Evaluating the impact of the biological control agent, *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), on *Pontederia crassipes* Mart. (Pontederiaceae) (water hyacinth) senescence and detritus formation at Hartbeespoort Dam

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Abstract

This study evaluated the biological control of *Pontederia crassipes* (water hyacinth) at Hartbeespoort Dam, South Africa, focusing on the impacts of plant senescence on water chemistry and detritus formation. Two studies were conducted, the first under laboratory conditions where the amount of detritus formed from senescing water hyacinth plants under biological and chemical control, as well as changes in water chemistry could be compared. This study found that water hyacinth produced increased amounts of detritus over time, compared to healthy plants across all treatments, indicating that senescing plants add to the sediment load of the water. Additionally, pH was found to decrease over time in the presence of senescing plants, restoring the acidity of water compared to alkaline water when water hyacinth plants are healthy. The second study was conducted *in situ* at Hartbeespoort Dam and monitored the effects of density of the biological control agent, *Megamelus scutellaris*, water hyacinth cover and eutrophic water on detritus formation. It was found that biological control effectively decreases water hyacinth abundance leading to the senescence of the plants, ultimately increasing the amount of detritus produced. Detritus production was significantly correlated to water hyacinth root length, plant abundance and root biomass. Findings indicate that while biological control effectively decreases plant abundance, it may influence nutrient concentrations in the eutrophic dam waters. This research suggests that the senescence of water hyacinth mats impacts sediment loading and nutrient water chemistry, these impacts are no different to water hyacinth under biological, chemical or no control at all.

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CHAPTER 1

General Introduction and Literature Review

1.1 Problem statement

Invasive Alien Plant (IAP) species are classified as non-native species introduced into an ecosystem, either intentionally or accidentally, that thrive and often drive out native species (Gentili et al., 2021) . Given enough time, IAPs can homogenise the flora in an area once rich in biodiversity (Roy et al., 2024). The loss of biodiversity as a consequence of invasion is an issue faced across the globe (Dueñas et al., 2021) and is second in importance only to habitat destruction (Roy et al., 2024). Due to their lack of natural enemies in new environments, IAPs can establish with little to no threat to their population expansion. Through this unhindered establishment, invasive communities outcompete native species for light, nutrients, water, and space resources. Additionally, under eutrophic water conditions, the growth of Invasive Alien Aquatic Plant (IAAP) species, particularly *Pontederia crassipes* Mart. (Pontederiaceae) (water hyacinth) is enhanced. This increased growth rate makes this IAAP very difficult to control.

South Africa is a water-scarce country and relies upon constructed dams and weirs for the majority of its water supply (Oberholster & Ashton, 2008; Donnenfeld et al., 2018). Hartbeespoort Dam is an example of a large impoundment constructed in 1923 to provide water to surrounding communities for household and agricultural use. Situated in the North West Province (approximately 53 km northwest of Johannesburg), the dam is fed by the Crocodile, Magalies and Swartspruit rivers, with the Crocodile River being the largest and most influential tributary into the dam (Matlala, 2023). The dam is located downstream from urban areas and is subjected to untreated sewage waste and agricultural runoff, leading to over-enriched nutrient (eutrophic) conditions.

Water hyacinth has been present on Hartbeespoort Dam since the 1960s and the infestation underwent mechanical and chemical control procedures, but these proved unsuccessful in the long term. In the 1990s, a biological control programme was initiated whereby numerous biological control agents were released onto the infestation but was ultimately hindered by extremely cold winters and herbicidal applications that continued until 2017 (Coetzee et al., 2022). In 2019, an inundative release programme was initiated whereby thousands of the biological control agent *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) were released onto the dam and showed signs of success with water hyacinth percentage cover declining to as low as 6% (Coetzee et al., 2022).

When water hyacinth dies, due to frost, chemical and/or biological control, the dead mat begins to senesce and sink, thus beginning the decomposition process. Water managers and local communities around Hartbeespoort Dam are concerned about the decomposition of the water hyacinth mat and have hypothesised that the dead material (detritus) sinks to the dam floor and accumulates, thus creating a thick "sludge layer". A further concern is that an increased sediment load could reduce the dam's water-holding capacity through displacement by sediment deposition. Additionally, sediment loading impacts water chemistry in dam ecosystems by reducing dissolved oxygen availability (Donohue & Garcia Molinos, 2009) and releasing total dissolved solids (Birhanie et al., 2020), phosphorous (Søndergaard et al., 2003), nitrates and carbon dioxide (Ikem & Adisa, 2011) into the water, increasing the concentration of these water chemistry parameters.

1.2 Invasive Alien Plant Species

Richardson & Van Wilgen (2004) state that the impact of an IAP can be summarised by the product of its potential geographical range, its abundance/density, and the effect that the species can have on a small scale. Most IAPs are successful due to their potential ability to invade large geographical areas and form dense populations in the newly invaded area. The consequences of an invasion in an area can vary, depending on the properties of the invader as well as the environment. Other than out-competing native species, they often transform the physical environment by, for example, promoting erosion or altering fire regimes, in the case of terrestrial invaders (Poona, 2008).

In freshwater ecosystems, native macrophytes are the drivers behind structuring shallow-water ecosystems (Hussner et al., 2017). In addition to providing a habitat for various organisms (from macroinvertebrates to fish), macrophytes retain nutrients, clarify water by trapping sediment and inhibiting algal blooms (Hussner et al., 2017). An invasion by alien macrophytes threatens macrophyte biodiversity as well as ecosystem services by out-competing native macrophytes, often removing them from the ecosystem completely (Hussner et al., 2017).

Artificial impoundments and dams are slow-flowing and are rarely disturbed by winds or waves, thus making them ideal ecosystems for free-floating aquatic IAPs (Coetzee & Hill, 2012; Hill & Olckers, 2001). This lack of kinetic disturbance along with an absence of natural enemies allows for IAAPs to establish and proliferate at a rapid pace, thus out-competing native species (Coetzee & Hill, 2012; Hill et al., 2020). In addition, eutrophication of these impoundments further exacerbates the proliferation of IAAPs. Eutrophication can be understood as a condition whereby increased nutrient concentrations, such as nitrogen and phosphorous, lead to algal blooms, thus degrading the quality of the water (Akinnawo, 2023). Eutrophic systems are more susceptible to IAAP invasion as the high nutrient load allows for rapid macrophyte growth (Coetzee & Hill, 2012). Due to the water scarcity that South Africa faces, dams were erected across the country to aid in achieving water security (Oberholster & Ashton, 2008). Most of these impoundments are located downstream from urban areas and thus receive high concentrations of nutrients from improperly treated sewage, acid mine drainage and agricultural runoff (Coetzee & Hill, 2012).

IAAPs were first identified in South African water bodies in the early 1900s (Hill et al., 2020). Their pathways of introduction are numerous and include the ornamental (horticulture) and aquarium trade as well as accidental introductions through the unintentional distribution of propagules (Hill et al., 2020; Nunes et al., 2020). The success of IAAPs in South Africa's waterways can be attributed to the slow-flowing, eutrophic nature of the country's water (Coetzee & Hill, 2012)

In South Africa, five significant IAAP species have been named the "Big Bad Five, " including *P. crassipes* (water hyacinth); *Salvinia molesta* D. Mitch. (Salviniaceae) (Kariba weed); *Pistia stratiotes* L. (Araceae) (water lettuce); *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) (parrot's feather); and *Azolla filiculoides* Lam. (Azollaceae) (red water fern) (Hill et al., 2020).

1.3 Impacts of Invasive Alien Plant Species

The negative impacts of IAAPs reach far beyond eliminating native plant species. The presence of IAAPs adversely affects the delivery of ecosystem services to those who rely upon the invaded dam/river for income, namely the loss of revenue from tourist activities such as water sports or fishing, the damage to infrastructure (namely piping or pumps) from nearby farms or industries, or the decreased crop or livestock yield due to compromised water quality and quantity (Keller et al., 2018). Through their rapid growth and expansion in the water body, IAAPs that form mats block light from penetrating the water's surface, thus depleting dissolved oxygen and nutrient concentrations, such as nitrates and phosphates (Hill et al., 2020). This has knock-on effects on community structure and functioning in the ecosystem, such as macro- and microinvertebrates, as well as phytoplankton communities. Additionally, the presence of

IAAPs further impedes the flow of water, resulting in a greater sediment load and increased siltation rate, thus altering the ecosystem to a great extent (Chamier et al., 2012).

1.4 Control Efforts Against Invasive Alien Aquatic Plant Species in South Africa

van Wilgen et al. (2020) reported that Working for Water, a South African government programme removing IAPs to enhance the country's waterways, had spent approximately ZAR 15 billion (USD 813 million) on IAP control in South Africa since 1995. This report focused on terrestrial IAPs but is a testament to the extensive control programmes running in South Africa. Water hyacinth has proven to be the most difficult IAAP to control, resulting in multiple control programmes being initiated to attempt to control the invasions (Hill & Coetzee, 2017). These projects include manual and mechanical removal, chemical control, biological control and integrated approaches (Coetzee & Hill, 2008; Hill et al., 2020; Hussner et al., 2017).

While the severity of water hyacinth invasions necessitates their control, it is important to note that while the control of the weed is important, it is a symptom of a larger problem - eutrophic water (Hill et al. 2020). If water quality can be improved, the control of water hyacinth would not be as big of an undertaking as it presently is. In addition, the rapid reproduction of the weed as a result of eutrophic conditions makes it difficult to control (Gentili et al., 2021).

1.4.1 Manual removal

The physical removal process can be done by hand or through the use of harvesting machinery whereby large portions of the mat are removed from the water's surface (Hussner et al., 2017). This method of control is most efficient when dealing with small infestations of water hyacinth coupled with early detection (Hailu & Degaga, 2019). This method is labour-intensive and often expensive when using harvesters and these costs do not justify having to constantly remove large populations. In the 1990s, an attempt was made to control the water hyacinth infestation on Lake Victoria in East Africa and after time, those involved found that the plant material weighed over 400 tons per hectare, thus making it too difficult to proceed with the programme (Albright et al., 2001).

1.4.2 Chemical control

Herbicides can be applied to populations of water hyacinth by boat or air or through manual spraying (Coetzee & Hill, 2008). Although this control method is less labour-intensive than mechanical removal, it is expensive and often uneven (some plants are coated with the herbicide while some are not), resulting in unsprayed plants that will proliferate and return the population to what it was pre-spray (Hill et al., 2020). Chemical control is a short-term solution, requires frequent re-application and rarely eradicates a population (Hailu & Degaga, 2019; Hill et al., 2020; Hussner et al., 2017).

In the past, herbicides used were extremely toxic to not only the target weed but also to the surrounding environment and organisms. The chemicals used in these herbicides could be fatal to fish, birds, aquatic invertebrates as well as other aquatic macrophytes (Karouach et al., 2022). Glyphosate-based herbicides are currently widely used and do not exhibit the same mortality in non-target species as other herbicides, making them more acceptable (Hill et al., 2021; Karouach et al., 2022). Glyphosate is also used as it degrades into its metabolite aminomethylphosphonic acid (AMPA) which is further degraded into carbon dioxide and phosphate (Covaci, 2014; Kostakis et al., 2017). While glyphosates are the more acceptable herbicides, surfactants (additives used to increase the efficiency of a herbicide) are often highly toxic to mammals and other organisms (Hill et al., 2021). Although glyphosate-based herbicides display lower mortality in target plants, there are non-target impacts on co-occurring flora (Karouach et al., 2022). When used safely and conscientiously, herbicides could work in tandem with biological control programmes, however, the long-term effects have yet to be studied (Hill et al., 2021).

1.4.3 Biological control

Biological control is a method whereby the natural enemy of an invasive species is introduced to manage the invader in an area population (Coetzee & Hill, 2008). Before releasing a biological control agent onto an invasion, rigorous host-specificity trials must be conducted (McClay & Balciunas, 2005). These tests determine the efficacy (the degree of damage to a natural enemy) as well as the specificity (the number of non-target species that might be damaged) of the agent to determine if it is a suitable candidate for release (McClay & Balciunas, 2005). An ideal biological control agent causes severe damage to its natural enemy and feeds only on that enemy (McClay & Balciunas, 2005). In addition to pre-release surveys (identifying invasions and host-specificity tests), post-release surveys are crucial in determining the success or failure of a biological control programme (Hill & Coetzee, 2017). In 2008, a programme was launched to assess the progress of IAAP biological control efforts across the country, focusing on parameters such as plant and insect metrics as well as weed density (Coetzee et al., 2011; Paterson et al., 2024). This programme highlighted the need for long-term evaluation of invaded sites to determine if the weed population is still rife or if the population has shifted to another weed.

Biological control is preferred as it is a natural approach towards weed control, is economically viable and requires minimal labour efforts (Hill et al., 2020). In an IAAP biological control programme, agents target specific areas of the macrophyte (roots, leaves, stems or flowers), either killing or damaging the plant (Coetzee et al., 2021). When a macrophyte's ability to reproduce or photosynthesize is compromised, it is forced to allocate energy towards survival mechanisms (longer root systems), rather than towards reproduction (flowering) (Coetzee et al., 2021). This decreases the ability of the macrophyte to reproduce, thus slowly reducing the size of the infestation over time (Coetzee et al., 2021).

Of the "Big Bad Five" named above, four are considered to be under complete biological control, namely Kariba weed, water lettuce, parrot's feather and red water fern (Hill et al., 2020). A weed is considered to be under complete biological control when the population is reduced and maintained at a level where it is no longer a threat to aquatic biodiversity and water utilisation (Hill & Coetzee, 2017). Additionally, the infestation would be under complete control in areas where no other control methods (mechanical removal or herbicides) have been implemented (Hill & Coetzee, 2017). It is important to note that successful biological control does not necessarily mean total eradication of the weed. Once the weed has surpassed the lag phase of invasion—when the plant is present but not yet widespread—eradication becomes impossible (Hill et al., 2020). The first biological control agent for water hyacinth, *Neochetina eichhorniae* Warner (Coleoptera: Brachyceridae), was released in South Africa in 1974 and reached some level of success (Cilliers, 1991; Coetzee et al., 2011, 2021). Since then, eight more biological control agents have been released against water hyacinth (Hill & Coetzee, 2017; Miller et al., 2023).

The case of New Year's Dam in the Eastern Cape, South Africa is an example of where water hyacinth was under complete biological control (Hill & Olckers, 2001). According to Hill & Olckers (2001), in 1990, water hyacinth covered 80% of the impoundment until 200 *N. eichhorniae* weevils were released. In four years, the infestation had been reduced to cover

10% of the water surface. The oligotrophic system has been considered to be under complete control since 2000 (Coetzee et al., 2011; Hill & Olckers, 2001; Hill & Coetzee, 2017).

There are constraints associated with biological control, which limit success under certain conditions. In winter, infestations often die off due to extreme conditions such as frost, consequently, the agent population also dies due to a lack of a food source as well as cold-induced mortality (Hill & Olckers, 2001; Karouach et al., 2022). In the spring/summer months, the seed banks germinate when conditions are favourable, resulting in water hyacinth proliferation to occur at an exponential rate while agent populations have yet to re-establish (Coetzee et al., 2022; B. Miller et al., 2023). To regain control over the infestation, agents must be re-released, but their population growth is slow in the beginning (lag phase) (Hill & Olckers, 2001; Karouach et al., 2022). Rainfall in impoundments has a large impact on biological agent success as heavy rainfall often removes weeds from the system, leaving the agents without a food source (Coetzee et al., 2011). Additionally, the high nutrient levels in South African water systems also pose a problem for biological control agents as the weeds proliferate much faster than the agent population, allowing the weeds to proliferate almost freely (Coetzee et al., 2011). Another problem faced by biological control programmes is the uncontrolled spraying and manual removal efforts as this removes the weed, i.e. the agent's food source, resulting in agent populations dying out (Hill & Olckers, 2001).

Studies have shown that an augmentive programme whereby agents are mass-reared over winter and high numbers are released onto the infestation when it begins to proliferate in spring/summer is successful with large invasions (Coetzee et al., 2022; Hill & Coetzee, 2017; Miller et al., 2023; Moffat et al., 2024). An augmentive programme aids in supplementing the small population of field agents that may have survived the winter and reduces the lag phase between agent and enemy population booms (Hill & Coetzee, 2017).

1.5 Study species: *Pontederia crassipes*

1.5.1 Origin and distribution

Water hyacinth is a perennial, free-floating aquatic weed originating from the Amazonian and Paraná Basins in South America (Sosa et al., 2005). The weed has since spread to over 50 countries across 5 continents situated in tropical, subtropical and warmer climates (Bhattacharya et al., 2015). First recorded as naturalised in KwaZulu-Natal in 1910, water hyacinth has since spread through the waterways of South Africa (Hill et al., 2020). Although the weed was first recorded in the early 1900's it was only in 1983 that the Conservation of Agricultural Resources Act (Act 43 of 1983) listed water hyacinth as a category 1 weed, prohibiting its ownership and distribution. In 2004, the National Environmental Management: Biodiversity Act (Act 10 of 2004) instructed all state institutions to manage and conserve biodiversity by controlling/eradicating all IAPs in their area. These acts were put into place to control water hyacinth invasions across the country; however, this has proven difficult due to the survival and proliferation properties of the weed.

1.5.2 Biology

A mature plant consists of petioles measuring up to 1 m in height and can be either inflated or attenuated, a result of the plasticity displayed by the weed (Center & Spencer, 1981; Penfound & Earle, 1948) (Figure 1.1b). The flowers are eye-catching and purple, and it is this that appeals to those looking for ornamental pond plants (Hill & Coetzee, 2017) (Figure 1.1a). One individual plant can have one spike with up to 15 flowers (Henderson, 2020). Water hyacinth has an extensive root system with many laterals, allowing the plants to form a tight, interlocking mat (Hailu & Degaga, 2019).

Water hyacinth can reproduce both sexually and asexually, although asexual reproduction is more common, resulting in a faster turnover rate (Barrett, 1980). When reproducing asexually, the mature plant produces a horizontal offshoot called a daughter plant or ramet (Penfound & Earle, 1948) (Figure 1.1b). Once a daughter plant matures, it breaks off and can produce more daughter plants; one mature plant can have many daughter plants at one time (Penfound & Earle, 1948). A water hyacinth plant can have up to 20 flowers which produce up to 3000 seeds (Albano Pérez et al., 2011). The seeds are contained in capsules (holding up to 300 seeds per capsule) that are released into the mat or sink into the sediment (Albano Pérez et al., 2011). The seeds produced by these plants can lie dormant in the sediment for up to 20 years until conditions are favourable for them to germinate (Hailu & Degaga, 2019). A fast turnover rate and multiple reproductive methods make water hyacinth nearly impossible to control once it has colonised an area (Hill et al., 2020).

