Post release evaluation of the distribution and efficacy of *Eccritotarsus catarinensis* and *Eccritotarsus eichhorniae* on *Pontederia crassipes* in South Africa.

by

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Declaration

All the work described in this thesis is my own and has never been submitted for examination with any University. It is being submitted to Rhodes University for the degree of Doctor of Philosophy.

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Abstract

Biological control involves the release of new species into the environment and therefore, needs to be carefully monitored through post-release assessments which have been largely neglected in the science. Post-release evaluations of biological control programmes reveal whether the control agent has established and if it impacts weed demography, while cost-benefit analyses require a different set of data that show the magnitude on return on investment.

The biological control effort on *Pontederia crassipes* in South Africa uses, amongst others, two species of mirid, *Eccritotarsus catarinensis* and *E. eichhorniae*. Initially, they were released as a single species, but were recently divided using molecular techniques. *Eccritotarsus catarinensis* was released in 1999, and *E. eichhorniae* in 2007. After many releases over two decades, there was need to assess where each species was established in the country. Molecular techniques proved to be valuable in identifying the two species as they are morphologically indistinguishable in the field. Therefore, molecular techniques should be routinely used for screening biocontrol agents, whether new or as re-introductions.

Annual surveys of the mirid release sites around South Africa were undertaken between 2016 and 2019. At each site both insect and plant parameters were measured. Only *E. catarinensis* is established in the field in South Africa despite the multiple releases of *E. eichhorniae* at over 70 sites across the country, and *E. catarinensis* has established at only 22 of the 45 release sites accessed during this study. This thesis tested climate, interaction with other agents already on *P. crassipes*, and direct competition between the two mirid species as reasons for the lack of establishment of *E. eichhorniae.*

The results of the country-wide surveys showed that climate and water trophic status were the major determinants in the establishment of *E. catarinensis*. Most of the establishment was recorded in the warmer regions of the country, however, a few populations of the mirid also established in cooler areas, thus demonstrating a degree of thermal plasticity, and possible microclimates as the mirids persisted at sites shaded by riparian vegetation. Stochastic events such as active herbicide campaigns, winter frosts, droughts and floods were responsible for the absence of the mirid at some sites. At some of the eutrophic sites, despite the abundance of *E. catarinensis*, plants still proliferated as the water trophic status facilitated plant growth, thus, plants were able to compensate for the damage inflicted by the mirid.

A more intensive, monthly, post-release evaluation was conducted on the Kubusi River, Eastern Cape Province between 2016 and 2019. This is regarded as one of the cooler water hyacinth sites. Populations of biological control agents at this site fluctuated seasonally. At this site, cold winters caused frosting of the leaves of *P. crassipes* with the exception of plants growing under overhanging vegetation that provided a refuge for the mirid. But, cool temperatures in the winter months (May to August) severely reduced the populations of *E. catarinensis* that required a long recovery phase in spring. The consequence of this was that the plants grew unchecked from the onset of the growing season forming dense mats. Of the four agents at the Kubusi River site, *Eccritotarsus catarinensis* recovered slowest after winter, with lag phases ranging from two months to several months of the three-year period. The release of a suite of agents has implications on the agents themselves, where interactions between the agents can be important. Interactions between pairs and even multiple agents can have implications for biocontrol, where agents are either complimentary or interfere with each other. In this case, because *E. catarinensis* recovered the slowest of the four agents at the site, plants were of a poor quality by mid-summer resulting in low mirid populations.

Competition in weed biological control could be expected to be strongest between pairs of agents that share the same niche, and this could be the reason why *E. eichhorniae* failed to establish at sites where *E. catarinensis* had already been established for several years. When the two mirids were combined in manipulated trials in a polytunnel, populations were lower compared to when the two mirids occurred separately. Under warm conditions, it is likely that *E. eichhorniae* would be the superior agent compared to *E. catarinensis*.

The evaluations discussed in this thesis highlighted gaps in agent release methodology in multispecies settings, as well as the need for strategic augmentation pre- and post-winter. It is important to release agents that will complement each other rather than compete, therefore, when releasing agents in a multispecies setting, niche differentiation needs to be considered. Here it is concluded that the best practice for dealing with the mirids is that they should be released individually, and at sites that have no other biological control agents in order to ultimately assess their efficacy.

Landscape level, long-term monitoring of biological control programmes shows the impact of the control programme at a broader scale and, are far more informative than short-term studies and at fewer sites. Long-term post-release evaluations should be mandatory in biological control programmes. Furthermore, these assessments will help develop new strategies or improve on existing ones, thus achieve greater success in control.

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Chapter 1 : General introduction and literature review

1 Introduction

1.1 Post release evaluation in biocontrol programmes

Global biodiversity is currently under threat from invasive alien plants whose impacts have far reaching consequences on both the biodiversity of ecosystems, and economic activities of various population groups, especially in Africa where the economies are mostly based on small-scale agriculture (Perrings, 2005). Measures are constantly taken to mitigate the negative effects of these invasions, and varying degrees of success have been achieved depending on the control measures used. Control measures used to manage invasive plant species, with special emphasis on biocontrol, need to be followed up in the form of post-release evaluations. However, this is typically neglected (Blossey and Skinner, 1999; Porter *et al.,* 2019), so biological control (hereafter referred to as biocontrol) programmes fail to reconcile pre-release predictions with post release assessments (Barratt *et al.*, 2006).

Post-release evaluations measure the success of biocontrol programmes through the assessment of changes in the invaded community (Carson *et al.*, 2008; CRC, 2008; Turner, 2008). The data gathered from post-release evaluations may be used to identify and address any limitations of a specific biocontrol programme, thereby informing future initiatives that improve effectiveness and success of the programme (CRC, 2008). For example, Porter *et al.* (2019) highlighted that post-release evaluations may bring out instances where an agent adapts to the environmental conditions of a new habitat contrary to pre- release climatic matching. Successful adaptation to a new environment by an agent has implications on the success of that agent bringing about effective control of the target weed (McEvoy and Coombs, 2000).

Carson *et al.* (2008) formulated a five-step protocol for comprehensive post-release evaluation of biocontrol programmes in the USA: (i) *establishment of r*and*omized release* and *non-release sites for comparison, spatially* and *temporally stratified coupled with biological* and *physical gradients*; (ii) *assessments into the abundance of the invasive species prior to release in both release* and *non-release sites*; (iii) *assessment of agent populations in both release* and *non-release sites*; (iv) *experimental suppression of agent populations on host plants on replicated subplots in release* and *no-release sites*; (v) *quantification of plant community response to invasion prior to agent releases in release* and *control sites*. Blossey and Skinner (1999) also used a similar protocol in the post-release assessment of the invasive plant purple loosestrife *Lythrum salicaria* Linnaues (Lythraceae) in the USA. Protocols should achieve a balance between scientific sophistication and ease of application by implementation managers, therefore the protocol by Blossey and Skinner (1999) was widely adopted by many implementation managers and students researching purple loosestrife. Due to the practical applicability of the protocol, declines in purple loosestrife populations were easily quantified (Blossey and Skinner, 1999).

A study by Turner (2008) highlighted that, biocontrol programmes aim to reduce invasive populations to levels where native plant communities recover. Understanding the biology of the agent and its requirements in the introduced range will significantly improve the success of a long-term, self-sustaining system of a biocontrol programme (Turner, 2008).

Furthermore, these post release evaluations help to provide data for a cost-benefit analysis (Zachariades *et al.*, 2017). This is important in sourcing funding for research into biocontrol of new and potential invasive species (Van Wilgen *et al.*, 2004; Zachariades *et al.*, 2017). The Department of Environmental Affairs in South Africa implemented a “National Strategy” aimed at dealing with biological invasions in 2014 (Zachariades *et al.*, 2017). This strategy was set to be assessed triennially starting in 2017, in order to determine its success in

combating biological invasions. The importance of monitoring and evaluation was highlighted, stressing the importance of accountability for public funds, with emphasis on reaping the most benefit from improved ecosystem services. Continuous monitoring and evaluation review the successes and shortfalls in attaining the goals set in any control programme. Furthermore, the evaluation of the management methods, including biocontrol, in relation to the set goals helps adjust management strategies (Department of Environmental Affairs, 2014).

South Africa has been invaded by a suite of aquatic weeds, which further exacerbate the problems associated with low water availability (Van Wilgen and Wilson, 2018). Control measures such as mechanical, chemical and biocontrol have been introduced to combat the spread, as well as the negative effects of these aquatic weeds (Byrne *et al.*, 2010; Van Wilgen and Wilson, 2018). In the thesis that follows, the biocontrol programme against *Pontederia crassipes* (Kunth.) Pellegrini and Horn (Pontederiaceae) is used as a model for post-release evaluation at varying scales from the laboratory to the landscape scale.

1.2.1 *Pontederia crassipes*

Pontederia (=*Eichhornia*) *crassipes* (Kunth.) Pellegrini and Horn (Pontederiaceae) is the most problematic aquatic weed in the world, currently in the top 100 invasive species list (Center *et al.*, 2002; Perrings, 2005; Coetzee *et al.*, 2007a; Albright *et al.*, 2016). This weed is originally from South America and has been spread across the world by gardeners and the aquarium industry due to its beautiful inflorescence (Cilliers, 1991; Center *et al.*, 2002; Coetzee *et al.*, 2014). In its exotic range, devoid of any natural enemies, this aggressive weed has proliferated and expanded its range unchecked, negatively impacting the environmental economy in invaded areas (Barrett, 1982; Cilliers, 1991; Coetzee *et al.*, 2007a; Coetzee and Madeira, 2008). *Pontederia crassipes* is typically a problem in still or slow flowing waters characteristic of most artificial impoundments (Coetzee and Hill, 2008). Riverine

communities are the most severely affected by the invasions (Center *et al.*, 2002; Albright *et al.*, 2016; Coetzee and Hill, 2008). Furthermore, economic losses have also been recorded in the agricultural sector as irrigation pipes and canals get clogged with the roots of the plant, hence lowering productivity and, further losses are incurred due to constant repairs to equipment (Center *et al.*, 2002; Albright *et al.*, 2016; Coetzee and Hill, 2008). Native biodiversity in the exotic range of the plant has also been negatively impacted as dense mats of *P. crassipes* colonise water ways and outcompete native aquatic flora (Cilliers, 1991). Rates of evapotranspiration, deoxygenation and siltation in water bodies are accelerated by the presence of *P. crassipes* (Fraser *et al.*, 2016; Arp *et al.*, 2017), which has wide ranging effects on primary consumers such as aquatic insects and fish, leading to reduced biodiversity (Midgley *et al.*, 2006; Villamagna and Murphy, 2010; Coetzee, 2012). Vectors of waterborne diseases also proliferate in dense mats, with cases of bilharzia, malaria and other diseases increasing (Bateman, 2001; Ajuonu *et al.*, 2009).

In a bid to ameliorate the impacts of this weed, several control programmes have been implemented, using mechanical, chemical and biocontrol methods (Center *et al.*, 2002; Coetzee *et al.*, 2011). Mechanical and chemical control operations are often costly and only effective for small infestations and rely on repeat applications raising the costs of control (Coetzee and Madeira, 2008; Téllez *et al.*, 2008). Biocontrol however, is more sustainable and cheaper in the long-run as host specific natural enemies of *P. crassipes* are used to keep the weed under acceptable levels (Center *et al.*, 2002). Biocontrol is considered the most effective control measure against *P. crassipes* (Coetzee *et al.*, 2009), however, long- term post-release evaluation is essential.

1.2.2 Description and biology of *Pontederia crassipes*

The genus *Pontederia* comprises eight species, with *P. crassipes* recognised as a pantropical aquatic weed (Coetzee *et al.*, 2009). The weed was collected as early as 1801 in Colombia,

and first described in 1824 by C.F.P. von Martius who named it *Eichhornia crassipes* (Aweke, 1994). A number of name combinations were tried by other taxonomists until in 1883, H. Solms-Laubach established the combination *Pontederia crassipes* (Penfound and Earle, 1948). There was often confusion between *P. crassipes* and *P. azurea* especially since their distribution overlapped in South and Central America. Five species of the genus *Pontederia* are found in South America, with one species (*Pontederia natans*) restricted to Africa (Penfound and Earle, 1948; Aweke, 1994; Coetzee *et al.*, 2017). Recently, the name was revised again by Pellegrini *et al.* (2018) from *Eichhornia crassipes* to *Pontederia crassipes*. This revision of the taxonomy of the plant came about due to the polyphyletic nature of the plants in the Pontederiaceae family. Because of morphological and molecular similarities, plants in this family have been re-classified to two genera, *Heteranthera* and *Pontederia*, which is a more taxonomically conservative way of classifying this group of plants. Furthermore, this method reduces the need to re-describe species using names rarely used in relevant taxonomic studies. Again, this classification method allows for easier identification of either fresh or preserved plant samples (Pellegrini *et al.*, 2018).

Pontederia crassipes displays two distinct morphologies depending on the immediate growth conditions (Center and Spencer, 1981). Elongate petioles and circular leaves are characteristic of the weed growing in dense stands in high nutrients in the absence of herbivores, while in low nutrient conditions and low plant density, the plants have bulbous petioles and kidney shaped leaves (Center and Spencer, 1981; Center *et al.*, 2002; Coetzee *et al.*, 2009). The petioles are packed with aerenchyma cells which give rise to its buoyancy, hence its floating habit, with submerged roots and rhizomes (Penfound and Earle, 1948; Center and Van, 1989). The equilibrium reached by the low centre of gravity of the floating section and the high centre of gravity of the submerged section enables the shoots to be erect as they grow (Center and Spencer, 1981; Center and Van, 1989). However, as the weed mat

increases, extensive intertwining of the plants keeps the shoot section erect and this takes the place of petioles in keeping the plants afloat (Center and Spencer, 1981).

The submerged parts of the plants consist of an adventitious rooting system which is always suspended in water, as well as rhizomes and stolons (Penfound and Earle, 1948; Center and Spencer, 1981). The rhizome consists of internodes along its length and these are pivotal in vegetative reproduction as these internodes give rise to new roots, shoots and inflorescence (Penfound and Earle, 1948). The rhizome size ranges from 1 - 2.5cm in diameter with length ranging from 1 - 30cm, where the reproductive tip of the plant is about 1cm in length for small plants and up to 4cm in larger plants (Penfound and Earle, 1948). The reproductive tips are cone shaped and have a conspicuous pink cap on the crown of the rhizome (Penfound and Earle, 1948). The stolons have similar diameter to rhizomes, with variable lengths, and they exhibit a purplish colour (Penfound and Earle, 1948). In high population densities, stolons average about 5cm in length, while in lower densities they can be as long as 25cm (Penfound and Earle, 1948).

Leaf arrangement is whorled with older leaves found at the outer edge of the rosette (Center and Spencer, 1981). The leaf has a thick petiole which is about 3cm in diameter, with a narrow isthmus found between the petiole and the blade, while the leaf lamina is broadly reniform to lanceolate. Leaf inclination of the new leaves at the centre of the rosette is vertical, while reflexing of the leaf base for the older leaves gives them lateral inclination (Center and Spencer, 1981). Plants which receive high levels of insolation, normally in low plant densities, have swollen leaf petioles that also function as floats. The blade of such leaves is not true leaf lamina but an extension of the petiole (Penfound and Earle, 1948). Apart from photosynthesis, leaves play a pivotal role in nutrient mobilization within shoots (Center and Van, 1989).

Pontederia crassipes reproduces both asexually and sexually. Lavender or violet flowers exhibiting a yellow patch in the perianth lobe are involved in sexual reproduction (Center and Spencer, 1981; Center *et al.*, 2002; Tellez *et al.*, 2008; Coetzee *et al.*, 2009). The flowers borne on spikes occur in clusters of more than 20 flowers per spike (Center *et al.*, 2002). *Pontederia crassipes* exhibits tristyly, where the flowers have three types of styles, short, medium and long, with the medium sized style plants more widespread in the exotic range (Barrett, 1977; Center *et al.*, 2002; Coetzee *et al.*, 2009). This morphological adaptation allows the plant to be pollinated by a variety of pollinators and enhances chances of legitimate pollen reaching the style (Barrett and Glover, 1985). These three types of styles are therefore adapted to the body shape of particular pollinators, thus facilitating cross polliantion (Charlesworth and Charlesworth, 1978; Barrett and Glover, 1985). After a 14-day flowering cycle, the flower stalks bends allowing the spike to be submerged, thereafter the seeds are released into the water (Center and Spencer, 1981; Center *et al.*, 2002). A single inflorescence can produce up to 3000 seeds per flowering cycle (Center *et al.*, 2002).

The seeds produced are long lived and may lie dormant in sediment for up to 20 years (Center *et al.*, 2002; Coetzee *et al.*, 2009). However, sexual reproduction is constrained by lack of compatible pollinators, hence reduced seed set (Center and Spencer, 1981; Coetzee *et al.*, 2009). Germination of *P. crassipes* seeds requires moist sediment and high light intensities, where shallow warm water close to sediment provides a rooting substrate for the seedlings (Center and Spencer, 1981; Malik, 2007; Albano Pérez *et al.*, 2011). The resultant seedlings root into the sediment producing about five linear leaves (Center and Spencer, 1981). Population growth via seedling recruitment is limited by lack of germination sites which are available when water levels drop, or on decaying mats after herbicidal applications (Center and Spencer, 1981; Center *et al.*, 2002; Malik, 2007). Successive leaves possess more aerenchyma tissue which gives rise to buoyancy, resulting in the seedling breaking some

roots and floating to the surface (Center and Spencer, 1981). A study by Albano Perez *et al.* (2011) showed that *P. crassipes* can deposit as many as 2500 seeds per square metre in the sediment, highlighting the importance of sexual reproduction. This has implications on the control programmes of *P. crassipes* as the large seed bank enables re-invasion of water bodies (Albano Perez *et al.*, 2011).

Growth and expansion of *P. crassipes* is by way of monopodial shoot growth with whorled leaf arrangement and sympodial stoloniferous growth giving rise to daughter plants (ramets) which forms the asexual reproductive pathway (Center and Spencer, 1981; Center *et al.*, 2002; Coetzee and Hill, 2008). Asexual reproduction is pivotal in the rapid spread of the weed, especially in high nutrient conditions and optimal temperatures (Malik, 2007). In the event that an inflorescence develops, monopodial shoot growth ceases, and sympodial growth from the axillary bud below the inflorescence takes over by way of a stolon, giving rise to an apical meristem from which shoot production proceeds leading to the formation of a new rosette away from the parent plant (Center *et al.*, 2002; Malik, 2007). Infestations have been known to spread at rates of 60 cm per month at the edge of a mat due to vegetative reproduction, with doubling times ranging from 6-18 days (Malik, 2007).

1.3 Environmental factors influencing plant growth

Pontederia crassipes tolerates a wide range of environmental conditions, including nutrients, pH and temperature (Malik, 2007). Optimal *P. crassipes* growth occurs under neutral pH though it tolerates pH of between 4 to 10 (Pieterse, 1978; Center *et al.*, 2002; Tellez *et al.*, 2008; Coetzee *et al.*, 2009). When pH fluctuates outside the neutral range, *P. crassipes* has been observed to regulate the pH of its immediate environment creating alkaline conditions (Téllez *et al.*, 2008). Salinity is another growth regulator for *P. crassipes* where plant growth and eventually mortality occur at concentrations of between 6 to 8% (Malik, 2007). Penfound and Earle (1948) reported that plant mortality occurred in 2% saline water after 22 days.

Mortality due to saline conditions is symptomized by epinastic curvature of leaves, chlorosis and necrosis of plant parts in contact with water (Penfound and Earle, 1948).

Temperatures ranging from 28 to 30°C encourage fast growth of *P. crassipes* resulting in dense mats of interwoven plants blanketing the water bodies (Reddy and Tucker, 1983; Center *et al.*, 2002; Malik, 2007; Coetzee *et al.*, 2009). Exposed parts of the plants are susceptible to frost; however, plants may survive frost episodes if stolons are sheltered from the frost (Center *et al.*, 2002). Furthermore, growth ceases when temperatures are below 10°C or exceed 40°C (Pieterse, 1978; Téllez *et al.*, 2008), while mortality results when temperatures drop below 5°C. In an early by Penfound and Earle (1948), *P. crassipes* subjected to low water levels succumbed to temperatures in excess of 35.5°C, leading to the conclusion that *P. crassipes* dies when exposed to full insolation at such high temperatures for more than four to five weeks (Penfound and Earle, 1948; Pieterse, 1978).

Eutrophic water coupled with high irradiance levels accelerate *P. crassipes* growth rates (Pieterse 1978; Reddy and Tucker, 1983; Center *et al.*, 2002; Malik, 2007; Coetzee *et al.*, 2009; Franceschini *et al.*, 2011). In comparison to terrestrial plants, *P. crassipes* has very low mineral nutrition requirements where 0.1mg phosphorus (P) is critical for growth (Center and Spencer, 1981; Pieterse, 1978). Furthermore, nitrogen to phosphorus (N: P) uptake rates range at 5-10: 1 which could mean the critical nitrate concentration for growth is 0.5-1.0mg $N\ L^{-1}$ (Pieterse, 1978). An increase in both N and P results in greater biomass accumulation (Coetzee and Hill, 2012). Eutrophic waters or those with lower nutrients but flowing gently provide a constant supply of nutrients to *P. crassipes* resulting in dense mats of the weed (Coetzee and Hill, 2012). High growth rates of *P. crassipes* were recorded at 21mg $N\ L^{-1}$, 62mg $P\ L^{-1}$ and 0.6mg $Fe\ L^{-1}$, while deficiency in other micronutrients like calcium (Ca) has a more negative impact on growth compared to N and P (Téllez *et al.*, 2008). Low Ca

availability leads to retarded vegetative and sexual reproduction where Ca is essential in seed formation (Téllez *et al.*, 2008).

Incident radiation reaching *P. crassipes* plays a significant role in the productivity of *P. crassipes* which is heliophilous (Malik, 2007; Li *et al.*, 2011). The highest growth rate of *P. crassipes* when nutrients are not limiting is at Photosynthetically Active Radiation (PAR) of 4 444 µmol.$m^{-2}s$-^{1}, while the minimum PAR requirement is 444 µmol.$m^{-2}s^{-1}$ (Téllez *et al.*, 2008). Furthermore, an increase in light availability coupled with nitrates leads to increased growth. Accelerated activity of nitrate reductase and glutamine synthetase in parent and daughter plants is key to the reproduction of the weed (Li *et al.*, 2011). Shading by the canopy of riparian vegetation reduces the available PAR and *P. crassipes* counters the effects of shading by producing daughter plants in less shaded zones under the canopy, thus facilitating further spread of the weed (Malik, 2007;Li *et al.*, 2011). Another strategy for increasing light interception in light limited conditions is via stem elongation and increase in specific leaf area (Li *et al.*, 2011). Nitrate is translocated from the parent plant to the daughter plants and this is enhanced by light availability (Li *et al.*, 2011).

1.4 Distribution and pest status

Pontederia crassipes has been spread from its native South America to many regions of the world including North America, Australia, China, Japan, Indo-China and Africa from the 19th and early 20th centuries (Penfound and Earle, 1948; Center *et al.*, 2002; Téllez *et al.*, 2008). It has been present in Africa since the 1870s where it was first recorded in Egypt and has spread to over 38 countries (Coetzee and Hill, 2008). *Pontederia crassipes* has invaded most tropical and subtropical regions globally (Figure 1.1), becoming a major conservation and economic problem since the 1940s (Winston *et al.*, 2014; Coetzee *et al.*, 2017).

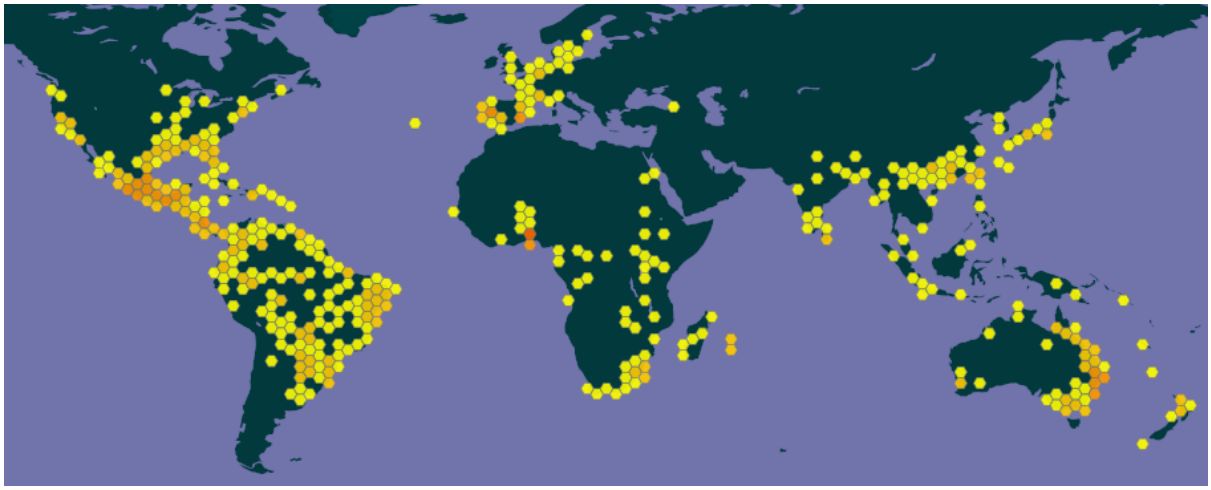

Figure 1.1: The global distribution of *Pontederia crassipes* (GBIF database, 2018). The different coloured points indicate multiple sites in close proximity.

In the United States, *P. crassipes* was introduced in 1884 in New Orleans where it successfully established and spread to the rest of the State of New Orleans (Penfound and Earle, 1948; Center *et al.*, 2002). *Pontederia crassipes* was recorded in South Africa in the early 1900s from KwaZulu-Natal Province, with the most notorious infestation in the Hartbeespoort Dam, in Gauteng Province (Cilliers, 1991; Byrne *et al.*, 2010). In South Africa the weed has affected Western Cape, KwaZulu-Natal, Mpumalanga, Gauteng, Eastern Cape, Free State, parts of Limpopo and North West provinces (Byrne *et al.*, 2010) (Figure 1.2). In Europe, *P. crassipes* was introduced as an ornamental plant to Portugal, first reported in 1939, and has since spread through irrigation channels to other areas of the country (Coetzee *et al.*, 2017).

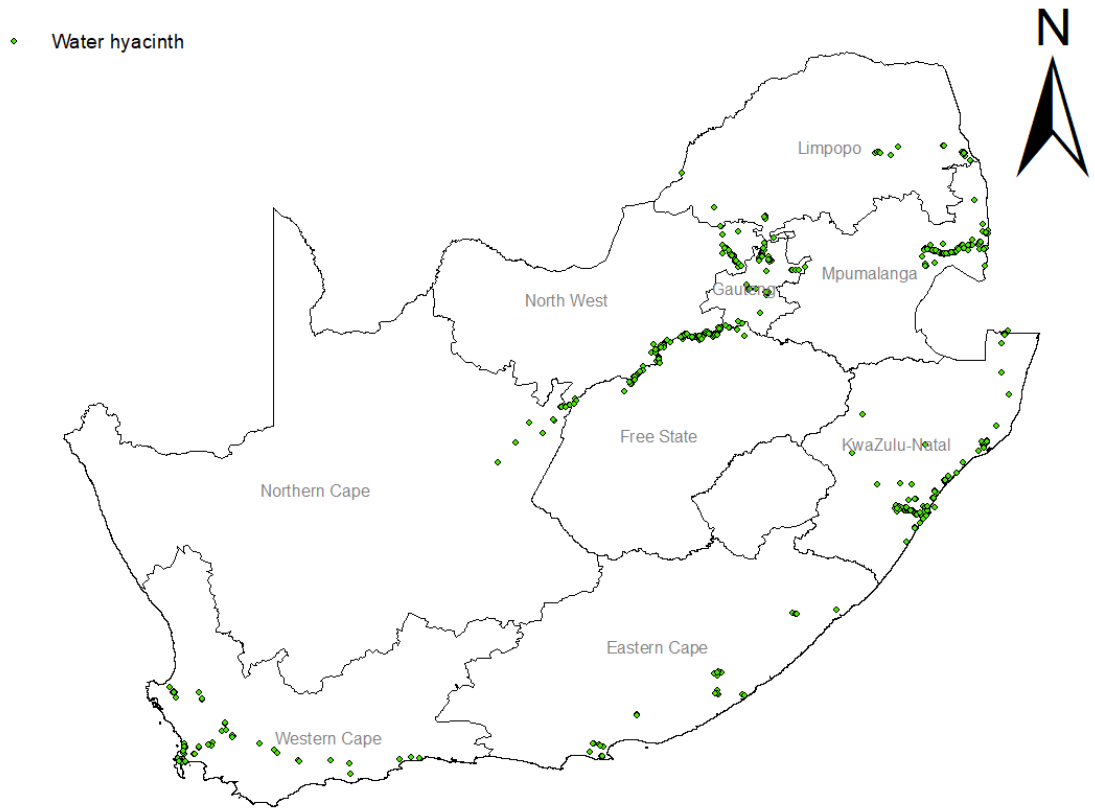

Figure 1.2: The distribution of *Pontederia crassipes* across South Africa. (Map data obtained from GBIF)**.**

In South Africa, *P. crassipes* has been listed as a National Environmental Management: Biodiversity Act (No. 10 of 2004) (NEMBA) category 1b weed, where it is prohibited to own, sell or move due to its invasiveness. In many countries such as Australia, Botswana, Burma, China, European Union, New Zealand and United States, appropriate legislation has been put in place to curb further spread of the weed (Coetzee *et al.*, 2017).

1.5 Impacts of invasion

A framework on plant invasions highlighted that for most plant invaders, a number of barriers must be overcome before they are naturalised and invasive (Blackburn *et al.*, 2011), most of

which are geographical in nature. However, for most invasions, the geographical barrier is overcome through anthropogenic means where humans transport a species beyond its natural geographic range (Blackburn *et al.*, 2011). *Pontederia crassipes* was spread throughout the world as an ornamental plant due to its attractive inflorescence (Tellez *et al.*, 2008). Invasions are also facilitated by environmental similarities in the exotic range, especially after overcoming the geographic barriers, as was the case with *P. crassipes* once it had escaped cultivation and spread to natural waterways (Tellez *et al.*, 2008). However, the environment of the exotic range may also act as a barrier to an invasion, where an incompatible climate reduces the chances of survival by the invader, hence hindering establishment (Blackburn *et al.*, 2011, 2014). In other cases, the invader may successfully overcome geographic and environmental barriers, and yet fail to establish successfully. There are instances where the invader may reproduce successfully in the introduced range, however, in the long term the population growth rate maybe negative, resulting in failure to establish and in some instances local population extinction (Blackburn *et al.*, 2011) (Table 1.1).

Invasion by *P. crassipes* mostly occurs in slow moving or still water (Harley, 1990; Malik, 2007; Coetzee and Hill, 2008), and invasion is successful due to its reproductive strategies, via clonal growth as well as seedling recruitment, with clonal growth being more important (Center *et al.*, 2002; Malik, 2007; Téllez *et al.*, 2008; Li *et al.*, 2011). Vegetative reproduction enables new plants to disperse from their parent plants via fragmentation where a complete fragment can give rise to new plants, which eventually form a mat (Harley, 1990; Greco and Freitas, 2002). Furthermore, during times of stress, such as droughts and herbicidal applications, seeds tend to germinate giving rise to new plants despite the perturbations, thus the plant poses a continual problem wherever it invades (Center and Spencer, 1981; Center *et al.*, 2002). Most dispersal is via anthropogenic means, but accidental introductions also occur via fishing boats and fishing gear as they are transported to pristine

areas via improperly cleaned fishing gear (Eiswerth and Johnson, 2002). Additionally, efforts to mechanically control *P. crassipes* cause fragmentation of the weed, facilitating spread.

Pontederia crassipes presents wide ranging ecological and socio-economic impacts in its invaded range. In the northern parts of Lake Victoria, Uganda, *P. crassipes* reduced abundance of aquatic invertebrates compared to open water (Masifwa *et al.*, 2001). Midgley *et al.* (2006) reported similar findings in New Year's Dam, South Africa, where there was higher benthic macroinvertebrate diversity in open water compared to beneath *P. crassipes* mats. The dense floating mats cause physical damage to submerged and riparian flora by attrition and scouring when the mats are moved by wind and water currents (Center *et al.*, 2002). Furthermore, due to the loss of native flora, aquatic insects as well as fish species are negatively affected due to the loss of their food supply, especially low abundances of phytoplankton (Center *et al.*, 2002; Eiswerth *et al.*, 2018). Dense mats of *P. crassipes* block out light from reaching the lower strata of water bodies thereby reducing productivity of the native plants (Center *et al.*, 2002). Oxygen starvation also results as the rotting plant matter from dead *P. crassipes* decomposes in the water, depleting oxygen in the water column (Cilliers, 1991; Masifwa *et al.*, 2001).

Commercial and subsistence activities on Lake Victoria where fishing is a major source of livelihood, were greatly affected as nets and boats could not be used over the invaded water leading to reduced productivity for the community (Center *et al.*, 2002; Malik 2007; Eiswerth *et al.*, 2018). Navigation for communities dependent on rivers for transport is severely restricted, cutting off access to essential amenities ((Eiswerth and Johnson, 2002; Malik, 2007). Hydroelectricity power generation is hampered due to blockages in the infrastructure where root and shoots of *P. crassipes* clog the machinery leading to lower productivity (Aweke, 1994; Center *et al.*, 2002; Malik, 2007). Agricultural output is reduced especially in irrigation schemes where irrigation channels are blocked and clogged by *P. crassipes*

(Aweke, 1994). Costs of health care and disease management increase as *P. crassipes* harbours vectors of water borne diseases such as malaria, bilharzia and encephalitis and filariasis (Pieterse, 1978; Aweke, 1994; Masifwa *et al.*, 2001; Center *et al.*, 2002). In Lake Victoria, the host for the bilharzia *Schistosoma mansoni*, the snail *Biomphalaria sudanica* was reported to be in high densities thus increasing incidences of the disease (Harley, 1990; Malik, 2007). Aesthetic value of major recreational water bodies diminishes as the sites are blanketed by mats of *P. crassipes*, hence negatively impacting the tourism sector (Center *et al.*, 2002; Téllez *et al.*, 2008; Eiswerth *et al.*, 2018).

Flooding is another socio-economic impact exacerbated by *P. crassipes* where waterways and drainage systems become blocked and, run off is impeded leading to a rise in backwater (Penfound and Earle, 1948; Harley, 1990; Center *et al.*, 2002). A study by Arp *et al.* (2017) on the Vaalharts Weir in Warrenton, South Africa, showed that at 100% *P. crassipes* cover, there was an estimated loss of between 20.3 to 45.3 million m^3 of water. When cover was at 50%, water loss ranged between 10 and 22 million m^3, while at 25% cover there was a range of 5 to 11.3 million m^3 water loss (Arp *et al.*, 2017). Furthermore, siltation of dams from these infestations results in the reduction of water holding capacity causing water shortages for domestic and commercial activities (Cilliers, 1991; Aweke, 1994). Water quality is also negatively affected, especially in areas where the source of water for households is rivers and dams (Eiswerth *et al.*, 2018).

1.6 Management and control measures

1.6.1 Mechanical control

Mechanical control entails physically removing the weed, often manually as in most developing countries, or using heavy machinery (Pieterse, 1978). This control method is

mostly feasible where the weed has invaded a small water body (less than 1 hectare) as there is greater success of eradication over a small area (Van Oosterhout, 2006). In South Africa, mechanical control was implemented as part of the integrated management strategy on the Enseleni and Mposa rivers. This strategy involved the use of 28mm steel cables which were anchored on trees and tied across the confluence of the two rivers, thus forming booms to contain the spread of the plant (Jones, 2001). More booms were installed at various points across the Enseleni River. The cables were submerged and fashioned with plastic flotations to provide buoyancy, enabling the booms to ensnare the roots of the plants. This method of controlled was ineffective during times of flood as the sheer weight of *P. crassipes* and force of water overcame the booms (Jones, 2001). Use of machinery such as harvesters is also a common mechanical control and, has been implemented in countries like Uganda (Port Bell and Owen Falls Dam) on Lake Victoria and the Shire River in Malawi, however with limited success (Coetzee *et al.,* 2017). Plants fragment during mechanical control and this facilitates the spread of the weed to other areas (Hussner *et al.*, 2017). The use of heavy machinery on the streambanks facilitates siltation of the affected water bodies. The costs of applying mechanical control are far higher than the benefits obtained due to the faster growth rate of the plant compared to the application of the type of management (Van Oosterhout, 2006; Hussner *et al.,* 2017).

In the Guadiana River, southern Spain, mechanical control measures were implemented, and this included the draining of the Montijo Dam via its bottom gates, thereafter the stranded plants were collected (Téllez *et al.*, 2008). Barriers in the form of steel grates with small apertures were used to prevent the migration of *P. crassipes* up two irrigation canals leading from the dam. A total 7200m of steel barriers was installed across the channels in order to contain *P. crassipes* from migrating (Téllez *et al.*, 2008). The contained plants were then removed from the channel using trucks that had cranes fashioned with grapples, backhoes

with buckets installed on them; and at inaccessible sites, boats were used to extract *P. crassipes* (Téllez *et al.*, 2008). However, after the initial removal of the weed, there was a resurgence attributable to the germination of the long-lived seeds, as well as from missed plants in sheltered areas of the river (Téllez *et al.*, 2008; Coetzee *et al.*, 2017). A further 5 tonnes of the weed was removed from the river in 2010, another 51 000 tonnes in 2012, while in 2015, *P. crassipes* was removed at a rate of 826 tonnes per day, and 170 000 tonnes was removed in 2016 driving the cost to USD 29.16 million (Coetzee *et al.*, 2017; Duarte, 2017). This mechanical control effort over 10 years (2005 to 2015) highlights how intensive and expensive mechanical control operations can get, where over 360 000 tonnes of water hyacinth were removed (Téllez *et al.*, 2008; Coetzee *et al.*, 2017). The extent of the problem is such that *P. crassipes* infestations have again spread along 150 km of the river, reaching as far as Portugal and Alqueva, the largest water reservoir in Europe, thus nullifying gains of the mechanical control effort (Téllez *et al.*, 2008; Duarte, 2017).

1.6.2 Chemical control

Chemical control has been effective to a certain extent and only in localized infestations on small water bodies. The most prevalent herbicides include amitrol, 2,4-dichlorophenoxyacetic acid, amine, diquat, glyphosate and paraquat (Téllez *et al.*, 2008). In Hartbeespoort Dam, South Africa, chemical control using a terbutryn herbicide started in 1970s and brought relief from the infestation (Byrne *et al.*, 2010), but surveys carried out in 2017 and 2018 revealed that the infestation had again covered the dam. Repeat herbicidal applications are expensive in the long-run and present non-target effects on native biodiversity. The cost of herbicides in the United States during 2000, ranged from half a million dollars in California to USD 3 million in Florida (Center *et al.*, 2002). In South Africa, costs of herbicide control averaged USD 140 per kilometre resulting in a total cost of USD 33 977 on the Crocodile River to in 2008 (Byrne *et al.*, 2010). Herbicides may contaminate water bodies, rendering water bodies

unusable for drinking water and for animal use. Due to contamination risks posed by herbicides, some countries have strict regulations over the use of herbicides, with total bans in EU countries. The USA prescribed post spray intervals where a water source may not be used until the herbicide has degraded (Julien *et al.*, 2009). Furthermore, after the spraying of herbicides, resurgences occur from the long-lived seed banks which germinate on the dying mats of the mature plants (Center and Spencer, 1981; Center *et al.*, 2002; Malik, 2007), rendering the control ineffective on its own, hence the need for follow up management (Téllez *et al.*, 2008).

1.6.3 Biocontrol

Biocontrol is the use of the natural enemies of the weed to keep its populations at acceptable levels (Coetzee and Hill, 2008; Messing and and Broduer, 2018). Reports on biocontrol as of 2012 have shown that, 468 biocontrol agents have been released against 175 weed species (Schwarzländer *et al.*, 2018). Impact evaluations of 313 biocontrol agents have shown that 175 species have caused variable, medium and heavy damage on the target weeds (Schwarzländer *et al.*, 2018). This method of control is relatively cheaper, sustainable in the long run and environmentally friendly compared to mechanical and chemical control (Pieterse, 1978; Center and Hill, 2001; Messing and and Broduer, 2018). Over the years, scientists have surveyed the native range of weeds to find host specific natural enemies (Center *et al.*, 2002). These natural enemies are put through a series of tests in the invaded range to ascertain their suitability to be part of the management strategies to control the weed (Center *et al.*, 2002). The most suitable control agents must pose minimal threat to native biodiversity, hence the use of specialist herbivores which will not feed on any other plant except the target (Pieterse, 1978).

Despite several biocontrol agents released on *P. crassipes*, it remains one of the worst aquatic weeds (Byrne *et al.*, 2010; Coetzee *et al.*, 2011). The most successful agents are the two

weevils, *Neochetina eichhorniae* Warner and *N. bruchi* Hustache (Coleoptera: Curculionidae) which leave feeding scars on the leaf lamina. The two weevils were first released in the United States in 1972 (*N. eichhorniae*) and in 1974 (*N. bruchi*) and have since established in 35 countries across the world (Winston *et al.*, 2014). In South Africa, other agents released include the moth, *Niphograpta albiguttalis* Warren (Lepidotera: Pyralidae); the mite, *Orthogulmna terebrantis* Wallwork (Acari: Galumnidae); the mirid, *Eccritotarsus catarinensis* and a new species of the mirid, *Eccritotarsus eichhorniae;* the leafhopper, *Megamelus scutellaris* Berg (Hemiptera: Delphacidae); the grasshopper,*Cornops aquaticum* Bruner (Orthoptera: Acrididae); and the fungus, *Cercospora pairopi* Conway (Hyphomycetes) (Coetzee and Hill, 2008; Byrne *et al.*, 2010; J. Coetzee pers. comm.) (Table 1.2).

Table 1.2: List of countries where different biocontrol agents have been released against *Pontederia crassipes* (Julien, 2000; Byrne *et al.*, 2010; Coetzee *et al.*, 2011; Tipping *et al.*, 2014).

Species	***Neochetina bruchi***	***Neochetina eichhorniae***	***Niphograpta albigutallis***	***Eccritotarsus catarinensis***	***Eccritotarsus eichhorniae***	***Orthogalumna terebrantis***	***Xubida infusellus***	***Cercospora piaropi***	***Cornops aquaticum***	***Megamelus scutellaris***
Countries										
Australia	1990	1975	1977				1981;1996			
Benin	1992	1991	1993	1999						
China	1996	1996		2000						
Congo	1999	1999								
Cuba	1995	1976	1995			1977				
Egypt	2000	2000								
Fiji		1977								
Ghana	1994	1994	1996							
Honduras	1989	1990								
India	1984	1983				1986				
Indonesia	1996	1979								
Kenya	1995	1993								
Malawi	1995	1995	1996	1996		1991;1996				
Malaysia	1992	1983	1996							
Mexico	1995	1967	1993							
Mozambique	1972	1972 ; 1985				1971				
Myanmar		1980								
Nigeria	1995	1993 ; 1984	2008							
Panama	1977		1977							
Philippines	1992	1992								
PNG	1993	1986	1994				1996			
Rwanda	2000	2000								
Solomon Islands		1988								
South Africa	1989	1974 ; 1996	1990	1996	1999	1989		1987	2011	2013
Sri-Lanka		1988								

Species	***Neochetina bruchi***	***Neochetina eichhorniae***	***Niphograpta albigutallis***	***Eccritotarsus catarinensis***	***Eccritotarsus eichhorniae***	***Orthogalumna terebrantis***	***Xubida infusellus***	***Cercospora piaropi***	***Cornops aquaticum***	***Megamelus scutellaris***
Countries										
Sudan	1979	1978	1980							
Taiwan	1993	1992								
Tanzania	1995	1995								
Thailand	1991	1979	1995				1999			
Uganda	1993	1993								
USA	1974	1972	1977			1968				2010
Vietnam	1996	1984								
Zambia	1997	1971 ; 1996	1971 ; 1997	1997		1971				
Zimbabwe	1996	1971	1994	1999		1996				
Niger		1993								
Ivory Coast	1997	1997								
Puerto Rico			1995							
Jamaica						1969				
Total	32	35	17	6	1	9	3	1	1	2

1.6.4 Factors affecting biocontrol

Biocontrol of weeds is influenced by several factors that determine the success and govern the rate at which control is achieved (Coetzee *et al.*, 2009). Across the introduced range, the level of biocontrol of *P. crassipes* varies where better control is realized in tropical regions in countries such as Malawi and Papua New Guinea, while in South Africa, where it is mostly temperate, the weed is still problematic (Coetzee and Hill, 2008; Coetzee *et al.*, 2009). In temperate zones, *P. crassipes* tends to persist because of the unfavourable temperature that the agents are exposed to. In winter, the emergent parts of plants succumb to frost while the crown survives and regenerates when a new season begins (Hill *et al.*, 1999; Hill and Olckers, 2001). The death of the emergent parts reduces agent populations. In South Africa, *P. crassipes* grows over a range of climatic conditions, which include high altitudes, where both plants and insects remain dormant during the cold months (Hill and Olckers, 2001). In summer when the plants recover, agent populations only reach sufficient populations mid-way through the season, hence the proliferation of the weed (Coetzee *et al.*, 2009).

Pontederia crassipes growth is dependent on available nutrients in the system within which it grows, and this subsequently affects performance of biocontrol agents (Heard and Winterton, 2000; Coetzee *et al.*, 2007a; Coetzee *et al.*, 2009). In high nutrient systems, characteristic of most South African systems, *P. crassipes* proliferates beyond the rate of insect reproduction and feeding intensity (Hill and Olckers, 2001; Coetzee and Hill, 2008; Center and Dray, 2010; Coetzee *et al.*, 2011). For example, Hammarsdale Dam in KwaZulu-Natal receives nutrient rich effluent from a wastewater treatment plant, resulting in high supply of nutrients for plants. In this system, the impact of two agents, *N. eichhorniae* and *E. catarinensis* is negligible due to high plant productivity despite the agent populations reaching very high levels (Coetzee *et al.*, 2009). High plant quality due to high nutrients may result in the establishment of the agent populations, however the impact of the agents may be reduced by rapid plant growth (Hill and Olckers, 2001; Center and Dray, 2010).

