.

Heino, J., Virkkala, R. and Toivonen, H., 2009. Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. *Biological Reviews* 84(1): 39-54.

Hejda, M., Pyšek, P., & Jarošík, V., 2009. Impact of invasive plants on the species richness, diversity and composition of invaded communities. *Journal of Ecology* 97 (3): 393-403.

Hernandez, P.A., Graham, C.H., Master, L.L. and Albert, D.L., 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography* 29 (5): 773-785.

Hierro, J.L., J.L. Maron, and R.M. Callaway., 2005. A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology* 93: 5-15.

Hill, M.P. and Coetzee, J., 2017. The biological control of aquatic weeds in South Africa: Current status and future challenges. *Bothalia-African Biodiversity & Conservation* 47 (2): 1-12.

Hill, M.P. and Olckers, T., 2001. Biological control initiatives against water hyacinth in South Africa: constraining factors, success and new courses of action. *In: Julien, M.H., Center, T.D., Hill, M.P. (Eds.), Proceedings of the Second Global Working Group Meeting for the Biological and Integrated Control Water Hyacinth*. October 9–12, 2000, Beijing, China. 102: 33–38. Australian Centre for International Agricultural Research, Canberra, Australia.

Howard-Williams, C., Schwarz, A.M. and Reid, V., 1996. Patterns of aquatic weed regrowth following mechanical harvesting in New Zealand hydro-lakes. *Hydrobiologia* 340 (1-3): 229-234.

Hofstra, D.E., Clayton, J., Green, J.D. and Auger, M., 1999. Competitive performance of *Hydrilla verticillata* in New Zealand. *Aquatic Botany* 63 (3-4): 305-324.

Hoveka, L.N., Bezeng, B.S., Yessoufou, K., Boatwright, J.S. and Van der Bank, M., 2016. Effects of climate change on the future distributions of the top five freshwater invasive plants in South Africa. *South African Journal of Botany* 102*:* 33-38.

Hudson, H.R. and Harding, J.S., 2004. Drainage management in New Zealand: *A review of existing activities and alternative management practices* 235: 39.

Hussner, A., Stiers, I., Verhofstad, M.J.J.M., Bakker, E.S., Grutters, B.M.C., Haury, J., Van Valkenburg, J.L.C.H., Brundu, G., Newman, J., Clayton, J.S. and Anderson, L.W.J., 2017. Management and control methods of invasive alien freshwater aquatic plants: a review. *Aquatic Botany* 136: 112-137.

Hussner, A., Smith, R., Mettler-Altmann, T., Hill, M.P. and Coetzee, J., 2019. Simulated global increases in atmospheric CO_2 alter the tissue composition, but not the growth of some submerged aquatic plant bicarbonate users growing in DIC rich waters. *Aquatic Botany* 153: 44-50.

Ikemoto, T. and Takai, K., 2000. A new linearized formula for the law of total effective temperature and the evaluation of line-fitting methods with both variables subject to error. *Environmental Entomology* 29 (4): 671-682.

James, C.S., Eaton, J.W. and Hardwic, K., 1999. Competition between three submerged macrophytes, *Elodea canadensis* Michx*, Elodea nuttallii* (Planch.) St John and *Lagarosiphon major* (Ridl.) Moss. *Hydrobiologia* 415:35-40.

Kass, J.M., Meenan, S.I., Tinoco, N., Burneo, S.F. and Anderson, R.P., 2021. Improving area of occupancy estimates for parapatric species using distribution models and support vector machines. *Ecological Applications* 31 (1): 02228.

Keane, R.M. and Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17 (4): 164-170.

Kearney M.R., Wintle B.A. & Porter W.P., 2010. Correlative and mechanistic models of species distribution provide congruent forecasts under climate change. *Ecology Letters* 3: 203-213.

Kelly, J., Tosh, D., Dale, K., and Jackson, A., 2013. The economic cost of invasive and non-native species in Ireland and Northern Ireland. *A report prepared for the Northern Ireland Environment Agency and National Parks and Wildlife Service as part of Invasive Species Ireland.*

Khanum, R., Mumtaz, A.S. and Kumar, S., 2013. Predicting impacts of climate change on medicinal asclepiads of Pakistan using Maxent modeling. *Acta Oecologica* 49: 23-31.

