The effect of biological control on the population dynamics of *Pontederia crassipes* Mart. (Pontederiaceae) and *Salvinia minima* Baker (Salviniales: Salviniaceae)

THESIS

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Abstract

Pontederia crassipes is widely regarded as the most damaging floating aquatic weed in terms of its invasive traits, and its impact on aquatic ecosystems. Biological control using host-specific natural enemies is widely used for its control, with the most recent agent released being a planthopper, *Megamelus scutellaris*, in South Africa and the USA. In South Africa, inundative releases of *M. scutellaris* have been shown to control the weed even at eutrophic, and high elevation cold sites, such as Hartbeespoort Dam, arguably Africa's most hypertrophic impoundment. However, subsequent to the control of *P. crassipes* on Hartbeespoort Dam, in 2021, a secondary invader, *Salvinia minima,* dominated the water system. *Salvinia minima* is only known from a handful sites near Hartbeespoort Dam and there is currently no approved biocontrol agent for it.

The population dynamics of *P. crassipes* and *S. minima* at the Dam were overall interchanging due to insect feeding on *M. scutellaris* reducing *P. crassipes* populations thus allowing *S. minima* to become dominant. Intense feeding from *M. scutellaris* resulted in the reduction of *P. crassipes* populations. During late autumn and winter, remaining *P. crassipes* plants experienced frost damage from cold temperatures. The decrease in *P. crassipes* allowed *S. minima* to successfully invade, potentially as the result of less competition from *P. crassipes*, and therefore available resources, such as space, nutrients, and light. However, as spring approached, *P. crassipes* regenerated from its seed bank, and *S. minima* populations diminished. The changes in dominance were observed in 2021 and 2022 but in 2023, this trend was not evident as *S. minima* did not dominate as in previous years.

This thesis investigated the combination of competition and herbivory by *M. scutellaris* on the vigour of *P. crassipes* and *S. minima* to understand the dynamics of these two highly invasive species. Competitive abilities of *P. crassipes* and *S. minima* were determined using an inverse linear model with plant weight as the yield variable. In the absence of herbivory, *P. crassipes,* was 4 times more competitive than *S. minima*, but as competitive when exposed to *M. scutellaris* feeding. *Salvinia minima* was 1.2 times as aggressive as *P. crassipes* in the absence of herbivory, but 2.6 times as competitive when *M. scutellaris* was established on *P. crassipes*. In the presence of herbivory on *P. crassipes*, interspecific competition coefficients from *P. crassipes* on *S. minima* were no longer statistically significant. These results indicate that the competitive ability of *P. crassipes* was reduced through herbivory when grown with *S. minima*, explaining the temporal dominance between the two species at Hartbeespoort Dam.

Some studies have reported that *P. crassipes* releases allelochemicals as a competitive strategy to algae and phytoplankton. Previously identified allelochemicals include N-phenyl-1-naphthylamine, N-phenyl-2-naphthylamine and linoleic acid. This thesis investigated the presence or absence of key chemical compounds released by *P. crassipes* during allelopathy with microbes and phytoplankton to determine its potential to inhibit *S. minima* growth. Methanol extracts from *P. crassipes* roots, leaves, and field samples underwent LC-ESI-MS/MS analysis, creating a molecular network to match chemical profiles. Of the investigated compounds, N-phenyl-1-naphthylamine and N-phenyl-2-naphthylamine were absent in *P. crassipes* and field samples, while linoleic acid was consistently found. Its presence suggests its potential defensive role against *S. minima*. Future research should explore allelochemical production in the presence of *S. minima* and algae to confirm if *S. minima* elicits an allelochemical response by *P. crassipes*, or whether the methods used here were insufficient to detect allelochemical production. Understanding whether *P. crassipes* employs allelochemicals, especially in the presence of *S. minima*, could shed light on its competitive advantage beyond its invasive nature at the Dam.

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CHAPTER 1

General Introduction and Literature Review

1.1 Problem statement

Anthropogenically-mediated activities, notably global trade, travel, and climate change have enabled the spread of organisms beyond their natural distribution boundaries (Mack et al. 2000, Banks et.al. 2015). Introduced species arrive in new environments and some have devastating consequences (Pejchar & Mooney 2009), an issue which has been declared a global concern (Mack et al. 2000, Dudgeon et al. 2006, Blackburn et al. 2011). However, the consequences of these introduced species extend beyond ecological concerns and can also impact human health and livelihoods in novel environments (Pyšek & Richardson 2010, Shackleton et al. 2019). The naturalisation of invasive species in novel ranges may threaten extant biodiversity, and food and water security as they often tend to perform better in novel environments due to a lack of natural enemies (Banks et al. 2001, Ricciardi & Cohen 2007).

A commonly accepted driver behind many exotic plant invasions is the Enemy Release Hypothesis (ERH). ERH states that after the introduction phase, invasive plant species proliferate in their introduced environments because they do not need to invest in defence structures (physical or chemical) due to reduced specialist herbivore pressure (Blossey & Notzold 1995). Following the establishment of an invasive plant in an environment, it begins to spread rapidly, out-competing native plant species for resources, including water, nutrients, space, and light. Invasive species are thus fundamental drivers of global change and ecosystem structure and function (Pyšek & Richardson 2010, Blackburn et al. 2011, Simberloff et al. 2013). Due to their ability to transform the structure and function of recipient ecosystems, sometimes irreversibly, invasive alien species,

together with habitat destruction, are ranked as number one on the list of the direct drivers of change in nature by the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) (Kumar Rai & Singh 2020).

Worldwide efforts are underway in managing invasive species, with biological control resulting in successful control of many species (McFadyen 1998, Culliney 2005, Van Driesche et al. 2010). Invasive plant management often succeeds in suppressing targeted invasive plants (Pyšek & Richardson 2010). However, a rapidly emerging problem is that of secondary invasion — an increase in the abundance of non-target exotics following the clearing of targeted invasive plants (Kettenring & Adams 2011, Pearson et al. 2016, Nsikani et al. 2019, 2020). Given that most ecosystems contain multiple species of invaders (Kuebbing et al. 2013), the threat of secondary invasion is a potentially ubiquitous problem requiring immediate attention (Reid et al. 2009, Ortega & Pearson 2011, Kettenring & Adams 2011, Skurski et al. 2013, Dickens et al. 2016, Pearson et al. 2016, Nsikani et al. 2020) and it is particularly relevant to the case of Hartbeespoort Dam (Coetzee et al. 2022b), one of South Africa's hypertrophic system, the topic of this thesis.

1.2 Invasive aquatic plants in South Africa

1.2.1 Brief history

Invasive alien aquatic plants have been a global challenge (Mack et al. 2000, Blackburn et al. 2011, Hussner et al. 2017) and South Africa is no exception since the introductions of these plants in the early 1900s (Hill 2003). The country is among one of the most invaded countries (Hill et al. 2020) and has faced the introduction and establishment of numerous invasive aquatic species, which have had significant negative impacts on its natural ecosystems, agricultural productivity, and human livelihoods (Coetzee & Hill 2012). Historically, groups of people from diverse areas across the world migrated to South Africa as early as 200 AD (Faulkner et al. 2020). These migrations

introduced alien invasive species from their natural range either intentionally aiming to establish them in agriculture, horticulture, as garden ornaments or forestry or accidentally through people, goods, and transport vessels (Padilla & Williams 2004, Cohen et al. 2007, Martin & Coetzee 2011). This phenomenon is known as biological invasion, which refers to the introduction pathways of non-native species into areas outside their natural range, as a result of human activities (Richardson & Pyšek 2010).

Aquatic invasive species are among the worst invasive species in South Africa which pose significant threats to aquatic ecosystems and biodiversity (Hill 2003, Hill et al. 2020a). The establishment of invasive alien aquatic species is in part due to favorable environmental conditions in the area of introduction (van Wilgen et al. 2020). Due to water scarcity in the country; South Africa has constructed impoundments to meet its water supply needs (Ilemobade et al. 2008). However, these impoundments are mostly constructed in densely populated urban areas and receive an inflow of phosphate and nitrate pollution from improperly treated waste and wastewater resulting in nutrient-enriched water systems which is an undesirable outcome (Van Ginkel et al. 2000, Hill 2003, Coetzee & Hill 2012). Consequently, human activities such as urbanization, industrial and agricultural pollution have also led to a rise in nutrient levels in South African waterbodies, termed eutrophication (Coetzee & Hill 2012). As a result of eutrophication, aquatic ecosystems in South Africa have become susceptible to invasive macrophytes. After the introduction, many aquatic invasive plants have been able to thrive in these eutrophic waterbodies including Hartbeespoort Dam (Hill 2003).

Hartbeespoort Dam, a significant artificial reservoir in South Africa, was constructed in 1923. Situated approximately 53 km northwest of Johannesburg and 30 km west of Pretoria, this large reservoir serves primarily for irrigation and domestic water supply (Hart & Matthews, 2008). Additionally, it has gained popularity as a recreational destination, attracting a substantial residential population, and becoming a favoured spot for tourists visiting the country. Located

downstream of densely populated urban areas, Hartbeespoort Dam receives substantial nutrient loads through its tributaries, namely the Crocodile, Hennops, and Magalies rivers. Over the years, these nutrient inputs have contributed to the dam's eutrophication, thus invasive aquatic plants have found favourable conditions for growth and are quickly established after introduction.

Hill & Coetzee (2017) highlighted that aquatic invasive macrophytes have key life history attributes which allow them to invade an introduced area, and these include rapid sexual and vegetative reproduction which aids population increase over a short period, high dispersal mechanisms and high phenotypic plasticity. To a greater extent, the lack of natural enemies, coupled with eutrophication, has allowed these invasive species to perform exceedingly well against native aquatic plants posing severe threats to native species (McFadyen 1998, Coetzee & Hill 2012).

Invasive aquatic weeds pose massive threats globally (Wilcove et al. 1998), but their effects are greatest in ecologically diverse and sensitive areas such as southern Africa (Latimer et al. 2004, Gibson et al. 2013). Since early introductions in the 1910s, South Africa's aquatic ecosystems have been invaded by many aquatic invasive plants including the "big bad five" (Hill et al. 2020a). These include *Pontederia crassipes* Mart. (Pontederiaceae), which remains problematic in South Africa, *Pistia stratiotes* L. (Araceae) (water lettuce); *Azolla filiculoides* Lam. (Azollaceae) (red water fern), *Salvinia molesta* D.S. Mitch. (Salviniaceae) (giant salvinia); and *Myriophyllum aquaticum* (Vell.Con.) (parrot’s feather) (Hill 2003, Hill & Coetzee 2017, Hill et al. 2020a). These invasive aquatic plants have been declared the "big bad five” due to their severe impacts on aquatic ecosystems globally and in South Africa (Hill et al. 2020a). However, of these five invasive species, with the exception of *P. crassipes* four are no longer considered invasive as they have been completely controlled using biological control (Coetzee et al. 2011).

1.3 Impacts of invasive aquatic plants on ecosystems

The impacts posed by aquatic invasive species are well studied and there is a vast amount of literature globally (e.g., Cilliers et al., 2003; Coetzee et., 2018). Aquatic invasive plants pose negative impacts on ecological systems (affecting floral and faunal diversity), social and economic activities where they occur (Hill 2003, McConnachie et al. 2003). Because invasive aquatic plants form dense mats, the impacts posed are quite similar, but some impacts may be greater on a large scale as some plants invade large-scale water bodies (Hill et al. 2020a). These impacts include reductions in domestic or recreational purposes, clogging of irrigation canals, drowning of livestock, breeding sites for vectors of human and animal disease, and reduction in light thus reducing oxygen levels altogether deteriorating aquatic macroinvertebrate biodiversity (Holm et al. 1969, Shillinglaw 1981, Midgley et al. 2006, Coetzee et al. 2014, Hill et al. 2020a). The high cost of control and change in stream ecology (Cilliers 1991a, Hill 2003, McConnachie et al. 2003) reduces the quantity and quality of irrigation water, increases danger of floods, increases siltation of rivers, dams, and wetlands (Clayton 1996, McConnachie et al. 2003, Clayton & Champion 2006). Such severe environmental impacts affect economic activities that depend on these resources (Coetzee & Hill 2012). In addition to causing physical disturbances, invader biomass can also emit allelopathic compounds that suppress and outcompete native macrophyte species (Corbin & D'Antonio, 2011), which is the subject of this thesis. Aquatic species differ in their morphological, physiological, and biochemical traits. Thus, the magnitude of impacts differs based on life traits and habitat (Hill et al. 2020a) and South Africa has played a significant role in understanding and managing invasive aquatic plants.

1.4 South Africa's approach to managing invasive aquatic plants.

Historically, South Africa has made significant contributions in understanding the ecology and management of biological invasions by empowering the development of an invasion science aimed at understanding invasive species and mitigating their impacts on ecological systems (Pyšek et al. 2008, van Wilgen & Richardson 2012, Moran et al. 2013, Hill et al. 2020b). South Africa has one of the most active communities in biological invasions in the world owing to strong collaborations, with strengths in theoretical and applied invasion science, and world-leading expertise in specific sub-disciplines (Abrahams et al. 2019). National-level efforts to address invasive species have included reports and discussions on the pathways of introduction (Zimmermann et al. 2004, Hulme 2015), how they spread (Faulkner et al. 2020), an overview of the status of alien species based on research evidence and published reports and papers (van Wilgen et at. 2018), an overview of invaded areas (van Wilgen et al. 2020), reports on the effectiveness of control and management measures (van Wilgen et al. 2018), an understanding of the knowledge gained over the past centuries and gaps in biological invasions and future directions (Hill et al. 2020b). These publications have addressed the development of invasion science and its applications in mitigating the impacts of invasive species on ecological systems. While South Africa has made progress in studying invasive alien species with varying degrees of success and setbacks, the country's efforts are commendable in terms of their contributions to the wider field of invasion science (van Wilgen et al. 2020).

In response to understanding invasion science and the detrimental impacts of invasive aquatic plants on ecosystems, active intervention measures that focus on prevention and control options were required. Several approaches to controlling aquatic invasive plants methods include mechanical and manual removal, chemical, biological and integrated control (Hussner et al. 2017,

Hill et al. 2020a). These approaches aid in reducing the impact of these aquatic invasive plants on ecosystems and can provide economic relief. Controlling invasive aquatic plants can be challenging, particularly in waterbodies that are fully infested. The implementation of control methods may be constrained and encounter setbacks in such situations. As such, there has been a shift towards an integrated approach that incorporates multiple methods to effectively manage these species over time (Cilliers et al. 1996, Coetzee et al. 2011, Hill et al. 2020a).

1.4.1 Control and management of aquatic invasive plants

1.4.1.1 Mechanical removal

Mechanical and manual removal of aquatic invasive plants involves the use of machines and manpower to remove invasive plant biomass physically (Hill 2003). The Working for Water programme is an example of an initiative that has practiced mechanical removal of invasive aquatic plants in South Africa by employing teams of workers to manually remove plants such as *P. crassipes*.

Although this method provides immediate improvement in infestation levels after removal, it is not viable for sustainable control of the highly invasive species due to the growth rate of most invasive aquatic plants making it difficult to manually control, such as *P. crassipes* and it recruits from seed, leading to new infestations and clonal spread (Hill 2003, Coetzee & Hill 2012, Hill & Coetzee 2017, Hill et al. 2020a). Also, there is a great possibility of regrowth from plants when natural enemies are destroyed during clearing and is thus not always compatible with biological control (Mailu 2001). Thus, this method is not efficient for *P. crassipes*. Additionally, this method can be labor-intensive and expensive, making it less cost-effective (Hill et al. 2020a).

In some instances, the inability of machines to move across the water body limits the success of mechanical control, as demonstrated in an international study at Lake Victoria in Kenya, where

attempts to harvest *P. crassipes* resulted in re-infestation within a short period. The mechanical removal of biomass in some instances destroys the plants, causing them to sink and decompose (Mailu 2001). This exacerbates their impacts on the loss of aquatic animals due to the increased activity of harmful and toxic bacteria that thrive on decaying plants and water deoxygenation (Villamagna & Murphy 2010).

Because the method only provides temporally relief from invasions, attempts to control some of the most aquatic invasive plants in South Africa seem impractical and unrealistic (Hill 2003). Therefore, this method is not promoted in South Africa although some managers do still deploy this method (Hill 2003, Hill et al. 2020a).

1.4.1.2 Chemical control

Herbicides provide another method for controlling aquatic invasive weeds and are relied upon by scientists and agricultural land managers to limit the spread of ecologically and economically threatening weeds (Hill & Coetzee 2008). The use of herbicides is widely used in South Africa (Hill 2003) and involves the application of a phytotoxic chemicals to plants, with active ingredients such as 2,4-D (2.4-Dicholorophenoxyaceticacid) and Glyphosate [N-(phosphonomethyl)glycine]. Upon application by water authorities in invaded areas, these active ingredients are absorbed by plant tissues and translocated into the internal vascular system of the plants (Bromilow et al. 1990) resulting in the death of the plant. Herbicidal control is also faster and more efficient than mechanical for controlling large mats of invasive water weeds, such as *P. crassipes* in South Africa.

However, while herbicides can be effective in controlling invasive species, there are concerns over their potential ecological impact, such as non-target species being affected, as herbicides lack specificity and the possible accumulation of toxins in the environment and biological control

agents. Hill et al. (2012) showed that several herbicides employed in South Africa can be toxic to certain biological control agents.

The use of herbicides can also lead to the evolution of resistance to the active herbicidal compounds, making them ineffective on the target weeds (Culliney 2005). Moreover, herbicidal control programmes often fail due to a lack of follow-up and commitment, and for any herbicide programme to succeed against water weeds, it is crucial to commit to a long-term operation (Hill & Olckers 2001, Coetzee et al. 2009). Finally, herbicide programmes are often costly, both financially and ecologically, as the chemicals are expensive to use over large proportions of water bodies, and often require aerial applications through airplanes or helicopters. Similar to the mechanical methods, after the application of chemicals, the dense mats of plant biomass sink and decompose at the bottom of the dam, leading to deoxygenation of the water, and damage to fish populations (Mailu 2001). In addition, populations regenerate from any plants not killed during the application.

Herbicides can be an effective method of controlling aquatic invasive weeds however, an integrated approach of chemical control and biological control is suggested to reduce invasive aquatic plant coverage altogether avoiding damage to native organisms (Jadhav et al. 2008).

1.4.1.3 Biological control

Biological control is considered the most viable method for managing invasive species globally and specifically in South Africa (Hill et al. 2020b). It involves the intentional release of host-specific herbivorous insects, mites, or pathogens, known as biocontrol agents, to control pest plants by reuniting them with their natural enemies (Center et al. 2001). This method is effective, environmentally friendly, and can provide a permanent solution for managing invasive water weeds (McFadyen 1998, Coetzee et al. 2011, Hill et al. 2020a, 2020b). A classical biological control programme aims to establish the introduced agents in their new environments and increase

their abundance to levels that damage the target species (Van Driesche et al. 2010). By reducing the reproductive output, competitiveness, and population growth of invasive species, biocontrol agents help keep them below ecologically, economically, and socially determined thresholds.

In South Africa, the use of biological control against invasive aquatic plants has grown since the implementation of the first biological control programme in 1974 against *P. crassipes* (Hill 2003). Numerous reviews and reports have been published to provide accounts of the progress made in this method (Coetzee et al. 2011, Moran et al. 2013, Hill & Coetzee 2017, Hill et al. 2020b, van Wilgen et al. 2020).

One of the advantages of biological control is its minimal impact on natural environments, as the released agents can effectively damage the weeds without requiring continual follow-up efforts, unlike other control methods (Van Driesche et al. 2010). Biological control is considered a low-risk practice, as it is unlikely to harm non-target species. Thorough host-specificity testing is conducted before the release of biocontrol agents to minimize the possibility of non-target effects (Tipping et al. 2011). Non-target effects are rare in biological control programmes, and termination of a programme may occur if the agent is not host-specific (Tipping et al. 2011). An example of this is the rejection of the moth, *Bellura densa* (Walker) as a biocontrol agent against *P. crassipes* in South Africa because it primarily feeds on *Pontederia cordata* and *Colocasia esculenta* (Center & Hill 2002).

In South Africa, researchers have focused on importing potential biocontrol agents, conducting pre-release host-specificity studies, releasing new agents, and evaluating their effectiveness against aquatic invasive plants. These efforts have been largely successful, with high establishment rates and reductions in target aquatic weed populations, and their consequent impacts on the environment (Coetzee et al. 2011, Hill & Coetzee 2017 & Hill et al. 2020b). The biological control programme against invasive floating weeds in South Africa has been highly successful, with four

of the major weeds (*P. stratiotes*, *S. molesta*, *M. aquaticum*, and *A. filiculoides*) considered to be under complete control using only one species of biocontrol agent per species (Hill & Coetzee 2017).

For an invasive species to be considered under complete control, no other control methods should be required to manage the weed populations at a level where they no longer impact aquatic biodiversity and water utilization (Hill 2003, Coetzee et al. 2011). Many other aquatic and terrestrial invasive plant species in South Africa have been targeted for biological control, with different degrees of success (Hill & Coetzee 2017). Partial successes of biological control programmes encompass scenarios where other control methods (chemical control) remain commonly required but with a significantly reduced frequency of application. Additionally, partial successes may also encompass situations where biological control achieves positive results only in specific areas of the weed-infested area (Hokkanen & Sailer 1985).

The degree of success of biological control requires long-term monitoring such as post-evaluation studies to monitor the populations of the invasive species. Key factors to observe in these evaluations encompass the establishment of biological control agents, decreases in the vigor of the host plants and finally the reductions in host population sizes following agent introductions. Hill and Coetzee (2017) propose that competitive interactions between species may be a key determinant of the success of biological control programmes.

1.5 Competitive interactions

Competitive interactions are crucial in shaping and maintaining ecosystem structure and function. Competition occurs between different species (interspecific) and among individuals of the same species (intraspecific) when they compete for limited resources (Lacoul & Freedman 2006). In invasion biology, competition plays a significant role, with successful invasions often attributed

to the absence of herbivory (Keane 2002), allowing invasive species to allocate more resources to growth and competitive ability. This advantage can lead to the outcompeting of native species, altering ecosystem dynamics. Aquatic systems are particularly influenced by competitive fitness, as it determines the success of invasive species establishment and the resilience of ecosystems to invasion (Joshi & Vrieling, 2005).

Several factors affect competitive interactions in aquatic ecosystems, including nutrient concentrations in sediment and water, light intensity, dissolved oxygen, temperature, climate, and species growth forms. Mony et al. (2007) examined the effects of season and sediment nutrient concentration on interspecific competition between two submerged macrophytes, *H. verticillata* and *E. densa*, in North America. In this study, interspecific competition was higher in spring compared to autumn. This study along with several others (Haller & Sutton, 1975; Van et al.1999) found a weakened competitive capacity of *H. verticillata* under low nutrient concentrations when competing against native species with similar growth forms. Additionally, competition from *H. verticillata* promoted resource allocation to the roots of *E. densa*, illustrating the effects of competition at both the community and species levels (Mony et al. 2006; Haller & Sutton, 1975; Spencer and Ksander, 2000). In determining competitive fitness, top-down mechanisms such as herbivory by natural enemies, including biocontrol agents, can also play a significant role (Callaway et al. 1999).

1.5.1 The effect of biocontrol agents on the competitive ability of plants

Biological control agents are applied to help control weed populations by feeding on them, thereby reducing their vigor and giving native species a competitive advantage (Callaway et al. 1999). However, it can be difficult to measure the feeding damage inflicted by biological control agents on plants, particularly when it does not result in direct mortality (Center et al. 2001). Several studies have shown that plant stressors from neighbouring plants, such as competition, can often

magnify the impact of herbivory (Coetzee et al. 2005). When grazed plants are competing with other plants for resources, the impact of herbivory becomes more noticeable (Center et al. 2001).

Previous studies have focused on the interaction between herbivory and plant competition, and their combined effects on plant performance, as a way of assessing the value of new agents (Ang et al. 1994, Van et al. 1999, Story et al. 2000). Center et al. (2001) suggested that the impact of invertebrate herbivory becomes more noticeable when the grazed plants are competing with other plants for resources, leading to a gradual reduction of the host plant's vigor. Additionally, Crawley (1989) noted that a herbivore's principal effect is not from completely eating plants to mortality, but more often from feeding modifying their relative competitive abilities of the plants with one another. As a result, any reduction in the competitive ability of targeted plants may make them more susceptible to competition from other plants (Whittaker 1979, Cottam 1986). Thus, using biological control agents to weaken the invader while native species gain a competitive advantage has been justified (Callaway et al. 1999). As a competitive strategy within aquatic ecosystems, attention has been drawn to the role of allelopathy as a competitive strategy in species abundance, which is one of the foci of this thesis.

1.5.2 Allelopathy in aquatic ecosystems

Aquatic plants of different species can have positive, negative, or neutral interrelations, which may lead to the preferential establishment and growth of certain species, outcompeting others (Agami & Reddy 1990). In addition to competing for limited nutrients, some aquatic plants use allelopathy as a competitive strategy. Allelopathy has long been intriguing as a theory explaining why certain plants or algal species predominate over others (Jin et al. 2003).

Allelopathy refers to chemical interactions between and among plants and microorganisms through the release of biologically active chemical compounds into the environment (Keating 1999). These

chemicals can either have a positive or negative effect on the surrounding organisms, and to some extent can affect the growth, survival, reproduction, health, and behaviour of surrounding plants or organisms (Chaib et al., 2021). Investigating the potential of an integrated approach to understanding allelopathy in invasive plants across South African waterbodies, especially on highly invasive species such as *P. crassipes*, is thus worth considering for better invasive species management strategies.

1.6 Study species 1: water hyacinth (*Pontederia crassipes*)

1.6.1 Origin and distribution

There is a vast amount of literature to date on *P. crassipes* globally owing to its highly invasive nature, and its impact on invaded aquatic ecosystems (Coetzee et al. 2011). *Pontederia crassipes* is native to the Amazon Basin, in countries including Brazil, French Guiana, Guyana, Surinam, and Venezuela (Wolverton & McDonald 1979, Wittenberg & Cock 2001). However, the plant has spread throughout the tropical, subtropical, and warmer temperate regions of the world since the late 1800s. In South Africa, *P. crassipes* was first recorded on the Cape Flats in 1908 and was introduced to KwaZulu-Natal at about the same time (Scott et al. 1979). Due to its attractive flowers, *P crassipes* was cultivated as an ornamental plant in South Africa in the early 1900s (Guillarmod 1979) which inevitably led to anthropogenic spread by aquarium owners, gardeners, and boating enthusiasts (Tipping et al. 2011). In South Africa, *P. crassipes* was classified as a serious invader in the 1970s, eventually becoming the worst invasive aquatic plant (Lowe et al. 2000), degrading the health and ecology of aquatic ecosystems (Holm et al. 1997; Cilliers 1991b). *Pontederia crassipes* has spread in South African waterbodies and is now found in both sub-tropical and temperate areas, and its invasive nature has resulted in its prohibition under the Conservation of Agricultural Resources Act ([CARA] Act 43 of 1983) and the National Environment Management: Biodiversity Act ([NEMBA] Act 10 of 2004) as a Category 1b

invasive species. These acts mandate control measures for *P. crassipes* in the country. However, the survival attributes of *P. crassipes* results make efforts to control the weed complicated.

