The tuber-feeding weevil *Listronotus frontalis* as a candidate biological control agent for the invasive semi-aquatic plant *Sagittaria platyphylla* within South Africa

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Abstract

Sagittaria platyphylla (Engelm.) J.G.Sm. (Alismataceae) is an invasive, aquatic macrophyte originating in the southern United States of America. In South Africa, the plant was first detected in Krantzkloof Nature Reserve, KwaZulu-Natal Province in 2008, and due to its known impact in other countries, it was listed as a Category 1a invader species under the National Environmental Management: Biodiversity Act 2004 (NEM:BA). This invasive plant has proved difficult to manage due to its varied growth forms and reproductive strategies, such as prolific seed and below ground tuber production. Due to the limitations of conventional control mechanisms, biological control is currently being considered as a potential control option. The tuber feeding weevil *Listronotus frontalis* LeConte (Coleoptera: Curculionidae) has been identified as a candidate biological control agent for this invasive species. The aims of this study were twofold; to firstly determine the importance of tubers to *S. platyphylla* populations growing in South Africa; and secondly, to determine the biology and suitability of *L. frontalis*, a tuber feeder, as a candidate biological control agent.

Surveys of *S. platyphylla* populations in South Africa showed that tubers were found in all sampled sites, except for Krantzkloof Nature reserve in KwaZulu-Natal Province. The highest number of tubers was 97.75 ± 10.62 (SE) m^{-2} recorded at Jonkershoek in the Western Cape Province. Monthly sampling from two sites in the Eastern Cape Province, the Makana Botanical Gardens and Maden Dam showed that neither season nor water depth affected tuber production. However, the mean number of tubers as well as mass of tubers sampled, were consistently higher ($F_{(1,179)} = 20.9542$, $P < 0.0001$) and heavier ($F_{(1,857)} = 585.7293$, $P < 0.0001$) at the Botanical Gardens than at Maden Dam, respectively. The study showed that tubers are an important life stage of *S. platyphylla* populations and may vary in size and abundance between and within sites.

The tuber feeding weevil was shown to develop from egg to ovipositing adult within just over 40 days. Females were recorded to lay up to 48 eggs within a period of one week. Impact studies showed that adult feeding led to a reduction in all but one of the 11 measured plant growth and developmental measurements, including a reduction in the mean mass of the above-ground plant material ($F_{(2,2743)} = 12.05$, $P = 0.002$) as well as a reduction in size and abundance of tubers ($F_{(2,58.47)} = 9.756$, $P = 0.0006$) and stolons($F_{(14.943)} = 8.7577$, $P = 0.003$). These results are encouraging and suggest that if the insect is released in South Africa, it may prove to be a valuable biocontrol agent.

It is concluded that, until suitable biological control options become available in South Africa, the chemical and mechanical control measures currently implemented should continue, however, controlling tubers should be considered during the planning and implementation of these strategies.

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Chapter 1: General introduction

1.1. Biotic invasions

Within the past few centuries the impact of human beings has brought about various imbalances to the natural world, with globalization being a prominent driver in this process. Globalization has increased the efficiency of movement between different regions, which has resulted in an exchange of species within and among countries and even between continents (Holmes et al., 2009). Through globalization, the number of biological invasions, both intentional and unintentional, has increased exponentially (Parker et al., 2013). This is especially true for the past half century as trade and commerce have expanded at an unprecedented rate (Pysek & Richardson, 2010; Keller et al., 2011). Through this increased translocation of organisms, there are very few ecosystems that are completely free of invasive species (or simply, invasives), with a growing number of biomes and ecosystems being overrun by invasives (Pysek & Richardson, 2010).

Although numerous species are being moved into novel areas on a daily basis, a very small percentage of these individuals are able to survive, and even fewer go on to become invasive (Blackburn et al., 2011). Only the species that are able to sustain self-replicating populations whilst having negative ecological and/or economic impacts are deemed invasive (Pysek & Richardson, 2010; Keller et al., 2011). Organisms that find themselves within novel ranges that do not have negative economic/ecological impacts are defined as alien species. Alien species are species that are present outside of their native range, and do not have negative ecological or economic impacts (Pysek & Richardson, 2010).

The impact invasive species can have on an environment will vary on a species-to-species basis, as well as the conditions present within the invaded range. The main reason invasives pose such a threat to ecosystems is due to their ability to unravel the processes that keep ecosystems functioning efficiently (Sousa, 2011). Such processes include the alteration of nutrient cycling, community dynamics and fire regimes, as well as the displacement of keystone species and a reduction in general biodiversity (Pysek & Richardson, 2010). Invasive species are considered to be one of the biggest threats to biodiversity worldwide, with some areas, such as islands, being particularly vulnerable to invasion (Pysek & Richardson, 2010; Simberloff, 2010; Keller et al., 2011).

Invasive species harbour tremendous costs to the global economy, and it has been estimated that per annum the United States of America (USA) loses between US$120-138 billion due to invasive species, while globally this figure is around US$1.5 trillion (Pimentel et al., 2005; Cook et al., 2007). Invasive species cause economic damage by negatively influencing ecosystem services, which are the benefits that humans receive from naturally occurring biological processes. The ecosystem services that invasives can alter include pollination services, natural biodiversity, as well as nutrient cycling (Vila et al., 2010). Such ecosystem services are crucial to various farming practices as well as ecotourism.

Examples of prominent invasive species will now be mentioned. The brown tree snake, *Boiga irregularis* Merrem (Colubridae), which has been held responsible for the extinction of at least 8 of the 11native forest birds present on the island of Guam (Wiles et al., 2003). The Nile perch, *Lates niloticus* Linnaeus (Latidae), which has led to the extinction of more than 200 endemic fish species within Lake Victoria through predation and competition for food (Pringle, 2005). Caulerpa Seaweed, *Caulerpa taxifolia* (Vahl) Agardh (Caulerpaceae), was introduced into theMediterranean in the 1980's and has since spread throughout most of the northern Mediterranean, where it's growth completely covers habitats and threatens native flora and fauna (Meinesz et al., 2001). The Small Indian Mongoose, *Herpestes javanicus* Saint-Hilaire (Herpestidae), was introduced into the West Indies, Hawaii, Fiji and Mauritius as a biocontrol agent for rats in the late 1800s, has since become invasive and has led to the extinction of various bird, reptile and amphibian species (Hays & Conant, 2007). The channeled apple snail, *Pomacea canaliculata* Lamarck (Ampullariidae), is most problematic within developing countries, specifically those where rice is the staple food source and rice farming is a prominent source of income (Horgan et al., 2014). Invasive alien plants are some of the most damaging invasive alien species and will now be examined in greater detail.

Invasive alien plants (or IAPs) are plant species found outside of their native range thatare causing economic and/or ecological damage. IAPs are a growing global concern as they have the ability to severely reduce biodiversity and impact ecosystem services that humans benefit from (Brooks et al., 2004). Ecosystem services can be grouped as either direct or indirect services received by humans from naturally occurring processes (Asah et al., 2014). Examples of direct services include provision of food as well as timber resources, while examples of indirect services include water purification, nutrient cycling, and air purification (Asah et al., 2014). *Miconia calvescens* D.C. (Melastomataceae) is an ornamental plant that was introduced into the island of Hawaii in the 1960s and has since become invasive (Kaiser, 2006). This plant has both economic and ecological adverse effects in Hawaii. For example, it has been shown not only to reduce biodiversity, but also to alter the habitat it is present in. Moreover, *M. calvescens* is slowly transforming the forest habitat from a multi-organismal and layered canopy, to a densely shaded monocultural ecosystem (Kaiser, 2006). This horticultural invasive also increases sedimentation and runoff, as well as using up groundwater resources. It is estimated that the costs of biodiversity lost from *M. calvescens* alone will be between $103–303 million per year, with this being said to be a conservative estimate (Kaiser,2006).

Richardson & van Wilgen (2004) have stated that the most troublesome IAPs are those that alter the ecosystem around them. Such ecological alterations can be grouped as either direct or indirect (Brooks et al., 2004). Examples of direct ecological alterations include the out competition of food and space, while examples of indirect ecological alterations include the alteration of nutrient cycling, fire regimes and the rate of soil erosion (Brooks et al., 2004; Richardson & van Wilgen, 2004), all of which influence the natural balance of an ecosystem and can lead to ripple effects throughout. For example, in South Africa (RSA), black wattle, *Acacia mearnsii* de Wild (Fabaceae), has been shown to use deep groundwater reserves, otherwise not used by native plants (Clulow et al., 2011). These invasive plants are thus able to alter the hydrology of such systems, which can lead to knock on effects within ecosystems (Clulow et al., 2011).

Plants that find themselves outside of their native range are thought to have two main ecological advantages that assist in them becoming invasive. Firstly, they are generally free from their co-evolved natural enemies, and secondly, they possess defences, either chemical or physical, that native species struggle to overcome (Parker et al., 2013). Although various hypotheses have been developed to help explain and predict invasiveness (Catford et al., 2009), the hypotheses that best describe these two ecological advantages have been condensed into: the ‘enemy release hypothesis’, and the ‘novel weapons hypothesis’ (Harvey et al., 2010). The ‘enemy release hypothesis’ assumes that plants in their native ranges are supressed by specialist consumers and/or pathogens, and when placed into alien areas, are free of such constraints and are thus able to proliferate to the carrying capacity of that system (Callaway & Ridenour, 2004; Harvey et al., 2010). Whereas, the ‘novel weapons’ hypothesis suggests that alien plants have biochemical defences that native herbivores, pathogens or plants competing for similar resources have not encountered before. Due to this independent evolution, native fauna and flora do not have co-evolved offences to overcome these new ‘weapons’ (Callaway & Ridenour, 2004; Harvey et al., 2010). For example, the Asian shrub, *Lonicera maackii* (Rupr.)

Maxim. (Caprifoliaceae), is invasive in the USA and appears to benefit from the absence of arthropod herbivores in its invaded range (Lieurance & Cipollini, 2012). Anecdotal evidence has suggested that in its invaded range *L. maackii* grows more abundantly and vigorously than it does in its native range. This has been attributed to a lack of co-evolved natural enemies present within its invaded range (Lieurance & Cipollini, 2012). Moreover, Svensson et al. (2013) looked to determine the influence of allelopathy in driving the invasive ability of *Bonnemaisonia hamifera* Hariot (Bonnemaisoniaceae) outside of its native range. These researchers found that the main secondary metabolite of *B. hamifera* had allelopathic qualities and aided this invasive in outcompeting native plants for space and resources. A large portion of the above mentioned examples consisted of terrestrial plants; however, aquatic invasive plants make up an important component of this field.

Aquatic invasive plants (AIPs) provide unique impacts to aquatic systems, which are dependent on their growth form, exposure to the correct resources, as well as the state and characteristics of the aquatic system itself. Although, in general, AIPs impact aquatic systems in the following ways: reduce biodiversity and water quality, facilitate water-borne diseases, limit utilisation of the resource for humans as well as limiting its recreational use, impede the movement of water through water channels, and obstruct hydroelectrical systems (Hassan et al., 2020; Havel et al., 2015). Many prominent AIPs are extremely challenging to manage due to their ability to reproduce both sexually as well as asexually. Other management challenges presented by AIPs include their ability to proliferate from plant fragments as well as the establishment of seed banks. The best example for all of these characteristics is water hyacinth, *Pontederia crassipes* (Mart.) Solms-Laub. (Pontederiaceae), which still evades complete control in most countries where it has invaded, including South Africa.

Aquatic plants have been shown to be more likely to become invasive than terrestrial plants within new environments, and therefore may require more urgent attention (Hoveka et al., 2016). Wang et al. (2016) found that AIPs have caused greater ecological and economic damage compared with terrestrial invasives. This could be due to the rapid rate at which many AIPs are able to proliferate (Zedler & Kercher, 2010). In some cases, empty nicheshave provided a vacant space for AIPs to take advantage of (Fleming & Dibble, 2015). A goodexample of this is how *P. crassipes* has invaded South African water bodies with such ease (Coetzee & Hill, 2012). South Africa is a country with very few natural still or slow-moving open water bodies. Being a water-scarce country, impoundments were constructed to create a more reliable and abundant source of water (Coetzee et al., 2014). As these still or slow-flowingsystems are not naturally present within South Africa, organisms, and specifically in this case native plants, have not been provided with enough time to evolve to take complete advantage of this new resource (Coetzee & Hill, 2012). Thus, very few floating macrophytes indigenous to South Africa occur in these open bodies of water, leaving these anthropogenic systems with a large and vacant niche (Coetzee et al., 2014).

The impact AIPs have on aquatic systems varies based on the growth form of the plant and the characteristics of the invaded system (Hill & Coetzee, 2017). For example, AIPs that form dense mats covering the water surface, such as *Salvinia molesta* D.S. Mitch. (Salviniaceae), can restrict light penetration into the water column, which ultimately reduces photosynthesis and can lead to trophic cascades; whereas, rooted-emergent invasive plants, such as *Iris pseudacorus* Linnaeus (Iridaceae), can cause sedimentation, which can alter natural flow regimes (Schultz & Dibble, 2012). Midgley et al. (2006) conducted a study where they looked into the biodiversity and abundance of aquatic invertebrates directly underneath water hyacinth mats and within open-water areas within the same system. They found that both the biodiversity and abundance of aquatic invertebrates directly beneath water hyacinth mats was significantly lower than within open-water areas of the dam. They concluded that through this reduction in biodiversity and abundance, various interspecific interactions were lost, which places the entire system in a vulnerable state to disturbance.

1.2. Management options

Ideally, the best way to avoid the issue of invasive species in general is to prevent their arrival into novel ranges in the first place (Hussner et al., 2017). However, this is often quite challenging as the pathways of introduction are plentiful and hard to monitor (Faulkner et al., 2016). A few primary pathways of introduction of alien plants include the horticultural trade, the farming industry, and the aquarium trade (Martin & Coetzee, 2011; Kaplan et al., 2017). Although broad studies have been conducted to better understand the pathways of introduction into South Africa, some pathways have received more attention than others (Faulkner et al., 2020). Kaplan et al. (2017) covered the topic of pathways of invasion into South Africa, where they focussed on invasion pathways of cacti. They determined the horticultural trade to be the main route for the translocation of cacti into South Africa, while other pathways included using cacti for food production as well as for animal fodder as well as through seeds and ‘hitchhikers’. This provides screening-officials with a challenge as it is hard to identify exactly which species arebeing brought into the country. As shown in the example above, the prevention of alien plants from entering into novelranges can be very tricky, and thus, this method often fails to succeed. Another way to mitigatethe establishment of invasive species is the 'early detection and rapid response' technique.

When faced with a novel alien plant population the ideal management strategy is 'early detection and rapid response' (Kaiser & Burnett, 2010). This is a strategy that aims to target alien, and potentially invasive populations early on in their colonisation, and eradicate them before being allowed the chance to establish and potentially become invasive (Simpson et al., 2009). Unfortunately, detecting early invasions is challenging and often management teams are not provided with enough resources to eradicate novel populations. This often leads to the establishment of an invasive species (Kaiser & Burnett, 2010).

Once an invasive population has established and proliferated, the early detection and rapid response strategy can no longer be considered (Kaiser & Burnett, 2010). From here on a suite of four management options are available, namely, mechanical control, chemical control, biological control (or simply, biocontrol) and integrated pest management (IPM) (Hussner et al., 2017). Each management option has its pros and cons (van Wilgen & de Lange, 2011), of which will be briefly discussed further in the context of AIPs.

1.2.1. Mechanical control

Mechanical control is any form of human assisted removal, whether it be the removal of aquatic plants by hand, tool, or machinery (Lancar & Krake, 2002). Mechanical control only truly works on small infestations, usually smaller than 1 ha in size (Hill, 2003). It is also a very costly control option without being particularly environmentally friendly. Mechanical control efforts will often damage more than just the target plant species, and some form of restoration will be required post-control (Lancar & Krake, 2002). Moreover, a poorly undertaken mechanical control programme can lead to the dispersal of weed propagules, aiding the expansion of the invasive plant (Martin et al., 2018).

Manual removal is the physical removal and disposal of plants by hand (Hussner et al., 2017). Manual removal is very labour intensive and can become an expensive control option as it requires a lot of labour and repeated removals to obtain the desired outcome (Hill & Coetzee, 2008). In countries such as South Africa where unemployment is a problem, this control option may be used to provide jobs for many individuals, but over the long-term it often fails to effectively control the target population on its own. Through the application of manual removal, and mechanical control in general, results are seemingly obtained immediately, but most AIPs will be able to re-establish their populations either through plants fragments left behind, or via seed beds (Hill & Coetzee, 2008; Van Wilgen & Wannenburgh, 2016). In conclusion, mechanical removal has its limitations, but if AIP populations are small enough, mechanical removal can work well to effectively aid in control.

1.2.2. Chemical control

Chemical control is a control option that can be effective if the herbicide being used has been well developed and if the application guidelines and application schedules are adhered to (Hussner et al., 2017). Often though, chemical control is deemed too environmentally damaging, through its non-target effects and ability to remain within a system for multiple years, and too expensive to warrant its use (Ervin & Jassaume, 2014). Although, Matthews *et al.* (2014) suggested that if used correctly, herbicides can be less environmentally harmful than the impact sustained from continuous mechanical control. Another concern with chemical control is that AIP populations are usually able to regenerate themselves, either from living propagules, or from a seed bank. This makes chemical control a short-term management option that is not sustainable. Having an over-reliance on herbicides can be risky as invasive plant populations may develop some form of resistance to these chemicals, which would make controlling such populations even harder (Ervin & Jassaume, 2014).

There are two main types of herbicides: contact herbicides and systemic herbicides (Sanudi et al., 2019). Systemic herbicides are intended for more long-term management programmes and are best suited for larger infestations, whereas contact herbicides are mainly used for short-term management programmes and are best suited for smaller infestations (Poovey et al., 2013; Sanudi et al., 2019). Contact herbicides are much stronger in their concentration and, unlike systemic herbicides, they need to come into direct contact with the target plant soon after being applied (Poovey et al., 2013; Sanudi et al., 2019).

Chemical control is an expensive control option that can risk the health of the aquatic system being targeted. It is a relatively short-term control strategy and is not sustainable. Although, when paired with other control programmes, such as biocontrol, it can provide promising results (Ervin & Jassaume, 2014).

1.2.3. Biocontrol

Biocontrol is the use of natural enemies to control populations of an invasive species below a certain threshold (McFadyen, 1998). The organisms responsible for controlling invasive populations are known as biocontrol agents. Biocontrol is not only an environmentally friendly control option, but it is also long-term and sustainable (Hill, 2003). In classic weed biocontrol programmes, once biocontrol agents have been released, biocontrol programmes do not require much management and scheduled re-applications, a negative characteristic shared by both chemical and mechanical control programmes.

A good example of an extremely successful biocontrol programme is the programme initiated against *Azolla filiculoides* Lam. (Azollaceae) in South Africa. *Azolla filiculoides*, commonly known as red water fern, is a floating aquatic fern native to South America (Hill, 2003). It was first identified in South Africa in 1948 (Hill, 2003). As a floating macrophyte, it is able to produce large, dense mats of plant material that cover the surface of water bodies (Hill, 2003). Through their colonisation of the water surface, *A. filiculoides* severely reduces the biodiversity of aquatic ecosystems and negatively influences the usability of water as a human resource (McConnachie et al., 2003). By 1999, *A. filiculoides* was recorded in 152 sites within South Africa, most of which were within the Free State Province (Coetzee et al., 2011). Biocontrol was selected as the preferred control method and in 1995 the weevil *Stenopelmus rufinasus* was imported into the country for further testing and by December 1997 *S. rufinasus* was first released in South Africa (McConnachie et al., 2003). After being released the weevils took, on average, 10 months to gain control over an infestation (Coetzee et al., 2011). In 2002 it was concluded that *A. filiculoides* no longer posed a threat to South Africa water bodies (Coetzee et al., 2011).

A major drawback to biocontrol is that once released, biocontrol agents can take a number of years to attain some form of control over the invasive target population. Moreover, they can be less effective within climatically unsuitable regions, as well as highly eutrophic systems, where theAIPs are able to expand their numbers without being hindered by the control agents (Reddy etal., 2019). A lot of resources, both time and financial, are also required before a biocontrol agent can be cleared for release.

There are a few steps that need to be taken before a biocontrol agent can be released. Firstly, once an invasive population has been identified, it is important to conduct genetic analyses on these populations to determine their origins (McFadyen, 1998). This will help to find a natural enemy that is more closely-evolved, and thus able to provide greater control to the invasive population in question. Next, field surveys need to be conducted in regions with the most closely related and climatically suitable populations when compared with the invasive populations (McFadyen, 1998). During field surveys all aspects of the plants should be assessed for damages, including their leaves, fruits, flowers, petioles, tubers, crown and even roots. Natural enemies that show promise as biocontrol agents must then be captured and kept alive and healthy for exportation, where further experimentation can be conducted. Three characteristics of the potential biocontrol agents then need to be assessed: their life history, impact on the target plant and their host specificity (McFadyen, 1998). The life history of a potential biocontrol agent needs to be assessed to be able to better understand how it might interact in the field. Moreover, knowing the life history of established biocontrol agent populations can assist in understanding why some agents are less effective than others. Such information can help biocontrol practitioners plan to gain better control of the invasive species in question. Impact studies are conducted to determine if the candidate control agent can provide sufficient damage to warrant its release (McFadyen, 1998). Host specificity tests are essential for the release of a biocontrol agent. These tests are conducted to make sure once these natural enemies are released, that they will not go on to adversely affect non-target organisms (McFadyen, 1998).

1.2.4. Integrated pest management

Integrated pest management is a sustainable management technique where chemical, mechanical, and biocontrol options are used together in an economically and environmentally healthy manner (Naranjo et al., 2015). A widely used definition of IPM is that given by the United States Department of Agriculture in 1996, it states that IPM is: “a sustainable approach to managing pests by combining biological, cultural, physical, and chemical tools in a way that minimizes economic, health, and environmental risks” (Barzman et al., 2015). The goal of IPM is to reduce the economic and ecological risks posed by invasive populations and by individual control options (Naranjo et al., 2015).

South Africa boasts one of the largest national-scaled IPM programmes on earth, the Working for Water (WfW) programme. The WfW programme was initiated in 1995 and has since focussed on the protection of South Africa’s water resources from alien plants (specifically within catchment areas, riparian systems, and conservation areas) as well as the promotion of job creation (Zimmermann et al., 2004; Turpie et al., 2008). Although the WfW programme has assisted in creating jobs for many South Africans, concerns have been raised regarding the efficacy of the programme itself (McConnachie et al., 2012). This is in part due to the lack of follow-up evaluations, but also due to the focus of this programme on job creation, rather than on well-structured ecological planning (Shackleton et al., 2017). The WfW programme has provided biocontrol with a platform to receive the political, regulatory as well as financial support that it requires (Zimmermann et al., 2004). In many ways, the WfW programme may be responsible for the invigoration of the practice of biocontrol within South Africa (Zimmermann et al., 2004). Biocontrol is comparatively cheaper and more efficient control method, and has allowed the WfW programme to be financially viable, whereas if mechanical and chemical control options were the focal control option, this would simply cost too much (Zimmermann et al., 2004; Zachariades et al., 2017). Between 1997 and 2006 it was estimated that, per annum, stream flow was increased by 46 million m^3 through the clearing of invasive alien plants from riparian zones by the WfW programme (Marais & Wannenburgh, 2008). Through the success this programme generates on both ecological and socio-economic platforms, it has been regarded as one of the best integrated management programmes worldwide (Hobbs, 2004), and has also been hailed as inspirational in terms of the restoration of national capital (Woodworth, 2006).

1.3. Invasive plants in South Africa

Within South Africa, invasive terrestrial and aquatic plants have proven to be extremely problematic (Chamier et al., 2012). Terrestrial invasives have a larger above and below ground plant biomass compared with native plants (Chamier et al., 2012). This is especially true within the fynbos biome of South Africa, where above ground biomass can be increased by three to ten times that of our native flora (Le Maitre et al., 1996). Moreover, terrestrial invasive plants have led to extreme water losses, increasing evapotranspiration rates that lead to decreased surface water runoff and groundwater recharge (Chamier et al., 2012). Furthermore, terrestrial invasives have altered fire and soil erosion regimes and have often reduced the usability of land resources (Chamier et al., 2012). Aquatic invasives within South Africa have increased evapotranspiration rates, degraded water resources, and limited their utilization (Hill, 2003).