Khosa, D., Marr, S.M., Wasserman, R.J., Zengeya, T.A. and Weyl, O.L., 2019. An evaluation of the current extent and potential spread of Black Bass invasions in South Africa. *Biological Invasions* 21 (5): 1721-1736.

Knevel, I.C., Lans, T., Menting, F.B., Hertling, U.M. and van der Putten, W.H., 2004. Release from native root herbivores and biotic resistance by soil pathogens in a new habitat both affect the alien *Ammophila arenaria* in South Africa. *Oecologia* 141*:* 502-510.

Kumar, S., Graham, J., West, A.M. and Evangelista, P.H., 2014. Using district-level occurrences in MaxEnt for predicting the invasion potential of an exotic insect pest in India. *Computers and Electronics in Agriculture* 103: 55-62.

Kwong, R.M., Sagliocco, J.L., Harms, N.E., Butler, K.L., Green, P.T. and Martin, G.D., 2017. Biogeographical comparison of the emergent macrophyte, *Sagittaria platyphylla* in its native and introduced ranges. *Aquatic Botany* 141*:* 1-9.

Kwong, R.M., Sagliocco, J.L., Harms, N.E., Butler, K.L., Martin, G.D. and Green, P.T., 2019. Could enemy release explain invasion success of *Sagittaria platyphylla* in Australia and South Africa?. *Aquatic Botany* 153*:* 67-72.

Lacoul, P. and Freedman, B., 2006. Environmental influences on aquatic plants in freshwater ecosystems. *Environmental Reviews* 14: 89-136.

Lajeunesse, M.J., 2013. Recovering missing or partial data from studies: a survey of conversions and imputations for meta-analysis. In J. Koricheva, J. Gurevitch, and K. Mengersen, editors. *Handbook of meta-analysis in ecology and evolution* 195-206. Princeton University Press, Princeton, New Jersey, USA.

Laranjeira, C.M. and Nadais, G., 2008. Eichhornia crassipes control in the largest Portuguese natural freshwater lagoon 1. *EPPO Bulletin* 38 (3): 487-495.

Larned, S.T., Snelder, T., Unwin, M.J. and McBride, G.B., 2016. Water quality in New Zealand rivers: current state and trends. *New Zealand Journal of Marine and Freshwater Research* 50 (3): 389-417.

Liu, H. and Stiling, P., 2006. Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions* 8: 1535-1545.

Long JL., 2003. Introduced mammals of the world: their history, distribution and influence. CABI Publishing, Oxford.

Louda, S.M., Pemberton, R.W., Johnson, M.T. and Follett, P., 2003. Nontarget effects-the Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. *Annual Review of Entomology* 48 (1): 365-396.

Madsen, J.D., Crosson, H.A., Hamel, K.S., Hilovsky, M.A. and Welling, C.H., 2000. Panel discussion-Management of Eurasian watermilfoil in the United States using native insects: State regulatory and management issues.

Mafokoane, D.L., Oberholzer, I.G. and Hill, M.P., 2007. The biology and laboratory host range of the weevil, *Listronotus marginicollis* (Hustache) (Coleoptera: Curculionidae), a natural enemy of the invasive aquatic weed, parrot's feather, *Myriophyllum aquaticum* (Velloso) Verde (Haloragaceae). *African Entomology* 15 (2): 385-390.

Maluleke, M., Fraser, G.C.G. and Hill M.P. 2021. Economic evaluation of chemical and biological control of four aquatic weeds in South Africa. *Biocontrol Science and Technology* 31 (9): 896-911.

Mangan, R., 2012. Pre-release evaluation studies of the leaf-mining fly, *Hydrellia lagarosiphon* (Diptera: Ephydridae) a candidate biological control agent of the submerged aquatic invasive weed *Lagarosiphon major* (Hydrocharitaceae). PhD Thesis, University College Dublin.

Mangan, R. and Baars, J.R., 2013. Use of life table statistics and degree day values to predict the colonisation success of Hydrellia lagarosiphon Deeming (Diptera: Ephydridae), a leaf mining fly of Lagarosiphon major (Ridley) Moss (Hydrocharitaceae), in Ireland and the rest of Europe. *Biological Control* 64 (2): 143-151.