1.6.2 Survival attributes of *Pontederia crassipes* and growth form

Pontederia crassipes is a free-floating herbaceous plant from the family Pontederiaceae (Pellegrini et al. 2018) which usually produces about 6–10 green leaves arranged in basal rosettes or elongated petioles. The general growth form of the shoot is monopodial, with the leaves being produced in a whorl. When many plants of *P. crassipes* emerge from one point, their petioles elongate and overlap to form dense, self-supporting mats that can cover the surfaces of dams, lakes, and rivers (Ray & Hill 2012).

Pontederia crassipes produces inflorescences of flowers with violet-blue petals (Figure 1.1), which produce prolific numbers of seeds. Each plant can produce over 3000 seeds annually (Barrett 1980). In some instances, it can recruit from the small, long-lived seeds (which may remain viable for up to 28 years), which sink and stay dormant in the sediment until they are ready to germinate when conditions become favourable (Matthews 1967, Gopal 1987). The seeds germinate mostly on moist sediments or in warm shallow water and flowering can occur 10-15 weeks thereafter (Barrett 1980). Failure to germinate limits seedling recruitment and populations increase mainly by vegetation means, through ramet production. Branching is sympodial with ramets (vegetatively produced plants) formed from axillary buds on stolons produced through the elongation of the internodes. As ramet production proceeds and crowding begins to occur, the newly formed leaves tend to become elongated rather than swollen. The vegetative growth form of *P. crassipes* is sympodial and individual ramets produce clones that form extensive floating mats supporting canopies that, in mature stands, extend a meter or more above the water surface (Center and Spencer 1981).

Figure 1.1: *Pontederia crassipes* plant with attractive flowers (Photo credit: David Taylor, Centre for Biological Control).

The stolons of *P. crassipes* produce numerous roots, with an abundance of fibrous roots (Penfound & Earle 1948, Center & Dray 2010, Nesic & Jovanovic 2013). New daughter plants are produced via the plant's stolons, and the ability of *P. crassipes* to reproduce both sexually and asexually results in rapid growth and spread, allowing it to cover a wide range within a short time (Ndimele & Jimoh 2011, Elenwo & Akankali 2016). According to Ashton et al. (1979), under ideal growth conditions, *P. crassipes* has a growth rate of up to 6% per day.

Several attributes of *P. crassipes* have contributed to its success as the world's worst invader in aquatic ecosystems. These include the ability to multiply through clonal reproduction, the high

mobility of its free-floating form, a very high rate of growth under favourable environmental conditions, the ability to re-infest via seed banks or from plant fragments and limited genetic diversity (Zhang et al. 2010). These growth characteristics of *P. crassipes* highly depend on the nutritional composition of water and space available for growth (Reddy & Sutton 1984, Sutton et al. 2016a, Coetzee et al. 2017). In the absence of its natural enemies, *P. crassipes* rapidly becomes invasive and damaging in nutrient-enriched waters. In South Africa, *P. crassipes* has taken advantage of nutrient-rich water systems posing severe ecological and environmental impacts (Coetzee et al. 2021).

1.6.3 Ecological and environmental impacts of *Pontederia crassipes*

Several studies in the literature provide valuable information on the ecological impacts of *P. crassipes* on aquatic systems around the world. In South Africa, the issues associated with *P. crassipes* include a reduction in water quality, the suppression of natural aquatic vegetation, loss of water from impoundments due to high rate of evapotranspiration, the obstruction of river flows, which may result in flooding and increased siltation. *Pontederia crassipes* also blocks sunlight penetration into the waterbody, outcompetes indigenous flora, and alters aquatic biodiversity (Jafari 2010, Villamagna & Murphy 2010).

Vilà et al. (2011) provide evidence that invasive plant species exert pressure on many ecological factors. The study suggests that invasive plant species, including *P. crassipes*, reduce local plant species diversity and increase the invader plant community's plant production. When invasive plants invade, some indigenous aquatic plants have difficulty surviving, thereby disrupting the aquatic ecosystem dynamics and resulting in trophic cascades within the aquatic community and causing extinctions over time (Chapungu et al. 2018).

The large mats of *P. crassipes* prevent the transfer of oxygen from the air to the surface and decrease oxygen production by other plants and algae because of limited photosynthesis in the water column (Villamagna & Murphy 2010). Oxygen deficiency in water bodies has detrimental effects on water quality, causing the death of plants and animals within the water bodies. The decomposing dead biomass further depletes the oxygen content in the water, and water quality deteriorates, which is linked to increased drinking water costs (Ndimele & Jimoh 2011). Furthermore, decomposing matter, including dead *P. crassipes* plants in the water bodies, is broken down by anaerobic bacteria and produces carbon dioxide. In solution, carbon dioxide forms carbonic acid which lowers the water's pH with lethal effects on the water inhabitants, affecting all fauna (Mironga et al. 2012). Moreover, the plant also provides breeding opportunities for mosquito larvae and a habitat for disease vectors such as bilharzia snails, posing a potential health risk and the limitation of recreational use of affected bodies (van Wyk and & Wilgen 2002).

1.6.4 Management measures of *Pontederia crassipes*

Although significant research has been conducted on the biology and ecology of *P. crassipes*, achieving complete control of this invasive species remains a challenge in the country (Hill & Coetzee 2017). Over the past few decades, extensive studies have focused on various aspects, including the plant's physiology, reproductive biology, ecology, environmental impacts, control options, and potential utilization.

An evaluation by van Wyk and van Wilgen (2002) assessed the cost-effectiveness of different management strategies for *P. crassipes*. The study revealed that herbicide application, despite being commonly used, was not the most cost-effective method of control. Herbicides necessitate ongoing commitment to follow-up measures and evaluation to prevent re-infestation from surviving plants or seeds. In contrast, biological control was suggested to be a more economical approach, as the biocontrol agents can establish themselves and sustain populations without

constant intervention (Hill 2003). However, the study proposed an integrated management that combines manual, chemical, and biological control methods, which currently represent the predominant management strategies employed in the country (Wyk and van Wilgen 2002)

1.6.5 *Pontederia crassipes* biological control agents

The biological control programme implemented against *P. crassipes* is the largest biocontrol programme for aquatic weeds in the country (Coetzee et al. 2011). Due to its wide distribution, rapid growth, and negative environmental impacts, extensive research and control efforts have been dedicated to combating this invasive species (Cilliers 1991b, Hill & Olckers 2001, Coetzee et al. 2011, Hill & Coetzee 2017, Hill et al. 2020b).

The biological control programme against *P. crassipes* was initiated in 1974 with the release of the leaf-chewing weevil *Neochetina eichhorniae* (Cilliers 1991a). However, the programme was temporarily terminated in 1975 due to heavy herbicidal control measures in place at the time. In 1985, following a request from the Department of Water Affairs and Forestry to explore alternative control methods, the biological control programme was reinstated, making it the longest-running biocontrol programme in the country (Cilliers 1991b). Since then, nine species of biocontrol agent have been released, with the most recent release in 2013, the water hyacinth planthopper, *Megamelus scutellaris* (Coetzee et al. 2014, 2021, Hill & Coetzee 2017).

However, despite the introduction of multiple agents, the control of *P. crassipes* remains problematic in some areas (Hill & Coetzee 2017). Several factors identified by (Hill & Olckers 2001) constrain the effectiveness of biological control agents against *P. crassipes.* These include the inappropriate use of herbicides (Hill et al. 2012), which have an adverse impact on biocontrol agents, as well as cold winters in temperate regions that restrict the population growth of these agents. Additionally, the presence of eutrophic waters enables *P. crassipes* to compensate for

herbivory, and the limited size of infested systems prevents the effective dispersion of agent-infested weed mats due to the lack of sufficient wind fetch.

To address these limitations, extensive research has been conducted to enhance the understanding of potential constraints and improve the effectiveness of biocontrol agents against *P. crassipes* in South Africa (Coetzee et al. 2011, Hill & Coetzee 2017, Hill et al. 2020a, 2020b). This has led to the release of additional agents, such as *M. scutellaris*, which can be established in cooler regions (Tipping et al. 2011). Furthermore, researchers have gained insights into the thermal requirements of both old and new agents and have emphasized the release of a high number of agents during optimal times, such as spring after herbicide application. Multiple mass-rearing facilities, including the Centre for Biological Control's Waainek Mass Rearing Facility at Rhodes University and the South African Sugar Research Institute, have been established to support the rearing and inundative release of agents (Hill et al. 2021).

Although *P. crassipes* is not yet fully controlled through biological means, some areas have shown successful biological control, while in other areas, it has resulted in reduced populations and impacts, necessitating the use of alternative control methods like herbicide applications (Cilliers 1999, Jones et al. 2018). Several studies have highlighted the varying effectiveness of biological control efforts and the need to keep releasing high numbers of effective biocontrol agents such as *M. scutellaris* (Coetzee et al. 2011, 2021, Hill et al. 2021).

1.7 Biocontrol agent: *Megamelus scutellaris*

1.7.1 Life history and biology

Megamelus scutellaris is a small (2.37 mm) multivoltine planthopper (Sosa et al. 2005) that was introduced as a biological control agent against *P. crassipes* in South Africa in 2013. *Megamelus scutellaris* is currently the most recent agent released against *P. crassipes* in South Africa, and it

has established well and damages *P. crassipes* in cooler areas of the country where other agents have traditionally struggled to establish and have an effect (Miller et al. 2021). The planthopper produces multiple overlapping generations per year and exhibits wing dimorphism with a macropterous long-winged dispersal form capable of flight and a brachypterous short-winged, non-flying form (Sosa et al. 2005).

The wing form in many Delphacids, including *M. scutellaris*, is influenced by density-dependent factors such as intraspecific competition (Denno et al. 1994). The wing formation of the planthopper is usually determined by several factors such as host plant quality, photoperiod, temperature, and density-dependent factors (Denno et al. 1994). However, it is challenging to confirm this in controlled environments (Fitzgerald & Tipping 2013). When habitats are persistent, the planthopper does not need to migrate to escape their environment, hence wingless forms may appear. Although wings are necessary for migration in resource-limited or overcrowded conditions, they can be a metabolic and reproductive hindrance to the planthopper. As a result, in persistent habitats, brachypters (no wings) are more likely to be more fecund, reproduce earlier, and have shorter intervals between oviposition events (Denno et al. 1991). Brachypterous females of many planthopper species, including *M. scutellaris*, have shorter preoviposition periods, reproduce at earlier ages, and are usually more fecund than their macropterous counterparts (Denno et al. 1991). Preliminary studies conducted in quarantine with *M. scutellaris* found limited macroptery despite high insect densities and lower-quality plants (Sosa et al. 2007). These findings suggest that even in less-than-optimal conditions, brachypters may be more advantageous for reproduction in persistent habitats.

Sosa et al. (2005) observed that adults of *M. scutellaris* are lighter in colour with blackish stripes on the abdomen and thorax. Adult *M. scutellaris* usually mate near the base of the emergent portion of the plant and on the upper leaves soon after ecdysis. While many species of Auchenorrhyncha use acoustic signalling for mating and territoriality, it is unclear whether *M. scutellaris* utilizes

acoustic signalling for these purposes (Claridge 1990). Following mating, females begin to oviposit within the apical portion of the petiole and the lamina of *P. crassipes* tissue, often leaving behind oviposition scars characterized by three parallel tracks. Each oviposition site typically contains two eggs (Tipping, unpublished data), which are ellipsoidal with a sharp cephalic apex and a rounded caudal end. They are milky white when laid and yellowish white with reddish eye spots before hatching (Sosa et al. 2005). Eggs are laid on scars on the leaf petioles of *P. crassipes* and nymphs emerge approximately 7-15 days after oviposition. The emerging nymphs shed an embryonic membrane and begin feeding at the base of the plant near the water surface. Nymphs develop through five instars and feed on abaxial and adaxial lamina surfaces and petioles. Maturation from the first to the fifth instar takes 15 days in a controlled environment and about 25 days under natural conditions (Sosa et al., 2005).

1.7.2 Impact of *Megamelus scutellaris* on *Pontederia crassipes*

Megamelus scutellaris is a host-specific insect that feeds on the phloem sap of its host plant, *P. crassipes*, through a three-segmented rostrum, which punctures the plant tissues and creates salivary sheaths (Sosa et al. 2005, Tipping et al. 2011) (Figure 1.2). When present in high-density numbers, *M. scutellaris* can cause chlorosis of the leaves, leading to plant stress and a decrease in leaf number and seed production, ultimately reducing the plant's vigour (Sosa et al. 2007). The insect's feeding activity also creates wounds that can serve as entry points for fungal pathogens, leading to further damage and reduction in plant vigour (Sutton et al. 2016). Thus, *M. scutellaris* is a valuable biological control agent for managing *P. crassipes* in South Africa, along with other agents, due to its host specificity and feeding behaviour.

Figure 1.2: Life stages of *Megamelus scutellaris* insects. (a) *Megamelus scutellaris* long-winged (macropterous) females, (b) *Megamelus scutellaris* wingless (brachypterous) female, and (c) Adults and nymphs on *Pontederia crassipes* leaf; (Photo credit: David Taylor, Centre for Biological Control).

Megamelus scutellaris was initially designated for release in the US to reinforce the impacts of *Neochetina* weevils, which had been released previously to control *P. crassipes*. This was effective as *M. scutellaris* can reproduce rapidly and have a relatively short generation time (Mattison et al. 2017). *Megamelus scutellaris* was then studied as a candidate for release in South Africa to determine if the agent can establish in cold winter temperatures. In their studies, May and Coetzee (2013) found that the agent was able to produce a different number of generations based on thermal tolerances and climate.

1.7.3 Factors affecting the performance of *Megamelus scutellaris* against *Pontederia crassipes*

Factors such as competition with other biocontrol agents for feeding sources in the plant, the nutrient quality of the plant, and temperature levels of the water affect the behaviour of *M. scutellaris* (Freedman & Harms 2017). Competition can either exert positive or negative impacts on the plant's feeding patterns. However, in South Africa, there are no negative interactions reported with *M. scutellaris* and other biocontrol agents. Temperature affects the performance and survival of *M. scutellaris* with both high and low temperatures having strong negative influences on the survival and establishment of this insect (Grodowitz et al. 2017). Grodowitz et al. (2017), observed lower populations of *M. scutellaris* at 18°C and 33°C treatment regimens than at an optimum temperature of 25°C. Plant nutritional quality is crucial to *M. scutellaris* fecundity; if the plant is low in nutrients, the insect is unlikely to establish successfully (Freedman & Harms 2017). However, despite these factors, *M. scutellaris* has adapted across South Africa, even in the country's temperate region (Miller et al. 2021).

1.7.4 Impact of *Megamelus scutellaris* at the Hartbeesport Dam

An inundative release strategy of *M. scutellaris* was implemented at Hartbeespoort Dam in an attempt to increase the probability of population build-up, particularly after winter. For the first time, in 2020 and 2021, these releases have shown that *P. crassipes* can be managed successfully using biological control, despite eutrophication and a temperate climate (Coetzee et al. 2022). The inundative releases of *M. scutellaris* effectively reduced the *P. crassipes* cover from over 40% to less than 10% in two consecutive years, as confirmed by Sentinel-2 satellite imagery and field surveys. The corresponding increase in *M. scutellaris* population density further demonstrated the effectiveness of this strategy. However, in January 2021, a latent population of common salvinia, *Salvinia minima* Baker (Salviniales: Salviniaceae), invaded the Hartbeespoort Dam in the absence

of *P. crassipes*, resulting in a secondary invasion that proliferated during the winter months in the absence of *P. crassipes*. Since then, the population dynamics of these two aquatic invasive plants have been interchanging at the dam (Coetzee et al. 2022b).

1.8 Study species 2: *Salvinia minima* (Common salvinia)

1.6.1 Origin and distribution

Salvinia minima, a free-floating aquatic fern, is an invasive species native to Mexico through Meso-America to northern Argentina (Mitchell & Thomas 1972) (Figure 1.3), but highly invasive in the southern USA (Jacono et al. 2001, Tewari & Johnson 2011). It was discovered in the Hartbeespoort Dam, North-West Province in December 2011 by Dr Carina Cilliers, a retired Agricultural Research Council entomologist and biocontrol scientist, during zooplankton studies of water samples from the dam (Henderson 2012). This species has a history of being sold in the nursery trade (Forno et al. 1983) and is differentiated from its relative, *Salvinia molesta* D. Mitch (giant salvinia), by the presence of divided hairs on the abaxial leaf surface that are free and not joined at the tips (Harley & Mitchell 1981).

Figure 1.3: *Salvinia minima* Baker (Salviniaceae), common salvinia (Photo credit: David Taylor, Centre for Biological Control).

1.8.2 Biology

Salvinia minima is characterized by horizontal branching rhizomes that bear leaves in whorls of three. Two of the leaves are floating, green, and usually ovate to cordate with distinct midribs, while the third leaf is submersed and highly divided (Mitchell 1972). The upper surface of the leaves has water-repellent hairs, while the undersurface has hairs as well. Submerged leaves support heterosporous sporangia (Jacono et al. 2001).

Reproduction in *S. minima* is primarily asexual, occurring through fast-paced fragmentation, leading to rapid surface coverage in water bodies (Miller & Wilson 1989; Jacono 2001). The plant exhibits three distinct growth stages. The initial stage involves isolated plants with flat leaves on

the water surface, marking the initial colonization. In the secondary stage, leaves curl upward, indicating more established growth. Finally, the tertiary stage is characterized by crowded plants with nearly vertical curled leaves, forming thick mats that hinder sunlight from reaching submerged plants (Jacono 2001).

Below the water surface, *S. minima* leaves undergo modifications to serve as a root system. The plant reproduces through fragmentation from attachment nodes, with up to five lateral buds per node, while spores are sterile (Jacono et al. 2001). The species exhibits remarkable expansion rates, with wild populations doubling every two weeks and greenhouse conditions doubling every five to six days, depending on nutrient availability (Al-Hamdani and Sirna 2008). In as little as six weeks after introduction, small ponds have been entirely covered by this weed.

Salvinia minima thrives in lentic freshwater areas, particularly marshes, low-lying forested woodlands, and slightly acidic, high-nutrient, slow-moving freshwater environments (Tipping et al. 2012). It can be found in streams, lakes, ponds, ditches, and even rice fields. The species displays resilience to low temperatures, water stress, and elevated pH levels (Olguín et al. 2002). The transportation of boats and vehicles, if not properly cleaned, contributes to the spread of *S. minima* between water bodies (Miller & Wilson 1989; Madeira et al. 2003). Furthermore, climatic conditions, particularly flooding, can lead to the fragmentation of mats, aiding the dispersal of *S. minima* (Harley and Mitchell 1981) thus causing severe impact to aquatic ecosystems.

1.8.3 Ecological and environmental impacts of *Salvinia minima*

The impacts of *S. minima* have been extensively studied in various regions, such as the United States (specifically Louisiana) (Montz 1989) but have yet to be measured in South Africa. In the United States, the uncontrolled growth of *S. minima* leads to the formation of dense mats that not only reduce the aesthetic appeal of aquatic areas but also limit their usability (Montz 1989). These

infestations result in decreased light availability, obstruction of waterways, alteration of pH levels, and reduced dissolved oxygen (Flores & Carlson 2006). Moreover, the persistent presence of these mats raises concerns for human health, as *Salvinia* spp. provide an ideal habitat for *Mansonia* spp. mosquitoes, which are known vectors of diseases such as West Nile Virus, St. Louis Encephalitis, and Venezuelan Equine Encephalitis (Lounibos et al. 1990).

The negative impacts caused by *S. minima* are similar to those associated with other floating aquatic weeds, as dense mats restrict the utilization of water bodies for agricultural, recreational, and conservation purposes (Cilliers 1991a). *Salvinia minima* has the potential to lower the dissolved oxygen levels in infested water, creating an environment conducive to pest species like mosquitoes (Gallardo et al. 1999). These thick floating mats also pose challenges for recreational activities such as boating and fishing, hindering navigation and potentially impeding the effectiveness of law enforcement agencies (Gallardo et al. 1999). Commercial activities such as rice and crawfish farming, water drainage, and electrical power generation can also be adversely affected by *S. minima* infestations (Charles Dugas, Louisiana Department of Wildlife and Fisheries, retired), and power generation can also be negatively impacted by *S. minima*. Due to the severe impacts of aquatic ecosystems, several management measures have been put in place (Tewari & Johnson 2011).

1.8.4 Management measures of *Salvinia minima*

Effective management strategies have been employed to address the proliferation of *S. minima*, including chemical control, mechanical control, and biological control methods. Chemical control, although non-selective, has been used; however, the application of herbicides can be costly (Tewari & Johnson 2011). On the other hand, mechanical control methods, such as physically removing the plants, are impractical due to the propensity of fragmented plants to generate additional vegetative growth, making manual removal unfeasible in most areas. Notably, classical

biological control programmes utilizing the *C. salviniae* species have demonstrated success in managing *S. molesta*, a close relative of *S. minima*, and have proven to be significantly more cost-effective compared to alternative control methods (Tewari & Johnson 2011).

1.9. Biocontrol agent: *Cyrtobagous salviniae*

1.9.1 Life history and biology

Cyrtobagous salviniae is a semi-aquatic weevil native to Brazil, Paraguay, and Bolivia, and has been intentionally introduced into 16 countries worldwide for the control of *S. molesta* (Room et al. 1981, Forno & Bourne 1985) including in South Africa (Cilliers 1991a, Coetzee et al. 2011). Although primarily released to manage *S. molesta*, *C. salviniae* also feeds on *S. minima* (Tipping & Center 2005). Initially misidentified as *Cyrtobagous singularis* (Hustache), *C. salviniae* populations from North and South America were observed to have a difference in size, leading to the identification of two distinct ecotypes: the smaller Florida ecotype, found on *S. minima* in Florida, and the larger Brazil ecotype, extensively used against *S. molesta* (Jacono 2001).

The smaller Florida ecotype is prevalent in *S. minima* populations throughout Florida, while the larger Brazil ecotype has been extensively used against *S. molesta* (Jacono 2001). Studies have indicated that the Florida ecotype causes more extensive damage to both *S. minima* and *S. molesta* compared to the Brazilian ecotype (Tipping et al. 2010). In Florida, *C. salviniae*, particularly the Florida ecotype, has successfully controlled *S. minima* populations. However, the absence of the weevil in Texas and Louisiana has resulted in the explosive growth of *S. minima* in these regions (Jacono et al. 2001; Tipping et al. 2012). The introduction of the Florida ecotype into Texas and Louisiana began in 1999 for the control of *S. molesta*, while the Brazilian ecotype was introduced starting in 2001 (Goolsby et al. 2000; Tipping et al. 2008).

Parys and Johnson (2013) conducted a study in Louisiana to assess the potential success of *C. salviniae* in controlling *S. minima* infestations. The results indicated successful establishment of *C. salviniae* in southern Louisiana, leading to a significant reduction in *S. minima* biomass in the released areas. However, complete control of the infestations was not achieved at most field sites. Sands et al. (1983) found that additional factors, such as nutrient limitations and interactions with other arthropods, may influence the effectiveness of *C. salviniae* feeding on *S. molesta* and these limitations could be the same for the Florida ecotype when feeding on *S. minima*. Despite the main research site not achieving complete control, the study demonstrated a significant decrease in biomass and an increase in the number of damaged terminal buds in southern Louisiana. According to Parys and Johnson (2013), the introduction of *C. salviniae*, coupled with native herbivores *Samea multiplicalis* and *Samea obliteralis*, holds promise as an ecologically and economically viable control option of *S. minima* management in southern Louisiana.

1.9.2 Impacts on *Salvinia minima*

Cyrtobagous salviniae has proven to be an effective biological control agent for suppressing *S. molesta* in various tropical and subtropical countries (Thomas & Room 1986; Cilliers 1991). This highly specialized insect exhibits a unique behaviour of tunnelling through rhizomes and feeding on buds, resulting in a significant reduction of large *S. molesta* infestations and the maintenance of low population levels (Sands et al. 1983; Room et al. 1981).

According to Forno et al. (1983) *C. salviniae* adults can be observed either on or beneath the leaves, within the leaf buds, or among the roots of *S. molesta* or *S. minima* (Forno et al. 1983, Russell et al. 2017). Eggs are laid individually and typically within cavities created as a result of adults feeding on the leaves, rhizomes, or "roots" (Forno et al. 1983). Adult *C. salviniae* individuals are known to feed on leaves, resulting in the formation of small, irregular holes. They may also target terminal buds, effectively inhibiting the growth of giant salvinia plants (Sands et al. 1983).

Furthermore, the feeding activities of *C. salviniae* larvae lead to the gradual darkening and subsequent shedding of leaves (Forno et al. 1983).

Cyrtobagous salviniae exclusively feeds on *Salvinia* species, highlighting its specificity as a control agent (Forno et al. 1983). This weevil is naturally distributed in southeastern Brazil, Bolivia, Paraguay, and northern Argentina (Calder & Sands 1985). Interestingly, *C. salviniae* was accidentally introduced to Miami, Florida, sometime prior to 1960, as the first collected specimen in the United States was initially identified as *Cyrtobagous singularis* Hustache (Kissinger 1966). However, the North American distribution of *C. salviniae* and its impact on *S. minima* have yet to be fully determined.

A study conducted by Tewari and Johnson (2011) investigated the status and occurrence of *Salvinia* in the southern United States and specifically examined the distribution of *C. salviniae* associated with *S. minima*. The findings revealed that treatments involving *C. salviniae* feeding on *S. minima* significantly reduced plant biomass (Tewari & Johnson 2011). A study by Parys & Johnson (2013) which investigated the impact of feeding of *C. salviniae* on *S. minima* reported that feeding from *C. salviniae* significantly caused substantial increase in the number of damaged terminal bud, decreasing the fresh weight biomass of *S. minima*. Moreover, from their findings, Jacono et al. (2001) and Tewari & Johnson (2011) highlighted that the presence of *C. salviniae* in Florida has helped in managing the population of *S. minima*, preventing its explosive proliferation observed in areas like Texas and Louisiana, where the weevil was absent. The results suggest the effectiveness of *C. salviniae* in managing *S. minima* populations.

