THE EFFECTS OF INVASIVE ALIEN AQUATIC PLANTS ON LARVAL MOSQUITO ABUNDANCES AND PREDATION.

BY

THORISO VANESSA MASALESA

A thesis submitted in fulfilment of the requirements for the degree of Master of Science in the Department of Zoology and Entomology, Rhodes University

SUPERVISORS: PROF. RYAN J WASSERMAN AND PROF. JULIE A COETZEE

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https://orcid.org/0000-0002-8048-584X

ABSTRACT

Invasive alien aquatic plants (IAAPs) pose significant threats to biodiversity by forming dense surface mats that limit light penetration, reduce species diversity, and impede water movement, adversely affecting freshwater ecosystems and the communities reliant on them. Climate change exacerbates these threats by facilitating the range expansion of IAAPs, amplifying their ecological and economic impacts globally. Despite extensive research on their broader effects, the role of IAAPs in facilitating mosquito proliferation is underexplored, particularly in South Africa, where conditions are ideal for both invasions and mosquito survival. Given the role of mosquitoes as vectors of various human, livestock and wildlife diseases, this represents a critical knowledge gap.

This study investigated the relationship between IAAPs and mosquito population dynamics through two experimental components. The first was a 42-day outdoor mesocosm experiment evaluating whether the IAAPs *Pontederia crassipes* (water hyacinth), *Pistia stratiotes* (water lettuce), *Azolla filiculoides* (red water fern), *Myriophyllum aquaticum* (parrot's feather), and *Salvinia molesta* (giant salvinia) differentially facilitate aquatic insect communities, with an emphasis on mosquito colonization. The second component comprised laboratory-based experiments testing predator-prey interactions between larvae of the mosquito, *Culex pipiens* and the predatory backswimmer *Anisops debilis* in the presence of IAAPs *Azolla filiculoides*, *M. aquaticum*, *Po. crassipes* and a native plant control, *Lemna minor*. Specifically, the first part of this component investigated the prey consumption rates under 100% cover of the IAAPs. The second part investigated the predator behaviour under the different treatment scenarios.

Results from the first component revealed that mosquitoes respond significantly differently to IAAPs, but that *Po. crassipes* and *M. aquaticum* facilitate the highest mosquito densities, while also having significant implications for predation and competition dynamics. Results from the second component showed that IAAPs significantly impede predation with *Po. crassipes* and *M. aquaticum* conferring the greatest mosquito prey risk reduction. Results from this second component also showed that the aquatic predator *An. debilis* adjusts its behaviour depending on the nature of the IAAP and that *M. aquaticum* in particular seems to be avoided, with implications for predator-prey encounter rates. These findings highlight the potential for specific IAAPs to exacerbate mosquito proliferation, posing risks for biodiversity and public health. This research underscores the need for targeted IAAP management strategies and opens avenues for future studies on mitigating mosquito-borne diseases in IAAP-invaded freshwater systems.

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Finally, to my friends and family, thank you for your unwavering support, love, and motivation throughout these challenging two years. Your belief in me gave me the strength to persevere, and for that, I am forever grateful.

DECLARATION

I, Thoriso Vanessa Masalesa, hereby declare that this thesis entitled “Investigating the potential relationship between Invasive Alien Aquatic Plants and mosquito population dynamics” is my original work carried out in the Department of Zoology and Entomology, Rhodes University under the supervision of Prof. Ryan J. Wasserman and Prof Julie. A Coetzee. All components of this thesis have not been submitted for any degree or examination at any other university or tertiary institution. This research was conducted under the Rhodes University Animal Research Ethics Committee (RU-AREC) permit numbers 2022-3842-7223, 2023-3842-8102 and 2024-8120-9068. All animals were collected under the Department of Economic Development, Environmental Affairs and Tourism, Eastern Cape (DEDEA-EC) permit numbers HO/RSH/ 05/2023 and HO/RSH/15/2024.

Signed: 12 December 2024

DEDICATION

This thesis is dedicated to the memory of my late mother, Linda Raisebe Masalesa whose passion for education was contagious and inspired me to pursue my academic dreams.

PREFACE

This work was presented at the following conferences (first and presenting author only):

- **Thoriso V. Masalesa**, Ryan J. Wasserman, Julie A. Coetzee. *Do floating alien invasive plants facilitate mosquito proliferation?* National Symposium for Biological Invasions, from 9 - 12 September 2024, Kimberly, Northern Cape, South Africa.
- **Thoriso V. Masalesa**, Ryan J. Wasserman, Julie A. Coetzee. *Do floating alien invasive plants facilitate mosquito proliferation?* South African Institute for Aquatic Biodiversity Student Symposium, 29 November 2024, Makhanda, Eastern Cape, South Africa

CHAPTER 1

GENERAL INTRODUCTION

Plate 1: Adult mosquito resting on *Pontederia crassipes* (water hyacinth) at an infestation site on the Schoonspruit river, in the North West province of South Africa. Photo credit: Prof Julie A. Coetzee, November 2024.

1.1 Biological invasions

Biological invasions are increasingly recognized as a significant driver of global environmental change (Pyšek et al., 2020; Turbelin et al., 2023). Invasive species, introduced through human activities, can disrupt ecosystems, reduce biodiversity (Bellard et al., 2016; Chaffin et al., 2016) and cause substantial economic damage (Pyšek & Richardson, 2010; Diagne et al., 2021). The impacts of these invasions are often insidious and long-lasting, making them a critical issue for environmental management and policy. Invasive species are defined as organisms present in a region where they do not naturally occur, often as a result of human actions, and they can have harmful impacts on both the environment and society by disrupting ecosystem services (Pyšek & Richardson, 2010; Pyšek et al., 2020). In addition to their ecological impacts, biological invasions can pose public health concerns by exacerbating human health risks through disease emergence and spread (Mazza et al., 2014). There are several pathways through which invasive species spread, with primary causes being human-mediated. Common entry points for invasions include the release of pets and aquarium animals, unintentional introductions via shipping and cargo transportation, intentional introductions for agricultural and horticultural purposes, and escapes from cultivation or captivity (Padaychee et al., 2017). These pathways facilitate the establishment of non-native species, allowing them to outcompete native species, particularly when combined with other stressors such as climate change and habitat degradation (Gutiérrez & Ponti, 2014).

During the last century, the rate at which species are translocated beyond their native ranges has substantially increased (Seebens et al., 2016). This anticipated rise in biological invasions globally is driven by interconnected factors, including global trade, climate change, and biodiversity loss. The expansion of trade and transportation networks facilitates the movement of species, often unintentionally introducing invasive species to new ecosystems (Hulme, 2009). Climate change exacerbates this issue by altering habitats, allowing invasive species to thrive while native species struggle to adapt (Bellard et al., 2013). Additionally, the decline in biodiversity due to habitat destruction and pollution reduces ecosystem resilience, making them more susceptible to invasions (Mills et al., 2010). However, quantifying the impacts and future extent of invasions remains challenging, as the patterns of invasions are often poorly documented (McGeoch et al., 2010) and their effects frequently manifest long after introduction (Essl et al., 2011). Patterns of biological invasions result from complex interactions among climate, land cover, economic, ecological, and demographic variables (Pyšek et al., 2010; Essl et al., 2011).