Mangan, R., Dirilgen, T. and Baars, J.R., 2015. Responses of adult *Hydrellia lagarosiphon* to a revised diet: implications for life cycle studies and laboratory culturing techniques. *Entomologia Experimentalis et Applicata* 157 (2): 164-169.

Mangan, R. and Baars, J.R., 2016. Can leaf-mining flies generate damage with significant impact on the submerged weed *Lagarosiphon major*?. *BioControl* 61*:* 803-813.

Mangan, R. and Baars, J.R., 2023. Risk assessment of the host range of *Hydrellia lagarosiphon* for the biological control of *Lagarosiphon major* in Ireland. *Biocontrol Science and Technology* 1-20.

Martin, G.D., Coetzee, J.A. and Baars, J.R., 2013. *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), a potential biological control agent for the submerged aquatic weed, *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae). *African Entomology* 21 (1): 151-160.

Martin, G.D. and Coetzee, J.A., 2014. Competition between two aquatic macrophytes, *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) and *Myriophyllum spicatum* Linnaeus (Haloragaceae) as influenced by substrate sediment and nutrients. *Aquatic Botany 114:* 1-11.

Martin, G.D., Coetzee, J.A. and Compton, S., 2018. Plant–herbivore–parasitoid interactions in an experimental freshwater tritrophic system: higher trophic levels modify competitive interactions between invasive macrophytes. *Hydrobiologia* 817*:* 307-318.

McClay, A.S. and Balciunas, J.K., 2005. The role of pre-release efficacy assessment in selecting classical biological control agents for weeds—applying the Anna Karenina principle. *Biological Control* 35 (3): 197-207.

McConnachie, M.M., van Wilgen, B.W., Ferraro, P.J., Forsyth, A.T., Richardson, D.M., Gaertner, M. and Cowling, R.M. 2016. Using counterfactuals to evaluate the cost-effectiveness of controlling biological invasions. *Ecological Applications* 26 (2): 475-483.

McGregor, P.G. and Gourlay, H., 2002. Assessing the prospects for biological control of lagarosiphon *(Lagarosiphon major* (Hydrocharitaceae)). *New Zealand: Department of Conservation.*

Mereta, S.T., Yewhalaw, D., Boets, P., Ahmed, A., Duchateau, L., Speybroeck, N., Vanwambeke, S.O., Legesse, W., De Meester, L. and Goethals, P.L., 2013. Physico-chemical and biological characterization of anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. *Parasites & Vectors* 6: 1-16.

Messing, R.H. and Wright, M.G., 2006. Biological control of invasive species: solution or pollution?. *Frontiers in Ecology and the Environment* 4 (3): 132-140.

Minuti, G., Stiers, I. and Coetzee, J.A., 2022. Climatic suitability and compatibility of the invasive *Iris pseudacorus* L.(Iridaceae) in the Southern Hemisphere: Considerations for biocontrol. *Biological Control* 169*:* 104886.

Morrissey, E., Meade, R., Matson, R., McCarthy, E. and Kelly, F.L., 2020. *Lagarosiphon major* Research on Lough Corrib. *Interim Report* 2018-2019.

Monacelli, Kathryn, and Douglas A. Wilcox., 2021. Competition between two floating-leaved aquatic plants. *Aquatic Botany* 172 (2021): 103390.

Mooney, H.A. and Cleland, E.E., 2001. The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences* 98 (10): 5446-5451.

Mozdzer, T.J., Zieman, J.C. and McGlathery, K.J., 2010. Nitrogen uptake by native and invasive temperate coastal macrophytes: importance of dissolved organic nitrogen. *Estuaries and Coasts* 33*:* 784-797.

Muskett, P.C., Paterson, I.D. and Coetzee, J.A., 2020. Ground-truthing climate-matching predictions in a post-release evaluation. *Biological Control* 144*:* 104-217.

Nachtrieb, J.G., Grodowitz, M. J. & Smart, R. M. 2011. Impact of invertebrates on three aquatic macrophytes: American pondweed, Illinois pondweed, and Mexican water lily. *Journal of Aquatic Plant Management* 49: 32–36.