1.6.5 Integrated management

In the face of confounding factors in the management of *P. crassipes* by a single method, integrated control may prove effective (Shea *et al.*, 2006). Researchers and implementation officers have concluded that there is no panacea for the eradication or control of *P. crassipes*, therefore an integrated approach may prove more successful (Téllez *et al.*, 2008). In South Africa, integrated management was successfully employed on the Enseleni River, KwaZulu-Natal since 1995, where herbicides and biocontrol were used in combination to achieve control of *P. crassipes*, resulting in 22km of river cleared of the weed (Jones, 2001, 2014). In an effort to develop an effective integrated approach using biocontrol and herbicides, Jadhav *et al.* (2008) explored the requisite retardant dose of glyphosate. In their experiment, they treated *P. crassipes* leaves with varying does of glyphosate, ranging from 0.1% to 1.5% and showed that at low doses, glyphosate does not interfere with feeding by *N. eichhorniae*, rather the stress induced by the herbicide makes the leaves more palatable, hence greater feeding.

Improper implementation of integrated management can lead to failure of the biocontrol effort, especially where the use of herbicides is involved (Hill and Olckers, 2001, Tellez *et al.*, 2008). Herbicide application at high doses has also resulted in the mortality of not only the weed, but the agents too. Furthermore, the sudden mortality of the agents' source of food leads to a crash in control agent populations, leading to resurgences of the weed via seedling recruitment (Center and Spencer, 1981; Hill and Olckers, 2001). Hill *et al.* (2012) showed that herbicides led to high mortality of the mirid and *N. eichhorniae*. Furthermore, when mechanical control is implemented, there is disturbance of biocontrol with both the plants and agents removed, thereby reducing agent populations and disruptions of the life cycles (Hill and Cilliers, 1999).

The hydrology of the systems where biocontrol measures have been implemented also has bearing on the success of the programme (Coetzee and Hill, 2008). Most persistent infestations occur in small, shallow and still or slow-moving water bodies, which are also sheltered from wind and wave action

(Coetzee and Hill, 2008). However, in deeper and larger water bodies exposed to greater wind fetch, the effect of control agents is complemented by wind and waves which help to fragment and sink plants already damaged by the agents (Hill and Olckers, 2001; Coetzee *et al.,* 2011).During flooding, both plants and insects are flushed from the system, and when resurgences occur via seedling recruitment, the plants grow unchecked due to lack of control agents, this is especially true in places such as India during the monsoon season (Julien *et al.*, 1996; Coetzee *et al.*, 2009)

1.6.6 Benefits of biocontrol

Economic and ecosystem benefits accrue from a successful biocontrol programme (Van Wilgen *et al.*, 2004). A report by Van Wilgen *et al.* (2004) revealed that benefits of more than USD 696,7 million savings in management costs could be accrued from biocontrol efforts of six invasive terrestrial plants in South Africa. Biocontrol of two terrestrial species, jointed cactus and golden wattle yielded economic benefits of between USD21 and USD250 per hectare per year for each species respectively (Van Wilgen *et al.*, 2004). Land invaded by terrestrial species such as lantana, jointed cactus and wattles depreciates in production value, leading to losses (approximately 2% per hectare loss in value where there is 100% invasion) by landowners (Van Wilgen *et al.*, 2004). Biocontrol has enabled land owners to reclaim their land, thus reaping benefits in terms of appreciation of land value (Van Wilgen *et al.*, 2004).

A cost-benefit analysis on water saving from biocontrol of *P. crassipes* on New Year's Dam, Eastern Cape, South Africa showed that there was a high benefit: cost ratio through the reduction in the amount of evapotranspiration caused by *P. crassipes* which resulted in great water savings (Fraser *et al.*, 2016). Ultimately, the application of biocontrol on New Year's Dam presented a low-cost, long-term solution for reducing water loss. Biocontrol has proven useful in South Africa which is a water scarce country due to erratic rainfall. Furthermore, an ever-increasing population has brought about the need for higher agricultural output that is dependent on the little available water, hence the

importance of biocontrol in water saving (Fraser *et al.*, 2016). The average cost saving attained from biocontrol of *P. crassipes* on the Vaalharts in South Africa, ranged between USD 3.81 million and USD 83.18 million, and this highlights the economic viability of the biocontrol programme (Arp *et al.*, 2017).

1.6.7 Biocontrol in South Africa

Most of the control programmes aimed at water weeds are led by Rhodes University through the Centre for Biological Control (CBC). The success of biocontrol has been attributed to the setup of large mass-rearing facilities which supply agents across the country (Hill and Coetzee, 2017). These mass rearing facilities are housed in the City of Cape Town, Rhodes University and SASRI (South African Sugar Research Institute) and supply agents across the country wherever they are needed (Hill and Coetzee, 2017). Biocontrol has led to recovery of ecosystems as well as accrual of socio-economic benefits (Hill and Coetzee, 2017). South Africa initiated biocontrol of *P. crassipes* in 1974 with the release of *N. eichhorniae* (Table 1.2), and since then, biocontrol programmes have largely been successful with 14 biocontrol established on six major aquatic weeds (Byrne *et al.*, 2010; Hill and Coetzee, 2017). The biocontrol agents released against *P. crassipes*, the most aggressive of all aquatic weeds, include seven insects and a mite, as well as two fungi.

1.7 Description and history of *Eccritotarsus catarinensis* in South Africa

The mirid, *E. catarinensis* was first described by Carvalho in 1957, and was collected in Rio de Janeiro as a potential control agent in 1989 (Hill *et al.*, 1999). After positive identification, it was again collected near Florianopolis, on the Island of Santa Catarina Brazil for importation into South Africa in 1992 (Hill *et al.*, 1999, 2012; Taylor *et al.*, 2011). This population underwent a genetic bottleneck while in quarantine in South Africa as all individuals of the population died out except for one gravid female (Taylor *et al.*, 2011). But following recovery of the population, and host specificity testing, permission for release was granted in 1996 (Hill *et al.*, 1999).

Eccritotarsus catarinensis has a high reproductive rate and can produce several generations in a year (Hill *et al.*, 1999; Center *et al.*, 2002). The adults range between 2-3mm in length and mirids are slender with black exoskeletons, pale legs, and hyaline patches on the wings (Hill *et al.*, 1999; Stanley and Julien, 1999; Center *et al.*, 2002). They exhibit sexual dimorphism with males having a slender abdomen with a yellow tip and asymmetric genitalia visibly displaced to one side, while females are bigger, having a black, rounded abdomen and distinct ovipositors (Hill *et al.*, 1999). The adult lifespan is about 50 days, while the nymphal stage lasts about 23 days with total life from egg to mortality being about 73 days (Hill *et al.*, 1999). Females oviposit eggs into leaf tissue on the underside of the leaf lamina, and upon hatching, nymphs go through four instars before reaching adulthood (Hill *et al.*, 1999). Both adults and nymphs feed gregariously on the underside of the leaves leading to chlorosis, with the palisade parenchyma suffering heavily from extraction of chlorophyll, and eventually mortality of the leaf (Hill *et al.*, 1999; Center *et al.*, 2002; Coetzee and Hill, 2008). Heavy feeding by the mirid can slow plant growth and infestation expansion where resources that would have been channelled for asexual reproduction are used to counter herbivory pressure (Hill *et al.*, 1999; Coetzee and Hill, 2008).

Initial establishment and performance of *E. catarinensis* was limited by cold winters in temperate South Africa (Coetzee *et al.*, 2007a; Taylor *et al.*, 2011). Therefore, another survey was undertaken in South America in 1999, where a population of *E. catarinensis* was found in Peru in the Amazon Basin on the Yarapa River (Taylor *et al.*, 2011). Because variable performance in the field by the Brazilian population was attributed to low genetic diversity, the introduction of the Peruvian population was aimed at increasing genetic diversity in the already established Brazilian population (Taylor *et al.*, 2011; Paterson *et al.*, 2016). After release, it was discovered that the two morphologically similar, but geographically separate populations of the mirid were cryptic species as laboratory trials revealed that the two populations do not interbreed (Paterson *et al.*, 2016). Genetic analysis comparing the Cytochrome oxidase region 1 (CO1 region) revealed a large genetic

divergence (5.2%) between the two populations which signified that these populations could cryptic species (Taylor *et al.*, 2011; Paterson *et al.*, 2016). As a result, a taxonomic re-appraisal to classify the two as separate cryptic species was formally done. The two species have now been classified as *Eccritotarsus catarinensis* (Brazilian) and *E. eichhorniae* (Peruvian) (Henry, 2017). Because the species was separated into two, the current distribution of both species in the field needs to be determined, and genetic techniques are the most appropriate as the two species are difficult to separate morphologically.

1.7.1 Distribution of *Eccritotarsus catarinensis* and *Eccritotarsus eichhorniae* in South Africa

The first releases of *Eccritotarsus catarinensis* in South Africa were made in 1996 after four years of quarantine host-specificity testing (Hill *et al.*, 1999). *Eccritotarsus catarinensis* was later released in Benin where it failed to establish, and in Malawi in 1999 (Cordo, 1999; Ajuonu *et al.*, 2007, 2009). In South Africa, insect establishment was confirmed at 14 sites (Coetzee *et al.*, 2007b), and to date establishment has been confirmed at over 20 sites according to release data as well as data from national surveys (pers. obs.). Establishment has been confirmed in the North-West Province, on the Crocodile River; the Gauteng Province, for example on Bon Accord Dam and Johannesburg (Delta Park); the Vaal River (Schuttes Eiland), Free State Province; and the Western Cape Province (Zeekoeivlei) (Coetzee *et al.*, 2007b). The establishment of the insect is strongly influenced by local climate where extremely low temperatures were initially thought to inhibit population growth (Coetzee *et al.*, 2007b). However, studies by Porter *et al.* (2019) reported that thermal plasticity of *E. catarinensis* enabled the population to establish across the country. The insects released on the Kubusi River, South Africa in particular have become the first cold-hardened strain of *E. catarinensis* as it has persisted at the coldest release site known for the species. In order to effect control, *E. catarinensis* needs to reach outbreak populations on the weed (Coetzee *et al.*, 2007b;

Paterson *et al.*, 2016). Attaining high insect population densities is therefore pivotal for adequate herbivory pressure to be exerted by *E. catarinensis* (Hill *et al.*, 1999).

1.9 Aims of the study

Biocontrol of weeds has made significant contributions towards combating the negative effects of invasive, alien plants on ecosystems as well as the economies of the invaded areas. The intervention has brought about sustainable and cost-effective control of the weeds, sometimes used on its own and at times used in conjunction with other methods like mechanical and chemical control. There is still the need to continuously evaluate and monitor the efficacy of the biocontrol agents on target weed species. Therefore, the aim of this study was to evaluate the current distribution and impacts of the two species of *Eccritotarsus* on *P. crassipes* across South Africa, as well as seasonal persistence at a single site for an extended period. Lastly, the thesis investigated the interaction of the two *Eccritotarsus spp*. where they may possibly converge in the field.

Chapter 2 of the study dealt with mapping the current distribution of the two-cryptic species of the mirid, *Eccritotarsus* across South Africa. This section of the study zoomed out and looked at the persistence of two very similar species, which occupy the same niche across a wider geographical range, that is, at national level. Since their release into the field, and subsequent discovery that they are cryptic species, no surveys have been carried out to map the distribution of each species in South Africa. A prior study by Coetzee *et al.* (2007b) focussed on predicting the distribution of *E. catarinensis* using thermal physiology of the mirids with respect to climatic regions within the country. Ground truthing was therefore necessary in order to assess the physical distribution of the two species. National surveys to collect insect specimens were therefore essential to ascertain the current distribution of the mirids, with molecular techniques employed to separate the two morphologically similar species. Mapping the distribution and the subsequent impacts of either

species will inform mass rearing and release efforts of the two insects. Knowledge of which species inhabit which parts of the country will help with climatic matching and adequate releases of the relevant species to those parts of the country, further enhancing the effectiveness of the control programmes.

Post release evaluation provides a measure of the efficacy of a particular biocontrol agent. Such evaluations may also be a measure of the cost-benefit analysis of any biocontrol programme undertaken. Furthermore, these evaluations help assess the establishment success, persistence or prevalence and abundance of the biocontrol agents in a particular site (CRC for Australian Weed Management, 2008). In Chapter 3, the thesis focussed on the population dynamics of *E. catarinensis* in the coldest release site in the country. It was important to get an understanding of the seasonal fluctuations in population density of the mirids and how these fluctuations relate to its efficacy at the release site. Since the mirids did not occur alone at the site, the interaction between the mirids and other insects was also investigated.

Chapter 4 of this thesis investigated the interaction between the two cryptic species. It is possible that although the two species were released at different sites, convergence may have occurred. The question that arose was whether there is a possibility of one displacing the other, or whether there is co-existence between the two species (niche partitioning), since they occupy the same niche. Therefore, this chapter investigated how the two species perform when they are in the same locale. The general discussion in Chapter 5 consolidated all the work in this thesis, highlighting the management implications of the findings of this thesis. Furthermore, the conclusion proposed possible future work to bridge the gaps identified in the the main discussion.

Chapter 2 : Distribution of *Eccritotarsus catarinensis* and *Eccritotarsus eichhorniae* on *Pontederia crassipes* in South Africa

2.1 Introduction

2.1.1 Distribution of biocontrol agents

The establishment and distribution of biocontrol agents of *P. crassipes* in South Africa, and elsewhere is largely regulated by temperature. Hill and Olckers (2001) reported that cold South African winters severely constrain the establishment of *Pontederia crassipes* biocontrol agents, which are typically sourced from the tropical Amazon Basin in South America. Laboratory studies by Coetzee *et al.* (2007b) investigated the thermal physiology of *Eccritotarsus catarinensis* in an effort to understand the then and potential distribution of the mirid. The use of degree day models showed that the distribution of *E. catarinensis* was limited by climate, where between 3 and 14 generations per year were completed depending on where in the country the insect had established. From these results, they predicted that the distribution and establishment of *E. catarinensis* would be restricted by the cold winter temperatures, and especially frost, in South Africa. Furthermore, Coetzee *et al.* (2007b) predicted that there will be lower establishment success in the cooler, elevated regions of South Africa. Only the warm tropical KwaZulu-Natal Province (KZN) was climatically matched with the native range of *E. catarinensis*, which suggested that the greatest success of establishment would be there (Coetzee *et al.*, 2007b).

Porter *et al.* (2019) then investigated the thermal physiology of *E. catarinensis*, after it had been present in South Africa for more than two decades. A population of *E. catarinensis* that had established at the coldest release site in South Africa, the Kubusi River in the Eastern Cape, was used in the trial. This population was compared to a laboratory-reared culture and it was shown that the field population was more cold-hardened compared to the laboratory-reared culture. After many

years of persisting at the site, this population has reportedly undergone a shift in thermal tolerance and acclimated to the low temperatures, but that this cold hardening was in fact plastic, and could be reversed within a single individual (Porter *et al.*, 2019). These findings have implications for future biocontrol efforts using *E. catarinensis*. It is now possible to collect insects from cool sites and release them at other cooler sites with a greater possibility of establishment success and greater efficacy on *P. crassipes*.

2.1.2 Releases of the two *Eccritotarsus* species in South Africa

Initial releases of *E. catarinensis* occurred in 1996, with subsequent releases until 2007 when *E. eichhorniae* was introduced. At some of the sites, both species were released, however at different times (Table 2.1; Figure 2.1). Different sites had different release rates ranging from 1000 insects to over 20 000 insects per site (Figure 2.1). Large insect releases were carried out on Misverstand Dam (Western Cape) with 22 500 insects; Enseleni River - 22 000; Howick and Umgeni both with 15 000 insects (KZN), Sandspruit (Gauteng) received 14 000 insects and Nelspruit (Mpumalanga) had 11 000 insects released (Table 2.1).

Table 2.1: Release data of sites where *Eccritotarsus catarinensis* and *Eccritotarsus eichhorniae* was initially released across South Africa. The number released is a cumulative total of insects from first release to the latest release. Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*, Both = sites where both species were released (M.Hill unpublished data; Centre for Biological Control, 2017).

Site	**Latitude**	**Longitude**	**Number released**	**Date released**	**Collection**	**Species**
Bamboo Canal	-29,5924	30,5659	6000	11-Nov	Present	Both
Bluff	-29,6027	30,8954	6000	16-Dec	Absent	Ec
Bluff nature reserve	-29,9381	30,9932	2000	17-Jan	Absent	Ec

Bon Accord Dam	-25,6421	28,1834	1500	1996 to 2000	Present	Ec
Bontebok 1, SanParks	-34,0761	20,4311	5000	15-Nov	Present	Both
Bontebok **2, SanParks**	-34,0762	20,4531	5000	15-Nov	Present	Both
Breede-Bontebok	-34,0761	20,4311	500	1996 to 2003	Present	Both
Bronkhorstspruit River	-25,7975	28,7483	1000	11-Nov	Present	Ee
Clairwood Quarry	-29,54	30,57		2000 to 2004	Present	Both
Croc River_**Brits**	-25,3948	27,4703		1996 to2001	Absent	Ec
Delta Park	-26,0729	28,0041	A	1998 to 1999	Absent	Both
Diep river	-33,957	18,2552	1000	12-Sep	Absent	Ee
Donald Dam	-29,2639	31,3558	10400	14-Oct	Absent	Ec
Dwarsweg, George	-34,0134	22,163	1000	15-Nov	Absent	Ee
Engelhadrt Dam	-23,5018	31,3815	3700	1997 to 2014	Present	Ec
Enseleni Game Reserve	-28,4038	32,0144	27000	1996 to 2001	Present	Both
Ezemvelo KZN	-29,9381	30,9932	1700	15-Aug	Absent	Ec
Wildlife						
Farmdam **Randburg**	-26,02	27,57			Absent	Ee
Glentana	-34,0305	22,2699	10000	17-Feb	Absent	Ee
Goukou	-34,1257	21,1711	5000	15-Nov	Present	Ee
Hammersdal	-29,482	30,3956		1996 to2001	Absent	Ec
Howick, **Umgeni**	-29,3223	30,1303	36500	17-Feb	Absent	Ec
water						
IsiThumba	-29,4038	30,422			Absent	Ec
Kaapmuiden	-25,5298	31,3507	3000	Present	Present	Ee
Kersefontein,	-32,5536	18,2953	3000	16-Mar	Absent	Ee
Moreesburg						

Klipeiland	-25,8466	28,6985	3900	14-Sep	Present	Ee
Klipfontein, Riversdale	-34,1908	21,2123	5000	16-Dec	Absent	Ee
Kubusi River	-32,5303	27,2819	3000	1999	Present	Ec
Letaba River	-23,4945	31,375	5000	11-Oct	Present	Ec
Lienfield park	-29,4139	30,299	600	14-Sep	Absent	Ec
Limpopo river,	-22,5886	28,5194	2000	15-Oct	Absent	Ec
Zanzibar						
Makhadzi **spruit**	-23,4945	31,375	1500	14-Sep	Present	Ec
Marianney Foley	-29,3905	30,4104			Absent	Ec
Mbozambo	-29,2123	31,1847			Present	Ec
Misverstand **Dam**	-33,5201	19,1854	22500	16-Nov	Absent	Ee
Mlalaan River	-29,2315	30,3344	5000	11-Oct	Absent	Ec
Mlazi	-29,82	30,46	200	1997	Present	Ec
Mooi Uitsig,	-33,5647	20,0229	1000	15-Oct	Absent	Ec
Bonnievale						
Mposa River	-28,4038	32,0109	3000	16-Jun	Present	Ec
Nahoon River	-32,9739	27,9257	1500	Present	Present	Ee
Nelspruit **Angling Club**	-25,4623	30,9648	4000	17-Jan	Present	Both
New Years **Dam**	-33,1744	26,0806	7500	1996 to 2004	Absent	Both
Nooitgedacht	-33,8345	18,7405	5000	17-Jan	Absent	Ee
Noord-kaap	-25,5831	30,919	4000	Present	Present	Ee
Patrysberg, Cederberg	-32,2017	18,5639	21000	17-Feb	Absent	Ee
Patrysberg, Noordhoek	-32,3364	18,9435	10000	17-Feb	Absent	Ee
Princess **Vlei**	-34,0252	18,2906			Absent	Ee

River **lodge, Viljoenskroon**	-26,5778	26,5551	4000	16-Apr	Absent	Ee
Riversdale	-34,1324	21,2751	1500	Present	Present	Ee
Riviersonderend	-34,0607	19,4148	4000	11-Sep	Absent	Ee
Rondevlei	-34,03	18,3			Absent	Ee
Sandspruit	-26,0018	27,5718	41000	15-Dec	Present	Ee
Schuttes Eiland	26,5445	27,252	600	1996 to 2002	Absent	Ec
Stones Hill, **GHT**	-33,3362	26,5684	4000	15-Oct	Absent	Ee
Tongati River	-29,5154	31,0512	1500	Present	Present	Ec
Umbokodweni **river**	-30,0084	30,9064	1300	15-Sep	Present	Ec
Umgeni River, **isiThumba**	-29,4038	30,422	3000	11-Oct	Absent	Ec
Umgeni River, **Saddles**	-29,3811	30,3944	2000	11-Oct	Absent	Ec
Umhlangane	-29,4828	30,5948	9000	11-Sep	Absent	Ec
Umlazi River	-29,9493	30,9385	1500	1997	Present	Ec
Umtata River	-31,3517	28,481	1600	2000 to 2013	Absent	Ec
Vaal river, **Oarkney**	-27,0161	26,6944	9000	17-Mar	Absent	Both
Vaal **river, Parys**	-26,8924	27,4709	9000	17-Mar	Absent	Both
Vet **River Hoopstad**	-27,5	25,55			Absent	Ee
Warrenton Weir	-28,0657	24,5531			Absent	Ee
Westlake	34,456	18,455	200	1999	Absent	Ec
Wolseley	-33,2526	19,1144	500	1999	Absent	Ec
Wrigglesdale Dam	-32,3505	27,2808	180	13-Oct	Present	Ec
Yarmona **Weir**	-23,8602	30,3922	5000	03-Oct	Present	Both
Yellowwoods	-32,5651	27,4891	500		Present	Both

Zanddrift weir **Brede river**	-33,5227	19,5944	3000		16-Mar	Absent	Ee
Zeekoeivlei	34,044	18,3123	1000	1999 to 2000	Absent		Ec
Zoar Park	-33,47	18,3	1000	2000	Absent		Ec
Hartebeespoort Dam	-25,7521	27,8331		2019	Present		Ee

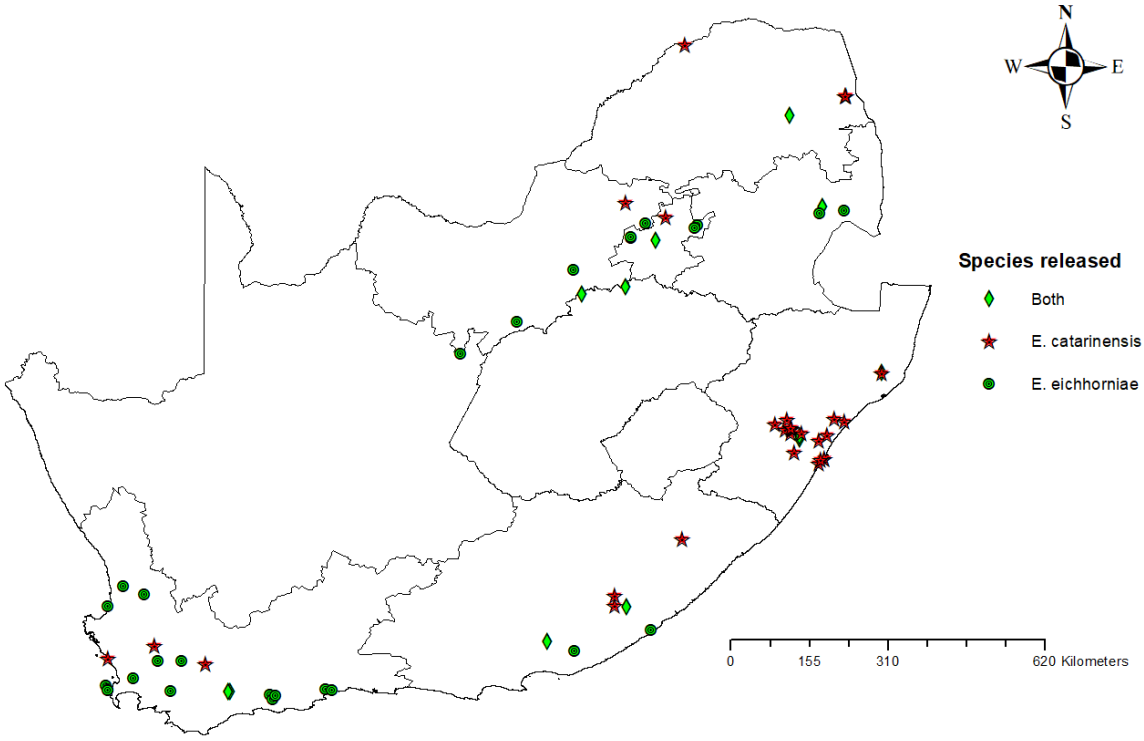

Figure 2.1: *Eccritotarsus* species releases across South Africa. The green diamonds represent sites where both species were released at different time frames, the red stars represent *Eccritotarsus catarinensis* while the green circles represent *Eccritotarsus eichhorniae*.

2.2 Cryptic species and genetic diversity within populations

The lack of clear, distinguishing morphological characteristics in cryptic species such as the two *Eccritotarsus* species has led to difficulties in identifying which one has established (Antonini *et al.*, 2008). Owing to these difficulties, molecular techniques have become very useful in the

identification of cryptic species (Antonini *et al.*, 2008; Gaskin *et al.*, 2011). There is evidence that cryptic species complexes are more common than originally thought in weed biocontrol, and these have been discovered through the application of molecular techniques combined with morphological, ecological and physiological data (Antonini *et al.*, 2008; Hendrichs *et al.*, 2015).

Through surveys of establishment sites of the mirids throughout South Africa, followed by molecular identification, this study aimed to determine the distribution of each *Eccritotarsus* species. Because the two species show variation in thermal tolerances, with *E. eichhorniae* being more warm adapted (Paterson *et al.*, 2019), I hypothesized that *E. catarinensis* would have established in both cooler and warmer regions, while *E. eichhorniae* would have established only in warmer parts of South Africa. I further suggest that there is potential for both species co-exist at certain sites, and this might inform their performance in those areas.

2.3 Methods

2.3.1 Field post-release evaluation

National field surveys were carried out throughout South Africa, visiting a subset of the *Eccritotarsus* spp. release sites at the peak and towards the end of the *P. crassipes* growing seasons, between February and April of 2017 and 2018. These were the time periods when there was a higher chance of finding the mirids at most of the sites (see Chapter 3).

The original release data were revisited and used to inform which sites to visit across South Africa. During the survey, 45 out of the 75 release sites were accessible as some sites were on private land which was not always readily accessible, while some sites were too dangerous to carry out any sampling (steep banks, potential threats from humans and wild animals such as hippos and crocodiles). Insects were found at only 22 release sites, and both insect and plant data were

subsequently collected from each of those sites (Figure 2.1; Table 2.1). Population densities of *Eccritotarsus* spp. were assessed using three quadrats (0.5 x 0.5m) randomly tossed onto a mat, where the number of insects per quadrat was counted and then converted to number of insects per square metre. In order to obtain insect samples for molecular identification, a 50m transect parallel to the bank of the water body (depending on the size of the water body) was set up, 1 - 2m from the bank, in the water. This was done in order to maximize the probability of obtaining both insect species should one be in low abundances compared to the other. A minimum of 30 insects (depending on insect abundances) was collected at random points along the transect from each site. Upon collection, samples were immediately preserved in 100% ethanol to preserve DNA for molecular identification. At some of the release sites, no mirids were found due to the drought of the 2016/2017 season where some sites were dried up, especially in the Western Cape such as the Cederberg sites on the Berg River. Some of the sites had been subject to herbicide applications and this was the case in Nooitgedacht and Misverstand Dam, Western Cape, and Sandspruit, Gauteng, however some samples were obtained at Sandspruit.

2.3.2 DNA extraction, sequencing and data analysis

DNA was extracted from at least 20 insects per site where each individual insect was counted as a sample. The Qiagen DNeasy Blood and Tissue kit (Qiagen Inc., Valencia, CA) was used to extract the DNA following the protocol prescribed by the manufacturer. The extracted DNA samples were stored in a freezer.

Polymerase Chain Reactions (PCR) following the CO1 protocol were run on the extracted DNA samples (Paterson *et al.*, 2016). The reactions were carried out in volumes of 20µl which comprised Primer (LCO1 and HCO1 primers) volumes of 0.8µl; $MgCl_2$ 0.8µl; Promega Mastermix solution 10µl (1U of Taq, 1.5 mM $MgCl_2$, and 0.2 lM dNTPs); water 5.4µl and 3 µl of DNA in each PCR

tube (Taylor *et al.*, 2011). In the CO1 protocol used, there was an initial 2 minute denaturation step at 94°C, which was followed by 35 cycles at 94°C for 30 seconds, 44 °C for 45 seconds and 72 °C for 90 seconds with a final extension of 20 min at 72 °C (Taylor *et al.*, 2011). Gel electrophoresis on Agarose gels (1%) was used to visualize the PCR products. The gel electrophoresis was run at 90V in 0.5 x Tris-boric acid-EDTA (TBE) buffer for 30 minutes, and thereafter stained with ethidium bromide. Bio-Rad ChemiDoc™ system (Bio-Rad, USA) was used to capture the images from the gel electrophoresis.

The products of the PCR were packaged and shipped to Macrogen (Europe) for sequencing. The resultant sequences were analysed in GeneStudio (GeneStudio^{TM} Professional Edition; version 2.2.0.0). Prior to sequence alignments, "noise" (false and irregular peaks) in the sequences was removed. The cleaned-out sequences were aligned using Clustal-W alignment method in GeneStudio. The aligned sequences were migrated to MEGA-X (Molecular Evolutionary Genetics Analysis) where they were re-aligned with "Muscle" method, and thereafter, a phylogenetic tree was drawn in order to visualize the separation of species. Similarity between samples was determined via the p-distance model to calculate the distance between two individuals using branches on a phylogenetic tree.

2.3.3 Sampling protocol for plant and environmental parameters

The sampling of plant growth parameters gave a measure of the state of the habitat of the biocontrol agents. At each site, 10 plants were selected at random across a stretch of water. The longest leaf petiole was measured from the crown of the plant to the base of the leaf lamina. The number of leaves per plant was counted on the main plant. Insect feeding damage, which is visible as chlorotic patches on the abaxial and adaxial surfaces, was recorded on leaf 4 which is the fourth oldest leaf. Leaf damage was an estimate of the proportion of the leaf that showed chlorosis relative to leaf area.

Leaf 4 was selected because damage is more pronounced on older leaves (Hill *et al.,* 1999), and, most of the mature plants had a minimum of four leaves. The maximum root length was measured from the base of the crown to the tip of the longest root. Plant fresh biomass was measured for different plant parts, where the plants were separated into emergent and submerged plant parts, and dead material. Plant biomass measures were obtained from the same three quadrats used for insect density measures, and thereafter the biomass was converted to mass per square metre. Plant densities were also calculated from those same three quadrats and converted to plants per square metre.

Environmental parameters such as nutrients and temperature influence plant growth, therefore, at each site, the physical and chemical properties of water were measured. The concentration of nitrate and ammonium was measured using Vernier Nitrate and Ammonium Ion-Selective electrodes. The pH, salinity, total dissolved solids and electrical conductivity were measured using a Multi-Parameter meter (PCSTestr 35 Series), while water oxygen content was measured using a dissolved oxygen meter (DO Pen 850045 Sper Scientific). Temperature and rainfall data for all the field sites were obtained from the South African Weather Services (SAWS, 2019).

2.3.4 Statistics

Data were analysed in R 3.6.0 (R core team 2019). Prior to analysis, data were checked for normality using the Shapiro test and the Levene's test of homogeneity, and the data failed both tests. Insect population data were not normal, therefore, a Generalized Linear Model (GLM) was used to test for the most influential factors on insect densities. The best fit model for the GLM was obtained by computing data through the "dredge" function in R which subsets the data from the global model to obtain the best fit model. Multivariate data analysis was carried out in R 3.6.1, where a Relate test was used to test the significance of the relationship between biological and environmental variables, and a Principal Component Analysis (PCA) biplot was used to visualize the relationship between the

environmental data and biological data. PERMANOVA was used to check for differences between sites and provinces. A similarity test following the Bray-Curtis scale in Primer 6 (Clarke and Warwick, 2001) was used to assess similarities in biological data between sites. Because the data were not normally distributed, they were transformed via the Log(X+1) transformation prior to analysis. On the Bray-Curtis scale, a value of 0 means the samples are the same, while a value of 100 represents the maximum dissimilarity between two samples in the data. The Bray-Curtis scale is robust to withstand absences of various values and is therefore recommended for ecological data. The BEST test in Primer6 was employed to show the best combination of environmental variables that explained the most variation in biological data. A PERMANOVA test of significance was run to show the differences between biological data in response to environmental factors, this was followed by an ANOSIM test which tested the significance of differences between sites and provinces. A Kruskal-Wallis ANOVA was used to test for differences in biomass, and other plant parameters (leaf number, root length) and damage sustained by plants across all sites. A correlation test was done in R to test for correlation between biomass and number of individuals per quadrat as well as with water physicochemical parameters. Analysis of Covariance (ANCOVA) was used to find the most influential predictors (physico-chemical parameters, rainfall and temperature) for plant biomass, ramet production and leaf numbers.

2.4 Results

2.4.1 Genetic sequence data analysis

During the survey, 22 sites had established insect populations (Figure 2.3) compared to the 45 accessible release sites (Figure 2.1). Sequence data was imported into MEGA-X for producing visualizations of the separation of the two species. A total of 540 base-pairs per sequence was obtained from sequence alignment process and these were used in the analyses. Because the data set was big (364 insect samples), only a single individual per site is presented on the phylogenetic tree

for clearer visualization (Figure 2.2). The field sequence data was compared with GeneBank sequence data for both *E. catarinensis* and *E. eichhorniae*. All the field specimens grouped with the branch of the tree which is aligned to *E. catarinensis* sequences (Figure 2.2). Pairwise distance tests showed high similarity between the field specimens and *E. catarinensis* sequence data (overall *p-distance* = 0.00186). One specimen was perfectly aligned to *E. eichhorniae* sequence data, (*p-distance* = 0.0000), revealing a perfect match (Figure 2.2). The only sample aligning with *E. eichhorniae* was from the laboratory reared culture of the species at the Rhodes University mass rearing facility. These results show that indeed there are two species of *Eccritotarsus* in South Africa, however, only one has successfully established in the field, *E. catarinensis* (Figure 2.2).

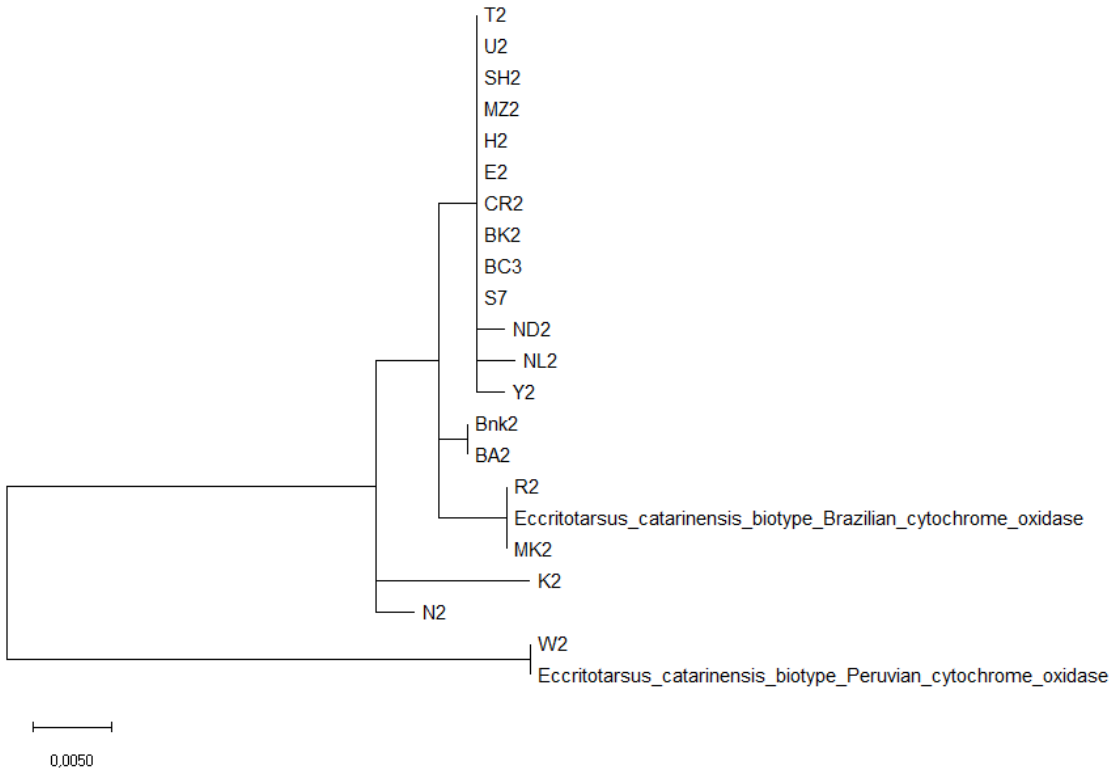

Figure 2.2: Phylogenetic tree showing the species by site. The Brazilian insects retained the name *Eccritotarsus catarinensis*, while the Peruvian insects assumed the new name, *Eccritotarsus eichhorniae*. Specimen W2 aligned with *E. eichhorniae* was obtained from the laboratory reared culture.

2.4.2 Establishment of *Eccritotarsus* spp. across South Africa

During the survey, only 45 out of over 70 release sites were accessible and *Eccritotarsus* spp. were present at only 22 sites. At 11 of the release sites, both *E. catarinensis* and *E. eichhorniae* had been released at different times, with *E. eichhorniae* being the latter species (Figure 2.1). Out of the 11 where both were released, only six sites had insects established. Most of the establishment sites were in the warmer regions of the country situated in the low-lying areas where mean annual temperature was ~20 °C (Figure 2.3). In the KZN, insect establishment was greatest, where eight sites had established insect populations. In this province, most of the sites were closer to the coast which had warmer temperatures compared to the inland areas. There were fewer establishment sites in the cooler, high altitude areas of the country, located mostly in the interior of the country (Figure 2.3). In Gauteng Province, despite the cool mean temperatures (<14 °C), insects had established at four sites, similar to the Eastern Cape where insects persisted in three cool sites subject to winter frost. In both the warm and cool sites, *E. eichhorniae* failed to establish (see below) (Figure 2.3).

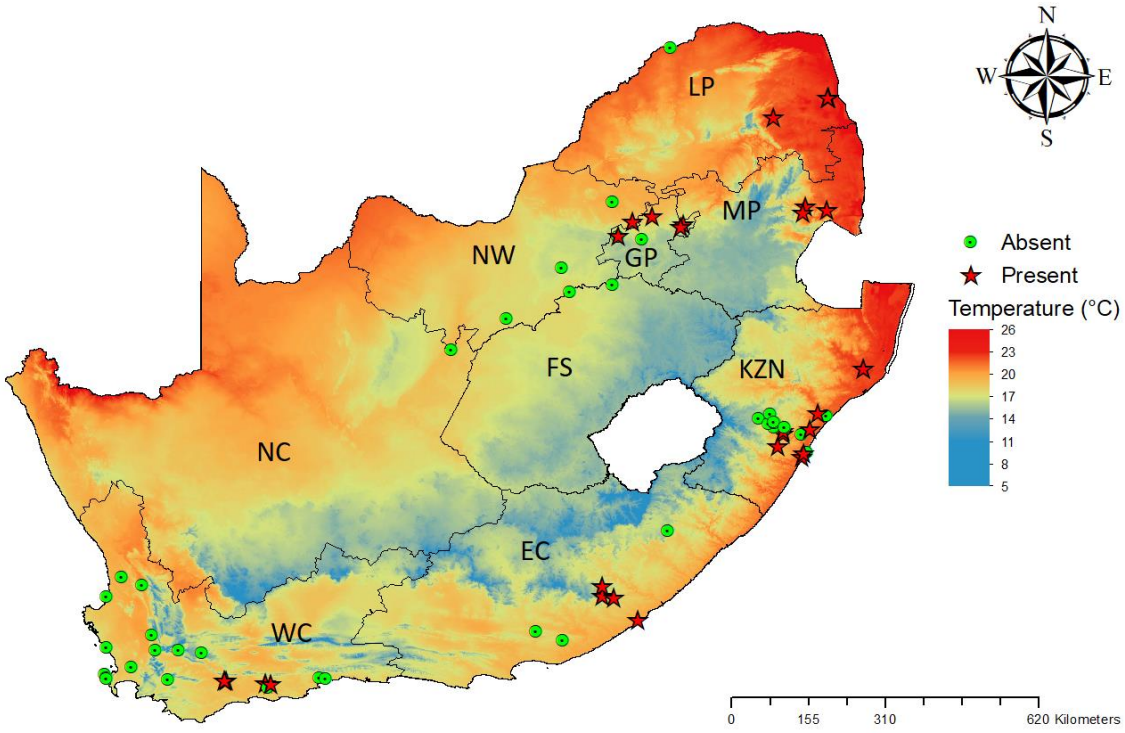

Figure 2.3: Distribution of *Eccritotarsus catarinensis* nationwide along a temperature gradient of mean daily temperatures from <5 °C to > 26 °C. Sites where insects were absent or unconfirmed are the green circles, while the red stars depict confirmed establishment. The abbreviations in the map represent the provinces; EC = Eastern Cape, FS = Free State, GP=Gauteng, KZN = KwaZulu-Natal, LP = Limpopo, MP = Mpumalanga, NC = Northern Cape, NW =North West, WC = Western Cape.

South Africa is subject to cold winters which also affect the populations and establishment of biocontrol agents. In South Africa, *P. crassipes* is susceptible to frosting in winter and this has implications on insect establishment and populations. Successful *E. catarinensis* establishment was found in areas subject to the least number of frost days, between 0 and 23 days annually (Figure 2.4). Sites in the provinces of KZN, Limpopo, Western Cape and Mpumalanga had the least frost days and subsequently KZN had the most establishment sites (8 sites) (Figure 2.4). Sites in Gauteng and two sites in the elevated parts of the Eastern Cape had greater durations of frost (between 23 and 58 days), however, *E. catarinensis* persisted at those sites (Figure 2.4). In the Western Cape, *E.*

catarinensis established in the warm coastal zones, with four confirmed establishment sites (Figure 2.4).

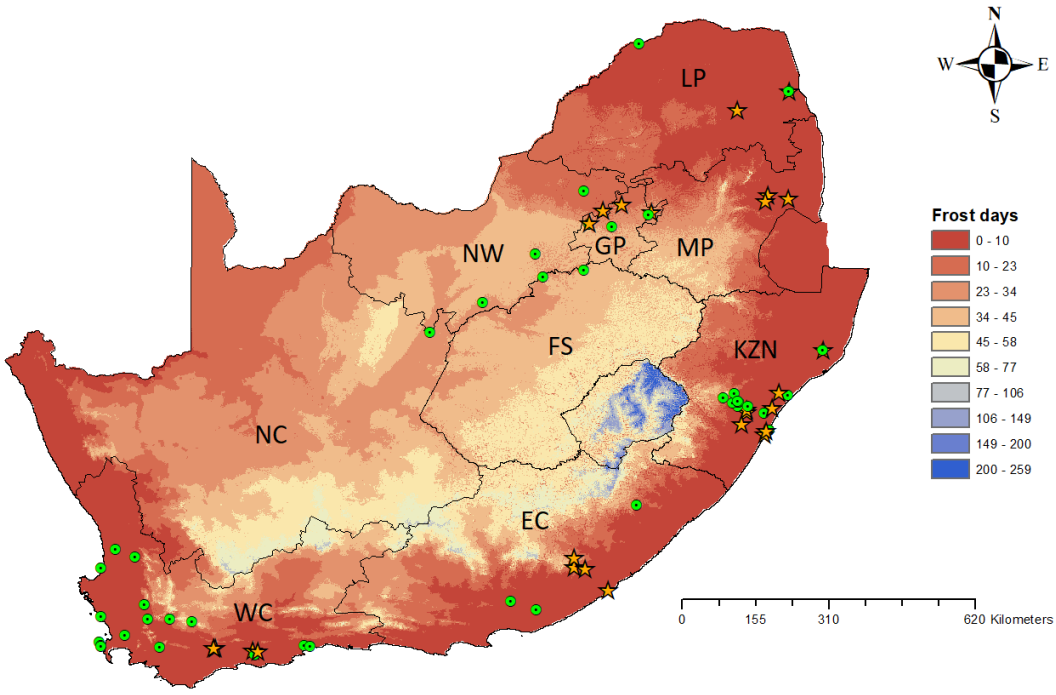

Figure 2.4: Establishment of *Eccritotarsus catarinensis* across South Africa along a frost gradient. Frost days are between 0 and 259 days. The green circles represent sites where insects failed to establish, while the gold stars denote sites with established insect populations. EC = Eastern Cape, FS = Free State, GP=Gauteng, KZN = KwaZulu-Natal, LP = Limpopo, MP = Mpumalanga, NC = Northern Cape, NW =North West, WC = Western Cape.

2.4.3 *Eccritotarsus catarinensis* densities across the country

Insect densities are important determinants of the success of a biocontrol programme, especially with regards to *Eccritotarsus* sp. which need to reach outbreak populations to effect control. Significant differences in insect densities were recorded across the country ($H_{(20)} = 53.412$, $P < 0.0001$) (Figure 2.5). The Eastern Cape sites had modest population sizes where Kubusi River and Laing Dam had similar densities (106±28 insects/ m^2), while lowest densities occurred at Nahoon River th (63±6 insects/ m^2). In Gauteng, the highest population densities were on Bon Accord Dam at 134±20

insects/ m^2, while on Hartbeespoort Dam densities averaged 89±7 insects/ m^2. The greatest insect densities in the country were recorded on Bamboo Canal, which is in a eutrophic zone KZN, with up to 276±22 insects/ m^2. Sites in the Western Cape recorded fairly high population densities with the Heidelberg site attaining the greatest densities (184±9 insects/ m^2) in the province, and the second highest nationally. Bontebok 2 also recorded some high populations (172±39 insects/ m^2) followed by Goukou and lastly Bontebok 1 with 99±31 insects/ m^2 (Figure 2.5). There was a weak relationship between insect and plant densities, and it was not significant (R_s= 7457.3, *Rho* = 0.24, *P* = 0.1324).