From here on, focus will be placed on aquatic invasives with South Africa specifically their management. South Africa has five main aquatic invasive plant species, namely: *P. crassipes*, water hyacinth; *S. molesta*, giant salvinia; *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae), parrot’s feather; *Pistia stratiotes* L. (Araceae), water lettuce; and *A. filiculoides*, red water fern (Hill, 2003). These five species are collectively known as the ‘Big Bad Five’ (Henderson & Cilliers, 2002). Biocontrol programmes have been implemented against invasives aquatic weeds in South Africa since 1973, with the first release of a biocontrol agent conducted a year later (Hill & Coetzee, 2017). Since 1973, 14 different biocontrol agents have been released against six aquatic weed species within South Africa (Hill & Coetzee, 2017). Importantly, four of the five 'Big Bad Five' species are now considered under complete biocontrol with the exception of water hyacinth (Hill & Coetzee, 2017). Complete control is achieved when no other control methods are required to maintain the population size of the weed under a certain threshold where the level of ecological and economic damage isacceptable (Hoffmann & Moran, 1995; Hill & Coetzee, 2017). Ironically, as biocontrol has achieved success against the floating invasives within South Africa, spaces and resources haveopened up a new channel for a second wave of aquatic invasives to take advantage of (Moran et al., 2013). This second wave of invasives are in their early stages of invasion and include submerged, emergent, rooted-floating and an assortment of novel free-floating weeds (Hill & Coetzee, 2017). Some examples of these new invasive aquatic weeds include: Brazilian waterweed, *Egeria densa* Planch. (Hydrocharitaceae); Mexican water lily, *Nymphaea mexicana* Zucc. (Nymphaeceae); *Salvinia minima* Baker (Salviniaceae); and Delta arrowhead, *Sagittaria platyphylla* (Engelmann) J.G. Smith (Alismataceae) (Hill & Coetzee, 2017).

1.4. Sagittaria platyphylla

Sagittaria platyphylla is a rooted emergent aquatic macrophyte (Fig. 1.1), native to the USA, more specifically to the southern and south-eastern regions of the country (Adair et al., 2012). The name 'Sagittaria' is derived from the Latin word 'sagitta', which means 'arrow'. This is with reference to the arrow-shaped leaves the plants within this genus possess (Adair et al., 2012).

This aquatic macrophyte has three leaf forms; namely, an emergent broad-leaf form (Fig. 1.1a), an emergent narrow-leaf form (Fig. 1.1b) and a submerged phyllodial form (Fig. 1.1c) (Adair et al., 2012). The leaf form of this plant is determined by the environmental parameters where it grows (Adair et al., 2012). The phyllodial submerged form is commonly found in deeper waters, while the two emergent forms are commonly found within more shallow waters (Adair et al., 2012). Moreover, the broad-leaf emergent form is typically found in slow flowing water systems, while the narrow-leaf emergent is found in still water systems (Adair et al., 2012).

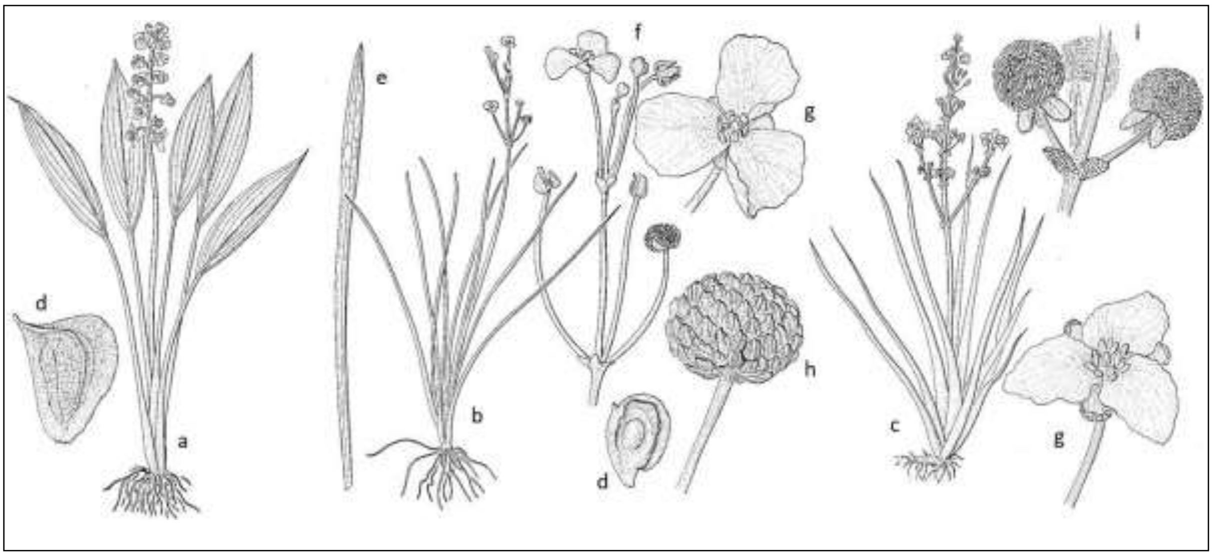

Figure 1.1. The following are all portions or forms of *Sagittaria platyphylla*. (a) Emergent broad-leaf form, (b) narrow-leaf emergent form, (c) submerged phyllodial form, (d) achenes, (e) a leaf from the emergent narrow-leaf form, (f) inflorescence, (g) male flowers, (h) mature fruiting body, (i) mature fruit arranged in a raceme structure. (Line drawing obtained from the Flora of North America Association).

Sagittaria platyphylla can reproduce both sexually, via achenes (Fig. 1.1d), and asexually, via tubers and stolons (Martin et al., 2018). Achenes are dry fruiting bodies that contain a single seed (Marzinek et al., 1996). Achenes of *S. platyphylla* can float for up to seven days and may be transported by birds (Adair et al., 2012). Flower (2004) found that the fruit (Fig. 1.1h) of *S. platyphylla* in northern Victoria, Australia produced, on average, 850 achenes, with an average of 6 900 achenes per inflorescence (Fig. 1.1f). Stolons, on the other hand, are produced by both the emergent and submerged *S. platyphylla* forms, but are not produced in waters deeper than 50cm (Adair et al., 2012). Tubers (sometime called corms) of *S. platyphylla* are round and fleshy starch-storing organs that are produced within the sediment (Adair et al., 2012). The production of these subterranean storage organs is highest prior to winter (Adair et al., 2012).

The climatic requirements of *Sagittaria platyphylla* are not well understood, but these aquatic macrophytes are mainly found in warm-temperate conditions (Adair et al., 2012). Frost damages the tops of these plants, but regrowth from submersed plants or subterranean organs will generally occur (Adair et al., 2012).

The dispersal of *S. platyphylla* from its home range has been attributed to its popularity as an aquarium and ornamental plant (Kwong et al., 2018). This aquatic macrophyte is highly invasive outside of its native range and has been found in a number countries, including

Panama, Indonesia, Australia, China, and South Africa (Martin et al., 2018). Characteristics that make *S. platyphylla* so invasive include its long-range dispersal ability and rapid growth, allowing it to dominate shallow water bodies (Kwong et al., 2014). The achenes are able to float downstream, which allows for this aquatic macrophyte to populate novel spaces (Martin et al., 2018).

Where invasive, *S. platyphylla* is damaging on both economic and ecological fronts. Infestations of this aquatic macrophyte can alter natural or man-made water systems through sedimentation and water flow restriction, which can lead to flooding (Martin et al., 2018). *Sagittaria platyphylla* infestations can influence the delivery of water to communities and farmlands (Adair et al., 2012); alter ecological balances and reduce biodiversity through the displacement of native flora and fauna (Kwong et al., 2014; Martin et al., 2018); and affect the recreational use of water systems, particularly fishing and boating (Adair et al., 2012).

This invasive was first recorded in South Africa in 2008 in KrantzKloof Nature Reserve, KwaZulu-Nata Province (Ndlovu et al., 2020). Since then *S. platyphylla* has been found in almost 90 different sites within three of the nine South African provinces, namely the Eastern Cape, Western Cape, and Kwa-Zulu Natal provinces (Ndlovu et al., 2020).

1.4.1. Control of Sagittaria platyphylla

Sagittaria platyphylla was initially targeted by the South African National Biodiversity Institute (SANBI) for eradication via mechanical control, but the populations grew too rapidly for this to be achieved (Martin et al., 2018). In Australia, *S. platyphylla* infestations are controlled using chemical and mechanical means, with biocontrol programmescurrently being considered (Kwong et al., 2014). Herbicides are the prominent control option utilised in Australia, but have received varied results, whereas mechanical control is only usedin extreme cases where the revival of the water system is extremely necessary (Kwong et al., 2014). Conventional control methods such as chemical and mechanical control have not sufficiently managed the spread of this invasive weed in South Africa, therefore biocontrol is being considered as a potential control option (Adair et al., 2012; Ndlovu et al., 2020).

Studies by various researchers including Deonier (1971 and 1998), Herlong (1979) and Brigham et al., (1982) suggested that a large suite of insects from the families Chrysomelidae (Coleoptera), Ephudridae (Diptera) and Pyralidae (Lepidoptera) are present on *Sagittaria* spp.

within the USA (Adair et al., 2012). Between 2010 and 2012, Kwong et al. (2014) conducted field surveys for natural enemies of *S. platyphylla* in southern USA. As the surveys were conducted, only one Dipteran species was found, while no Chrysomelidae or Pyralidae individuals were found. However, four species of weevil within the genus *Listronotus* were observed and collected from *Sagittaria* spp. (Adair et al., 2012). Each species differed in its life history and impact to *S. platyphylla*, with weevils found attacking the tubers, crowns, and fruiting heads (Adair et al., 2012). The four weevil species found were, *Listronotus sordidus* Gyllenhal 1834 (Coleoptera: Curculionidae), *Listronotus lutulentus* Boheman 1843 (Coleoptera: Curculionidae), *Listronotus appendiculatus* Boheman 1842 (Coleoptera: Curculionidae) and *Listronotus frontalis* LeConte 1876 (Coleoptera: Curculionidae) (Adair et al., 2012). The weevil *L. appendiculatus* was the most common and abundant of the four species (Adair et al., 2012).

In 2014, researchers from the Centre for Biological Control (CBC), Rhodes University, South Africa brought back the above mentioned *Listronotus* spp. from the Mississippi Basin for further testing (Martin et al., 2018). *Listronotus sordidus* was found to extensively damage the crowns of *S. platyphylla*, but did not oviposit on plants that were inundated, a common characteristic of the *S. platyphylla* populations within South Africa (Martin et al., 2018). *Listronotus lutulentus* was deemed not damaging enough for release and the population was terminated (Ndlovu et al., 2020). Currently the CBC is working on two candidate control agents, *L. appendiculatus* and *L. frontalis* against *S. platyphylla*. Studies conducted by Kwong et al. (2018) suggested that *L. appendiculatus* could be a good biocontrol agent in Australia for *S. platyphylla* as it reduces the number of achenes produced by the plants, limiting their spread and reducing the seed bank, mitigating future re-establishment. Impact trials and host specificity testing conducted on *L. appendiculatus* in South Africa have also suggested it should be a good biological control agent and an application for its release has been submittedto the Department of Environment, Forestry and Fisheries (G. Martin, pers. comm.) *Listronotus frontalis* is closely associated with the crown and root system of *S. platyphylla* and is thought to feed on tubers to complete its life cycle (Kwong et al., 2014). However before it can be considered, the life history, impact on target-plant as well as host-specificity will have to be determined for *L. frontalis.*

1.5. Aims of this study

The aims of this study were to determine the importance of tubers to *S. platyphylla* populations growing in South Africa, with a specific focus on their presence within different water depths, as well as their response to seasonality within South Africa. In addition, the study aimed to collect quantitative pre-release data of individual plant as well as population parameters of *S. platyphylla* at two sites in the Eastern Cape Province that could then be used to compare post-release data to if biological control agents are released in South Africa

In addition, the study aimed to determine the life history of the tuber feeding weevil *L. frontalis,* as well to quantify its impact on *S. platyphylla*, with the ultimate goal of providing valuable data which may assist in acquiring permission to release of the candidate agent. This, in turn, may result in a reduction of the negative effects associated with the invasions of *S. platyphylla* in aquatic ecosystems of South Africa.

Chapter 2: Population dynamics of Sagittaria platyphylla in South Africa

2.1. Introduction

2.1.1. Plant population biology

Population biology refers to the general study of populations, with common focus placed on their structural compositions and how they alter over time (Silvertown & Charlesworth, 2009). A population is a group of individuals within the same species that are growing and developing within the same spatial region (Husband & Barrett, 1996). Individual plants within a population are thus competing with each other for various resources including space, nutrients, and light (Silvertown & Charlesworth, 2009). Individual plant populations can grow quite uniformly as they share the same resources and incur the same ecological pressures (Weiner et al., 2001; Silvertown & Charlesworth, 2009). However, plants are also phenotypically plastic, which is their ability to be structurally distinct, despite being genetically identical (Nicotra et al., 2010). This enables plants, in particular, different populations, to develop discretely under contrasting ecological pressures and resources (Gratani, 2014).

Population ecology and population dynamics are branches of population biology. Population ecology tries to better understand how biotic and abiotic factors influence certain aspects of a population (Begon et al., 2009). Biotic factors can include interactions with other plants that compete for similar resources; animals that feed on the plants themselves; various pollinators; or even fungi that infect the plants (Wood et al., 2012), while abiotic factors include sediment type; water pH; wind intensity; sunlight intensity; and water depth (Wood et al., 2012). Population ecology tries to better understand how these factors influence a population's growth rate, growth form and investment in different reproductive strategies (Begon et al., 2009). Essentially, population ecology is concerned with the ecological reasons for the distinctions found between populations of the same species. However, population dynamics looks to better understand how the changes brought about by such ecological pressures alter population characteristics, with a particular focus on population variability in response to seasonality (Crawley & May, 1987). More relevant for this study, population dynamics components willfocus on determining if/how reproductive characteristics of *S. platyphylla* populations respond to seasonal fluctuations.

2.1.2. Population ecology and dynamics in terms of biocontrol

Biocontrol programmes require a large investment of time and resources before a control agent can be released (McFadyen, 1998). Although many successful biocontrol programmes have been implemented, for example programmes against *Hypericum perforatum* Linnaeus (Hypericaceae) in the USA (Huffaker & Kennett, 1959) and *Opuntia ficus-indica* in South Africa (Zimmermann & Moran, 1982), there have been less successful programmes too. For example, two control agents were released against *Ageratina adenophora* (Sprengel) R. King & H. Robinson (Synonym: Eupatorium adenophorum Sprengel) in South Africa in the 1980s, that have gone on to become established; however, control efforts have been negligible (Morris, 1991; Klein, 2011). Moreover, biocontrol efforts against *Macfadyena unguis-cati* L. (Bignoniaceae) have seen the release of five control agents in South Africa since the late 1990s, with establishment of four of these agents confirmed; however, the level of control achieved by the agents is regarded as negligible (King et al., 2011; Klein, 2011). Unsuccessful biocontrol programmes, besides having the potential to cause ecological harm, utilise an abundance of resources that could have been allocated elsewhere (McFadyen, 2003; Suckling & Sforza, 2014). Moreover, they erode societal and economical trust in biocontrol as a whole (Blossey et al., 2018). Given the risks and costs that encompass such programmes, it is worthwhile investing in methods that help to reduce their shortcomings (Briese, 1993). Kriticos et al. (1999) suggested that population ecology surveys of target weeds could have great benefits. Although these surveys currently seem to be a luxurious expense, they should be viewed as a way to reduce the number of failed programmes and lessen the misuse of resources (Kriticos et al., 1999).

A prominent reason why population ecology and dynamics are so important in the context of biocontrol is that they provide biocontrol practitioners with an enlarged scope of the problem (Briese, 1993). Instead of focussing on how a few control agents impact a few individual plants directly, population ecology and dynamics help to figure out under what ecological pressures plants flourish, how other ecological pressures influence their growth, development, and reproductive investments, and how all of these factors change with seasonality (Begon et al., 2009). This allows biocontrol practitioners to understand different components of populations better and to assess the best method whereby the release of a biocontrol agent, or perhaps multiple agents, would lead to the greatest control, rather than the greatest damage (Briese, 1993). Spectacular damage does not necessarily lead to effective control (Briese, 1993). Biocontrol agents need to significantly lessen both survivorship and recruitments rates below a given threshold to obtain control (Briese, 1993). These targets can only be better prepared for if practitioners have a greater understanding of populations as a whole (Kriticos et al., 1999).

Pre-release and post-release studies can play extremely important roles in the overall betterment of biocontrol (McFadyen, 1998), and are being included more routinely in biocontrol programmes. Pre-release studies enable biocontrol practitioners to understand the ecology of different invasive populations, helping to select agents with a greater likelihood of success and also providing baseline data for post-release evaluations to be compared against (McFadyen, 1998; Barratt et al., 2006). Without pre-release data, data from post-release evaluations can be very ambiguous, and evaluating success levels may become inconclusive (Swope et al., 2017; Blossey et al., 2018). Moreover, without such evaluations, it would be hard to convince regulatory agencies and funders for their continued support and investment in biocontrol (McFadyen, 1998; Blossey et al., 2018). Pre-release data can help to target the more sensitive life history transitions in plant demography (Davis et al., 2006). This will help to determine agent(s) release parameters and when augmentative releases could be conducted, if required (Davis et al., 2006). The more prominent incorporation of pre- and post-release evaluations into biocontrol programmes will require a fundamental shift in their operation and funding (Davis et al., 2006). While host-specificity tests and impact studies are important, pre- and post-release evaluations should not be considered as an auxiliary assessment (Davis et al., 2006). To illustrate this with an example, DeClerck-Floate (1996) used pre-release data of the target plant *Cynoglossum officinale* Linnaeus (Boraginaceae) to predict the possible long-term impact of *Mogulones cruciger* Pallas (Coleoptera: Curculionidae) following its release. Through this analysis, it was determined that for optimum control agent establishment, three characteristics of *C. officinale* populations were required. Populations were required to have predominantly large plants, a high proportion of the plants needed to be flowering plants and the populations needed either to be increasing in size or to be already well established. Of the agents released in 1997, it was later found that all released agent populations established (DeClerck-Floate et al., 1996), and *M. cruciger* has since gone on to rapidly reduce populations of *C. officinale* through its high establishment rates and heavy impact (Winston et al., 2014).

Another benefit of conducting pre-release studies is to determine differences between invasive populations themselves. There are various invasive plants that develop and reproduce differently within their invasive ranges compared to within their native ranges (Edwards et al., 1998). For example, invasive populations of *Centaurea solstitialis* Linnaeus (Asteraceae) have been shown to grow larger, produce larger seeds and flower earlier in comparison to native individuals (Barker et al., 2017). In their native range in South America, *E. densa* and *M. aquaticum* are dioecious aquatic plant species capable of both sexual and asexual reproduction, however, as invasives within South Africa, only female plants occur, and thus these populations are limited to asexual reproduction only (Coetzee et al., 2011).

The same kinds of differences can also be observed between populations within their invasive range. For example, *Decodon verticillatus* (Linnaeus) Elliot (Lythraceae) is an invasive plant within the USA. While the majority of *D. verticillatus* populations reproduce both sexually and asexually, a few select populations only reproduce asexually (Dorken & Eckert, 2001). Such information can help save biocontrol practitioners a lot of time and money when each invasive population is assessed individually.

Linking population ecology with biocontrol can essentially help to select more appropriate control agents; increase the likelihood of agent establishment; and support greater predictability of the interactions between the host and control agent populations (De Clerck-Floate & Bourchier, 2000). Population ecology studies will thus benefit biocontrol programmes in a more general sense, whereas understanding invasive species' population dynamics may help biocontrol programmes in more targeted scenarios.

Assessing the population dynamics of an aquatic invasive plant involves determining predictable changes over time, and more importantly, linking these changes to seasonality. This is made more challenging with the variety of systems *S. platyphylla* has invaded within South Africa. This invasive plant has populated both lentic and lotic water bodies in the form of streams, irrigation canals and dams (Ndlovu et al., 2020). Moreover, *S. platyphylla* has established populations within three South African provinces (Ndlovu et al., 2020). This expansive range means that *S. platyphylla* populations also vary in terms of climate and altitude within South Africa. These variant contributors, along with the phenotypic plasticity of these plants, has allowed for distinct differences to arise between populations of *S. platyphylla* within South Africa. The assessment of such distinctions can allow practitioners to group together similar populations and treat them subjectively, with the hope that programmes initiated against them will be more effective.

Although having a greater understanding of an invasive species creates the potential for a more successful control programme, this greater knowledge does not always guarantee success. *Hydrilla verticillata* (Linnaeus filius) Royle (Hydrocharitaceae) is an invasive aquatic plant in the USA and after its introduction, a large amount of research was conducted to improve understanding of its biology and population biology (Arias et al., 2005). Despite the plant itself being very well known and understood, complete control of *H. verticillata* still evades biocontrol programmes (Purcell et al., 2020). Some invasives may elude biocontrol efforts through biological and ecological complexities, however, possessing more information on an invasive species always allows for more accurate control agent evaluations and strategy developments, thus increasing the chances of successful control.

2.1.3. Population ecology and dynamics of *Sagittaria platyphylla*

While aquatic invasive plants are able to grow uniformly under similar conditions (Silvertown & Charlesworth, 2009), phenotypic plasticity may allow different populations of the same species to display very different characteristics (Nicotra et al., 2010). This is very applicable to *S. platyphylla* as it has three different growth forms, one of which is completely submerged (Adair et al., 2012). From a biocontrol perspective, this makes executing a biocontrol programme quite challenging as different growth forms come with different control requirements. Moreover, determining if variation exists between plant characteristics of the different growth forms will be required knowledge for a biocontrol programme, but may also make developing the programme more challenging. For example, the submerged growth form of *S. platyphylla* does not produce fruits, therefore a fruit-feeding control agent, such as *L. appendiculatus* will provide no control over this growth form whatsoever (Adair et al., 2012). In addition, understanding if subtle differences exist between the two emergent growth forms, such as differences in the size and abundances of tubers and fruit, could help to understand which control agent(s) would yield the greatest results.

Based on anecdotal evidence, it is thought that water depth might influence the growth form, population size and number of tubers produced by *S. platyphylla*. Various characteristics of *S. platyphylla* are well understood in terms of their growth and development under different water depths. Kwong et al. (2017) conducted a thorough biogeographical study that incorporated the influence of water depth when the following characteristics of *S. platyphylla* were assessed: population size, percentage cover, plant density, plant height, number of fruiting bodies and number of achenes per fruiting body. However, thus far, no studies on the tuber dynamics of *S. platyphylla* have been conducted. This is particularly important as one of the candidate agents currently under consideration is the weevil *L. frontalis*. *Listronotus frontalis* larvae feed only on the tubers of *S. platyphylla* while the adults feed on the new leaves, flowers, and pollen of the plant. Although a better understanding of the abundances, viability, and seasonality of *S. platyphylla* tubers would greatly assist biocontrol efforts, they would also help to develop a better understanding of the basicpopulation biology of the plant itself.

Understanding the population dynamics of *S. platyphylla*, and how ecological pressures influence these dynamics seasonally could help to determine when releases should be conducted to optimise agent establishment. Moreover, if control agents are to be used augmentatively as 'green-herbicides', such information would also help determine the most suitable time for such releases.

Collecting *S. platyphylla* population data prior to any agent release will help to predict their establishment and impact. Determining the relationship between seasonality and *S. platyphylla* fruit production would, for example, help to assess when best to release *L. appendiculatus*. Similarly, determining the relationship between *S. platyphylla* tuber production and seasonality would provide the same assessment ability for *L. frontalis*. Such knowledge can aid in release strategies; however, it can also helpto determine, with greater accuracy, the levels of control. For example, if *S. platyphylla* generally produces fewer tubers in summer, this ecological trend will not be misunderstood aseffective control efforts from *L. frontalis*. This will give practitioners very precise assessmentabilities and from the trends pre- and post-release, decisions can be made with regards to current management strategies. Although pre-release data may not be available for all *S. platyphylla* populations, having these data for a few populations may enable more accurate presumptions to be made for populations without such data.

Therefore the aims of this study were to determine the population dynamics of a number of populations of *S. platyphylla* within South Africa. Additionally, tuber production of different *S. platyphylla* populations was assessed against the abiotic drivers, specifically water depth. Furthermore, the growth rate of *S. platyphylla* under different types of sediment was also assessed.