NEMBA, 2004. National Environmental Management: Biodiversity Act (No. 10 of 2004). *Government Gazette* 26436.

Netherland, M.D., 2014. Chemical control of aquatic weeds. *Biology and control of aquatic plants. A best management practices handbook. 3rd edition.* Aquatic Ecosystem Restoration Foundation: Marietta (GA) 71-88.

Oberholster, P.J. and Ashton, P.J., 2008. State of the nation report: An overview of the current status of water quality and eutrophication in South African rivers and reservoirs. *Parliamentary Grant Deliverable. Pretoria: Council for Scientific and Industrial Research (CSIR)* 2006.

Obermeyer, A.A. (1964). The South African species of *Lagarosiphon*. *Bothalia* 8 (2), 139-146.

Olckers, T., Coetzee, J.A., Egli, D., Martin, G.D., Paterson, I.D., Sutton, G.F. and Wood, A.R., 2021. Biological control of South African plants that are invasive elsewhere in the world: A review of earlier and current programmes. *African Entomology* 29 (3): 1005-1029.

Paynter, Q., Overton, J.M., Hill, R.L., Bellgard, S.E. and Dawson, M.I., 2012. Plant traits predict the success of weed biocontrol. *Journal of Applied Ecology* 49 (5): 1140-1148.

Paynter, Q., 2015. Biocontrol of aquatic weeds. *Conference proceedings, Lakes Water Quality Society* 109-116.

Paynter, Q., Fowler, S.V., Hayes, L. and Hill, R.L., 2015. Factors affecting the cost of weed biocontrol programs in New Zealand. *Biological control* 80: 119-127.

Petruzzella, A., van Leeuwen, C.H., van Donk, E. and Bakker, E.S., 2020. Direct and indirect effects of native plants and herbivores on biotic resistance to alien aquatic plant invasions. *Journal of Ecology* 108 (4): 1487-1496.

Phillips, S.J., Anderson, R.P., Dudík, M., Schapire, R.E. and Blair, M.E., 2017. Opening the black box: An open-source release of Maxent. *Ecography* 40 (7): 887-893.

Pimentel, D., Zuniga, R. and Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological economics* 52 (3): 273-288.

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD., 2020. The PRISMA statement: An updated guideline for reporting systematic reviews. *BMJ* 372: 71.

Prior, K.M. and Hellmann, J.J., 2013. Does enemy loss cause release? A biogeographical comparison of parasitoid effects on an introduced insect. *Ecology* 94 (5): 1015-1024.

R Core Team., 2023. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing, Vienna, Austria.*

Rahel, F.J. and Olden, J.D. 2008. Assessing the effects of climate change on aquatic invasive species. *Conservation Biology* 22 (3): 521-533.

Rattray, M.R., Howard-Williams, C. and Brown, J.M.A., 1994. Rates of early growth of propagules of *Lagarosiphon major* and *Myriophyllum triphyllum* in lakes of differing trophic status. *New Zealand Journal of Marine and Freshwater Research* 28 (3): 235-241.

Redekop, P., Gross, E.M., Nuttens, A., Hofstra, D.E., Clayton, J.S. and Hussner, A., 2018. *Hygraula nitens*, the only native aquatic caterpillar in New Zealand, prefers feeding on an alien submerged plant. *Hydrobiologia* 812: 13-25.

Redekop, P., Hofstra, D. and Hussner, A., 2016. *Elodea canadensis* shows a higher dispersal capacity via fragmentation than *Egeria densa* and *Lagarosiphon major*. *Aquatic Botany* 130: 45-49.

Reeves, J.L., 2017. Climate change effects on biological control of invasive plants by insects. *CABI Reviews* 2017: 1-8.

Regulation, C., 2016. Commission Implementing Regulation (EU) 2016/1141 of 13 July 2016 adopting a list of invasive alien species of Union concern pursuant to Regulation (EU) No 1143/2014 of the European Parliament and of the Council. *Official Journal of the European Union* 189*:* 4-8.

Rejmánek, M., 2000. Invasive plants: approaches and predictions. *Austral ecology* 25 (5): 497-506.