Effective strategies may include early detection and rapid response protocols, habitat restoration efforts to enhance ecosystem resilience, and public awareness campaigns to prevent further introductions (Higgins et al., 2017). Moreover, urbanization creates fragmented habitats that favour invasives, and gaps in research and monitoring hinder effective management strategies (Higgins et al., 2017). Collectively, these factors indicate that biological invasions are likely to increase, posing significant risks to biodiversity and ecosystem functioning (Davidson et al., 2011). In addition, the presence of invasive species has been shown to facilitate the establishment and spread of additional invasive species, thereby exacerbating ecological disruptions and biodiversity loss (Simberloff, 2006). This is known as invasion meltdown, and occurs when invasive species alter habitat conditions, such as modifying nutrient cycles or changing light availability, creating a more favourable environment for other invaders (Boulanger et al., 2019). For example, in freshwater ecosystems, invasive macrophytes can enhance nutrient availability, which supports the proliferation of invasive algae and invertebrates, leading to shifts in community dynamics and decreased native species abundance (Preston et al., 2018; Peng et al., 2023). The interaction between invasive species can lead to complex ecological cascades, making management efforts more challenging and increasing the ecological footprint of invasions (Davis et al., 2011). Both these concepts make it hard to quantify the impacts and future extent of invasions, confounding management and control.

The effects of biological invasions on ecosystems are extensive, altering ecological functioning and endangering native species and their habitats (Vila, 2011). Native species can go extinct as a result of invasive species' ability to outcompete them for resources, which reduces biodiversity (Holmes et al., 2009). Introduced rats, for example, have caused the extinction of more than 37 species and subspecies of island birds throughout the world (Simberloff, 2010). Invasive species can change water flow and fire patterns, impair the quality of habitats, and affect nutrient cycling (Simberloff, 2010). Invasive animals may feed on or outcompete local species, upsetting the complex web of ecological interactions (Stewart, et al., 2021). An example of an invasive species that has caused detrimental changes to the habitat it invaded is *Procambarus clarkii* (red swamp crayfish). Originally from Mexico, the red swamp crayfish has been introduced and become invasive to the Iberian Peninsula (Conde & Dominguez, 2015). By consuming and destroying aquatic macrophytes and decreasing macroinvertebrate abundance, it reduces food resources and habitat complexity, such as refugia and breeding places for fish and amphibians (Ficetola et al., 2012). It is also an effective predator of a broad variety of aquatic fauna, including insects, crabs, snails, and both fish and amphibian eggs and larvae (Ilhéu et al. 2007).

Biological invasions also have important societal and economic repercussions (Lodge et al., 2009). They can harm fisheries, forestry, and agricultural resources, which can cause significant financial losses. In the 1890s, rinderpest, a viral disease of ungulates brought to southern Africa from Arabia or India infected many native ungulates and caused mortality rates in some species that reached about 90%, affecting the wildlife tourism sector (Simberloff, 2010). Additionally, invasive species can harm human health by spreading diseases/pathogens or triggering allergic reactions (Hulme, 2014).

1.2 Climate change and biological invasions

One of the most urgent problems facing our world right now is climate change, as a result of human activity (Montgomery, 2009). Climate change is defined as a phenomenon that involves changes in environmental factors such as temperature and precipitation over a period of years (Skendzic et al., 2021). Climate change affects the movement of people as the increase in temperatures might influence them migrating into cooler regions. As they migrate to new countries, they might carry invasive species with them, introducing them to a new region where they might pose serious threats (Robinson, et al., 2020). Invasions may also be influenced by humans as the need to grow drought resistant plants will increase.

Climate change affects individual species and their interactions with other species and their environments, altering the structure and function of ecosystems (Weiskopf et al 2020). For an invasive species to establish, it needs to survive and reproduce rapidly at the point of introduction. And as physiological processes are often regulated by environmental factors such as temperature changes, the success and performance of both native and invasive species will be affected (Robinson, et al., 2020). Current literature assumes that a consequence of climate change is that invasive species will not be as negatively affected as native species as the range of native species represents optimal conditions, and any environmental change may represent challenges that may reduce native species survival rates (Hellmann, et al., 2008; Thuiller, et al., 2008). Furthermore, alterations in global temperatures and rainfall regimes may favour an increase in the distribution, number, and impact of invasive alien species (Vila et al, 2006). Its involvement in promoting and changing the dynamics of biological invasions is a crucial part of this impact. Invasive species pose serious dangers to native biodiversity, ecosystems, and human well-being because of how climate change acts as a catalyst for their establishment and proliferation in new areas (Hulme, 2017). As wind and ocean currents are altered due to a changing climate, there may be greater opportunities for invasive alien species from neighbouring regions to migrate and establish in new environments (Hulme, 2017).

Climate change has two effects on biological invasions. For starters, it makes it easier for invasive species to survive and establish themselves in new ecosystems (Hulme, 2017). As temperatures rise, formerly unsuited places become ideal for invading species survival and reproduction (Gutierrez & Ponti, 2014; Robinson, et al., 2020).

This can be seen in Europe as the Asian tiger mosquito (*Aedes albopictus* Skuse [Diptera: Culicidae]) has invaded through importation and is thriving as the temperatures now favours their establishment (Bhaumik, 2013; Stachowicz, et al., 2002). Warmer temperatures can lengthen the growing season, giving invasive plants more opportunity to spread and outcompete native species. Furthermore, changes in precipitation patterns, such as excessive rainfall or drought, can cause ecological disruptions that favour the introduction of invasive species (Bogale & Tolossa, 2021; Weltzin, et al., 2003). Second, climate change has an impact on the interactions between invasive and native species. It has the potential to disturb ecosystems processes, making them more vulnerable to invasion (Dutta, 2018). Because of climate change, native species may endure increased stress and reduced resilience, leaving them more vulnerable to competition from alien species (Dutta, 2018). Furthermore, climate change has the potential to alter species phenology, affecting the timing of crucial life cycle events such as flowering, migration, and reproduction (Hulme, 2017). As a result, the synchronization between native species and their ecological partners may be interrupted, undermining ecosystem stability and allowing invading species to spread (Hulme, 2017).