A generalized linear model tested the effects of environmental conditions and plant parameters on insect densities. There was a significant influence of site and minimum temperature (Table 2.2). The significant values indicated a pronounced influence of location or region on the establishment of the insects. Minimum temperatures and insect densities were significantly different between sites (Table 2.2, Figure 2.7, Figure 2.5).

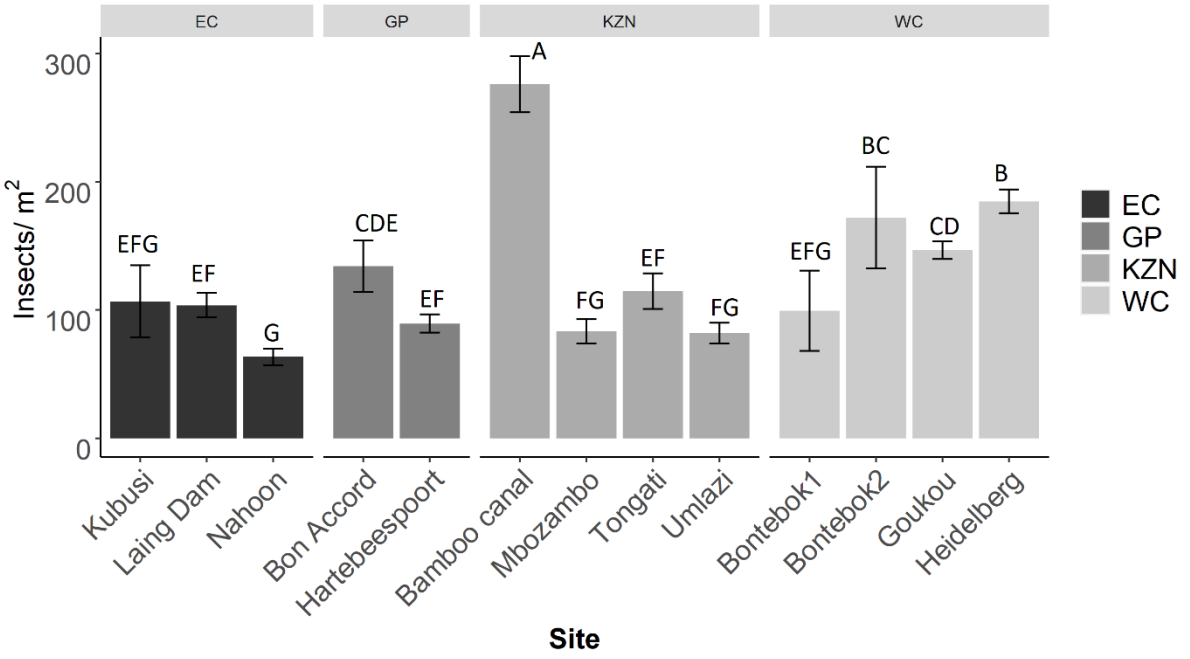

Figure 2.5: Insect densities across 21 sites and 6 provinces (EC- Eastern Cape, GP- Gauteng, KZN- KwaZulu-Natal, LP- Limpopo, MP- Mpumalanga, WC- Western Cape). Means with similar letters are not significantly different, error bars represent standard error.

Table 2.2: Influence of spatial factors (Site), environmental factors (TempMin- minimum temperature, conductivity, rainfall) and plant parameters (Roots-root length, Leaves- leaf number) on insect densities. The values in bold are significant

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
(**Intercept**)	**1.289e+01**	**2.997e+00**	**4.300**	**1.71e-05**
Conductivity	-8.785e-5	2.221e-04	0.396	0.692449
Nitrate	-2.384e-3	3.095e-02	0.077	0.938600
Leaves	-3.701e-3	5.880e-03	0.629	0.529081
Roots	-1.553e-2	1.332e-02	-1.166	0.243667
TempMin	**-3.802e-1**	**1.140e-01**	**-3.336**	**0.000851**
Rainfall	-2.798e-2	2.374e-02	-1.179	0.238582
SiteBon Accord	**-2.008e+0**	**6.187e-01**	**-3.245**	**0.001173**
SiteBontebok1	**-1.218e+0**	**1.174e-01**	**-10.380**	**< 2e-16**
SiteBontebok2	**-6.451e-1**	**1.076e-01**	**-5.996**	**2.02e-09**
SiteGoukou	**-1.079e+0**	**3.853e-01**	**-2.800**	**0.005107**
SiteHartebeespoort	-7.065e-1	7.794e-01	0.907	0.364635
SiteHeidelberg	**-9.083e-1**	**4.124e-01**	**-2.202**	**0.027648**
SiteKubusi	1.587e+00	2.670e+00	0.594	0.552264
SiteLaing Dam	-1.166e+0	8.762e-01	-1.331	0.183196
SiteMbozambo	3.542e+00	3.046e+00	1.163	0.244847
SiteNahoon	**-1.590e+0**	**4.272e-01**	**-3.721**	**0.000198**
AIC	515.94			
DF	22			
Residual deviance	225.21			
Dispersion	1			

2.4.4 Insect feeding damage on *Pontederia crassipes*

Eccritotarsus catarinensis induced plant damage by reducing the plants photosynthetic capability through chlorophyll extraction. There were significant differences in the amount of damage inflicted by the two species across the country ($H_{(15)} = 83.524$, $P < 0.0001$). In the Eastern Cape, the greatest amount of damage was recorded on the Nahoon River (50.5±6.85%) while plants on Laing Dam sustained the least damage (5.4±1.87%) (Figure 2.6). Plants on the Bon Accord Dam in Gauteng sustained the most damage nationally and provincially (62±5.33%). Bronkhorstspruit and Hartbeespoort plants sustained much lower damage (27.5±6.15%) compared to Bon Accord (Figure 2.6). Bamboo Canal in the KZN recorded the highest damage in the province which was also the second most heavily damaged plants across the country (56±5.2%). The rest of the sites in KZN sustained modest damage, with the plants on the Enseleni River recording the least damage (2±0.81%) compared to all the sites nationwide (Figure 2.6).

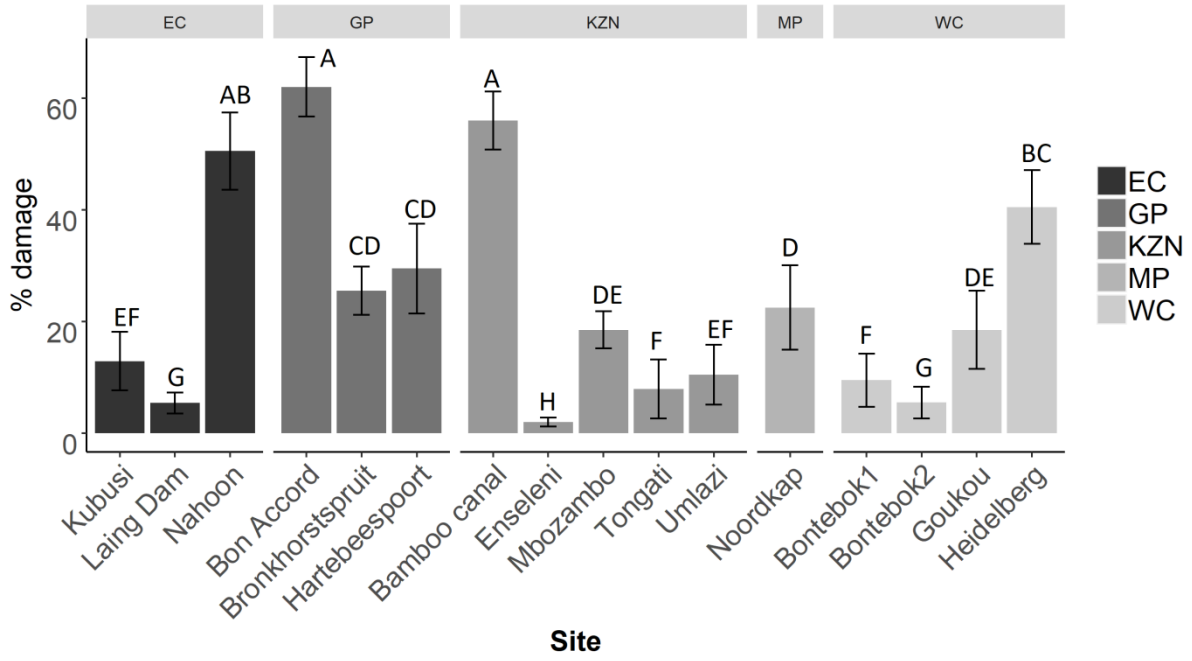

Figure 2.6: Leaf damage inflicted by *Eccritotarsus catarinensis* on water hyacinth at each site. Different letters depict significant differences.

2.5 Factors that influence the establishment of *Eccritotarsus catarinensis* and *Eccritotarsus eichhorniae*.

2.5.1 Climate data

Climate has a profound influence on the establishment success of biocontrol agents, with temperature and precipitation being important factors. Several studies have highlighted the importance of climate in the establishment of biocontrol agents (Julien *et al.*, 1996; Hill and Cilliers, 1999; Hill and Olckers, 2001; Coetzee and Hill, 2008; Coetzee *et al.*, 2009). Warmer climates favour most of the tropical biocontrol agents when introduced into a host country such as South Africa. In the nationwide survey investigating the establishment and distribution of *Eccritotarsus* sp., KZN recorded the warmest mean maximum temperatures between February and April 2018 with Tongati reaching 29.3°C, while in the Eastern Cape, the lowest mean maimum temperatures were recorded at Kubusi River, 22.6°C (Figure 2.7). Furthermore, KZN recorded the warmest mean minimum temperatures compared to the rest of the country with temperatures ranging from 14.5°C to 19.3°C, while Gauteng sites had low mean minimum temperatures ranging from a low of 7.8°C to 12.2°C (Figure 2.7). Temperatures in the Western Cape were similar across all sites with mean maximum and minimum temperatures at 26.4°C and 14.3°C respectively. Mpumalanga was warm with minimum and maximum temperatures of 15.9°C and 24.9°C respectively.

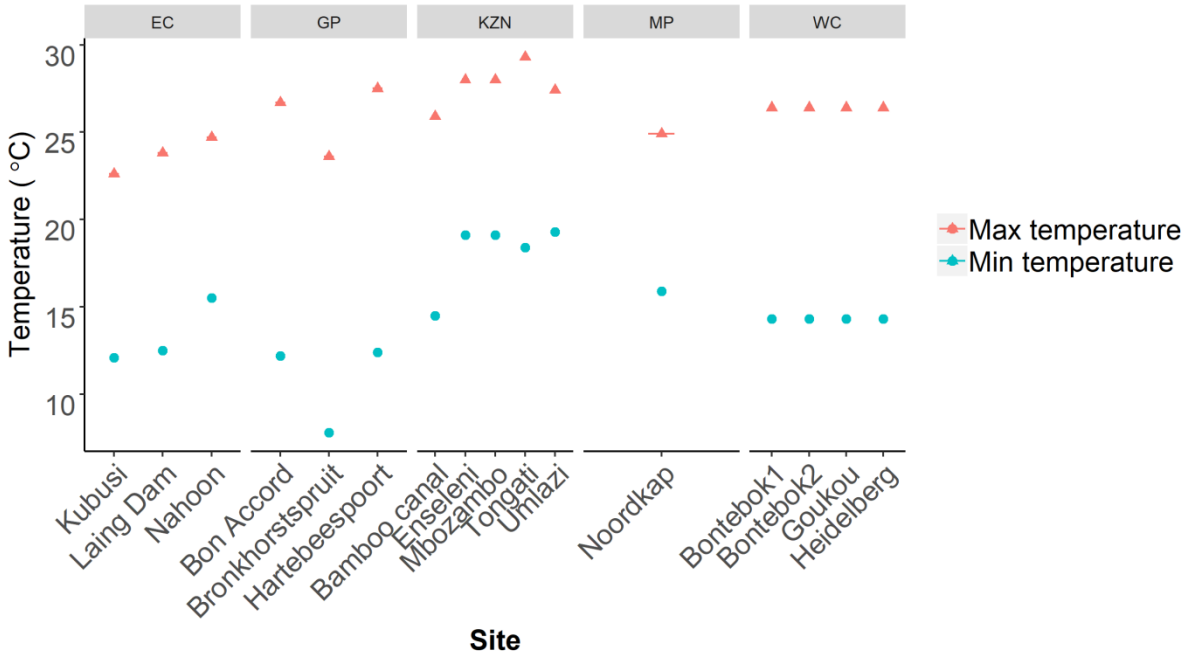

Figure 2.7: Maximum and minimum temperatures for sites where *Eccritotarsus catarinensis* had established across five provinces. EC-Eastern Cape, GP- Gauteng, KZN-KwaZulu-Natal, MP-Mpumalanga, WC, Western Cape.

Rainfall data revealed different trends to those of temperatures, with Kubusi River in the Eastern Cape receiving the highest mean precipitation (178.6 mm) between February and April 2018 (Figure 2.8). Two sites in the KZN (Enseleni River and Mbozambo Swamp) also recorded significant amounts of precipitation at 162.3 mm during the same period, however, two sites of the same province received the least rain for the month of April 2018 recording 36.2 mm (Figure 2.8). The rest of the sites in the country recorded similarly lower rainfall not exceeding 80 mm with the exception of Hartbeespoort (Gauteng) and Noordkaap (Mpumalanga) with 90 mm and 110 mm respectively (Figure 2.8). The Western Cape sites received low rainfall across all sites.

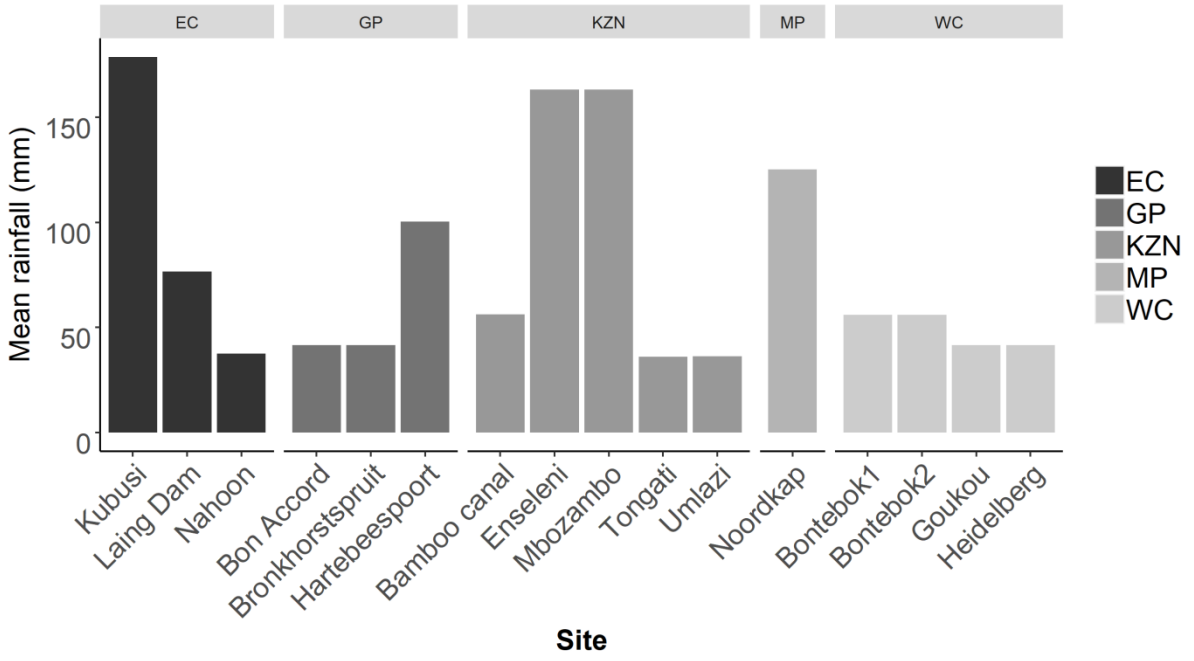

Figure 2.8: Mean monthly rainfall for March and April 2018 across sites in five provinces. EC-Eastern Cape, GP- Gauteng, KZN-KwaZulu-Natal, MP-Mpumalanga, WC, Western Cape.

2.5.2 Influence of water chemistry on *P. crassipes* growth

Plant quality is influential in the establishment of biocontrol agents. Healthier, high quality plants ensure successful insect development (Coetzee *et al.*, 2007). Plant quality is in turn influenced by the prevailing abiotic factors such as physical and chemical conditions of water. The BEST function in Primer 6 following a stepwise model selection revealed combinations of variables that best explain variation in plant parameters.

The relationship between environmental and biological data was analysed via a Principal Components Analysis (PCA) in R3.6.0. The most influential environmental variables on plant parameters were water electrical conductivity and ambient minimum temperature on PC1 and PC2 respectively (Figure 2.9; Table 2.3). Conductivity was significantly correlated to dimension 1 (R= 0.90, P <0.0001) and explained 27.1% of variation in plant data, where conductivity is often associated with nutrients in the water column. Minimum temperatures explained 14.3% along

dimension 2 with a significant correlation (R= 0.87, *P* <0.0001) to dimension 2 (Figure 2.9). Increase in conductivity led to reduced root length along dimension 1, while lengths of petioles and leaf damage increased with increase in conductivity along the same dimension. Nahoon River, Eastern Cape and Heidelberg in the Western Cape were significantly separated from the rest of the sites in their respective provinces which were significantly influenced by dissolved oxygen and nitrate (Figure 2.9). Sites in the Gauteng Province were clustered in the middle, with little spread from the centre. In KZN, temperature was an important factor in the grouping of sites as plant parameters were governed by the prevailing minimum temperatures (Figure 2.9). A PERMANOVA test with 999 permutations followed by an ANOSIM test was used to test for differences in the relationships between plant and environmental variables between provinces. Gauteng and Western Cape, as well as KZN and Gauteng were not significantly different (Table **2.4**).

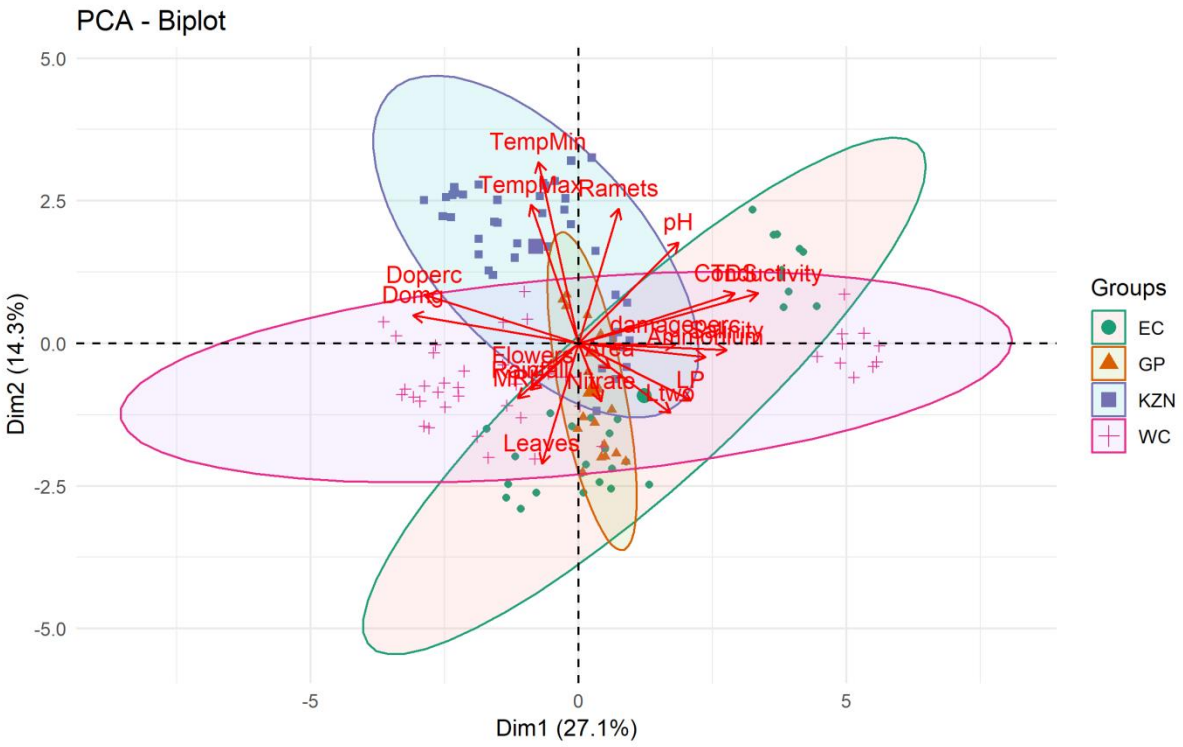

Figure 2.9: The effect of various environmental factors on the parameters of different parts of *P. crassipes* across provinces. Groups are 4 provinces: EC- Eastern Cape, GP- Gauteng, KZN- KwaZulu-Natal, WC- Western Cape. The biplot shows the contribution of dimension 1 and dimension 2 to the variation in plant parameters across provinces.

Table 2.3: PCA Eigen vectors summarising the most influential variables in principal components 1 to 5. Value in bold indicate the variables that explain the most variation.

Variable	PC1	PC2	PC3	PC4	PC5
TempMax	-0.175	0.544	-0.196	0.026	**0.079**
TempMin	-0.119	**0.558**	0.166	-0.019	-0.332
Rainfall	-0.092	-0.337	-0.083	-0.674	-0.524
pH	0.257	0.267	**0.584**	-0.170	-0.140
Conductivity	**0.421**	0.193	-0.016	0.106	-0.188
TDS	0.390	0.187	-0.080	-0.106	-0.195
Salinity	0.368	0.002	-0.301	0.039	-0.317
Nitrate	0.063	-0.332	0.268	**0.653**	-0.445
Ammonium	0.277	0.047	-0.606	0.133	-0.015
Domg	-0.416	0.079	-0.129	0.208	-0.279
Doperc	-0.402	0.130	-0.184	0.062	-0.373

Table 2.4: Permanova followed by Anosim test of significance. Values with asterisk are significantly different.

Groups	R Statistic	Significance level (%)	Actual Permutations
EC, GP	0.507	0.1*	999
EC, KZN	0.405	0.1*	999
EC, WC	0.285	0.1*	999
GP, KZN	0.05	24.9	999
GP, WC	0.135	24.9	999
KZN, WC	0.213	0.2*	999

2.5.3 Plant parameters

Leaf petioles

Eccritotarsus catarinensis is a leaf feeder and is affected by the quality and availability of leaf material. Increased petiole length is characteristic of a mature stand of *P. crassipes* where crowding is prevalent. Water hyacinth plants increase leaf petioles in crowded conditions to avoid intraspecific competition for light. There were significant differences in leaf petiole length between sites ($F_{(15, 144)}$= 11.8, $P < 0.0001$) (Figure 2.10). In the Eastern Cape, plants on the Nahoon River were the tallest (41.56±2.09cm) while on the Kubusi River, the plants were shortest at 35.9±0.84cm. In Gauteng, plants were the second tallest with plants on Bon Accord measuring 55.4±1.4 cm in height (Figure 2.10). In KZN, the tallest plants were on the Enseleni River with an average 52.1±4.11cm, followed by Bamboo canal (49.8±1.46 cm) while Mbozambo plants were the shortest at 30.9±2.79cm (Figure 2.10). Plants on Bontebok 2, Western Cape were the shortest of all the Western Cape sites with an average of 31.5±1.04 cm, while Heildeberg plants were the tallest (59.8±1.99 cm) (Figure 2.10). In Mpumalanga, plants were the shortest with average lengths of 20.9±2.26 cm recorded on Noordkap River compared to the rest of the sites surveyed (Figure 2.10).

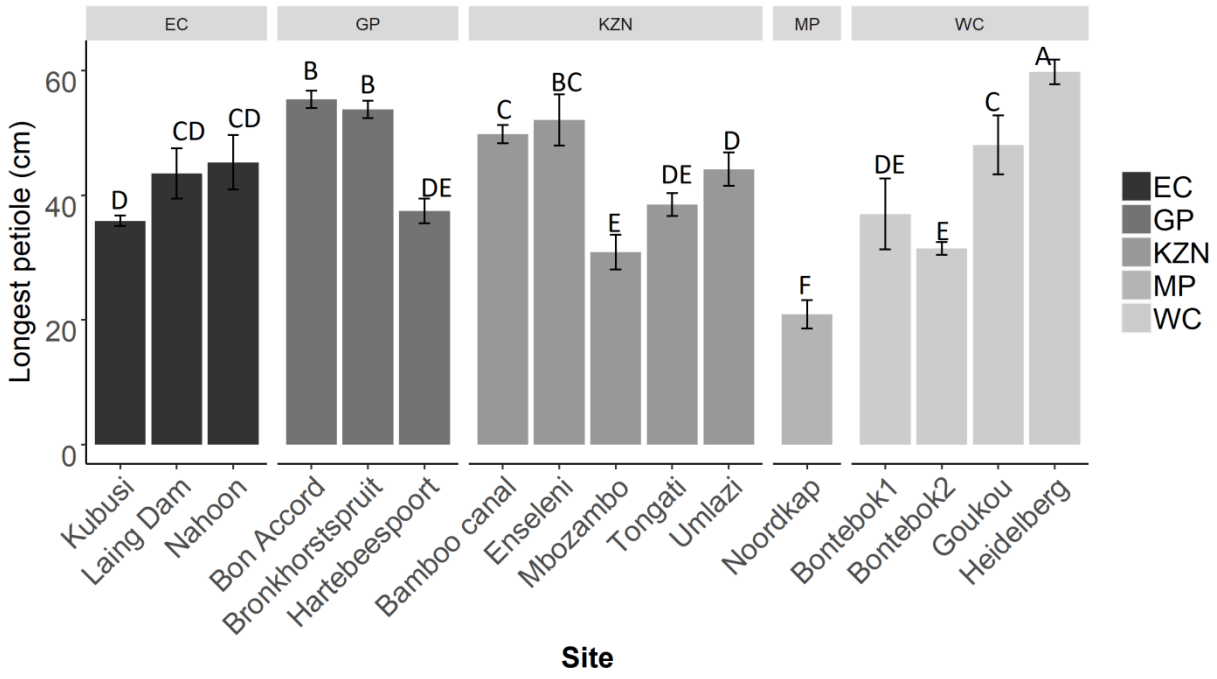

Figure 2.10: Differences in the longest leaf petiole across sites in all provinces. Sites with similar letters are not significantly different. EC = Eastern Cape, FS = Free State, GP=Gauteng, KZN =

KwaZulu-Natal, LP = Limpopo, MP = Mpumalanga, NC = Northern Cape, NW =North West, WC = Western Cape. Error bars represent standard error.

Number of leaves produced per plant

Leaf production was significantly different across sites in the country, ($H_{(15)} = 57.531$, $P < 0.0001$). Plants on the Bon Accord Dam (Gauteng) produced the most number of leaves (8.8±0.53 leaves per plant), while plants on Kubusi River (Eastern Cape) and Goukou (Western Cape) averaged 8.6±0.58 leaves/ plant (Figure 2.11). On the Nahoon River and Mbozambo Swamp plants produced the least number of leaves with averages of (5±0.44 leaves/ plant) and (5.6±0.4 leaves/ plant) respectively (Figure 2.11). In KZN, on the Enseleni River, plants produced 7.7±0.8 leaves per plant, the most in the province (Figure 2.11). There were no significant differences in leaf production with province.

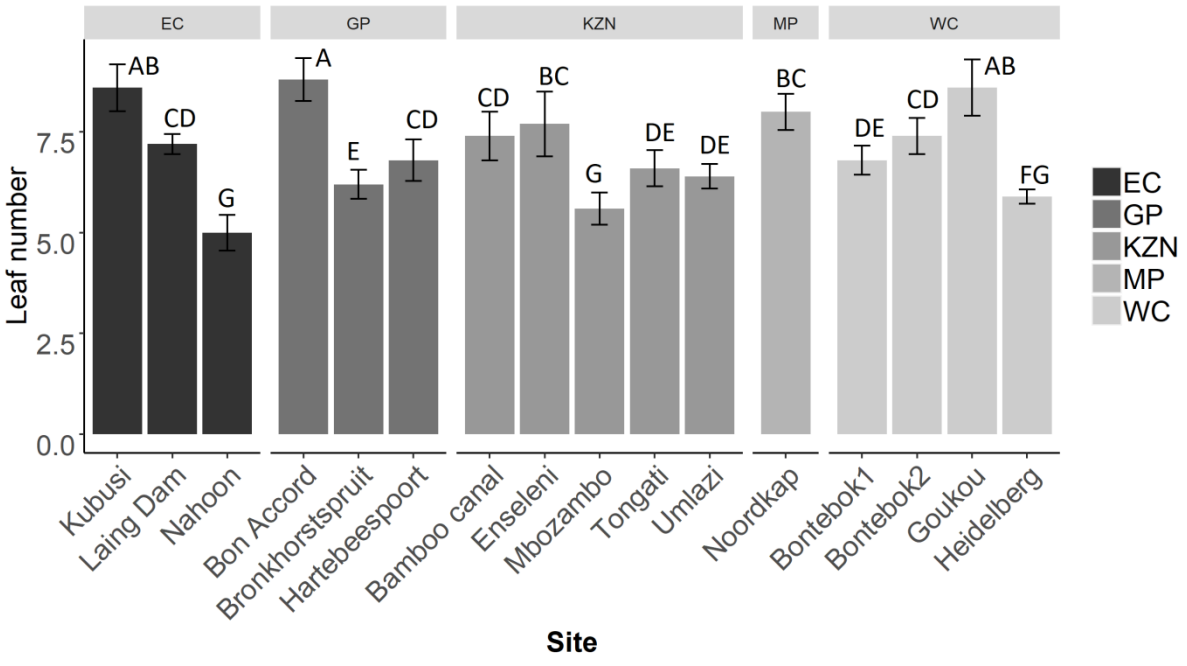

Figure 2.11: Average number of leaves produced per plant in each site.Groups on the legend represent provinces: EC = Eastern Cape, FS = Free State, GP=Gauteng, KZN = KwaZulu-Natal, LP = Limpopo, MP = Mpumalanga, NC = Northern Cape, NW =North West, WC = Western Cape. Different letters depict significant differences. Error bar represent standard error.

Root length

Root length is an indication of water nutrient status, with *P. crassipes* developing longer roots in nutrient deficient water. There were significant differences in root lengths across sites ($H_{(15)}$= 119.81, $P < 0.0001$). On the Nahoon River, the plants had the longest roots in the Eastern Cape, with an average length of 71±2.25 cm (Figure 2.12), whileplants on Laing Dam had the shortest roots in the province (26.7±2.4 cm). In Gauteng, the shortest roots averaging 20.7±2.38 cm and 22.5±2.05 cm were measured on Hartbeespoort Dam and Bronkhorstspruit River respectively (Fig. 2.7). In KZN, plants from three sites had some of the shortest roots nationally with an average 19.5±2.5 cm on the Bamboo Canal, Mbozambo and Tongati; while on the Enseleni River, plants had relatively longer roots (72.9±5.33 cm). Noordkaap River in Mpumalanga also recorded short roots (32.1±1.38 cm) (Fig. 2.7). Plants found in two sites of the Western Cape, Bontebok 1 and 2 had the longest roots nationally 83.7±6.6 and 101±14.9 cm respectively, while Heidelberg had the shortest root-lengths nationally (16.2±1.53 cm) (Figure 2.12). The results further revealed that the longer the roots, the shorter the leaf petioles, while the shorter the roots the longer the petioles (Figure 2.12 and Figure 2.10)

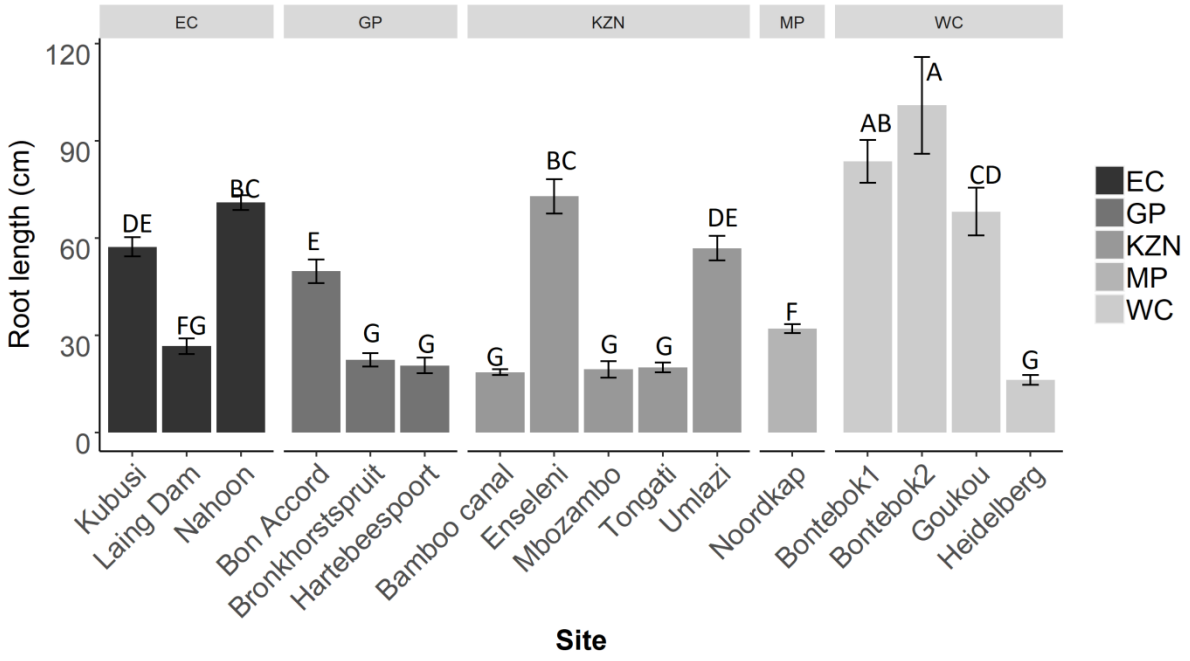

Figure 2.12: Measures of root length by site across 5 provinces: EC = Eastern Cape, FS = Free State, GP=Gauteng, KZN = KwaZulu-Natal, LP = Limpopo, MP = Mpumalanga, NC = Northern Cape, NW =North West, WC = Western Cape. Different letters above the bars mark sites which are significantly different. Error bars represent standard error.

2.5.4 Plant biomass measures

Emergent plant parts

There were significant differences in plant biomass accumulation across all sites ($H_{(12)} = 30.177$, $P < 0.005$). In the Eastern Cape, Laing Dam plants had the greatest amount of biomass (11.66±2.01 kg/ m^2) compared to Kubusi and Nahoon rivers (5.66±0.33 kg/ m^2) (Figure 2.13). In Gauteng, plants in two sites recorded similar biomass measures where Bon Accord and Hartbeespoort dams had 13.33±0.88 and 12.66±0.66 kg/ m^2. Bamboo Canal plants recorded the greatest amount of biomass in KZN while the least was recorded on the Umlazi River (Figure 2.13). Plants in the Western Cape recorded some of the lowest biomass values where the Bontebok 1 and 2 sites had an average 6.33±0.33 and 5.33±1.33 kg/ m^2 respectively (Figure 2.13). However, the plants on the Goukou River recorded the greatest biomass compared to all other sites in the country with an average

19.8±5.67 kg/ m^2. In some sites, the water was inaccessible, therefore, biomass values were not recorded.

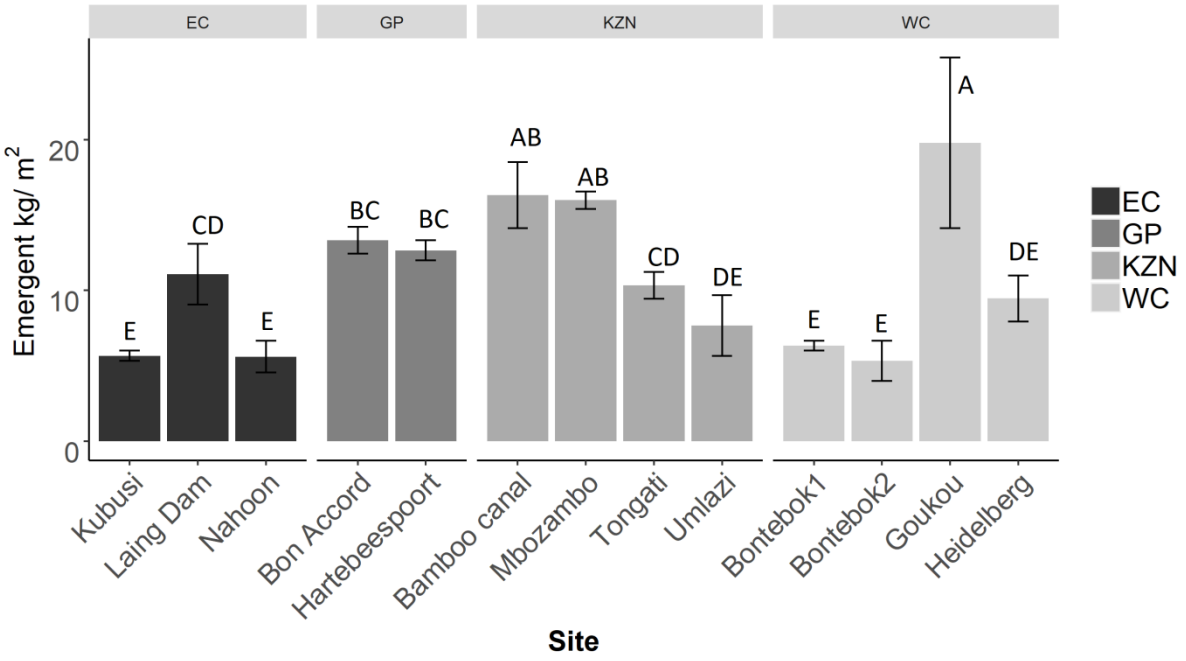

Figure 2.13: Differences in the fresh mass of emergent plant parts with across sites. Error bars represent standard error and means with similar letters are not different.

Submerged plant parts

The biomass of submerged plant parts was significantly different across all sites ($F_{9,26)} = 5.091$, $P < 0.001$). On the Kubusi River, Eastern Cape plants recorded a lot of root mass with 10±1 kg/ m^2, and this site recorded the greatest amount of root biomass compared to other sites in the country (Figure 2.14). The Laing Dam plants also in the Eastern Cape recorded some of the lowest root biomass values nationally with an average 2.26±0.26 kg/ m^2. In Gauteng, Bon Accord and Hartbeespoort Dams had significantly less root biomass of 6.53±0.74 and 4.66±0.88 kg/ m^2 respectively compared to Kubusi River in the Eastern Cape, however the biomass was very similar to most sites in other provinces (Figure 2.14). Root mass values in KZN were uniform between sites where plants on Mbozambo Swamp recorded the greatest root biomass (6±0.57 kg/ m^2) while plants on Tongati and

Umlazi rivers had the least (4.2±0.41kg/ m^2). Bontebok 1 plants in the Western Cape recorded the least root biomass (1.86±0.89 kg/ m^2) in the province as well as nationally (Figure 2.14). Goukou River plants had the greatest root biomass (7±1.52 kg/ m^2) in the Western Cape while plants in Bontebok 2 and Heidelberg had similar values, 3.66±1.2 and 2.66±0.33 kg/ m^2 respectively (Figure 2.14).

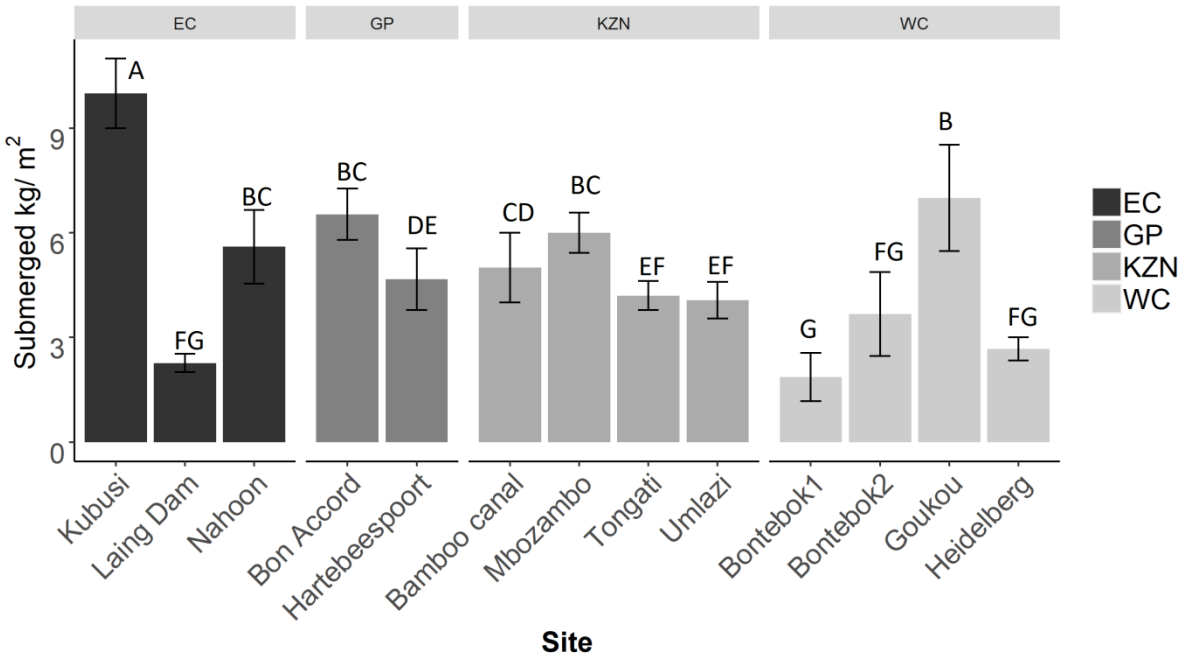

Figure 2.14: Differences in the amount of fresh root-mass across sites. Error bars represent standard error while means with similar letters are not different.

Plant densities

Plant densities were significantly different across all sites ($H_{(12)} = 27.188$, $P < 0.005$). The greatest plant densities were on Goukou River (69.3±3.52 plants/ m^2), Bon Accord (58.6±4.8 plants/ m^2) and Hartbeespoort dams (60±8.32 plants/ m^2) (Figure 2.15). On Umlazi River (22.6±1.33 plants/ m^2) in KZN and Bontebok 1 (24±4 plants/ m^2), plant densities were lowest. Sites in the Eastern Cape and KZN had similar plant densities (Figure 2.15).

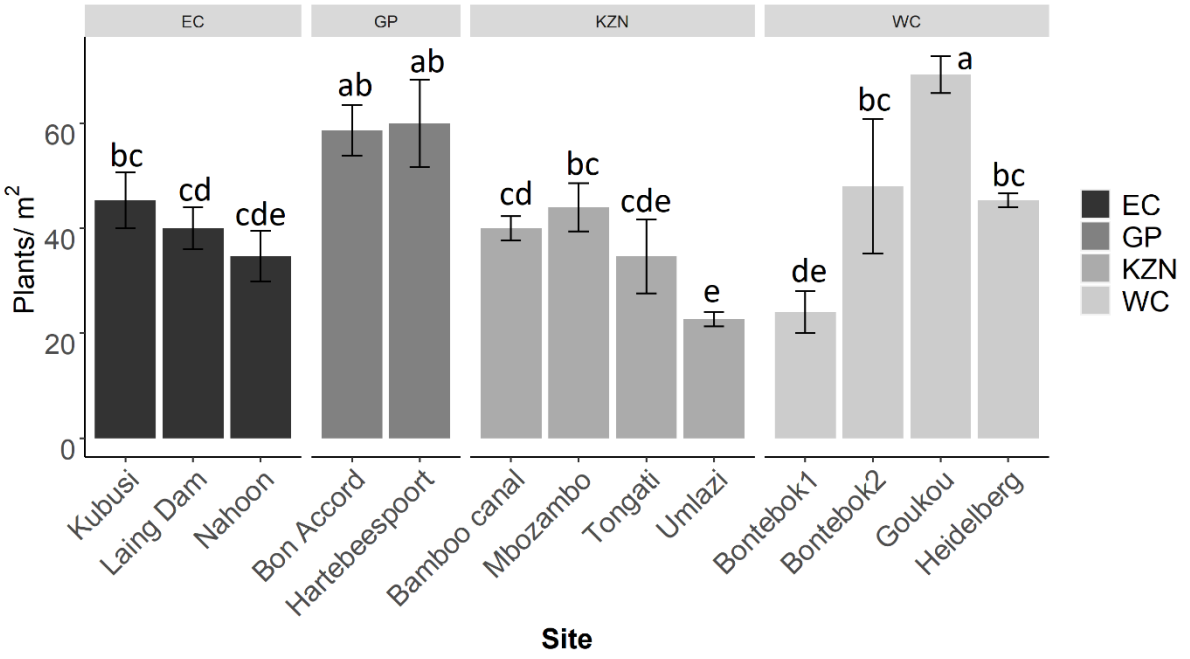

Figure 2.15: Plant densities across the different sites. Error bars denote standard error while means with similar letter are not significantly different.

2.6 Discussion

Following the release of both *E. catarinensis* and *E. eichhorniae* into the field, and subsequent discovery of the fact that they were cryptic species, a post-release evaluation was essential. Despite the two species being released across the country and with more *E. eichhorniae* being released in the last decade, the insects had not established at the sites visited during this study. A combination of factors such as plant quality and climatic conditions could be responsible for the establishment patterns discussed in this study.

Although *E. catarinensis* and *E. eichhorniae* were released at over 70 sites, less than half of these had successfully established populations. Furthermore, the establishment of only one species was surprising, especially given that two species have been released in high numbers for close to two decades, and because their biologies are very similar (Paterson *et al.*, 2019). However, because the two species evolved under different climatic conditions, there may have been differences in their

ability to acclimate and establish in the South African climate. *Eccritotarsus catarinensis* evolved under a subtropical climate, while *E. eichhorniae* evolved under a tropical climate, hence they possess different thermal physiologies. Most of the sites where *E. catarinensis* established were located along the low-lying regions of the country. These low-lying areas are much warmer than the inland areas and are almost matched with the climate of the native range of the insects. This was especially true in the province of KZN, which has a warm subtropical climate similar to that of the native range. This result consolidates the predictions by Coetzee *et al.* (2007b), where they stated that KZN is the most climatically matched province for *E. catarinensis*. Subsequently, the greatest number of establishment sites were from this province. Therefore, *E. catarinensis* favours the warmer areas compared to the cooler areas. However, *E. catarinensis* successfully established at a few cold sites in the interior of the country. It is likely that the insects have gone through microevolution that has enabled them to occupy the more temperate zones of South Africa.

