Managing the invasive aquatic plant *Sagittaria platyphylla* (Engelm.) J.G. Sm (Alismataceae): problems and prospects

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By

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Abstract

Sagittaria platyphylla (Engelm.) J.G.Sm. (Alismataceae), commonly known as Delta arrowhead, is an invasive aquatic macrophyte native to southern United States of America (USA) that has become a serious weed in freshwater systems in South Africa, New Zealand, Australia, and recently China. 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 listing required mechanical and chemical control methods to be implemented by the South African National Biodiversity Institute's (SANBI), Invasive Species Programme (ISP), with the aim of eradicating the weed. Despite the eradication efforts, by 2016, the weed was recognized as one of the country's top 10 worst and fastest spreading invasive alien plants. Since its introduction in 2008, the plant has spread both within and between sites in South Africa, increasing from one site in 2008 to 72 sites by 2019. Once introduced into lotic systems, the plant spread rapidly downstream, in some cases up to 120km within six years, with an average of 10 km per year. Extirpation over the last ten years was only possible at a limited number of sites. Under the current management approach, the invasion is foreseen to spread to new sites within a 5 km radius of the current populations. Due to the failure of conventional control mechanisms, biological control is currently being considered as a potential control option.

Four potential biological control agents are under investigation, but none have been released. Amongst them is the fruit and flower feeding weevil *Listronotus appendiculatus* Bohm. (Coleoptera: Curculionidae) which showed most potential as a suitable biological control agent. This study demonstrated that *L. appendiculatus* herbivory negatively influenced the overall fitness of *S. platyphylla* by reducing the plant's growth rate and above ground biomass. *Listronotus appendiculatus* herbivory also reduced the plant's size and the potential to kill adult plants. Most importantly, *L. appendiculatus* larval feeding damage significantly reduce viable-germinating seeds, the weed's primary dispersal mechanism. Therefore, a biological control programme is advised to be integrated within the current management plan.

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CHAPTER 1:

GENERAL INTRODUCTION

1.1 INVASIVE AQUATIC PLANTS IN SOUTH AFRICA

Invasive alien plants pose one of the greatest threats to the world's biodiversity and ecosystem services and have significant economic costs (Foxcroft *et al.,* 2017; Henderson & Wilson, 2017). They cause harm by altering ecosystems, out-competing native species for resources and altering genetic diversity (McGeoch *et al.,* 2010; Bellard *et al.* 2016). The economic costs incurred are the result of management efforts, as well as lost production (Van Wilgen *et al.* 2011; Pratt *et al.* 2017). South Africa is no exception, with nearly two million hectares of land invaded by alien plants. This is estimated to cost the country ZAR 6.5 billion (US$ 445.96 million) in ecosystem services per annum (Van Wilgen & De Lange, 2011; Clusella-Trullus & Garcia, 2017). There are currently 773 alien plant taxa and 87 000 records of species atlased in the South African Plant Invaders Atlas (SAPIA), with an increase of 50% plants per quadrant from 2006-2016 (Henderson & Wilson, 2017). Most of these invasions have higher infestations along the country's coastal belt and spread gradually inland (Henderson, 2007; Henderson & Wilson, 2017). In addition to terrestrial weeds, South Africa hosts several the world's worst invasive aquatic plants (Hill, 2003; Hill & Coetzee, 2017).

Invasive aquatic plants are exotic plants that complete their life cycle in water, perpetually wet places or seasonal, inundated areas, and are grouped as: free floating plants (e.g. red water fern, *Azolla filiculoides* Lam. (Azollaceae)); floating attached plants (e.g. Mexican water lily, *Nymphaea mexicana* Zuccarini (Nymphaeaceae)); submerged or emergent feathery plants (e.g. parrot's feather, *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae)); submerged, not feathery (e.g. Brazilian waterweed, *Egeria densa* Planch. (Hydrocharitaceae)); and emergent broad leaved (e.g. pickerelweed, *Pontederia cordata* L. (Pontederiaceae)) (Henderson & Cilliers, 2002). Most aquatic invasive plants are introduced through the horticulture and aquarium trades (Martin & Coetzee, 2011; Hill & Coetzee, 2017). For example, *Pontederia* *crassipes* (Mart.) Solms (Pontederiaceae), commonly known as water hyacinth, was introduced in South Africa for its aesthetic and horticultural value while *E. densa* was introduced for aquaria use (Martin & Coetzee, 2011). The invasion success of aquatic plants in South Africa is promoted by the lack of co-evolved natural enemies and the presence of nutrient (high nitrate- and phosphate-) enriched waters from pollution associated with urbanization (Colautti *et al.,* 2004; Hill & Coetzee, 2017). Additionally, manmade impoundments that alter hydrological flow further promote population build-up of invasive aquatic plants (Hill & Coetzee, 2017). These environmental conditions aid invasive aquatic plants to rapidly establish and grow at an exponential rate in South Africa (Coetzee & Hill, 2012).

When invasive aquatic plants spread and become dominant within an aquatic system, they impact their introduced range in four negative ways: ecological impact, economic impact, public health and social impact. Ecological impacts occur when invasive aquatic plants affect local biodiversity and alter ecological processes (Chamier *et al.,* 2012). For example, Midgley *et al.* (2006) demonstrated benthic invertebrate communities and algal mass to be significantly lower under *P. crassipes* mats than under water hyacinth-free zones; while Coetzee *et al.* (2014) demonstrated how macroinvertebrate biodiversity in a conserved area infested by *P. crassipes* was negatively impacted by the presence of water hyacinth, because water hyacinth is known to alter water clarity, reduce phytoplankton production, and change contaminant concentrations, such as dissolved oxygen, nitrogen, phosphorous, and heavy metals (Villamagna & Murphy, 2010). Other forms of ecological impacts include formation of surface canopies that block sunlight from reaching the native submerged macrophytes and macro-invertebrates, reduction in water flow, surface water availability and water quality, and destruction of native food webs through habitat alteration (Henderson & Cilliers, 2002; Molnar *et al.,* 2008; Broadhurst & Chong, 2011; Foxcroft *et al.,* 2017).

Economic impacts occur when the impacts caused by invasive plants lead to economic loss and through management costs. The Department of Water Affairs and Forestry (DWAF) established the national ‘Working for Water’ (WfW) programme in 1995, now known as the National Resources Management (NRM) Programme of the Department of Environmental Affairs (DEA), to manage and control invasive alien species (the programme is still referred to as ‘WfW’ and ‘WfW’ will be used throughout this document) (Wilson *et al.,* 2013; Henderson & Wilson, 2017). According to van Wilgen (2009), the programme is the biggest and most ambitious conservation project in Africa. The initial grant set to clear 750,000 ha per year for 20 years was R 25 million (US$ 1.72 million) (Bek *et al.,* 2017). Over the years, the programme has grown with spending on control operations estimated to be R3.2 billion (US$ 219. 49 million) and an estimated annual budget of R500 million (US$ 34.29 million) for clearing invasive plants (Chamier *et al.,* 2012; Van Wilgen *et al.,* 2012). Historical records show that South Africa spent between R10 million (US$ 685918) and R15 million (US$ 1.03 million) on clearing aquatic weeds between 1986 and 1999 (Van Wyk & Van Wilgen, 2002), and later on, Turpie (2004) found that the cost of controlling aquatic weeds was estimated to be between R80 million (US$ 5.49 million7351) and R200 million (US$- 13.72 million) per catchment cleared. Recently, Maluleke (2019) evaluated the economics of chemical and biological control methods on four aquatic weeds (*Pista stratiotes* L. (Araceae), *Salvinia molesta* D.Mitch. (Salviniaceae), *Azolla filiculoides Lam.* (Azollaceae) and *M. aquaticum*) in South Africa. The results estimated the management cost of biological control on four aquatic weeds to be R7, 8 million US$ 53. 50 million), where else management costs of chemical control by boat application to be R149, 5 million (US$ 10.25 million), pick-up truck application to be R268.2 million (US$18.3 million) and knapsack application to be R881, 7 million (US$ 60.48 million).

Social impacts linked to socio-economic activities occur when invasive aquatic plants threaten communities near infested water bodies. For example, *P. crassipes* infestations on Lake Victoria and Lake Tana obstructed fishing activities by entangling boat propellers and blocking boats from reaching fishing grounds, and reducing the feeding grounds of fish (Patel, 2012; Asmare *et al.,* 2017). The infestations resulted in reduced fish catches which affected not only the poorest communities who relied on fishing activities, but also affected sustainable development in those regions (Asmare *et al.,* 2015). Fish catch in those communities serve as a source of direct cash for more than 5400 fishers in Lake Tana alone which was lost due to *P. crassipes* infestations (Asmare *et al.,* 2017). Additionally, large macrophyte infestations destroy natural landscapes of invaded areas, which in turn reduce the potential of tourism attraction and leisure activities (McCormick *et al.,* 2009).

Finally, invasive aquatic plants also have secondary impacts on public health by forming surface canopies which host insects that host pathogens which cause diseases. An increase of vector borne diseases (malaria, dengue fever and elephantiasis) in Sri Lanka was linked to *S. molesta* invasion which provided breeding grounds for mosquitoes (Chamier *et al.* 2012). Another example is invasion of *Nasturtium officinale* W.T. Aiton (Brassicaceae) which provides habitats for parasites and diseases such as bilharzia-carrying snails (Madsen, 2005).

Due to the serious ecological impacts and economic consequences that invasive aquatic plants have in South Africa, national policy frameworks and legislations were set up to conserve and protect South Africa's biodiversity from invasive plants.

1.2 *ENVIRONMENTAL LEGISLATION OF INVASIVE AQUATIC PLANTS IN SOUTH AFRICA*

In South Africa, the National Environmental Management: Biodiversity Act (NEM: BA) regulations were promulgated in 2004, which legislate that all invasive plants must be contained and destroyed according to their stage of invasion (Poona, 2008). The Conservation of Agricultural Resources Act, Act 43 of 1983 (CARA) provided NEM: BA with a list of three legislated categories used to describe the invasion stage and management options of identified invasive plant species. The categories are:

- **Category 1a** (prohibited): Invasive species that cannot be grown or kept legally. These species are immediately prioritized for eradication. Permits are not issued. Example: *Sagittaria platyphylla* (Engelm.) J.G. Sm (Alismataceae) (Delta arrowhead)
- **Category 1b** (prohibited/destroyed, if in possession/under control): Invasive species that must be immediately prioritized for eradication. These species have high invasive potential. Permits are not issued. Example: *P. cordata*
- **Category 2** (permit required): Invasive species that are recognized as problematic species with commercial and/or beneficial function. These species can be grown in controlled populations with permits. Example: *N. officinale*
- **Category 3** (prohibited): Invasive species that are kept as ornamentals plants but cannot be grown within 30 meters of watercourse. Example: Pink porcelain lily, *Alpinia zerumbet* (Pers.) B. L. Burtt and R. M. Smith (Zingiberaceae)

- **The 'Species Under Surveillance–Possible Eradication or Containment Targets' (SUSPECT) list**: Invasive species that are not listed under NEM: BA but are identified as potential risks (Wilson *et al.,* 2013). Example: *Spartina alterniflora* Loisel. (Poaceae).

In 2014, the regulation on Alien and Invasive species (A & IS regulations 2014) were published in terms of Section 97(1) of the NEMBA, Act 10 of 2004 (Wilson *et al.,* 2017). With the regulations came the publication of the Alien and Invasive Species List. The Invasive Species List identifies taxonomic identities of introduced invasive species in the country and assigns a legislated category based on their invasive stage and location. The WfW programme initiated the South African National Biodiversity Institute (SANBI)'s Early Detection and Rapid Response (EDRR) Programme, now called the Invasive Species Programme (ISP). Their priority is to detect invasive species, assess the risk they pose to South Africa and attempt eradication of Category 1a high risk species (Wilson *et al.,* 2013). Additionally, non-governmental organizations such as the Duzi-Umgeni Conservation Trust (DUCT) and awareness programmes were also initiated to reduce invasive plants in local river systems and water bodies. These programmes not only aim to reduce existing invasions with the guidance of the NEM: BA regulations, but also try to reduce the introduction of new invasive plants and try to restore invaded systems (Pysek & Richardson, 2010). The NEM: BA, Act 10 of 2004 places all South African landowners under legal obligation to identity invasive alien plants on their property, implement correct methods to manage them and also report on the status and efficacy of the control measure (Foxcroft *et al.,* 2017).

1.3 *MANAGEMENT OPTIONS FOR CONTROL OF INVASIVE AQUATIC PLANTS*

In South Africa, and all over the world, there are different multiscale programmes and initiatives set up to prevent and manage invasive aquatic plants. These management innovations consider four important topics to guide them through the management processes, including risk assessment, pathway and vector management, early detection and rapid response, and application of eradication techniques (Pyke & Richardson, 2010).

Management strategies start with risk assessments, which aim to prevent introduction of new species that have a high risk of becoming invasive. Risk assessments act as the first line of defence which prevent invasive species from entering the country's borders (Maynard & Nowell, 2009). This includes detecting invasive plants species through biosecurity measures such as pre-border screening in major transport hubs (Molar *et al.,* 2008; Keller & Kumschick, 2017). Multiple threats invasive plant introduction exist in South Africa given that South Africa shares land borders with six African countries and has a coastline stretching more than 2,500 kilometres (Faulkner *et al.,* 2017). Pathway and vector management aim to prevent the introduction of new invasive plants by identifying and targeting high-risk pathways most sensitive to the introduction of new invasive plant (McGeoch *et al.,* 2016). A study by Martin & Coetzee (2011) identified pet stores, aquarists, and the internet trade as major modes of introduction of prohibited invasive macrophytes not permitted for import by the NEM: BA, Act No. 10 of 2004, into South Africa.

Once invasive plant species are identified and detected, management approaches such as eradication are taken in response to the threat. Detecting and treating invasive plants in their earlier stages with the intent of eradicating them is the best control option used to control smaller populations before spread occurs (Olckers, 2004; Maxwell *et al.,* 2009; DiTomaso *et al.,* 2010; McGeoch *et al.,* 2016). Different control methods are used for invasions that are well established in their invaded range. South Africa has adopted four control methods to manage established populations, namely: herbicide control, manual and mechanical control, biological control, and integrated pest management. Under NEM: BA regulations, invasive plant species under category 1a are mainly assigned herbicide and manual control and biological control is usually assigned for managing category 1b invasive plant species which are already well established in the country (Zachariades *et al.,* 2017). The advantage and disadvantages of using each control measure on aquatic invasive plants is discussed.

1.3.1 Herbicide Control of Invasive Aquatic Plants

Herbicide control is a management option that aims to control invasive species by directly applying and spraying registered herbicides. Herbicides for aquatic plants can be applied as foliar or submersed treatments with contact or systemic activity (Netherland, 2009). Herbicides with contact activity only damage the plant tissues they come in contact with, while herbicides with systemic activity are slowly distributed throughout the plant, killing the plant completely (Petty, 2005; Hoare, 2014). During application of foliar herbicides, herbicides are mixed with water and adjuvants, and directly applied or sprayed onto emergent and free-floating aquatic plants, while herbicides for submerged aquatic weeds are applied to the entire waterbody. In South Africa, there are two recommended herbicides registered for aquatic invasive plants, Diquat (dibromide) and glyphosate (ammonium; isopropyl amine; potassium and sodium). Diquat and glyphosate are both foliar treatments, diquat has contact activity and glyphosate has systemic activity (Carey *et al.,* 2016).

The advantages of herbicide control of aquatic plants include:

- Effectively killing targeted plants in a short period of time, given that the correct registered herbicide and concentration dosage is applied to targeted invasive species (Van Wyk & Van Wilgen, 2002).
- Is less labour intensive when compared to mechanical control (Motooka *et al.,* 2003).
- Can be integrated with biological control (Jadhav *et al.,* 2008; Byrne *et al.,* 2010).

The disadvantages of herbicide control of aquatic plants include:

- Residue build up from additives can be harmful to the environment (Adair *et al.,* 2012; Kwong, 2014).
- Contamination of water systems that may threaten human and animal health (Hoare, 2014).
- Killing only the exposed plant material above the water level and not the plant material below the water level during spraying application (Allendorf & Lundquist, 2003; Petty, 2005), which promote the regrowth of submerged plants due to lowered plant competition (Vincent *et al.,* 2009; Weidenhamer & Callaway, 2010; Strange *et al.*, 2018).

- The high cost associated with specialized training of people to apply herbicides (Rocha-Ramirez *et al.,* 2007).

Herbicides effective only on foliage such as *P. crassipes,* have led to re-infestation in some treated sites through isolated plants missed during herbicide application and from seed germination following sinking of the mat (Hill & Olckers, 2001).

1.3.2 Manual and Mechanical Control of Invasive Aquatic Plants

Manual and mechanical control of aquatic plants is a management option that aims to control invasive species by physically or mechanically removing them (Vincent *et al.,* 2009; Rebek *et al.,* 2012). Manual removal and mechanical control are mostly successful in controlling aquatic plants in small populations, where all plant material is completely removed (Vincent *et al.,* 2009; Nelson, 2009).

The advantages of manual and mechanical control of aquatic plants include:

- Immediately opening waterbodies for fishing and recreation activity when correct procedures under appropriate conditions are used (Villamagna & Murphy, 2010).
- Improving water flow which increases dissolved oxygen in the water body (Perna & Burrows, 2005) and since no chemicals are used, there is no contamination of water with chemicals, as opposed to herbicide control (Hoare, 2014).
- Creating high employment opportunities through invasive plant removal (Bek *et al.,* 2017; Hill & Coetzee, 2017).

The disadvantages of manual and mechanical control of aquatic plants include:

- The high cost associated with disposing of harvested material, and cost of machinery and equipment (Villamagna & Murphy, 2010).
- The use of large machinery damaging river banks and being non-selective to desirable aquatic organisms (Gettys, 2014).

- The method being time consuming and labour intensive (Motooka *et al.,* 2003; Nelson, 2009; Hill & Coetzee, 2017).
- The method being unsuitable or impractical for larger infestations (McConnachie *et al.,* 2003).

There is also a high risk of spreading the infestation by leaving plant fragments that can regenerate and re-establish in sites, and movement of plants caught in machinery, vehicles and shoes used during the removal of the plants (Hoare, 2014). Furthermore, some invasive water plants that can reproduce asexually are stimulated by mechanical control to produce more vegetative propagules (Broadhurst & Chong, 2011). *Egeria densa* and *Hydrilla verticillata* Royle. (Hydrocharitaceae) are examples of aquatic plants known to regenerate through fragments and increase in infestation under mechanical control (Dayan & Netherland, 2005).

1.3.3 Biological Control of Invasive Aquatic Plants

Biological control is a management option that aims to control invasive species using their natural enemies (McClay & Balciunas, 2005; Moran *et al.,* 2013; Westcott *et al.,* 2013). When invasive weeds are introduced in their new range, natural enemies that regulate the weed's population level are usually left behind. Without the natural enemies, invasive weeds become more abundant and widely distributed in their introduced range compared to their native range (Kwong *et al.,* 2017). This is supported by the Enemy Release Hypothesis (ERH) which states that plant species in introduced regions increase in distribution and abundance due to the lack of regulation by herbivores (Keane & Crawley, 2002; Hierro *et al.,* 2005). Biological control, therefore, intentionally imports the weed's natural enemies from the weeds native range and introduce them into the introduced region where weeds are introduced in order to regulate the weeds population.

South Africa started practising classical weed biological control as a management strategy on invasive weeds in 1913 with the release of *Dactylopius ceylonicus* Green (Hemiptera: Dactylopiidae) to control *Opuntia ficus-indica* (L.) Mill. (Cactaceae) (sweet prickly pear) (Moran *et al.* 2013). Before the biological control programme was implemented, sweet prickly pear had invaded 1 million ha of land, predominantly in the Karoo regions of the Eastern Cape Province (Moran, 1978). *Dactylopius ceylonicus* was able to bring the weed to less than 5% of the original infestation (Zimmermann *et al.,* 2009), and it is now regarded as being under complete control (Moran *et al.,* 2003). In South Africa, 77 of the 773 alien plant species recorded in SAPIA have been targeted for biological control, but only 13 are regarded as being under complete control (Henderson and Wilson, 2017). Ninety-three control agents including insects, plant pathogens and mites have established on 59 invasive alien species (Zachariades *et al.,* 2017). Thirteen biological control agents (11 insects, one mite, and 1 pathogen) have been released on the six worst invasive aquatic plants in South Africa (Hill & Coetzee, 2017). They have resulted in complete control of four of the big five floating aquatic species namely: *Pistia stratiotes* L. (Araceae) (water lettuce), *S. molesta*, *M. aquaticum* and *A. filiculoides* (Henderson & Wilson, 2017; Hill & Coetzee, 2017; Zachariades *et al.,* 2017).

The most successful biological control programme on aquatic invasive plants in South Africa is the control of *A. filiculoides* by the biological control agent, *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae), which was released in 1997, on a one hectare dam in a bird sanctuary in Pretoria (Hill, 2003). The dam was completely covered with *A. filiculoides* and in just two months, the entire mat had collapsed and sunk because of the weevil (Hill, 2003). Currently *A. filiculoides* is no longer listed as one of the top five most important aquatic plants (Hill & Coetzee, 2017), and it has been suggested that it is removed from NEM: BA as it is no longer seen as a threat to South African fresh water systems (Zachariades *et al.,* 2017).

Stages of a biological control programme

A typical biological control programme follows a series of sequential steps. Most biological control programmes start with identifying the target weed and exploration of potential control agents in the native range (Briese, 2000). Surveys for potential agents are one of the most important aspects of a biological control programme (Van Klinken & Raghu, 2006). The aim of this study is to determine the most promising control agent that has the potential to significantly damage the invasive weed, and avoid the cost of testing unsuitable herbivorous arthropod species associated with the weed (McFadyen, 1998; Paterson *et al.,* 2014). Potential control agents are then imported into quarantine facilities where they undergo host-specificity and host-range studies (Kenis *et al.,* 2017). Host-specificity studies determine the potential control agent's feeding group, ranging from a specialist to a generalist feeder, while host-range studies sum up the number of plant species (relative to the host plant) suitable for the potential control agents (Van Driesche *et al.,* 2010). A good biological control agent reduces the plant's competitive ability, damages flowering heads, plant tissue and/or crown (Oberholzer *et al.,* 2007). Host-specificity and host-range studies predict the potential biological control agent's capability in the field, which decreases the potential risk of releasing a control agent that poses a threat to indigenous plants and crops (Murdoch *et al.,* 1985). Control agents that prove to be host specific and damaging to the invasive plants are released after permits are granted for their release. In South Africa, the permission to release a control agent is granted by the Department of Agriculture, Forestry and Fisheries and the Department of Environmental Affairs. Once the control agents are approved for release, they are mass reared in mass rearing facilities, and released into invaded habitats (Morin *et al.,* 2009).

Several assessments have shown biological control to have good returns on investment. These include returns on water supply in the system, long-term ecological safety, biodiversity, recreational activities and the reduced management costs of 20% (R1.38 billion) (van Wilgen & De Lange, 2011; Fraser, 2016; Zachariades *et al.,* 2017). In South Africa, alien plant taxa under biological control programmes with agents released prior to 2000 halved their rate of spread and showed a decrease in cover over the years, especially aquatic plants (Henderson & Wilson, 2017).

The advantages of biological control of aquatic plants include:

- Excellent return on investments with low management cost over the long term (Van Wyk & Van Wilgen, 2002; Hill & Coetzee, 2017).
- Is regarded as the most environmentally friendly and self-sustainable control method (Zimmermann *et al.,* 2004; Moran *et al.,* 2013; Schwarzländer *et al.,* 2018).
- Protects threatened indigenous species by being host specific to the targeted species and has minimal risk on non-target species (Suckling & Sforza, 2014; Zachariades *et al.,* 2017), and can access areas that are difficult to reach by other methods.

The advantages of biological control of aquatic plants include:

- The efficacy of the control agent on desired invasive plants can be negatively affected by insects' biology and environmental factors that may act against it (Grevstad, 1999; Zachariades *et al*., 2017).
- The limitation of available biological control agents for all targeted species (Ghosheh, 2005).
- Inaccurate release strategies resulting in failure of control agents to establish. For example, releasing a small number of control agents with a small number of releases (Hopper & Roush, 1993).
- The longer period of time for a control agent to show visible reduction in population size (Zachariades *et al.,* 2017).
- Some infestations staying as a part of the system under biological control instead of being under complete control (Moran *et al.,* 2013; Hoare, 2014).

It is crucial to understand that the aim of biological control is not to result in eradication, but to keep the invasive weed populations at low levels thereby reducing the impact on the ecosystem they inhabit (Ghosheh, 2005; Hill & Coetzee, 2017).

1.3.4 Integrated Pest Management of Invasive Aquatic Plants

Integrated Pest Management (IPM) is a long-term management programme designed to combine or sequentially use more than one control method to effectively manage invasive plants (DiTomaso *et al.,* 2010). IPM manages invasive plants at an ecosystem-centred level which not only targets the invasive plant, but includes information gathered from pest biology and the environment in order to control the invasive plant using the most cost effective control design that poses the least possible risk to the ecosystem (Masters & Sheley, 2001; DiTomaso *et al.,* 2010). An example of IPM in South Africa is the integration of biological and herbicide control against the *P. crassipes*. In the initial stages of IPM development for *P. crassipes*, the applied herbicide concentration affected the biological control agents' efficacy by either killing them (Hill *et al.,* 2012) or through habitat destruction (Ainsworth, 2003). A study by Jadhav *et al*. (2008) identified a retardant dose of glyphosate (0.8% concentration) to be integrated with the biological control of water hyacinth. This allowed the weed to be suppressed by the herbicide but allowed the biological control agents to survive as well. In some cases, the agents move from herbicide treated plants onto plants missed by the herbicide which increased the numbers of biological control agents on the untreated plants (Jadhav *et al.,* 2008). The identified retardant dose (sub-lethal dose) of glyphosate is now recommended in an integrated control for *P. crassipes* without killing *Neochetina eichhorniae* Warner and *N. bruchi* Hustache (Coleoptera: Curculionidae) (Jadhav *et al.,* 2008). Tipping *et al*. (2017) reported that in the USA, integrating the two methods suppress *P. crassipes* infestations more than each control method would have alone. A study by Van Wyk & Van Wilgen (2002) compared the cost-effectiveness of all four control methods of water hyacinth South Africa, showed that IPM is more cost-effective at R277/ha, while herbicide control is five times less cost effective compared to either biological or IPM control at R1 481/ha. Managing invasive plants by IPM is the best long-term solution for invasive aquatic plants management (Hill & Coetzee, 2017; Zachariades *et al.,* 2017).

Design of a successful management programme, management strategies require a good understanding of the plant species' biology, its effect on the ecosystem and its invasion processes that drive it to be a successful invader (Gettys *et al.,* 2014; Martin *et al.,* 2018). Understanding the theory behind a plant's invasion can significantly assist in anticipating the risk species with the potential to be successful invaders, so that the vulnerable stage is targeted to prevent further invasion.