1.3 Invasive alien aquatic plants

The pathways through which aquatic invasive weeds spread are diverse, including those associated with the ornamental, horticultural and aquarium trade (Coetzee, et al., 2017). When introduced into new ecosystems, these non-native plant species have the potential to spread quickly and outcompete native plants, having detrimental effects on the environment, the economy, and society (Barrett, 1986; Chamier, et al., 2012). This may be because they do not have natural enemies, have rapid growth rates and a high phenotypic plasticity which helps them establish quickly in new habitats (Hill, 2003).

Understanding the invasion biology of an organism is important for its effective management and control. Bauer (2012) and MacDougall & Turkington (2005) have identified and categorized invasive species into three groups based on their relationship with disturbance, which can be used to understand IAAPs. The three categories are as follows: 1. passengers who are dependent on disturbances for establishment and should the disturbance be removed; the invasion and all its impacts will disappear; 2. drivers of biodiversity loss which are species that do not need disturbances to establish; and 3. backseat drivers which is what has been oberved in South Africa. Backseat drivers occur when there is an initial disturbance that causes the invasive species to establish and once established, they continue to invade and impact the ecosystem even when the initial disturbance is removed (Bauer, 2012). IAAPs in South African waterbodies rely on disturbances such as slow-flowing permanent waterbodies, caused by impoundments, to facilitate their establishment and gain an advantage over native indigenous aquatic plants (Coetzee & Hill, 2012; Hill & Coetzee, 2017). Perhaps the most important driving factor, however, for the successful establishment of these IAAPs is the nutrient levels of the waterbodies they invade. South African waterbodies have elevated levels of nutrients due to the poor state of wastewater treatment works that are a result of the growing population (Coetzee et al., 2009; Herbig, 2019; Adams et al., 2020). Poorly treated water enters waterbodies which then raises the levels of nitrogen and phosphorus, creating ideal environments for IAAPs (Adams et al., 2019). Elevated levels of phosphorus and nitrogen stimulate photosynthesis and in turn this eutrophication allows the plant to grow rapidly and form dense mats. This along with the absence of natural enemies causes invasive aquatic plants to be a significant problem.

Invasive alien aquatic plants have serious detrimental effects to freshwater ecosystems which include loss of native fauna and flora, thereby altering the biodiversity of that region which may destabilise the system (Coetzee et. al., 2018). Invasive waterweeds form dense mats which limit light penetration and reduce or limit primary production by phytoplankton, decreasing the dissolved oxygen concentration (Schultz & Dibble, 2012). Decreased dissolved oxygen concentrations lead to death of aquatic fauna and their decomposition further requires oxygen causing the oxygen content in the water to further decrease (Moreira, et al., 1999). Decomposition of these species increases the nutrient concentration of the system, further favouring the growth of invasive aquatic weeds, which continue to flourish in the presence of these nutrients until they occupy the entire system (Yang et al., 2021). It is clear that invasions in freshwater habitats alter species composition affecting biodiversity, the structure and functioning of the ecosystem, as well as the livelihoods of people who utilize freshwater systems for survival by either fishing or using the water for domestic purposes (Coetzee et al., 2018). For example, *Egeria densa* Planch., (Hydrocharitaceae), a submerged, mat-forming IAAP can modify and dominate aquatic habitats, outcompete native plants and modify natural ecosystems (Chamier, et al., 2012).

Of the IAAPs, there are relatively few floating IAAP species that have invaded freshwater ecosystems (Coetzee et al., 2018). However, where they have invaded, floating IAAPs have often caused significant ecological impacts to receiving environments, through limiting light penetration, altering water quality and disrupting native biodiversity (Pysek et al., 2020). In areas where hydropower is used, floating invasive aquatic plants can affect power production as they can end up clogging turbines (Aloo et al., 2013). They have the capacity to accumulate into dense mats or stands, altering the physical makeup of bodies of water and obstructing water movement, both of which have an adverse effect on aquatic life, including fish, amphibians, and invertebrates (Orwa, et al., 2015). An example is the South American water hyacinth (*Pontederia crassipes* Mart., (Pontederiaceae)) which has invaded all continents except for Antarctica. It covers parts of Lake Victoria in Africa, many lakes and rivers in the southeastern United States and various waterbodies in Asia and Australia (Aloo, et al., 2013; Coetzee, et al., 2017), making life difficult for those who depend on invaded waterbodies for survival. Another example is *P. stratiotes* which has invaded two aquatic systems in Botswana producing seeds that germinate after seasons if draught making its control hard (Kurugundla, 2014).

1.4 Floating IAAPs in South Africa

South African waterways have been invaded by invasive aquatic weeds since the late 1800s when water hyacinth was introduced (Hill & Coetzee, 2017). Since then, other invasive species, mostly from South America, have also managed to invade freshwater systems, causing major problems to biodiversity and the economy. Invasions of these species was made easy as the waterways in South Africa are nutrient rich due to an increasing population which caused an increase in nutrient-rich effluents ending up in the waterways (Coetzee & Hill 2012). South Africa has a limited presence of indigenous floating aquatic plants due to the scarcity of natural lakes (Hill et al., 2020). Consequently, its freshwater ecosystems have not developed extensive floating plant species. Among the few floating aquatic plants found in South Africa, species such as *Lemna minor* (Araceae), known as common duckweed, have short root systems and small leaves, and are competitively inferior to the larger floating IAAPs.

The most problematic species in South Africa are some of the floating aquatic plants from the Amazon basin (Hill et al., 2020). In particular, *Po. crassipes* (water hyacinth), *Pistia stratiotes* L. (Araceae) (water lettuce), *Azolla filiculoides* Lam. (Azollaceae) (red water fern), *Myriophyllum aquaticum* (Vell. Conc.) Verd. (Haloragaceae) (parrot’s feather) and *Salvinia molesta* D.S. Mitch. (Salviniaceae) (giant salvinia) do particularly well in nutrient rich systems throughout the region. These species are all managed as invasive in South Africa as they do not have natural enemies and rely on disturbances such as eutrophication which allow them to establish and have a competitive advantage over native plants (Hill et al., 2020). An overview of each species is outlined below.

Pontederia crassipes (water hyacinth)

Pontederia crassipes is a floating aquatic plant native to the Amazon basin in South America (Hill, 2003). The primary mode of its spread was originally for ornamental purposes as it has attractive flowers (Barrett, 1989). It has a rapid growth rate and can double its biomass in about 14 days if the conditions are ideal (Hill & Coetzee, 2017). In its native environment, its growth is controlled by its arthropod and pathogen natural enemies, but in environments which it invades, it grows uncontrollably due to the lack of these natural enemies (Coetzee & Hill 2012).

Pistia stratiotes (water lettuce)

Pistia stratiotes is a free-floating aquatic plant that is native to Brazil, South America and has also invaded most continents (Langeland & Burks, 1999). It has invaded many waterbodies with similar harmful implications as water hyacinth. Like water hyacinth, it forms dense mats on the water surface which limits light penetration which decreases productivity and in turn decreasing biodiversity (Moore & Hill, 2012). Water flow is decreased due to the dense mats it forms (Hill, 2003).