A study by Griffith *et al.* (2019) reported adaptation by *E. catarinensis* in two climatically different sites in South Africa. They suggested that the persistence of *E. catarinensis* at both the cold and warm sites was due to microevolution developed over many generations of the insect since 1996. This adaptive response by *E. catarinensis* has positive implications for future biocontrol effort as these biocontrol agents will be able to occupy sites previously thought to be beyond their thermal range. In the current study, the greatest establishment of *E. catarinensis* (44%) occurred in the subtropical parts of the country, especially in KZN, double that of Mediterranean and the temperate interior plateau. However, recent studies have shown that *E. catarinensis* has successfully managed to adapt to cooler climates in South Africa. At a cold field site in South Africa, *E. catarinensis* has successfully persisted and achieved a lower CT_{min} from the initial 1.2 ºC reported by (Coetzee *et al.*, 2007b) to -0.2 ºC (Porter *et al.*, 2019). This therefore explains why this species successfully established in both the cool and warm regions of South Africa.

The failure of *E. eichhorniae* to establish could be due to its evolutionary history where this species evolved under very warm conditions characteristic of the equatorial zone (Paterson *et al.*, 2019). In contrast, South Africa has a warm temperate climate compared to the country of origin. Climate has been identified as a major determinant of success in weed biocontrol, where about 50% failures in weed biocontrol are due to climate mismatches (Cuda *et al.*, 2008; May and Coetzee, 2013). The climate of the Western Cape is Mediterranean, and establishment occurred at only 22% of the release site in that province, however, *E. eichhorniae* was absent. From the Eastern Cape to KZN and Mpumalanga, the climate transitions from Mediterranean to subtropical, and this further shows a climatic mismatch for *E. eichhorniae*, hence the absence. Release data showed that in the subtropical KZN province, only *E. catarinensis* has been released, except on the Enseleni River where both species have been released. Despite the two species being released on one system, *E. eichhorniae* failed to establish. The failure to establish on the Enseleni is contrary to the predictions by Paterson *et al.* (2019) that *E. eichhorniae* would perform better in warmer areas. Therefore, from this current study, we can conclude that *E. eichhorniae* failed to adapt to even the subtropical climatic conditions.

The biocontrol effort in some instances is disrupted by stochastic events such as droughts and implementation of other control measures which form part of the integrated pest management (IPM) systems. For example, adverse climatic conditions which prevailed prior to the first survey in 2017 could have played a role in the establishment patterns. South Africa was in the grip of a severe drought in 2016 and many water bodies dried up (Vogel and van Zyl, 2016; Blamey *et al.*, 2018). Most of the release sites in the Western Cape were dry during the 2017 survey and this ultimately meant local extinctions of these host specific agents. Although the drought was detrimental to the biocontrol effort, it proved that these biocontrol agents are host specific as they did not move onto

any other plants growing nearby. Subsequent visits to the formerly dried up sites in 2018 revealed that there were no biocontrol agents at these sites, only water hyacinth which had resurged via the long-lived seed bank. Extreme weather conditions such as droughts are accelerated by climate change and have implications for weed biocontrol (Diez *et al.*, 2014). Droughts are also characterized by extreme temperatures which may exceed the thermal physiological adaptations of the biocontrol agents, thus reducing their fitness and efficacy.

During the surveys, some of the sites had been sprayed with herbicide (part of IPM) which destroys the resource base of the biocontrol agents, hence the absence of the two mirids. For example, on the Misverstand Dam in the Western Cape, herbicides are applied indiscriminately despite the release of more than 22 000 *E. catarinensis* and *E. eichhorniae* until 2016. A similar case occurred at the Sandspruit site where herbicide had been used, however, I managed to obtain insect specimens on a few live plants. Mechanical removal of *P. crassipes* at a site in Mpumalanga had been conducted, and such operations remove both the plant and biocontrol agents from the systems. Ideally, the use of IPM is supposed to be properly planned and timed such that the methods do not interfere with each other (Center *et al.*, 1999; Byrne *et al.*, 2010). An effective IPM programme usually leaves a refuge for the insects to build up populations should there be a resurgence of *P. crassipes* (Jones, 2001; Byrne *et al.*, 2010), however, this was not the case at several sites surveyed. Therefore, there is need to implement well-coordinated IPM strategies in the management of *P. crassipes*.

Densities of *E. catarinensis* were variable across the country with most sites showing similarity. This similarity in densities could be becasue most of the establishment sites were in the low-lying area, thus making climatic conditions more uniform for most sites. There were a few differences in densities with a few sites having significantly higher densities. This could have been a function of both prevailing temperatures and water nutrient status which gave rise to vigorous plants, ideal for

insect reproduction (Room *et al.*, 1989). Some of the inland sites like the Bronkhorstspruit River and Hartbeespoort Dam, in Gauteng, had cool temperatures and were also eutrophic. This abundance of nutrients accounted for the high quality plants ideal for insect reproduction, however, cool temperatures led to the moderately high densities. Studies have shown that, *P. crassipes* growing under eutrophic conditions grow faster and provide an ideal habitat for biocontrol agents such as *Neochetina bruchi* (Heard and Winterton, 2000). Healthy *P. crassipes* plants allow biocontrol agents to achieve greater fecundity and accelerate insect development (Heard and Winterton, 2000). In some areas such as Laing Dam, Eastern Cape, although plants were healthy, there were low densities of *E. catarinensis*, likely due to plant growth outstripping insect populations. This is attributed to eutrophic water characteristic of most South African aquatic systems. Hill and Coetzee (2017) alluded to the fact that plants such as *P. crassipes* exhibit compensatory growth responses to herbivory especially in eutrophic systems. Furthermore, minimum temperatures below the insect developmental threshold (10.3ºC) could have suppressed population growth at the cool eutrophic sites (Byrne *et al.*, 2010).

The patterns of insect feeding damage mostly corresponded with the insect density patterns. This result corresponds with previous studies that show feeding damage is an indicator of biocontrol agent density (McEvoy and Coombs, 2000; Schooler and McEvoy, 2006). These studies showed that feeding damage caused by *Galerucella pusilla* Duftschmid (Coleoptera: Chrysomelidae) on purple loosestrife *Lythrum salicaria* Linnaues (Lythraceae), could be translated into insect density. In the current study, there were high levels of damage in sites with high insect densities. This is attributable to high plant quality, which favoured insect reproduction and ultimately high densities. In sites where there were low densities such as Enseleni River, Laing Dam and Kubusi River, correspondingly low damage was observed. However, there were sites which did not show these trends, such as Goukou where insect density was high, but damage was low. This is contrary to most studies where

phytophagous insects prefer plants with high nitrogen (Room *et al.*, 1989; Heard and Winterton, 2000).

Plant growth and quality informs biocontrol agent population establishment and growth. Growth of *P. crassipes* is strongly dependent on water nutrient status and prevailing climatic conditions (Malik, 2007). In South Africa, *P. crassipes* is found in both the warm lowland areas and the cool, elevated inland areas. This means that although *P. crassipes* is a tropical plant, it is tolerant of a wider range of thermal conditions. Therefore, for biocontrol to be effective, biocontrol agents should also be adapted to the conditions in which *P. crassipes* grows. In the current study, water nutrient status and minimum ambient temperatures were the most important growth regulators. This is in line with other studies that have investigated the *P. crassipes* abiotic growth regulators (Penfound and Earle, 1948; Reddy and Tucker, 1983; Heard and Winterton, 2000; Center *et al.*, 2002; Malik, 2007). Plants grew more vigorously in areas that had high nutrient input, such as in Heidelberg, Western Cape, Bamboo canal and Mbozambo in KZN as well as on the Bon Accord Dam in Gauteng. The most eutrophic sites were close to human industrial and agricultural activity thus, human activity exacerbates or fosters the persistence of *P. crassipes* despite control interventions being put in place.

Two congeneric weevil species, *Neochetina eichhorniae* and *N. bruchi* are the most widespread biocontrol agents of *P. crassipes* in South Africa (Coetzee and Hill, 2011). These two weevils have established at all the release sites where they have been released in South Africa (Byrne *et al.*, 2010). As in the case of the two mirids, the weevil feeding patterns are similar where larvae of both species mine through leaf petioles while adults feed on the leaf lamina. These two weevils normally coexist in most field sites where there is some level of resource partitioning between them. *Neochetina eichhorniae* performs well in plants with variable nitrogen content, while *N. bruchi* prefers more nitrogen rich plants (Heard and Winterton, 2000). Compared to the two weevils, the mirids have a

more restricted geographical range of establishment as shown in this study, where the mirids established in about 30% of the release sites. Furthermore, the mirids were not released at as many sites as the weevils due to the limited predicted geographical range where they were likely to establish (Byrne *et al.*, 2010). The weevils and mirids have variable thermal tolerances where the weevils have a lower lethal temperature (LT_{50} = -7.4 ºC) while that of *E. catarinensis* is -3.5 ºC (DeLoach and Cordo, 1976; Coetzee *et al.*, 2007b). Therefore, climate was a major contributing factor to the distribution of the mirid as predicted by Hill and Olckers (2001).

2.7 Conclusion

The distribution of the two species as outlined in this study is confounding because both species were released in high numbers in seemingly suitable environments, with only one being able to establish. Minimum temperatures were shown to be the major determinant of establishment, consistent with previous reports (Hill & Olckers, 2001; Coetzee *et al.*, 2007b). Further research needs to be implemented with regards to why *E. eichhorniae* has failed to establish in the country. South Africa has some warm regions particularly in the north-east where the temperatures may be more favourable. Therefore, it would be expected that there would be greater chances of establishment in those regions. According to the release data, *E. eichhorniae* was released at only one site in KZN, which is the warmest province. Therefore, in future, there needs to be releases of this insect in KZN as it has the most favourable temperatures. The role of possible competitive interactions between these two species could also explain the failure of *E. eichhornae* to establish and this was be investigated in Chapter 4.

Chapter 3 : Seasonal population dynamics of *Eccritotarsus catarinensis,* and its interaction with three other biocontrol agents of *Pontederia crassipes* at a cold temperate site in South Africa

3.1 Introduction

3.1.1 Seasonal plant-insect population dynamics in biocontrol

In weed biocontrol, the effect of season may limit the range of insects selected as control agents (Byrne *et al.*, 2003; Dalin *et al.*, 2010) because mismatches between the agent and the host's environment can result in poor control (Byrne *et al.*, 2003; Goolsby *et al.*, 2006; Coetzee *et al.*, 2007b; Dalin *et al.*, 2010). The host specific nature of the agents used in weed control means that there is close synchrony between the weed and the agent, and this is particularly important for perennial weeds such as *P. crassipes*, (Dalin *et al.*, 2010; Gichuki *et al.*, 2012)). Perennial plants often exhibit compensatory growth responses to herbivory; this is especially evident in periods when the agent is in low abundances such as at the onset of summer, where the target weed tends to grow exponentially while the agent population is yet to build up (Dalin *et al.*, 2010).

Pontederia crassipes is currently the most difficult aquatic weed to manage in South Africa and globally. Seasonal changes significantly influence its growth, where plant growth is optimal in water temperatures between 13°C and 40°C (Wilson *et al.*, 2001; Eid and Shaltout, 2017). During the summer season, characterised by high temperatures, *P. crassipes* accumulates biomass as dry matter (DM) at a rate of 0.044g/DM/day and can accumulate as much as 680g/DM/m^2 (Eid and Shaltout, 2017). During low winter temperatures, plant mortality mostly occurs when there is frost damage, leading to a decline in plant biomass This has implications for the populations of the all of the control agents used against the weed, including *Neochetina eichhorniae* and *N. bruchi*, where weevil larvae and eggs are killed by frost in the emergent leaves and petioles (Wilson *et al.*, 2001). However, the late instar larvae of the weevils

overwinter in the submerged rootstock of the plants. In areas where there is no frost, while the plants may persist for longer, the agent population development and their feeding are suppressed by the low temperatures (Wilson *et al.* 2001). Spencer and Ksander (2005) highlighted that in winter, *P. crassipes* plants can be as small as 10cm in height, while at the onset of spring, growth spurts may result in plants reaching heights of 80cm. At the onset of spring, *P. crassipes* populations recover faster than the agent populations, thereby escaping agent damage for a certain period due to the lag in agent population recovery (Wilson *et al.*, 2001; Eid and Shaltout, 2017). A study by Byrne *et al.* (2010), showed that life stages of all the water hyacinth biocontrol agents are prone to frost, further exacerbating the winter bottleneck due to low insect survival. In such scenarios, insect population recovery is slow, with negligible impact on the recovering plants. For example, in the national survey, I discovered that only one of the cryptic species of mirid (*E. catarinensis*) was established across the country (Chapter 2). There was however lack of conclusive evidence as to how temperature influenced this insect, especially that some populations had established in temperate areas, while most were in the warmer regions.

Several studies have investigated the effect of climate on the biocontrol of aquatic weeds, some of which are summarized below. *Alternanthera philoxeroides* (Mart) Griseb (Amaranthaceae), an invasive weed native to South America (Lu *et al.*, 2015), has been a target for biocontrol using *Agasicles hygrophila* Selman and Vogt (Coleoptera: Chrysomelidae), which was released in China in 1987. *Agasicles hygrophila* is restricted to lower latitudes which have warmer winters and are conducive for insect survival during winter months, while the weed has a broader climate range. Lu *et al.* (2015) investigated the effect of climate change on both the target weed and the control agent, where they highlighted that *A. hygrophila* has expanded its range since a 2012 survey. This range expansion is attributed to the higher latitudes experiencing significant warming of 0.55°C per decade since 1987 (Lu *et al.*, 2015) which has enabled *A. hygrophila* to

overwinter successfully, and hence maintain viable populations to exert control on the weed in the summer months (Lu *et al.*, 2015).

Thermal tolerance limits were tested for two biocontrol agents of aquatic weeds in South Africa, *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae) on *Pistia stratiotes* Linnaeus (Araceae) and *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) on *Azolla filiculoides* Lam. (Azollaceae) (Mvandaba *et al.*, 2019). *Stenopelmus rufinasus* was collected from a warmer climate in Gainesville, USA which is a subtropical climate, while *N. affinis* was collected in Brazil in a region characterised by a humid, subtropical climate. Laboratory studies showed that the two biocontrol agents had wide thermal tolerances where the range of *N. affinis* was between 5.5°C and 44.5°C, and the range of *S. rufinasus* was from 5.3°C to 44°C (Mvandaba *et al.*, 2019). Due to these wide thermal tolerances, the two species have established in most of the range of the target weeds, which are in some instances in the cool temperate zones of South Africa. Furthermore, these two species of biocontrol agents go through several generations per year, with *N. affinis* producing between 4 and 9 generations compared to the 5 to 14 generations by *S. rufinasus* (Mvandaba *et al.*, 2019). Owing to the successful establishment of these two biocontrol agents across a range of temperatures within South Africa, the two weeds have been brought under complete biocontrol (Coetzee *et al.*, 2011).

In another study, overwintering by *Euhrychiopsis lecontei* Dietz (Coleoptera: Erirhinidae), the biocontrol agent of Eurasian watermilfoil, *Myriophyllum spicatum* Linnaeus (Haloragaceae) was investigated at Smith's Bay, Lake Minnetonka, and on Lake Auburn in the USA, with both lakes subject to icing over during winter (Newman *et al.*, 1996). In summer, *E. lecontei* produced between 3 to 6 generations on submersed watermilfoil. In spring, there were significantly more adults in the lake compared to autumn for the three year study period (Newman *et al.*, 1996). At

the onset of winter, adults moved onshore, and densities of adults ranged from 43 to 125 adults/ m^2, where they overwintered in leaf litter, and survival of adults at the end of winter was greater than 60% (Newman *et al.*, 1996). At the beginning of spring, adults moved back to the water where the females developed and laid eggs when the water temperature ranged between 10°C and 15°C (Newman *et al.*, 1996). It was also postulated that the last generation produced at the end of summer does not reproduce, but rather overwinters to reproduce in the next spring when conditions were more favourable (Cuda *et al.*, 2008). Ultimately, overwintering habitat had a role to play in the population dynamics of *E. lecontei* where in certain areas of the shoreline, like the sandy areas, winter habitat was a limiting factor (Moody *et al.*, 2016).

A four-year study by Ray (2015) explored the population dynamics of two biocontrol agents of *P. crassipes* on various water bodies in India. The study revealed that the populations of the two weevils, *N. eichhorniae* and *N. bruchi* were significantly influenced by temperature and humidity as well as water nutrient status. There were more weevils during the summer, while during the monsoon season, *N. bruchi* was more abundant than *N. eichhorniae* due to the eutrophic conditions of the water bodies as well as high humidity. Furthermore, the monsoon season presented conditions favourable to insect development for both species. The results from Ray (2015) were contrary to the report that *N. bruchi* is more abundant under lower temperature conditions (DeLoach and Cordo, 1976). Populations of both *P. crassipes* and *N. eichhorniae* are affected by prevailing temperatures during certain times of the year (Wilson *et al.*, 2000). After winter, *P. crassipes* recovers while *N. eichhorniae* completes development after overwintering in the larval stage, thus the plants build up large populations before the insects are able to catch up (Wilson *et al.*, 2001). During the dry seasons and incidence of drought, *P. crassipes* plant populations decline, and ultimately the populations of *N. eichhorniae* decline. After the onset of the rainy season, the dormant seeds of *P. crassipes* germinate and boost the plant populations

before the weevil populations can recover (Wilson *et al.*, 2001). Such variations in habitat conditions of biocontrol agents influences their population dynamics. Hill and Olckers (2001) reported that biocontrol agent establishment in South Africa is mostly restricted by climate, especially due to severe winters experienced in South Africa. Because biocontrol agents are sourced from different climatic zones compared to their areas of introduction, it is important to understand their thermal requirements.

The study site used in this chapter has four biocontrol agents coexisting on water hyacinth; the two weevils, *Neochetina eichhorniae* and *N. bruchi*, the planthopper *M. scutellaris* and the mirid, *E. catarinensis* (topic of the thesis). The planthopper was most recent agent to be released on this river system. Initial studies by Byrne *et al.* (2010) showed that the mirid was the dominant species on the Kubusi River. However, subsequent work on the same system showed that prior to the introduction of the plant hopper in 2013, there was a spatial separation between weevils and mirid along sections of the river system (Weyl and Hill, 2012). The Kubusi River site is the coldest long-term monitoring site for biocontrol at which quantitative post-release evaluations were conducted in South Africa between 2003 and 2018 (Byrne et al 2010; this study). Furthermore, the agents on this river have varying thermal requirements, which may influence establishment success and persistence at the site. The mirid, *E. catarinensis* has a lower developmental threshold of 10.3 ºC (Coetzee *et al.*, 2007b), while that of *N. bruchi* is 15 ºC (Byrne *et al.*, 2010). The planthopper, *M. scutellaris* has a minimum developmental threshold of 11.46 ºC (May and Coetzee, 2013). Despite these differences in thermal requirements, they have established and coexist at a number of sites across South Africa, where one of the sites is the current study site.

Because this study was carried out in a multispecies setting, there were bound to be interactions between the co-existing biocontrol agents at the site (see below for detail). Furthermore, the study site is a cold, temperate site, and temperature may influence the biocontrol agents as well as their interaction. Climate influences the lifecycle of biocontrol agents as well as the rate of reproduction (May and Coetzee, 2013; Krechemer *et al.*, 2015). In warmer climates, insect development is accelerated, leading to higher populations and more generations per unit time (Coetzee *et al.*, 2007b; May and Coetzee, 2013; Krechemer *et al.*, 2015). Successful biocontrol programmes are dependent on insect populations, and thus climate also has a bearing on the success of the biocontrol by influencing insect abundances and survival (McClay, 1996; Nooten *et al.*, 2014; Catton *et al.*, 2016; Abhishek Pareek *et al.*, 2017). For example, at sites where there are fewer frost days, overwintering by biocontrol agents is more successful (Selvaraj *et al.*, 2013). Successful overwintering reduces the lag phases between the recovery of the biocontrol agent and the target weed after winter, thus effecting control on the target from the onset of the growing season (Selvaraj *et al.*, 2013). In the currently changing environment due to climate change, it has been shown that as the climate warms, there is change in community composition of insect herbivores on host plants (Nooten *et al.*, 2014). Warming may progressively favour one species over the other, causing displacement of one species while another becomes dominant (Nooten *et al.*, 2014; Krechemer *et al.*, 2015). A study by Weyl and Hill (2012) showed that there was a negative interaction between *N. eichhorniae* and *E. catarinensis*, where there was less leaf feeding by *N. eichhorniae* when *E. catarinensis* was present. A subsequent study showed that when in combination, *N. eichhorniae* and *E. catarinensis* perform well in reducing different plant parameters (Marlin *et al.*, 2013). In combination, there were no negative interactions reported as the two did not affect each other's feeding (Marlin *et al.*, 2013). Furthermore, the study by Marlin *et al.*, (2013) showed that there were similar *E. catarinensis* densities between the treatment with weevils and mirids, and where the mirids occurred alone. A

study by (Petela, 2017) investigated the interaction between *M. scutellaris* and *E. catarinensis*. Their study showed that *M. scutellaris* complements feeding by *E. catarinensis*, with no negative relationship observed between the two species. In this chapter, the seasonal variation in abundances of and interaction between four biocontrol agent populations on *P. crassipes* at a single site was investigated. Temperature as an abiotic factor, and host plant population dynamics as well as interaction between the biocontrol agents themselves likely influenced the population dynamics.

3.2.1 Materials and methods

Population dynamics of water hyacinth control agents were investigated at a sampling site located on a cattle ranch, the Featherstone Farm through which the Kubusi River passes, in the Eastern Cape Province. The river meanders past several farms before the sampling site, with a weir about 10 km from the sampling site. The town of Stutterheim lies further upstream. The biocontrol effort on *P. crassipes* on the Kubusi River (-32.93417, 27.43861) (Figure 3.1) was initiated with the release of two weevils, *Neochetina eichhorniae* and *N. bruchi*, both leaf feeders during the adult stage, in the mid-1990s. The programme was followed with the release of *E. catarinensis*, a sap sucker, in August 1999, with subsequent annual releases until 2003. *Megamelus scutellaris* was the most recent agent to be released in October 2013.

The climate of the site is warm temperate and has an altitude of approximately 823 m above sea level. Precipitation averages 598mm per annum, with most of the precipitation occurring during summer. Maximum temperatures at midday average between 17.9 ºC in winter, with the coldest month being August and 25.7 ºC in summer (March) (SAWS, 2018).

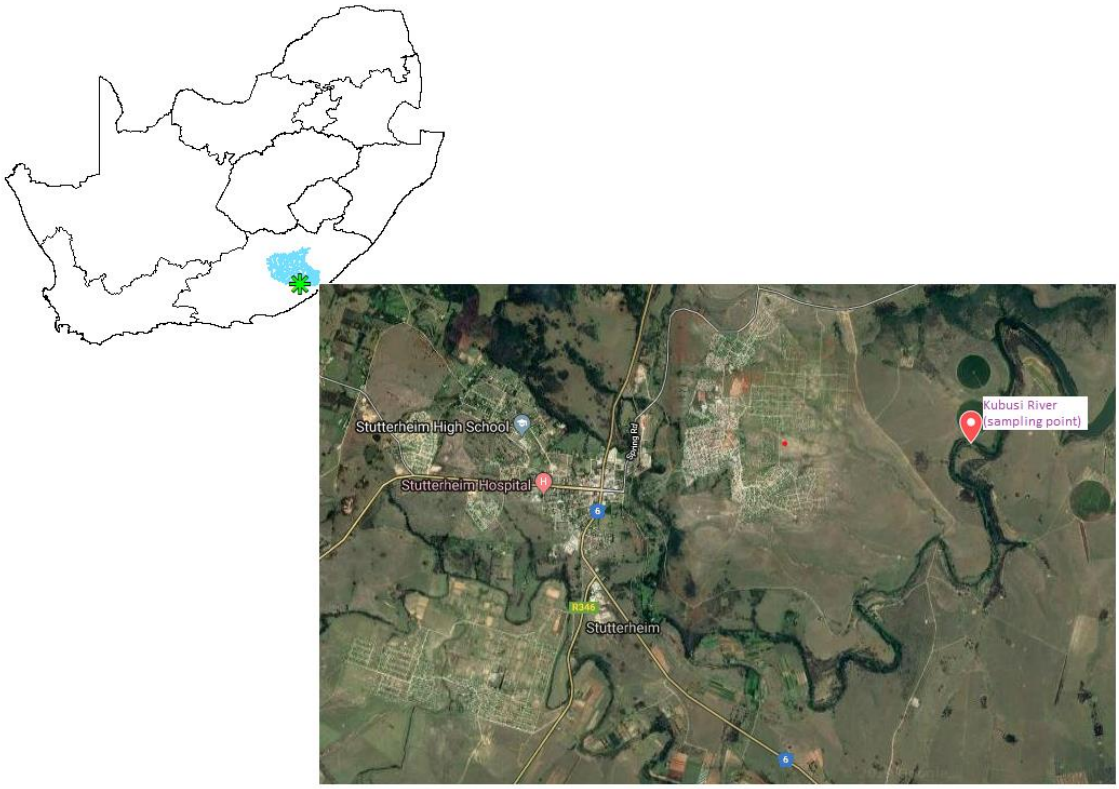

Figure 3.1: Location of the Kubusi River in the Eastern Cape. The river passes through the town of Stutterheim and feeds into the Wriggleswade Dam. The red pin represents the sampling site, while the blue patch on the full map represents the catchment area where Kubusi River is located.

3.2.2 Field data collection

Monthly field trips were conducted from May 2016 until April 2019. Each month, *P. crassipes* biomass ($kg.m^{-2}$) was recorded from three random quadrats (0.25 m^2) thrown into the mat, and the collected plants were separated into live emergent plant parts, live submerged plant parts and dead plant material, and thereafter weighed, for each individual quadrat. In each of the three quadrats, the number of individual plants was also recorded. Plant parameters from ten random plants were recorded (longest leaf, leaf area, maximum root length, number of ramets (daughter plants), number of flowers and number of leaves) as outlined in (Coetzee and Hill, 2012).

Percentage damage by *E. catarinensis* was recorded from leaf 4 of the same ten random plants. Leaf 4 was selected because leaf damage from mirid feeding is more pronounced in older leaves (Hill *et al.,* 1999), and because the water hyacinth plants at the site consistently had a minimum four leaves. On the same ten plants, the number of feeding scars inflicted by the weevils, *N. eichhorniae* and *N. bruchi* were counted from leaf 2 of the plants. Furthermore, the number of adult weevils present on each of the 10 plants was recorded, and the total number of adult *E. catarinensis* per square metre from 10 random quadrats on the river were recorded.

Megamelus scutellaris was sampled in a similar manner to *E. catarinensis*; however, a specialised sampler was used for *M. scutellaris* instead of using a quadrat. The sampler was constructed from an 80L dustbin with the base cut off, with a wire cross attached 30cm from the base, adapted from "the Minteer Method" (Minteer *et al.*, 2016; Lake and Minteer, 2018). The sampler was used to completely submerge plants *in situ*, forcing *M. scutellaris* to float to the water surface and onto the sides of the bin, allowing quantification. Only insects floating on the water surface and on the sides of the sampler were counted. Because climate is a major determinant of insect establishment, temperature and rainfall data were obtained from the South African Weather Services (South African Weather Services, 2019). These data were obtained for the whole study period, from May 2016 until April 2019.

3.2.3 Statistical analyses

Data were analysed in R 3.6.0 and RStudio 1.2.1335-1 (R Core Team, 2019). Linear and non-linear models explored the relationship between plant and insect parameters in response to minimum temperatures. Emphasis was placed on minimum temperatures in the analyses because the study site is known to be a cold continuous monitoring site, and the effect of cold temperature on population dynamics was the focus of this study. Generalized Least Squares

(GLS) models with repeated measures were used to explore the most influential factors that affected insect populations at the site. The GLS models (Auto-regressive models with heterogenous variances) were used to account for the heterogeneity of variance found in the data, as well as to account for the temporal correlation of the data. Auto-correlation due to temporal dependence was explored using the Auto-Correlation Factor (ACF) plot with alpha = 0.05 in conjunction with the Durbin-Watson (DW) test of auto-correlation. The DW test statistic was less than 2, revealing positive auto-correlation in all the models tested. The successive difference contrasts (SDC) was applied for successive pairwise comparisons between seasons, thus revealing differences between each successive season. The "contr.sdif" function in R was applied to call the SDC parameters on the models. All data visualizations were produced in RStudio using ggplot, tidyverse and ggpubr packages (R Core Team, 2019) and the map was produced in ArcGIS 10.5.1.

3.3 Results

3.3.1 Climatic conditions on the Kubusi River

The climate of the study site varied seasonally from May 2016 (end of autumn) to April 2019 (mid-Autumn). In the 2016-2017 growing season, the peak rainfall was 88mm in February 2017, while the mean maximum temperature was 26.7°C recorded in March 2017 (Figure 3.2). In the 2017-2018 growing season, there was an increase in temperature from November 2017 and a peak was recorded in January 2018 (27.2°C), a month earlier than the peak in 2017, which was in February (Figure 3.2). Similarly, in 2019, the temperatures peaked in January (27.2°C), giving a constant annual trend. Overall, the temperatures peaked in the last two summer months over the three-year study period. During the winter seasons, mean minimum temperatures were 5.5 °C, and for two of the three years (2017 and 2019), the lowest temperatures were recorded in August. Precipitation followed similar trends where there were varied fluctuations in rainfall between the

warmer and cooler months of the year. In 2017, peak rainfall was received in October (125mm), while in 2018 and 2019 the peaks were reached in March (178mm) and April (89mm) respectively. The most rainfall was recorded in 2018 reaching over 178mm in March, the highest over the course of three years (Figure 3.2).

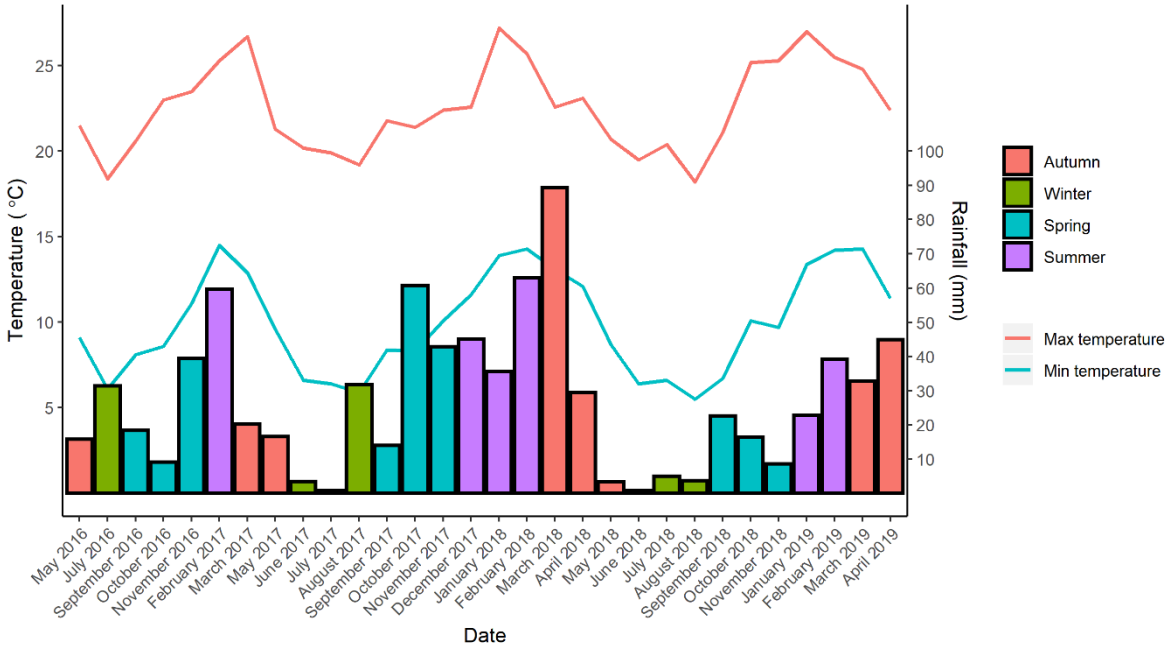

Figure 3.2: Climate data showing patterns of precipitation, maximum and minimum temperatures at Kubusi River from May 2016 to April 2019.

3.3.2 Plant biomass and populations of *Pontederia crassipes*

Plant growth fluctuated widely over the course of the three-year study period. Initially emergent plant wet biomass (6.6kg/ m^2 ±2.1) in 2016-2017 (Figure 3.3A), was less than the submerged plant wet biomass (15.46kg/ m^2 ±0.71) in the same season (Figure 3.3B). The biomass of submerged plant parts first peaked in January 2017, while biomass of the emergent plant parts weighed approximately less than half (Figure 3.3B and Figure 3.3A). Thereafter, the biomass of submerged plant parts steadily declined throughout 2017, while emergent parts remained constant, albeit low (Figure 3.3A and Figure 3.3B). The amount of dead material and emergent

plant parts was similar during the winter months of 2017 (7.66kg/ m^2 ±1.44), as well as during early summer in December 2017 (Figure 3.3C and Figure 3.3B).

The biomass of emergent plant parts was highest in January 2018 with a mean fresh biomass of 16.6kg/ m^2 ±2.24 (Figure 3.3A). Thereafter, biomass declined until the end of winter 2018 with a mean of 1.5kg/ m^2 ±0.38 by August 2018. The plants started to recover in October 2018 and exponential growth was realised until mid-Autumn in April 2019 with biomass averaging about 25.5kg/ m^2 ±1.98 (Figure 3.3A). Dead material increased at the same rate as the emergent plant parts, while there was slightly less submerged plant biomass (18.46kg/ m^2 ±2.78) by April 2019. At the end of the third year (October 2018-April 2019), there was an exponential increase in the fresh mass of the plants where biomass of emergent, submerged and dead material increased sharply (Figure 3.3). The increases and declines in plant biomass characteristically followed the temperature and rainfall patterns (Figure 3.2 and Figure 3.3).

Plant density declined during the winter of 2016 until mid-spring in October 2016, when the lowest plant density was recorded over the three-year study period (Figure 3.3D). Recovery was only recorded from November 2016, with plants reaching highest densities in March 2017 (64±2 plants/ m^2), and thereafter, steadily declined to fewer than 40±6 plants/ m^2 in October 2017, similar to the previous year where the lowest plant density (18±1 plants/ m^2) was also recorded in October (Figure 3.3). Thereafter, plant density increase in the summer of December 2017 led to another peak with 74±9 plants/ m^2 which was maintained at the same level until February 2018 (Figure 3.3D). Population decline ensued, beginning in March 2018 until a low of 29±4 plants/ m^2 was recorded in July 2018, with this trend persisting until September 2018 (Figure 3.3D). Recovery began in October 2018 with peak densities in November 2018 at 130±15 plants/ m^2, and this marked the highest density recorded in the study (Figure 3.3D). Plant populations

(Figure 3.3D) showed similar trends to those observed in plant biomass where peak populations were recorded in March 2017 (64±2 plants/ m^2), November 2018 (130±15 plants/ m^2) and April 2019. The plant biomass and density trends coincided with low water levels in the river as well as a narrow river channel, owing to low precipitation (Figure 3.2).

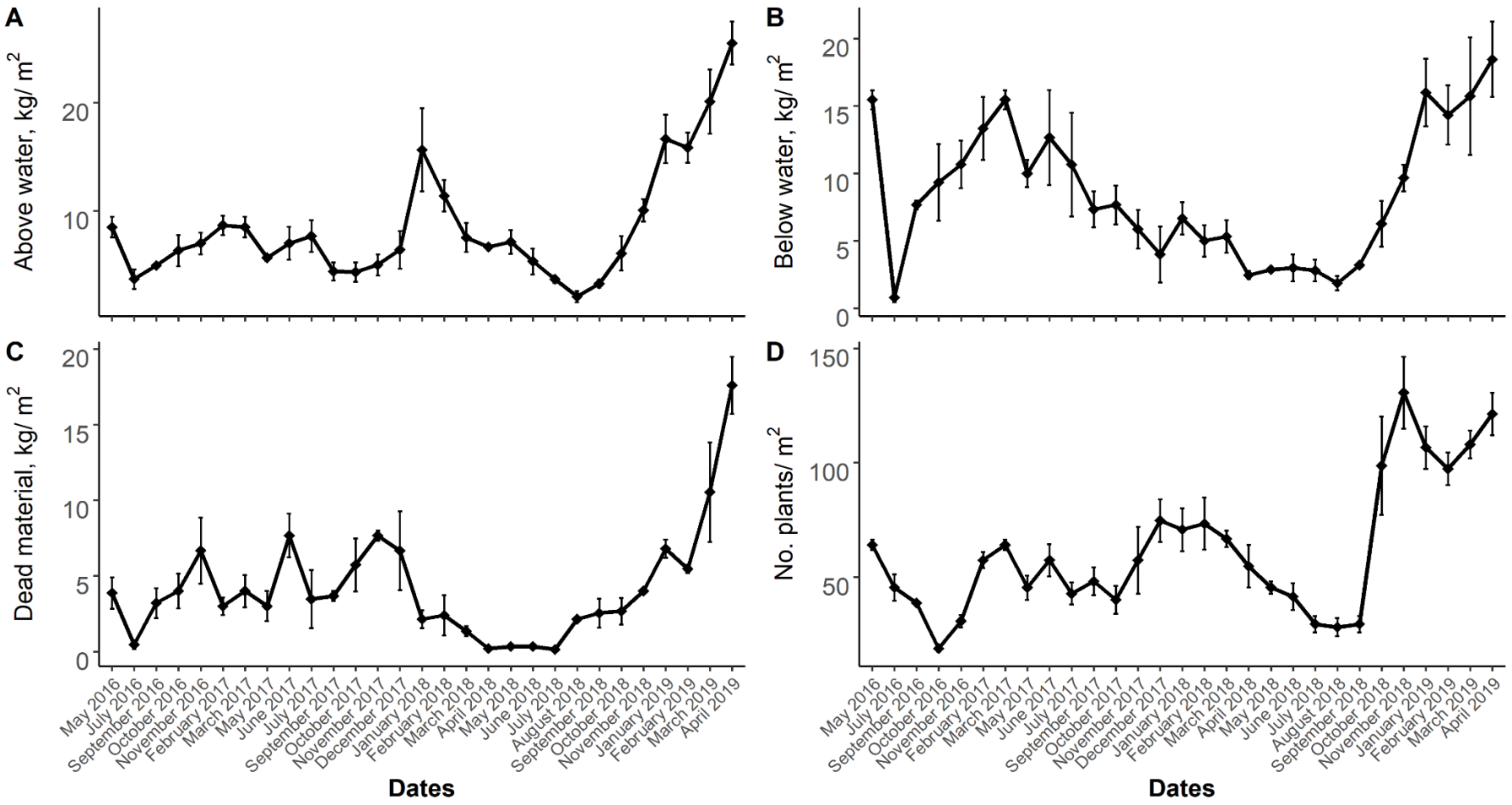

Figure 3.3: Fresh biomass of *Pontederia crassipes* (A-emergent parts; B-submerged parts; C-dead material) and plant density (D) from May 2016 to April 2017. Error bars represent standard error.

During winter, there was a significant and positive relationship between the emergent and submerged biomass ($y = 2.9+3.8x$, $R^2= 0.49$, $P < 0.005$), while in spring, there was an even stronger correlation between emergent and submerged biomass ($y = 1.6+0.55x$, $R^2= 0.57$, $P < 0.005$) (Figure 3.4). In summer and autumn, the relationships were not as strong, but remained significant (summer: $y = 7.4+0.51x$, $R^2 = 0.33$, $P < 0.05$; autumn: $y = 3.11+0.73x$, $R^2 = 0.36$, $P < 0.005$) (Figure 3.4). Less than 40% of variation was explained by the correlation between emergent and submerged biomass during autumn, even though the highest biomass was recorded in autumn. In all the seasons, there was an increase in emergent biomass with increase in submerged biomass, presenting a positive correlation between the two. Biomass of emergent and submerged plant parts was positively correlated when all seasons were combined (Figure 3.5). Total plant biomass was positively correlated with mean minimum temperature. ($y = -0.21+2.2x$, $F_{1, 85}=25.31$, $R^2= 0.23$, $P < 0.001$).

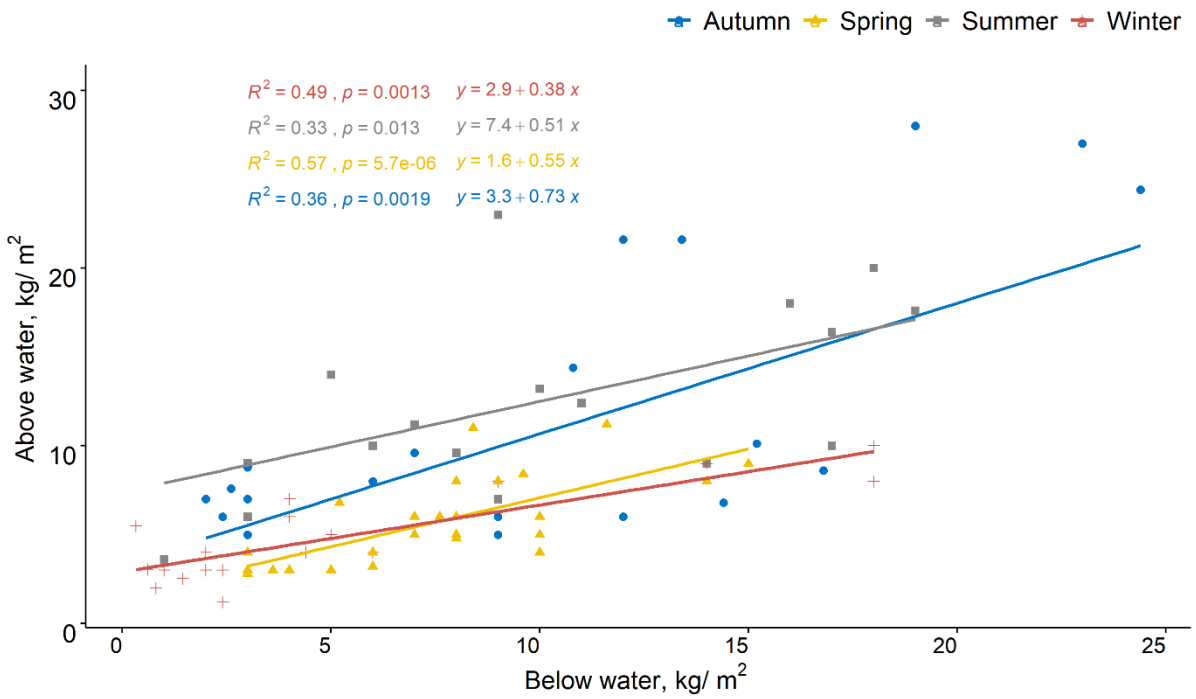

Figure 3.4: Relationship between the wet biomass of *Pontederia crassipes* emergent plant parts (above water) and submerged (below water) across seasons.

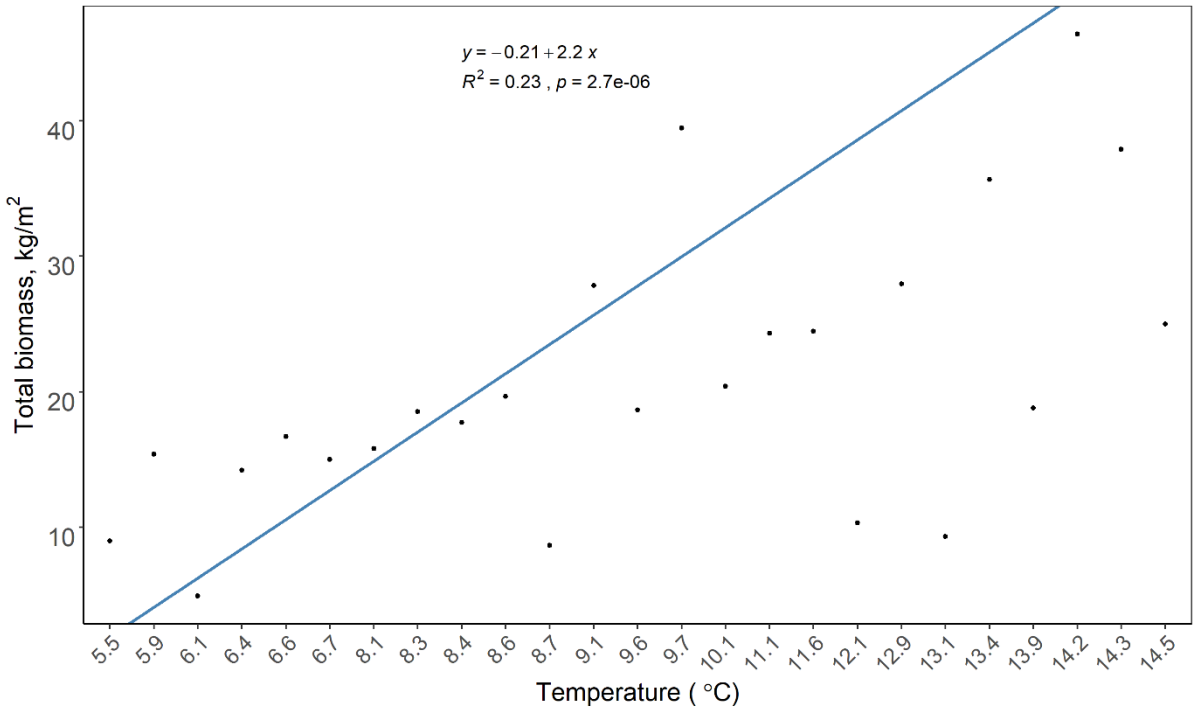

Figure 3.5: Relationship between total plant biomass accumulation and minimum temperatures on the Kubusi River, n=26.

Plant density increased with mean minimum temperature, ranging from fewer than 20 plants at 8°C to more than 100 plants / m^2 at 14°C (Figure 3.6). The relationship was weak, but significant (y = 16+4.7x, R^2=0.19, $P < 0.001$). Although minimum temperatures had a significant effect on plant populations, it accounted for less than 20% of the variation in the model.