Ricciardi, A., Blackburn, T. M., Carlton, J. T., Dick, J. T., Hulme, P. E., Iacarella, J. C., and Pyšek, P., 2013. Invasion science: a horizon scan of emerging challenges and opportunities. *Trends in Ecology & Evolution* 28 (6): 329-336.

Riis, T., Lambertini, C., Olesen, B., Clayton, J.S., Brix, H. and Sorrell, B.K., 2010. Invasion strategies in clonal aquatic plants: are phenotypic differences caused by phenotypic plasticity or local adaptation?. *Annals of Botany* 106 (5): 813-822.

Riis, T., Olesen, B., Clayton, J.S., Lambertini, C., Brix, H. and Sorrell, B.K., 2012. Growth and morphology in relation to temperature and light availability during the establishment of three invasive aquatic plant species. *Aquatic Botany* 102: 56-64.

Roy, H.E., Lawson Handley, L.J., Schönrogge, K., Poland, R.L. and Purse, B.V., 2011. Can the enemy release hypothesis explain the success of invasive alien predators and parasitoids?. *BioControl* 56: 451-468.

Rodríguez-Merino, A., Fern´andez-Zamudio, R., García-Murillo, P., Mu˜noz, J., 2019. Climatic niche shift during *Azolla filiculoides* invasion and its potential distribution under future scenarios. *Plants* 8 (10): 424.

Room, P.M. and Thomas, P.A., 1985. Nitrogen and establishment of a beetle for biological control of the floating weed Salvinia in Papua New Guinea. *Journal of Applied Ecology* 139-156.

Schooler, S., Clech-Goods, C. and Julien, M., 2006. Ecological studies to assess the efficacy of biological control on populations of alligator weed and lippia. *Australian Journal of Entomology* 45 (4): 272-275.

Schultz, M., Kozersjki, H.P., Pluntke, T. & Rinke, K. 2003. The influence of macrophytes on sedimentation and nutrient retention in the lower River Spree (Germany). *Water Research* 37: 569-578.

Serra, M.N., Albariño, R. and Díaz Villanueva, V., 2013. Invasive *Salix fragilis* alters benthic invertebrate communities and litter decomposition in northern Patagonian streams. *Hydrobiologia* 701: 173-188.

Sheldon, S.P. and Creed Jr, R.P., 1995. Use of a native insect as a biological control for an introduced weed. *Ecological Applications* 5 (4): 1122-1132.

Sheldon, S.P. and Creed Jr, R.P., 2003. The effect of a native biological control agent for Eurasian watermilfoil on six North American watermilfoils. *Aquatic Botany* 76 (3): 259-265.

Sheppard, A.W., Shaw, R.H. and Sforza, R., 2006. Top 20 environmental weeds for classical biological control in Europe: a review of opportunities, regulations and other barriers to adoption. *Weed research*, 46 (2): 93-117.

Schutz, L., 2008. Insect communities associated with three submerged aquatic macrophytes, with emphasis on the phytophagous species. MSc thesis, University of the Witwatersrand.

Silveira, M.J. and Thiébaut, G., 2020. Effect of density and neighbours on interactions between invasive plants of similar growth form. *Aquatic Ecology* 54: 463-474.

Smith, R., 2017. The potential of *Hydrellia Egeriae* Rodrigues (Diptera: Ephydridae) as a biocontrol agent for *Egeria densa* Planch. (Hydrocharitaceae) in South Africa. Rhodes University Msc Thesis.

Smith, R., Mangan, R. and Coetzee, J.A., 2019. Risk assessment to interpret the physiological host range of *Hydrellia egeriae*, a biocontrol agent for *Egeria densa*. *BioControl* 64 (4): 447-456.

Smith, R., 2021. The biological control of *Egeria densa* Planch. (Hydrocharitaceae) in South Africa. Rhode university PhD Thesis.

Smith, R., Coetzee, J.A. and Hill, M.P., 2022. Best of both worlds: The thermal physiology of *Hydrellia egeriae*, a biological control agent for the submerged aquatic weed, *Egeria densa* in South Africa. *BioControl* 67 (3): 365-374.

Soliman, T. and Inglis, G.J., 2018. Forecasting the economic impacts of two biofouling invaders on aquaculture production of green-lipped mussels *Perna canaliculus* in New Zealand. *Aquaculture Environment Interactions* 10: 1-12.