Azolla filiculoides (red water fern)

Azolla filiculoides is a small, heterosporous, floating aquatic fern native to South America and has spread out of its native habitat and invaded all continents (McConnachie et al., 2004). It was first introduced to South Africa in 1948 as a pond plant but spread throughout the country due to its rapid uncontrollable growth and the absence of natural enemies which could help in regulating its population (McConnachie & Hill, 2005). It inhabits still and slow-moving water, forming a dense layer on the surface which prevents light penetration and can degrade the quality of water. The dense mats also affect how water is utilized by preventing boats from moving though the water, clogging drains and hydropower pumps (Hill, 1997).

Myriophyllum aquaticum (parrots feather)

Myriophyllum aquaticum is an emergent rooted aquatic plant that is native to South America (Rojas-Sandoval, 2024). It was introduced to South Africa in 1919 as an ornamental plant for aquariums and ponds and has since then been classified as an invasive species affecting freshwater ecosystems (Wely & Coetzee, 2013). Like other invasive aquatic species, it has serious ecological impacts to water bodies. It outcompetes native species for light, nutrients and space, decreasing the biodiversity of that waterbody it invades (Barrett, et al., 1993). Its dense growth can clog waterways, alter the habitat by decreasing flow of water and diversity of that water body. It can also affect water quality by altering oxygen levels, sedimentation rates and nutrient cycling (Yang, et al., 2011).

Savinia molesta (giant salvinia)

Salvinia molesta is a free-floating fern, also native to South America, that invades still and slow-moving freshwater ecosystems. It was introduced to South Africa in the early 1900s (Hill, 2003). Its impact on waterbodies it invades are the same as the other invasive aquatic plants, forming dense mats which limit light penetration and primary production in the waterbodies (Hill, 2003). It clogs waterways affecting how the communities use the waterbody dependent on it and decreases biodiversity (Hill, 2003).

These five major invaders of South African water systems show characteristics of problematic invasive weeds characterised by rapid growth rates and dense mat accumulation, outcompeting native species and homogenizing systems they invade (Hill, 2003). As they can alter ecosystems, it is important to understand the full suite of ecosystem services disruptions associated with these plants, as well as ecosystem disservice provisions. One aspect of floating IAAP ecology that is underexplored is the notion that they may potentially facilitate the success of other invasive or problematic species, such as mosquitoes.

1.5 Invasive mosquitoes

Arthropods are one of the few taxa that have invaded many freshwater ecosystems (Coetzee et al., 2018). Their primary cause of invasion is through aquaculture and transportation. However, there are several factors that contribute to the success of invasive insects. These include climate change as it can provide suitable environmental conditions for invasive insects, allowing them to establish in new regions that they have not established in yet (Kiritani, 2006). Habitat disturbance can also facilitate invasion as it can create favourable conditions for invasive insects to thrive (Kiritani, 2006). The majority of studies on the invasion potential of arthropods unfortunately do not cover species that harbour infectious diseases.

For years, insects have been known for spreading infectious diseases in both human and animals, damaging forests, destroying infrastructure, ruining crops, and food stocks, and altering ecosystem functions, thereby weakening the resilience of ecosystems to other disturbances (Hulme, 2009). They are among the worst invasive taxa due to the multiple effects they have on ecosystems and various communities (Bradshaw, et al., 2016). Their capacity for quick establishment and spread has the potential to have catastrophic effects on native species, ecosystems, human activities, and human and animal health (Hulme, 2014). Some notable examples include the Emerald Ash Borer (*Agrilus planipennis*, Fairmaire, Coleoptera, Buprestidae) in North America, which has devastated ash tree populations, and the Brown Marmorated Stink Bug (*Halyomorpha halys*, Stal, Hemiptera, Pentatomidae), an agricultural pest that has invaded North America and Europe (Suckling & Sforza, 2014).

One of the worst invasive insects are mosquitoes which are a concern as they transmit multiple diseases as they carry pathogens (Juliano & Lounibos, 2005). Over the past years, these species have spread further and further from their place of origin due to transportation, trade, and climate change (Montarsi, et al., 2015). An example of this is the tiger mosquito *Aedes albopictus* that was first imported to Italy in the early 1990s, responsible for an outbreak of chikungunya virus in 2007 (Angelini, et al., 2007; Montarsi, et al., 2015). Another global invader is *Aedes aegypti* Linnaeus (Diptera: Culicidae) which originates and central Africa and has been spread to many regions, including Southern Africa (Iwamura et al., 2020). These and other mosquitoes in particular pose a serious hazard to human life since they are vectors of various pathogens that can lead to disease transmission, such as dengue fever, yellow fever and zika virus. There are estimates of 390 million infections every year, with up to 3.9 billion people potentially at risk of illness resulting from mosquito related pathogens (Mclaughlin & Dearden, 2019).

1.6 Invasive aquatic plants and mosquitoes

Invasive aquatic vegetation may have an impact on the habitat quality of larval mosquitoes, an issue that has received little attention as some water weeds attract mosquitoes, while others repel them (Portilla et al., 2021). Aquatic plants can provide ideal habitats for larval mosquito populations by offering a safe haven from predators, reducing the oxygen content in water to keep predators and competitors that are dependent on high concentrations of dissolved oxygen away (Portilla et al., 2021). These plants are also a food source for larval mosquitoes by providing fine particulate matter produced through decay or by promoting the growth of bacteria and other microbes which exude chemical cues (volatiles) that attract mosquitoes (Afify & Galizia, 2015). Mosquitoes use various cues to assess whether a habitat is conducive for reproduction, possibly including the presence of stimuli from aquatic macrophytes (Turnipseed , et al., 2018). Flowering plants also attract adult mosquitoes encouraging oviposition as they have nectar which is a food source for mosquitoes (Portilla, et al., 2021). Wetlands with water lettuce (*Pi. stratiotes*) are preferred host sites for many mosquito species (Silver, 2008) due to the chemical cues they exude. In 2018, Turnipseed et al. ran a study on the behavioural response of gravid mosquitoes to aquatic macrophyte volatiles. The results showed that aqueous infusions from water hyacinth and water lettuce possess attractive properties that facilitate *Culex quinquefasciatus* Say (Diptera: Culicidae) and *A. aegypti* mosquito oviposition more than two other macrophytes evaluated which were *Hydrocotyle vulgaris* Linnaeus, (Araliaceae) (pennywort) and parrot's feather. These attractive volatiles may include specific attractive chemicals or a blend of chemicals in a ratio and concentration that gravid female mosquitoes associate with preferred oviposition sites (Rejmánková et al., 2005) . Attractive plants, such as water hyacinth and water lettuce presumably produce concentrations and mixtures of volatile chemicals optimal for the attraction of mosquitoes.