1.4 *INVASION THEORY OF INVASIVE WEEDS*

Managing and assessing invasive plants is difficult because of their impact ecosystems and humans at different spatial and temporal scales (Millennium Ecosystem Assessment, 2005). Due to the complexity of managing invasive species, invasion biology and ecology is explored as a field of study, which aims to understand the aspects of biological invasion, highlighting the processes of species invasion and stages of invasion. These findings are used to predict and prevent new invasions and help eradicate and manage invasive species according to their importance and stage of invasion (Pysek & Richardson, 2010).

1.4.1 Biological Invasion Theory

Several different conceptual frameworks that explore invasion processes have been proposed to better understand and explain the biology of invasive species. (Williamson, 1996; Richardson *et al.,* 2000; Blackburn *et al.,* 2011). Blackburn *et al*. (2011) proposed a biological invasion framework that effectively merge two previously proposed invasion frameworks. This was done so that the unified framework could be explored by both plant and animal ecologists. The merged frameworks were originally proposed by Richardson *et al.* (2000) to explore plant invasions and Williamson's (1996) invasion framework used to explore animal invasion. Richardson *et al*. (2000) describe invasion as a series of barriers an alien species has to overcome to become either naturalised or invasive, while Williamson (1996) describe invasion as a series of stages an alien species passes through from its being native to being invasive. The unified framework can be applied to all human-mediated biological invasions and is divided into a series of stages separated by barriers that a must species overcome to get to the next stage (Fig. 1.1).

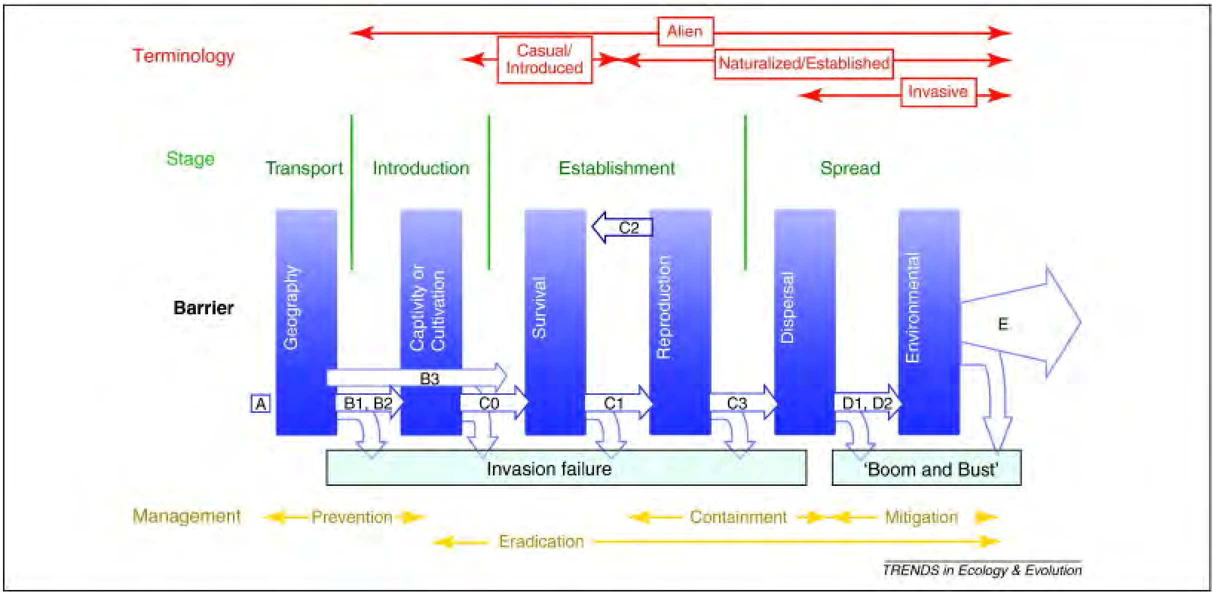


Figure 1. 1: The proposed unified framework for biological invasion (Blackburn *et al.,* 2011).

According to Blackburn's framework, the first stage of an alien species invasion process is the transport stage in which an alien species must overcome a geographical barrier. The worldwide increase in trade, tourism and transport has resulted in increased movement of goods and people, which facilitates the movement of propagules of alien species to non-native regions (Faulker *et al.,* 2017). Once transported to a new geographic range, an alien species must overcome geographic and captivity barriers that may restrict it from further invasion (Blackburn *et al.,* 2011). The introduction stage is mostly related to animal and plant species that are kept in captivity or are cultivated before they are directly released (or escape) into the new environment. According to Blackburn *et al*. (2011), most alien plant species skip the introduction stage and are directly released to the new environment, and go straight to the third stage, namely the establishment stage where they overcome survival and reproduction barriers. In the establishment stage, individuals that survived the earlier stages must persist and reproduce successfully until they establish and maintain a self-sustaining population (Blackburn *et al.,* 2011). The final stage in Blackburn's framework is the spread stage, where an alien species becomes fully invasive through dispersal by overcoming the environmental barriers (Fig. 1.1). By overcoming these barriers, the invasive species must have a high propagule pressure and be able to spread and maintain populations in their new found environments. Propagule pressure is an 'event-level' characteristic that is the total number of individuals and the number of times species are introduced to form a new founding population that can sustain itself (Lockwood *et al.,* 2005).

Blackburn *et al*. (2011) framework also guides managers with the implementation of appropriate management interventions depending on each invasion stage (Fig. 1.1). Alien species that are between the transport and introduction stage are assigned prevention interventions, which aim to prevent high risk alien species from being introduced in the first place. The aim of prevention interventions is to remove and destroy high risk species that may lead to harmful invasions, for example, running pre-border weed risk assessment in airports (Wittenberg & Cock, 2001). Alien species transitioning between the introduction stage towards the establishment stage and those in the spreading stage are assigned eradication interventions. The aim of eradication interventions is to destroy alien species by using different control methods such as herbicide application, mechanical and manual removal (Wittenberg & Cock, 2001). Alien species at the establishment stage are assigned containment interventions, which aim to restrict the spread by containing the population in a defined geographical range (Wittenberg & Cock, 2001). Finally, alien species at the spread stage are assigned mitigation interventions, which focus on conserving affected native species (Wittenberg & Cock, 2001).

1.4.2 The Invasion Curve

The invasion curve demonstrate how an alien species invasion progresses over time through three typical phases, starting from the point of introduction, namely: the 'lag phase' described by a slow initial growth with a relatively stable population size; followed by the 'exponential growth phase' described by the rapid exponential population growth and the invader becoming more wide-spread and abundant. Finally, the 'stationary or equilibrium phase' described by the invader's population spread reaching its maximum occupation and remaining at a plateau (Fig. 1.2) (Akins, 2012; Harvey & Mazzotti, 2014).

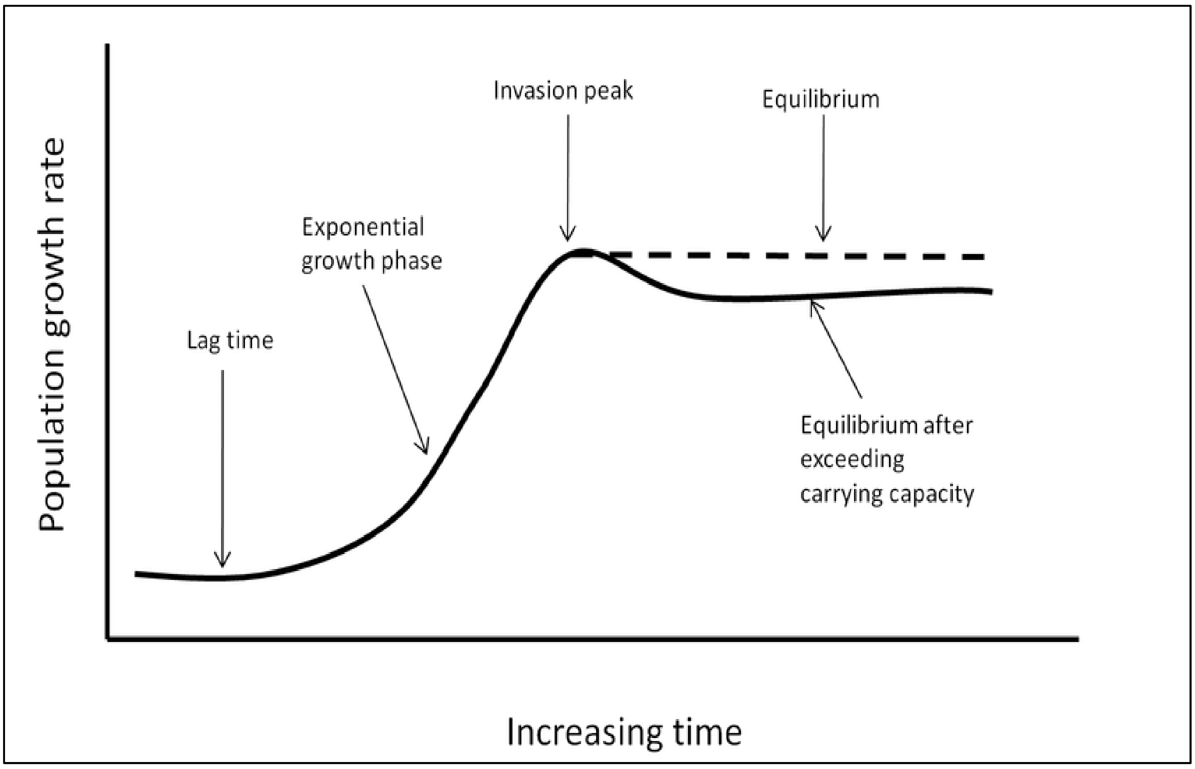


Figure 1.2: Theoretical line-plot depicting a simplified population growth curve for invasive species (Akins, 2012).

Chippendale (1991) developed a guide widely used in management for invasive aquatic plants based on the species' position in the invasion (Fig. 1.3). Chippendale (1991) suggests species prevention through pre-border risk assessments are implemented and if the invader passes through the border and is introduced into a country, detection and eradication of small localized populations is then implemented in the lag phase. As the invader approaches the exponential phase, public awareness is initiated and eradication involving manual and mechanical removal and herbicide control is applied, and intensifies as the population increases. As the population approaches the stationary/equilibrium phase, eradication is unlikely to be successful, the management efforts shift from prevention and eradication to long-term management and population suppression which may include the addition of biological control and integrated management (see also Hobbs & Humphries, 1993; Harvey & Mazzotti, 2014). Chippendale's (1991) guide also demonstrated how management costs of the control efforts increases as the area of infestation increases, which also helps managers to predict when to reduce cost on management efforts that are no longer cost effective based on the invader's invasion stage (Fig. 1.3).

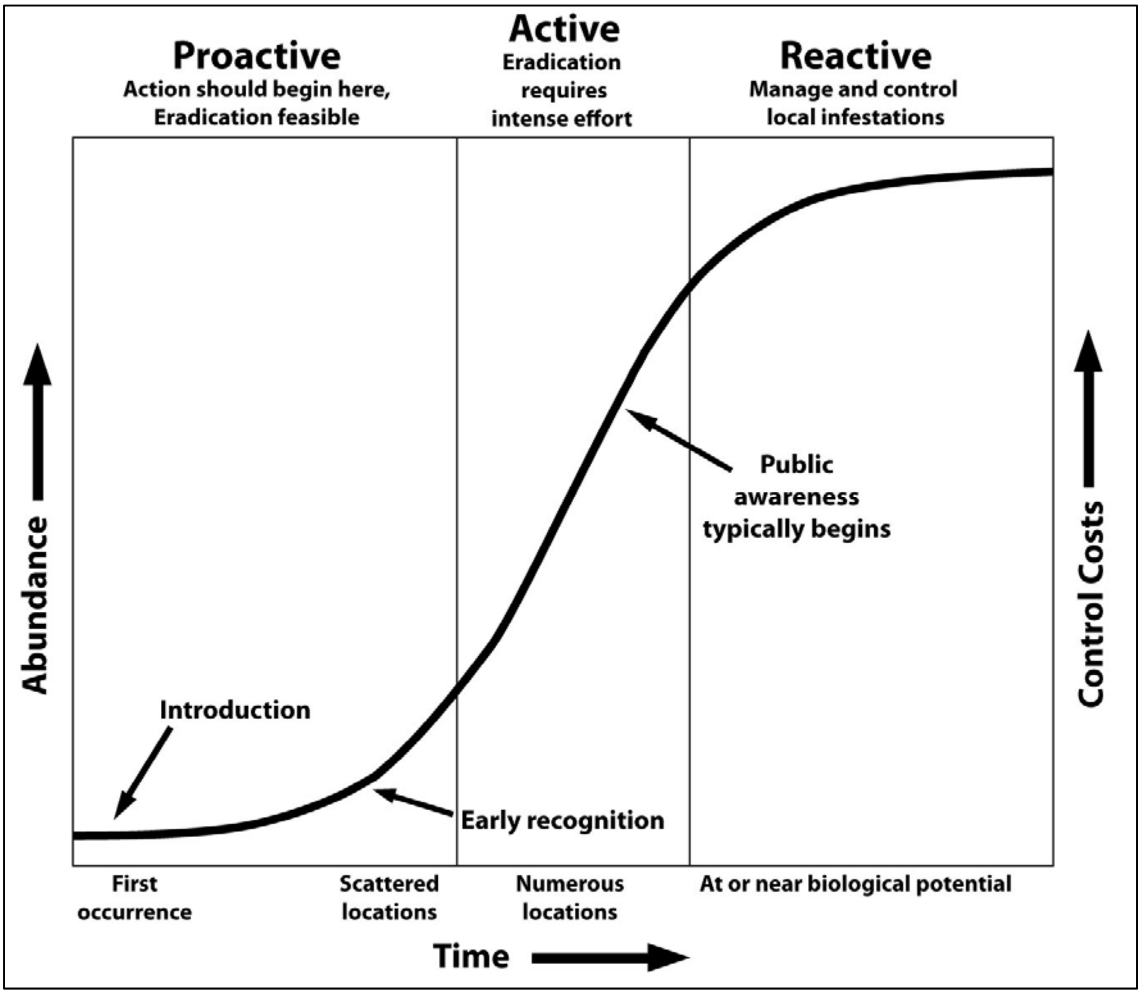


Figure 1.3: Phases of invasive species invasion and control (Chippendale, 1991).

This study looks at the invasion of the rooted macrophyte *Sagittaria platyphylla* Engelm.) J.G. Sm (Alismataceae), (Delta arrowhead) in South Africa over the last 10 years and gives insights into potential management options.

1.5 *STUDY SPECIES:* SAGITTARIA PLATYPHYLLA

1.5.1 Study Species

Sagittaria platyphylla belongs to the aquatic plant family Alismataceae, in the order Alismatales (Chapman & Dore, 2009). Globally, the Alismataceae consists of 12 genera and 81 species, occurring mostly in the Northern Hemisphere tropics and subtropics (Adair *et al.,* 2012). There are five genera and seven species in the Alismataceae family native to southern Africa, and only two species are recorded in the northern borders of South Africa (Cook, 2004). The two indigenous Alismataceae species are *Burnatia enneandra* Hochst. and *Lymnophyton obtusifolium* (L.) Miq (Martin *et al.,* 2018). Other sub-Saharan indigenous species in the Alismataceae include *Butomopsis latifolia* (D. Don) Kunth, *Caldesia reniformis* (D. Don) Makino, *L. obtusifolium* and *Wiesneria schweinfurthii* (Dalzell) M. Micheli, which are mainly found in seasonal pans, the Okavango swamps of Botswana and in the Zambezi valley of Zimbabwe (Martin *et al.,* 2018). Other cultivated and exotic (to South Africa) species in the Alismataceae found in South Africa include: *Sagittaria latifolia* Willd, *Echinodorus cordifolius* (L.) Griseb and *Alisma plantago-aquatica* (L.).

Under the South African NEM: BA, Act 10 of 2004, regulations, *Sagittaria platyphylla* is listed as a category 1a, *S. latifolia* category (the suspect) plant and *A. plantago-aquatica* as a category 1b invasive species (Wilson *et al.,* 2013; Chapter 1, section 1.2 for relevant NEM: BA categories). *Sagittaria platyphylla* and *A. plantago-aquatica* have the broadest distribution in South Africa, while *S. latifolia* and *E. cordifolius* are recorded from a few localities (Martin *et al.,* 2018).

1.5.2 Species Description

Sagittaria platyphylla can grow up to 1.5m tall (Kwong *et al.,* 2014) however, it may grow taller (pers. obs.; Fig. 1.4a). In its native range, it grows in moist soil close to water sources such as shallow swamps and slow streams (Flower, 2004). The plant is semiaquatic and can be found in two morphological growth forms, a fully submerged rosette under the water surface (Fig. 1.4c), and emergent plants above the water surface, as either broad-leaved or narrow-leaved emergent plants (Fig. 1.4 a-b) (Adair *et al.,* 2012; Kwong *et al.,* 2014). *Sagittaria platyphylla* displays phenotypic plasticity in the different types of leaves it can produce; large emergent leaves (10-48cm long and 2-10cm wide) which have long triangular petioles (in cross-section) with narrowly, egg-shaped pointed tips (Fig. 1.4a), as well as submerged leaves (50cm long and 10-25mm wide) which have flattened petioles that form a rosette shape below the water surface (Fig. 1.4c) (Kwong *et al.,* 2014). Large emergent leaf types occur in slow moving water bodies while submerged leaf types occur in deep water bodies and are often found after herbicide application (Adair *et al.,* 2012). The change in leaf morphology is dependent on environmental and management drivers. This allows *S. platyphylla* to survive in environments that have drastic water level fluctuations (Martin & Shaffer, 2005; Adair *et al.,* 2012; Kwong *et al.,* 2014).

a.

a.

Figure 1. 4: Two morphological growth forms of *Sagittaria platyphylla*: broad-leaved emergent plant above water (a), narrow-leaved emergent plant above water (b) and fully submerged rosette (c) (photograph c: Ernita van Wyk, 2018).

Sagittaria platyphylla is a monoecious plant that has both male and female flowers which flower at the same time (Kwong *et al.,* 2017b; Fig. 1.5). Whorled flowers are arranged in 2-12 groups of three near the top of the raceme (Fig. 1.5) (Adair *et al.,* 2012). Male flowers have three white petals and three sepals with round yellow stamens. Female flowers have a flattened green stigma and no petals. Flowering season is mostly during spring and autumn (Forrest *et al.,* 2011), but in South Africa, some populations are observed flowering throughout the year (Martin, unpublished data). Pollen is transferred to the female reproductive organs by generalist bees, syrphids and other small insects that are attracted to pollen (Adair *et al*., 2012). The fruits of the plant are small rounded fruitlets covered by densely arranged one-seeded segments (Adair *et al.,* 2012). Each seeded segment is flattened, winged, and consists of a single achene (Kwong *et al.,* 2014) (Fig. 1.5).

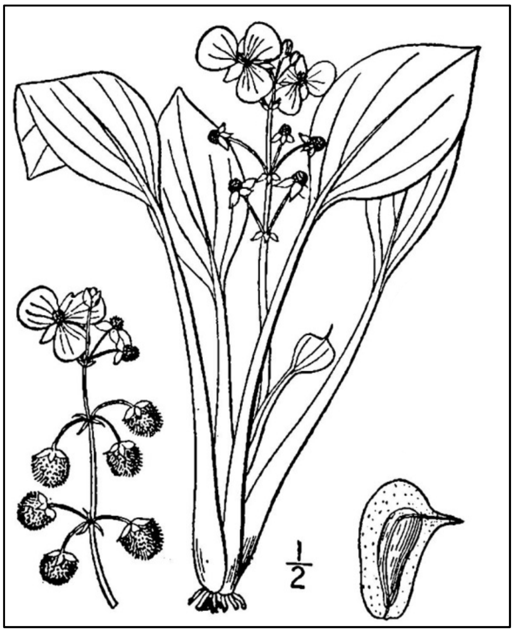


Figure 1.5: Line drawing of *Sagittaria platyphylla* showing a raceme and an achene (NL Britton and A Brown 1913, USDA-NRCS Plants Database).

1.5.3 Plant Reproduction and Dispersal Mechanism.

Sagittaria platyphylla reproduces sexually and asexually. Sexual reproduction involves the fertilization of male and female gametes that grow into achenes (Fig. 1.6a). Seeds are produced prolifically, mostly in summer and autumn (Kwong, 2012). The spread of *S. platyphylla* is assumed to be primarily promoted by the dispersal mechanism of achenes (Kwong *et al.,* 2018), which have spongy pericarp wings that provide buoyancy, allowing them to float and disperse through wind and water (Adair *et al.,* 2012). This feature allows the seeds to be transported downstream and establish long distances from founding populations (Adair *et al.,* 2012; Martin *et al.,* 2018). They also adhere to animals or are attached to mud and machinery which may also promote establishment of new populations far away from founder populations (Adair *et al.,* 2012). Furthermore, records of enhanced seed germination after being ingested by ducks has been reported (Rogers, 1983). 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 its native range in USA (Kwong *et al.,* 2017a). Additionally, the plant is observed to flower and produce fruits throughout the year but produces smaller sized fruits during winter (pers. obs.), compared to plants growing in south-eastern Australia and Queensland Australia which both have a shorter flowering and fruiting period (Stephens & Dowling, 2002; Flower, 2004).

Asexual reproduction consists of vegetative propagules, such as underground stem fragments, daughter plants (runners), stolons, and tubers (Fig. 1.6b-d) (Broadhurst & Chong, 2011). Ramets and tubers are produced at the terminal end of stolons (Adair *et al.* 2012). Tubers are fleshy, rounded outgrowths that grow from an underground stem or rhizome (Flower, 2003). They act as storage organs that store the plant's nutrients such as carbohydrates and are mostly produced just before winter (Adair *et al.,* 2012; Kwong, 2014). Stolons produced by mother plants also act as runners that can grow into new plantlets which appear as ramets. Regeneration of vegetative propagules and seed germination can occur anytime when the conditions are favourable (Broadhurst & Chong, 2011).

(a)

(b)

(c) (d)

Figure 1.6: *Sagittaria platyphylla* reproduction outputs: fruits and flowers (a), tubers (b), daughter plant/runners (c) and stolon (d). (Photographs (a & d) Ernita van Wyk, 2018).

1.5.4 Invasion history of Sagittaria platyphylla *in South Africa*

Blackburn *et al.'s* (2011) unified framework for biological invasions was selected to explore the biological invasion processes of *S. platyphylla* invasion success in South Africa.

Transport stage

Sagittaria platyphylla, was transported intentionally beyond the limits of its normal geographic range as an ornamental and aquarium plant (Martin & Coetzee, 2011; Kwong *et al.,* 2018). In the aquarium trade, it is advertised as a plant that is undemanding to keep in any sized aquarium, can take a wide range of pH parameters and can be used as a foreground plant in larger aquariums (Aqua-Fish.Net, 2014). It is sold in pet stores in South Africa (Martin & Coetzee, 2011).

Introduction stage

In South Africa, it is speculated that *S. platyphylla* was introduced through dumping of aquarium contents due to the locations of the first recorded populations (Hill *et al.,* 2018). The first recorded locations were in a pond in the Krantzkloof conservation region, KwaZulu-Natal Province followed by the second record in the Makana Botanical Gardens, Eastern Cape Province (G.D. Martin pers. comm.).

Establishment stage

The success in the establishment stage of *S. platyphylla* in South Africa could be supported by its morphological and reproductive traits as well as by ERH (Kwong *et al.,* 2018). The different growth forms also allow the plant to establish in a wider range of environments (Richards *et al.,* 2006; Gallagher *et al.,* 2015). The different reproductive mechanisms of *S. platyphylla* increase the number of reproductive outputs released in the environment, which promote establishment of small populations (Kwong *et al.,* 2018; Martin *et al.,* 2018). Additionally, South Africa's disturbed aquatic systems have high levels of nitrate and phosphate associated with agriculture, urbanization and industrial pollution (Coetzee *et al.,* 2009), and have also been manipulated to control their water flow by construction of impoundments, low-water bridges, water channels, and weirs. This has resulted in slow-moving, nutrient rich permanent waters which facilitate population build up and rapid establishment of invasive species growing at an exponential rate (Hill & Coetzee, 2017), especially *S. platyphylla*.

Additionally, in its native region, *S. platyphylla* occurs in temperate and tropical regions similar to the invaded regions in South Africa (Adair *et al.,* 2012). The success in controlling free-floating aquatic plants in South Africa has resulted in opening up new niches and nutrients to be exploited (Coetzee *et al.,* 2011), which could have promoted *S. platyphylla*'s establishment success. All the above factors are assumed to have contributed to the establishment of *S. platyphylla* in South Africa. Furthermore, new invasion sites are continuously identified during national water body surveys (L. Henderson, 2018, pers. comm.). Therefore, the aim of the study is to identify water bodies where *S. platyphylla* has fully established in the country and to update the current distribution records of *S. platyphylla* in South Africa.

Spread stage

The first record of *S. platyphylla* in South Africa was in 2008, at Krantzkloof Nature Reserve, KwaZulu-Natal Province, by Rene Glen (ex. SANBI) (Martin *et al.,* 2018). Since then, the invasion has been recorded in three South African eastern, coastal provinces, namely, KwaZulu-Natal, Eastern Cape, and Western Cape Provinces. The latest distribution map of *S. platyphylla* in South Africa has 18 sites occurring in three provinces (Fig. 1.7), yet the total number of *S. platyphylla* independent populations in South Africa is currently unknown (SAPIA, 2018). Over the years, a total of 23 independent *S. platyphylla* sites were surveyed between 2008 and 2014 (Martin *et al.,* 2018), yet new populations are continuously identified and recorded during national surveys (L. Henderson, pers. comm.). Some of the sites are in close proximity, located downstream of founder populations, indicating dispersal between and within sites.