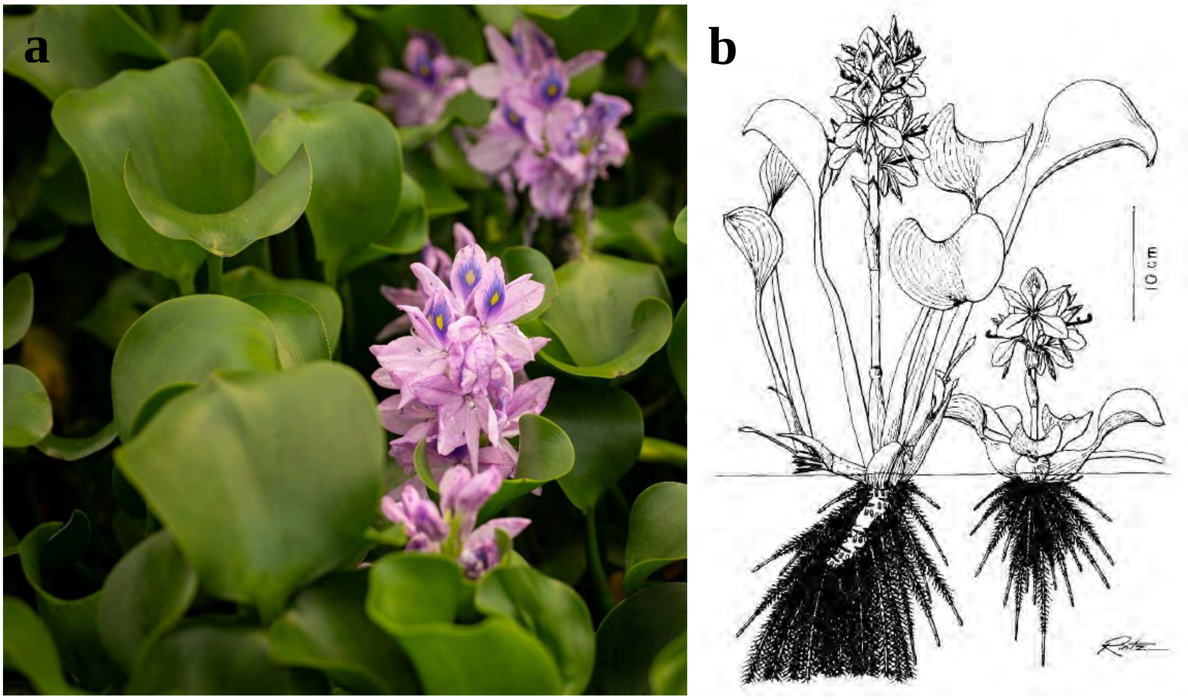

Figure 1.1: (a) Water hyacinth mat displaying attractive flowers (Photo credit: David Taylor, Centre for Biological Control) and **(b)** a diagram of a water hyacinth plant with one daughter plant (From Center and Spencer, 1981).

1.5.3 Impacts

Globally, water hyacinth has large socioeconomic consequences (Chamier et al., 2012). Invasions pose many threats to farming and fishing communities. Fisherpeople face challenges such as the inability to access the water as the mat is too dense for boats to pass through and even if they can fish, their nets and lines often get tangled or break amongst the weeds (Damtie et al., 2022). These obstacles lead to increased time and effort for often decreased economic rewards which threaten food security (Damtie et al., 2022). Water hyacinth infestations impact irrigation systems used by farmers as they block canals used to deliver water to farms for crops or livestock (Damtie et al., 2022). This impact also threatens food security and economic return. By blocking canals, water hyacinth also impedes waterways used for transportation, often leading to increased transport costs for locals and governments (Lubembe et al., 2023). Water hyacinth can do great damage to infrastructure around dams, such as jetties (Hill et al., 2020). Infestations have been known to block large pipes, eventually breaking them and where hydroelectricity is implemented, the generators are at a high danger risk as the water hyacinth is likely to get stuck in the pumps, ultimately destroying the equipment (Harun et al., 2021). Infestations impact tourism on various scales - small businesses (boat tours, fishing excursions, water sports) are often unable to conduct business as a result of the mat blocking access to the water. Similarly, towns along infested sites (rivers, dams and lakes) lose multiple revenue sources, such as restaurants and hotels, as people no longer want to visit the area (Chamier et al., 2012; Hill et al., 2020). The nature of the interlocked mats provides a steady and almost unmoving breeding ground for disease vectors such as mosquitos and snails, thus increasing the risk of diseases such as malaria and cholera (Harun et al., 2021). This not only harms the quality of life of locals but also the economic productivity of the area.

In the African context, Lake Tana in Ethiopia is an example of a water hyacinth infestation impacting rural communities (Damtie et al., 2022). According to the case study by Damtie et al. (2022), over 88% of the population surrounding the lake relies on subsistence agriculture. They found that crop (namely rice) production was on average 1 245 kg less in areas relying on an infested part of the lake compared to an area that did not have water hyacinth. These differences were due to reduced irrigation caused by the water hyacinth infestation. Similarly, households keeping livestock for food, income or labour, relying upon the infested water, held significantly less livestock than other households. This is because they are unable to provide water and irrigation for grazing to the animals. Damtie et al. (2022) noted that the lake's fish production is 13 times less than its potential and this can be attributed to the water hyacinth. This is partly because fishing is now a secondary income for most people in the community and most have stopped fishing completely. Reasons for decreased fishing include a decreased fish population (death due to anoxic conditions) and the inability of boats to access the water (Figure 1.2).

Figure 1.2: Fishing boat stuck in a water hyacinth mat on Lake Tana, Ethiopia (From Damtie et al. 2022).

The most severe impact of water hyacinth on an area is its environmental damage. Dense interlocked mats block sunlight, stopping submerged plants and algae from photosynthesising, reducing dissolved oxygen levels, and harming aquatic life and fisherpeople's livelihoods (Auchterlonie et al., 2021; Damtie et al., 2022). The lack of light below the water decreases temperature variation, thus decreasing the biodiversity of primary production in the ecosystem (Dallas, 2008; Harun et al., 2021). Dallas (2008) found that diatoms dominate when temperatures are below 20°C, while blue-green algae are the dominant species in temperatures greater than 30°C. When water hyacinth decomposes, the bacteria responsible for the decomposition utilise most of the available oxygen in the water, reducing dissolved oxygen concentrations, and leading to the death of organisms from multiple trophic levels (Auchterlonie et al., 2021).

Lake Victoria, Africa’s largest lake is in Tanzania, Uganda, and Kenya. Water hyacinth was first introduced here in the 1980s and has had severe economic and ecological impacts on the people relying on the basin as well as the health of the lake (Güereña et al., 2015). Since its introduction at the lake, water hyacinth has reduced the biodiversity of indigenous algae and other aquatic plants, ultimately reducing endemic fish populations. Masifwa et al. (2001) found that large mats of water hyacinth on Lake Victoria were associated with reduced oxygen concentrations resulting in decreased abundance and diversity of invertebrate species and fish deaths.

1.5.4 Biological control agents

The control of water hyacinth has a long history in South Africa, with the first agent, *N. eichhorniae*, being released in 1974 on multiple infestations across the country(Cilliers, 1991; Hill & Cilliers, 1999). The programme was terminated due to flooding and herbicidal application but resumed in 1985 when *N. eichhorniae* was re-released and populations re-established (Hill & Cilliers, 1999). In 1990, another weevil, *Neochetina bruchi* Hustache (Coleoptera: Curculionidae), was released due to its ability to withstand cold winters and damage water hyacinth plants in eutrophic water, where *N. eichhorniae* is often unsuccessful (Hill & Cilliers, 1999). Since the release of *N eichhorniae* in 1974, eight more biological control agents have been released in order to achieve full control of water hyacinth, including a mite, *Orthogalumna terebrantis* Wallwork (Acarina: Sarcoptiformes: Galumnidae) in 1989; a moth *Niphograpta albiguttalis* Warren (Lepidoptera: Crambidae) in 1990; a fungal pathogen, *Cercospora piaropi* (Mycosphaerellales: Mycosphaerellaceae) in 1992; two leaf-sucking mirids *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae) and *Eccritotarsus* sp. nov (Hemiptera: Miridae) in 1996 and 2008 respectively; a grasshopper *Cornops aquaticum* Brüner (Orthoptera: Acrididae) in 2011; and the most recent release: a planthopper *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) in 2013 (Table 1).

Despite the complement of biological control agents released, complete control of water hyacinth remains difficult to achieve in some areas across the country. Hartbeespoort Dam is one such area where the plant infestation is problematic due to herbicide application, extreme winters and hypereutrophic water, all of which restrict the population growth of agents (Hill & Olckers, 2001; Hill & Coetzee, 2017). It is therefore important to look into integrated control plans, integrating biological control with chemical or mechanical control to keep water hyacinth populations low, reducing its impacts on the area (Hill & Coetzee, 2017).

Table 1: The history of biological control agents released onto water hyacinth in South Africa and the damage they inflict on the plant.

Biological control agent	Year of release in South Africa	Area of plant damaged	References
Neochetina eichhorniae Warner (Coleoptera: Curculionidae)	1974	Leaves, petioles and crown	(Cilliers, 1991)
Orthogalumna terebrantis Wallwork (Acarina: Sarcoptiformes: Galumnidae)	1989	Leaves	(Cilliers, 1991)
Cercospora piaropi (Mycosphaerellales: Mycosphaerellaceae)	1992	Leaves	(Hill & Coetzee, 2017)
Neochetina bruchi Hustache (Coleoptera: Curculionidae)	1990	Leaves, petioles and crown	(Cilliers, 1991)
Niphograpta albiguttalis Warren (Lepidoptera: Crambidae)	1990	Petioles	(Cilliers, 1991)
Eccritotarsus catarinensis Carvalho (Hemiptera: Miridae)	1996	Leaves	(Coetzee et al., 2021)
Eccritotarsus sp. nov (Hemiptera:	2008	Leaves	(Hill & Coetzee, 2017)

Miridae)			
Cornops aquaticum Brüner (Orthoptera: Acrididae)	2011	Leaves and flowers	(Coetzee et al., 2021)
Megamelus scutellaris Berg (Hemiptera: Delphacidae)	2013	Leaves and petioles	(Coetzee et al., 2011, 2021)

1.6 Biological control agent: *Megamelus scutellaris*

1.6.1 Life history and biology

Originating from Paraguay and Argentina, *Megamelus scutellaris* is a dimorphic, delphacid species that solely feeds on water hyacinth (Sosa et al., 2005). Oviposition takes place a few days after mating and occurs between the petiole and the leaf lamina, typically laying two eggs per oviposition scar (Sosa et al., 2005). Eggs are characterised by being a milky white colour when laid and turning yellowish white when hatching, with hatching occurring 7 days after oviposition. *M. scutellaris* nymphs develop through 5 instars in approximately 25 days in outdoor conditions (Figure 1.3) (Goode et al., 2019; Sosa et al., 2005). The nymphs are vagile, thus decreasing time spent within the water hyacinth plant in the larval phase, thus increasing their probability of population growth and establishment due to their ability to disperse from undesirable conditions (manual or chemical disturbances) (Goode et al., 2021; Sosa et al., 2005).

Figure 1.3: Two *M. scutellaris* eggs oviposited on water hyacinth (Photo credit: David Taylor, Centre for Biological Control).

M. scutellaris adults display wing dimorphism where individuals are either macropterous (having wings) or brachypterous (having underdeveloped wings) (Figure 1.4) (Denno & Roderick, 1992b). Wing formation depends on several factors including host plant quality, temperature and population density (Denno & Roderick, 1992b). When habitats are persistent, populations of *M. scutellaris* populations largely consist of brachypterous adults as there is no need to migrate to locate a more optimal environment, while macropterous forms develop when population densities are too high, or host plant quality is low (Denno et al., 1991; Denno & Roderick, 1992b, 1992a). It has been noted that while the formation of wings is advantageous in resource-limited or overpopulated conditions, it decreases reproductive and metabolic functions of the insect - brachypterous females have been found to be more fecund and reproduce earlier (Denno et al., 1991; Denno & Roderick, 1992b).

Figure 1.4: Macropterous (top right) and brachypterous (bottom left) *M. scutellaris* individuals on a water hyacinth leaf (Photo credit: Benjamin Miller, Centre for Biological Control).

1.6.2 Impacts on water hyacinth in South Africa

Adult and nymph of *M. scutellaris* feed on water hyacinth by piercing the plant and extracting the sap, thus resulting in waterlogging of the plant reducing its buoyancy (Moffat et al., 2024). Damage to the plant is visible by feeding scars, rotting tissue and brown, shrivelled leaves. As noted by Moffat et al. (2024), the greatest damage to water hyacinth was achieved when *M. scutellaris* population numbers were at their highest.

M. scutellaris was first released in South Africa in 2013 on the Kubusi River in the Eastern Cape and has since been released onto several systems across the country. In South Africa, water hyacinth infestations can be found in Mediterranean (warm, dry summers and wet winters), humid subtropical (warm summers and mild, dry winters) and subtropical highland (hot summers and cold, dry winters) climates in the Western Cape, KwaZulu-Natal and Gauteng/North-West provinces respectively (Council for Scientific and Industrial Research, 2015; Miller et al., 2023). There is a climatic mismatch between water hyacinth and insect enemies, especially in the colder regions of South Africa, namely in the Highveld, which is characterised by extreme, frosty winters. With a critical thermal range of 11.5℃ to 33℃ (May & Coetzee, 2013), *M. scutellaris* should be unsuited to the Highveld, however, they have successfully established in this area (Coetzee et al., 2021, 2022; Hill & Coetzee, 2017; Moffat et al., 2024). It should be noted that *M. scutellaris* displays thermal plasticity in relation to host plant quality – individuals exposed to plants grown in nutrient-rich water displayed a wider thermal range (Owen et al., unpublished data). Thermal plasticity is a trait displayed by multiple biological control agents owing to the range of temperatures they were exposed to over its evolutionary history in the native range (Griffith et al., 2019; B. Miller et al., 2023).

1.6.3 Impact at Hartbeespoort Dam

At Hartbeespoort Dam in the North-West Province, South Africa, the water hyacinth infestation has been notable and has severely impacted ecosystem services as well as the tourism economy of the area. The biological control programme has faced many challenges in attempting to control the weed, namely the rapid growth of the macrophytes due to the hypertrophic water (Coetzee et al., 2022).

In 2018, *M. scutellaris* was released onto the dam due to its rapid reproduction rate and its ability to recover from cold periods faster than other agents already released onto the dam (Coetzee et al., 2022). As detailed by Coetzee et al. (2022), an inundative release programme began at the dam in 2019 whereby *M. scutellaris* agents were released onto the dam, with majority being collected from the Centre for Biological Control's (CBC) Waainek Mass Rearing Facility, at Rhodes University, Makhanda, South Africa and the rest of the releases were made from three satellite rearing stations. A satellite rearing station consists of one to four greenhouse tunnels containing pools of water hyacinth where *M. scutellaris* individuals are reared – to date, there are nine situated around the dam (Moffat et al., 2024) (Figure 1.5). Since 2019 those operating the stations were able to release the agents onto the dam in early spring - timed with the germination of water hyacinth. Through this programme, it was found that the percentage cover of water hyacinth on the dam decreased significantly during the summer months (36% in November 2020 decreased to 6% in April 2021). They also found that the decrease in water hyacinth percentage cover was correlated to the increase in *M. scutellaris* density. This programme has continued and in 2023, approximately 684 000 *M. scutellaris* agents were reared and released by the satellite stations in late winter/early spring to close the gap between water hyacinth growth and agent population buildup (Moffat et al., 2024).

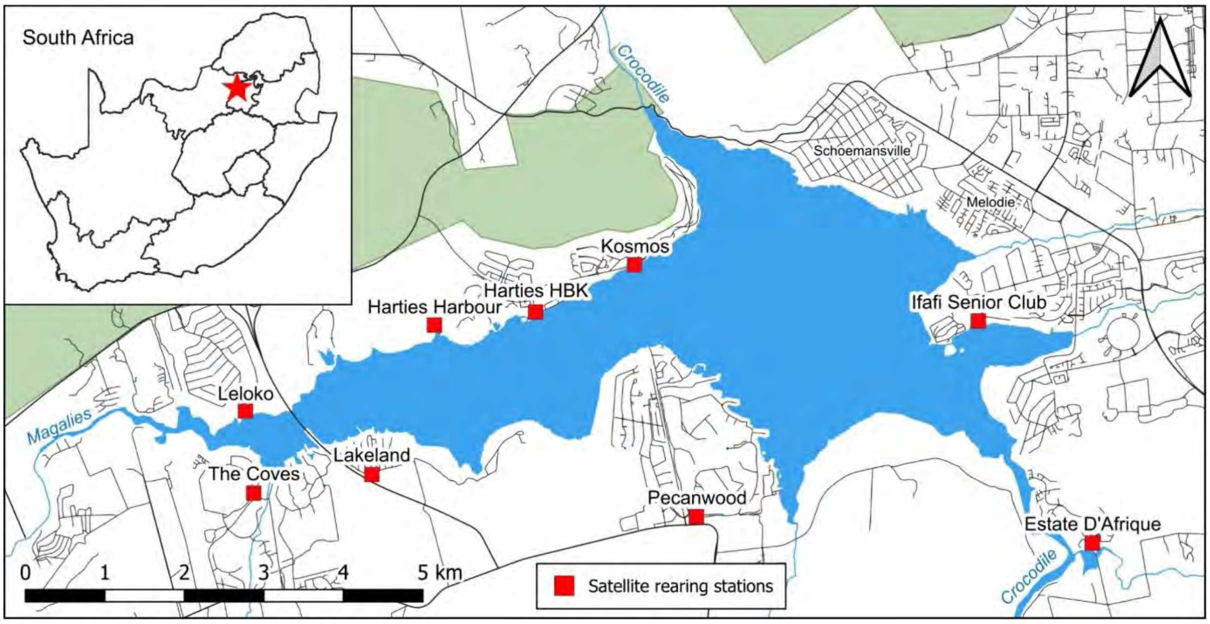

Figure 1.5: Map of Hartbeespoort Dam indicating satellite rearing stations (From Moffat et al., 2024).

Moffat et al. (2024) noted that high densities of *M. scutellaris* occurred in populations of brown, damaged plants. When *M. scutellaris* is released in high numbers, it can be extremely damaging to water hyacinth (B. Miller et al., 2019). Agent damage is difficult to detect initially (B. Miller et al., 2019), but once significant *M. scutellaris* damage has been incurred, stands of water hyacinth begin to brown, with leaves drying out and petioles and crowns becoming waterlogged (Figure 1.6). Once stands of water hyacinth are waterlogged, they begin to sink, thus reducing the size of the mat (Moffat et al., 2024). Unlike herbicidal applications whereby water hyacinth dies and sinks rapidly, the process of *M. scutellaris* damage is slower, thus allowing the water hyacinth mat to sink more gradually (Hill et al., 2021).

Figure 1.6: Damage inflicted by *M. scutellaris* on water hyacinth at Hartbeespoort Dam (Photo credit: Rochelle Bessinger).

1.7 Decomposition

The decomposition process of plant matter in aquatic systems comprises three stages: leaching, biotic and abiotic processes (Zhou et al., 2023). Once a plant has died, it begins to senescence, whereby it begins to fragment and form litter/detritus. This process is often aided by abiotic factors such as frost damage, wind or rainfall (Zhou et al., 2023). The process of leaching involves the release of soluble compounds (nitrogen, phosphorous and sugars) into the water (Zhou et al., 2023). Leaching increases the amount of dissolved organic material (DOM) in the water, making it available for microbes in the next phase of the decomposition process (Zhou et al., 2023). Aerobic bacteria and fungi utilise oxygen to break down complex organic molecules, such as cellulose and lignin, into simpler molecules such as carbon dioxide and water, however, some of these compounds cannot be reduced and fall to the sediment as solids (Masifwa et al., 2004). Anaerobic bacteria are found in hypoxic environments and use electron acceptors such as nitrates, sulphates and carbon dioxide to form harmful compounds such as methane, hydrogen sulphide and ammonia (Masifwa et al., 2004).