On the other hand, aquatic species may also reduce mosquito populations. They limit light penetration as they form dense mats on the water surface which prevents larval mosquitoes from accessing atmospheric oxygen and may also decrease the temperature of the water which slows down their development (Walton et al., 2013).

They can also prevent female adults from ovipositing their eggs (Portilla, et al., 2021). Floating macrophytes shade the water, decreasing algae growth, which is a food source for larval mosquitoes (Portilla, et al., 2021), and may also attract predators such as fish and competitors that are tolerant of the harsh environmental conditions they create (Reiskind & Wilson, 2004(a)). An example of this is the eared water fern (*Salvinia auriculata* Aubl. [Salviniaceae]) which represses larval mosquito populations by limiting access of gravid females to the water surface (Hobbs & Molina, 1983). Portilla et al. (2021) found that the submerged *E. densa* inhibits mosquitoes as it inhibits phytoplankton through allelopathy therefore reducing food for the mosquito larvae. Parrot's feather and pennywort produce the same chemicals but in a different ratio than those produced by water hyacinth and water lettuce, and this makes them less attractive to mosquitoes (Turnipseed et al, 2018). Soares et al. (2021) found that *Mansonia* larvae attach to submerged invasive *Pistia* sp. to get oxygen and nutrients which increases their survival and proliferation. Therefore, controlling invasive aquatic weeds may have an impact on mosquito populations by either increasing or decreasing their populations.

Invasive aquatic plants can significantly affect mosquito populations through several mechanisms. These plants can attract mosquitoes for oviposition by altering habitats to create stagnant water, reducing flow, and forming ideal breeding sites (Bownes & Pringle, 2019). For example, the dense mats formed by water lettuce provide habitats for mosquito genera such as *Anopheles*, the invasive *Aedes*, and *Culex*, allowing them to oviposit and develop throughout their larval stages (Juliano & Gravel, 2002). This creates public health concerns, as these mosquitoes are vectors for diseases like yellow fever and malaria (Teng, 2016). Invasive plants may also promote mosquito recruitment and survival by offering food sources and refuge. Their long roots and dense leaves can protect mosquito larvae from predators, while symbiotic relationships with blue-green algae provide additional food sources (McConnachie & Hill, 2005). Furthermore, invasive species often reduce biodiversity by outcompeting native plants and aquatic organisms. This decline in biodiversity reduces competition and predation pressures, further favouring mosquito populations (Van der Velde et al., 2017). The habitat changes caused by plants like parrot's feather exemplify how these factors synergistically influence mosquito ecology. A study done by Midgley et al., (2006) showed the detrimental effects that water hyacinth has on the abundance and diversity of benthic invertebrates. They found that the presence of water hyacinth mats decreased the biodiversity of benthic invertebrates which decreased the stability of the ecosystem. Water hyacinth alters water chemistry and composition creating a suitable habitat for larval mosquitoes and this might also get rid of predators or competitors by creating hostile habitats for them. A study done by Portilla et al. (2020) found that water hyacinth, indeed, provided an ideal breeding ground for mosquitoes by offering protection from predators and limiting competition or predation by decreasing the dissolved oxygen content of water.

1.7 Knowledge gaps, aims and objectives.

There is a significant knowledge gap regarding the complex and long-term interactions between invasive species and other organisms in the ecosystem, whether native or invasive. The detrimental effects of invasive species tend to worsen over time, as evidenced by numerous studies (Mack, et al., 2000; Aloo, et al., 2013; Coetzee, et al., 2019). Invasive species have been well-documented to cause alterations in ecosystem structures, functions, and services (Russo & Boyd, 2018). While immediate and direct impacts, such as the displacement of native species, have been investigated, the consequences of ongoing and long-term interactions of various taxa with invasive species remains largely unknown and warrants further exploration. An example of such overlooked interactions is those between floating invasive aquatic plants and mosquitoes. The potential facilitation of mosquito populations by key invasive and widespread invasive plant species is a major knowledge gap in the mosquito ecology and biological invasions literature, with major direct implications for healthcare burden in invaded areas. Exploring these interactions is relevant for mitigating potential negative health impacts on human populations, wildlife, domestic animals, and the overall environment. This knowledge is key to developing strategies that protect our ecosystems and promote public health.

The overall aim of this study was to investigate if mosquito populations are facilitated by floating IAAP species . The specific objectives of the study were to:

Objectives:

i) determine whether floating IAAP species facilitate mosquitoes' proliferation in water bodies.

ii) determine whether floating IAAP species alter predator-prey interaction outcomes.

iii) determine whether avoidance behaviour of key predators is evident in relation to floating IAAP species.

1.8 Structure of the thesis

To address these objectives, the study employed a combination of outdoor mesocosm, and indoor microcosm experiments. These are presented as discrete data chapters (Chapter two & Chapter three, respectively) and are succeeded by the general discussion chapter (Chapter Four). The last section is the combined references of all the chapters

To address the study's objectives, a combination of outdoor mesocosm and indoor microcosm experiments was employed. The thesis is organized into four chapters:

- **Chapter One** (present chapter) provides a general background to the study, outlining the knowledge gaps, research aim and objectives.
- **Chapter Two** investigates whether invasive alien aquatic plants (IAAPs) attract mosquitoes and favour their survival. This chapter is structured into sections covering the introduction, methods, results, and discussion of key findings.
- **Chapter Three** examines how IAAPs influence predator-prey interactions. Similar to Chapter Two, it includes detailed sections on the introduction, methods, results, and discussion.
- **Chapter Four** consolidates the findings from Chapters Two and Three, providing a general discussion and drawing overarching conclusions from the study.

A single **References** section at the end of the thesis presents all the references used in this study in a single location to reduce repetition.

CHAPTER 2

Do floating invasive aquatic alien invasive plants facilitate mosquito proliferation?

Plate 2. Mesocosm experimental set up outside the Rhodes University Department of Zoology and Entomology. Photo credit: Thoriso V. Masalesa, November 2023.

ABSTRACT

Floating invasive alien aquatic plant (IAAP) species are problematic in many lentic and slow-flowing lotic environments globally. In southern Africa, there are very few examples of native floating aquatic plants and so invasive counterparts have the potential to disproportionately alter invaded ecosystems through provision of novel characteristics. One aspect of floating IAAPs ecology that has received no attention in Southern Africa is the potential role these plants play in facilitating mosquito proliferation. The present study, using a mesocosm approach, assesses if the IAAPs *Pontederia crassipes* (water hyacinth), *Pistia stratiotes* (water lettuce), *Azolla filiculoides* (red water fern), *Myriophyllum aquaticum* (parrot's feather) and *Salvinia molesta* (giant salvinia) differentially facilitate discrete aquatic insect communities over time, with an emphasis on mosquito colonization dynamics. The 42-day experiment revealed that at an alpha-diversity level, the treatments had limited effects on community structure. However, the floating IAAPs indeed facilitated discrete community dynamics at the treatment, time and treatment × time levels. These effects were driven primarily by *Cx. pipiens* mosquito numbers. In particular, *Po. crassipes* and *M. aquaticum* treatments were favoured by mosquitoes, while *A. filiculoides* and *S. molesta* treatments were largely avoided. In addition to treatment level effects, there was also evidence for indirect competitor and predator-level effects on *Cx. pipiens* numbers across treatments over time. This study highlights additional ecosystem disservices associated with floating IAAPs in southern Africa and offers further insight for triaging of species-level mitigation measures.