Figure 1.4: The Florida biotype of *Cyrtobagous salviniae* (Photo credit: David Taylor, Centre for Biological Control)

19.3 Current status of *Salvinia molesta, Salvinia minima* and *Crytobagous salviniae* in South Africa

Salvinia molesta was considered the second most problematic weed after *P. crassipes* during the 1980s. As a result, the South African Department of Water Affairs initiated a biological control programme against *S. molesta*, which proved to be successful (Coetzee & Hill 2020). The introduction of the weevil *C. salviniae* rapidly controlled *S. molesta* infestations across its invaded range, and by the late 1990s, control of *S. molesta* in South Africa was considered complete (Moran et al. 2021). Motitsoe et al. (2020) presented findings that highlight a crucial outcome: a notable restoration of aquatic biodiversity which occurred in waterbodies where *S. molesta* populations declined through the feeding activity of *C. salviniae*. Ongoing management efforts involve the redistribution of the *C. salviniae* to newly infested sites (Hill et al. 2021).

Considering the success of the biological control programme against *S. molesta* in South Africa and other regions, and the effective biological control of *S. minima* in the USA, it is evident that biological control can also be effective in managing populations of *S. minima* in South Africa.

Field surveys have confirmed the presence of *S. minima* in four sites in South Africa, with the largest invasion occurring in the highly polluted Hartbeespoort Dam. Recent surveys in 2021 revealed the presence of *S. minima* downstream of the impoundment on the Crocodile River in Roodekoppies Dam, as well as in two disconnected systems, the Bon Accord and Roodeplaat dams. Previously, records of S. *minima* were limited to ponds on housing estates around the dam and Hartbeespoort Dam (Coetzee et al. 2022b). The spread of *S. minima* to these disconnected systems is likely attributed to the movement of recreational boaters' and anglers' equipment, a common method of spreading aquatic invasives (Coetzee et al. 2009).

In South Africa, the smaller Florida ecotype weevil, *C. salviniae*, is being considered for biological control of *S. minima*. Currently, this weevil is undergoing host specificity testing under quarantine conditions (Coetzee et al., 2022b). It is important to note that the population dynamics of *S. minima* and *P. crassipes* at the Hartbeespoort Dam have been interchanging. Following a decrease in *P. crassipes* in late Autumn of 2021, *S. minima* cover rapidly increased, reaching a maximum of 38% cover (692 hectares) in midwinter, July 2021 (Coetzee et al., 2022b) (Figure 1.5). However, during the following spring months of September to November 2021, *P. crassipes* reappeared as the dominant invader due to seed germination, causing fluctuations in *S. minima* cover (Coetzee et al., 2022b). As of December 2022, *P. crassipes* was reported to be the dominant macrophyte in the dam, with a coverage of 7.8% (Coetzee et al., 2022b). Therefore, it is worthwhile to investigate the competitive dynamics and interactions between these two species, which is the central focus of this thesis.

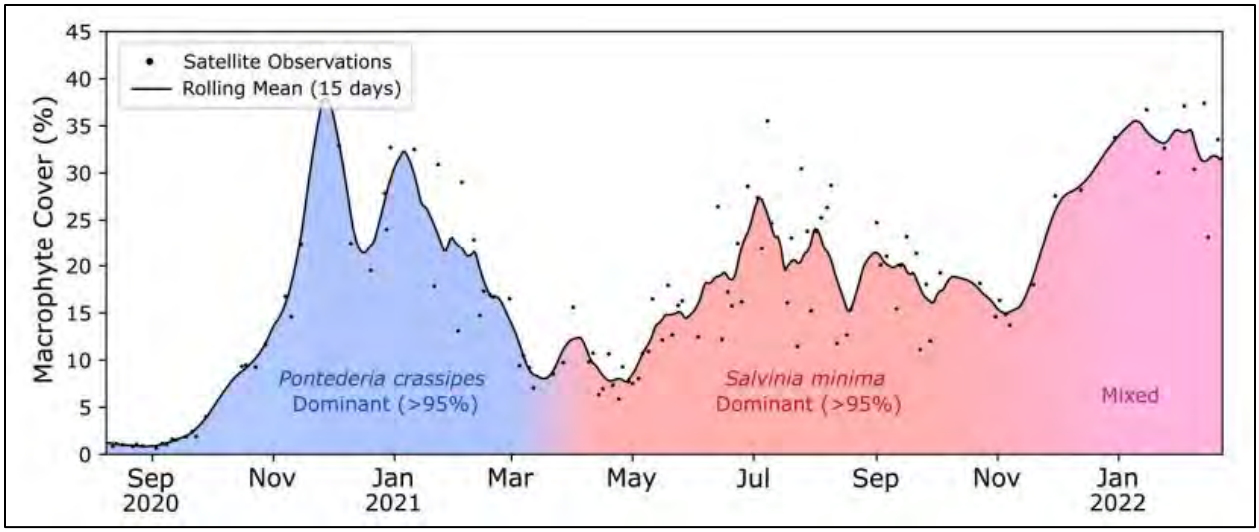

Figure 1.5: Time-series of macrophyte (*Pontederia crassipes* and *Salvinia minima*) cover on Hartbeespoort Dam, derived from Sentinel-1 (Coetzee et al., 2022b)

1.10 Study rationale and aims

The reduction of *P. crassipes* populations at the Hartbeespoort Dam led to secondary invasion of another existing invasive species, *S minima* (Coetzee et al. 2022b). Aquatic invasive plants have been known to co-occur in South Africa's water systems, but the interactions occurring between *P. crassipes,* and *S. minima* have not been subjected to research efforts yet. How these species interact with one another, and the likelihood of one or more of these species becoming dominant under conditions of invasion was addressed in this study. This thesis is centred around the secondary invasion of *S. minima* at the Hartbeespoort Dam and aimed to assess the competitive interactions between *P. crassipes* and *S. minima* when grown in combination with one another at different planting densities, in the presence or absence of biological control. I also investigated the possibility of allelopathy by *P. crassipes* on *S. minima* as a competitive strategy.

1.11 Thesis Layout

This thesis consists of five chapters. In Chapter 1, a comprehensive review of the relevant literature is provided, establishing the necessary background information to justify the research conducted in this study. Chapter 2 investigates the population dynamics of *P. crassipes* and *S. minima* at the Hartbeespoort Dam since invasion of *S. minima*. Chapter 3 investigates the effect of the biological control agent, *M. scutellaris,* on the competitive ability of *P. crassipes*. Chapter 4 investigates the potential allelopathic effects of *P. crassipes* on *S. minima* as a competitive strategy. Finally, Chapter 5 integrates the findings from Chapters 2, 3 and 4, offering a synthesis of the information acquired throughout the study. This chapter identifies persistent research gaps, highlights key findings, and discusses the implications of the study for effective *P. crassipes* control and the management of *S. minima* populations in Hartbeespoort Dam.

CHAPTER 2

Population dynamics of *Pontederia crassipes* and *Salvinia minima* at Hartbeespoort Dam

2.1 Introduction

Increasing rates of biological invasions require an urgent imperative to mitigate their negative impacts and restore native biodiversity (Hulme 2006, Pyšek & Richardson 2010). Global initiatives are actively underway in attempting to clear invasive species (McFadyen 1998, Culliney 2005, Van Driesche et al. 2010). Invasive plant management often succeeds in suppressing targeted invasive plants (Pyšek & Richardson 2010); however, a newly emerging problem is that of secondary invasion which has been defined by Pearson et al. (2016) as an increase in the proliferation of non-target exotics following efforts to suppress dominant target invaders be it temporal (Pearson et al. 2016).

Theoretically, controlling the initial invader in an ecosystem should allow for ecosystem recovery (Blanchard & Holmes 2008). However, various studies have indicated that secondary invaders are commonly the principal beneficiaries after the removal of the initial invader, limiting the recovery of native species (Kettenring & Adams 2011, Pearson et al. 2016, Nsikani et al. 2019, 2020). Buckley et al. (2007) and Kettenring & Adams (2011) reported that what qualifies for secondary invasions is often the removal of target invaders since it creates space that favours secondary invaders.

When the primary invader is removed, the decrease in competition and the release of nutrients, primarily high nitrogen often triggers a secondary invasion by either a different plant species or a

reinvasion of the initial invader in the cleared zone (Nsikani et al. 2018). Secondary invaders occupy the available space as their propagules were already present in surrounding areas and when they invade in the cleared areas, their proliferation is aided by their ability to establish rapidly (Nsikani et al. 2018).

Several studies have demonstrated the phenomenon of secondary invasions (Symstad 2004, Ogden & Rejmánek 2005, Pearson et al. 2016). These studies have reported that increases in secondary invasions correlated significantly with reductions in targeted invaders and in turn present significant barriers to native biodiversity restoration (Torres et al. 2018, Nsikani et al. 2019). Therefore, given that most ecosystems host multiple invasive species (Kuebbing et al. 2013), the threat of secondary invasion is widespread and demands immediate attention. A crucial factor lies in comprehending the population dynamics of invasive species within these ecosystems.

Studying aquatic ecosystems aims to explain community structure patterns, such as species richness and relative abundance, by understanding the population dynamics of plants occupying that ecosystem (Crawley & May 1987). Population dynamics, as defined by Fernandez-Quintanilla (1988) involves the study of fluctuations in numbers within animal and plant populations in their natural environments. Understanding population dynamics within ecosystems is essential for two key reasons: it quantifies population fluctuations and growth/decline patterns, and it investigates the underlying biological and physical processes responsible for changes in plant numbers over space and time (Watkinson 1997, Juliano 2007, Burlakova et al. 2010).

Similarly, understanding the population dynamics of invasive species within aquatic ecosystems is crucial for identifying factors governing species fluctuations, and spatial dispersion, and, most importantly, for developing effective control strategies (Burlakova et al. 2010). In recent times, remote sensing methods have been extensively employed in various studies, such as estimating above-ground biomass across diverse ecosystems and monitoring agricultural fields (Isbaex et al.

2021). Satellite image sensors have proven instrumental, providing comprehensive insights into vegetation cover by capturing intricate details related to growth patterns, vigour, dynamics, and the overall diversity of vegetation (Askar et al. 2018, Carranza et al. 2019). When examining invasive aquatic plant populations, two essential factors stand out: density and percentage coverage. These parameters play a crucial role not only in understanding ecosystem dynamics but also in facilitating the monitoring and control of invasive aquatic species (Wilson et al. 2001).

In South Africa, the Hartbeespoort Dam has provided a suitable habitat for invasive aquatic plants primarily due to its nutrient-rich waters, with *P. crassipes* and several other invasive plants posing challenges in the past (Hill & Coetzee 2017). However, over the past five years, there have been dedicated efforts to reducing the severity of aquatic invasive infestations at the Hartbeespoort Dam, with a strong emphasis on the implementation of biological control (Hill & Coetzee 2017, Hill et al. 2020b, Coetzee et al. 2022a). Remarkably, biological control has proven effective in managing the population of *P. crassipes* within the dam, notably through the introduction of the planthopper, *M. scutellaris*, along with other biocontrol agents (Hill et al. 2020b).

Megamelus scutellaris, released at the Hartbeespoort Dam in 2018 established well and reduced *P. crassipes* populations. through frequent inundative releases of *M. scutellaris* since the beginning of 2019 (Coetzee et al. 2021). These releases, conducted by the Centre for Biological Control (CBC) from rearing facilities near the dam and Waainek Research Facility have resulted in significant damage to *P. crassipes* at the Dam as reported by Coetzee et al. (2022a) where, for the first time, there was a significant decline in *P. crassipes* population over the summer of October 2019 and February 2020 with a decrease from 37% to 3% (Figure 2.1).

The success of the CBC programme lies in rearing and consistent inundative releases of the biocontrol agents, resulting in significant control over *P. crassipes* populations despite the dam's eutrophic status and the region's temperate winter climate (Coetzee et al., 2022a).

However, despite the effectiveness of *M. scutellaris* in managing *P. crassipes* populations at the Dam, challenges persist. The temporary absence of *P. crassipes* over the winter months of 2021 led to the secondary invasion by *S. minima*, a previously less prominent invasive plant species (Figure 2.1) (Coetzee et al. 2022b).

Given the prevalence of *P. crassipes* as the dominant species at the Hartbeespoort Dam (Coetzee & Hill 2012, Coetzee et al. 2014, Hill & Coetzee 2017), it is essential to investigate the population dynamics of both *P. crassipes* and *S. minima* as this will provide critical insights for effective management strategies. This involves addressing critical questions, such as the periods during which each invasive species dominates, the factors driving this dominance, and the circumstances under which these two species coexist, if at all. Therefore, this chapter aimed to provide a comprehensive understanding of the population dynamics of *P. crassipes* and *S. minima*, since the increase in prevalence of *S. minima* shedding light on the invasion dynamics at play in the Hartbeespoort Dam.

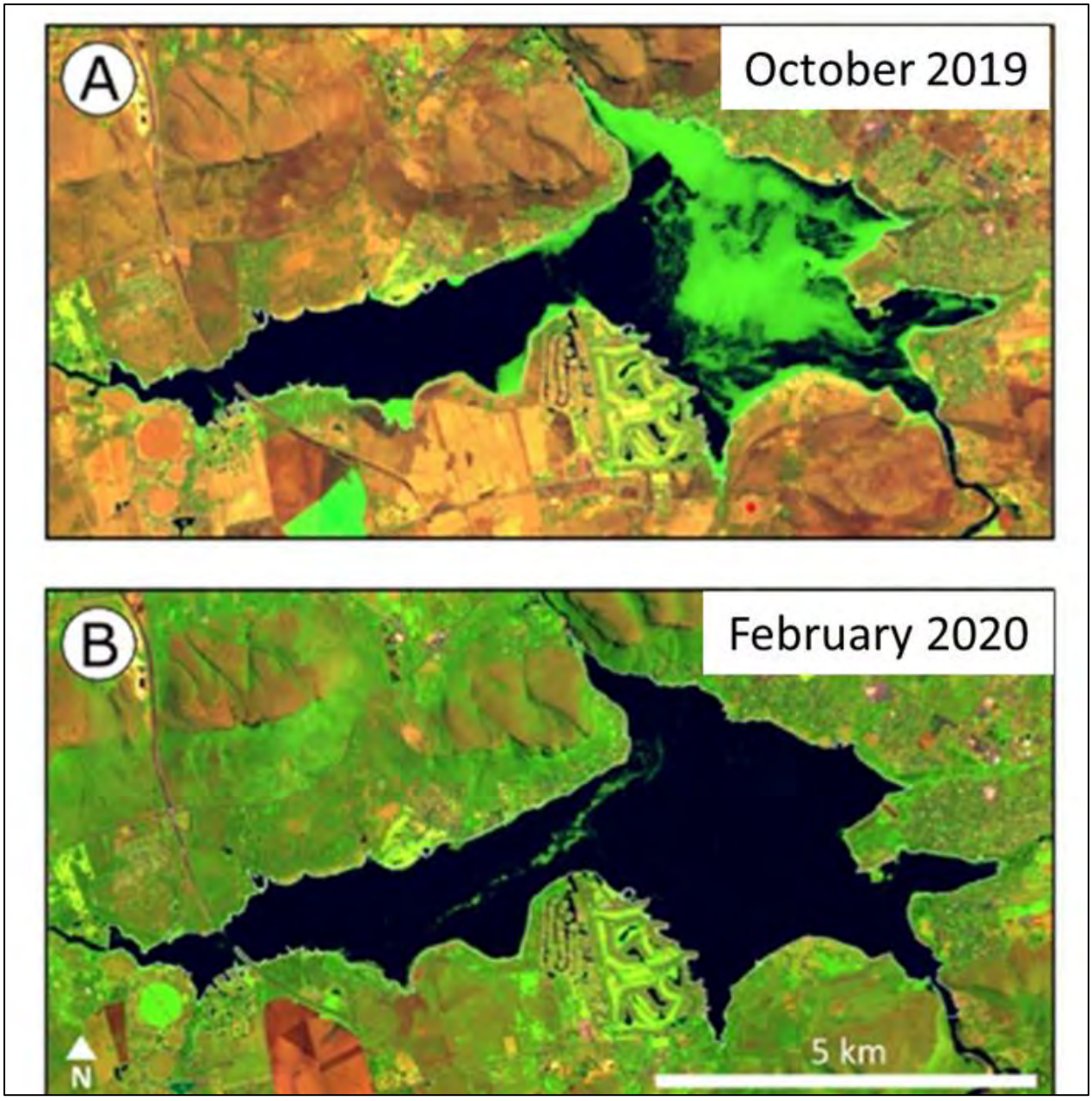

Figure 2.1: Decline in *Pontederia crassipes* coverage due to high-density inundative releases of *Megamelus scutellaris* from (a) October 2019 to (b) February 2020 (Coetzee et al., 2022a)

Figure 2.2: *Salvinia minima* at the Hartbeespoort Dam in October 2022 (Image: T. Chikodza)

2.2 Methods and materials

2.2.1 Sampling sites

Sampling for *S. minima* biomass on Hartbeespoort dam was done at six different sites located around the dam. The sites were named based on their respective locations. Three of the sites, Kurperood, Kosmos, and Magalies Park were located on the northern side of the dam. Magalies Park was close to the Magalies River that feeds into the western side of the dam. The remaining three sites, Coves, Lakeland, and Ifafi were located on the southern part of the Dam (Figure 2.3).

2.2.2 Sampling protocol

A simple sampling procedure was conducted where *S. minima* biomass within 0.25 m^2 was collected using a a square quadrat (0.5 x 0.5m) (Figure 2.4). All the plant material within the quadrat was collected, and excess water was allowed to run off for 2 minutes before being weighed. This procedure was replicated three times per site. Sampling was done in February, March, May, June, and October 2022, and subsequently in February, June, and July 2023.

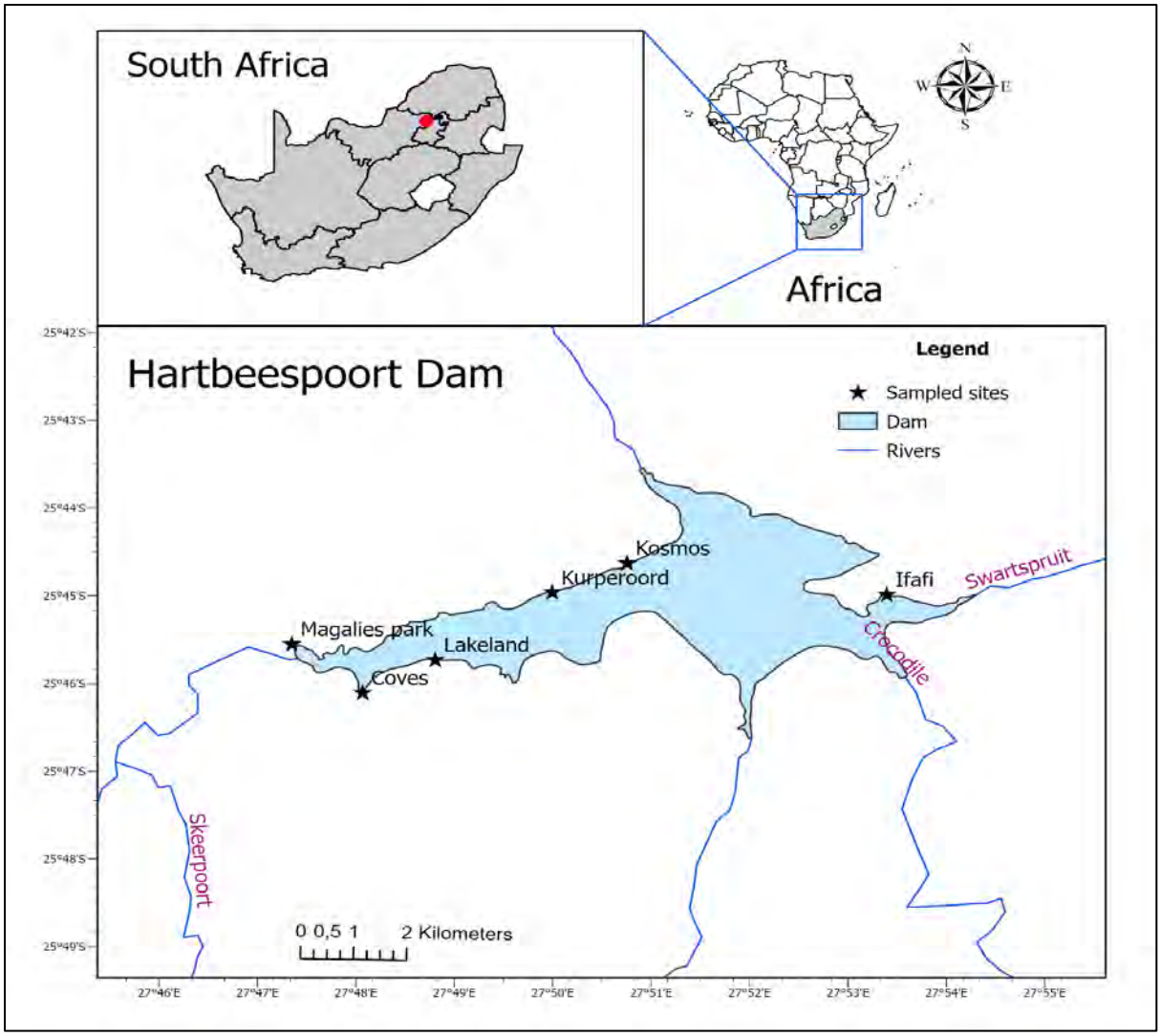

Figure 2.3: Sampling sites for *Salvinia minima* biomass around Hartbeespoort Dam

Figure 2.4: *Salvinia minima* biomass collection a) random placement of a quadrat within a site, b) plant material collection from within a quadrat.

2.2.3 Climate data

Climate data including rainfall, and maximum and minimum temperatures recorded from January 2021 to September 2023 were sourced from the South African Weather Services (https://www.weathersa.co.za/). Understanding correlations between climatic factors and population dynamics of species helps identify patterns and potential causal relationships (Selonen et al. 2021). As a result, these weather parameters were analyzed to explore potential correlations between these variables and the population dynamics of both *P. crassipes* and *S. minima*.

2.2.4 Estimating macrophyte cover using remote sensing data.

Pontederia crassipes and *S. minima's* area coverage data were acquired through Sentinel-2 satellite image analysis, accessed at no cost through the following WebApp: https://davidkinsler123.users.earthengine.app/view/macrophytemonitoring-tool (Kinsler, 2023 (unpublished data)). To estimate *P. crassipes* and *S. minima* coverage on Hartbeespoort Dam, a

random forest image classifier was trained on Sentinel-2 MultiSpectral Instrument (MSI) satellite images, performed within the Google Earth Engine remote sensing platform (https://earthengine.google.com). Sentinel-2 produces multispectral images at a ground resolution of 10 meters, with a temporal frequency of at least 5 days (reference ESA website). To produce a time series, every cloud-free image of the dam within the study period (n = 254) was classified with the random forest classifier, and area values for each species were calculated. To account for the high spatial-temporal variability, a centered rolling mean of 20 days was applied to the data.

2.2.4 Data Analysis

Data were analysed using R software version 4.2.3 (R Core Team 2023) and STATISTICA ver. 13.0 (StatSoft Inc., 2015). Before analyses, *S. minima* biomass data were first checked for normality and homogenous variance using both the Shapiro-Wilk and Bartlett tests respectively. The data did not meet the linear model assumptions of ANOVA as they were not normally distributed and were analysed using a Kruskal Wallis test to determine differences in biomass across sampling events. Following the Kruskal Wallis test, a Dunn test was conducted using the "bonferroni" method as the data's non-normal distribution didn't meet the requirements for parametric testing, allowing for a non-parametric approach to assess differences among the field-collected biomass.

The effect of three fixed factors: rainfall, temperature, and *P. crassipes* coverage, along with their interactions on *S. minima* coverage was examined using a General Linear Model (GLM) employing the Gaussian family (Table 2.1). The selection of these fixed effects was based on their significance and relevance to the study. Specifically, *P. crassipes* was chosen due to its co-occurrence with *S. minima* at Hartbeespoort Dam (Coetzee et al. 2022b). Rainfall and Temperature data were selected as abiotic factors influencing the coverage dynamics of *S. minima*. According

to Xia et al. (2022), abiotic factors such as air temperature and hydrology have a significant impact on macrophyte community short-term seasonal structure and biodiversity patterns.

To identify the most influential model affecting *S. minima* coverage, an information-theoretic model selection approach was employed. Models were ranked using Akaike's information criteria corrected for small sample sizes (AICs) scores, with the model having the lowest AICs recognized as the most plausible (Harrison et al. 2018). Once the best model was identified, the relative importance of fixed effects was determined using two methods: (i) model averaging, along with the calculation of conditional parameter estimates, and (ii) the summation of wi (Akaike weights) across all models within the plausible model set in which each predictor appeared (Andersson et al. 2018).

The "DHARMa" package (Hartig & Lohse 2020) was used to ensure the model's adequacy and fitness. Subsequently, model selection was carried out using the 'MuMIn' package (Barton 2015) to identify the most relevant variables or the best-fit model. Following this, an Analysis of Variance (ANOVA) Type III was performed to assess the significance of interaction terms encompassing rainfall, temperature, and *P. crassipes* coverage within the model. This analysis aimed to comprehensively evaluate the collective impact of these interactions on the occurrence of *S. minima*.

Table 2.1: Description, data type and range of values for fixed effects.

Model term	Description	Data type	Range
Rainfall	Total monthly rainfall received.	Continuous	0 – 150
Temperature	Mean monthly temperature.	Continuous	0 – 34
P. crassipes	Mean month coverage	Continuous	0 – 100

2.3 Results

2.3.1 *Salvinia minima* biomass

Mean biomass varied significantly across the sampled months with a mean minimum of 0.1kg/1m^2 and a mean maximum of 10.52kg/1m^2 ($F_{7,16}$= 4.645, P = 0.016). Notably, biomass was significantly higher in February 2023 (summer) than June 2023 (winter). Although there were fluctuations in mean biomass across other months, they did not differ significantly (Figure 2.5).

