

**Post-Release Evaluation of *Megamelus scutellaris*
Berg. (Hemiptera: Delphacidae): A biological
control agent of water hyacinth *Eichhornia crassipes*
(Mart.) Solms-Laub (Pontederiaceae) in South
Africa**

A thesis submitted in the fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Of

Rhodes University

By

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Abstract

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laub. (Pontederiaceae) is a free-floating aquatic macrophyte from South America that was introduced to South Africa in the 1900s for its attractive ornamental flowers. The plant was classified as a serious invader in the country in the 1970s, eventually becoming the worst invasive aquatic plant in South Africa. Biological control is widely regarded as the most effective method of managing water hyacinth, as it is ecologically safe, cost-effective, and self-sustaining. To date, nine biological control agents have been released in South Africa against water hyacinth, including eight arthropods and a pathogen.

Due to the cumulative effects of highly eutrophic waterbodies, which mitigate the damage caused by biological control, and the cold winters which inhibit the rate of biological control agent population build up, South Africa currently has more biological control agents released on water hyacinth than anywhere else in the world. The need for a cold-tolerant agent that can reproduce and develop quickly, while still being damaging to water hyacinth in eutrophic systems, led to the introduction of the most recently released water hyacinth biological control agent, the planthopper *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), which was initially collected from Argentina.

This thesis formed the first post-release evaluation of *M. scutellaris* since its release in South Africa in 2013. It included a greenhouse experiment to measure the agent's feeding damage in relation to different nutrient levels and stocking rates, as well as a field component to evaluate both the post-winter recovery of *M. scutellaris*, and a nationwide survey to measure the establishment of the agent around the country in relation to climate, water quality, and plant health.

In the greenhouse experiment, the feeding damage was quantified using measurements of plant growth parameters and chlorophyll fluorometry. It was found that, like other biological control agents of water hyacinth, *M. scutellaris* was most damaging when released in high numbers on plants grown at medium nutrient levels, and less effective on plants grown at elevated nutrient levels.

A water hyacinth infestation on the Kubusi River was selected for the evaluation of the post-winter recovery of *M. scutellaris*. The Kubusi River is both the first site where *M. scutellaris* was released, and the coldest site where water hyacinth biological control agents have established successfully in South Africa. Monthly visits tracking seasonal plant health characteristics and agent population densities indicated that the populations of *M. scutellaris* were impacted most significantly by the season. Low temperatures led to the water hyacinth plants being of poor quality during the winter, which had a subsequent negative effect on the agent populations. The agents could only fully recover by late summer, which meant that the plants were without any significant biological control through the initial phases of the growing season, when they were most vulnerable, and a significant lag-phase occurred between the recovery of the plants and the recovery of the agent population after the winter bottleneck.

A survey of all sites where *M. scutellaris* had been released in South Africa yielded 16 sites where the agents had successfully established, having survived at least one full winter. Among these sites were four sites where the agents were found without them having been released, indicating that they can disperse unaided to new sites. The temperature was a major factor responsible for the success or failure of establishment, with very few agents surviving in the hot areas of South Africa or in areas with a high frost incidence. The density of *M. scutellaris* was higher in nutrient-rich water, and on plants with more leaves, suggesting that the quality of the plants also contributed to establishment.

The results of this thesis showed that *M. scutellaris* is able to establish successfully in South Africa, and that the agents are capable of causing significant damage to water hyacinth, making it a promising addition to the biological control programme. Novel methods of measuring subtle insect feeding damage in plants and quantifying agent populations are also discussed, along with suggestions for the future implementation of *M. scutellaris* in South Africa.

Acknowledgements

I would like to thank my supervisors, Distinguished Professor Martin Hill and Professor Julie Coetzee, for their support, comments on drafts of my thesis, and for inviting me to be a part of this project. I also thank the NRF, Working for Water, Rhodes University and the Centre for Biological Control (CBC) for funding the project and for the use of their facilities.

I also wish to express my thanks to these people, without whom the completion of this thesis would not have been possible:

- Mr. Zolile Maseko, for being there every step of the way in the field and in the office. Thank you for your mentorship and friendship, for answering my barrages of stats questions and for putting up with me on the road for so long.
- Mr. Lulama Poni, Ms. Maretha Boshoff, and the team at the Waainek Mass Rearing facility for helping me with my greenhouse experiments, assisting with data collection, and for maintaining the insect cultures.
- Dr Candice Owen and Dr Grant Martin for your help with statistics and mapping.
- All the Biodiversity Officers that I met with in the field who took time to help me find and access sites.
- The members of the Rhodes Taekwon-Do club, for your dedication, hard work and friendship.

I also thank my varsity friends and my partner, Meg. The endless support and companionship you have given me made this possible.

Finally, to my parents, Arnie and Toni Miller, thank you for your guidance and for instilling in me a passion for the outdoors. This is for you.

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Chapter 1: General Introduction

1.1. The Spread of Alien Species in South Africa

Alien invasive species pose one of the most significant threats to the environment after large-scale habitat loss and global climate change. However, none of these factors are mutually exclusive, and each one is associated with the other (Wilcove *et al.* 1998; Bright 1999). Prior to the era of globalisation, organisms were often located in restricted ranges and separated by insurmountable biogeographical barriers that lead to allopatric speciation. Historically, geographic separation guided organisms toward their respective evolutionary trajectories. However, these barriers are no longer sufficient to stem the tide of human intervention in the environment. Due to the increased rates of travel and the wide-spread trade of commodities, there has been a marked rise in the numbers of propagules of alien species reaching new areas, with a positive correlation between the degree of trade and the number of invasive alien species in a region (Westpahl *et al.* 2008). At the current rate of global trade, the influence that higher rates of invasion by alien species will have on natural areas will only be exacerbated. Alien invasive species have global impacts, but the effects are likely to be greatest in ecologically diverse and potentially sensitive areas such as southern Africa, particularly in the Cape Floristic Region, a biodiversity hotspot (Latimer *et al.* 2004; Richardson & van Wilgen 2004; Gibson *et al.* 2013).

The introductions of plants and animals into new and non-native areas have been facilitated by people for hundreds of years, specifically in previously colonised countries. Many of these species were introduced on or near water, as these were common transport routes (Washitani 2001; Richardson & Rejmánek 2011). Hence, non-indigenous and/or invasive alien species can now be found on every continent, island, and coastal region where human activity has occurred (Bright 1999).

Biological invasions lead not only to the competitive exclusion of native species and the loss of species diversity in natural environments, but they can also have much greater impacts on ecological processes and ecosystem services. Water, for example, is a precious resource in semi-arid to arid regions such as South Africa (Cowling *et al.* 1994). Therefore, any alteration of streamflow – a known side-effect of increased evapotranspiration by dense infestations of plants, such as invasive *Acacia* spp. in riparian zones (Dye & Poulter 1995), and large floating macrophyte invasions on the water surface (van der Weert & Kamerling 1974) – may directly impact the viability and longevity of the artificial impoundments that provide water to nearby communities.

Many alien invasions can be modelled within the framework of the Fluctuating Resource Hypothesis (FRH) which assumes that communities are at risk of invasion when resources are unused (Davis *et al.* 2000). The FRH also accepts that the invasion potential within a given area is a factor of both the environment and the invading species itself, where the environment needs to be sufficiently susceptible to becoming invaded (therefore having a suitable climate or recent disturbance), and the invading organism must be able to successfully naturalise and propagate in the area (Davis *et al.* 2000). According to Blackburn *et al.* (2011), species must first pass a series of stages (transport to a new environment, introduction, establishment and spread) before they can be considered invaders. Failure to surpass any of these barriers means that the organism will not be able to move onto the next stage towards naturalizing and invading any given area (Fig 1.1). However, once an organism is established, it may become invasive if it is able to spread rapidly and outcompete native species and the following invasion hypotheses will elucidate the pathways by which plants can become invasive in new habitats.

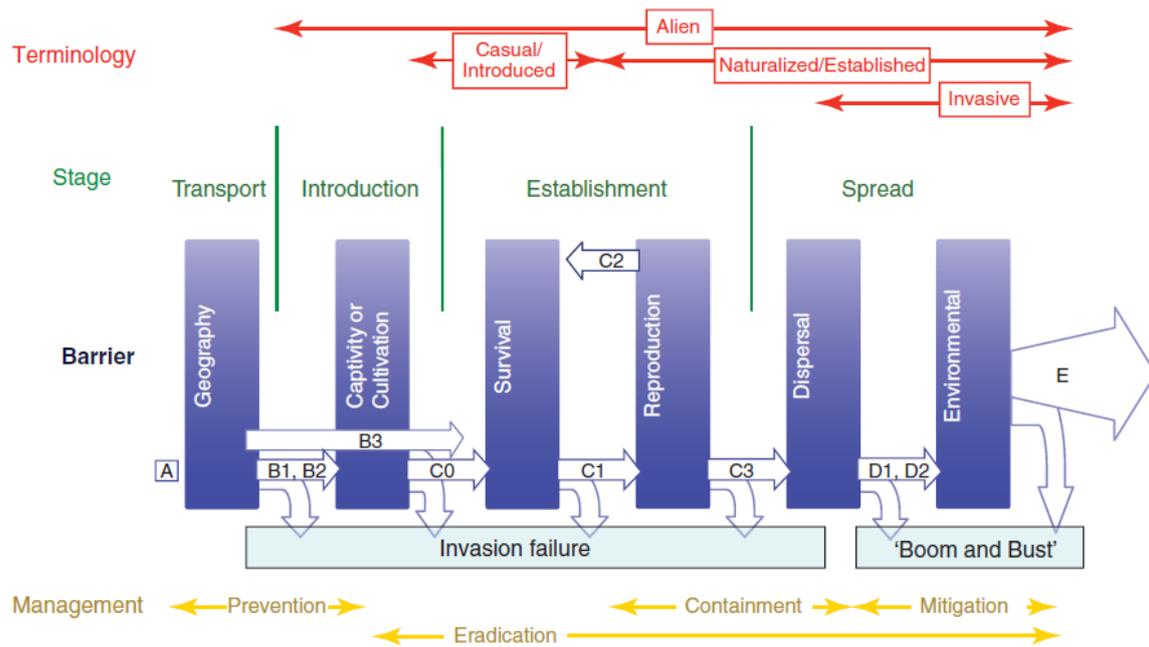


Figure 1.1. A hypothetical pathway for invasions by biological organisms showing the stages of an invasion, with the barriers associated with each stage (from Blackburn *et al.* 2011)

For invading organisms to become established, they need to proliferate. The potential for proliferation by non-indigenous species is, in turn, dependent on the propagule pressure of the invader, and is subject to “the tens rule”. The tens rule specifies that of all introduced species that arrive in a novel area, only ~10% make their way into natural habitats. Of these, only ~10% escape and become established in these native environments, and only ~10% become invasive in this environment (Williamson & Fitter 1996; Westpahl *et al.* 2008). According to this rule, ~10% can be expected to be anywhere between 5 and 20% (Williamson & Fitter 1996). In short, only a very few of the total numbers of propagules that enter an area will surpass all barriers to become invasive (Blackburn *et al.* 2011).

Non-indigenous plants that become invaders usually arrive in the new host range without their complement of host-specific natural enemies, a central premise of the Enemy Release Hypothesis. This hypothesis predicts that many non-indigenous plants establish more effectively when they experience a release from their natural herbivores and pathogens, which

may have been excluded during the introduction phase (Keane & Crawley 2002; Mitchell & Power 2003). Therefore, these plants can benefit from untapped resources and much less top-down regulation from natural enemies compared to their native counterparts. Where there has been a recent disturbance, the majority of plants will be ruderal pioneer species which, according to Grime's triangle, will not be highly competitive (Grime 1974). Less competitive plants will leave residual unexploited nutrients in the environment, which can then be taken up by the invading plants and may lead to an invasion (Davis *et al.* 2000). Moreover, non-indigenous plants are often not as water-use efficient as native species, and many invasive species show elevated magnitudes of carbon assimilation across different habitat types, suggesting greater photosynthetic rates and degrees of resource usage (Funk & Vitousek 2007). This means that established weed populations can out-compete native populations for resources, including water, space, nutrients and light.

Some research suggests that invading plant species have greater biomass and fecundity in their adventive ranges. The Evolution of Increased Competitive Ability (EICA) states that the absence of specialist herbivores and a reduction in the allocation of metabolites to defence in favour of growth and reproduction is responsible for the competitive superiority of some weed species. The EICA also suggests that natural enemies of the plants will be more effective in the non-native range, as the plants will be less well defended against specialist herbivores (Blossey & Nötzold 1995).

As a direct result of the deleterious impacts that invasive plant populations have on their new environments, it is imperative that methods of monitoring, managing, and where applicable, eradicating the invaders be implemented. This is necessary to maintain the natural biodiversity and aesthetics of habitats, and hence, ensuring that the environment remains accessible to people and native species.

1.2. Methods of Weed Management

1.2.1. Chemical control

The first documented records of invasive plant infestations come from the mid-1800s (Randall 1996), but only really became well understood in mainstream science after the publication of Charles Elton's (1958) book "The Ecology of Invasions by Plants and Animals". This work likens invasions to 'explosions' that are "bursting out from control of forces" that may have otherwise exacted control on them in their native ranges (Elton 1958). To limit the spread of weeds which are ecologically or economically injurious, scientists and land managers typically rely on chemical and/or mechanical means. Chemical or herbicidal control usually entails the application of a phytotoxic substance to the plants or their substrate. The active ingredients of the herbicides are often compounds such as 2,4-D (2,4-Dichlorophenoxyacetic acid) and Glyphosate [*N*-(phosphonomethyl)glycine]. These xenobiotics are absorbed by the plant tissues and then translocated within the internal vasculature of the plants (Bromilow *et al.* 1990). Non-selective herbicidal compounds have been used extensively for managing invasive plants in natural areas and in agricultural crops.

Glyphosate is a more modern chemical than 2,4-D, as it only became widely used in the 1970s. It works by inhibiting the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) enzyme of the shikimate pathway in plants, which is important for the biosynthesis of certain aromatic amino acids (Herrmann & Weaver 1999). However, the commercially available form of glyphosate (Roundup, Monsanto Co.) was found to be significantly damaging to anuran communities in a series of short-term laboratory experiments, though this is thought to be due to the surfactant in the product (polyethoxylated tallow amine; POEA), not the active herbicidal ingredients themselves (Relyea 2005). Research is aiming to find a dose of the herbicide that is less damaging to non-target species, particularly biological control agents (Jadhav *et al.*

2008). This type of research is still ongoing but highlights some of the potential threats that extensive herbicide use poses in natural environments (Newbold 1975; Graymore *et al.* 2001; Cedergreen & Streibig 2005).

Among the aquatic weeds such as water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laub. (Pontederiaceae), the subject plant of this thesis, spraying large mats with herbicides is an important method of managing large infestations. Nevertheless, extensive herbicide use is costly – both financially and ecologically – as the chemicals themselves are expensive to use over large bodies of water (and often require aerial applications with helicopters or aeroplanes), and dense mats of plant biomass sink and decompose on the bottom of the water body, which typically leads to critical deoxygenation of the water. Low dissolved oxygen in the water will adversely affect the native flora and fauna of the water body and can lead to extensive fish die-off (Chu *et al.* 2006). Furthermore, chemical control requires intense follow-up regimens as the weeds quickly reproduce both from seeds and asexually from unsprayed plants that remain behind (Coetzee *et al.* 2009).

There are also many cases of herbicides becoming ineffective on target weeds due to the evolution of resistance to the active herbicidal compounds (Culliney 2005), while the lack of specificity in herbicide formulations leads to damage to non-target species – particularly when spraying on a large-scale – and the impact on the environment can be long-lasting. Therefore, while herbicidal control of invasive plants is a useful method of managing infestations, it is evidently not without major drawbacks.

1.2.2. Mechanical and manual control methods

Mechanical and manual control methods, which include using manpower or machines to physically remove invasive plant biomass, are often employed in the management and control of several problematic weed species. However, many weeds, such as water hyacinth, are

capable of coppicing or re-sprouting, and they may have a long-lived seed bank from which new infestations can develop. Machine harvesters used to mechanically control aquatic weeds are also limited by the size of the waterbody and the weed mat. If the weed mat does not cover the entire water body, any attempt to remove the plants may cause fragmentation, and therefore lead to new mats forming in previously uninvaded areas of the waterbody (Clayton 1996). As a result, these methods are often insufficient for achieving complete control of infestations, as the weeds often reappear shortly after the clearing is complete. This was the case in Lake Victoria where local people were incentivised to help clear out small patches of water hyacinth. Machines were also used to chop up the water hyacinth where it grew in larger mats. The outcome was that the manual removal was only useful in strategic areas (i.e. near piers and fish landing zones), but that the success depended on the attitudes held by the riparian communities toward the work (Mailu *et al.* 1998).

In terms of mechanically controlling the water hyacinth in Lake Victoria, the machine used to chop the weed was limited by its ability to move across the lake. Furthermore, merely chopping up the plants in a water body does not address the damage caused by the invasion, nor does it prevent future invasions, as the plant fragments can often regrow quite rapidly. The growth rate of water hyacinth is logistic, being highest when the plants are sparse, usually after winter and slowing as the density of plants increases into a mat (Center & Spencer 1981). When exposed to eutrophic conditions, water hyacinth can attain biomass at a rate of 20 g/m each day, and an increase in total biomass of up to 73% over a single week during the initial stages of the growing season has also been recorded (Wolverton & McDonald 1979; Center & Spencer 1981). The other consequence of physically destroying the plants (similar to chemical controlling the weeds) is that the remaining plant fragments will sink and decompose (Mailu 2000). Allowing plants to die and decompose in the water will lead to further loss of aquatic animals as a result of water deoxygenation as well as the increased activity of harmful and toxic

bacteria that thrive on decaying plants (Villamagna & Murphy 2010). Furthermore, mechanical methods can be expensive, and manual methods are labour intensive. With the follow-up required for these methods to be successful, this practice is costly and inefficient in the long-term, while often proving futile as the infestations continually reappear. Manual and mechanical methods also often remove large populations of established biocontrol agents which gives the weed a short-term release from herbivory while the population of the agent catches up, facilitating rapid plant regrowth (Mailu 2000).

In the case of the weed hydrilla, *Hydrilla verticillata* (Lf) Royle (Hydrocharitaceae), a highly invasive submerged aquatic weed in the United States, mechanical removal was not as beneficial as expected for the long-term management of the weed. Researchers found that mechanical removal was effective temporarily, as it facilitated the removal of biomass and also allowed for the recolonisation by some fish guilds previously excluded by the dense beds of submerged macrophytes. However, long-term monitoring showed that mechanical harvesting of the weeds had an undesired effect in that, instead of reducing the density of hydrilla, a ‘pruning’ effect led to an increase in the overall growth and biomass of the weed as it began to coppice from where it was chopped (Serafy *et al.* 1994). The mechanical harvesting of the weed also led to other problems: fish became entangled in the submerged vegetation when it was removed, leading to multiple species of fish being killed during harvesting. Though the impact on biodiversity was not significantly deleterious in the study by Serafy *et al.* (1994), there are likely to be more far-reaching consequences in larger water bodies with more frequent harvesting.

1.2.3. Biological control

Chemical and mechanical control methods are still employed successfully in many invasive plant management schemes, but they are less effective as independent management solutions

due to the need for constant follow up and monitoring. Classical biological control, which is the intentional release of host-specific pathogens, herbivorous insects and/or mites to damage a target weed, is a more permanent method of controlling weed growth and spread in the field when implemented correctly (McFayden 1998). The advantages of biological control are many, as biological control agents are cost-effective and self-sustaining in the long-term if managed correctly. Using biological control has less impact on natural environments, and the agents act continuously, often without the need for a continual follow-up effort (Fowler *et al.* 2000). Furthermore, the biological control of weeds is a low-risk practice, where any non-target effects are usually mitigated by the positive impact of decreasing the biomass of invasive plants. One unusual case where non-target effects were recorded occurred in the USA with the flowerhead weevil, *Rhinocyllus conicus* (Froelich) (Coleoptera: Curculionidae) which was released to control invasive rangeland thistles. The weevil was known to be oligophagous when released and was subsequently reared on multiple species of the native *Cirsium* thistles post-release (Turner *et al.* 1987). This was the result of a study that showed that *R. conicus* could survive on other hosts, but from the results, it was not expected that it would have such an effect (Louda 1998; Fowler *et al.* 2000). The likelihood of non-target effects occurring were significantly reduced with the inclusion of the centrifugal testing method as standard practise, as this method of pre-release testing eliminates the likelihood of non-target effects occurring on closely related or economically important plant species (Wapshere 1974).

Non-target effects are rare in biological control studies, and the potential for agents to damage non-host plants are thoroughly examined prior to release. The possibility of non-target effects can lead to the termination of the programme if the agent is not sufficiently host-specific. For example, the plan to release *Eccritotarsus eichhorniae* Henry (Hemiptera: Miridae) in the USA was terminated because *E. eichhorniae* could complete its lifecycle on pickerelweed, *Pontederia cordata* L., a native plant in the north, south, and central USA (Tipping *et al.* 2018),

however, this same agent was safe for release in South Africa (Paterson *et al.* 2016), as *P. cordata* is a naturalised alien in the country.

Many invasive and ecologically injurious weed species have been controlled using their arthropod natural enemies. As an example, alligator weed, *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae) from South America, was at one time a devastating and noxious invasive in the southern United States. Research from the native range of alligator weed found three agents suitable for release: the flea beetle *Agasicles hygrophila* Selman & Vogt (Coleoptera: Chrysomelidae), a stem-boring moth *Vogtia malloi* Pastrana (Lepidoptera: Phycitidae), and the thrips *Amynothrips andersoni* O'Neill (Thysanoptera: Thripidae). The three agents, particularly the flea beetle, successfully established and facilitated the control of the weed between initial releases in the mid-1960s and 1970s to the 1980s when it was believed that control was achieved in the USA (Buckingham 1996).

In South Africa, biological control practices have been intentionally used against invasive plants since the early 20th century. The most notable early triumph was the successful control of prickly pear cacti (*Opuntia* sp.) after the introduction of the cochineal bug *Dactylopius ceylonicus* Green (Hemiptera: Dactylopiidae) in 1913 to control *Opuntia monacantha* Haw. (Cactaceae). Further introductions of the cactophagous moth *Cactoblastis cactorum* Berg. (Lepidoptera: Pyralidae) in 1933 and other cochineal species like *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) lead to a decrease of approximately 80% of the 1 million hectares of land invaded by *Opuntia ficus-indica* (L.) (Bromilow, 2010; Moran *et al.* 2013). Another notable, more recent biocontrol success in South Africa is the effective control of red water fern, *Azolla filiculoides* Lamarck (Azollaceae), controlled by the frond feeding weevil *Stenopelmus rufinasis* Gyllenhal (Coleoptera: Eriirhinidae) (McConnachie *et al.* 2004). The introduction of the weevil has led to the local extinction of the weed in the majority of sites where it has been released (McConnachie *et al.* 2004), and the weed is currently listed as

being under complete biological control (Hill & Coetzee 2017). According to the standards set out by Hoffmann (1995), in order for an invasive weed to be listed as being under complete biological control, no other measures should be required to maintain sufficiently low densities of the plants to keep the populations below economic and environmental injury levels. Invasive plant species designated as being under substantial biological control are those that require other methods of management (such as herbicides) to achieve an acceptable population density, though at a decreased amount due to the damage caused by biocontrol agents. Any species listed under negligible control are managed almost entirely through non-biological control measures due to minimal impact by introduced biological control agents, even though the agents may have established (Hoffmann 1995; Zachariades *et al.* 2017; Hill & Coetzee 2017).

Many other invasive plant species, aquatic and terrestrial, have been targeted for biological control in South Africa (Zachariades *et al.* 2017), with each programme achieving varying degrees of success (Moran *et al.* 2013). The water hyacinth programme is the largest aquatic weed biocontrol programme in the country, mainly due to the weed's wide distribution, deleterious impact on the environment, and vigorous growth rate (Lowe *et al.* 2000; van Wyk & van Wilgen 2002; Wilson *et al.* 2005; Villamagna & Murphy 2010). A number of other floating aquatic weeds have been targeted for biological control in South Africa and are considered to be under complete control (Table 1.1).

Table 1.1. Overview of the agents and current level of control of the five major aquatic weeds in South Africa currently. The biological control agent listed in bold font is a pathogen, the rest are arthropods.

Plant	Agent	Control level	Reference
<i>Pistia stratiotes</i> L. (Araceae)	<i>Neohydronomous affinis</i> Hustache (Coleoptera: Eriirhinidae)	Complete	Cilliers (1991c)
<i>Azolla filiculoides</i> Lam. (Azollaceae)	<i>Stenopelmus rufinasis</i> Gyllenhal (Coleoptera: Eriirhinidae)	Complete	McConnachie <i>et al.</i> (2004)
<i>Salvinia molesta</i> D.S. Mitch. (Salviniaceae)	<i>Cyrtobagous salviniae</i> Calder & Sands (Coleoptera: Eriirhinidae)	Complete	Cilliers (1991b), Coetzee <i>et al.</i> (2011)
<i>Myriophyllum aquaticum</i> (Vell.) Verdc. (Haloragaceae)	<i>Lysathia</i> sp. (Coleoptera: Chrysomelidae)	Complete	Coetzee <i>et al.</i> (2011)
<i>Eichhornia crassipes</i> (Mart.) Solms-Laub. (Pontederiaceae)	<ul style="list-style-type: none"> • <i>Neochetina eichhorniae</i> Warner (Coleoptera: Eriirhinidae) • <i>Neochetina bruchi</i> Hustache (Coleoptera: Eriirhinidae) • <i>Megamelus scutellaris</i> Berg (Hemiptera: Delphacidae) • <i>Eccritotarsus eichhorniae</i> Henry (Hemiptera: Miridae) • <i>Eccritotarsus catarinensis</i> (Carvalho) (Hemiptera: Miridae) • <i>Cornops aquaticum</i> (Brüner) (Orthoptera: Acrididae) • <i>Orthogalumna terebrantis</i> Wallwork (Acarina: Galumnidae) • <i>Nipohograpta albiguttalis</i> Warren (Lepidoptera: Crambidae) • <i>Cercospora piaropi</i> Tharp. (Mycosphaerellales: Mycosphaerellaceae) 	Substantial	Cilliers (1991a), Hill <i>et al.</i> (1999), Paterson <i>et al.</i> (2016), Hill & Coetzee (2017)

1.3. Water Hyacinth (*Eichhornia crassipes*) in South Africa

1.3.1. Water hyacinth impact and status

Water hyacinth, *E. crassipes*, is a floating perennial macrophyte from the family Pontederiaceae. The plant, a native of South America, has invaded many tropical and subtropical areas of the world and its impact on the health and ecology of aquatic systems is so severe that it is placed in the top 100 of the World's Worst Invasive Alien Species (Lowe *et al.* 2000), and is regarded as the worst aquatic weed (Holm *et al.* 1977; Cilliers 1991b).

Water hyacinth primarily reproduces vegetatively by producing ramets but can also recruit from seeds, which can stay dormant in the sediment until they are ready to germinate when conditions become favourable. Invasive populations of water hyacinth display limited genetic diversity, and this is as a result of its life-history traits: extensive clonal reproduction, fast growth rate, high degrees of phenotypic plasticity and its ability to disperse clones great distances (Zhang *et al.* 2010). Therefore, genetic recombination is less likely to be an important factor in maintaining healthy populations in the adventive range (Zhang *et al.* 2010). The low diversity of genes also supports the hypothesis that most water hyacinth infestations are derived from small founder populations.

In South Africa, where water hyacinth was cultivated as an ornamental plant in the early 1900s for its attractive flowers (Jacot Guillarmod 1979), the weed has spread to many of the major rivers and catchments in the country (Fig 1.2). The now highly invasive plant is categorised as a declared weed that must be controlled or eradicated where possible by law, according to the Conservation of Agricultural Resources Act ([CARA] Act 43 of 1983), and more recently the National Environment Management: Biodiversity Act ([NEMBA] Act 10 of 2004). This categorisation of water hyacinth has led to the widespread use of herbicides to control its spread and impact in South Africa, particularly to combat large and aggressive blooms (Ashton *et al.*

1979). van Wyk & van Wilgen (2002) estimated the cost per hectare of different management methods and determined that herbicide use was not the most cost-effective method of controlling water hyacinth, costing approximately ZAR 1481.00 per hectare in 2002 to clear with herbicide which, accounting for inflation, would equal approximately ZAR 3624.00 per hectare in 2018 (the following cost value estimates account for changing inflation cost from 2002 to 2018). Herbicides also require constant reapplication and evaluation to prevent re-infestation from dormant seeds and/or surviving plants, which further increases the number of annual applications and elevating costs. On the other hand, biological control is markedly cheaper in the long-term, costing approximately ZAR 780.00 per hectare in 2018. However, the study advocated that an integrated management scheme, employing both chemical and biological control, would optimise the cost efficiency (ZAR 699.50 per hectare) and the levels of control that can be achieved (van Wyk & van Wilgen 2002). Biological control is also self-sustaining if the agents establish effectively, meaning that managers would not need to revisit sites where biological control is applied as often as they would if herbicide use was the only method of management being used.

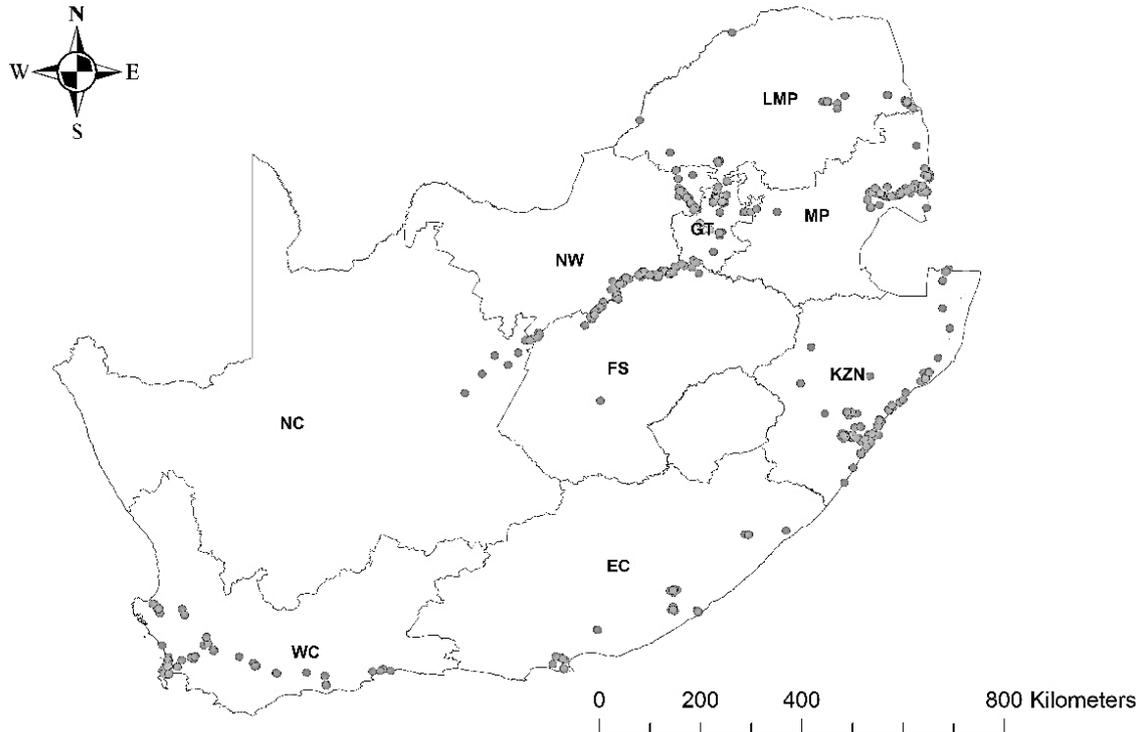


Figure 1.2. All currently known infestations of water hyacinth in South Africa (data from SAPIA database)

1.3.2. Water hyacinth biological control agents

As a result of the ecological and economic advantages of using natural enemies to control problem plants, and the many difficulties associated with the long-term management of water hyacinth, great emphasis has been placed on an extensive biological control programme for the weed. The first agent, the leaf-chewing weevil *Neochetina eichhorniae* (Warner) (Coleoptera: Eirirhinidae) was released in South Africa in 1974 and successfully established soon after (Cilliers 1991a). Since then, other agents have been released including *Neochetina bruchi* Hustache (Coleoptera: Eirirhinidae), a congener of *N. eichhorniae*, which was released in 1989. *Neochetina bruchi* feeds in a similar way to *N. eichhorniae*, but has a shorter generation time and is more effective in eutrophic systems (Cilliers 1991a, Heard & Winterton 2000). The weevil larvae mine the petioles and tunnel into the crown of the plant, which also causes

damage and leads to petioles becoming water logged (Julien 2000). Other agents that have been released on water hyacinth include the moth *Niphograptia albiguttalis* Warren (Lepidoptera: Crambidae), released in 1990, the mirid *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae), released in 1996 (Hill *et al.* 1999), and a cryptic congener, *Eccritotarsus eichhorniae*, (Paterson *et al.* 2016). The semi-aquatic grasshopper *Cornops aquaticum* (Bruner) (Orthoptera: Acrididae) was subjected to speculation over its host specificity as it was able to feed and develop on *P. cordata* (pickerelweed), *Heteranthera callifolia*, and *Canna indica* (canna). However, many of the plants that were fed on outside of the Pontederiaceae could not support egg development or were themselves potentially invasive aliens, sufficient motivation for the release of the grasshopper in South Africa, though it has yet to establish effectively (Hill & Oberholzer 2000; Oberholzer & Hill 2001).

Some of the other biological control agents that are effective on water hyacinth had a more obscure mode of entry into South Africa and may have entered undetected with other agents. An example of this is the mite, *Orthogalumna terebrantis* Wallwork (Acari: Galumnidae), which was originally brought into quarantine in South Africa for research as a prospective agent (Cilliers 1991a). The mite oviposits on the leaf surface, and the majority of the damage is caused by the formation of galleries, which are tunnels excavated in the leaf by the larvae (Cilliers 1991a). Research on the impact caused by the mite in the field is limiting, but a study has shown that the mite, though not as damaging to the tissues of water hyacinth as previously thought, does have a significant impact on the efficiency of photosynthesis and increases plant stress characteristics (Marlin *et al.* 2013). This makes the mite a useful addition to the current suite of biological control agents already on water hyacinth in South Africa.

Another agent that was not initially released intentionally is the fungal pathogen, *Cercospora rodmanii* (= *piaropi*) Conway (Mycosphaerellales: Mycosphaerellaceae) which was found to have established on some South African water hyacinth infestations by 1987 (Cilliers 1991a;

Coetzee *et al.* 2011). The pathogen is relatively widespread in the region where it causes foliar necrosis. *Cercospora rodmanii* is often benefitted by the feeding of insect agents, as the feeding wounds provide entry points for the pathogen to infect the plants (Moran 2005). Other pathogens are also present on water hyacinth infestations in the region, including *Acremonium zonatum* (Sawada) W.Gams (Ascomycotina) and *Alternaria eichhorniae* Nag Raj & Ponnappa (Ascomycotina). These pathogens have been found on water hyacinth but have had limited impacts and restricted distributions and, as a result, their effect on infestations is negligible (Morris *et al.* 1999).