Keywords: *Culex pipiens*; habitat selection; oviposition, *Pontederia crassipes* (water hyacinth), *Myriophyllum aquaticum* (parrot's feather).

2.1 INTRODUCTION

The relationship between vegetation (aquatic and terrestrial) and mosquitoes in general, particularly under natural conditions is well-studied. For example, aquatic plants can provide ideal habitats for larval mosquito populations by offering safety from biotic threats through, for example, reduction of oxygen content in water reducing fully aquatic predator and competitor utilization of these zones (Portilla, et al., 2021). These plants may also serve as a food source for larval mosquitoes by providing fine particulate matter produced through decaying or by promoting the growth of bacteria and other microbes which exude chemical cues (volatiles) that attract mosquitoes (Afify & Galizia, 2015). Mosquitoes use various cues to assess whether a habitat is conducive for reproduction, possibly including the presence of stimuli from aquatic macrophytes (Turnipseed, et al., 2018). Flowering plants also attract adult mosquitoes encouraging oviposition as they have nectar which is a food source for mosquitoes (Portilla, et al., 2021). Conversely, plant species may also reduce mosquito populations by limiting light penetration as they form dense mats on the water surface which prevents larval mosquitoes from accessing atmospheric oxygen and may also decrease the temperature of the water which slows down their development, or prevent female adults from ovipositing (Portilla, et al., 2021). They also shade the water, decreasing algae growth, which is a food source for larval mosquitoes (Portilla, et al., 2021). Aquatic plants may also attract predators such as fish and competitors that are tolerant of the harsh environmental conditions they create (Reiskind & Wilson, 2004(b)). Leaf litter from certain plant species has been shown to serve as a repellent for mosquito oviposition, with implications for control measured (Cuthbert et al., 2019). As a result, the relationship between plants and mosquitoes is complex, largely due to the variation in factors such as plant structure, decay rates, production of seed and secondary metabolites and other characteristics across species. There are numerous gaps in our understanding on even the fundamentals of plant-mosquito interactions (Silver, 2008; Rejmánková et al., 2013). In relation to interactions between floating invasive alien aquatic species specifically, there are certainly knowledge gaps.

Invasive alien aquatic plants (IAAPs) often aggressively colonize water bodies, disrupting ecosystems, human activities, and biodiversity (Hussner et al., 2017; Macedo et al., 2024). These plants often thrive due to the lack of natural predators and competitors in their new environments as well as anthropogenic environmental changes which promote proliferations (Lowe et al., 2000). In many freshwater environments, these plants have serious detrimental effects on ecosystems, which include loss of native fauna and flora and associated decreases in regional biodiversity, with implications for ecosystem stability and in many instances, ecosystem services (Havel et al., 2015). Floating IAAPs can be particularly problematic in certain parts of their invasive range given that they can represent a largely novel plant type and functional group for the receiving environment (Strayer, 2012; Aloo et al., 2013). While work addressing how floating IAAPs compromise aquatic ecosystems and service provisions is prevalent, progress in the evaluation of associated ecosystem disservices is lagging. One aspect that is still poorly understood is if and how floating IAAPs potentially facilitate increases in problematic or nuisance species.

Floating IAAPs often form dense mats on the surface of lentic and slow-flowing lotic environments, irrespective of the depth of the water (Madsen et al., 1991; Vilamagna & Murphy, 2010). One of the main direct effects of these IAAPs on the physicochemical environment is that the dense mats typically significantly limit light penetration, resulting in reductions in primary production by phytoplankton and subsequent decreases in the dissolved oxygen concentration of the water column (Higgins & Vander Zanden, 2010). This has implications for secondary productivity dynamics as there is less food for primary consumers such as zooplankton and secondary consumers such as zooplanktivores. The reduction in dissolved oxygen concentrations also limits the extent to which secondary and tertiary consumers can proliferate (Moreira, et al., 1999). Floating IAAPs in lentic habitats also increase the plant edge ecotonal surface area dynamics, with implications for microhabitat availability (Pieczyńska, 1990). Decomposition of IAAPs when they die, has further implications for nutrient concentrations and the availability of detrital material, further shifting food webs from phytoplankton- (green food web) to a detrital-based (brown food web) systems (Thomaz & Bini, 2003).

One such ecosystem disservice anecdotally linked to floating IAAP proliferation, is that of facilitation of mosquito success. Physical and chemical changes driven by IAAPs could promote mosquito recruitment by changing nutrient properties in waters, increasing predator-free space, or providing increased resting habitat availability for adults. While some studies have alluded to the link between certain floating IAAP species and mosquitoes (e.g. Stone et al., 2018, Turnipseed et al., 2018, Portilla et al, 2021), few studies have attempted to quantify this relationship (Cuthbert et al., 2020). Indeed, there is generally a significant knowledge gap regarding the complex and long-term direct and indirect interactions between invasive species and other organisms in the ecosystem, whether native or invasive. While immediate and direct impacts, such as the displacement of native species, have been thoroughly investigated, the consequences of ongoing and long-term interactions with invasive species and mosquito populations remain largely unknown and warrant further exploration.

One of the most problematic insect groups are mosquitoes, many of which are either nuisance species, of healthcare concern as vectors for various diseases, or both (Cuthbert et al., 2020). From a healthcare perspective, mosquitoes are implicated as leading causes of morbidity and mortality worldwide through their ability to vector pathogens that cause serious diseases, such as West Nile Virus, onchocerciasis, and malaria (Farajollahi et al., 2011; Rejmánková et al., 2013). From a non-healthcare nuisance perspective, the disservices of mosquitoes and mosquito control measures are likely considerable. Globally, there is historic and ongoing spread of various species of mosquito, driven by many of the same factors that promote floating IAAPs, including climate change (Montarsi, et al., 2015). Similarly, in many compromised areas, even indigenous problematic mosquito species are proliferating due to environmental modification (Gubler, 2002, Juliano & Lounibos, 2005). Given the semiaquatic nature of mosquitoes, environmental considerations for investigating mosquito proliferation dynamics need to incorporate aquatic drivers (Buxton et al., 2020). It is well known that proliferation of floating IAAPs has significant impacts on freshwater systems, their productivity levels, food web pathways and other dynamics, therefore it is not unlikely that such changes may have implications for mosquito success or failure.