Various factors affect the rate of decomposition of plant litter in a water body (Song et al., 2021; Wang et al., 2024). Song et al. (2021) showed that plants containing more lignin decomposed faster than plants with a lower lignin composition and plants with higher nitrogen and phosphate ratios decomposed faster than those with less nitrogen and phosphorous. Additionally, they found that more fragmented litter decomposed faster, due to larger surface areas, compared to litter that had not been fragmented. Lastly, it was found that litter in eutrophic or hypereutrophic waters decomposed at a significantly slower rate than plants in less nutrient-rich environments. They hypothesise that this decrease in decomposition rate is due to a decrease in microbial abundance and species richness.

Large mats of water hyacinth create detritus from senescing plant parts, frost damage, and damage from control (chemical or biological) (Balasubramanian et al., 2012; Reddy & DeBusk, 1991). This detritus may be trapped in the roots of the healthy plants or sink into the sediment (Reddy & DeBusk, 1991), where decomposition takes place. The rate of decomposition depends on factors such as detritus composition, microbial composition and availability, water quality as well as the amount of detritus present to be decomposed, as stated above (Masifwa et al., 2004).

The most pertinent of these factors is water quality, namely eutrophic/hypereutrophic waters. The release of nutrients into the water through leaching is controlled by the uptake of these nutrients by healthy water hyacinth plants (Masifwa et al., 2004). Therefore, the nutrient load in the water is lessened through remaining healthy water hyacinth plants.

Water hyacinth proliferates rapidly in the presence of nutrient-rich water and creates a hypoxic environment by covering the water's surface, thereby blocking sunlight from reaching submerged photosynthesising plants, thus reducing dissolved oxygen concentrations in the water. When a water hyacinth mat begins to senesce, it remains on the water's surface for 15-20 days, maintaining a hypoxic environment, thus anaerobic respiration is often the main mechanism of detritus decomposition (Gamage & Asaeda, 2005). Anaerobic respiration, which occurs in the absence of oxygen, depletes dissolved oxygen further promoting hypoxic/anoxic conditions, and releases methane, hydrogen sulphide and ammonia – biogases that are toxic to aquatic species (Masifwa et al., 2004; Zhou et al., 2023). The decomposition rate is increased with increased detritus, therefore a larger stand of senescing water hyacinth will decompose faster, thus resulting in a greater reduction in dissolved oxygen at one time (Masifwa et al., 2004). It can therefore be surmised that it is more beneficial to have slower senescence rather than the immediate death of a large mat, with dissolved oxygen reducing gradually.

A large die-off (senescence) of a water hyacinth mat due to chemical or biological control or a frost event during winter adds to the sediment load in the water (Balasubramanian et al., 2012). Artificial impoundments, like Hartbeespoort Dam, have a shorter water retention time than natural lakes, often resulting in decreased water levels (Kebedew et al., 2023; Mitchell & Crafford, 2016). Lower water levels coupled with an elevating dam floor due to increased sediment loading could result in the impoundment drying up completely (Kondolf et al., 2014). After senescence, decomposition occurs and irrespective of anaerobic or aerobic decomposition, organic matter is reduced to simple compounds. This suggests that detritus released from a senescing water hyacinth mat will ultimately be decomposed, reducing the amount of solid organic matter (detritus) sinking to the bottom of the water column (Balasubramanian et al., 2012). A decrease in the amount of sediment collecting on the dam floor mitigates the threat of a rising dam floor and decreased water levels.

1.8 Study aims and rationale

The study presented in this thesis aimed to monitor the senescence of water hyacinth and how this process results in detritus formation both in laboratory conditions and *in situ* (Hartbeespoort Dam) over a growing season. In this study, the detritus formation rate was compared between water hyacinth plants controlled by both biological control agents and herbicidal application. Additionally, water chemistry parameters were studied before and after senescence to understand how the addition of detritus may alter the chemistry of the water column. The results will help determine how the senescence of large mats impacts sediment loading and the possible formation of a "sludge layer", and whether sediment loading changes the eutrophic water in Hartbeespoort Dam.

CHAPTER 2

Methods and Materials

Two studies were conducted to quantify the detritus released from senescing water hyacinth under different conditions and to assess its impact on water chemistry.

The first study was conducted under controlled laboratory conditions, allowing for precise measurement and comparison of detritus production between water hyacinth plants subjected to biological control (herbivory by *M. scutellaris*) and chemical control (herbicide application), and those not under control. Water chemistry parameters were analysed before and after senescence to determine the effects of detritus decomposition on water quality.

The second study was conducted *in situ* at Hartbeespoort Dam, where detritus production from water hyacinth under biological control, and in open water, was monitored. The amount of detritus produced was compared to various factors, including plant characteristics, *M. scutellaris* density, water hyacinth cover, and water chemistry, to evaluate the influence on detritus accumulation in the system.

2.1 Mesocosm experiments

Mesocosm studies were conducted at the Centre for Biological Control’s (CBC) Waainek Mass Rearing Facility, Makhanda, South Africa (33° 19' 12.20" S, 26° 30' 27.80" E) to determine the difference in detritus production of water hyacinth between chemical and biological control programmes as well as their impacts on water quality.

2.1.1 Experimental design

Healthy water hyacinth plants were placed in 70 L refuse bins (552 mm wide) filled with tap water and 30 mg/L iron chelate (13% Fe) was added to maintain plant health and prevent yellowing of leaves. To emulate hypereutrophic water (15 mgN/L), 250 mg/L Culterra Multisol® 6.1.3 (44) Foliage fertilizer was added as per Miller et al. (2019). Five water hyacinth plants were placed into each bin, ensuring that the surface was completely covered, and each bin was enclosed with a mesh net to ensure no biological control agents were able to enter or leave the mesocosm (Figure 2.1). Four treatments were studied: negative control (water only), positive control (untreated water hyacinth), water hyacinth treated with herbicide, and water hyacinth treated with biological control agents. Each treatment had 10 replicates.

Figure 2.1: Experimental bins arranged randomly.

Plants were left for a month to acclimatise to their new environment and grow into larger plants. After one month, the treatments were applied to the mesocosm replicates. The plants in the chemical control treatment were dosed with Kilo Max 700 WSG (Volcano Agroscience Ltd., Verulam, South Africa) and received the glyphosate herbicide at 1.56% concentration, applied as per label, and it was ensured that every part of the plant was sprayed. The plants in the biological control treatment received 15 *Megamelus scutellaris* insects per plant (75 insects per bin) to ensure a large enough population to achieve plant death. After all treatments were applied, the plants were re-covered. Every 2 weeks, 10 insects were added per plant (averaging 50 per bin) to the plants in the biological control treatment to ensure that the control agent population was sufficiently large for the duration of the experiment. The water in the bins was topped up when necessary, ensuring that the bins were always full.

2.1.2 Data collection

2.1.2.1 Detritus sampling

The first detritus sampling (time point one) took place once the plants in the chemical control treatment had died (3 weeks after inoculation) (Figure 2.2a). At this point, 5 bins from each treatment were randomly selected to determine detritus formation. The process entailed removing any healthy plants from the bin and passing the remaining contents through a metal sieve with an aperture of 500 microns. The water was discarded, and the wet weight of the detritus remaining in the sieve was weighed using a hanging balance (5 g accuracy: 2 decimals). The second detritus sampling (time point two) took place once the plants in the biological control treatment had died (2 months after time point 1), and detritus from the remaining 5 bins from each treatment was measured (Figure 2.2b).

Figure 2.2: Water hyacinth plants in the biological control treatment at **(a)** time point one and **(b)** time point two.

2.1.2.2 Water chemistry measurements

Water chemistry was measured monthly from the time that the treatments were established using a handheld multiparameter probe (YSI Pro1030, YSI Inc., USA). The following parameters were measured: pH, temperature, dissolved oxygen, total dissolved solutes, and nitrates. Water was also taken in 10 ml centrifuge tubes from each bin and tested for phosphates using a multiparameter bench top photometer (Hanna Instruments; HI83399-01).

2.2 Field study

2.2.1 Site

Three sites across Hartbeespoort Dam were selected due to their accessibility and spatial variance between the other sites (Figure 2.3), including the Coves (25°46'4.66"S, 27°48'4.45"E) and Ifafi Senior Club (25°44'59.15"S, 27°53'23.94"E) where two community rearing stations were established at the dam, and Kurperoord (25°44'57.32"S, 27°49'59.78"E), an open-access site on the dam.

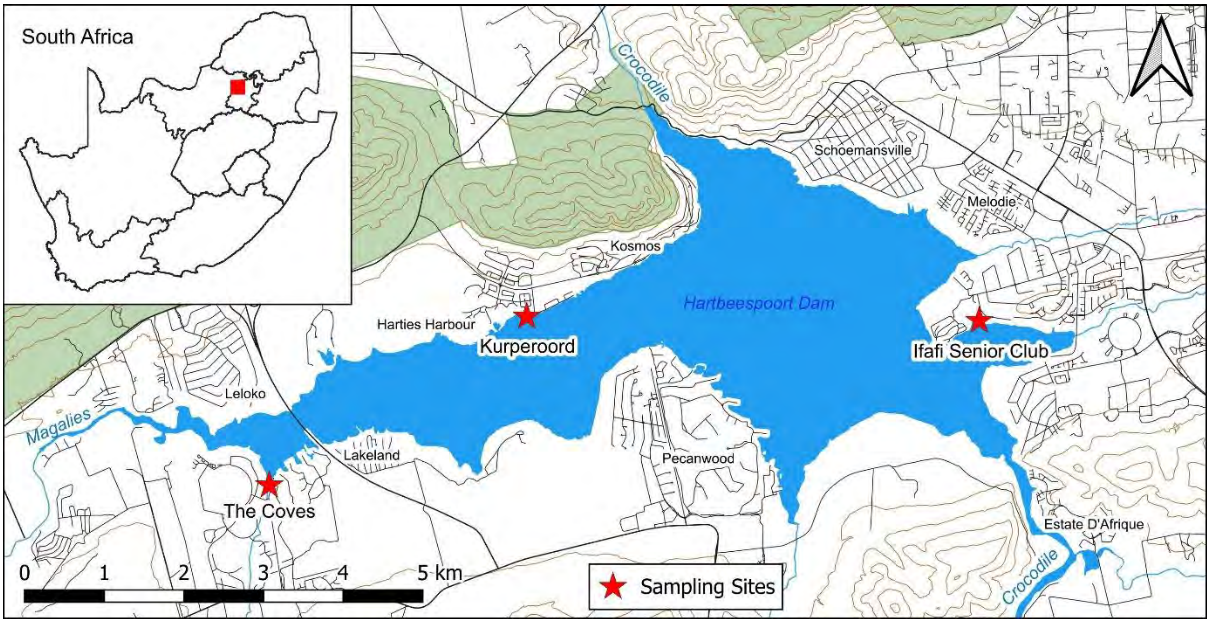

Figure 2.3: Map of Hartbeespoort Dam indicating the three sampling sites used in this project (map courtesy of David Kinsler).

2.2.2 Experimental design

The study was conducted from November 2023 until April 2024. Healthy water hyacinth plants from the dam were placed into five wire cages at each selected site, covering the surface of the cage. Each cage consisted of a welded metal frame measuring 50 cm x 42 cm, constructed with 50 mm x 50 mm mesh. A mesh cone (80% shade cloth), measuring 60 cm x 50 cm, was attached to the outside of the frame. This mesh was attached to the frame by piercing the fabric with the mesh and folding the metal over the fabric to secure it. To ensure buoyancy, 30 cm sections of cylindrical flexible, buoyant polyethylene foam (pool noodles) were secured inside the frame with cable ties. Depending on the site, cages were either anchored with bricks (Coves and Ifafi) or tied to a jetty (Kurperoord), allowing them to remain buoyant while preventing them from drifting away.

Between November and December 2023, five treatment cages (containing water hyacinth) and five control cages (containing no water hyacinth) were installed at the Coves, Kurperoord and Ifafi study sites between December 2023 and January 2024 (Figure 2.4).

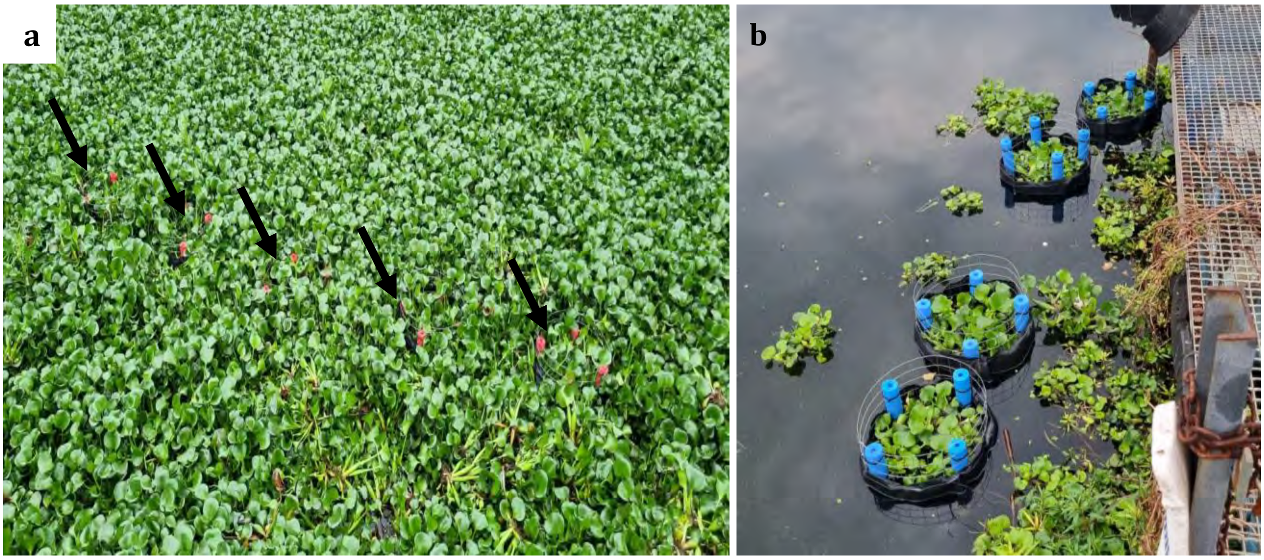

Figure 2.4: Treatment cages at **(a)** the Coves as indicated by arrows and **(b)** Kurperoord.

2.2.3 Data collection

2.2.3.1 Detritus sampling

Detritus sampling was conducted monthly by removing the mesh cone from each frame and shaking it gently to collect all detritus at the base. Once the detritus had settled at the cone's base, it was removed and weighed using a 1 L tub on a kitchen scale (Figure 2.5). After recording the mass, the detritus was discarded back into the dam, and the mesh was reattached to the frame before placing it back in the water. This method allowed the water hyacinth to remain in place, eliminating the need for plant replacement.

Figure 2.5: Detritus collected from a mesh cone.

2.2.3.2 Plant parameter measurements

To assess the establishment of biological control agents and the extent of herbivory, water hyacinth plant parameters were measured monthly throughout the study period, following the methods of Coetzee & Hill (2012) and Miller et al. (2021)**.** Ten water hyacinth plants were randomly selected from each site, and several growth parameters were recorded. These included the length of the longest petiole, the length of the petiole of the second leaf (the second leaf counted outwards from the centre of the plant), the surface area of the second leaf, and the length of the longest root, all measured using a 5 m tape measure. Additionally, the number of ramets (daughter plants) and the number of photosynthetically active leaves were counted. To measure the biomass of water hyacinth plants at a site, three randomly selected 0.25 m^2 quadrats of plants were removed from the site. After the number of individual plants per quadrat was recorded, the water hyacinth plants from each quadrat were separated into above-water biomass (living green leaves and petioles), below-water biomass (roots) and dead biomass (extremely unhealthy or dead material) and were weighed using an Alvinlite electronic hanging scale (USA; up to 40 kg) to determine the wet weight per unit area.

2.2.3.3 Water chemistry measurements

Water samples were collected monthly from each site and stored in 100 ml bottles, which were then frozen until analysis could be conducted at Rhodes University. The same water chemistry parameters used in the laboratory study were measured for these samples.

2.2.3.4 *Megamelus scutellaris* density measurements

As in Miller et al. (2021), a 70 L black plastic bin, modified with the base removed and replaced by a cross of wires, was used to estimate the abundance of *M. scutellaris* at each of the study sites. This bin was pressed down onto sections of water hyacinth plants, submerging them, and prompting the insects to jump onto the inside surface of the bin. The insects (adults and nymphs combined) were easily counted due to their light colour contrasting against the black interior of the bin. The counts were later converted to *M. scutellaris* density/m^2. This procedure was repeated five times at each site to ensure consistency.

2.2.3.5 Water hyacinth cover

Water hyacinth coverage between November 2023 and January 2024 was mapped using Sentinel-2 MultiSpectral Instrument (MSI) satellite imagery from the European Space Agency, via the Google Earth Engine application, a cloud-based platform for remote sensing, https://davidkinsler123.users.earthengine.app/view/macrophyte-monitoring-tool. The app uses a decision tree classification method, using spectral indices, to distinguish between open water, cyanobacteria, and water hyacinth. Thresholds for the decision tree were determined using a K-means clustering algorithm. Google Earth Engine was used to generate a detailed time series of water hyacinth coverage, with images captured every five days, weather permitting. To reduce variability caused by wind-driven movement of the floating mats, a 15-day centred rolling mean was applied to smooth the time series data (Moffat et al., 2024).

2.3 Statistical analyses

All statistical analyses were conducted using R version 4.4.1 (R Core Team, 2024). The data from the mesocosm experiments were modelled using a Gaussian general linear model (GLM) with a logarithmic link function, accommodating the effects of treatment and time, as well as their interaction. The model fitting was executed through the GLM function, with subsequent analysis of variance using the CAR package (Fox & Weisberg, 2019) to test the hypothesis that parameters significantly differed across treatment groups and time points. Post-hoc comparisons were performed with the Estimated Marginal Means package (Lenth, 2025) to pinpoint where significant differences lay.

The same packages were utilised to analyse the data collected from the field study to determine which factors (water hyacinth cover, water chemistry and *M. scutellaris* population counts) had significant effects on detritus accumulation. To identify the most influential plant parameters affecting detritus formation, they were ranked using Akaike's information criteria (AIC) scores, using the MASS package (Ripley, 2009). A one-way analysis of variance (ANOVA) Type I was performed to assess differences in water chemistry parameters across sites using the stats package (R Core Team, 2024). Tukey's honest significant difference (HSD) test was applied to identify pairwise differences. The assumptions of normality and homogeneity of variances were assessed using the Shapiro-Wilk test and Levene's test from the CAR package (Fox & Weisberg, 2019).

Principal component analysis (PCA) from the FactoMineR package (Lê et al., 2008) was performed to reduce the dimensionality of water chemistry variables and identify key gradients driving variation in detritus formation. The analysis was conducted using a correlation matrix of standardized water chemistry variables (pH, dissolved oxygen, total dissolved solids, nitrates, and phosphates). Before analysis, variables were scaled to ensure comparability, and observations containing missing values were removed. Eigenvalues were examined to determine the proportion of variance explained by each principal component, and the first principal component (PC1) was retained for further analysis, as it explained more than 85% of the total variance. A linear regression model was then used to evaluate the relationship between PC1 and detritus formation in treatment mesocosms, and model fit was assessed using summary statistics. (Kassambara & Mundt, 2020).