Despite a large number of biocontrol agents released on water hyacinth in South Africa, more than any other country where water hyacinth is invasive (May & Coetzee 2013), complete control has yet to be achieved. However, in other areas, such as Papua New Guinea and Lake Victoria in Uganda, complete control of water hyacinth has been accomplished using only the two weevil species *N. eichhorniae* and *N. bruchi* (Hill & Olckers 2000, Wilson *et al.* 2007). In South Africa however, even though more than 90% of invaded sites have successfully established populations of at least one biocontrol agent (Hill & Coetzee 2017), the level of control is highly variable. This is due to varied and complicated factors relating to South Africa's climate, water nutrient status, problem plant control measures (herbicide use), and hydrology (Hill & Olckers 2000).

Water hyacinth in South Africa is typically located in one of three climatic zones: high altitude temperate sites with summer rainfall and cold winters that often experience frost, sub-tropical, coastal areas also with summer rainfall, and Mediterranean sites that experience winter rainfall but no frost. These highly variable South African climate zones do not match the warm, low-altitude tropical climate of the plant's native South American range (Hill & Olckers 2000; May & Coetzee 2013). While water hyacinth has managed to overcome the climatic variability in South Africa by re-sprouting in the summer after most of the canopy has been destroyed by the

frost (in higher-altitude temperate sites), biocontrol agents cannot always respond in the same way – particularly at the beginning of the growing season when the quality of the above-water biomass is particularly poor and persisting agent populations are low. This leads to a lag in agent population density build-up by the end of the winter, and some time is required for any remaining agents to re-populate the infested area to effect any significant damage on the plants, usually peaking near the end of the growing season (Hill & Olckers 2000).

The lack of biocontrol agent efficacy in some scenarios is coupled with the second issue, which is that many of the water bodies where water hyacinth is present are highly eutrophic (2.5-10 mgN l⁻¹) or even hypertrophic (>10 mgN l⁻¹) (DWAF 2006), and the surplus of nitrates and phosphates in the water facilitate rapid growth by the plants. High nutrient inflow leads to greater biomass and shoot/root ratio in water hyacinth. The increase in shoot biomass over roots suggests that the plants are healthy and growing in the absence of major stress or nutrient deficiency (Coetzee & Hill 2012). Hence, plants which grow in high nutrient water display a rate of leaf turnover and compensatory growth that often exceed the damage that can arise from even large populations of host-specific herbivores in most situations (Reddy *et al.* 1989, 1990; Hill & Olckers 2000). Water nutrient manipulation experiments involving *E. catarinensis* and *C. aquaticum* showed that, while the insects were better able to reproduce and establish on plants grown at high nutrients, their effect on plant growth was not significant (Ripley *et al.* 2006; Coetzee *et al.* 2007a; Bownes 2009). Furthermore, higher nitrogen content in plants satiates insects' hunger more rapidly, meaning that the biocontrol agents can acquire the necessary nutrients for growth and reproduction with less feeding effort (Horsfield 1977; Mattson 1980; Minkenberg & Ottenheim 1990). However, studies have also shown that the heavy metals absorbed by water hyacinth tissues can have strong negative impacts on reproduction in some of the biocontrol agents feeding on these tissues (Jamil & Hussain 1992). Therefore, the passing of legislation and a formulation of methods to reduce the anthropogenic

impacts on South Africa's limited waterways should be prioritised if we are to successfully manage invasive aquatic macrophytes. Eutrophic waterways are a major concern for the health of South African ecosystems and their associated biodiversity. In many ways, high nutrient input into the water is hypothesised to be the driver of invasive plant infestations and severely impacts the management schemes put in place to mitigate these problems. Therefore, invasive plants could be considered to be 'backseat drivers' – the symptoms of a far greater problem (Bauer 2012). The most effective way to control invasions by aquatic weeds in South Africa is to have more stringent controls on the quality of water that enters dams and rivers (Heard & Winterton 2000; Hill & Olckers 2000).

However, given the nutrient-enriched status of water hyacinth plants in South Africa at present, finding new herbivores that supplemented the natural enemy complex already released on the weed was crucial. Prospective agents needed to be better able to damage the weeds, colonize new sites, and potentially be capable of overwintering at colder sites in South Africa. It was also important that the new candidate agents had shorter generation times and reproduced quickly to facilitate rapid colonisation upon release, particularly during early spring plant regrowth. The water hyacinth planthopper, *Megamelus scutellaris* Berg. (Homoptera: Delphacidae) was selected for this purpose and subsequently tested and declared safe for release into South Africa in 2013 (Coetzee 2013).

1.4. *Megamelus scutellaris*

1.4.1. Life history

Megamelus scutellaris is the most recent addition to the water hyacinth biological control programme in South Africa where it is considered to have established (Hill & Coetzee 2017). The multivoltine insect is relatively small at 2.37 mm (± 0.09 mm) in length as an imago (total of 5 instars with overlapping generations). The eggs are laid in scars on the leaf petioles of water hyacinth near the water's surface and the nymphs emerge approximately one week after

oviposition. First instar nymphs undergo an intermediate moult at the point of hatching when they leave behind the embryonic membrane that covered them inside the egg. Maturing from first to the fifth instar takes 15 days in a controlled environment and about 25 days under natural conditions (Sosa *et al.* 2005). Once matured into an adult, the insect can display one of two wing morphologies, a phenomenon called wing dimorphism. The insects will either be of the short-winged flightless form (brachypterous), or the long-winged migratory form (macropterous). The wing form present in the population is usually determined based on plant host quality, photoperiod and temperature, as well as other density-dependent factors (Denno *et al.* 1991), although this has proved difficult to emulate in controlled environments (Fitzgerald & Tipping 2013). When habitats are persistent, migration is not required to escape the habitat, hence wingless forms appear. The presence of wings, though necessary to migrate under certain conditions when resources are limited or overcrowding occurs, impose a metabolic and reproductive hindrance. Therefore, when habitats are persistent, brachypters are likely to be more fecund, reproduce earlier, and experience shorter intervals between oviposition events (Denno *et al.* 1991).

1.4.2. Impact on water hyacinth

Megamelus scutellaris is a host-specific sap-sucking insect that feeds on phloem sap in the leaves via a small, three-segmented rostrum that it uses to puncture the tissues of the plant, creating salivary sheaths (Sosa *et al.* 2005; Tipping *et al.* 2011). When in sufficiently high densities (typically more than 100 individuals per plant [Fitzgerald & Tipping 2013]), *M. scutellaris* can cause chlorosis of the leaves and plant stress, leading to an overall reduction in leaf number, seed production and ultimately, plant vigour (Sosa *et al.* 2007). This made *M. scutellaris* a potentially valuable agent for the classical biological control of water hyacinth in South Africa. Additionally, the damage caused by the agent's rostrum also offers a mode of

entry for fungal pathogens to enter the plant and cause further damage and reduce the overall vigour of the plants (Sutton *et al.* 2016).

1.4.3. Climate matching of *Megamelus scutellaris* in South Africa

As a biological control agent, *M. scutellaris* was initially designated for release in the US to bolster the impacts of the previously released *Neochetina* weevils, particularly as the insects can reproduce rapidly and have relatively short generation times and should theoretically be better able to recolonise infested sites after herbicides have been applied than other agents (Mattison *et al.* 2017). Later, *M. scutellaris* was studied as a candidate for release in the higher altitude water hyacinth infestations in South Africa, as this insect is from temperate latitudes in Argentina. As a result, *M. scutellaris* was expected to be more suited to the cold winter temperatures associated with the Highveld regions of South Africa, which are often exposed to colder and drier winters than the coastal regions. However, according to May & Coetzee's (2013) findings, the actual result was quite the opposite. Based on the thermal tolerance of the insects in a laboratory *M. scutellaris* was able to produce between zero and 10 generations per year, where other agents like *E. catarinensis* and *N. albiguttalis* underwent 3-14 generations and 4-11 generations per year respectively. May & Coetzee (2013) also mapped the expected number of annual generations of *M. scutellaris* that would be produced in each of the sites based on thermal tolerances and climate mapping (Fig. 1.3). It is predicted that *M. scutellaris* will be more fecund in coastal areas like KwaZulu-Natal, where the winter temperatures are typically warmer than in the high-altitude inland areas of South Africa.

A recent study by Grodowitz *et al.* (2017) found that populations of *M. scutellaris* were negatively affected at both very high and very low temperatures, and they found insect fecundity and vigour decreased when plants were grown at 18°C and when plants were grown at 33°C, compared to significantly more robust populations when reared at 25°C. This was

related to water temperature and air temperature, both of which had an impact on insect survival. The results of this study could explain both the limited establishment of *M. scutellaris* in the warmer parts of the USA as well as its overwintering performance in South Africa in relation to temperature. Furthermore, *M. scutellaris* was not projected to be a particularly cold tolerant biological control agent (May & Coetzee 2013), but work by Porter *et al.* (In Press) on the thermal physiology of the water hyacinth mirid *E. catarinensis* has shown that some insects possess the ability to acclimate to cooler areas and to, therefore, maintain populations even over winter. Both *E. catarinensis* and *M. scutellaris* (as well as the weevils *Neochetina eichhorniae* and *N. bruchi*) have established long-term populations at the coldest biological control site in South Africa: the Kubusi River in the Eastern Cape Province (32°34'35.4" S 27°28'56.8" E), which shows that the insects can survive the cool temperatures and poor host plant quality during the winter. In the higher altitude sites of its native range, *M. scutellaris* nymphs have been collected from plants where the leaves and petioles have decayed from the frost in winter, suggesting that they may overwinter at the base of the petioles which are slightly more protected from frost (Sosa *et al.* 2005).

Cold tolerance is a key factor for the efficacy of a biological control agent released in South Africa, but agents must also be able to survive the sometimes extreme heat. To this end, some research has found that *M. scutellaris* is capable of thermoregulation when temperatures are extremely warm by residing on the petioles nearest to the water's surface (Grodowitz *et al.* 2014). The petioles themselves are cooled by the transpiration of water through the xylem and shaded by the canopy, while the cooler water further reduces the temperature under the canopy, making variable microhabitats on the plant. This also provides a possible explanation as to why the insects oviposit near the surface of the water, as the eggs are highly sensitive to any temperature fluctuations (Grodowitz *et al.* 2014).

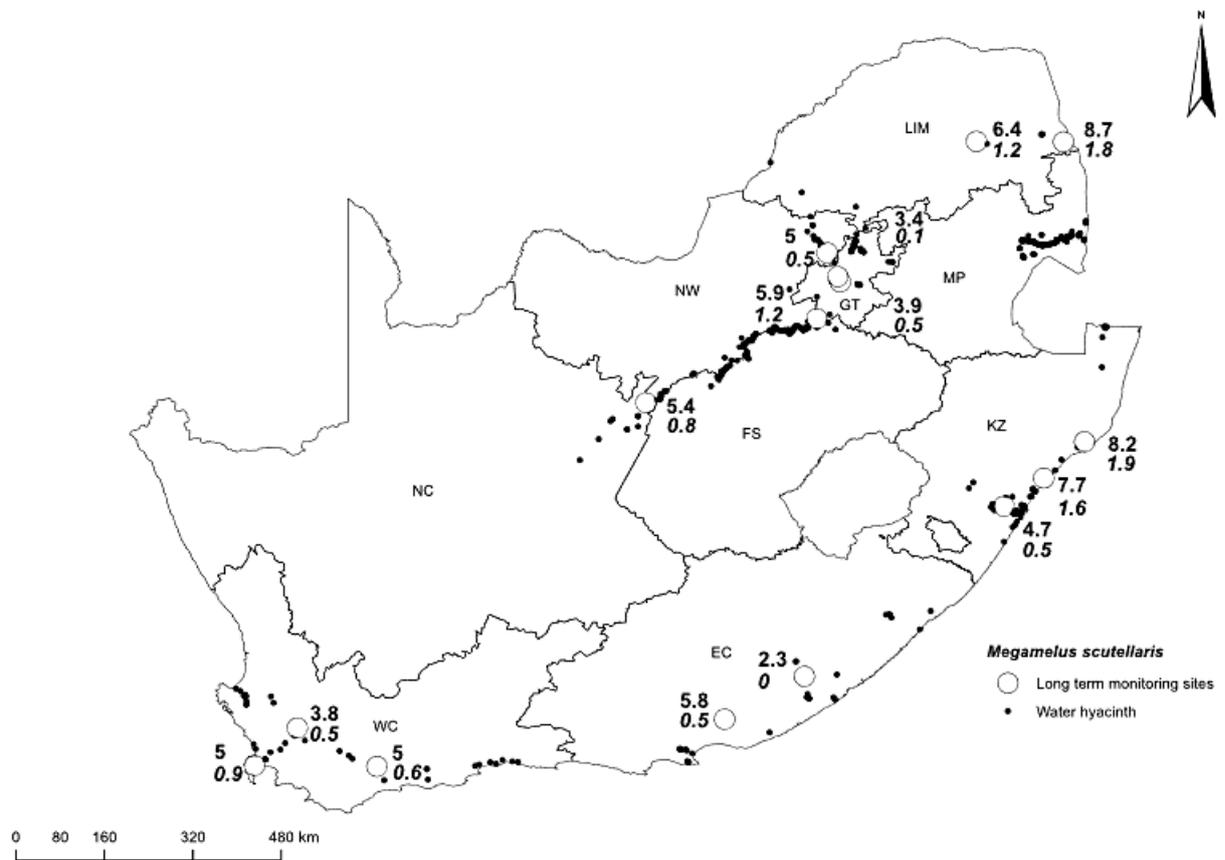


Figure 1.3. The number of expected generations of the agent *Megamelus scutellaris* produced per annum (top value), and critically during winter (bottom value) in South Africa (taken from May & Coetzee 2013)

1.4.4. The potential impact of *Megamelus scutellaris* in eutrophic water bodies

In terms of nutrient status, all of the river systems in South Africa are negatively impacted in some way by highly accelerated eutrophication. The nutrient levels of these rivers exceed the recommended guidelines for healthy and sustainable natural biodiversity and safe human consumption (de Villiers & Thiar 2007) and the eutrophication of the waterways often has knock-on effects which lead to large blooms of toxic algae and cyanobacteria (Oberholster & Ashton 2008). Excessive nutrients in the water may also have many deleterious implications for people that sustain themselves and their livelihoods using the water. But, of potentially

greater significance in the context of this thesis, the excessive nutrient concentration of the water enables accelerated growth and increased vigour in water hyacinth plants, which readily take up the nutrients. Water hyacinth is well suited to assimilate excess amounts of nitrogen and phosphorous (Gosset & Norris 1971; Reddy *et al.* 1989, 1990), which drives increased plant growth and vitality but leads to a reduction in agent impact (Heard & Winterton 2000).

Herbivorous insects may derive metabolic benefits from feeding on nutrient-enriched plants. This was shown to be true for the cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae), another hemipteran sap-feeder and a major pest of cotton and other crops. When exposed to plants grown in soils with different nitrogen levels (0%, 50%, 100%, 150% enrichment), the insects were significantly more fecund, maintained more weight and were more vigorous when grown at higher nutrient levels compared to those grown at standard or low nutrient levels. The population densities of the aphids also showed a positive correlation with nutrient level (Nevo & Coll 2001). However, the population growth rate is not infinite, and other sap-sucking insects, like *E. catarinensis*, established and survived more successfully on water hyacinth plants grown at medium nutrient levels than at high levels (Coetzee *et al.* 2007a), perhaps pointing towards an increased level of metabolic plant defense facilitated by the extra nutrients.

Furthermore, Coetzee *et al.* (2007a) posited that the presence of high nutrients means water hyacinth plants may have more nitrogen than they require for their photosynthetic needs. This implies that the feeding damage from sap-sucking insects, like the mirid *E. catarinensis*, may be nullified by the high nitrogen content of the leaves. At elevated nutrients, the rate at which water hyacinth plants are able to produce new leaves also outweighs the damage caused to the leaves by mirid feeding (Coetzee *et al.* 2007a). What this shows is that, in many cases, water nutrients play a greater role in determining the extent with which water hyacinth grows than does herbivory by biological control agents, even if the insects are able to establish and proliferate (Heard & Winterton 2000; Ripley *et al.* 2006; Coetzee *et al.* 2007a; Coetzee & Hill

2012). In more hypertrophic systems, high levels of feeding by natural herbivores will be of minor consequence to the overall vitality and health of the water hyacinth plants due to the excess nutrient stores (Coetzee & Hill 2012). Plants grown in high nutrients may also benefit from a greater ability to produce secondary metabolites (like phenolics) for defence against herbivores, the production of which will be magnified in hypertrophic water, and may offer an explanation for the reduction in feeding and survival (Center & Wright 1991; Coetzee *et al.* 2007a). In this regard, *M. scutellaris* and *E. catarinensis* could potentially be used as proxies of plant health and water nutrients, based on their population densities in the field on water hyacinth plants.

1.5. Thesis outline and aims

The aim of this thesis was to determine how effectively *M. scutellaris* had established in South Africa since its release in 2013, both spatially and temporally, as well as determining the impact that the agent had on water hyacinth. Cumulatively, these data add to our understanding of the interactions between biological control agents and their host-plants as well and provide a method to focus the usage and efficiency of *M. scutellaris* as part of the suite of biological control agents already used against water hyacinth in South Africa. Using a more holistic approach combining controlled greenhouse experiments and nationwide field data, this research aimed to justify the number of resources and the amount of effort currently being used to mass rear and release this agent as part of the solution to suppress water hyacinth growth in the face of highly eutrophic waterbodies, phenological variability, and variable host-plant quality in South Africa.

Chapter 2 investigated how the relationship between *M. scutellaris* stocking density and water nutrients impacted the effectiveness of the agents on the photosynthetic productivity, growth and biomass. This chapter also aimed to quantify the impact that *M. scutellaris* had on water hyacinth in a controlled environment, as well as adding insight to how many insects should be

considered as effective in future release efforts for optimal damage and to ensure the best chance of successful establishment.

Post-release evaluations, though often overlooked, are of huge importance to biological control programmes to determine how effective they are at meeting their goals of reducing the impacts of invasive species (for an example, see McConnachie *et al.* 2004). Chapter 3 aimed to monitor simultaneous temporal changes in both water hyacinth growth and biomass and *M. scutellaris* population densities at one site over one year. This was compared with temperature and rainfall data to determine seasonal population dynamics at the coldest site of water hyacinth biological control in South Africa, and Chapter 4 dealt specifically with where *M. scutellaris* had established in the field and estimations of agent density are presented along with water hyacinth plant growth parameters, biomass, and water quality.

Chapter 5 forms a review and discussion of the *M. scutellaris* biological control programme in South Africa, which looked at combining other research as well as the findings of this thesis to formulate recommendations for the future use and implementation of this agent as an integral part of water hyacinth management in South Africa, and potentially into Africa.

Chapter 2: The effect of nutrient level and *Megamelus scutellaris* density on water hyacinth growth and photosynthetic performance under glasshouse conditions

2.1. Introduction

Studies of the interactions between herbivorous insects and plants are common in the literature. This is particularly true in the field of weed biological control, where the success of a biological control programme depends on demonstrating a strong negative influence on the growth or reproductivity of an invasive alien plant by its natural enemy (insect, pathogen, or another arthropod). For controlled laboratory or glasshouse type experiments, researchers usually measure broad-scale changes in the plant-growth parameters and biomass and these can be supported by finer-scale measurements of changes at the cellular level, which requires dedicated plant efficiency tools (e.g. Ripley *et al.* 2006, 2008; Anitha & Ramani 2016). Where sub-lethal levels of herbivory are difficult to accurately quantify in the short time frames of laboratory experiments (for example feeding damage caused by some phytophagous Hemiptera), measurements at a cellular level become more useful, particularly if combined with broad-scale measurements of plant growth, reproduction, and biomass. Measuring the chlorophyll fluorescence emission of a plant provides a fast and non-intrusive method of gathering reliable and objective data on the plant's photosynthetic efficiency (Maxwell & Johnson 2000, Bussotti *et al.* 2010).

2.1.1. Chlorophyll-*a* fluorescence as a tool to measure plant efficiency

Kautsky & Hirsch (1931) were among the first to discover the link between chlorophyll fluorescence (ChlF) and photosynthesis. Since then, our understanding of these processes have greatly improved, and chlorophyll fluorescence remains a practical and efficient method of testing how effectively a plant can use solar radiation to drive photochemistry in the photosynthetic pigments of its leaves (Strasser *et al.* 2000). The basic principle of measuring

ChlF is based on the assumption that a photon of actinic (photosynthetically active) light entering a leaf can follow one of three pathways: it can drive photochemistry, therefore producing energy for the plant, or it is released as either heat or light (fluorescence) (Maxwell & Johnson 2000). These pathways are mutually exclusive, and therefore, a change in one assumes that the others will vary proportionally (Maxwell & Johnson 2000). Consequently, if photochemistry decreases (due to some stress on the leaf), more energy will be dissipated as heat and/or fluorescence, and the fluoresced light can then be measured. ChlF has been used to demonstrate how damaging many abiotic factors, such as limiting or excess light, extremely low or high temperature, and the presence of above-normal concentrations of heavy metals can be to plant efficiency. For example, similar methods have been used to reveal the inhibitory impacts of low temperature and light intensity on the efficiency of photosystem II (PSII) (measured as F_v/F_m ; see Table 2.1 for a description of ChlF parameters) in a bamboo species, *Phyllostachys aureosulcata* McClure (Poaceae: Bambusoideae) (Van Goethem *et al.* 2015). Extremely high temperature has also been shown to negatively affect PS II efficiency through a sudden increase in the production of reactive oxygen species (ROS) in wheat plants, *Triticum aestivum* L. (Poaceae: Pooideae), which were exposed to constant high temperatures (Luo *et al.* 2018).

Temperature and light are not the only abiotic factors that affect leaf fluorescence; certain heavy metals, like lead (Pb) and arsenic (As), can also cause toxic effects in plants which inhibit their photochemical output (Kumar & Prasad 2015; Wang *et al.* 2016). Plant health and growth are influenced by biotic stresses, like herbivory and pathogens, which can also have an impact on the efficiency of the photosystems.

Table 2.1. Explanation of the different ChlF parameters used

Fluorescence parameter	Definition
F_o	Minimal fluorescence
F_m	Maximal fluorescence
F_v	$(F_m - F_o)$ Variable fluorescence
F_v/F_m	The maximum photochemical efficiency of PS II (P680)
TR_o/RC	The maximum rate of Q_A reduction
DI_o/RC	Rate of heat dissipation in an active reaction centre (RC)
ET_o/RC	Rate of electron transport in an active RC
ABS/RC	Photon absorption per RC
PI_{ABS}	Performance index

Many plant biomes occur in climates that can facilitate much higher biomass accumulation than is actually present, and this is thought to be due to consumer-driven effects from herbivores (Bond 2005). Insect herbivores, in particular, can be extremely damaging to plants, but the ways in which different insect feeding guilds cause damage can vary significantly. Some, like the mandibulate insects, cause very obvious damage through scarring or defoliation. The water hyacinth biological control agents *C. aquaticum* and adult *Neochetina* spp. are examples of mandibulate leaf feeders that use their large mandibles to remove the leaf tissue on which they feed (Cilliers 1991b; Bownes 2009, Bownes *et al.* 2013) (Chapter 1, Table 1.1). Other insect guilds cause more subtle damage to plants, particularly the phytophagous hemipterans, which feed through a thin straw-like rostrum which they insert into their preferred plant tissues. The Russian wheat aphid, *Diuraphis noxia* (Homoptera: Aphididae), a pest of crops such as wheat (*T. aestivum*) and barley (*Hordeum vulgare* L. (Poaceae: Pooideae)), and

the mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), a pest of tomatoes, both reduce the photosynthetic efficiency of susceptible host plant cultivars when they occur in high numbers, and the damage that they caused was measured using ChlF (Burd & Elliot 1996; Huang *et al.* 2013).

The effects that water hyacinth biological control agents have on their host plant has also been tested using ChlF techniques. Marlin *et al.* (2013) showed that damage caused by the gallery forming water hyacinth mite *O. terebrantis* lead to a reduction in the quantum efficiency of PSII, as well as the performance index (PI_{ABS}) and the density of reaction centres per cross-sectional leaf area (RC/CS). These effects were noticeable in the absence of any significant reduction in the chlorophyll content of the leaves, which was suggested to be the result of physiological damage at the cellular level that may not be obvious at the plant level, particularly over the short-term (Marlin *et al.* 2013). When chlorophyll feeding insects, like *E. catarinensis* fed on water hyacinth leaves, the damage they caused lead to measurable differences in ChlF and the PI_{ABS}, but these impacts varied between nutrient treatments with plants grown in higher nutrients being less affected by herbivory (Ripley *et al.* 2006).

The experiments mentioned above have made use of controlled laboratory-type experiments to control or manipulate certain variables in order to test specific changes in the physiological responses of the test subjects in question. Mesocosm experiments thus form an important part of biological control studies, testing how various changing elements will impact both the plants and the biological control agents while eliminating the stochasticity that is characteristic of purely field-based research, as this may confound the data. There is great value in using mesocosm-type experiments in biological control research, particularly with water hyacinth. Glasshouse mesocosm studies have been used to empirically test how biological control agents impact the competitive ability of aquatic weeds like water hyacinth (Center *et al.* 2005; Coetzee *et al.* 2005), how water hyacinth biological control agents interact with each other (Petela

2017), and how nutrients can affect the growth of the plants, and the effectiveness of the biological control agents used against them (Heard & Winterton 2000; Ripley *et al.* 2006; Coetzee *et al.* 2007a; Canavan *et al.* 2014).

The aim of the experiment reported in this chapter was to determine how nutrient level and insect density influences the growth, reproduction, and photochemical efficiency of water hyacinth plants that were exposed to herbivory from *M. scutellaris*.

2.2. Materials and Methods

2.2.1. Experimental plants

Insect free plants were sourced from cultures maintained at Rhodes University (Grahamstown, South Africa) and placed singularly into individual mesocosms (each mesocosm was a 70L cylindrical bin measuring 50 cm at its widest part and 60 cm tall) filled with 60 litres of tap water and sprayed with foliar insecticides (Efekto[®] Oleum white oil emulsion, and Efekto[®] Malasol, an organophosphate) to remove any insect pests. The plants were of similar size and all dead material and daughter plants were removed for the initiation of the mesocosms, where the single plants were allowed to grow into a mat thereafter. The mesocosms (n=90) were randomly separated into three nutrient treatments: hypertrophic (15 mgN l⁻¹), eutrophic (5 mgN l⁻¹) and oligotrophic (no nutrients added). These treatments were representative of field conditions at sites around South Africa (Byrne *et al.* 2010) and classified according to the guidelines proposed by the Department of Water, Agriculture and Forestry (DWAFF, 1996). The nutrients, applied as CULTERRA Multisol 'N' – 6.1.3 (44) water-soluble fertilizer (CULTERRA, Johannesburg, South Africa), were re-applied monthly. Commercial iron chelate (13% Fe EDTA) was also added monthly (1 heaped 10 mL spatula per mesocosm) to maintain plant health and prevent chlorosis and yellowing of leaves. The plants were enclosed with purpose-built 0.5 mm mesh covers that fitted over a wire frame attached to the top of the

mesocosm and secured 15 cm from the rim of the bin with Velcro to stop insects moving between treatments and to prevent unwanted pests from contaminating the plants. Plants and insects were accessed via a 25 cm zipper installed in the top of the covers (Fig 2.1). The plants were left for approximately 6 months until most of the mesocosms had grown enough biomass to cover most of the surface of the water (the low nutrient plants were not able to form mats and remained predominantly single plants).

2.2.2. Experimental insects

The insects used in this experiment, *M. scutellaris*, were reared at the Waainek insect mass rearing facility at Rhodes University (33°19'12.2"S 26°30'27.8"E). Two insect stocking rates were used during the experiment and plants were inoculated with a total of either fifteen insects (low insect density) or 60 insects (high insect density) from three separate inoculations, with releases occurring weekly over three weeks (3x5 insects for low insect density or 3x20 insects for high insect density). The insects were removed from their rearing cages using an aspirator and placed onto the plants that were grown in their appropriate nutrient media. Only brachypterous adult insects were collected and a sex ratio of 1:1 was assumed. Each treatment was replicated 10 times, and treatments were distributed according to Table 2.2.

Table 2.2. Experimental treatment allocations. Each control/treatment replicated 10 times.

Treatment	Nutrients	Insect density
Control 1	High	None
Control 2	Medium	None
Control 3	Low	None
Treatment 1	High	Low
Treatment 2	Medium	Low
Treatment 3	Low	Low
Treatment 4	High	High
Treatment 5	Medium	High
Treatment 6	Low	High

2.2.2. Data collection

The temperature in the polytunnel was monitored every 30 minutes *in situ* using three iButton temperature data loggers which were placed in pill vials and surrounded by tissue paper. The data loggers were suspended from support beams in the tunnels on a wire loop and were positioned at the front, middle, and rear of the tunnel.

Plants were measured weekly over 13 weeks to measure changes in plant growth parameters and biomass accumulation in response to different insect densities and nutrient levels. The wet biomass (kg) of the entire mat in each mesocosm was weighed using a handheld scale at the beginning and end of the experiment. Biomass and root length measurements were made prior to insect release and at the end of the experiment to mitigate insect losses as the plants would have to be removed from the mesocosms for these to be measured. Stress and herbivory data were collected using a Handy PEA chlorophyll fluorometer (Hansatech, Kings Lynn, UK). The Handy PEA was set with a saturating light intensity of $3500 \mu\text{mol m}^{-2} \text{s}^{-1}$, measuring over 1 second with a fixed gain (1x). The fourth leaf (or oldest leaf if less than four leaves were present) was dark adapted using two leaf clips on either side of the fourth fully expanded leaf

to relax the photochemical pathways of PSII, thus halting the light-dependent reactions of photosynthesis (Fig 2.1). Leaves were dark-adapted for 30 seconds prior to measurement by closing the metal slide on the leaf clips. Although dark adaptation is usually done over longer periods – upward of 15 minutes (Ripley *et al.* 2006; Anitha & Ramani, 2016) – preliminary measurements of healthy water hyacinth leaves (data not shown) indicated that the fluorescence response was sufficiently similar after 30 seconds of dark adaptation when compared to the responses after longer dark adaption times. Therefore, the shorter time was used for convenience and time efficiency in measuring the plants. To perform a measurement, the sensor head was attached to the leaf clip and the leaf was illuminated with a saturating red light of pre-set wavelength ($3500 \mu\text{mol m}^{-2} \text{s}^{-1}$). The fluorescence response is measured primarily as the variable fluorescence over the maximum fluorescence (F_v/F_m), where $F_v = F_m - F_o$, which is a relative measurement of the potential quantum efficiency of photosystem II (PSII), and can therefore be used to measure the performance of photosynthesis within a leaf, where an F_v/F_m value near to 0.8 is regarded as healthy in most plants (Maxwell & Johnson 2000).



Figure 2.1. A mesocosm indicating the placement of the dark-adaptation leaf clips prior to measuring chlorophyll fluorescence.

2.2.3. Chlorophyll content analysis

Chlorophyll content was measured twice per leaf, measuring the fourth leaf or oldest if fewer than four leaves were available. The measurement was performed on either side of the adaxial surface using the hand-held Apogee CCM-200 plus chlorophyll meter (ADC BioScientific Ltd., Hoddesdon, United Kingdom). Chlorophyll content was only measured at the end of the experiment to minimize any disturbance on the plants.

2.2.4. Data analysis and statistics

Data were graphed using the `ggplot2` package in RStudio v 3.4.3 (R Core Team 2017). Differences in plant parameters between the treatments were tested using factorial ANOVAs in RStudio. The *post hoc* Tukey's HSD test was used where appropriate ($\alpha = 0.05$). Low nutrient plants were removed from the analyses due to high rates of mortality from nutrient stress.

2.3. Results

2.3.1. Temperature

The temperature was monitored constantly over the experimental period. The highest temperature recorded did not exceed the CT_{max} of *M. scutellaris*, which is 39.1°C (J. Coetzee, *pers comm*). The average temperature remained above the t_0 of *M. scutellaris* which was reported as 11.5°C (May & Coetzee 2013) (Fig 2.2).

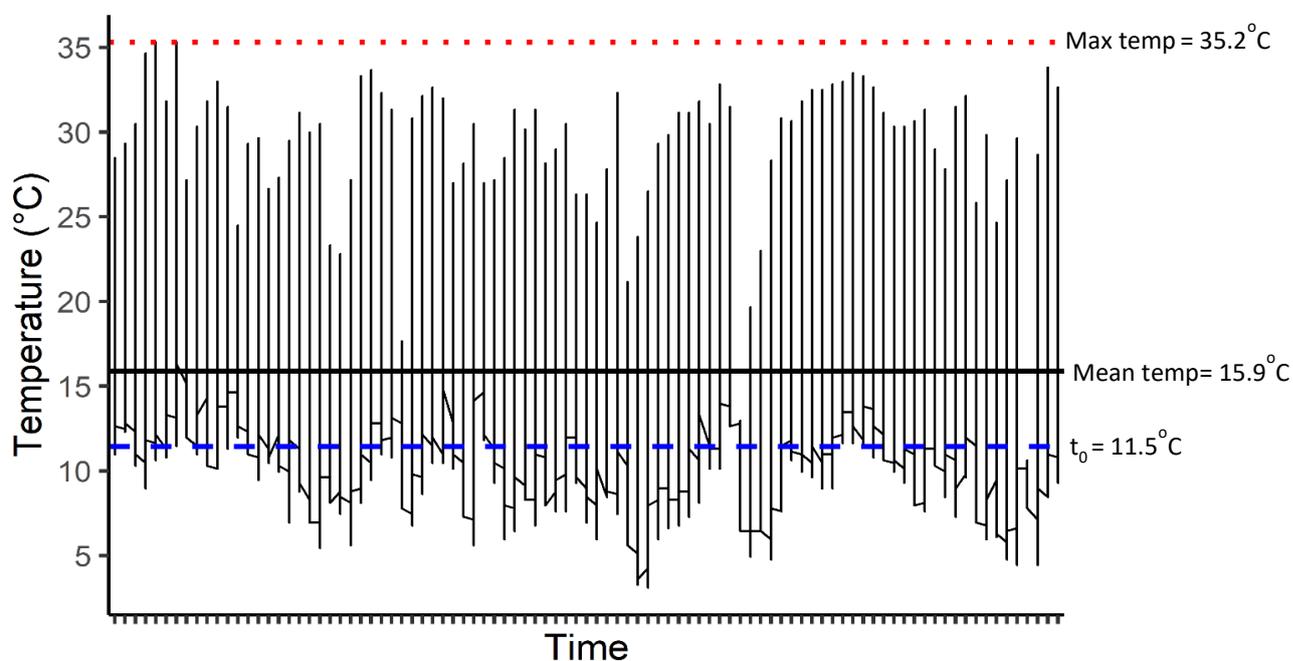


Figure 2.2. Ambient temperature recorded in the polytunnel from 13 May 2018 to 13 August 2018. The black line represents the mean temperature, the red dashed line shows the maximum recorded temperature (35.3°C), and the blue line is the lower developmental threshold (t_0) of *Megamelus scutellaris* (May & Coetzee 2013).

2.3.2. Plant measurements

All plants decreased in size during the experiment, shown as a decrease in the length of the longest petiole over time (Fig 2.3). The longest petioles on all plants were significantly shorter after 13 weeks than at the beginning of the experiment, showing a steady decrease in size throughout, with no significant effect of insect density ($F_{2,107} = 1.29$, $P = 0.28$). There were significant differences in petiole length over time ($F_{1,107} = 504.48$, $P < 0.0001$), and between nutrient levels ($F_{1,107} = 111.45$, $P < 0.0001$). The maximum petiole length was also significantly influenced by the interaction between nutrients and time ($F_{1,107} = 42.90$, $P < 0.0001$). The statistical tests only included the high and medium nutrient treatments due to the majority of the low nutrient plants having died before the termination of the experiment, due mostly to the lack of nutrients.