In southern Africa, there are very few examples of native floating aquatic plant species (Seymour, 2014). As such, it can be argued that floating IAAPs have the potential to disproportionately impact ecosystems of the region, or other regions where floating plants are only marginally part of the native aquatic plant community. This ecological novelty is compounded by regional habitat transformations, where substantial portions of aquatic systems have been human-altered by dams and artificial connections. These considerations also have implications for human communities directly reliant on invaded freshwater ecosystems, as traditional knowledge on ecosystem service extraction may no longer apply to the compromised systems invaded by floating IAAPs (Coetzee et al., 2018). Furthermore, given that regional ecosystem disservice research on the floating IAAPs is lagging, the extent to which these plants affect human communities regionally is likely underestimated. The aim of the study was to investigate the potential relationship between the presence of key regionally and globally problematic floating IAAPs and mosquitoes. Specifically, using an experimental mesocosm approach, aquatic community development over time with an emphasis on early life-stage mosquito numbers, was determined across treatments containing cover of floating IAAP species (*Pontederia crassipes*, *Pistia stratiotes*, *Azolla filiculoides*, *Myriophyllum aquaticum* and *Salvinia molesta*). It was hypothesized that selected floating IAAPs would 1) facilitate the development of distinct aquatic communities, and that 2) differences in mosquito abundances would be a key driver of community differences and succession dynamics. The study, therefore, has implications for management of both IAAPs and mosquito populations, offering valuable information for the ecological intervention and public health strategies.

2.2 METHODS AND MATERIALS

2.2.1 Mesocosm setup

Thirty-six 70 L outdoor mesocosms (6 treatments × 6 replicates) were employed for the study over a 42-day experimental period during November and December 2023. The mesocosm array was established on Rhodes University campus, in a field behind the Life Sciences Building (33°18'37.08"S; 26°31'7.47"E). Each mesocosm was a 70 L polypropylene oval-shaped wash tub (75 cm high × 55 cm wide × 32 cm high) (Figure 2.1a), filled to a depth of 23 cm, with 50 L of borehole water from on site and the water level marked. Five floating IAAP treatments and one control treatment were established. Each mesocosm treatment was inoculated with 2.48g of fertilizer (Multisol (N), Culterra Pty Ltd), resulting in similar Day 0 concentrations of nitrates, ammonia, and phosphates across the different treatments. The different invasive plants; *Po. crassipes*, *Pi. stratiotes*, *M. aquaticum*, and *S. molesta* were added to each mesocosm, covering 50% of the surface area. A PVC conduiting tube divided each mesocosm in half, to ensure that the plants stayed on one side of the mesocosm only (Figure. 2.1b). All plants were obtained from the Waainek Research Center at Rhodes University, with plants carefully rinsed before transport and transfer to mesocosms and any semiaquatic organisms removed. Controls were the same as the plant treatments, but with an absence of plants. Nitrate levels at the start of the experiment were 19.1 (± 4.4) mg/L in the control, 11.6 (± 3.5) mg/L in *Po. crassipes*, 13.8 (± 5.1) mg/L in *Pi. stratiotes*, 14.5 mg/L in *A. filiculoides*, 16.78 (± 5.2) mg/L in *M. aquaticum*, and 14.2 (± 4.8) mg/L in *S. molesta* treatments. Ammonia levels at the start of the experiment were 3.2 (± 0.5) mg/L in the control, 2.7 (± 0.3) mg/L in *Po. crassipes*, 2.6 (± 0.2) mg/L in *Pi. stratiotes*, 2.7 (± 0.3) mg/L in *A. filiculoides*, 3.1 (± 0.5) mg/L in *M. aquaticum*, and 2.8 (± 4.4) mg/L in *S. molesta* treatments. Phosphate levels at the start of the experiment were 7.1 (± 2.0) mg/L in the control, 7.4 (± 0.8) mg/L in *Po. crassipes*, 6.4 (± 3.5) mg/L in *Pi. stratiotes*, 4.8 (± 4.0) mg/L in *A. filiculoides*, 8.3 (± 1.9) mg/L in *M. aquaticum*, and 7.1 (± 2.1) mg/L in *S. molesta* treatments.