CHAPTER 3

Results

3.1 Mesocosm experiment

3.1.1 Detritus sampling

The interaction between treatment and time had a significant effect on detritus formation (χ^2 = 66.50, df = 3, $P < 0.001$). The water hyacinth and biocontrol treatments showed the largest increase in detritus across the two time points (1.17 g ± 0.20 and 1.36 g ± 0.24 respectively), but they were not significantly different from each other (Figure 3.1). Detritus remained low in the control treatment across both time points. The herbicide treatment was the only treatment to show a decrease in detritus from time point one to two (0.20 g ± 0.12). Additionally, the herbicide treatment had more than double the amount of detritus than any treatment at time point one. Still, by the end of time point two, the biocontrol and water hyacinth treatments accumulated more detritus than the control treatment but were not different from each other (Figure 3.1).

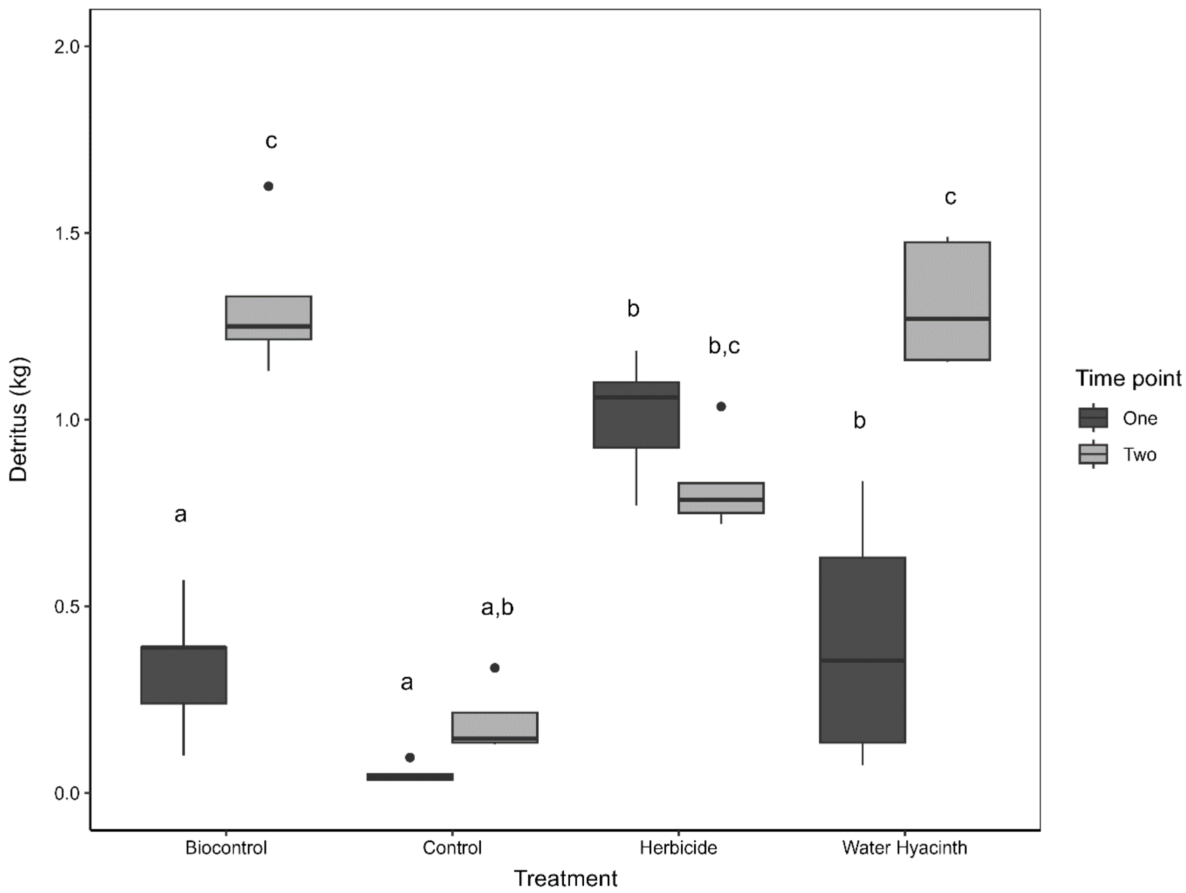

Figure 3.1: Detritus measured across four treatments at two time points. Each treatment had 5 replicates (n=5) at each time point. Boxes denote the interquartile range; whiskers are drawn to the maximum and minimum values and outliers are represented by points. Different letter codes represent significant differences.

3.1.2 Water chemistry

A significant interaction was observed between treatment and time for pH levels (χ^2 = 24.02, df = 3, P < 0.001). At time point one, the water hyacinth and control treatments had significantly higher pH values than the herbicide and biocontrol treatments. The pH values decreased within treatments over time, particularly in the water hyacinth treatment, where pH decreased by 0.12 (± 0.02), however, this value was not significantly different to the pH values in the biological control and herbicide treatments at time point two (Figure 3.2a). The pH in the herbicide treatment decreased the least across the treatments (0.03 ± 0.02) (Figure 3.2a).

There was no significant interaction between treatment and time for dissolved oxygen (χ^2 = 3.67, df = 3, P = 0.3). However, there was a significant difference in dissolved oxygen across time points (χ^2 = 851.28, df = 1, P < 0.001), with a decrease in DO concentration over time (Figure 3.2b).

The interaction between treatment and time for total dissolved solids (TDS) was not statistically significant (χ^2 = 1.40, df = 3, P = 0.71). TDS showed significant increases over time (χ^2 = 148.91, df = 1, P < 0.001), but the treatment did not significantly impact the TDS (χ^2 = 9.90, df = 3, P = 0.02) (Figure 3.2c).

The analysis of nitrate levels revealed no significant interaction between treatment and time (χ^2 = 0.02, df = 3, P = 1.00), suggesting that the effect of treatment on nitrate concentration is consistent over different time points. Despite the lack of interaction, significant main effects were found for both time (χ^2 = 60.93, df = 1, P < 0.001) and treatment (χ^2 = 17.35, df = 3, P < 0.001). The significant effect of time indicates that nitrate levels decreased across time points, independent of the treatment applied. Similarly, the significant effect of treatment on nitrate levels demonstrates that different treatments influence nitrate concentrations independently of the time factor (Figure 3.2d).

No significant interaction between treatment and time was found for phosphates (χ^2 = 2.04, df = 3, P = 0.56). Neither treatment (χ^2 = 10.00, df = 3, P = 0.02) nor time (χ^2 = 0.61, df = 3, P = 0.44) had a significant effect on phosphates (Figure 3.2e).

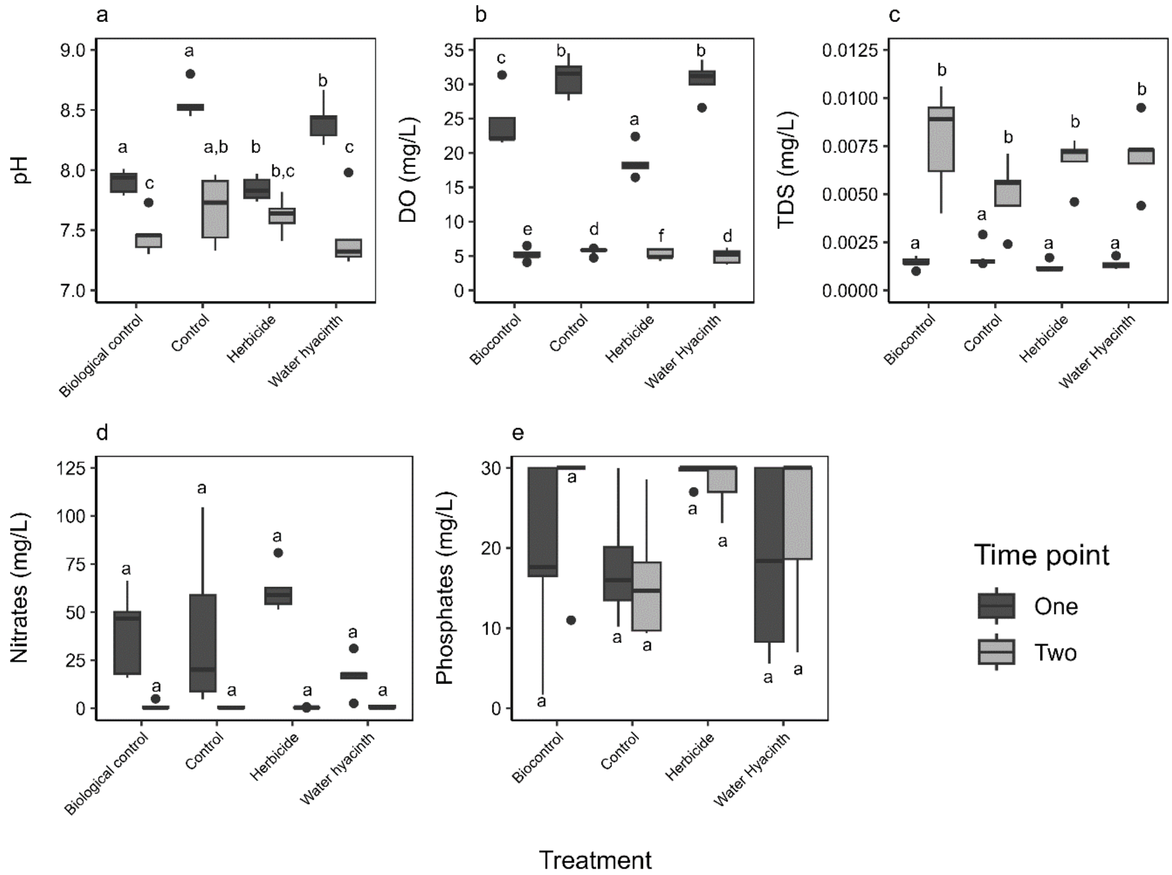

Figure 3.2: Water chemistry parameters measured across four treatments at two time points. Each treatment had 5 replicates (n=5) at each time point. The parameters include **(a)** pH, **(b)** dissolved oxygen (mg/L), **(c)** total dissolved solids (mg/L), **(d)** nitrates (mg/L), and **(e)**, phosphates (mg/L). Boxes denote the interquartile range; whiskers are drawn to the maximum and minimum values and outliers are represented by points. Different letter codes represent significant differences.

This section has presented the effects of various treatments over time on detritus formation and water chemistry. The analyses revealed significant interactions between treatment and time in the formation of detritus and pH levels. During time point one, detritus formation in the water hyacinth (control) and biological control treatments did not differ significantly from each other but were significantly lower than that of the herbicide treatment. Detritus formation decreased in time point two in the herbicide treatment only, while it increased in the water hyacinth and biological control treatments. The amount of detritus formed in the water hyacinth and biological control treatments were not significantly different at the end of the experiment (time point two).

For pH, a general decrease was noted across treatments, with the largest decrease observed in the water hyacinth (control) treatment as plants continued to grow. In contrast, no significant interaction between treatment and time was detected for dissolved oxygen and total dissolved solids, although both parameters showed significant changes over time alone. Despite showing large differences over time, the concentrations of nitrates and phosphates were not significantly different due to large variations in the data.

3.2 Field experiment

3.2.1 Water chemistry

At the Coves, the highest pH was recorded in January 2024 (8.3), followed by a decline in February 2024 (7.9) and a slight increase in March 2024 (8.1). At Ifafi, pH levels peaked in January 2024 (8.2) before decreasing to 7.80 in February 2024 and stabilizing in March 2024 (8.0). Similarly, at Kurperoord, pH reached a maximum in January 2024 (8.2), followed by a decline in February 2024 (7.8) and March 2024 (7.9) (Figure 3.3a). pH values were significantly different across sites ($F_{(2, 107)} = 8.04$, $P < 0.001$) and pH at Kurperoord was significantly lower than at Ifafi ($P < 0.001$). No significant differences were observed between Ifafi and the Coves ($P = 0.15$) or between Kurperoord and the Coves ($P = 0.062$).

Dissolved oxygen (DO) concentrations showed seasonal peaks at all sites in February 2024. At the Coves, DO peaked in February 2024 (53.20 mg/L) and decreased in March 2024 (47.31 mg/L). At Ifafi, DO was highest in February 2024 (50.50 mg/L) and dropped to 45.00 mg/L in March 2024. At Kurperoord, the highest DO value was recorded in February 2024 (48.00 mg/L), with a decline to 42.75 mg/L in March 2024 (Figure 3.3b). DO was significantly different across sites ($F_{(2, 107)} = 18.00$, $P < 0.001$). DO at Ifafi was significantly lower than at the Coves ($P = 0.012$). DO at Kurperoord was significantly higher than at the Coves ($P = 0.0036$). DO at Kurperoord was significantly higher than at Ifafi ($P < 0.0001$). Dissolved oxygen at sites can be ranked as follows: Kurperoord > Coves > Ifafi.

Total dissolved solids (TDS) concentrations peaked in February 2024 across all sites. At the Coves, TDS was highest in February 2024 (0.17 mg/L), decreasing in March 2024 (0.13 mg/L). At Ifafi, TDS reached a maximum in February 2024 (0.16 mg/L) and decreased in March 2024 (0.12 mg/L). Similarly, at Kurperoord, TDS peaked in February 2024 (0.16 mg/L) and declined in March 2024 (0.12 mg/L) (Figure 3.3c). TDS differed significantly between sites ($F_{(2, 107)} =$

18.57, $P < 0.001$) and TDS at Kurperoord was significantly higher than at the Coves ($P < 0.001$) and at Ifafi ($P < 0.0001$). No significant difference in TDS was found between Ifafi and the Coves ($P = 0.13$).

Phosphate concentrations showed a peak in February 2024 at all sites. At the Coves, the highest phosphate concentration was recorded in February 2024 (5.27 mg/L), which decreased in March 2024 (2.23 mg/L). At Ifafi, phosphates also peaked in February 2024 (4.75 mg/L) and decreased in March 2024 (2.00 mg/L). At Kurperoord, the phosphate concentration was (4.50 mg/L) in February 2024 and declined in March 2024 (1.80 mg/L) (Figure 3.3d). There was a significant difference in phosphate concentration across sites ($F_{(2, 107)} = 5.74$, $P < 0.01$). Phosphate concentrations at Ifafi were significantly lower than at the Coves ($P = 0.018$). Phosphates at Kurperoord were significantly higher than at Ifafi ($P < 0.01$). No significant difference in phosphate concentrations was found between Kurperoord and the Coves ($P = 0.89$).

At the Coves, nitrate concentrations were highest in January 2024 (40.48 mg/L), decreasing to 21.92 mg/L in March 2024. At Ifafi, nitrate levels peaked in January 2024 (38.50 mg/L) and declined to 20.75 mg/L in March 2024. At Kurperoord, nitrates also reached their maximum in January 2024 (35.25 mg/L), with a decrease to 18.50 mg/L by March 2024 (Figure 3.3e). Nitrate concentrations differed significantly between sites ($F_{(2, 107)} = 14.39$, $P < 0.01$). Nitrate concentrations at Kurperoord were significantly lower than at the Coves ($P < 0.0001$). Nitrate concentrations at Kurperoord were also significantly lower than at Ifafi ($P < 0.01$). There were no significant differences found in nitrate concentrations between Ifafi and the Coves ($P = 0.11$).

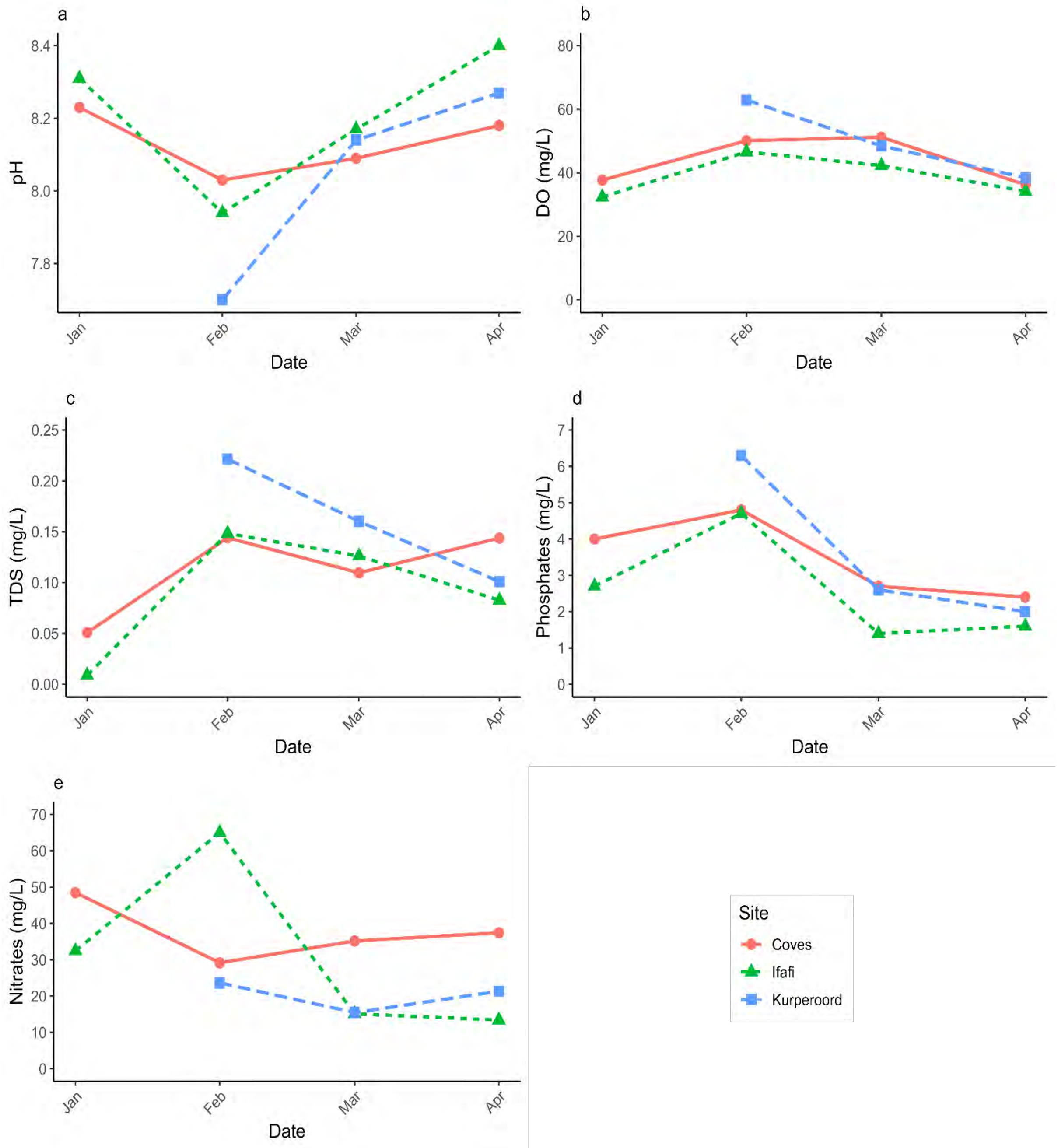

Figure 3.3: Time series of water chemistry parameters taken at the three study sites at Hartbeespoort Dam between January and April 2024. These parameters include **(a)** pH, **(b)** dissolved oxygen (mg/L), **(c)** total dissolved solids (mg/L), **(d)** phosphates (mg/L) and **(e)** nitrates (mg/L).

3.2.2 Plant parameters

Mean below-water biomass (root material) peaked in January at the Coves (1541.67 g), followed by a sharp decline in February 2024 (655 g). The below-water biomass at Ifafi declined by December 2023 (668.33 g) with a slight recovery in January 2024 (708.33 g). The biomass decreased to its lowest point in March 2024 (1070 g). At Kurperoord, below-water biomass began at 345 g in November 2023, increased steadily through December 2023 and January 2024, and peaked in February 2024 (491.67 g, 848.33 g and 1068.33 g respectively). By March 2024, it had decreased to 740 g (Figure 3.4a). In March 2024, the site at the Coves had little to no water hyacinth present and was occupied by a population of *Ludwigia palustris* (L.) Elliott. In April 2024, the water hyacinth mat had receded from the site at the Coves, making it impossible to gather plants for sampling.