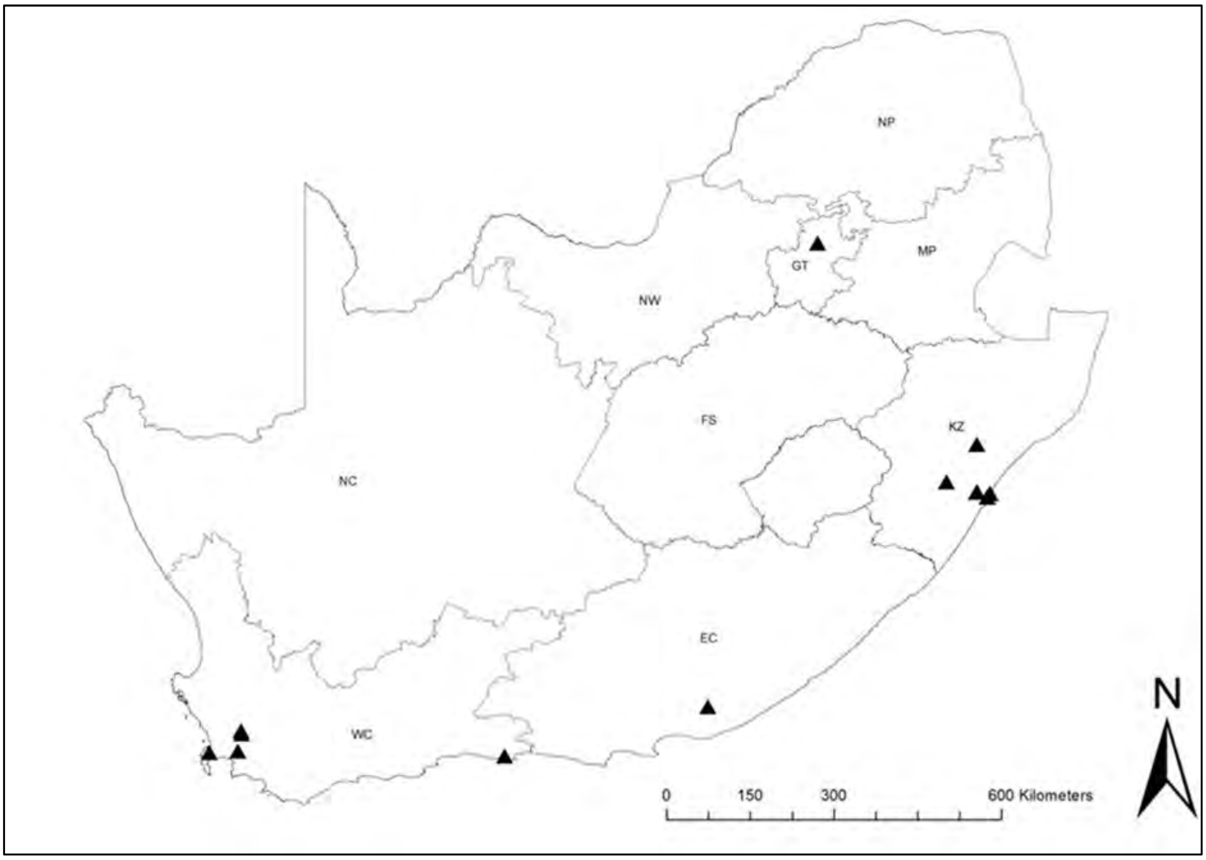


Figure 1. 7: Distribution map of *Sagittaria platyphylla* in South Africa according to the SAPIA database (Henderson, 2018).

According to Blackburn's unified framework, *S. platyphylla* has transitioned from being an alien plant transported to South Africa as an ornamental and aquarium plant, to a fully invasive species with potential individuals dispersing, surviving and reproducing at different sites across a range of different habitats (Fig. 1.1). According to the invasion curve (Fig. 1.2), the invasion stage at which *S. platyphylla* is in, is currently unknown but based on its invasion success, it has surpassed the lag stage.

Due to visible spread of *S. platyphylla*, management options were implemented as an attempt to control the invasion, soon after it was identified as a category 1a invader species under NEM: BA.

1.5.5 Management Options of Sagittaria platyphylla *in Australia and South Africa.*

In Australia, different control mechanisms have been attempted to control *S. platyphylla,* but most have been ineffective (Adair *et al.,* 2012). In South Africa, the Early Detection and Rapid Response (EDRR) programme of the South African National Biodiversity Institute (SANBI) attempted to eradicate *S. platyphylla* but was unsuccessful due to the plant's rapid re-growth after control attempts (Martin *et al*, 2018). These attempts involved herbicide field trials, mechanical control and raising awareness (SANBI, 2012). However, the effectiveness of attempted control mechanisms must be further investigated.

Herbicide control

In Australia, foliar spray herbicides have been used as an attempt to control *S. platyphylla* on different infestation types. The herbicides applied included the 360g L^{-1} glyphosate salts sprayed on populations; 2,4-D amine sprayed on channels and drains; and amitrole sprayed in drains on large populations (Chapman & Dore, 2009; (Adair *et al.,* 2012). However, the herbicides killed the above plant material but also stimulated submerged plants and tubers to grow due to reduced competition (Forrest *et al.,* 2011; Broadhurst & Chong 2011). Adair *et al.* (2012) noted that it was difficult to apply herbicides in infested areas with shallow water, and continued application in these areas increases the risk of exceeding the maximum residue specified limit under minor use permits of Australia. Furthermore, the submerged form of *S. platyphylla* and its underground reproductive organs (tubers and rhizomes) may not be affected by the application of foliar spray herbicides (Chapman & Dore, 2009). The use of these herbicides only suppressed infestations, and in the long run ended up being ineffective

(Vincent *et al.,* 2009). In addition, *S. platyphylla* has reportedly developed herbicide resistance in Australia (R. Kwong pers. comm.).

In South Africa, Arysta Life Science (2011) initiated field trials against *S. platyphylla* using Kilomax and Diquat herbicides which are recommended herbicides for aquatic species (SANBI-Ernita van Wyk, pers. comm.). Kilomax (glyphosate based) mainly attacks emergent and floating plants while Diquat kills submerged plants. Kilomax applied to *S. platyphylla* causes the plant to decay from the bottom up, leaving unsightly infested areas. Field trials resulted in initial suppression of *S. platyphylla*, which were, however, followed by regrowth (SANBI & DUCT-conv, 2017). In KwaZulu-Natal, SANBI's Invasive Species Programme (ISP) and DUCT applied Kilomax in accessible sites (SANBI & DUCT-conv, 2017). Currently, there are no registered herbicides specifically used to control *S. platyphylla* in South Africa (Martin *et al.,* 2018), and it is unknown whether South African populations have developed resistance to herbicide applications.

Manual and mechanical control

In Australia, attempts to control *S. platyphylla* manually have been attempted but deemed impossible, especially in large populations (Adair *et al.,* 2012). There is high risk of using manual and mechanical control for *S. platyphylla* as broken plant pieces, floating seeds and underground tubers left behind can regenerate and quickly re-infest systems (Forrest *et al.*, 2011).

In South Africa, manual and mechanical control of *S. platyphylla* involves extracting seedlings and smaller plants by machinery or manual digging, bagging the plant material and then burning it (M. Nxumalo, pers. comm.). In some sites, like Krantzkloof Nature Reserve, manual control is applied as an environmentally friendly management approach compared to herbicide control (M. Nxumalo, pers. comm.). Manual control of *S. platyphylla* has been effective in small, isolated and enclosed populations (M. Nxumalo, pers. comm.). However, there is no evidence that shows that manual and mechanical control has been effective in larger populations. The total cost and high risk of spreading *S. platyphylla* associated with using manual and mechanical control still needs to be investigated. Due to the ineffectiveness and running costs of mechanical and herbicide control, biological control of *S. platyphylla* was considered as a potentially environmentally friendly strategy to control the weed (Kwong, 2014; Martin *et al.,* 2018).

Biological Control

The Centre for Biological Control (CBC) (Rhodes University), collaborated with the Australian Victoria Department of Primary Industries (DPI) and the United States Army Corps of Engineers (USACE), Mississippi, to find a solution against *S. platyphylla* using biological control (Hill &Coetzee, 2016; Kwong *et al.,* 2017a). In 2014, a native range survey was conducted in the USA to collect potential biological control agents for *S. platyphylla.* The survey resulted in the collection of four *Listronotus* weevils (Curculionidae: Brachycerinae: Rhytirrhinini) identified as: *Listronotus lutulensis* (Boheman), *Listronotus sordidus* (Gyllenhal), *Listronotus frontalis* (LeConte) *and Listronotus appendiculatus* (Boheman) (Fig. 1.8) (Hill & Coetzee, 2017).

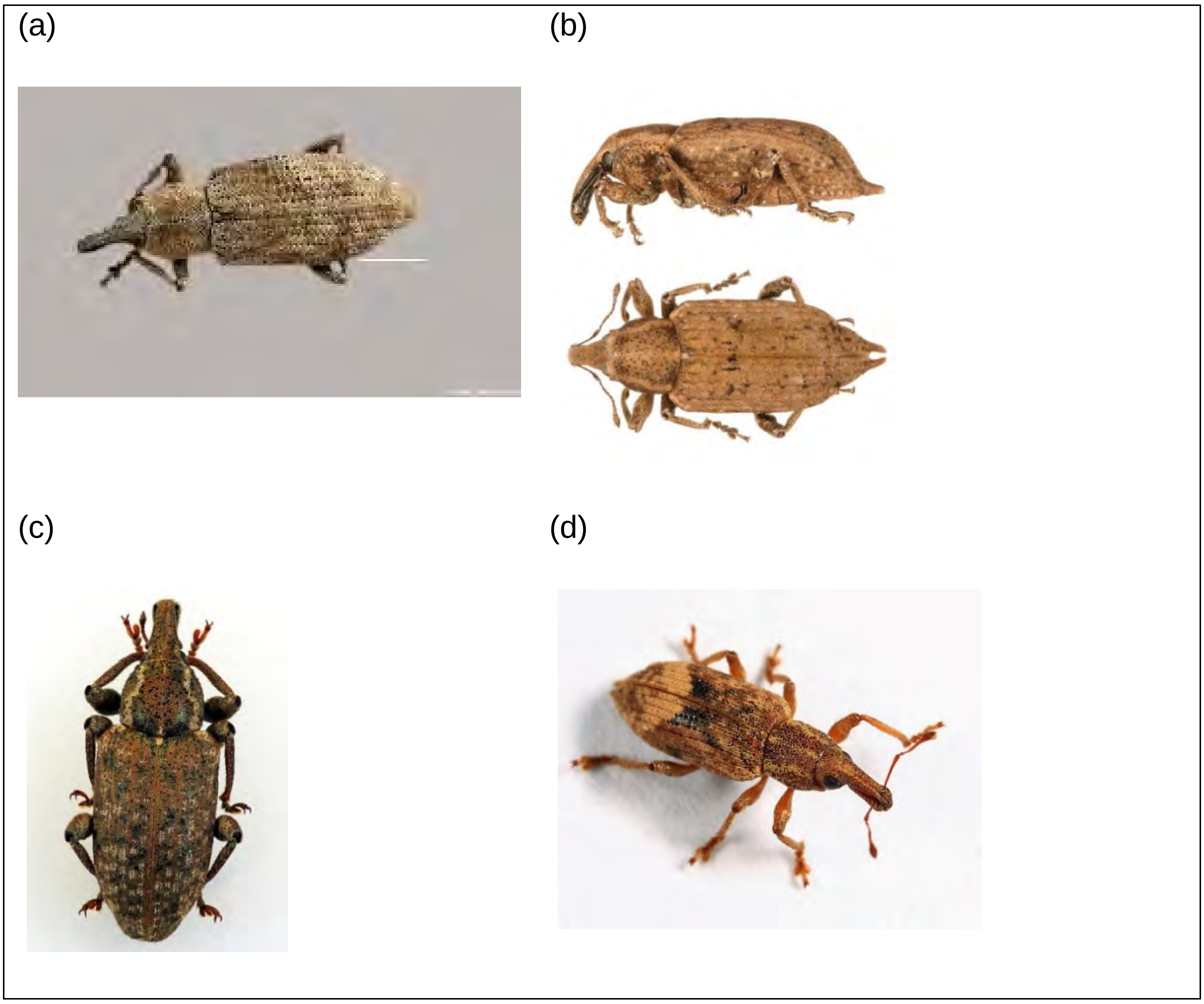


Figure 1.8: The four potential biological control agents for *Sagittaria platyphylla*:

Listronotus lutulensis (a), *Listronotus sordidus* (Martin *et al*, 2018) (b), *Listronotus frontalis* (Ott, 2010) (c), and *Listronotus appendiculatus* (Murray, 2014) (d).

Cultures of four *Listronotus* weevils were collected in September 2014 from a small artificial dam on Tara Wildlife Reserve (32.491; -91.060), near Vicksburg, Mississippi in the United States of America. They were imported into the CBC's Quarantine Facility on the 26th September 2014. The potential biological control agents have gone through host specificity testing to determine if any are suitable biological control agents. The studies also included determining the weevils' life histories and methods for culturing them under quarantine conditions. The status of the *Listronotus* weevils as potential biological control agents are:

- *Listronotus lutulensis* is a foliage feeders which feed on the plant's leaf tissue. *Listronotus lutulensis* had limited impact on *S. platyphylla* (G.D. Martin, pers. comm.). Due to this, the weevil was shelved as a potential control agent for *S. platyphylla* in South Africa.

- *Listronotus sordidus* is a root-crown feeders (Adair *et al.,* 2012; Martin *et al.,* 2018). After the investigation of the weevils' life history and host specificity testing, *L. sordidus* was also not considered as a potential biological control agent due to its inability to impact plant populations growing in high water levels where most *S. platyphylla* populations occur in South Africa (Martin *et al.,* 2018).

- *Listronotus frontalis* is a crown and tuber feeders that have a potential to reduce subterranean carbohydrate storage organs, damage growth tips and cause plant death (Adair *et al.,* 2012; Kwong *et al.,* 2014). Host range and host specificity testing are still being conducted for this agent.

- *Listronotus appendiculatus* is a fruit and flower weevil that feeds on fruiting heads of the plant (Adair *et al.,* 2012; Kwong *et al.,* 2014). *Listronotus appendiculatus* is currently regarded as the most promising potential biological control agent for *S. platyphylla* because of its impact and specificity (Hill &

Coetzee, 2017). *Listronotus appendiculatus* has also been considered for release in Australia (R. Kwong, pers. comm.2018). During the host range and host specificity testing*, L. appendiculatus* showed host specificity and the potential to damage the plant's achenes (Hill & Coetzee, 2017).

1.6 AIMS AND OBJECTIVES

The main concern with the *S. platyphylla* invasion is that since it was first identified in South Africa in 2008, and targeted for eradication under SANBI in 2013, it has spread and persisted over the last 10 years under herbicide and mechanical control. These methods have been costly, unsustainable, and have not reduced the spread of *S. platyphylla*. The other concern is the risk and threat it holds in spreading further into the agricultural sector and other major river catchments while it remains under the same management plans that have failed to spread in the environmental sector over the past years. Additionally, there is little data and information on problems and prospects associated with managing *S. platyphylla* in South Africa. Research on *S. platyphylla*, as an emergent aquatic weed has concentrated on understanding its invasion biology and population genetics, focusing mainly on the invasion in Australia (Kwong *et al.,* 2004; Kwong *et al.,* 2004; Adair, *et al.,* 2012; Kwong *et al.,* 2017a, Kwong *et al.,* 2017b). During a recent study that assessed the impact of eight aquatic invasion plants in South Africa, *S. platyphylla* scored a 1.3 impact level on both environmental and socio-economic impact using data captured from non-South African review papers and chapters (Hill & Coetzee, 2017). Based on Nentwig *et al.*, (2016) level 1 impacts are described as minor impacts, only locally, only on common species, with negligible economic loss.

The aim of this study is to explore the current distribution, problems and prospects associated with managing *S. platyphylla* in South Africa. Furthermore, assess whether a biological control programme could be added as an additional management tool by determining if *L. appendiculatus*, a potential biological control agent, is suitable for controlling *S. platyphylla* in South Africa.

More specifically, the objectives of this study are to:

- Determine the current distribution, density, and rate of spread of *S. platyphylla* in South Africa.

- Determine the potential impact of the candidate biological control agent, *L. appendiculatus* on *S. platyphylla,* focusing mainly on its reproductive outputs.

CHAPTER 2:

SAGITTARIA PLATYPHYLLA INVASION IN SOUTH AFRICA

2.1 INTRODUCTION

Introduced invasive plant species, regardless of their taxonomic identity, generally follow a similar growth pattern which is relatively faster compared to their native counterparts (James & Drenovsky, 2007). This growth pattern demonstrates the invasion spread over time and is typically known as ‘the invasion curve’ (see Chapter 1: Fig 1.2) (Harvey & Mazzotti, 2014). Population growth and spread of invasive plants is driven by three fundamental rates, namely: survival, fecundity, and growth (Sakai *et al.,* 2001; Ramula *et al.,* 2008). Most successful invaders have characteristics that promote these rates such as the ability to reproduce both sexually and asexually, the ability to self-fertilize, the ability to grow rapidly from seedling to sexual maturity, and having phenotypical plasticity traits that allow the plant to adapt to different environmental conditions (Baker, 1965; Sakai *et al.,* 2001; Arim *et al.,* 2006; Griffith *et al.,* 2016). Therefore, the lag time between initial colonization and exponential population growth, and the way in which the population behaves around the population’s plateau varies in plants due to the plant’s growth characteristics and evolutionary adaptations in the introduced range (Kowarik, 1995). Population models, such as the invasion curve model are studied to offer insight into the stage of the invasion and the population’s severity over time (Griffith *et al.,* 2016). Furthermore, population size and infestation level demonstrated in invasive plant population models can be used to select appropriate and feasible management techniques based on the knowledge of the invasion stage (Chippendale, 1991; Wilson *et al.,* 2013).

Management of invasive populations based on their growth stage is incorporated in model frameworks for invasion biology of invasive species (Richardson *et al.,* 2000; Pysek & Richardson, 2010). Blackburn *et al*. unified framework for biological invasions and Chippendale’s (1991) invasion curve identified population categories based on invasion stages and linked them to different management interventions that can be applied to specific population category (see Chapter 1: section 1.5.3). This was supported by a simulation study done by Maxwell *et al*. (2009) which suggested prioritizing management of source populations using the early detection and rapid response (EDRR) approach, one of the most successful strategies for reducing the increase in total number of invasive populations. Chippendale's (1991) management design and Blackburn's *et al.* (2011) unified framework can be used as guidelines for managing invasive weeds before and after first occurrence. However, not all management options are permitted in certain countries (Wittenberg & Cock, 2001). Therefore, other factors like the country's legislation and stakeholders directly involved must be considered (Wilson *et al.,* 2013; Coetzee *et al.,* 2019).

In South Africa, the Department of Agriculture manages quarantine services and conducts pre-border risk assessments in collaboration with the National Plant Protection Organization with the aim to prevent and detect potential invasive species before introduction (Wilson *et al.,* 2013). When the invasive species becomes established in its new found range and the plant's population gradually increases in the lag phase, the South African National Biodiversity Institute's (SANBI) Invasive Species Programme (ISP) detects new invaders, conducts post-border risk assessment and either develops or implements eradication plans (Wilson *et al.,* 2013). As a management goal, eradication techniques aim to eliminate every single individual in each population and is mostly practical on small populations (Savoie & Van Randen, 1998). Eradication techniques include application of registered herbicides, and mechanical and manual removal of invasive plants. When the population is at the exponential phase or stationary phase, eradication is not cost-effective and the Department of Environmental Affairs (DEA)'s Working for Water Programme (WfW) implements ongoing management, which includes the addition of biological control and integrated pest management (Wilson *et al.,* 2013).

In South Africa, management is structured by government legislation. The primary government legislation with regards to invasive species is the National Environmental Management: Biodiversity Act (NEM: BA, Act 10 of 2004). Under the South African NEM: BA regulations, invasive plant species under category 1a are mainly assigned herbicide application, mechanical and manual removal while biological control is usually assigned for managing category 1b invasive plant species which are well established in the country (Zachariades *et al.,* 2017). Well established invasive species are those that are at a spreading stage with populations with dispersing individuals that survive and reproduce in various sites and habitats (Blackburn *et al.,* 2011) such as *S. platyphylla.*

Sagittaria platyphylla was first recorded in South Africa in 2008, and due to its known invasive tendencies in other countries, it was immediately listed as a category 1a invader species (Martin *et al.,* 2018). Therefore, it was targeted for eradication (EDRR approach) through herbicide and manual control. However, the plant was able to spread to additional sites. A biogeographical study conducted in 2014 by Kwong *et al*. (2017a) showed that *S. platyphylla* populations in South Africa were larger in size than initially thought, with half the populations being more than 500m in length, occupying a greater percentage cover (40%) compared to populations in the plant's native region. Furthermore, South African populations had similar population densities to populations in the plant's native region at around 75 plants/m^2 (Kwong *et al.,* 2017a). Previous surveys conducted to monitor the spread, density and distribution of the plant in South Africa, showed an increased number of invaded sites in 2008 to be 16 sites in 2010 (the majority down-stream from the original infestation), and 19 sites in 2013 (SANBI-ISP meeting, 2018). There is no current information on the overall population structure and potential risk of the *S. platyphylla* invasion, and how the management of *S. platyphylla* in South Africa has performed over the years under the current management plan driven by the SANBI-ISP eradication approach.

The SANBI-ISP eradication efforts were initiated in 2012 nationwide and included public awareness at the early stages of the programme, then eradication using herbicide applications, manual and mechanical removal (R. Lalla SANBI, pers. comm.). These were applied to accessible known infested sites. In KwaZulu-Natal, efforts of EDRR through public awareness and eradication techniques were mostly applied by SANBI-ISP and the Duzi-Umgeni Conservation Trust (DUCT) using manual removal, herbicide application (Kilomax) and mechanical removal by private land owners. In the Eastern Cape, herbicide application was applied at one site (Maden Dam, King Williams Town); in the Western Cape, mechanical removal was applied at one site by a private land owner (Stark-Conde Wines, Stellenbosch); and in Gauteng, manual removal by SANB-ISP at one site.

The largest and most numerous infestations are in KwaZulu-Natal. Most of the management that has occurred under the SANBI-ISP has also occurred in this province and the best records of management are also from this province (Fig. 1.5). However, the effectiveness of the management efforts, the management practices, along with research costs conducted by the efforts of the Centre for Biological Control (CBC), Rhodes University for its biological control potential, require further investigation.

The aim of this chapter was to determine the current geographical range and population abundance of *S. platyphylla* in South Africa since its first introduction. In addition, it aimed to assess the outcome of the control mechanisms used on *S. platyphylla* populations and determine the potential risk of currently known populations. This information will determine the overall state of the *S. platyphylla* invasion in South Africa, which will assist in its management.

2.2 MATERIAL AND METHODS

2.2.1 Compilation of Distribution Data

Surveys of South African freshwater systems invaded by *S. platyphylla* were conducted between 2008-2019 (2013 had no recorded data gathered) (Fig. 2.1) by different stakeholders which included government and research institutions (Table 2.1). The intention of these surveys ranged from collection of distribution data (SAPIA dataset), monitoring and application of eradication techniques (SANBI and DUCT datasets), national waterweeds status (CBC datasets) and a biogeographic comparison study (Biosciences Research dataset). Most surveys were conducted in the summer of each year, during the plant's flowering season. The process of data collection and the procedures used during surveys across all different stakeholders was conducted according to their own objectives in relation to species invasion. Therefore, the different datasets were combined according to each site's location, water system type and year in which the data was recorded, cleaned to detect and remove errors and inconsistencies in different datasets, and standardized to make sure that the datasets were comparable to each other and meet this study's objectives.

This was the only way to consolidate information on the invasion of *S. platyphylla* from 2008 till 2018, without leaving out important data to the study.

Table 2. 1: Datasets collected from different stakeholders.

Year	**Principal Collector**	**Stakeholder: Organization**	**Dataset Name**
2008, 2018	Leslie Henderson	Research: Southern African Plant Invaders Atlas (SAPIA) database	• *Sagittaria platyphylla* SAPIA database • *Sagittaria platyphylla* Copy of 2018-05-03_122848322-brahmsonlinedata
2009, 2015- 2019	Menzi Nxumalo and Reshnee Lalla	Government: South African National Biodiversity Institute (SANBI)	• 2009 Management Plan *Sagittaria* spp RL and MN 14 March 2017 • *Sagittaria platyphylla* RR data 2019 final
2010 2012	Jabu Sithole	Government: South African National Biodiversity Institute (SANBI)	• 2010 *Sagittaria* localities from other Provinces (1,2) • 2012 localities with Sag invasion in KZN
2014	Dr Raelene Kwong and Dr Grant Martin	Research: Biosciences Research, AgriBio, La Trobe University: Centre for Biological control, Rhodes University	• *Sagittaria* natural enemy surveys complete
2017-2018	Mpilonhle Ndlovu and Dr Grant Martin	Research: Centre for Biological control (CBC), Rhodes University	• *Sagittaria*dat-COLLECTION • *Sagittaria* Sites2017-2018

			• Ms*sagittaria* Sites2008-2019
2017	Ernita van Wyk	Government: Former-South African National Biodiversity Institute (SANBI)	• WP 2017 complete data (1)
2017	Bart Fokkens	Government: Duzi-Umgeni Conservation Trust (DUCT)	• Duct*sagittariaplatyphylla* 2017

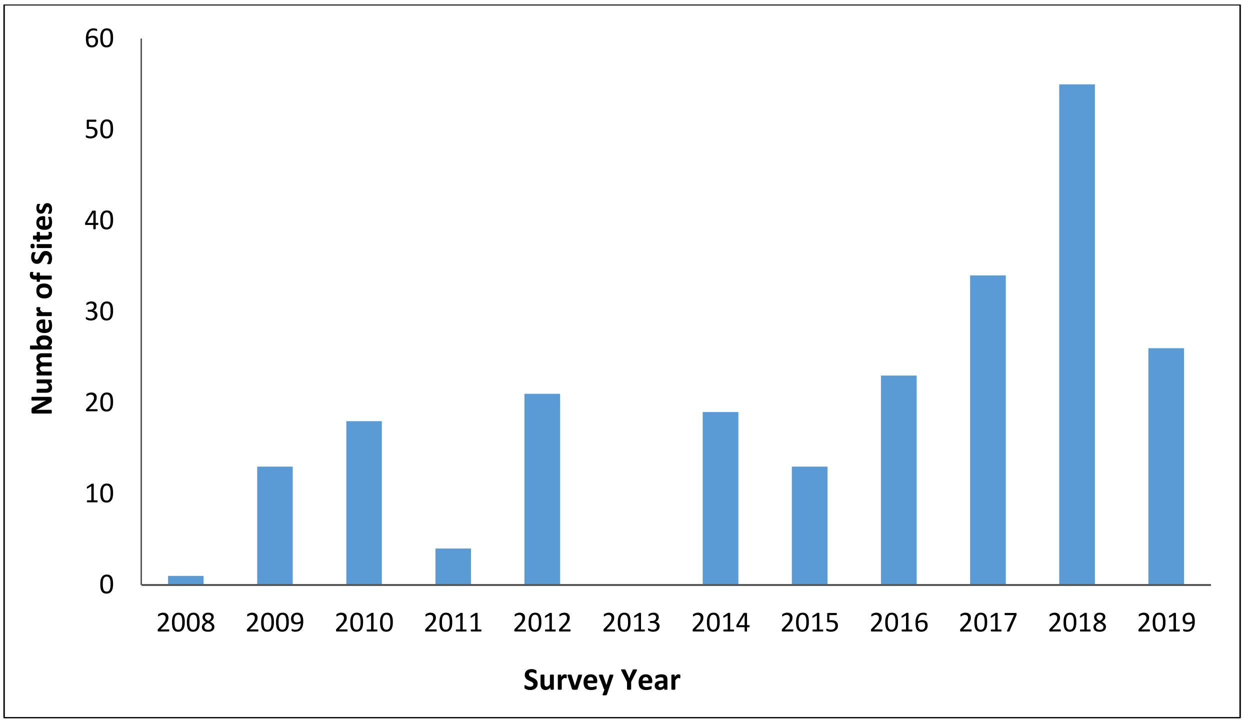


Figure 2. 1: Number of sites colonized by *Sagittaria platyphylla* during the years 2008-2019 of in South Africa**.**

In addition to the surveys described above, surveys were conducted by the Centre for Biological Control, Rhodes University, in 2014, 2017 and 2018 (Table 2.1). During these surveys, sites were surveyed below and above known infestations to identify new established populations in the same water systems (e.g. river system). New sites were treated as new populations. During these surveys, data recorded at each site included: site characteristics (year surveyed, longitude and latitude, location, water body type (lentic or lotic water system), population size, and plant density (Table 2.2). Population size was estimated by determining the length and width of the population (m^2), while plant density at each site was measured by randomly placing 10 quadrats (1 m^2) at each site and counting the number of plants in each. During the compilation of the dataset, the annual mean value of population size and plant density were recorded for each site. To standardize the data, sites with visually estimated plant density and population size were assigned plant density values, and sites with population length as the estimated population size were assigned population width to calculate the population size in m^2 (Table 2.2).