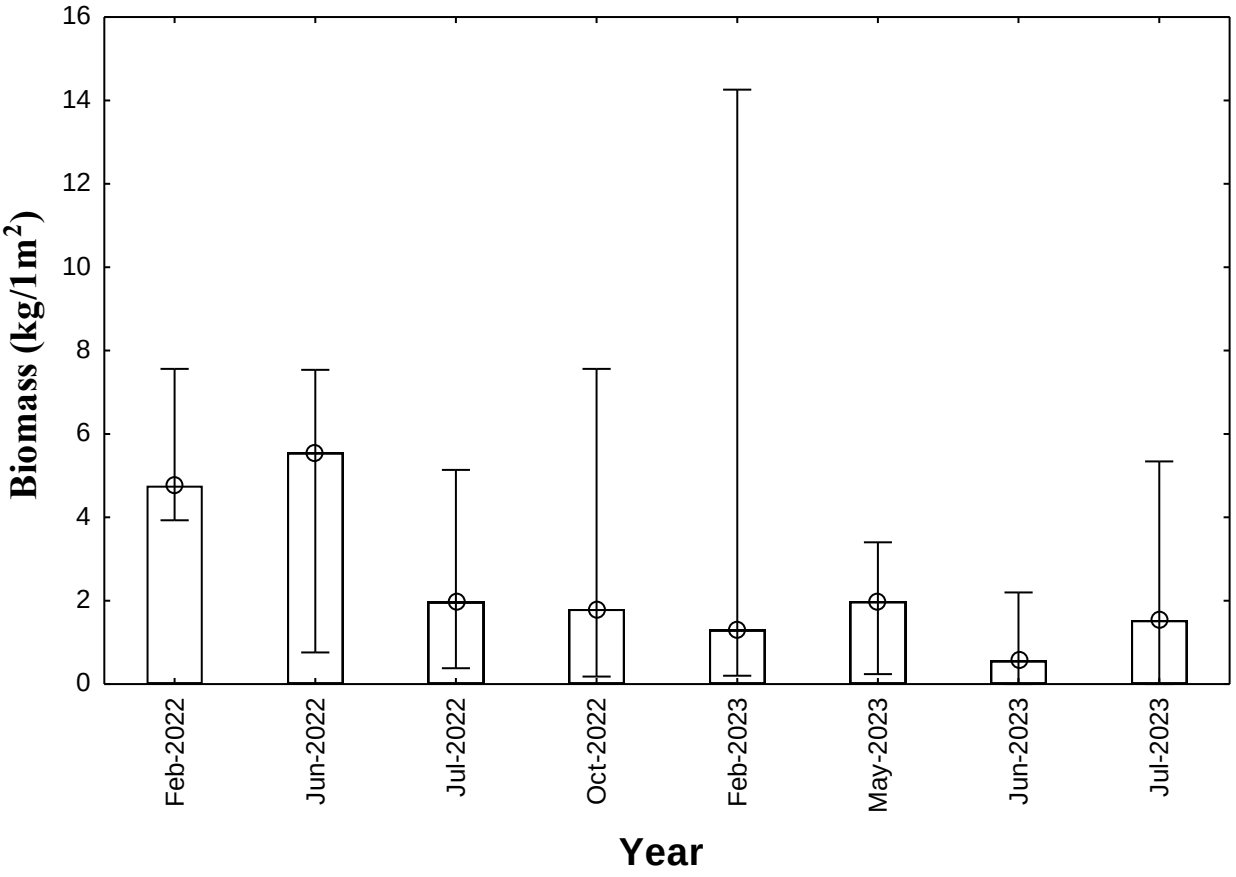

Figure 2.5: *Salvinia minima* biomass (kg/1m^2) from March 2022 to September 2023. Nonparametric error bars denote median values and whiskers denotes quartiles.

2.3.2 Climate data

Rainfall and temperature fluctuated seasonally from August 2019 to September 2023. Highest rainfall was recorded between December 2021 and April 2022, while the lowest recorded rainfall periods were experienced in July 2021 and August 2023. The data also illustrated cyclic temperature variations, with the highest temperatures from November to February annually. January 2023 marked the highest peak in mean maximum temperature, reaching 34.3°C, whereas the lowest mean minimum temperature, at -1.7°C, was recorded in July 2022 (Figure 2.6).

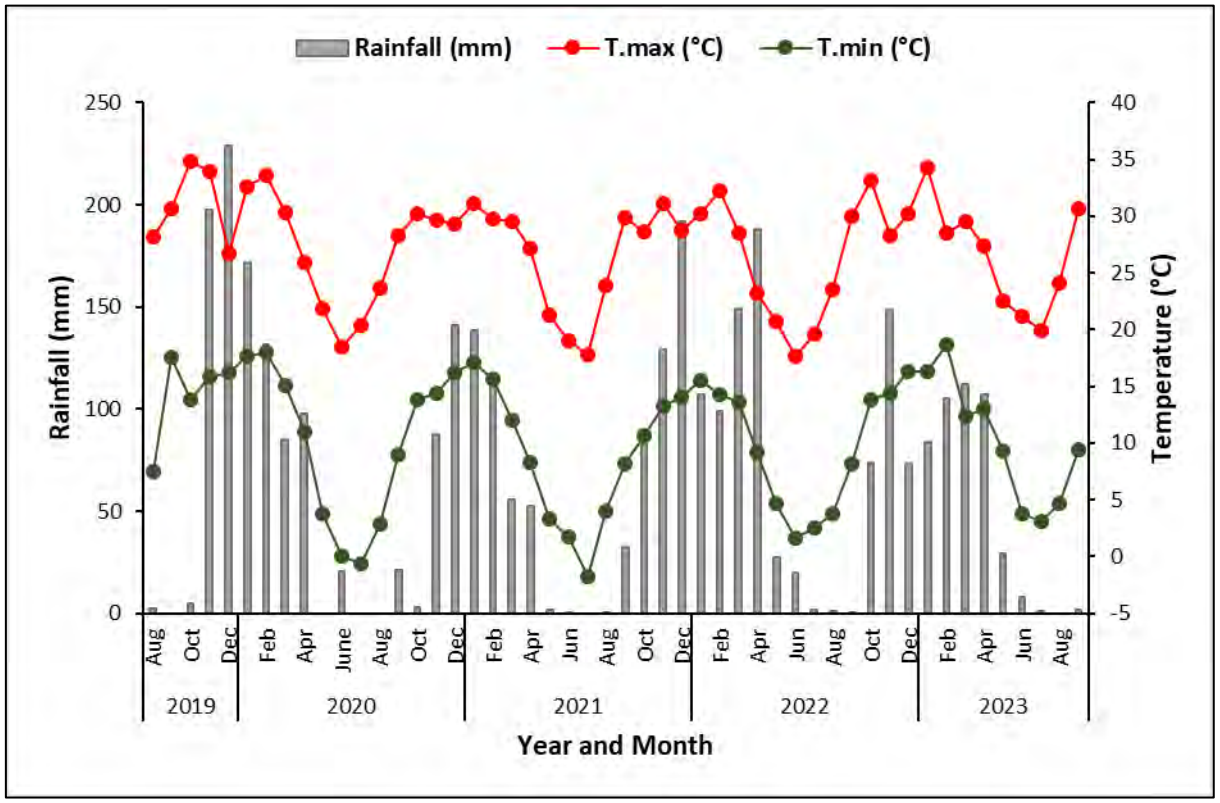

Figure 2.6: Weather station data from Hartbeespoort Dam, showing rainfall, and mean minimum and maximum temperatures, from August 2019 to September 2023 (https://www.weathersa.co.za/).

2.3.3 Aerial coverage

In September 2019, (early autumn), *P. crassipes* coverage fluctuated (Figure 2.7). However, a substantial decline in *P. crassipes* as a result of *M. scutellaris* feeding occurred from October 2019-September 2020 (Figures 2.1 and 2.7). *Pontederia crassipes* coverage began to increase in the following spring once again (September and October 2020) to a maximum of approximately 30% coverage in December 2020 (Figure 2.6). However, *P. crassipes* cover declined over the height of summer from January 2021 to May 2021.

Following the decrease in *P. crassipes* in late Autumn (May 2021), *S. minima* cover increased rapidly, reaching a maximum of 38% cover in midwinter (July 2021). Satellite data derived from a September 2021 Sentinel-2 image revealed that *S. minima* covered more than 50% of the dam area, while *P. crassipes* cover was only 7.8% (Figure 2.8) (Coetzee et al., 2022b). During spring (September- November 2021), *P. crassipes* reappeared once again as the dominant invader.

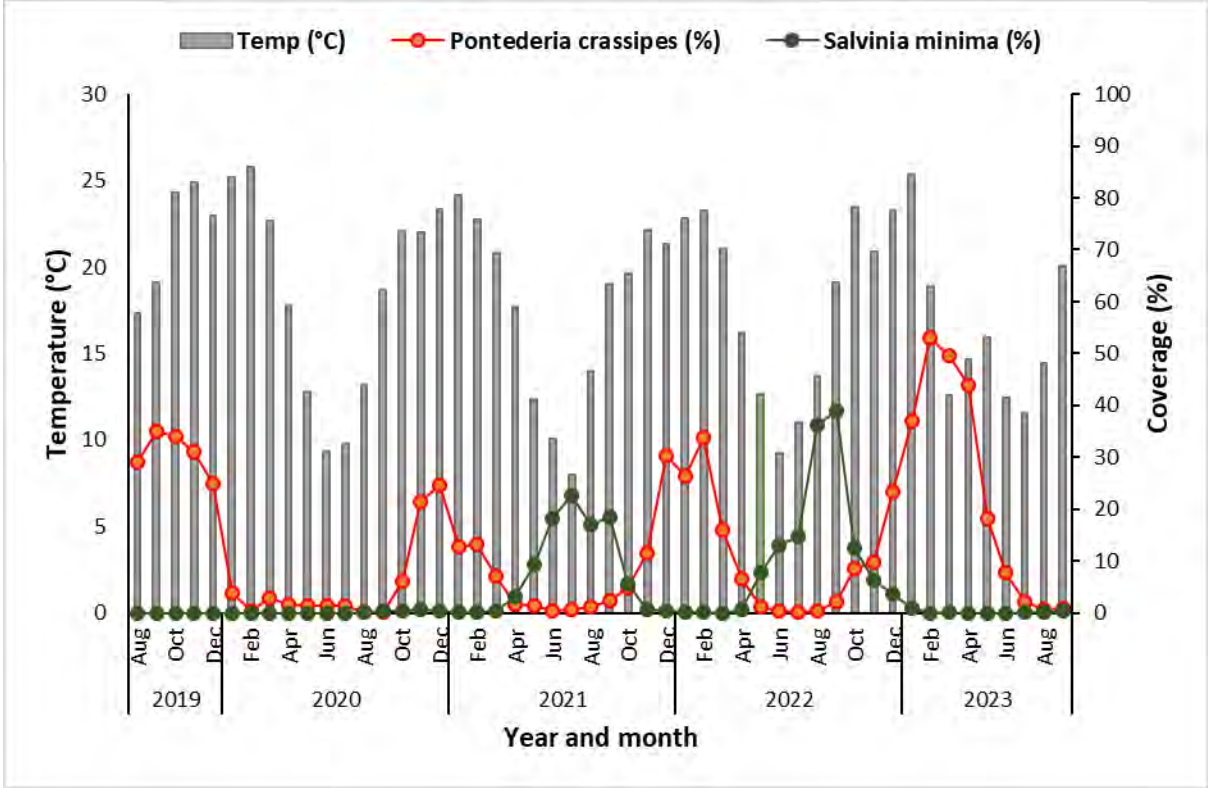

Figure 2.7: *Pontederia crassipes* and *Salvinia minima* cover at Hartbeespoort Dam, from August 2019, until August 2023. Mean monthly temperature is included (error bars excluded to reduce clutter).

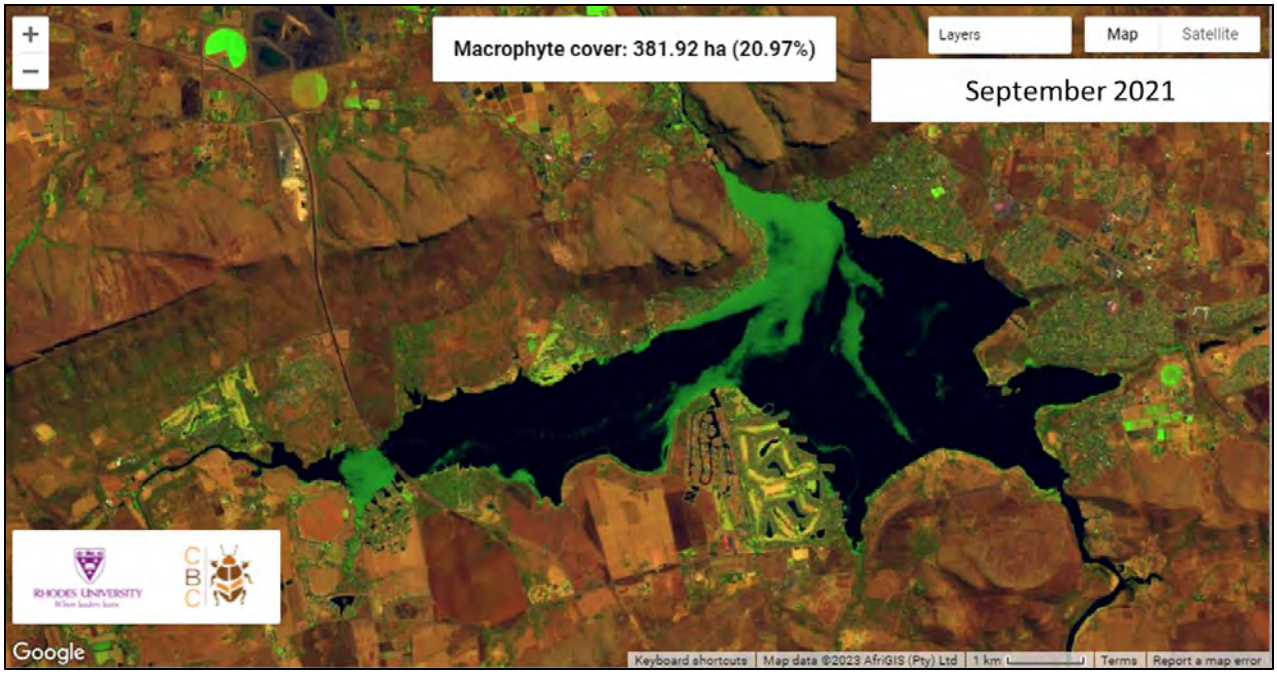

Figure 2.8: Coverage of *Pontederia crassipes* in September 2021 at the Hartbeespoort Dam in both hectares and percentages obtained from the macrophyte monitoring WebApp: https://davidkinsler123.users.earthengine.app/view/macrophytemonitoring-tool .

In October and November 2022, following heavy rainfall, *S. minima* cover was drastically reduced (Figure 2.9). In the absence of *M. scutellaris* and potential competition from *S. minima*, *P. crassipes* dominated the waterbody by January 2022.

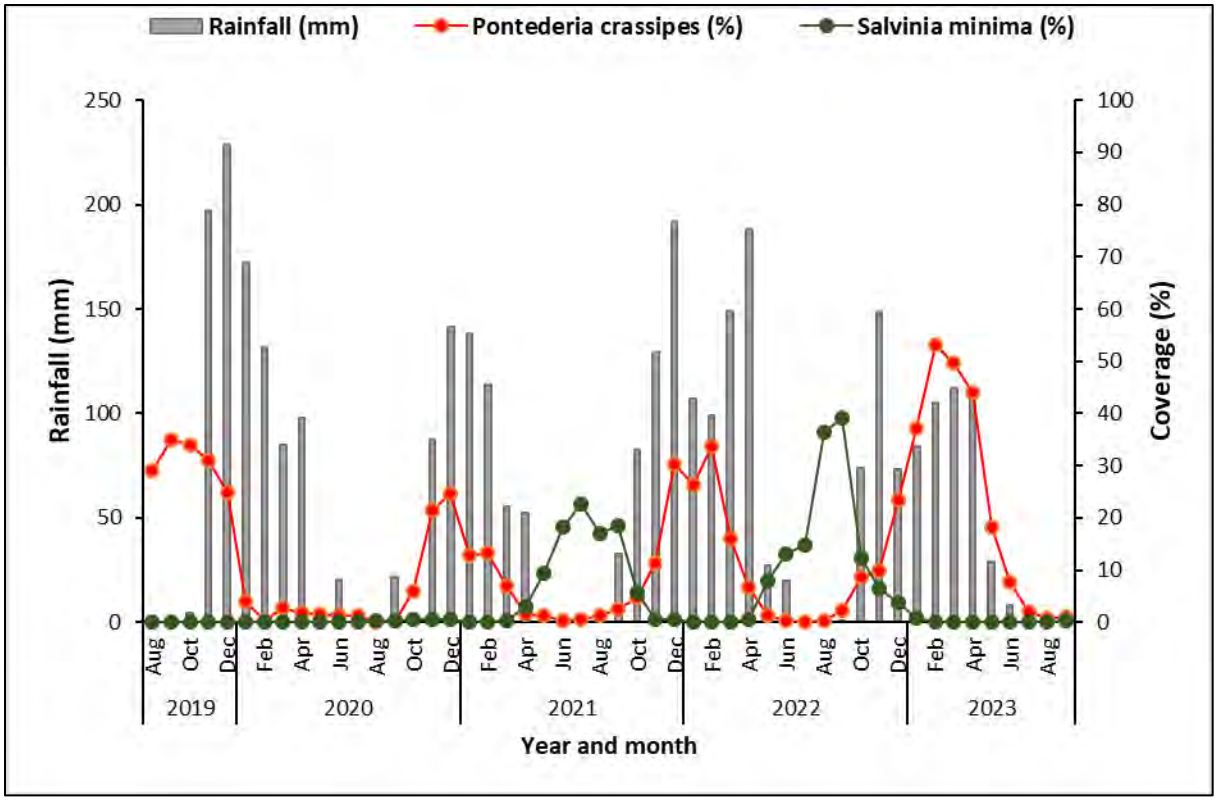

Figure 2.9: *Pontederia crassipes* and *Salvinia minima* cover at Hartbeespoort Dam, from August 2019, until August 2023. Mean monthly rainfall is included (error bars excluded to reduce clutter).

As summer progressed at the dam, the biocontrol agent populations proliferated, resulting in *P. crassipes* populations crashing (Fig. 2.7) as a result of intense feeding damage (Coetzee et al. 2022b). In the absence of *P. crassipes*, *S. minima* dominated once again over the winter months (Figure 2.7). A similar cycle as the previous year was evident in the spring (September) of 2022, where *S. minima* cover decreased while *P. crassipes* cover increased exponentially, reaching a maximum cover in February 2023. Interestingly, this trend was not evident in the spring of 2023; although *P. crassipes* cover decreased drastically by the end of summer as a result of biological control (April 2023), *S. minima* did not increase as in previous years.

2.3.4 Model selection

The model containing *P crassipes* (model 4) was chosen as the most parsimonious model affecting *S. minima* coverage. *Pontederia crassipes* had a sum of weights of 0.65 (Table 2.2).

Table 2.2: Plausible models (ΔAICs < 248) explaining *Salvinia minima* coverage at Hartbeespoort Dam. Parameter estimates are provided for each model term across all plausible models based on Maximum Likelihood estimation.

	Model rank	1	2	3	4	5	6	7	8
	ΔAICc	0	0.42	0.85	2.15	2.54	3.12	3.16	3.77
	Akaike weight (w_i)	243.6	244.1	244.5	245.8	246.2	246.8	246.8	247.4
$\Sigma_{(wi)}$									
0.83	Rain	-0.09	-0.06	-0.10		-0.84		-0.06	-0.11
0.65	*P. crassipes*		-0.18	-0.62	-32		-0.27	-0.17	0.12
0.3	Temp					-0.10	-0.44	0.089	0.65
0.22	Rain: *P. crassipes*			0.005					.0.005
0.04	Rain: temp								
0.04	Temp: *P. crassipes*								
<0.01	Rain: temp: *P. crassipes*								

Coverage of *S. minima* was significantly influenced by *P. crassipes* ($\chi 2 = 8.879$; df = 31; $P = 0.00557$) (Figure 2.9). When *P. crassipes* populations were low, *S. minima* coverage increased. The equation: *Salvinia minima* = 11.1713 - 0.3163 * *Pontederia crassipes* + ε was obtained from the generalised linear model. The symbol ε, the error**,** represents the deviation between the model's predictions and the actual observed values in a regression model. This equation illustrates the negative impact of *P. crassipes* on *S. minima* coverage. As the coverage of *P. crassipes* increased, there was a corresponding decrease in the coverage of *S. minima*. The coefficient for *P. crassipes*

(-0.3163) indicates that for each unit increase in *P. crassipes*, *S. minima* is estimated to decrease by 0.3163, holding other factors constant. This suggests a significant negative relationship between the presence of *P. crassipes* and the presence of *S. minima* in the given context. Figure 2.9 demonstrates a negative relation between *P. crassipes* and *S. minima* coverage. Additionally, it illustrates a critical threshold: when *P. crassipes* coverage surpasses 10%, *S. minima* populations decline.

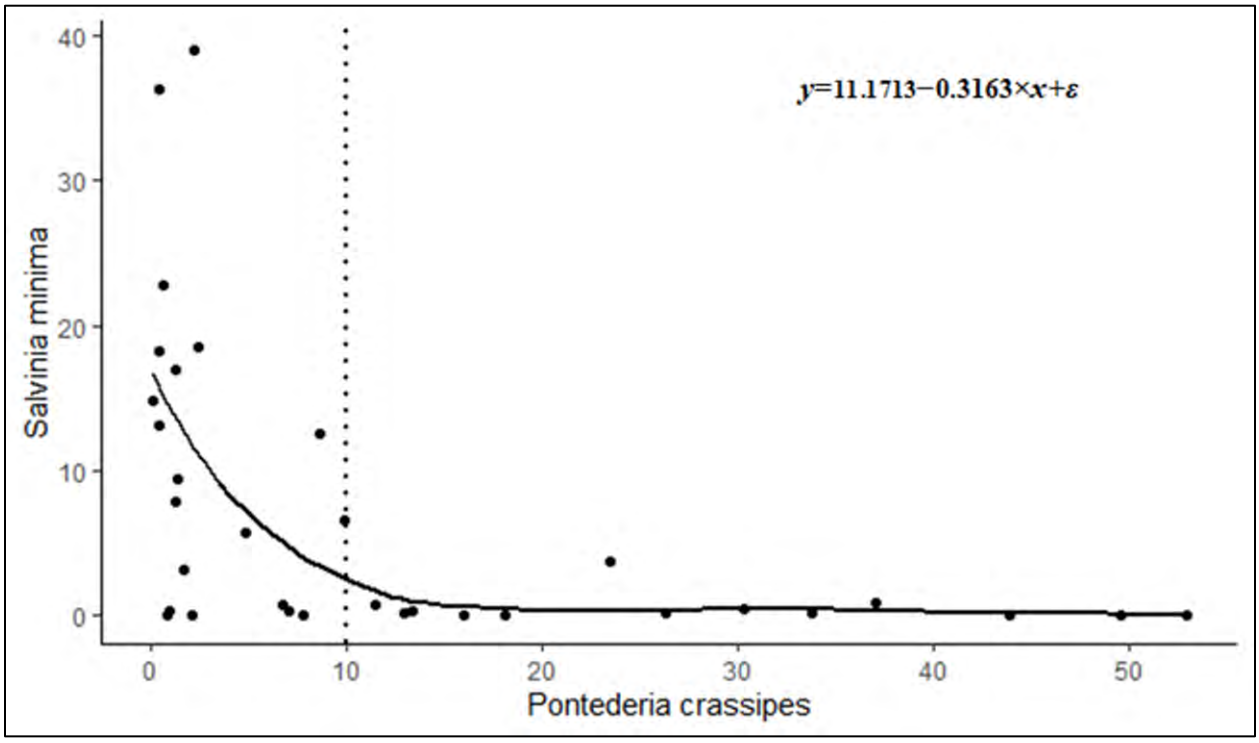

Figure 2.10: Negative relationship between *Pontederia crassipes* and *Salvinia minima* coverage at Hartbeespoort Dam. The dotted line illustrates a threshold indicating that when *Pontederia crassipes* suppress 10% coverage, *Salvinia minima* populations decline.

2.4 Discussion

Field-collected *S. minima* biomass varied over the sampling period, with biomass in February 2023 at a maximum, and a minimum in June 2023. The significant difference in mean biomass between February and June 2023 biomass is likely related to anticipated seasonal variations as recorded by previous studies (Coetzee et al., 2022b). February, the height of summer, usually had high *P. crassipes* and low *S. minima* populations while, in contrast, in June, at the peak of winter, *S. minima* populations tended to increase due to decreased *P. crassipes* populations. Therefore, results from the field-collected biomass data contradict observations from the aerial coverage data as biomass collected in February was high and biomass collected in June was low.

The contradiction between the aerial coverage, determined through Sentinel-2 satellite image classification, and the field-collected biomass of *S. minima* biomass can be linked to several factors. Firstly, the aerial image coverage through Sentinel-2 satellite image classification reflects the entire dam area, whereas biomass was specifically collected from areas where *S. minima* was present and accessible. This disparity or variation in the sampled areas could contribute to the differences in field-collected *S. minima* biomass coverage from the Sentinel-2 satellite image. Consequently, this could mean that field-collected biomass might not be an accurate measure of *S. minima* populations, while remote sensing, such as the use of satellite imagery, emerges as a potentially more reliable estimator due to its ability to cover larger areas and capture data beyond the sampled areas.

In the present study, the population dynamics of *P. crassipes* and *S. minima* at Hartbeespoort Dam obtained from the aerial coverage data were overall interchanging which aligns with findings from Coetzee et al., (2022b). The observed shifts in dominance between *P. crassipes* and *S. minima* point to the concept of alternative stable states within the Hartbeespoort Dam. In the context of invasive aquatic plants, alternative stable states illustrate different stable conditions or ecological

regimes coexisting within the same ecosystem (Scheffer 1990, Beisner et al. 2003). These states can persist or change, depending on the invasive species' presence or absence. Importantly, alternative stable states often feature distinct community structures and ecological functions, indicating the adaptable nature of ecosystems affected by invasive species (Beisner et al. 2003).

The reduction of *P. crassipes* populations at the dam was due to intense feeding from *M. scutellaris* as a result of the implementation of an inundative release programme (Coetzee et al. 2022a, Miller et al. 2023). During late autumn and winter (May to August), remaining *P. crassipes* plants experience frost damage from cold temperatures which also affects the biological control agents (Miller et al. 2021). Miller et al. (2021) revealed a seasonal trend in *M. scutellaris* populations, with numbers declining during winter months due to the absence of *P. crassipes* for feeding.

The decrease in *P. crassipes* allowed *S. minima* to successfully invade, potentially as the result of less competition from *P. crassipes*, and therefore available resources, such as space, nutrients and light. This finding agrees with Nsikani et al. (2020), which highlighted that secondary invaders often appear after the clearing of targeted species.