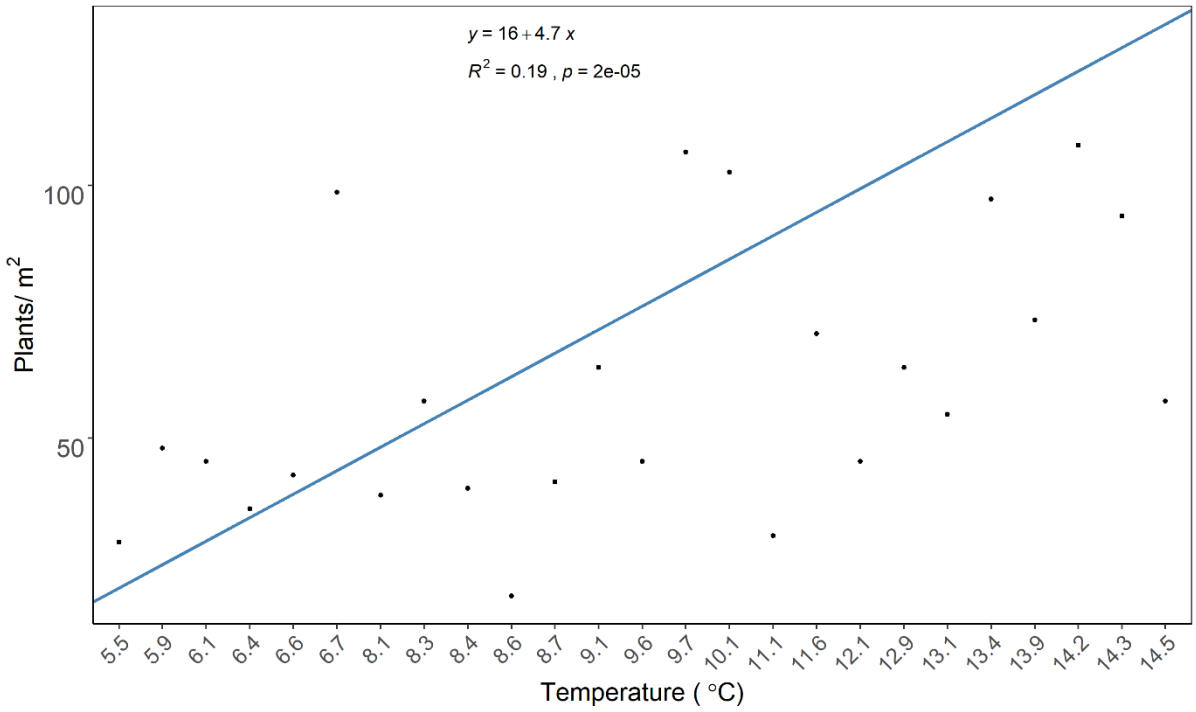

Figure 3.6: Relationship between plant population and minimum temperatures, n=26.

3.3.3 Plant growth parameters across seasons

At the beginning of the study, petiole length declined from May to July (from 44±0.35 cm to 10±0.23cm) (Figure 3.7A). In the spring of 2016 (September), plants had short leaf petioles averaging 13.8 ± 1.29 cm in length (Figure 3.7A). Leaf length increased in November until December when growth tapered off and was constant, averaging 30.4±2.79cm, until July 2017 when there was high leaf mortality as the temperatures dropped (Figure 3.7A). Leaf petiole length declined from August 2017 until recovery was observed from December 2017, with plant height peaking in January 2018 with average petiole length of 50.8±2.87 cm (Figure 3.7A). Another decline was observed from March 2018, followed by recovery in October 2018, and rapid growth was observed until January 2019 where plant height averaged 79.4±1.79 cm, the highest recorded over the three-year study (Figure 3.7A). Furthermore, the longest leaf petioles over the three-year study aligned with the greatest biomass of the emergent plant parts (live

leaves and leaf petioles) (Figure 3.7A and Figure 3.3A). Plant growth tapered off with similar petiole lengths measured in March and April 2019, 78.2±1.59 cm (Figure 3.7A).

Root length declined at the beginning of the study (May 2016 to July 2016), similar to the trend observed with the leaf petioles (Figure 3.7B). However, at the break of spring in September, there was a steady increase with slight fluctuations, until March 2017 where an initial peak of 57.2±2.95 cm was recorded. The longest root length in 2017 was recorded in December with an average of 105.28±8.78 cm (Figure 3.7B). Thereafter, root length declined and remained low from January 2018 until September 2018. However, the longest root length recorded was in the summer of December 2017, the longest root measures recorded across all summer seasons, while in the summer of January 2019 plants had the shortest roots (69.9±1.9 cm) (Figure 3.7B) The shorter roots in January 2019 were because the plants shed off the previous season's roots. Interestingly, the longest root length coincided with lowest root mass in December 2017 (Figure 3.7B and Figure 3.3B).

Ramet production was highest in the spring of 2016, during September and October (Figure 3.7C). This was the highest production of ramets (9.6±0.95 ramets) produced in any season throughout the course of the study. Ramet production decreased considerably in March 2017 (0.1±0.1 ramets per plant) until September 2017, followed by an increase in October with an average of 1.5±0.15 ramets per plant (Figure 3.7C). Another increase in ramet production was recorded in April 2018, 3.16±0.29 ramets per plant, which was the second highest ramet production over three years (Figure 3.7C).

Leaf production was initially high (8.7±0.47 leaves per plant) at the beginning of the study between May and July 2016, which was confounding in that it was during winter months where

the minimum temperature recorded in July was 6.1°C, (Figure 3.7D and Figure 3.2). Normally, in winter there is dieback and senescence of the emergent plant parts, therefore it was unexpected that there would be high leaf production. Leaf production remained high until May 2017, followed by a rapid decline, which was the lowest in three years of the study, characteristic of winter between June (0.3±0.15 leaves per plant) and August 2017 (Figure 3.7D). Steady increase in leaf production was observed from September 2017, peaking in February 2018 (7.5±0.52 leaves per plant) (Figure 3.7D), thereafter leaf production remained at a constant 6.4±0.37 leaves per plant from March to May 2018. Again, as winter set in, leaf production declined until recovery was recorded in September 2018, where production increased and peaked in February 2019 (9.0±0.49 leaves per plant), similar to the previous two years which also had peaks in February (Figure 3.7D).

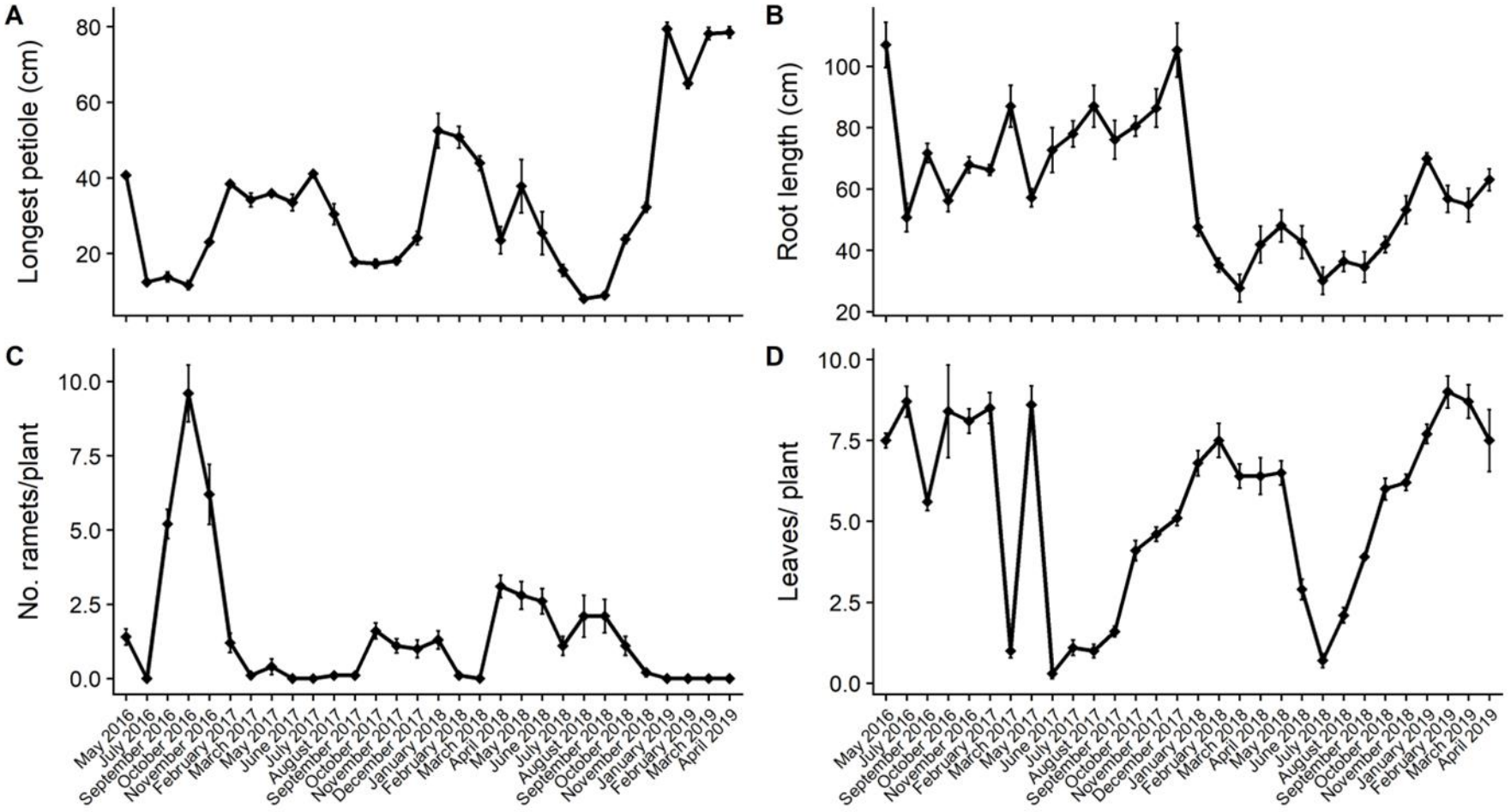

Figure 3.7: Plant parameters over three years; A- average longest leaf petiole; B- average root length; C- mean number of ramets per plant; D- mean number of leaves produced per plant. Error bars represent standard error.

There was a weak relationship between the number of leaves produced by plants and the minimum temperatures ($y = 33+11x-0.99x^2 +0.031x^3$). Increase in the minimum temperature did not translate into increased leaf production as there is a finite number leaves produced by each plant (Figure 3.8).

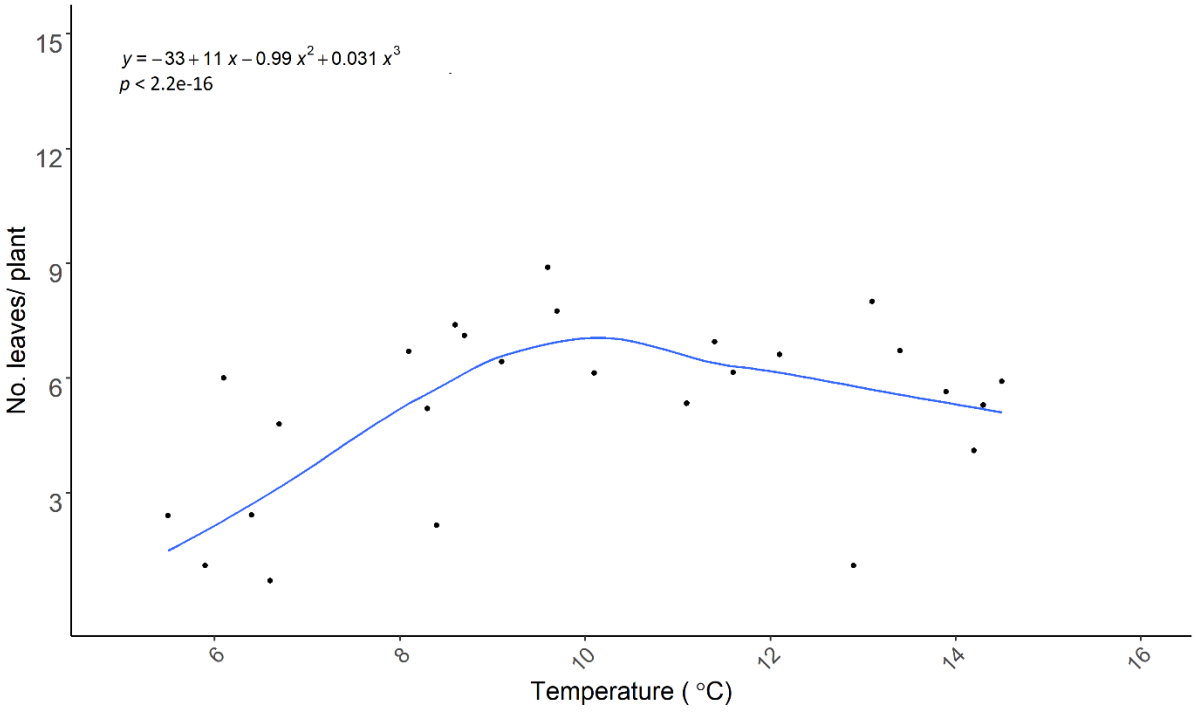

Figure 3.8: The relationship between leaf production and minimum temperatures, n=26.

3.4.1 Population patterns of *Neochetina eichhorniae* and *Neochetina bruchi*

Because establishment of biocontrol agent populations is largely regulated by climatic conditions in the introduced zones; populations of the agents found on *P. crassipes* on the Kubusi River were expected to be governed by the prevailing seasonal climatic conditions. The weevil populations closely followed plant populations initially with about one-month lag phase after the spring recovery of the plants (Figure 3.9). After the winter of 2016, plants started to recover in November 2016 while weevil populations remained low until February 2017, and this represented a lag phase of 3 months before weevil populations started to recover (Figure 3.9). Plant populations reached their peak in March 2017, while weevil populations peaked in June of

the same year, again, still 3 months behind the plant population (Figure 3.9). Mean plant density was 74±9 plants/ m^2 while *N. eichhorniae* and *N. bruchi* averaged 1±0.5 and 1.4±0.7 weevils per plant respectively (Figure 3.9). Weevils reached their highest populations in January 2018 and thereafter declined with the decline in plant populations until September 2018 when both the plants and weevils were at their lowest. The plants then recovered rapidly where the highest populations were achieved within two months of the start of spring, reaching the highest density in November 2018 (130±15 plants/ m^2). Considering this rapid plant population recovery, weevil populations remained low with *N. eichhorniae* only reaching 2±0.7 insects/ plant, *N. bruchi* was not recorded for the rest of the study period (Figure 3.9). The patterns of the weevils and plant density revealed that initially there was a long lag phase between plant recovery and weevil population build up (3 months) in 2017 and 2018, however this was reduced to one month in 2019 (Figure 3.9). It is worthy to note that although the lag phase between the plants and weevils was reduced in 2019, the weevil populations declined with each successive year on the Kubusi River (Figure 3.9). In each season, there was no relationship between weevil population and temperature (R^2=0.024, $P < 0.001$). The relationship between *N. eichhorniae* and plant density was weak, however it was positive and significant (DF = 298, $R^2 = 0.33$, $P < 0.0001$). With an increase in plant density, there was a corresponding increase in the population of *N. eichhorniae* (Figure 3.9).

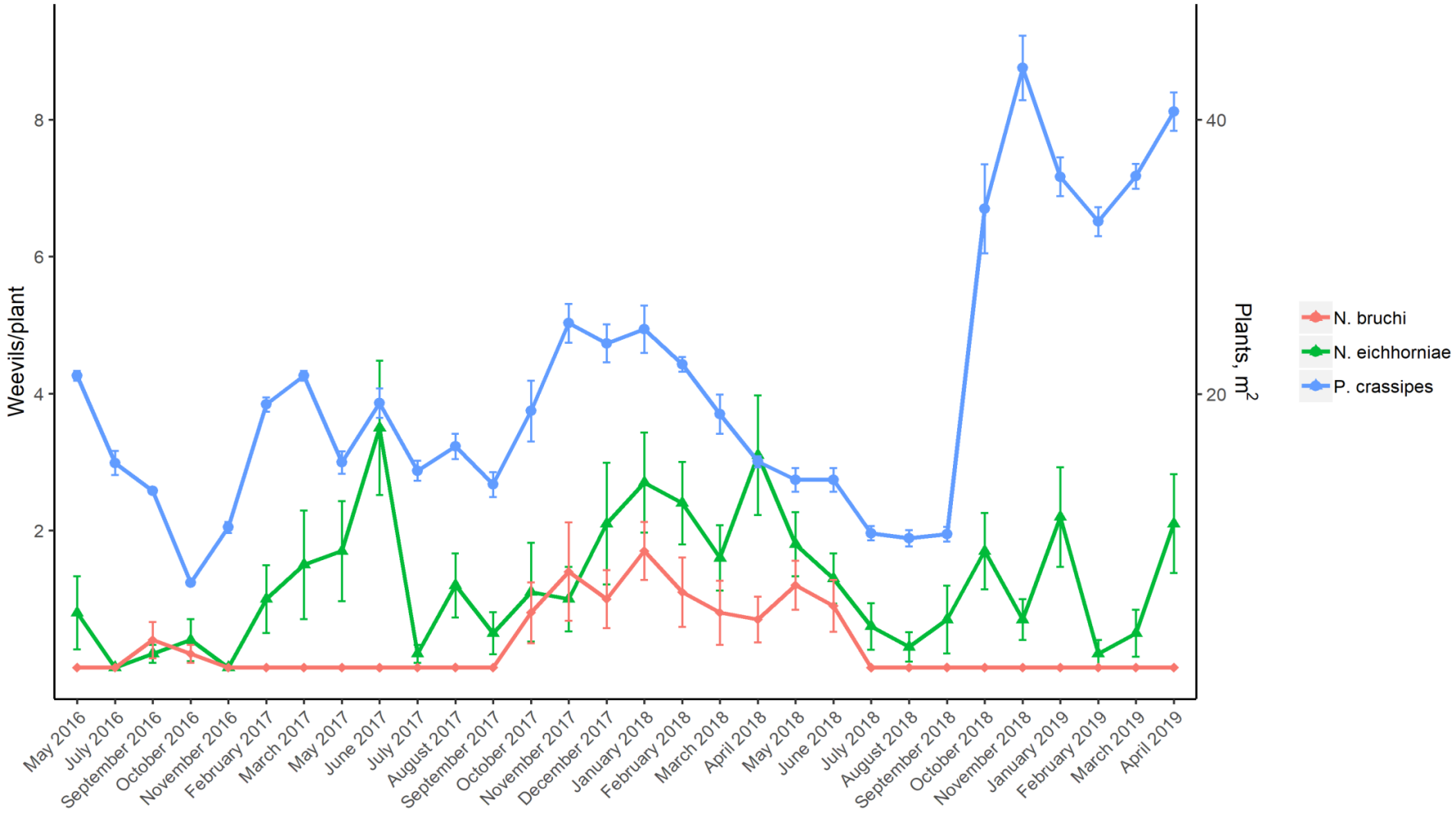

Figure 3.9: Patterns of the two weevils, *Neochetina eichhorniae* and *Neochetina bruchi* following plant population patterns.

3.4.2 Weevil feeding patterns

In the winter of 2016, there was no weevil feeding due to leaf frosting and die back. Active insect feeding by both the weevils was recorded in late spring (November 2016) and was sustained until late autumn (May 2017) (Figure 3.10). The weevil feeding damage for the 2016-2017 growing season peaked in February 2017 and was sustained at the same level until March 2017 (92.6±27.1 scars per leaf). A decline in feeding was recorded from late autumn in May 2017 (77.4±14.8 scars per leaf), and finally, no feeding was recorded at the beginning of winter in June 2017 (Figure 3.10). Once again, there was no visible feeding damage throughout winter (June to August) until early spring in September 2017 (Figure 3.10). Weevil feeding was then recorded in October 2017 (14.9±1.9 scars per leaf) and rose steadily until a peak in March 2018 (121±14.1), which was sustained until May 2018 (111.8±11.7 scars per leaf) (Figure 3.10). Thereafter, feeding declined from June 2018, again due to frosting of leaves, with no feeding occurring in July and August of the same year (Figure 3.10). Negligible feeding was observed at the break of spring in September 2018, with 12.5±5.4 scars per leaf, but this increased until a peak in feeding damage was reached in March 2019 (129.8±14.7 scars per leaf), similar to the previous year (Figure 3.10). There was a sigmoidal relationship between weevil feeding and minimum temperature ($y = 300\text{-}110x+13x^2\text{-}0.42x^3$, $P < 0.0001$). At temperatures below 9°C, there was suppressed feeding by the weevils, while feeding damage increased with increase in the minimum temperatures (Figure 3.11). Most of the feeding damage was inflicted at temperatures above 11.5°C.

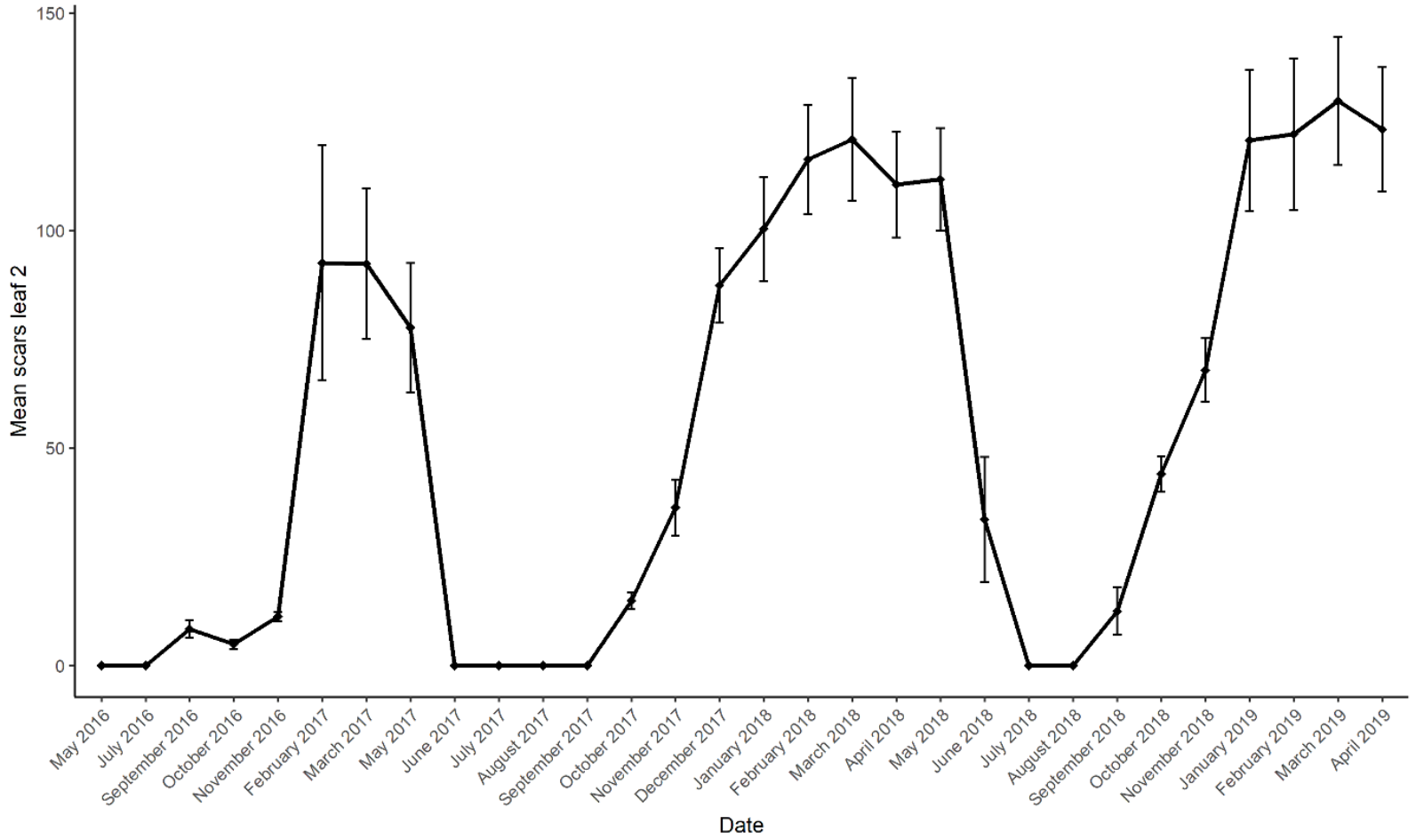

Figure 3.10: Patterns of weevil feeding damage on leaf 2 over three years. Error bars represent standard error.

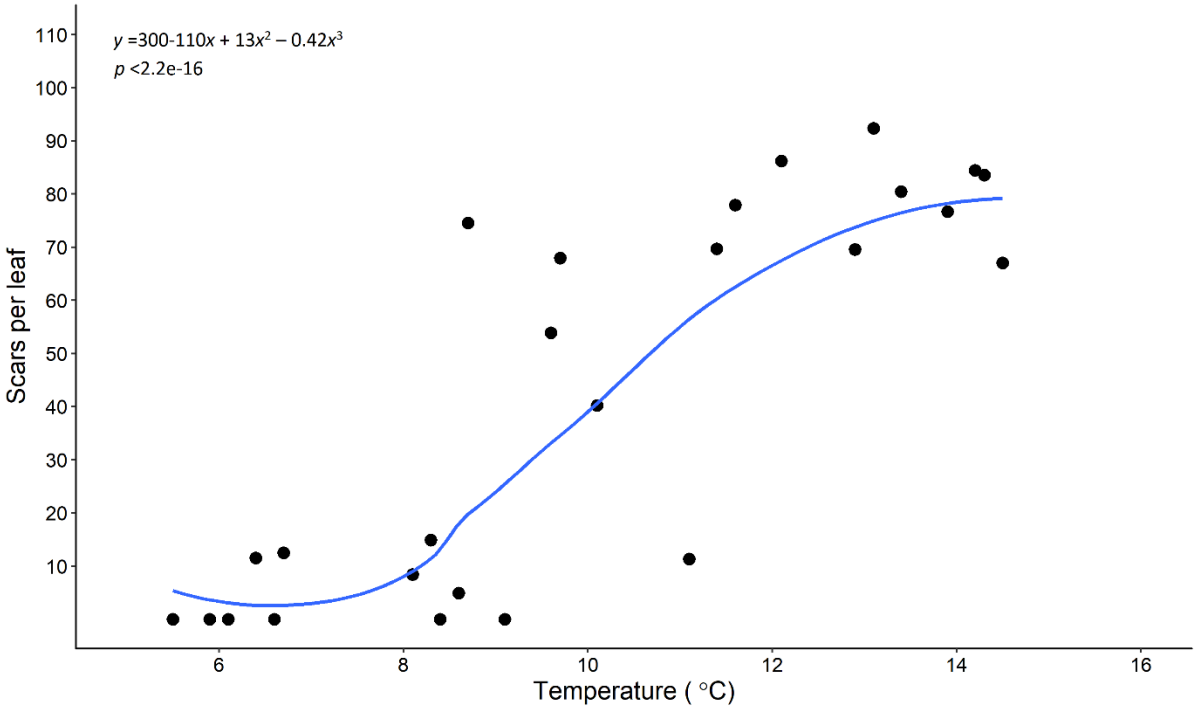

Figure 3.11: Relationship between weevil feeding and minimum temperatures on the Kubusi River, n = 26.

3.4.3 Effect of seasonality and abiotic factors on weevil populations

Seasonality had a strong effect on the populations of the four biocontrol agents. Both season and the interaction of season and minimum temperature significantly affected *N. eichhorniae* (Table 3.1). The low temperatures experienced during the winter season led to significantly low adult weevil numbers at the site (Figure 3.12). Plant parameters did not significantly influence the abundance of *N. eichhorniae* (Table 3.1).

Table 3.1: Generalised Least Squares model summary on *Neochetina eichhorniae* as affected by season (Sn) and various plant parameters; number of leaves (Leaves), root length (Roots), number of ramets (Ramets) and length of longest petiole (Longest). Values in bold are significant.

	Value	**Std Error**	**t-value**	***P*-value**
(Intercept)	-4.382043	10.013789	0.4376009	0.6620
Longest	0.000278	0.009625	0.0288576	0.9770
Leaves	0.087692	0.052525	-1.6695502	0.0961
Roots	0.001709	0.005843	0.2924043	0.7702
Ramets	0.009971	0.057858	0.1723314	0.8633
Rainfall	0.002763	0.003845	0.7187773	0.4729
TempMin	0.151547	0.141317	-1.0723916	0.2844
Dates	0.000471	0.000572	0.8231241	0.4111
SeasonSpring	-3.625062	2.260428	-1.6037062	0.1099
SeasonSummer	1.380786	4.414936	0.3127534	0.7547
SeasonWinter	**-10.232341**	**4.498034**	**-2.2748477**	**0.0237**
TempMin:SeasonSpring	0.257076	0.223050	1.1525481	0.2501
TempMin:SeasonSummer	0.068089	0.328745	0.2071191	0.8361
TempMin:SeasonWinter	**1.385800**	**0.692098**	**2.0023188**	**0.0462**
Phi	0.107868			
Residual Standard Error	2.103849			
Degrees of freedom	300			
Residual DF	285			
AIC	1289.3			
Log-Likelihood	-624.65			

Neochetina bruchi was not affected by season, nor did any environmental parameter influence the population of these weevils. However, there was a nearly significant effect of rainfall on the abundance of *N. bruchi* (Table 3.2). Plant parameters also did not influence populations of *N. bruchi* (Table 3.2).

Table 3.2: Generalized Least Squares summary of the effects of season, leaf number (leaves), root length (Roots) and length of longest petiole (Longest) on *Neochetina bruchi* abundance.

	Value	**Std.Error**	**t-value**	**p-value**
(Intercept)	-19.826491	16.746129	-1.1839447	0.2374
Longest	0.007065	0.004579	-1.5427989	0.1240
Leaves	0.014077	0.025753	0.5465920	0.5851
Rainfall	0.004076	0.002195	1.8570194	0.0643
TempMin	1.379947	1.838338	0.7506493	0.4535
Dates	0.001146	0.000952	1.2043812	0.2294
SeasonSpring-Autumn	-1.584563	1.456765	-1.0877276	0.2776
SeasonSummer-Spring	2.699375	3.291782	0.8200346	0.4129
SeasonWinter-Summer	-5.078819	3.543151	-1.4334189	0.1528
TempMin:Dates	0.000075	0.000105	0.7135786	0.4761
TempMin:SeasonSpring-Autumn	0.141476	0.145258	0.9739667	0.3309
TempMin:SeasonSummer-Spring	0.190620	0.254948	0.7476826	0.4553
TempMin:SeasonWinter-Summer	0.592660	0.349600	1.6952503	0.0911
Phi	0.335133			
Residual Standard Error	0.837854			
Degrees of freedom	300			
Residual DF	285			
AIC	848.5036			
Log-Likelihood	-404.2518			

3.4.4 *Eccritotarsus catarinensis* populations

The population of *E. catarinensis* was characterised by three peaks throughout the duration of the study (Figure 3.12). In 2016, populations remained low, with population recovery recorded in November 2016, and the first peak in February 2017 (58.4±23.6 insects/ m^2) (Figure 3.12). This population peak coincided with the mean temperature peak (19.9°C) of that same year (Figure 3.2). Populations started to decline at the beginning of autumn, in March 2017, and by mid-winter, populations had declined to zero. No insects were recorded from July 2017 until May 2018 when the next population peak was recorded (64±15 insects/ m^2), at the end of autumn, which meant that the populations would soon crash again as with the previous winter (Figure 3.12).

With warmer temperatures recorded in November 2016 leading up to the temperature peak in February 2017 (Figure 3.2), there was a rapid increase in the population of *E. catarinensis* which recovered faster than the plants and reached peak population in February 2017 (58.4±23.6 insects/ m^2) while plants reached their peak in March 2017 (74±9 plants/ m^2). At the point where the plants reached their peak, *E. catarinensis* was already in rapid decline, while the plants declined steadily (Figure 3.12). By July 2017 (mid-winter), *E. catarinensis* was virtually absent at the site until it was again recorded 10 months later in May 2018, the end of the autumn season (Figure 3.12). This sudden population boom of *E. catarinensis* appeared six months after plants reached peak population, a less than ideal for biocontrol. The rapid population increase so late in the season was followed by a rapid crash yet again at the onset of winter, within two months of the peak (Figure 3.12). After the winter of 2018, plants again recovered faster in October 2018; reaching their peak in November 2018, while *E. catarinensis* only appeared three months later, another long lag phase (Figure 3.12). There was no relationship between increase in minimum temperatures and increase in populations of *E. catarinensis* (R^2 =0.046, DF = 298, $P < 0.001$)

(Figure 3.13). The patterns of the population of *E. catarinensis* were quite sporadic when compared to those of the weevils throughout the course of the study as *E. catarinensis* did not maintain a consistent pattern of recovery with plant patterns (Figure 3.12 and Figure 3.9).

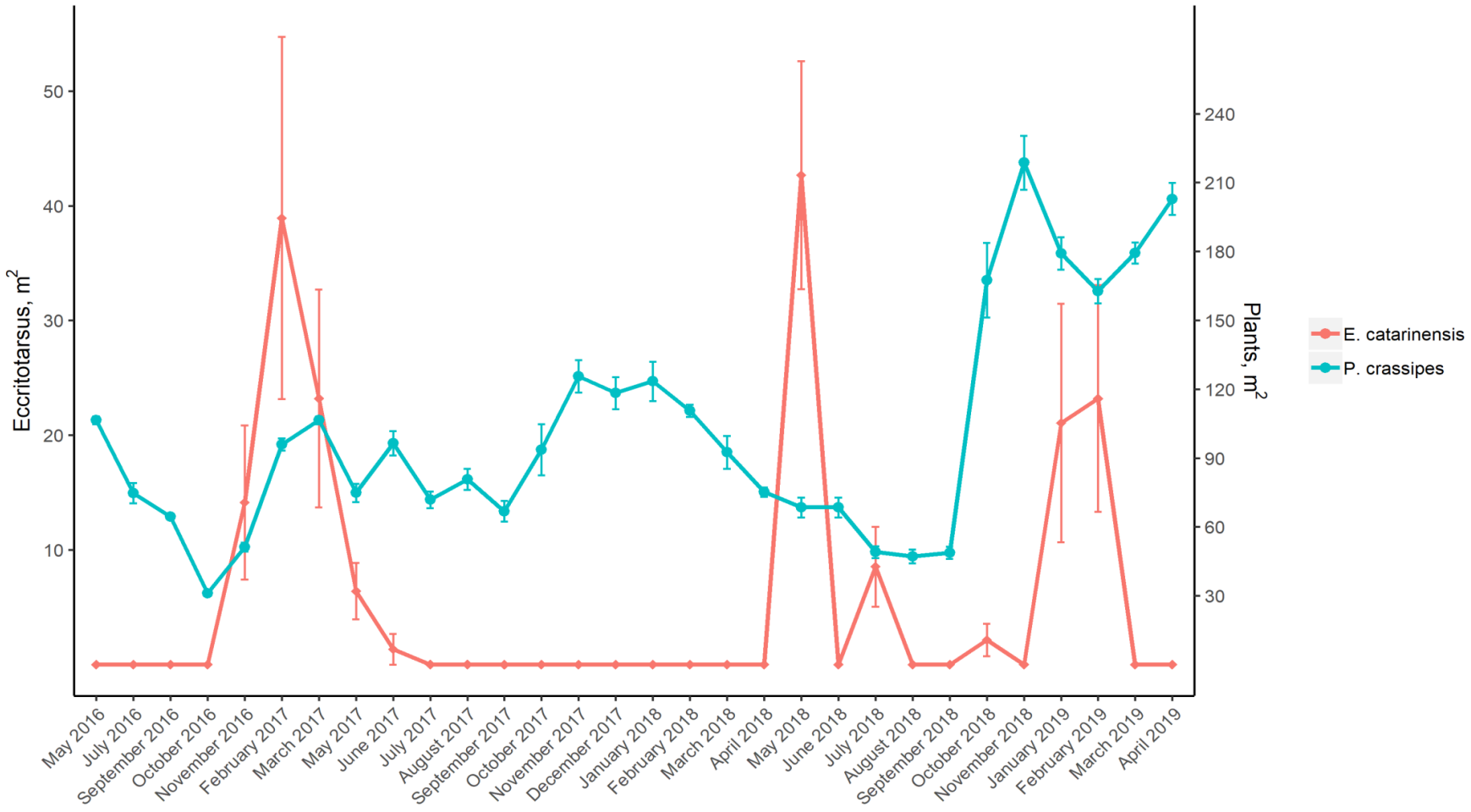

Figure 3.12: Population patterns of *Eccritotarsus catarinensis* and *Pontederia crassipes*. Error bars represent standard error.

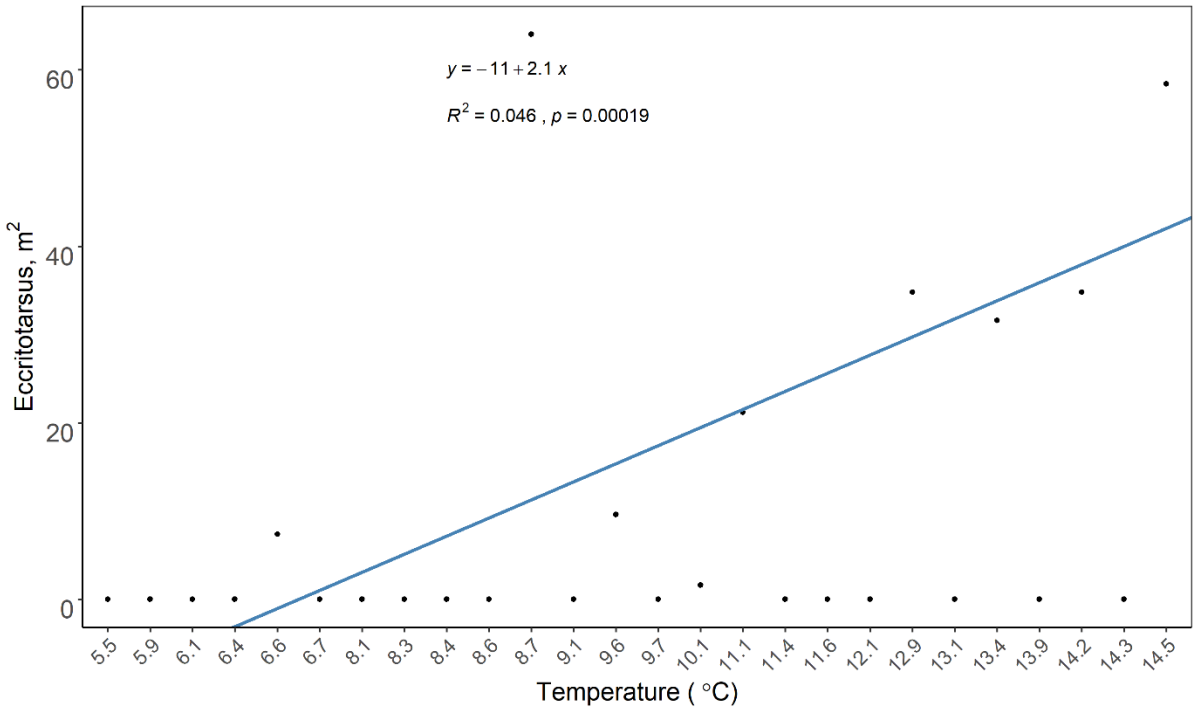

Figure 3.13: Population response of *Eccritotarsus catarinensis* to minimum temperatures, n =28.

3.4.5 Feeding patterns of *Eccritotarsus catarinensis*

At the beginning of the study in May 2016, no feeding damage by the mirid was evident until October 2016. In November 2016, there was a rapid increase in feeding which consequently resulted in the highest feeding damage (25±5.7 %) recorded in the 2016-2017 growing season (Figure 3.14). This peak in mirid damage was reached 3 months earlier than peak weevil feeding (Figure 3.14 and Figure 3.10), and thereafter there was a gradual decline in feeding by *E. catarinensis* until May 2017, where 12.5±5.6 % leaf damage was recorded (Figure 3.14). A sudden population crash of *E. catarinensis* occurred due to leaf frosting, resulting in the absence of feeding damage throughout winter, and into spring and the following summer (Figure 3.14). *Eccritotarsus catarinensis* only briefly recovered in autumn of 2018 (April and May) when feeding damage peaked at just 14.2±5.6 % leaf damage (Figure 3.14). After the brief two-month appearance of *E. catarinensis*, the population again crashed due to the cold temperatures and lack of food after the leaves of *P. crassipes* frosted over. *Eccritotarsus catarinensis* again remained

absent through the winter of 2018, with the population recovering in mid-summer, February 2019, resulting in peak feeding damage (15±5.8 % leaf damage) recorded for the 2018-2019 growing season (Figure 3.14). Overall, *E. catarinensis* persisted for shorter durations compared to the weevils, and hence inflicted damage for only short durations each growing season (Figure 3.14 and Figure 3.10). The amount of feeding damage declined from the first growing season (2016-2017) compared to the last growing season (2018-2019) (Figure 3.14). Insect herbivores are significantly affected by temperature, and feeding may stop below certain temperature thresholds. There was no relationship between feeding by *E. catarinensis* and the minimum temperature (y = -6.5+x, R^2 = 0.098, P <0.0001) (Figure 3.15). The best fit line was fitted using the intercept and slope obtained from a robust fitting linear model to minimize the effects of the zero inflated data (Figure 3.15). Feeding damage was variable with little or no feeding even as minimum temperatures increased (Figure 3.15).

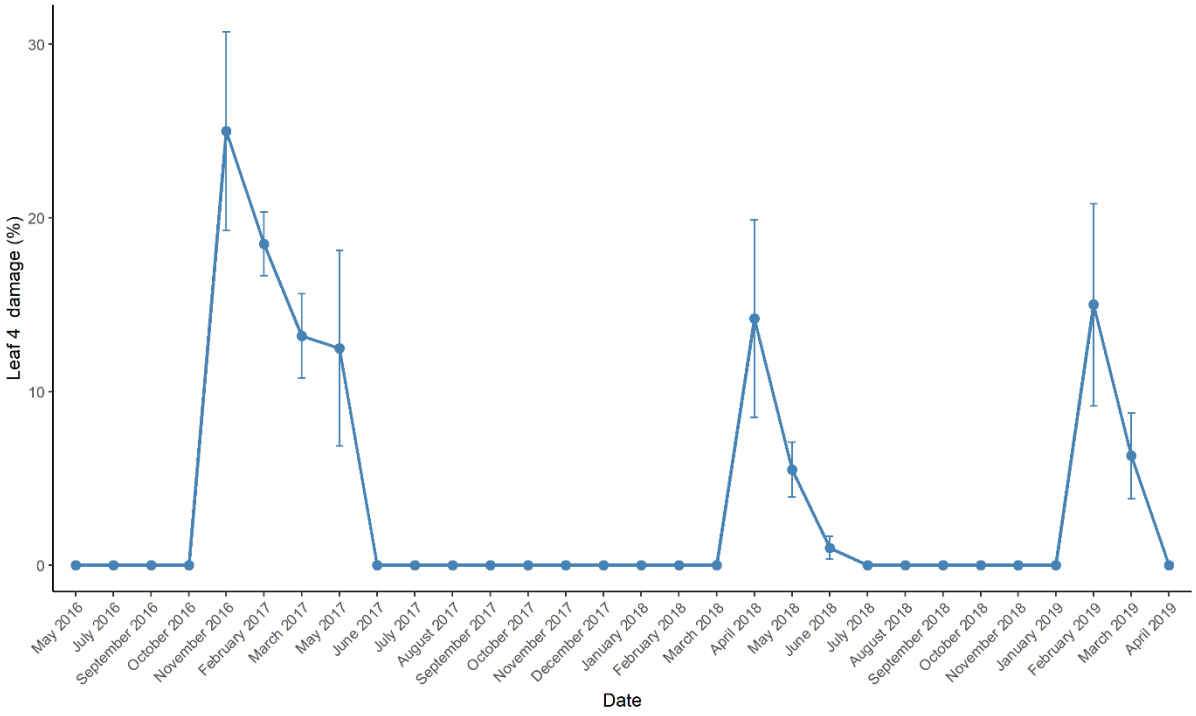

Figure 3.14: Feeding damage on unfurled leaf 4 by *Eccritotarsus catarinensis* over three years on the Kubusi River. Error bars denote standard error.

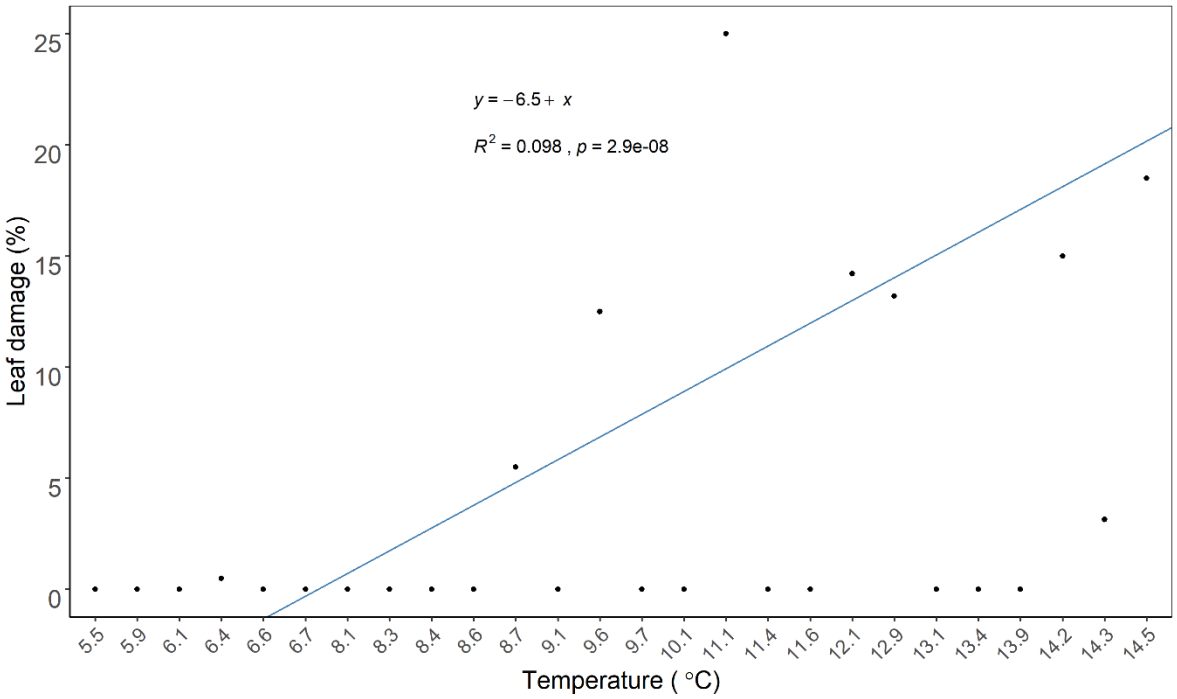

Figure 3.15: The relationship between *Eccritotarsus catarinensis* feeding and minimum temperature, n = 26.