Table 2. 2: Classification of *Sagittaria platyphylla* population size and plant density. Sites with population length as the estimated population size were assigned population width to calculate the population size in m^2

Population size in m^2 (infestation length : assigned diameter)	**Plant density (number of plants/m^2): assigned plant density values***
>450m: 4m	>75 plants/m^2: 95 plants/m^2
205-450m: 3m	45-75 plants/m^2: 65 plants/m^2
50-250m: 2m	15-45 plants/m^2: 35 plants/m^2
<50m : 1m	<15 plants/m^2: 5 plants/m^2

*Data standardization used to assign plant density with estimated plant density values.

*2.2.2 S*agittaria platyphylla *in South Africa*

Current distribution of S. platyphylla *in South Africa*

To map the change in the distribution *of S. platyphylla* over the surveyed years, the Geographic Information System (GIS) software, ArcGIS 10.5.1. (ESRI: Redlands, California, United States) was used to transform the distribution data into a digital map with number of sites recorded annually. The distribution map was narrowed down to three invaded provinces (KwaZulu-Natal (KZN), Eastern Cape (EC) and Western Cape provinces (WC)). The longest invaded lotic water system in each province was singled out to estimate the spread of infestation over the years. Additionally, a general linear model with Poisson errors and a log link function was conducted in R version 3.6.0 (R Core Team: Auckland, New Zealand) to determine whether the number of *S. platyphylla* sites identified in South Africa increased over time, by determining the relationship between time in years and the number of sites recorded every year.

Sagittaria platyphylla's *population demographic*

To visualize the change in *S. platyphylla*'s population demographics over the years of survey, the trend over time of the overall population size and plant density of infestations were evaluated by grouping population size and plant density values recorded for each year into categories ranging from smallest to largest for each province (KwaZulu-Natal, Eastern Cape and Western Cape provinces) (Table 2.3). During the surveys, sites where control had been attempted were assigned a qualitative measure: 'extirpated' sites where complete removal of the population through control methods was achieved for a period longer than three years; 'attempted control' sites in which the weed persisted in spite of the control method applied; 'not controlled' sites where no control was applied. Furthermore, a negative binomial general linear model was conducted to determine whether the population size and plant density changed significantly over time, by determining the relationship between time in years and the average population size and plant density recorded every year.

Table 2. 3: Population size and plant density categories of *Sagittaria platyphylla* sites.

	Population size (m^2)	**Plant density (plant /m^2)**
Extirpated sites*	-	-
Temporary cleared sites[$]	0	0
Small	5-100	5-35
Medium	100-500	35-65
Big	500-1000	65-95
Large	>1000	>95

*Sites cleared for more than the past three years

$Sites that had been cleared for less than two years

2.2.3 Management cost in South Africa

The management cost spent on controlling infested sites was compiled from data supplied by South African National Biodiversity Institute (SANBI), private landowners and research costs of the Centre for Biological Control, Rhodes University. The management techniques used for managing *S. platyphylla* across invaded sites were evaluated to determine their effectiveness. Herbicide control involved spraying Kilomax (glyphosate based) on emergent plants, and manual and mechanical control of *S. platyphylla* involved extracting seedlings and smaller plants by machinery or manual digging, bagging the plant material, and burning it (Private landowners Pers. comm). To give an overall indication of management sites under similar eradication techniques (herbicide, manual, and mechanical control), sites were grouped together for each province to determine the outcome of the control mechanisms used.

2.2.4 Potential Spread of Sagittaria platyphylla *in South Africa*

The potential spread of *S. platyphylla* was mapped in Arc GIS 10.3 by creating buffer zones of 1, 2.5, and 5 km around the locations. These buffer zones were then overlaid on the South-Africa-Lesotho-waterways-shape file, and OSM_WaterLine and OSM_WaterPoly shape files. Waterbodies free of *S. platyphylla* located within these buffer zones were deemed to be threatened locations and were tabulated and provided with a risk index based on their proximity to the infestation (Table 2.4).

Table 2. 4: The risk Index used to assign the risk of threatened water bodies.

Level of Importance	**Distance from site (km)**
High (H)	1
Medium (M)	2.5
Low (L)	5

2.3 RESULTS

*2.3.1 S*agittaria platyphylla *in South Africa*

Current distribution of S. platyphylla in South Africa

The first recorded site of *S. platyphylla* in South Africa was in Krantzkloof Nature reserve in KwaZulu-Natal Province in 2008. There are now 72 known sites, distributed between the three coastal provinces namely: KwaZulu-Natal, Eastern Cape and Western Cape (Fig. 2.2). Successfully eradicated sites were not included in the distribution map.

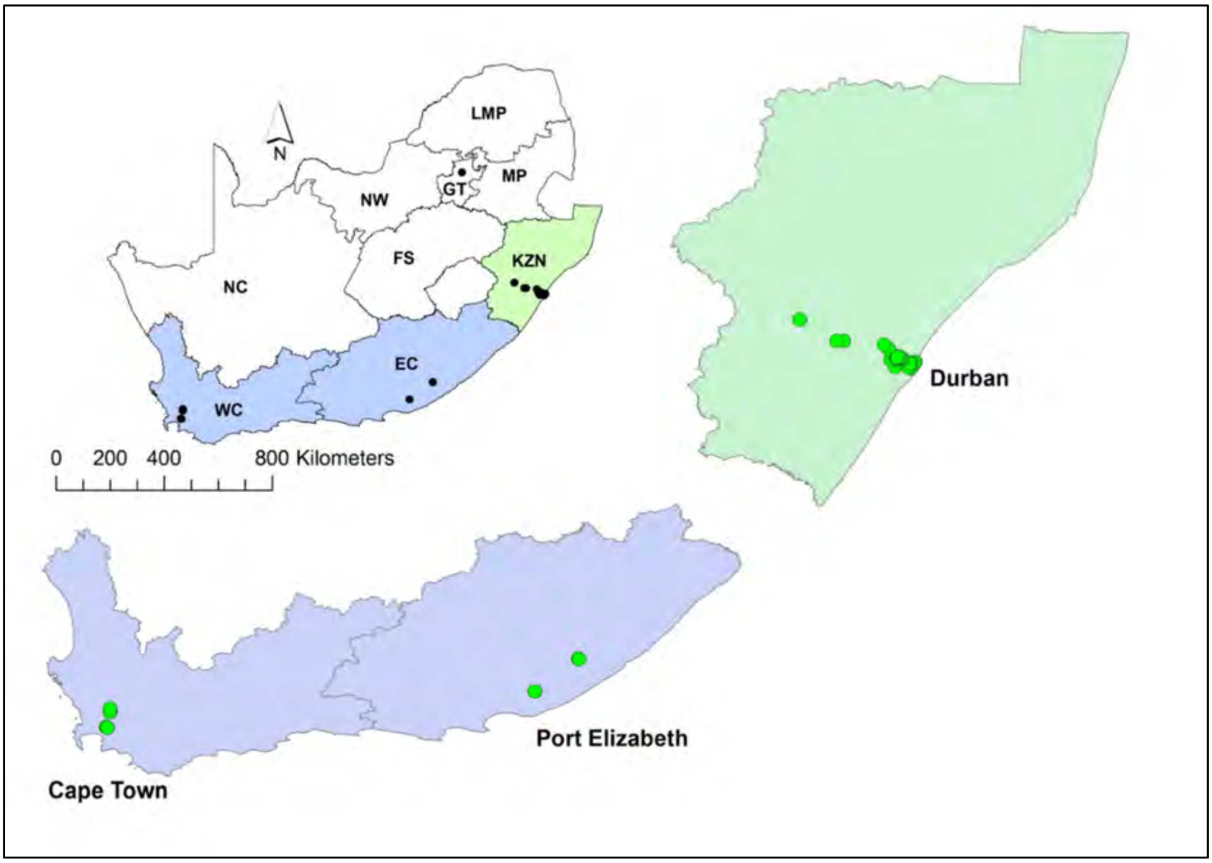


Figure 2. 2: *Sagittaria platyphylla* localities in South Africa. Gauteng (GT), Mpumalanga (MP), Limpopo (NP), North West (NW), KwaZulu-Natal (KZ), Eastern Cape (EC), Western Cape (WC), Northern Cape (NC) and Free State (FS).

Additionally, the cumulative number of *S. platyphylla* sites recorded per year in South Africa resulted in a significant increase in number of sites over the years ($F_{-1,\ 10}$ = 0.222, *P* = 0.001) (Fig. 2.3).

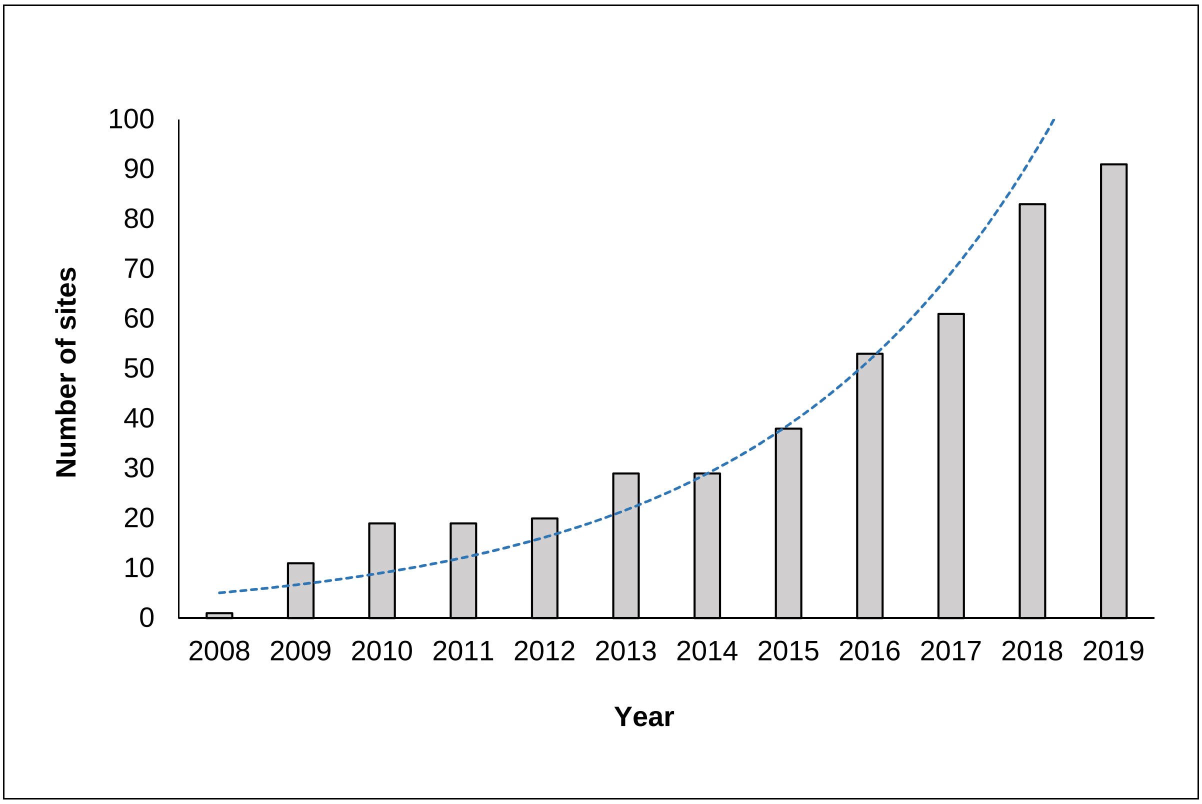
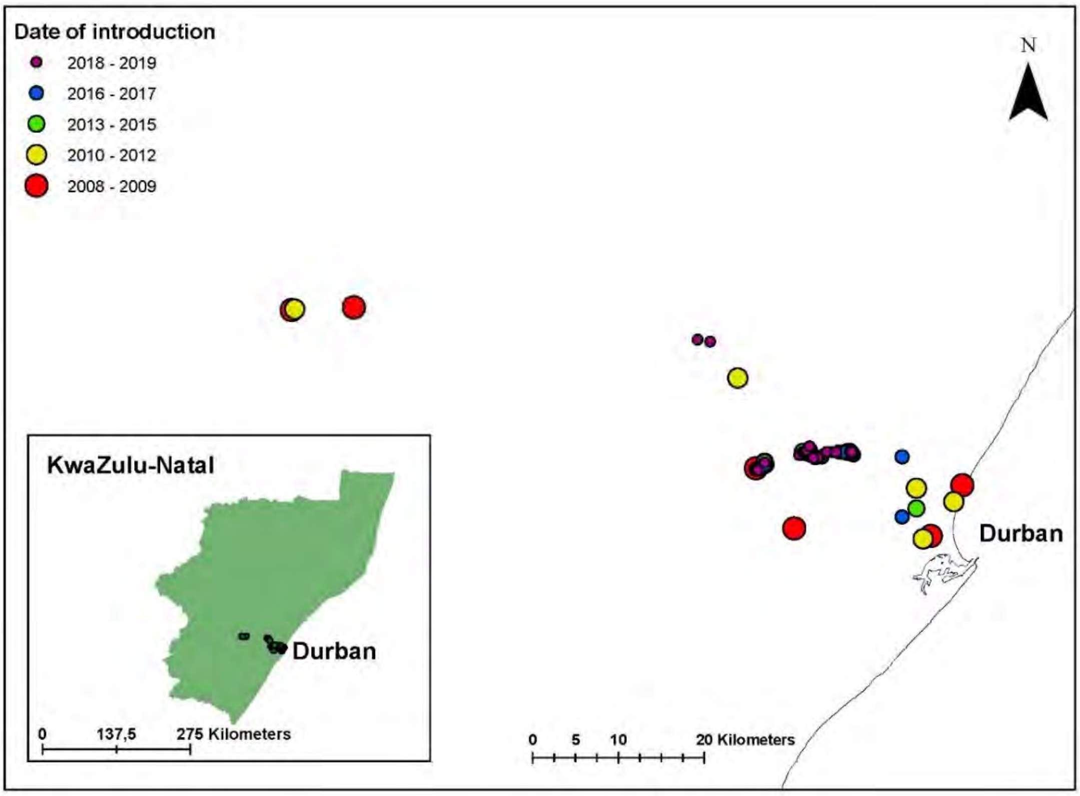


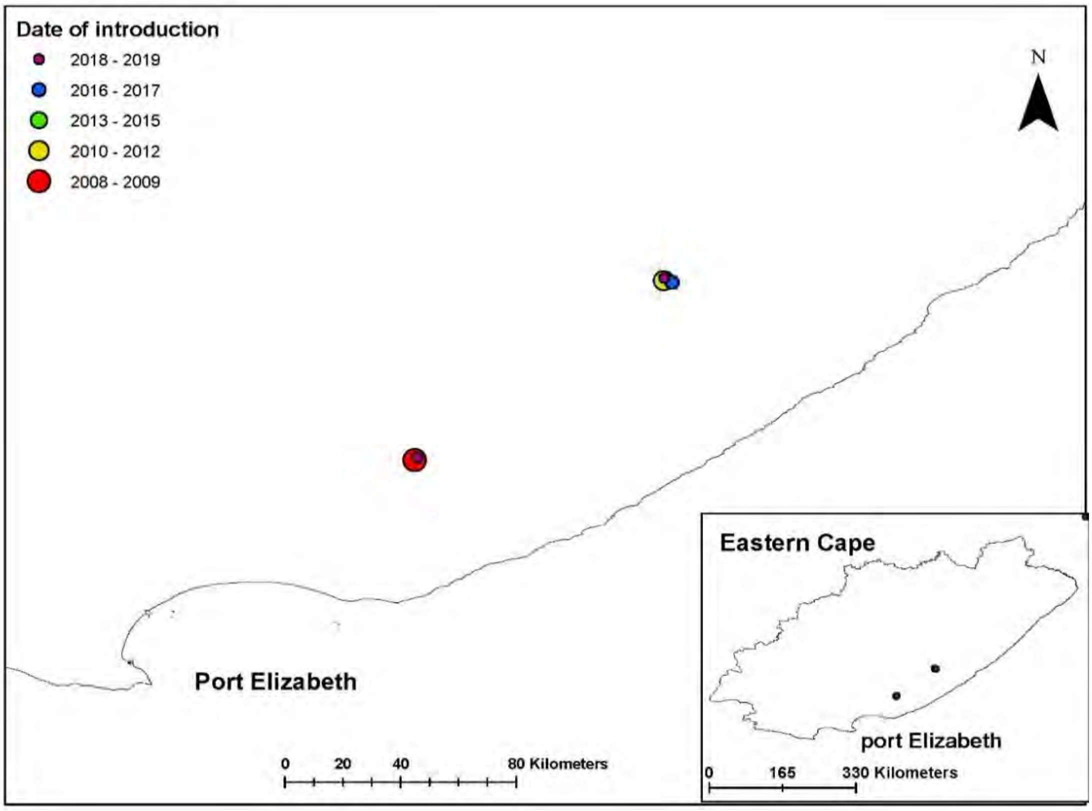
Figure 2. 3: Significant increase in cumulative number of *Sagittaria platyphylla* sites recorded per year in South Africa (GLM; $F_{1,\ 10}$ = 0.222, *P* = 0.001).

Over the past ten years, the infestation has spread within and between sites in each of the three provinces. The weed has spread within a number of these lotic systems since it was first recorded: In KwaZulu-Natal, the infestation in the Umgeni catchment, *viz.* the infestation from the Kranzkloof Nature Reserve and Ascot Bush Lodge, has spread 120km over the last 10 years (Fig. 2.4a). In the Eastern Cape, the weed has managed to spread downstream 6.6 km from Maden Dam to Rooikrans Dam. Additionally, in the Eastern Cape, the weed has spread from one of the first recorded localities, in the Makana Botanical Garden stream, to 1.3 km downstream since its first record in 2009 (Fig. 2.4b). Finally, in the Western Cape, in the Berg River, the infestation has spread 13.1 km downstream since its first record in 2009 (Fig. 2.4c).

(a)



(b)


Date of introduction
2018 - 2019
2016 - 2017
2013 - 2015
2010 - 2012
2008 - 2009
N
Port Elizabeth
0 20 40 80 Kilometers
Eastern Cape
port Elizabeth
0 165 330 Kilometers

(c)

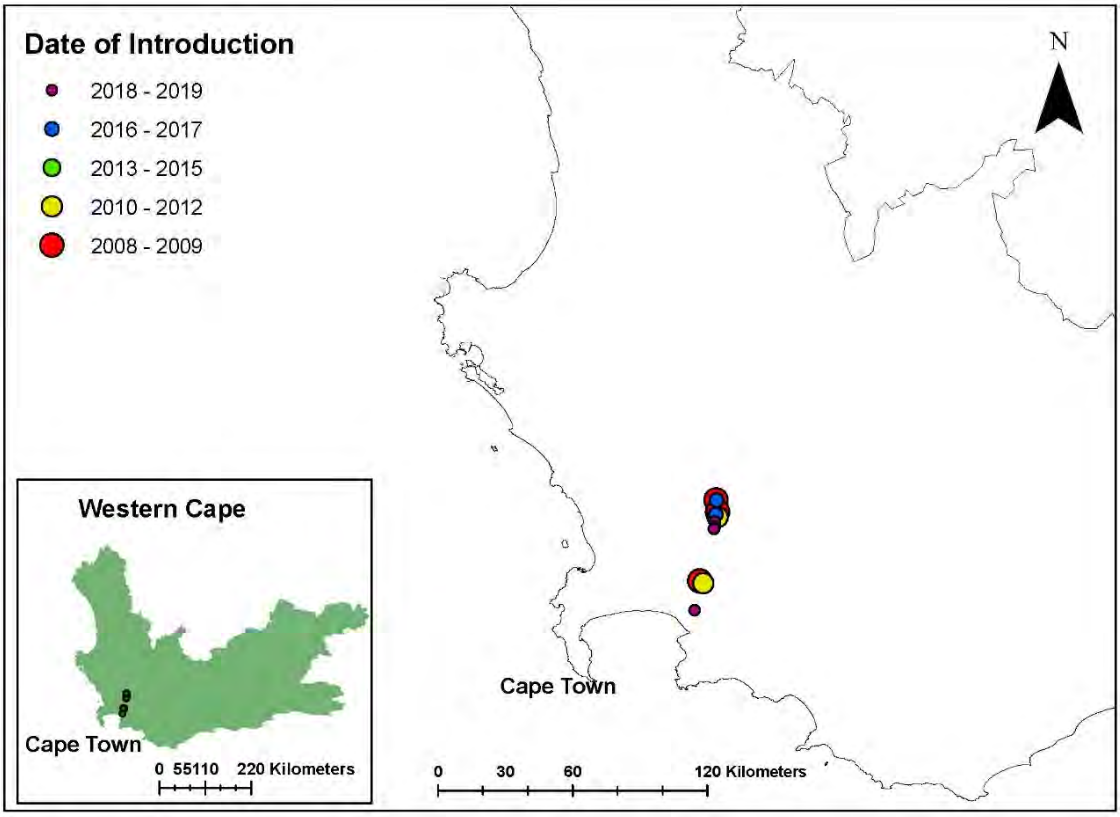

Date of Introduction
2018 - 2019
2016 - 2017
2013 - 2015
2010 - 2012
2008 - 2009
N
Western Cape
Cape Town
0 55110 220 Kilometers
Cape Town
0 30 60 120 Kilometers

Figure 2. 4: The spread of *Sagittaria platyphylla* between 2008 and 2019 in KwaZulu-Natal Province (a), Eastern Cape Province (b) and Western Cape Province (c).

Sagittaria platyphylla in South Africa is mostly found in lotic water systems with 80% of sites being in lotic water systems compared to 20% in lentic systems. Water systems in KwaZulu-Natal were 83% lotic and 17% lentic, in the Eastern Cape Province, 60% lotic and 40% lentic, and in the Western Cape, 73% lotic and 27% lentic (Fig. 2.5). Most sites were located in slow moving water bodies such as the Molweni and Umgeni Rivers in KwaZulu-Natal and the Berg River, Western Cape.

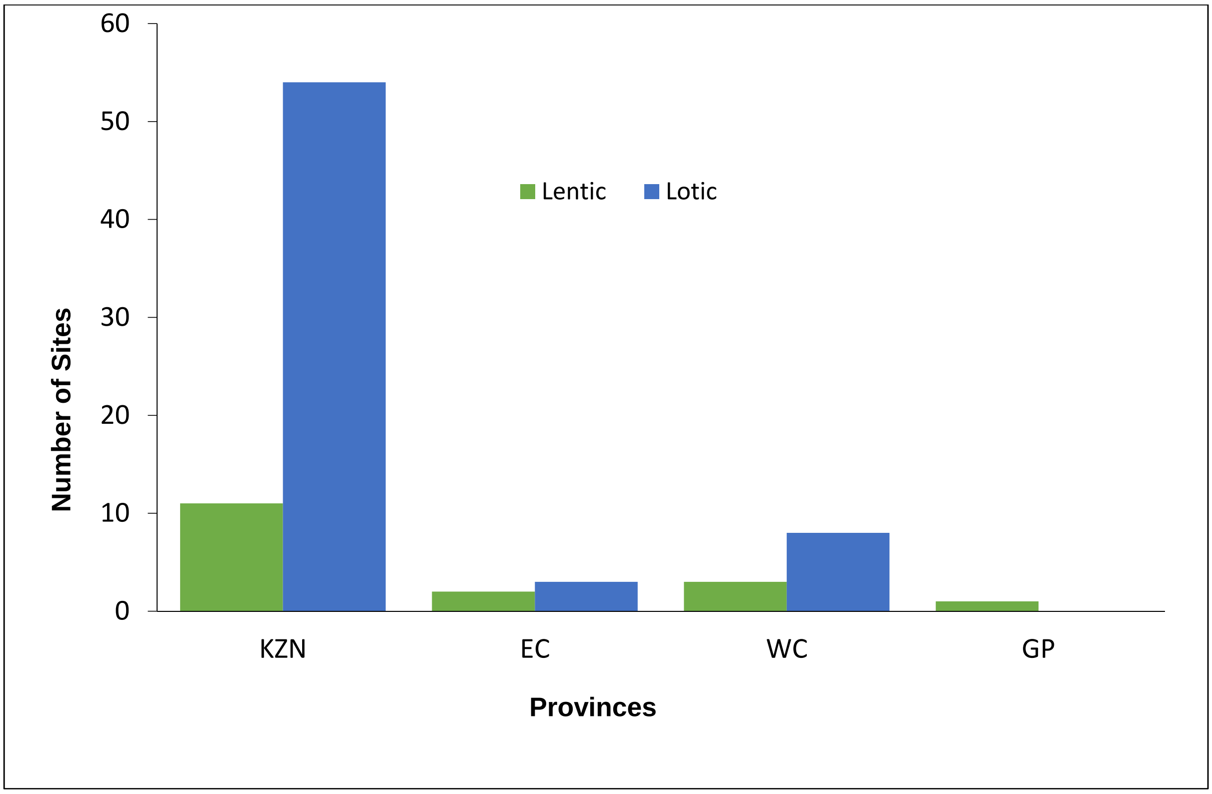


Figure 2. 5: The total number of sites infested with *Sagittaria platyphylla* between lentic and lotic water systems. KwaZulu-Natal (KZN), Eastern Cape (EC), and Western Cape (WC) provinces.

Population demographics of S. platyphylla *in South Africa*

The cumulative number of *S. platyphylla* sites recorded per year in South Africa resulted with an annual significant increase in population size (F1, 10 = 0.109, P = 0.003) (Fig. 6a). There was no significant difference in plant density measured over the years (F1, 10 = 0.028, P = 0.476) (Fig. 2.6b) but the overall trend revealed a gradual increase in plant density from 2008 to 2016 followed by a sudden decrease from 2016 to 2019 since first record of invasion.