Spitters, C.J.T., 1983. An alternative approach to the analysis of mixed cropping experiments. 1. Estimation of competition effects. *Netherlands Journal of Agricultural Science* 31: 1-11.

Steele, L., Ray, C. and Guidone, M., 2018. High phenolic content fails to deter mesograzer consumption of *Myriophyllum spicatum* (Eurasian watermilfoil) in New England. *Aquatic Ecology* 52: 255-267.

Stephens, A.E., Srivastava, D.S. and Myers, J.H., 2013. Strength in numbers? Effects of multiple natural enemy species on plant performance. *Proceedings of the Royal Society B: Biological Sciences* 280 (1760): 2012-2756.

Stiers, I., Njambuya, J. and Triest, L., 2011. Competitive abilities of invasive *Lagarosiphon major* and native *Ceratophyllum demersum* in monocultures and mixed cultures in relation to experimental sediment dredging. *Aquatic Botany* 95 (2): 161-166.

Strange E. 2017. Evidence for a biological control induced regime shift between floating and submerged invasive plant dominance in South Africa. PhD Thesis, Rhodes University, South Africa.

Strange, E.F., Landi, P., Hill, J. and Coetzee, J., 2019. Modelling top-down and bottom-up drivers of a regime shift in invasive aquatic plant stable states. *Frontiers in Plant Science* 10: 889.

Strong, D.R. 1977. The insects of British trees: community equilibration in ecological time. *Annals of the Missouri Botanical Garden* 61: 692–701.

Sutton, G.F., 2019. Searching for a needle in a haystack: Where to survey for climatically-matched biological control agents for two grasses (Sporobolus spp.) invading Australia. *Biological Control* 129: 37-44.

Symoens, J.J. and Triest, L., 1983. Monograph of the african genus Lagarosiphon Harvey (Hydrocharitaceae). *Bulletin du Jardin Botanique National de Belgique/Bulletin van de Nationale Plantentuin van Belgie* 441-488.

Thiébaut, G., Rodriguez-Perez, H. and Jambon, O., 2019. Reciprocal interactions between the native *Mentha aquatica* and the invasive *Ludwigia hexapetala* in an outdoor experiment. *Aquatic Botany* 157: 17-23.

Titus, J.E. and Adams, M.S., 1979. Coexistence and the comparative light relations of the submersed macrophytes *Myriophyllum spicatum* L. and *Vallisneria americana* Michx. *Oecologia* 40: 273-286.

Van, T.K., Wheeler, G.S. and Center, T.D., 1999. Competition between *Hydrilla verticillata* and *Vallisneria americana* as influenced by soil fertility. *Aquatic Botany* 62 (4): 225-233.

Van Ginkel, C.E. 2011. Eutrophication: Present reality and future challenges for South Africa. *Water SA 3*7 (5): 693-702.

Van Noort, S., Smith, R. and Coetzee, J.A., 2021. Identity of parasitoid wasps (Hymenoptera, Braconidae and Eulophidae) reared from aquatic leaf-mining flies (Diptera, Ephydridae) on invasive Brazilian waterweed *Egeria densa* in South Africa. *African Invertebrates* 62: 287.

Vevea, J.L. and Hedges, L.V., 1995. A general linear model for estimating effect size in the presence of publication bias. *Psychometrika* 60*:* 419-435.

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software* 36 (3): 1–48.

Vilà, M., Maron, J.L. and Marco, L., 2005. Evidence for the enemy release hypothesis in *Hypericum perforatum*. *Oecologia* 142*:* 474-479.

Vilà, M., Corbin, J.D., Dukes, J.S., Pino, J. and Smith, S.D., 2007. Linking plant invasions to global environmental change. *Terrestrial Ecosystems in a Changing World* 93-102. Springer, Berlin, Heidelberg.

Vilà, M., et al. (2011). Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities, and ecosystems. *Ecology Letters* 14 (7): 702–708.

Vitousek, P. M. (1990). Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos* 57 (1): 7-13.

Weeks, E. and Cuda, J., 2014. *Hydrilla* leaf mining flies (unofficial common name) *Hydrellia* spp. (Insecta: Diptera: Ephydridae): EENY590/IN1034, 4/2014. EDIS, 2014 (6).