The number of leaves on each plant was affected primarily by time ($F_{1,107} = 52.28$, $P < 0.0001$), although there was some interaction between the combination of nutrients and time ($F_{1,107} = 3.51$, $P = 0.06$) as well as an interaction between nutrients and insects ($F_{2,107} = 2.78$, $P = 0.07$, Fig 2.4). Although some of these interactions fall outside of the standard 95% confidence interval for statistically significant relationships, it is possible that greater rates of *M. scutellaris* establishment could have yielded more significant results. Furthermore, between the high and medium nutrient conditions, there was a noteworthy decline in the number of photosynthetic leaves on plants in the medium nutrient and high insect treatment, where leaf number declined from 6.6 ± 0.4 leaves per plant to 4.1 ± 0.5 leaves per plant. This reduction coincides with observed trends on some of the plants where *M. scutellaris* had established well and their feeding caused considerable damage to these plants. The plants grown in low nutrients had very few leaves as a result of the nutrient-stress, and the leaves were often smaller and prone to browning and premature senescence. Very few insects established on low nutrient plants, with only one plant in the low nutrient treatment maintaining insects for the duration of the

study. More often, plants grown in low nutrients would sink and die prematurely, irrespective of whether or not insects were present.

In terms of reproductive output, few flowers were produced by the plants in either treatment (low nutrient plants produced no flowers), which is typical of greenhouse experiments. However, ramets were common among all plants in the medium and high nutrient treatments and were significantly different between the nutrient levels ($F_{1,106} = 9.87$, $P = 0.002$, Fig 2.5). Very few ramets were recorded from low nutrient plants, with a maximum of two ramets recorded on the last surviving plant in the low nutrient low insect treatment. No other significant interaction was recorded, although there was some relationship between the number of ramets with insect treatment and time ($F_{2,106} = 2.77$, $P = 0.07$).

The root:shoot ratio in plants is indicative of a range of growth-influencing factors, including the prevailing nutrient conditions or the presence of biotic or abiotic stresses. A high root:shoot ratio (where the roots are longer than the shoots) usually indicates that plants are nutrient limited or exposed to high intraspecific competition. There was no significant difference between the root:shoot ratios of the test plants between the nutrient and insect treatments ($F_{2,108} = 1.81$, $P = 0.17$), but time did have a significant effect ($F_{1,108} = 58.71$, $P < 0.0001$, Fig 2.6). All treatments had a higher root:shoot ratio at the end of the experiment compared to at the beginning, but this was only significantly higher in the medium nutrients with low insect treatment and in the control for high nutrients and resulted from the reduction in petiole length (Fig 2.3). The root:shoot ratio of the medium nutrients high insect treatment changed the least over the course of the experiment, with a mean difference of 2.09 ± 0.67 . The contribution of the change in root length to the root:shoot ratio is shown below (Fig 2.7a).

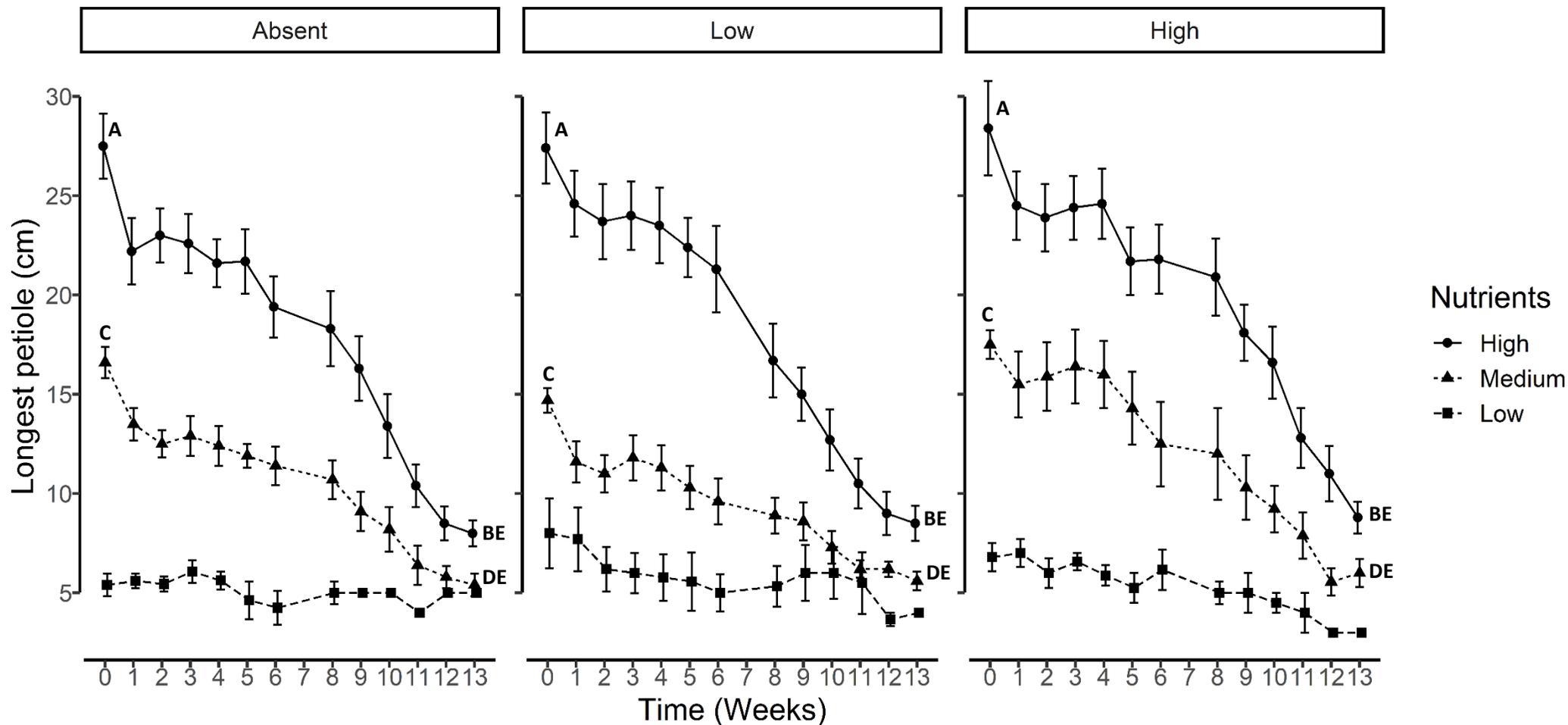


Figure 2.3. The change in mean length of the **longest petiole** by nutrients (within facet) and by *Megamelus scutellaris* density (between facets) over time (\pm SE). Different letters indicate significant difference (Tukey's HSD, $P < 0.05$). Due to high plant mortality, the statistics only include the H and M nutrient treatments, although all nutrients levels are displayed.

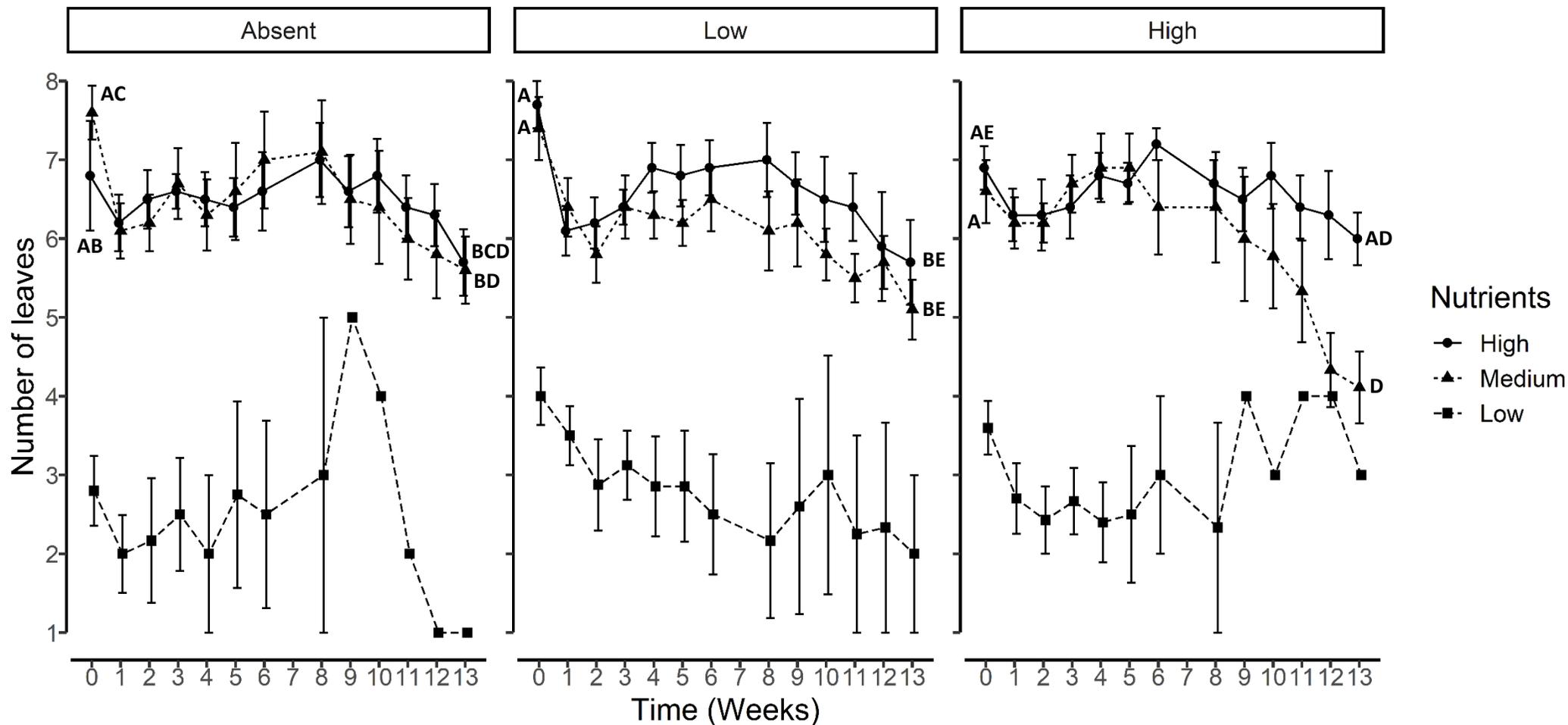


Figure 2.4. The change in **mean number of leaves** by nutrients (within facet) and by *Megamelus scutellaris* density (between facets) over time (\pm SE). Different letters indicate significant difference (Tukey's HSD, $P < 0.05$). Due to high plant mortality, the statistics only include the H and M nutrient treatments, although all nutrients levels are displayed.

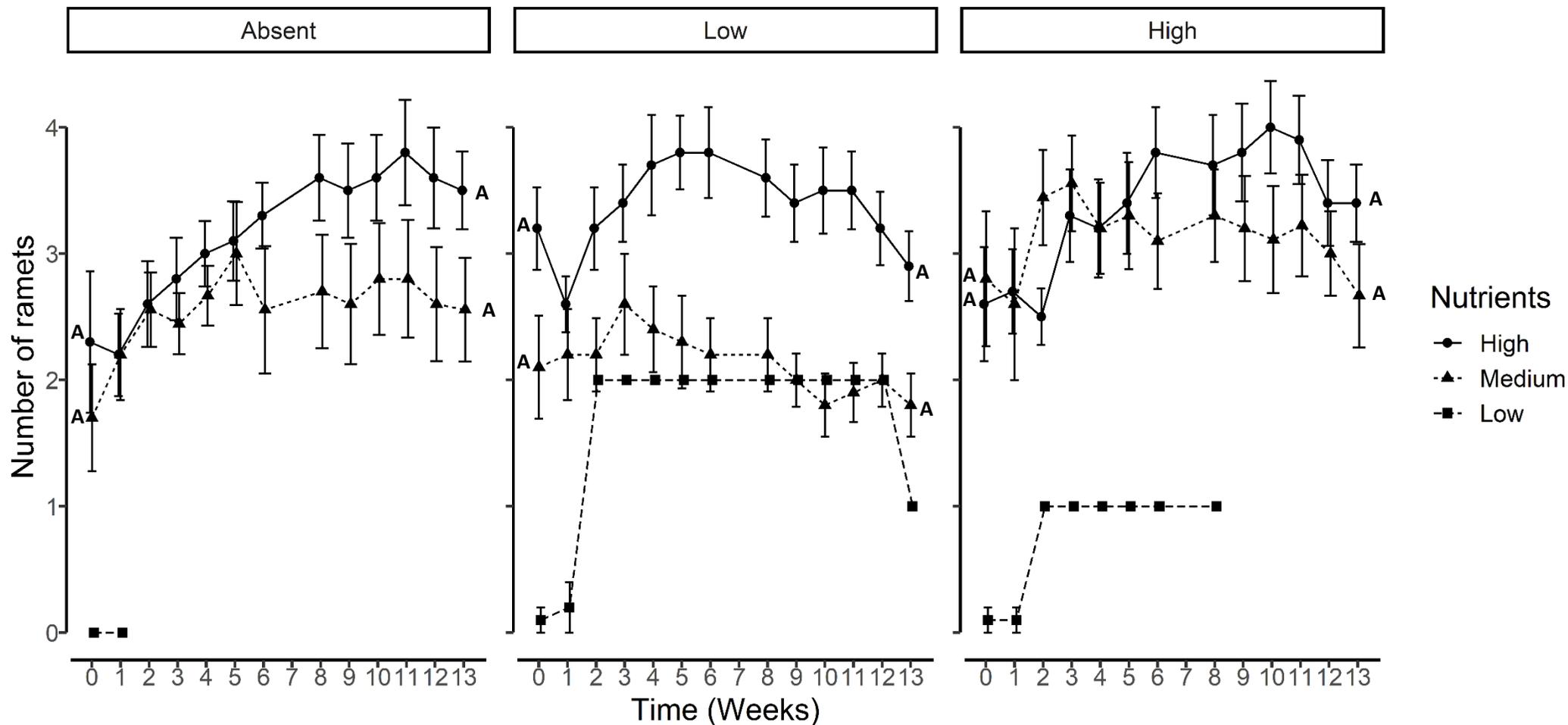


Figure 2.5. The change in the **mean number of ramets** by nutrients (within facet) and by *Megamelus scutellaris* density (between facets) over time (\pm SE). Different letters indicate significant difference (Tukey's HSD, $p < 0.05$). Due to high plant mortality, the statistics only include the H and M nutrient treatments, although all nutrients levels are displayed.

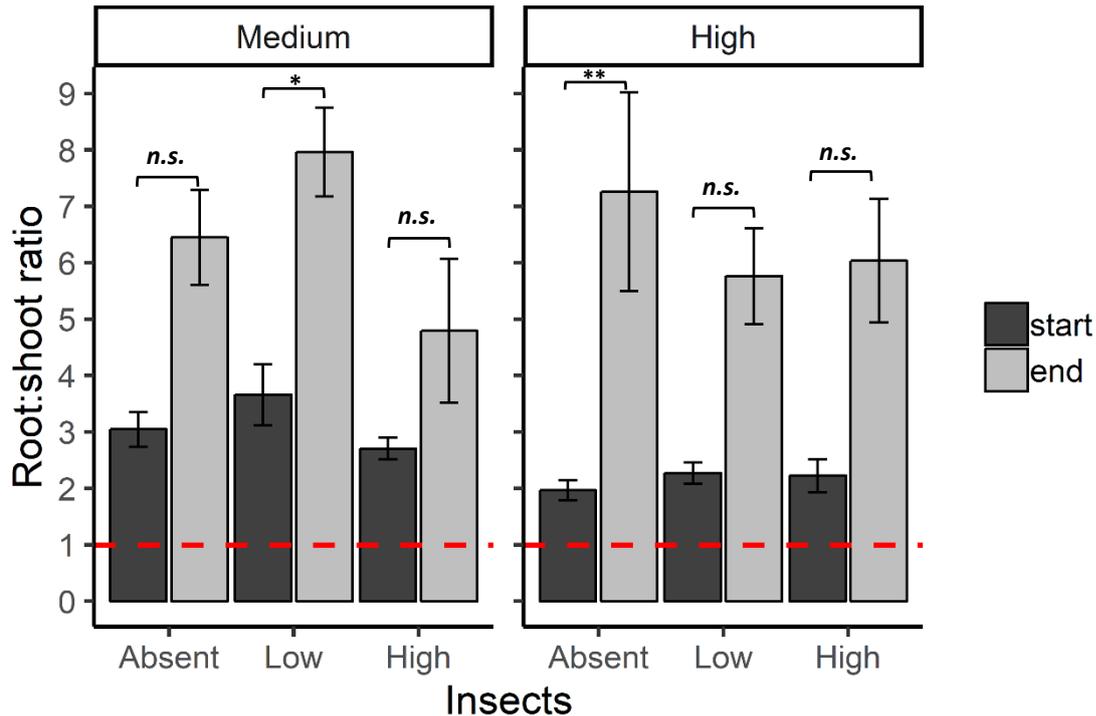


Figure 2.6. Change in **mean root:shoot ratio** over time (different coloured bars) by nutrients (between facets) and by *Megamelus scutellaris* density (within facet) (\pm SE). Significant differences between time indicated above bars (Tukey's HSD, $P < 0.05$). Due to high plant mortality, only H and M nutrient treatments are included. Roots = shoots indicated by red dashed line.

The high nutrients control was the only treatment that had a net increase in mean root length, increasing by $3.44 \text{ cm} \pm 9.13$ (Fig 2.7a), which would account for the significant increase in the root:shoot ratio for this treatment. The combined effects of biocontrol and different nutrients had no significant effect on the changes in either root length or biomass ($F_{2,54} = 0.666$, $P = 0.52$; $F_{2,54} = 0.351$, $P = 0.71$ respectively)

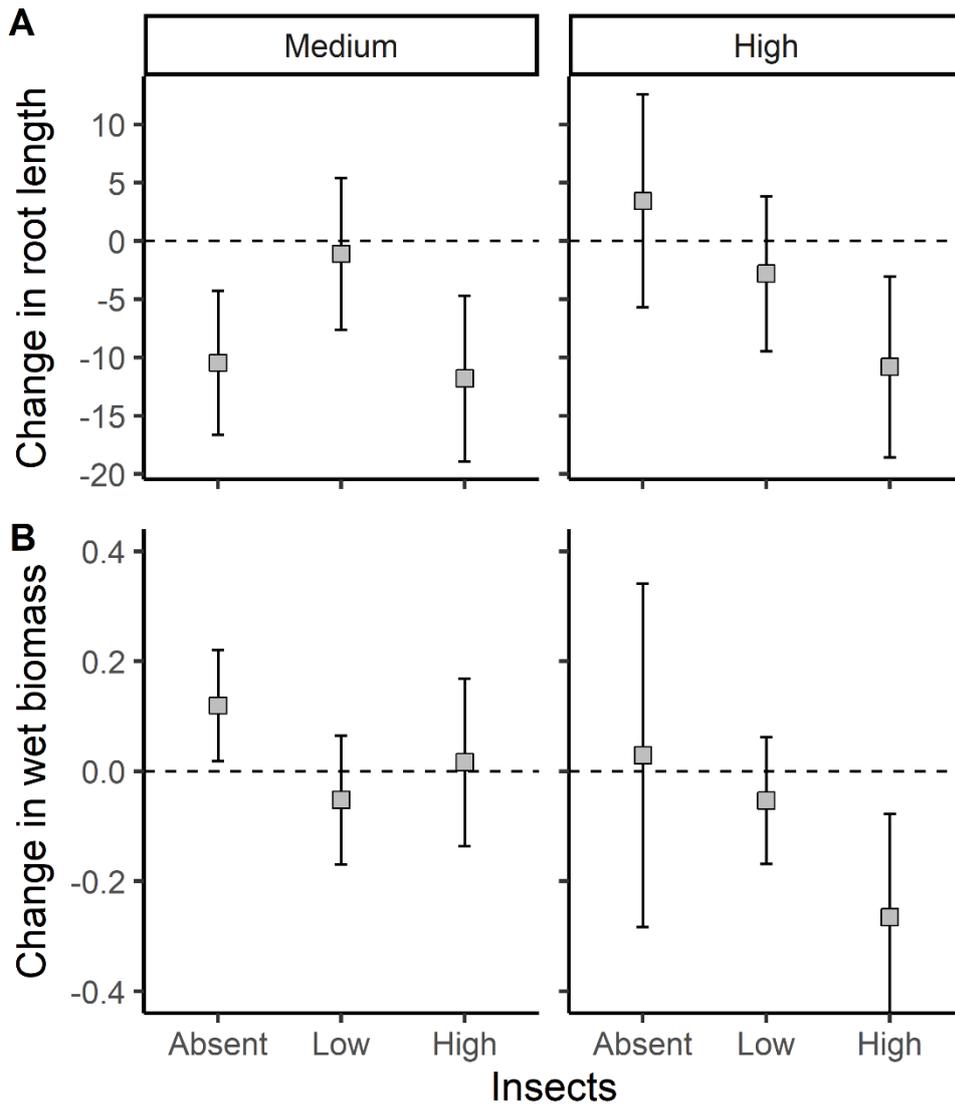


Figure 2.7. Change in (A) **mean root length (cm)** and (B) **wet biomass (kg)** by nutrients (between facets) and by *Megamelus scutellaris* density (within facet) (\pm SE). Due to high plant mortality, only H and M nutrient treatments are included. The dashed line indicates zero change.

2.3.3. Chlorophyll fluorescence

The quantum efficiency of photosystem II, measured as Fv/Fm, was significantly affected by time ($F_{1,223} = 19.67$, $P < 0.0001$) and nutrients ($F_{1,223} = 16.85$, $P < 0.0001$). The greatest increase in Fv/Fm was recorded in the medium nutrient control (insects absent), which increased Fv/Fm by 7.3% over time. Where insects were released, the Fv/Fm increased by 3.3% and 0.5% respectively for the low and high inoculations of *M. scutellaris* at the medium nutrient level.

Compared to the control, neither of the medium nutrient plants showed any significant increases in Fv/Fm, however, the Fv/Fm for the medium nutrient plants with high insect densities remained below 0.8 at the end of the experiment, which suggests that the leaves were experiencing stress.

Between nutrient levels, high nutrient plants typically had greater Fv/Fm values, and these were not affected significantly by the presence of *M. scutellaris* (Nutrients: insects $F_{21,223} = 2.49$, $P = 0.08$). The high nutrient treatment plants showed no significant change in Fv/Fm, regardless of insect level, and there were no significant higher order interactions between time, *M. scutellaris* density, and nutrients ($F_{21,223} = 1.91$, $P = 0.15$).

The performance index (PI_{ABS}), which is derived from the combination of the density of reaction centres (RC/ABS), the efficiency of excitation trapping or Q_A reduction (TRo/RC), and the rate of electron transport (ETo/RC) (Strasser *et al.* 2000), was significantly influenced by nutrients, insect density, and time ($F_{21,223} = 5.27$, $P = 0.006$) (Fig 2.8b). The PI_{ABS} of plants across all treatments increased significantly over time, except in the medium nutrient high insect treatment. The medium nutrient high insect treatment also had a significantly lower PI_{ABS} than the other treatments by week 13, except the medium nutrient control, although its total PI_{ABS} score is well below that of the control.

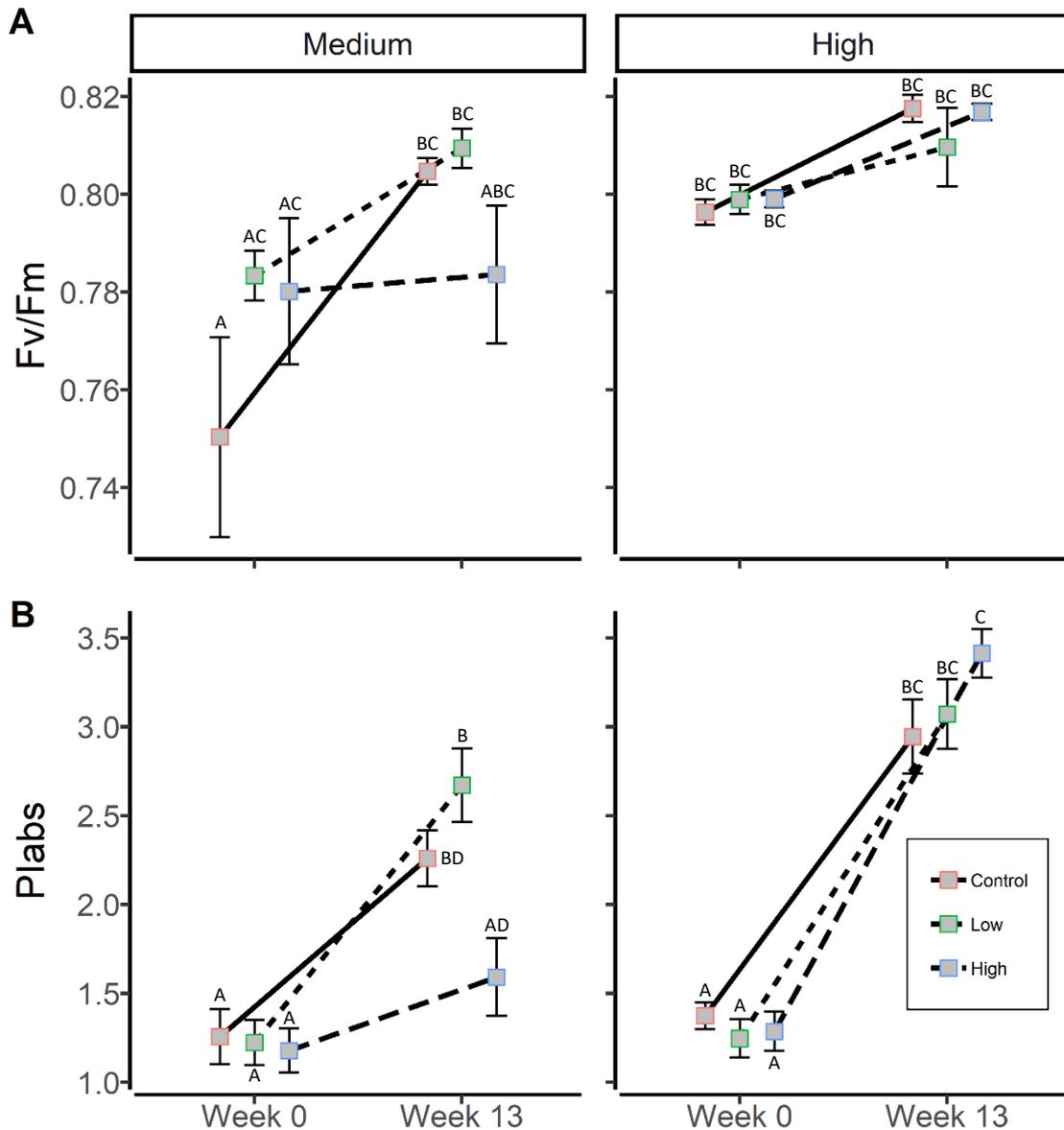


Figure 2.8. Change in (A) F_v/F_m and (B) Performance Index (PI_{ABS}) by nutrients (between facets) and by *Megamelus scutellaris* density (within facet) (\pm SE) over time. Due to high plant mortality, only H and M nutrient treatments are included. Significant differences indicated by different letters (Tukey's HSD, $P < 0.05$)

The JIP-test parameters for the specific fluxes (Figs 2.9 a-d) show that there was a significant interaction between nutrients and time in the number of photons absorbed by chlorophyll molecules per active reaction centre (ABS/RC) ($F_{21,223} = 1.19$, $P = 0.01$). In the high nutrient treatments, there was a significant decrease in ABS/RC over time in all high nutrient treatments, with the decrease suggesting that more reaction centres (RCs) were activated. The

same trend was recorded with regards to plants grown in medium nutrients, with the exception that the medium nutrient plants exposed to high *M. scutellaris* herbivory decreased significantly less in ABS/RC over time (nutrients: insects $F_{2,223} = 4.74$, $P = 0.01$) and was significantly higher than all other treatments at the end of the study (13 weeks), suggesting that the RCs were becoming inactive. The same trend was found with regard to the trapping rate of PSII (TRo/RC) (Fig 2.9b), and the rate of heat dissipation per reaction centre (DIo/RC) (Fig 2.9d) where the rate of decline over time was significantly different (time: $F_{1,223} = 205.644$, $P < 0.0001$ and $F_{1,223} = 36.08$, $P < 0.0001$ respectively), however the medium nutrient plants inoculated with high *M. scutellaris* densities declined at a significantly slower rate than in the other treatments. The higher ABS/RC, DIo/RC and TRo/RC compared to other treatments at the end of the experiment were due to the inactivation of some of the PSII reaction centres, and this is evidenced by the reduced PI_{ABS} , to which these fluxes contribute. The three-way ANOVA results indicated that nutrients, time, and *M. scutellaris* herbivory all have an effect on TRo/RC ($F_{2,223} = 3.16$, $P = 0.04$), while only the effect of nutrients ($F_{2,223} = 18.23$, $P < 0.0001$) and time ($F_{1,223} = 36.08$, $P < 0.0001$) influenced DIo/RC.

There was a significant effect of insects ($F_{2,223} = 5.97$, $P = 0.003$) and the interaction of nutrients and time ($F_{1,223} = 26.81$, $P < 0.0001$) on the rate of electron transport per active reaction centre (ETo/RC). However, ETo/RC was not significantly different between any of the treatments after 13 weeks (Fig 2.9c).

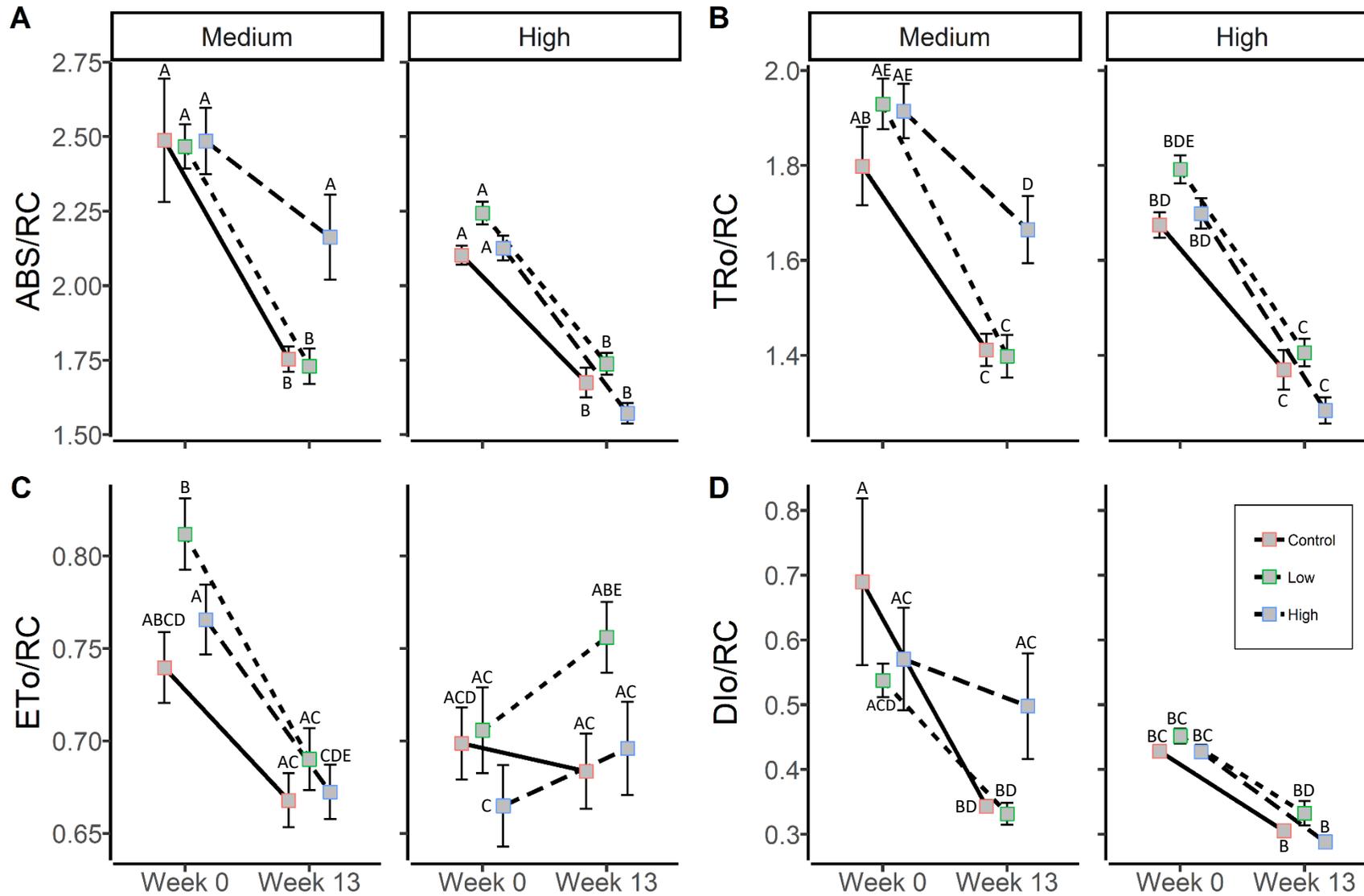


Figure 2.9. Change in the specific fluxes (A) ABS/RC (B) TRo/RC (C) ETo/RC, and (D) DIo/RC by nutrients (between facets) and by *Megamelus scutellaris* density and time (within facet) (\pm SE). Due to high plant mortality, only H and M nutrient treatments are included. Significance level indicated by different letters (Tukey's HSD, $P < 0.05$)

The chlorophyll-a fluorescent transients for the treatments are presented below (Fig 2.10) and these data were used to calculate the JIP-test parameters explored above (Figs 2.8 and 2.9). The induction curves show that nutrients have the greatest effect on the photosynthetic performance of PSII, however, there was an effect of herbivory shown in the medium nutrient treatment with a high *M. scutellaris* population (Fig 2.10a). There was a decrease in fluorescence at the J, I and P stages for the medium nutrient high insect treatment (Fig 2.10a, red line with squares), which then presented as a decrease in F_v , which is the difference between F_m (P) and F_o (O). Importantly, this difference was not mirrored in the high nutrient treatments.