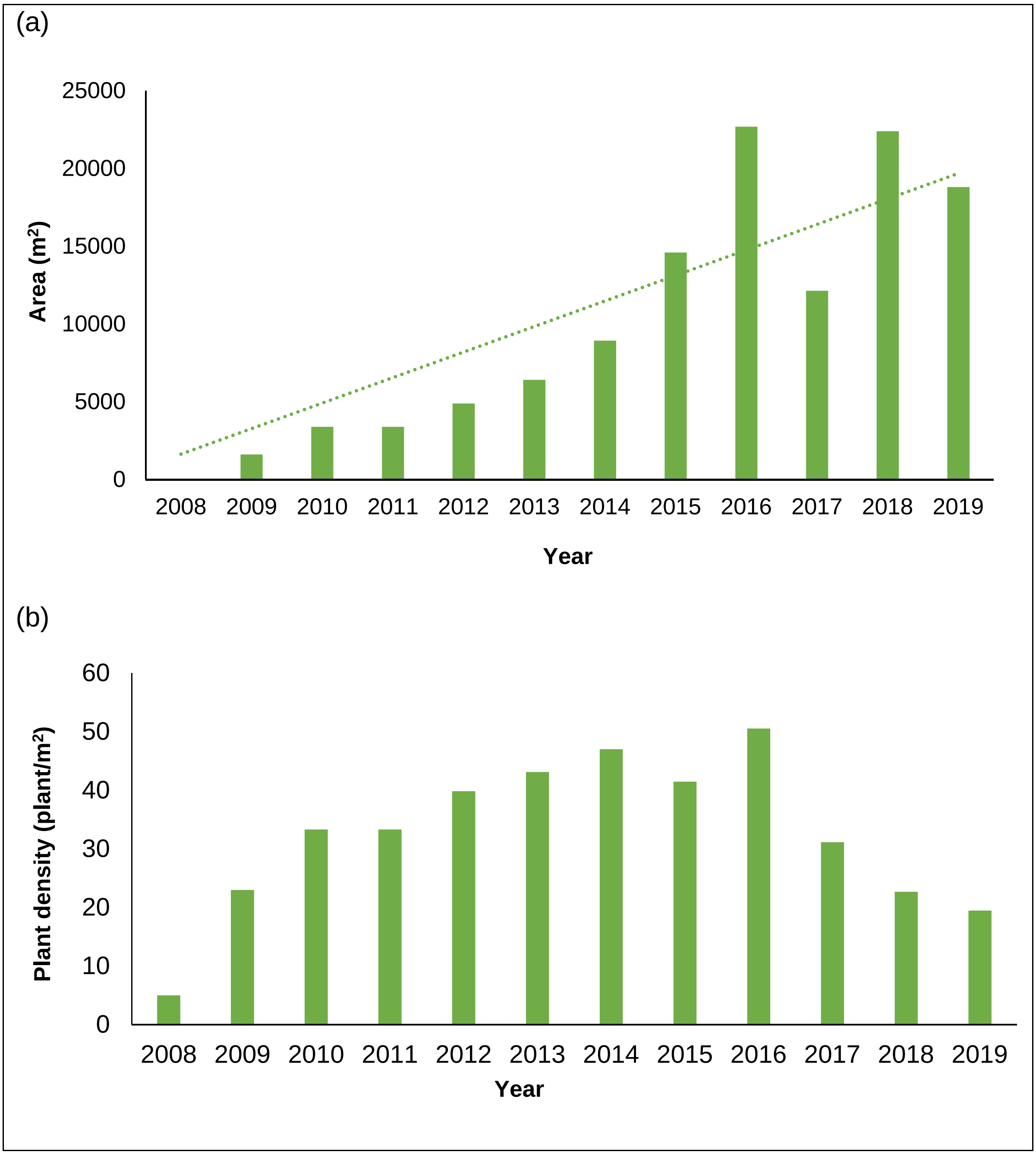


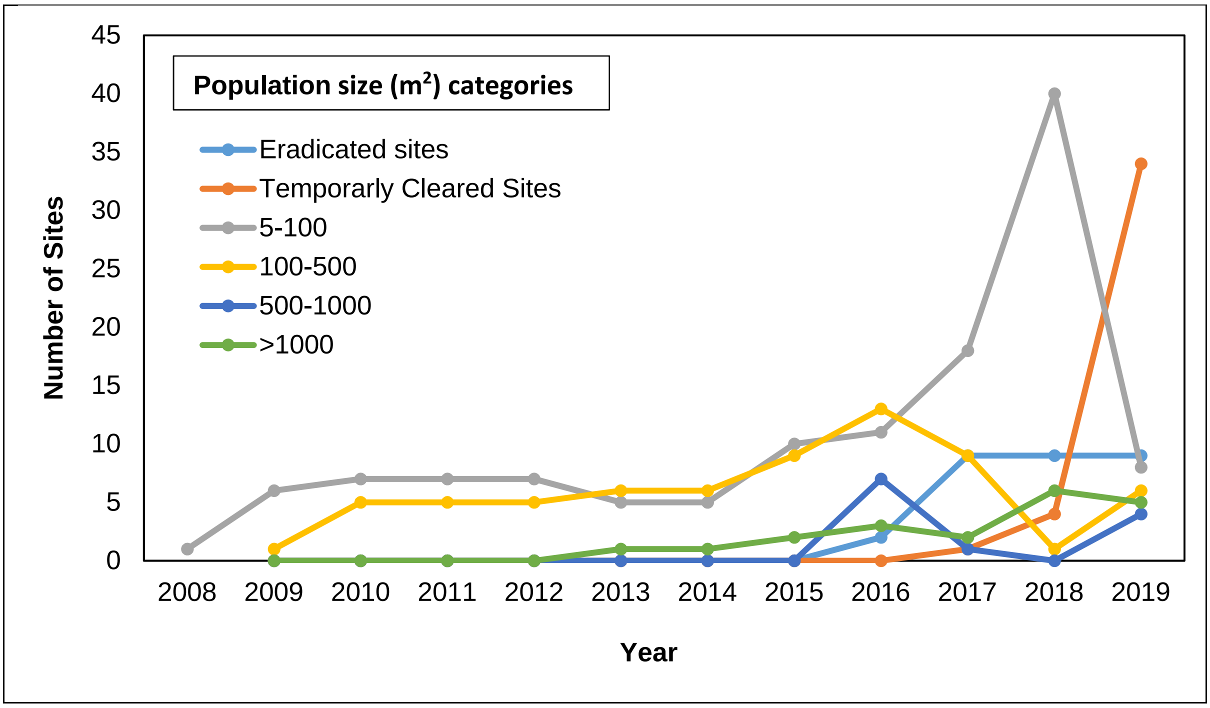
Figure 2.6: Significant increase in mean population size of *Sagittaria platyphylla* per

year in South Africa (GLM.NB; $F_{1, 10}$ = 0.109, P = 0.003) (a). Mean plant density of *Sagittaria platyphylla* per year in South Africa (GLM.NB; $F_{1, 10}$ = 0.028, P = 0.476) (b).

KwaZulu-Natal Province

The first record of *S. platyphylla* was in KwaZulu-Natal province, and by 2019, there were 66 sites distributed in the province. From the time of first record until 2012, there was a steady increase in the number of small sized populations, ranging between 5-100 m^2 and medium sized populations between 100-500 m^2 (Fig. 2.7). In 2013, sites with larger population >1000 m^2, were recorded, and by 2015, population between 500-1000m^2 were also recorded (Fig. 2.7). By 2016, the population size was at its highest, covering an infestation area of 22 685 m^2 from a total of 33 sites, with population sizes ranging between 100-500m^2 dominating the infestations. Additionally, two sites were recorded as being successfully eradicated through control efforts (Kingfisher lake, Pietermaritzburg (-29.605; 30.348) and the Japanese Garden, Pinetown (-29.835; 30.871). In 2017, the number of sites with population size between 100 m^2 -1000 m^2 decreased, while the number of sites with smaller population size between 5-100 m^2 increased. Additionally, the number of eradicated sites increased by six sites and the number of temporary cleared sites were recorded for the first time. In 2018, the provincial population size decreased slightly from 22 685 m^2 recorded in 2016 to 22 396.48 m^2, but the number of sites still increased from 33 to 54 sites. The population ranged between 5-100m^2 and covered 74.07% of the provincial population (Fig. 2.7). The number of sites with large population size >1000 m^2 gradually increased while the number of sites with population between 100-1000m^2 continued to decrease from the previous year. Additionally, the number of eradicated sites increased by seven sites, and the number of temporary cleared sites started to gradually increase, while no new eradicated sites were recorded. In 2019, the provincial population size continued to drop from the previous year to 18 804 m^2, but the number of sites continued to increase (Fig. 2.7). Temporarily cleared sites were recorded for the first time in 2017 and continued to rise in 2018 reaching the highest number of sites in 2019. The number of sites with population between 5-100 m^2 which dominated the provincial population sizes in 2018, drastically dropped from 74.07% to 12.12% (Fig. 2.7). No new eradicated sites were recorded but remained with the total of nine eradicated sites. The dramatic change in infestation sizes were the result of clearing efforts.

The provincial plant density within populations followed a similar pattern as population size, with plant density slowly increasing from 2008-2015, then the trend changed in 2016 and started fluctuating until 2019, because of management (Fig. 2.7). The number of sites with low plant density ranging from 5-35 plants/m^2 was the most common throughout the sampling period. The number of sites with 35-65 plants/m^2 gradually increased from 2009 to 2016 and decreased in 2017, and remained low in 2018 and 2019. The number of sites with 65-95 plant/m^2 remained low until 2016, and decreased from 2017, just to increase again in 2019. The first site with >95 plants/m^2 was recorded in 2013 and remained low throughout the period of surveys.



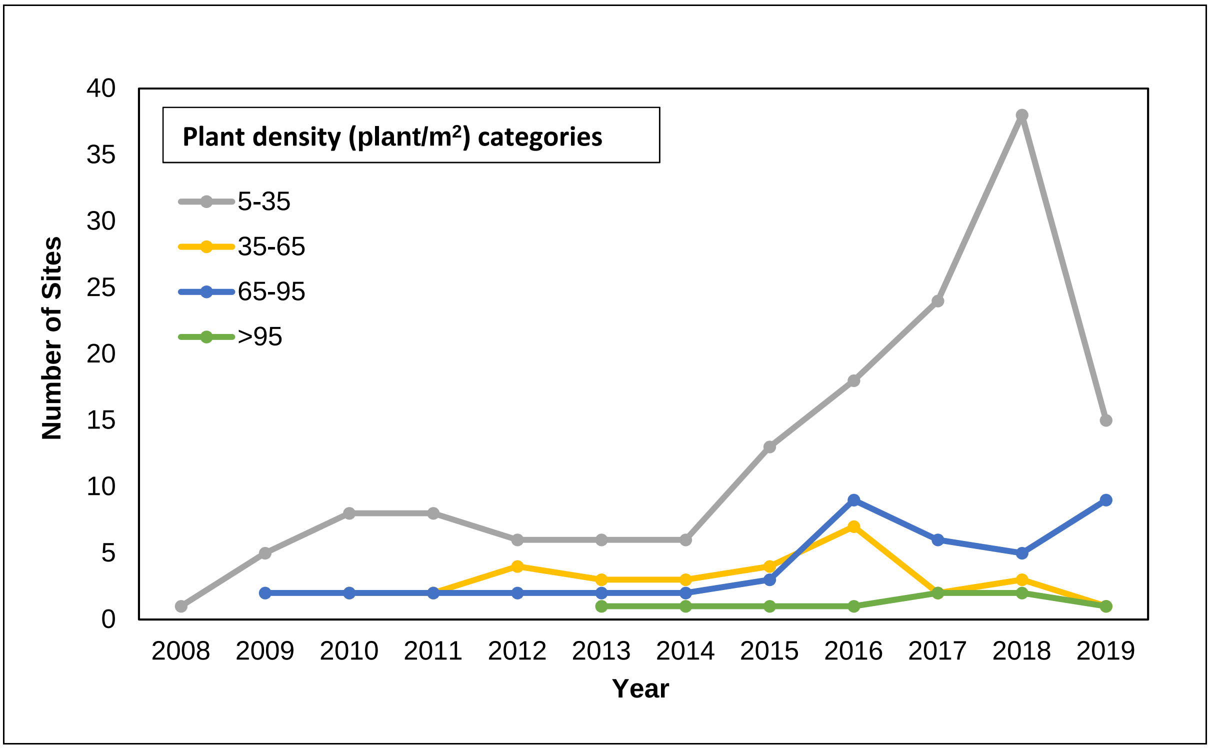


Figure 2. 7: The population size (a) and plant density (b) trends of *Sagittaria platyphylla* infestations in KwaZulu-Natal Province during 2008-2019. Y-axis represents the total number of sites with a size range recorded each year.

Eastern Cape Province

The first record of *S. platyphylla* in the Eastern Cape was one population, 100-500m^2 in size, in 2009. The number of sites increased to five sites by 2019, with the dominant population size >1000 m^2 (Table 2.5). The first site with population of >1000 m^2 was recorded in 2012. No sites were cleared due to eradication efforts, but one site was cleared due to natural causes in 2016 and remained the only cleared site until 2019. The changes in population structure are presented in Table 2.5.

In 2009, the only recorded site had a plant density between 5-35 plants/m^2 which increased to 35-65 plants /m^2 from 2010-2011. In 2012, one site had a plant density of 35-65 plants /m^2 and another 65-95 plants /m^2 until 2013. In 2014, the number of sites with plant density between 65-95 plants/m^2 increased and remained so until 2016. Also, in 2014, one site was recorded with plant density of 5-35 plants/m^2 until 2016 and was cleared due to natural causes. In 2017, sites with plant density of 65-

95 plants/m^2 decreased to one site with a density > 95 plants /m^2 and remained as one site until 2019.

Western Cape Province

Sagittaria platyphylla was first recorded in the Western Cape in 2009, and by 2019, the number of infestations grew to 11 sites, with the dominant population size ranging between 5-100 m^2. From 2009, only one site had a population between 500-1000m^2, until 2018, when this increased to two sites by 2019. In 2018, two sites were recorded as temporarily cleared sites due to natural causes, no sites were eradicated, and no new infestations were recorded. The population structure and density are presented in Table 2.5. Sites with plant density between 5-35 plant/m^2 increased from one site to three sites by 2016, increased to six sites in 2017 than seven in 2018 and remain unchanged in 2019. A single site with a density of 35 - 65 plant/m^2 was recorded between 2009 and 2013 and the site increased to two between 2014 and 2016. The number sites were reduced to a single site in 2017 and none was recorded since 2018. A new site with a density of >95 plant/m^2 was recorded in 2017 but no change was observed in number of sites until 2019.

Table 2. 5: The population size and plant density of *Sagittaria platyphylla* infestation in Eastern Cape Province (EC) and Western Cape Province (WC) between the years 2009-2019. Values represent the total number of sites with a size range experience within each year.

Year	Population size (m^2)											Plant density ($plant/m^2$)						
	Eradicated sites		Temporarily Cleared Sites		5-100		100-500		500-1000		>1000	5-35		35-65		65-95		> 95
	EC	WC	EC	WC	EC	WC	EC	WC	EC	WC	EC	EC	WC	EC	WC	EC	WC	EC
2009	0	0	0	0	0	1	1	1	0	1	0	1	1		1		1	
2010	0	0	0	0	0	2	1	2	0	1	0		3	1	1		1	
2011	0	0	0	0	0	2	1	2	0	1	0		3	1	1		1	
2012	0	0	0	0	0	2	1	2	0	1	1	1	3		1	1	1	
2013	0	0	0	0	0	2	1	2	0	1	1	1	3		1	1	1	
2014	0	0	0	0	1	2	0	2	0	1	2	1	3			2	2	
2015	0	0	0	0	1	2	0	2	0	1	2	1	3			2	2	
2016	0	0	0	0	1	2	0	2	0	1	2	1	3			2	2	
2017	1	0	0	0	0	5	0	2	0	1	2	0	6			1	1	1
2018	1	0	0	2	0	5	0	2	0	1	2	0	7		1	1		1
2019	1	0	0	2	2	5	0	2	0	2	2	2	7		2	1		1

2.3.2 Management cost in South Africa

The efforts of *S. platyphylla* eradication were nearly, entirely implemented by SANBI-ISP. In 2019, in KwaZulu-Natal, nine sites were recorded as extirpated sites, 51 sites remained present after eradication attempts, and six sites were not recorded as being under any form of management (Fig. 2.8). In the Eastern Cape, eradication was attempted at only one site (Maden Dam: -32.748; 27.297) by Amathole District Municipality, while three sites were not under any form of management. In the Western Cape, one site (Stark-Conde Wines: -33.954; 18.910) was mechanically controlled by a private landowner in 2016, however this was unsuccessful as the weed returned the following year. In Gauteng, the only site (Pretoria National Botanical Gardens: -25.739: 28.272) recorded in 2009 was eradicated by SANBI-ISP in 2013 through manual control. Sites that were cleared due to natural reasons were not included in the count of successfully eradicated sites. By 2019, following six years of management, 64 sites (excluding eradicated and non-managed sites) were actively managed but only ten sites, were successfully extirpated. The weed persisted at most sites.

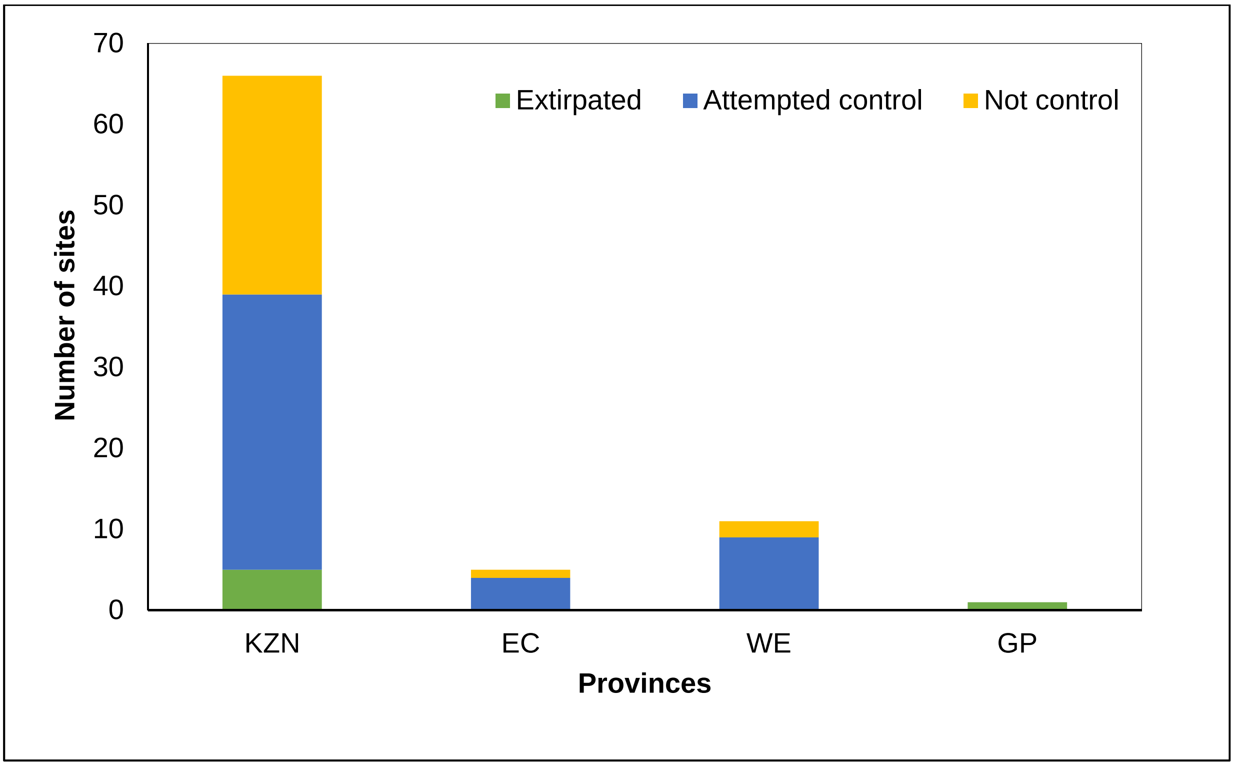


Figure 2. 8: Management of sites infested with *Sagittaria platyphylla* in KwaZulu-Natal (KZN), Eastern Cape (EC), Western Cape (WC), and Gauteng (GP) provinces under different management techniques (herbicide application, manual and mechanical removal). 'extirpated', were sites which had complete removal of the population; 'attempted control' were sites that had populations which still prevailed in spite of the eradication control method applied; 'no control', were sites that had no eradication method applied to them.

Even though there were some examples of extirpated sites, there was a general increase in the number of sites within managed sites. In 2013, when eradication was initiated by SANBI-ISP, *S. platyphylla's* population size covered an area of 3 035 m^2, however by 2019, this had increased to 9 847 m^2 and the number of the sites increased from 19 to 70 sites (Fig. 2.9). This suggested that even though some eradication efforts were successful in terms of extirpated sites, the weed spread rapidly despite management efforts, suggesting that these management efforts are currently insufficient to manage the species.

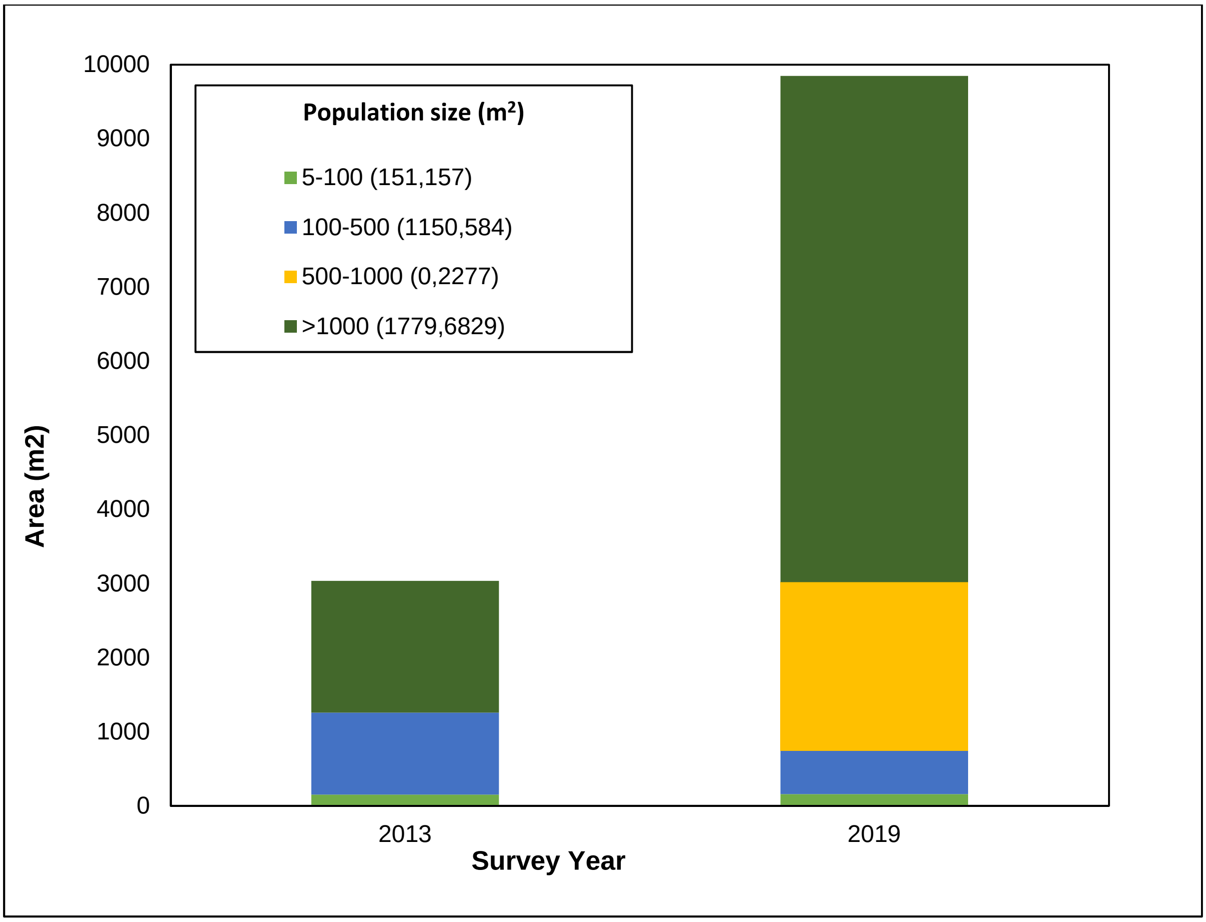


Figure 2.9: The area occupied by *Sagittaria platyphylla* infestation composed of different population sizes in South Africa at the initiation of eradication control project in 2013, and after six years of eradication application in 2019. Population size range from small (5–100), medium (100–500), big (500–1000) and large (>1000).

The total management cost spent on *S. platyphylla* in South Africa from 2010 to 2019 was estimated to be ZAR 1,720,000 (US $118,388) (Table 2.6). The biggest items on the budget were herbicides and salaries of local contractors employed to spray and, where appropriate, manually remove *S. platyphylla* populations. As no biological control agents have been released yet, no comparison could be made regarding the cost incurred vs. management, but the cost spent on the research for the weed's biological control was included.

Table 2.6. The total management cost of *Sagittaria platyphylla* in South Africa from 2010 to 2019.

Organisation	Type of Control	Time Frame	Overall Management Cost (R)	US$ (1R = $0.07)
Government (SANBI-BID)	Mechanical/Chemical	2010–2019	1,000,000	66,959
NGO	Chemical	2013–2017	60,000	4286
Private Landowner 1	Mechanical/Chemical	2017–2019	600,000	42,857
Private Landowner 2	Mechanical	2016	60,000	4286
Total Cost			1,720,000	118,388
Government (Centre for Biological Control)	Biological Control	2013–2019	701,000	50,071

The total management cost used to control *S. platyphylla*, calculated every three years, showed an increase with time (Fig. 2.10). In 2013, when eradication techniques were initiated by SANBI BID, *S. platyphylla* infestations covered a total area of 3035 m^2, at a cost of ZAR 32.95 (US$2.21)/$m^2$, but by 2019 the infestation cover had increased to 9847 m^2 and the number of sites from 19 to 70, therefore only ZAR 10.16 (US $0.68)/$m^2$ was available for management.

2.3.3 Potential Spread of Sagittaria platyphylla *in South Africa*

Thirty-eight threatened water bodies were recorded around known invaded sites in South Africa. Ten were within a 1km buffer, 15 within a 2.5 km buffer and 38 within a 5 km buffer (Table 2.7).

Table 2.7: Waterbodies threatened by the *Sagittaria platyphylla* invasion within a buffer zone of 1, 2.5 and 5km applied around known invaded sites in South Africa.

Province	Water Type	Name of Threatened Water Bodies	1km Buffer	2.5km Buffer	5km Buffer
Kwazulu-Natal	Lentic	Aquarium			*
Western Cape		Banghoek			*
Kwazulu-Natal		Blackborough Spruit	*	*	*
Eastern Cape	Lotic	Buffalo River	*	*	*
Kwazulu-Natal	Lotic	Camps Drift			*
Kwazulu-Natal	Lotic	Diepgat River			*
Eastern Cape	Lentic	Dolphin Stadium			*
Kwazulu-Natal	Lentic	Dorpspruit			*
Kwazulu-Natal	Lentic	Duck Pond			*
Western Cape	Lotic	Franschhoek Tunnel		*	*
Eastern Cape	Lentic	Grey Dam	*	*	*
Western Cape	Lotic	Helderberg Nature Reserve Reservoir		*	*
		Hugos	*	*	*
		Kwapata			*
	Lotic	Lang River			*
Kwazulu-Natal	Lentic	Lions Dam		*	*
		Lourens	*	*	*
Eastern Cape	Lentic	Mbongozi			*
Kwazulu-Natal	Lotic	Mhlatuzana River		*	*
Western Cape	Lentic	Model Yacht Pond	*	*	*
Kwazulu-Natal		Mpofana			*
Kwazulu-Natal		Nkutu	*	*	*
Kwazulu-Natal	Lentic	Oceanwalker			*
	Lentic	Old Arts Bokkie Pond			*
	Lotic	Outside Stream			*
Western Cape	Lotic	Palmiet River		*	*
Kwazulu-Natal	Lentic	Penguin Rookery			*
		Sentrale Kloof			*
Kwazulu-Natal	Lentic	Shark Dive			*
Kwazulu-Natal	Lentic	Snorkel Lagoon			*

Kwazulu-Natal	Lentic	The Bird Sanctuary			*
Eastern Cape	Lotic	The Mill Stream			*
Kwazulu-Natal	Lotic	Town Bush Stream			*
Eastern Cape		Tyusha	*	*	*
Kwazulu-Natal	Lotic	Umbilo River	*	*	*
Kwazulu-Natal	Lotic	Umsundusi River	*	*	*
Kwazulu-Natal	Lentic	Ushaka Pond			*
Western Cape	Lotic	Wilgerfontein River			*

* The buffer zone distance in which the Waterbodies threatened by the *Sagittaria platyphylla* invasion is located.