2.2. Materials and methods

2.2.1. Description of sampled sites

Currently, there are almost 90 recorded *S. platyphylla* sites within South Africa and to gain a better understanding of their population dynamics, seven sites were sampled (Fig 2.2.1; Table 2.2.1). Within the Western Cape Province, three *S. platyphylla* sites were sampled,two of which were located on wine farms, Stark-Condé Wines, just outside of Stellenbosch, and Lourensford Wine Estate, Somerset West, and the third growing at the defunct JonkershoekTrout Hatchery, also just outside of Stellenbosch. These three sites are referred to as 'Stark- Condé', 'Lourensford' and 'Jonkershoek' from here on. Within the KwaZulu-Natal Province, two *S. platyphylla* populations were sampled, both within protected areas, the Krantzkloof Nature Reserve, Durban, and the KwaZulu-Natal National Botanical Gardens in Pietermaritzburg. These sites are referred to as 'Krantzkloof' and 'Kingfisher Lake'. In the Eastern Cape Province, two *S. platyphylla* sites were sampled, the first at Maden Dam, locatedclose to Stutterheim, and the second site within the Makana Botanical Gardens in Makhanda (previously known as Grahamstown). These two sites will be referred to as 'Maden Dam' and 'GHT Bots'.

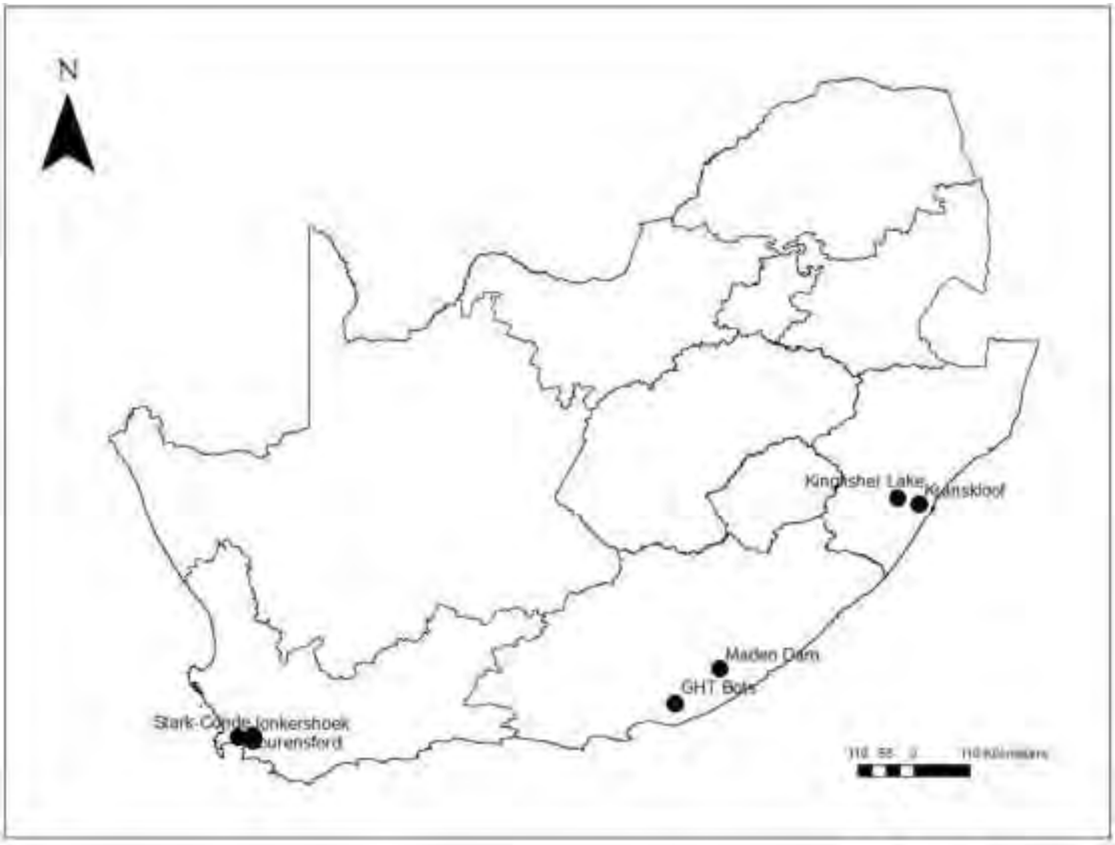

Figure 2.2.1. *Sagittaria platyphylla* sites sampled within South Africa for the nationwide survey.

Table 2.2.1. Locality details of the *Sagittaria platyphylla* populations sampled across South Africa for this study.

Site name	**Area containing site**	**Province**	**Latitude**	**Longitude**
Lourensford	Lourensford Wine Estate	Western Cape	-34.070912	18.88966
Stark-Condé	Stark-Condé Wines	Western Cape	-33.954367	18.909758
Jonkershoek	Jonkershoek Trout Hatchery	Western Cape	-33.963365	18.925493
GHT Bots	Makana Botanical Gardens	Eastern Cape	-33.317352	26.522201
Maden Dam	Maden Dam	Eastern Cape	-32.738975	27.298423
Kingfisher Lake	KZN National Botanical Gardens	KwaZulu-Natal	-29.604706	30.347779
Krantzkloof	Krantzkloof Nature Reserve	KwaZulu-Natal	-29.772815	30.830941

2.2.2. Nationwide surveys

Nationwide surveys were conducted to determine if variability existed between the *S. platyphylla* populations present within South Africa. Specifically, populations that experience different environmental pressures and climatic differences were sampled to better understand the influence such variability has on the growth, development, and reproductive investment of *S. platyphylla* populations.

Sampling events did vary slightly due to the variability in growth and population sizes of different *S. platyphylla* populations. Sites were sampled using 50 x 50 cm quadrats and each site was visited once during the summer period in 2019. Quadratswere placed as randomly as possible, as populations often did not expand across the entire system, either due to greater water depths experienced, the presence of other plant species or even uninhabitable zones. The number of quadrat measures taken per site did vary, and this wasdue to the limited size of some of the populations sampled. In particular, quadrat measures taken at Krantzkloof and Stark-Condé were limited to only six and eight measures respectively,both due to the small sizes of the populations, whereas most of the other sites sampled were done so with 20 quadrat measures. For the nationwide survey, focus was placed on the influence of water depth on tuber characteristics. Therefore, half the quadrat measures taken per site were done so in shallow sections of the populations, while the other half were taken within deeper sections. Per quadrat measure, the following characteristics were recorded: ‘water depth’, ‘number of plants’, and ‘number of tubers’. Additionally, an individual plant nearby to each quadrat sample taken was randomly selected and dug up out of the sediment, and the following parameters were recorded: ‘plant height’, and ‘ number of leaves’.

Tubers were collected per quadrat by pumping sediment with an angling prawn pump through a sediment sieve to separate the sediment from the tubers so that they could be counted and collected. Sediment was pumped out nine times per quadrat, as this worked out to sample 625 cm^2 of the 2 500 cm^2 quadrat.

2.2.3. Continuous monitoring

Monthly surveys were conducted between September 2019 and August 2020 (excluding March and April 2020 due to the nationwide Covid-19 lockdown) at Maden Dam and GHT Bots. This continuous monitoring was conducted in order to determine the seasonal variability of the growth, development, and reproductive investment of *S. platyphylla* populations. Maden Dam is a large body of lentic water while GHT Bots is, in comparison, a small-and-narrow slow-flowing stream. Key differences between the two sites other than one being lentic and the other lotic include the sediment and the exposure to direct sunlight. The sediment at Maden Dam mostly consisted of clay-like soil with high sand content, while the sediment at GHT Bots had a very soft, mud-like consistency with high levels of organic matter. The population of *S. platyphylla* at GHT Bots were also very well shaded, whereas the population at Maden Dam, for the most part, was constantly exposed to direct sunlight.

For the monthly surveys, two main types of measures were conducted; those within a 50 x 50cm quadrat and those conducted on individual plants. Per month, each site was sampled once, where 10 random quadrat measures, and measures on 10 randomly selected individual plants were taken. The randomness of the samples taken was limited to where patches of *S. platyphylla* grew. The measurements taken per quadrat were: ‘water depth’, ‘number of plants’, ‘number of emergent leaves’, ‘number of flowers’, and ‘number of tubers’. The measures taken per individual plant were: ‘plant height’, ‘root length’, ‘crown width’, ‘number of leaves’, ‘number of fruit’, ‘number of flowers’, ‘number of tubers’, and ‘number of stolons’. Tubers were collected in the same manner as for the nationwide surveys, by pumping sediment through a sieve, nine times. Tubers and fruit sampled within the quadrats were kept in brown paper bags and transported back to the Centre for Biological Control (CBC) laboratory, Rhodes University

(RU) Makhanda, where the weight of tubers and the width of the fruits were measured. In addition, soil samples were taken at both GHT Bots and Maden Dam. 500g of sediment was collected per site and then sent to Bemlab Laboratory in Strand, Western Cape, South Africa, for physical and chemical analysis.

As temperature was used to determine its influence on various parameters of *S. platyphylla* growth and development, temperature data were obtained from the South African Weather Services (SAWS, 2020). Daily minimum temperature data was used as cold temperatures are predicted to be most limiting to tuber dynamics and plant growth.

2.2.4. Plant growth comparison in different sediments

An experiment was set-up to determine if different types of sediment would influence the growth of *S. platyphylla* plants. This experiment was conducted as field observations suggested varied growth of *S. platyphylla* in different sediment types, notably a compact clay-like sediment and a loose sandy sediment. Three sediment types were tested: sand sediment obtained from a local construction company, a pond sediment with high organic content obtained from a local dam and a combination of the two. These sediments were used as they were continuously available. For the purposes of this study, the three sediment types are referred to as 'builder's sand', 'pond sediment', and 'mixed sediment'.The builder's sand had high fine sand percentages and a low soil organic Carbon of 0.21 %. This sand also had low pH values of around 6 (Martin 2013). The pond sediment was loam andalso had a low soil organic Carbon of 0.08 % and a low pH of 3.9 (Strange 2018). Per treatment,30 20-L buckets were used, each with a 25 x 20 cm plastic pot filled with the relevant sediment. 15g of controlled slow-release fertiliser (which had an NPK ratio of 15:7:15 and released nutrients over a period of eight months) was added to the sediment and mixed into the top five cm by hand. Two *S. platyphylla* tubers were added to each pot. These tubers were collected at GHT Bots and weighed so that only tubers weighing between two and three grams were used in thisexperiment. Water was added to the buckets so that the pots were inundated, the water level was then maintained throughout the experiment. Plant height was recorded on a weekly basis for a period of four weeks.

2.2.5. Statistical analyses

All statistical analyses were carried out through R version 3.6.3 and RStudio version 1.3.1056. The normality of the residuals from the nationwide survey data were visually inspected through plots of residuals versus fitted values and quantile-quantile plots of model residuals. Homoscedasticity was tested using a Levene's test. Response variables were log-transformed when necessary for satisfying assumptions of the Analysis of variance (ANOVA). An ANOVA was used to test how number of leaves and water depth varied between the sampling sites. Welch's ANOVA was performed to determine significant differences in, plant height, number of plants and tubers between the sites. Welch's ANOVA is used when the data are normally distributed but violate the assumption of homogeneity of variances i.e. the variance is not assumed to be equal. Tukey's HSD multiple comparison tests were used to detect pairwise differences between the sites. I also applied a Bonferroni's correction for adjusting the significance levels to control for Type I error in a multiple testing situation. A pairwise comparisons post-hoc t-test was used for data analysed through Welch's ANOVA. P-adjusted values were calculated using Benjamini and Hochberg (BH) method.

For the plant parameters recorded in the monthly surveys of Maden Dam and GHT Bots, the Shapiro-Wilk normality test was used to determine normality of the data. All the data were then analysed with a Factorial Analysis of Variance to determine if significant differences were found between the sampled months and between the two sites sampled. From all of the survey data collected, correlations between the fruit and tuber numbers and various environmental variables were tested. Again, all the data were run through normality tests using the Shapiro-Wilk normality test. The relationship of those data found to be normally distributed were determined using Pearson's product-moment correlation, while non-normally distributed data were analysed using Spearman's rank correlation. An analysis of covariance (ANCOVA) was used to determine the influence of sampled site against plant growth patterns while controlling for temperature. To make sure the data were appropriate for this analysis, 'quantile-quantile' and 'residuals vs fitted' plots were used. All of the data used were in agreement with the assumptions.

Data from the tuber growth experiment were analysed using a Chi-squared test to determine significant differences.

2.3. Results

2.3.1. Nationwide survey

Unsurprisingly, the mean water depths measured between each site were significantly different ($F_{(6,97)} = 19.45$, $P < 0.0001$), with Krantzkloof experiencing, on average, the deepest waters and Jonkershoek the most shallow, from the sites sampled (Fig. 2.3.1). The mean number of plants also differed significantly ($F_{(6,97)} = 14.798$, $P < 0.0001$), with Krantzkloof having the least per quadrat (Fig. 2.3.1). The mean number of tubers per site differed significantly ($F_{(6,97)} = 31.45$, $P < 0.0001$), with Jonkershoek producing the most tubers and Krantzkloof the least (Fig. 2.3.1). In addition, the mean number of leaves ($F_{(6,97)} = 9.886$, $P < 0.0001$) and the mean plant height ($F_{(6,97)} = 63.566$, $P < 0.0001$) of individual plants were significantly different between the sites sampled (Fig. 2.3.2). Krantzkloof and Jonkershoek again showed very opposing trends, with the former producing the most leaves and greatest plant height of all the sampled sites, while the latter population displayed the inverse.

When tuber abundance and water depth were correlated, very similar trends were found throughout the sites sampled in South Africa. Very weak, slightly negative, and not significant relationships were found between water depth and tuber abundance at Stark-Condé ($R^2 = 0.0096$, $P = 0.8173$), GHT Bots ($R^2 = 0.0037$, $P = 0.7989$), Maden Dam ($R^2 = 0.0369$, $P = 0.4167$), and Kingfisher Lake ($R^2 = 0.0646$, $P = 0.2796$) (Fig. 2.3.3). A weak, positive, and significant relationship was found between water depth and tuber abundance at Lourensford ($R^2 = 0.399$, $P = 0.0087$) (Fig. 2.3.3). Whereas, no correlations could be made for the sites at Jonkershoek and Krantzkloof as the former did not have any water present, while the latter did not have any tubers present (Fig. 2.3.3). When all the water depth and tuber abundance data were pooled together and correlated, their relationship was found to be very weak, slightly negative and not significant ($R^2 = 0.1877$, $P < 0.0001$). From these correlations run, it can be seen that, bar extreme water depths (e.g. Krantzkloof) or situations where sites experienced drought (e.g. Jonkershoek), water depth did not have much of an influence over the production of tubers made by *S. platyphylla* within South Africa.

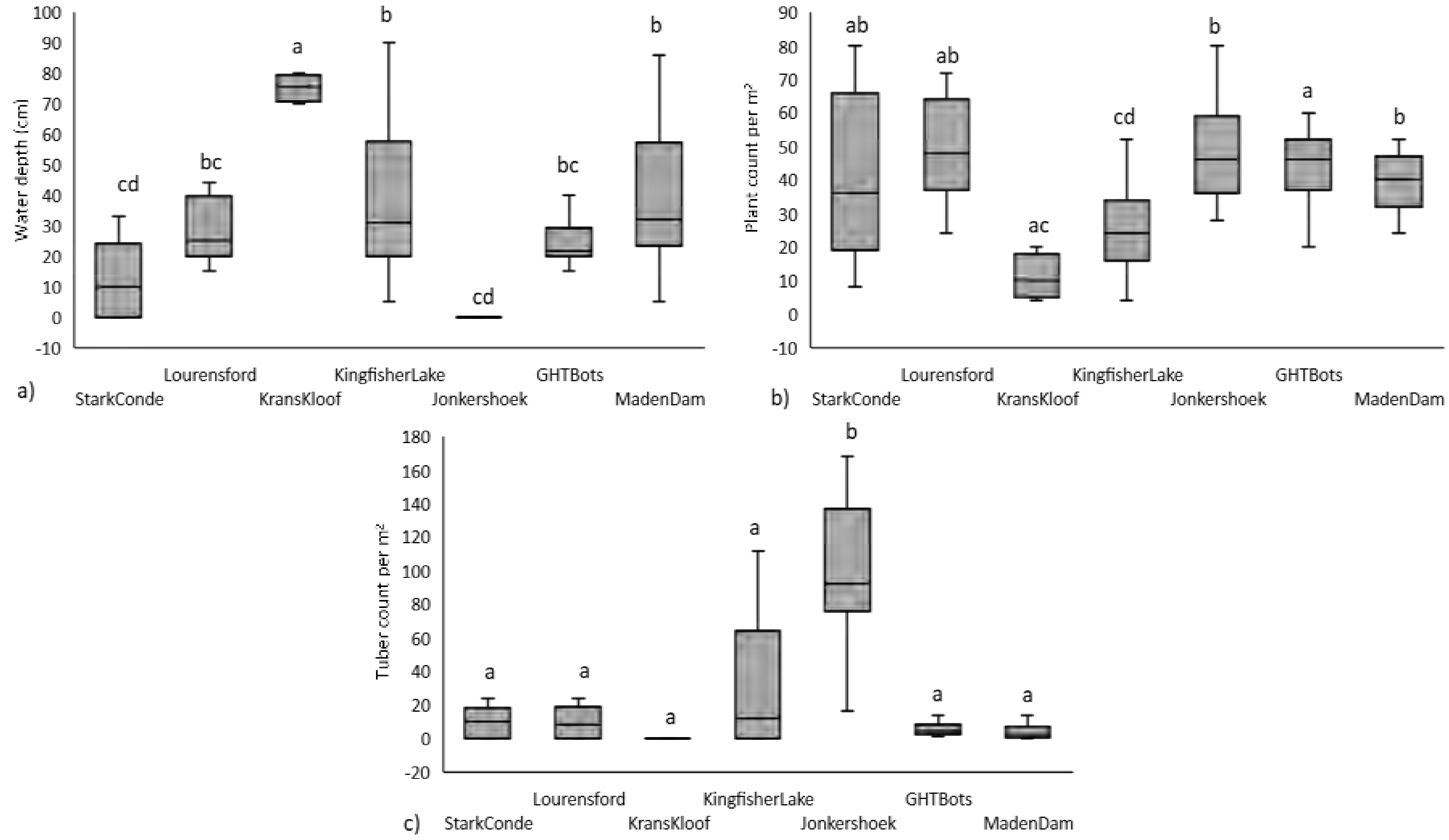

Figure 2.3.1. The median, lower and upper quartiles, lowest and highest observations of a) water depth, b) number of plants and c) number of tubers per m^2 of *Sagittaria platyphylla* populations sampled in South Africa. Letters denote levels of significance.

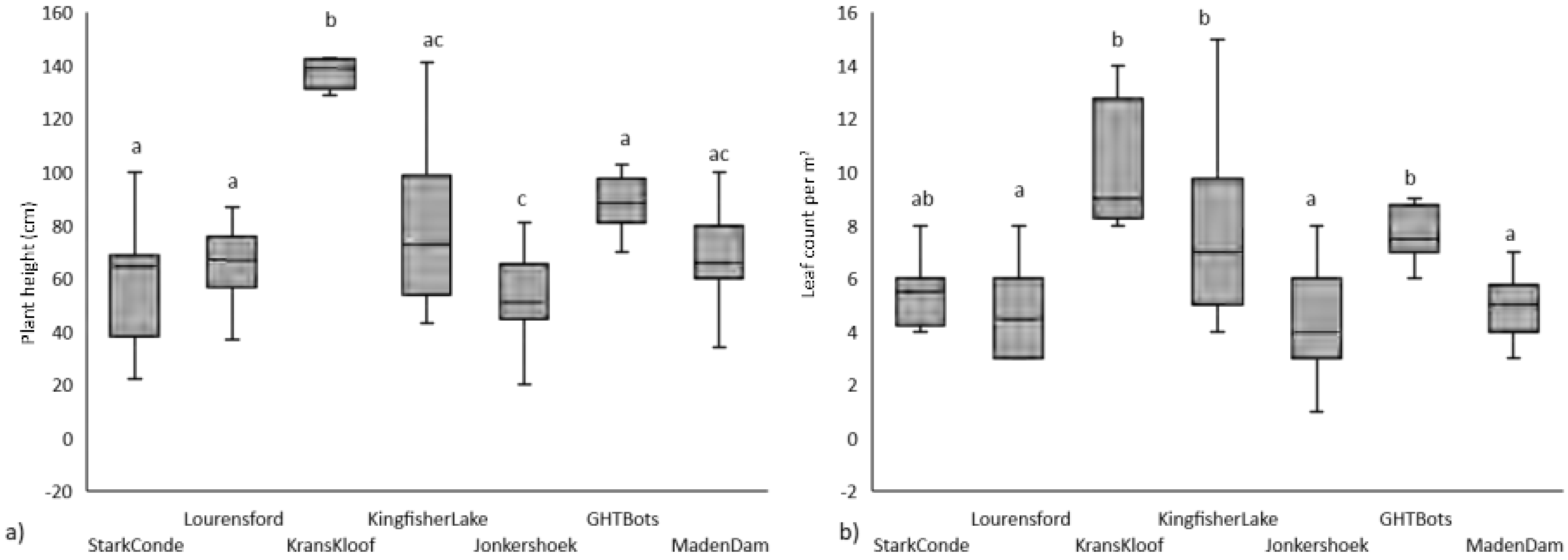

Figure 2.3.2. The median, lower and upper quartiles, lowest and highest observations of a) plant height and b) number of leaves from individual *Sagittaria platyphylla* plants sampled at various sites in South Africa. Letters denote levels of significance.

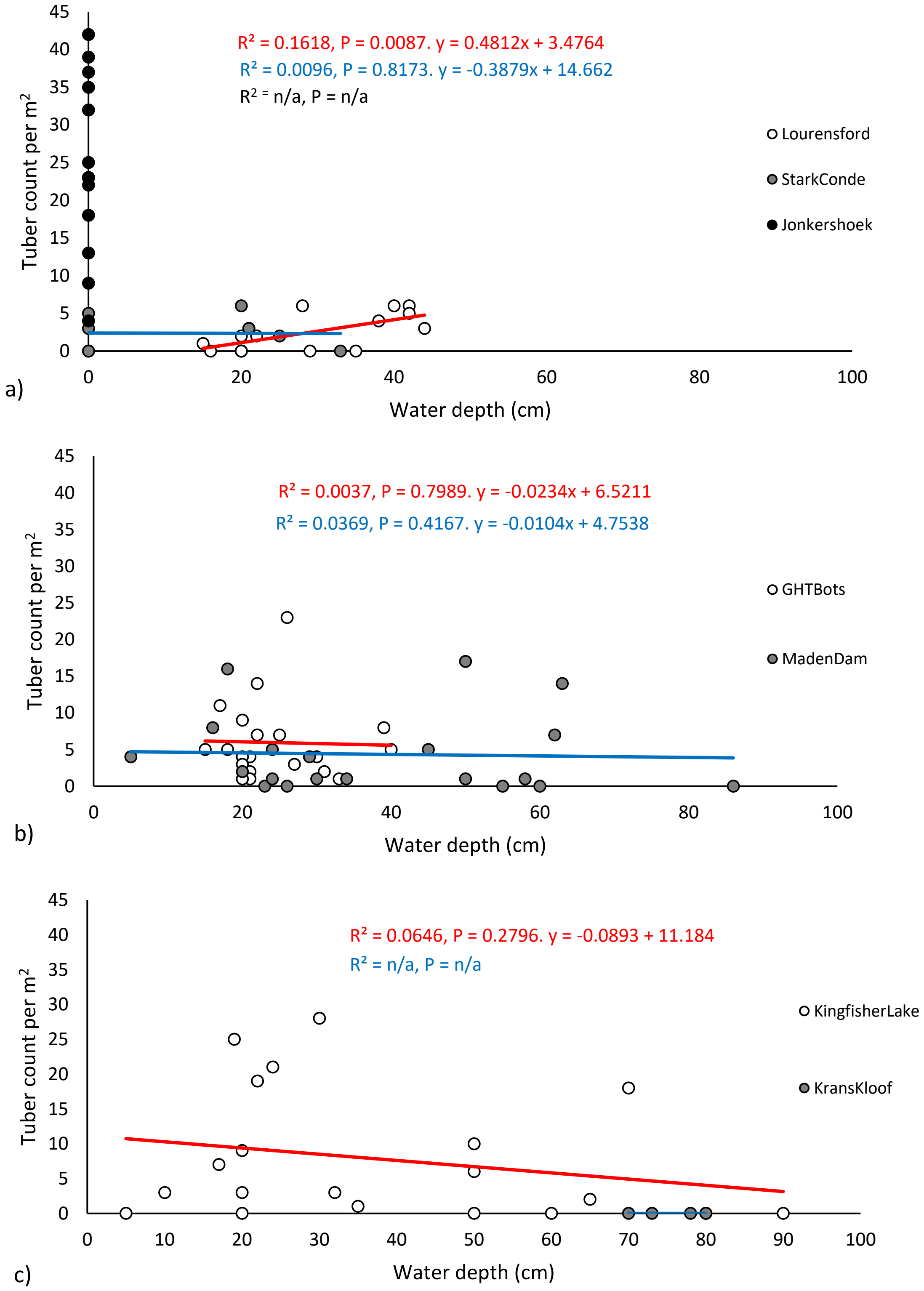

Figure 2.3.3. The relationship between water depth and number of *Sagittaria platyphylla* tubers sampled at sites within the a) Western Cape (n=3), b) Eastern Cape (n=2) and c) KwaZulu-Natal (n=2) provinces. The order of both the trend line equations and legends are matched to denote which lines represent which sites.