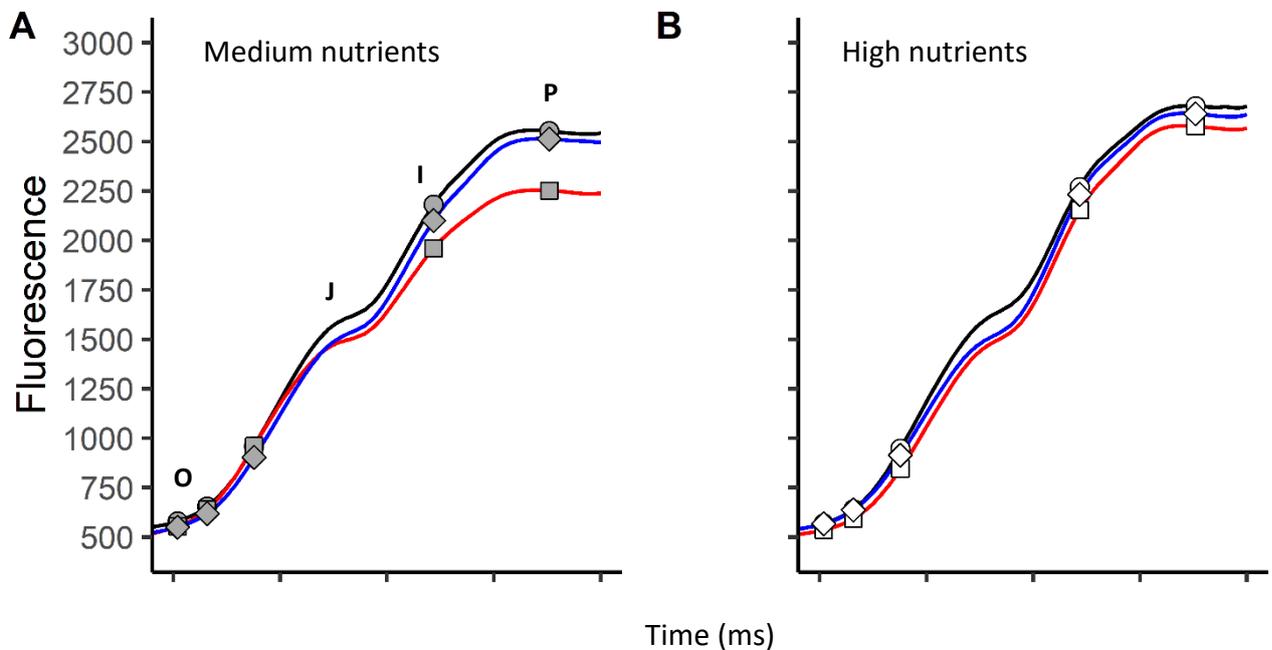


Figure 2.10. Mean chlorophyll fluorescence induction curves from dark-adapted water hyacinth leaves for (A) **medium nutrient** and (B) **high nutrient** plants plotted on a log scale from $50\mu s - 1 s$. Points indicate values at predetermined time marks, $T_1 = 50\mu s$, $T_2 = 100\mu s$, $T_3 = 300\mu s$ (K step), $T_4 = 2 ms$ (J step) and $T_5 = 30 ms$ (I step). O, J, I, and P steps are labelled. Circular markers with blue line = control, square marker with red line = high insects, diamond-shaped marker with blue line = low insects.

2.3.4. Chlorophyll content analysis

The chlorophyll content of the leaves was significantly different between the different nutrient levels ($F_1 = 23.14$, $P < 0.0001$), but not between different *M. scutellaris* densities ($F_2 = 0.07$, $P = 0.93$), or the interaction of nutrients and insect density ($F_2 = 1.08$, $P = 0.35$).

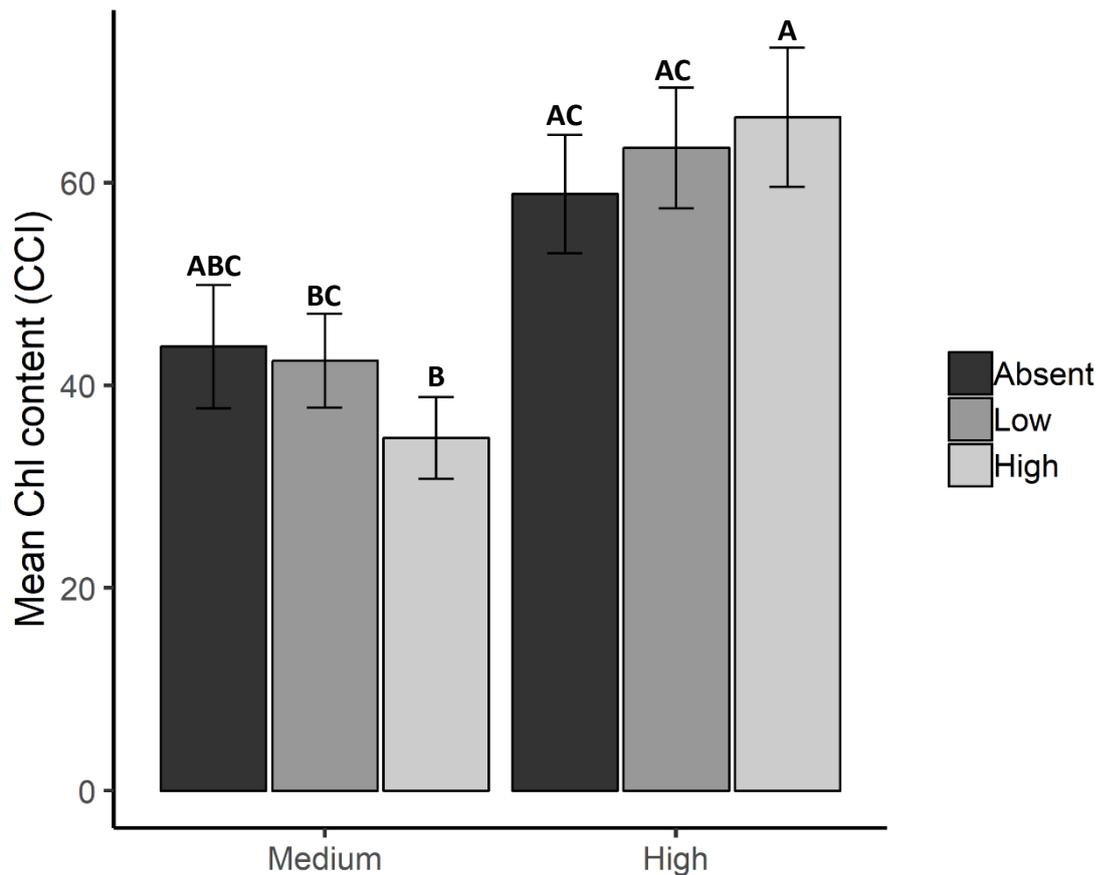


Figure 2.11. Mean chlorophyll content index (CCI) (\pm SE) of the fourth leaf on water hyacinth plants grown at different nutrient levels with different *Megamelus scutellaris* densities. Means compared by factorial ANOVA. Significant differences between nutrients and insect level indicated by different letters (Tukey's HSD, $P < 0.05$).

2.4. Discussion

This study has reiterated that nutrient input is more influential to the growth of water hyacinth than herbivory by a host-specific biological control agent (Heard & Winterton 2000; Ripley *et al.* 2006; Hill & Coetzee 2012; Marlin *et al.* 2013; Canavan *et al.* 2014). For *M. scutellaris* to

have the most impact, the results suggest that establishing a large population of insects on plants grown at medium nutrients is the most effective combination for any reduction in water hyacinth growth rate to occur. The idea that high densities of *M. scutellaris* per plant are required to initiate a stress response by the plant was initially proposed by Fitzgerald & Tipping (2013) who reported that water hyacinth does not exhibit signs of stress unless there is a population of more than 100 insects per plant. The data presented in this chapter concur that high numbers of *M. scutellaris* are required to cause damage to water hyacinth. Fortunately, due to the rapid reproductive rates of *M. scutellaris*, attaining high densities of the planthoppers is not necessarily difficult to accomplish given the correct combination of nutrient-enriched plants and optimal temperatures (Grodowitz *et al.* 2017).

The effect that *M. scutellaris* had on the plants was variable, and much of the variation in the plant parameters was due to changes over time, differences between nutrient levels, or a combination of both. Furthermore, all of the plants decreased in size (explained by the reduced length of the longest petiole), irrespective of nutrients or the presence of *M. scutellaris*, over the course of the experiment which may have been caused by cooler temperatures and/or reduced daylight hours over winter, or possibly as a result of a reduction in photosynthetically active radiation (PAR) reaching the leaves. PAR was reduced by filtering through the mesh covers placed over the plants which prevent the movement of *M. scutellaris* between different treatments and to avoid contamination by other species. The reduction in PAR from the mesh has been previously recorded to be as great as 40% (Heard & Winterton 2000). It is also possible that, by growing mats of water hyacinth in each mesocosm, which typifies natural water hyacinth infestations, intraspecific competition between water hyacinth plants could have also played a role in some of the changes recorded, as increases in the densities of water hyacinth per unit area have been shown to have an effect on the size of the plants, the number of leaves per rosette, as well as the plant phenostage (whether it has elongated or bulbous

petioles) (Center & Spencer 1981). The decrease in petiole length was greatest in high nutrient treatments compared to medium nutrients, and this may provide further evidence for a decrease in size in response to intraspecific competition.

The number of leaves per plant at the end of the experiment was not significantly different between any of the treatments, although the reduction in leaves in the medium nutrient treatment with high *M. scutellaris* was reduced by the greatest amount, from 6.6 ± 0.4 leaves per plant to 4.1 ± 0.5 while none of the other high or medium nutrient treatments ever had fewer than 5 leaves per plant on average. The feeding damage by *M. scutellaris* had less impact on plants grown at high nutrients, as the elevated nutrients meant that the plants were able to compensate for the damage caused by the feeding, or were otherwise able to attain biomass at a rate that exceeded the planthopper's ability to destroy them. Water hyacinth has been shown to be able to compensate for low but sustained feeding damage at all nutrient levels, but the compensatory response decreases when there is high herbivore pressure (Soti & Volin 2010). However, there is evidence that the concentration of phenolic compounds increases when water hyacinth is grown in elevated nutrients, and phenolics are likely to be induced by sustained herbivory (Center & Wright 1991). If secondary plant metabolites were present, this could have affected the insects' ability to feed and survive in the high nutrient treatments (Center & Wright 1991).

The decrease in size for many of the plants was not likely to be due to increased stress, as many of the stress-indicating parameters (eg Fv/Fm, PI_{ABS}) increased instead of decreased over time, suggesting that the plants were more efficiently converting photons of light into energy, even in the presence of *M. scutellaris*. However, the performance index and Fv/Fm were lower when *M. scutellaris* was present in high densities at medium nutrients indicating that light energy was being dissipated from the leaf as heat, light (increased fluorescence), or moved as energy to other parts of the plant (Mathur *et al.* 2011), instead of being used to drive photochemistry.

An increase in energy dissipation was shown by the increase in DI_o/RC which rises when there are more inactive RCs.

The trapping flux, TR_o/RC , decreased significantly in all treatments over time, showing that Q_A was efficiently transferring electrons to Q_B . However, TR_o/RC decreased significantly less in the medium nutrient high *M. scutellaris* treatment, indicating that *M. scutellaris* feeding damage (A) caused the reaction centres to become inactive, and (B) that the feeding had an effect on the efficiency by which Q_A transferred electrons, and therefore more energy was lost as the Q_A was reduced but unable to oxidise back to its original state efficiently.

The variation in the F_v/F_m and other fluxes between insect levels could not be explained by significant changes in chlorophyll content, as these were not different between treatments within nutrient levels. However, there were some differences between nutrient levels, with high nutrient treatments always having more chlorophyll, and this variation is expected and accounted for in the results.

The initial damage caused by delphacids is subtle and often difficult to quantify unless it is measured by analysing some aspect of photosynthetic efficiency, as the data in this chapter have shown. Through measurements of photosynthetic rates, the feeding by the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), which is another hemipteran species and a serious pest of rice in Asia, was found to reduce the growth and yield of the plants by removing photosynthates from the leaves. High densities of *N. lugens* also led to plant mortality (Watanabe & Kitagawa 2000). Similar studies have yielded comparable results, showing that a range of phytophagous hemipteran families can reduce photosynthesis in plants through sustained feeding. This includes the Russian Wheat Aphid on wheat (Haile *et al.* 1999), and the spittlebug *Mahanarva fimbriolata* (Cercopidae) on sugarcane (Soares *et al.* 2017), both of which caused reduced crop yields and decreased plant growth.

The feeding damage caused by salivary sheath feeding delphacids causes a condition called hopper burn, which leads to chlorosis of the host plant. Brentassi & Maldonado (2002) showed that planthopper saliva from a major maize pest, *Delphacodes kuscheli* Fennah, contains digestive enzymes that can damage the cell walls of its host plant, and that the mechanical probing by the rostrum of planthoppers can damage the chloroplasts. Damage to chloroplasts could explain how the RCs became inactive and why Fv/Fm is decreasing in heavily damaged water hyacinth plants infested with *M. scutellaris*.

2.5. Conclusion

The results of this chapter found that, at the insect densities used, and for the time period monitored, herbivory by *M. scutellaris* is not sufficient to damage plants in highly eutrophic water and that this agent is more likely to have an effect at a medium to low nutrient level if the population density is high. Furthermore, using chlorophyll fluorometry as a method of detecting the initial stages of damage by sap-sucking insects has been shown to be effective in a glasshouse setting and could provide useful avenues of research in the future. *Megamelus scutellaris* is likely to be an effective biological control agent of water hyacinth in South Africa, provided that the nutrient status of the river systems and other water bodies is addressed. However, the way in which phenology and seasonal changes can affect the populations of *M. scutellaris* in relation to its host plant is important, and is explained in the following chapter.

Chapter 3: Seasonal population dynamics and phenology of *Megamelus scutellaris* and water hyacinth on the Kubusi River (Stutterheim, Eastern Cape)

3.1. Introduction

The lack of adequate biological control agent establishment in many colder, high-altitude sites in South Africa has been a major hindrance to the control of water hyacinth in South Africa (Hill & Olckers 2000). One of the reasons suggested by Hill & Olckers (2000) was that water hyacinth often grows in colder areas in South Africa than it would in its tropical South American native range, leading to a climatic mismatch of the insects associated with the plant. Climatic incompatibility of biological control agents in introduced ranges is a major reason for the failure of the insects to establish on their target weeds (McEvoy and Coombs 2001). The difference in the temperature negatively impacts the survivorship, reproductive output, and feeding rates of beneficial insects introduced for biological control (Kingsolver 1989).

In much of the literature, the impacts that temperature and season have on both plant quality and insect populations have been overlooked (Gassmann 1996). However, Byrne *et al.* (2004) outlined the benefit of using degree-day models and the insect-specific thermal physiology data of several biological control agents to predict their modelled post-release distribution compared to their actual distribution. In addition, Paterson *et al.* (2014) successfully used climate matching to locate the most climatically favourable areas in South America to prioritize the search for potential biological control agents to manage the invasive creeping cactus *Pereskia aculeata* Miller (Cactaceae) in South Africa. For wide-spread weeds, like alligator weed *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae), CLIMEX modelling was used to predict where the flea beetle, *Agasicles hygrophila* Selman and Vogt (Coleoptera: Chrysomelidae) would be successful outside of the USA where it has already established. In the event of the weed spreading to new areas, this information can facilitate the implementation

of a biological control programme in areas outside of the USA, where the weed is already problematic (Julien *et al.* 1995). These methods have proven useful for determining which localities are climatically the most likely to support the successful establishment of biological control agents outside of their native ranges (Byrne *et al.* 2004). However, even in ideal temperature conditions, many host-specific insect herbivores are dependent on their host-plants for all stages of their life cycles. Hence, the seasonal availability and quality of the host-plants are important for the establishment of self-sustaining biological control agent populations in the field, particularly because host-specific herbivores are likely to seek out the highest quality host plants for feeding and oviposition, implying that establishment is likely to be largely bottom-up regulated. However, the quality of the plants is influenced by seasons, with periods of extreme growth usually only occurring in specific growing seasons (Price 2000).

3.1.1. Seasonal effects on plant host quality

Plants growing in areas with distinct seasons undergo temporal phenotype changes with shifting seasons. These variations typically involve a reduction in the concentrations of nutrients and important elements (e.g. carbon, nitrogen, phosphorous, magnesium) in the leaves, and these changes usually occur as the leaves begin to senesce at the end of the growing season (Adams *et al.* 1990). The changes are brought about by subtle fluctuations in the ambient temperature and photoperiod which are recognised by an endogenous, hormone-mediated mechanism within plants called the circadian clock (Searle & Coupland 2004), which is involved in the seasonal induction of flowering and bud burst in plants. As the photosynthetic tissues become less nutritious, the plant is likely to become less favourable to some insect herbivores.

Studies of phloem-feeding hemipterans have shown that the amount and quality of nitrogen in the phloem is directly related to the fecundity of the herbivore. Dixon (1970) (also, reviewed

in Awmack & Leather 2002) showed that, at the start of the growing season, the leaves of the sycamore tree (*Acer pseudoplatanus* L.) contained a higher concentration of amino acids than at the end. The reduction in amino acid content as the leaves matured had a direct, and negative, effect on the population density and overall fecundity of the host-specific herbivore, the common sycamore aphid *Drepanosiphum platanoidis* (Schr.) (Hemiptera: Aphididae), which feeds exclusively on the leaves of the Sycamore tree (Dixon 1970). However, unlike many other types of folivorous insects, aphids can survive on plants with lower nitrogen levels due to the presence of bacterial *Buchnera* symbionts which live in their guts and provide them with essential nutrients, like amino acids. Certain amino acids are either not present or present in very low amounts in the phloem sap of plants (Douglas 1998), and there are some suggestions that delphacid planthoppers, such as *M. scutellaris*, may also derive some important nutrients from microbial symbionts in their guts. However, other hemipteran species cannot extract these nutrients as efficiently as aphids (Denno & Roderick 1990; Awmack and Leather 2002) and they are thus more reliant on their host plant being of greater quality for their survival and reproduction. However, plant quality will fluctuate between seasons (Price 2000), so host specific herbivores need to adapt to this.

3.1.2. Seasonal effects on folivorous insects

Many herbivorous insects undergo diapause or states of reduced activity during certain times of the year when conditions are less favourable. Diapause, either obligate or facultative, is usually triggered by the interactions of photoperiod and temperature, however, host-plant quality has also been shown to induce diapause (Takagi & Miyashita 2008). Facultative diapause occurs in many multivoltine insects that live in environments with unpredictable seasons, where host plant quality can fluctuate seasonally and between years (Hunter & McNeill 1997). The microhabitat of the host plant leaf surface is of greater consequence to the insect than the greater environment in determining if insects undergo facultative diapause, and

in some polyphagous insects, the quality of the host plant they are feeding on is also an important deciding factor in how long they stay active (Hunter and McNeill 1997). Research has shown that some insects, such as the butterfly *Byasa alcinous* (Klug) (Lepidoptera: Papilionidae), are more likely to undergo pupal diapause when the larvae feed on tougher leaves with a higher C:N ratio than larvae that feed on soft, more nutritious leaves. The tough leaves also decrease rates of pupal survival and increase the length of the pupal stage of *B. alcinous* (Takagi & Miyashita 2008).

Delphacid planthoppers also undergo facultative diapause, and research has shown a significant response to daylight length and temperature on diapause induction in the major crop pest *Laodelphax striatellus* (Fallén) (Hemiptera: Delphacidae) which diapause as 3rd or 4th instar nymphs, measurable as a distinct developmental delay over nondiapausing individuals (Wang *et al.* 2014). Most delphacid planthoppers overwinter as nymphs, but in the colder, northern latitudes they can also overwinter as eggs (Denno & Roderick 1990). The benefit of overwintering as a nymph, as opposed to an egg, is that the nymphs can begin to feed as soon as the climate is suitable, forgoing the development time required for an egg to hatch (reviewed in Denno & Roderick 1990). This seems to be consistent with field observations of *M. scutellaris* in its native range, whereby the nymphal stage is the most commonly encountered during winter (Sosa *et al.* 2005), although the insects can often experience high winter mortality (reviewed in Denno & Roderick 1990). High winter mortality in biological control agents has obvious negative implications as fewer insects post-winter means that the ability of the insects to colonize and feed on the new flush of the growing season is compromised by the small seasonal founding population.

The thermal physiology of *M. scutellaris* was determined by May & Coetzee (2013) prior to its release in an effort to determine if the insects, which originated from the cooler areas of Peru and Argentina (Tipping *et al.* 2008) would be able to survive and reproduce effectively in

South Africa. May & Coetzee (2013) concluded that *M. scutellaris* was climatically incompatible with certain high altitude sites around South Africa which experienced very cold winters. However, Tipping *et al.* (2014) noted that, while *M. scutellaris* performed poorly in areas in the USA with very hot summers, the agents overwintered for at least three consecutive years in Gainesville, Florida despite multiple days of below-freezing winter temperatures. Therefore, this chapter aimed to ground-truth these predictions by monitoring *M. scutellaris* population dynamics on the Kubusi River near the town of Stutterheim through all four seasons to document plant growth parameters, plant biomass, and insect density. The Kubusi River was selected as the site for this study for two main reasons: it is the first release site of *M. scutellaris* on water hyacinth in the field in South Africa, where the insects have established a continuous population since being first released in November 2013, and this is also the coldest site where a water hyacinth biological programme has been implemented in South Africa, which was initially selected as a long-term monitoring site in the report on integrated water hyacinth management by Byrne *et al.* (2010). Being the coldest site, and given that cold weather limits the establishment of biological control agents, this site allows us to rigorously test the winter survival and post-winter recovery of *M. scutellaris* in the field.

3.2. Materials & Methods

3.2.1. Study Site

The site selected for this study was on the Kubusi River near Stutterheim in the Eastern Cape Province of South Africa (S 32°34'35.4" S 27°28'56.8" E) (Fig 3.1). The site lies at 774 m AMSL and is heavily infested with water hyacinth. The Kubusi River is the coldest site where biological control agents have established on water hyacinth in South Africa until present (Coetzee 2012) but has fewer frost days than the Highveld sites, which makes the site thermally important, but less physiologically stressful on the biological control agents (Byrne *et al.* 2010).

The Kubusi River falls within the Afromontane biome of the Amathole mountain region, which is a high altitude forest biome with patches of grassland (Lubke *et al.* 1986), although much of this has been cleared for forestry and agriculture. The climate of the area is characterised as temperate, with year-round rainfall and cold winters which can experience up to 56 days of heavy frost each year (Byrne *et al.* 2010).

The frost leads to the die-back of the above water biomass each winter and reduced availability of leaves for insects to feed on (Fig 3.2a). The biomass grows back vigorously in Summer (Fig 3.2b) and quickly recolonizes the available water surface.

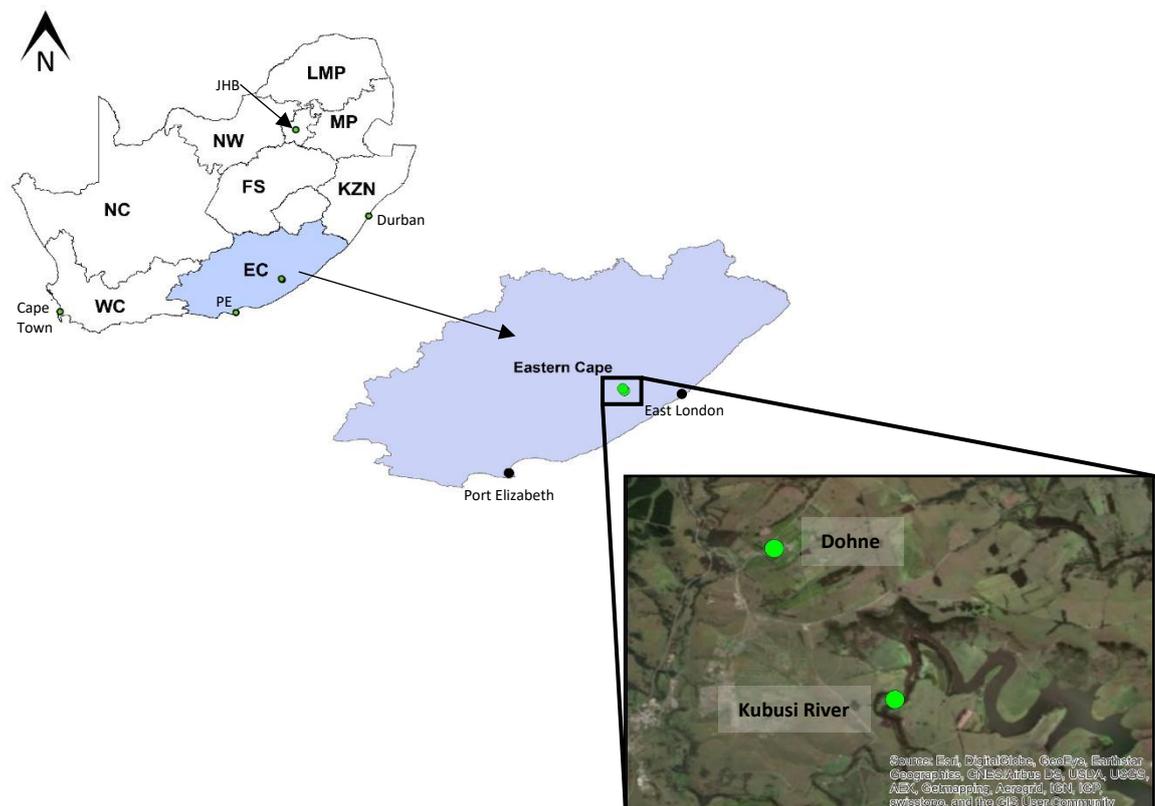


Figure 3.1. The position of the Kubusi River site in relation to the Dohne weather station in the Eastern Cape Province of South Africa.



Figure 3.2. Fixed point images of the Kubusi River in late winter (A) and mid-summer (B) in 2017

3.2.2. Data collection

A section of the Kubusi River (-32.56503, 27.48915) was sampled monthly for 15 months from May 2017 until August 2018. Plant and insect data were collected from the same site on the system each month and the data were compared with temperature and rainfall data received from the nearby Dohne weather station which is approximately 3 km away from the site where the data was collected (Fig 3.3) (S 32°32'02.4" E 27°28'01.2", altitude 901 amsl). The climate data from the Dohne weather station, which were provided by the South African Weather Service, were not significantly different from the climate of the river and were therefore used as a proxy for the climate at the Kubusi River (Coetzee 2012).

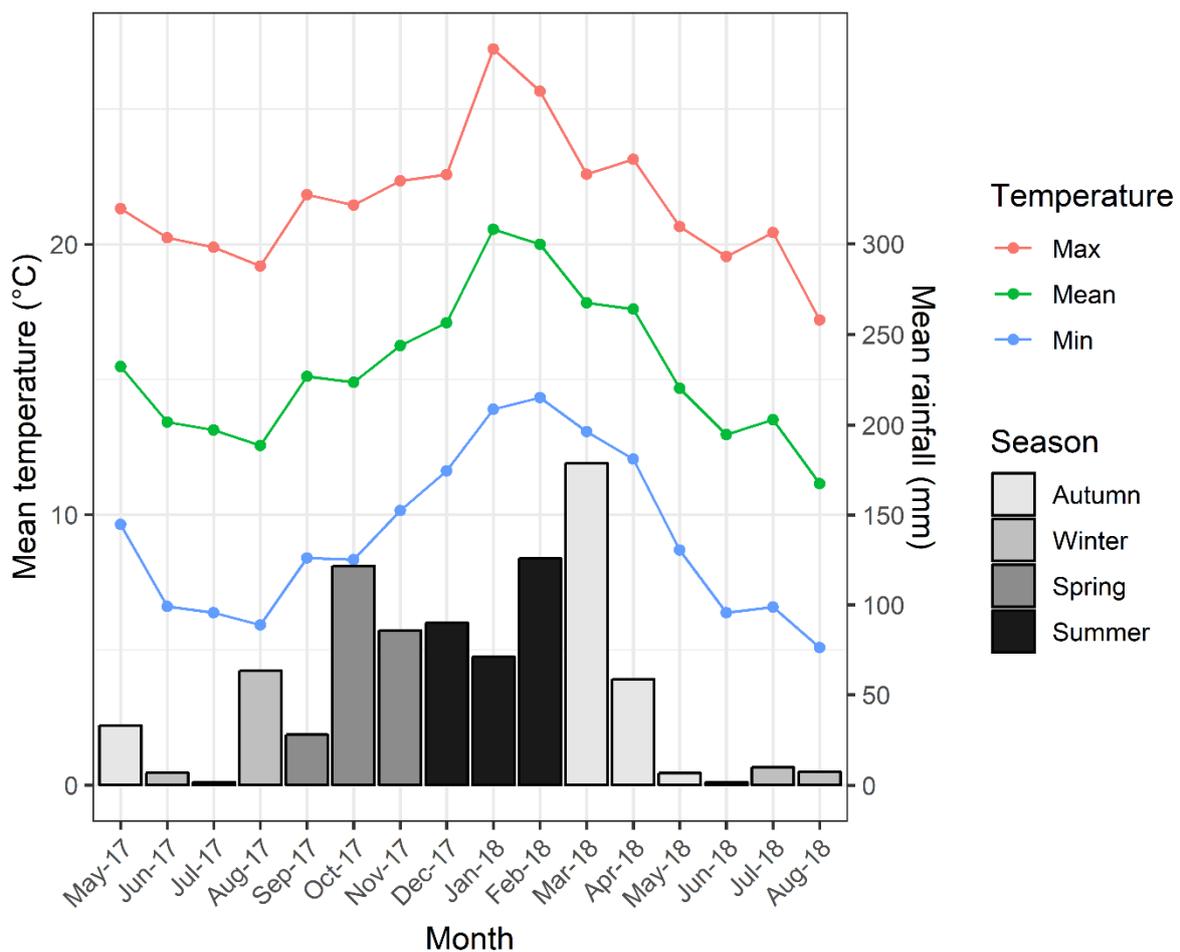


Figure 3.3. Monthly minimum, maximum and mean temperature with total monthly rainfall by season from the Dohne weather station near the Kubusi River over the duration of the study.

3.2.2.1. Plant health and fecundity measurements

The population structure and establishment potential of a biocontrol agent are determined, in part, by the quality and quantity of its host plant. Therefore, plant parameters were measured from 10 randomly selected water hyacinth plants from within the site each month. The measured plant parameters were adapted from Coetzee & Hill (2012), and are listed as follows: (1) the length of the longest live petiole, which is a measure of plant height, (2) total root length which is an indication of nutrient status and intra-species competition, (3) the number of ramets (daughter plants) which determine the extent of clonal reproduction, and (4) the number of photosynthetically active leaves on each plant, which is important for host-plant quality and *M. scutellaris* feeding. The number of flowers present is an important measure of sexual reproduction and seed set in water hyacinth, but these data are not presented due to low flower numbers at the site (only three flowers recorded in 15-month sampling effort, all of which occurred between January and February 2018).

Each month, three randomly selected 0.25m² quadrats of biomass were also removed from within the water hyacinth mat, and the contents of each quadrat were separated into above water biomass (green leaves and petioles), below water biomass (roots), and dead biomass, and weighed to determine the wet weight. The number of individual plants per each quadrat was also recorded. These quadrat data were multiplied by 4 to get a value per square metre.

3.2.2.2. *Megamelus scutellaris* population measurements

The density of *M. scutellaris* present at the site on the Kubusi River was determined using a purpose-built sampler, which was adapted from the “Minteer Method” (C. Minteer, unpublished) which has been used to measure aquatic insect numbers on floating aquatic macrophytes in the USA. The sampler used in this chapter was devised from an 80 L black plastic bin from which the bottom was removed and into which a thin metal wire frame was

built (Fig 3.4). The sampler was placed over water hyacinth plants in the water and pushed down, submerging the plants. The wires kept the plants underwater, allowing *M. scutellaris* nymphs and adults present on the water hyacinth to float up onto the water's surface and the side of the bucket where they could be counted. No differentiation was made between the nymphs or adults in the analysis. This was repeated 10 times in different parts of the site where plants were most accessible.



Figure 3.4. Sampler for estimating the population density of *Megamelus scutellaris* in the field.

3.2.2.3. Statistical analyses

An ANCOVA was used to determine how plant parameters and seasonal variables affected the density of biological control agents on the Kubusi River. To find a comparative method of measuring the insect density, the data were transformed from a measure of insects/sample to insects/m² by equating the circular area of the base of the sampler (Fig 3.3) to a square metre, yielding a conversion factor of

$$\frac{\bar{X}_{\text{insect number per sample}}}{0.093} = \text{insects. m}^{-2}.$$

A Kruskal-Wallis test with a post hoc Nemenyi-test for multiple comparisons of (mean) rank sums of independent samples using the Tukey method was used to determine if there were differences in the insect population density between seasons. Linear regression models were used for correlations.

The statistical analyses were completed using RStudio statistical software v 3.4.3 (R Core Team 2017).

3.3. Results

3.3.1. Plant health and fecundity measurements

Over the 15 month sampling period, the length of the longest petiole fluctuated greatly depending on the season. On average, the plants were tallest during the mid-summer in January 2018 (54.48 ± 4.38 cm), and shortest during the late-winter in August 2018 (8.00 ± 0.80 cm) (Fig 3.5A). The mean number of leaves per plant was greatest in May 2017, with a mean of 8.6 ± 0.58 leaves per plant. However, in both years, leaf number decreased rapidly after May as the winter frost began to set in in June, reducing the number of viable leaves in the canopy to the annual minimum of 0.8 ± 0.33 leaves per plant. The annual minimum in 2018 occurred a month later than in 2017, with 0.7 ± 0.21 leaves per plant in July 2018 (Fig 3.5B).

The ramet production, which is a representation of fecundity, was reduced to almost zero over the winter of 2017, with only a few plants bearing any daughter plants. However, the number of ramets produced over the winter of 2018 was much higher, with a mid-winter mean of 2.8 ± 0.47 and 2.6 ± 0.43 ramets for May and June 2018 respectively, whereas only 0.4 ± 0.27 were recorded in May 2017, and none in June 2017 (Fig 3.5C). The highest number of ramets were recorded in April 2018, with a mean of 3.1 ± 0.38 ramets per plant.

Root length varied over time and between seasons. The longest roots were recorded in December 2018, during the mid-summer (105.28 ± 8.78 cm), while the shortest roots occurred in the late-summer of March 2018 at 27.7 ± 4.48 cm (Fig 3.5D).

The above water biomass peaked in January 2018, mid-summer. At the highest point, the above water biomass was 15.67 ± 3.84 kg/m² (Fig 3.6A). There was a marked decrease in the above water biomass as winter set in, leading to annual minima of 4.33 ± 0.88 kg/m² in October 2017 and 2.07 ± 0.52 kg/m² in August 2018 (although August was the last sampling period for 2018, and the biomass may have decreased further afterwards). However, the below water biomass, which is an indicator of nutrients and intraspecific competition, was variable between months, but there was a generally decreasing trend in the root biomass over the duration of the study. The maximum root biomass of 12.67 ± 3.53 kg/m² was measured in June 2017, decreasing steadily to a mean minimum in August 2018 of 1.87 ± 0.92 kg/m² (Fig 3.6B). The lowest root biomass also coincided with the lowest above water biomass in August 2018.

Old leaves and petioles that had senesced or been damaged by winter frosts formed the dead biomass. The amount of dead biomass present at each sampling interval was variable and did not follow a seasonal trend. The mass of dead material did, however, decrease sharply in January 2018 to 2.13 ± 0.58 kg/m², which coincided with a marked increase in live biomass (Fig 3.6C). The least dead biomass was recorded in July 2018 (0.13 ± 0.13 kg/m²), and the greatest mass of dead material was recorded equally in June and November 2017, both measuring 7.67 kg/m² (± 1.45 and ± 0.33 respectively).

The number of plants per square meter is an indication of the density of plants in the mat and followed a seasonal trend. The highest density of plants was recorded during the mid-summer in December 2017, with 74.67 ± 9.33 plants/m², while the lowest plant density occurred at the end of winter in August 2018, with only 28.00 ± 4.00 plants/m².