Most of these new waterbodies are in KwaZulu-Natal and include uninfected rivers around the uMgeni catchment such as Diepgat River, Mhlatuzana River, Umbilo River and Umsundusi River (Fig. 2.11).

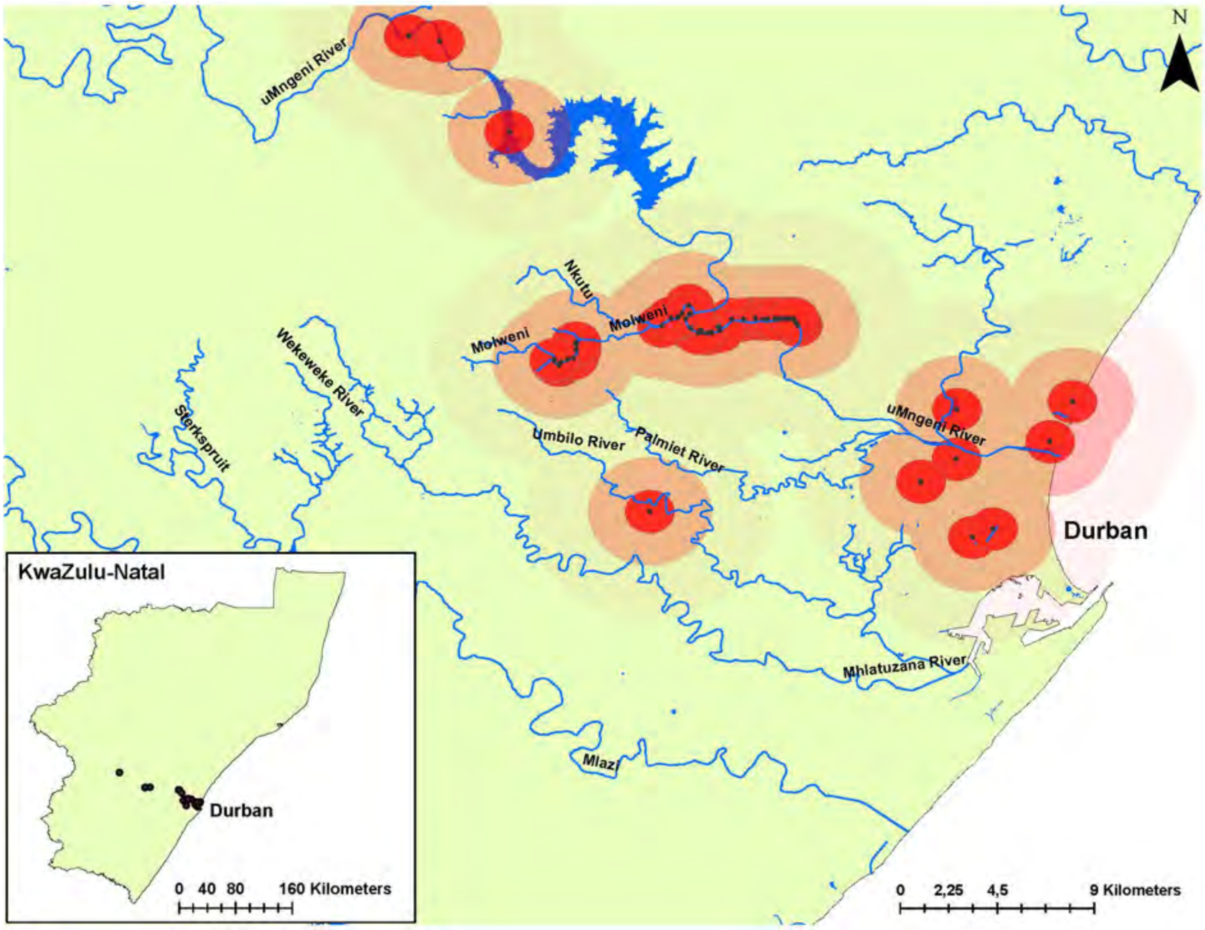


Figure 2.11: Waterbodies threatened by the *Sagittaria platyphylla* invasion within a buffer zone of 1, 2.5 and 5km applied around known invaded sites in in KwaZulu-Natal Province.

The most threatened river catchments include the Umgeni River catchment in KwaZulu-Natal, Buffalo River in Eastern Cape and the Berg River catchment in Western Cape.

2.3 DISCUSSION

Sagittaria platyphylla has spread within and between freshwater systems in South Africa since it was first recorded in 2008. This chapter describes the eradication approaches that were attempted against the weed, which form an important case study for invasive aquatic plant control, in particular species targeted for eradication. To date, despite significant financial investment, *S. platyphylla* continues to spread exponentially with invasions becoming more widespread and abundant. The invasion in South Africa is at the stage where it is outgrowing eradication efforts. Additionally, several new sites are in danger of being invaded by the weed.

To eradicate the weed under the SANBI-ISP programme, extensive management has been applied against populations of the weed for over 10 years. This has resulted in some populations being cleared and suppressed. However, in general this has not been successful with the number of sites increasing dramatically. In addition, the infested area has tripled since management applications were initially implemented in 2013, suggesting that there are more sites and a greater area to manage, making the current management process unsuitable and eradication no longer feasible.

Drivers of Sagittaria platyphylla *invasion*

In its native range, *S. platyphylla* is mostly found inhabiting shallow flooded or marshy areas in natural or semi-natural habitats like wetlands, riverbanks and along the margins of lakes (Adair *et al.,* 2012). In countries where it is introduced such as Australia and South Africa it is found favouring ruderal habits (Kwong *et al*., 2017a;

Martin *et al.,* 2018). A previous study showed that 65% of populations in both introduced ranges were found in ruderal habitats such as irrigation supply channels, drainage ditches and artificial impoundments (Kwong *et al*., 2017a). This study showed that 80% of populations in South Africa occur in lotic water systems, mainly along riverbanks, with only 20% in artificial impoundments (ponds and dams). Kwong *et al.* (2017a) also showed that there was 40% greater vegetative cover and more extensive vegetative spread in both introduced ranges compared to native range, which was supported by this study. In South Africa, several sites have developed along 120 km of the Umgeni River catchment, KwaZulu-Natal Province. Similar large infestations are also recorded from Australia where infestations can be found over 11 900 km of creeks, drains and channels of the Goulburn-Murray Irrigation district (Chapman & Dore, 2009). South African populations produce 40% more achenes per fruiting body compared to its native range (Kwong *et al.,* 2017a). This has increased the number of seeds that may spread within lotic water systems, which is the main driver of the *S. platyphylla* invasion. Recreational activities and water movement also allow the high number of seeds to disperse further downstream. Furthermore, the seeds may be trapped in the soil and can be dispersed by animals, birds and recreation equipment (Martin *et al.* 2018). Additionally, South African populations are exacerbated by the presence of nutrient (high nitrate and phosphate concentrations) enriched waters from pollution associated with urbanization from the Umgeni River and Berg River, which has promoted the invasion success and spread of *S. platyphylla* (Hill & Coetzee, 2017).

The outcome of management techniques

Eradication of an invasive species is the second line of defense after prevention, it aims to eliminate every single individual, including vegetative propagules (Myers *et al.* 1998; Beric & MacIsaac, 2015). This is only practical just after the species is detected, and if there is sufficient funding and resources for the management application (Simberloff, 2003; Zimmerman, 2011). Previous South African studies have shown several plant and animal eradication approaches applied on invasive species which were recorded as being unsuccessful (Wilson *et al.,* 2013), until recently on a substantial control in South Africa of *Spartina alterniflora* Loisel. (Poaceae), an aggressive ecosystem engineer. The small population of the plant (<1 ha) was first detected in the Great Brak Estuary, Western Cape Province, in 2004, and through regular herbicide treatment during the growing period, the plant was successfully extirpated (Riddin *et al*., 2016). The success of this programme was attributed to the small, accessible localised infestation, and appropriately timed herbicide application (Riddin *et al*., 2016).

Sagittaria platyphylla was one of several invasive species in South Africa targeted for eradication in 2013 at an initial cost of R90 000 (Wilson *et al.,* 2013). By 2017, *S. platyphylla* was no longer considered for eradication due to its spread, but even so, eradication techniques were still applied at a rising cost. In the first years of the species identification and detection, a site in located Pretoria National Botanical Gardens, Gauteng (-25.739; 28.272) was manually removed in 2012 and never recorded again. This proved eradication techniques to be more feasible when infestation is small and not in lotic water systems where they are most likely to spread through seed dispersal. In three years of eradication attempts that followed (2013-2016), *S. platyphylla* increased from 6 406 m^2 to 22 685 m^2 under these efforts, but in the second, three year cycle (2016-2019), the invasion decreased in 2017, increased in 2018 just to decrease again in 2019, leaving the overall invasion at 18 804 m^2. Additionally, throughout the six years of eradication application, there was a steady increase in the number of recorded sites, and the number of large infestations (> 1000 m^2) recorded. Therefore, this study's results showed that the eradication approach of *S. platyphylla* has not been effective in the past ten years. In South Africa, eradication is possible in small population sizes of terrestrial invasive weeds but can be challenging for aquatic invasive weeds due to application techniques (Wilson *et al.* 2013; Hill & Coetzee, 2017). For example, manual removal and mechanical control of aquatic plants such as *Hydrilla verticillata* and *Egeria densa* are known to lead to fragmentation of the weed mat which results in successive dispersal, thereby increasing the weeds' infestation (Dayan & Netherland, 2005). Herbicide application to invasive species with non-registered herbicides, such as *S. platyphylla* in South Africa, is also known to be ineffective in the end as it does not affect the root zone of the plants. This was observed on an experimental approach of *Myriophyllum aquaticum invasion* when two glyphosate-based herbicides that were not registered against the weed were applied and resulted in temporarily controlling the weed, but did not affect the root zone of the plants (Cilliers *et al.,* 2003).

Herbicide application on *S. platyphylla* kills the emergent plant material but also stimulates submerged plants and tubers to grow into emergent plants due to reduced competition (Forrest *et al.,* 2011; Broadhurst & Chong 2011). When manual removal and mechanical control of *S. platyphylla* is applied, it has a high risk resulting from broken plant pieces, floating seeds and underground tubers remaining in the system, which have the ability to regenerate and quickly re-infest systems (Forrest *et al.,* 2011). Both these reasons could explain the re-appearance of plants on supposedly eradicated sites seen in the field.

The best management options for controlling submerged invasive weeds are mostly driven by the management goal, traits of species in question, type of invaded habitat and national legislation for management (Coetzee *et al.,* 2019). The control management options for controlling *S. platyphylla* were driven by the national legislation for management of Category 1a species under NEM: BA regulations (see Chapter 1, section 1.2). This was applied in 2013 when the population size was small with 18 known sites covering an infested area of 6 406 m^2, but over the past six years, those numbers have drastically increased. The failure to achieve eradication of *S. platyphylla* in South Africa could have resulted from simple management errors. For example, it is likely that by 2013, the *S. platyphylla* infestation was already too widespread for eradication. Eradication is advised to be implemented right after the invader species is detected, on small infestations (Myers *et al.,* 1998: Vincent *et al.,* 2009; Beric & MacIsaac, 2015). This minimizes the time for the invasion to further spread before eradiation applications are implemented (Willby, 2007).

The infestation spreading through the Umgeni River and the Berg River catchments in KwaZulu-Natal is concerning as both these rivers are valuable water supplies to their respective provinces, and are already prone to invasion by invasive alien plants (Macdonald, 2004; Turpie *et al.,* 2008). While eradication was not successful, management of the species should remain a priority. If *S. platyphylla* is left to invade without any intervention, it would increase the negative impacts on the environment, which include reduction of stream flow, displacement of native plant species, an increase in water consumption and habitat transformation

If *S. platyphylla* management continues under the same management regime, it is expected that the plant will further spread into additional water bodies, which will in turn increase its management cost. Recategorizing *S. platyphylla* from a category 1a invader species to a category 1b will change the current management approach used to manage *S. platyphylla* in South Africa, by shifting it from an eradication target to managing it through containment and impact reduction strategies of local infestations. *Sagittaria platyphylla's* invasion is at the stage where an integrated weed management approach seems to be the only practical method that can achieve a long-term, cost effective management approach, including the release of host-specific and damaging biological control agents, such as *Listronotus appendiculatus*.

CHAPTER 3:

IMPACT OF *LISTRONOTUS APPENDICULATUS* ON *SAGITTARIA PLATYPHYLLA*'S.

3.1 INTRODUCTION

Several invasion hypotheses have been proposed to explain why some plants become invasive in their introduced ranges when compared to their native range. One of many supported hypotheses is the Enemy Release Hypothesis (ERH), which proposes that in a plant's introduced range, it can become more abundant and widely distributed due to the lack of natural enemies (Keane & Crawley, 2002; Kwong *et al.,* 2017a, Kwong *et al.,* 2018). The hypothesis is based on three principles: (1) natural enemies are important regulators of plant populations, (2) natural enemies have a greater impact on native species than on exotic species, and (3) plants are able to benefit from a reduction or absence of natural enemies, resulting in increased plant reproduction and population growth (Keane & Crawley, 2002).

Kwong *et al.* (2017a) conducted a biogeographical study on *Sagittaria platyphylla* (Engelmann) J.G. Smith (Alismataceae) which aimed to determine if the ERH explained its invasion success in the introduced range (namely, South Africa and Australia). The study compared individual plant as well as population parameters between the two invaded countries and the native range, the United States of America (USA). It also measured the levels of herbivory and disease associated with the plant in both the invaded and native range. The study showed higher levels of plant cover in both introduced range populations compared to the native range population but found no difference in plant densities between the native population and introduced range populations (Kwong *et al.,* 2017a). The plant's morphological traits differed only in South Africa, which had 50% taller plants compared to Australia and the native range, while plant height in Australian populations was similar to plants in the native range (Kwong *et al.,* 2017a). However, there was a consistent difference observed in the sexual reproductive traits between both introduced range and native range. In the introduced range, *S. platyphylla* produce 40% more achenes per fruiting body, which also weighed 50% more, compared to the native range (Kwong *et al.,* 2017a). The change in sexual reproductive traits were taken as evidence that biotic stressors, such as specialist herbivory, influenced seed size in the native range (Kwong *et al.,* 2017a).

Kwong et al. (2014) also showed that plants in the introduced range had fewer arthropods and pathogens associated with it, compared to plants in the native range, which had a diverse community of natural enemies that targeted different parts of the plants, including endophagous flower and fruit feeders (Kwong *et al.,* 2014). In the native range, natural enemies damaged fruiting heads by 46%, leaves between 33-57%, and petioles and inflorescence by 56% and 43%, respectively (Kwong *et al.,* 2018). This was compared to plants in both introduced ranges which only had 10% damage (Kwong *et al.,* 2018). Kwong *et al.,* (2017 a) suggest that the lack of natural enemies has promoted the plant's invasion in its introduced ranges, thereby supporting the ERH for *S. platyphylla* in both South Africa and Australia. Kwong *et al*. (2017a, 2018) go on to suggest that pre-dispersal seed predation may play an important role in the plant's management. The enemy release hypothesis, along with the biotic resistance hypothesis, which proposes that herbivory regulation (pathogens, natural enemies) is able to suppress population growth of invasive species by reducing plant reproduction and population growth (Wilbur *et al.,* 2013), can be used to explore the effect of insect herbivory on population growth and plant fitness of invasive species using biological control (Bigger & Marvier, 1998).

During *S. platyphylla* growth and development, resource allocation is balanced between three major functions, namely growth, reproduction and defence (Gomez *et al.,* 2007). When plants are influenced by insect herbivory, the balance of plant resource allocation may change in favour of rapid defence responses, thereby reducing resource allocation in growth and reproduction functions (Gomez *et al.,* 2007; Schultz *et al.,* 2013). Empirical studies have shown that responses that are always present in plants, known as constitutive defence mechanisms, are often costly to the plant's growth (Elle *et al.,* 1999); while plant defence theories suggest that responses produced in reaction to damage or stress caused by herbivores, or inducible defence mechanisms, have evolved to reduce this cost by matching resource allocation between the three functions (Agrawal *et al.,* 1999). This plasticity in inducible defence responses, reducing the cost of constitutive defence responses begs to question if herbivore attack does indeed suppress population growth of invasive species by reducing plant reproduction and population growth. Understanding how plant resource allocation is managed during herbivore attack can be complicated (Schultz *et al.,* 2013), but it can be expressed or measured in terms of decreased plant fitness (Wilbur *et al.,* 2013). Plant fitness is generally measured in terms of reproduction success which is generally seed production (Strauss *et al.,* 2001). According to the resource allocation theory, this can be tested under low-resource environments (Gomez *et al.,* 2007), but may also depend on how environmental conditions predicted by environmental change, and how related the phenotypic and genetic costs are affected by these changes (Van Dam & Baldwin, 2001). Therefore, the effect and impact of insect herbivory can be measured by assessing the plant's reproductive outputs between herbivory treated environments and herbivory free environments (Gomez *et al.,* 2007).

Sagittaria platyphylla is a hermaphroditic plant that has both male and female reproductive organs, and reproduces asexually through tuber and ramet production (Fig. 1.3, Chapter 1) (Flower, 2004). In South Africa, the plant produces heavier seeds than in its native range (Kwong *et al*., 2017a) and has a longer flowering duration period which increases the number of seeds and the probability that more seeds get released into the environment (pers. obs.). According to Kwong *et al*. (2017a) and Martin G.D., (pers. comm. 2018), seed production is assumed to be the primary mechanism for *S. platyphylla* dispersal and spread in both Australia and South Africa. Due to the high number of seeds produced by the plant and the ease of its spread through the environment, the initial attempts to eradicate the plant using herbicide and mechanical efforts are unsuccessful (Martin *et al*., 2018).

Due to the failure of the eradication programme against *S. platyphylla* in South Africa, a classical biological control programme was initiated as a potential solution for the management of *S. platyphylla* (Martin *et al.,* 2018). Classical biological control is based on the fundamentals of the enemy release hypothesis (Liu & Stiling, 2006). Therefore, surveys for natural enemies were conducted in the USA which resulted in the collection of four *Listronotus* weevils. Amongst these was *Listronotus appendiculatus* Boheman (Coleoptera: Curculionidae), which was collected as a potential seed-attacking biological control agent, prioritized for reducing seed production of *S. platyphylla*. The adults feed on the flowers and fruits, and the larvae feed on the receptacle and developing achenes on fruiting heads of *S. platyphylla* (Adair *et al.,* 2012). As the larva develops, it mines through the flower stalk and petioles, damaging the plant vascular tissue. The larvae pupate in the petiole (Rogers, unpublished). *Listronotus appendiculatus* was the most common and damaging insect identified during surveys conducted for natural enemies in the native range (Martin pers. comm.; Kwong, 2014, Kwong *et al*, 2014). Therefore, *L. appendiculatus* was prioritised as a pre-dispersal seed attacking potential control agent that could potentially play an important role in suppressing population growth of *S. platyphylla* by reducing the plant's reproduction and population growth in South Africa.

The aim of this study was to determine the potential impact of *L. appendiculatus* on *S. platyphylla*, focusing on the impact on plant morphology and both reproductive traits. Furthermore, determining seed viability and seed germination on insects damaged seeds. This could then be used to predict whether *L. appendiculatus* can reduce the number of viable seeds released into the environment and in turn, aid in limiting the spread of *S. platyphylla* in South African.

3.2 METHODS AND MATERIALS

3.2.1 Study Species: Listronotus appendiculatus

Listronotus appendiculatus is a small slender (4.2-6.5mm long) weevil from the Curculionidae family that is native to North America (Blatchley & Leng, 1916; Alonso-Zarazaga & Lyal, 1999). The biology of *L. appendiculatus* has been previously studied on both *Sagittaria latifolia* Willdenow. (Alismatacea) by Muenchow & Delesalle (1992), and on *Sagittaria platyphylla* by Rogers *et al*. (2018 unpublished). The biology on both plant species appears to be similar (Kwong *et al.,* 2014), for this study I describe *L. appendiculatus*' biology based on Rogers' *et al*. (2018 unpublished) laboratory-based study.

I. Adults

The taxonomic description can be found in Henderson (1940) and O'Brien (1981). Under the ventro-abdomen structures: the second and third abdominal sternites are concave in female adults and convex in male adults; the second abdominal sternite is rounded on its anterior side in male adults and flat in female adult. Additionally, female adults have larger and longer last three abdominal sternites compared to male adults. Insect mass and ventro- abdomen structures can be used to differentiate between sexes. Female adults are slightly larger and weigh more than male adults. The average development time (from egg to adult) of *L. appendiculatus* under laboratory conditions was calculated to be 41.2 days ± 2.16 (S.E.).

II. Egg

Female *L. appendiculatus* oviposit eggs deeply in between the achenes in older fruits, on the surface of the fruit, and on the base of the receptacle. They are mostly laid in clusters, containing two or more eggs, but no more than five eggs, which change from a cream colour to a metallic black colour with time. The eggs weigh 0.052 mg ± 0.001 (SE) with an average length and breadth of 0.86 mm ± 0.01 (SE) and 0.32 mm ± 0.00 (SE), respectively. The average time (from oviposition to neonate larvae) under laboratory conditions is 5.1 days ± 0.13 (SE) days.

III. Larvae

Neonate larvae are initially translucent but become creamy yellow in colour as they mature. Neonate larvae mine through the carpels of the fruits and into the receptacle. As they mature, they leave the fruiting body and crawl down to the petioles and inflorescence stalks where they mine, causing the above leaf to wilt, they remain in the petiole until they pupate. The weevil has four larval instar stages, and the development time from hatching into neonate larvae to pupation under laboratory condition was 11.45 days ± 0.63 (SE).

IV. Pupae

Larvae pupate in the base of inflorescence stalks or leaf petioles. Initially pupae are creamy white in colour but darken with age. Pupae weigh between 7.5-11 mg (n= 6).

3.2.2 Laboratory Cultures

Listronotus appendiculatus

Listronotus appendiculatus weevils used for the study originated from the insect culture collected on the 17th September 2014 from a small artificial dam on Tara Wildlife Reserve, near Vicksburg, Mississippi in the United States of America (32.490289: -91.060123). This culture was imported (import number: P0066302) into the Rhodes University Quarantine Facility on the 26th September 2014. The conditions in the quarantine facility were kept at a constant temperature of +/- 26˚C, with 16/8-hour day/night schedules.

The culture was reared under the following conditions: Adult weevils were kept in eight litre clear plastic containers containing racemes, placed on a moist paper towel. This allowed the adults to feed on the flowers, mate and oviposit on the fruiting heads. The fruiting heads were left in the container until the eggs hatch and larvae develop within the fruits. Once the larvae were in their second or third instar, they were moved to two litre plastic containers (30 x 20 cm) layered with petioles placed on dry paper towel where they completed their development. The pupae were kept in (30 x 20 cm) two litre plastic containers, placed within moist paper towel until they enclose, then moved into the adult box (boxes where adult insects were kept). The culture was handled during the day.

Sagittaria platyphylla

Forty *S. platyphylla* plants were collected from the Makana Botanical Garden, Grahamstown, Eastern Cape, South Africa (33.316; 26.521), pruned and replanted into plastic pots (25 x 20 cm). The pots contained building sand mixed with Osmocote fertilizer which had an NPK ratio of (19-6-12) and nutrient release duration time of four months. They were then placed in plastic lattice pools at the Waainek Mass Rearing

Facility, Rhodes University, to standardize their growth. Plants were allowed to grow until they reached a similar standard height after approximately four weeks.

3.2.3 Impact Study: plant morphological traits and reproductive outputs

To determine the potential impact of *L. appendiculatus* on *S. platyphylla*, an impact study was conducted to compare plant morphological and reproductive traits between plants grown under insect herbivory exposure and plants grown under the absence of insect herbivory. The study looked at the impact of insect feeding and development on plant morphological traits, reproductive outputs and the resulting first generation (F^1) reproductive outputs.

The study was conducted in the Rhodes quarantine facility because *L. appendiculatus* was still under investigation and not yet released for the control of *S. platyphylla* in South Africa. Before the study was conducted, the mean number of reproductive outputs and the morphological traits of the starting population (n= 6) were measured by destructively sampling the plants. The traits measured in the beginning of the experiment were determined as the traits of the starting population which was compared to the traits measured after the experiment from the different herbivory treatments.

The morphological traits measured included:

- The height of each plant (measured from the base of the crown to the tallest leaf).
- The number of healthy leaves per plant (based on visual observations).
- The number of racemes per plant.
- The number of fruits on the selected raceme observed to have the biggest fruits.
- The length of the selected raceme (measured from oldest pedicels to the raceme’s growth tip).
- The number of tubers per plant.

Twenty plants were then moved to Rhodes University's Quarantine Facility and randomly divided into the herbivory treatment and controls. The same plant parameters as mentioned above were then measured, however, the number of tubers was not counted as this would be too destructive for the plants to survive. Two pairs of mating weevils were then added to each of the plants comprising the herbivory treatment (n=10) and no insects were placed on the controls (n=10). All plants were then covered with a white fabric mesh sheet, held by four dowels and tied with string around the pot plant to ensure no insects could escape (Fig. 3.1). The experiment 's duration was conducted for five weeks.

Figure 3. 1: Impact study design showing white fabric mesh sheet used to contain *Listronotus appendiculatus* weevils onto potted *Sagittaria platyphylla* plants.

After five weeks, the plant's morphological traits (mentioned above) were re-measured. Additionally, the direct impact on reproductive outputs (number of germinated seeds and ramets before F^1 generation), and the impact on F^1 reproductive outputs (raceme (number and size) and tubers (number and weight) were measured. The herbivory and control measurements were then compared to the starting population data.

STATISTICA © ver. 13.0.4.14. was used to analyse the data and to determine if there was a significant difference between the treatments. A General Linear Model (LM) was used to compare the mean difference of measured plant traits between the initial population, control treatment and herbivory treatment, followed by Tukey HSD tests to determine where the significant difference between the treatments were. A Student's *t*-test compared the mean difference of measured plant traits between control treatment and herbivory treatment plants.