3.4.6 Influence of season and abiotic factors on *Eccritotarsus catarinensis*

Eccritotarsus catarinensis was significantly influenced by minimum temperature, as well as by season and the interaction of season and minimum temperature (Table 3.3). The interaction of season and minimum temperature informed the breeding and feeding cycles of *E. catarinensis* (Table 3.3). *Eccritotarsus catarinensis* was absent during winter seasons due to restricted resource availability when water hyacinth leaves had frosted over (Figure 3.16A). *Eccritotarsus catarinensis* populations were low during spring, except for the spring of 2017 (Figure 3.16A).

Table 3.3: Generalized Least Squares summary on the influence of season, leaf number (Leaves), root length (Roots) and length of longest petiole (Longest) on populations of *Eccritotarsus catarinensis*. Values in bold are significant.

	Value	**Std.Error**	**t-value**	**p-value**
(Intercept)	**-738.6696**	**332.7144**	**-2.2201308**	**0.0272**
Longest	0.0380	0.0904	0.4206759	0.6743
Leaves	0.3824	0.4614	0.8288497	0.4079
Roots	0.0087	0.0385	0.2270841	0.8205
Ramets	0.7396	0.4132	1.7898941	0.0745
Rainfall	0.0335	0.0392	0.8543316	0.3936
TempMin	**85.7819**	**40.0430**	**2.1422439**	**0.0330**
Dates	**0.0450**	**0.0185**	**2.4361792**	**0.0155**
SeasonSpring	**-82.4164**	**33.3368**	**-2.4722368**	**0.0140**
SeasonSummer	-190.3037	126.6798	-1.5022416	0.1341
SeasonWinter	**-113.8902**	**39.1120**	**-2.9118972**	**0.0039**
TempMin:Dates	**0.0051**	**0.0022**	**-2.2648482**	**0.0243**
TempMin:SeasonSpring	**6.6434**	**3.0158**	**2.2029088**	**0.0284**
TempMin:SeasonSummer	15.1040	9.3618	1.6133635	0.1078
TempMin:SeasonWinter	**13.8246**	**4.7897**	**2.8863046**	**0.0042**
Phi				
Residual Standard Error	30.40749			
Degrees of freedom	300			
Residual DF	285			
AIC	2574.085			
Log-Likelihood	-1267.043			

3.4.7 Population dynamics of *Megamelus scutellaris*

The monitoring of *M. scutellaris* began in June 2017, the beginning of the winter season. Plant populations were also in decline at this stage, while *M. scutellaris* was not recorded until March 2018. *Megamelus scutellaris* was absent from the site from June 2017 until March 2018, when an average 63.2±8.4 insects/ m^2 were recorded (Figure 3.16). The population of *M. scutellaris* persisted until June 2018, when plants were already in decline (Figure 3.16). The population peaked in April 2018 with an average 225.2±41.6 insects/ m^2 (Figure 3.16). After the initial population recovery, there was again a decline from June 2018 due to the crash in plant population during the winter of 2018. In the early summer of 2018, the *M. scutellaris* population recovered, reaching a peak in January 2019 (638±62 insects/ m^2). Thereafter a small decline between February and March 2019 was recorded, with a final peak in April 2019, the highest population peak recorded over the course of the study (1861±212 insects/ m^2) (Figure 3.16). An outlier was recorded following a sudden population explosion of *M. scutellaris* in April 2019. To account for the effect of the outlier, a "robust fitting linear model" (rlm) was used instead of a "linear model". The coefficients obtained from the rlm were used to produce the line of best fit in the linear regression. Similar to *E. catarinensis*, there was a very weak, positive relationship between populations of *M. scutellaris* and increase in the minimum temperatures where $y = -290+47x$, $R^2 = 0.1$, $P < 0.001$ (Figure 3.17 and Figure 3.15).

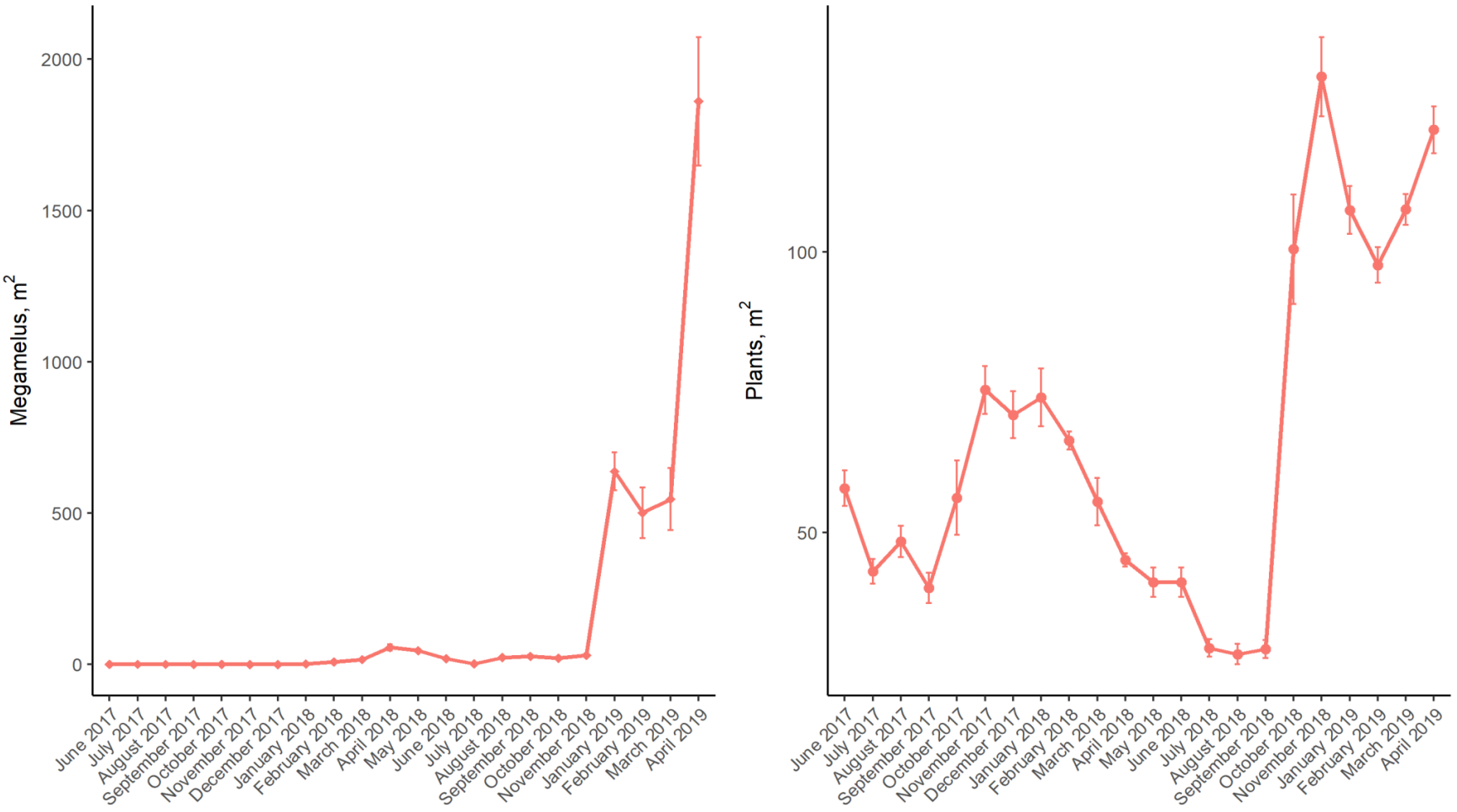

Figure 3.16: Patterns of *Megamelus scutellaris* and *Pontederia crassipes* on the Kubusi River.

Error bars represent standard error.

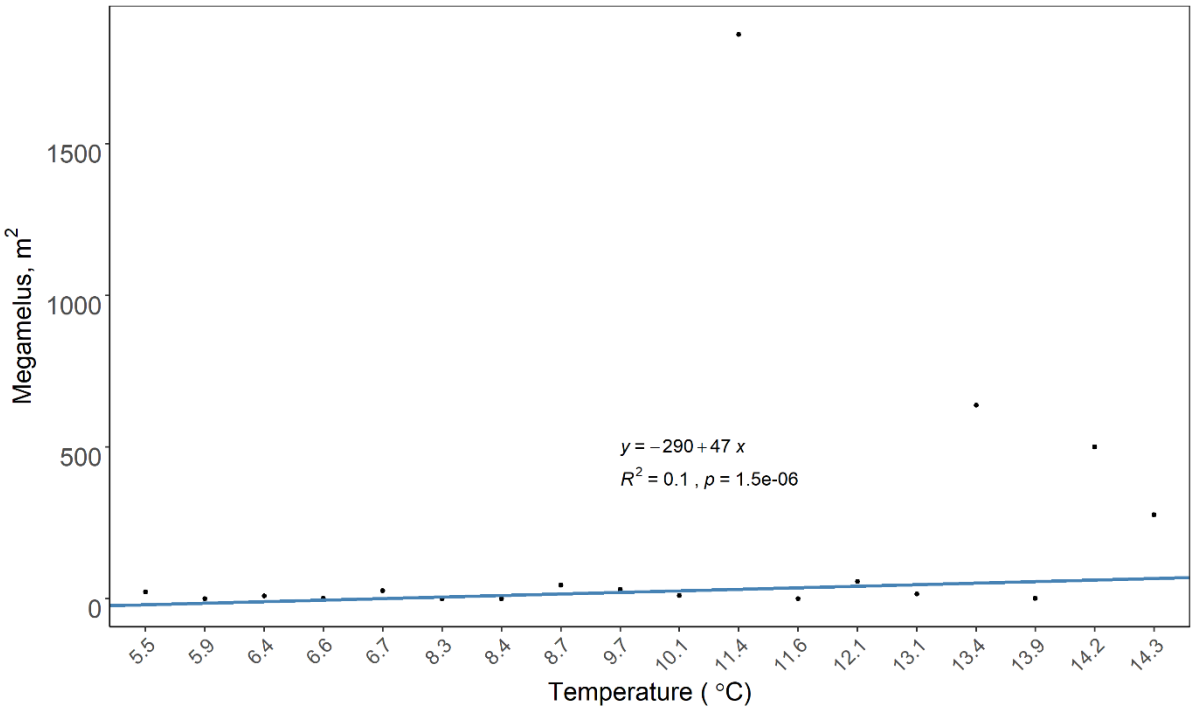

Figure 3.17: Response of *Megamelus scutellaris* to minimum temperatures on the Kubusi River, n =18.

3.4.8 Effects of season and biotic factors on insect populations

Populations of *M. scutellaris* were significantly affected by the longest leaf (Table 3.4). This insect predominantly feeds on leaves, and adults and nymphs are both found on the leaf petioles as well as the leaves, therefore the availability of such leaves has a profound influence on the populations. However, there were no significant interactions between any of the plant and environmental parameters, as well as season, on the population of *M. scutellaris* (Table 3.4).

Table 3.4: Summary of the influence of season combined with leaf number (Leaves), root length (Roots) and length of longest petiole (Longest) on the population growth of *Megamelus scutellaris*. Values in bold are significant.

	Value	**Std.Error**	**t-value**	**p-value**
(Intercept)	726.0978	1800.0372	0.4033794	0.6871
Longest	**0.1909**	**0.0948**	**2.0138191**	**0.0453**
Leaves	0.7146	0.8633	0.8277254	0.4088
Roots	0.0344	0.0346	0.9949521	0.3209
Ramets	0.0129	0.5987	0.0214869	0.9829
Rainfall	0.1178	0.1024	-1.1508870	0.2511
TempMin	-163.9474	209.8879	0.7811186	0.4356
Dates	0.0240	0.0718	0.3340265	0.7387
SeasonSpring	-260.1176	1257.1222	0.2069151	0.8363
SeasonSummer	-819.6820	1632.4757	0.5021098	0.6161
SeasonWinter	-221.9855	1257.5571	0.1765212	0.8601
TempMin:Dates	0.0102	0.0102	1.0008122	0.3181
TempMin:SeasonSpring	-18.8177	104.2176	0.1805617	0.8569
Phi	0.734423			
Residual Standard Error	9.095818			
Degrees of freedom	220			
Residual DF	205			
AIC	2276.891			
Log-Likelihood	-1118.446			

3.5 Interactions between the biocontrol agents on Kubusi River

The study was carried out on a multispecies system with four biocontrol agents, and because there was more than one species on the site, therefore, there were possible interactions between these agents. There was significantly less *E. catarinensis* feeding damage compared to *N. eichhorniae* between seasons ($F_{(3, 592)} = 88.28$, $P < 0.0001$) and between the two species ($F_{(1, 592)} = 304.98$, $P < 0.0001$) (Figure 3.18). There was no seasonal difference in feeding damage between autumn and

summer for either species (Figure 3.18). Furthermore, the interaction between season and insect species was significant ($F_{(3, 592)} = 28.36$, $P < 0.0001$). There was however no relationship between *N. eichhorniae* and *E. catarinensis* populations ($Rho_{(220)} = -0.045$, $P = 0.4531$). The relationship between weevil feeding and *E. catarinensis* feeding was weak, however, it was significant ($Rho_{(220)} = 0.216$, $P < 0.0005$). Populations of *E. catarinensis* and *M. scutellaris* were weakly correlated ($Rho_{(220)} = 0.2760$, $P < 0.0001$). However, *M. scutellaris* had a significant effect on the populations of *E. catarinensis* during summer (Table 3.5). Feeding by *M. scutellaris* is very subtle and was therefore not recorded. There was no relationship between populations of *M. scutellaris* and *N. eichhorniae* ($Rho_{(220)} = 0.067$, $P = 0.3204$). The presence of *N. eichhorniae* had no individual effect on *E. catarinensis*, however, the interaction *of N. eichhorniae, M. scutellaris* and season had a significant, negative effect on the populations of *E. catarinensis* (Table 3.5). There was an effect of season on the populations of *E. catarinensis* with populations recovering between spring and summer (Table 3.5).

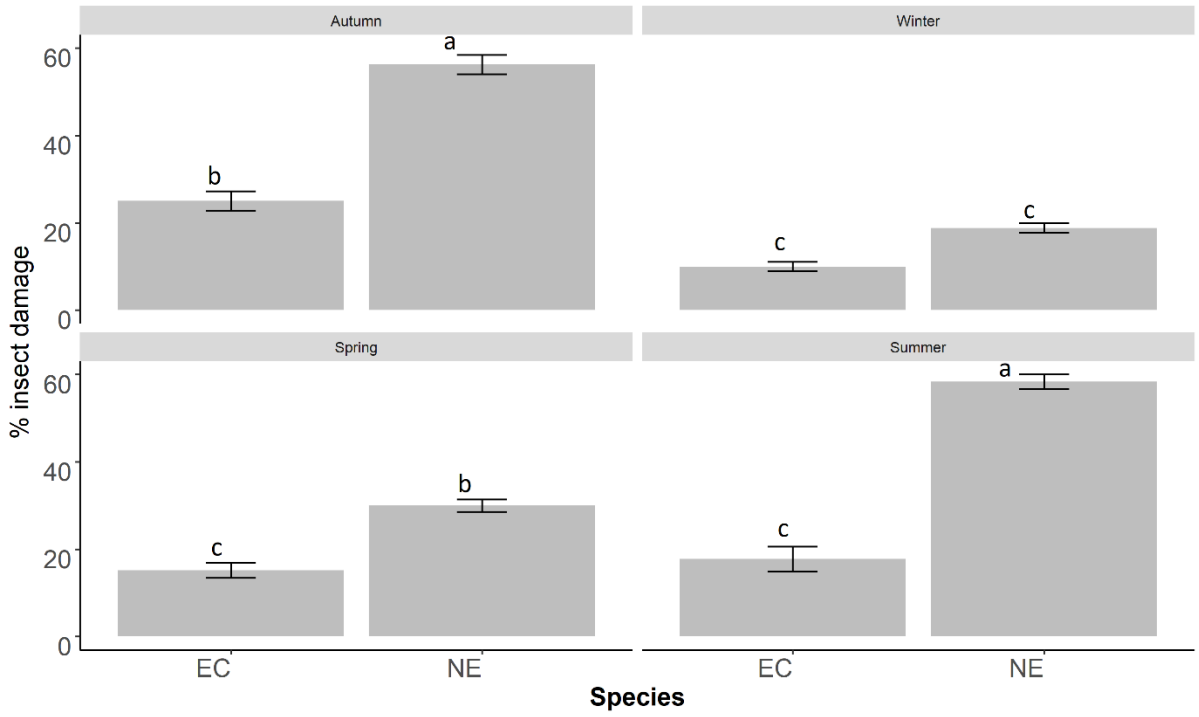

Figure 3.18: Comparison of insect performance between *Eccritotarsus catarinensis* (EC) and *Neochetina eichhorniae* (NE). Means with different letters are significantly different.

Table 3.5: Effect of the interactions between *Neochetina eichhorniae* (Ne), *Megamelus scutellaris* (Ms) and season on the populations of *Eccritotarsus catarinensis*.

	Value	**Std.Error**	**t-value**	**p-value**
(Intercept)	**16.215890**	**7.893653**	**2.054295**	**0.0412**
Ne	0.675474	2.646475	0.255235	0.7988
Ms	0.008962	0.008082	-1.108855	0.2688
SeasonWinter	-10.929149	8.090584	-1.350848	0.1782
SeasonSpring	**-15.969115**	**7.920866**	**-2.016082**	**0.0451**
SeasonSummer	**-23.750022**	**9.421394**	**-2.520861**	**0.0125**
Ne: Ms	0.000011	0.002211	0.005114	0.9959
Ne:SeasonWinter	-1.757182	2.725706	0.644671	0.5199
Ne:SeasonSpring	0.545001	2.662686	0.204681	0.8380
Ne:SeasonSummer	0.412707	2.991958	0.137939	0.8904
Ms:SeasonWinter	0.213543	0.140608	-1.518703	0.1304
Ms:SeasonSpring	0.004583	0.033916	0.135114	0.8927
Ms:SeasonSummer	**0.121920**	**0.015238**	**8.001249**	**0.0000**
Ne: Ms:SeasonWinter	0.053541	0.114328	0.468309	0.6401
Ne: Ms:SeasonSpring	0.015070	0.017492	0.861570	0.3899
Ne: Ms:SeasonSummer	**0.023019**	**0.004650**	**-4.949938**	**0.0000**
Phi	0.2152469			
Residual standard error	8.221782			
Degrees of freedom	220			
Residual DF	204			
AIC	1703.802			
Log-Likelihood	-830.9012			

3.6 Discussion

In this study, the seasonal population patterns of four biocontrol agents of *P. crassipes* coupled with host plant phenology, were measured over a three-year period. In biocontrol systems, environmental factors such as temperature play a significant role in the success of the control effort (Hill and

Olckers, 2001). In multispecies biocontrol programmes, insect-insect interactions are also important determinants of insect performance. Environmental factors are fluid, subject to seasonal change, inducing different responses from both the host plants and their associated herbivores. Winter presents a population bottleneck for most insect herbivores, in this case the biocontrol agents of *P. crassipes*, where winter causes mortality of both host plants and agents. It is important in biocontrol for agent populations to recover with the host plants following winter, however, the environmental factors determine the rate at which the agents will recover (Johnson *et al.*, 2016). In the case of *P. crassipes*, the plants tend to undergo rapid recovery and growth spurts at the onset of spring, while agent populations mostly have a lag phase and, in some instances, the agent populations lagged behind the plants considerably. The long-term monitoring effort at the cold site presented in this chapter, is in line with the predictions made by Hill and Olckers (2001) and further gives insights on the seasonal patterns of both agents and host plants.

Plant biomass and *population density*

Biomass was used as a proxy for plant productivity while the density gave an indication of the rate of spread and increase of *P. crassipes*. On the Kubusi River, the biomass and plant populations were influenced by temperature. The variation in the shoot to root ratios indicated that there were periods of low and high nutrient input into the system coinciding with the precipitation patterns observed. In high nutrient conditions, *P. crassipes* invests more in emergent plant parts and less on roots; while in nutrient deficient water they invest more in roots to increase nutrient uptake (Reddy and Tucker, 1983; Reddy *et al.*, 1989, 1990). Plant biomass was highest during the spring and summer possibly due to nutrients and favourable temperatures. The density of *P. crassipes* has been shown to increase during the warmer months. (Thamaga and Dube, 2019) showed that during the warm, wet season, surface coverage by *P. crassipes* increased up to 68% on the Letaba River, Limpopo Province in South Africa. During the drier, and cooler season, surface coverage declined to less than 30%. These

dynamics are similar to what was observed in the current study on the Kubusi River which is in a much cooler region than the Letaba River.

In the last growing season (2018-2019), the high plant density and therefore biomass was the result of a plant growth spurt. This growth trend is attributable to the recession of the river water levels leaving plants growing in a much narrower channel of water. The narrowing of the channel caused crowding of plants, hence the dense stand of plants per unit area of river channel. The crowding in the narrow stretch of water, may have led to competition for light, which induced greater leaf production and development of elongated petioles, resulting in taller plants and greater biomass. The results of the current study were therefore consistent with findings by Center and Spencer (1981) who reported that intraspecific competition in *P. crassipes* induced increases in plant density and size. The crowding also presented competition for nutrients, leading to dense roots as individual plants strove to outcompete adjacent plants. In the last growing season, there was an increase in both leaf and root lengths. This was an unexpected result because normally, when roots are short, then leaf petioles are longer, and the opposite is true. When *P. crassipes* has elongated petioles, roots tend to be shorter, however, this was not the case in the last growing season. This trend can again be attributed to the narrowing of the channel which intensified intraspecific competition. Furthermore, there was reduced vegetative reproduction by *P. crassipes* as there was lack of open space into which to spread, as evidenced by the absence of ramets during the last growing season of 2018-2019. It is therefore possible that resources which would otherwise have been channelled towards reproduction were utilized in petiole elongation and leaf area increases due to intraspecific competition. The phenology of *P. crassipes* on the Kubusi River was therefore informed by seasonal changes at the site. The plants experienced periods of senescence and dormancy characteristic of the winter season, while the summer season was characterized by actively growing plants.

Seasonal insect population patterns

Weevil population patterns were weakly correlated to the plant densities at the study site. The lack of correlation stemmed from the lag experienced by the weevil population because the weevil populations reached their peaks when the plants were already in decline. Higher weevil populations during the spring and summer seasons were the result of healthier plants and warmer temperatures favourable for weevil development. Healthy, actively growing plants are mostly characterised by dark green, glossy leaves. Maximum weevil oviposition and feeding occur at 30°C for both *N. eichhorniae* and *N. bruchi* (De Loach and Cordo, 1976; Byrne *et al.*, 2010). In the current study, maximum temperatures averaged approximately 27°C, which is close to the maximum oviposition threshold of 30°C, hence weevil numbers increased during the warmer seasons. In the current study, the results indicated that temperature restricted population growth at the site where minimum temperatures in winter were as low as 5°C; which is significantly below the lower developmental threshold of 15°C, and the lower oviposition threshold of 10°C (Byrne *et al.*, 2010). Furthermore, temperature changes associated with seasonal change also influence resource availability, where in winter lower temperatures cause plant mortality, hence a reduction in food resources (Johnson *et al.*, 2016). The study by Johnson *et al.* (2016) supports the results of the current study where plant mortality in winter restricted insect populations, while in summer, there was an increase in the abundance of the adult stage of the agents with the recovery of *P. crassipes*.

There were visible differences in the abundance of the two weevil species as *N. bruchi* was only present during the 2017-2018 growing season. *Neochetina bruchi* is reportedly more cold tolerant compared to *N. eichhorniae* (DeLoach and Cordo, 1976), however, in this current study, the results were contrary to these reports. On the Kubusi River, *N. bruchi* was absent in the colder months while *N. eichhorniae* persisted at the site. The absence of *N. bruchi* for two growing seasons could be the result of spatial competition from *N. eichhorniae* and plant quality. Plant quality plays a significant

role in weevil population dynamics, where *N. bruchi* performs poorly at low nutrient conditions compared to *N. eichhorniae* (Heard and Winterton, 2000). This is supported by the results from the current study where roots were longest for two growing seasons (2016-2017 and 2018-2019). Root length is a proxy for water nutrient status where long roots indicate low water nutrient quality (Reddy and Tucker, 1983; Reddy *et al.*, 1989). Furthermore, weevil feeding reduces plant quality (Tipping *et al.*, 2014), therefore, intense feeding on the plants on Kubusi River meant reduced quality. Because *N. bruchi* performs well in high nutrient conditions, then the reduced plant quality had negative effects on its populations. Although *N. eichhorniae* remained relatively abundant, the trends over the three growing seasons revealed a gradual decline in the population of the weevils. This trend again points to reduced plant quality because of weevil feeding, hence plants were less suitable for insect development. The situation on the Kubusi River is in contrast to that reported on the Sacramento River where *N. bruchi* managed to establish while *N. eichhorniae* was absent (Akers *et al.*, 2017). The absence of *N. bruchi* on the Kubusi River could be a factor of that the weevils were in very low abundances such that it was difficult to detect. However, a study investigating suppression of *P. crassipes* by biocontrol agents in Florida, USA (Tipping *et al.*, 2014), showed a trend similar to the one on the Kubusi River, in that about 99% of the weevils established on *P. crassipes* were *N. eichhorniae*. It is interesting that there was more *N. eichhorniae* than *N. bruchi* in Florida, however, no clarification was given as to the possible reasons why this was so. An earlier study by (Center and Dray, 1992), showed that at 22 sites where they sampled for insects in Florida, USA, there was consistently more *N. eichhorniae*. This was attributed to plant phenostage where tall plants in crowded conditions, stressed by herbivory had lower plant quality, while *N. bruchi* need high quality plants (Center and Dray, 1992). They proposed that since *N. bruchi* is more sensitive to plant quality, then they disperse to more vigorous sites, thus leaving *N. eichhorniae* more abundant in most sites.

In the current study, a near two-month lag phase in population recovery of *Neochetina* weevils and *E. catarinensis*, was similar to reports by Byrne *et al.* (2010). Such a scenario is detrimental to biocontrol as plants obtain ample time to build up populations while suffering minimal damage. In biocontrol, it is more desirable to have agents build populations soon after the recovery of the host plant, thus attaining a short lag phase. Hill and Olckers (2001) stated that the lag phase experienced by biocontrol agents of *P. crassipes* post-winter has contributed to the variable success in the control of *P. crassipes* in South Africa. In the current study, weevil populations in the growing season remained low (approximately 24 adults/ m^2) compared to the maximum reported in the USA. Maximum weevil population per unit in Wallisville, a subtropical region in the USA have been reported to be 55 adults/ m^2 in the growing season, while in winter the figure drops to approximately 8 adults/ m^2 (Grodowitz *et al.*, 1991).

Weevil recovery after winter was usually faster than the sap suckers on the Kubusi River. Although both the weevils and *M. scutellaris* overwinter in their immature stages (Wilson *et al.*, 2005; Sosa *et al.*, 2006), on the Kubusi River, the weevils always recovered faster. This is likely due to weevils having a lot of larvae compared to the nymphal stages of *M. scutellaris*. This is because *N. eichhorniae* overwinters better than the sapsuckers, where the last instar larvae take refuge in the root stalk and crown of the plants and overwinter as larvae (Wilson *et al.*, 2005). *Megamellus scutellaris* also overwinters in the immature stages, much like the weevils (Sosa *et al.*, 2006). In the current study, intense weevil feeding occurred from spring until end of autumn throughout the three growing seasons. Despite suffering damage, *P. crassipes* continued growing aggressively throughout all the three seasons. Insect feeding at times leads to plant mortality, however, in most cases, suppressed plant growth results from herbivory (Catton *et al.*, 2016; Myers and Sarfraz, 2016). In certain areas, *P. crassipes* grows beyond 1m tall depending on the nutrients and temperature, yet on the Kubusi River average height was 52.28±3.37 cm. This could have been the result of the presence of the

biocontrol agents as well as intraspecific competition , consistent with previous studies on the phenology and biocontrol of *P. crassipes* (Center and Spencer, 1981; Myers and Sarfraz, 2016).

In the first growing season, *E. catarinensis* maintained high populations, similar to *N. eichhorniae*; and during that period, plant population density was lower than the other growing seasons. A competition study was set up to investigate the effect of herbivory on competitive abilities of two aquatic weeds, *P. crassipes* and *Pistia stratiotes* in South Africa. The study highlighted that the interaction of *E. catarinensis* and various stress factors such as intraspecific competition, reduced productivity of *P. crassipes* (Coetzee *et al.*, 2005). The competitive ability of *P. crassipes* was reduced from 23 times more without the mirid to 10 times more than *P. stratiotes* when *E. catarinensis* was present (Coetzee *et al.*, 2005). Therefore, the presence of *E. catarinensis* and *N. eichhorniae* early in the season suppressed plant growth. Winter severely affected *E. catarinensis* where populations consistently plummeted at the onset of winter with long periods of absence. Such a scenario meant that the whole biocontrol effort or system on the Kubusi River was one agent short, hence less herbivory pressure on *P. crassipes*. The most negative impact on the population of *E. catarinensis* was due to reduced resource availability in winter, as reported by Johnson *et al.* (2016), that agent populations decline when limited by resources. On the Kubusi River, massive plant leaf die-back occurred every winter season, leaving *E. catarinensis* stranded on a few small patches of live material. Reduced resource availability due to the winter bottlenecks severely reduced populations to low levels such that there was always a long lag phase during the growing season. Most of the feeding by *E. catarinensis* was towards end of summer and beginning of autumn across the three growing seasons of the study. Insect activity increases with increase in temperature where both feeding and development of *E. catarinensis* occur optimally between 20°C and 30°C (Hill *et al.*, 1999). The damage patterns of *E. catarinensis* closely followed the patterns exhibited by the weevils which also showed intensive feeding behaviour throughout the spring and summer seasons.

Although the insect had persisted on the site for about two decades, in this current study there were short-lived durations of *E. catarinensis* persistence on the Kubusi River over three seasons. This is interesting because it was initially predicted that *E. catarinensis* would not establish at the site because of the cool climate (Coetzee *et al.*, 2007b). Because of the population patterns of *E. catarinensis*, the damage inflicted by the mirid was less compared to the weevils. However, *E. catarinensis* inflicts sublethal damage to *P. crassipes*, therefore, acting together with the weevils they function to reduce plant productivity. Only in the first growing season did we observe similar damage patterns and durations for both the weevils and the mirid. Although *E. catarinensis* was recently reported to be thermally plastic (Griffith *et al.*, 2019), it was absent at the site for most of the second growing season after winter. This was confounding because seasonal climatic conditions (temperatures) were similar at the site across the three years of the study and; we would have expected to have similar population and damage patterns in the successive seasons. It is however likely that on the successive season, there was complete destruction of the refugia at the site, hence absence of the insect for a longer period in the second season. The recovery of the insect was therefore related to precipitation which would have flushed down a mat of *P. crasssipes* which had an established population of *E. catarinensis*.

Although *M. scutellaris* was released at the Kubusi site in 2013, population counts for *M. scutellaris* were only initiated at the onset of winter in 2017 when a sampling protocol was put in place. During this time period, *M. scutellaris* was undetectable at the site, and this was due to the low temperatures and lack of habitat due to frosting of plants. May and Coetzee (2013) reported that low temperatures impact negatively on the development of *M. scutellaris* where development is slowed down to 65 days at temperatures below 19°C. The long absence of *M. scutellaris* from the site for most of the second growing season, coincided with that of *E. catarinensis*, hence only the weevils were available

to exert some level of control on *P. crassipes*. This absence of both sapsuckers was confounding, especially that the winter temperature were not significantly different from the previous winter. After recovery of *M. scutellaris* in the second growth season, the plant hopper remained more abundant than *E. catarinensis* until the end of the study. In the winter of 2018, *M. scutellaris* did not completely disappear from the site despite the cold temperatures. This scenario enabled *M. scutellaris* to gain a head start for the following growing season (2018-2019) where there was a population explosion. *Megamelus scutellaris* was therefore available throughout the growing season effecting control on the plants. It is likely that, the crowding of plants owing to a narrower river channel, offered a better refuge to the insects. The insects were therefore buffered from fluctuations in temperature, hence a constant environment which fostered greater developmental rates. Furthermore, the population increase of *P. crassipes* led to an increased resource base, which enabled exponential growth of *M. scutellaris*. Moran (2004)highlighted that the height of *P. crassipes* plants tends to decrease as biocontrol agent populations build up. However, this was not the case on the Kubusi River, where despite the increase in agent populations, particularly *M. scutellaris*, the height of *P. crassipes* was not affected. This is likely because *P. crassipes* compensated for herbivory, coupled with intraspecific competition due to crowding on the river channel (Myers, 1984; Coetzee *et al.*, 2005).

On the Kubusi River, the biocontrol programme had 4 insect species, and there was some interaction between them. In the presence of *N. eichhorniae*, there was less *E. catarinensis* feeding. It is likely that, since weevils cause scarring on the leaf surface, they reduced the leaf surface area and quality for feeding by the mirid. At times, in multi agent species settings, host plant quality is reduced due to feeding by one agent, making the host less suitable for the other agents (Weyl and Hill, 2012; Tipping *et al.*, 2014). Reduced mirid feeding on the Kubusi River is converse to the report by Weyl and Hill (2012), where they showed that in a controlled experiment, feeding by *N. eichhorniae* is

reduced when *E. catarinensis* is present. Alternatively, weevil feeding may have consolidated the feeding damage by the mirids hence reducing the visually evident damage inflicted by *E. catarinensis*. Interestingly, weevil feeding was most pronounced in summer and autumn, while that of *E. catarinensis* was pronounced in autumn only. This trend was because this was the time when insect populations were at their peak. The relationship between weevil and mirid populations was neutral and did not conform to the relationship in feeding damage. This may be because of their diel habits where *N. eichhorniae* is nocturnal and *E. catarinensis* is diurnal, thus reducing chances of their physical interaction for most of the day (Marlin *et al.*, 2013). The study showed that the interaction of weevils, *M. scutellaris* and season had a significant impact on the population of *E. catarinensis*. This was evident in the summer and autumn of the last growing season when there was a population explosion of *M. scutellaris*, which is also a sap sucker. It is therefore possible that the presence of the two other agents reduced the competitive ability of *E. catarinensis*. On the Kubusi River, weevil populations mostly recovered month before those of *E. catarinensis*, meaning that there were a lot of old feeding scars on the leaves by the time the mirid recovered. Ajuonu *et al.* (2007) showed that numerous old feeding scars reduced survival of the mirid compared to when there were fresh scars. The trend on the Kubusi River refuted the cumulative stress hypothesis where successive addition of biocontrol agents on a target weed has been conventionally viewed to increase the level of stress that is exerted on and reduce growth of the target (Myers, 1984; Schulz *et al.*, 2019). The combined effect of the biocontrol agents on the Kubusi River failed to reduce the growth of the plant during the study period.

Overall, *N. eichhorniae* was consistently present in detectable numbers throughout the study period compared to the other three agents. *Neochetina eichhorniae* consistently recovered two months after the plants recovered from the winter season, with the exception for the last growing season. The weevils consistently showed earlier recovery compared to the sapsuckers, which have a faster

generation time than the weevils. The long lag phases recorded by *E. catarinensis* and *M. scutellaris* are detrimental to the biocontrol effort on the Kubusi River as the insects fail to build up sufficient populations to halt the growth and persistence of the infestation. Furthermore, the cold winters negatively affected all the four insect species as there was a population bottleneck induced by the winter frost on the insects. However, the sapsuckers consistently experienced long delays in population recovery after the winter season especially for the last two years of the study. This is likely due to that vestigial pockets of *P. crassipes* which did not get frosted supported very few insects, leading to a long lag before the insects recovered. Many insect species overwinter in sheltered areas, while with some species, only the immature stages overwinter while adult stages perish from lack food (Center *et al.*, 1999). Another possibility is that of insects overwintering on non-target plants which would not have succumbed to frosting (Wang *et al.*, 2019). Such refugia provide a microclimate that may be above the CTmin of the biocontrol agents. The recovery of the insects after winter ultimately depends on the availability of refugia for overwintering (Center *et al.*, 1999). In the current study, insect recovery was shown to be slow post winter.

3.7 Conclusion

Seasonality and the prevailing environmental conditions inform host plant phenology, abundance and quality. Host plant patterns in turn inform the abundance of their associated insect herbivores. In this study, resource availability, which is subject to seasonal change and associated temperatures, affected the population dynamics of all the biocontrol agents on the Kubusi River. Both the plant and insect abundances fluctuated with change of season throughout the study period. The patterns shown in this study point towards need for further management interventions after the winter season. Augmentative releases are therefore crucial at the beginning of each growing season in order to establish viable populations from the onset and reduce the lag phase shown in this study. It would be advantageous for the biocontrol effort at the site to release the weevils and the planthopper post

winter each year. This will ensure the presence of the insects in high nimbers from the onset of the growing season/ Therefore, insect mass-rearing during winter should be intensified in order to have sufficient numbers to release at the onset of spring. Furthermore, insect interactions indicated a degree of interference competition, resulting in the suppression of populations of *E. catarinensis*. Although insect interactions have been studied in the laboratory, there is need to further investigate these interactions in the field.

Chapter 4 : The interaction between *Eccritotarsus catarinensis* and *Eccritotarsus eichhorniae* on *Pontederia crassipes*

4.1 Introduction

4.1.1 Insect-insect interactions

Thus far, this study has shown that *E. eichhorniae* has not established in South Africa despite having been released in high numbers (Chapter 2). Furthermore, the thermal requirements for optimal insect development of the congeneric species overlap at 26 ºC (Paterson *et al.*, 2019). However, *Eccritotarsus eichhorniae* has a narrower thermal range compared to *E. catarinensis*, which has established in one of the cooler *P. crassipes* sites in South Africa, even in the presence of other biocontrol agents that have been released in this country (Chapter 3). Thus, the lack of establishment of *E. eichhorniae* could be due to direct competition with *E. catarinensis*.

The biocontrol programme against *P. crassipes* worldwide presents an analogous situation where the two weevils, *N. eichhorniae* and *N. bruchi* have very similar biologies and yet occur together (Winston *et al.*, 2014). Furthermore, there are reports that the two weevil species have hybridised (Hopper *et al.*, 2019). A study on the *Neochetina* spp. weevils by Center and Dray (2010) revealed that the two species performed equally well in terms of population growth when they occurred in combination, compared to when they were separate. Here, the weevils were reared on plants in low and high nutrient conditions, and in the mixed treatments, it was shown that the weevils produced more offspring unlike when the two species were separate. Therefore, the conclusion was that there was no interspecific competition between the two (Center and Dray, 2010). They further highlighted that *N. eichhorniae* had consistently high populations in high and low nutrient treatments, however, *N. bruchi* performed nearly as well in high nutrient conditions. An earlier study by Heard and Winterton (2000) found that *N. bruchi* was more fecund on high nutrient plants compared to *N.*

eichhorniae, alluding to the fact that *N. bruchi* has higher nitrogen requirements than *N. eichhorniae*. This report was in contrast to what was reported by Center and Dray (2010). The study showed that in highly eutrophic systems, *N. bruchi* performs better due to its higher fecundity, ultimately outcompeting *N. eichhorniae* demographically; furthermore, *N. eichhorniae* failed to reduce plant growth in the eutrophic systems (Heard and Winterton, 2000). Both Center and Dray (2010) and Heard and Winterton (2000) showed that *N. bruchi* has higher nitrogen requirements even though different levels of nitrogen enrichment were used.

Another study by Weyl and Hill (2012) on the interaction of three control agent species (*Neochetina eichhorniae, N. bruchi* and *E. catarinensis*) on *P. crassipes* showed that there was negative interaction between the two weevils, *N. eichhorniae* and *N. bruchi* when tested together. The amount of feeding damage was significantly reduced when the weevils occurred together. However, when *E. catarinensis* was combined with either weevil species, there was a synergistic effect with no significant negative interactions observed (Weyl and Hill, 2012). The interaction of the two weevils revealed that there was a decrease in the number of leaf feeding scars when they occurred together (Weyl and Hill, 2012). The negative interactions were mostly due to niche overlap between the two weevils, despite their coexistence in their native range (Weyl and Hill, 2012). The study revealed that *N. eichhorniae* performed significantly better when occurring alone compared to the combination with *N. bruchi*. Furthermore, *N. bruchi* mined more petioles than *N. eichhorniae*; however, since the feeding scars are similar, it was impossible to identify which weevil was adversely affected (Weyl and Hill, 2012). Here, the results were contrary to Center and Dray (2010) who found that there was no negative interaction between the two weevils.

The two mirids, *E. catarinensis* and *E. eichhorniae* occupy the same niche as they have similar feeding habits. Because they feed in a similar manner on the same parts of the plant, there may be the

possibility of competition between the two species, which may lead to the reduced performance, or competitive exclusion of one of the species. It is also possible that they complement each other. In this chapter, I explored the performance of the two mirid species in both single and multispecies contexts. The aim of the study was to determine whether either species performed significantly better on its own, or whether there was a synergistic or antagonistic interaction between the two.

4.2 Experimental design

The experiments were conducted in a greenhouse at the Waainek mass rearing facility, Rhodes University, and investigated which interaction between *E. catarinensis* and *E. eichhorniae* achieved the greatest impact on the weed. The experiment ran for 15 weeks from April to August 2019.

Four flow through systems comprising five interconnected tanks, with a sixth tank in the series assigned as a feeder tank were used for the experiment (Figure 4.1). The positioning of the flow through systems was such that System 1 was the midmost system in the greenhouse, while System 4 was the most peripheral system in relation to the entrance of the tunnel. Furthermore, the positioning of System 4 meant that it received most of the incident radiation in the late afternoon as it was less exposed to shading during that time of the day.

Figure 4.1: Arrangement of each flow through system where 1 = the feeder tank that supplies nutrient enriched water to the other 5 tanks in the series; 2 = the UV filter to reduce algal growth in the water supplied to the rest of the flow through system; 3 = delivery pipes from the feeder tank to the other tanks; 4 = return pipes from the other tanks to the feeder tank.

In each flow through system, water nitrogen status was manipulated via the feeder tank to achieve low and high nutrient conditions (two low nutrient and two high nutrient systems). Nutrients were added to the water (borehole water) at a rate of 6mg N L^{-1} (High), 1.6mg N L^{-1} (Low), 1.5mg P L^{-1} and iron chelate at 11mg/L Fe EDTA for each respective flow through system. This nutrient regime reflects the water nutrient status of South African waters (Coetzee *et al.*, 2012). Insect free plants were cultured in a greenhouse, located 2.5km away from the Waainek research station to ensure that the plants remained insect free before the experiment commenced. The plants were cultured to a mature stand in 1000L pools with 15mg/L N, 11mg/L Fe EDTA. Thereafter, plants were transferred

to the flow through system at the Waainek mass rearing facility at the beginning of April 2019 where they acclimated and formed mats before inoculation with insects. Mat formation was allowed in order to simulate field conditions of the plant monocultures. Plants were left to grow in the respective nutrient conditions (high and low) for 3 weeks prior to inoculation with the mirids. Water was pumped from the feeder tank and distributed to the other five tanks so as to achieve uniform nutrient concentrations throughout each system (Figure 4.1). A completely randomized design was used to reduce confounding factors associated with positioning of the various treatments.

In each flow through system, insect treatments were arranged in the following manner: (i) insect free control, (ii) *E. catarinensis*, (iii) *E. eichhorniae*, (iv) mixed populations, (v) procedural control; and these were randomly allocated tanks in each flow through system. *Eccritotarsus* spp. have a sex ratio of 1:1, therefore there was a balance in the number of males and females (Coetzee *et al.*, 2005). Each Population x Nutrient treatment had 4 replicates making the total number four 1000L tanks per treatment. Four of the six tanks in each flow through system were covered with mesh netting. Frames of 20mm PVC piping were constructed over the tanks, and thereafter mesh netting was used to cover these frames, such that a series of cages was formed over the flow though system (Figure 4.2). The dimensions of the cages were 120cm x 110cm x 50cm (length x width x height), while the volume of each tank was one cubic metre. A double curtain secured with Velcro was used to separate two tanks in each series of cages to prevent insect migration. Velcro was secured with silicon around all the tanks which were covered with netting as a way of insect proofing the cages to prevent escape of insects during the experiments. The procedural control was left uncovered in order to provide a comparison of plant growth to assess if the netting influenced plant growth due to light interception. After three weeks of acclimation and mat forming, plants in the cages were inoculated with the respective insects at a rate of 300 adults per square metre. *Eccritotarsus eichhorniae* was obtained

from Waainek mass rearing facility, while *E. catarinensis* was reared at a separate greenhouse, 2.5 km away from the mass rearing facility.

Figure 4.2*:* Flow through system covered with mesh netting to prevent escape of insects and contamination of species.

4.3 Data collection

Ambient temperature data were collected using Thermachron iButtons (Climastats Environmental Monitoring software, Version 4), while water temperature was collected with Multi-Parameter meter (PCSTestr 35 Series). Ambient temperatures were recorded at 1 hour intervals over the course of the experiment. The iButtons were suspended above the mat within the midmost cage for each flow through system. The temperature data were recorded from when the plant acclimation began until the end of the experiment. Physico-chemical properties of the water (pH, dissolved oxygen, conductivity, total dissolved salts [TDS] and salinity) were recorded at 3 week intervals using a

Multi-Parameter meter (PCSTestr 35 Series) and a dissolved oxygen meter (DO Pen 850045 Sper Scientific).

Every 21 days, a population census of the mirids was conducted within each cage to assess the population densities. Half a square metre per tank was sampled using four 0.25m x 0.25m quadrats randomly placed in each tank and a visual estimate of the populations was recorded. In two of the flow through systems, there was high insect mortality by the third week, prompting a replacement of the insects, the two systems were high and low nutrient systems. At the end of the experiment, the insects still alive were collected with an aspirator and counted and, then compared between treatments. At the end of the experiment, two of the systems had severely reduced populations, therefore, DNA data were limited to compare between the populations of the two species in the mixed species set up. However, molecular tests were used to verify the species in each cage, following the methods used in Chapter 2. No feeding damage or insects were detected on the controls, and this confirms that the insect-proofing measures were successful. This further affirms that there was no contamination between populations, therefore each respective species remained where it was initially inoculated.