2.3.2. Monthly trends in *Sagittaria platyphylla* population parameters

Sagittaria platyphylla population parameters were sampled monthly, between September 2019 and October 2020, at Maden Dam and GHT Bots. The mean water depth (cm) fluctuated significantly over the sample period at Maden Dam ($F_{(9, 90)} = 4.1773$, $P < 0.0001$), between the two sites sampled ($F_{(1, 179)} = 75.2897$, $P < 0.0001$), but not over the months sampled at GHT Bots ($F_{(10, 89)} = 0.5013$, $P = 0.8849$) (Fig. 2.3.4). The *S. platyphylla* population grew within deeper waters at Maden Dam, whereas more shallow and uniform water depths were recorded at GHT Bots.

Plant density per m^2 changed over the sample period at both Maden Dam ($H_{(9)} = 28.738$, $P < 0.0001$) and GHT Bots ($F_{(10,89)} = 7.2225$, $P < 0.0001$), and between the two sites ($F_{(1,179)} = 42.2326$, $P < 0.0001$) (Fig. 2.3.4). Plant densities were at their peak in the warmer months of the year for both sites (between December and February) while the plant density at GHT Bots was higher. The mean number of emergent leaves per m^2 also differed significantly over the months sampled at both Maden Dam ($H_{(9)} = 74.049$, $P < 0.0001$) and GHT Bots ($F_{(10,89)} = 15.23$, $P < 0.0001$), as well as between the sites sampled ($F_{(1,179)} = 201.1215$, $P < 0.0001$) (Fig. 2.3.4). There were higher numbers of emergent leaves per m^2 at GHT Bots compared to Maden Dam. Moreover, the number of emergent leaves per m^2 peaked during the warmer months for both populations and while these number reduced over winter between June and August at Maden Dam, the same decrease was not evident at GHT Bots.

Flower production differed over the months sampled at both Maden Dam ($H_{(9)} = 50.868$, $P < 0.0001$) and GHT Bots ($H_{(10)} = 59.271$, $P < 0.0001$), as well as between the two sites sampled ($F_{(1,179)} = 42.623$, $P < 0.0001$) (Fig. 2.3.5). The number of flowers peaked for both populations around January, and while Maden Dam experienced the absence of flowers in September and again between June and August, flowers were always present at GHT Bots. The mean number of fruit was significantly different over the months sampled at both Maden Dam ($H_{(9)} = 63.034$, $P < 0.0001$) and GHT Bots ($H_{(10)} = 74.334$, $P < 0.0001$), and between the sites sampled ($F_{(1,179)} = 89.862$, $P < 0.0001$) (Fig. 2.3.5).The dynamics of fruit produced by both populations was similar to that of the flowers produced, however, the population at Maden Dam produced more fruit in December than the other months, whereas the population at GHTBots produced more fruit over a sustained period of time, with fruit numbers being higher inJanuary through to March.

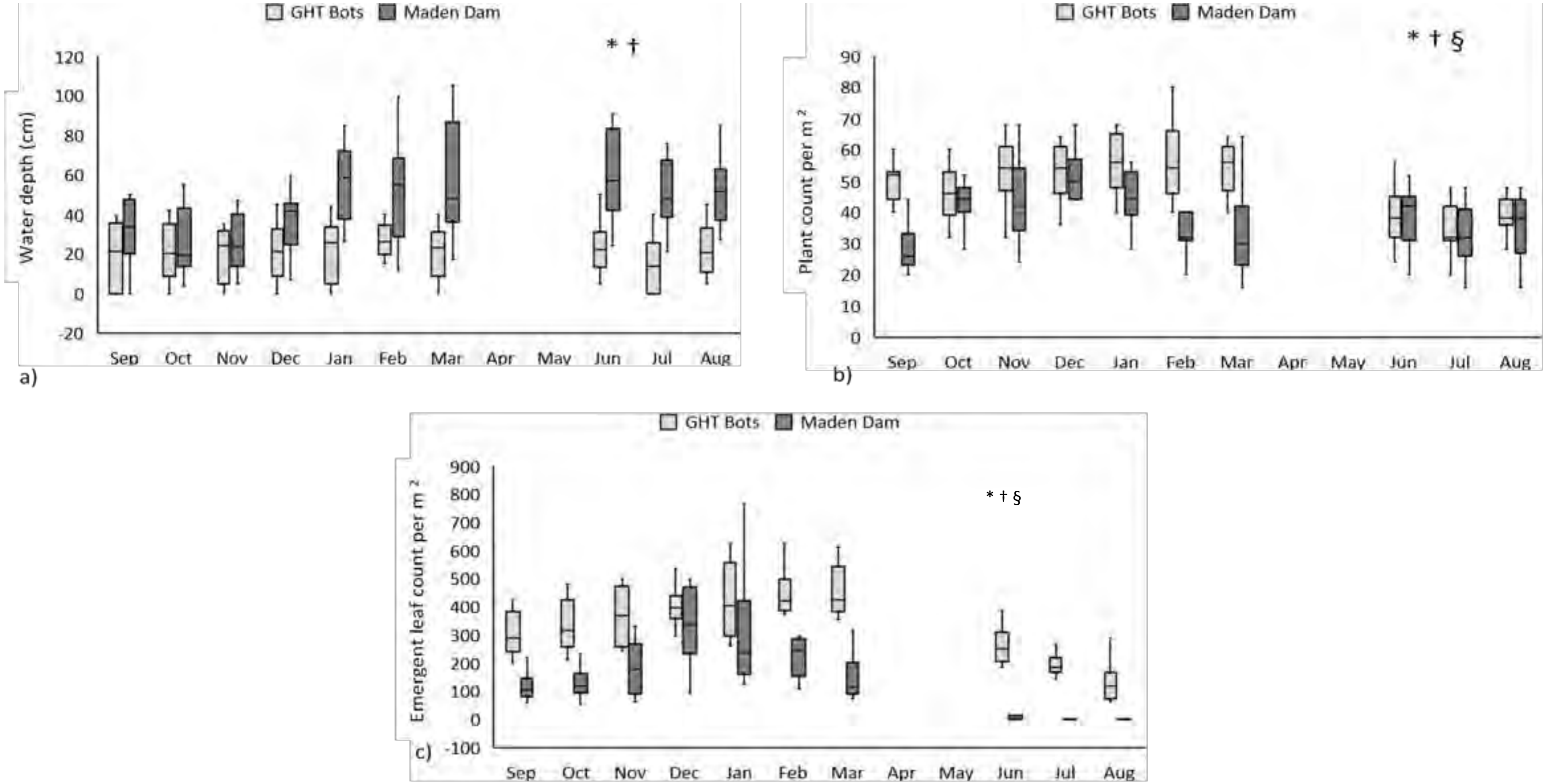

Figure 2.3.4. The median, lower and upper quartiles, lowest and highest observations of a) water depth at sampling points, as well as the mean (±SE) b) number of plants and c) emergent leaves per m2 of *Sagittaria platyphylla* sampled at both GHT Bots and Maden Dam. The symbol '*' denotes significant differences between the two populations, while the following denote differences between months sampled at GHT Bots '†' and Maden Dam '§'.

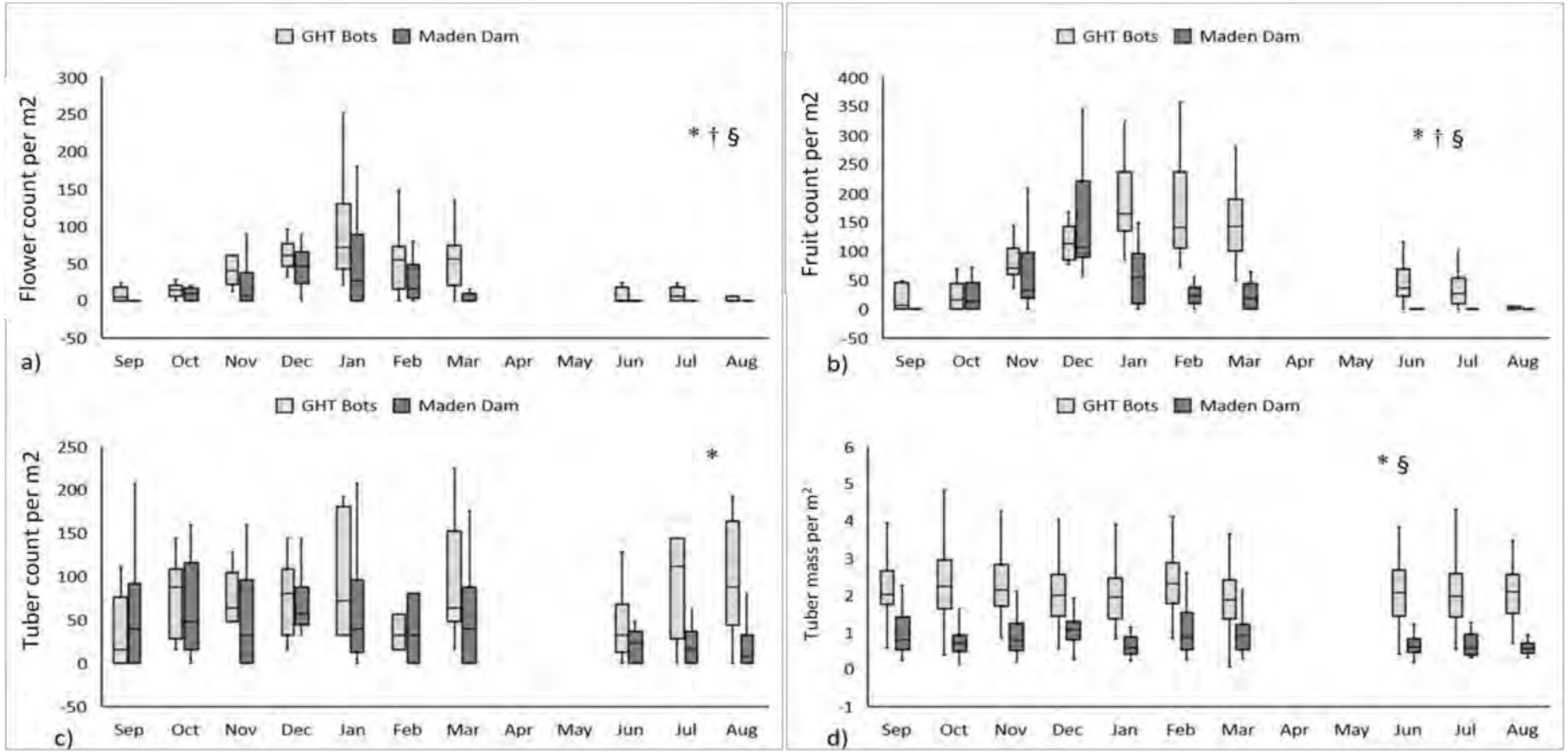

Figure 2.3.5. The median, lower and upper quartiles, lowest and highest observations of a) number of flowers, b) number of fruit, c) number of tubers and d) tuber mass per m2 of *Sagittaria platyphylla* sampled at GHT Bots and Maden Dam in the Eastern Cape Province. The symbol '*' denotes significant differences between the two populations, while the following denote differences between months sampled at GHT Bots '†' and Maden Dam '§'.

The mean number of tubers sampled differed significantly between the two sample sites ($F_{(1,179)} = 20.9542$, $P < 0.0001$), but not over the months sampled at both Maden Dam ($H_{(9)} = 13.086$, $P = 0.1588$) or at GHT Bots ($H_{(10)} = 16.234$, $P = 0.0931$) (Fig. 2.3.5). The population at GHT Bots invested more resources towards tuber production than the population at Maden Dam. However, both populations produced tubers year-round. The mean mass (g) of tubers was constant over the months sampled at GHT Bots ($H_{(9)} = 11.726$, $p = 0.2292$), but varied significantly at Maden Dam ($H_{(9)} = 37.221$, $P < 0.0001$), as well as between the two sites sampled ($F_{(1,\ 857)} = 585.7293$, $P < 0.0001$) (Fig. 2.3.5). Tubers produced at GHT Bots were consistently larger than those produced at Maden Dam. Additionally, their sizes remained very similar throughout the year, whereas tubers produced at Maden Dam were smaller between June and August and largest in February.

The mean number of leaves from plants at Maden Dam varied significantly over the sample period ($F_{(9,90)} = 2.0879$, $P = 0.0387$), but did not differ at GHT Bots ($H_{(10)} = 9.2295$, $P = 0.5105$). nor between the two sites sampled ($F_{(1,179)} = 0.3072$, $P = 0.5801$) (Fig 2.3.6). Plants at Maden Dam produced fewer leaves during the warmer months, in particular, significantly fewer leaves were produced in January, whereas significantly greater numbers of leaves were produced in September per plant. The mean plant height (cm) also changed over the months sampled at both Maden Dam ($F_{(9,90)} = 6.5037$, $P < 0.0001$) and GHT Bots ($F_{(10,89)} = 4.2498$, $P < 0.0000$), as well as between the two sites sampled ($F_{(1,179)} = 16.7862$, $P < 0.0001$) (Fig. 2.3.6). Plant height between the two populations followed similar patterns over the months sampled. However, plant height was lower at Maden Dam, particularly in September as well as July and August. The mean number of stolons differed significantly over the months sampled at GHT Bots ($H_{(10)} = 22.04$, $P = 0.0149$) but not at Maden Dam ($H_{(9)} = 12.153$, $P = 0.2048$), while significant differences were not found between the two sites sampled ($F_{(1,179)} = 9.2270$, $P = 0.0027$) (Fig. 2.3.6). *Sagittaria platyphylla* at GHT Bots produced more stolons within the warmer months sampled (from January through to March).

The relationship between water depth and tuber abundance was weakly negative and significant ($R^2 = 0.1079$, $P = 0.0028$) at GHT Bots while at Maden Dam, there was no relationship ($R^2 = 0.0003$, $P = 0.8639$) (Fig. 2.3.7). Something to note from this is that tubers were found in similar abundances in both deep and shallow waters at Maden Dam. There was also no relationship between water depth and tuber mass at both GHT Bots ($R^2 = 0.009$, $P = 0.0217$) and Maden Dam ($R^2 = 0.0244$, $P = 0.0045$) (Fig. 2.3.7). Mean monthly minimum

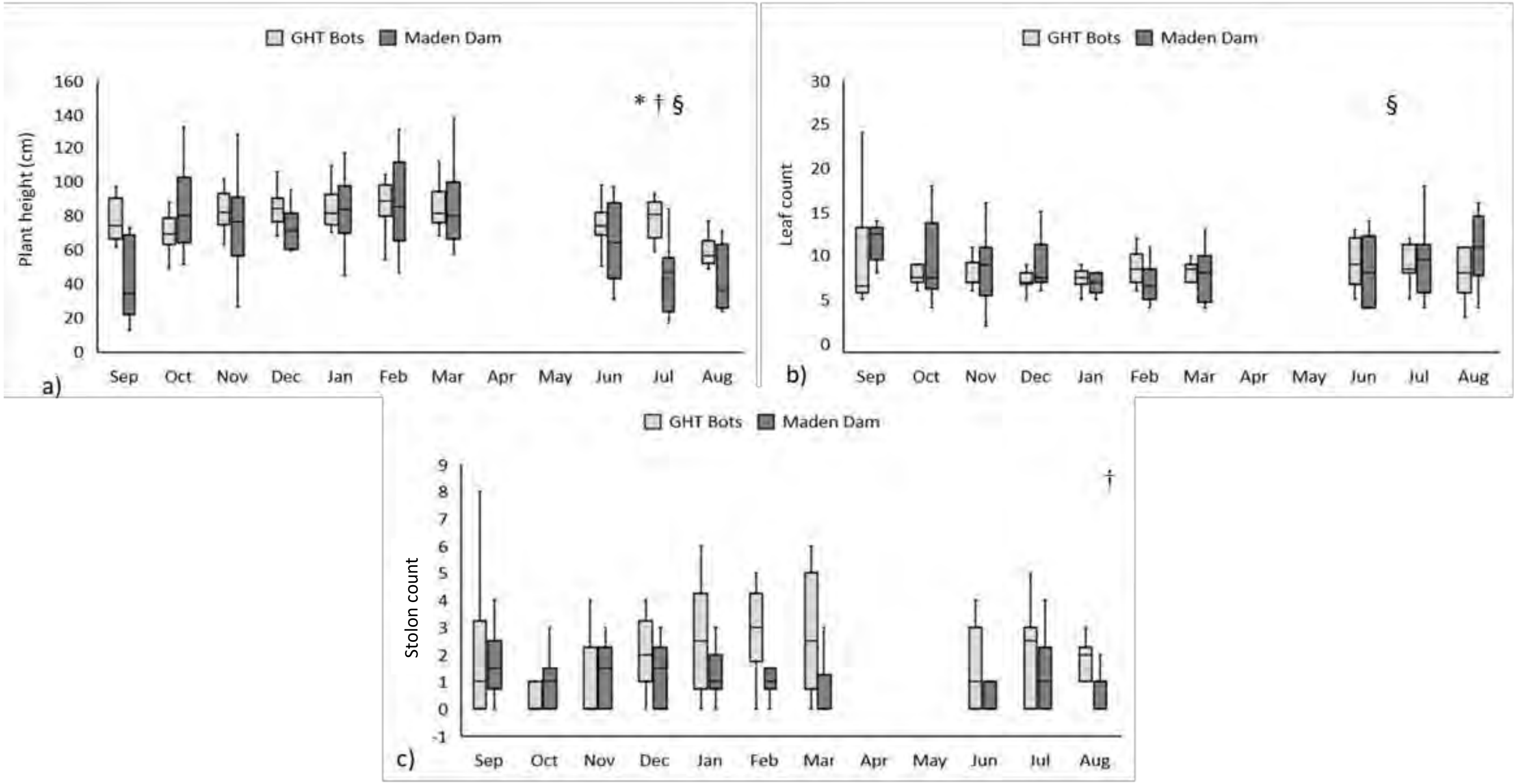

Figure 2.3.6. The median, lower and upper quartiles, lowest and highest observations of a) plant height, b) number of leaves and c) number of stolons per individual *Sagittaria platyphylla* plant sampled between Maden Dam and GHT Bots. The symbol '*' denotes significant differences between the two populations, while the following denote differences between months sampled at GHT Bots '†' and Maden Dam '§'.

temperature did not correlate with total number of tubers collected per month at GHT Bots ($R^2 = 0.0225$, $P = 0.679$), but at Maden Dam, there was a positive and significant relationship ($R^2 = 0.4748$, $P = 0.0275$) (Fig. 2.3.7). This result shows that temperature has a stronger influence over the number of tubers produced at Maden Dam, whereas GHT Bots plants are not as affected by temperature in terms of tubers produced. Tuber mass was also not correlated with mean monthly minimum temperature at either GHT Bots ($R^2 < 0.0001$, $P = 0.0019$), or Maden Dam ($R^2 = 0.0076$, $P = 0.1297$) (Fig. 2.3.7). While tuber size was not influenced by temperature, fruit production was at both GHT Bots ($R^2 = 0.8504$, $P = 0.0002$), and at Maden Dam ($R^2 = 0.6049$, $P = 0.0081$) (Fig. 2.3.8). Both populations produced more fruits as temperatures increased, with GHT Bots producing more than three times the number of fruits when both these populations' fruit production peaked. On the other hand, the relationship between mean monthly minimum temperature and fruit size was weak at GHT Bots ($R^2 = 0.1618$, $P < 0.0001$), while at Maden Dam, there was no relationship ($R^2 = 0.0435$, $P < 0.0001$) (Fig. 2.3.8).

To determine the influence of temperature on the general growth patterns of *S. platyphylla*, temperature was modelled against the dependent variables 'plant height' and 'number of emergent leaves per m^2' at both monthly surveyed sites, Maden Dam and GHT Bots (Fig. 2.3.9 and 10). Temperature had a significant effect on plant height ($F_{(1,197)} = 41.079$, $P < 0.0001$), as did site ($F_{(1,197)} = 24.323$, $P < 0.0001$), as well as a significant interaction between temperature and sites sampled ($F_{(1,196)} = 11.1$, $P < 0.001$). Similarly, the number of emergent leaves was significantly affected by temperature ($F_{(1,196)} = 146.13$, $P < 0.0001$), as did site sampled ($F_{(1,196)} = 234.74$, $P < 0.0001$), while no significant interaction was found between temperature and site sampled ($F_{(1,196)} = 0.1577$, $P = 6917$) on the number of emergentleaves per m^2. While both GHT Bots and Maden Dam soil types were very similar in terms of % clay, silt and sand, there were differences in nutrients (Table 2.3.1). P was 5 times, K 3 times and Fe 6 times, higher at GHT Bots than at Maden Dam, which could explain the differences in plant population parameters as a result of nutrient availability at GHT Bots.

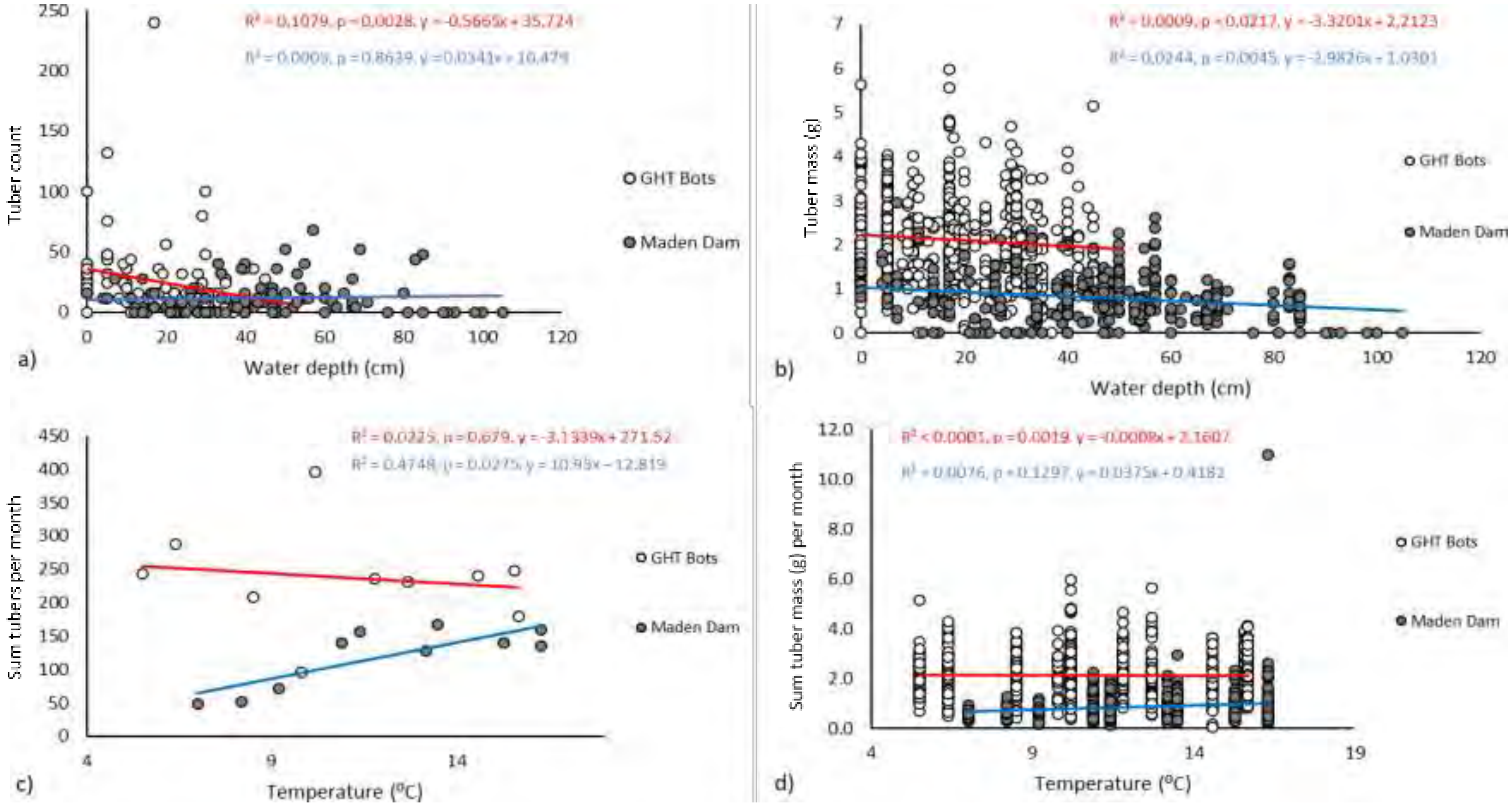

Figure 2.3.7. The relationships between water depth and a) tuber abundance and b) tuber mass as well as the relationships between monthly mean minimum temperatures and c) sum number of tubers sampled per month and d) tuber mass of *Sagittaria platyphylla* sampled at GHT Bots and Maden Dam. The order of both the trend line equations and legends are matched to denote which lines represent which sites.