3.2.4 Impact Study: seed viability and germination

To determine the impact of larval damage by *L. appendiculatus* on *S. platyphylla's* seed production, several seed viability and germination trials were conducted on seeds damaged and not damaged by the insect.

Seed Viability Test

Six mature plants collected and cultured in the same methods as above were moved to Rhodes University Quarantine Facility for seed viability studies. The experimental design involved placing a field density of two pairs of mating weevils per plant onto three separate plants (herbivory treatment) and no insects on three plants (control). All experimental plants were then covered with a white fabric mesh sheet held by four dowels and tied down by a string around the pot plant base (Fig. 3.1). When signs of larval damage on the fruits were recorded, the adult insects were removed. The racemes were then covered with fabric mesh tied with a string to ensure all seeds produced could be collected (Fig. 3.2).

Figure 3. 2: Experimental design showing white fabric mesh sheet used to contain *Sagittaria platyphylla* racemes under *Listronotus appendiculatus* larval impact*.*

After the two-week period, all seeds were collected from the herbivory treatment and control treatment, and left to air dry at room temperature, for seven days. The seeds from the herbivory treatment were then inspected under a dissecting microscope and divided into two groups, seeds that had direct mechanical larval damage, and undamaged seeds. The seeds in the control treatment were also collected. Due to the high number of seeds, a sub-sample of 100 seeds was taken from both the herbivory treatment (both groups) and the control.

A seed viability test using a 1% tetrazolium (TZ) assay for viability according to Verna and Majee (2013) was then conducted. The 2, 3, 5 triphenyl tetrazolium chloride or bromide is a colourless redox indicator widely used to indicate the presence of cellular respiration during seed viability trials. Respiring cells reduce the colourless chemical into a red compound thereby staining the living parts of a seed, proving it viable. After sub-sampling seeds between the treatments, the seeds were scarified by soaking in 1ml scarification solution for 8 minutes under shaking conditions of 150 RT using a Labcon shaker (model no. spompis4upf75, SN.L228822). The scarification mixture was made from a mixture of 20 ml commercial bleach and 100 µl Triton X-100 in 100 ml autoclaved distilled water. The scarification process encouraged germination by weakening and altering the seed coat to permit access of the TZ solution to the internal seed tissue. After scarification process, the seeds were soaked and rinsed in distilled water to remove the scarring solution, then placed between dry paper towels to remove excess water. The seeds were then stained by soaking them in 20 ml of 1% TZ solution, which was stored in 100 ml amber colour bottles. The bottles were placed in a black container and covered by a dark tray for 30hrs in a 26 ºC control-environmental chamber. After the staining process, seeds were submerged in a cleaning agent for 1-2hrs. The cleaning agent was made from a mixture of lactic acid: phenol: glycerine: water in a ratio of 1:1:2:1. The seeds were then observed under the microscope to evaluate the TZ staining pattern, allowing for a comparison between the herbivory treated and control samples to be made.

The TZ stain did not stain the seeds with a bright red stain as expected but resulted in distinctively staining the embryo's darker than its cotyledon when observed under the microscope. Therefore, the TZ staining pattern was determined by rating the visible contrast between the achene's embryo and cotyledon. To clearly visualise this, different seeds were inspected under the Leica EZ4D stereo microscope set at 94% brightness, 1.60 gamma and 198.00 saturation. Seeds with a clear healthy, visible embryo and cotyledon were recorded as viable seeds and seeds which had a partially visible embryo and cotyledon were recorded as abnormal/partially viable seeds, and the seeds which had no visible contrast and/ mined embryo were recorded as not viable or dead seeds (Fig. 3.3). The percentage in the number seed stain patterns between the herbivory treatment and control treatment was calculated. A Chi-squared test was then used to determine the significant difference in seed viability between the herbivory and control treatments.

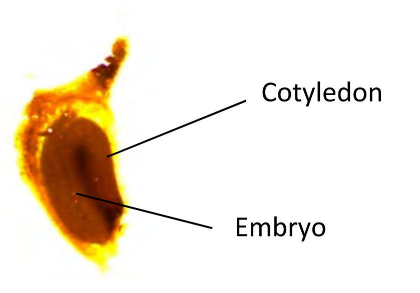
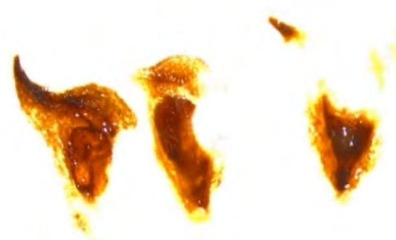
Viable Seed	**Abnormal/Partially Viable Seed**	**Dead or Non-Viable Seed**
		

Figure 3. 3: Different pattern of 1% tetrazolium (TZ) staining showing viable seed, abnormal/partially viable seed and dead or non-viable seeds of *Sagittaria platyphylla* under the impact of *Listronotus appendiculatus* larval damage, after 30hrs staining period in a 26 ºC controlled environment chamber.

Germination trial

To determine the best method to test seed germination of *S. platyphylla,* several different trials were attempted following procedures recommended for other *Sagittaria* and aquatic plant species (Giles, 2004). The method of germinating seeds in water, under +/- 26˚C yielded the highest germination rates in these trials. Fifty seeds per treatment sample were randomly selected and soaked in water-filled 10 ml glass vials (n=10/ glass vial) and placed at a constant temperature of +/- 26˚C, with 16/8-hour day/night schedules. Seeds from both treatments were randomly selected to allow all seeds (even the undamaged seeds in the treatment sample) to germinate as they would in the field. After a two-week germination period, the percentage of germinated seeds per treatment sample was calculated. A Chi-squared test was used to determine the significant difference in % germination between the herbivory and control treatments.

3.3 RESULTS

3.3.1 Impact Study: plant morphological traits and reproductive outputs

Plant morphological traits

Feeding damage by *L. appendiculatus* significantly reduced plant height ($F_{(2, 20)} = 5.25$; $P = 0.015$). The control treatment had significantly taller plants (57.45 cm ± 0.83 S.E.) compared to plants measured in the initial population (43.35cm ± 0.84 S.E.) and herbivory treatment (43.7 cm ± 1.80 S.E.) (Fig. 3.4a). At the end of the experiment, the control treatment also had significantly fewer dead leaves (1.44 ± 0.22 S.E.)

compared to plants measured in the herbivory treatment (5.40 ± 0.38 S.E.) ($t_{(17)}$ = 0.012) (Fig. 3.4b).

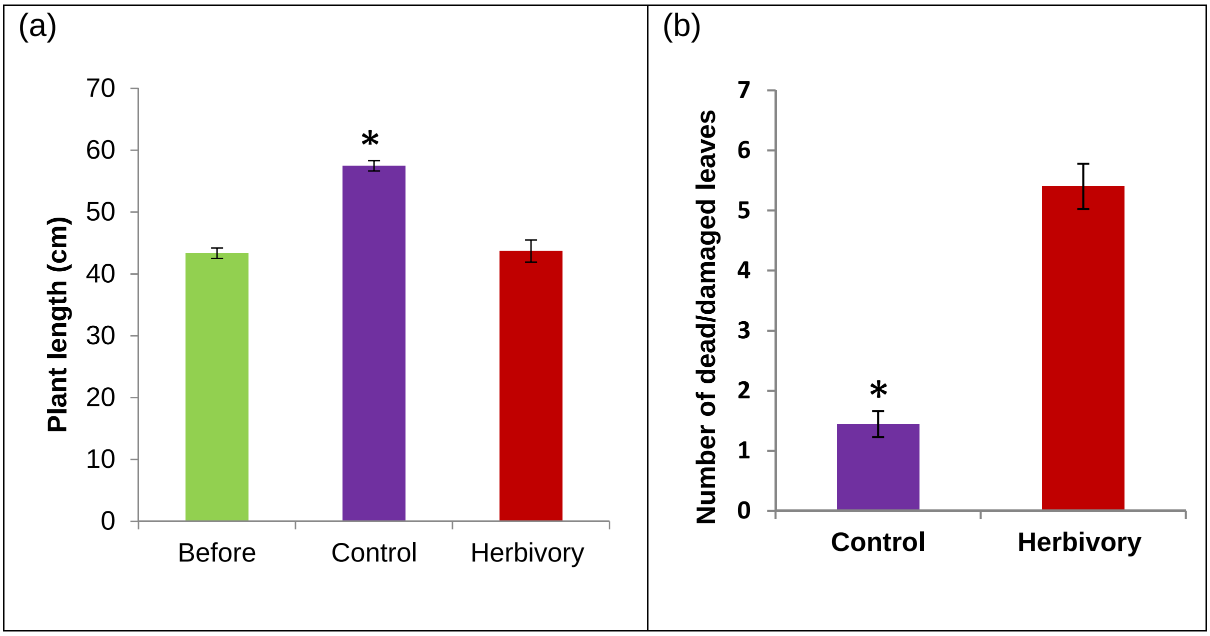


Figure 3. 4. The morphological difference in *Sagittaria platyphylla* plant height (a) and number of dead/damaged leaves (b) between initial plant populations (before), control treatment and herbivory treatment. Error bars indicate standard error, * represents significant difference (Tukey HSD, $P<0.05$).

After all dead/damaged leaves (including larval damaged petioles) were removed, visual inspection revealed 20% of plants under the herbivory treatment were dead and a visible reduction of the plant fitness was observed between the herbivory and control treatments (Fig. 3.5).

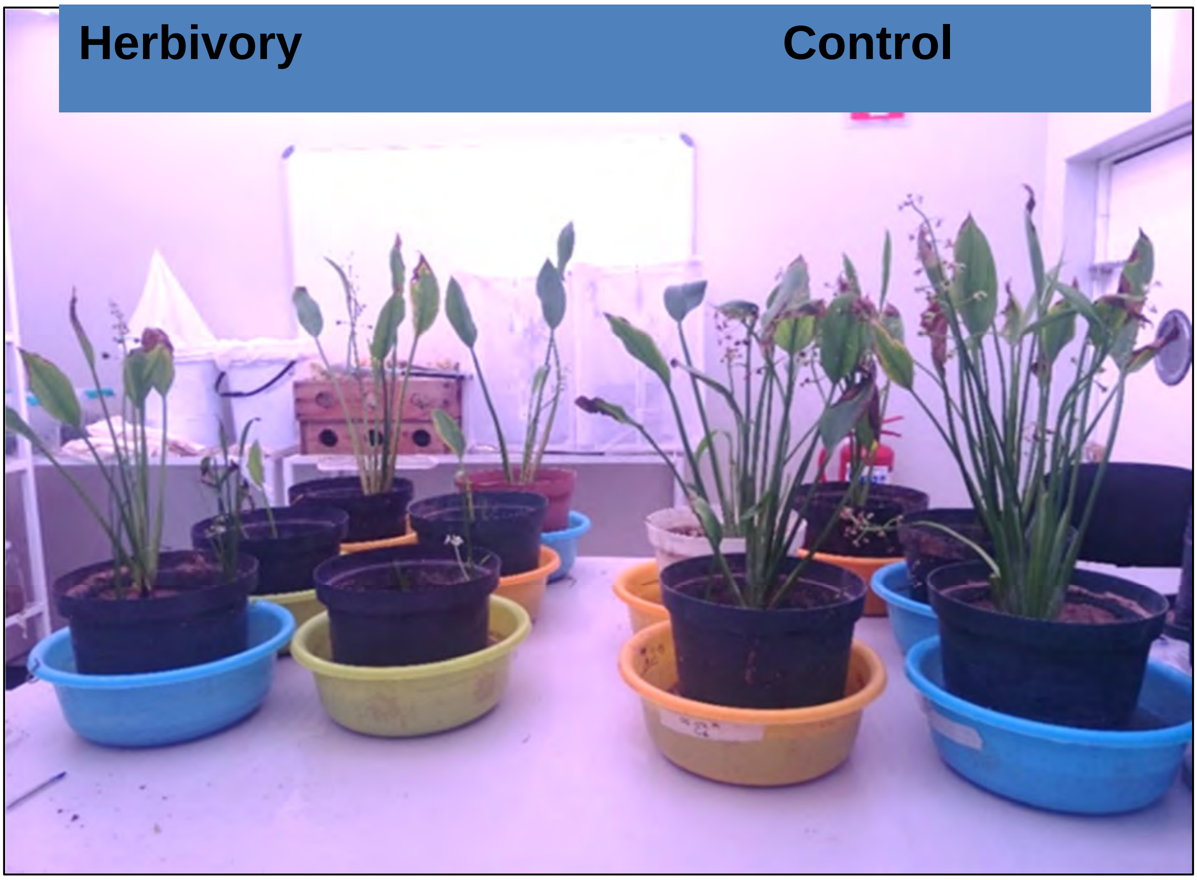


Figure 3. 5: The structural differences in *Sagittaria platyphylla* plants after removal of dead/damaged leaves between herbivory (left) and control (right) treatments at the end of a 5-week experiment, under the impact of *Listronotus appendiculatus* feeding.

Direct impact on reproductive outputs

The direct impact of *L. appendiculatus* feeding on the reproductive outputs of *S. platyphylla* resulted in a higher number of germinated seeds and ramet production, on herbivory treated plants compared to the control plants. Insect herbivory treated plants under the feeding of *L. appendiculatus* had a higher number of germinated seeds (42.6 ± 5.99 S.E) and ramet production (2.3 ± 0.21 S.E.), compared to the insect-free plants which had an average of 28.33 ± 3.52 S.E. germinated seeds and 1.7 ± 0.16 S.E. ramets (Fig. 3.6a-b).

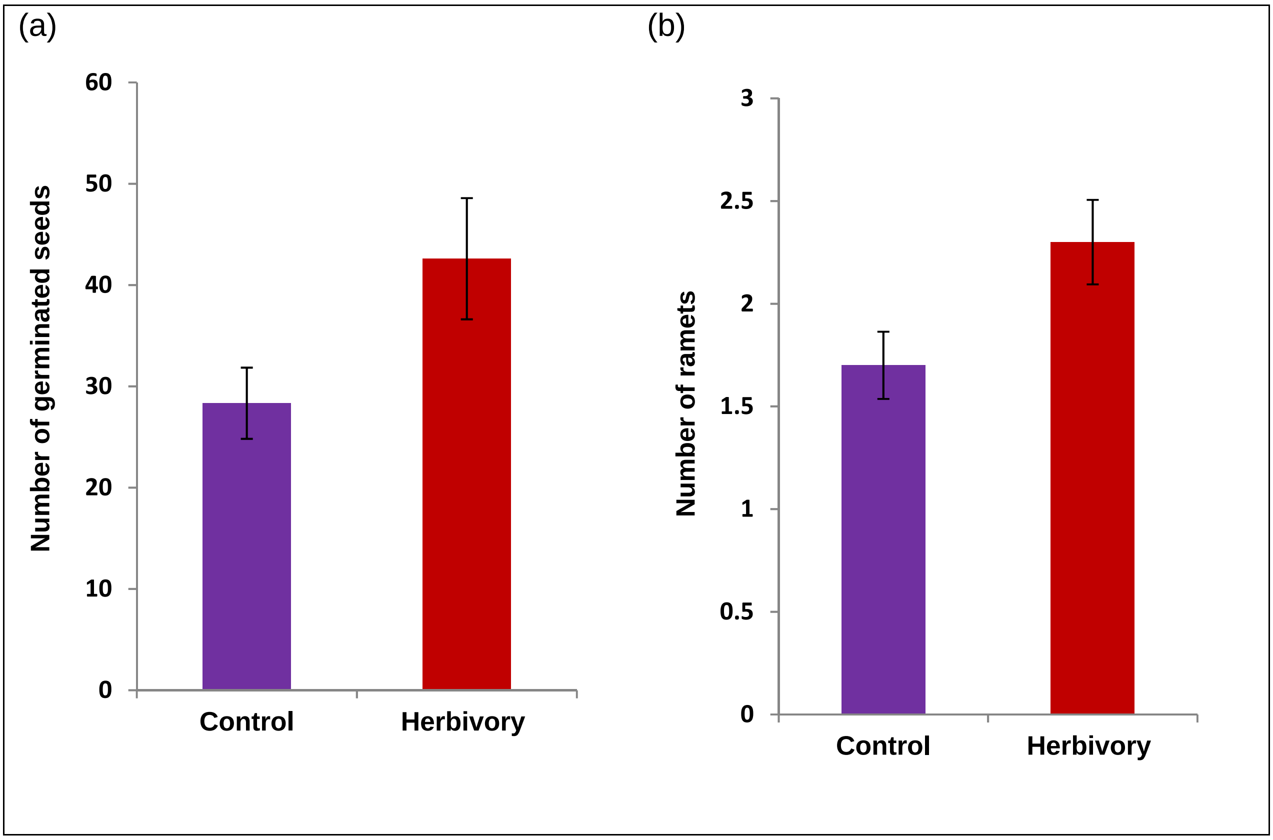


Figure 3. 6: Direct impact on reproductive outputs of *Sagittaria platyphylla* between control treatment and herbivory treatment at the end of a 5-week experiment, under the impact of *Listronotus appendiculatus*, for: (a) the number of germinated plant length and (b) number of ramets.

The impact on F^1 sexual reproduction outputs (raceme)

Listronotus appendiculatus feeding damage significantly reduced the number of racemes per plant ($f_{(2,22)}$=10,24; p=0.00072), length of raceme ($f_{(2,22)}$=17,71; p=0.00003), and number fruits per raceme ($f_{(2,22)}$=15,001; p=0.00008). This is compared to the herbivory treated plants which produced fewer racemes per plant (0.5 ± 0.07 S.E.) that were smaller in size (2.67cm ± 0.38 S.E.), and had fewer fruits per raceme (1.4 ± 0.24 S.E.) compared to the initial population and control treatment (Fig. 3.7 a-c). In the herbivory treated sample, only 40% of plants produced racemes and within them 75% produced fruits. These fruits were smaller compared to fruits on plants in the initial population sample and the control.

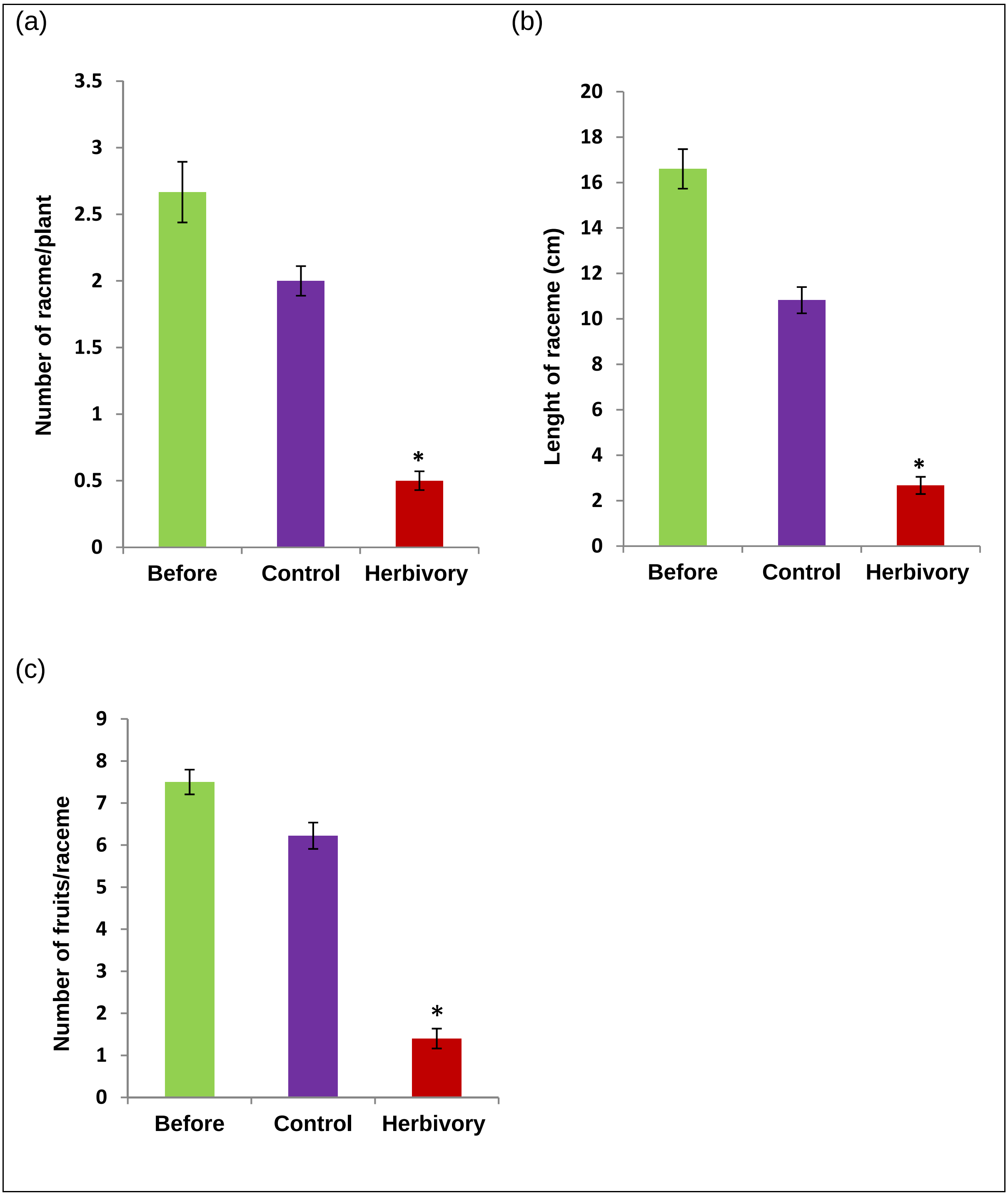


Figure 3. 7: Differences in *Sagittaria platyphylla* sexual reproduction output (raceme) between the initial plant populations (before), control treatment and herbivory treatment at the end of a 5 week experiment, under the impact of *Listronotus appendiculatus* for: (a) number of raceme per plant, (b) length of raceme, and number fruits per raceme (c). Error bars indicate standard error.* represents significant difference (Tukey HSD, $P<0.05$).

The impact on F1 asexual reproduction outputs (ramets)

Listronotus appendiculatus feeding reduced the number of tubers produced (13.33 ± 1.18 S.E.) compared to the control treatment which produced a mean of 20.4 ± 2.87 S.E. tubers (Fig. 3.8a). The tubers produced in the herbivory treatment also weighed less (1.54g ± 0.013 S.E.) than the control treatment (1.55g ± 0.011 S.E.) (Fig. 3.8b).

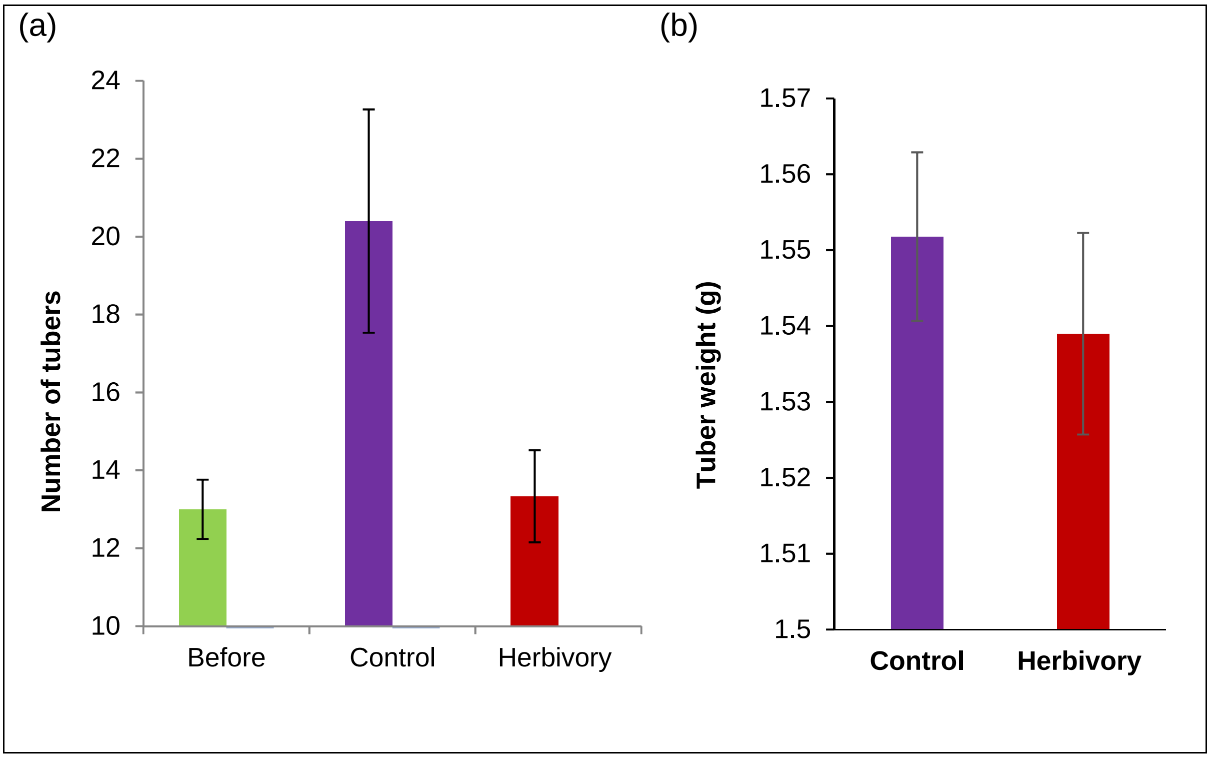


Figure 3. 8. Differences in *Sagittaria platyphylla* asexual reductive output (tubers) between initial plant populations (before), control treatment and herbivory treatment at the end of a 5-week experiment, under the impact of *Listronotus appendiculatus* for: (a) number of tubers and (b) tuber weight. Error bars indicate standard error.

Inferring all experimental plants in the initial population had a mean of 13.33 ± 0.76 S.E. tubers per plant based on the six samples destructively sampled at the start of the experiment, the mean number of tubers produced in the herbivory treatment increased by 0.33 ± 1.18 S.E. compared to the control treatment which increased by 7.4 ± 2.86 S.E. tubers. Furthermore, the herbivory treatment had a higher percentage of small sized tubers weighing from 0-2 g compared to the control treatment (Table 3.1).

Table 3. 1: The tuber percentage calculated per tuber size of *Sagittaria platyphylla's* under the impact of *Listronotus appendiculatus*, measured between the herbivory treatment and control treatment, at the end of a 5-week experiment.

Tuber size	**Category weight (g)**	**Herbivory Treatment (n=80)**		**Control Treatment (n=102)**	
Small	0-2	56/80	**70%**	67/102	**66%**
Medium	2-3	13/80	16%	24/102	24%
Large	3+	11/80	14%	11/102	10%

3.3.2 Impact study: seed viability and germination

Larval damaged seeds

Damaged seeds that were selected from the herbivory treatment, had a significantly reduced percentage of viable seeds ($\chi^2{}_2$=24.4: *P*= 1. 29 $^{-20}$). Under the herbivory treatment, 86% of seeds were dead, no seeds were viable and 14% were partially viable compared to the control treatment which had no dead seeds, 6% of partially viable seeds and 94% of viable seeds.