Feeding damage data were collected at 3-week intervals on leaf 2, 3 and 4 from 7 randomly selected plants in each tank. The 3-week intervals were chosen in order to correlate with insect density measurements recorded at the same interval. Percentage leaf damage was scored by estimating the amount of leaf tissue exhibiting chlorosis relative to the total leaf area. The percentage damage was graded on a scale of 0 to 5 (0=0%, 1=<5%, 2= 5-25%, 3= 25-50%, 4= 50-75%, 5= 75-100%) on both abaxial and adaxial surfaces with five being severely damaged leaves exhibiting a yellow or white appearance. The following formula, adapted from Ray and Hill (2015), was used to calculate percentage damage per tank in addition to the total damage per plant:

Plant % damage:

$$\frac{(0 \times N_0) + (1 \times N_1) + \ldots\ldots + (5 \times N_5) \times 100}{\text{Total } n_0 \text{ leaves x maximum damage rating}}$$

N_0 = number of leaves with score 0, … , N_5 = number of leaves with score 5.

Plant photosynthetic efficiency was used as a measure of plant performance subject to herbivory. A Handy PEA (Hansatech, Kings Lynn, UK) chlorophyll fluorescence analyser was used to measure plant photosynthetic efficiency. Leaves were initially dark adapted for 5 to 7 minutes using leaf clips before fluorescence measures were recorded (Figure 4.3). A saturating light pulse (3500 µmol m^{-2} s^{-1}) with a fixed gain of 1x was then emitted by the analyser. The analyser recorded various measures such as maximum fluorescence (F_m), variable fluorescence (F_v), minimum fluorescence (F_o), plant performance index (PI_{abs}) and density of reaction centres (RC). Plant performance index (PI_{abs}) was used as a measure of plant photosynthetic performance (Strasser, 1997; Strasser *et al.*, 2004), subject to herbivory and different nutrient levels. Plant photosynthetic performance was tested in order to ascertain the physiological impact the two mirid species have on *P. crassipes*. Other plant parameters measured were longest leaf petiole, area of leaf 2 (the second youngest unfurled leaf): the longest and widest dimensions of the leaf were measured, number of ramets and number leaves per parent plant. These parameters were recorded from the same 7 randomly selected plants in each tank at 3-week intervals. Plant wet biomass was measured at the end of the experiment to get a comparison of biomass accumulation between treatments. The plants were separated into emergent plant parts, roots and dead material, and these were weighed separately. The roots were drained of excess water by gently squeezing out water prior to weighing.

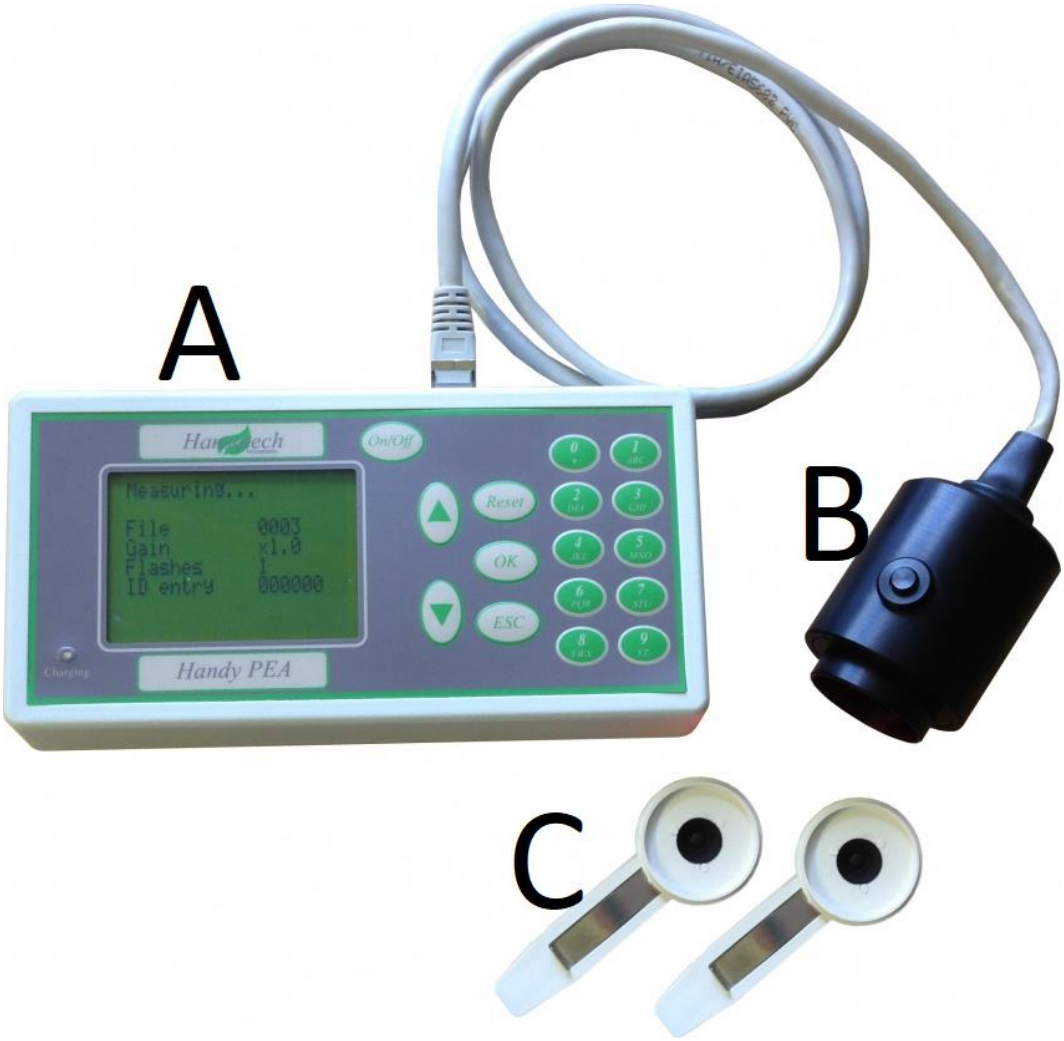

Figure 4.3: Hanstech Handy PEA unit- A = PEA control unit; B = sensor head housing sensor array which consists of 3 x 650nm LED's, and a filtered PIN photodiode for fluorescence detection; C = leaf clips for dark adapting leaves.

4.4 Statistical analysis

Data were analysed in R 3.6.0 (R Core team, 2019). Data were first tested for normality using the Shapiro and the Kolmogorov tests in R, followed by a Levene's homogeneity test. A Generalized Linear Model (GLM) (family = poisson, link = log) was used to analyse insect population data. Plant photosynthetic performance over time was analysed using a Generalized Least Squares model (autoregressive model with heterogenous variances), following the method outlined in Chapter 3. Plant relative growth rates per week were calculated for leaf petiole lengths and leaf area using the formula *ln(L2)- ln(L1)/(t2-t1)* where L2 = second measurement after a time period, L1 = initial

measurement while t1 and t2 are initial and final time of measurement respectively. Change in ramet and leaf production per week was calculated as *R2-R1/ t2-t1*. A factorial ANOVA was used to test for differences in temperature and leaf length. Other plant parameters were analysed using gaussian (link = "identity") GLM's because they are more robust to deviations from normality. The model building process utilized an initial complex model that was tested for the variance inflation factor (VIF), where variables with a VIF greater than 5 were eliminated from the model. Perfectly correlated variables were also eliminated from the model after using the "alias" function to identify such variables. Thereafter the "dredge" function for model selection was called on the model. This function used an iterative stepwise deletion and addition of the variables from the global model in order to identify the best combination of explanatory variables. The best model had the lowest AIC and delta score of less than 3 and was subsequently used for the analyses. Model fit was then assessed using plots of fitted versus residual values where randomly scattered points indicated a good model fit, while a pattern forming arrangement of points indicated a poor fit. All data visualization were made using "tidyverse" and "ggpubr" in R.

4.5 Results

4.5.1 Temperature

Temperature influences the rate of insect development and feeding behaviour (See chapter 3). Temperature data were collected for 18 weeks because plants were set up three weeks prior to inoculation. There were fluctuations in temperature over 15 weeks of the study. The highest temperatures (23.2±0.24 ℃) were recorded in the second week of the experiment (Figure 4.4). The trends revealed a gradual decline of temperature from the third week, with the lowest mean temperature of 14.2℃ recorded in week 12 (Figure 4.4). Thereafter, a sharp increase in temperature was recorded at the beginning of week 16 with mean temperature reaching 18±0.45 ℃, and there was a more gradual increase thereafter until the end of the experiment when temperatures peaked at

19.8±0.63°C (Figure 4.4). Although System 4 was exposed to more radiation for most of the day, the temperature was not significantly higher compared to the rest of the systems. A One-Way ANOVA was used to test for differences in temperature between the four systems, and there were no significant differences ($F_{(3,84)} = 1.562$, $P = 0.205$).

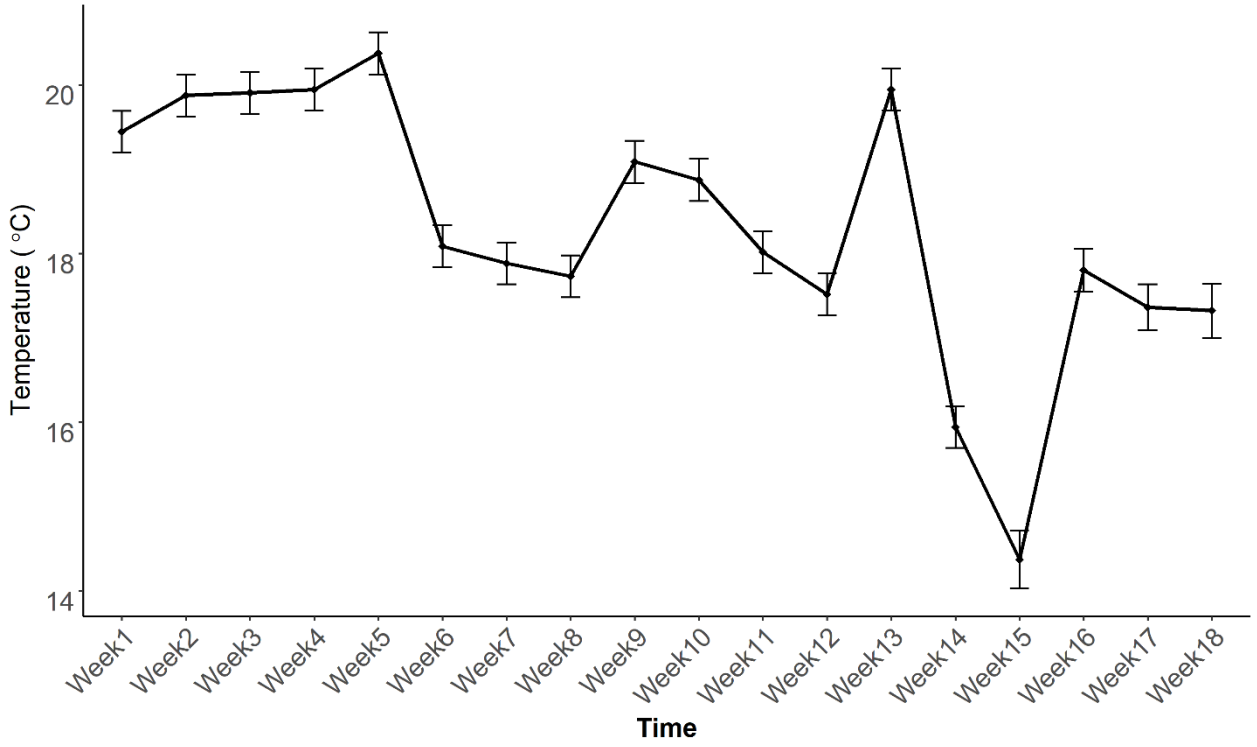

Figure 4.4*:* Mean ambient temperature in the greenhouse for all four flow through systems of the experiment.

4.5.2 Insect populations and feeding

Insect populations declined through most of the study under both high and low nutrient conditions. At the end of the 15 week study, the *E. eichhorniae* (Ee) treatment had a significantly higher population compared to either mixed or *E. catarinensis* (Ec) treatments under high nutrients (Table 4.1; Figure 4.5). Under low nutrient conditions, there were significantly fewer insects in the mixed treatment compared to the *E. eichhorniae* treatment, while the populations from the mixed and *E. catarinensis* treatments were the same (Table 4.1; Figure 4.5). All three treatments under low nutrients showed an initial decline in populations, then the populations fluctuated until the end of the

experiment (Figure 4.5). During the last three weeks of the study, under both high and low nutrients insect populations increased (Figure 4.5). Temperature had a significant influence on insect population of the mixed species treatment (y = -140+9.1x, R^2 = 0.55, P < 0.0001) (Figure 4.6). There was a weak, significant relationship which was positive, between temperature and population size for the other herbivory treatments due to variability in the data (Figure 4.6).

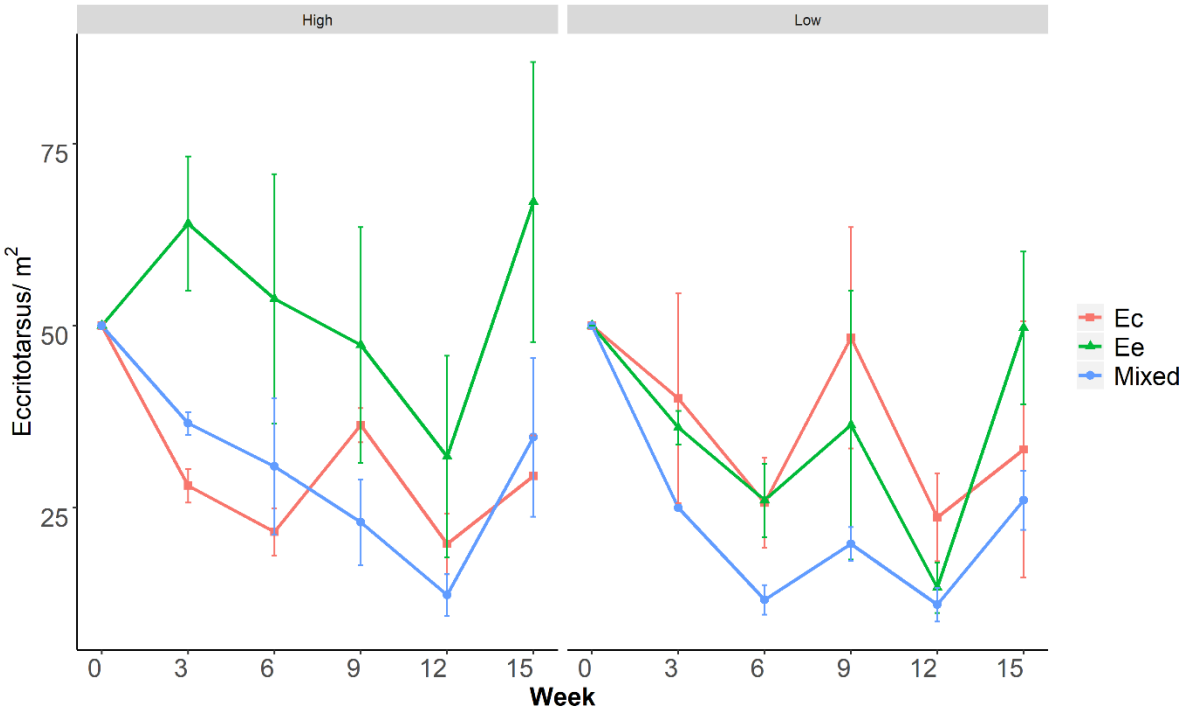

Figure 4.5: Change in the population of the two species of *Eccritotarsus* over a 15-week period under high and low nutrients. The insect treatments presented in the legend are: Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*. Error bars are standard error.

Table 4.1: Response of insect populations to abiotic factors. Species represent herbivory treatments1: Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*. Values in bold are significant.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	**21.9791979**	**2.7433395**	**8.012**	**1.13e-15**
NutrientsLow	**1.0303033**	**0.1414595**	**7.283**	**3.26e-13**
SpeciesEe	**0.8947937**	**0.0907133**	**9.864**	**< 2e-16**
SpeciesMixed	**0.2748893**	**0.1029395**	**2.670**	**0.007576**
Temperature	**0.3379624**	**0.0919715**	**-3.675**	**0.000238**
Conductivity	**0.0083268**	**0.0008551**	**-9.738**	**< 2e-16**
NutrientsLow:SpeciesEe	**0.3682055**	**0.1305458**	**-2.821**	**0.004795**
NutrientsLow:SpeciesMixed	**0.8118746**	**0.1565890**	**-5.185**	**2.16e-07**
AIC	265.15			
Residual DF	28			
Dispersion	1			

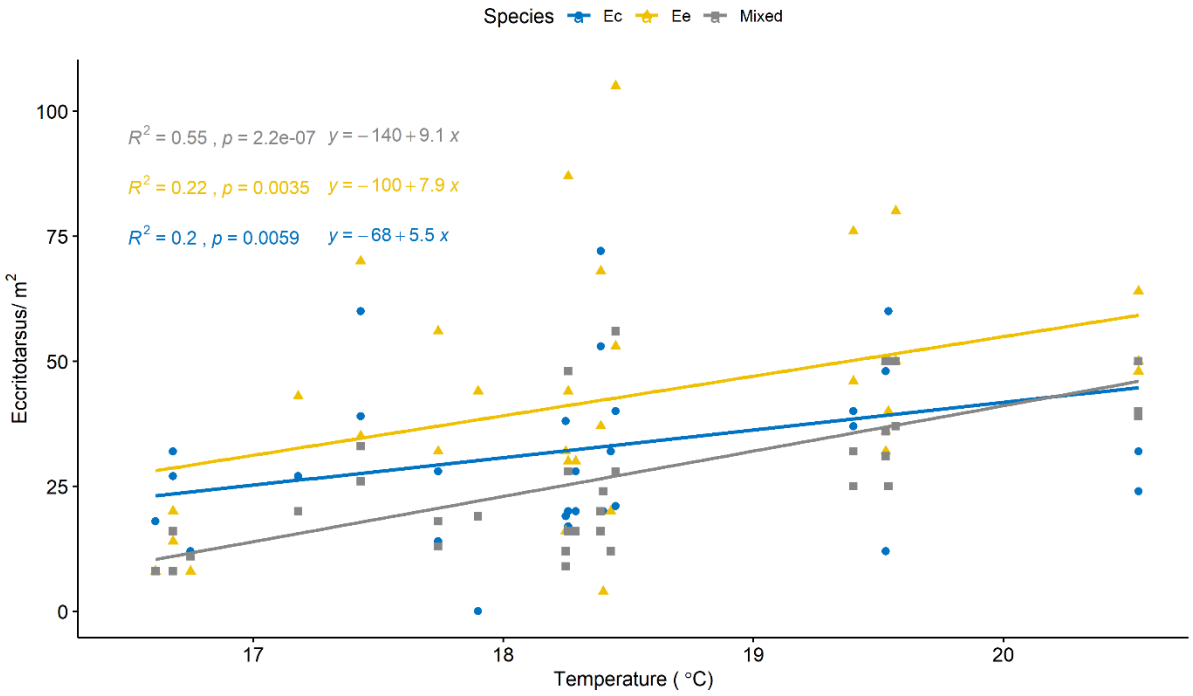

Figure 4.6: Relationship between insect population and temperature. Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*.

Insect feeding damage on P. crassipes.

Patterns in insect feeding damage fluctuated throughout the duration of the study. There was an initial increase in feeding damage under high nutrients during the first 3 weeks and thereafter a decline until week 6, thereafter fluctuations until the end of the study (Figure 4.7). At the end of the experiment, there was significantly more feeding damage in the *E. catarinensis* treatment compared to the mixed treatment under high nutrients (Figure 4.7). Under low nutrients, there was significantly less feeding damage in the mixed treatment compared to the other two treatments (Figure 4.7). These patterns were informed by water temperature, ambient temperature and nutrients (Table 4.2). In the single species herbivory treatments, *E. catarinensis* and *E. eichhorniae*, there were similarities in damage patterns, however they had significantly higher damage values compared to the mixed treatment (Figure 4.7).

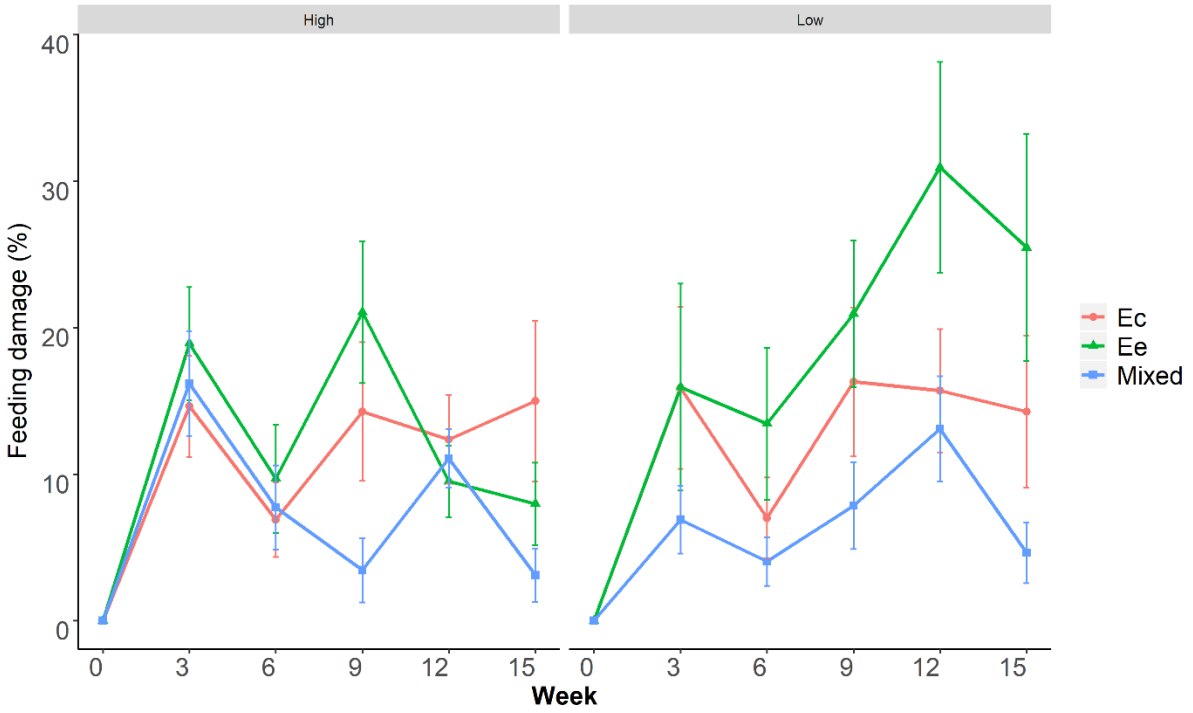

Figure 4.7: Insect feeding patterns under high and low nutrients. Herbivory treatments are Ec= *Eccritotarsus catarinensis*, Ee= *Eccritotarsus eichhorniae*, Mixed. Error bars are standard error.

Table 4.2: Summary of the differences in insect damage between the herbivory (Species) treatments under high and low nutrients. Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*,. Values in bold are significantly different.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	**544.27305**	**252.43676**	**2.156**	**0.034328**
NutrientsLow	**30.72190**	**8.34618**	**3.681**	**0.000439**
SpeciesEe	-3.64260	4.92798	0.739	0.462144
SpeciesMixed	**-8.65313**	**4.24453**	**-2.039**	**0.045057**
Temperature	**-23.76720**	**5.59359**	**-4.249**	**6.18e-05**
Longest	0.26439	0.14651	1.805	0.075216
Conductivity	**0.22644**	**0.06652**	**-3.404**	**0.001075**
Water temperature	**15.32750**	**5.23693**	**2.927**	**0.004547**
NutrientsLow:SpeciesEe	**14.36808**	**6.71239**	**2.141**	**0.035607**
NutrientsLow:SpeciesMixed	**-16.59645**	**6.89548**	**-2.407**	**0.018586**
AIC	653.23			
Reisdual DF	74			
Dispersion	121.93			

4.5.3 Plant photosynthetic performance

Plant photosynthetic performance is a measure of the efficiency of the plants' light harvesting mechanism in photosystem II (PSII). The two *Eccritotarsus* species feed on leaves, thereby extracting chlorophyll, potentially reducing the amount of photosynthetic reaction centres. At the beginning of the study, there was similar plant performance across the treatments (Figure 4.8). There was a decline in plant performance for 12 weeks of the study, with an increase recorded in the last 3

weeks. In week 9 under high nutrients, there was significantly higher plant performance in the control ($t_{(299)} = -5.312$, $P < 0.05$), while there was significantly lower performance in the other treatments (Figure 4.8). By week 12, there was significantly higher plant performance in the procedural control, however, at the end of the experiment, there was the lowest performance in the procedural treatment (Figure 4.8). There was a significant interaction between herbivory, nutrients and time on plant performance in the mixed ($t_{(299)} = -3.225$, $P < 0.005$) and *E. catarinensis* ($t_{(299)} = -3.815$, $P < 0.0001$) treatments compared to *E. eichhorniae* by week 9 in the high nutrient conditions (Figure 4.8). Similar to the high nutrient conditions, trends showed a general decline in plant performance across treatments under low nutrients (Figure 4.8). The procedural control and mixed treatment had the lowest plant performance under low nutrient conditions ($t_{(299)} = -5.415$, $P < 0.0005$). At the end of week 15, there was similar plant performance between *E. catarinensis*, *E. eichhorniae* and the control under low nutrient conditions, while there was significantly lower plant performance in the mixed treatment and procedural control (Figure 4.8). There was a weak, but non-significant positive relationship between insect feeding damage and plant performance under high and low nutrients ($R^2 = 0.3$, $P = 0.11$; $R^2 = 0.31$, $P = 0.095$ respectively).

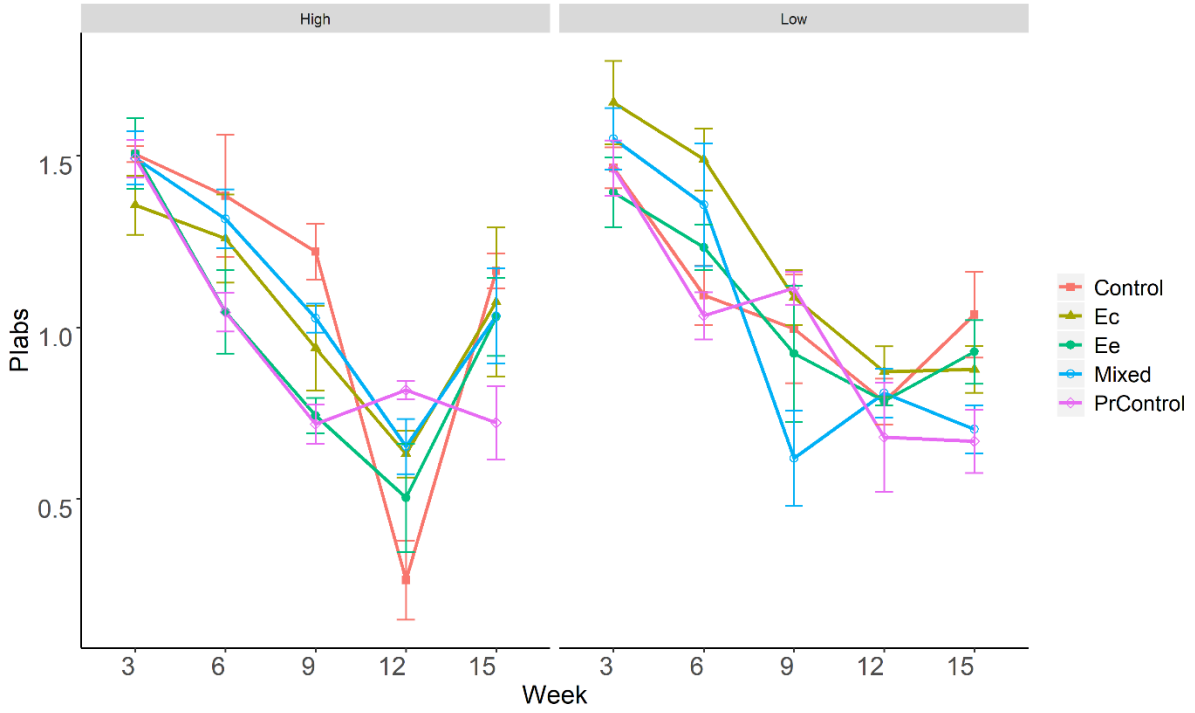

Figure 4.8: Patterns of plant performance across herbivory treatments (Ec= *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*, Mixed) under high and low nutrient conditions. PIabs represents plant Performance Index. Error bars denote standard error.

4.5.4 Plant growth parameters

Longest petiole

Plant relative growth rates were calculated and used in the analyses because the plants were not the same size at the beginning of the experiment. The length of leaf petioles was significantly affected by the interaction of nutrients and herbivory where there were significantly shorter leaf petioles under low nutrients in the *E. catarinensis* treatment compared to the *E. catarinensis* treatment under high nutrients (Figure 4.9; Table 4.3). Plants in the procedural control and *E. eichhorniae* treatment were significantly shorter at high nutrients compared to the rest of the treatments (Figure 4.9; Table 4.3). Leaf area had a significant effect on the length of leaf petioles (Table 4.3). Number of leaves, herbivory treatment, pH and temperature did not have a significant effect on leaf petiole length, however conductivity was nearly significant (Table 4.3).

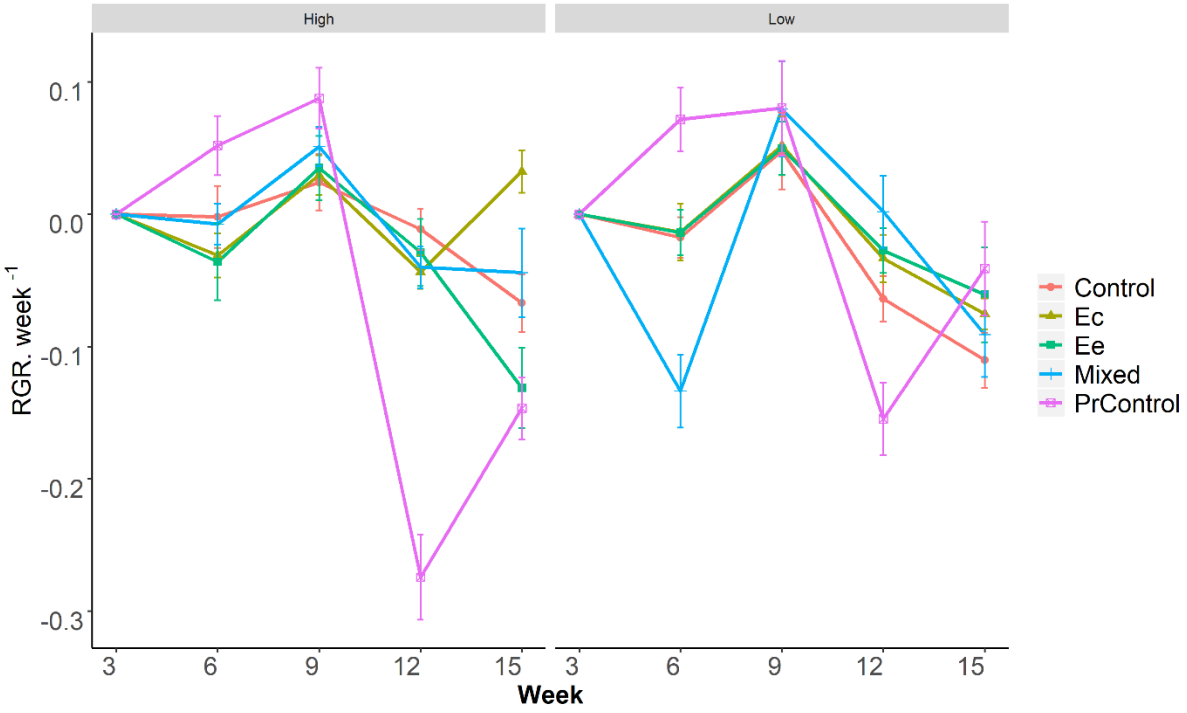

Figure 4.9: Relative growth rate of the longest leaf petioles under high and low nutrients subject to different herbivory treatments- Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*, ProControl = procedural control. Error bars represent standard error.

Table 4.3: Summary of the effects of nutrients, herbivory (Species) and temperature on rates of increase in leaf petiole length. Values in bold are significant.

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Nutrients	1	0.0006	0.00064	0.066	0.797502
Species	**4**	**0.1091**	**0.02726**	**2.811**	**0.028307**
Ambient Temperature	1	0.0068	0.00676	0.697	0.405309
Conductivity	1	0.0374	0.03745	3.861	0.051646
Water temperature	1	0.0062	0.00617	0.636	0.426599
Number of ramets	1	0.0204	0.02044	2.107	0.149111
Number of leaves	1	0.0005	0.00054	0.055	0.814358
Leaf area	**1**	**0.0786**	**0.07862**	**8.107**	**0.005162**
Nutrients:Species	**4**	**0.2275**	**0.05687**	**5.864**	**0.000235**
Residuals	124	1.2026	0.00970		

Leaf production

The rate of leaf production fluctuated throughout the duration of the study under both high and low nutrients (Figure 4.10). In both high and low nutrient conditions, leaf production was accelerated in the last 3 weeks of the experiment (Figure 4.10). There was significantly higher leaf production in the mixed treatment under high nutrients by the end of the experiment (Figure 4.10). The interaction between nutrients and herbivory in the mixed treatment was significant (Table 4.4). Leaf production was similar across herbivory treatments under low nutrients at the end of the experiment (Figure 4.10).

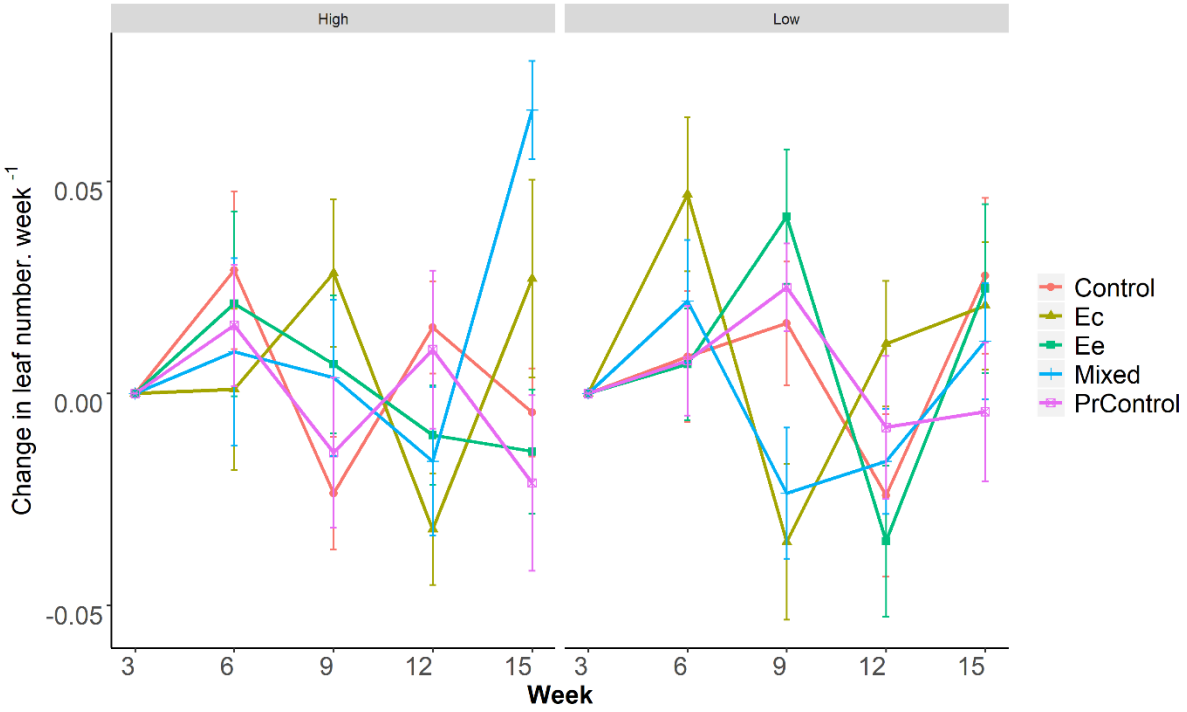

Figure 4.10: Rate of leaf production at high and low nutrients under different herbivory levels (Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*). Error bars represent standard error.

Table 4.4: Summary of the response of leaf production to the effect of nutrients, herbivory (Species), temperature and plant parameters. Values in bold are significant.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.788e-01	8.888e-01	0.201	0.84088
NutrientsLow	3.310e-02	3.665e-02	0.903	0.36819
SpeciesEc	3.321e-02	2.516e-02	1.320	0.18934
SpeciesEe	-1.254e-2	2.456e-02	0.510	0.61071
SpeciesMixed	**7.089e-02**	**2.482e-02**	**2.856**	**0.00504**
SpeciesPrControl	-1.989e-2	2.527e-02	0.787	0.43278
Temperature	-2.074e-2	1.835e-02	-1.131	0.26043
Conductivity	-2.888e-5	2.337e-04	0.124	0.90185
Leaf area	-1.340e-3	5.256e-02	0.025	0.97970
Number of ramets	-4.919e-3	3.035e-02	0.162	0.87151
Leaf length	-2.864e-2	5.829e-02	0.491	0.62403
NutrientsLow:SpeciesEc	-4.146e-2	3.524e-02	-1.176	0.24168
NutrientsLow:SpeciesEe	7.753e-03	3.554e-02	0.218	0.82766
NutrientsLow:SpeciesMixed	**-8.923e-2**	**3.697e-02**	**-2.413**	**0.01727**
NutrientsLow:SpeciesPrControl	-1.064e-2	3.552e-02	0.299	0.76510
AIC	-355.43			
DF	139			
Dispersion	0.409			

Leaf area

Leaf size is an important determinant in plant photosynthetic performance where the total surface area determines the amount of light captured. Leaf area was significantly affected by the length of the petiole and conductivity (Table 4.5). In the high nutrient conditions, plants with insects on them initially had slow rates of change leaf size in the first 3 weeks after inoculation (Figure 4.11). Thereafter, there were fluctuations in the rates of change in leaf size. Plants grown in low nutrients

had a significant decline in the rate of change in leaf size from week 6 until the end of the experiment (Figure 4.11; Table 4.5). There were no significant differences in the rate of change in leaf size between the herbivory treatments (Figure 4.11). The interaction of herbivory and nutrients was not significant (Table 4.5). Ambient temperature had a nearly significant effect on leaf size (Table 4.5). There was a positive and significant relationship between petiole length and leaf area under high and low nutrients ($R^2 = 0.51$, $P < 0.0001$; $R^2 = 0.4$, $P < 0.0001$).

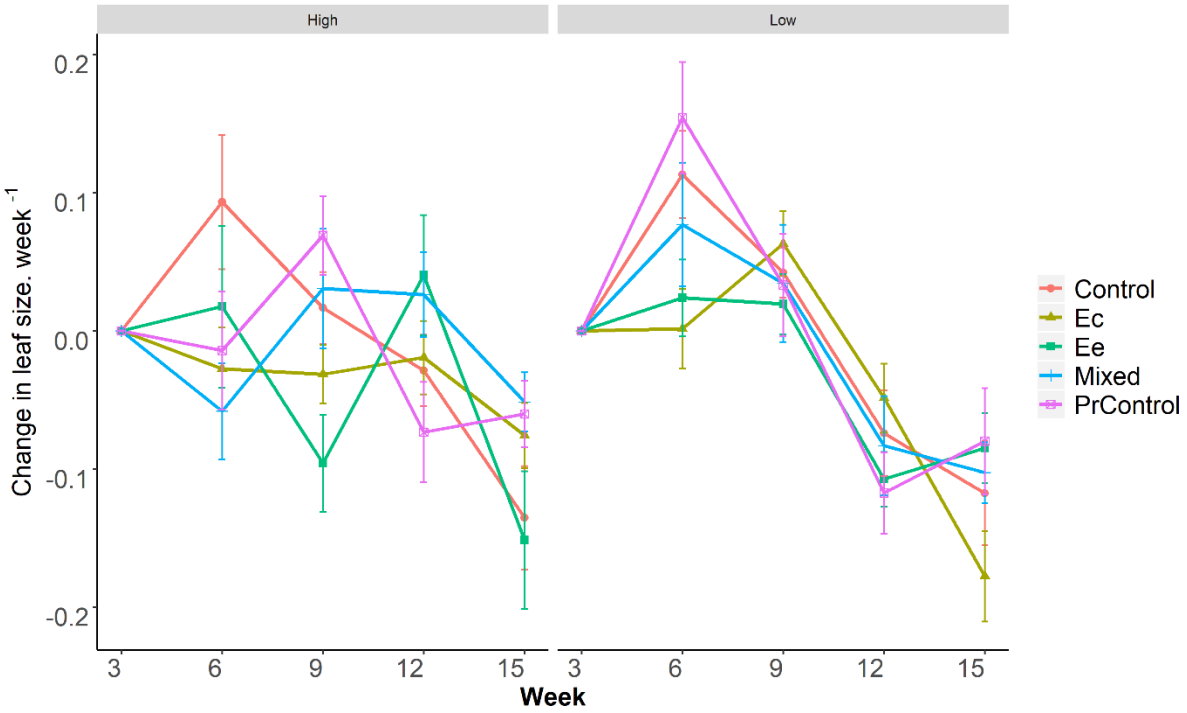

Figure 4.11:Changes in leaf sizes at high and low nutrient conditions, subject to herbivory treatments: (Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*). Error bars represent standard error.

Table 4.5: Response of leaf size to nutrients, herbivory (Species) and environmental factors. Ec = *Eccritotarsus catarinensis*, Ee= *Eccritotarsus eichhorniae*, ProControl = procedural control. Values in bold are significant.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	**-3.9422518**	**1.4768582**	**-2.669**	**0.008617**
NutrientsLow	**0.1550469**	**0.0612546**	**-2.531**	**0.012618**
SpeciesEc	0.0314981	0.0431930	0.729	0.467229
SpeciesEe	0.0020102	0.0420080	0.048	0.961911
SpeciesMixed	0.0626697	0.0434208	1.443	0.151455
SpeciesPrControl	0.0727370	0.0427900	1.700	0.091664
Temperature	0.0602990	0.0310351	1.943	0.054291
Conductivity	**0.0014600**	**0.0003771**	**3.871**	**0.000174**
Water temperature	0.0367097	0.0431168	0.851	0.396187
Number of ramets	0.0444028	0.0517015	0.859	0.392091
Number of leaves	0.0039111	0.1534206	0.025	0.979703
Petiole length	**0.2336774**	**0.0974528**	**2.398**	**0.017983**
NutrientsLow:SpeciesEc	0.0963970	0.0599219	-1.609	0.110223
NutrientsLow:SpeciesEe	0.0181606	0.0607045	0.299	0.765316
NutrientsLow:SpeciesMixed	0.0208192	0.0646072	0.322	0.747812
NutrientsLow:SpeciesPrControl	0.0760886	0.0603152	-1.262	0.209491
AIC	-205.27			
DF	139			
Dispersion	0.11			

Ramet production

Ramet production was significantly affected by temperature and conductivity (Table 4.6). All other continuous and discrete predictors had no significant effect on the production of ramets (Table 4.6). Under low nutrient conditions, *E. eichhorniae* and the mixed treatment had significantly lower rates of ramet production compared to the control (Figure 4.12). There were no significant differences in ramet production under high and low nutrients (Figure 4.12). However, there was a significant effect of the interaction between nutrients and the herbivory treatments (Table 4.6). There was a weak, negative relationship between temperature and ramet production under high and low nutrients (R^2 = -0.31, $P < 0.0001$; R^2 = -0.22, $P < 0.0001$) as the mean temperatures (<20 ºC) were below the optimal growth conditions (Figure 4.4).

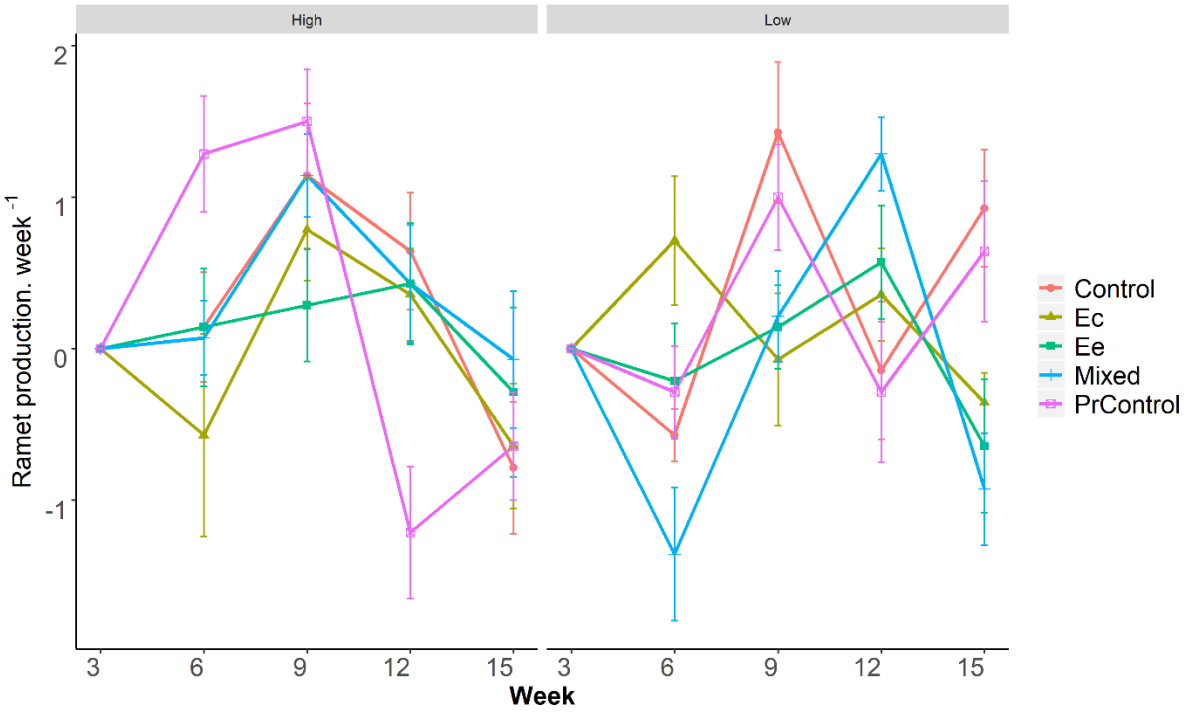

Figure 4.12: Changes in the rate of ramet production over the course of the experiment under high and low nutrients subject to different herbivory treatments. Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*. Error bars denote standard error.