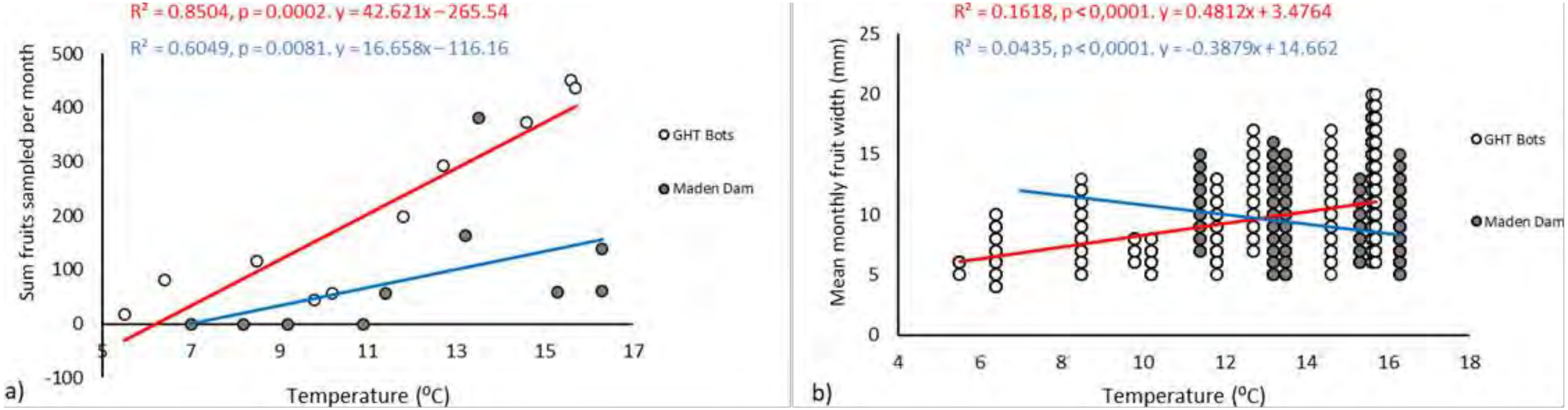

Figure 2.3.8. The relationship between the monthly mean minimum temperature and a) total number of fruits sampled per month and b) the monthly mean fruit size of *Sagittaria platyphylla* sampled at GHT Bots and Maden Dam. The order of both the trend line equations and legends are matched to denote which lines represent which sites.

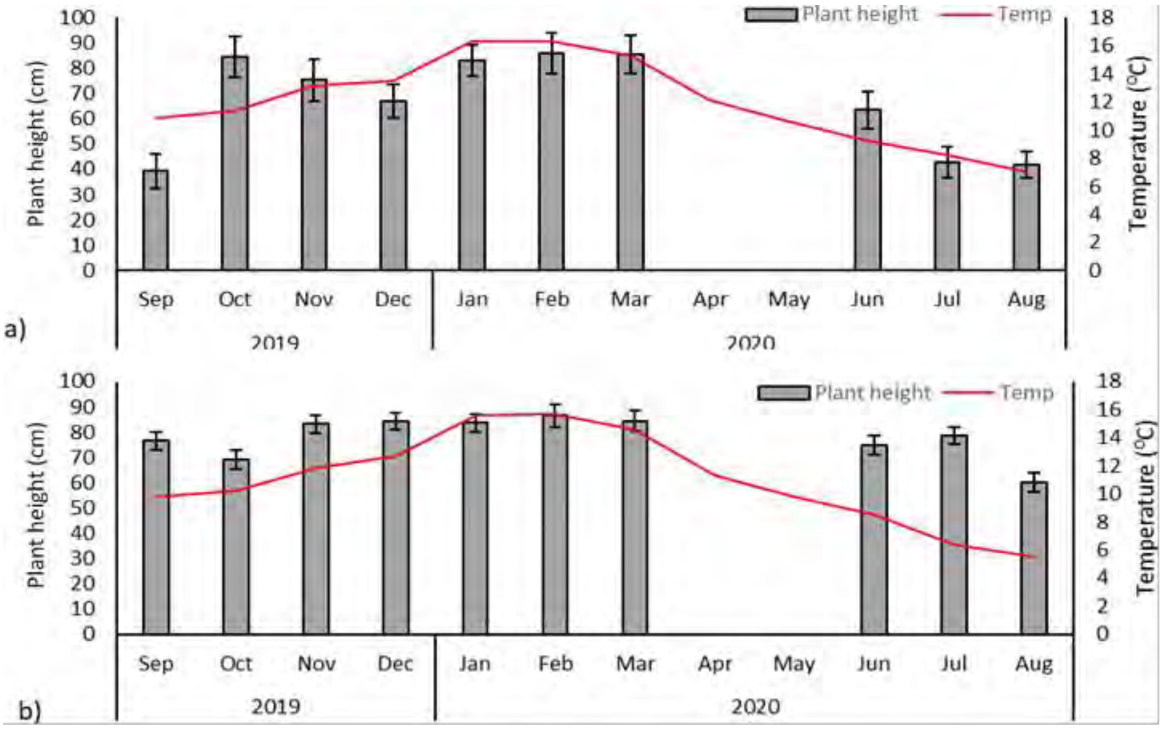

Figure 2.3.9. The monthly mean minimum temperature and the mean (±SE) height of *Sagittaria platyphylla* plants at a) Maden Dam and b) GHT Bots.

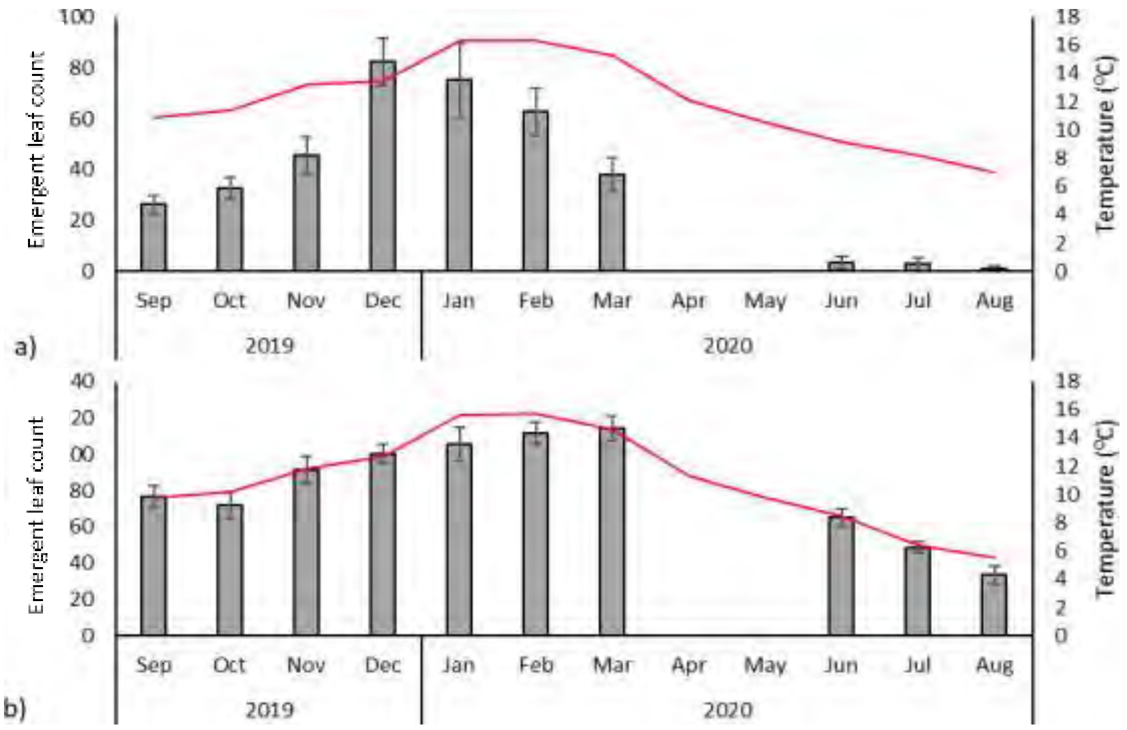

Figure 2.3.10. The monthly mean minimum temperature and the mean (±SE) number of emergent *Sagittaria platyphylla* leaves at a) Maden Dam and b) GHT Bots.

Table 2.3.1. Various sediment measurements recorded from the populations of *Sagittaria platyphylla* within the Makhanda Botanical Gardens and Maden Dam.

Site	Measured sediment characteristics							
	Soil type	Clay (%)	Silt (%)	Sand (%)	P (mg/kg)	K (mg/kg)	Fe (mg/kg)	C (%)
GHT Bots	Loam	11	18	71	16	150	3352	2.62
Maden Dam	Loam	9	14	77	3	45	548	1.23

2.4. Discussion

2.4.1. Nationwide survey

From the nationwide survey conducted, *S. platyphylla* populations were found to grow within a range of depths between the different populations sampled. This was expected as Kwong *et al.* (2017) showed how invasive populations of *S. platyphylla* grow in deeper waters compared to native populations in the USA. For example, *S. platyphylla* were found to grow within depths of up to 40 cm in the USA and more than 90 cm in Australia (Kwong et al., 2017). In this study, populations of *S. platyphylla* have been found in depths of more than one meter. The deepest waters *S. platyphylla* plants were sampled atwere more than a meter, at Krantzkloof. On the other hand, plants surveyed within Jonkershoek were foundwithin dry sediment, with no inundation. It is worth noting that this site looked like it recentlyhad water and was slowly drying out. A study by Sheldon & Boylen (1977) determined the maximum depths at which 28 species of rooted macrophytes grew, and found that each plant species had a preferred range of depth for growth. Moreover, Sheldon & Boylen (1977) emphasised the influence of light penetration, not just water depth, on limiting the depth at which plants were able to grow at. For instance, plants would be able to grow at greater depthsin oligotrophic systems as light is able to penetrate deeper than within eutrophic systems. Thismay explain why *S. platyphylla* was able to grow at greater depths at the Krantzkloof site, as this was theonly site which had flowing water and is located within a nature reserve, which could suggestthe water could be less turbid than some of the other sites sampled.

Most of the sites displayed similar densities per m^2 of *S. platyphylla*. The two major exceptions included the populations at Kingfisher Lake and Krantzkloof. The population at Kingfisher Lake had been mechanical cleared in the last 10 years and had received some herbicidaltreatment prior to sampling which reduced the plant density that this population wouldnormally display. However, the population at Krantzkloof was not subject to any form of control prior to sampling. The lower density could be the result of a number of variables, including flowing water, lower nutrient levels, and water depth at the site. As mentioned previously, the deepest site was Krantzkloof and Sheldon & Boylen (1977) found that for the 28 species they assessed, abundance was considerably lowerat the maximum depth at which each species was found. Thus, the population of *S. platyphylla* at Krantzkloof may be close to the maximum depth threshold for the species itself,or had to increase their height in order to emerge from the water body. Kwong *et al*. (2017) did record populations of *S. platyphylla* growing at depths just shy of one meter withinAustralia. Grace (1989) suggested that emergent aquatic plants will generally maintain a positive carbon balance and thus it would be expected for plants to alter their morphology to grow taller, produce more leaves and invest fewer resources to flowering, vegetative reproductive structures as well as to root tissues. This holds true when the vegetative growth of *S. platyphylla* populations at Jonkershoek and Krantzkloof (the most shallow and deepest sampled sites, respectively) are compared. The population located within the deepest waters at Krantzkloof displayed more than double the plant height and number of leaves per individual plant when compared against the population at Jonkershoek. Although these responses to increased water depth are expected, aquatic macrophytes do vary in other morphological aspects when water depths alter (Grace, 1989). For example, with increased water depth *Zizania aquatica* Linnaeus (Poaceae) did not alter investment in seeds (Thomas & Stewart, 1969), while *Bolboschoenus maritimus* (L.) Palla (Cyperaceae) allocated more to seed production rather than vegetative reproduction as water depth increased (Lieffers & Shay, 1981).

Tuber abundances varied greatly between sample sites. Krantzkloof, Lourensford and Stark-Condé produced significantly fewer tubers compared to the other four sites sampled. Of the sites sampled, the deepest waters *S. platyphylla* were found to grow in were at Krantzkloof. This could have influenced the reproductive allocation of this population as greater water depths have a negativeinfluence on the conditions, which will influence tuber growth. Moreover, not many samples were taken from this population as it was a very small population, and tubers could have beenmissed. Grace (1989) found that for two emergent aquatic plants, *Typhya latifolia* L. (Typhaceae) and *Typha domingensis* Pers. (Typhaceae), both reduced allocation to vegetative reproductive structures as water depths increased. This is a general trend with emergent aquatic plants, while allocation to sexual reproductive structures varies greatly between species withinincreased water depths (Grace, 1989). Similarly, the population of *S. platyphylla* at Stark- Condé was also small in size and limited samples were taken. However, the Stark-Condé population was regrowing after control efforts and had not yet established properly and may have been investing more resources into growth before allocating such resources towards reproductive outputs. Interestingly enough, the population at Lourensford was a well- established population and the above-ground plant structures were well developed and abundant. The main difference this population displayed was that these plants were growing within man-made ponds constructed with pond-lining, and the depth of sediment was very shallow. Such characteristics may have promoted a greater investment in dispersal via achenesrather than persistence through tubers as these unnatural conditions may reduce the ability of tubers to stay within the sediment for long periods of time. The other four populations assessed produced significantly more tubers. The population at Jonkershoek displayed the highest density of tubers per m^2. This population was located at an abandoned trout hatchery, and the area it was growing in was almost completely dry. This population invested more in resourcesthat would have allowed for re-growth if/when more favourable conditions returned, as tubershave the ability to survive unfavourable conditions (An, *et al*., 2018). GHT Bots, Maden Damand Kingfisher Lake displayed quite similar external pressures. All populations were present in sessile water bodies, had access to deep sediment and the *S. platyphylla* plants themselves were inundated. The Kingfisher Lake population produced fewer tubers than at GHT Bots and Maden Dam, and this could be put down to the herbicidal control used at Kingfisher Lake priorto our sampling efforts.

Tubers were found in each of the *S. platyphylla* sites sampled in this survey, except for Krantzkloof. The presence of tubers within the majority of *S. platyphylla* sites, in spite of the environmental and climatic variability experienced between these sites, shows the importance of these structures for populations of *S. platyphylla*. As they are prominent components of *S. platyphylla* populations, they have to become prominent targets of any successful management programme.

Water depth did not influence tuber abundance at any of the field sites, except at Lourensford, suggesting that populations of *S. platyphylla* produce similar numbers of tubers no matter the depth at which they grow. A study focussing on two emergent macrophytes found that *Z. aquatica* did not differ in their allocation to vegetative reproductive structures with increased depth, while *B. maritimus* invested more resources to seed production, and fewer to vegetative reproductive structures (Grace, 1989). Although water depth is often seen as an influential variable that may affect tuber production, it works together with both water clarity and thus light penetration to influence the ability of plants to produce tubers (Kautsky, 1990) . This study however suggests that this does not apply to *S. platyphylla*.

The plant parameters ‘number of leaves’ and ‘plant height’ were relatively similar between sample sites, except at the deepest site, Krantzkloof, where leaf production and height was significantly higher than the other populations, and those at the most shallow site, Jonkershoek, were lowest. These two populations displayed very contrasting environmental pressures. The Jonkershoek population was facing drought and seemingly needed to invest resources elsewhere, which is why less investment wasmade towards the growth of the plants themselves and rather the development of tubers. Meanwhile, the population at Krantzkloof had not yet established within this site following control, thus more resources were invested in growth than any form of reproductive output. As water depth increases, emergent macrophytes are expected to invest more resources towards maintaining a positive carbon balance, and producing more leaves and taller plants is a common response to increased water depth (Grace, 1989).

2.4.2. Continuous monitoring

Although there are various influential factors that control the distribution and abundance of aquatic plants, phenotypic and biomass plasticity is a feature various aquatic plants use in response to varied environmental pressures (Miller & Zedler, 2003; Thouvenot, *et al.*, 2013). Phenotypic plasticity and local adaptations improve the survival and dispersal of invasive plant species, especially if these plastic responses display a greater advantage to the species of the invaded community (Thouvenot *et al.*, 2013). For example, *Ludwigia grandiflora* (Michx.) Greuter & Burdet (Onagraceae) is able to grow within different water levels and light levels through its plasticity, providing this plant with a strong adaptive advantage (Thouvenot *et al.*, 2013; Harms, 2020). Along with plasticity, the flexibility of reproductive modes provided to many aquatic macrophytes can also help to adapt to heterogenous aquatic environments (Eckert *et al.*, 2016).

Sediments are the main source of nutrients for rooted aquatic macrophytes (Qian *et al.*, 2014) and therefore sediment type has a strong influence on the kind of macrophytes able to grow and develop within them (Bornette & Puijalon, 2011). It has been observed that for plants growing within systems exposed to different types of sediment, the level of wave-exposure along with the sediment type are the main forces that act to influence the growth form, growth stature, distribution, and biomass of aquatic macrophytes (Idestam-Almquist & Kautsky, 1995). Wave-exposure has a strong influence on sediment type, with regions of greater wave-exposure more likely to have sandy sediment, while more sheltered regions a more muddy sediment, which then in-turn influences the plant composition (Idestam-Almquist & Kautsky, 1995). From the continuous monitoring conducted here, sediment type seemingly does influence the growth of *S. platyphylla*. The sediment at GHT Bots was higher in P, K and Fe than the sediment at Maden Dam. A section of the Maden Dam population grows within muddy sediment with high organic content, very similar to that present within GHT Bots, and the individuals present here displayed a broad-leaf emergent growth form, with similar plant densities too. Where Maden Dam had more clay-like sediment, the *S. platyphylla* individuals grew as the narrow-leaf form, with greatly reduced plant densities. Similar growing trends were found with *Potamogeton pectinatus* (L.) Böerner (Potamogetonaceae), which grows better within muddy sediment compared with sandy sediment (Idestam-Almquist & Kautsky, 1995). Although sediment type influences plant growth, various other environmental pressures have to be taken into account as well.

Water depth is a very influential variable concerning the growth of aquatic plants, more so when light penetration and temperature are incorporated with water depth alterations (Sheldon & Boylen, 1977; Barko *et al*., 1986; Bornette & Puijalon, 2011). If light is not limited with depth, then water temperature is what it thought to be a limiting factor for plant growth. Although changes in water depth did not have any significant correlations with regards to growth parameters, *S. platyphylla* at Maden Dam grew as their submerged phyllodial growth form within the deeper regions of this water system. The seemingly limited influence of water depth on the growth and composition of *S. platyphylla*, particularly at Maden Dam, suggests that water turbidity is low and temperature gradients are not strongly defined.

Aquatic plants have predictable growth patters when such patterns are looked at with the changing of seasons. During the winter months, plants will look to survive such colder conditions by storing resources in overwintering stems or other storage structures, such as tubers (Madsen, 1991). Early spring is when plants typically display their highest resource levels, before which such resources are allocated to the growth of the plants and later on to the development of propagules (Madsen, 1991). Flower and seed formation coincides with the development of propagules, after which plants will then begin to prepare for overwintering (Madsen, 1991). For the above-ground plant growth characteristics assessed, significant differences were found between the sampled sites as well as over the months sampled within each sites, with greater proportions of above-ground structures being most prominent within summer. An exception to this was the number of leaves per plant did not vary significantly over the months sampled at GHT Bots. Various environmental factors influence the productivity of aquatic macrophytes (Henry-Silva *et al*., 2008). Such environmental variables include temperature, light, pH, plant density, nutrient availability, and sediment composition (Henry-Silva, *et al*. 2008). The two sites sampled displayed varied environmental pressures, which would have influenced the varied growth patterns of *S. platyphylla* (Hangelbroek *et al*., 2002). Maden Dam experienced colder temperatures, greater exposure (i.e. lack of shelter from surrounding vegetation), greater water depth as well as more clay-like sediment, compared to that of GHT Bots. The mean number of emergent leaves was reduced over the colder months at both sampled sites. However, for the majority of *S. platyphylla* at Maden Dam, no leaves were found to grow above the water level during winter and had died off due to the cold conditions. An exception to this was found within the sheltered cove at Maden Dam, where *S. platyphylla* were still able to produce leaves, just as the population at GHT Bots was able to. Such areas could help biocontrol agents sustain their populations during more harsh conditions. Moreover, if releases of adult weevils are conducted, they should be done so after frosting events, when populations regrow. Concerns for overwintering of agents are had, especially within sites that experience frosting as does Maden Dam, however, agents could be released inundatively as ‘green-herbicides’ as populations grow back. At Maden Dam, the number of leaves per individual plant peaked at the beginning of spring and dropped at the end of summer, which is uncharacteristic for aquatic macrophytes, which usually experience their greatest growth toward the end of summer, like *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae) (Engloner, 2009). It can be seen that *S. platyphylla* grows best when conditions are not at their coldest or hottest within Maden Dam. This is something that could be attributed to the exposed nature of this population, as GHT Bots plants do not experience such draw-backs in growth during their coldest and warmest months.

In trying to determine the influential factors that related to the growth parameters of *S. platyphylla*, temperature was modelled against plant growth, as temperature is an important variable that influences the growth of aquatic macrophytes (Henry-Silva *et al*., 2008). Temperature was taken into consideration for a number of studies which looked at various growth aspects of *P. australis*. As expected, patterns varied depending on what structures were assessed. Some studies suggested that temperature did not have a strong influence on biomass produced by *P. australis*, while others suggested that inflorescence height was strongly linked with temperature and shoot growth (McKee & Richards, 1996; Zemlin *et al*., 2000). From this study, the influence of temperature on leaf production at GHT Bots was greater than at Maden Dam. The leaves that died off over winter at Maden Dam would have influenced the strength of this relationship. Moreover, when mean plant height was considered, the population at Maden Dam displayed a stronger relationship with temperature than GHT Bots did. This speaks to the stability of the population at GHT Bots, as the plant height observed here did not vary greatly over the months sampled. Seasonality will influence how plants will react to different environmental pressures (Thouvenot *et al*., 2013).

Tubers were always present within both sampled sites; however, tuber mass did vary over the months sampled at Maden Dam and between the sites, and tuber abundances differed between the sites sampled. Tubers are used as carbon storage structures, and their use can change with seasonality. For example, plants have been shown to store carbon within tubers over the winter months and when needed, carbon reserves are used for respiration, seasonal growth and flower and fruit production during the warmer months (Klimesova & Klimes, 2008). Although tubers at Maden Dam may have been used to store carbon over the winter months, their increase in mass over the warmer months shows that the plants could be limited to growth and development within the warmer months only. At GHT Bots, this trend is not observed, as both the abundances and mass of tubers did not vary greatly with seasonality. Investment in tubers is more successful in habitats which display greater stability (Liu *et al.*, 2009). Although investment in tubers can be influenced by sediment type, nutrients, and water quality (Sutton & Portier, 1985), environmental stability could be considered to be the most influential factor in the differences in tuber production between the two sampled sites.

Tuber abundance and tuber mass were not correlated with water depth at both GHT Bots and Maden Dam. Water depth is an environmental variable that affects the regenerative strategies of aquatic plants as changes in water depth bring about changes in light conditions, as well as oxygen and nutrient content (An *et al.*, 2018). Although water depth can be an influential variable for the production of tubers and growth of plants, water quantity and quality is considered to be more important. Bowes *et al.* (1979) assessed the seasonal growth and develop parameters of *H. verticillata* within three different lakes. They found that shorter days promoted the production of tubers, while longer days and lower CO_2 levels within the sediment promoted tuber germination. However, an exception was found at one of the sites. This site displayed a well-established population of *H. verticillata* and, although tubers were found present within this population, fluctuations in tuber densities were less variable here than the other two sites. This was put down to the limited light available as well as the higher CO_2 levels within the sediment. The population of *S. platyphylla* within GHT Bots is comparable to this, as their population density is high and competition for resources, such as light, is strong. Thus, there is less need for these individuals to over-invest in tubers as the tuber bank is stable and the already present tubers probably cannot germinate unless resources are provided with which to do so. A further consideration is the greater mean water depths within Maden Dam and the shallower conditions within GHT Bots. Light limitation would not be as influential at GHT Bots, whereas the opposite would be found within Maden Dam.