In addition to reduced competition and the presence of abundant nutrients from the Hartbeespoort Dam, identifying specific traits facilitating the successful invasion of *S. minima* into this water system is crucial. The success of an exotic plant species in invading ecosystems relies on its functional traits (Drenovsky et al. 2012, Liao et al. 2021). *Salvinia minima* has become widely distributed in tropical and subtropical regions due to its adaptability to diverse temperatures (Jacono et al. 2001, Parys & Johnson 2013). As such, various studies have reported the productivity and growth of *S. minima* over varying climatic conditions.

Dickinson and Miller (1998) studied the competitive interactions among *S. minima*, *Azolla caroliniana* Willdenow (Salviniaceae), and *Spirodela punctata* Meyer (Lemnacee) in Florida during summer (mid-June to early August) and autumn (early October to late November). *Salvinia*

minima dominated in summer but was dominated by the other two species in autumn. This shift in dominance was reported to be a result of extreme cold winter conditions and floods which might have caused a decline in the population of *S. minima*. Contrary to the assumption of vulnerability to cold, Olguín et al. (2002) highlighted *S. minima*'s resilience to low temperatures, enhancing its adaptability despite harsh conditions. A study by Al-Hamdani & Ghazal (2009) investigated the effects of different temperatures (15, 23, and 35°C) on the growth of *S. minima*. Their findings revealed that *S. minima* salvinia growth was the highest at 23 °C and 35°C and lowest at 15°C but highlighted that the plants could survive temperatures of 15 °C. Notably, *S. minima* has been found to survive in winter conditions in the southeastern United States (Parys 2013). This is consistent with the cold tolerance observed in the related species, *S. molesta* (Owens et al. 2004). Thus, the tolerance of *S. minima* to low temperatures observed in the previous studies explains its prevalence during winter at the Hartbeespoort Dam, where temperatures often drop below 15°C. The plant's ability to survive such cold conditions aligns with its observed presence in these colder climates.

However, as spring approaches (September 2021 and 2022), *P. crassipes* regenerates from its seed bank as *P. crassipes* is reported to have a considerable seed bank reservoir (Albano Pérez et al. 2011). *Pontederia crassipes* population growth is rapid during these periods due to the delayed recovery of *M. scutellaris* populations (Miller et al., 2021). Therefore, concerted efforts in implementing an inundative release control agent release programme during early Spring were necessary to increase insect population, thereby reducing the delay between *P. crassipes* regrowth and biocontrol agent population recovery (Hill et al.,2021 Miller et al., 2021, Coetzee et al., 2022a).

Robichaud & Rooney (2021) studied a similar ecological phenomenon related to the European common reed, *Phragmites australis* (Cav.) Trin. ex Steud (Poaceae), a highly invasive species in North American wetlands and marshes (Catling & Mitrow 2011). Once established, *P. australis* negatively impacts the environment by creating tall, dense canopies that shade out other wetland

plants (Hirtreiter & Potts 2012, Yuckin & Rooney 2019, Lei et al. 2020). After successfully removing *P. australis* using a glyphosate-based herbicide, a secondary invasion occurred. European frog-bit, (*Hydrocharis morsus-ranae* L.), a non-native aquatic plant, invaded the area due to high lake-water levels, illustrating the dynamics following secondary invasion. *Hydrocharis morsus-ranae* is a small, free-floating aquatic plant native to Europe, Asia, and Africa, and its presence at low levels increased to 33.6% after one year of *P. australis* removal, and further to 48% after two years (Robichaud & Rooney 2021). This study sheds light on the changes in population dynamics following secondary invasion, mirroring observations in the present study.

Although similar patterns were observed in 2021 and 2022 between *P. crassipes* and *S. minima*, interestingly, in 2023, the dynamics did not follow the same trend. While there was a decrease in the *P. crassipes* population during the winter months, similar to previous years, *S. minima* did not invade as extensively during this period. This shift will be thoroughly explored in Chapter 4 of this thesis, where I investigate whether allelopathy or other factors might be the cause of this change.

Secondary invasions of non-target species are increasingly noticeable in ecosystems post-removal of target species, be it temporal, particularly in communities with multiple invasive species (Pearson et al. 2016, Nsikani et al. 2019, Coetzee et al. 2022). This study highlights the secondary invasion of *S. minima* during low *P. crassipes* periods since its invasion in 2021 at the Hartbeespoort Dam, altering the population dynamics of both aquatic plants as reported by Coetzee et al., (2022).

These findings offer crucial insights into the population dynamics of *P. crassipes* and *S. minima* at Hartbeespoort Dam. Moreover, the findings highlight the effectiveness of biological control and shed light on the ecological interactions within the Dam. These insights are crucial for developing resilient and sustainable strategies to manage these invasive plants within Hartbeespoort Dam, and elsewhere where these species co-occur.

Although having established the dynamics between these invasive species, the role of competition in these population dynamics is in question as two species cannot coexist within the same space without competing for resources. The following chapter investigates the role of competition in the population dynamics between *P. crassipes* and *S. minima* exploring the effect of herbivory by the biological control agent *M. scutellaris* on the competitive ability of *P. crassipes* and *S. minima.*

CHAPTER 3

Impact of herbivory by the biocontrol agent *Megamelus scutellaris* on competitive interactions between water hyacinth, *Pontederia crassipes* and common salvinia, *Salvinia minima*

3.1 Introduction

The introduction, spread, and establishment of invasive aquatic species have captured significant attention regarding the main drivers behind their invasions (Martin & Coetzee 2011). In the case of invasive aquatic plants, their establishment in ecosystems is influenced by various factors, including their success in colonizing, spreading, and becoming dominant (James et al. 1999). However, the possibility of these invasive species spreading and establishing largely depends on their interactions with established populations, in addition to their reproductive and spreading abilities. Understanding the interactions between co-occurring plant species and how they shape the structure, dynamics, and evolution of plant communities has been a central focus for ecologists (Grace & Wetzel 1981). Aquatic plant communities are structured by multiple factors such as competition, herbivory, water quality, hydrology, and their intricate interactions (Grime 1973, Tipping et al. 2009). While the introduction of invasive species has increased with globalization, the probability of successful invasion depends on a variety of factors including resource competition (Grace & Wetzel 1981).

Competition is among the various factors that plays a significant role in aquatic environments, particularly among species with similar growth forms that occupy similar niches (Grace & Wetzel

1981, Gopal & Goel 1993). Several studies have emphasized the occurrence of competition in aquatic ecosystems due to the overlapping resource requirements of these species (Gopal & Goel 1993, Zhonghua et al. 2007, Monacelli & Wilcox 2021). Crawley (1989) suggests that interspecific competition is a crucial factor influencing the success of invasion and the dynamics of plant communities in aquatic ecosystems.

When invasive plant species are introduced to new environments, they compete with native and other invasive species for vital resources such as water, light, nutrients, and space (Bowes 1987). The outcome of this competition depends on the invasive species' ability to outcompete its counterparts and efficiently acquire limited resources in the new environment. If the invasive plant species demonstrates superior competitive traits, it can cause significant shifts in species composition by reducing the abundance of established species and increasing its population. On the other hand, if an invasive species lacks competitive advantages in the new environment, its chances of establishing are very unlikely (Grime 1973, Keane 2002, Richardson & Pyšek 2012).

Competition studies on free-floating macrophytes have primarily been centered on evaluating their relative growth rates within pure and mixed cultures as a means to evaluating their competitive abilities (Reddy & Debusk 1984, Tipping et al. 2009). These studies have indicated that species superior growth rates tend to establish dominance during initial succession stages (Goldberg 1990). Competitive outcomes are influenced by various additional factors: phylogeny, which refers to evolutionary relatedness; competition within the same species, resource availability, herbivory, and positive and negative interactions between species (Grime 1973, Crawley 1989, Bertness & Callaway 1994, Maherali & Klironomos 2007, Harpole & Suding 2007).

Competition among plants can have direct effects on their growth and fitness, but it can also indirectly influence the plants' fitness by affecting the amount of herbivore damage suffered by competing plants in the field (Grace & Wetzel 1981, Coetzee et al. 2005). Research supporting

these theories in natural ecosystems stems from observing differences in species, character displacement and the overlap of niches following competition. These observations help substantiate the impact of competitive interactions among plants on their growth and fitness (Connolly et al. 2001).

A widely acknowledged approach for evaluating the competitive interaction between species is the inverse linear model proposed by Spitters (1983) and Pantone et al. (1989), used initially in agricultural planting models, and extended to aquatic plant studies. This model incorporates an addition series, allowing the evaluation of relative competitive capabilities among plant species by means of reciprocal-yield models of mean plant dry mass. The Spitters' model has been employed in studies evaluating competition among different aquatic species with varying growth forms inhabiting diverse environmental conditions (e.g., Van et al. 1999, Coetzee et al. 2005, Mony et al. 2007).

Studies conducted by Van et al. (1999), Center et al. (2001, 2005), Coetzee et al. (2005), and Martin & Coetzee (2014) have followed Spitters' model, demonstrating its effectiveness in assessing the effects of competition on plant population ecology. These studies provide evidence that competition plays a crucial role in shaping the structure and dynamics of aquatic plant communities. However, along with competition, herbivory also plays a crucial role in the ability of species to compete with surrounding species (Harper 1977, Crawley 1989).

Interspecific competition and herbivory play significant roles in shaping plant populations and community-level patterns (Crawley 1989, Carson & Root 2000). Both processes can result in reductions in biomass, growth, reproduction, and other fitness-related measures in plant species (Cipollini & Bergelson 2002). For example, a study by Center et al. (1999) demonstrated that sustained herbivory in the field resulted in *P. crassipes* plants allocating proportionately less biomass to floral structures compared to plants that were less affected by herbivory. Herbivory

does not only directly affect plant species through feeding damage, but also indirectly by modifying the competitive abilities of plants, which can alter their positions in competitive hierarchies (Crawley 1989).

In dense plant systems where intra- or interspecific competition is intense, insect feeding is more likely to increase plant mortality (Kim et al. 2013). The indirect effects of herbivory, such as reduced plant vigour and impaired competitive ability, are considered crucial factors influencing plant population dynamics, particularly in the context of biological weed control (Crawley 1989). A spectrum of insect herbivores, such as defoliating insects, phloem-feeding aphids, whiteflies, scale insects, sap-feeding Heteroptera, as well as stem-boring flies, moths, and beetles, have been implicated in reducing the competitive ability of their host plant to an extent that they become unable to compete with more vigorous neighbouring plants (Van et al. 1999, Coetzee et al. 2005, Center et al. 2005, Martin & Coetzee 2014).

Herbivory can have direct effects on the plant species they consume, but rarely cause plant extinctions. Instead, herbivory can affect the distribution and abundance of species by altering competitive dynamics among plants (Crawley 1989). This influence can occur in two ways: (i) by causing greater damage to dominant competitors, or (ii) by imposing uniform damage across species, allowing more tolerant species to prevail. In the first case, herbivores may specialize in consuming dominant competitor species (the case for biocontrol agents) or exhibit density-dependent feeding behaviour, leading to greater damage to the more abundant species. This can result in a shift in competitive advantage, favouring the less damaged or less abundant species. In the second case, herbivory acts as a disturbance or stressor, affecting all species equally. In the presence of this stress, more tolerant species are likely to increase in abundance compared to less tolerant species (Louda et al. 1990). Herbivory can also impact competitive fitness by affecting plant growth, fecundity, and community composition, inducing defence mechanisms, and reallocating nutrients within individuals (Crawley 1989, Louda et al. 1990, Olff & Ritchie 1998,

Maron & Crone 2006, Kim et al. 2013). The influence of herbivores on competition outcomes can be subtle but significant. In cases where acute effects lead to the death of the host plant, additional factors like plant competition usually come into play (Center et al. 2001).

Center et al. (2001) conducted a study focusing on the effect of herbivory by two congeneric specialist weevils, *N. eichhorniae* and *N. bruchi*, on the competition between their host plant, *P. crassipes,* and *P. stratiotes*. In their study, Center et al. (2001) manipulated planting densities, the presence of one or both weevil species, and nutrient levels to investigate the interactions among herbivory, competition, and plant performance. The results demonstrated that in the absence of herbivory, intraspecific competition within *P. crassipes* was 41 times more intense than interspecific competition in terms of biomass yield. However, the presence of herbivores shifted the competitive outcomes, aligning intraspecific and interspecific competition. Both weevil species, *N. eichhorniae* and *N. bruchi*, significantly reduced *P. crassipes* biomass and flowering. Moreover, *N. bruchi* also had an additional effect of lessening clonal expansion, whereas *N. eichhorniae* did not. A nutrient limitation was found to affect plant performance but did not alter the overall pattern of competitive interactions. Interestingly, under low nutrient conditions, the differences between the weevil species became less pronounced, as both weevils seemed equally damaging to the plant.

A study conducted by Coetzee et al. (2005) supported and expanded upon the previous research on herbivory and competition by examining the impact of the biological control agent *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae) on the competitive performance of two aquatic plant species: *P. crassipes* and *P. stratiotes*. *Eccritotarsus catarinensis* is a sap-feeding mirid that feeds on the chlorophyll in *P. crassipes* leaves but does not typically cause lethal damage (Hill et al. 1999). The study aimed to investigate how herbivory on *P. crassipes* affected the competitive interaction between the two invasive plant species in South Africa. Using the Spitters' model, the results showed that in the absence of herbivory, *P. crassipes* was 23 times more

competitive than *P. stratiotes*. However, when exposed to *E. catarinensis*, *P. crassipes* became 10 times more competitive to *P. stratiotes*. In the absence of herbivory, *P. stratiotes* was only 0.9 times as aggressive as *P. crassipes*, but when *E. catarinensis* were present, it became 1.5 times more competitive than *P. crassipes*. This study demonstrated that biological control through herbivory reduced the vigour and competitive ability of *P. crassipes* (Coetzee et al. 2005).

The findings of Center et al. (2001) and Coetzee et al. (2005) provide valuable insights indicating that herbivory, both directly and indirectly, can impact plant performance by changing the competitive dynamics between invasive plant species. The specific response to herbivory varied depending on the herbivore species and nutrient availability. Therefore, the influence of herbivores on competitive interactions should be considered when evaluating the invasive potential of exotic plant species and assessing the effectiveness of biological control agents (Coetzee et al. 2005, Martin & Coetzee 2014).

While there is extensive theoretical and empirical research on the separate effects of competition and herbivory (Hambäck & Beckerman 2003), investigations into how these two processes interact to alter population dynamics and community-level patterns have been relatively limited. Therefore, further studies exploring the interactive effects of competition and herbivory are required to elucidate the complex interactions between these two factors and their combined influence on population dynamics and community patterns. Moreover, the complexities arising from the interactions between herbivory, competition, and nutrient availability highlight the need for clear understanding to predict these dynamics, particularly at the Hartbeespoort Dam where the population dynamics of *S. minima* and *P. crassipes* have been interchanging.

This study, therefore, assessed the competitive abilities of *P. crassipes* and *S. minima* when grown in combination with one another at different planting densities, in the presence or absence of herbivory by the planthopper *M. scutellaris*. The hypotheses tested were as follows: (i) in the

absence of herbivores, *P. crassipes*, known for its dominance in the field (El-Seed 1978, Coetzee et al. 2005), would outperform *S. minima*; (ii) herbivory would influence the competition outcome by affecting the competitive ability of the dominant competitor; and (iii) both competition and herbivory would negatively impact *P. crassipes'* plant performance.

3.2 Methods and Materials

3.2.1 Experimental setup

In order to determine the competitive interactions between *P. crassipes* and *S. minima,* an experiment was conducted inside a greenhouse tunnel at the Waainek Research Facility at Rhodes University in Makhanda, South Africa, from 25 May – 16 August 2022. *Pontederia crassipes* and *S. minima* were grown in 75L plastic tubs (Figure 3.1). Healthy and insect-free mature plants of *P. crassipes* and *S. minima* were obtained from stock cultures at the Waainek Research Facility. Before the start of the experiment, the plants were thoroughly washed to remove any insect pests and then placed in the tubs. Initial wet weights were recorded for each species. A two-week acclimation period was allowed before the commencement of the experiment. Dead material and daughter plants were removed, and the remaining plants were of similar size.

The control agents used in the experiment*, M. scutellaris*, were reared at the Waainek Research Facility. Only adult insects were collected, and the male-to-female ratio was not controlled since *M. scutellaris* exhibits a naturally balanced 50:50 sex ratio, as mentioned by Sosa et al. (2005). Thirty adults per *P. crassipes* plant were released, and the number of insects released in a tub depended on the treatment ratios specified at the beginning of the experiment (Table 3.1). To sustain and increase the insect populations, regular releases of insects were conducted every three weeks to mimic field population levels. During the initial four weeks of the experiment, visual monitoring was performed to assess feeding damage to the *P. crassipes* plants and to ensure that *M. scutellaris* had established and was actively feeding on *P. crassipes*.

Figure 3.1: Experimental setup of a particular planting combination of *P. crassipes* and *S. minima*.

Table 3.1: Planting ratios with different insect densities

Treatment	***P. crassipes*: *S. minima* ratios**	**Insect density (*M. scutellaris*)**
Control 1	0:3	0
Control 2	0:9	0
Control 3	3:0	0
Control 4	3:3	0
Control 5	3:9	0

Control 6	9:0	0
Control 7	9:3	0
Control 8	9:9	0
Treatment 1	0:3	0
Treatment 2	0:9	0
Treatment 3	3:0	30
Treatment 4	3:3	30
Treatment 5	3:9	30
Treatment 6	9:0	90
Treatment 7	9:3	90
Treatment 8	9:9	90

3.2.2 Experimental design

The experimental design followed an additive series by Spitters (1983) of factorial combinations of different planting densities of the two species in a randomized block design to elucidate the outcomes of competitive interactions between these species. A Completely Randomized Design (CRD) of 8 treatments was employed in this study. *Pontederia crassipes* and *S. minima* were planted in eight different planting densities (including mixed and monocultures), at ratios of 0:3, 3:3, 3:0, 3:9, 9:9, 9:3, 0:9, and 9:9 (Table 3.1). In total, two density matrices of *P. crassipes* and *S. minima* were created. One series was the control without insects, while the treatment included the presence of *M. scutellaris*. Each planting matrix was replicated five times, resulting in 80 tubs with different combinations of plants.

Figure 3.2: The experimental setup.

Each tub was filled with 60l of tap water, and CULTERRA Multisol 'N' –6.1.3 (44) water-soluble fertilizer (CULTERRA, Johannesburg, South Africa) was added at a rate of 3.36g/L, with monthly reapplication. The addition of Multisol 'N' at this specific dosage aimed to simulate representative field site conditions around water bodies in South Africa, providing 10mg/L of N in a 60L water tub. Commercial iron chelate (13% Fe EDTA) was also added monthly (1 heaped 10 mL spatula per tub) to maintain plant health and prevent leaf chlorosis and yellowing.

To prevent insect movement between treatments and unwanted pests from entering the tubs, the tubs were enclosed with purpose-built 0.5 mm mesh covers. These covers were fitted over a wire

frame attached to the top of the tubs and secured 15cm from the rim of the tub using Velcro. Access to the plants and insects was facilitated through the mesh cover. The plants were left to grow for 12 weeks, allowing them to reach a substantial biomass that covered most of the water surface in the tubs before being harvested and measured. The final measurements obtained included the total wet biomass of each species in each combination and the number of daughter plants that had emerged during the 12-week period.

3.2.3 Data collection

At the end of the experimental period, *P. crassipes* and *S. minima* plants were harvested from the tubs (Figure 3.3). In each tub, the total biomass (fresh weight), including daughter plants, of each species was measured using a scale. As the plants' starting weights were not identical, many of the plants experienced transplant shock whereby their growth and survival capabilities were impaired (Close et al. 2005), it was not appropriate to evaluate the performance of the plants based on their final wet weights, nor based on the amount of biomass they had accumulated during the growth period. Thus, to address the variations caused by transplant shock and differing starting weights, the wet weight values were then divided by the initial plant density to obtain the mean wet weight. This normalized the data providing a standardized measure for comparing and evaluating plant growth. Dead plant material was not removed prior to weighing. As fresh weight and dry weight are highly correlated for *P. crassipes* (Coetzee et al. 2005), fresh weight was preferred instead of dry weight.

Figure 3.3: Plant harvesting after the experiment.

3.2.4 Data analysis

The biomass data were analyzed using inverse linear models, which demonstrated the competitive abilities of each species when competing against the other plant. The magnitude of the relationship between plant density and biomass accumulation was analyzed using Spitters' (1983) reciprocal-yield-model, which involves linear regression formulas as used by Coetzee et al. (2005) and Martin and Coetzee (2014):

$$\frac{1}{W_p} = a_{p0} + a_{pp}d_p + a_{ps}d_s$$

$$\frac{1}{W_s} = a_{s0} + a_{ss}d_s + a_{sp}a_p$$

Where; $\frac{1}{W_p}$ and $\frac{1}{W_s}$ represent the inverse of the dry biomass of *P. crassipes* and *S. minima* respectively. The planting densities for *P. crassipes* and S. *minima* are expressed as $d_p$and d_s. The partial regression coefficients $a_{pp}$and a_{ss} estimate the degree of intraspecific competition occurring, while the coefficients a_{ps} and a_{sp}estimate the degree of interspecific competition. The intercepts of the plot a_{p0} and a_{s0}were used to determine the reciprocal of the isolated plants' maximum masses. The ratio of the inter- and intraspecific competition coefficients a_{pp}/a_{ps} and a_{ss}/a_{sp} measured the effects of inter- and intraspecific competition by species x or species y on its own species' yield, as well as on that of the competing species. This information is presented in the form of multiple regression planes, which indicate the effects of interspecific and intraspecific competition on the reciprocal of the mean wet weight of *P. crassipes* and *S. minima.* Two planes were produced per experiment, indicating the effects of competition on both species involved.

To evaluate the rate at which *P. crassipes* and *S. minima* were accumulating biomass over the period of the experiment, relative growth rates (RGR) were obtained using the following formula:

RGR = (ln(W2) - ln(W1)) / (t2 - t1)

Here, W2 and W1 represent the final weight and initial weight of the plants respectively at times t2 and t1, respectively, both measured in days as described by (Hunt 2012). A factorial ANOVA within a General Linear Model was used to determine whether there were significant differences in the mean end-yields of treatments (control and treatment) as well as to determine if there were noteworthy distinctions in the mean RGRs of the two species among the treatments. All statistical analyses of the biomass data were performed using STATISTICA ver. 13.0 (StatSoft Inc., 2015).

3.3 Results

3.3.1 Competition

After a period of 4 weeks, a visual assessment was conducted on each tub where *M. scutellaris* had been released. The assessment confirmed the presence of feeding damage to the leaves of *P. crassipes*. By the end of the 12-week experimental period, it was observed that *M. scutellaris* adults and nymphs were present in every tub. This showed that the insect populations had established and caused extensive damage to the *P. crassipes* plants (Figure 3.4).

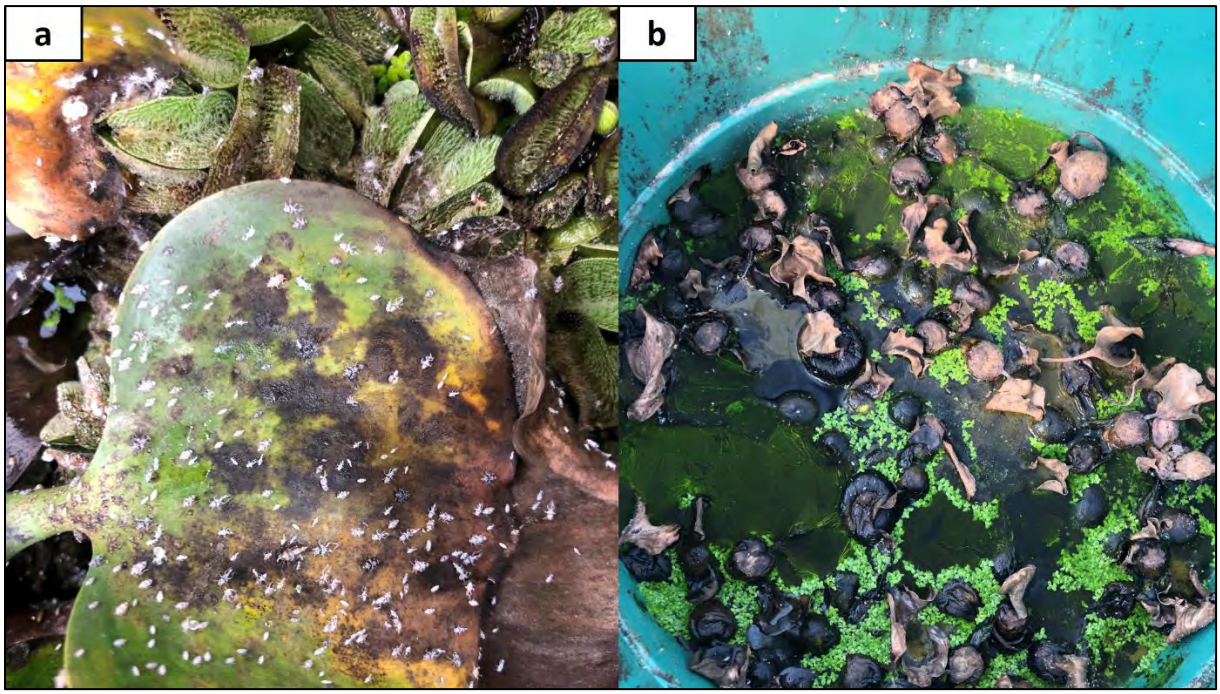

Figure 3.4: (a) *Megamelus scutellaris* damage on *Pontederia crassipes* and (b) the impact to *Pontederia crassipes* (image: T. Chikodza)

The impact of *M. scutellaris* was clearly evident, as the plants without the insect showed healthy growth, while those with *M. scutellaris* exhibited extensive damage (Figure 3.5). At the end of the experiment, it was observed that *P. crassipes* emerged as the dominant species in the absence of *M. scutellaris* feeding. The multiple regression analysis demonstrated that the biomass production of *P. crassipes* was significantly influenced by intraspecific competition but not by interspecific competition from *S. minima* (3.6a). The competition ratio (a_{pp}/a_{ps}) for *P. crassipes* biomass yield indicated that intraspecific competition was 4.5 times stronger than interspecific competition (Table 3.2). In other words, the addition of a single *P. crassipes* plant had an equivalent impact on biomass yield as four *S. minima* plants. However, when exposed to herbivory by *M. scutellaris*, the competitive ability of *P. crassipes* against *S. minima* decreased, as evidenced by the respective competition coefficients ratios (a_{pp}/a_{ps}) (Table 3.2). The presence of sustained damage from *M. scutellaris* feeding resulted in *P. crassipes* being only 1.26 times competitive than *S. minima*, indicating a 75% decrease in its competitive ability (Table 3.2).