At the Coves, the mean above-water biomass (living plant material) followed a similar trend to below-water biomass, with the highest value recorded in January 2024 (3565 g) and declined sharply thereafter, reaching its lowest point in February 2024 (313.33 g). The above-water biomass at Ifafi increased steadily through December 2023 (1330 g) and January 2024 (1511.67 g), where it peaked. By March 2024, the above-water biomass declined sharply to 490 g, marking the lowest value recorded at this site. Similarly, the above-water biomass at Kurperoord increased steadily in December 2023 (916.67 g) and peaked in January 2024 (1478.33 g). A slight decline followed in February 2024 (1336.67 g), with the lowest value recorded in March 2024 (261.67 g) (Figure 3.4b).

The longest petiole length peaked at the Coves in December 2023 (71.2 cm ± 2.9 cm) before declining steadily in the subsequent months. By January 2024, the petiole length had decreased to 55.4 ± 2.0 cm and further in February 2024 (50.2 cm ± 2.7 cm). The lowest value was recorded in March 2024 (41.4 cm ± 2.9 cm). At Ifafi, a similar trend was observed, with the longest petiole length reaching a maximum in December 2023 (64.9 cm ± 3.1 cm). By January 2024, the mean length had decreased to 44.5 cm ± 2.1 cm and continued to decline through February 2024 and March 2024 (35.4 cm ± 2.3 cm and 31.3 cm ± 2.4 cm respectively). At Kurperoord, the longest petiole length started at 32.7 cm ± 2.2 cm in November 2023 and increased steadily, peaking in January 2024 (55.4 cm ± 2.0 cm). However, by February 2024, it had decreased to 50.2 cm ± 2.7 cm and further in March 2024 (41.4 cm ± 2.9 cm) (Figure 3.4c).

For the leaf 2 petiole, the trends were similar across the sites. At the Coves, the mean leaf 2 petiole length peaked in December 2023 (64.9 cm ± 3.1 cm), followed by a significant decline in January 2024 (44.5 cm ± 2.1 cm). The length decreased further to 35.4 ± 2.3 cm in February 2024 and reached its lowest point in March 2024 (31.3 cm ± 2.4 cm). At Ifafi, the leaf 2 petiole length began at 27.2 cm ± 2.4 cm in November 2023 and peaked in December 2023 (64.9 cm ± 3.1 cm). From there, it decreased consistently in February and March 2024 (35.4 ± 2.3 cm and 31.3 cm ± 2.4 cm respectively). At Kurperoord, the leaf 2 petiole length followed a similar trajectory, increasing from 27.2 cm ± 2.4 cm in November 2023 to a peak of 44.5 cm ± 2.1 cm in January 2024. It then declined in February 2024 and March 2024 (35.4 cm ± 2.3 cm and 31.3 cm ± 2.4 cm respectively) (Figure 3.4d).

The mean surface area of the second leaf was largest at the Coves in December 2023 (108.0 cm^2 ± 4.2 cm^2), followed by a steady decline in January 2024 (90.0 cm^2 ± 3.5 cm^2). The area continued to decrease through February 2024 (72.0 cm^2 ± 3.2 cm^2) and reached its lowest point in March 2024 (60.0 cm^2 ± 4.0 cm^2). At Ifafi, the leaf 2 area displayed a similar pattern, with a peak in December 2023 (108.0 cm^2 ± 4.5 cm^2) and subsequent declines in February 2024 and March 2024 (72.0 cm^2 ± 3.2 cm^2 and 60.0 cm^2 ± 4.0 cm^2 respectively). At Kurperoord, the leaf 2 area followed a comparable trajectory, increasing from 60.0 cm^2 ± 3.0 cm^2 in November 2023 to a peak of 108.0 cm^2 ± 4.2 cm^2 in December 2023, and then decreasing to 72.0 cm^2 ± 3.2 cm^2 in February 2024 and 60.0 cm^2 ± 4.0 cm^2 in March 2024 (Figure 3.5a).

The mean root length at the Coves increased from 18.0 cm ± 1.5 cm in November 2023 to a peak of 26.0 cm ± 1.8 cm in December 2023. By January 2024, it had decreased and reached its lowest value in March 2024 (24.0 cm ± 1.2 cm and 18.0 ± 1.0 cm respectively). At Ifafi, root length peaked at 26.0 cm ± 1.8 cm in December 2023 before declining through February 2024 (20.0 cm ± 1.5 cm) and March 2024 (18.0 cm ± 1.0 cm). Similarly, at Kurperoord, root length increased to its highest value in December 2023 (26.0 cm ± 1.8 cm) before declining in February 2024 and March 2024 (24.0 cm ± 1.2 cm and 18.0 cm ± 1.0 cm respectively) (Figure 3.5b).

The mean number of ramets was highest at the Coves in December 2023 (3.0 ± 0.2) and gradually declined in February 2024 and March 2024 (2.0 ± 0.1 and 1.0 ± 0.1 respectively). At Ifafi, ramet numbers displayed a similar trend, peaking at 3.0 ± 0.2 in December 2023 before decreasing in February 2024 and March 2024 (2.0 ± 0.1 and 1.0 ± 0.1 respectively). At Kurperoord, the ramet count followed the same pattern, increasing to 3.0 ± 0.2 in December 2023 before declining steadily in February 2024 and March 2024 (2.0 ± 0.1 and 1.0 ± 0.1 respectively) (Figure 3.5c).

The average number of leaves peaked at the Coves in December 2023 (6.0 ± 0.4), decreasing slightly by January 2024 (5.0 ± 0.3) and February 2024 (3.0 ± 0.2), with the lowest value observed in March 2024 (2.0 ± 0.2). At Ifafi, the trend was similar, with a peak in December 2023 (6.0 ± 0.4), followed by declines in February 2024 and March 2024 (3.0 ± 0.2 and 2.0 ± 0.2 respectively). At Kurperoord, the number of leaves increased from 3.0 ± 0.2 in November 2023 to 6.0 ± 0.4 in December 2023, then declined steadily through February 2024 and March 2024 (3.0 ± 0.2 and 2.0 ± 0.2 respectively) (Figure 3.5d).

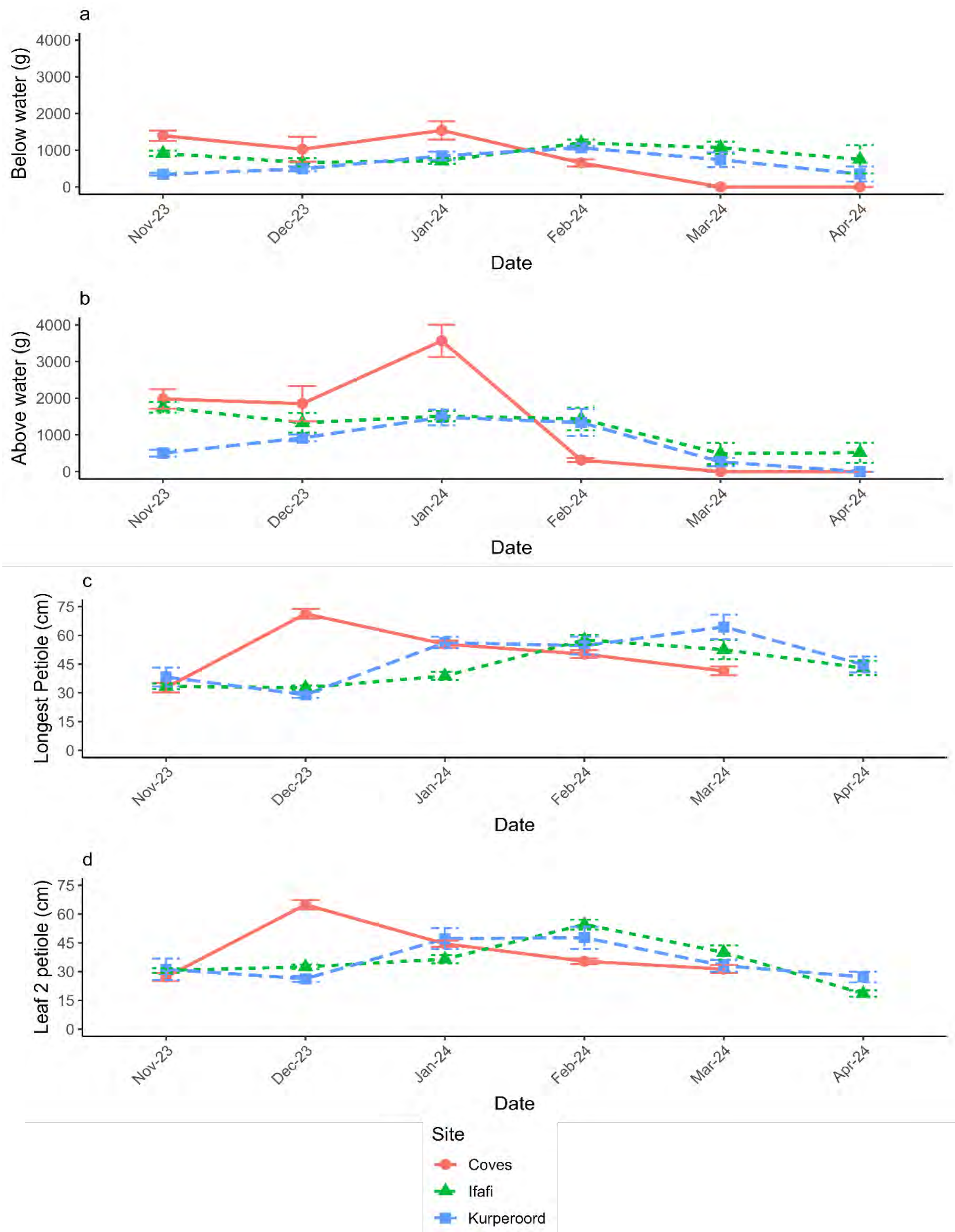

Figure 3.4: Mean of the **(a)** below-water biomass (g/m^2), **(b)** above-water biomass (g/m^2), **(c)** length of the longest petiole (cm) and **(d)** length of the leaf 2 petiole (cm) of water hyacinth plants at the three study sites at Hartbeespoort Dam between November 2023 and April 2024. Error bars = ± S.E.

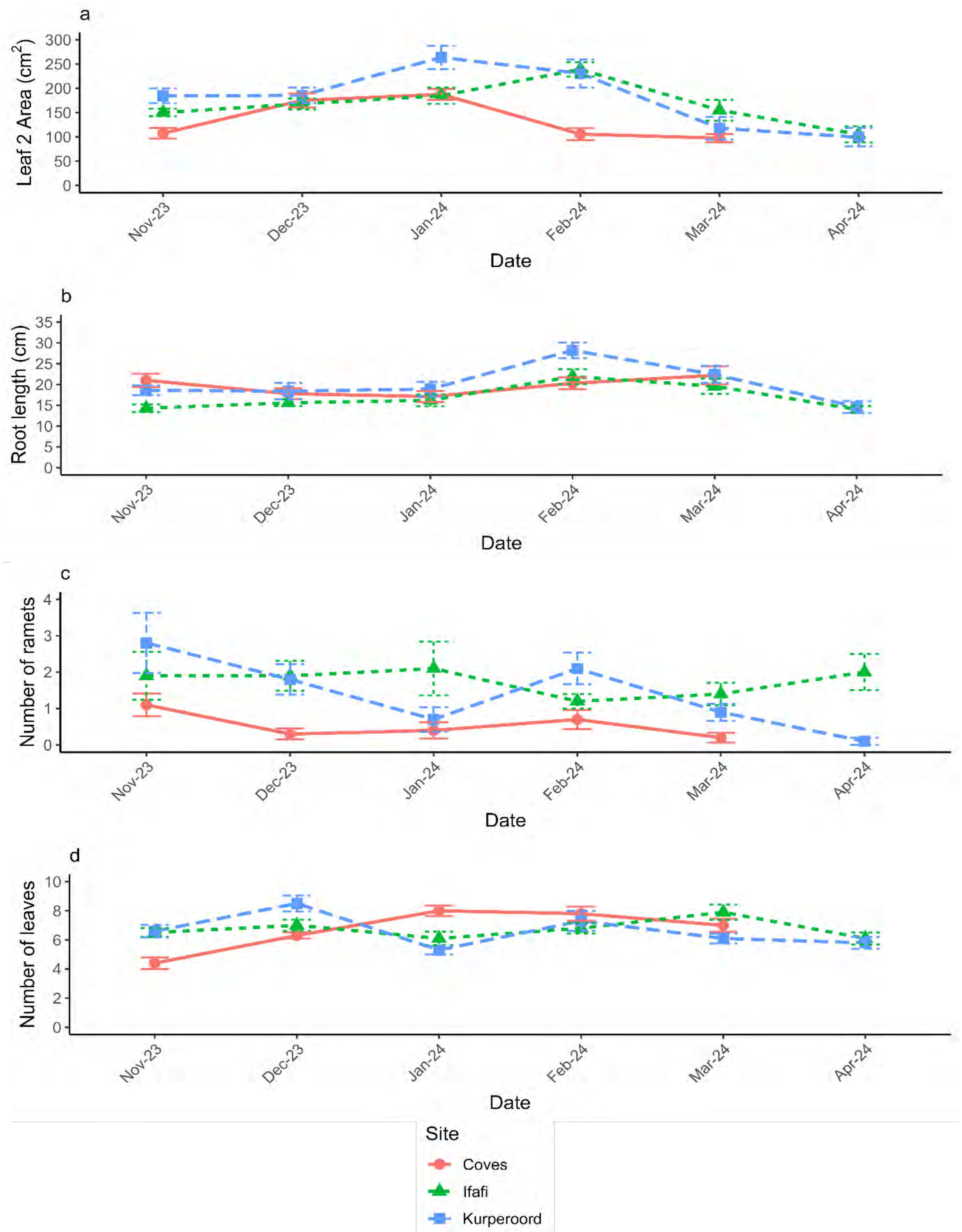

Figure 3.5: Mean of the **(a)** leaf 2 area (cm^2), **(b)** root length (cm), **(c)** number of ramets per m^2 and **(d)** number of leaves per m^2 of water hyacinth plants at the three study sites at Hartbeespoort Dam between November 2023 and April 2024. Error bars = ± S.E.

The biomass of dead material was highest at the Coves in January 2024 (866.67 g ± 25.0 g). A decline was observed in February 2024 (655.00 g ± 20.0 g) and March 2024 (490.00 g ± 15.0 g). At Ifafi, dead material followed a similar trend, with the highest value recorded in January 2024 (848.33 g ± 22.0 g). This declined to 740.00 g ± 18.0 g in February 2024 and 527.50 g ± 14.0 g in March 2024. At Kurperoord, dead material increased steadily from November 2023 (345.00 g ± 10.0 g) to a peak in January 2024 (848.33 g ± 22.0 g). A decline was recorded in February 2024 (740.00 g ± 18.0 g), with the lowest value in March 2024 (527.50 g ± 14.0 g) (Figure 3.6a).

The average water hyacinth plant density was consistent during the early stages of the study at the Coves, peaking in December 2023 ($15.33/m^2 \pm 0.5/m^2$). A gradual decline was observed in February 2024 ($10.67/m^2 \pm 0.4/m^2$) and further in March 2024 ($8.33/m^2 \pm 0.3/m^2$). At Ifafi, the pattern was similar, with plant density reaching a peak in December 2023 ($15.33/m^2 \pm 0.5/m^2$) before decreasing in February 2024 and March 2024 ($10.67/m^2 \pm 0.4/m^2$ and $8.33/m^2 \pm 0.3/m^2$ respectively). At Kurperoord, plant density increased slightly from November 2023 to December 2023 ($12.00/m^2 \pm 0.4/m^2$ and $15.33/m^2 \pm 0.5/m^2$ respectively), followed by a decline in February 2024 and March 2024 ($10.67/m^2 \pm 0.4/m^2$ and $8.33/m^2 \pm 0.3/m^2$ respectively) (Figure 3.6b).

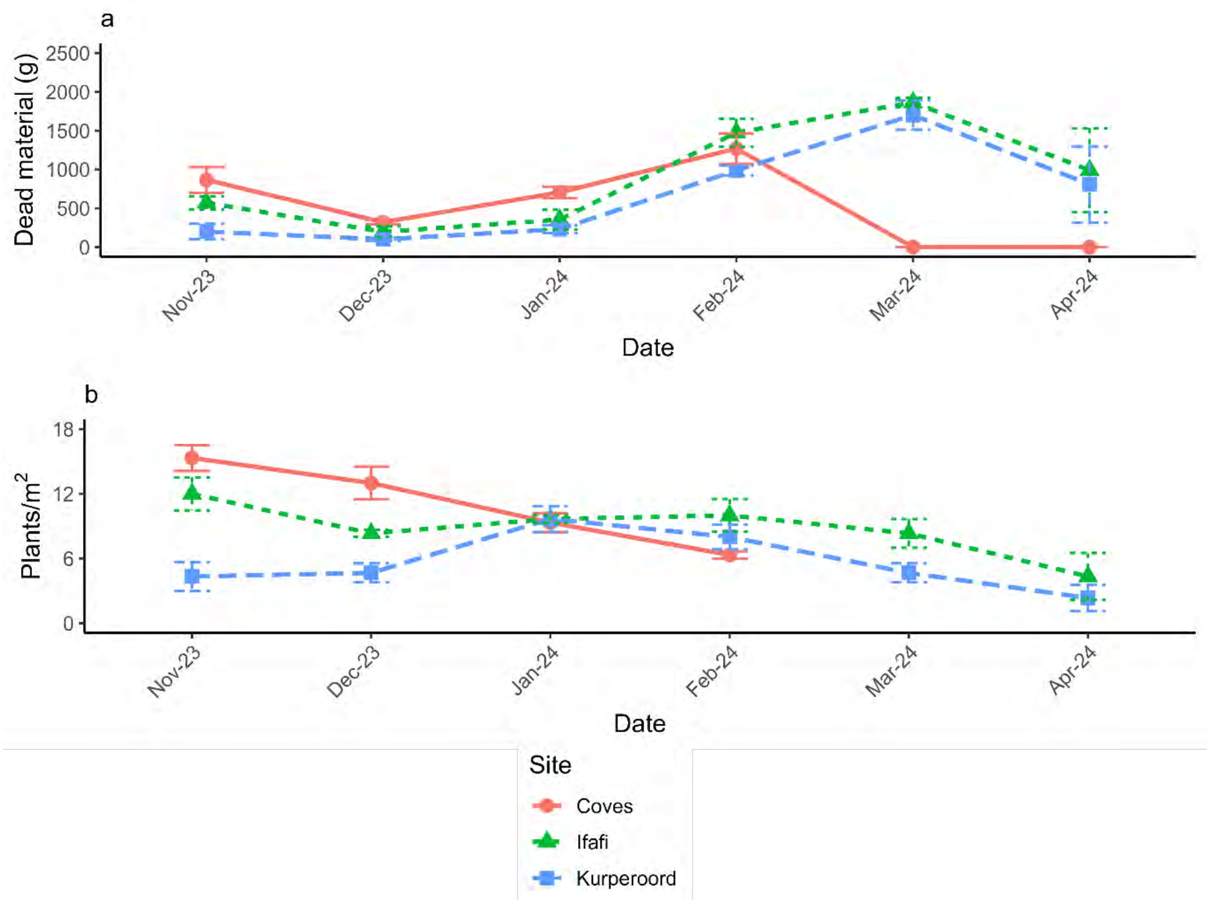

Figure 3.6: Mean of the **(a)** dead material biomass (g/m^2) and **(b)** number of plants per m^2 of water hyacinth plants at the three study sites at Hartbeespoort Dam between November 2023 and April 2024. Error bars = ± S.E.