Wells, R.D., de Winton, M.D. and Clayton, J.S., 1997. Successive macrophyte invasions within the submerged flora of Lake Tarawera, central North Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 31 (4): 449-459.

Wells, R., 2015. Success studies and the way forward. Conference Contribution. *Proceedings of LakesWater Quality Society*.

Weyl, P.S.R. and J.A. Coetzee., 2013. An integrated remote sampling approach for aquatic invertebrates associated with submerged macrophytes. *African Journal of Aquatic Science* 38: 337-340.

Wheeler, G.S. and Center, T.D., 1996. The Influence of Hydrilla Leaf Quality on Larval Growth and Development of the Biological Control Agent *Hydrellia pakistanae* (Diptera: Ephydridae). *Biological control* 7 (1): 1-9.

Whiting P., Savović J., Higgins J.P.T., Caldwell D.M., Reeves B.C., Shea B., Davies P., Kleijnen J., Churchill R., 2016. ROBIS: A new tool to assess risk of bias in systematic reviews was developed. *Journal of Clinical Epidemiology* 69:225-234.

William, P.A and Timmins, S. 2002. Economic impacts of weeds in New Zealand. *Biological invasions: economic and environmental cost of alien plant, animal, and microbe species* 177-184.

Williamson, M. and Griffiths, B. 1996. *Biological Invasions*. Springer Science & Business Media, London.

Willis, A.J., Kilby, M.J., McMaster, K., Cullen, J.M. and Groves, R.H., 2003. Predictability and acceptability: potential for damage to non-target native plant species by biological control agents for weeds. Improving the selection, testing and evaluation of weed biological control agents. *Glen Osmond: South Australia CRC for Australian Weed Management* 35-49.

Wilson, J.R., Ajuonu, O., Center, T.D., Hill, M.P., Julien, M.H., Katagira, F.F., Neuenschwander, P., Njoka, S.W., Ogwang, J., Reeder, R.H. and Van, T., 2007. The decline of water hyacinth on Lake Victoria was due to biological control by Neochetina spp. *Aquatic Botany* 87 (1): 90-93.

Winston, R.L., Schwarzländer, M., Hinz, H.L., Day, M.D., Cock, M.J. and Julien, M.H., 2014. Biological control of weeds: A world catalogue of agents and their target weeds. *Biological Control of Weeds: a world catalogue of agents and their target weeds* (5th Ed.). USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, West Virginia. *FHTET* 2014 (04): 838.

Xu, M., Wang, Z., Duan, X. and Pan, B., 2014. Effects of pollution on macroinvertebrates and water quality bio-assessment. *Hydrobiologia* 729: 247-259.

Yan, Y., Oduor, A.M., Li, F., Xie, Y. and Liu, Y., 2022. Opposite effects of nutrient enrichment and an invasive snail on the growth of invasive and native macrophytes. *Ecological Applications* 2737.

Yarrow, M., Marin, V.H., Finlayson, C., Tironi, A., Delgado, L.E. and Fischer, F., 2009. The ecology of Egeria densa Planchon (Liliopsida: Alismatales): A wetland ecosystem engineer?. *Revista Chilena de Historia Natural* 82 (2): 299-313.

Zangerl, A.R. and Rutledge, C.E., 1996. The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *The American Naturalist* 147 (4): 599-608.

Zehnsdorf, A., Hussner, A., Eismann, F., Rönicke, H. and Melzer, A., 2015. Management options of invasive Elodea nuttallii and Elodea canadensis. *Limnologica* 51: 110-117.

Zhang, D., et al. (2019). Effects of invasive alien aquatic plants on ecosystem services: A review. *Science of the Total Environment* 661: 332–344.

Zhang, X., Yu, H., Lv, T., Yang, L., Liu, C., Fan, S. and Yu, D., 2021. Effects of different scenarios of temperature rise and biological control agents on interactions between two noxious invasive plants. *Diversity and Distributions* 27 (12): 2300-2314.

Zimmermann, H.G., Hoffmann, J.H. and Moran, V.C. 2004. Biological control in the management of invasive alien plants in South Africa, and the role of the Working for Water Programme: working for water. *South African Journal of Science* 100 (1): 34-40.