The influence of minimum monthly temperatures on tuber characteristics were not significant, except for at Maden Dam, where an increase in minimum monthly temperature increased tuber abundance. Therefore, at Maden Dam the *S. platyphylla* population invested more resources toward the production of tubers within the warmer periods than it did during the colder periods. Again, this points to the stability and stronger establishment of the population at GHT Bots. The population at Maden Dam is under stress from the more exposed cold conditions faced, which could open up resources previously taken up for tubers to germinate. This could be why lower tuber number are found within this site over the winter months and these numbers increase over the warmer months. (Bowes *et al*., 1979).

The investment in fruit was the same for both populations of *S. platyphylla* assessed here. As temperatures increased, fruit abundance as well as size increased. An exception for this was found at Maden Dam, where fruit size decreased with increasing temperatures. Due to the exposed nature of the population at Maden Dam the plants here may have struggled to produce larger fruits at higher temperatures due to the warmer conditions they were exposed to. More exposed conditions could have contributed to fruits breaking up into achenes sooner as direct sunlight may have been more harsh on the fruiting bodies themselves while stronger winds could have also contributed to this. *Sagittaria platyphylla* producing greater number and larger sized fruiting bodies during the warmer months is expected. Sexual reproduction in aquatic plants is often tied in with variable environmental factors, such as light availability, water availability and temperature, which act as cues informing the plants when the best period to invest in sexual reproduction would be (Calero *et al*., 2017).

Pre-release studies that assess the population dynamics of the target species can help to pin-point when releases should be made as well as what life stage of the biocontrol agent should be released. Such data will also be useful for post-release evaluations to be compared against with greater accuracy, which can allow for the manipulation of management programmes to attain greater levels of success while also showing tangible differences to keep the support of funders.

2.4.3. Soil growth experiment

No significant differences were found between plant height or tuber growth of *S. platyphylla* grown in different sediments after four weeks, The opposite was found of *Stuckenia pectinata* L. (Potamogetonaceae) *(syn. Potamogeton pectinatus),* commonly called sago pondweed, which was shown to grow better in muddy sediment, compared to sandy sediment (Hangelbroek et al., 2002).Something overlooked in this experiment was the initial nutrient concentrations present withinthe sediments themselves, which may have skewed the results of this experiment. This experiment should be redone to allow for a longer growing period as well as assessing moregrowth and development structures upon completion of the study.

2.5. Conclusion

It was already well known from Kwong *et al*. (2018) that sexual reproduction was very important to this species and driving its invasiveness outside of its native range. However, the contribution of tubers was not known. This study has shown that tubers are abundant and found at most *S. platyphylla* sites across the invaded range of South Africa. Sampling at two sites in the Eastern Cape Province also showed that while tubers were present in the soil throughout the year, size, mass and abundance vary between sites. These results suggest the tubers are part of the *S. platyphylla* ecology potentially contributing to its resilience and therefore tubersshould be considered when management is being planned for or implemented.

Chapter 3: The life history of *Listronotus frontalis*

3.1. Introduction

3.1.1. The value of life history information

Life history studies of candidate biocontrol agents are a fundamental component of most biocontrol programmes (McFadyen, 1998; Stiling & Cornelissen, 2005). Understanding the life history data of a candidate biocontrol agent allows researchers to develop appropriate host specificity and impact trials. For example, in South Africa *L. sordidus* was considered to be a potential biocontrol agent of *S. platyphylla,* however, the life history data collected on the candidate agent showed that *L. sordidus* was not able to develop on inundated *S. platyphylla* plants. *Sagittaria platyphylla* primarily grow in inundated systems within South Africa, which ultimately ended in *L. sordidus* being rejected for release (Martin *et al*., 2018). Additionally, knowing the life history of candidate control agents on their host plant allows researchers to accurately interpret the results of host specificity and impact trials. It is standard practice to determine the developmental time of an agent on its host plant and compare it to secondary hosts, while insect size and mass may also be used as a measurement of host suitability (McFadyen, 1998).

The life history of a candidate biological control agent can provide biocontrol practitioners with information to better predict their establishment and control efficacy (Davis *et al*., 2006). Life history traits are thus often seen as good indicators of the success a biocontrol agent may have (Southwood & Henderson, 2000). Moreover, having a good understanding of the fecundity, longevity and developmental time can provide critical information required for the development of successful mass-rearing techniques when the release of agents are being prepared (Duan *et al*., 2014).

3.1.2. The genus *Listronotus*

Listronotus weevils fall within the order Coleoptera and the family Curculionidae. Species within this genus are within the subfamily Cyclominae and the tribe Listroderini (Aidar et al., 2012). The genus *Listronotus* was first described by Henderson in 1938 and further revised by O'Brien in 1981. This genus comprises 62 described species, all of which are found within North America (O'Brien, 1981).

Most species of *Listronotus* are not only nocturnal, but are also associated with either aquatic or semi-aquatic plants (O'Brien, 1981). Exceptions include *L. appendiculatus* Boheman (Coleoptera: Curculionidae) and *L. oregonensis* LeConte (Coleoptera: Curculionidae). It is unclear as to whether *L. appendiculatus* is nocturnal or diurnal, while *L. oregonensis* has been shown to be associated exclusively with terrestrial plants (O'Brien, 1981). Many *Listronotus* species, including *L. appendiculatus*, *L. sordidus*, *L. novellus* Blatchley (Coleoptera: Curculionidae), and *L. frontalis* have all been shown to have unique swimming abilities (O'Brien, 1981). These weevils propel themselves forwards with their forelegs while it is thought that their hindlegs are responsible for controlling the direction in which they swim (O'Brien, 1981).

In general, not much is known about species within the *Listronotus* genus. Information on these weevils is only collected if they pose an economic threat or if they can be utilised in biocontrol programmes. For example, the carrot weevil, *L. oregonensis* is a major pest of carrots, parsley, and celery in north-eastern USA (Martel et al., 1976; Justus & Long, 2019), and is an economic threat to the USA as the value of the above mentioned crops is estimated at around US$ 1.3 billion per annum. The bluegrass weevil, *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae) is a pest of annual bluegrass, *Poa annua* Linnaeus (Poaceae). This weevil poses a large threat to golf course owners, especially within the north-eastern region of the USA (Diaz & Peck, 2007). *Listronotus maculicollis* is extremely destructive and has been shown that, as larvae, can feed on up to 20 grass stems of *P. annua* throughout an individual's development (Diaz & Peck, 2007). As mentioned previously, *L. appendiculatus* is a potential biocontrol agent for the invasive aquatic plant *S. platyphylla* (Kwong et al., 2014).

3.1.3. Associations between *Sagittaria platyphylla* and *Listronotus* weevils

There are 26 known species of *Listronotus* associated with various *Sagittaria* spp. within the USA (Kwong *et al*., 2014). Despite this strong association between the two genera, not much biological information has been developed for the weevils (Aidar *et al*. 2012). From what is known about this genus, *Listronotus* larvae are typically endophytic, and feed within the petioles of several aquatic plants (Morrone, 2013), while the adults have been generally shown to feed externally on various parts of their host plant(s), including leaves and flowers (Adair *et al.*, 2012).

As mentioned in Chapter 1, in a series of surveys conducted between 2010 and 2012 to find candidate biocontrol agents for the invasive *S. platyphylla*, from various insects collected, four promising weevils were selected for further examination, namely, *L. sordidus*, *L. lutulentus* Soheman (Coleoptera: Curculionidae), *L. appendiculatus* and *L. frontalis* (Adair *et al*., 2012). *Listronotus sordidus* larvae were shown to extensively damage the crown of invasive *S. platyphylla* plants in South Africa, but could not complete their life cycle on inundated *S. platyphylla* plants, a common growth characteristic of the populations within South Africa (Martin *et al*., 2018). *Listronotus lutulentus* is a weevil that feeds primarily on theleaves of *S. platyphylla* (Adair *et al*., 2012), however, after initial investigation, they were declared not damaging enough to warrant their release, and the culture was terminated (Ndlovu, 2020). *Listronotus frontalis* is a tuber-feeding weevil within its larval stages and as an adult, itfeeds on the leaves and flowers of *S. platyphylla* (pers. obs.). *Listronotus appendiculatus* is a fruit and flower feeding weevil within both its larval and adult life stages (Adair *et al*., 2012). This weevil was also found to be the most abundant of the weevils sampled on *S. platyphylla* during the surveys conducted to find potential biocontrol agents (Kwong *et al*., 2019).

The biology of *L. appendiculatus* is very well understood. Muenchow (1998) and Muenchow & Delesalle (1992) contributed a lot of work towards better understanding *L. appendiculatus*, where its biology was determined on one of its hosts, *Sagittaria latifolia* Willdenow (Alismataceae), while more recently, Rogers *et al*. (2018, unpublished) conducted life history work on *L. appendiculatus* on another of its hosts, *S. platyphylla*. Moreover, Kwong *et al*. (2018) also investigated *L. appendiculatus* on *S. platyphylla*, determining various aspects of its relationship with this aquatic macrophyte, specifically the damage it inflicts to the fruiting bodies. *Listronotus appendiculatus* is thought to have strong potential as a biocontrol agent for a few reasons: firstly, it was found in various different climatic regions within the USA; secondly, it is able to attain very high densities on *S. platyphylla* populations; and thirdly, it appears to have a limited host range (Kwong *et al*., 2018). Kwong *et al*. (2018) concluded that the weevil *L. appendiculatus* could work well as a biocontrol agent to reduce the dispersal ability of *S. platyphylla* through the damage it inflicts on their fruiting-bodies. However, it has been suggested that this weevil will not be enough on its own to control *S. platyphylla* infestations in the long-term, but rather should be seen as a biocontrol agent that can work well with a suite of other control agents that attack other life history stages of this invasive weed (Kwong *et al*., 2018).

Listronotus frontalis is a weevil that is associated with the crown of *S. platyphylla* plants (Kwong *et al*., 2019). It is thought to complete its life cycle through the consumption of the plants' subterranean tubers within their larval stages, while pupation is thought to take place underground as well (Kwong *et al*., 2014). The biology of *L. frontalis* has not been documented; however it is currently being considered as a candidate agent in both South Africa and Australia for the control of *S. platyphylla* due to the damage it inflicts to the crown, roots, and tubers of the plant (Kwong *et al*., 2014).

Therefore, the aim of this chapter is to determine the life history of *L. frontalis*. In particular I aimed to determine the weevils' developmental rate on *S. platyphylla*, female fecundity, as well as adult longevity. Additionally, I aimed to determine the impact of adult *L. frontalis* feeding on the overall plant health as well as the impact on tubers.

3.2. Materials and methods

3.2.1. Insect culture

The *L. frontalis* culture was collected from a population of *S. platyphylla* growing in a small artificial dam in Tara Wildlife Reserve (32.490289; -91.060123), near Vicksburg, Mississippi, USA in 2014. The consignment of insects was imported (import number: P0066302) to the Centre for Biological Control (CBC), Rhodes University (RU) quarantine facility in Grahamstown, Eastern Cape Province, South Africa, where a culture was established. This quarantine facility was kept at a constant 26ºC, 70% humidity and maintaineda 16: 8 day: night cycle. All of the plant material required for the maintenance of the culture was collected on a weekly basis from a population of *S. platyphylla* growing in the Makana Botanical Gardens (-33.317737; 26.522536), Eastern Cape Province, with the material being used the day of collection. All of the investigations that involved the use of *L. frontalis* took place within the CBC, RU quarantine facility.

The culture of *L. frontalis* was handled differently depending on the life stage. As adults, the insects were kept in 30 x 20 x 10 cm white plastic containers with perforated lids for ventilation, or 'adult boxes' (Fig. 3.2.1). No more than 20 adults, of equal sex ratios, were kept in a single adult box to prevent the spread of *Beauveria* sp. Vuill. (1912) (Cordycipitaceae), which occasionally developed in the cultures. Adult boxes were lined with moistened paper towelling to reduce desiccation. The bottom portion of a single *S. platyphylla* leaf with roughly five cm of petiole still attached and a portion of a raceme that contained a couple of fruiting bodies were wrapped in moist paper towelling and placed into the container. Having the petiole of the leaf wrapped with paper towelling encouraged *L. frontalis* to oviposit and also provided them with an area to seek shelter in. A few more pieces of cut leaves were added, along with some male *S. platyphylla* flowers. Pollen from these flowers is thought to provide female weevils with additional protein with which helped them produce eggs (G. Martin, pers. comm.). To this, a small handful of moistened Nutrigro coco peat was added to the base of the wrapped petiole, which also helped reduce desiccation. Water was then sprayed into the prepared adult boxes and then left for a week, after which the whole process was repeated. As the adult boxes were changed, the vegetative material (in particular the petiole portion of the leaf and the raceme) and the paper towelling (on both the base of the container and wrapped around the leaves' petiole) were examined for *L. frontalis* eggs. Eggs were collected with the use of a fine-bristled paint brush which helped to easily manipulate them without incurring any damage.

A different setup was used to allow *L. frontalis* to develop from egg to pupa. The same containers were used as for the adult boxes. To these containers, called 'larval boxes', a thin layer of moistened coco peat was added, to which *S. platyphylla* tubers were placed and then covered with another thin layer of moistened coco peat. The layers of peat helped to reduce desiccation of both the tubers as well as the eggs. Eggs collected from the adult containers were then lightly sprinkled onto the peat. The larval boxes were sealed with perforated lids and placed into a larger dark plastic container which was covered with 70% shade cloth. This helped mimic the dark conditions of being underground, where the egg-to-pupal stages of *L. frontalis* develop in natural conditions. These larval boxes were checked on a weekly basis so that any emerged adults could be transferred to the adult boxes.

3.2.2. Life history determination

To determine the life history of *L. frontalis*, a single pair of adult weevils were kept in 15 x 5 x 3 cm plastic containers with a perforated lid. These weevils were provided with moistened paper towelling that lined the container, peat, a leaf with its petiole wrapped in moistened paper towelling, a raceme with fruiting bodies attached was added within the wrapped portion of the leaf petiole, additional pieces of cut leaves as well as male *S. platyphylla*flowers. Eggs were collected on a daily basis so that the age of the developing larvae could easily be estimated to within less than a day. Collected eggs were then placed into petri dishes lined with filter paper. This filter paper was sprayed with water to prevent desiccation and thepetri dishes were then sealed with parafilm to maintain the moisture levels and to prevent neonate larvae from escaping. Once the eggs had hatched, the number of days they took to hatch was recorded as well as the head capsule width of the larvae themselves.

In order to determine the number of larval instars of *L. frontalis*, neonate larvae were transferred to 50 ml vials which were lined with moistened paper towelling containing a 10 mm thick slice of a *S. platyphylla* tuber. The tubers were cut into thin discs to allow for *L. frontalis* larvae to be easily removed without harming them. The lids of these vials had perforated holes to allow for some ventilation. Dyar's law suggests that head capsule width is a good indicator of the different stages some groups of insects go through as larvae, as the head capsule sizes develop at a standard and predictable rate (Panzavolta, 2007). Therefore, to determine the number of larval instars, larvae were checked every second day and their head capsule widths were recorded. Prior to head capsule width measurements, larvae were briefly placed into a freezer to reduce their mobility which allowed for head capsule width to be

Figure 3.2.1. A typical adult box that allowed adult *Listronotus frontalis* access to *Sagittaria platyphylla* leaves for feeding and petioles to oviposit into.

measured with greater ease and accuracy. Head capsule widths were recorded using a Bestscope® BS-3040 Zoom Stereo Microscope.

Once pupation had occurred, pupa were kept in 50 ml vials with perforated lids between two pieces of moistened paper towelling. This was done to mimic the increased moisture levels of their natural development within sediment. Once pupae had eclosed, pairs of adult weevils were placed in 15 x 5 x 3 cm containers which were provided with the same materials provided to the cultured insects within the adult boxes. Adult weevils were then monitored until they died to determine their longevity. These containers were also checked on every second day until an egg was found to determine their pre-oviposition period.

3.2.3. Effect of sediment type on *Listronotus frontalis* larval development

Personal observations suggested that certain types of sediment may prohibit *L. frontalis* neonates from reaching subterranean tubers and developing through to adults. Therefore, a study was conducted to determine the effect of sediment type on neonate larval survival. In order to test this, three different types of sediment, with different degrees of coarseness, were used as growing medium. These were: a more coarse commercial building sand, called 'builder's sand', a more fine sediment called 'pond sediment' (collected from Jameson Dam, Eastern Cape, South Africa, -33.190072, 26.260240) and a combination of the two, called 'mixed sediment'. The builder's sand had high fine sand percentages and a low soil organic Carbon of 0.21 %. This sand also had low pH values of around 6 (Martin 2013). The pond sediment was loam and also had a low soil organic Carbon of 0.08 % and a low pH of 3.9 (Strange 2018). Plants were grown from tubers collected from the population of *S. platyphylla* within the Makana Botanical Gardens, Eastern Cape Province. Tubers were weighed and those weighing between two and three grams were selected and grown. Tubers were grown within three different sediments within 25 x 20 cm pots that were placed within 20L buckets (Fig. 3.2.2). 15g of the slow-release Osmocote fertilizer was used per pot, and mixed within the top layer of the sediment. Osmocote has a NPK ratio of 19:6:12, respectively, and releases nutrients for up to four months. Enough water was added to the buckets to inundate the sediment. Once plants had fully developed, three pots per sediment type were moved into the CBC, RU quarantine facility at a time. A single pot was considered a replicate and thus this experiment was replicated three times. Plants were then trimmed at the crown and 10 *L. frontalis* eggs were added to the sediment of each pot. These pots were then left for four weeks to allow *L.frontalis* to develop. At this point, the sediment was removed from the pots and any developmental stages of *L. frontalis* were searched for. This experiment was then replicated once more.

Figure 3.2.2. Growing setup of *Sagittaria platyphylla* used for experimentation. This picture displays plants being grown in pond sediment for the experiment

concerning the development of *Listronotus frontalis* on different types of sediment.

3.2.4. Impact study

In order to determine the impact that adult *L. frontalis* feeding had on *S. platyphylla* individuals, 50 *S. platyphylla* plants were grown under the same methods and conditions as the plants grown for the *L. frontalis* development within different sediments experiment (Fig. 3.2.2). The only difference was that only builder's sand was used as a growing medium. Plants were grown until mature, which took around three months as they were grown within colder winter months. Once plants had developed fully, 30 were selected for the experiment based on similar plant sizes and parameters. Per pot, the largest and healthiest individual was kept while the other plants which had developed within the same pot were trimmed off at the crown. This was done so that any weevils placed onto plants only had a single plant as a source of food and any impact received was restricted to that individual, making later deductions more comparable. 10 of the 30 pots were then selected, and the individual plant not removed through cutting off of the above-ground portions at the crown as mentioned previously, was destructively sampled. These 10 plants were the pre-experiment controls. For each of the pre-experiment control plants, the following parameters were measured, the: number of leaves, number of flowers, number of fruit, number of stolons, number of tubers, number of daughter plants, maximum plant height, maximum root length, mass of below-ground plant material, mass of above-ground plant material as well as the mass of the tubers (Fig. 3.2.3). The remaining 20 plants were then moved into the CBC, RU quarantine facility, where 10 were used as controls, while the remaining 10 were exposed to *L. frontalis* adults. These pots were kept within the 20L buckets and the water levels were maintained at the sediment level for both treatments. This was done to allow the weevils the ability to move to other sections of the plant with greater ease.

Furthermore, to mitigate any disturbances or misplacement of the adult weevils, the 10 plants exposed to *L. frontalis* were placed within insect cages (1.2 x 0.6 x 0.6 m). For the plants exposed to *L. frontalis* feeding, a single pair of *L. frontalis* weevils were added to each of the 10 plants. The experiment was allowed to run for a period of eight weeks, at which time the insects were removed, and the above-mentioned plant parameters were assessed within both treatments. During the course of the experiment, the *L. frontalis* exposed plants were checked every few days to replace any adults that had died, while the water levels were topped up as required for plants within both treatments. After eight weeks, the plants were destructively sampled, and the same parameters as the pre-experiment controls were measured.

Figure 3.2.3. The various *Sagittaria platyphylla* plant parameters assessed during the impact study. The a) leaves, b.i) male flowers, b.ii) female flowers, c) fruiting-bodies, d) stolons, e) tubers, and f) daughter plants.

3.2.5. Statistical analyses

To determine the relationship between larval instar and head capsule width, a Spearman's rank correlation was run in R version 3.6.3 and RStudio version 1.3.1056 after the data were found to be not normally distributed by running a Shapiro-Wilk normality test. No statistical analyses were required for the experiment where *L. frontalis* development was tested within different types of sediment. The impact study data were analysed in R version 3.6.3 and RStudio version 1.3.1056 and after testing these data for normality with the Shapiro-Wilk normality test and equal variance with Bartlett's test, data were either analysed using a One-Way ANOVA or a Welch's ANOVA. Moreover, some of the data were log transformed, this is all indicated in Table 3.3.4.

3.3. Results

3.3.1. The biology of *Listronotus frontalis*

Female *L. frontalis* were observed to probe into potential oviposition sites using their rostrum. When in the adult boxes, they mainly probed into the paper towelling that was wrapped around the petioles of the *S. platyphylla* leaves in search of oviposition sites. Moreover, these probed holes were often made in linear arrangements. When adult *L. frontalis* were placed onto potted *S. platyphylla* plants, they probed the surrounding sediment instead of the plant material. Eggs were eventually laid within holes probed by the female weevils.

Newly laid eggs were creamy-yellow in colour (Fig. 3.3.1a), while more mature eggs became black and shiny (Fig. 3.3.1b). On overage, it took *L. frontalis* eggs 8.00 ± 0.09 (SE) days until they hatched, and, on average, they were 1.05 mm ± 0.03 (SE) long and 0.65 mm ± 0.02 (SE) wide.

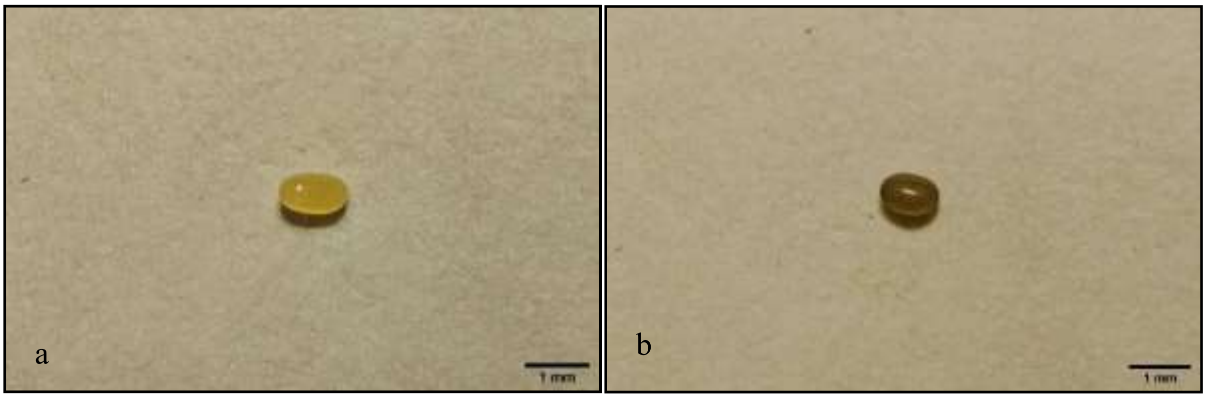

Figure 3.3.1. A newly laid *Listronotus frontalis* egg (a) and a mature egg (b).

Listronotus frontalis have four larval instar stages (Fig. 3.3.2). All of the instar stages had a red-to-orange head while the rest of the body varied in colour between a creamy-white and a creamy-yellow. The first three instars developed and fed within *S. platyphylla* tubers, while fourth instar larvae were typically found within the substrate external to the tubers, priorto pupation. Morphologically, the four instar stages looked the same, and only differed in size. These size discrepancies were best highlighted with the differing head capsule widths (Table 3.3.1). The first instars had a head capsule width of 0.42 ± 0.01 (SE) mm, the second 0.68 ± 0.01 (SE) mm, the third 1.05 ± 0.01 (SE) mm and the fourth 1.55 ± 0.01 (SE) mm. The growth ratio between the instar stages ranged between 1.48 and 1.6 (Table 3.3.1). The relationship between the different instar stages was significant ($R^2=0.9208$, $P < 0.0001$).