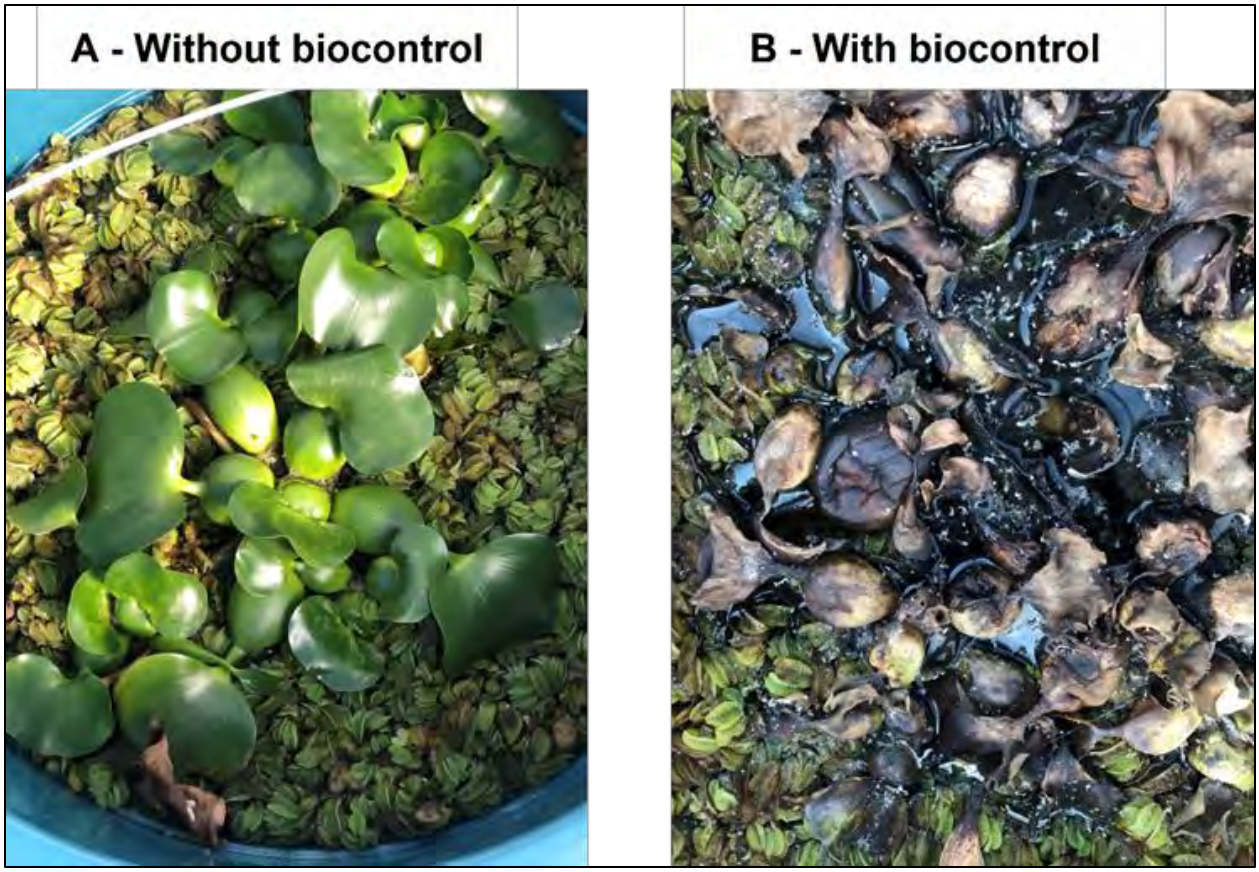

Figure 3.5: (a) Healthy plants with no *Megamelus scutellaris* feeding and (b) damaged plants by *M. scutellaris* feeding, at the end of the experimental period.

Table 3.2: Multiple regression analyses of the effects of *Megamelus scutellaris* herbivory and plant density on the reciprocal yield of *Pontederia crassipes* and *Salvinia minima*.

	Intraspecific[1] competition	**Interspecific[2] Competition**	**Ratio of [3] competition coefficients**	**Intercept**	**R^2**	**F value**
Pontederia crassipes						
M. scutellaris absent	0.0024	0.011	4.583333	0.0528	0.608	21.02*
M. scutellaris present	0.0407	0.0516	1.267813	-0.0695	0.294	5.64*
Salvinia minima						
M. scutellaris absent	0.0423	0.0518	1.224586	-0.1403	0.678	28.52*
M. scutellaris present	0.0129	0.0344	2.666667	0.0086	0.817	60.409*

[1] Intraspecific competition: represented by regression coefficients a_{pp} for *Pontederia crassipes* and a_{ss} for *Salvinia minima*

[2] Interspecific competition represented by regression coefficients a_{ps} for *Pontederia crassipes* and a_{sp} for *Salvinia minima*

[3] The ratio of competition coefficients (a_{pp}/a_{ps} for *Pontederia crassipes* and a_{ss}/a_{sp} for *Salvinia minima*) quantifies the impact of intraspecific competition within a species compared to the effects of interspecific competition from the other species on their respective biomass.

*P< 0.01

To visualise the data, three-dimensional surface-response planes were constructed, where the slope in one direction depicts the relationship between *P. crassipes* yield and its own density and the other slope depicts the density of *S. minima* on *P. crassipes* (Figure 3.6). In the absence of herbivory, the yield of *P. crassipes* was primarily influenced by intraspecific competition, with minimal impact from *S. minima* (Figures 3.6a and 3.6c). However, the presence of *M. scutellaris* altered this pattern, as interspecific competition from S. *minima* started to align more closely with intraspecific competition from *P. crassipes* (Figures 3.6b and 3.6d).

Salvinia minima was the weaker competitor. In the absence of insects, both *P. crassipes* and *S. minima* planting densities had a significant influence on the wet weight of *S. minima*, as indicated by the steep gradients in both directions (Figure 3.6c). However, when *M. scutellaris* was present, only intraspecific competition had a significant effect on *S. minima* yield (Figure 3.6d), suggesting an increase in its competitive ability, while the competitive ability of *P. crassipes* decreased.

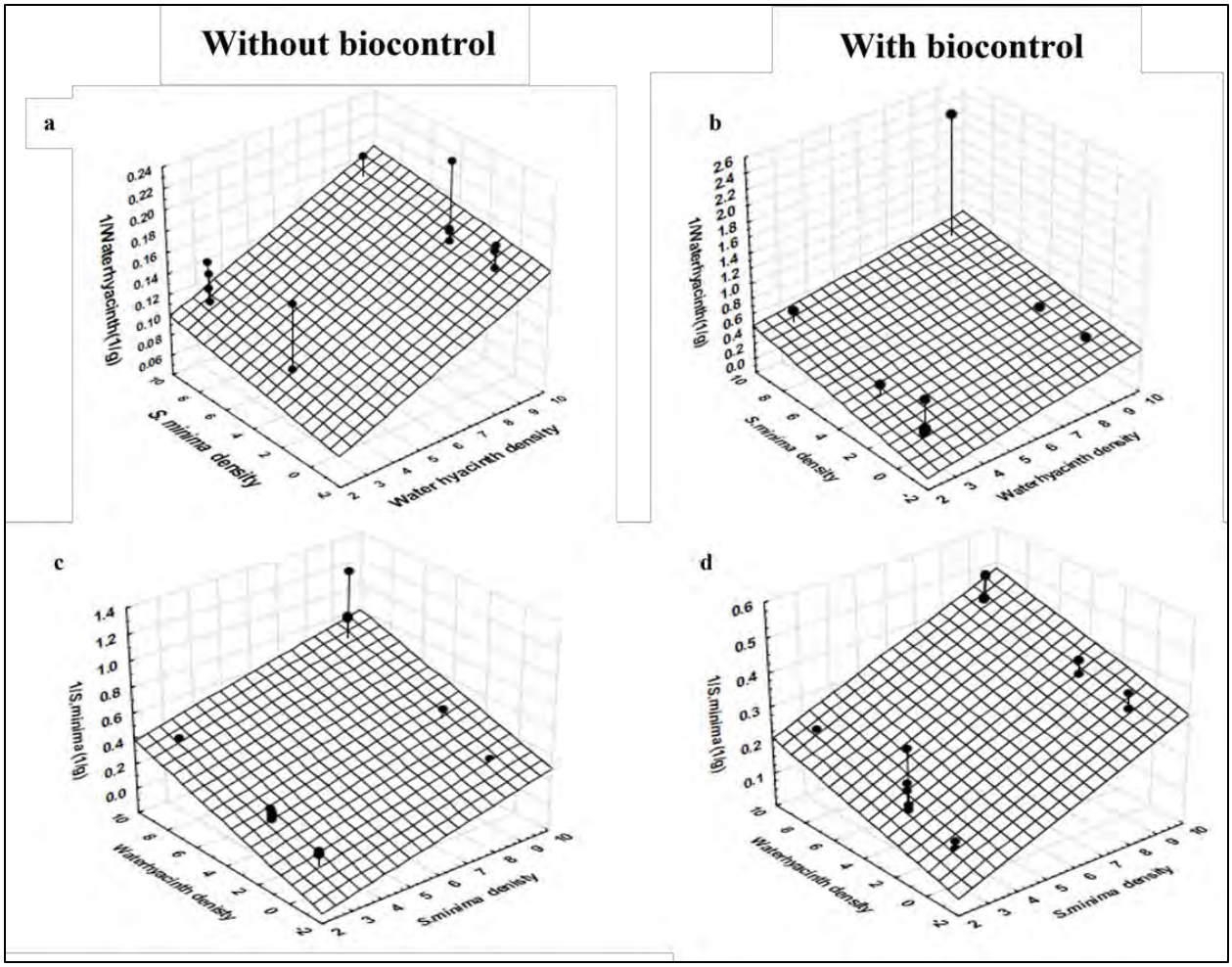

Figure 3.6: The multiple regression planes illustrate the combined effects of planting densities for *Salvinia minima* and *Pontederia crassipes* on the reciprocal of the mean wet weight (1/g) of one *P. crassipes* ($\frac{1}{W_p}$) and one *S. minima* plant ($\frac{1}{W_s}$) The graphs (a, c) and (b, d) compare the competitive abilities of *P. crassipes* in the absence and presence of *Megamelus scutellaris* feeding damage, respectively, both without and with biocontrol. X and Y axes represent the initial planting densities of *Pontederia crassipes* and *Salvinia minima*, respectively, while the Z-axis represents the inverse yield. Each point represents an observation, and the vertical lines between points depict residuals.

3.3.2 Relative Growth Rate

In the control treatment, *P. crassipes* consistently showed lower RGRs at low densities (3:0), while *S. minima* had higher RGRs (Figure 3.7). This trend held when grown together at low densities (3:3) and with different planting density combinations. At higher densities (9:0 and 9:9), *P. crassipes* had higher RGRs. In the insect treatment, *P. crassipes* generally had higher RGRs than *S. minima* across all planting density scenarios (Figure 3.7). This trend remained even when both were grown together at higher densities (9:9).

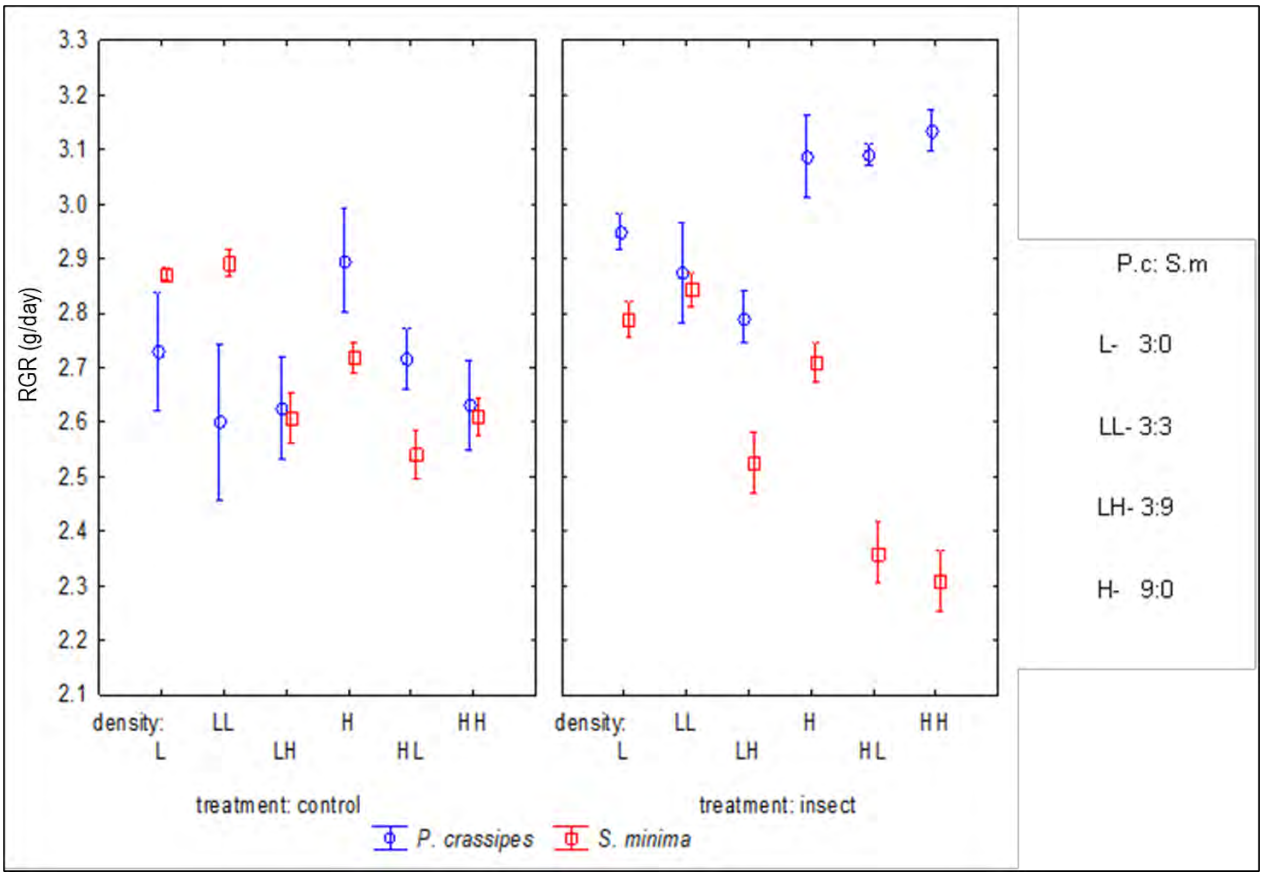

Figure 3.7 Relative growth rate of *Pontederia crassipes* and *Salvinia minima* at different densities for control and insect treatment respectively.

Statistical analysis revealed notable significant differences within the experimental factors (Table 3.3). There was a significant difference between the control treatment and insect treatment ($F=10.62$; $df=1$; $P<0.05$). Additionally, significant differences were observed between the two plant species ($F=56.14$; $df=1$; $P<0.05$). Moreover, planting densities demonstrated significant differences ($F=8.67$; $df=5$; $P<0.05$), highlighting the density's impact on outcomes. The interaction between treatment and plant yielded a significant difference ($F=60.05$; $df=1$; $P<0.05$), emphasizing their combined influence. Plant and planting density interaction displayed significance ($F=13.05$; $df=5$; $P<0.05$), indicating varied responses. Finally, the combined impact of treatment, plant type, and density showed significance differences ($F=3.25$; $df=5$; $P<0.05$), highlighting intricate factor interactions.

Table 3.3: Univariate tests of Significance for Relative Growth Rate

Effect	SS	DF	MS	F	p
Intercept	905.2411	1	905.2411	44014.84	0.000000
Treatment	0.2185	1	0.2185	10.62	0.001546
Plant	1.1546	1	1.1546	56.14	0.000000
density	0.8914	5	0.1783	8.67	0.000001
treatment *Plant	1.2351	1	1.2351	60.05	0.000000
treatment*density	0.0169	5	0.0034	0.16	0.975040
Plant*density	1.3425	5	0.2685	13.05	0.000000
treatment*Plant*density	0.3341	5	0.0668	3.25	0.009393
Error	1.9744	96	0.0206		

3.4 Discussion

Competition theory predicts that two ecologically similar species cannot coexist indefinitely when competing for the same limiting resource (Hardin 1960, Goldberg 1990, Connolly et al. 2001). This concept is particularly relevant for terrestrial plants where habitat space serves as a critical limiting resource due to the immobility of individuals. However, floating aquatic plants present a unique case as they are capable of passive movement, often through fragmentation, which enables them to evade spatial restrictions and mitigate competition (Room et al. 1981). In the context of this study system, *P. crassipes* is as an aggressive floating weed that possesses a structural advantage over other floating weeds. This advantage can be attributed to various factors, including rapid vegetative reproduction and the presence of seedbanks containing large seeds with high germination rates. These characteristics contribute to the success and competitiveness of *P. crassipes* in its environment (Hill 2003, Coetzee et al. 2005, Albano Pérez et al. 2011).

Previous studies have demonstrated the competitive nature of *P. crassipes* when grown in the presence of *P. stratiotes* (El-Seed 1978, Agami & Reddy 1990, Coetzee et al. 2005). The morphological plasticity and high productivity of P. crassipes enable it to shade and stress aquatic plants, including P. stratiotes (Agami & Reddy 1990, Coetzee et al. 2005). The results obtained in the present study align with the findings of other researchers (El-Seed 1978, Agami & Reddy 1990, Center et al. 1999, Coetzee et al. 2005), confirming that *P. crassipes* is a strong competitor under both intra- and interspecific competition, particularly in the absence of herbivory. This supports our first hypothesis that *P. crassipes*, a dominant competitor in the field (Center et al. 1999, Coetzee et al. 2005), outperforms *S. minima* in the absence of herbivores. The structural advantage of P. crassipes allows it to shade *S. minima* from sunlight, and its ability to thrive in high-nutrient waters enables it to acquire most resources, thereby causing stress to *S. minima*.

Herbivory by *M. scutellaris* reduced the vigour of *P. crassipes*, and therefore its competitive ability as measured by biomass production. The presence of *M. scutellaris* rendered the effects of interspecific competition from *P. crassipes* on *S. minima* yield negligible, indicating a reduction in the competitive ability of *P. crassipes* as shown by the flat regression plane (Figure 2.6). There was a substantial 75% reduction in intraspecific competition by *P. crassipes* when subjected to herbivory. These findings align with previous research suggesting that herbivory by biological control agents can diminish the competitiveness of host plants (Coetzee et al. 2005, Center et al. 2005, Martin & Coetzee 2014). Another significant finding of this study in particular is the ability of *M. scutellaris* in managing *P. crassipes* populations, reducing their overall competitive advantage, thereby improving the competitiveness of *S. minima* against *P. crassipes*. Mechanisms that might have contributed to *S. minima's* improved competitiveness include reduced competition for resources, increased access to light, or altered ecological conditions, such as a reduction in allelochemical concentration from *P. crassipes* if present.

Previous studies have suggested that herbivory can alter competition outcomes by differentially affecting dominant competitors (Louda et al. 1990). This present study provides empirical support for this proposition, demonstrating that herbivory does indeed influence the outcome of competition between these two invasive aquatic plants. These findings are consistent with recent research indicating that insect herbivory can affect the intensity and outcome of competition (Coetzee et al. 2005, Center et al. 2005, Martin & Coetzee 2014, Monacelli & Wilcox 2021). Therefore, my second hypothesis, which posits that herbivory modifies competition outcomes through its selective impact on dominant competitors, is supported.

Interestingly, when subjected to herbivory, *P. crassipes* showed higher RGR across all planting densities. This could potentially be attributed to a compensatory mechanism triggered in response to herbivory. It was also noted that the presence of herbivory does not appear to significantly reduce *P. crassipes* yield. This absence of yield reduction in *P. crassipes* could be linked to the

herbivory compensation phenomenon, as suggested by Coetzee et al. (2005). Generally, interactions between plants and herbivores are viewed as antagonistic due to the negative direct effects of biomass consumption by herbivores (De Mazancourt & Loreau 2000). However, an intriguing hypothesis challenges this notion. The grazing optimization hypothesis suggests that under specific conditions, herbivores can enhance plant primary production. According to this hypothesis, primary production increases at low grazing intensity, reaches a maximum at moderate grazing intensity, and then declines with further increases in grazing intensity (Hilbert et al. 1981, Dyer et al. 1986, De Mazancourt & Loreau 2000, Yamauchi & Yamamura 2004).

Coetzee et al. (2005) identified several factors that could account for the limited impact of herbivory on the yield of *P. crassipes* in the study. Firstly, while most tubs exhibited severe feeding damage from *M. scutellaris*, it could be possible that some tubs contained plants with moderate feeding due to fluctuations in insect populations influenced by environmental conditions. This spatial variation in herbivory intensity suggests that the extent of damage varied. Secondly, the compensatory response of *P. crassipes* to herbivory may have been a short-term phenomenon, implying that a longer duration of the study would likely reveal a more pronounced reduction in *P. crassipes* yield, as observed in natural systems at the Hartbeespoort Dam. This highlights the importance of considering the temporal dynamics of herbivory effects. Lastly, the presence of high nutrient concentrations in the nutrient media may have influenced the response of *P. crassipes* to herbivory. Nutrient availability is known to modulate plant responses to herbivory, and the elevated nutrient levels in the media could have facilitated the compensatory growth and resilience of *P. crassipes* when exposed to herbivory.

Previous studies have indicated that herbivory can impact competition outcomes, particularly if it affects the dominant competitor (Louda et al. 1990). In line with these findings, this study also found that herbivory influenced the competitive dynamics between *P. crassipes* and *S minim*a. This finding aligns with numerous other studies that have suggested the ability of insect herbivory

to influence the magnitude and outcome of competition (Coetzee et al. 2005, Kim et al. 2013, Martin & Coetzee 2014). In this study, the planthopper *M. scutellaris* effectively neutralized the competitive advantage that *P. crassipes* had over *S. minima*, demonstrating its efficacy as a biocontrol agent. The effects of herbivory on competitive interactions observed in this study can help explain the patterns observed in the field at the Hartbeespoort Dam.

In this study, *M. scutellaris* effectively countered the strong competitive advantage held by *P. crassipes* over *S. minima*. This finding confirms the efficacy of M. scutellaris as a biocontrol agent in mitigating the dominance of *P. crassipes* over surrounding species. The results of the study contribute to our understanding of competition between invasive macrophytes and shed light on the establishment and spread of such invasive species. By investigating the competitive ability of *P. crassipes* when grown with *S. minima* and examining the impact of biological control, the study provided valuable insights into the dynamics of competition between these invasive plant species. It became evident that herbivory, both directly and indirectly, affected plant performance by altering the competition between *P. crassipes* and *S. minima*. However, it is important to note that the competitive response varies depending on herbivore species and nutrient availability. Despite understanding, the impact of herbivory on the population dynamics of these invasive aquatic plants, it is important to explore whether allelopathy plays a central role in *P. crassipes*' competition with *S. minima* as previous studies have reported the allelopathic effects of *P. crassipes*. The upcoming chapter investigates the potential of allelopathy as a driving mechanism behind the competitive strategies of *P. crassipes*.

CHAPTER 4

Assessing the potential allelopathic effects of *Pontederia crassipes* on *Salvinia minima*

4.1 Introduction

Within the complex interactions of natural ecosystems, resource competition and allelopathy play a crucial role in shaping the dynamics of plant communities (Muller 1966, 1969, Harper 1977). As such, a growing body of research has underscored the significance of allelopathy as a competitive strategy employed by certain plants to outcompete surrounding plants (Gopal & Goel 1993). Allelopathy involves chemical interactions between and among plants and microorganisms, via the release of biologically active compounds into the surrounding environment (Levin 1976, Inderjit & Keating 1999).

The concept of allelopathy, derived from the Greek words "allelos" (mutual) and "pathos" (suffering), was first explored by Molisch (1937), an Australian physicist in his book "The Influence of One Plant on Another: Allelopathy", and later redefined by Rice (1985) as the direct or indirect impact that a plant exerts on another by releasing chemical compounds. Rice (1985) further explored the phenomena of allelopathy and termed the plant releasing the allelochemicals the ‘donor’ and the plant being influenced by the allelochemicals the ‘target’. Allelopathy is a well-known phenomenon in terrestrial plant communities and has also been found to occur in macrophytes (Gross 2003).

Research has highlighted the central role of allelopathy in shaping interrelationships among organisms (Rice 1995; Mulderij 2006) with both field observations and laboratory studies strongly

supporting the occurrence of allelopathy across aquatic habitats, ranging from marine to freshwater environments (Gross 2003). Shading by epiphytic and planktonic primary producers, alongside competition for limited resources such as nutrients, moisture, temperature, pathogens, and light, may be the ultimate cause of allelopathic interaction interfering with multiple physiological processes of another plant (Lovett et al. 1989, Gross 2003, Cronin and Lodge 2003, Weidenhamer 2006, Albert et al. 2009).

Chromatographic and spectroscopic analyses have shown that macrophytes can release fatty acids, alkaloids, phenols, and tannins (Saito et al. 1989, Gross 2003, Hilt 2006). These analyses are instrumental tools for understanding allelopathy within aquatic ecosystems, shedding light on the specific compounds that mediate allelopathic effects. By separating and identifying the distinct compounds released by plants into their environment, chromatographic and spectroscopic analyses provide evidence of the biochemical mechanisms involved.

According to Inderjit & Keating (1999), plants release allelochemicals into the environment through root exudation, foliar leaching, and residue decomposition, with root exudates as the most common pathway of allelochemical production by several plants. The effectiveness of allelopathic compounds in aquatic ecosystems relies on their concentration within water (Kato-Noguchi & Ino 2001). Consequently, the production, excretion, and distribution of these active compounds determine their impact (Lewis, 1986). Both biotic and abiotic stressors significantly influence the expression of allelopathy, with environmental stressors potentially leading to increased allelochemical production (Lovett et al. 1989, Einhellig 1994).

The biomolecules involved in allelopathic processes can be categorized as secondary metabolites, which are not directly involved in an organism's fundamental physiological processes, or primary metabolites, which play essential roles in normal growth, development, and reproduction (Molisch 1937, Weidenhamer 2006). These substances, produced by plants, consist of biomolecules that

influence the growth, survival, reproduction, health, and behaviour of neighbouring organisms (Chaïb et al. 2021, Weidenhamer 2006).