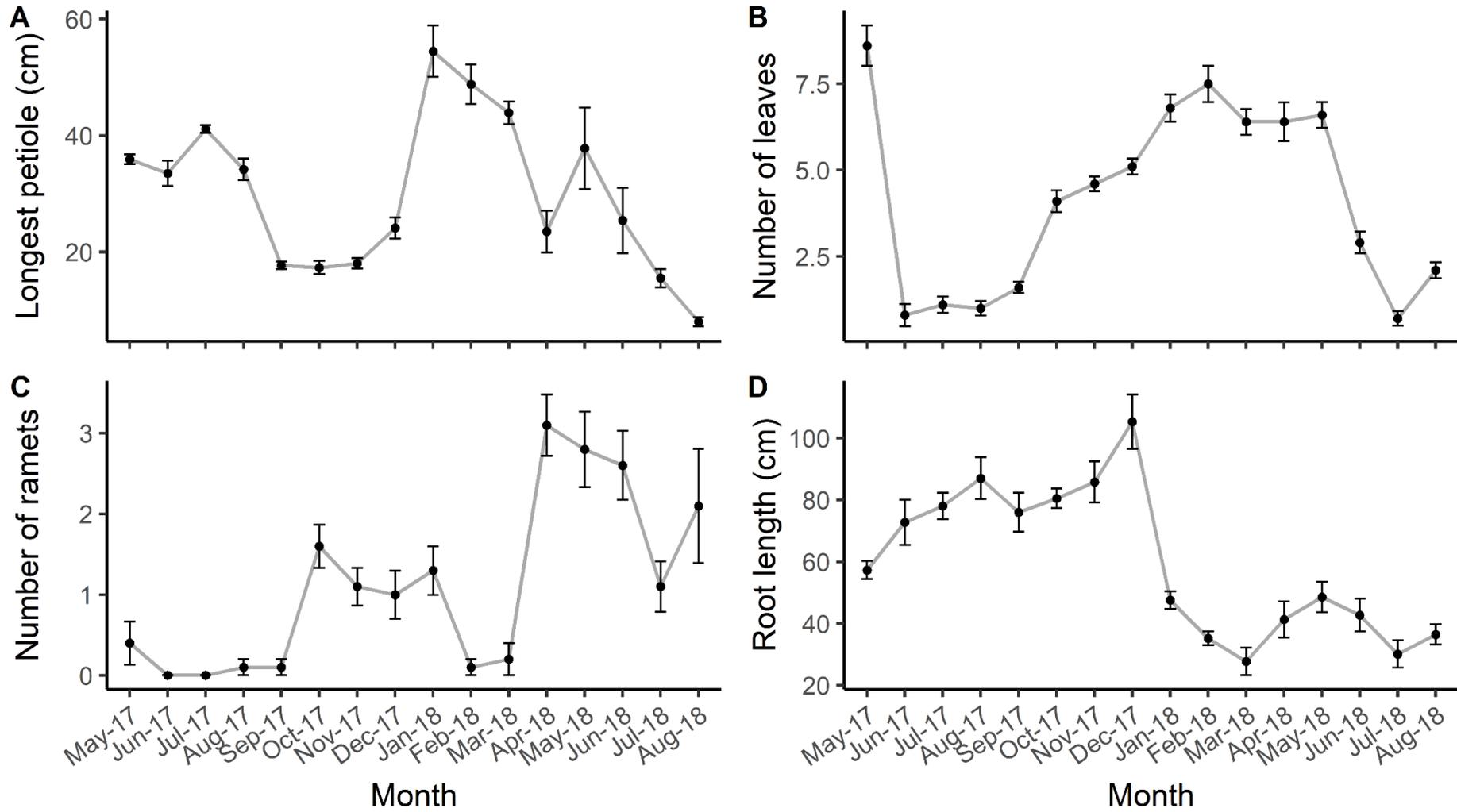


Figure 3.5. Mean of (A) the length of the **longest petiole (cm)**, (B) the **number of leaves/plant**, (C) the **number of ramets/plant** and (D) the **root length** of water hyacinth plants in the Kubusi River between May 2017 and August 2018. Error bars = \pm S.E.

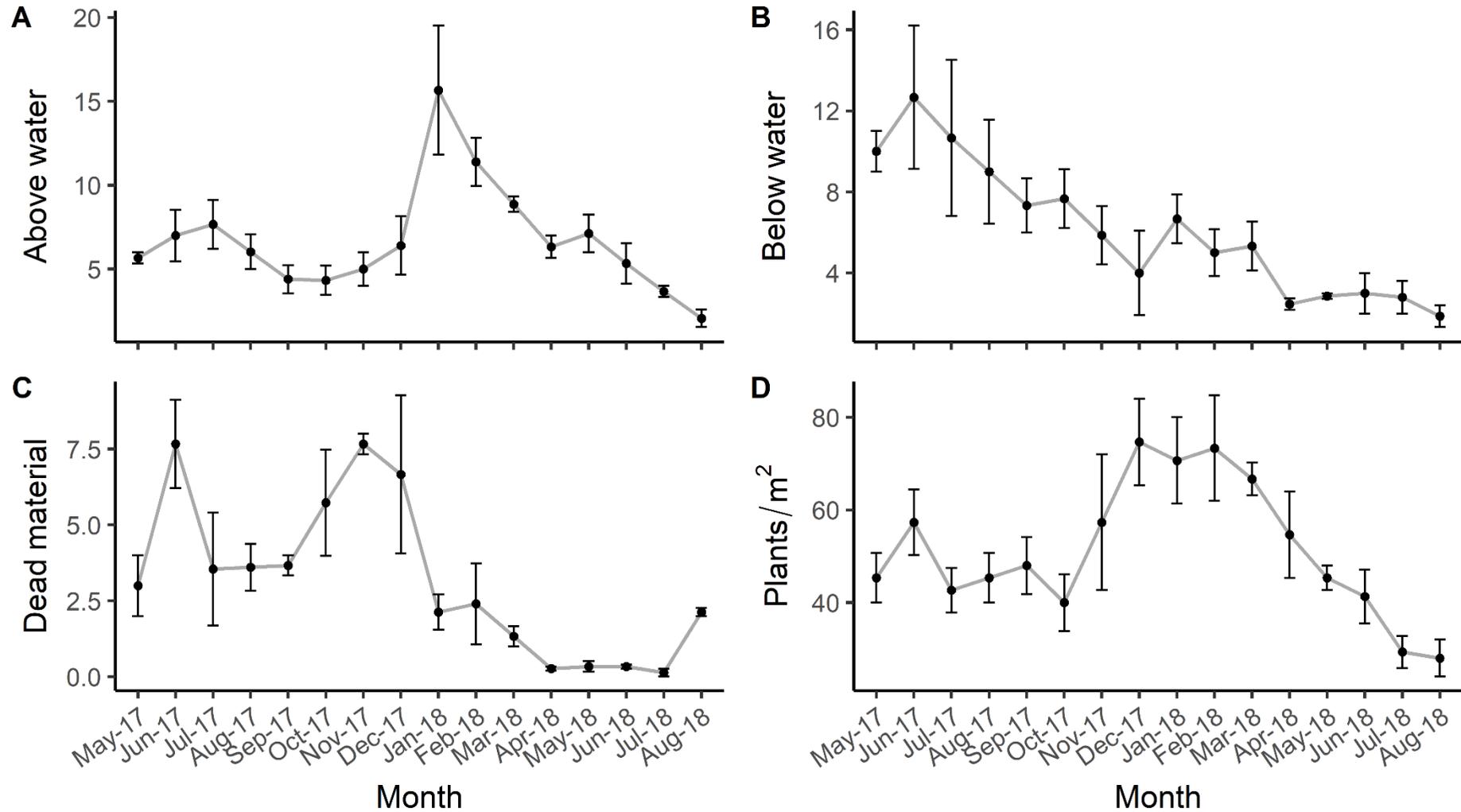


Figure 3.6. Mean of (A) the above water biomass (kg/m²) (B) the below water biomass (kg/m²), (C) the dead biomass (kg/m²) and (D) the number of plants per m² of water hyacinth plants in the Kubusi River between May 2017 and August 2018. Error bars = ± S.E.

The root:shoot biomass ratio indicates seasonal changes in the above water biomass against the below water biomass. During the summer months, there was a highly positive correlation between above and below water biomass modelled by $y = 1.61x + 2.7$ ($R^2 = 0.50$, $F_{1,7} = 8.98$, $P = 0.02$) (Fig 3.7). There was also a highly supported positive correlation between the two variables in winter, except that the slope of the modelled line was not as steep in winter, suggesting that for every cm of growth in the roots, the above water biomass growth was not as great as it was during the summer. The winter root:shoot ratio was modelled by $y = 0.39x + 2.6$ ($R^2 = 0.74$, $F_{1,16} = 49.79$, $P < 0.0001$). Less well-supported linear models occurred in the spring ($y = 0.39x + 1.9$, $R^2 = 0.32$, $F_{1,7} = 4.82$, $P = 0.06$) and autumn ($y = -0.1x + 7.5$, $R^2 = -0.05$, $F_{1,10} = 0.46$, $P = 0.51$).

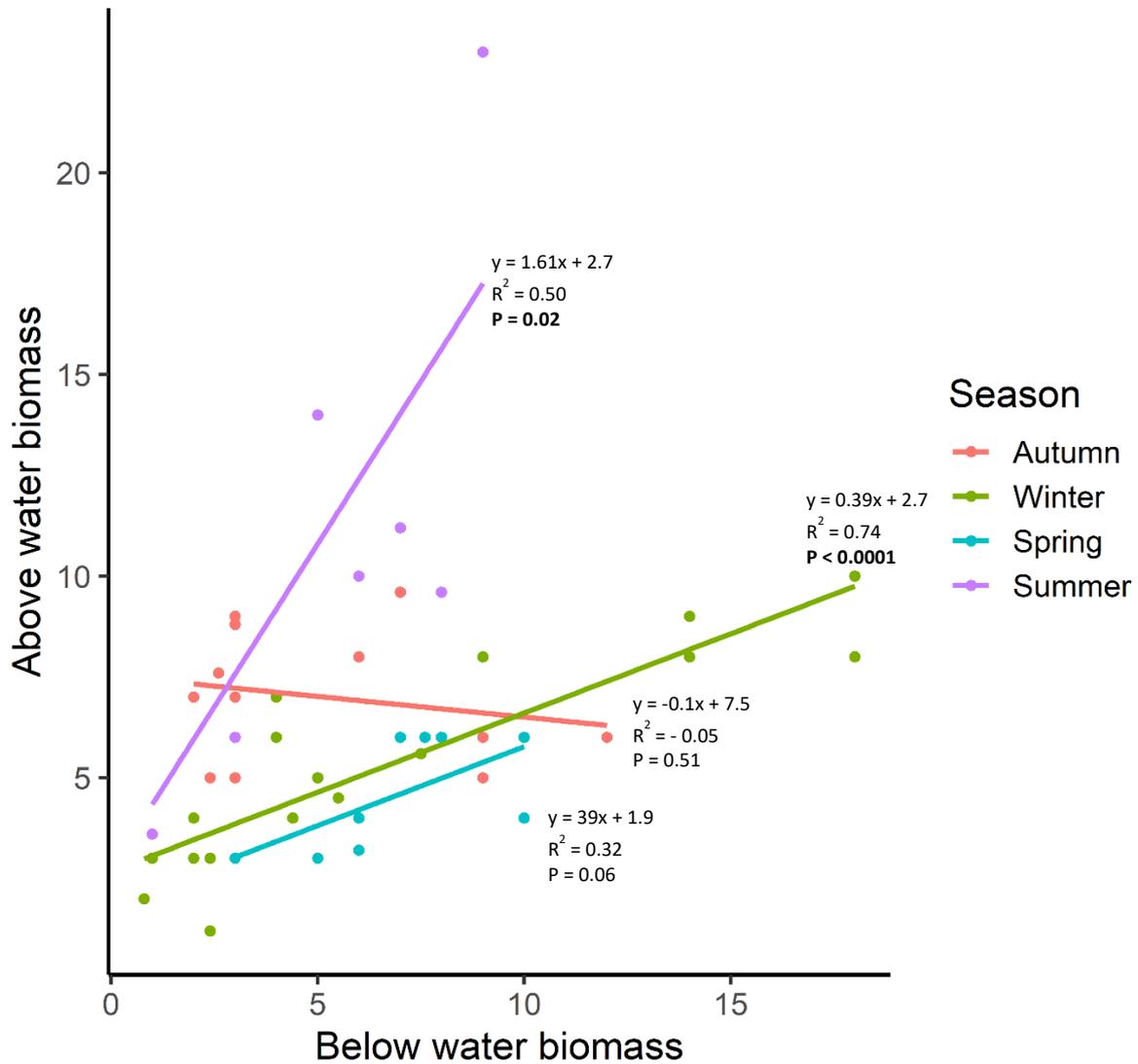


Figure 3.7. The **root:shoot ratio** of water hyacinth plants in the Kubusi River between May 2017 and August 2018. The trend lines indicate the best fit linear models.

Both the length of the longest petiole ($y = 2.39x + 8$, $F_{1,14} = 5.876$; $p = 0.03$; $R^2 = 0.25$) (Fig 3.8A) and the number of leaves per plant ($y = 0.72x - 2.5$, $F_{1,14} = 25.85$; $p = 0.03$; $R^2 = 0.62$) (Fig 3.8B) were significantly correlated with minimum temperature. Unlike the longest petiole, the linear equation predicting the number of leaves accounted for more of the variation in the data, indicating that the number of leaves present on each plant more closely tracked the seasonal changes in the minimum temperature. The minimum temperature was used as low minimum temperatures are limiting factors for both water hyacinth growth as well as *M. scutellaris* survival and reproduction.

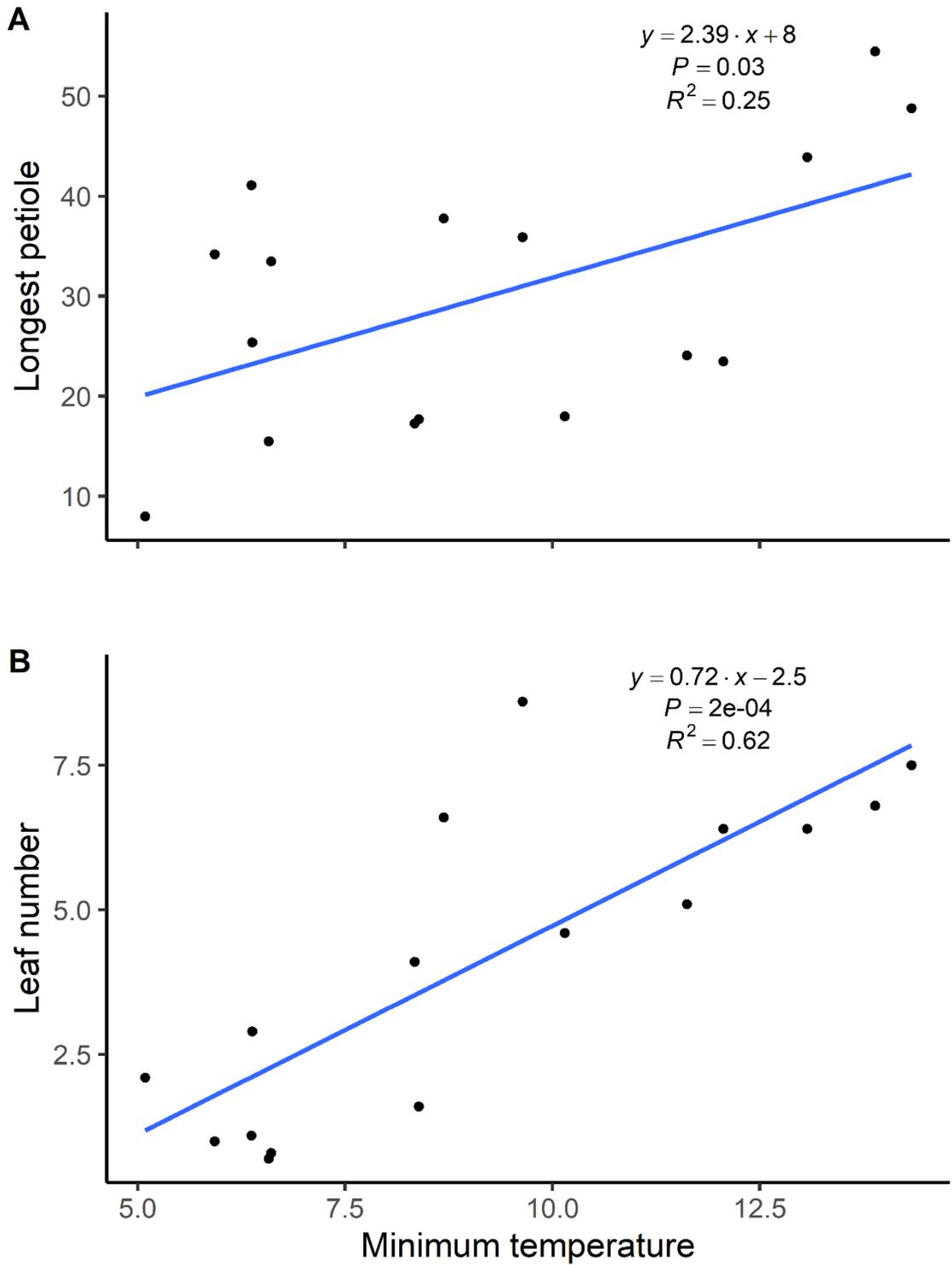


Figure 3.8. The interaction between (A) the length of the longest petiole (cm) and (B) the number of leaves per plant against increasing minimum temperature (°C).

Increases in minimum temperatures also correlate with increases in above water biomass ($y = 0.76x - 0.3$; $F_{1,14} = 14.16$; $p = 0.002$; $R^2 = 0.47$) (Fig 3.9 A) and the number of plants per m^2 ($y = 4.07x + 14$; $F_{1,14} = 35.13$; $p < 0.0001$; $R^2 = 0.69$) (Fig 3.9B) respectively. The steep gradient of the line modelling the increase in the number of plants per m^2 is of particular interest, as it proposed that for every 1°C increase in the minimum temperature, the number of plants per m^2 will increase by just over 4 plants.

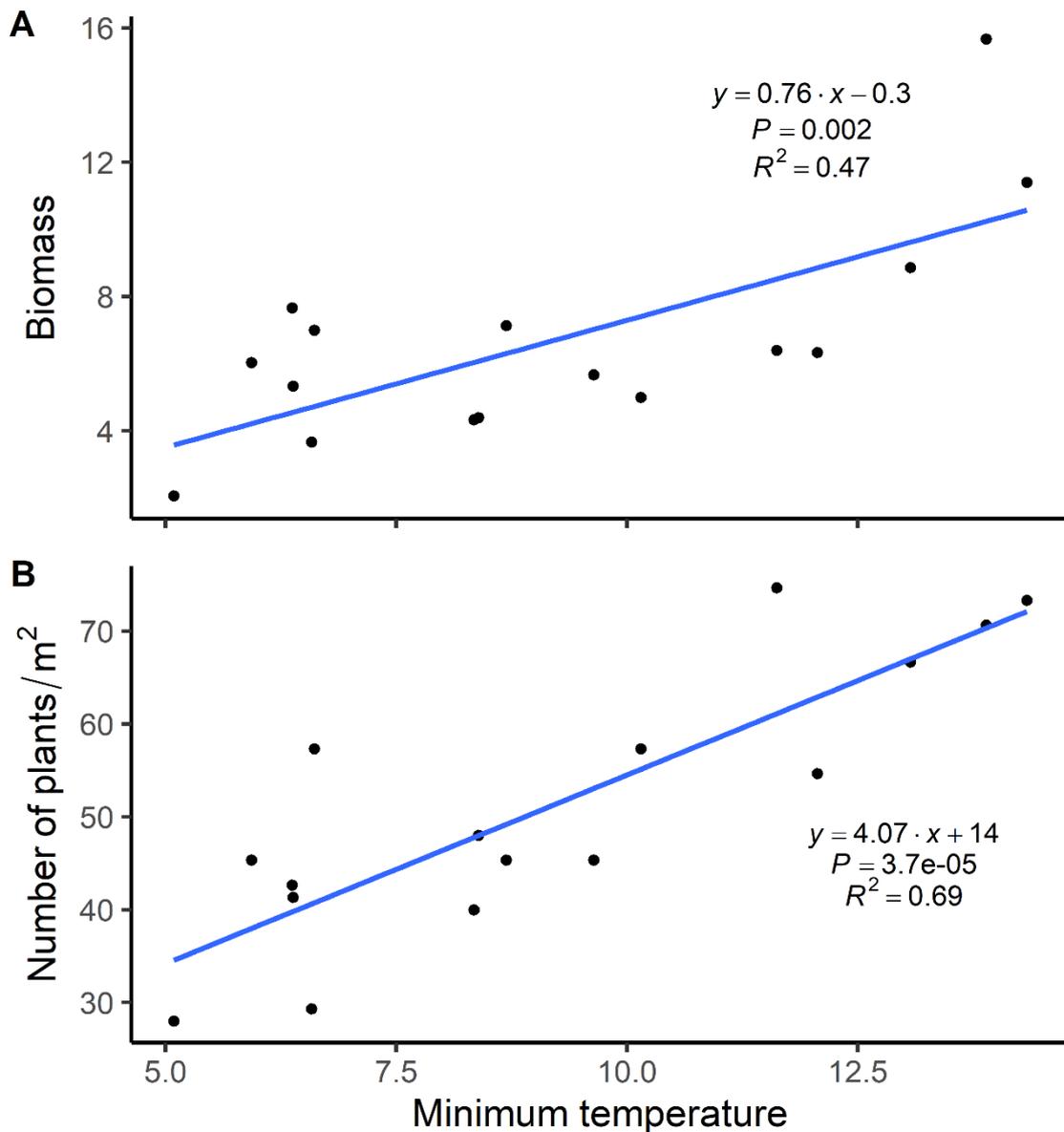


Figure 3.9. The interaction between (A) the above water biomass (kg/m^2) and (B) the number of plants per m^2 against increasing minimum temperature ($^\circ\text{C}$).

3.3.2. *Megamelus scutellaris* population measurements

The number of insects per metre squared follows seasonal and climatic trends (see Fig 3.3). During the spring of 2017, the insect density was extremely low (with mean numbers of insects/m² ranging between 0 and 1.08 insects between September and December 2017) (Fig 3.10). By April 2018, which is late summer, the insect numbers had increased to their maximum recorded density of 605.34 ± 111.91 insects/m². The insect numbers were also higher in the winter months of 2018 than they were in the winter of 2017, with a mean of 11.47 ± 3.92 insects/m² in 2017 compared to 230.45 ± 16.57 insects per m² in 2018, possibly suggesting that the insects were not induced into diapause.

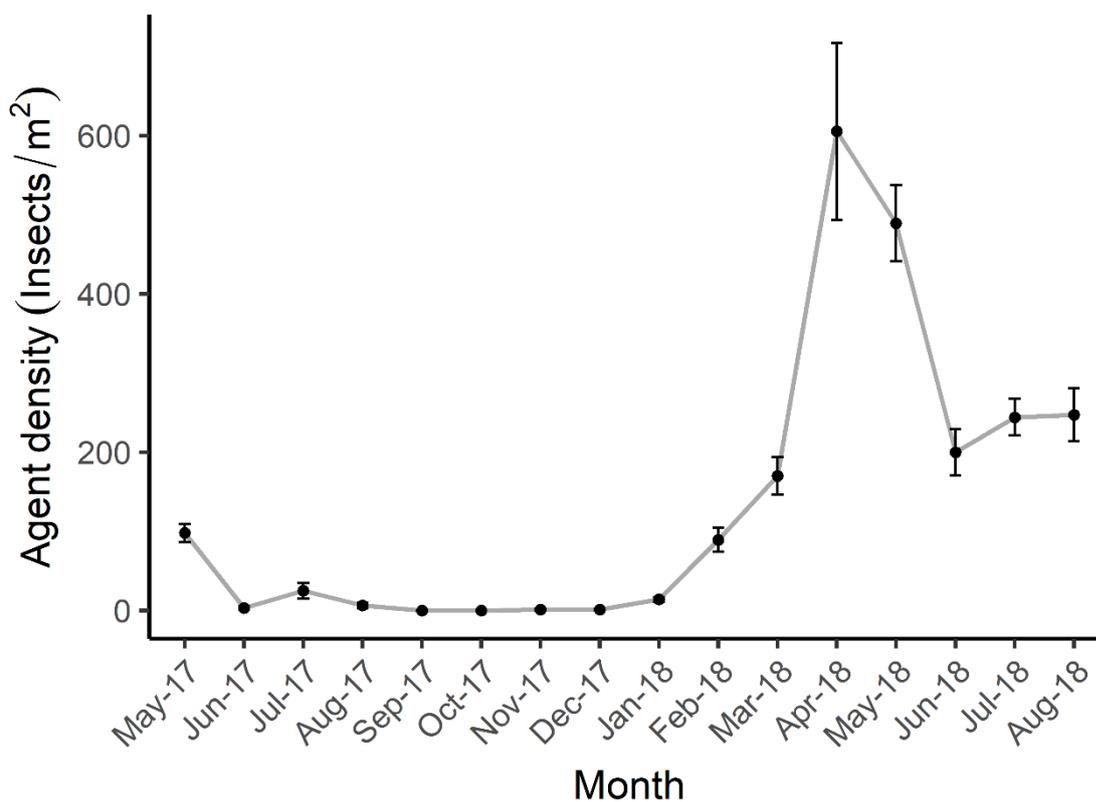


Figure 3.10. Mean monthly insect density per m² at the Kubusi River between May 2017 and August 2018. Error bars indicate S.E.

The change in the density of *M. scutellaris* was associated with both the number of leaves per water hyacinth plant and the above water biomass (Fig 3.11A). The number of leaves increased

rapidly post-winter, showing signs of recovery between September and October 2017, while the *M. scutellaris* population recovery lagged behind until it reached a maximum population density in April 2018 (Fig 3.11A). Biomass decreased initially in early summer as the new plants began to grow, but, by the late summer, the biomass rapidly increased (Fig 3.11B). Importantly, the maximum density of *M. scutellaris* lagged until after the biomass and the number of leaves had reached their maxima. Both the insect density and the number of leaves declined sharply at the start of winter in June of 2017 and 2018. Biomass began to decline in autumn, prior to the first frosts.

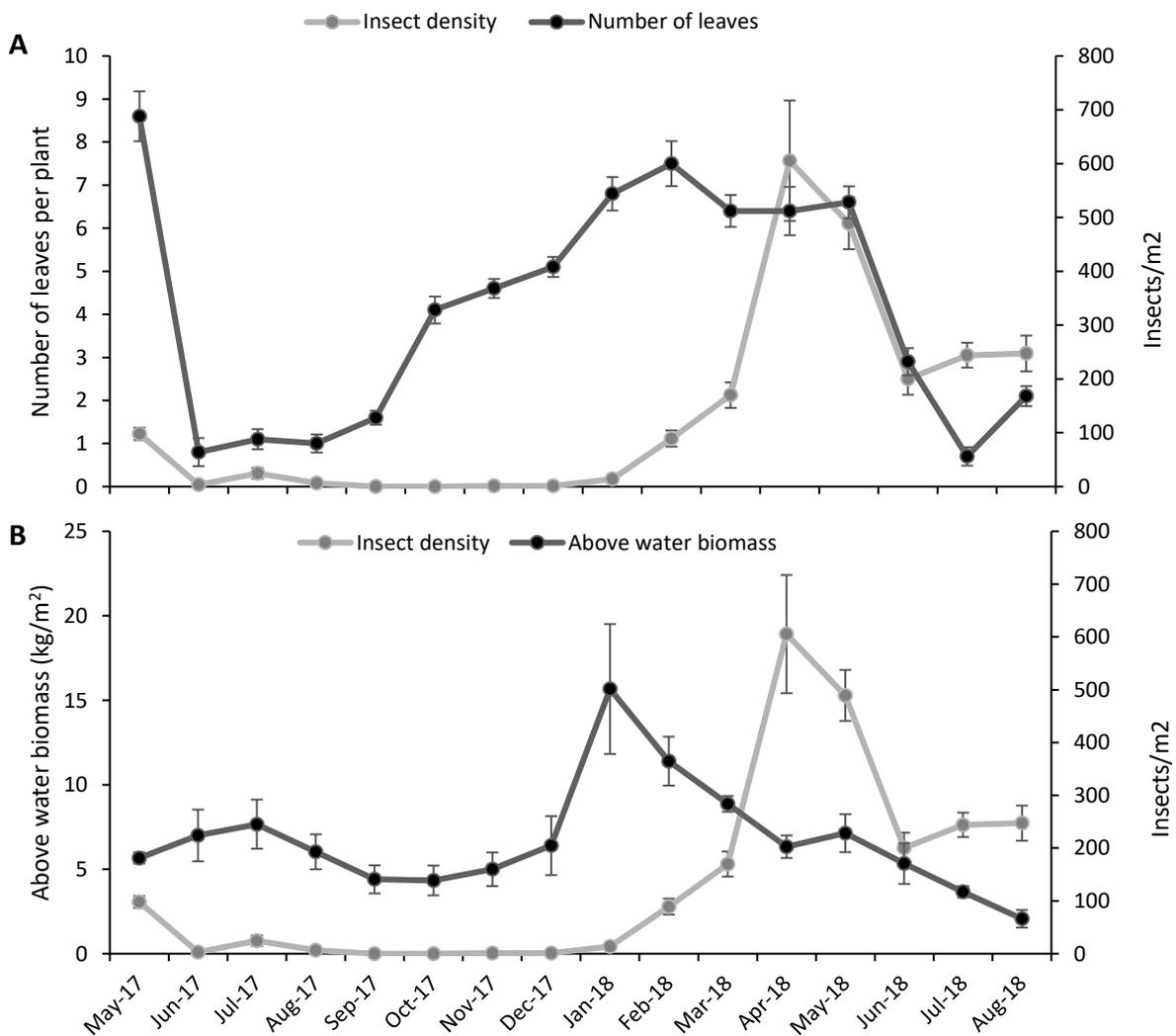


Figure 3.11. The interaction of *Megamelus scutellaris* density, (A) leaf number and (B) above water biomass on Kubusi River between May 2017 and August 2018. Error bars indicate S.E.

Season had a significant effect on the the *M. scutellaris* population size (Table 3.1). A significantly higher density of *M. scutellaris* occurred in autumn (340.00 ± 45.31 insects/m²) than in any other season ($H_3 = 81.236, P < 0.0001$), with fewer insects per square metre in spring than any other season (0.36 ± 0.36 insects/m²). There was no significant difference between the mean insect population density in summer (34.77 ± 8.76 insects/m²) and winter (Mean = 120.96 ± 16.57 insects/m²).

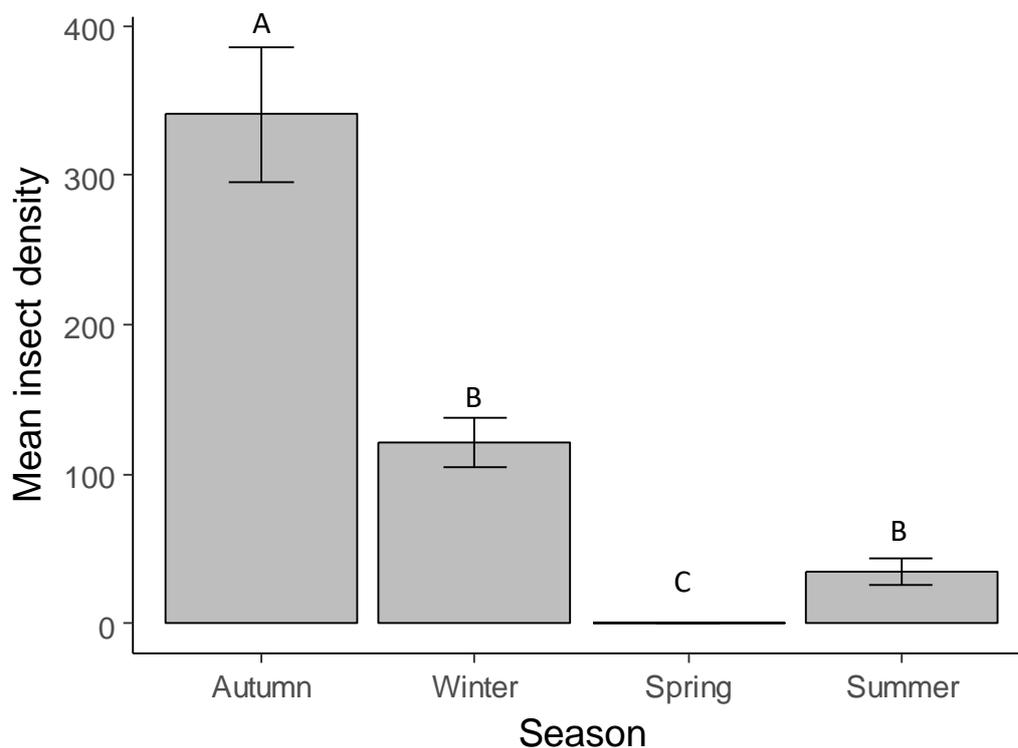


Figure 3.12. Seasonal mean insect density per m² at the Kubusi River between May 2017 and August 2018 Error bars indicate S.E. Significant differences are indicated by a different letter ($P < 0.05$).

The number of *M. scutellaris*/m² did not correlate with the minimum temperature ($y = 2.9x + 110$; $F_{1,14} = 0.0316$; $p = 0.86$; $R^2 = -0.07$) (Fig 3.13). This result is not unexpected given that, unlike the plant measurements, the natural enemy populations undergo a significant lag phase before they begin to increase in late summer/early autumn, indicating that they depend more on plants than the minimum temperature for population growth (Figs 3.10 & 3.11).

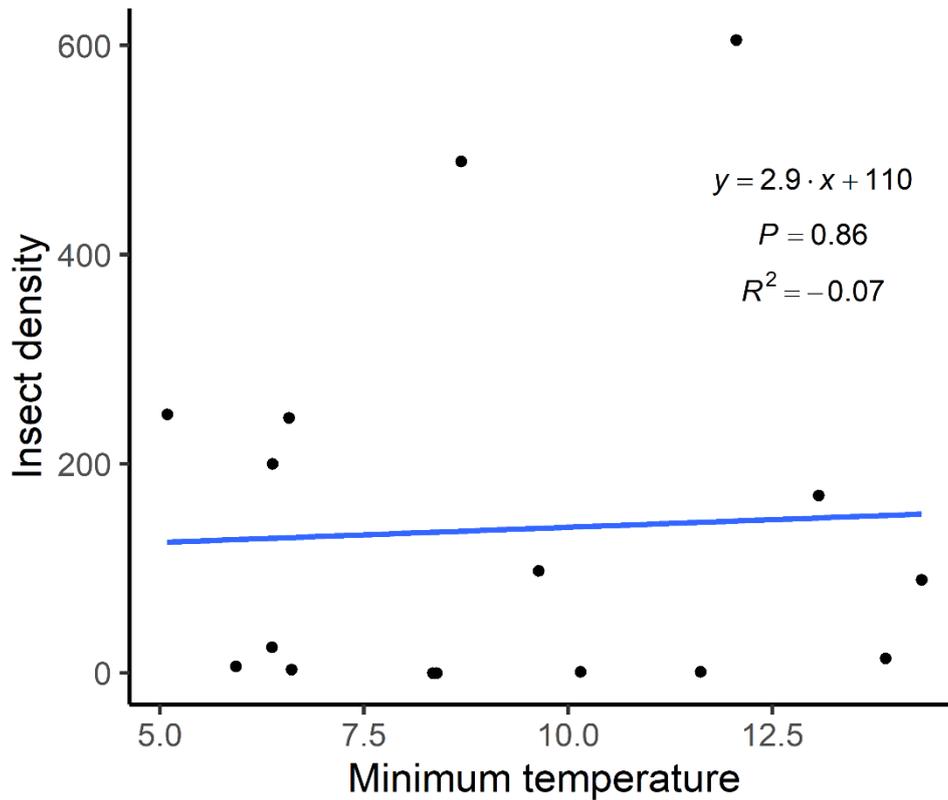


Figure 3.13. The interaction between the **number of *Megamelus scutellaris* (insects/m²)** and increasing minimum temperature (°C)

The density of *M. scutellaris* on the Kubusi River was dependent on the season (Table 3.1, Fig 3.12) as well as various plant parameters, including root length and the number of leaves per plant. The interactions of the longest petiole and the root length, and the number of leaves and season were also significant. The root length and the interaction of the longest petiole and the root length can be extrapolated to be indicators of water nutrient quality, where long roots occur when nutrients are poor, and short roots when nutrients are not limiting (Reddy & Tucker 1983).