3.2.3 *Megamelus scutellaris* abundance

Mean *M. scutellaris* numbers peaked in January 2024 at the Coves (7875/m^2) and peaked in March 2024 at Kurperoord and Ifafi (7775/m^2 and 8375/m^2 respectively). At the Coves, agent counts decreased and reached a minimum in March 2024 (810/m^2) because majority of the plants had died. *M. scutellaris* counts were lowest in April 2024 at Kurperoord and Ifafi (22.5/m^2 and 67.5/m^2 respectively) and no insects were recorded at the Coves in April 2024 as the water hyacinth mat had receded from the site (Figure 3.7).

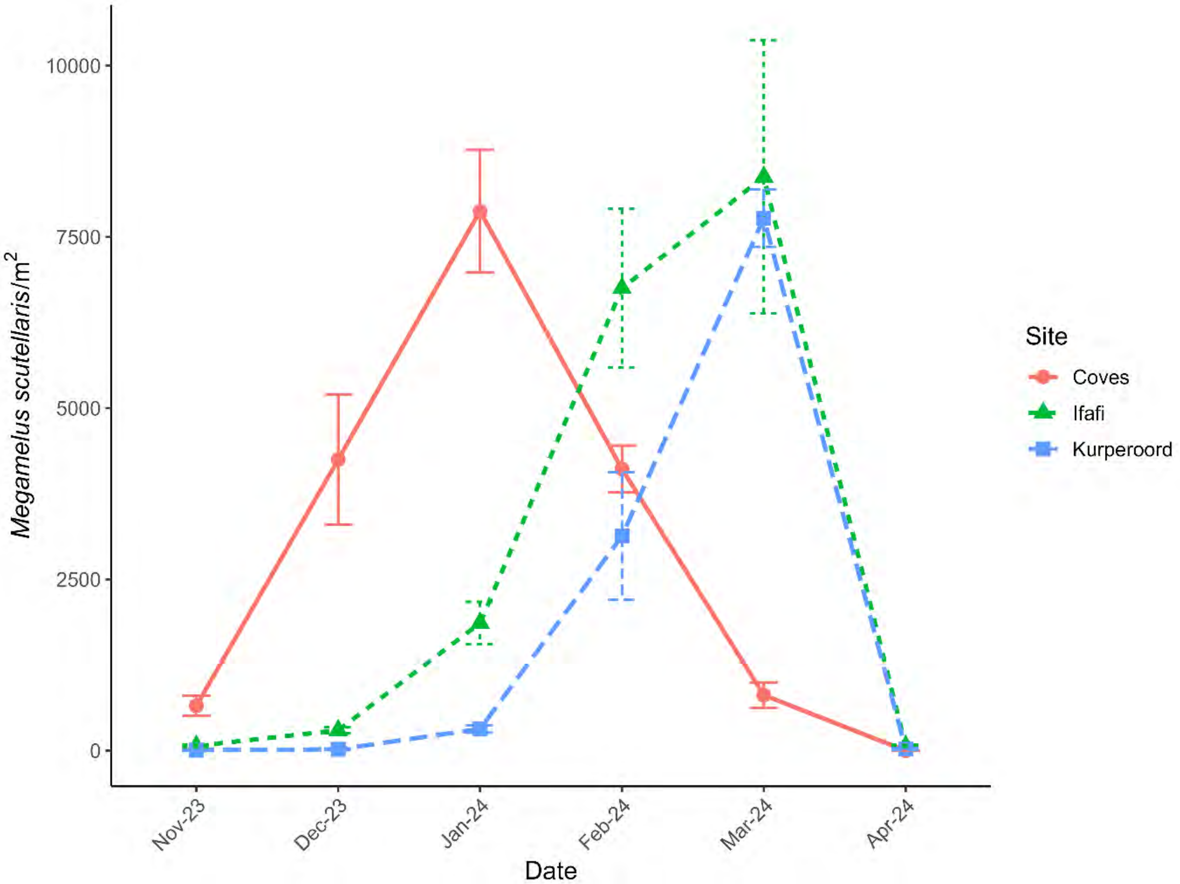

Figure 3.7: Mean *M. scutellaris* per m^2 at the three study sites at Hartbeespoort Dam between November 2023 and April 2024. Error bars = ± S.E.

M. scutellaris density/m^2 had a significant effect on the root biomass ($P < 0.001$, adjusted $R^2 = 0.21$) and the biomass of dead material ($P < 0.001$, adjusted $R^2 = 0.34$) of water hyacinth plants. Insect density was positively correlated with root biomass (Figure 3.8a) and dead material biomass (Figure 3.8b).

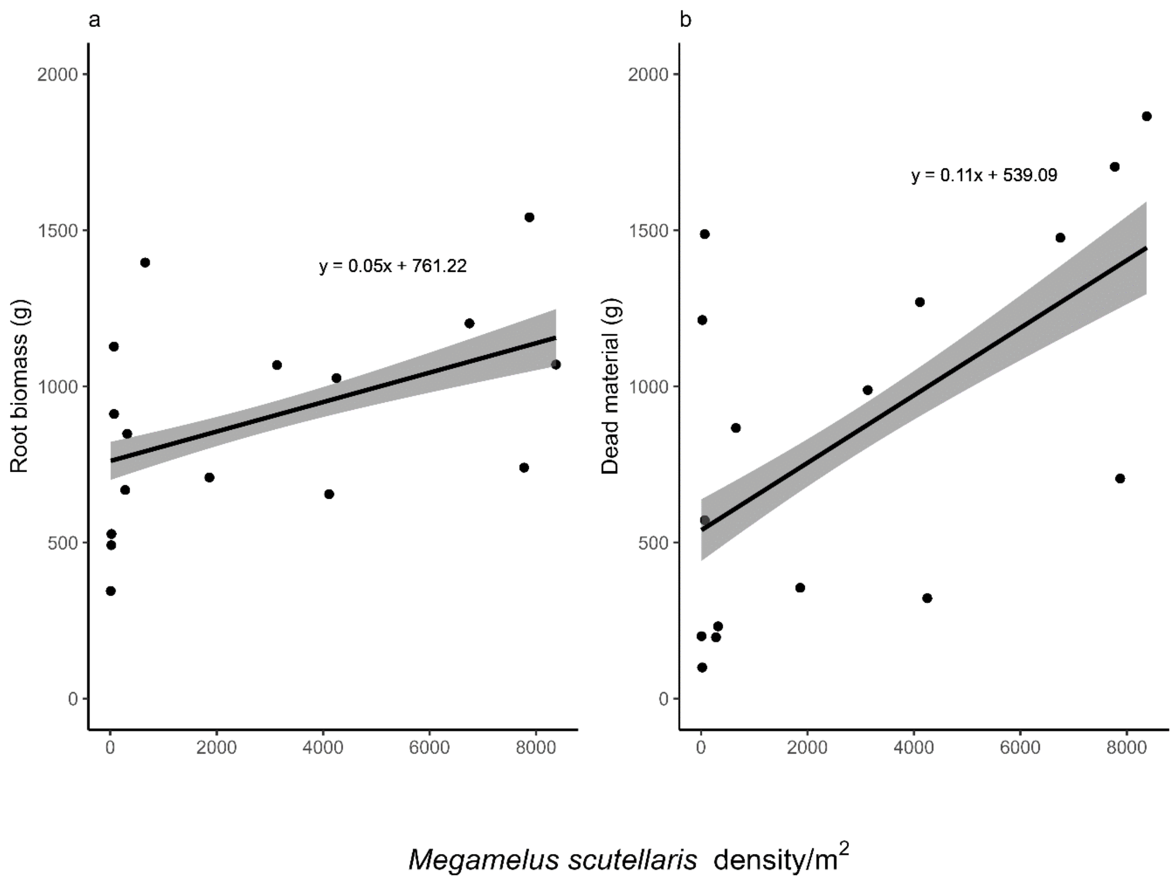

Figure 3.8: The relationships between *M. scutellaris* density/m^2 on Hartbeespoort Dam and **(a)** the below water (roots) biomass (g) and **(b)** the biomass of dead material (g) of water hyacinth plants measured from November 2023 to February 2024.

3.2.4 Water hyacinth percentage cover

During the study period, water hyacinth coverage fluctuated (Figure 3.9). Between December 2023 and February 2024, the summer months, cover began to increase, reaching its maximum in February 2024 (26.52% ± 1.62%). The coverage decreased in the subsequent months, reaching its minimum in April 2024 (2.19% ± 0.22%). Water hyacinth reached its peak coverage on 10 February 2024 at 29.98% (Figure 3.10).

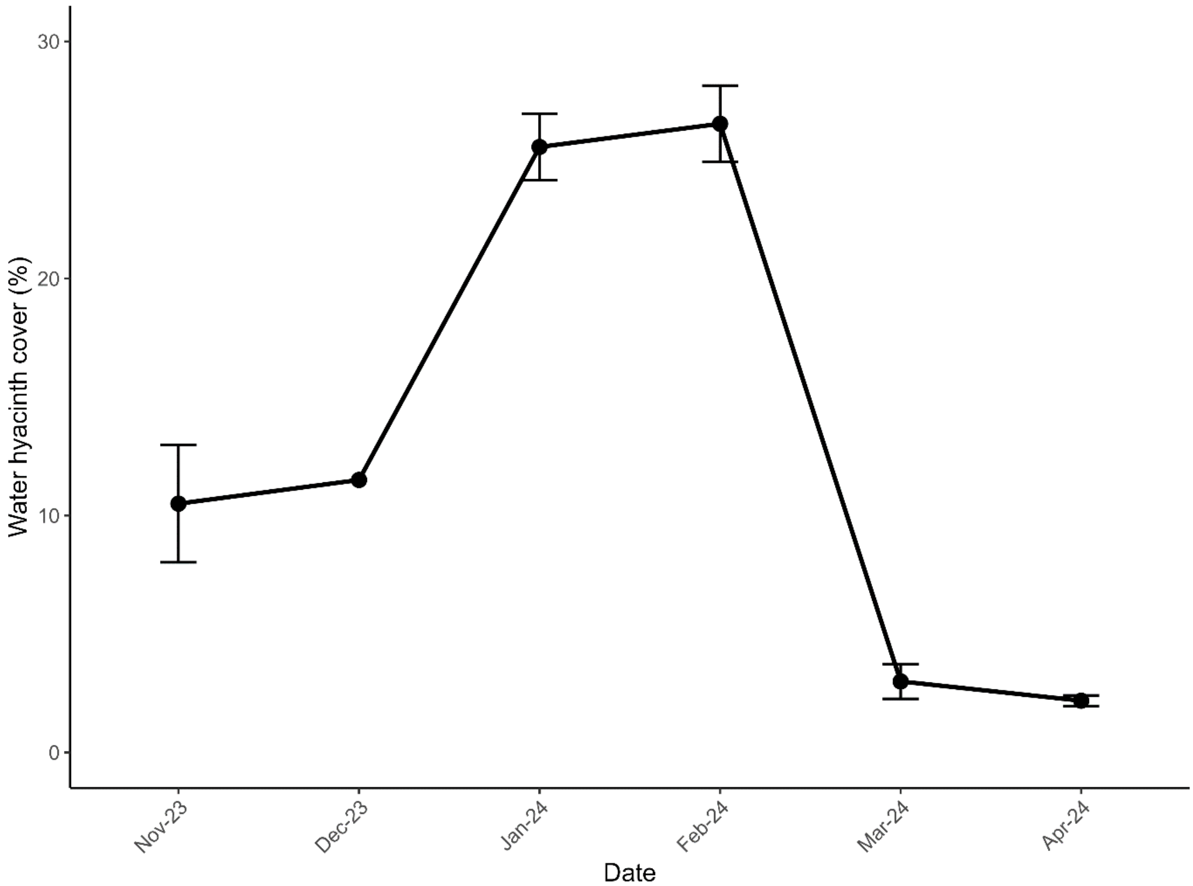

Figure 3.9: Mean water hyacinth percentage cover percentage at Hartbeespoort Dam between November 2023 and April 2024. Error bars = ± S.E.

Figure 3.10: Water hyacinth cover was ~30% and 546 ha on 10 February 2024 at Hartbeespoort Dam, obtained from the macrophyte monitoring WebApp: https://davidkinsler123.users.earthengine.app/view/macrophyte-monitoring-tool.

The population density of *M. scutellaris* insects began to increase in November 2023 (244.17 ± 90.20) and peaked in March 2024 (5653.33 ± 1113.58), with a sharp decrease in water hyacinth coverage. Insect populations peaked one month after the water hyacinth coverage peaked in February 2023. *M. scutellaris* densities decreased sharply and reached a minimum in April 2024 (30.00 ± 10.12) (Figure 3.11).

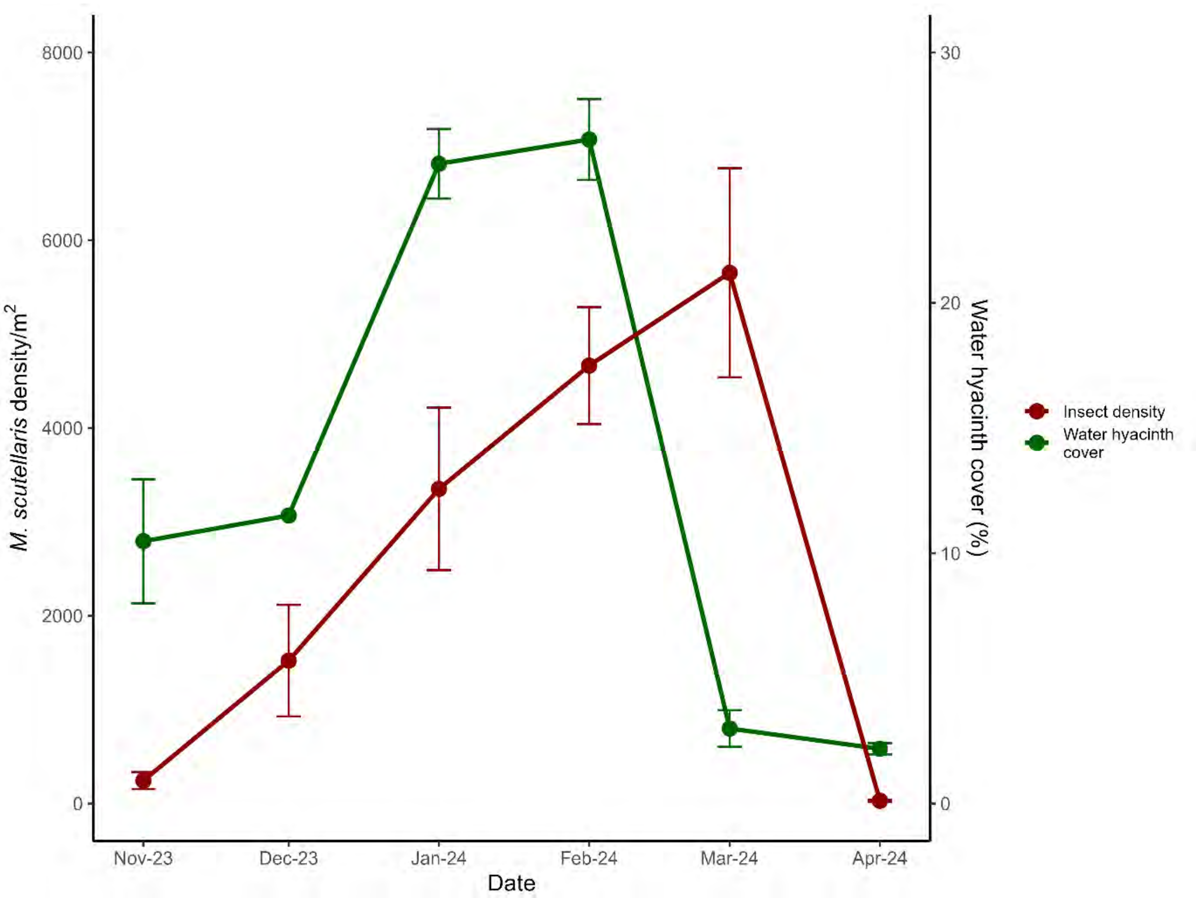

Figure 3.11: Mean *M. scutellaris* population density and water hyacinth cover at Hartbeespoort Dam between November 2023 and April 2024. *M. scutellaris* densities represent combined counts from the three sites (The Coves, Kurperoord, and Ifafi). Error bars = ± SE.

3.2.5 Detritus formation

No data were recorded initially, for the control cages between November and December 2023 as they were installed during December 2023 and data could only be recorded one month after installation. The mean detritus collected from the treatment mesh cages was highest in April 2024 (847.30 g ± 89.10 g; Kurperoord and Ifafi combined). There was a notable peak in detritus collected from the treatment cages in January 2024 at the Coves, Kurperoord and Ifafi (627.20 g ± 87.72 g, 482.00 g ± 39.72 g and 234.20 g ± 23.23 g respectively). In November 2023, the treatment mesh cages at the Coves and Kurperoord yielded their lowest amount of detritus (77.40 g ± 27.65 g and 66.80 g ± 21.60 g respectively), while the treatment cages at Ifafi were lowest in December 2023 (38.80 g ± 12.94 g). The detritus collected from the control cages was always less than the detritus collected in the treatment cages. At Kurperoord and Ifafi, detritus peaked in April 2024 (571.25 g ± 149.02 g and 261.67 g ± 29.67 g respectively) (Figure 3.12).

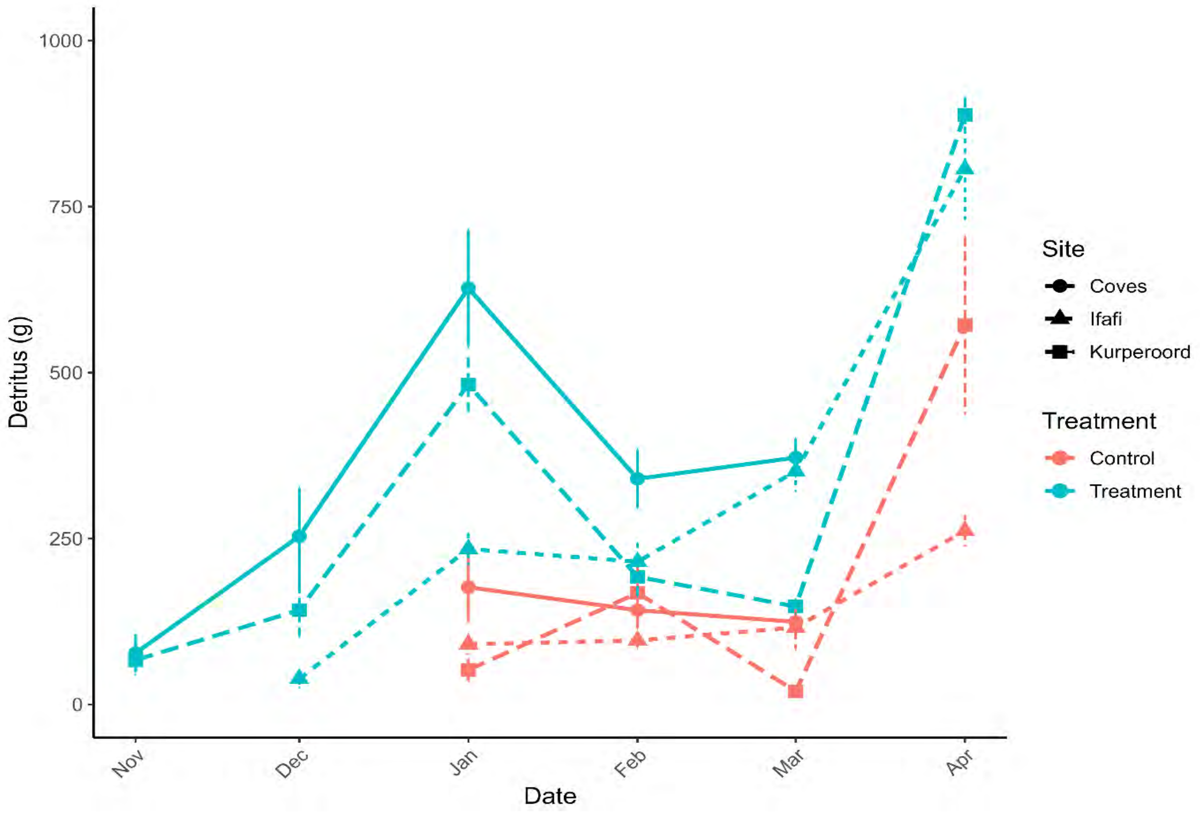

Figure 3.12: Mean amount of detritus (g) collected from treatment and control mesh bags at the three study sites at Hartbeespoort Dam between November 2023 and April 2024. Error bars = ± S.E.