Table 4.6: Summary of the effects of nutrients, herbivory (Species), plant parameters and temperature on ramet production. Values in bold are significant

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	5.9610253	2.5750308	2.315	0.0223
NutrientsLow	**0.2741335**	**0.1059649**	**2.587**	**0.0108**
SpeciesEc	0.0943127	0.0744819	1.266	0.2078
SpeciesEe	0.0327556	0.0726907	0.451	0.6531
SpeciesMixed	0.0527163	0.0756773	0.697	0.4874
SpeciesPrControl	0.0216575	0.0749370	0.289	0.7731
Temperature	**0.1298335**	**0.0532983**	**-2.436**	**0.0163**
Conductivity	**0.0013101**	**0.0006814**	**-1.923**	**0.0500**
Leaf area	0.1331703	0.1550600	0.859	0.3921
Number of Leaves	0.0430575	0.2656667	0.162	0.8715
Leaf length	0.2860479	0.1707156	-1.676	0.0963
NutrientsLow:SpeciesEc	0.1782735	0.1036208	-1.720	0.0878
NutrientsLow:SpeciesEe	**0.2051232**	**0.1035403**	**-1.981**	**0.0498**
NutrientsLow:SpeciesMixed	**0.2230649**	**0.1101268**	**-2.026**	**0.0450**
NutrientsLow:SpeciesPrControl	0.0267717	0.1050947	0.255	0.7993
AIC	-50.7			
DF	139			
Dispersion	0.358			

4.5.5 Plant biomass measures

There were significant differences in plant leaf biomass with nutrients (Figure 4.13, Table 4.7). The interaction between nutrients and herbivory was significant for both *E. catarinensis* and mixed treatments under low nutrients (Table 4.7). There was significantly more biomass under high nutrients in the *E. catarinensis* treatment compared to either the control or the procedural control, however, there were no significant differences with neither mixed nor *E. eichhorniae* treatments. Nutrients significantly affected plant biomass, where there was significantly less biomass in the low nutrient treatments (Figure 4.13, Table 4.7). Ambient temperature had a significant effect on biomass of the emergent plant parts (Table 4.7).

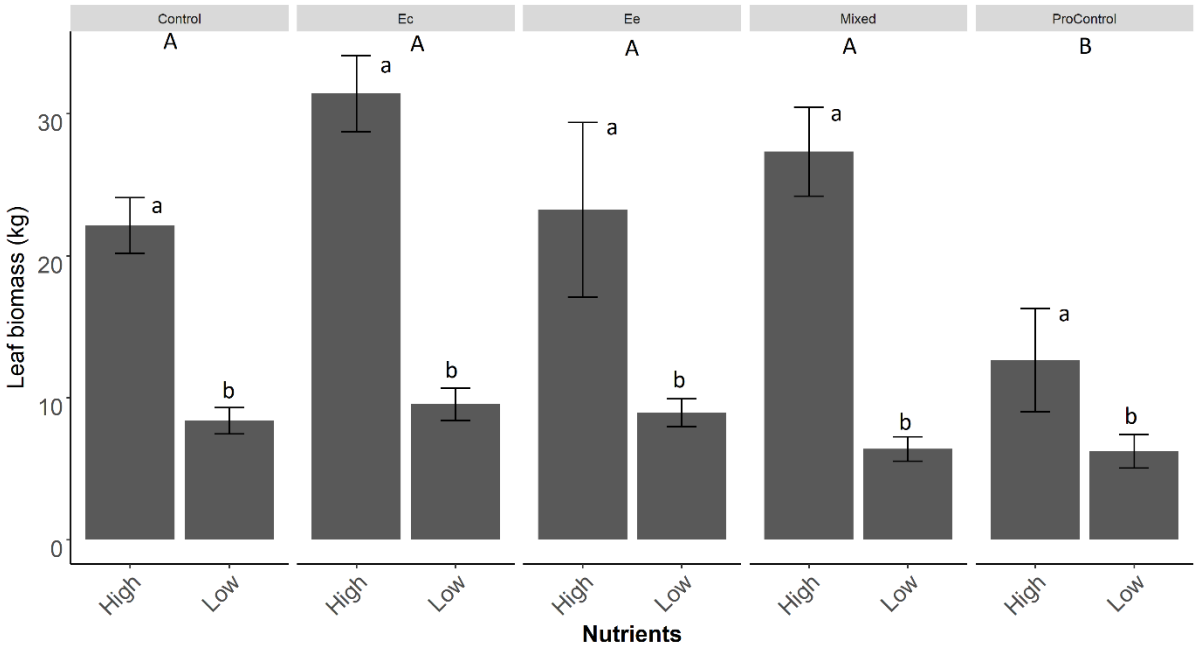

Figure 4.13: Mean biomass measures of emergent plant parts, faceted according to herbivory treatments subject to high and low nutrients. Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*, ProControl = procedural control. Error bars denote starndard error, while means with similar letters are not different.

Table 4.7: Differences in the biomass of the emergent plant parts. Ec= *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*, ProControl = procedural control. Values in bold are significant.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	147.840791	94.812395	1.559	0.12609
Nutrients-Low	-9.285677	5.062427	-1.834	0.07339
Species-Ec	**15.297967**	**4.454306**	**3.434**	**0.00131**
Species-Ee	7.777369	4.348007	1.789	0.08055
Species-Mixed	**11.311547**	**4.327684**	**2.614**	**0.01221**
Species-ProControl	3.167019	4.717299	0.671	0.50550
Ambient temperature	**-5.996156**	**2.978482**	**-2.013**	**0.05004**
Roots	0.111181	0.266927	0.417	0.67905
Dead matter	0.042370	0.196268	0.216	0.83008
Conductivity	0.005191	0.026575	0.195	0.84604
Oxygen	0.223978	0.517170	0.433	0.66707
Water temperature	0.681972	1.321386	0.516	0.60837
NutrientsLow:SpeciesEc	**-14.324562**	**5.998610**	**-2.388**	**0.02130**
NutrientsLow:SpeciesEe	-7.225784	6.162814	-1.172	0.24731
NutrientsLow:SpeciesMixed	**-13.256471**	**6.100306**	**-2.173**	**0.03520**
NutrientsLow:SpeciesProControl	-5.199607	6.368441	0.816	0.41863
AIC	419.66			
DF	59			
Dispersion	49.400			

Biomass of submerged plant parts

Root biomass was significantly greater in the procedural control compared to the control, *E. catarinensis* and *E. eichhorniae* under high nutrients (Figure 4.14; Table 4.8). Root biomass was similar for all the other treatments under both high and low nutrients (Figure 4.14; Table 4.8).. There was a significant effect of temperature on root biomass (Table 4.8).

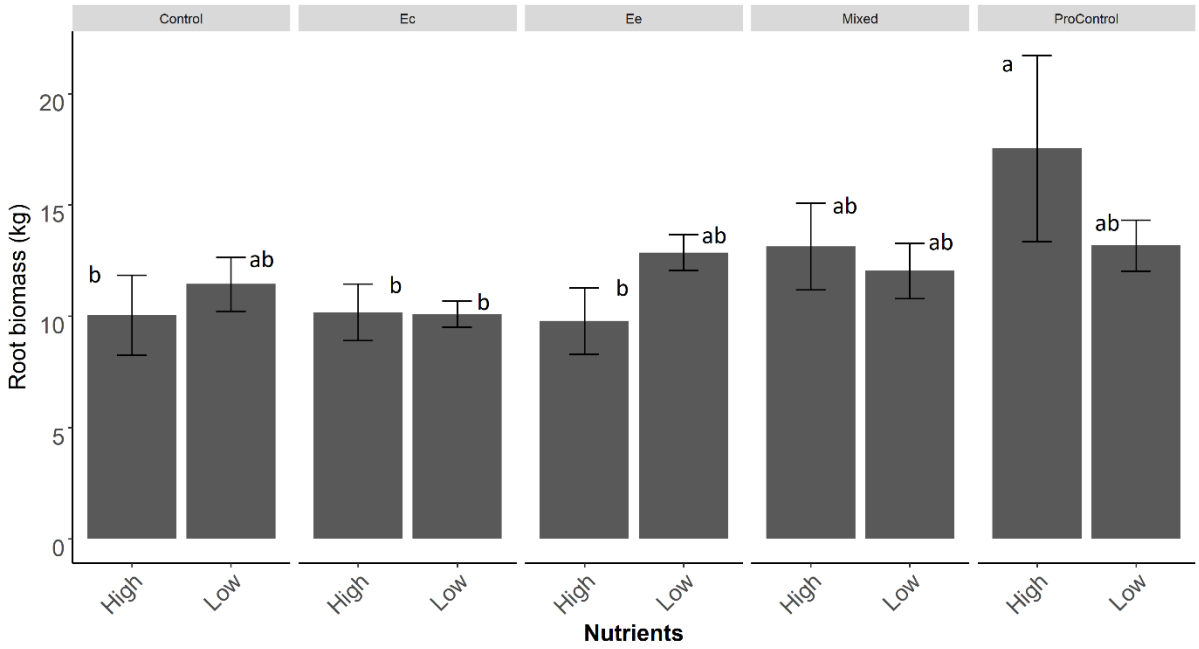

Figure 4.14: Mean root biomass at the end of the experiment faceted according to herbivory treatments under high and low nutrients. Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*, ProControl = procedural control. Error bars denote standard error and means with similar letters are different.

Table 4.8: The effect of biotic and abiotic factors on root biomass accumulation. Species represents herbivory treatment where Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*, ProControl = procedural treatment. Values in bold are significant.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-5.8476925	2.1440336	-2.727	0.00895
NutrientsLow	**0.3371112**	**0.1645045**	**2.049**	**0.04604**
SpeciesEc	0.0504965	0.1602292	0.315	0.75404
SpeciesEe	0.0181759	0.1610607	0.113	0.91063
SpeciesMixed	0.2960663	0.1643744	1.801	0.07809
SpeciesProControl	**0.5157779**	**0.1634737**	**3.155**	**0.00280**
Temperature	**0.3090559**	**0.1211283**	**2.551**	**0.01404**
Conductivity	0.0002962	0.0006836	0.433	0.66681
NutrientsLow:SpeciesEc	0.4026164	0.2348018	-1.715	0.09299
AIC	29.334			
DF	59			

Dispersion	0.07

Dead material biomass accumulation

There was significantly less dead material in the *E. eichhorniae* treatment under high nutrients compared to the control (Table 4.9; Figure 4.15). The amount of dead material was significantly influenced by ambient temperature (Table 4.9). There were no differences in dead material between the herbivory treatments under low nutrients, nor was there an influence of the rest of the variables (Figure 4.15; Table 4.9).

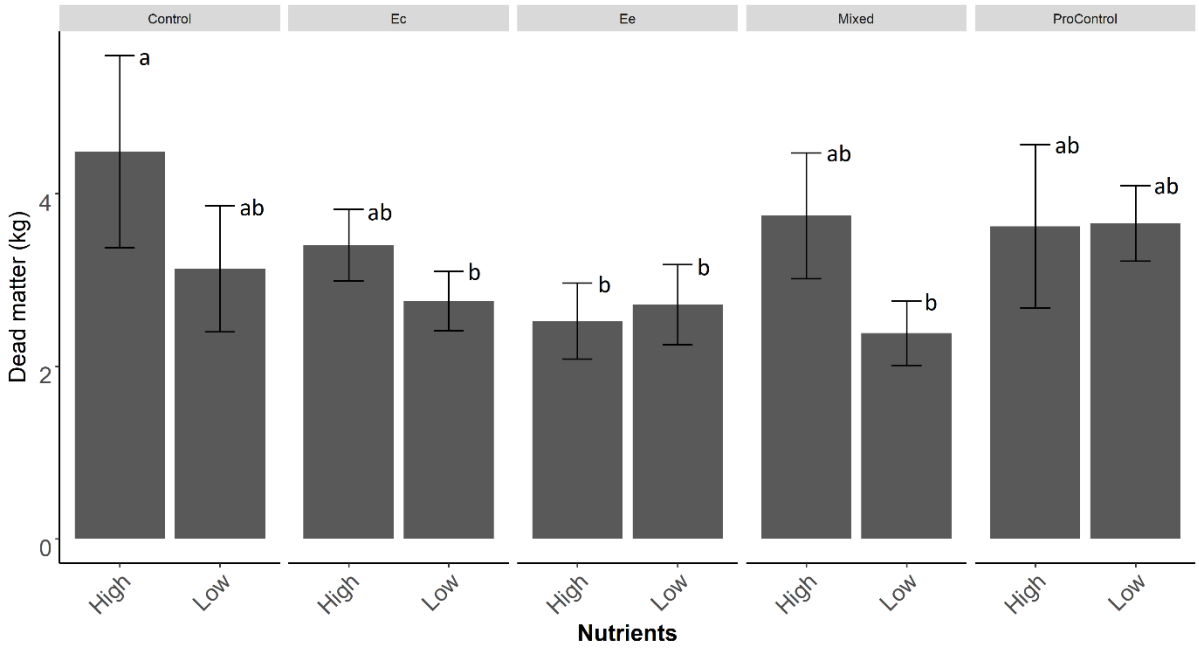

Figure 4.15: The accumulation of dead material on the plants at the end of the experiment under the different herbivory and nutrient treatments. Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*, ProControl = procedural control. Error bars represent standard error, while means with similar letters are not significantly different.

Table 4.9: The influence of biotic and abiotic factors on biomass accumulation of dead material. Ec = *Eccritotarsus catarinensis*, Ee = *Eccritotarsus eichhorniae*, ProControl = procedural control. Values in bold are significant.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-16.332810	11.451317	-1.426	0.1604
NutrientsLow	0.895561	0.878621	-1.019	0.3133
SpeciesEc	-1.210939	0.855787	-1.415	0.1637
SpeciesEe	**-1.953040**	**0.860228**	**-2.270**	**0.0278**
SpeciesMixed	0.914972	0.877926	-1.042	0.3027
SpeciesProControl	-1.071487	0.873116	-1.227	0.2259
Temperature	**1.408950**	**0.646948**	**2.178**	**0.0345**
Conductivity	0.001005	0.003651	0.275	0.7843
NutrientsLow:SpeciesEc	0.149455	1.254080	0.119	0.9056
NutrientsLow:SpeciesEe	0.422753	1.359832	0.311	0.7573
NutrientsLow:SpeciesMixed	0.446608	1.312383	0.340	0.7351
NutrientsLow:SpeciesProControl	1.139093	1.249375	0.912	0.3666
AIC	230.38			
DF	59			
Dispersion	2.18			

4.6 Discussion

The extent to which some weed species are problematic necessitates the introduction of several biocontrol agents. The notion behind releasing multiple agents follows the cumulative stress hypothesis where each successive agent adds to the stress inflicted by an already established agent (Myers, 1984). In such instances, there may be some interactions between the agents (Strong *et al.*, 1984; Price, 2000). A target plant will often offer several niches, which each individual species occupies (Marlin *et al.*, 2013), however, in this study, the two insects occupy the same niche. Therefore, the way biocontrol agents interact often has a bearing on their performance in controlling

the weeds. In this study, I explored the performance of the two species, in both single and multispecies contexts.

Insect populations and *feeding*

Insect populations in this study were similar in the two single species treatments. The similarities were possibly due to favourable temperature and nutrient conditions prevailing in the controlled set up. In high temperatures, *E. eichhorniae* has been reported to do well, mostly because this species is more warm adapted due to its evolutionary history in a tropical environment (Paterson *et al.*, 2019). The mean temperatures in the greenhouse (17 - 20 ºC) were however in the optimal temperature range of the native distribution of *E. catarinensis* (16 - 26 ºC). Although *E. eichhorniae* evolved under a warmer environment (26 - 27 ºC), it increased its population at rates similar to *E. catarinensis* despite the mean temperatures in the greenhouse. There is a likelihood that after almost two decades of being reared in a greenhouse across several seasons, *E. eichhorniae* has adapted to those temperatures, in a similar way to the more thermally plastic *E. catarinensis*. At the latter stage of the study, insect populations increased as the winter season was ending. This is likely because the plants were now recovering from slowed growth during winter and vigorously growing (Casotti and Bradley, 1991; Staley *et al.*, 2011). At the beginning of the growing season, nitrogen is mobilised and allocated to growing parts of plants and this makes nitrogen more available for herbivores (Roháček *et al.*, 2008; Staley *et al.*, 2011). It is interesting that in the multispecies treatment, there were low populations of insects. Although the temperatures and nutrient conditions were similar, populations were low. It is likely that although the two species are reproductively isolated, there might have been some mating interference between the two leading to low populations. Mnguni (2019) investigated the mating preferences of the two species and, showed that there were incidences where male *E. eichhorniae* and female *E. catarinensis* mated, and this is a possible case of interference that may have occurred in the current study. The mating between the species has been

reported not to produce offspring, while mating between female *E. eichhorniae* and male *E. catarinensis* resulted in very few offspring (Paterson *et al.*, 2016). This could have occurred in the current study leading to lower populations in the mixed treatment. Insect populations also inform how the biocontrol effort will progress where higher agent populations, result in greater levels of damage and control on *P. crassipes* (Coetzee *et al.*, 2007a; Center and Dray, 2010). Since low populations were recorded in the mixed treatment, feeding damage was low as a result.

There was greater insect damage in the single species treatments, and the rates of damage recorded in the single species treatments were similar, as both species inflicted similar levels of damage. However, in the mixed species treatment, there was less damage inflicted due to low populations and potential competitive interaction between the two. In a meta-analysis, Stephens *et al.* (2013) reported that there are possible antagonistic effects when two species of biocontrol agents occupy the same niche. A similar result was reported by Weyl and Hill (2012), where there was a marked reduction in the number of feeding scars when the two weevils, *N. eichhorniae* and *N. bruchi* were coexisting on *P. crassipes*. In this study, there is insufficient evidence to suggest resource competion between the two mirids since there were populations in the mixed treatment. If resource competition occurred between the two species, it is likely that it occurred at the beginning of the study, hence resulting in the low populations recorded. These results however, corresponded with the population patterns where fewer insects in the mixed treatment, resulted in less feeding damage. This is likely the case because the two insects occupy the same niche, and therefore, may be competing for the same resource. This kind of negative interaction between two biocontrol agents was classified as an inhibitory interaction (Hatcher, 1995; Hatcher *et al.*, 2008) where less damage is inflicted when two agents are combined compared to when the weaker one acts alone (Hatcher, 1995). The amount of feeding was also related to the temperatures in the greenhouse. Insect feeding has been reported to

increase with increase in temperature until a maximum threshold is reached depending on plant quality (Ismail *et al.*, 2017).

Plant performance

Plant performance is a measure of plant photosynthetic efficiency. In biocontrol, agents released on plants function to reduce plant productivity and growth and, in some cases, cause mortality. Some insects have very subtle visual effects on the target plant but may have physiological impacts, therefore, use of plant performance index via chlorophyll fluorescence is used to assess damage. Sap suckers like *Megamelus scutellaris* inflict very subtle damage that is difficult to detect visually (Miller *et al.*, 2019). Miller *et al.*, 2019 demonstrated that plant performance was significantly reduced at high feeding intensities by *M. scutellaris*, especially under low nutrient conditions. *Eccritotarsus* spp. are also sap suckers, however chlorosis is evident from their feeding. In the current study, plant performance was increased under high nutrient conditions despite insect feeding. It is likely that plants were able to compensate for the loss of chlorophyll. This is consistent with reports by Miller *et al.* (2019) that photosynthetic performance of *P. crassipes* under high nitrogen is not affected by herbivory. However, it has been shown that reduced photosynthetic performance due to *E. catarinensis* feeding does not necessarily translate to reduced biomass or other plant parameters (Marlin *et al.*, 2013). A study by Ripley *et al.* (2006) showed that feeding by *N. eichhorniae* significantly reduced *P. crassipes* photosynthetic rates. We see a similar pattern in this study where there was a decline in plant performance throughout the study, with greater performance in the insect-free procedural control. In this study, plant performance declined significantly over the duration of the study period. This is likely due to the seasonal effects under which the experiment was conducted, where plant growth and performance is slowed down during winter despite favourable nutrient conditions (Pieterse, 1978; Téllez *et al.*, 2008). The current study further showed that over a prolonged period, when *P. crassipes* is subjected to herbivory, plant performance is likely

to be reduced both under high and low nutrients. Similar to other plant growth responses, plant performance improved towards the end of the study, again showing the importance of seasonality on plant growth as the plants recovered towards the end of winter season, a similar scenario observed in Chapter 3. There was a marked increase in performance in the eutrophic conditions, thus highlighting the importance of nutrients to plant performance (Ripley *et al.*, 2006). Interestingly, in the procedural control, the lowest plant performance occurred in both the low and high nutrients, largely because this treatment was not covered by mesh cages. Mesh netting has a light interception effect which reduces the amount of incident radiation reaching the plants. It is therefore likely that the other treatments were forced to increase efficiency due to a combination of stressors, herbivory and reduced incident radiation.

Plant growth response to herbivory and *nutrients*

The presence of *E. eichhorniae* significantly suppressed rate of plant height increase, and this was shown also in the mixed treatment. Acting alone, *E. catarinensis* failed to suppress plant growth. The results therefore show that *E. eichhorniae* was the superior agent in slowing plant growth. Similarly, plant reproduction was significantly slowed down in the *E. eichhorniae* and mixed treatments, although in all treatments there was a reduced rate of vegetative reproduction. There was a synergistic relationship between the two species, where in the mixed treatment, the two species managed to suppress both plant growth and reproduction. However, this contradicted the feeding results that showed that there was less feeding when the two species were combined. The study therefore showed that the two species are effective at slowing down the rate of spread of the plant as there was a continuous decline in the rate of ramet production throughout the study. Feeding by *E. catarinensis* was previously reported to reduce the number of ramets produced (Coetzee *et al.*, 2007a), similarly in this study, *E. eichhorniae* reduced ramet production. Furthermore, nutrients also played a significant role where in low nutrient conditions, there was a greater effect of the insects on

reducing the rate of spread. Previous studies have highlighted that under high nutrients, there is exponential growth of *P. crassipes* which allows it to overcome herbivory (Heard and Winterton, 2000; Coetzee *et al.*, 2007a; Bownes *et al.*, 2010). This result therefore has management implications where there is need to reduce the amount of nutrients coming into infested systems in order to complement the impact of the biocontrol effort. In most cases where there is variable biocontrol success, *P. crassipes* usually has a constant supply of nutrients which hence, the plant escapes the effects of herbivory by compensating for the damage (Coetzee *et al.*, 2005).

Interestingly, in the mixed treatment, there was a higher rate of leaf production compared to when the species were separate. This is similar to rates of feeding damage where there was less feeding damage in the mixed treatment. Coetzee *et al.* (2003) reported that, at high feeding intensities, there is reduced leaf production by *P. crassipes.* This report consolidates the findings of this study where, low feeding intensity led to increased leaf production. The effects of the low grazing intensity reported in this current study is similar to what was reported by Dyer *et al.* (1986)where low grazing intensity potentially increase plant primary productivity. The higher rates of leaf increase could be an indicator of plants growth response to herbivory where feeding induced plants to produce new shoots faster. A mathematical model postulated that after a grazing episode, plant physiological responses may lead to greater interaction between the plants and the environment (Hilbert *et al.*, 1981). This model theorised that physiological cues induced by herbivory may lead to plants taking up more nutrients in order to increase productivity. In the case of *P. crassipes*, under high nutrient conditions, there is increased growth even in the presence of biocontrol agents (Coetzee *et al.*, 2005). The slowest rate of leaf production was again recorded in the *E. eichhorniae* treatment, and this was similar to what was observed in the plant height where the species slowed the rate of plant height increase. This consolidates the notion that this species is the superior agent in a set of conditions. Biocontrol agents have been shown to effectively reduced certain aspects of plant growth, but not

holistically halt plant growth in all aspects (Marlin *et al.*, 2013). Despite the increase in the rate of insect population growth towards the end of the experiment, damage levels were low, and this would explain why there was an increase in the rate of leaf production. This suggests that although the insect population increased, it did not reach sufficient levels to halt leaf production. This is therefore an indication of interference between these two agents as there is reduced performance in more than one aspect.

Plant biomass in the presence of herbivores

Plant biomass is another measure of plant productivity aside from growth parameters like plant height and ramet production. In this study, plant biomass was significantly influenced by nutrients as well as temperature, however, herbivory did not impact biomass accumulation. High nutrient inputs in a system facilitate biomass accumulation by *P. crassipes* (Heard and Winterton, 2000; Coetzee *et al.*, 2005; Ripley *et al.*, 2006), and in this study it was demonstrated that nutrients have a significant role in biomass accumulation. These effects were most pronounced in the emergent plant parts where there was a lot of leaf biomass in high nutrient conditions. In eutrophic systems, *P. crassipes* develops more emergent plant parts compared to the submerged (Reddy *et al.*, 1989; Ripley *et al.*, 2006). In the low nutrient conditions, the root to shoot ratio was proportional as the plants invested in root development in order to maximize nutrient uptake. Dead material was not related to herbivory, rather plants parts were undergoing senescence naturally. The damage caused by *E. catarinensis* has been reported to be superficial and rarely affects plant biomass, although it reduces other parameters like height and ramet production (Marlin *et al.*, 2013). Therefore, the results in the current study are consistent with previous findings where the mirid does not reduce plant biomass.

4.7 Conclusion

In this study, I showed that the success of the two biocontrol agents is dependent on the nutrient status of the systems where they are released. In order to effectively reduce the extent of *P. crassipes*, there must be catchment level management interventions. There is need for stricter regulation of effluent emission into our aquatic systems if control of aquatic weeds like *P. crassipes* is to be achieved (Coetzee and Hill, 2012). In a closed setup, *E. eichhorniae* proved to be superior to *E. catarinensis*, probably due to more conducive environmental conditions. However, this is not what was shown in the field in Chapter 2 where only *E. catarinensis* has established in South Africa. This is could be because *E. eichhorniae* performs better in warmer regions, which is not the case in South Africa, where climate is less favourable to this species. Furthermore, future releases of the insects should be separate as it was shown that the two species perform well singly. Releases of *E. eichhorniae* should target the warmest regions of South Africa. The interaction of the two still needs to be investigated further in order to ascertain if they have synergistic or antagonistic effects on each other. The time span of the experiment was possibly too short to come up with conclusive results, plus the season during which the experiment was conducted could have confounded the results since it was conducted from late autumn throughout winter. A future experiment will therefore ideally traverse all seasons and thus give a good representation of the actual dynamics at play.

Chapter 5 : Discussion

5.1 Introduction

The outcomes of the biocontrol programme against *P. crassipes* in South Africa have been varied and, in some cases, challenging (Hill and Coetzee, 2017). The intervention measures implemented have been very successful in certain areas and less so in others. However, biocontrol has still made a significant contribution to the control of this plant where multiple biocontrol agents have, in most cases, reduced the rate of growth and spread of the plant, and thus reduced reliance on other control methods (Center *et al.*, 2002; Winston *et al.*, 2014). However, *P. crassipes* is resilient and still poses a threat to South African waters despite the suite of biocontrol agents that have been released. Post-release evaluations are an essential component of invasive alien plant management programmes using biocontrol (Beanland *et al.*, 2003; Morin *et al.*, 2009), and without post-release evaluations, the reasons for the outcomes of biocontrol efforts are unknown (Schulz *et al.*, 2019). The post-release evaluation in the current study gave insights into the establishment, persistence and population dynamics of the two mirid species released in South Africa. The main focus of this study was to determine the distribution of the two mirids, *E. catarinensis* and *E. eichhorniae* in South Africa and to investigate the factors that have resulted in this distribution, and the results of the studies conducted in this thesis are contextualized below.

5.2 Value of molecular techniques in biocontrol

The incidence of cryptic species in biocontrol is not unique to this study. However, misidentification of cryptic species may have unintended consequences for biocontrol. The lack of clear, distinguishing morphological characteristics in species can lead to difficulties in identifying species (Antonini *et al.*, 2007). It is therefore important to select the correct candidate for each target plant in order to achieve the best results and prevent any possible non-target effects. It is also important in

these cases to know which species is contributing to the control of a weed. Therefore, owing to these difficulties, molecular techniques have become very useful in the identification of cryptic species (Antonini *et al.*, 2008; Gaskin *et al.*, 2011). Molecular techniques are currently broadly applied in biocontrol in determining the origins of target weeds; which helps with matching control agents with the target (Antonini *et al.*, 2008; Gaskin *et al.*, 2011; Paterson *et al.*, 2016). However, they are less often used in the identification of the agents, which largely relies on traditional morphological taxonomic identifications. In the current study, molecular techniques were necessary in distinguishing between the two mirid species, thereby determining their distribution in South Africa. Through the use of molecular techniques, I discovered that only *E. catarinensis* was established (Chapter 2). This discovery was despite initial expectations that both species would be established in the field due to the number of releases made over the years, and their performance in the laboratory and mass-rearing station.

In cases where there are new introductions or re-introductions of biocontrol agents, there needs to be rigorous screening of these agents via molecular means, if necessary, to verify their identity. This will be important in minimizing the risk of releasing a cryptic species that may have non-target effects on native flora. For example, Gaskin *et al.* (2011) reported that in the biocontrol effort against yellow starthistle, *Centaurea solstitialis* Linnaeus (Asteraceae); the survey for the potential agent was difficult because of a species complex of flea beetles. The stem-boring flea beetle, *Psylliodes* cf. *chalcomerus* Berthold (Coleoptera: Chrysomelidae) was identified as the potential control agent, and a survey spanning from Spain to Russia was conducted to collect specimens across 10 populations (Gaskin *et al.*, 2011). DNA extractions and sequencing were conducted on the specimens using the mitochondrial DNA markers as a reference point, and the results showed genetic differentiation separating the populations into three groups, where two were specialist herbivores on certain plants, while the third group were largely generalists (Gaskin *et al.*, 2011). These differences were important in the host specificity tests that were conducted as this would have explained the different feeding

behaviours of the three groups even though they were morphologically similar (Gaskin *et al.*, 2011). In another study in the yellow star thistle programme, molecular techniques were employed in the identification of immature stages of the biocontrol agents where *P. chalcomerus* and *Ceratapion basicorne* Illiger (Coleoptera: Apionidae) larvae and pupae were found in plants but could not be reared to adulthood (Antonini *et al.*, 2007). These examples illustrate the value of incorporating molecular studies in weed biocontrol for not only target weed identification, but also to tease out agent population genetics and cryptic species.

5.3 Determining insect distributions, establishment and impact

The establishment of only *E. catarinensis* was a surprising finding, especially because there have been repeated releases of *E. eichhorniae* at various sites across South Africa with different climates. Climate incompatibility is reported to be responsible for about 44% of the failures of establishment in biocontrol (Byrne *et al.*, 2003; Marchetto *et al.*, 2018). Thus climate, and in particular, low winter temperatures with frost (Chapter 3) might explain the lack of establishment of *E. eichhorniae*. The laboratory-based thermal physiology of these two species is well studied and it has been shown that the two species favour warm climates, while *E. catarinensis* was able to withstand slightly cooler temperatures (Coetzee *et al.*, 2007b; Griffith *et al.*, 2019; Paterson *et al.*, 2019; Porter *et al.*, 2019). Thus, there are sites in the north-east region of South Africa that might be more suitable and where *E. eichhorniae* has not been released in high numbers. It will be prudent in future to target these warm subtropical areas where there was a high establishment rate of *E. catarinensis*. In this thesis (Chapter 4), I showed that these two insects were able to build up similar populations under similar favourable conditions in the green house tunnel. There is potential that in the warmer regions of the country, *E. eichhorniae* may outperform *E. catarinensis* as it was shown that the former performed better in reducing some plant parameters (Chapter 4). However, those results were inconclusive due to challenges that occurred during the experiment. Furthermore, the study did not factor in all

seasons and therefore, these results only showed how the insects perform during winter when both plant growth and insect development was considerably slowed down.

It was also concerning that there was less than 50% establishment of *E. catarinensis* across the country (Chapter 2). *Eccritotarsus catarinensis* has been observed to favour riparian zones with good tree cover (M. Hill pers. comm.). Riparian vegetation has frost buffering functions and provides winter refugia for semi-aquatic insects (Dallas and Ross-Gillespie, 2015; Ramey and Richardson, 2017) and this is likely why *E. catarinensis* may favour such sites. In the current study, *E. catarinensis* was collected from both open and shaded sites, where 60% of the sites were shaded or had dense riparian vegetation. It is surprising that despite the large numbers of insects released, and in many cases with repeated releases, the insects still failed to establish. Some studies have recommended that for successful establishment to be attained, several, small releases should be carried out, while others suggest that a large single release is sufficient to attain establishment (Memmott *et al.*, 1996; Memmott *et al.*, 1998; Shea and Possingham, 2000; Shea *et al.*, 2006). Both these strategies were employed with the release of both *E. catarinensis* and *E. eichhorniae*, however with variable success.

Temperature was the major factor considered in this study and I showed that minimum temperature significantly influenced establishment. Another aspect that came out in this study was seasonality, where, as expected, seasonal cycles influenced both plant and insect populations. Many insects undergo winter diapause due to harsh temperatures as well as limited resources (Strong *et al.*, 1984). Similarly, in biocontrol, insect activity is severely constrained by winter when food resources are scarce, while other insects succumb to the direct effects of low temperatures (Pratt *et al.*, 2004; Johnson *et al.*, 2016). This is a major challenge for the biocontrol agents of *P. crassipes*, which mostly feed on the emergent plant parts. In areas where winter frosts occur, irrespective of water trophic status, leaf frosting in the exposed parts of the waterbody causes biocontrol agents to be stranded and mortality results due to lack of resources. Populations are severely reduced and may

only be found on small patches of within thermal/frost refugia where they overwinter (Johnson *et al.*, 2016). On *P. crassipes*, the weevils, *N. eichhorniae* and *N. bruchi* overwinter as third instar larvae in the root stalk of the plants (Wilson *et al.*, 2001), while *M. scutellaris* also overwinters as nymphs (Sosa *et al.*, 2006). The success of overwintering therefore determines the rate at which the insect populations recover at the onset of the growing season. In this study I showed that there is a long recovery phase between the plants and the insects (Chapter 3). Byrne *et al.* (2010) reported similar results on *P. crassipes* where insects lagged behind the plants by two months. A predictive model on the interaction between *Myriophylum spicatum* and a biocontrol agent, *Euhrychiopsis lecontei* in the USA, showed that in the warmer seasons, there were high populations of the agent with multiple generations (3-6 generations) (Miller *et al.*, 2011). They also showed that low initial populations resulted in a lag of several months before the agent populations reached the peak stocking rates after winter (Miller *et al.*, 2011). In the current study, I showed similar results where low populations after winter resulted in a two month lag phase before the mirid was able to build up to high and damaging populations (Chapter 3). To reduce the lag phase, it will be important to carry out augmentative releases at the onset of the growing season (spring) in order to boost insect populations.

Both climate and water nutrient status have a bearing on the establishment and performance of biocontrol agents as these factors also influence the habitat of the agents. *Eccritotarsus eichhorniae* had greater efficacy on plants growing in eutrophic conditions (Chapter 4). Ismail *et al.* (2017) reported that *E. catarinensis* performed well on high quality plants, but in the current study, *E. eichhorniae* was more effective at reducing plant growth compared to *E. catarinensis* where the plants were fertilized in the green house tunnel. It is possible that this could be transferred to the field, as the biocontrol effort of *Salvinia molesta* required plants with high levels of nitrogen to sustain large populations of the biocontrol agent *Cyrtobagous salviniae* (Room *et al.*, 1984; Room, and Thomas, 1986), successful biocontrol was achieved on Lake Moondarra in Australia only after nitrogen enrichment (Room *et al.*, 1989). However, in South Africa, infestations of *P. crassipes* grow

in eutrophic waters (Coetzee *et al.*, 2007a; Coetzee and Hill, 2012), so plant nutrition should not be problematic. At a number of eutrophic sites identified in this study, there were high numbers of biocontrol agents, however, their impact was nullified as *P. crassipes* is efficient at nutrient uptake and assimilation (Reddy *et al.*, 1990; Coetzee and Hill, 2012). But this still does not explain the establishment of *E. catarinenesis* at only half of the sites it has been released at, and the non-establishment of *E. eichhorniae*.

Short-term solutions such as herbicide application, which was recorded at several sites during this study, also lead to the reduced success of biocontrol (Center *et al.*, 1999). Hill *et al.* (2012) showed that *E. catarinenesis* was especially sensitive to herbicide application on *P. crassipes* and, might well have been the reason for the lack of persistence of this agent at a number of sites. Thus, the implementation of a more appropriate integrated management system is required to ensure that *E. catarinensis* can be more effective.

5.4 Insect-insect interactions

How many different species of agent that should be released on a target weed to effect control has puzzled biocontrol practitioners. Further, will introducing two species, such as *E. catarinensis* and *E. eichhorniae* that occupy the same niche on the plant have an additive effect? The cumulative stress hypothesis proposes that the addition of a subsequent biocontrol agent onto a system which has an already established agent has additive effects on the stress induced by herbivores (Stephens *et al.*, 2013; Schulz *et al.*, 2019). For example, in the biocontrol of spotted knapweed, the efficacy of two closely related control agents of knapweed, *Urophora affinis* Frauenfeld (Diptera: Tephritidae) and *U. quadrifasciata* Meigen (Diptera: Tephritidae) was tested. The two agents showed similar damage patterns separately and, when the two were in combination, 35% more damage was recorded, hence a complimentary effect was observed (Myers, 1984). The variable control in the temperate areas necessitated the release of more agents with the view of one subsequently controlling the plant

successfully. This scenario aligns with the lottery model where, with the addition of subsequent agents, it is hoped that one would be successful in controlling the target weed (Denoth *et al.*, 2002; Nordblom, 2003). However, Myers (2008) argues that one agent usually provides sufficient control on a target weed, similar to what was shown in the biocontrol of aquatic weeds in South Africa with the exception of *P. crassipes*.

In the biocontrol of *P. crassipes,* there is a lot of overlap between the agents as all of them use the leaf, with only the moth and weevil larvae additionally mining through the petioles. In this study, I investigated five biocontrol agents (the two *Eccritotarsus* species, the two *Neochetina* species and *M. scutellaris*) and their interaction with *P. crassipes*. Perhaps the disadvantage of the multiple agents on *P. crassipes* is the overlap in the area attacked. Agents with varied niches should provide a more broad herbivory pressure on the plant, where seed and flower feeders would reduce the amount of seed that goes into the system (Müller-Schärer and Schroeder, 1993; Stephens *et al.*, 2013). A study into the biocontrol of knapweeds in North America, showed that when a combination of agents was used, the co-existence of *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) and *U. quadrifasciata* resulted in an increase in the number of damaged seed heads of knapweed when compared to the effect of *U. quadrifasciata* on its own (Evans, 2016). Both of these insects are seed feeders and although there was some interspecific interaction, the overall result showed synergy between the two species. This, however, is different from what this study showed where one agent performed worse in combination with others (Chapters 3 and 4). Therefore, in the biocontrol of *P. crassipes*, interactions in the field between agents need to be investigated further in order to determine the best combinations of agents in order to reduce interference and maximize efficacy. Laboratory studies have shown complementary feeding by *M. scutellaris* and *E. catarinensis*, as well as *N. eichhorniae* and *E. catarinensis*, but the field studies in this thesis showed suppression of *E. catarinensis*.

Multispecies biocontrol agent releases create a community of insects on the plants, therefore, the individuals within that community are bound to interact (Denoth *et al.*, 2002; Seastedt, 2015). In addition to population growth and agent performance, interactions between the biocontrol agents were investigated in this study. I showed that the populations of *E. catarinensis* were suppressed in the presence of the weevils and *M. scutellaris* (Chapter 3). This is supported in the biocontrol of purple loosestrife in the USA, where it was shown that there was interference competition between three of the four agents. Foliar feeding by *Galerucella calmariensis* Linnaeus (Coleoptera: Chrysomelidae) and *G. pusilla* Duftschmidt (Coleoptera: Chrysomelidae) inhibited flower production, utilized by a third agent, *Nanophyes marmoratus* Goeze (Coleoptera: Curculionidae). This indirect competition led to the decline of *N. marmoratus*, thus reducing the efficacy of this insect. Similarly, in this study, weevil feeding also interfered with the mirids, leading to lower populations of *E. catarinensis*. However, this low population cannot only be attributed to the presence of the other insects, but also season. Because the other agents recovered faster than *E. catarinensis* after winter, the mirid was spatially outcompeted, with interference competition from the weevils and *M. scutellaris* taking place. In Benin, Ajuonu *et al.* (2007) showed that the mirid failed to establish when introduced to an area that had high levels of old weevil feeding scars. The two cryptic species here performed poorly when in combination, since they utilized a shared resource, with similar feeding habits. Other studies have shown that the release of multiple agents has the advantage of spreading the efficacy of the biocontrol over a wider geographic range as different agents perform differently in different areas (Impson *et al.*, 2008). Hence, in the biocontrol of *P. crassipes*, the use of multiple species with different thermal tolerances seeks to facilitate control in different climatic zones in South Africa.

Many weed biocontrol programmes have been successful with the introduction of a single species (Myers, 2008). The biocontrol of *Azolla filiculoides* was successful in South Africa with the release the weevil *Stenopulmus rufinasus* Gyllenhal (Coleoptera: Erirhinidae) (Hill *et al.*, 2004). The weevil

was released in 1997, and within three years had caused local extinction of the plant at most sites and the weed was no longer considered a problem. The success of the control of *A. filiculoides* may also be attributed to the thermal physiology of the agent which has a wide range of thermal tolerances (Mvandaba *et al.*, 2019). The biocontrol efforts against *S. molesta*, *Pistia stratiotes* and *M. aquaticum*, are all examples of successful biocontrol with single species in South Africa (Hill and Coetzee, 2017; Martin *et al.*, 2018). Therefore, the need to use multiple agents on *P. crassipes* testifies to the complexity of the threat posed by this plant.

The lack of niche differentiation between *M. scutellaris* and *E. catarinensis* in the field may have resulted in the delphacid excluding, or at least reducing the populations of *E. catarinesis*, as *M. scutellaris* is more cold adapted compared to *E. catarinensis* (May and Coetzee, 2013), and its populations increase more rapidly in spring. This warrants further investigation.

Winston *et al.* (2014) produced a catalogue of biocontrol agent releases across the world. The data were classified into three categories: single species, dual species (2 species per target) and multiple species (>2 species per target). The data were further divided into the aquatic and terrestrial environments. In South Africa, 135 biocontrol agents have been released against 59 plants, with 93 of the agents establishing on the target weeds. On aquatic weeds, 15 biocontrol agents have been released and of these, 10 have been released against a single target (*P. crassipes*), while the other five agents have been released on five different targets. Four biocontrol agents in the single species releases have been successful while for one, *Hydrellia egeriae* Walsh (Diptera: Ephydridae)) on *Egeria densa* Planch (Hydrocharitaceae), it is too early to tell as it is a recent release. However, despite multiple agents on *P. crassipes*, there has been variable success. The bulk of the biocontrol agent releases (120 species) were in the terrestrial environment where single species releases contributed 42%, while dual species accounted for 38% of the releases, with 20% of the releases being multispecies releases. The study further showed that 19 out of 33 (58%) established single species cases had moderate to heavy impact on their hosts, while 7 out of 18 (39%) in the dual

species setting, reported moderate to heavy impact. However, in the multispecies setting with the combined action of all agents involved; only 3 out of 11 (27%) of the cases showed moderate to heavy impact on their targets. Interestingly, in the multispecies examples, usually one agent in the suite of agents had impacts on the target weed, where in 6 out of 11 (55%) multispecies examples, a single agent showed the greatest impact. The results from the multispecies releases align with the lottery model where one agent out of a suite of agents has the greatest impact. This shows that in most cases, single species have been more successful in establishing and impacting weeds. Therefore, these data suggest that in many instances, only a single species is necessary to control a target weed. We can further conclude that there are exceptional cases where the target is problematic, for example in South Africa, 19 species of biocontrol agents established on *Lantana camara* and the problem still persists (Winston *et al.*, 2014; Schwarzländer *et al.*, 2018). Similarly, *P. crassipes* is still challenging in South Africa despite the 9 species of biocontrol agents released against it.

5.5 Conclusion

Long-term post release evaluation is vital in biocontrol as it gives a more accurate measure of the effect of biocontrol compared to short-term evaluations. A 10-year post-release evaluation on the biocontrol of *S. molesta* across the whole of South Africa showed a significant reduction in the extent of the plant that would have been missed had the evaluation period been shorter, or limited to a few sites (Martin *et al.*, 2018). In our study, the monitoring process was only three years long, which still is relatively short. Longer term surveys consider both the aspects of seasonal and possibly climate change, especially with the more pronounced effects of climate change which have been reported to impact biocontrol efforts (Samways *et al.*, 1999; Bale and Hayward, 2010). The need for post-release evaluation of biocontrol in New Zealand prompted the drafting of the National Assessment Protocol (Hayes, 2015). This protocol outlined the methodology for collecting field data

at regional and national levels. Data collection at this scale is similar to what was presented by Martin *et al.* (2018). However, the frequency of these monitoring efforts also needs to be determined, for example, should evaluation be carried out annually, biannually or every 5 years, or several times within a year. The frequency of these evaluations is usually subject to funding and resource availability but, should be based on the phenology of the target weed and the agent. Understandably there are funding constraints where funders are reluctant to invest money into a problem that "has been solved". Based on the results of this thesis, long-term monitoring should form an integral part of biocontrol, where my study gave insights on the distribution of the two cryptic species as well as seasonal population dynamics of biocontrol agents.

Methodologies for insect release in multispecies systems need to be developed and well defined. The methods needed to study the feeding mechanism of each species and predict how intense feeding by one species may affect the other species must be developed (Bale and Hayward, 2010; Catton *et al.*, 2016). These methods also need to be able to predict in which order to release the agents, similar to what was done with the control of the invasive *Acacia* in South Africa (Impson *et al.*, 2008). Therefore, the release of the agent that will reduce the rate of plant reproduction needed to be released first in order to slow down the reproduction of the plant; while the supplementary agent which destroyed the seed bank was ideally released later. This sequence ensured a reduction in the rate of plant dispersal and regeneration via seed. Furthermore, insect release technology should consider the time of releases. In Chapter 3 of this study, I showed that there is a consistent lag phase between plant regrowth and insect population recovery. I therefore suggest that it is necessary to bridge that gap using a few insect release strategies. For example, inundative releases of biocontrol agents late in the growing season may be enough to stress plants such that plant survival during winter is reduced, thereby removing the ability to regenerate in spring. Alternatively, releases can be carried out at the onset of the growing season for the insects to induce herbivory pressure on the plants as they regrow in order to suppress plant growth. Another strategy could be the use of the two

previously suggested strategies where the pre-winter releases reduce winter survival while the post-winter releases act on the regrowth that survived winter. Emphasis should also be placed on the strict control of water quality in order to successfully combat aquatic weeds, otherwise the resources utilized in research on control measures are wasted.

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