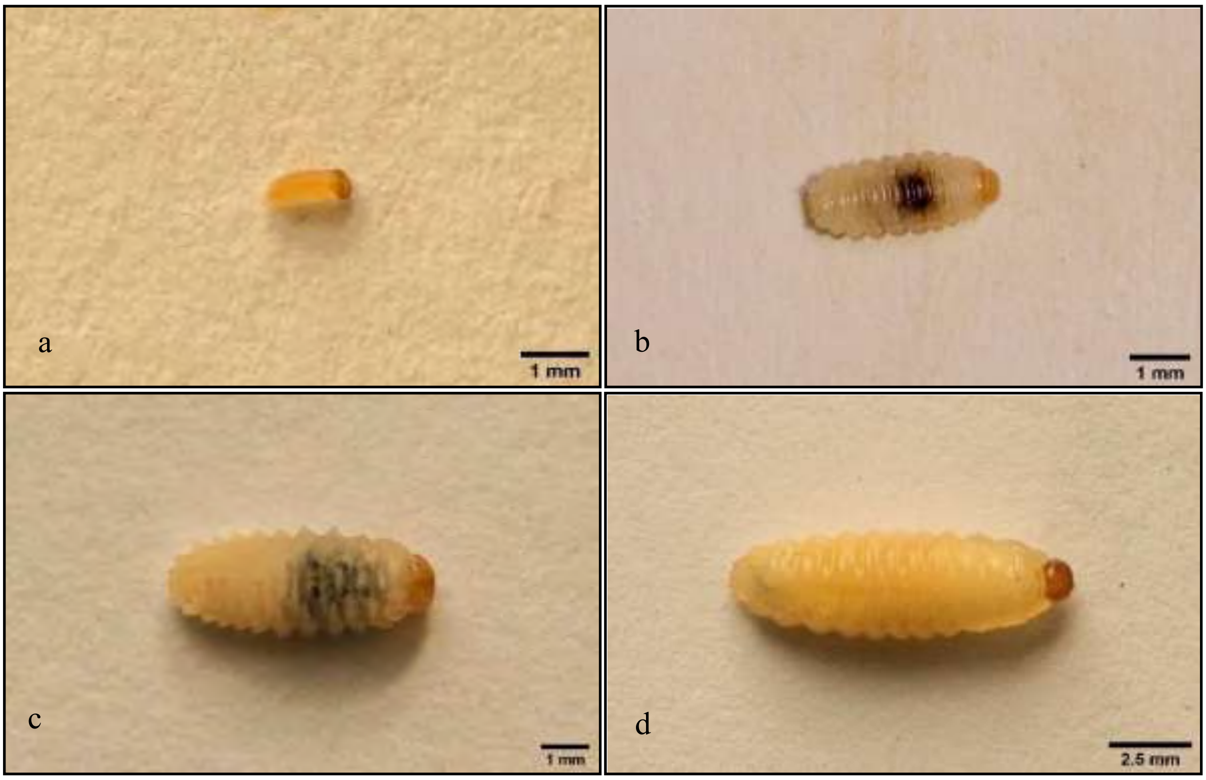

Figure 3.3.2. The four different instar stages of *Listronotus frontalis*: a) first instar, b) second instar, c) third instar, and d) fourth instar larvae.

Table 3.3.1. The mean head capsule width (mm) (± standard error) for each larval instar stage of *Listronotus frontalis*. The growth ratio between the larval instar stages is provided.

Larval instar	Mean head capsule width (mm) ± SE	Growth ratio
1st	0.42 ± 0.01	-
2nd	0.68 ± 0.01	1.6
3rd	1.05 ± 0.01	1.54
4th	1.55 ± 0.01	1.48

Figure 3.3.3. A *Listronotus frontalis* pupa.

Once the *L. frontalis* larvae pupated, the pupae developed within the substrate surrounding the subterranean plant material. Pupae typically took 8.18 ± 0.48 (SE) days until they eclosed into adult weevils. *Listronotus frontalis* pupae are a rich, creamy-yellow colour (Fig. 3.3.3) and darken slightly as they mature. Moreover, with maturation, the adult features they will eclose into became more prominent.

Adult *L. frontalis* weevils were dark brown to grey in colouration (Fig. 3.3.4). Their tarsi and antennae are a combination of orange, red or dark brown. They have mottled features all across their body. They have two light brown lines that run vertically down their thorax. The females are slightly larger (11.4 mm ± 0.25 (SE)) than the males (7.9 mm ± 0.22 (SE)) (Fig. 3.3.4b). Female weevils display a slightly convex shape on their third and fourth sterna (Fig.

Figure 3.3.4. Adult *Listronotus frontalis* weevils. A single adult female weevil (left) and a pair of adults (right), with the female on the left and the male on the right.

3.3.5a), while the same structures on the males are deeply concave (Fig. 3.3.5b) (Henderson, 1940). The adults feed on the leaves of *S. platyphylla* as well as petioles and flowers. Adult *L. frontalis* weevils, on average, took 8.50 days ± 0.51 (SE) (Table 3.3.2) until they were able to oviposit, and, on average, they lived for 67.50 days ± 7.10 (SE) (n = 10) (Table 3.3.2).

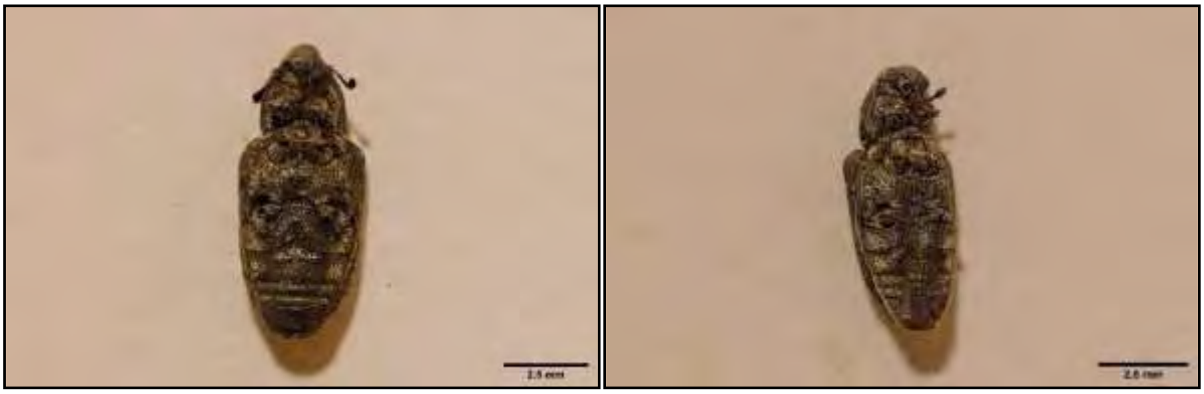

Figure 3.3.5. Ventral view of an adult female (left) and male (right) *Listronotus frontalis* weevil.

On average, *L. frontalis* took 42.67 ± 1.39 (SE) days to develop from egg to ovipositing adults (Table 3.3.2). Moreover, the percent mortality of the different life stages (Table 3.3.3) of *L. frontalis* was found to be highest within the larval stages (64.52%) and lowest within the pupal life stage (18.18%).

The maximum number of eggs laid by a single female weevil over a period of one week was 48. This varied greatly, and the weevils went through periods where fewer eggs were laid.

Table 3.3.2. The mean number of days (± standard error) for each life stage of *Listronotus frontalis* to develop. Sample size (n) is also provided.

Stage	Mean duration (days) ± SE	n
Egg - 1st instar	8.00 ± 0.09	31
1st instar - 2nd instar	2.52 ± 0.16	31
2nd instar - 3rd instar	2.43 ± 0.17	23
3rd instar - 4th instar	2.53 ± 0.24	17
4th instar - Pupae	8.31 ± 0.43	13
Pupa - Adult	8.18 ± 0.48	11
Adult – Oviposition	8.50 ± 0.51	6
Adult Longevity	67.5 ± 7.10	10
Total (Egg - Oviposition)	42.67 ± 1.39	6

Table 3.3.3. Life table of *Listronotus frontalis* at a constant temperature of 26 ^{o}

Life stage	Number of individuals entering stage	Number of individuals dying in stage	Percentage (%) mortality
Eggs	41	10	24.39
Larvae	31	20	64.52
Pupae	11	2	18.18
TOTAL	41	32	78.05

3.3.2. Effect of sediment type on *Listronotus frontalis* larval development

The development of *L. frontalis* within different sediment types was shown to differ (Fig. 3.3.6). From the plants grown within builder's sand, 10 % of *L. frontalis* eggs placed within the sediment were able to develop to at least their fourth instar, five % of eggs were found to develop within the mixed sediment, while no individuals were found to develop from eggs placed within the pond sediment. However, tuber mining was observed in a single tuber within the pond sediment treatment.

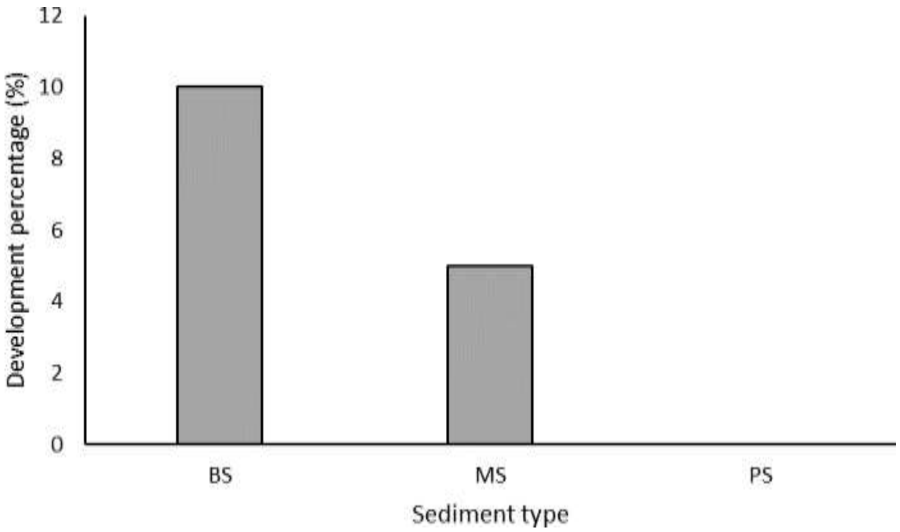

Figure 3.3.6. The developmental percentage of *Listronotus frontalis* from eggs within the varied sediment *Sagittaria platyphylla* individuals were grown in. The abbreviations within this graph represent the three sediment types used, which were: builder's sand (BS), mixed sediment (MS) and pond sediment (PS).

3.3.3. Impact study

An impact study was conducted to determine the level of damage caused by adult *L. frontalis* feeding on various growth and developmental structures of *S. platyphylla* plants. Three treatments were tested within this study, a pre-control, post-control, and an insect-exposed treatment. Significant differences were found between the three groups sampled for the following plant variables: leaves, flowers, fruit, stolons, tubers and daughter plants (Table 3.3.4; Fig. 3.3.7). Most of the variables mentioned above increased significantly within the post-control group, except for the mean number of leaves, however, all the above-mentioned variables were lower with the presence of adult *L. frontalis* feeding when compared with the post-control treatment. From the plant height and root length measures taken, significant differences were found between the three different treatments (Table 3.3.4; Fig. 3.3.8). Feeding by *L. frontalis* adults reduced the root length of *S. platyphylla* by more than half the length, compared with plants in the post-control treatment. From the mass measurements taken in this study, significant differences were found between the treatments for the above-ground plant material and the tubers, while no significant difference was found for the below-ground plant material (Table 3.3.4; Fig. 3.3.9). In particular, the mean mass of above-ground plant material was greatly reduced by the feeding of adult *L. frontalis*.

Table 3.3.4. The morphological parameters of *Sagittaria platyphylla* tested for differences between the pre-control, insect-exposed and post-control treatments of the impact study. Highlighted values indicate no significant differences. '*' represents data that were log transformed while '**' represents data that were analysed through a Welch's ANOVA.

Morphological parameters	**Df**	**Sum Sq**	**Sum sq res**	**F-value**	**P-value**
Number of leaves	2	385.8	95.4	54.59	< 0.0001
Number of flowers	2	22.47	68.5	4.428	0.0217
Number of fruit	2	18.87	81.00	3.144	0.0392
Number of stolons**	14.943			8.7577	0.00304
Number of tubers*	2	58.47	80.90	9.756	0.0006
Number of daughter plants	2	178.9	176.6	13.67	< 0.0001
Plant height	2	373.0	872.9	4.274	0.0285
Root length	2	2763.8	686.2	54.37	< 0.0001
Above ground plant material	2	2743	3073	12.05	0.00018
Below ground plant material*	**2**	**0.742**	**4.92**	**2.036**	**0.15**
Tuber mass*	2	5.75	94.2	4.794	0.0093

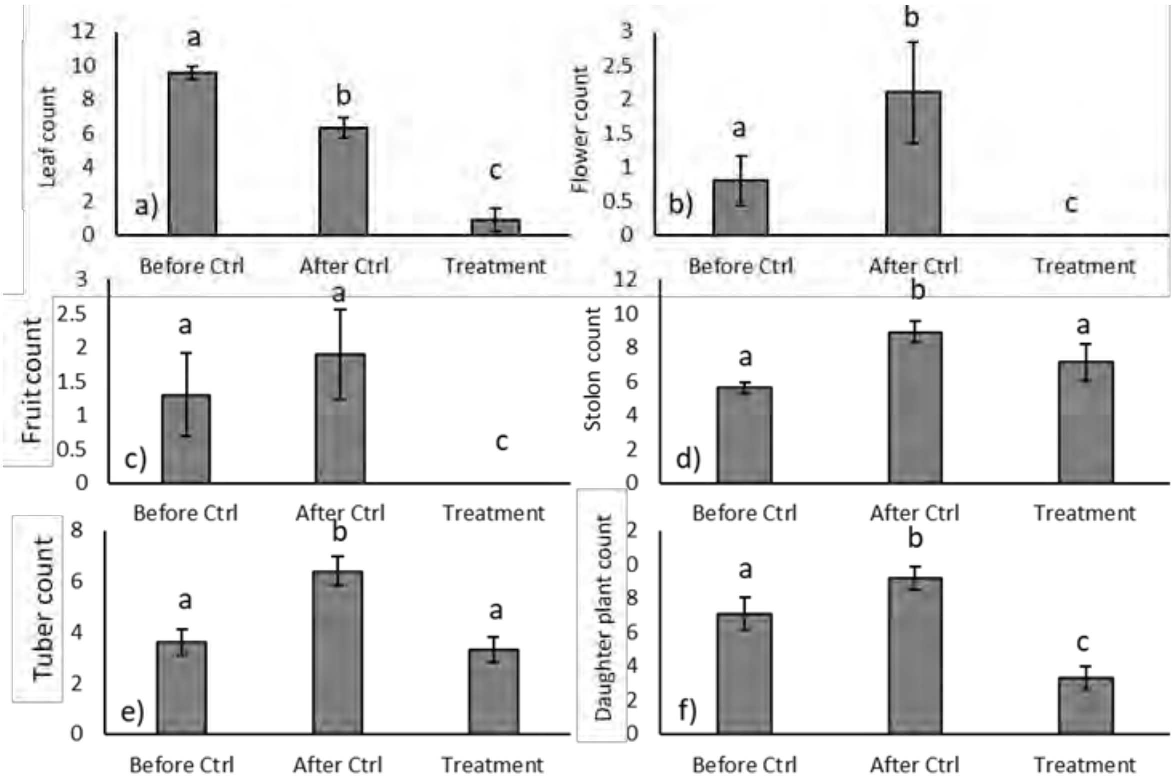

Figure 3.3.7. Plant parameters recorded from an impact study to determine the level of damage inflicted by *Listronotus frontalis* on *Sagittaria platyphylla*. The plant parameters recorded are the: a) mean number of leaves, b) mean number of flowers, c) mean number of fruit, d) mean number of stolons, e) mean number of tubers and f) mean number of daughter plants from each test plant. The error bars represent standard error. Letters above bars represent significant differences.

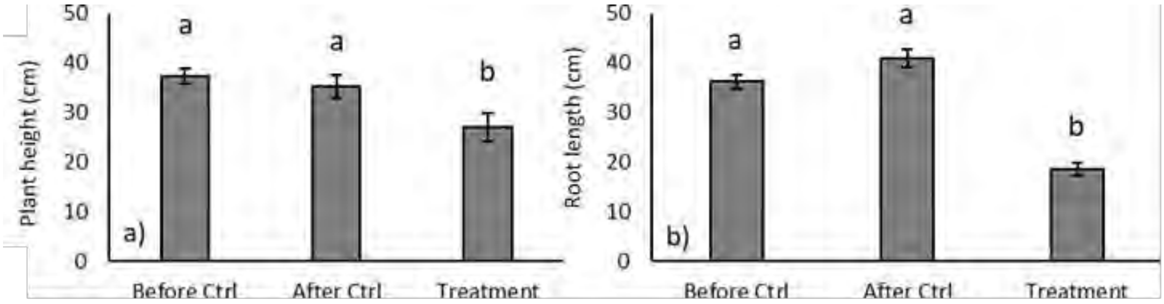

Figure 3.3.8. Plant parameters recorded from an impact study to determine the level of damage inflicted by *Listronotus frontalis* on *Sagittaria platyphylla*. The plant parameters recorded are the: a) mean plant height and b) mean root length. The error bars represent standard error. Letters above bars represent significant differences.

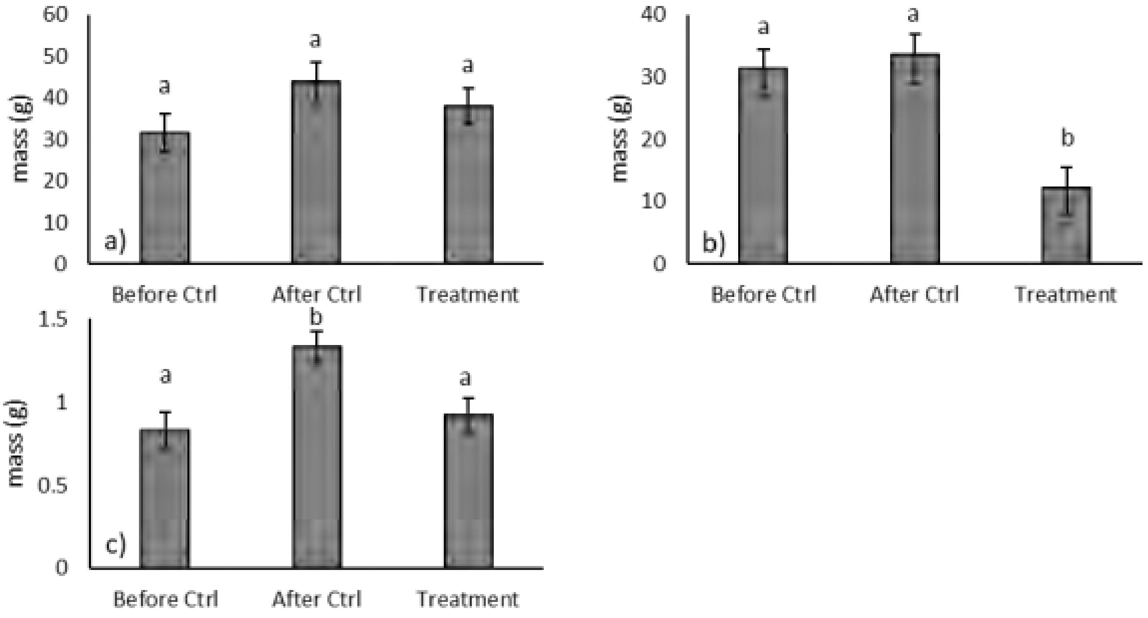

Figure 3.3.9. Plant parameters recorded from an impact study to determine the level of damage inflicted by *Listronotus frontalis* on *Sagittaria platyphylla*. The plant parameters recorded are the: a) mean mass of below-ground plant material, b) mean mass of above-ground plant material and the c) mean mass of tubers. The error bars represent standard error.

3.4. Discussion

Adult *L. frontalis* weevils oviposit within the sediment surrounding *S. platyphylla* plants. Once the eggs have hatched, neonate larvae bury into the sediment where they feed on and develop within tubers. Larvae then exit the tubers to pupate within the surrounding sediment. Once eclosed, adult weevils emerge from the soil where they feed on the fruits, flowers and leaves of *S. platyphylla*. From egg to ovipositing adult, development takes, on average, 42.67 ± 1.39 days (n = 6).

Listronotus frontalis was shown to undergo four larval instar stages and develop from egg to ovipositing adult in just over 40 days. This is similar to the other candidate agent *L. appendiculatus*, which is currently being considered for release against *S. platyphylla* in both South Africa and Australia (D. Rogers, unpublished). *Listronotus appendiculatus* also have four instar stages and also took just over 40 days to develop from egg to ovipositing adult when being reared under quarantine conditions (D. Rogers, unpublished). If released, their relatively quick developmental time, in combination with suitable habitats and sufficient plant material could facilitate rapid population increases.

The physiological and metabolic process of insects are strongly influenced by their immediate temperature. Biocontrol is thus strengthened when potential control agents are sourced from similar climates to where releases are planned. Within Mississippi, USA (where the initial *L. frontalis* weevils were collected), winters are temperate and temperatures occasionally fall below-freezing, while summers are both humid and hot, often exceeding 30ºC. *Sagittaria platyphylla* populations within South Africa fall under various climatic regions none of whichreceive winter snow. For the most part, *S. platyphylla* populations are found in moderate temperatures near-coastal regions within the KwaZulu-Natal, Eastern Cape, and Western Capeprovinces. The Western Cape Province has a Mediterranean climate with warm and dry summers, and mild and rainy winters, while the other two provinces experience subtropical climates. As the constant 26 ºC experienced within the CBC, RU quarantine facility is representative of temperatures expected within the field, this does give hope to any potential releases against *S. platyphylla* which should allow biocontrol agents to firstly establish but also build up generations relatively quickly.

This kind of population increase could lead to reductions in weed populations which has been experienced in South Africa previously with the release of the weevil *Neohydronomus affinis* Hustache 1926 (Coleoptera: Curculionidae), a biocontrol agent that was released in 1985 in South Africa to control the invasive floating plant *Pistia stratiotes* L. (Araceae) (Coetzee *et* *al*., 2011). This control agent rapidly built up in numbers and gained complete control over *P. stratiotes* in all regions where the plant had invaded South Africa. *Listronotus frontalis* has a comparable developmental time period to *N. affinis*, which has three larval instars and takes between four to six weeks to develop from egg to adult under optimal conditions (Moore, 2005). Similar rapid build-up in insect numbers and resultant weed control was also experienced with the release of *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) on *Azolla filiculoides* Lam. (Azollaceae) in South Africa (Coetzee *et al*., 2011). However rapid control of populations across the whole country did rely on the insect's ability to spread to new infestations. The dispersal abilities of *L. frontalis* is not well understood, and if found to be poor, mass rearing and co-ordinated releases should be done so to facilitate the spread of the agent to new sites, if possible.

3.4.1. Damage by Listronotus frontalis

Listronotus frontalis adults were shown to reduce almost all of the measured plant parameters of *S. platyphylla*, indicating their potential to reduce both growth and development of *S. platyphylla*. Besides the above-ground plant material being heavily fed on and stunted, the reduction in size and abundance of tubers as well as stolons was a very promising result. Although tubers are of strong importance with regards to the survival of a number of invasive species, there has not been much investigation into how foliage-feeders influence their dynamics (Kleinjan *et al*., 2004). However, a couple of studies have looked into this. A study conducted by Kleinjan *et al*. (2004) determined the impact that the foliage feeding hemipteran *Zygina* sp. (Hemiptera: Cicadellidae) had on a southern African form of *Asparagus asparagoides* (L.) Druce (Asparagaceae). The above-ground feeding of *Zygina* sp. reduced both the vegetative and reproductive output of *A. asparagoides*. A similar study conducted by Piqueras (1999) determined the impact of above-ground invertebrate herbivory on the tubers of the plant *Trientalis europaea* L. (= *Lysimachia europaea* (L.) U.Manns & Anderb.) (Primulaceae), but failed to find a significant relationship between the two. Although the findings from the above-mentioned studies do differ, it is important to keep in mind that there are various influential factors that will determine how plants respond to damage. Such factors include timing of damage, the type of tissue attacked as well as the degree and frequency of the damage itself (Piqueras, 1999).

A reduction in the vegetative reproductive abilities of *S. platyphylla* as a result of *L. frontalis* adult feeding is promising and similar to the biocontrol programme initiated against

Hydrilla verticillata (Lf) (Royle) (Hydrocharitaceae) in the USA. The stem-boring weevil *Bagous hydrillae* O'Brien (Diptera: Ephydridae) was found to develop on both the stem and the tubers of *H. verticillata* in the laboratory. After being released, the same trends were observed when these weevils had access to exposed sediment. When *B. hydrillae* were able to develop and feed on the tubers of *H. verticillata,* they placed additional stress onto populations of *H. verticillata* by reducing future propagation via tubers (Wheeler & Center, 2007). The same would be expected of *L. frontalis* if released onto *S. platyphylla* within South Africa.