Table 3.1. ANCOVA results illustrating the effects of various plant parameters and season on *M. scutellaris* density. Values in bold indicate significant differences. LP = longest petiole, RL = root length, and LN = number of leaves/plant.

Effect	Sum Sq	DF	Mean Sq	<i>F</i>	<i>P</i>
Longest petiole (LP)	76490	1	76490	3.534	0.06
Root length (RL)	1174318	1	1174318	54.251	0.00
Number of leaves (LN)	307497	1	307497	14.206	0.00
Season	1609018	3	536339	24.778	0.00
LP:RL	126751	1	126751	5.856	0.02
LP:LN	19154	1	19154	0.885	0.35
RL:LN	37	1	37	0.002	0.97
LP: Season	103998	3	34666	1.602	0.19
RL: Season	126227	3	42076	1.944	0.13
LN: Season	330832	3	110277	5.095	0.00
LP: RL: LN	16536	1	16536	0.764	0.38
LP: RL: Season	2616	3	872	0.040	0.99
LP:LN: Season	73390	3	24463	1.130	0.34
RL: LN: Season	47523	3	15841	0.732	0.53
LP: RL: LN: Season	3466	3	1155	0.053	0.98
Residuals	2770677	128	21646		

3.4. Discussion

This chapter investigated *M. scutellaris* population changes over time in relation to seasonal changes and host plant quality. Understanding how various biotic and abiotic factors influence the population dynamics of a biological control agent is key to the success of invasive plant management programmes and these results show that the population of *M. scutellaris* can vary significantly over time and between seasons. The post-winter recovery of *M. scutellaris* was highly dependent on both the season and the quality of the host plants, which was considerably reduced in the winter time, particularly with the onset of frost, which had deleterious effects on plant vigour and leaf quality. Grodowitz *et al.* (1991) found the same trend with the onset of winter in Texas, which lead to a dramatic decrease in the number of *Neochetina eichhorniae* weevils from a maximum population density of 55 weevils/m² to a minimum of 8 weevils/m²

for a period of 5 months, however the *N. eichhorniae* over-wintered as larvae, the population of which increased over winter.

Following winter, the water hyacinth plants were able to recover rapidly at the Kubusi River, but *M. scutellaris*, which is dependent on the plants to survive, was unable to mirror the rapid population growth rate of their host plants until later in the growing season. The mirid *Eccritotarsus catarinensis* and *Neochetina* spp. weevils also only re-emerge effectively after an extended post-winter lag period, with adult mirids and weevils only modelled to emerge by late October, showing an almost two month lag between the onset of spring and the recovery of the natural enemy populations (Byrne *et al.* 2010). Both Tipping *et al.* (2014) and Moran *et al.* (2016) found that *M. scutellaris* was able to overwinter successfully through multiple winters at cooler sites in the United States, particularly if there was riparian vegetation offering shaded areas to the insects to buffer the effects of rapid diel temperature fluctuations.

The lag period in post-winter recolonization is not specific to *M. scutellaris*, and it was initially proposed by Hill & Olckers (2000) to be a hindrance to all of the water hyacinth natural enemies released in South Africa, and one of the reasons that complete control of water hyacinth has not been achieved through biological control as yet. The lower developmental threshold (t_0) reported by May & Coetzee (2013) for *M. scutellaris* is 11.458°C, below which development ceases. However, the minimum temperature did not exceed t_0 between May 2017 and December 2018, and only between January and April 2018 was the mean temperature high enough to facilitate development. This short time period of development also coincides with, and may explain, the greatest increases in the populations of the insects at the Kubusi River site. A similar study which monitored the seasonal population density of *Neochetina* sp. on the Kubusi River found results that closely mirrored those presented in this chapter, where the adult weevil density remained at or near zero over winter and spiked during late Summer and early Autumn, only to collapse again with the first frosts (Byrne *et al.* 2010). Low winter

temperatures may also explain why there is no significant positive correlation between the population size of *M. scutellaris* and the minimum temperature (Fig 3.19). The same trend was reported by Hopper *et al.* (2017) who also found *M. scutellaris* population densities to be temperature dependent in California, peaking between October and November. The peak in density coincided with late summer/early autumn in the northern hemisphere, and the population declined again over winter when temperatures were close to the reported t_0 . Hopper *et al.* (2017) also determined that, among other water hyacinth biological control agents, the presence of a climatic mismatch as well as the lag phase in post-winter agent recovery are major factors responsible for the lack of water hyacinth management by biological control methods alone in the USA. In parts of the USA and in South Africa, the natural enemy populations on water hyacinth often only reach densities that are damaging at the end of the summer, merely to be reduced once again with the onset of winter, as projected initially by Hill & Olckers (2000).

However, one of the major benefits of using *M. scutellaris* as a natural enemy of water hyacinth in South Africa is that it is multivoltine with a fast generation time. Yet, the low temperatures experienced by these insects in the field still has the effect of prolonging their developmental time to as much as 65.6 days (at 19°C) from egg to adult, where, under ideal conditions, *M. scutellaris* can complete its development in 39.42 days at 27°C (May & Coetzee 2013). Furthermore, from observations in the field, *M. scutellaris* was often the first biological control agent to appear post-winter, and in the greatest numbers – particularly after major environmental perturbations, like drought conditions. *Megamelus scutellaris* has been shown to be able to co-exist with other biological control agents, and can even increase the damage that they cause (Petela 2017). Therefore, competition is unlikely to be the reason that they were able to recolonize faster than the other water hyacinth biological control agents at the site. Furthermore, evidence from sites in California suggests that the presence of *M. scutellaris* was

suspected to have contributed to a 27% reduction in overall leaf number on water hyacinth at a temperate site in Folsom, California, USA (Moran *et al.* 2016).

Interestingly, the populations of *M. scutellaris* did not decline as much in the winter of 2018, even though the minimum temperature was below the insect's reported t_0 , suggesting that *M. scutellaris* undergoes facultative diapause. This may have a beneficial effect on the use of this insect as a biological control agent because if, like in the winter of 2018, the insects remain throughout the winter without undergoing diapause, then the post-winter recovery rates are likely to be significantly quicker, allowing the insects to attain higher densities in the following summer growing season, as the overwinter population bottleneck will be less severe on the size of the breeding population.

Various cues for diapause induction have been studied – typically photoperiod and temperature – which are likely to influence the induction of diapause in *M. scutellaris*, however, it is just as likely to be influenced by the changes in the quality of their host-plants, particularly in areas where host-plant quality is seasonally variable. Similar relationships between the quality of the host plant and the propensity for insects to induce diapause have been described by Hunter & McNeil (1997) who found that diapause induction of the polyphagous oblique-banded leafroller *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) larvae under constant temperature and photoperiod was dependent on the quality of their host plants.

3.5. Conclusion

This chapter has determined that *M. scutellaris* can overwinter successfully in the coldest water hyacinth biological control site in South Africa, and that diapausing individuals faced a distinct lag-period before their population density built up to a sufficient size in the late summer, as a result of temperatures below the developmental threshold of the insect, as well as due to the reduction in the winter host-plant quality on which *M. scutellaris* feeds. It is also concluded

that, as a result of facultative diapause, certain factors can lead to the insects remaining throughout the winter, which is ideal for the insect population to recover much quicker in the warmer months that follow.

This chapter investigated temporal changes in *M. scutellaris* population sizes in relation to seasons and host plant quality. The next chapter explores the factors that affect the spatial distribution of *M. scutellaris* in South Africa.

Chapter 4: The establishment of *Megamelus scutellaris* populations in relation to climate and host-plant dynamics in South Africa

4.1. Introduction

For classical biological control programmes to be successful, candidate agents must be able to successfully establish self-sustaining populations in the areas where their host plants have become non-native invaders. During the prospecting phase in the native region of the target weed, climate matching methods are used to focus on specific areas to search for potential agents from the most climatically similar regions to the region of intended release (Wilmot Senaratne *et al.* 2006). Climate matching is done in an attempt to ensure that the candidate biological control agents have the best chances of establishing as they would be adapted to a mostly homologous environment (Hoelmer & Kirk 2005). A climate that is not suitable for a biological control agent to persist in is one of the main reasons why some biological control programmes fail (McEvoy and Coombs 2001; Byrne *et al.* 2004). A matching climate, however, may not always be enough to ensure agent establishment and the subsequent control of the target species (McFayden 1998), and a matching climate does not guarantee establishment.

The range of an insect cannot always be accurately predicted based on a matching climate, and even though technologies exist that can model where a species is likely to establish, the models are still only predictive. In a study of the globally translocated ladybird genus *Chilocorus* (Coleoptera: Coccinellidae), the ranges of each translocated species could be accurately determined with complete certainty in only a quarter of all attempts using the predictive models (Samways *et al.* 1999). Much of the variation in the establishment of the ladybirds was attributed to factors other than the climate, such as the time of release, losses due to predators, and the weather at the time of the release, all of which are contributing factors to establishment likelihood (Samways *et al.* 1999). Furthermore, modelling climate over large spatial scales

does not account for variations in the microclimate, which can be influenced by topography and vegetation structure, and these can have significant effects on insects.

When an invasive plant species, such as water hyacinth, is as widespread as it is in South Africa, the affect that the climate will have between different sites will be variable, and this will effect where an agent will establish and how successful it will be. According to Rapoport's rule, insect species evolving closer to the equator will have a smaller latitudinal distribution and therefore will be less likely to survive in highly variable climates typical of higher latitudes (Addo-Bediako *et al.* 2000). If these assumptions hold, they imply that tropical species will lack the physiological and behavioural adaptations to persist in more temperate regions (King 2011). Many tropical species are disadvantaged by temperatures that fluctuate throughout the day, such as in the interior regions of South Africa. This was demonstrated by the moth *Hypocosmia pyrochroma* Jones (Lepidoptera: Pyralidae), which is a biological control agent of the invasive cat's claw creeper *Dolichandra unguis-cati* (L.) Lohman (Bignoniaceae) that was released in both Australia and South Africa. The moth was found to be most successful at temperatures between 20-30°C, and large fluctuations in diel temperatures were deleterious to its survival and fecundity. Climate matching studies demonstrated that *H. pyrochroma* is more likely to establish in the coastal areas of South Africa, but not inland in the Highveld regions where a large proportion of the moth's host plant is located (Dhileepan *et al.* 2013).

Since 2013, *M. scutellaris* has been released in eight of the nine provinces in South Africa (Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North-West, and Western Cape) (J. Coetzee, *pers comm*). However, since release, there is very little data to determine where the insects have established successfully, and what factors, if any, have had an impact on establishment. Getting biological control agents to establish in a new, non-native range is not always straight-forward and is often hampered by a range of factors, such as climate, stochastic events, and the frequency and size of releases (Grevstad 1999). Initial pre-

release research on *M. scutellaris* predicted that minimum temperature at a site will be the most important factor limiting the establishment and spread of the agent in South Africa (May & Coetzee 2013). The study found that *M. scutellaris* had a lower developmental threshold (t_0) of close to 11.5°C, and in cold areas such as the Highveld, was modelled to be able to produce between 0 and 10 generations per year, thus indicating that cold temperatures may influence where the planthoppers can establish (May & Coetzee 2013). Low winter temperatures are not the only constraining factor on the establishment of *M. scutellaris*, as particularly high temperatures have been shown to affect the Argentinian biotype of *M. scutellaris*, which is currently the only strain present in South Africa, and this strain has a CT_{max} of 39.1°C (J. Coetzee, *pers comm*). The Paraguayan biotype, which is as yet not released in South Africa, has been shown to be more heat tolerant in controlled laboratory experiments (Foley *et al.* 2016).

This chapter investigated where *M. scutellaris* has established around South Africa since its release and compared this to the pre-release predictions from May & Coetzee (2013). Where *M. scutellaris* had established, this chapter aimed also to determine what environmental factors may have contributed to this. The intention is that these data can be used to predict where future populations will be able to establish in order to focus future release efforts on sites with the greatest likelihood of success.

4.2. Materials and Methods

4.2.1. Assessment of *Megamelus scutellaris* establishment in South Africa

A national field survey was conducted based on the release records housed at the Centre for Biological Control at Rhodes University (Eastern Cape, South Africa) listing the sites where *M. scutellaris* had been released in all provinces (except the Northern Cape where no sites existed) and the release effort. As many of these sites as possible were sampled between

03/04/2018 and 19/04/2018. Sampling occurred in autumn as this is when the populations of biological control agents were expected to be at their seasonal maximum (Chapter 3). At sites where the presence of water hyacinth was confirmed, a physical search for the presence of *M. scutellaris* was conducted. At sites where *M. scutellaris* was present, insect and plant data were collected using the same techniques outlined in the protocol in 3.3. The plants on the Suid Kaap River site in Mpumalanga were not measured in 2018 due to the presence of crocodiles at the site, but the establishment of *M. scutellaris* was confirmed in a separate field trip in November 2017 (pers. obs).

4.2.2. Plant data

Upon successfully identifying *M. scutellaris* in the field, 10 water hyacinth plants were randomly selected from the mat and measured, using a tape measure, to determine growth parameters. The measured parameters were adapted from Coetzee & Hill (2012), and are as follows: (1) the length of the longest petiole, (2) root length, (3) the number of ramets, and (4) the number of leaves on the main plant. Due to the low numbers of flowers recorded, this measurement was not included in the data set. See 3.2.2.1 for an explanation of each parameter.

Wet biomass was also determined by randomly removing three 0.25 m² quadrats of water hyacinth from within the mat and separating the above and below water biomass as well as the dead biomass. These were then weighed and recorded. The number of plants per quadrat was also counted, and these data were multiplied by 4 to present a comparable per metre squared (m²) unit. Herbicides had been applied at two of the sites (Gauteng and KwaZulu-Natal), and therefore biomass and the plant parameters were not measured due to the influence of the herbicide.

4.2.3. Insect data

At each site, a thorough visual inspection looking for insects or recent exuviae determined if *M. scutellaris* was present at the site. If present, the density of *M. scutellaris* was calculated using the same sampling device and protocol described in the previous chapter and replicated 10 times (see Fig. 3.4).

4.2.4. Environmental variables

4.2.4.1. Water quality

The water quality at each site was tested by collecting three water samples from each site within the water hyacinth mat at a depth of 30 cm under the water. The samples were tested within 10 hours of collection using the Oakton PCtestr 35 multiprobe (Eutech/Oakton Instruments, Singapore) to test for pH, conductivity, total dissolved solids (TDS), and salinity, while dissolved oxygen in the water was measured using a Dissolved Oxygen Pen DO 850045 (Sper Scientific, Taiwan). Nitrate and ammonia concentrations were measured using the NO₃-BTA Nitrate Ion Selective Electrode and the NH₄-BTA Ammonium Ion Selective Electrode respectively (Vernier, Oregon).

4.2.4.2. Study sites

The sites where *M. scutellaris* were successfully located and measured are listed in Table 4.1. Sites where the agents dispersed naturally are not listed in the table, and data were not collected from these sites due to them being identified during different seasons, making the data incomparable.

4.2.4.3. Climate

Climate data were retrieved from the South African Weather Service (SAWS). Weather stations were selected based on proximity to sites where *M. scutellaris* was still present after

having overwintered successfully over at least one year since release. The data retrieved included the total rainfall, and the daily minimum and maximum temperatures.

4.2.5. Statistical analyses

All statistics were conducted in RStudio v3.4.3 (R Core Team 2017). A Principal Components Analysis (PCA) determined which variables were most influential on the spread of the data, and differences between the plant parameters, biomass, and insect density were calculated with a GLM using a Poisson distribution and a log link function. Differences between sites were analysed with a Tukey post hoc test and linear regression analyses were used to analyse correlations. An ANCOVA was used to determine which factors influenced the density of *M. scutellaris* recorded at each site.

4.3. Results

4.3.1. Climate data

The sites where *M. scutellaris* had established successfully are displayed below, along with the closest available weather station where the climate data was retrieved, and the approximate distance from the site (Table 4.1, Fig 4.1).

The highest mean maximum temperature measured between February and April 2018 (coinciding with the end of summer and beginning of autumn) was $28.93^{\circ}\text{C} \pm 0.48$ recorded at the Tygerhoek weather station in the Western Cape (Fig 4.2A). The lowest mean maximum temperature recorded during the allocated period was at the George weather station, with a mean maximum temperature of $23.70^{\circ}\text{C} \pm 0.39$, meaning that the Western Cape Province had both the highest and the lowest maximum temperatures recorded during this period, showing the variability of the climate in the area. However, the Dohne weather station in the Eastern Cape was only negligibly warmer with a maximum temperature of $23.79^{\circ}\text{C} \pm 0.50$. The mean

minimum temperature at the Dohne weather station was the lowest of all during the study period $13.18^{\circ}\text{C} \pm 0.26$. The Nelspruit weather station in Mpumalanga recorded the highest mean minimum temperature, with a mean temperature of $17.17^{\circ}\text{C} \pm 0.19$.

Table 4.1. Sites where *Megamelus scutellaris* overwintered successfully with associated closest weather station from where the climate data was retrieved.

Province	Site	Nearest weather station	Approximate distance from site (km)
Western Cape	Bontebok National Park (NP)	Tygerhoek	50
Western Cape	Goukou River	Stilbaai	15
Western Cape	Dwarsweg	George	11
Eastern Cape	Kubusi River	Dohne	3
Eastern Cape	Laing Dam	Bisho	18
KwaZulu-Natal	Broadmore	Pietermaritzburg	28
KwaZulu-Natal	Camelot	Greytown	35
KwaZulu-Natal	Doornkop	Pietermaritzburg	30
Gauteng	Sandspruit	JHB Botanical Gardens	18
Mpumalanga	Noord-Kaap River	Nelspruit	9
Mpumalanga	Suid-Kaap River	Nelspruit	26

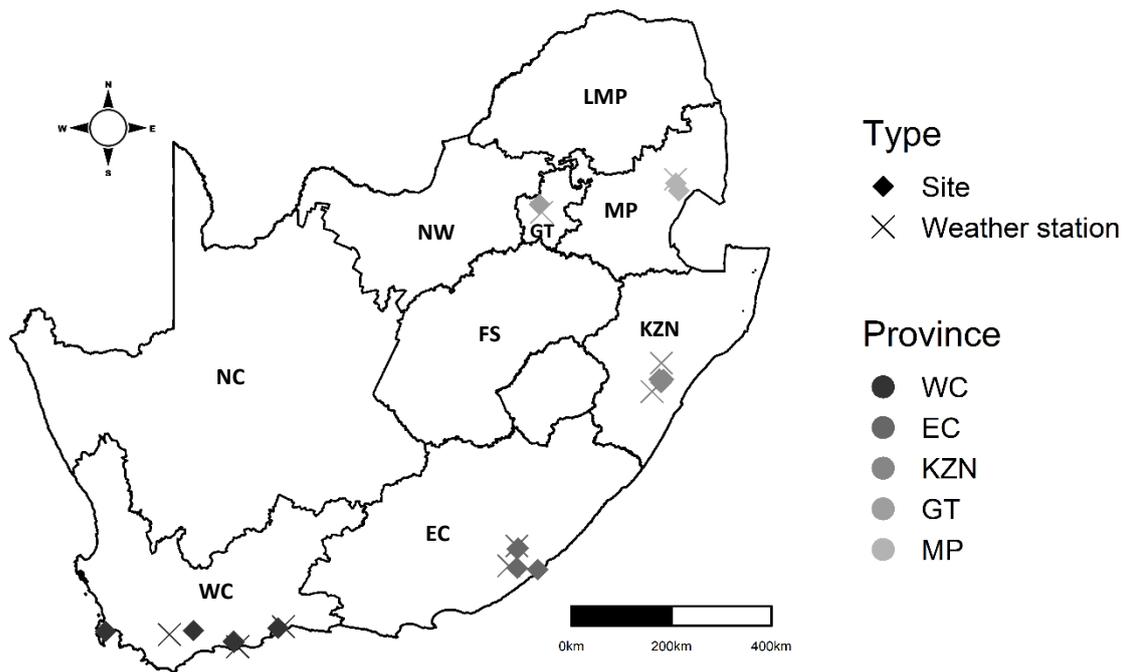


Figure 4.1. Distribution of *M. scutellaris* in South Africa in relation to **the nearest weather station**. The diamond shapes are sites where *M. scutellaris* established after release, and the crosses are the weather stations. LMP = Limpopo, MP = Mpumalanga, GT = Gauteng, KZN = KwaZulu-Natal, NW = North-West Province, FS = Free State, NC = Northern Cape, EC = Eastern Cape, WC = Western Cape

The percentage change in temperature from the mean minimum to the mean maximum demonstrates the types of diel fluctuations that biological control agents experience in the field. The weather station at Nelspruit experienced only a $54.8\% \pm 2.2$ change in daily temperature, whereas the Tygerhoek weather station recorded a $118.8\% \pm 7.2$ change in mean daily temperature (Fig 4.2B). This shows the climatic variability experienced by biological control agents in South Africa.

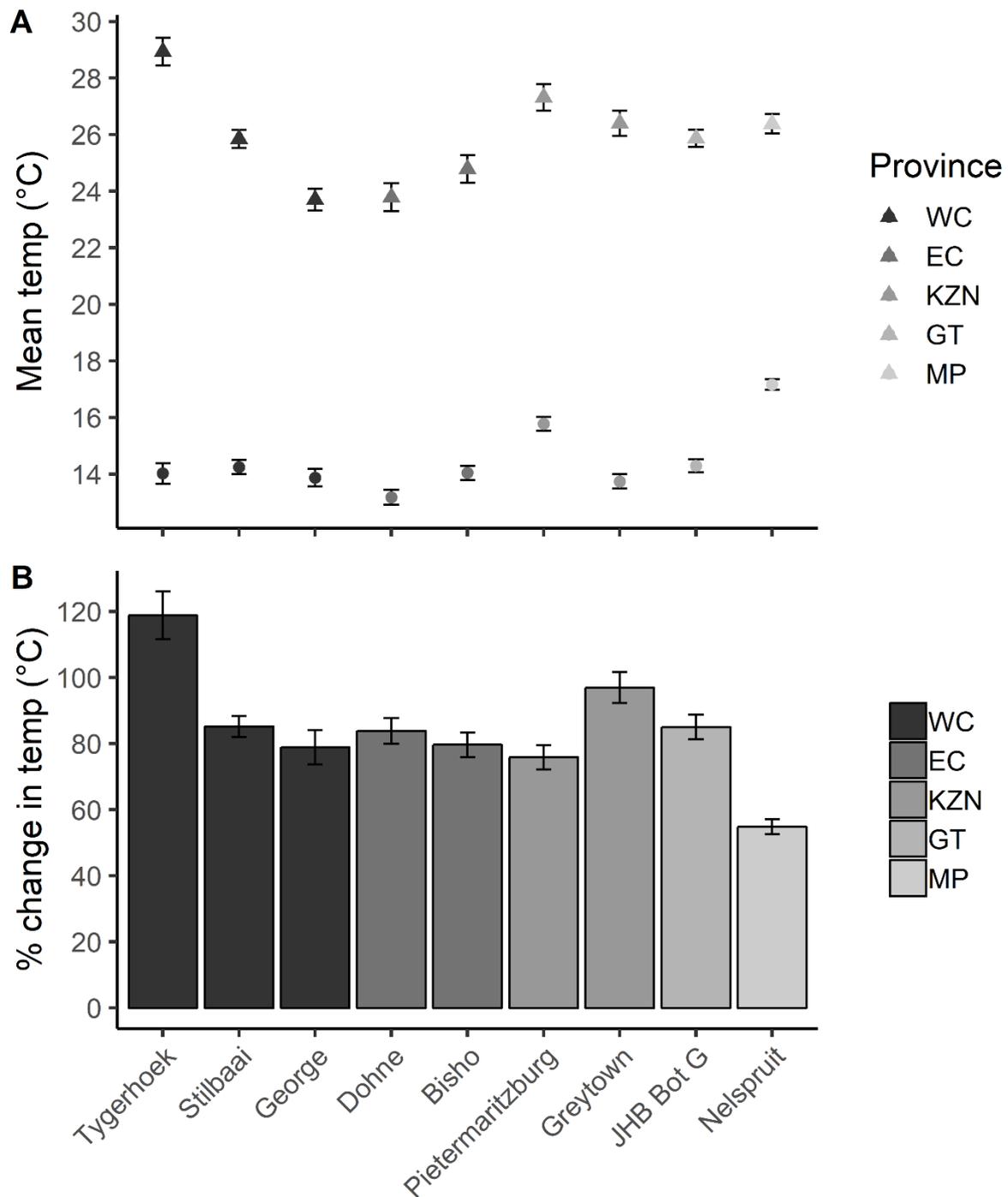


Figure 4.2. (A) Mean minimum and maximum temperatures (\pm SE) and (B) Mean percentage change in minimum and maximum temperature between February and April 2018 (\pm SE). Each weather station relates to a site in Table 4.1. EC = Eastern Cape, GT = Gauteng, KZN = KwaZulu-Natal, MP = Mpumalanga, WC = Western Cape.

The highest rainfall recorded during February and April 2018 was at the Dohne weather station in the Eastern Cape, which recorded a total of 363.2 mm of rain (Fig 4.3). The Western Cape

stations recorded the lowest rainfall of all the provinces, with Stilbaai recording only 44.0 mm of rain during this three-month period of time.

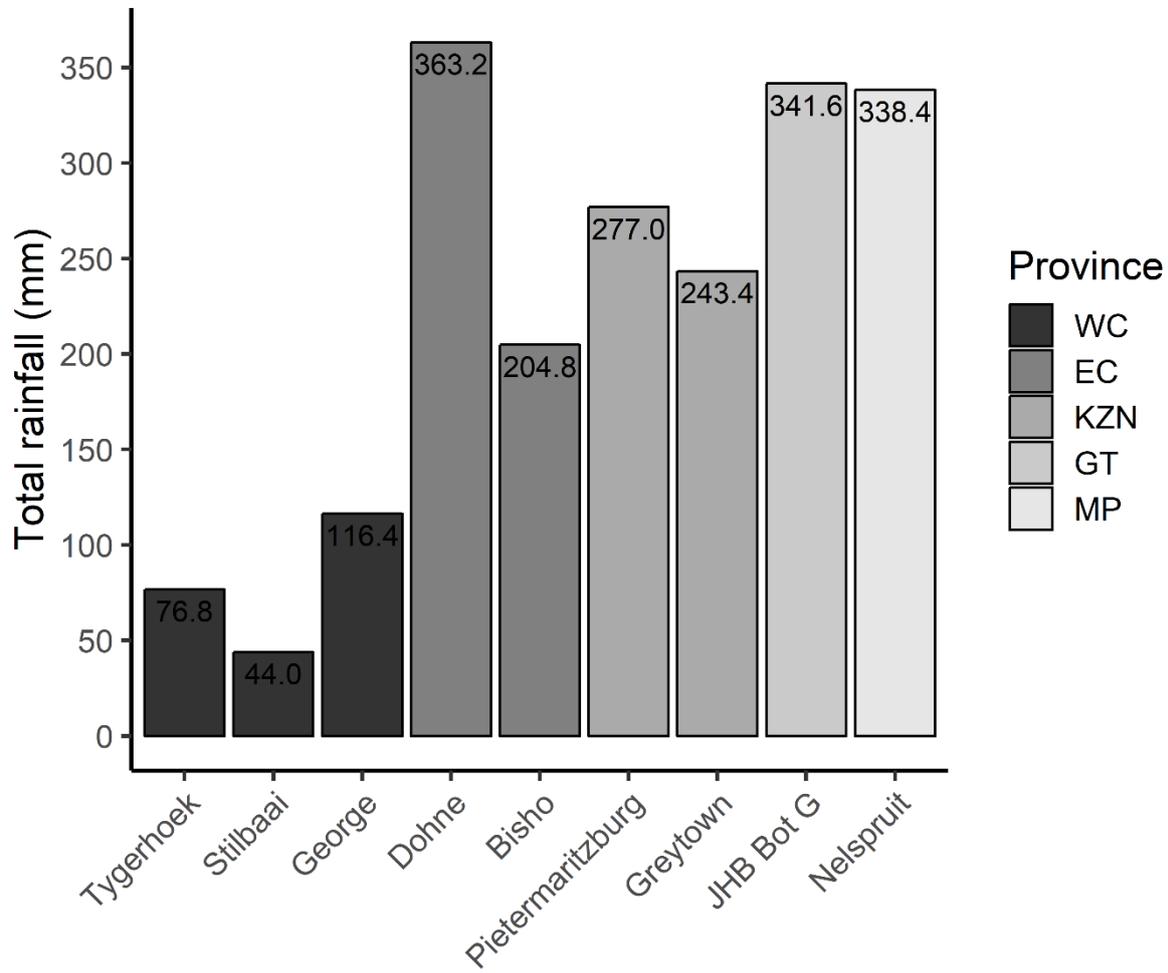


Figure 4.3. Total rainfall accumulated between February and April 2018. Each weather station relates to a site in Table 4.1. Province represented by colour. EC = Eastern Cape, GT = Gauteng, KZN = KwaZulu-Natal, MP = Mpumalanga, WC = Western Cape. The number on the bar indicates total rainfall.

4.3.2. Release effort

The numbers and frequency of agents released at each site are displayed in Table 4.2, as well as the time in between the first and last release. The highest release effort was at a small dam in Gauteng near Sandpruit (26°00'16.2"S 27°57'14.2"E) where a total of 48 000 *M. scutellaris* were released between March 2015 and November 2017 in 6 separate releases. Even though

the agent did successfully establish here, population density was low, and this is most likely due to a combination of harsh winters, periodic flooding and the injudicious use of herbicides by land-owners bordering the site. Both the Mpumalanga sites had single releases of only 4000 insects, and both have very well established *M. scutellaris* populations that have persisted since the end of 2014. Many of the sites only had a single release performed, indicating that multiple release efforts are not required to establish a population, although this does not take into consideration stochastic events.

Table 4.2. Release effort and time since first and last releases at sites where the establishment of *Megamelus scutellaris* has been confirmed.

Province	Site	Date of first release	Date of last release	Total number of <i>M. scutellaris</i> released	Total number of releases
Eastern Cape	Kubusi	20-Nov-13	15-Oct-15	15500	5
Eastern Cape	Laing Dam	15-Oct-15	15-Oct-15	13000	1
Gauteng	Sandspruit	18-Mar-15	05-Nov-17	48000	6
Western Cape	Bontebok NP	13-Nov-15	13-Nov-15	5000	1
Western Cape	Dwarsweg	25-Nov-15	25-Nov-15	5000	1
Western Cape	Goukou	13-Nov-15	13-Nov-15	5000	1
KwaZulu-Natal	Broadmore	10-Nov-15	10-Nov-15	5000	1
KwaZulu-Natal	Camelot	10-Nov-15	10-Nov-15	5000	1
KwaZulu-Natal	Doornkop	10-Nov-15	10-Nov-15	5000	1
Mpumalanga	Noord-Kaap River	11-Dec-14	11-Dec-14	4000	1
Mpumalanga	Suid-Kaap River	11-Dec-14	11-Dec-14	4000	1

Since the middle of 2014, there has been a major effort to release *M. scutellaris* at sites around South Africa, culminating in the release of thousands of these insects. The six month period from October 2015 to March 2016 saw the release of 205 000 *M. scutellaris* at water hyacinth infestation sites, which was the highest release effort on record. This extreme release did lead

to a collapse in the mass rearing colony which meant no releases were performed between April 2016 and March 2017, with a total of 58 000 released in the year following this collapse.

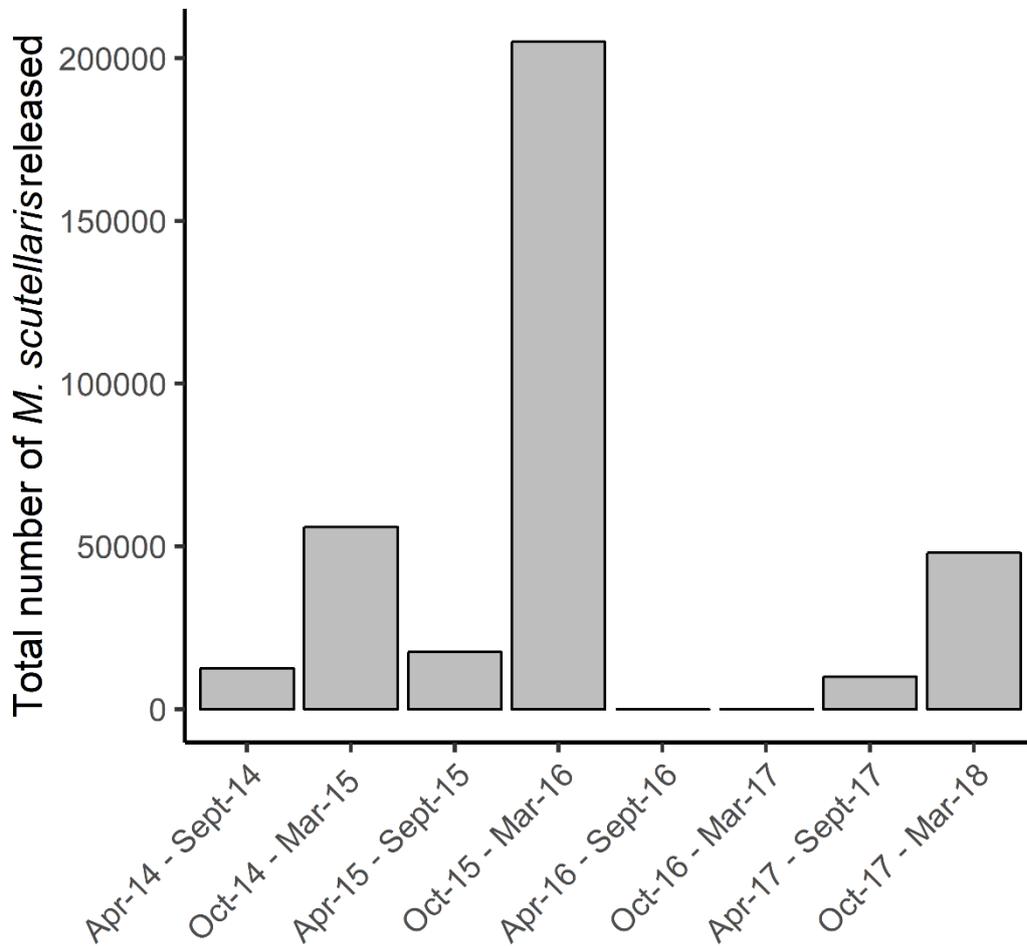


Figure 4.4. Total number of *M. scutellaris* released per 6-month period from April 2014 – March 2018.