There was a significant difference in detritus collected from the water hyacinth cages in comparison to the empty cages for the entire study duration ($\chi^2 = 41.56$, df = 1, $P < 0.005$). Not surprisingly, the cages containing water hyacinth plants produced more than double the amount of detritus (386.09 g ± 0.12 g) than the cages without plants (157.35 g ± 0.12 g) (Figure 3.13).

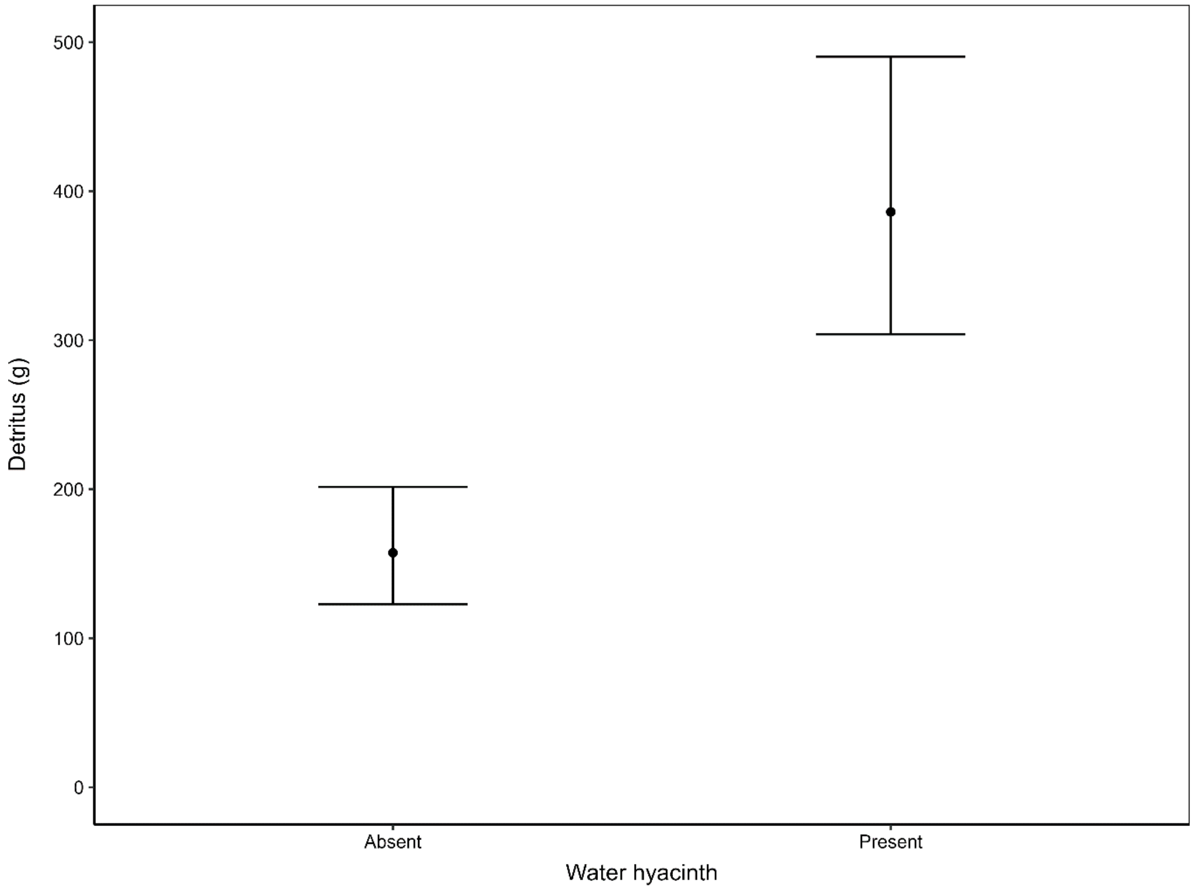

Figure 3.13: Mean detritus collected from Hartbeespoort Dam over the sample period (November 2023 - April 2024) from cages with or without water hyacinth.

PC1 distinguishes between sites with higher PH/lower DO-TDS-Phosphates (PC1 positive) vs lower PH/higher DO-TDS-Phosphates (PC2 negative). Detritus was positively correlated with higher PH vs lower DO/TDS/Phosphate values ($X^2 = 9.00$, df = 1, $P = 0.003$) (Figure 3.14). The concentration of nitrates in the water did not show a significant correlation with the amount of detritus collected.

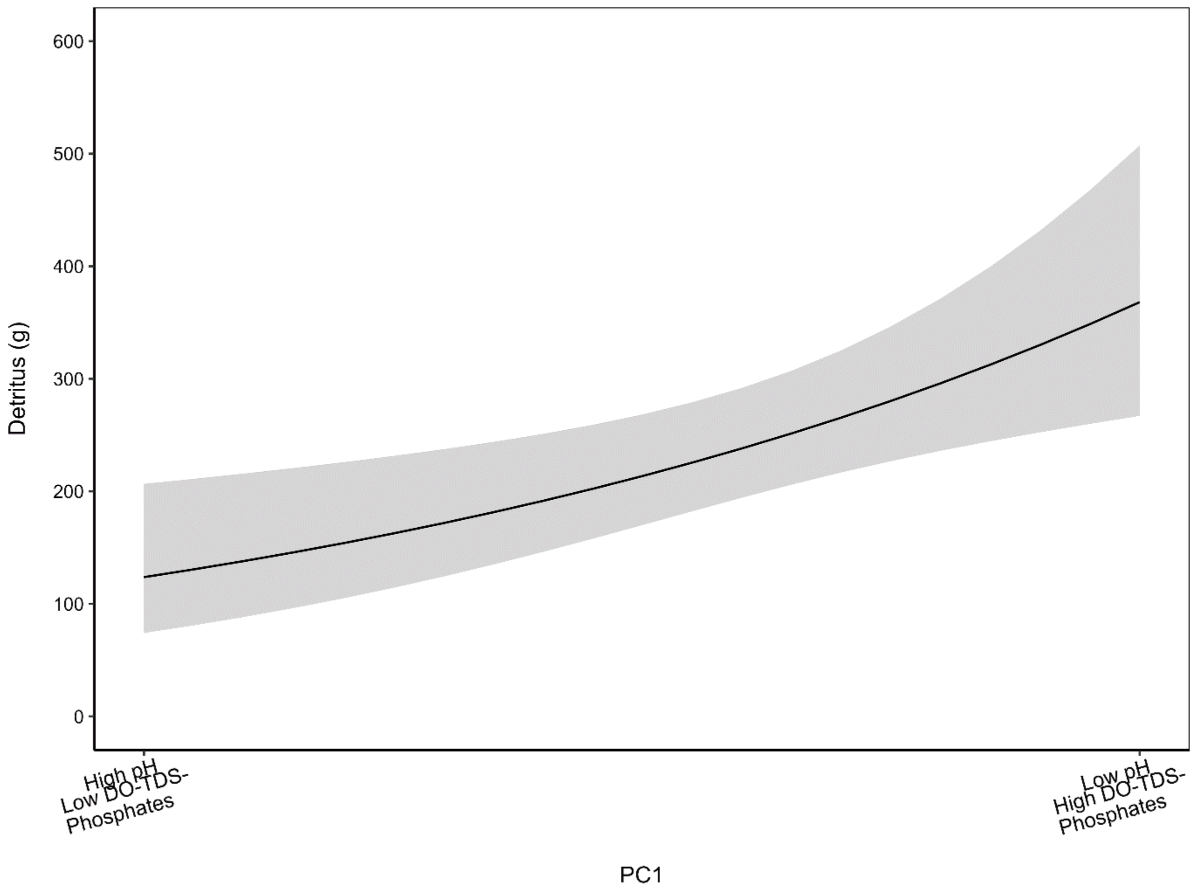

Figure 3.14: The relationship between detritus collected monthly from Hartbeespoort Dam (November 2023 - February 2024) and the first PCA axis of water chemistry parameters.

The multiple linear regression model examining the effects of plant parameters on detritus accumulation was significant ($F_{(8, 101)} = 5.484$, $P < 0.001$), explaining 30.3% of the variance in detritus accumulation ($R^2 = 0.303$, adjusted $R^2 = 0.248$). Among the predictor variables, root (below-water) biomass, root length, and plant density had significant effects on detritus accumulation. Root length had a significant negative effect (β = -14.90, t = -3.28, P = 0.001), indicating that longer roots were associated with lower detritus accumulation (Figure 3.15a). Plant density (plants/m^2) also showed a strong negative effect (β = -48.74, t = -3.72, $P < 0.001$), meaning that higher plant densities corresponded with lower detritus accumulation (Figure 3.15b). Root biomass had a significant positive effect (β = 0.75, t = 2.74, P = 0.007), suggesting that an increase in root biomass is associated with higher detritus accumulation Figure 3.15c). Other variables, including above-water biomass**,** dead material biomass, number of ramets, petiole length, and leaf 2 petiole length, were not statistically significant predictors of detritus accumulation ($P > 0.05$) and were therefore not considered for further analysis.

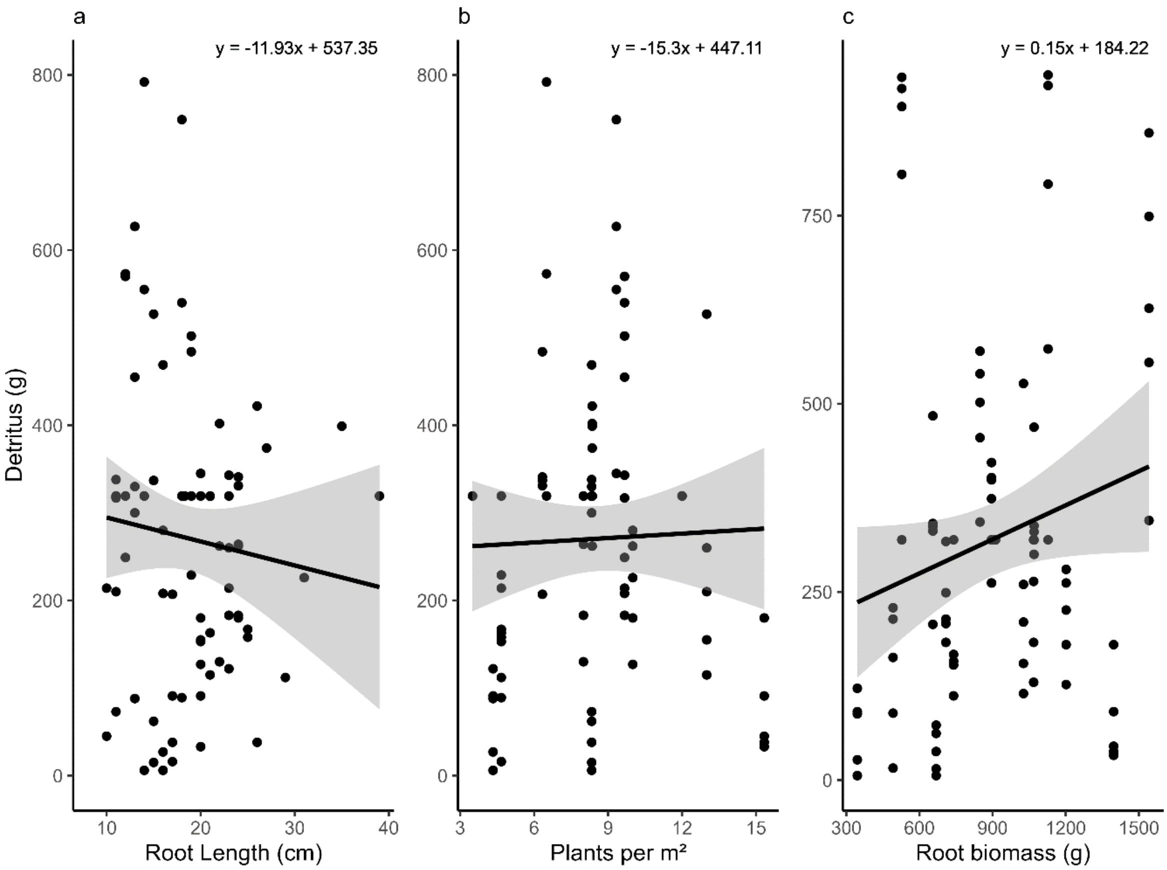

Figure 3.15: The relationships between detritus collected monthly from Hartbeespoort Dam (November 2023 - February 2024) and **(a)** the root length (cm), **(b)** plants per m^2 and **(c)** the root biomass (g) of water hyacinth plants.

The relationship between *M. scutellaris* density on the dam and the amount of detritus collected was statistically significant ($\chi^2 = 10.88$, df = 1, $P = 0.001$). The two variables were negatively correlated (Figure 3.16). For every *M. scutellaris* individual added onto the dam, detritus decreased by 0.02g.

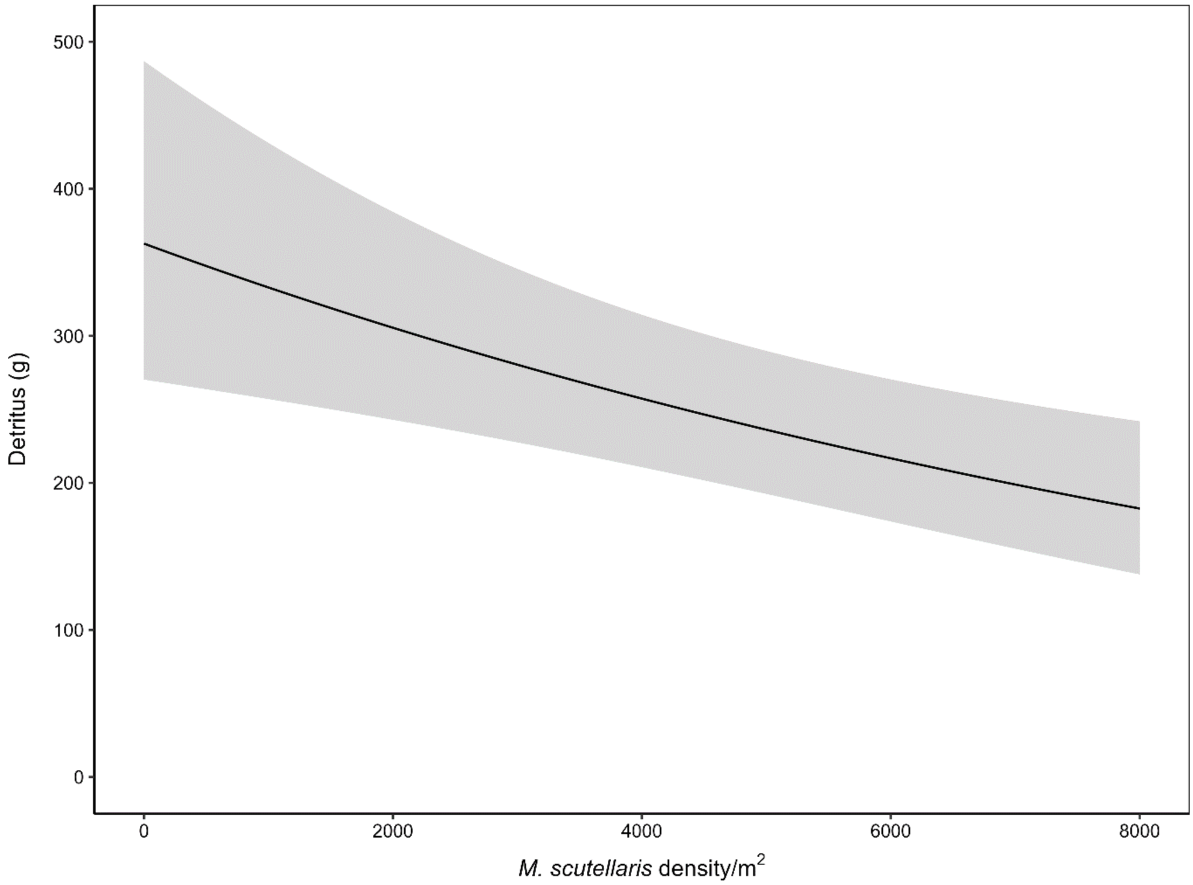

Figure 3.16: The relationship between detritus collected monthly from Hartbeespoort Dam (November 2023 - February 2024) and the density of *M. scutellaris* insects on the plants.

There was no significant relationship between water hyacinth cover (%) across Hartbeespoort Dam and the amount of detritus collected in the cages ($\chi^2 = 0.04$, df = 1, $P = 0.85$). (Figure 3.17). For every 1% increase in water hyacinth cover, detritus increased by 0.55 g.