As with most studies conducted under artificial environments it is hard to extrapolate the results from the quarantine study to what might actually happen in the field. It is not known what densities *L. frontalis* population can attain, especially considering they were not found in great abundances during the natural enemy surveys between 2010 and 2012. Having said this, based on the damage inflicted by *L. frontalis*, may not necessarily need to attain such high densities to be able to gain high levels of control over *S. platyphylla* populations. Moreover, the evolution of increased competitive ability, or EICA, hypothesis predicts that invasive plant populations provide a greater platform for therapid population build-up of biocontrol agents, as the plants are hypothesised to have reduceddefences (Wang *et al*., 2011).

The destructive feeding of *L. frontalis* larvae on *S. platyphylla* tubers was not directly quantified in this study due to the development of tubers within the sediment, such a study proved challenging to accurately assess. However, based on laboratory observations, the level of damage inflicted by larvae on tubers was often very high and damaged tubers also showed reduced developmental ability (pers. obs.). Gosiki *et al.* (2018) mentioned that promising control agents display the ability to inhibit the generative abilities of their host plant, which the larvae of *L. frontalis* seemingly display.

3.4.2. Reason for high larval mortality

During life history studies, the mortality rates of *L. frontalis* were high, and there are a couple of reasons why this could be the case. Firstly, head capsule width sampling of *L. frontalis* required manipulation of the larvae, and, not surprisingly, the larval stages showed the highest levels of mortality, with almost two-thirds not completing development through to pupation. In a study conducted by Ungsunantwiwat & Mills (1979), the effect of mechanical disturbance on four *Sitophilus* populations from three *Sitophilus spp.* (Coleoptera: Curculionidae) was tested. These weevils were disturbed every two days while they were still larvae, and it was found that these disturbance events significantly reduced the number of individuals that were able to reach the adult stage. Secondly, most of the developmental examinations were done so with the individual being placed within small containers, which made it difficult to balance the moisture and humidity levels within the warm quarantine facilities. Since these weevils naturally develop within very moist conditions, larvae are probably very comfortable at high moisture content levels, however, keeping plant material from rotting and mitigating fungal infections was challenging. Thirdly, due to the way plant material, specifically tubers, was presented to *L. frontalis* larvae, the nutritional content of said tubers may have been of a lower quality than required for healthy development of *L. frontalis* larvae. The most visually healthy tubers were always selected for the larvae to feed on, however, the tubers provided as food to the larvae were sliced into discs, which may have altered their chemistry.

Proteinase inhibitors (PIs) are a class of plant chemical defences that are strongly present within storage organs, such as tubers (War *et al*., 2012). These PIs reduce the efficacy of digestive enzymes within insects' guts, which leads to slower rates of development, and even starvation (War *et al*., 2012). However, insects have been shown to be able to defendthemselves against PIs, either by producing PI-insensitive protease or by inactivating already ingested PIs. War *et al*. (2012) found evidence of induced upregulation of PIs within plants inresponse to wounding. This could suggest that the cut tubers provided to *L. frontalis* larvae may have been harder to extract high-quality nutrients from.

Although mortality rates were found to be quite high, it must be mentioned that these weevils were kept in artificial conditions and did not have access to the more natural structures they would be exposed to in their native range. For example, it is thought that in their native range, *L. frontalis* oviposit within the sediment surrounding *S. platyphylla* plants. This was not made available to the weevils kept in quarantine and may have hindered their oviposition levels. In a similar study conducted by Herrick *et al*. (2011), very low oviposition rates for *Eucryptorrhynchus brandti* Harold (Coleoptera: Curculionidae) under quarantine conditions were observed. They attributed such low levels of oviposition to various factors, one of which was the use of tree cuttings as a source of food as opposed to whole trees. Moreover, another factor to consider for *L. frontalis* oviposition rates is the possible limitation of oviposition sites. *Listronotus frontalis* weevils very much favoured the leaf petiole wrapped in paper towelling over other potential oviposition sites. Although this oviposition site was relatively large, numbers of weevils did fluctuate, and this may have led to intraspecific competition for oviposition sites.

3.4.3. Female oviposition

Under quarantine conditions female fecundity seemed to be erratic and females often laid eggs in batches and sometimes stopped laying eggs for long periods of time. It is thought that fecundity is linked to the availability of pollen from male *S. platyphylla* flowers (G. Martin, pers. comm.), which becomes limited during the colder months. However, in general, *L. frontalis* females laid high numbers of eggs under quarantine conditions. for example a single female was able to lay 48 eggs during a seven day cycle. The female weevils were observed to probe plant materials and substrates with their rostrum before deciding on oviposition sites. The eggs were securely laid within plant material within the oviposition boxes and when laid in sediment they were done so at a shallow depth, which could help lower parasitism of the eggsin the field. Moreover, the larvae and pupae all feed and develop underground, which should limit predation and parasitism of these life stages (Gosiki *et al.*, 2018).

With endophagous insects, there is always a concern around intraspecific competition for space and resources. As mentioned by Prischmann-Voldseth *et al.* (2016), "oviposition decisions can impact the survival and fitness of offspring". Although no experiments have looked at the reasons behind *L. frontalis* oviposition preference, the number of probed holes without eggs having been laid within them indicates that the females assess potential oviposition sites before deciding on where to deposit their eggs. Whether these are linked to temperature, humidity, structural integrity or available resources, the oviposition selection made by *L. frontalis* does appear to show some level of 'planning' for the next generation. A study conducted by Desouhant (1998) looked to determine the oviposition selection reasoning made by *Curculio elephas* Gyllenhal (Coleoptera: Curculionidae) on European chestnut, *Castanea sativa* Miller (Fagaceae). From this study it was thought that the main selective process made by these weevils was to select hosts, i.e. plant structures, of the highest feeding quality for the larvae.

3.4.4. Soil development experiment

Although this experiment showed that more *L. frontalis* larvae were found to develop within the finer sediment, the low number of replicates used means these results should be treated with caution. Another aspect of concern was the differing soil pH levels, as some h studies have shown thatsoil pH may affect insect development(Brown & Gange 1990). For example Johnson et al (2010) found that different levels of soil pH affected the hatching rate of *Sitona lepidus* Gyllenhaal

(Coleoptera: Curculionidae) eggs, but not the number of hatched eggs.

3.5. Conclusion

Under quarantine condition *L. frontalis* adults significantly reduced a number of *S. platyphylla* growth parameters. Additionally, the observed damage inflicted by larvae suggest theweevil shows a lot of promise as a biocontrol agent for invasive populations of *S. platyphylla* within South Africa. Moreover, their short developmental time and high fecundity add to their potential as a control option for *S. platyphylla* in South Africa. Although more data regarding the host specificity of *L. frontalis* as well as their ability to develop on inundated *S. platyphylla* is required, this species may yet provide a useful management optionfor *S. platyphylla* in South Africa.

Chapter 4: General discussion

4.1. Thesis synthesis

This thesis aimed to determine the plant population biology of *Sagittaria platyphylla* (Engelmann) J.G. Smith (Alismataceae) within South Africa, as well as the life history of the candidate control agent *Listronotus frontalis* LeConte 1876 (Coleoptera: Curculionidae) and the interactions between the agent and the plant under quarantine conditions, in order to make an informed decision about the suitability of *L. frontalis* as a control agent for this invasive macrophyte.

In order to determine the population dynamics of *S. platyphylla*, different populations were sampled from around South Africa growing in differing climates and waterbody types, because different environmental conditions affect modes of reproduction and growth (Ndlovu *et al*., 2020). As expected, there were differences in growth form, proliferation, density, and investment in reproductive modes of *S. platyphylla* between the seven field sites. One of the main aims of this thesis was to determine if there was a need for a tuber feeding agent, hence the tuber dynamics of *S. platyphylla* were of prominent importance. Water depth did not influence tuber production as tubers grew in both deep and shallow waters. It was also shown that while tuber size varied between sites, tubers were consistently produced throughout the year. This is a significant finding because *L. frontalis* would then be able to complete its lifecycle on plants growing in more shallow waters as tubers would firstly be present and secondly, they would be large enough for *L. frontalis* development within these regions.

Chapter 3 showed that *L. frontalis* developmental time was 43 ± 1.39 days, fecundity was relatively high for a *Listronotus* weevil (the highest recorded number of eggs from a single weevil was 48, whereas *Listronotus bonariensis* (Coleoptera: Curculionidae) was found to produce an average of 20 eggs per week (Malone, 1987), and once eclosion to adult occurred, the pre-oviposition period was slightly longer than a week. Moreover, the impact study revealed the significant amount of direct and indirect damage inflicted by *L. frontalis* adults, i.e. the direct damage they provided towards the structured they directly fed on, such as the leaves, and the indirect developmental damage they caused to the structures they did not feed on, such as the reduction in tuber mass.

These findings suggest that *L. frontalis* is sufficiently damaging to *S. platyphylla* and should not be limited by environmental conditions at infested sites in South Africa. If it is suitably host specific, an application for its release should be submitted to the relevant decision making parties.

4.2. Achenes vs tubers

Aquatic plants have various ways which they can disperse, and such options include asexual propagules such as ramets, turions, corms and tubers, as well as sexual organs such as seeds and achenes (Santamaria, 2002). Since *S. platyphylla* was identified as invasive in South Africa more than a decade ago, it has rapidly increased from just 16 sites in 2009 to 72 unique sites by 2019 (Ndlovu *et al*., 2020). To date, it is assumed that the primary mechanism of dispersal between sites is via achenes (Ndlovu *et al*., 2020). On average, *S. platyphylla* can produce 850 seeds per fruit and 6900 achenes per inflorescence (Adair *et al*., 2012). In South Africa, the number of achenes per fruiting body and individual achene weigh 50% more than those in its native range in USA (Kwong *et al*., 2017a). *Sagittaria platyphylla* achenes have specific qualities that enable them to be well adapted to aid in dispersal. Such qualities include their ability to stay buoyant for up to seven days and to attach to animals via a sticky outer surface (Adair *et al*., 2012). This, along with the knowledge that tubers generally allow persistence of aquatic plants within a habitat (Li, 2014), further points to the more favourable dispersal method of sexually produced achenes by *S. platyphylla* in South Africa.

In addition to being easily dispersed, achenes are sexually produced reproductive structures that have greater genetic variability than clonal propagules (Li, 2014). This enables achenes a greater adaptive advantage within newly invaded areas, which suggests that individuals will be well equipped to respond to the selective pressures applied to them within novel regions. Thus, if *S. platyphylla* within South Africa disperses via achenes, they would have a strong ability to grow and establish in novel areas with diverse selective pressures, which might not hold true for tubers dispersing to new environments. Establishment success between tubers and achenes was not studied here, however due to the infrequency of tubers moving long distances, except during floods when seeds would also be transported, it is not considered significantly important for the spread of *S. platyphylla* in South Africa (Berkovic *et al*., 2014).

Although dispersal does need to be considered for management programmes, the ability of an invasive to persist is of major concern. Tubers are produced by *S. platyphylla* within the sediment of habitats it occupies and is unlikely to contribute to their spread unless management efforts translocate tubers or soils containing tubers to new sites. For example, a private landowner at Oude Nektar Wine Estate in the Western Cape Province moved sediment from a dam infested with *S. platyphylla* into a nearby waterbody, the Eerste River, which allowed the plant to establish in the River (pers. obs.).

This study has shown that the majority of populations in South Africa produce tubers, which has implications for management. Whatever management plans are conducted, they need to do so with the knowledge that tubers are present, further confounding management efforts. For example, water was drained from Kingfisher Lake, KwaZulu-Natal Province, for a period of 10 years in an attempt to eradicate *S. platyphylla* (G. Martin, pers. comm.). However, when water was added back to the system, the population of *S. platyphylla* re-established, most likely from the tubers which remained in the pond sediment. This displays the ability of tubers to withstand harsh conditions where seeds might not be able to survive. Seed viability for *S. platyphylla* is not currently know, however, seed viability of the closely related *Sagittaria calycina* Engelmann (Alismataceae) was found to reduce from 54% in their first year to 45% after three years (Adair *et al*., 2012). Parallels can also be drawn to the management of *S. latifolia*, an emergent alien plant in South Africa that only reproduces vegetatively and does not produce seeds, as only male plants are found in the country (G. Martin, pers. comm.). Only two populations of *S. latifolia* are present within South Africa and they are both relatively small, each occupying shallow ponds less than 10 x 10m, and each containing fewer than 100 individual plants. Although these populations, given their sizes and lack of seeds, do seem to be easy targets for eradication, management efforts through hand pulling have been in place for more than 10 years, yet both these populations persist. Although tubers have been challenging to manage through chemical and mechanical control options, they are structures that require targeting, and biocontrol could provide a sustainable solution.

4.3. Biological control options for tubers

Tubers were found throughout the sampled populations of *S. platyphylla* in South Africa, except at one site. This is important if *L. frontalis* is released as a biological control agent in South Africa as it requires tubers to complete its life cycle. Moreover, having a better understanding of the tuber dynamics can help biocontrol practitioners to co-ordinate releases when tuber densities are highest, or at sites with larger tubers, which could lead to increased establishment of *L. frontalis*.

Tuber-feeding biological control agents are not very well represented within biological control programmes. A few examples do exist, such as the programme initiated against *C. esculentus* (Morales-Payan *et al*., 2005), *Hydrilla verticillata* (Wheeler & Center, 2007) and *Macfadyena unguis-cati* L. (Bignoniaceae) (Williams *et al*., 2008). While the tuber-feeding agents, *S. phoeniciensis* and *B. affinis*, were released against *C. esculentus* and *H. verticillata*, respectively, establishment of the agents failed, for different reasons. *Sphenophorus phoeniciensis* did not attain sufficiently high densities when released and failed to establish (Poinar Jr, 1964), whereas *B. affinis* failed to establish due to a lack of exposed sediment (Purcell *et al*., 2019). Water level fluctuations are a seasonal trend where *H. verticillata* grows in it native range (Purcell *et al*., 2019), allowing *B. affinis* access to the sediment, where their eggs are then deposited and their larvae are able to access, develop within and damage the tubers of *H. verticillata* (Purcell *et al*., 2019). Both of these scenarios could also affect *L. frontalis* establishment of *S. platyphylla* if released in South Africa.

The ability of *L. frontalis* to find tubers in South African systems to complete its life cycle is a significant concern. Kwong et al. (2017) compared the population and environmental parameters of *S. platyphylla* between the native range and the invaded range in South Africa, highlighting the significant differences in habitat, specifically with regard to water depth. In South Africa, water depth in invaded sites was deeper in comparison to water bodies in the native range. They also showed that majority populations of *S. platyphylla* growing in the USA occur in natural or semi-natural habitats such as wetlands, riverbanks and along the margins of lakes. In contrast, about 65% of *S. platyphylla* populations in South Africa occur in ruderal habitats such as irrigation supply channels, drainage ditches and artificial impoundments (small ornamental lakes, sewerage, and fisheries ponds (Kwong *et al*., 2017)). This difference has already resulted in the termination of a candidate agent in South Africa. During laboratory studies, it was shown that the candidate agent *L. sordidus* did not oviposit on inundated plants, potentially nullifying the impact of the insect on South African populations (Martin *et al*., 2018). Even though the insect larvae were damaging and specific, its limited impact on inundated plant populations in South Africa did not justify the inherent risk associated with the release of a biological control agent. The main difference between *L. sordidus and L. frontalis* is that unlike *L. sordidus, L. frontalis* adults were shown to be damaging to above and below ground structures of *S. platyphylla* while *L. sordidus* adults did not (Martin *et al.,* 2018). Thereby, suggesting that if released *L. frontalis* like *L. frontalis* larvae may not prove to be sufficiently damaging to warrant release however the damage inflicted by *L. frontalis* adults may warrant it consideration for release.

Another biological control programme that has struggled to deal with similar reproductive strategies is the control programme initiated against *Cyperus esculentus*. This weedy species, commonly known as yellow nutsedge, is a prominent pest plant of agricultural importance within the USA (Poinar Jr, 1964). Initial attempts to control *C. esculentus* included various mechanical control as well as limited chemical control methods (Poinar Jr, 1964). Neither provided sustainable long-term solutions, while chemical control options were deemed far too expensive for large scale use (Poinar Jr, 1964). Biological control was sought, and various biological control agents were released in attempts to control *C. esculentus*, however, no releases were deemed successful (Morales-Payan *et al*., 2005). A tuber-feeding billbug, *S. phoeniciensis*, was at one point considered a potential control agent for *C. esculentus*, as this invasive species was able to re-establish itself after the application of other control options (Poinar Jr, 1964). However, *S. phoeniciensis* was not able to establish aftermultiple release efforts (Poinar Jr, 1964).

Although there are concerns with regards to the establishment and success of tuber-feeding control agents, Wheeler & Center (2007) investigated the ability of control agents of *H. verticillata* to feed and develop on structures of the plant other than what they are commonly known to do. Larvae of *B. affinis* primarily feed and develop on the tubers, while *B. hydrillae* larvae feed and develop on the stems of *H. verticillata.* Studies showed that larvae of *B. affinis* could only feed and reproduce on *H. verticillata* tubers, however, *B. hydrillae* larvae displayed the ability to not only feed and develop on the stems of *H. verticillata*, but the tubers too (Wheeler & Center, 2007). Although the tuber-feeding *B. affinis* could only develop on tubers, the same may not be true of *L. frontalis*. The ability of *L. frontalis* to oviposit within *S. platyphylla* petioles (pers. obs.) could suggest that this weevil is able to develop within structures other than the tubers of *S. platyphylla*. Although the development of *L. frontalis* larvae within *S. platyphylla* petioles would mean tuber banks remain free of direct damage by these larvae, this could aid in the establishment of *L. frontalis* and allow these weevils to persist until favourable conditions allowed them to oviposit, feed and develop on the tubers of *S. platyphylla*. Other favourable management outcomes may be provided by the feeding and development of *L. frontalis* on structures other than the tubers of *S. platyphylla*. Similar predictions were made by Wheeler & Center (2007) with regards to the ability of *B. hydrillae* to feed and develop on both the tubers and stems of *H. verticillata*.

4.4. Biological control options

Common concerns of biological control programmes are the number of control agents that should be released. However, in terms of weed biological control, lower levels of interspecific competition have been found between control agents, whereas, greater levels of interspecific competition are observed for arthropod biological control, something that negatively influences the overall success of a control programme (Denoth *et al*., 2002). Although greater concern is had when more control agents are released, the quantity of releases should not influence the quality of the releases. This is to say that if control agents are developed with enough precision, non-target effects should not be of great concern. Increasing the number of releases is thought to feed into two predictive models, the lottery model, and the cumulative stress model (Denoth *et al*., 2002). The lottery model predicts that with a greater number of releases there are higher chances of releasing the 'right' agent, while the cumulative stress model predicts that multiple agent releases will be needed to sufficiently damage the target species (Denoth *et al*., 2002).

Focus on the biological control of *S. platyphylla in* South Africa has shifted from four candidate control agents to only two, *L. frontalis* and *L. appendiculatus*. As these two weevils feed and develop within two separate structures of *S. platyphylla* (Kwong *et al*., 2014), it is predicted that they would be able to act synergistically to reduce production of sexual and asexual reproductive structures, reduce population growth rates, and do so without competing with one another for the same resources. In contrast, when two weevils that occupy the same feeding niche (*N. eichhorniae* and *N. bruchi*) were assessed for their interactive effects on *Pontederia crassipes* (Martius) 1823 [≡*Eichhornia crassipes* (Martius) Solms-Laubach 1883] (Pontederiaceae), their feeding levels were found to be significantly lower than when treated individually. Interactions between biocontrol agents will depend on the agents themselves and how they feed and develop (Petela, 2016), which may allow *L. frontalis* and *L. appendiculatus* to complement rather than diminish impact.

An application for permission to release *L. appendiculatus* was submitted in late 2020, so it could be cleared for release in South Africa in the near future, however, additional studies on the suitability of *L. frontalis* need to be completed before it can be considered for release. Above-ground feeding by *L. appendiculatus* reduced the size and number of tubers produced by *S. platyphylla* (Ndlovu 2020), which may potentially limit the tubers available to *L. frontalis*, either hindering the establishment of *L. frontalis* through interspecific competition, or increasing the stress both control agents could apply on *S. platyphylla*. The feeding and development niches of these agents differ greatly, therefore interaction studies between these two agents will elucidate whether they can operate in unison to reduce the vigour of *S. platyphylla,* or if one species will outcompete the other.

If the release of *L. frontalis* were to be rejected, attention could refocus on the leaf-feeding weevil *L. lutulentus*. Although the culture of *L. lutulentus* was terminated because its impact on *S. platyphylla* was regarded as insufficient, this agent could be reconsidered for release, following interaction studies with *L. appendiculatus* which could demonstrate a synergistic effect of herbivory, resulting in significant damage to *S. platyphylla*. While individual *L. lutulentus* weevils do not inflict considerable damage to *S. platyphylla*; they can occasionally obtain attain high densities in their native range (G. martin, pers. comm.). Thus, for the control of *S. platyphylla* within South Africa, the release of multiple control agents, specifically *L. frontalis* and *L. appendiculatus*, could lead to greater success, when compared to the release of only one agent.

4.5. Future studies

While the biology of *L. frontalis* is known, an understanding of its host-specificity is required prior to its potential release within South Africa. To ensure the accuracy of host-specificity results, different life stages of candidate control agents should be tested on whole plants. However, getting *L. frontalis* to develop on living potted *S. platyphylla* individuals, has been challenging because oviposition and larval development occur below-ground. Additionally, having more information that could assist mass-rearing efforts should be investigated. Such information could include determining a sex ratio that yields greater levels of fecundity.

4.6. Pre-release surveys

Although the majority of biological control programmes do not conduct any quantitative pre-release surveys prior to agent releases, such surveys are extremely important for the overall evaluation of success of any management programme (Morin *et al*., 2009). Pre-release data can allow for better synchrony of releases of agents, but, more important to the broader outcome, they provide greater accuracy of assessment to how successful control efforts have been (McClay, 1995). Moreover, they can enable biocontrol practitioners the ability to determine possible limiting factors to agent releases within different sites (Morin *et al*., 2009).

For the purposes of long-term monitoring of *S. platyphylla* within South Africa, this study has provided management efforts with baseline data that outlines how *S. platyphylla* populations act without the presence of any biocontrol agents. Moreover, more pre-release data has been acquired by other researchers as well (Kwong *et al*., 2017; Ndlovu *et al*., 2020). After the release of any agents within South African populations, their impact on growth as well as both forms of reproduction can not only be assessed through post-release evaluations, but importantly, this information can be compared against these pre-release surveys to accurately assess the influence the released biocontrol agents are having on populations of *S. platyphylla* (Clay & Balciunas, 2005). It would be ideal to have pre-release evaluation data from a range of *S. platyphylla* populations that experience both different habitat types as well as climates. This could enable future post-release data to be compared back to pre-release data from similar sites, even without the exact site itself having been surveyed for pre-release data. Even if such surveys were only conducted seasonally, this information could be valuable not only to investors but also to management co-ordinators.

Typical biological control programmes heavily skew their investment of time and resources towards the search for natural enemies and the testing of candidate control agents for their suitability for the host species, whereas, comparatively fewer investments are made towards assessing the effectiveness of candidate biocontrol agents in supressing the target species, both pre- and post-release (McClay & Balciunas, 2005). Post-release evaluations are particularly important for the continual improvement of biocontrol programmes as they allow practitioners to slightly adjust components of management plans through the novel information acquired. For example, once post-release evaluations are made and compared back to baseline pre-release evaluation data, biocontrol practitioners can determine if other agents may be required or the inclusion of alternative management practices could be needed to improve overall successes (Morin *et al*., 2009). Moreover, quantitative data displaying successes of biocontrol programmes can help to improve the public opinion of biological control while also maintaining funding from investors (Morin *et al*., 2009).

4.7. Conclusion

Mechanical and chemical control of *S. platyphylla* do not provide sustainable control over a long period of time as they also come with high costs and repeated applications (Ndlovu *et al*., 2020). Along with the non-target concerns chemical control options come with, herbicide resistance is another consideration, and there are concerns that within Australia, some populations of *S. platyphylla* are developing resistance to the herbicides being applied to them (Clements *et al*., 2018), something that still needs to be investigated within South Africa. This study has shown that tubers are found within almost all *S. platyphylla* sites in South Africa and their presence is not affected by variable climates or habitat types. Moreover, it was shown that adult *L. frontalis* provides significant levels of damage to both above- and below-ground *S. platyphylla* structures. However, more work is required to determine the ability of *L. frontalis* to develop on inundated *S. platyphylla* plants as well as to conduct host-specificity tests. With these findings, *L. frontalis* should be able to provide a valuable option for the management of *S. platyphylla* within South Africa.

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