4.3.3. Water chemistry

The results of the PCA, which was used to determine which components of the dataset were responsible for the variation in the data, showed that first two principal components (PCs) accounted for 64.4% of the total variance, with the first 5 PCs making up a total of 93.4%. Conductivity was the highest loading factor on PC1 (0.409) and was therefore responsible for

the majority of the variation. Dead biomass loaded highest on PC2 (0.483), and these results are summarized in Fig 4.3.

Table 4.3. The Eigenvectors and proportion of variance of the first five principal components (PC) of the water chemistry and biomass variables. Parenthetical values show the proportion of variance for each PC. The first 5 PC make up 93.4% of the total variance. The highest loading factor in each PC is in bold.

Variable	PC1 (39.2%)	PC2 (25.2%)	PC3 (15.5%)	PC4 (9.9%)	PC5 (3.6%)
pH	0.262	-0.214	0.237	-0.553	0.390
Conductivity	-0.409	-0.261	0.096	-0.172	-0.035
TDS	-0.376	-0.188	0.048	-0.431	-0.355
Salinity	-0.394	-0.190	0.254	0.186	-0.109
Nitrate	-0.366	-0.248	0.005	0.367	0.319
Dissolved O ₂ (mg.L ⁻¹)	0.209	-0.266	0.564	0.153	-0.111
Dissolved O ₂ (%)	0.308	-0.178	0.491	0.132	-0.338
Plants/m ²	-0.277	0.418	0.151	-0.375	-0.353
Above water biomass	-0.368	0.122	0.337	0.029	0.403
Below water biomass	0.004	0.478	0.371	-0.129	0.380
Dead biomass	-0.081	0.483	0.187	0.333	-0.220

Conductivity, which was the highest loading score on PC1, is directly related to water nutrient status (Daddy *et al.* 2002). The variation in the data from the Laing Dam, Kubusi and Goukou sites were driven by conductivity, however, in other sites such as Noord Kaap, Broadmore, and the Bontebok NP, other factors were more influential on the variability of the data, such as pH and dissolved O₂, showing that these sites were not as eutrophic (Fig 4.5).

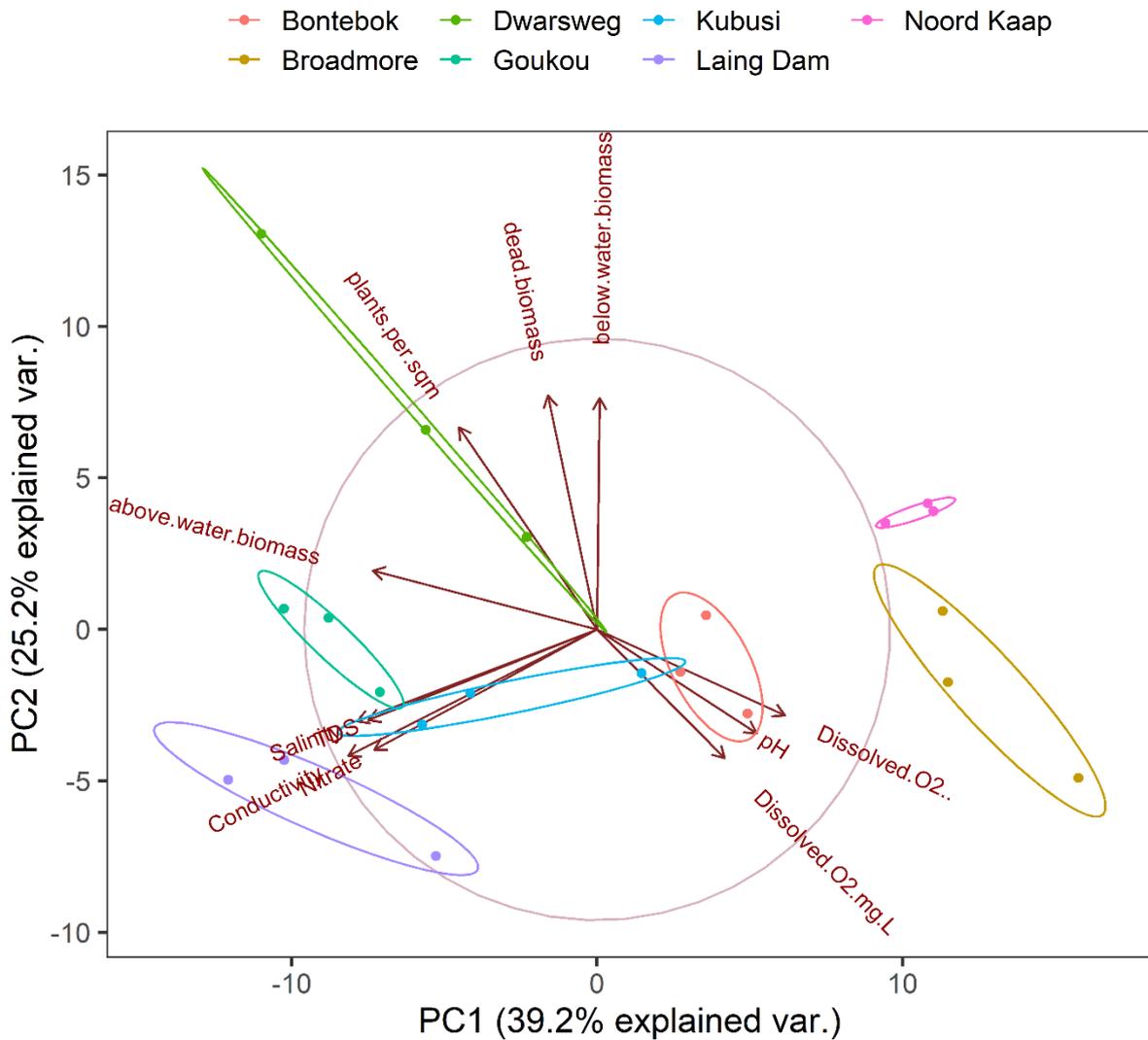


Figure 4.5. PCA showing component 1 against component 2 performed on the data of 11 water chemistry and plant biomass variables grouped by site (Table 4.2.)

4.3.4. Plant data

The length of the longest petiole was variable between the sites. The tallest plants were found at Goukou, with a mean petiole length of $48.1 \text{ cm} \pm 4.73$, but these were not significantly taller than the plants at Laing Dam, which had mean petiole lengths of $43.5 \text{ cm} \pm 4.04$ ($Z_{72,79} = 63.82$, $P < 0.0001$) (Fig 4.6A). The shortest petioles were sampled at the Broadmore Dam in

KwaZulu-Natal, where the mean of the longest petioles was $14.1 \text{ cm} \pm 1.55$. The mean number of ramets recorded at each site was also highly variable.

Root length is a reliable indicator of the nutrient status of a water body and/or the level of competition between plants, where short roots indicate eutrophic conditions and low competition, and longer roots are usually a sign of low nutrients and/or high levels of competition for limited resources (Xie & Yu 2003). The plants with the longest root lengths were both found in the Western Cape (Fig 4.6B). These plants were measured on the Goukou River ($68.2 \text{ cm} \pm 7.41$) and at the Bontebok National Park ($71.0 \text{ cm} \pm 4.71$). Both of these sites had roots that were significantly longer than roots at any other site ($Z_{72,79} = 113.58, P < 0.0001$). Plants measured at Laing Dam had the shortest roots on average, with mean root lengths of $26.7 \pm 2.40 \text{ cm}$.

Most of the plants had similar numbers of leaves between sites, ranging from a mean of 4.1 ± 0.31 leaves per plant at Camelot in KwaZulu-Natal to 8.7 ± 0.68 leaves per plant on the Goukou River in the Western Cape, which were significantly more than Camelot ($Z_{72,79} = 15.57, P < 0.0001$) (Fig 4.6C). At all the other sites, however, leaf number was relatively consistent and were not statistically different. Furthermore, there was evidence of a recent glyphosate herbicide application at the Camelot site, which may explain the reduced number of leaves we recorded. Plants at the Dwarsweg site in the Western Cape recorded no ramets, while a mean of 3.1 ± 0.37 and 2.9 ± 0.64 ramets per plant was recorded at the Kubusi and Bontebok National Park sites respectively, which was significantly greater than the Goukou, Noord-Kaap and Camelot sites which all had a mean of less than one ramet per plant ($Z_{72,79} = 5.73, P < 0.0001$) (Fig 4.6D).

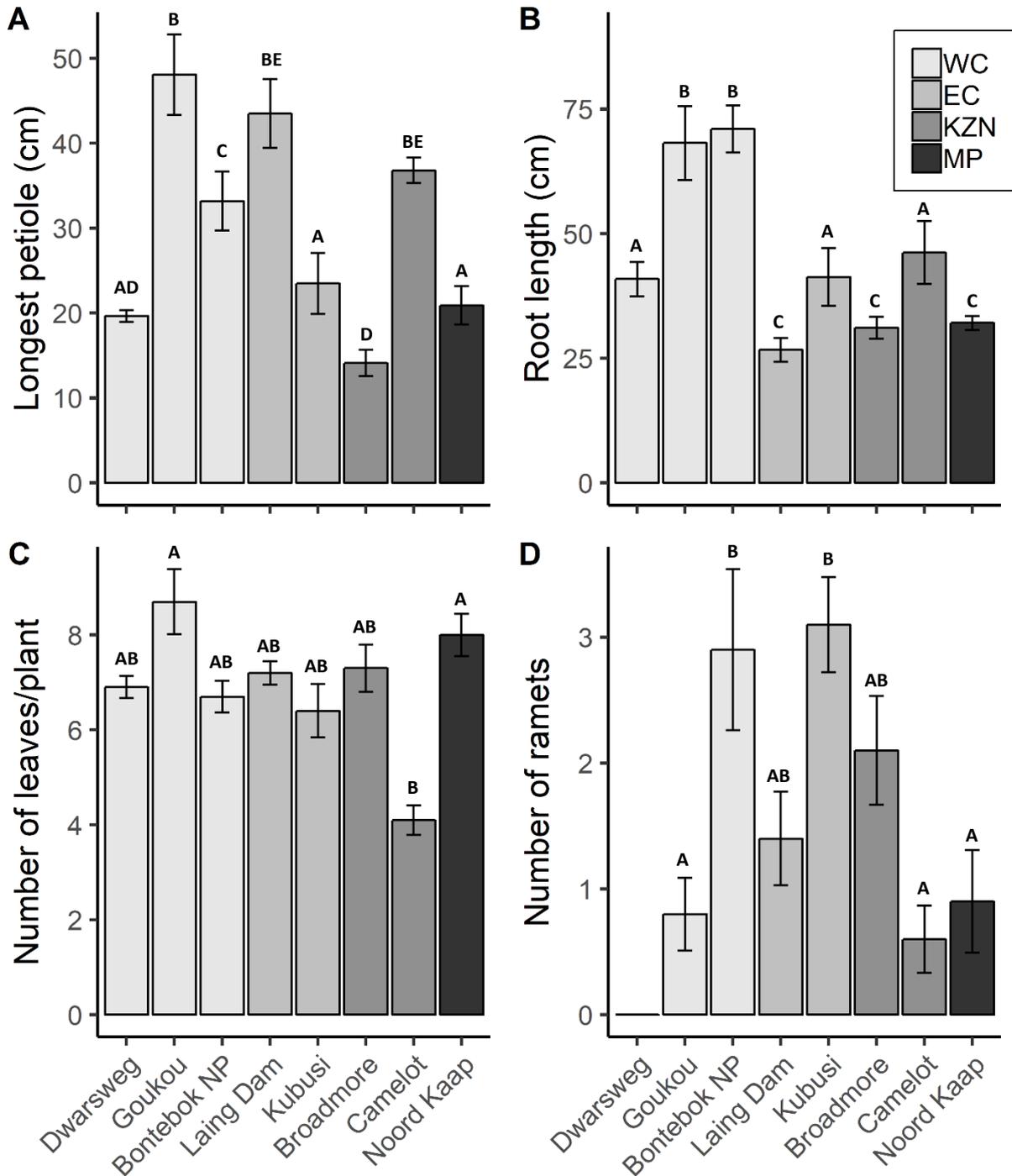


Figure 4.6. (A) Mean length of the **longest petioles**, (B) the mean **maximum root length**, (C) the mean **number of living leaves** per plant, and (D) the mean **number of ramets** per plant at each site (\pm SE). Provinces are represented by colour. WC = Western Cape, EC = Eastern Cape, KZN = KwaZulu-Natal, MP = Mpumalanga. Significance level represented by letter.

Similar to the root length, the root:shoot biomass ratio is an indication of plant health and water nutrient status, where a root:shoot biomass ratio <1 means that the plant had greater shoot biomass, and a root:shoot ratio >1 indicates that the root biomass made up more of the total

biomass than the shoots. The greatest root:shoot ratio was found at Broadmore in KZN, which was a low nutrient site (Fig 4.5). The plants at Broadmore were mostly small plants with bulbous petioles and the quotient of $\frac{roots}{shoots} = 2.45 \pm 0.33$. The root:shoot value was significantly higher than plants from Laing Dam, which were typically made up of very tall plants, and yielded a root:shoot ratio of 0.64 ± 0.06 ($Z_{72} = 4.12$, $P < 0.0001$) (Fig 4.6B). None of the other sites were significant different. Plants at Goukou had very long roots and a high root:shoot ratio (1.52 ± 0.18) which may indicate a nutrient poor system, but this is likely to have been due to other factors, such as competition, as the water the plants were sampled from was nutrient rich (Fig 4.5). In addition, the plants at Goukou were the tallest of all the sites visited (Fig 4.6A). Laing Dam was a highly eutrophic water body which had high conductivity and a high concentration of nitrates (Fig 4.5) and hence, the nitrophilous water hyacinth were able to convert the extra nutrients into long photosynthetic shoots. The stacked bar in Fig 4.7A clearly shows the limited contribution of the roots to the overall size of the plants, and this site is the only site recorded where the quotient of roots and shoots was <1 (Fig 4.7B).

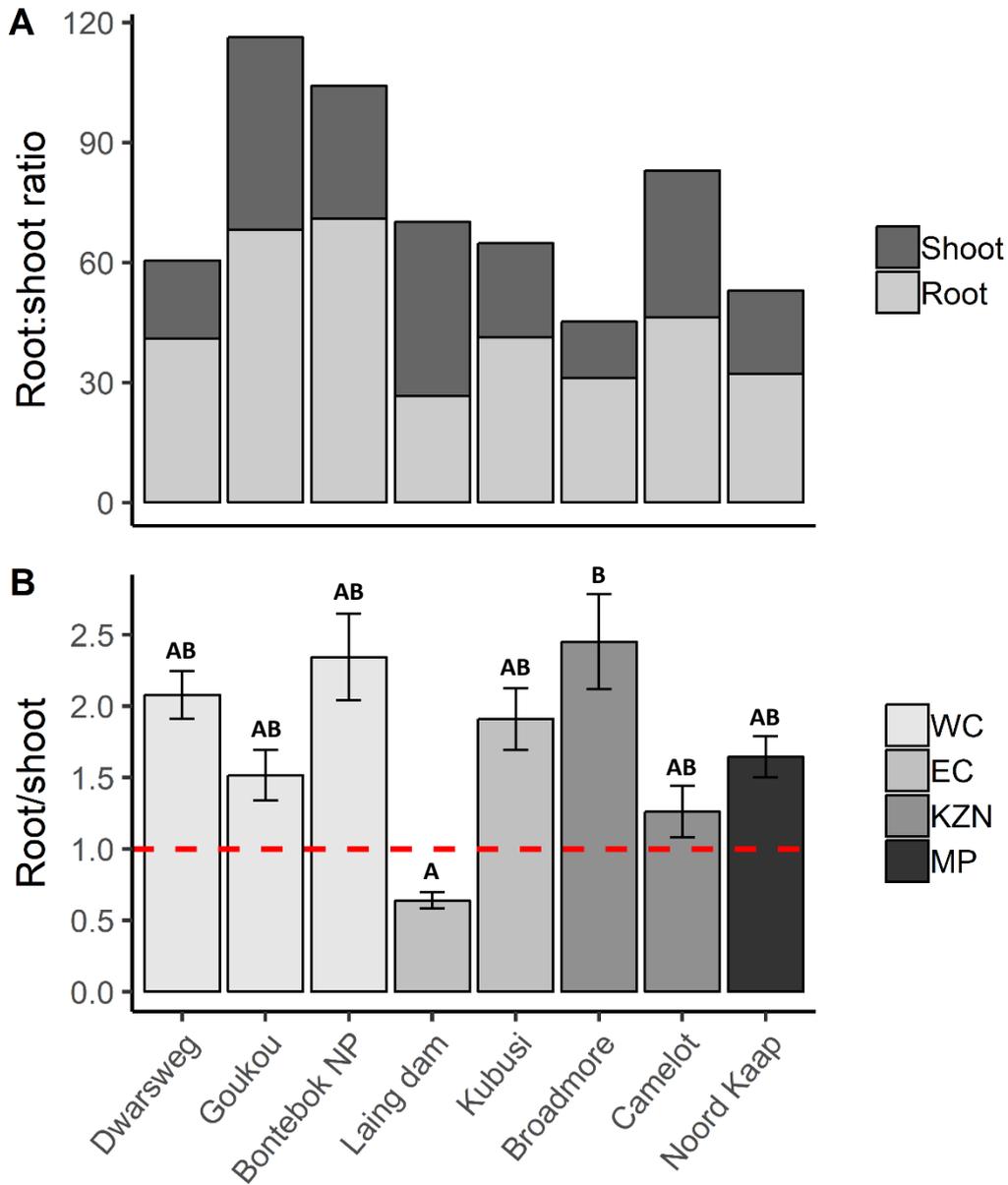


Figure 4.7. (A) The **root:shoot ratio** at each site, represented by the mean contribution of the root (light grey) to the shoot (dark grey) and **(B)**, the **root:shoot ratio** at each site (\pm SE), represented by the quotient of the root length and the length of the longest petiole. Significant differences represented by different letter. The red dashed line indicates the point at which the root length = shoot length. WC = Western Cape, EC = Eastern Cape, KZN = KwaZulu-Natal, MP = Mpumalanga.

The above water biomass, which is a measure of the wet weight of the photosynthetic material above water, was significantly higher at Goukou ($19.8 \pm 5.67 \text{ kg/m}^2$) than all sites except Laing Dam ($Z_{14,20} = 7.06$, $P < 0.0001$) (Fig 4.8A). The Goukou site was an impoundment of the Goukou River on a cattle farm that accessed water from the site. This site had a particularly

dense infestation that covered 100% of the water surface and had a high nutrient inflow from the surrounding farmlands, indicated by the high conductivity and nitrate concentration (Fig 4.5). The Broadmore site had the lowest above water biomass, with a mean of 2.33 ± 0.77 kg/m², made up mostly of smaller plants which lined the marginal areas of the dam, and this site was not influenced as greatly by nutrients.

Dwarsweg had the highest below water (root) biomass but also had the most variability in the biomass (9.67 ± 6.17 kg/m²) (Fig 4.8B). Both sites representing the Eastern Cape showed the lowest mean root biomass (Kubusi: 2.47 ± 0.29 kg/m²; Laing Dam: 2.27 ± 0.27 kg/m²), although this was not significantly lower than the below water biomass at the Bontebok NP (3.00 ± 1.00 kg/m²) ($Z_{14,20} = 3.30$, $P = 0.001$). Along with the highest root biomass, Dwarsweg also had the greatest mean amount of dead material (13.13 ± 2.42 kg/m²), exceeding the next highest site (Noord Kaap: 3.73 ± 1.10 kg/m²) by 71.6% (Fig 4.8 C). Dead biomass was relatively uniform between the sites, with the exception of the Dwarsweg site ($Z_{14,20} = 0.46$, $P = 0.64$).

The final parameter measured was plant density/m². Dwarsweg had the greatest number of plants/m², with a mean of 88 ± 24.11 plants/m², while Laing Dam, Broadmore and Kubusi had the lowest values for this measurement, with a mean of 40 ± 4.00 plants/m² for both Laing Dam and Broadmore and 54.67 ± 9.33 plants/m² for Kubusi ($Z_{14,20} = 39.37$, $P < 0.0001$) (Fig 4.8D). However, even though both Laing Dam and Broadmore had the same biomass, the plants themselves were different, as were their spatial arrangements on the water, with plants at Broadmore being smaller and growing only on the margins of the dam, while plants at Laing Dam were very large and tightly clustered. Therefore, this measurement was not an accurate indicator of either nutrient status or plant size.

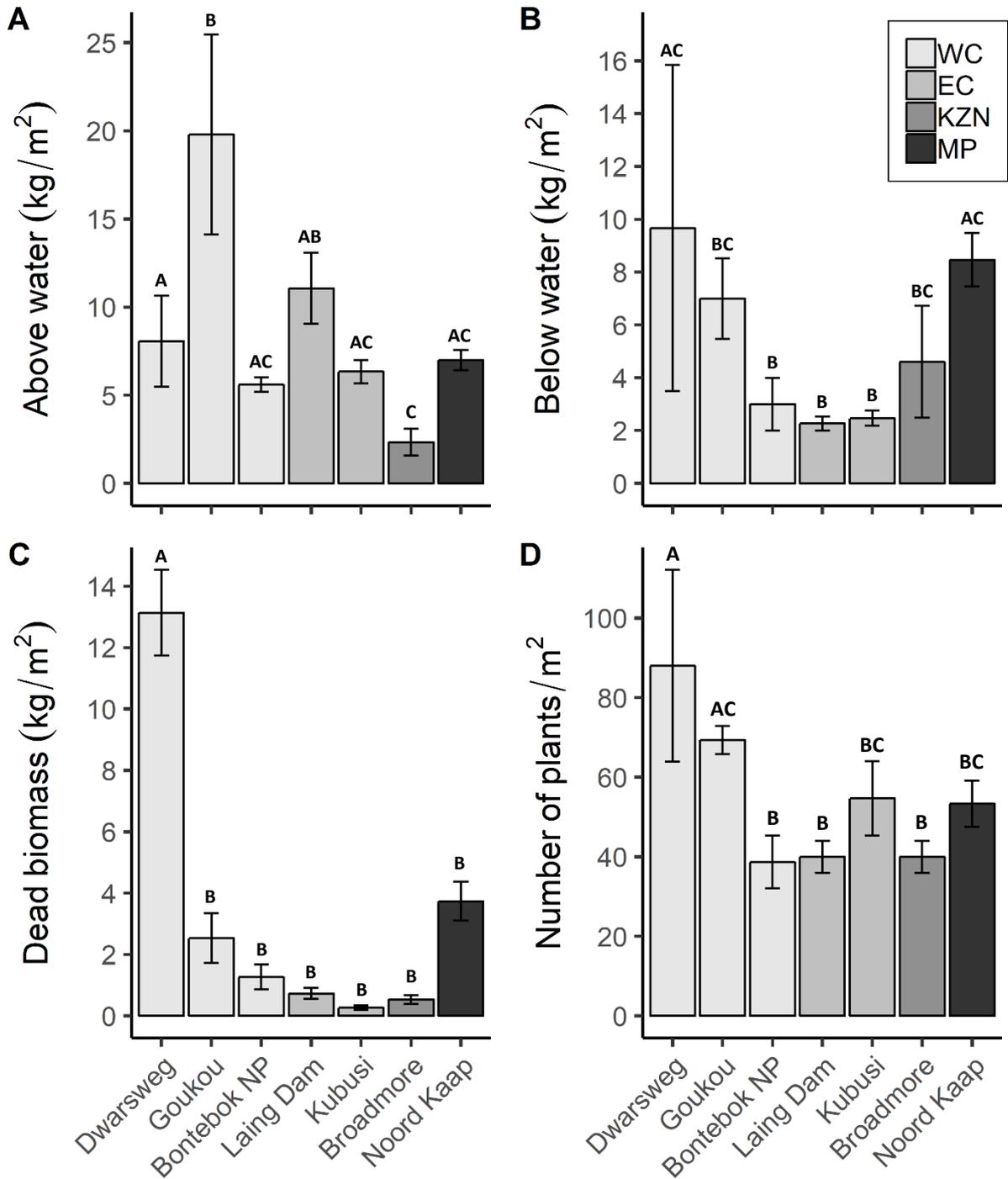


Figure 4.8. The mean (A) above water biomass, (B) the below water biomass, (C) the dead biomass, and, (D) the number of plants per m² (\pm SE). Provinces are represented by colour. EC = Eastern Cape, KZN = KwaZulu-Natal, MP = Mpumalanga, WC = Western Cape. Significant differences are represented by different letters.

4.3.5. Insect data

Megamelus scutellaris was successfully identified at 16 sites in South Africa, where 12 of those were sites where the insects had overwintered at least once, and four sites had insects that had dispersed naturally, without any releases having been made (Figs 4.9 & 4.10). The sites where the agents dispersed without release were the Portmann Spruit in KwaZulu-Natal (-29.3876, 30.5622), the Nahoon River in the Eastern Cape (-32.9637, 27.9129), and in the Western Cape, *M. scutellaris* passively dispersed to two new sites: the lower part of the Goukou River (-34.2910, 21.3076) and the Strandfontein Sewage Works (-34.0910, 18.5131).

The distribution of *M. scutellaris* was limited to cooler areas of South Africa, with no establishment recorded in the hotter north-east of South Africa where the mean annual temperature is above 22°C (red areas, Fig 4.9), while establishment was recorded in the Mediterranean climate of the Western Cape at four sites, with two of those being sites where the agents had dispersed to and established without any intentional releases made. The establishment of *M. scutellaris* in the Free State (FS) and Gauteng (GT) provinces was hampered by the cold temperatures and frost (Fig 4.10), although one site in the Farmall area of Gauteng did have successful establishment over a winter season, however the population growth was hampered by frequent herbicide applications.

Frost is known to affect the establishment of all water hyacinth biological control agents, and *M. scutellaris* is also susceptible to frosts. During the survey, *M. scutellaris* had not established at sites where frost occurred frequently during winter, and this included all of the major Highveld sites, with the exception of one site in Gauteng which gets between 1 and 30 days of frost per year, as opposed to sites in the Free State and the southern parts of Gauteng that get frosts on between 31 and 60 days per year (Fig 4.10). More successful establishment occurred in sites that had little to no frost events in a year.

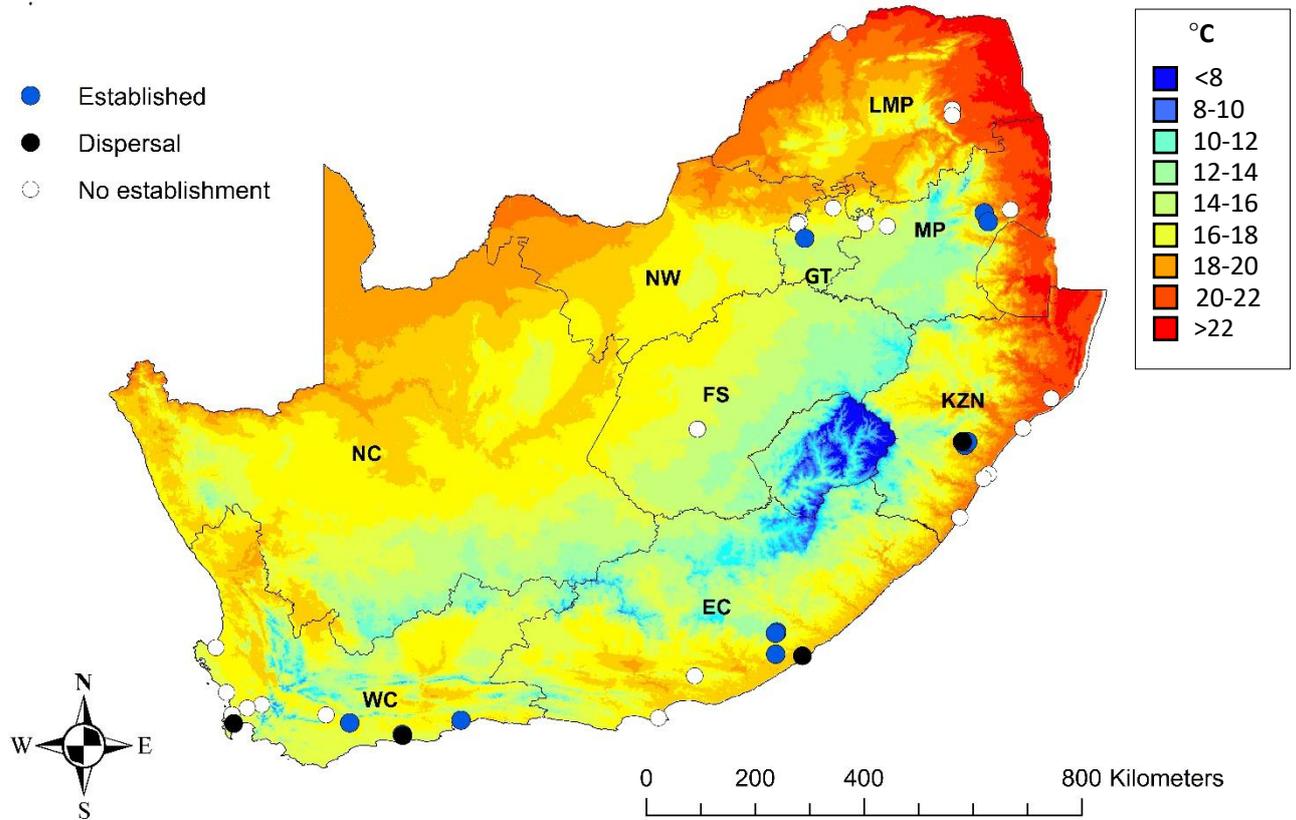


Figure 4.9. Distribution of *M. scutellaris* in South Africa in relation to **mean daily temperature**, low (blue) $\leq 8^{\circ}\text{C}$, high (red) $\geq 22^{\circ}\text{C}$. Blue circles are sites where *M. scutellaris* established after release, and the black circles are sites where *M. scutellaris* dispersed naturally. White circles represent sites where establishment failed or was unconfirmed. Climate data from Schulze (1997). LMP = Limpopo, MP = Mpumalanga, GT = Gauteng, KZN = KwaZulu-Natal, NW = North-West Province, FS = Free State, NC = Northern Cape, EC = Eastern Cape, WC = Western Cape

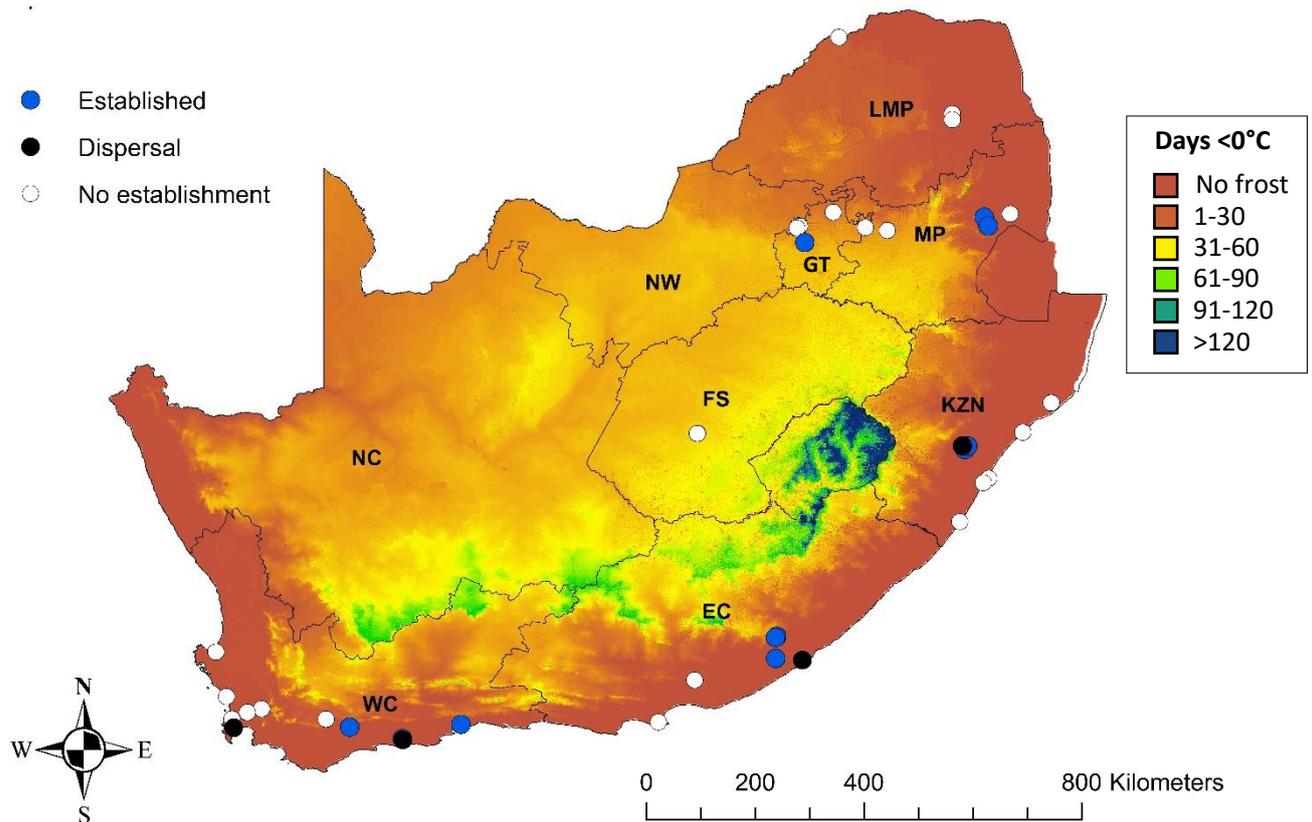


Figure 4.10. Distribution of *M. scutellaris* in South Africa in relation to **the average number of days with heavy frost (days <0°C)**, low (red) = 0 days, high (blue) ≥ 120 days of frost. Blue circles are sites where *M. scutellaris* established after release, and the black circles are sites where *M. scutellaris* dispersed naturally. White circles represent sites where establishment failed or was unconfirmed. Climate data from Schulze (1997). LMP = Limpopo, MP = Mpumalanga, GT = Gauteng, KZN = KwaZulu-Natal, NW = North-West Province, FS = Free State, NC = Northern Cape, EC = Eastern Cape, WC = Western Cape

The mean density of *M. scutellaris* was significantly different between all sites except the two Eastern Cape sites: Laing Dam (605.38 ± 84.07 insects/m²) and the Kubusi River (605.38 ± 111.92 insects /m²) ($Z_{72} = 135.34$, $P < 0.0001$) (Fig 4.11). The most densely populated site was the Noord Kaap River, which had a mean density of 769.89 ± 118.85 insects /m², where the lowest number of *M. scutellaris* was collected at the Camelot site in KZN, which had a mean density of 34.41 insects /m² ± 8.30 . The density of *M. scutellaris* was also highly significantly different between provinces (indicated by lower case letters), showing that there is a high degree of spatial variability in *M. scutellaris* populations ($Z_{76} = 704.86$, $P < 0.0001$).