Aquatic plants exhibit adaptability, employing both competitive and allelopathic strategies under varying circumstances and when interacting with different plant species. A good example of this adaptability is *P. crassipes*, a species known for its tendency to dominate a range of aquatic environments (Shanab et al. 2010, Chapter 3). Studies have been conducted to investigate whether *P. crassipes* dominates other organisms in its habitat solely through competition, where it competes for and reduces available resources in the environment, or if it also exerts allelopathic effects (Yu et al. 1992, Jin et al. 2003, Gross et al. 2007). Multiple studies have recognized *P. crassipes* as an allelopathic plant, particularly due to its release of allelochemical compounds (Sharma 1985, Yu et al. 1992, Jin et al. 2003, Chen et al. 2005, Shanab et al. 2010, Pei et al. 2018). These studies have shown that *P. crassipes* releases allelochemicals in the presence of different species of green algae to inhibit their growth in eutrophic water bodies. As a result, the removal of *P. crassipes* in nutrient-rich water bodies results in the presence of blue-green algae.

Sharma (1985) reported that both the leaf leachate and root litter of *P. crassipes* showed inhibitory effects on algae. In contrast to this finding, Sun et al. (1993) reported that mainly the roots of *P. crassipes* showed inhibitory activities on algae. Pei et al. (2012) further investigated the allelopathic effects of *P. crassipes* on *Microcystis aeruginosa* tracing the algicidal active parts (algae inhibitory activity) using roots and whole plant. Results revealed that although the roots of *P. crassipes* have similar algicidal activity on *M. aeruginosa* as the whole plant did, *P. crassipes* inhibits the growth of *M. aeruginosa* mainly through its root system.

A comprehensive study by Sun et al. (1990) investigated the potential of *P. crassipes* to regulate phytoplankton communities through allelopathy. This study was the first to lead to the isolation and identification of specific allelochemicals from *P. crassipes* root exudates. Among these

compounds are N-phenyl-1-naphthylamine (P1NA) (compound 1), N-phenyl-2-naphthylamine (P2NA) (compound 2), linoleic acid (compound 3), and glycerol-1,9-12(ZZ)-octadecadienoic ester (compound 4) which have been highlighted to be key allelochemicals (Figure 4.1) (Shanyuan et al. 1992, Sun 1993). Previous research highlighted that the structural variance between P1NA and P2NA solely involves the placement of anilino-N substitutions at the 1 and 2 positions within the naphthyl. Previously, there were uncertainties whether there were different toxicity levels between P1NA and P2NA. A study by Cheng et al. (2017) sought to fill this gap by comparing the biotoxicity of P1NA and P2NA on cyanobacteria *M. aeruginosa*. This comparative study revealed that exclusively P1NA exhibited high toxicity towards *M. aeruginosa*, while P2NA did not demonstrate the same level of toxicity.

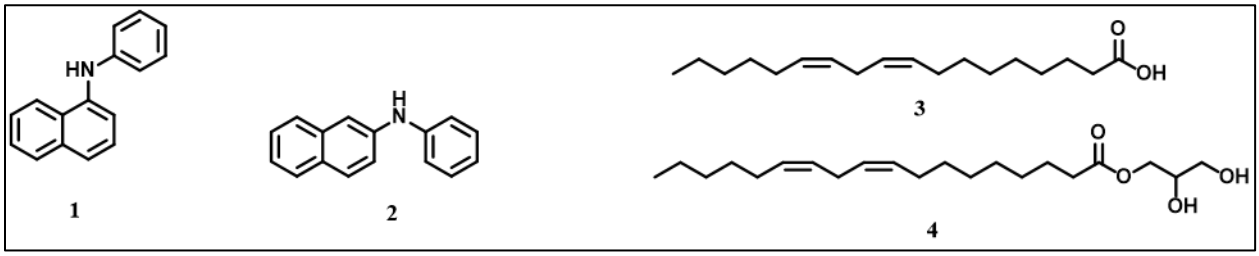

Figure 4.1: (1) N-phenyl-1-naphthylamine (2) N-phenyl-2-naphthylamine (3) Linoleic acid and (4) glycerol-1,9-12(ZZ)-octadecadienoic ester.

Notably, all four of these compounds exert negative effects on various microbes, including phytoplankton (Sun 1993, Jin et al. 2003, Shanab et al. 2010, Pei et al. 2018). P2NA extracted from *P. crassipes* exhibited a strong inhibitory effect on cyanobacteria (Shanyuan et al. 1992). This discovery of P2NA in the culture water where *P. crassipes* was cultivated hints at the possibility of its biosynthesis within the plant's roots, with subsequent excretion into the aquatic environment, thereby exerting its inhibitory effects on algae (Shanyuan et al. 1992). Additionally, Brack et al. (1999) examined P2NA concentrations in sediment and freshwater near *P. crassipes*

production sites in Germany, revealing significantly elevated levels, reaching as high as 15.8 mg/kg of P2NA, within the sediment surrounding these production areas.

Mironga (2006) identified specific compounds that underlie *P. crassipes'* allelopathic effects on microbes within Lake Naivasha, the largest freshwater lake in Kenya. The outcomes of this study showed similar compounds P1NA, P2NA and linoleic acid found by Shanyuan et al. (1992) and Sun (1993). Mironga (2006) further reported a notable reduction in phytoplankton productivity in the presence of *P. crassipes*, suggesting that *P. crassipes* not only shapes the lake's ecological dynamics but also brings about alterations in species composition and biodiversity through its allelopathic actions.

While various case studies have underscored *P. crassipes'* allelopathic influence on neighbouring algae, its effects on adjacent aquatic vegetation remain uncertain. This chapter addresses this knowledge gap by investigating the potential of *P. crassipes* to inhibit the growth of *S minima*, a co-occurring aquatic invasive macrophyte on Hartbeespoort Dam. This chapter's objectives were twofold: (i) to extract the chemicals within *P. crassipes* when grown in the same environment as *S. minima* and when grown alone. (ii) to determine the presence or absence of chemical compounds released by *P. crassipes* during allelopathy with microbes and phytoplankton (particularly algae) are also released when interacting with *S. minima*. This outcome will evaluate the potential of *P. crassipes* releasing allelochemicals, not only to algae but neighbouring plants such as *S. minima*.

4.2 Methods and materials

4.2.1 Chemicals used for matching *P. crassipes* extracts

Previously identified allelochemicals from *P. crassipes* were bought from the Merck Pharmaceutical company in South Africa to match their similarities with compounds within *P. crassipes*. Glycerol-1,9-12(ZZ)-octadecadienoic ester was not found and thus was not tested. Chemical. Methanol for extracting the compounds from the plants was bought at the Rhodes University Chemistry store.

4.2.2 Experimental design

The same *Pontederia crassipes* plants used in the competition experiment (Chapter 3) were tested for allelopathic compounds in this study. Following the additive series by Spitters model described in Chapter 3's methods, tubs were labelled C1-C8 (control without insects) and T1-T8 (treatment with insects) based on varied planting densities thereby providing a spectrum of potential competition outcomes that could influence allelochemical production. Tubs C1, C2, T1, and T2 were omitted as they contained *S. minima* without *P. crassipes* (Table 3.1 in chapter 3).

4.2.3 Plant processing and extraction

At the end of the competition study, following weighing, the *P. crassipes* plants were taken to the laboratory and washed to eliminate all impurities. The fresh plant material (separate leaves and roots, Figure 3.2 c) was then air-dried at room temperature for five weeks, and ground to powder as per Jin et al., (2003) and Pei et al., (2018) using a commercial blender (Philips HR2056/90) for five minutes. The powder was weighed and mixed in 350 ml of methanol (solvent) in a conical flask to extract the secondary compounds. Methanol is a commonly used solvent to extract

compounds as described by (Gross 2003). Plant extract solutions were prepared at a standard concentration (10mg/ml), following the methodology described by Elsayed (2020).

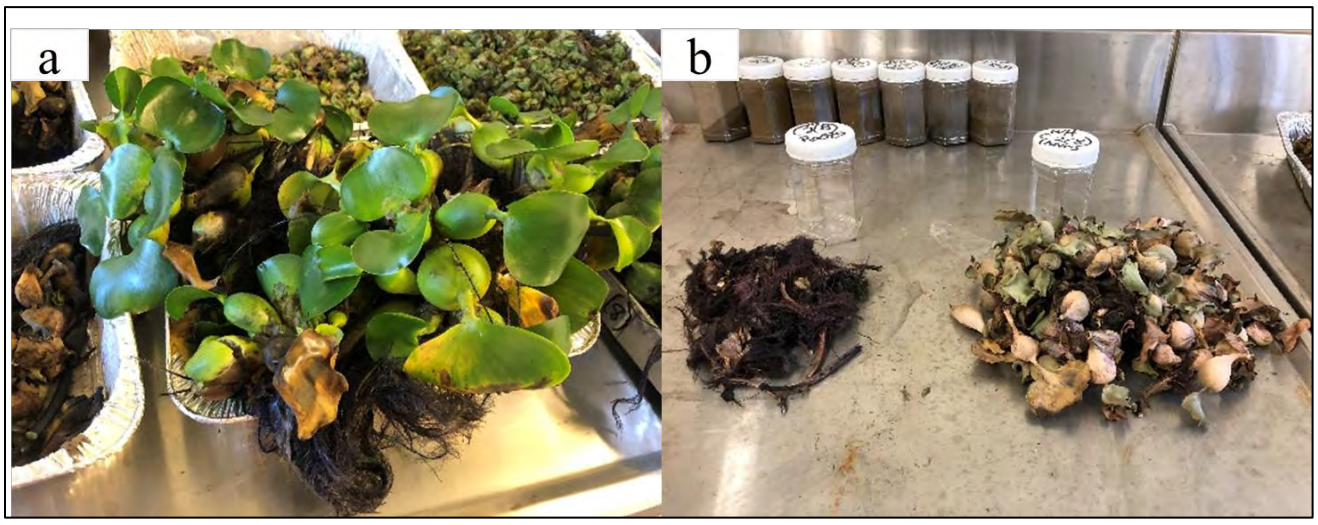

Figure 4.2: (a) Fresh samples of *P. crassipes* before drying and (b) dry leaves and roots of *P. crassipes*.

The flask was then placed on a rotary shaker for two days to extract the compounds from the plant material (Figure 4.3a). Subsequently, the mixture was filtered using suction filtration with a Büchner funnel containing filter paper attached to the flask to separate the methanol from the ground powder (Figure 4.3b). The filtered product was weighed on a balance, and the solvent was evaporated in a fume hood as described by Alves et al. (2011) and Elsayed (2020). Subsequently, the solvent underwent further evaporation using a rotary at 40 degrees Celsius following the method outlined by Elsayed (2020). The resulting crude extract from the fume hood was weighed and then dissolved in methanol in a plastic beaker to achieve the desired concentration (10mg/ml) (Elsayed 2020). Following this, an LC-ESI-MS/MS was used to identify the active compounds in the *P. crassipes* extracts.

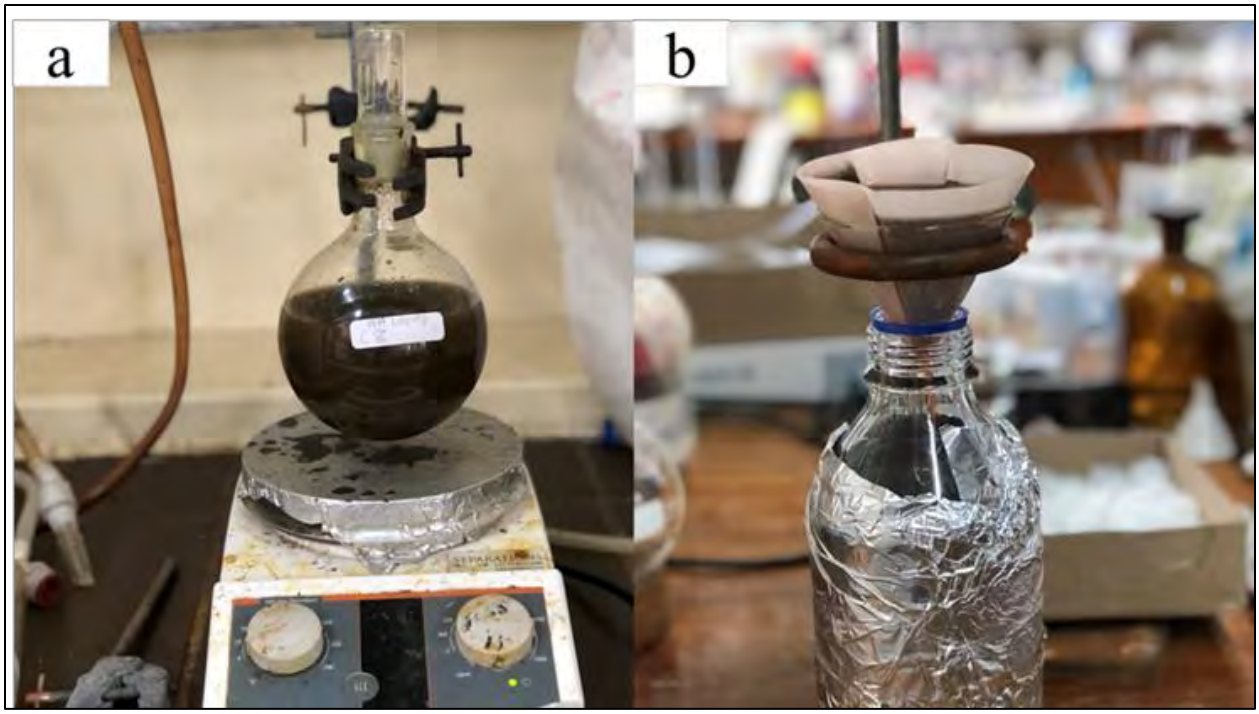

Figure 4.3: (a) Extract solution on a rotary shaker for mixing and (b) the filtration of the mixture.

4.2.4 Data analysis

4.2.4.1 LC-ESI-MS/MS acquisition

Samples were resuspended in methanol at 1 mg/mL and analyzed in a Bruker Compact ESI-MS/MS, coupled to a Dionex Ultimate 3000 UHPLC equipped with a Water Xselect CSH C18 column (2.1x150 mm, 2.5 µm). The injection volume was 5 µL and the mobile phase consisted of water (A) and acetonitrile (B), both acidified with 0.1 % formic acid. The mobile phase gradient program was as follows with a flowrate of 0.4 mL/min: min 0-2 = 85/15 (A/B), min 2-3 = ramp to 60/40 (A/B), min 3-5 = hold, min 5-6 = ramp to 30/70 (A/B), min 6-8 = hold, min 8-10 = ramp to 0.1/99.9 (A/B), min 10-13 = hold, min 13-16 = re-equilibration.

The electrospray source was operated in positive ionization mode with an End Plate Offset voltage of 500 V, a capillary voltage of 4500 V, a nebulizer pressure of 3 bar, a dry gas flow of 9 L/min and a dry temperature of 200 °C. A mass range from 50 to 2000 *m/z* was set for acquisition and collision energies for MS2 collision-induced dissociation were set to 30 and 60 eV (Multi-CE) and an isolation window width of 1.5 *m/z.* Number of precursors was set to 10 with active exclusion after 6 spectra and release after 0.5 min or when current intensity/previous intensity ≥ 2. The trigger threshold was set to 1350 cts.

4.2.4.2 Data preprocessing

Data were converted to mzXML format with Bruker Compass software and then processed in MZmine3 (Schmid et al. 2023). Mass detection was carried out with noise levels of 1000 for MS1 and 50 for MS2, followed by chromatogram assembly with the ADAP Chromatogram builder with a minimum of 3 consecutive scans, a minimum intensity for consecutive scans of 2500 and a minimum height of 5000. *M/z* tolerance was set to 0.001 *m/z* or 10 ppm. Following this, the chromatograms were deconvoluted using the local minimum feature resolver with a chromatographic threshold of 10 %, a minimum search range of 0.1 min, a minimum height of 10000, a minimum edge top peak ratio of 1.2 and a peak duration of 0-3 min. MS2 spectra were linked to MS1 features with a tolerance of 0.05 *m/z* and 0.2 min.

Next, the isotopic peaks finder was used with the elements C, H, N, O, P, S, Cl, and Br, with a maximum charge of 1 and a tolerance of 0.001 *m/z*. The 13C isotope filter was used to remove isotope signals with a tolerance of 0.1 min and 0.001 *m/z* as well as a requirement for monotopic shape and a charge of 1. The feature lists were then merged with the Join Aligner with a tolerance of 0.2 min and 0.001 *m/z*. Weight was set to 80 for *m/z* and 20 for retention time. The aligned feature list was then filtered to only keep features between 1.2-14 min and with an associated MS2 spectrum. Gap-filling was carried out with the Peak finder module with tolerances of 25 % for

intensity, 0.2 min, and 0.001 *m/z*. Next, the Feature list blank subtraction module was used to remove all features present in a methanol blank, unless their peak area in one of the samples was more than 300 % compared to the blank. The Duplicate feature filter was then used with tolerances of 0.2 min and 0.001 *m/z*, followed by reordering of the feature IDs and finally, export of the final feature list files for GNPS, web-based mass spectrometry ecosystem and SIRIUS5, a java-based software framework to analysis of LC-MS/MS data of metabolites and other. In both cases, MS2 spectra were merged across samples with the weighted average mode, a mass tolerance of 0.001 *m/z* or 10 ppm, a minimum cosine of 0, a signal count threshold of 20 %, and an isolation window width of 1.5.

4.2.4.3 Spectral matching and molecular networking with GNPS

A molecular network was created with the Feature-Based Molecular Networking (FBMN) workflow on GNPS (Nothias et al. 2020). The mass spectrometry data were first processed with MZMINE2 (Nothias et al. 2020) and the results were exported to GNPS for FBMN analysis. The precursor ion mass tolerance was set to 0.01 Da and the MS/MS fragment ion tolerance to 0.02 Da. A molecular network was then created where edges were filtered to have a cosine score above 0.7 and more than 6 matched peaks. Further, edges between two nodes were kept in the network if and only if each of the nodes appeared in each other's respective top 10 most similar nodes. Finally, the maximum size of a molecular family was set to 100, and the lowest-scoring edges were removed from molecular families until the molecular family size was below this threshold. The analog search mode was used by searching against MS/MS spectra with a maximum difference of 100.0 in the precursor ion value. All matches kept between network spectra and library spectra were required to have a score above 0.7 and at least 4 matched peaks. The DEREPLICATOR was used to annotate MS/MS spectra (Mohimani et al. 2018) The molecular networks were visualized using Cytoscape software (Shannon et al. 2003). The GNPS results can be accessed at:

https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=b1a7925ce5ae4df4ad7aae3e68799c0b.

4.2.4.4 SIRIUS5 in silico structure and compound class prediction

The results were further analyzed through the SIRIUS5 suite to achieve compound class and molecular structure predictions for annotation of ion features that did not provide spectral matches through GNPS (Dührkop et al. 2019). The default parametrization was utilized except for the selected possible elements being C, H, N, O, P, S, and Cl with possible ionizations of $[M+H]^+$ and $[M+Na]^{+,}$ and for *in silico* structure prediction all databases were selected. Finally, two-sided T-test were carried out with the MetaboAnalyst (Pang et al. 2021) with missing values being replaced by one fifth of the minimum value per feature and the data was log-transformed (base 10) and features were scaled (auto scaler, mean-centered and divided by the square root of the standard deviation of each variable). PERMANOVA for group significance was carried out using the scikit-learn and scikit-bio packages in python.

4.3 Results

A network of all the samples was obtained with a presentation of chemical metabolites present in each sample (Figure 3.4). Annotations in italics are based on *in silico* predictions from SIRIUS5/CANOPUS, those in normal font are based on spectral matching to GNPS library spectra. Node labels correspond to precursor *m/z* values. Node sizes increase with precursor intensity and edge width increases with spectral similarity. Red rounded rectangular node borders indicate the top 5 features that are enriched in either leaves or roots, red octagonal borders indicate the top 5 features enriched in control or treatment samples, black rectangular node borders indicate features enriched in samples from *P. crassipes* co-planted with *S. minima* (C/T 3 and 6), black dashed border (**V**) shows feature most enriched in *P. crassipes* only samples.

Flavonoids were putatively identified through spectral matching with GNPS and *in silico* structure predictions with SIRIUS5 (**A** and **B**). A subcluster of these **B** was predominantly detected in the root samples, while the other (**A**) appeared relatively more abundant in treatment leaves. Amyl amines were annotated predominantly in treatment roots and field samples (**C**). Putative chlorins (pheophorbide related (**D**) were detected predominantly in leaf samples, while putative naphtoquinones (**E**) appeared more abundant in root samples. Numerous putative linoleic acid derivatives were detected throughout the samples, including spectral matches for linoleic acid and pinolenic acid (**F**). Linoleic acid was identified by comparison to authentic standard. Similarly, features putatively identified as steroids (**G**) were detected throughout the samples.

Putative cinnamic acid-related compounds were detected predominantly in the root samples **(H).** Features annotated as anisoles were detected through the samples (**I**), and similar observations were made for the clusters **J** and **K** annotated as alkaloids. Features annotated as hydroxycinnamic acid-related compounds (**L**) showed inconsistent profiles (possibly a result of low intensities near the detection limit). Putative plasticizers (**M**, phthalates, and citrate esters) were detected in some

samples, which is likely a result of leaching from containers used during sample processing. Several of the features annotated as ceramides (**N**) were found to be more abundant in treatment samples. Features annotated as *N*-acyl amines were predominantly detected in field samples (**O** and **U**). The two clusters **P** and **Q** were annotated as alkaloids and detected throughout the samples. The features in cluster **R** were putatively identified as octadecanoids and present mostly in selected treatment samples. Putative glycerophosphocholines **(S)** were detected throughout the samples. The features in cluster **T**, annotated as linoleic acid-related glycerides were predominantly detected in control leaf samples. Features annotated as *N*-phenethylhexadecanamide-related compounds **(V)** were detected throughout the samples with one feature appearing to be mostly present in root samples. Putative phenylalanine-related features (**W**) were mostly present in control leaf samples (phenylalanine itself) and the field leaf sample. Treatment leaf samples appeared enriched in features matched to polanrazines (**X**). These are fungal metabolites, and they may indicate fungal infection. The features in cluster **Y** were annotated as alkaloids. Harmane and Norharmane (**Z**) were putatively identified, and the respective features were found most abundant in field samples. All singleton features without partner features with sufficiently similar fragmentation spectra were omitted except for a feature matched to the synthetic pesticide primicarb **(1),** mostly detected in treatment samples and control root samples, the feature annotated as *N*-acetyltyramine **(2),** mostly detected in selected treatment leaf and root samples and the files samples, as well as putatively identified vanillin **(3),** present predominantly in treatment root samples and the field root samples as well as lower relative abundance in the field leaf sample. No N-phenylnaphthylamines (P1NA and P2NA) were detected.

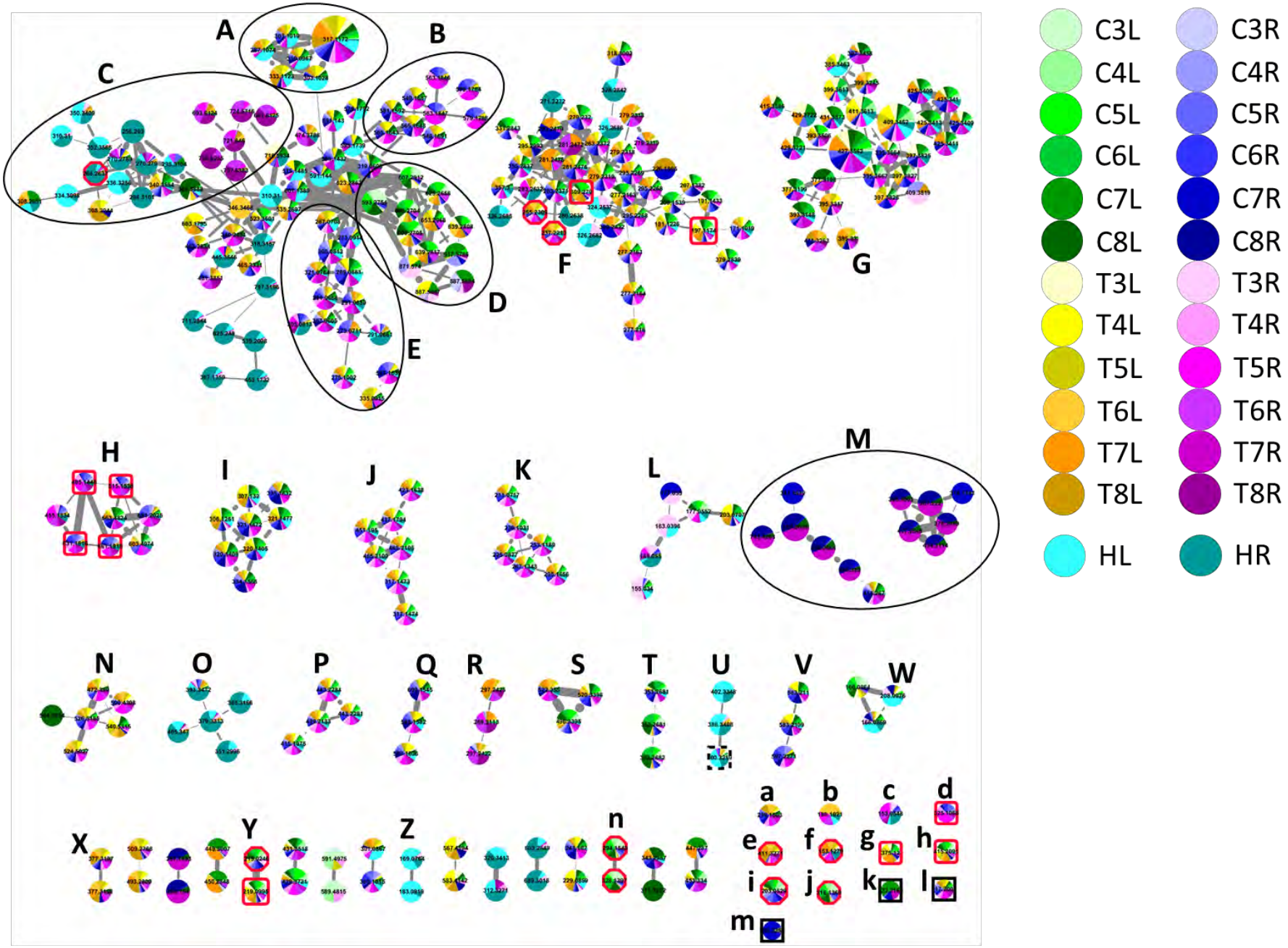
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Figure 3.4 **A** *flavonoids* **B** isoschaftoside related flavonoids **C** amyl amines **D** chlorins **E** *naphtoquinones* **F** linoleic acid and analogs **G** steroids **H** *cinnamic acid derivatives* **I** *anisoles* **J+K** *alkaloids* **L** *hydroxycinnamic acid related* **M** plasticizers **N** *ceramides* **O** *N-acyl amines* **P+Q** *alkaloids* **R** *octadecanoids* **S** *glycerophosphocholines* **T** *linoleic glycerides* **U** *N-acyl amines* **V** *N-phenethylhexadecanamide* related **W** phenylalanine and related **X** polanrazine related **Y** *alkaloids* **Z** harmane and norharmane **a** primicarb (pesticide) **b** N-acetyltyramine **c** vanillin **d-m** statistically significant singleton features n *N*-(1-Deoxy-1-fructosyl) phenylalanine and related feature.