Undamaged seeds that were selected from the herbivory treatment, had a significantly reduced percentage of viable seeds ($\chi^2{}_2$=17.07: *P*= 2. 07^{-05}). Under the herbivory treatment, there was the same percentage of partially viable and viable seeds at 37%, and 26% of dead seeds compared to the control treatment which had 89% viable seeds, 11% partially viable seeds and 0% dead seeds. Additionally, although some of the seeds from the herbivory treatment appeared viable, they were smaller (visual estimation) in size compared to the viable seeds in the control treatment (Fig. 3.9).

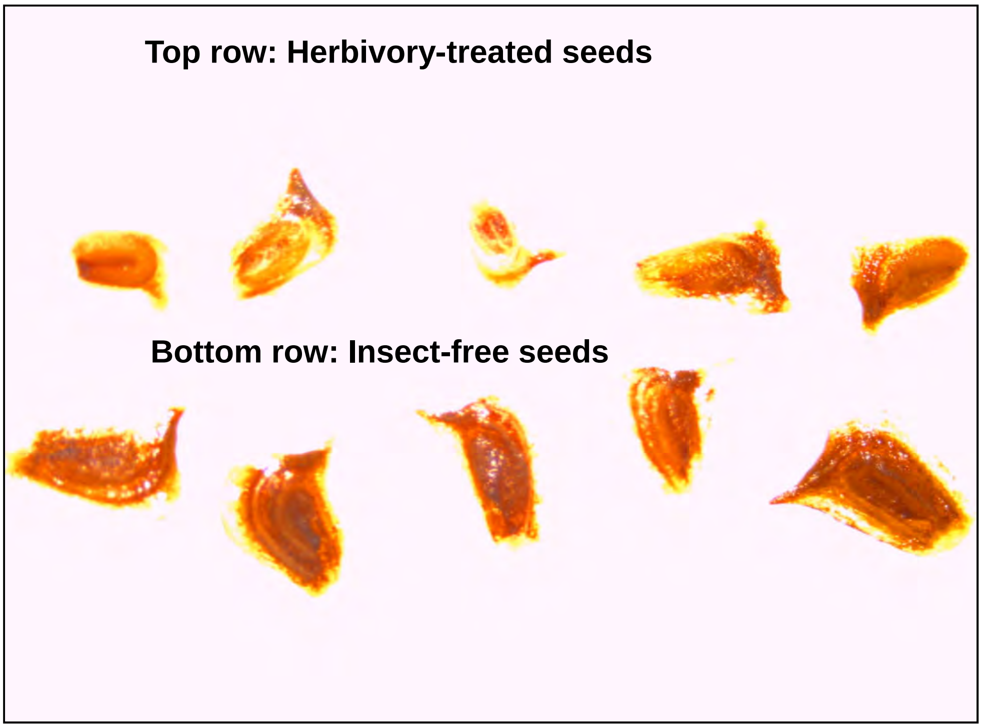


Figure 3. 9: The difference in 1% Tetrazolium (TZ) test stain pattern of *Sagittaria platyphylla* seeds under the feeding of *Listronotus appendiculatus* larvae, compared between undamaged seeds from the herbivory treatment (above) and insect-free seeds (bottom), after 30hrs staining period in a 26 ºC control-environmental chamber

Germination trial

Listronotus appendiculatus' larval damage significantly decreased the number of germinating seeds (χ^2_1=22.42: *P*= 2. 22^{-05}). In the control treatment 78% seeds germinated compared to the 39% of seeds that germinated from the herbivory treatment.

3.4 DISCUSSION

Listronotus appendiculatus negatively influenced the overall fitness of *S. platyphylla* and reduced its reproductive outputs by reducing the number and size of racemes produced. Additionally, *L. appendiculatus'* larval stage significantly reduced the percentage of viable and germinating seeds that would potential be released into the environment.

Sagittaria platyphylla is an invasive plant with two reproduction modes that contribute to the plant's establishment, population growth and spread. Seed production is regarded as the primary driving mechanism for its spread (Kwong *et al.,* 2017a), while tuber production enables the plant to persist during unfavourable conditions such as winter season, water level fall back and weed management application (Adair *et al.,* 2012). In this study, *L. appendiculatus* brought about substantial impact on both reproductive outputs of *S. platyphylla*. As a potential biological control agent, *L. appendiculatus* has the potential to reduce the amount as well as size of viable, germinating seeds. This suggest that if released the agent could reduce the current spread of *S. platyphylla* in South Africa. In addition, the aboveground feeding of *L. appendiculatus* has the potential to reduce tuber production and tuber biomass, thereby reducing the plant's ability to persist during unfavourable conditions.

Seed-attacking insects are recognised as "successful agents" as they have the ability to damage invasive plants until they no longer produce seeds, which is the highest scored characteristic for selecting a potential control agent (Goeden, 1983; Kremer, 2000). The Chrysomelidae and Curculionidae families are known to be successful agents capable of reducing plant size (Crawley, 1989, Crewley *et al.,* 2012), and some of the *Listronotus* weevils from the Curculionidae have demonstrated similar damaging impacts. A laboratory-based study investigating the impact of, *L. setosipennis* Hustache (Coleoptera: Curculionidae), released in Australia for the control of *Parthenium hysterophorus* L. (Asteraceae) showed that the weevil had a significant negative impact on parthenium rosette stage and was able to reduce plant height by 51%, the number of leaves by 78%, and plant biomass by 54% (Dhileepan, 2003). Another case is *L. marginicollis* Hustache (Coleoptera: Curculionidae) which was studies as a potential control agent for controlling parrot's feather, *Myriophyllum aquaticum* (Veloso) Verde (Haloragaceae) which significantly damaged a site in Argentina resulting to the weed's dieback (Cordo & DeLoach 1982). *Listronotus appendiculatus* similarly demonstrated negative impact to *S. platyphylla* fitness.

Direct impact on reproductive output

The direct feeding damage on *S. platyphylla*'s reproductive output resulted in an increased number of germinating seeds and growing ramets. The increased number of germinating seeds can be explained by the stimulation of mechanical damage to the seeds protective outer coating which increases germination rate. Initiation of seed germination can be triggered by different environmental factors including mechanical damage that leave seeds sacred or chipped (Manzano *et al.,* 2005; Bareke, 2018). Therefore, the larval damage of seeds could have made small cuts or thinned some part of the seed coats which allowed water to enter more easily and initiate germination in damaged seeds. But the larval damage on these seeds could have decreased the amount of nutritional reserves allocated for the initial seedling growth. Additionally, later in the study, undamaged seeds collected from the herbivory treated plants were smaller in size which could have explained the increased number of germinated seeds from the herbivory treated plants because small-sized seeds generally germinate faster when compared to bigger seeds (Baskin & Baskin, 1998; Souza & Fagundes, 2014). This is because small sized seeds may ultimately be less vigorous when they germinate due to reduced resource (Souza & Fagundes, 2014).

The increased number of ramets can be explained as a defence mechanism response in stoloniferous plants shared between the inter-ramet connections. When plants are under attack, or are stressed, they release volatile signals that allow communication within and between plants which may either be beneficial or costly to the plant (Newingham & Callaway, 2006; Karban, 2011). Stoloniferous plants have inter-ramet connections which are used for sharing resources and/or signals in response to environmental conditions (Hartnett & Bazzaz, 1984; Gomez *et al.,* 2007; Sui *et al.,* 2009). Therefore, the volatile cues within the plant could have resulted in an increase in ramet production which is common in stoloniferous plants under herbivory attack or in stressed environments. A study conducted by Sui *et al*. (2011) showed how the influence of mechanical stimulation significantly increased the growth of proximal ramets of *Leymus secalinus* (Georgi) Tzvel. Similarly, Liu *et al*. (2007) showed an increase of *Potentilla reptans* L. (Rosaceae) stolon's which were under the influence of spectral shading and mechanical stress.

Additionally, volatile cues released into the surrounding environment could have encouraged seed germination of undamaged seeds in the herbivory treatment. Fincheira *et al*. (2017) demonstrated how volatile organic compounds released (2-

nonanone, 2-undecanone and 2-tridecanone) by soil bacteria were able to germinate the seeds of *Lactuca sativa* L. (Asteraceae). The study design had experimental plants covered with fabric mesh sheet creating an experimental enclosure which could have intensified the volatile cue concentration, resulting to the increase of ramet and seed germination in herbivory treated plants. This explanation is subject to differ under different experimental conditions because plant responses are not fixed but differ under various abiotic and biotic stress, and plant communication can be influenced by distance between neighbouring plants and stress period in which the plant is under (Pierik *et al.,* 2013; Yamawo *et al.,* 2014; Ninkovic *et al.,* 2019). However, the influence of volatiles were not directly studied in this experiment and may form the basis of further studies.

Impact on reproductive outputs

The adult stage of *L. appendiculatus* significantly reduced the reproductive outputs of *S. platyphylla* by reducing the number and size of racemes produced (Chapter 3, section 3.2.3). The larval stage significantly reduced the percentage of viable and germinating seeds released into the environment (Chapter 3, section 3.2.4). Smaller sized seeds produce weak seedlings which has a disadvantage in plant establishment during the early development stages of the plant (Baskin & Baskin, 1998; Souza & Fagundes, 2014). Small sized seeds tend to produce weak seedlings because they have low amounts of nutritional reserves that are allocated for the initial seedling growth (Primack, 1987; Yanlong *et al.,* 2007). The impact on sexual reproductive output was expected and in agreement with previous field studies. A field study conducted by Kwong *et al*. (2017a) showed how *L. appendiculatus* significantly reduced the number of achenes in fruiting heads by 60% in 2011 and 61% in 2012. This study further quantified how viable the damaged achenes can be and how many seeds are able to germinate after larval damage. Furthermore, the aboveground feeding of both adult and larval stages also affected the asexual reproductive output of *S. platyphylla* by reducing the number and weight of tubers produced. In potato farming, studies have shown that smaller seed tubers exhibit lower physiological growth (Barry *et al.,* 2001; Masarirambi *et al.,* 2012). Therefore, small size tubers can produce plants with reduced seedling vigour, which has disadvantages for plant establishment. The results from this study were similar to a study conducted by Smith and Hough-Goldstein (2014) who showed that the viable seeds of *Persicaria perfoliata*

(L.) H. Gross (Polygonaceae) were reduced by 35% and clusters reduced by 37%, when both adult and larval *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae) weevils fed on whole plants in the greenhouse. The importance of these findings is that different stages of *L. appendiculatus* can affect both reproductive outputs, thereby demonstrating how *L. appendiculatus* impact on *S. platyphylla* can be greater than initially expected.

Listronotus appendiculatus *as a potential biological agent*

This study showed that *L. appendiculatus* herbivory negatively influenced the overall fitness of *S. platyphylla* and reduced the plants reproductive outputs. More specifically the feeding damage reduced *S. platyphylla*'s growth rate and above ground biomass, and was able to kill 20% of plants within a five-week period.

However, this was a laboratory-based study, which are known to have limited value and aggrandized results as they may not represent what may happen in the field because impacts on individual plants may not be matched with impacts on a population scale (McClay, 1995; Morin *et al.,* 2009). It is always important to study the impact of an agent once released or within its native range. *Listronotus appendiculatus* has shown to be just as damaging in the field (Kwong *et al.,* 2017a). In its native range, *L. appendiculatus* was recorded as the most common, abundant fruit-feeding insect associated with *S. platyphylla,* and its larvae were observed to further damage *S. platyphylla's* internal petiole and scape (Adair *et al.*, 2012; Kwong 2014; Kwong *et al.,* 2018). Therefore, as much as this laboratory-based study demonstrated the potential impact of *L. appendiculatus* on *S. platyphylla,* it may be combined with the results from the field studies to generate a stronger prediction of *L. appendiculatus* efficacy as a biological control agent.

The impact of seed mortality in relation to a weed's population dynamics has the potential to influence the distribution, abundance and reproduction of the weed (Hill & Stevens, 1981), thereby slowing or halting the spread of the weed's invasion. *Listronotus appendiculatus* seems to be a promising control agent with an added advantage of not only directly attacking seeds, but also the potential to impact the overall plant fitness and its underground reproductive outputs. Therefore, it should be prioritized for the biological control of *S. platyphylla* in South African. As an added weed management tool, biological control can be applied or integrated with the current control mechanisms to control and manage *S. platyphylla* invasion in areas where herbicide and mechanical control has failed to be effective and sustainable. Additionally, biological control will be ecologically viable and sensitive in *S. platyphylla* populations that are in protected areas like nature reserves where chemical control is prohibited, and areas not easily accessible for the use of mechanical and herbicide control.

This study's findings should be combined with host-specificity tests, habitat preference studies and climate-based models mapping the potential thermal distribution of the agent before the decision of releasing *L. appendiculatus* for managing the invasion of *S. platyphylla* in South Africa is considered.

CHAPTER 4:

GENERAL DISCUSSION

This thesis presents the problems and prospects of managing the invasive aquatic plant *S. platyphylla* in South Africa. In chapter 2, the study explored the distribution and spread of *S. platyphylla* since its first record of invasion. In addition, it investigated the success of the management options implemented against this Category 1a invasive. The chapter showed that *S. platyphylla* has continued to spread, despite the current eradication approach, to the "exponential phase" as the weed is now widespread and abundant in three provinces. Additionally, there is potential for the weed to spread to several new sites identified within a 5 km radius of the current populations. In chapter 3, a possible biological control option was considered for the management of the species. The chapter showed that the flower and fruit feeding weevil *L. appendiculatus* decreased the overall fitness of *S. platyphylla* by reducing the plant's growth rate and above ground biomass. Most importantly, *L. appendiculatus* larval feeding damage significantly reduced the number of viable germinating seeds produced into the environment, the weed's primary dispersal mechanism. Therefore, integrating biological control with the current management strategies may offer a long-term, cost effective management approach by releasing *L. appendiculatus* as a potential control agent for controlling *S. platyphylla*.

In order to achieve sustainable management of *S. platyphylla* in South Africa, some additional actions need to be considered: The failed current management approach used against *S. platyphylla* in South Africa; *S. platyphylla'*s ranking under NEM: BA regulations should be changed from category 1a to 1b; monitoring ecosystem recovery and initiating habitat remediation on previous cleared sites; and considering the effect of climate change on *S. platyphylla*. These suggested actions and considerations are discussed.

4.1 Eradication approach of emergent aquatic weeds in South Africa.

Invasive plants in South Africa are controlled by preventing introductions, eradicating taxa that manage to get into the country and strategically managing established infestations (Wilson *et al.,* 2010). Previously, management of invasive aquatic weeds such as *P. crassipes* were implemented years, and sometimes decades after their introduction (Cilliers, 1991), once the weeds had a broad distribution covering several sites. At this stage, prevention and/or eradication were no longer management options. Recently, the management of *S. platyphylla*, along with several other newly recorded emerging species, namely, *Sagittaria latifolia* Willd. (Alismataceae); *Lythrum salicaria* L. (Lythraceae) (purple loosestrife), *Nasturtium officinale* W.T. Aiton. (Brassicaceae) (watercress); *Iris pseudacorus* L. (Iridaceae) (yellow flag); *Hydrocleys nymphoides* (Humb. & Bonpl. ex Willd.) Buchenau (Alismataceae) (water poppy); *S. alterniflora* (Hill & Coetzee, 2017), have challenged researchers and resource managers to re-evaluate the known management strategies for invasive species. New approaches to control need to be implemented for these species before they become too widespread in South Africa using prevention and eradication approaches.

In invasive species management, early detection and rapid response are usually key to successful control (Rejmanek & Pitcairn 2002). This is intuitive as the smaller and more localised the infestation, the more resources can be targeted to ensure control is achieved. As invasions become larger, finances and resources become increasingly dissolved and eradication less likely (Rejmanek & Pitcairn 2002). An excellent example of eradication in South Africa is the successful management of the aggressive ecosystem engineer, *S. alterniflora*, commonly known as smooth cordgrass. The small population of the plant, less than 1ha, was also first detected in 2004 along with *S. platyphylla*, and through regular herbicide treatment during the growing period, the plant was successfully eradicated from the Great Brak Estuary in the Western Cape Province (Riddin *et al*., 2016). The success of this programme was due to the small area of infestation, appropriately timed herbicide application and access to the entire weed population (Riddin *et al*., 2016). This study highlights that when responding to new plant invasions, two key practices are essential to ensure eradication, firstly, plant removal and/or destruction must be greater than population growth (Rejmanek & Pitcairn, 2002); and secondly, management must be continued until no more propagules are present within the system. This is often the biggest challenge in newly detected species management, which can result in failed eradication and a waste of resources.

In 2009, after the year of first detection, *S. platyphylla* was already recorded in four provinces (KZN, EC, WC and GP) with a total of 15 sites. Hindsight suggests the population size at a national scale was already too large to consider eradication, especially when eradication resources and efforts were only conducted in KwaZulu-Natal Province, and one site in Gauteng Province, allowing populations in other provinces to spread unmanaged. In addition, three years after first detection, *S. platyphylla* was still sold in South African pet shops even though it was listed as a prohibited invasive aquatic weed under the draft NEM: BA regulations (Martin *et al.,* 2011).

Once herbicide control was selected as the best management option, the glyphosate based Kilomax was used to manage the weed. The herbicide was not registered for controlling *S. platyphylla* and had been shown to be in-effective in eradicating *S. platyphylla* in Australia (Adair *et al*., 2012). Underground biomass was not targeted by the herbicide, and prolific seed production throughout the year allowed regeneration despite the herbicide applications, which were only applied during the summer seasons.

The delay in management in South Africa potentially allowed the weed to spread past a point where eradication was possible. This was concerning as in Australia, during the same time period, the final strategic plan for *S. platyphylla* was already drafted with the aim to control an infestation of 11 900 km in a channel system administered by Goulburn Murray Water (Chapman & Dore, 2009). This was one year after the first record of *S. platyphylla* in South Africa, which could have motivated the urgency to provide eradication resources in all invaded provinces at its very earliest stages. Shared information from other countries on biological invasions, and impact risk assessments of new invasive plant species that are detected in South Africa, should be used as motivation for intensifying eradication application at the point of the species detection, and to allocate more funding for eradication application. This should result in a more effective eradiation approach by minimizing the period between detection and eradication attempts, further saving on management costs.

The strategic approach used to control new aquatic weeds in South Africa should be more proactive at the early stage of invasion when these species population sizes are suitable for prevention of further introductions, and eradication. In South Africa, some emerging invasive species, such as *S. latifolia* and *H. nymphoides,* remain in small contained areas of infestation where successful eradication can be achieved, yet they remain uncontrolled. Targeting these species could not only improve the success of eradication of emerging aquatic weeds, but also save on future management cost that has accumulated over the years, as presented in Chapter 2.

When considering eradication, an understanding of the plants morphological and reproductive traits is essential to ensure management is achieved. *Sagittaria platyphylla* has several morphological and reproductive traits which make its management difficult. Out of its native range, especially in more subtropical environments such as South Africa, *S. platyphylla* can produce high numbers of achenes as well as underground tubers continuously throughout the year because fruiting and plant growth is not limited to a single season. In these areas, *S. platyphylla* can continuously produce sexually mature plants capable of producing thousands of viable seeds (Kwong, 2014). Management must therefore be implemented almost continuously to ensure no plants reach maturity. In South Africa, once the plant had escaped from its initial points of invasion, occurring in high densities in lentic systems, this kind of intensive management became impossible. The sheer number of continuously produced achenes allows rapid repopulation of a system after management if a single plant remains. The achenes are also easily transported within and between systems, making containment of populations nearly impossible. The underground tubers also allow the plant to survive drought, water drawdown, frost and chemical and mechanical management (Adair *et al*., 2012). Management must consider how to remove these propagules or how to manage them once they grow into emergent plants again capable of setting seeds. In South Africa, the removal of below-ground tubers was not attempted, as management focused on emergent plants, allowing regeneration from the tubers (Menzi Nxumalo, pers. comm).

Eradication is possible for emerging aquatic weeds, like the successful eradication of *S. alterniflora* from the Brak River, where several factors contributed to this success. The weed occurred in a small contained area; there was persistent and continuous chemical application under good supervision and monitoring that reduced both above-and below-ground biomass; and sufficient funding was allocated to support the programme (Rejmanek & Pitcairn, 2002; Maxwell *et al*., 2009; Riddin *et al*., 2016). The eradication of S*. platyphylla* from South Africa is no longer an option and management should change appropriately.

4.2 Recategorizing S. platyphylla *under NEM: BA regulations in South Africa*

Unfortunately, the programme against *S. platyphylla* in South Africa is unlikely to receive increased financial support. In 2013, approximately R 78 ($5.5) was used to manage every m^2 of *S. platyphylla,* but by 2019, the amount available was reduced to approximately R 43 ($3) per m^2. It seems unlikely that eradication will be achieved in South Africa under the current management practices as it is no longer a feasible management goal. Therefore, the invasive status of *S. platyphylla* should be changed from category 1a to category 1b under NEM: BA regulations. Management efforts will then shift from eradication alone to implementing long-term management options focussing on population suppression and including biological control as an integrated management tool. Importantly this will move *S. platyphylla* out of the SANBI-ISP programme into the larger WfW Programme which has more resources available.

Biological control is a sustainable cost-effective control method that can manage invasive plant populations over a large scale, restore natural food webs and protect natural ecosystems (De Lange & van Wildgen, 2010; Hill & Coetzee, 2017; Schwarzländer *et al.,* 2018). Choosing an effective biological control agent involves many steps. These steps are imperative to reduce the risk of releasing a biological control agent that may not be host specific or damaging to the plant. Experimental data from this study showed that *L. appendiculatus* decreased the fitness of *S. platyphylla* through reductions in its reproductive outputs *viz.* the number and size of racemes produced. Additionally, the larval stage of *L. appendiculatus* significantly reduced the percentage of viable and germinating seeds released into the environment. However, further host-specificity tests, and climate-based models mapping the potential thermal distribution of the agent should be conducted before the decision to release *L. appendiculatus* for the biological control of *S. platyphylla* in South Africa is made.

Integrating biological control with the current management options would be beneficial in areas that are not easily accessible or where herbicide applications are not permitted (e.g. the Krantzkloof Nature Reserve), and in areas that may interfere with the persistence of biocontrol agents such as highly disturbed aquatic environments (Kwong *et al.,* 2014). Integrating all three management options in the aim of fighting aquatic weeds is often the best long-term solution (Hill & Coetzee, 2017; Zachariades *et al.,* 2017).

4.3 Bioclimatic envelope models

Climate change and biological invasions are co-occurring global threats with deteriorating impacts on both biodiversity and the environment (Rahel & Olden, 2008; Stephens *et al*., 2019). Climate change in South Africa is anticipated to enhance plant competitiveness due to elevated carbon dioxide levels, intensify weather change, increase the temperatures and decrease humidity levels in western region of the country, and increase both temperatures and humidity levels in the Kwazulu-Natal coast (Prince, unpublished; Hovekaab *et al*., 2016; Stephens *et al*., 2019). These changes are predicted to affect aquatic plants more than terrestrial plants by increasing the potential number of new invasions and increasing the geographic range of currently known invasions (Coetzee *et al*., 2009; Verlinden *et al*., 2014). Therefore, management of these invasions must formulate strategic plans that consider the change in plant activity in terms of behaviour, relating to the change in climate using bioclimatic envelope modelling.

In the prospect of managing *S. platyphylla* in South Africa, bioclimatic envelope models can be produced to predict future spread of *S. platyphylla* by identifying areas that are prone to the invasion and currently invaded areas that may be no longer be climatically suitable. Should *L. appendiculatus* be released, bioclimatic envelope models may be used to determine the control agent's efficiency and thermal tolerance under different climates. Intense weather changes that could result in floods would allow further increase of achene dispersal and facilitate the spread of *S. platyphylla* into vulnerable water bodies. The ability to survive in environments that have drastic water level fluctuations by changing its leaf morphology (Martin & Shaffer, 2005; Kwong *et al*., 2014) and the regrowth of submerged and subterranean organs after cold seasons (Adair *et al*., 2012), would allow *S. platyphylla* to thrive under changing environments. Therefore, future management plans should include developing bioclimatic envelope models for both *S. platyphylla* and *L. appendiculatus*, if released, especially for the invasions in subtropical regions, such as KwaZulu-Natal, where climate change is anticipated to be the breeding ground of future invasive weeds due to its habitat type, high nutrient availability in water bodies and human disturbance (Chytrý *et al.*, 2008; Hill & Coetzee, 2017).

4.4 Monitoring of ecosystem recovery and future restoration plans on cleared sites

There are many studies that assess the negative impacts of aquatic weeds in South Africa which assume that controlling the weed will result in benefits of a similar magnitude, but this is not always the case as ecosystems do not revert to the original state after control (Maxwell *et al.,* 2009; Coetzee *et al.,* 2011; Van Wilgen, & De Lange, 2011). Management success can be demonstrated by the recovery of the ecosystem when native vegetation and aquatic macrophytes and macroinvertebrates are seen returning to previously invaded areas (Covich *et al.,* 1999; Bakker *et al.,* 2013; Motitsoe, unpublished). The eradication of *S. alterniflora* was so successful that native salt marsh vegetation began to grow after the first year of herbicide application and covered 95% of the previously invaded area by the second year (Riddin *et al.,* 2016). In aquatic systems, ecological recovery is identified by the return of macroinvertebrate and aquatic microalgae communities after management (Bakker *et al.,* 2013). A recent study by Motitsoe (unpublished) illustrated ecological recovery of aquatic macroinvertebrate functional diversity at sites previously invaded by *Salvinia molesta* in South Africa, after integrated management. Unfortunately, in this study on *S. platyphylla*, only percent cover, density, size of infestation, number of sites and amount spent on management were considered so it will be difficult to determine management success based on any social, economic or environmental parameters, and this should be considered in future, especially if management strategies are to change.

Incorporating restoration into management may ensure environmental recovery after management. Ecological restoration in freshwater and marine habitats may include restoring connectivity and fish passage in rivers; restoring water flow and sediment dynamics to promote ecosystem recovery. Transformation restoration includes restoring previously damaged ecosystems with native plants that have a wider ecoregion that may not have previously occupied the ecosystem, but have the potential to bring it back to its maintain ecosystem function (Harris *et al.,* 2006). This kind of management has not been attempted in any aquatic systems in South Africa but should be considered as a valuable tool within the management toolbox available for freshwater ecosystems.

Therefore, in the prospect of managing *S. platyphylla* invasions, additional integrated modeling, monitoring and experimental work is needed to assess whether native species can reoccupy currently cleared sites before they become climatically unsuitable, test different viable species assemblages for transformative restoration, and predict potential retreat areas that may be targeted for restoration due to climate change. It is crucial to monitor ecosystem recovery and to implement restoration plans as *S. platyphylla* is being managed, thereby closing the time gap before reinvasion by other invasive species, and the risk of invasion expansion due to climate change.

4.5 Conclusion

The results from this thesis strongly suggest that the implementation of eradication approach as the only management strategy is no longer suitable for the infestation of *S. platyphylla* in South Africa. Therefore, the integration of a biological control programme is advised, with the release of *L. appendiculatus* because it can significantly reduce viable-germinating seeds produced into the environment. Additionally, bioclimatic envelope models involving the change in plant behaviour in relation to the change in temperature and monitoring of ecosystem recovery with future restoration efforts on cleared sites should be included in future management plans to ensure the restoration of functional aquatic ecosystems.

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