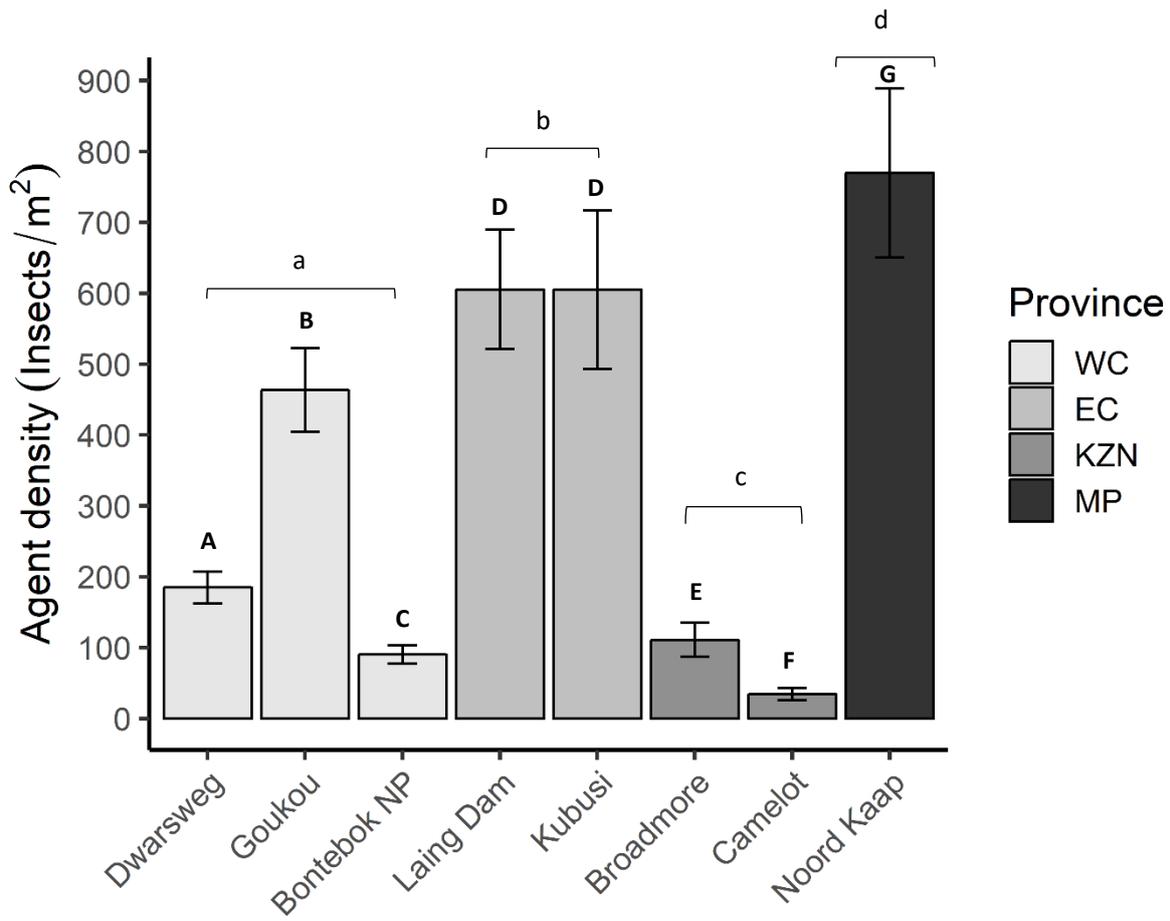


Figure 4.11. The mean density of *Megamelus scutellaris* (insects/m²) (\pm SE) on water hyacinth at each site. Provinces are represented by colour. EC = Eastern Cape, GT = Gauteng, KZN = KwaZulu-Natal, MP = Mpumalanga, WC = Western Cape. Significance level between sites represented by the upper case letter, and significance between provinces represented by lower case letter.

The insect populations responded positively to increasing conductivity, a proxy for water quality (Daddy *et al.* 2002), with the insect density increasing by 0.71 insects/m² per unit increase in conductivity ($F_{1,68} = 6.39$, $R^2 = 0.07$, $P = 0.01$) (Fig 4.12A). The nutrients most likely affect the insect density via an increase in host-plant biomass as opposed to a direct influence on *M. scutellaris*, shown by the increase in above water biomass as conductivity increases ($F_{1,19} = 7.253$, $R^2 = 0.24$, $P = 0.01$) (Fig 4.13). The number of leaves on each plant had a highly significant positive interaction on the numbers of *M. scutellaris* present at each site ($F_{1,78} = 9.706$, $R^2 = 0.1$, $P = 0.003$), with more leaves per plant yielding more *M. scutellaris* per square metre (Fig 4.12b; Table 4.4).

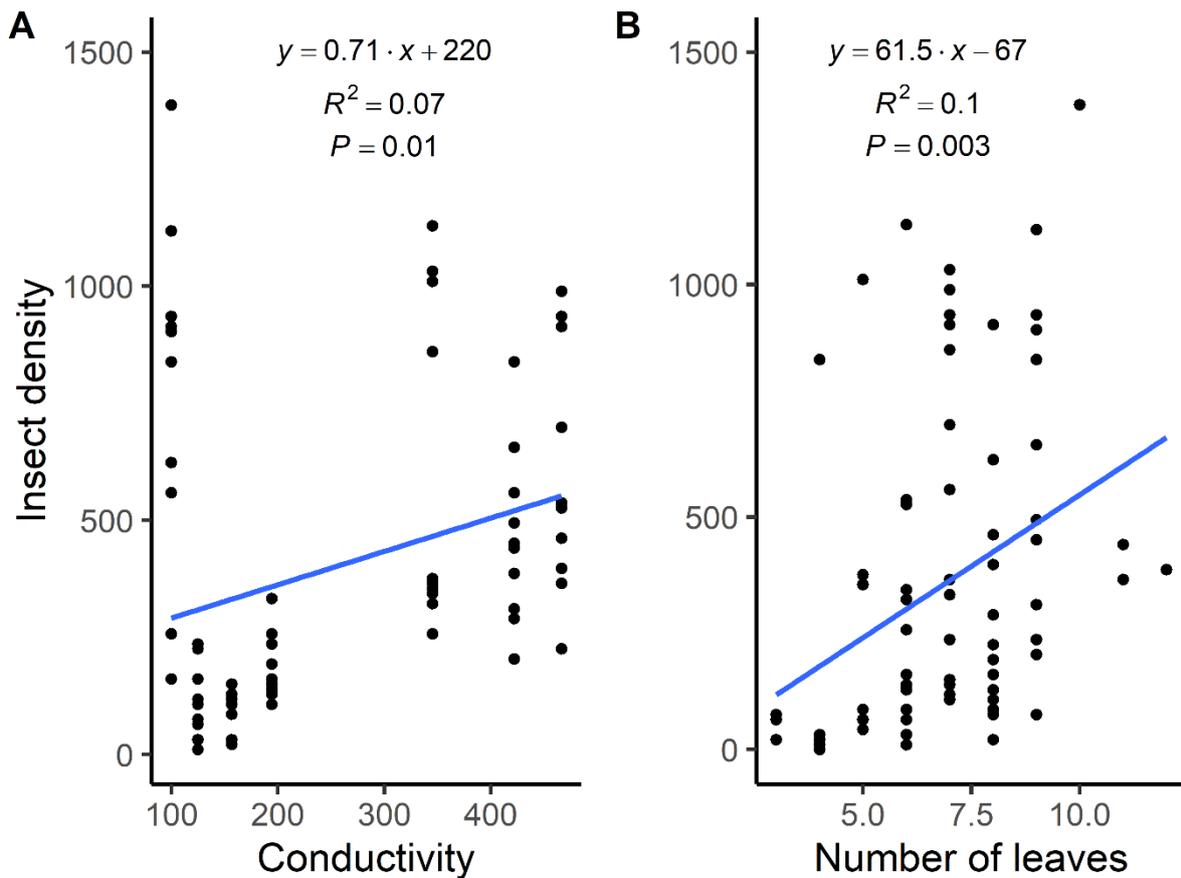


Figure 4.12. The interaction between (A) conductivity and (B) the number of leaves on insect density (insects/m²)

There was a highly significant interaction between conductivity and the wet weight of water hyacinth above water biomass ($F_{1,19} = 7.253$, $R^2 = 0.24$, $P = 0.01$) (Fig 4.13). The increased nutrients have a direct impact on water hyacinth growth and this interaction was most likely to have been cause of the increase in insect density seen in Fig 4.12A.

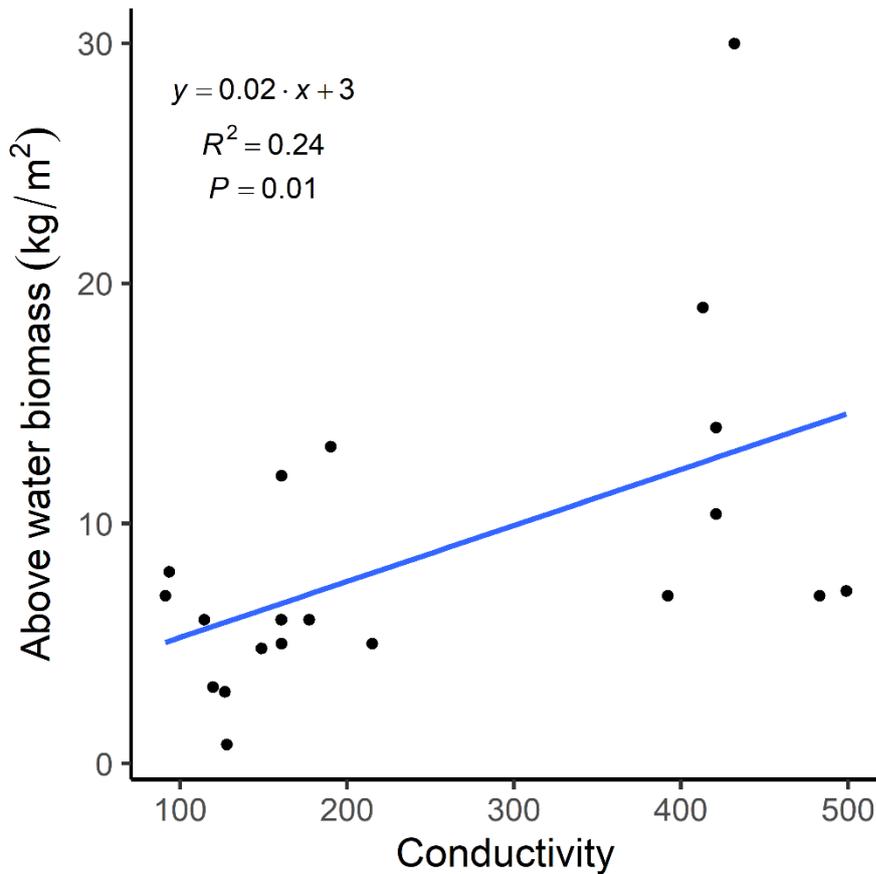


Figure 4.13. The interaction between **conductivity** and **above water plant biomass** (kg/m²)

The population densities and establishment potential of *M. scutellaris* in South Africa were dependent on the root length of their host plants, as well as the number of photosynthetically active leaves on each plant, the province, and the interaction between the number of leaves and the province in which the plants were sampled (Table 4.4). These results were very similar to those extracted from the seasonal data (Table 3.1), suggesting that the likelihood of establishing a robust and self-sustaining population of *M. scutellaris* was dependent on the water quality (indicated by root length), the availability of healthy leaves to feed on (indicated by leaf number), and spatial location (indicated in Figs 4.9 & 4.10).

Table 4.4. ANCOVA results illustrating the effects of various plant parameters and spatial separation on the establishment of *Megamelus scutellaris*. Values in bold indicate significant interactions. LP = longest petiole, RL = root length, LN = number of leaves, NR = number of ramets.

Effect	Sum Sq	DF	Mean Sq	F	P
Longest petiole (LP)	36797	1	36797	2.800	0.24
Root length (RL)	430936	1	430936	32.789	0.03
Number of leaves (LN)	1107160	1	1107160	84.242	0.01
Province	3652429	3	1217476	92.636	0.01
Number of ramets (NR)	1492	1	1492	0.114	0.77
Site	552210	4	138053	10.504	0.09
LP:RL	21289	1	21289	1.620	0.33
LP:LN	105683	1	105683	8.041	0.11
RL:LN	54487	1	54487	4.146	0.18
LP: Province	494515	3	164838	12.542	0.07
RL: Province	149226	3	49742	3.785	0.22
LN: Province	857298	3	285766	21.744	0.04
LP:NR	112694	1	112694	8.575	0.10
RL:NR	36690	1	36690	2.792	0.24
LN:NR	37038	1	37038	2.818	0.24
Province: NR	59984	3	19995	1.521	0.42
LP: Site	68347	4	17087	1.300	0.48
RL: Site	116482	4	29120	2.216	0.33
LN: Site	116441	4	29110	2.215	0.33
NR: Site	87168	3	29056	2.211	0.33
LP:RL:LN	10075	1	10075	0.767	0.47
LP:RL: Province	75462	3	25154	1.914	0.36
LP:LN: Province	28426	3	9475	0.721	0.62
RL:LN: Province	46846	3	15615	1.188	0.49
LP:RL:NR	63224	1	63224	4.811	0.16
LP:LN:NR	11429	1	11429	0.870	0.45
RL:LN:NR	1734	1	1734	0.132	0.75
LP: Province: NR	15502	3	5467	0.393	0.77
RL: Province: NR	56254	3	18751	1.427	0.44
LN: Province: NR	161184	2	80592	6.132	0.14
LP:RL: Site	344750	4	86188	6.558	0.14
LP:LN: Site	172658	4	43165	3.284	0.25
RL:LN: Site	61213	4	15303	1.164	0.51
Residuals	26285	2	13143		

These results have shown that, in terms of the spatial establishment of *M. scutellaris*, many factors play a role in where these insects will be able to survive and maintain long-lasting populations. Suitable climate, water quality and the availability of food are the most influential

factors in determining where the insects can establish, and the number of releases seems to be less influential unless the area is liable to experience stochastic events such as flooding, frost, or excessive herbicide applications.

4.4. Discussion

The most influential factor in any biological control programme is whether or not a biological control agent can establish and suppress weed densities in novel environments (McFayden 1998). The data presented here demonstrate that there are a myriad of contributing factors that determine whether *M. scutellaris* is able to establish a population that is self-sustaining and capable of overwintering, with climate being the major factor contributing to the success or failure in establishment. Extremely hot temperatures negatively impact the Argentine biotype of *M. scutellaris* that was released in South Africa, as it is not as heat tolerant as the other Paraguayan genotype which has not yet been released, and this might limit the establishment in parts of the country that have higher mean annual temperatures (Foley *et al.* 2016). High temperatures are particularly limiting to insect establishment if they rise above the CT_{max} of *M. scutellaris* which, for the Argentine biotype, is 39.1°C (J. Coetzee, *unpub. data*). Extremely high temperatures are not uncommon in some parts of South Africa during the summer months, particularly in the Limpopo, Mpumalanga, and KwaZulu-Natal provinces where, in the hottest areas, *M. scutellaris* has failed to establish. In other areas where populations have established, *M. scutellaris* has developed some behavioural adaptations to deal with warmer temperatures by migrating to lower regions of the petiole, where the cooler water buffers the high temperatures, providing a more suitable microclimate for the insects (Grodowitz *et al.* 2014). The canopy of water hyacinth plants can also be up to 3.5 °C cooler than the surrounding air which may provide thermal refugia for the insects when the air temperature rises close to the thermal tolerance levels of *M. scutellaris* (Grodowitz *et al.* 2014, 2017).

To date, *M. scutellaris* populations have not established in Limpopo, even though the release records state that approximately 17 000 individuals have been released on water hyacinth infestations around the province. Degree day studies of *M. scutellaris* predict that the planthoppers should be able to produce upwards of six generations per year at sites in Limpopo and along the coast of KwaZulu-Natal, but as yet this is not the case and no establishment has been recorded (May & Coetzee 2013). Contrastingly, the sites where *M. scutellaris* populations were recorded in both Mpumalanga and KwaZulu-Natal fall within midlands areas where the higher altitude results in lower mean temperatures that are more conducive to the survival of the planthoppers. Consistently hot temperatures favour high levels of plant growth but are often detrimental to *M. scutellaris* growth, survival, and reproduction, with the ideal temperature for these processes to occur shown to be within the range of 25°C (Grodowitz *et al.* 2017). Temperatures that were either below 18°C or above 33°C were determined to lead to significantly reduced numbers of the insects (Grodowitz *et al.* 2017). Where the Argentine biotype of *M. scutellaris* was originally released in Louisiana and some sites in Florida in the USA, the establishment was limited as a result of high temperatures, which affect the nymphs in particular (Grodowitz *et al.* 2014, 2017). However, the presence of shaded areas in the sites was thought to provide buffer zones where the temperature did not fluctuate as greatly, and these may have aided establishment in some sites (Tipping *et al.* 2014).

Extreme climatic variability is a contributing factor to establishment failure in weed biological control programmes, and temperature incompatibility alone accounts for 34.5% of failed biological control programmes globally (Stiling 1993). Similar to *M. scutellaris*, many biological control agents have preferences for areas that are climatically similar to their country of origin. Cowie *et al.* (2016) found that the weevil *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), a South American biological control agent of the Bugweed *Solanum mauritianum* Scop. (Solanaceae), was only able to establish in two South African

localities, in Mpumalanga and KwaZulu-Natal, which more closely mirrored the climate of its native range. The weevil, which has a CT_{min} as an adult of approximately 4°C, could not establish in the Highveld where the winter temperature and humidity were too low to support the populations.

Alongside temperature, plant quality was an important factor contributing to the rate of establishment among biological control agents in the field, and more highly fertilized plants yielded greater populations of herbivorous insects. These results support the conclusions of Heard and Winterton (2000) and Awmack & Leather (2002) who determined that the nutrient status of the water had a direct impact on both plant health and the fecundity and vitality of associated biological control agents. Furthermore, Freedman & Harms (2017) found that greenhouse colonies of *M. scutellaris* attained higher densities on plants grown in high nutrient water, and this supports the results of this study, as sites with higher water nutrients were more likely to support higher populations of planthoppers. So important is the relationship between water nutrients and the populations of host-specific herbivores that Room & Thomas (1985) advocated for the release of fertilizers in infestations of *Salvinia molesta* DS Mitch. (Salviniaceae) in Papua New Guinea to increase the nutrient content of the leaves. The higher nitrogen content promoted higher populations of the weevil *Cyrtobagous salviniae* Calder & Sands (Coleoptera: Eirirhinidae) and therefore, helped to reduce the cover of the weed on the waterways. The relationship between nutrients and insect density eventually becomes harmful to the biological control programme, whereby the increased plant growth and leaf turnover in eutrophic water is greater than the rate at which the insects can damage them, making the effects of nutrients greater than the effects of herbivory (Heard & Winterton 2000; Ripley *et al.* 2006; Hill & Coetzee 2012).

In laboratory studies, *M. scutellaris* was found to be damaging to water hyacinth at all nutrient levels. *Megamelus scutellaris* also possesses the ability to kill water hyacinth outright, unlike

any other agent currently released in South Africa (Coetzee 2013). The fact that *M. scutellaris* has established viable populations around South Africa, particularly in the cooler areas which have been historically problematic for the establishment of biological control agents, shows that this agent is a viable option for the control of water hyacinth in the future. Of particular interest is the fact that multiple populations have been recorded in the Western Cape, which is a winter rainfall area with a Mediterranean-style climate that can be problematic to insect establishment. Not only have insects established, but the long-distance dispersal to the Strandfontein Sewage Works site is a positive sign that this agent will not only establish around Cape Town in the future but that eventually, this agent will be able to passively disperse to new sites without human intervention, reducing further the costs involved in mass rearing and releasing biological control agents.

At present, four sites exist where there is no record of active releases of *M. scutellaris*, suggesting that the insects dispersed passively. Some sites, such as the Goukou site, are on the same system where the insects were released, but the insects have been able to move downstream, far beyond their initial release site. Other sites, such as the Portmann Spruit in KwaZulu-Natal, the Strandfontein Sewage Works in the Western Cape, and the Nahoon River in East London are sites that are not connected to any systems where *M. scutellaris* is currently established. The Nahoon River lies more than 40 km away from Laing Dam, which is the nearest site of successful establishment. Unaided dispersals of this distance have not yet been recorded with introduced populations of *M. scutellaris*, with some suggesting that this insect is predicted to disperse only 50 m per year (Moran *et al.* 2016). However, the non-dispersing brachypterous form of *M. scutellaris* is the most common and is the likely reason for the low recorded dispersal distances.

4.5. Conclusion

Megamelus scutellaris is capable of establishing large, self-sustaining populations in South Africa, even in areas that experience cold, frosting winters, so long as the plants are of a suitably high quality to maintain the insects. Constant high temperatures are limiting to the establishment of the insects, and while this should not mean that attempts to establish populations of *M. scutellaris* in the hotter areas of South Africa should necessarily be abandoned, maximum temperatures should be considered. The agent has also shown the ability to disperse, without human intervention, to novel sites which, added to the overwintering capabilities, the high rate of increase and multi-voltinism, could make this a highly effective biological control agent of water hyacinth in the future.

Chapter 5: General discussion

Biological control is the most cost-effective and environmentally safe practice for the management of invasive weeds (McFayden 1998; van Wyk & van Wilgen 2002). However, for a biological control programme to be successful, the agents must be effective, host-specific, and capable of establishing self-perpetuating populations that are large enough to inflict significant damage to weed populations in the adventive range (McFayden 1998). The biological control of water hyacinth in South Africa has been bolstered in recent years by the addition of new agents to manage the spread and damage that the weed causes (Hill & Coetzee 2017). The objectives of this study were to assess the damage caused by *M. scutellaris*, the most recent addition to the South African water hyacinth biological control programme, at different stocking rates and at different nutrient levels in a controlled greenhouse experiment, as well as to conduct the first post-release evaluation of the agent's establishment since it was released in 2013.

5.1. The effectiveness of *Megamelus scutellaris* as a biological control agent of water hyacinth

In the greenhouse experiment, *M. scutellaris* was most damaging to water hyacinth when released in high densities on plants grown under medium nutrients, but less damaging to plants that were grown in high nutrients (Chapter 2). On the medium nutrient plants, the subtle feeding damage caused by the agent was responsible for significant declines in both leaf production and photosynthetic efficiency (efficiency was measured using chlorophyll fluorometry). Water hyacinth, being a nitrophilous plant, will readily establish in high nutrient waterways (Reddy & Tucker 1983; Coetzee & Hill 2012), and high nutrients are one of the main drivers of water hyacinth biomass accumulation and growth (Chapter 4). However, high water nutrients facilitate a faster rate of leaf turnover and biomass accumulation, and this was found to be

greater than the impact of herbivory. This result echoed a growing number of studies which indicate that South Africa's highly eutrophic waterways have a negative impact on the biological control of water hyacinth (Coetzee & Hill 2012), as the effectiveness of some of the agents is reduced under high nutrient conditions (Heard & Winterton 2000; Ripley *et al.* 2006; Coetzee *et al.* 2007; Marlin *et al.* 2013; Canavan *et al.* 2014). Controlled experiments are important for quantifying the effectiveness of an agent under a range of different scenarios which may occur in the field, particularly for more recently released agents, however, there is a need for more post-release studies on biological control agents to determine how successfully these agents perform in the field (McFayden 1998; Blossey & Skinner 2000; Morin *et al.* 2009). To this end, most post-release evaluations measure success by the reduction in the target plant's cover or productivity (Blossey & Skinner 2000; Martin *et al.* 2018a), and this is not yet a viable approach for measuring success with *M. scutellaris* due to how recently it has been released. In general, biological control programmes are inherently long-term management schemes and success is typically achieved between 10 and 20 years after introduction (Hofmann 1995; McFayden 1998). Therefore, in the early stages of its release, establishment was the indicator by which success was measured in this study.

5.2. Evaluating the establishment of *Megamelus scutellaris*

Megamelus scutellaris has successfully established in at least 16 sites in South Africa covering five provinces and it has also successfully overwintered at all of these sites (Chapter 4). The distribution of *M. scutellaris* was limited by both frost in winter and high temperatures in summer, and this excluded the insects from much of the high altitude interior which is prone to frosts in winter, as well as the hotter northeastern parts of South Africa where the mean annual temperatures are typically above 22 °C (Chapters 3 and 4). This contradicted some of the conclusions of May & Coetzee (2013) which suggested that *M. scutellaris*, based on

laboratory results, would be most successful in warmer areas such as the KwaZulu-Natal and Limpopo provinces. However, unlike the other water hyacinth biological control agents released in South Africa, *M. scutellaris* established well in the cooler areas where, historically, other agents have struggled (Hill & Olckers 2000; Coetzee *et al.* 2007b), and this is promising for the biological control of this weed.

5.3. Temperature effects on the distribution of *Megamelus scutellaris*.

Climate had a significant impact on the distribution of *M. scutellaris* in South Africa, influencing its population densities both temporally (Chapter 3) and spatially (Chapter 4). It is possible that the establishment of *M. scutellaris* was better than expected by May & Coetzee (2013) in cooler areas, such as the Kubusi River, as a result of thermal plasticity which allows for a degree of cold-weather acclimation, as has been shown in at least one species of water hyacinth biological control agent, the mirid *E. catarinensis* (Porter *et al.* in press). Thus, while there are some benefits of pre-release climate modelling as a method of predicting the potential distribution of biological control agents in the field, ground-truthing of these predictions is important, so that future releases can be prioritized, to either augment the population if the climate is suitable, or stopped if the climate is unsuitable.

Having originally been released in the USA, *M. scutellaris* did not establish permanent populations in the hotter southern States, such as Texas and Louisiana (Grodowitz *et al.* 2014). However, successful establishment has occurred at some hot sites in Florida and California (Grodowitz *et al.* 2014; Moran *et al.* 2016). Furthermore, glasshouse experiments in the USA determined that rearing *M. scutellaris* at temperatures exceeding 33°C lead to significantly lower insect yields compared to rearing them at 25°C, indicating that high temperatures can cause increased mortality and reduced fecundity (Grodowitz *et al.* 2017).

The potential for thermal plasticity also questions whether there is a need to import different genotypes of biological control agents from different climatic regions into South Africa. For example, the Paraguayan genotype of *M. scutellaris* has been shown to be better adapted to survive in warmer areas than the Argentine genotype and was thus introduced into southern Florida (Foley *et al.* 2016). Indeed, the results of this thesis have indicated that the Argentine genotype of *M. scutellaris*, which was the one released into South Africa, may be limited by extremely hot temperatures, and this could warrant the release of the Paraguayan genotype here to establish in warmer provinces. However, given the results of the work by Porter *et al.* (in press), acclimation of the currently released genotype to warmer areas prior to release could be possible. Acclimating the agents to other climates should be explored first before importing a new genotype, particularly if the genotype could potentially be a cryptic species, as was the case with the mirids, *E. catarinensis* and its cryptic congener *E. eichhorniae*. Even though both *Eccritotarsus spp.* were safe to release in South Africa, the potential for cryptic species warns that the importation of new consignments of biological control agents should be conducted only when necessary (Paterson *et al.* 2016).

Changes in the temperature and host-plant characteristics leading up to winter had a profound effect on the population density of *M. scutellaris*, initiating the onset of diapause in 2017 at the Kubusi River when plant quality was low, but not in the winter of 2018 when the plants were less damaged by frosts during winter (Chapter 3). This suggests that season, which includes stochastic events like frosts and drought, had a major impact on biological control agent populations post-release, particularly in South Africa where winter temperatures are limiting to the establishment of biological control agent populations (Hill & Olckers 2000).

As the seasons warmed after winter, the plants were able to grow back rapidly, however, at the Kubusi River, there was a substantial lag period where the density of *M. scutellaris* remained low. The asynchrony of the post-winter recovery of the weed and its suite of biological control

agents means that water hyacinth was able to accumulate biomass and new foliage, unchecked by biological control (Chapter 3). This seasonal lag-phase was initially predicted by Hill & Olckers (2000) and prompted the inclusion of a biological control species that could reproduce quickly when the climate was favourable.

Unlike the *Neochetina* weevils, which require 984 degree days ($^{\circ}\text{D}$) to complete their development (King 2011), *M. scutellaris* develops from egg to adult in 502.96 $^{\circ}\text{D}$ (May & Coetzee 2013), 51% faster than the development time of the weevils at all temperatures. Even though the weevils can begin their development at a lower temperature than *M. scutellaris* ($t_0 = 9.6^{\circ}\text{C}$ and 11.5°C respectively), the fact that *M. scutellaris* is multivoltine and capable of producing multiple overlapping generations means that the density that they can achieve in a shorter space of time is significantly higher than many other biological control agents in the field.

Comparing pre-release predictions of development and establishment potential with the realised distribution in the field is a powerful method of: (a) ground truthing the predictions that were made pre-release, in order to transform biological control practice from an empirical to a predictive science (Goolsby *et al.* 2004); and (b) analysing the establishment data in order to prioritise areas for future release where establishment is most likely (McClay 1996), which is important for addressing one of the initial aims of this thesis, to justify the resources being used to mass rear and release *M. scutellaris* in South Africa. Ensuring that the agents that are released have the highest chance of establishing and becoming effective is the most parsimonious method of ensuring that the risk is worth the reward for this programme.

Climate is a critical barrier to the successful establishment of a biological control agent, and this barrier must be surpassed in order for the insect population to persist, but it is likely that insects are thermally plastic to a degree, allowing them to establish in areas where they may

not initially have been thought able to do so. Once established though, control of the problem species is not guaranteed, and there are multiple influences from other highly variable and stochastic processes that occur in nature and, in particular, in waterways, such as the nutrients status of the water.

5.4. *Megamelus scutellaris* as part of the integrated solution to managing water hyacinth in South Africa

Megamelus scutellaris was released as part of a suite of biological control agents to reduce the spread, growth, and reproduction of water hyacinth in South Africa (Coetzee 2013). The interactions of these different biological control agents are not antagonistic and are, in some instances, synergistic (Petela 2017). However, there are phenological differences in the post-winter recovery of different species of biological control agents, with *M. scutellaris* being the first to recolonize plants after winter, followed by the weevils, and then *E. catarinensis* (at the Kubusi River; Z. Maseko, RU CBC, *pers comm.*).

Releasing multiple biological control agents on an invasive plant increases the odds of success (Denoth *et al.* 2002) and, looking at multispecies releases within the framework of the Enemy Release Hypothesis (ERH), the addition of multiple natural enemies is more likely to have a greater impact on the weed. According to the ERH, invasiveness is caused by a lack of natural enemies and, therefore, by releasing multiple species of natural enemies, the invasiveness should be reduced more than if only one species was released (Keane & Crawley 2002). Further, Sutton *et al.* (2016) demonstrated that *M. scutellaris* was able to facilitate the transport and infection of various phytopathogens to water hyacinth tissues, and insect-borne phytopathogens can contribute significantly to a reduction in plant vigour (Venter *et al.* 2013). Hence, by measuring only the damage caused by the biological control agent on the photosynthetic rates of the target weed, the data could be misleading by attributing reductions

in vitality to feeding damage alone (Ripley *et al.* 2008) and, as a result, multitrophic interactions including host-specific pathogens should be investigated further (Ray & Hill 2016).

Although *M. scutellaris* can cause significant damage to water hyacinth, major caveats to the complete success of the biological control of water hyacinth in South Africa using *M. scutellaris* are present, and these were identified as: (a) successfully establishing field populations throughout South Africa; and (b) getting the population densities of the agents to high enough levels at the start of the growing season for them to inflict the maximum damage on water hyacinth before winter. Therefore, the correct implementation of biological control becomes important.

How effectively a weed management scheme is implemented is crucial to its success. Implementation, in the context of *M. scutellaris*, includes the mass-rearing, transport, and release of *M. scutellaris* in the field, followed by post-release monitoring. Each of these steps is important to ensure that the agents remain healthy prior to their release, giving them the greatest possibility of establishing effectively. Each province in South Africa has a government-appointed biodiversity officer who is tasked with implementing the management scheme for water hyacinth in the region (Martin *et al.* 2018b). Consistent monitoring, release, and effective handling are likely to significantly increase the establishment rates of *M. scutellaris* in the field. Reducing the distance that agents are transported will also go a long way towards decreasing the mortality of insects during transport.

Currently, the main mass-rearing facilities in South Africa for *M. scutellaris* are located at Rhodes University (Grahamstown, Eastern Cape) and at the City of Cape Town (Western Cape), meaning that the distance between the mass-rearing facilities and the targeted site for the release of the agents can often be vast. In some instances, this has been rectified through the formation of smaller mass-rearing facilities at schools near to large infestations, such as at

the Hartbeespoort Dam in the North-West Province of South Africa (K. Weaver, RU CBC, *pers comm.*). The benefits of this are many: the agents can develop and acclimate in the climate in which they will be released, and transport distances are likely to be significantly shorter which should reduce the mortality of the agents prior to release. Furthermore, involving the community in the rearing process has the added benefits of changing their perceptions of biological control and invasion biology (Weaver *et al.* 2017).

The major role that eutrophication plays in all aspects of water hyacinth management in South Africa cannot be overlooked (Coetzee & Hill 2012). Although they have managed to significantly slow growth and reproduction, none of the biological control agents currently released has yet been able to completely control water hyacinth in eutrophic water. However, there are methods that can be used to increase their effectiveness, including the combined application of non-lethal doses of registered herbicides to slow the initial post-winter plant growth coupled with augmentative releases of *M. scutellaris* at the beginning of the growing season to increase the post-winter populations of biological control agents (Hill & Coetzee 2008; Byrne *et al.* 2010). This will increase the probability of the insect populations reaching damaging levels before the onset of winter, while reducing the extent of the insect recruitment lag after winter, in order to best control the weed using an integrated pest management (IPM) scheme. It is important that the augmentative releases are focussed on sites where the climate is suitable, and where the level of implementation is sufficient to ensure that the insects are collected, transported, and released in a way that mitigates losses due to mortality, while maximising the potential for the dispersal of healthy insects within the system in order to avoid the unnecessary use of resources.

5.5. Long-term monitoring of biological control agents

Further investigations into the most efficacious use of *M. scutellaris* as a biological control agent of water hyacinth in South Africa are still required, and it is likely that this agent will be a good candidate for use on water hyacinth where it has become invasive throughout Africa. The field-based post-release aspects of this study focused mainly on the establishment of *M. scutellaris* in the field, and did not attempt to evaluate the extent of the damage that the agent has caused as, having only been released since 2013, it is most likely too early for significant damage to have occurred in so short a space of time. Furthermore, establishment studies benefit from long-term data, which can account for both temporal changes and changes in plant and animal communities over time (Blossey 1999). Further, long-term data enable researchers to measure the increase in establishment of biological control agents. For example, *C. salviniae*, the biological control agent of the Kariba weed (*S. molesta*), was monitored annually over 10 years and the data generated from those field surveys enabled the researchers to measure the success of biological control at *S. molesta* infested sites (Martin *et al.* 2018a). Since the weevil had been released in 1985 (Cilliers 1991a), there had been sufficient time for the agent to establish, and this enabled the researchers to determine which factors had reduced the efficacy of the biological control agents at certain sites where control was limited, and what factors have enabled complete control. This facilitated the formation of site-specific management plans which included augmentative releases, and this demonstrates the benefits of long-term monitoring data on the *in-situ* management of aquatic weeds in South Africa (Martin *et al.* 2018a).

5.6. General Conclusion

In conclusion, it is recommended that the methods used in this thesis to measure establishment and quantify agent densities in the field be continued in the future to monitor changes in

establishment and, in particular, to look for areas of natural dispersal where biological control agents have established without having been actively released. Chlorophyll fluorometry was also found to be an effective measure of the success of biological control at the cellular level, and the use of this technique should be expanded on in the future, particularly for agents that inflict more subtle types of damage, such as *M. scutellaris*, *E. catarinensis*, *E. eichhorniae*, and *O. terebrantis*. The work conducted has provided a framework for the future monitoring of *M. scutellaris* in South Africa, as well as a record of its effectiveness. The intention is that post-release evaluations such as this become standard practice for the cost-effective and ecologically safe biological control of water hyacinth in the future.

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