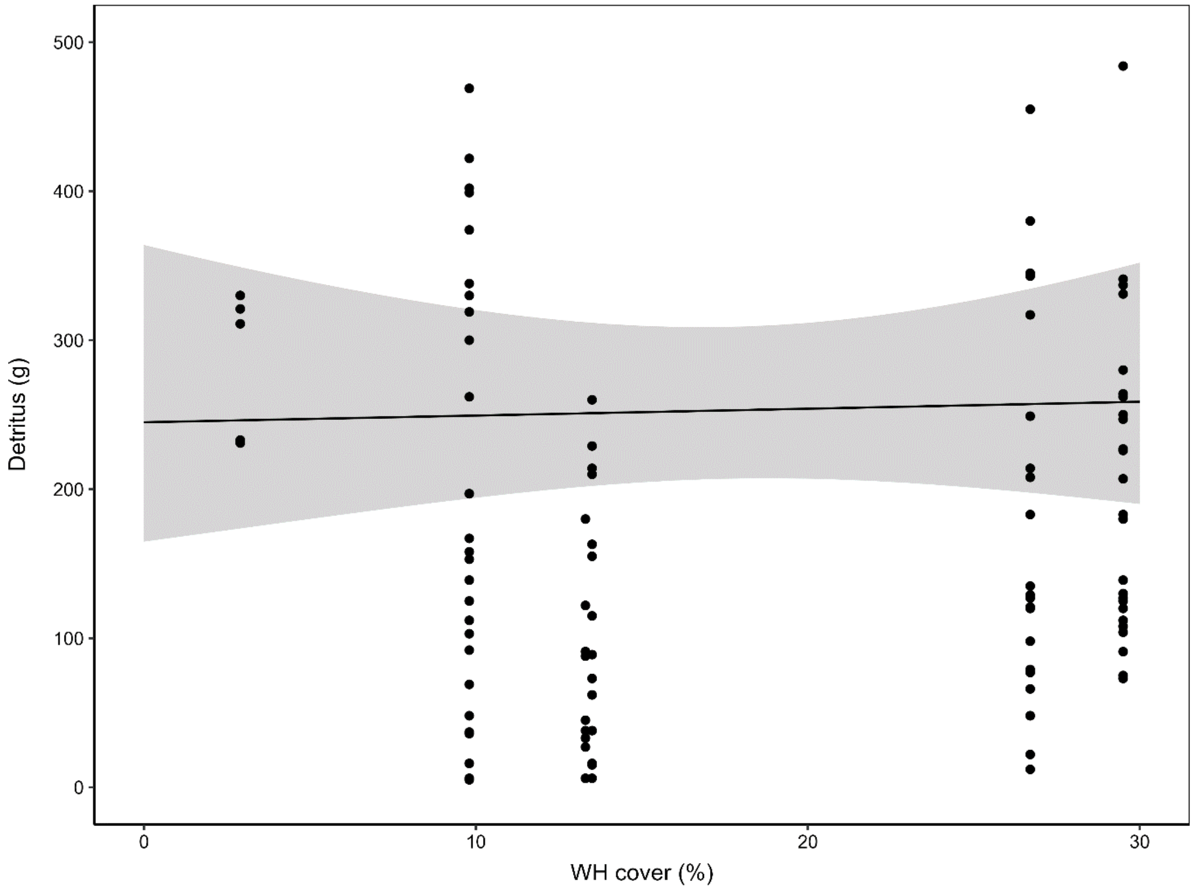

Figure 3.17: The relationship between detritus collected monthly from Hartbeespoort Dam (November 2023 - February 2024) and the percentage of water hyacinth covering the dam.

Overall, water chemistry parameters varied significantly across sites over time. pH levels showed seasonal fluctuations, with significant differences observed between sites, particularly between Kurperoord and Ifafi. Dissolved oxygen (DO) concentrations peaked in February 2024 across all sites, with Kurperoord exhibiting the highest values. Total dissolved solids (TDS) and phosphate concentrations also varied significantly, with Kurperoord generally having higher values than the other sites. Nitrate concentrations were significantly lower at Kurperoord compared to the Coves and Ifafi.

Plant parameters followed similar seasonal trends, with below- and above-water biomass, petiole length, leaf area, and root length peaking in the summer months before declining towards March 2024. The presence of *M. scutellaris* was strongly linked to plant decline, with peak insect abundance observed at different times across sites. Water hyacinth cover increased during summer, peaking in February 2024, before declining sharply after *M. scutellaris* densities peaked in March 2024.

Plant parameters significantly influenced detritus formation, with higher root biomass linked to increased detritus, while longer roots and higher plant density were associated with lower detritus accumulation. Additionally, *M. scutellaris* density was negatively correlated with detritus levels, suggesting that increased insect herbivory contributed to plant senescence. Nitrate concentrations and water hyacinth cover were not correlated with detritus formation.

CHAPTER 4

Discussion and Conclusion

4.1 Introduction

Water hyacinth has invaded many of South Africa's waterways including major dams and rivers (Auchterlonie et al., 2021; Coetzee & Hill, 2012; B. Miller et al., 2023). One such system is Hartbeespoort Dam in the North-West Province where the weed has been a cause for concern since the 1960s. (Moffat et al., 2024; van Wyk & Van Wilgen, 2002). Varying levels of control have been achieved on the system since, and between 1977 and 2001, herbicide use was the main control method for water hyacinth (Moffat et al., 2024; van Wyk & Van Wilgen, 2002). This programme yielded successful results for short periods before reapplication of the chemical was necessary to combat the reoccurring mat. In 2016, the chemical control programme ceased at Hartbeespoort Dam due to a lack of funding (Mitchell & Crafford, 2016). The Hartbeespoort Dam Remediation Programme (HRDP) or the "Harties Metsi a Me" programme ran from 2006 to 2016, where water hyacinth and other IAAPs were removed from the dam (Mitchell & Crafford, 2016). The project also aimed to restructure the food web by removing invasive fish species and algae and constructing floating wetlands. The HRDP removed 213 296 m^3 of water hyacinth and 67 947 m^3 of algae (Mitchell & Crafford, 2016). Unfortunately, the eutrophic conditions in the water continued to promote the rapid growth of water hyacinths increasing the labour required for effective weed removal. Consequently, the programme was discontinued in 2016 (Mitchell & Crafford, 2016).

In 2017, after the Metsi a Me programme and chemical control programmes had ceased, biological control became the sole control method employed at the dam. The Centre for Biological Control (CBC) launched an augmentative control programme in 2018 whereby *M.*

scutellaris were mass-reared at the CBC's Waainek Mass Rearing Facility in Makhanda, and released onto water hyacinth plants inundatively and frequently. From 2021, through community engagement, satellite rearing stations were established with local stakeholders around the dam, who boosted the release effort, particularly early in spring when the plants germinated from seed (Moffat et al., 2024). This programme resulted in drastic mat die-offs for five consecutive years. Unlike the previously mentioned programmes, this initiative does not aim to act as a "quick fix" for the water hyacinth infestation at Hartbeespoort Dam for several reasons (Moffat et al., 2024). Firstly, the seed bank in the sediment has been accumulating for decades, indicating that controlling the water hyacinth is a long-term endeavour rather than a short-term solution achievable within one or two growing seasons (Albano Pérez et al., 2011; Moffat et al., 2024). Secondly, the water in Hartbeespoort Dam is highly eutrophic, primarily due to factors such as acid mine drainage, agricultural or industrial runoff, and untreated sewage entering the system (Moffat et al., 2024). Consequently, water hyacinth is merely a symptom of the larger issue of nutrient-rich water.

The substantial die-off and subsequent sinking of the water hyacinth mat achieved through the augmentative biological control programme raised concerns among local community members about the impact of the dead and decomposing organic matter on the water column and, ultimately, on the health of the aquatic ecosystem. Due to the success of biological control in controlling the water hyacinth infestations, locals began to believe that biological control may be impacting the amount of detritus being added to the sediment load of the dam and possibly creating a "sludge layer" on the dam floor. This study highlights the effect that a biological control programme had on the senescence of water hyacinth plants, how the senescence process added to the detritus load and the impact that it had on water chemistry.

4.2 The relationship between detritus formation and water chemistry

Several studies have analysed the impact of water hyacinth on water chemistry (Bayu Zeleke et al., 2024; Hounkpe et al., 2022). Lower amounts of detritus can be related to healthy plants and a large mat. In aquatic systems, pH is generally higher in the presence of healthy macrophytes due to the uptake of carbon dioxide and release of OH^- ions during photosynthesis, thus creating an alkaline environment (Hounkpe et al., 2022). In this study, lower amounts of detritus were associated with high pH. When water hyacinth plants become unhealthy and are senescing, the amount of detritus entering the water column increases. During the decomposition of detritus, carbon dioxide is released into the water column, increasing acidity, and thus lowering the pH. This trend was seen in the mesocosm and field experiments where pH declined over time when plants had entered senescence. Lower pH in the presence of senescing water hyacinth plants was also seen in the study conducted by Masifwa et al. (2004) at Hartbeespoort Dam. At the end of the mesocosm experiment (time point two), there was no significant difference between the pH of the water in the bins containing water hyacinth controlled by *M. scutellaris*, herbicide and water hyacinth not under any control, indicating that the method of control does not impact the pH of the water once water hyacinth plants have died.

In the field experiment, lower amounts of detritus were associated with low dissolved oxygen concentrations. Water hyacinth infestations often reduce the dissolved oxygen concentration of water as plants cover the water's surface, not allowing light to penetrate and inhibiting photosynthesis in submerged plants (Lakane et al., 2024), opposing the trend seen at Hartbeespoort Dam. In the mesocosm experiment increased dissolved oxygen concentration was associated with increased detritus. This can be attributed to several factors: the absence of water flow, which stagnated the water; the coverage of the water's surface by algae or yet-to-sink dead material, which blocked gas exchange; the absence of submerged plants that perform photosynthesis; and the consumption of oxygen through aerobic respiration, which is necessary for the decomposition of organic matter. Any of the above factors could explain the trend seen at Hartbeespoort Dam and the latter was seen in the study conducted by Masifwa et al. (2004). At the end of the mesocosm experiment (time point two), although dissolved oxygen concentrations differed significantly, the values were fairly similar between the bins containing water hyacinth controlled by *M. scutellaris*, herbicide and water hyacinth not under any control, indicating that the method of control does not impact the dissolved oxygen concentration of the water once water hyacinth plants have died.

Lower detritus was associated with low total dissolved solids concentration in both studies. The concentration of total dissolved solids in aquatic systems is a combination of natural compounds such as salts, minerals, and organic matter and is an indication of detritus/sediment concentrations. A study by Elizabeth et al. (2020) explored the relationship between healthy water hyacinth plants and the total dissolved solids concentration in the water. They found that the concentration of TDS is low when water hyacinth mats are healthy as the plants perform phytoremediation and filter organic compounds. This finding supports the low concentration of TDS in the water as little detritus had entered the water column and was filtered out. The concentration of total dissolved solids increases with increased concentration of organic matter and therefore increases with increased detritus, as seen by Birhanie et al. (2020). At the end of the mesocosm experiment (time point two), there was no significant difference between the TDS concentration of the water in the bins containing water hyacinth controlled by *M. scutellaris*, herbicide and water hyacinth not under any control, indicating that the method of control does not impact the TDS concentration of the water once water hyacinth plants have died.

In the field experiment, low phosphate concentrations were associated with lower amounts of detritus. Auchterlonie et al. (2021) explored the phytoremediation capabilities of water hyacinth, finding that healthy plants can absorb significant amounts of phosphorus during their growing season. When water hyacinth plants are healthy and growing, they actively remove phosphates from the water, using them to support their growth. Consequently, when the water hyacinth mat is thriving and detritus levels are low, phosphate concentrations in the water decrease. The concentration of phosphates increased with increased amounts of detritus, suggesting that when plants enter senescence, they release absorbed phosphates back into the water (Masifwa et al., 2004). It is important to note that the uptake and release of phosphorous by water hyacinth only accounts for ~1% of the total phosphate concentration in the dam (Carroll & Curtis, 2021). Additionally, the phosphate concentrations in the mesocosm experiment were not significantly different between treatment or time, indicating that the method of control does not impact the phosphate concentration of the water once water hyacinth plants have died. This also confirms that the uptake and release of phosphates by water hyacinth plants are negligible.

Although the relationship between detritus formation and nitrate concentration was not significant in either study, general trends can be identified. In the field experiment, higher amounts of detritus were associated with increased nitrate concentrations and can be explained by the release of nitrates into the water during the decomposition process (Thorén et al., 2004). As with phosphates, the concentration of nitrates retained and released by water hyacinth, ultimately, has a negligible effect on the dam (Carroll & Curtis, 2021). In the mesocosm experiment, nitrate concentrations decreased over time while the amount of detritus increased. When the water in the experimental bins was topped up, no extra fertiliser was added alongside it, resulting in a decrease in nitrate concentration over time. The concentration of nitrates also decreased in the control treatment, a possible effect of nitrate-consuming algae in the experimental bins (Lachmann et al., 2019). Additionally, the phosphate concentrations in the mesocosm experiment were not significantly different between treatment or time, indicating that the method of control does not impact the nitrate concentration of the water once water hyacinth plants have died. This also confirms that the uptake and release of nitrates by water hyacinth plants are negligible.

The take home message from these results is that the chemistry of the water column returns to the same level regardless of the control method, suggesting that inputs to Hartbeespoort Dam are more influential on the water chemistry than the presence of water hyacinth, and its control.

4.3 The relationship between detritus formation and biological control

Since the start of the inundative biological control programme in 2018, approximately 480 500 *M. scutellaris* individuals from the CBC's mass-rearing facility have been released onto Hartbeespoort Dam as of 2023 (Moffat et al., 2024). In 2021, satellite rearing stations began releasing *M. scutellaris* onto the dam and released 2 600 individuals that year and in 2023 the stations released approximately 684 000 individuals onto the dam (Moffat et al., 2024). This programme has proven successful in reducing water hyacinth cover at Hartbeespoort Dam with large mat die-offs seen every summer since 2020. The annual die-offs of large water hyacinth stands have resulted in concerns regarding the relationship between the amount of detritus formed and the biological control programme.

In the mesocosm experiment, water hyacinth plants under control programmes (biological and chemical) and those without a control programme produced similar amounts of detritus after senescence. This is an indication that, regardless of any control method, mats of the same size will produce the same amount of detritus, but at different rates. Water hyacinth plants under chemical control entered senescence much sooner (time point one) than those in other treatments and the amount of detritus formed at the end of the experiment decreased over time. This decrease in detritus formation suggests that the plants had died completely and possibly began to decompose. Time point two represented the senescence of water hyacinth plants under biological control, confirming that this programme is a slower process compared to a chemical control programme. By the time that plants under biological control entered senescence, some water hyacinth plants under no control still had healthy petioles but were in declining health. Overall, this experiment highlights the rate of detritus formation of water hyacinth under various control programmes, as noted by (Hill et al., 2021), and confirms that the amount of detritus formed by senescing water hyacinth plants is independent of control methods.

Water hyacinth plants display plasticity when subjected to herbivory pressure as seen in a few plant parameters when *M. scutellaris* populations were highest. Plant parameters are an indication of plant health – unhealthy plants generally reduce the number of ramets and leaves produced as well as petiole length (Miller et al., 2021). Additionally, unhealthy water hyacinth stands are in the beginning stages of senescence and will therefore produce and release more detritus into the water (Balasubramanian et al., 2012; Reddy & DeBusk, 1991).

Root length, root biomass and plants per square meter were three parameters that were significantly correlated to detritus production. The findings by Lakane et al. (2024) indicate that stress leads to a decrease in the root length of water hyacinth plants, supporting similar observations made in other studies. This response is a physiological adaptation to stress, where the plant reallocates resources from growth to more critical survival processes. This pattern of root shrinkage under stress highlights the plant's sensitivity to environmental changes, which can be key to developing effective control strategies. The root length of water hyacinth plants decreased when insect populations peaked and showed a negatively correlated relationship with detritus. Under herbivory pressure, water hyacinth plants reduce root length to conserve energy.

Decreased root length, therefore, is an indication of unhealthy plants (possibly in senescence), thus increasing the amount of detritus produced.

When under herbivory pressure, water hyacinth plants expend energy into a denser root system to increase the surface area from which water and nutrients can be absorbed. This was seen in the positive correlation between *M. scutellaris* density and root biomass. A positive correlation between insect density and root biomass further explains the positive correlation between detritus production and root biomass, as detritus production increased with increasing root biomass, indicating the senescence of the plants.

The amount of detritus collected was negatively correlated to the number of plants per square meter. This parameter was measured by counting the number of healthy plants in a quadrat, meaning that more plants/m^2 represented a healthy water hyacinth mat. This suggests that fewer plants/m^2 represent an unhealthy mat undergoing senescence, thus producing more detritus.

The water hyacinth stand at the Coves experienced a different timeline (growth and death) compared to the other sites on the dam. This was due to the sheltered conditions, resulting in little variation from currents and adverse weather, and more frequent insect releases by the associated rearing station. This was seen in the earlier peak of water hyacinth and *M. scutellaris* population growth. With *M. scutellaris* populations peaking earlier than other sites on the dam, the water hyacinth population at the Coves entered senescence sooner, resulting in no plants and therefore zero data in March and April 2024.

4.4 The relationship between detritus formation and water hyacinth cover

Using the Sentinel-2 MultiSpectral Instrument (MSI) satellite imagery and *M. scutellaris* counts, there was a clear lag phase in insect population growth one month after the peak of water hyacinth coverage. Additionally, after the peak of insect populations, water hyacinth cover began to decrease due to herbivory. Insect populations subsequently decreased significantly after this due to reduced food availability. Moffat et al. (2024) highlighted the success achieved through an augmentative biological control programme at Hartbeespoort Dam where they found that there was a lag phase between the peak of water hyacinth cover and the peak of *M. scutellaris* populations. It was also found that releasing insects in early spring as the water hyacinth begins to germinate (primarily young plants) reduced the size of the water hyacinth mat and resulted in a large die-off at the peak of summer.

Although water hyacinth cover was not significantly correlated to detritus production, a decrease in water hyacinth cover is indicative of dying plants and a reduction in the size of the water hyacinth mat, thus increasing the amount of dead plant material being released from the mat. This trend is corroborated by a negative correlation between the density of plants and the quantity of detritus accumulated. The significant negative correlation between detritus and *M. scutellaris* population can be explained by the death of the insect population when water hyacinth plants are no longer healthy and are in senescence (Moffat et al., 2024). There was thus a small lag phase between detritus production and the death of biological control agent populations. These trends were seen at two sites at Hartbeespoort Dam – Kurperoord and Ifafi. Water hyacinth cover reached a maximum on the dam in February 2024, while *M. scutellaris* densities were rapidly increasing at both sites. Between February and March 2024, *M. scutellaris* densities at the sites peaked while water hyacinth cover drastically decreased on the dam. In the following month, both water hyacinth cover and *M. scutellaris* populations reached a minimum, while the amount of detritus increased sharply, reaching a peak.

4.5 Conclusion and further recommendations

This study has provided a detailed analysis of the management and ecological impact of water hyacinth at Hartbeespoort Dam, with a particular emphasis on the consequences of plant senescence facilitated by biological control methods. The augmentative biological control programme using *M. scutellaris* effectively reduced the water hyacinth coverage, thereby initiating significant ecological changes in the dam's water column and sedimentation dynamics. The decrease in water hyacinth coverage correlates with increased detritus levels, suggesting that as plants die off, they release previously absorbed nutrients back into the water. This release of nutrients back into the water proves insignificant when accounting for the total nutrient concentration of the dam.

The results underscore the dual impact of water hyacinth management: while the biological control strategy successfully hinders the proliferation of the weed, it does increase the rate of detritus formation slightly, but the amount of detritus formed is not different to water hyacinth under no control programme. This accumulation of organic matter has raised concerns about the potential formation of a sludge layer on the dam floor. The results show that while the detritus load increases with senescence, over time, decomposition processes reduce the sediment load. This suggests that any “sludge layer” that may be formed will be reduced during decomposition.

In conclusion, the management of water hyacinth through biological control in an artificial reservoir has demonstrated significant ecological impacts. Although the release of detritus through the senescence process is initially a cause for concern, the sediment load is decreased during decomposition and is no different to the sediment load that would result from water hyacinth under chemical control or no control at all. It is important to note that chemical control results in rapid mass die-offs of water hyacinth mats, which could negatively impact sediment deposition if the detritus decomposition rate is not as rapid. Additionally, water hyacinth invasions threaten the ecology of these reservoirs by altering water chemistry, such as increasing alkalinity and decreasing the concentration of dissolved oxygen available for life cycles. After senescence, despite increasing the sediment load, these water chemistry parameters recovered, making the water more suitable for aquatic life.

Future studies should consider performing this study over multiple growing seasons to fully understand the sedimentation dynamics that occur during the die-off of large water hyacinth mats. Additionally, it would be valuable to conduct soil studies to determine what proportion of the sediment layer on the dam floor is composed of water hyacinth detritus compared to other types of sediment.

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