Three comparisons were made by carrying out two-sided T-tests with the Metaboanalyst suite (i) Roots vs Leaves, (ii) Control vs. Treatment, and (iii) *P. crassipes* grown by itself vs grown with *S. minima*. These comparisons help to identify any significant differences in metabolite profiles between groups. For each comparison, the top 5 most significantly enriched features per group were highlighted in the molecular network (for comparison 3, there were only four reported overall, because there were only very few features with significant differences between samples, and the differences were not very pronounced. This means there does not seem to be much of a detectable influence of *P. crassipes* co-existing with *S. minima*). Field samples from the dam were excluded from this comparison as the trends from the field samples are clear in the network and there were only two samples.

For comparison 1 (Leaves vs Roots), there were significant differences observed ($p<0.047$). The top 5 most enriched features in leaves included two features in cluster (**F)** (the one on the right was matched to linoleic acid, the one on the left to eicosadienoic acid), as well as one feature in cluster (**Y)** (predicted to be an ornithine alkaloid) and the features **g** (predicted to be a sulfonamide) and (**h)** (precited to be a lignan). The features enriched in root samples included 4 features in cluster **H** (predicted to be cinnamic acid glycoside analogs) and feature (**d)**, precited to be an anthraquinone.

Similarly for comparison 2 (Control vs Treatment), there were significant differences observed ($p<0.05$). The top features most significantly enriched in treatment samples were a putative amyl amine (predicted to be a heptadecenamide) that was most abundant in the root field samples (but still enriched in treatment over control), two features in cluster **F** (Linoleic acid related fatty acids) and features (**e)** (predicted to be a fatty amide) and **f** (precited to be a fatty acyl). The top 5 features enriched in the control samples compared to the treatment comprised a putative alkaloid (**Y**), a cluster of two features **(n)** that includes a match to N-(1-Deoxy-1-fructosyl) phenylalanine (bottom node), as well as feature (**i)** (no prediction or match) and **j** (predicted to be a nucleoside).

For Comparison 3 (*P. crassipes* grown alone vs. grown with *S. minima*), there were no significant differences (P= 0.298). Although no significant differences were observed from the overall network, three enriched features were found in *P. crassipes* samples grown with *S. minima*: **k** (predicted as a pyrrolidine), **l** (predicted to be a flavonoid), and **m** (with no prediction but notably more abundant in one sample than others). The limited number and nature of these enriched features suggest minimal differentiation between these growth conditions. Additionally, a single feature was identified in samples solely from *P. crassipes* growth (cluster **U**, dashed rectangular borders), identified as N-phenethylhexadecanamide. Notably, this feature showed the highest abundance in field samples, suggesting its prevalence in natural conditions.

4.4 Discussion

Various studies have reported that macrophytes such as *P. crassipes* can release bioactive allelochemical secondary metabolites in quantities sufficient to inhibit the growth of surrounding organisms, particularly algae (Saito et al. 1989, Aliotta et al. 1991, Nakai et al. 1996). This study detailed the chemical composition of *P. crassipes* leaves and roots under different growth conditions: alone and with *S. minima*. Additionally, the study also examined field samples from Hartbeespoort Dam. This allowed the identification of chemicals present under different conditions: with competition from *S. minima*, without competition, and variations due to insect presence or absence.

Of the investigated compounds, N-phenyl-1-naphthylamine and N-phenyl-2-naphthylamine, were absent in *P. crassipes* leaves and roots and field samples. However, linoleic acid was detected. Linoleic acid (**F**) was found in all the samples (both roots and leaves) including the field samples. Linoleic acid is listed among the allelopathic compounds from *P. crassipes* by Shanyuan et al. (1992) and Sun (1993). The presence of this compound in all samples highlights the potential that *P. crassipes* uses this compound for defence purposes.

A similar study by Aliotta (1991) investigated the potential allelopathic effects of another macrophyte, *Pistia stratiotes* L. (Araceae) (water lettuce) on microalgae. Among the active compounds observed to potentially inhibit the growth of microalgae was linoleic acid. The collective finding from this study and along with that of Aliotta (1991), Shanyuan et al. (1992), and Sun (1993) highlight that linoleic acid, a fatty acid is employed by certain macrophytes, including *P. crassipes*, as an allelopathic defence compound.

Another interesting finding observed was the presence of alkaloids throughout all the leaf and root samples in the control and treatment (**J+K, P+Q**) including field samples (only leaves) in **P+Q**. A study by Shanab et al. (2010) which investigated allelopathic effects of *P. crassipes* reported that the crude extracts from the whole plant (leaves and roots) contained 0.98%

alkaloids which exhibited antialgal, antibacterial, and moderate antifungal activities. The presence of alkaloids in the samples could potentially mean that alkaloids are present to aid in plant defence.

Previous research on *P. crassipes* allelochemicals consistently found phenolics, as highlighted by Shanab et al. (2010), indicating a 4.35% presence. The absence of N-phenyl-1-naphthylamine and N-phenyl-2-naphthylamine in the current study might result from differing compositions between plant species or specific growth conditions. Another plausible explanation could relate to the existence of linoleic acid, potentially serving as a defence mechanism. Chowdhary et al. (2021) states that plants might not need multiple defense compounds if one compound adequately fulfils its role. Therefore, in this case, *P. crassipes* may not require the aforementioned compounds if linoleic acid adequately fulfils its functional role.

The identification of Ceramides (**N**) in treatment samples of *P. crassipes* (roots and leaves) not prominent in control samples, is intriguing despite prior unreported findings. According to Li et al. (2022), ceramides are bioactive lipids that are involved in plant defence and plant development. Li et al. (2022) reported that a high accumulation of ceramides in the plasma membrane of the plant activates the NADPH (Nicotinamide Adenine Dinucleotide Phosphate) oxidase RbohD (Respiratory Burst Oxidase Homolog D**)** and promotes hydrogen peroxide which results in a defence-related gene activation. The presence of ceramides in treatment samples is possibly due to stressors like herbivory by *M. scutellaris* and competition from *S. minima*. This also indicates the potential use of ceramides by *P. crassipes* as defence mechanisms aiding its competitive ability. Despite this defence, intense herbivory by *M. scutellaris* ultimately results in plant death.

Another interesting finding was the presence of N-acyl amine (**U**) observed in the field samples. While the precise roles of N-acyl amines in plants remain under investigation, studies such as that of Blanaflor et al. (2014) suggest its potential in microbial defense. The presence of N-

acyl in these field samples might indicate its role in defending against microbial threats, contributing to the plant's defence mechanism.

From the comprehensive network analysis reported in this study, there were differences between metabolites in Roots vs Leaves. Leaves contained significant enrichment, notably in (**F**) linoleic acid, (**Y**) alkaloids, (**g**) sulfonamide, and (**h**) lignan. Conversely, root samples showed enrichment in (**H**) cinnamic acid glycoside analogs and (**d**) anthraquinone. The distinct metabolite compositions potentially suggest specialized functions of each organ. Leaves, being photosynthetic organs, emphasize compounds involved in photosynthesis, defence such as linoleic acid while roots may prioritize compounds related to nutrient uptake and responses to environmental cues.

The differences in metabolic profiles between the control (no insect) vs treatment (with insects feeding on the plant) suggest distinct responses triggered by insect presence. In the treatment, notable enrichments like **c**-amyl amine (predicted to be a heptadecenamide), **F** (Linoleic acid related fatty acids), and features **(e)** (predicted to be a fatty amide) and **f** (precited to be a fatty acyl) were observed. The prevalence of linoleic acid in the treatment plants indicates potential plant defence mechanisms activated under herbivory-induced stress. Other detected compounds might signal specific responses unique to herbivory, deserving further investigation (Smit et al. 2021). Conversely, control samples exhibited enriched features like alkaloid (**Y**), N-(1-Deoxy-1-fructosyl) phenylalanine **(n),** as well as feature (**i)** (no prediction or match) and **j** (predicted to be a nucleoside). Alkaloids, for instance, could play a role in plant defence mechanisms against herbivores (Shanab et al. 2010). The distinct compound enrichments in control versus treatment plants suggest potential differential responses in plant metabolism triggered by insect presence or absence, highlighting their involvement in plant defence mechanisms against herbivory.

The difference observed between *P. crassipes* grown alone vs *P. crassipes* grown with *S. minima* suggests limited alterations in the metabolic profile due to the presence of *S. minima*

during growth. When comparing the two growth conditions, only three distinct features were significantly enriched in *P. crassipes* samples grown with *S. minima*, indicating a minor impact on metabolite composition: **k** (predicted to a pyrrolidine), **l** (predicted to be a flavonoid), and an unidentified compound showing notable abundance variation (**m**). This scarcity and type of enriched feature imply minor variations between these growth conditions.

As previous studies sought to investigate whether *P. crassipes* dominates other organisms in its habitat solely through competition, or if it also exerts allelopathic effects (Yu et al. 1992, Jin et al. 2003, Gross et al. 2007), the present study only shows the presence of some allelochemicals from *P. crassipes* previously noted in the literature. Yet it does not directly confirm their inhibition of *S. minima* growth. Instead, it suggests the potential for such an effect. Future research testing the production of allelochemicals by *P. crassipes* in the presence of *S. minima* and *M. aeruginosa* would confirm whether *S. minima* elicits an allelochemical response by *P. crassipes*, or whether the methods used here were insufficient to detect allelochemical production. This exploration could reveal whether *P. crassipes*, beyond controlling algae and phytoplankton, also hinders neighbouring plant growth. Such findings would shed light on how *P. crassipes*, uses allelochemicals for defence and competitive advantage.

CHAPTER 5

General discussion and concluding remarks.

5.1 Introduction

Biological invasions remain a growing concern on a global scale due to the combined effects of anthropogenic activities and climate change (Vilà et al. 2011, Vantarová et al. 2023, Worischka et al. 2023). Coetzee & Hill (2012) reported that, anthropogenic activities, particularly environmental nitrogen pollution, have been increasing on a global scale reaching aquatic ecosystems, resulting in numerous environmental impacts, including eutrophication which thus promotes aquatic invasions.

In response to this challenge, many countries have turned to biological control as a cornerstone in the management of both terrestrial and aquatic invasive alien species (Schwarzländer et al. 2018). Notably, South Africa has made significant contributions to combatting biological invasions through substantial investments in research, as evidenced by Hill & Coetzee (2017), Hill et al. (2020), and van Wilgen et al. (2020). As such, existing literature highlights that biological control continues to be regarded as the most environmentally friendly, sustainable, and cost-effective approach to diminish invasive populations by feeding on the host plants (Van Wyk & Van Wilgen 2002).

Pontederia crassipes remains a global menace in many water bodies and has received considerable research to aid in controlling the spread of this invasive aquatic weed in introduced areas (Cilliers 1991, Hill & Olckers 2001, Hill & Coetzee 2008, Segbefia et al. 2019). To aid in the control of *P. crassipes* in invaded areas, several studies have been conducted to understand the physiology of the plant, population and community dynamics, the ecosystem ecology of the plant and to understand the interspecific competition of the plant overall highlighting achievements and challenges (Gao & Li 2004).

Biological control remains the sustainable, long-term strategy against *P. crassipes* globally. While biological control stands as a significant long-term strategy, the integration of control measures is considered the most effective strategy in the country (Hill & Coetzee 2017, Schwarzländer et al. 2018, Hill et al. 2020). The biological control of *P. crassipes* in South Africa has seen recent additions of new agents, including the recent release of *M. scutellaris* to aid in managing the spread and damage that this weed causes (Hill et al. 2020, 2021, Coetzee et al. 2021, 2022a).

The Centre for Biological Control's inundative release programme to aid in the intense feeding from the insects *M. scutellaris* has significantly damaged *P. crassipes*, indicating potential success in managing the weed in South Africa.This programme has been successful, indicating potential success in managing *P. crassipes* in South Africa (Coetzee et al. 2022a). Although there is evidence of population declines of *P. crassipes* at Hartbeespoort Dam (Coetzee et al. 2022a, Miller et al. 2023), this study highlights, a rapidly emerging problem, after removal of the plant has led to a concerning issue - secondary invasion.

5.2 Secondary invasion

The results presented in Chapter 2 showed a significant ecological phenomenon: secondary invasions of *S. minima* during periods of low *P. crassipes* populations, leading to distinct population dynamics for both invasive aquatic plants. Secondary invasions are not a new concept and have been observed in previous research (Pearson et al. 2016, González et al. 2017, Nsikani et al. 2018, 2019, 2020). While existing literature has addressed secondary invasions in South Africa, much of the focus has been on terrestrial secondary invasions (Nsikani et al. 2019, 2020). This study examined secondary invasions within an aquatic context, presenting valuable insights into this aspect of invasive species dynamics.

Secondary invasions pose a significant threat to ecosystems because, after temporarily removing an invasive species, biodiversity recovery is anticipated. Nsikani et al. (2020),

highlighted that secondary invaders often appear after clearing targeted species, a phenomenon not frequently reported. The presence of another alien species within an ecosystem be it in small proportions, is a prerequisite for secondary invasions (Kuebbing et al. 2013; Pearson et al. 2016). In the context of the Hartbeespoort Dam*, S. minima*, although less prevalent initially, gained prominence after the biological control of the dominant species, as there was less competition from *P. crassipes* but still an abundance of nutrients, the primary driver of macrophyte invasions.

Chapter 2 reported periods during which each invasive species dominates and the factors driving dominance. The decline in *P. crassipes* was attributed to intensive feeding by *M. scutellaris* (Hill et al. 2021, Coetzee et al. 2022a, Miller et al. 2023). Late autumn and winter bring frost damage to remaining *P. crassipes* due to cold temperatures, impacting biological control agents (Miller et al. 2020). *Salvinia minima* favoured by reduced competition during low *P. crassipes* populations in winter, dominated. This observation contributes to our understanding of the nature of the occurrence of this secondary invader in this water system, aiding to the management strategies for controlling *S. minima*. The functional traits of *S. minima* which include high tolerance of cold winter temperatures allows it to prevail during winter periods at the dam. It was noted however, that during spring, the regeneration of *P. crassipes* diminishes *S. minima's* dominance, as *P. crassipes* exhibit rapid growth, a fast reproductive nature, and the ability to germinate from seeds when conditions are favorable (as documented by Coetzee & Hill in 2012). These cycles were observed in 2021 and 2022.

Interactions among nonnative species are relatively understudied, despite the high likelihood of co-occurrence (Kuebbing et al. 2013). Thus, this thesis further investigated the competitive interactions among these two invasive plants and determined the effect of biological control, *M scutellaris* on the population's dynamics of these two aquatic weeds and what the outcomes are in Chapter 3.

5.3 Effect of *Megamellus scutellaris* on *Pontederia crassipes*

Insect herbivory significantly influences interactions among invasive alien species as these plants lack robust defense mechanisms against them (Parker et al. 2006). *Megamelus scutellaris,* introduced in 2013 to combat *P. crassipes* in South Africa, has remarkably adapted to the diverse climates of South Africa (Coetzee et al. 2013). A study by May & Coetzee (2013) showed that this planthopper has not only thrived in cooler regions where biological control was applied but has also endured warmer temperatures, challenging initial assumptions regarding its temperature tolerance.

Intensive feeding activity by *M. scutellaris* inflicts substantial cellular damage, leading to a significant reduction in the photosynthetic efficiency of *P. crassipes* (Tipping et al. 2011). Therefore, as with previous studies, Sosa et al. (2007), Coetzee et al. (2022a) and Miller et al. (2023), this planthopper has demonstrated its capacity to drastically reduce *P. crassipes* populations (Chapter 3). Notably, at Hartbeespoort Dam, *M. scutellaris* has achieved a remarkable reduction of *P. crassipes* coverage (Coetzee et al. 2022, Miller et al. 2023), with certainty of managing *P. crassipes* in the country.

Moreover, recent research by Coetzee et al. (2022a) underscores the effectiveness of inundative release of *M. scutellaris* as a strategic approach to enhance the population growth of this insect, particularly after winter. The implementation of the inundative release strategies, commencing early in spring, has proven instrumental in accelerating the recovery of this biological control agent (Hill et al. 2021, Coetzee et al. 2022a). Additionally, mass releasing the insects shortly after winter facilitates a boost in their populations in the field, ((Hill et al. 2021).

In the competition experiment, *M. scutellaris* intensively damaged *P. crassipes* plants (Chapter 3), showing the ability of this planthopper as a biocontrol agent. Therefore, as with previous studies, this planthopper has demonstrated its capacity to drastically reduce *P. crassipes* populations (Chapter 3) by feeding on *P. crassipes* resulting in plant stress and alternatively

reducing the plant's population (Sosa et al. 2007, Fitzgerald & Tipping 2013, Miller et al. 2021, Coetzee et al. 2022).

Although *P. crassipes* is not completely under control at Hartbeespoort Dam, it is important to note the impressive effort of the biological control agents that have been released at the Hartbeespoort Dam and all parts of the country. Biological control programmes are usually extended management strategies, often taking 10 to 20 years to show success post-introduction (McFadyen, 1998). Thus, the establishment of *M. scutellaris* and its significant impact on *P. crassipes* populations can be seen as a positive step forward.

5.4 Limiting factors to complete control.

Many of South Africa's waterways, including Hartbeespoort Dam, face invasion from floating aquatic weeds, of which *P. crassipes* remains the worst despite decades of interventions to mitigate its spread (Coetzee et al. 2021). Established biological control agents such as *M. scutellaris* in South Africa have proven effective against *P. crassipes* (Miller et al. 2021, Coetzee et al. 2022). Despite this, achieving complete eradication of *P. crassipes* remains a challenge.

Pontederia crassipes' reproductive characteristics contribute to its invasiveness, but the role of the seed bank in its success in new environments has been emphasized in various studies (Téllez et al. 2008). Cronk & Fennessy (2016) highlighted the weed's potential to produce up to 3000 seeds per inflorescence, varying by site and season, impacting long-term control strategies. These seeds possess longevity, posing challenges for the complete eradication of *P. crassipes* (Gunnarsson & Petersen 2007).

In South Africa, Albano Pérez et al. (2011) conducted a pioneering report on *P. crassipes* seed banks, revealing a considerable reservoir in various water bodies. This study emphasized that factors like water fluctuations, eutrophication, and seed decomposition collectively influence seed dispersal and persistence, facilitating re-infestation despite eradication efforts. Similarly,

Richardson & Kluge (2008) explored the seed banks of invasive Australian *Acacias* with a focus on the role of the seed bank in the invasiveness of these species and improvement in the management of options. The study reports that persistent seed stores significantly contribute to the success of invasive plant species, including the Australian *Acacias*. Further, the authors highlight in their review that reducing seed banks is crucial for the success of expensive management initiatives against these invasive species.

Studies on the invasive species *P. crassipes* emphasize the significant role of seed banks in invasiveness (Téllez et al. 2008, Albano Pérez et al. 2011, Cronk & Fennessy 2016). However, biological control methods play a crucial role in mitigating seed bank accumulation as herbivory feeding affects the plant's reproductive cycle by destroying its flowers and fruits, preventing seed production. Biological control hinders the formation of buds, flowers, and young pods, consequently limiting seed production and the accumulation of seed banks. The implementation of biological control measures is considered the most effective strategy for exhausting existing seed reservoirs and should be an integral part of invasive species management strategies (Gioria et al. 2012). Biological control not only limits the production of seeds but also addresses the existing seed reservoirs, crucial for successful invasive species management.

Another critical aspect impeding the complete eradication of *P. crassipes* in South Africa is eutrophication (Hill & Olckers 2001, Hill & Coetzee 2008, 2017). Coetzee & Hill (2012) highlighted that South Africa's water bodies rank among the most eutrophic globally, promoting the proliferation of invasive aquatic plants like *P. crassipes* due to the abundance of nutrients. These excess nutrients act as fertilizers, facilitating rapid growth not only of *P. crassipes* but also of other invasive aquatic species (Coetzee et al. 2007, Coetzee & Hill 2012). Eutrophic conditions, observed in water bodies such as Hartbeespoort Dam, create an ideal environment for aquatic invaders to thrive (Hill 2003, Coetzee & Hill 2012). The high nutrient levels persist even after the targeted removal of invasive species, allowing for subsequent

invasions. The invaders are no longer limited by nutrient availability, leading to secondary invasions, as documented in Chapter 2 of this thesis.

5.5 Competition Interactions

In the absence of herbivory, *P. crassipes* exhibited dominance over *S. minima* due to its fast growth rate and high biomass accumulation, outcompeting *S. minima*. However, the introduction of herbivory by *M. scutellaris* resulted in a reduction of *P. crassipes'* vigour and competitive ability, allowing *S. minima* to exhibit increased resilience. Similar findings from various studies (El-Seed 1978, Agami & Reddy 1990, Coetzee et al. 2005) support *P. crassipes'* dominance in the absence of herbivory and the competitive ability of *P. crassipes*. The observation that herbivory from *M. scutellaris* altered the competitive interaction of *P. crassipes* and *S. minima* aligns with a study by Center et al. (2005).

This observation highlights how biological control mechanisms, particularly herbivory, can diminish the competitive ability of host plants, providing a competitive advantage to surrounding species as indicated by (Callaway et al. 1999, Center et al. 2005, Coetzee et al. 2005). It contributes to understanding the competitive dynamics of invasive plants and how biological control strategies influence their interactions. Understanding these dynamics aids in the management and development of effective control strategies for these invasive aquatic weeds. The findings further contribute valuable insights into the intricate nature of invasive aquatic plant interactions, their biology, and competitive abilities, facilitating the development of improved management strategies.

Given the fast-growth rate and dominance of *P. crassipes* when grown with other species, *P. crassipes* outcompeted *S. minima* in the absence of herbivory by *M. scutellaris.* However, the introduction of herbivory by *M. scutellaris* reduced the vigour and competitive ability of *P. crassipes*. Similar findings of *P. crassipes* maintaining its dominance in the absence of herbivory have been reported (El-Seed 1978, Agami & Reddy 1990, and Coetzee et al. 2005).

This result helps in the understanding of how biological control alternatively reduces the competitive ability of the host plants thus allowing the surrounding plants to gain a competitive advantage. While this result helps understand the competitive nature of invasive species, it also contributes valuable insights into the complex dynamics of invasive aquatic plants and their management. In addition to the competitive nature of *P. crassipes* both in the field and experiments, several studies have reported its ability to release allelochemicals as a competitive ability against algae and phytoplankton (Sharma 1985, Sun 1993, Jin et al. 2003, Pei et al. 2018). Therefore, Chapter 4 extracted the chemicals previously identified as key allelochemicals to determine their presence within *P. crassipes* when grown with *S. minima* as discussed below.

5.6 Allelopathy

Previous studies have focused on the allelopathy of *P. crassipes* on surrounding algae and phytoplankton communities, with a scarcity of studies on its impact on neighbouring macrophytes. Allelopathy remains a captivating concept in explaining species dominance over time (Yu et al. 1992, Inderjit & Keating 1999, Gross 2003).

In this chapter, among the previously identified allelochemicals—N-phenyl-1-naphthylamine, N-phenyl-2-naphthylamine, and linoleic acid—only linoleic acid was detected in *P. crassipes* leaves, roots, and field samples. Its presence hints at a potential role as an allelochemical against *S. minima*. However, further tests involving *P. crassipes*, *S. minima*, and algae are necessary to confirm whether *S. minima* elicits an allelochemical response by *P. crassipes*, or whether the methods used here were insufficient to detect allelochemical production. Additionally, the study noted the presence of N-acyl amine and Ceramides in *P. crassipes* samples, indicating potential roles in defence mechanism (Blancaflor 2014, Li et al, 2022). Although not predicted as allelochemicals against algae, these chemicals should be investigated to detect if they hold direct effects on surrounding plants. This will determine whether *P.*

crassipes, beyond controlling algae and phytoplankton, also inhibits the growth of neighbouring plants.

5.7 Conclusion and Future recommendations

In conclusion, this thesis and other studies highlight the emerging issue of secondary invasions (Pearson et al. 2016, Nsikani et al. 2019, Coetzee et al. 2022b, Shen et al. 2023). It reveals the high possibility of secondary invaders dominating after removal of targeted species while the primary driver remains. Furthermore, the results presented in this thesis demonstrates the efficacy of *M. scutellaris* in controlling *P. crassipes* and suppressing its competitive abilities, as supported by various studies (Sosa et al. 2007, Tipping et al. 2011, Miller et al. 2021, Coetzee et al. 2022). The detection of secondary invasions emphasizes the need for early control to prevent further spread. Reaser et al. (2020) highlight that early responses to invasions are more successful when the species is still contained and becomes increasingly challenging and costly when infestations become more widespread.

Finally, with effective inundative release strategies and increased public awareness and support through community engagement, the battle of species invasions is manageable. Notably, the success of the Centre for Biological Control which lies in rearing insects has shown promising outcomes in controlling invasive species (Weaver et al. 2017). The success and establishment of biological control agents on aquatic weeds in South Africa is highly affected by plant quality, which is determined by the nutrient's status of water in which they grow (Coetzee et al. 2007, Coetzee & Hill 2012). The secondary invasion of *S. minima* signifies persistent pollution fostering further invasions. While the waterbodies such as Hartbeespoort Dam remain highly polluted, invasive species continue to invade, and the systems allow for secondary invaders.

Therefore, managing aquatic invasive species requires proactive measures notably reducing nutrient influx into ecosystems and preventing invasive species entry (Hill et al. 2003). And thus, the Centre for Biological Control continues to rear and release biological control agents

throughout the country, conducting post release evaluations to determine efficiency in managements strategies. These evaluations also allow for early detection of new invasions or secondary invasions. The Centre for biological control's success also stems from public education initiatives on invasive species, their impacts, and combined efforts through community engagement. Collectively, these efforts significantly contribute to effective biological invasion management, offering promising prospects for the future.

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