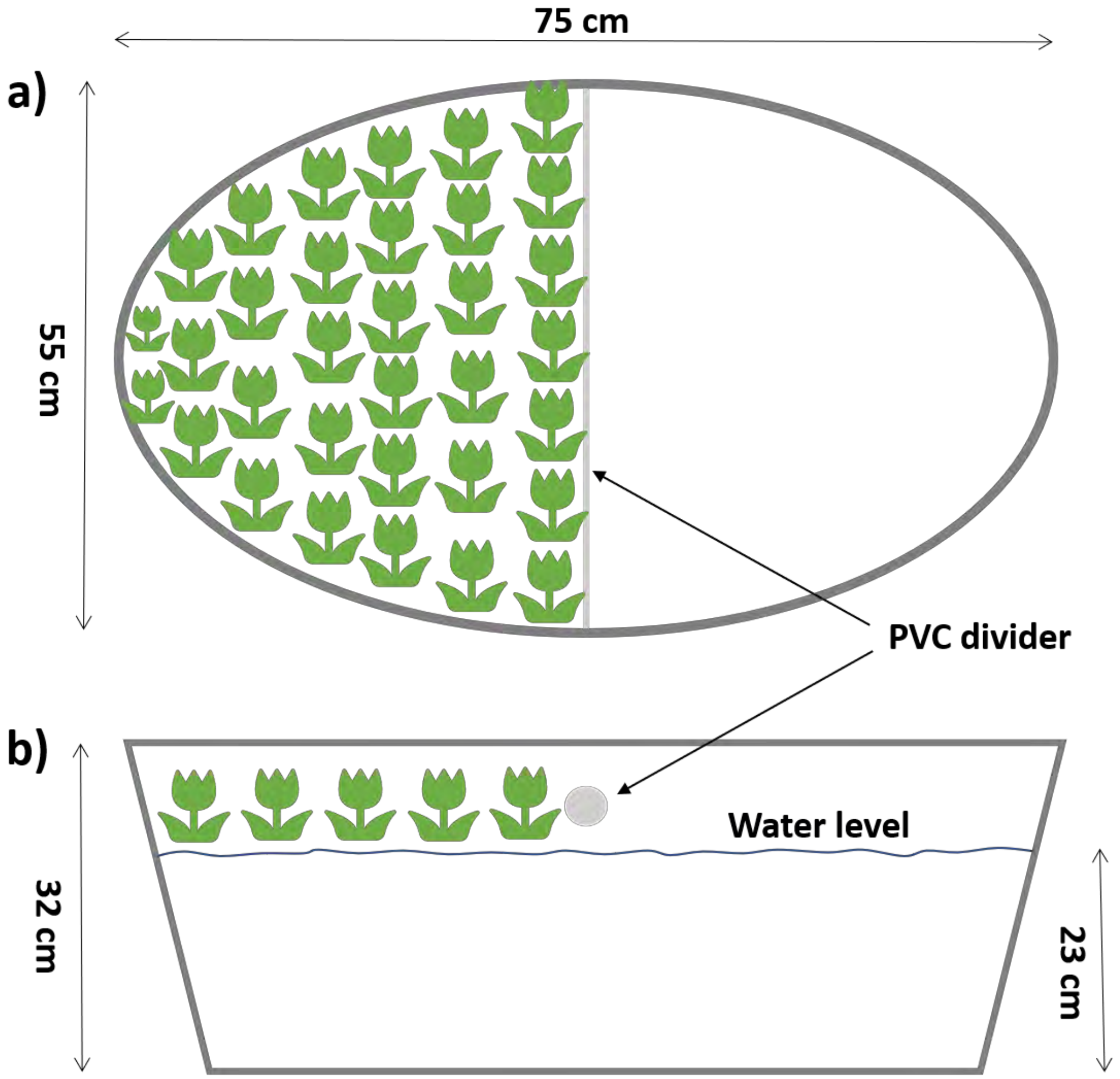
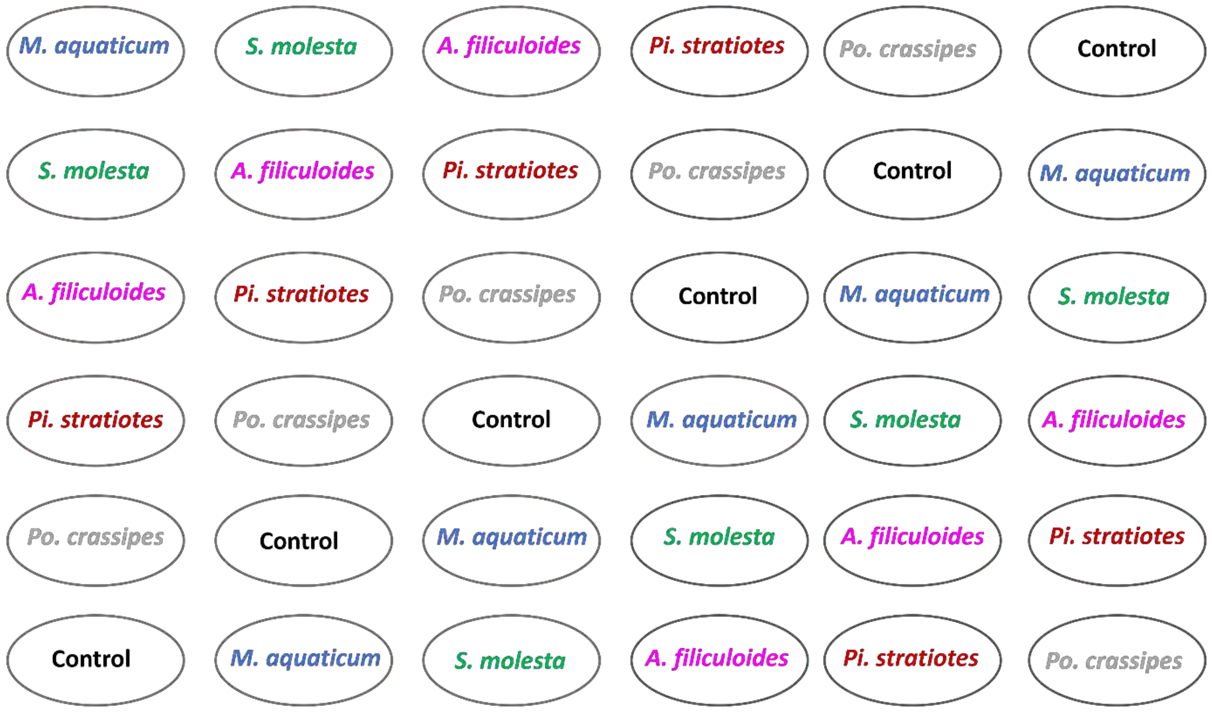


Figure 2. 1: Schematic representation of each mesocosm highlighting a) an aerial view the mesocosm with 50% of the water surface covered by a floating invasive alien aquatic plant species, kept in place with a PVC pipe divider, and b) a side-view of the mesocosm and the level to which water was maintained throughout the experiment.

The 36 mesocosm grid array involved an alternating pattern within each row, whereby one replicate of each treatment was represented in each row and in each column (Figure 2.2). Mesocosms were checked daily, and water levels maintained with borehole for the duration of the 42-day period. Sampling took place on days 0, 7, 14, 21, 28, 35 and 42 over the experimental period, with Day 0 serving as the commencement of the experiment when the mesocosms were established. Per sampling event, physico-chemical parameters of the water from each mesocosm were measured using an Aquaread AP-5000 (Aquaread Ltd., UK) handheld water quality meter. These were temperature (°C- surface water and bottom of mesocosm), pH, dissolved oxygen (mg. L^{-1}), electrical conductivity (µS cm^{-1}), total dissolved solids (mg. L^{-1}) and turbidity (NTU). Nitrate levels were also determined *in situ* per sampling event, using a YSI ProQuatro Multiparameter Meter *(*YSI Inc., Model ProDSS*)*. Similarly, water samples (50 ml) were collected from each mesocosm per sampling day for phosphate and ammonia determination in the laboratory, with an additional water sample (250 ml) collected for chlorophyll-*a* determination. These water samples were placed on ice in a dark cooler box prior to analyses in the laboratory within 6 hours of collection. Biological sample collection was done on days 7, 14, 21, 28, 35 and 42. Sampling involved the careful transfer of the floating weeds to a 70 L polypropylene holding container with clean borehole water. A sweep net approach was then used, whereby a standardized net haul was employed to collect aquatic fauna in each mesocosm. A half-moon shaped, flat bottom-rimmed sweep net (30 cm × 50 cm, 500 µm mesh sieve) was hauled three times through each of the top, middle and bottom waters of each mesocosm, with the composite as the sample for the mesocosm per sampling event. Invertebrate samples were transferred from the net into 70% ethanol in 250 ml honey jars until further analysis in the laboratory. Upon completion of sampling, the same floating weeds were gently transferred back into the original mesocosm. The holding container was carefully screened for any invertebrates that may have been transferred during this process, and if so, were added to the respective sample.



M. aquaticum	*S. molesta*	*A. filiculoides*	*Pi. stratiotes*	*Po. crassipes*	Control
S. molesta	*A. filiculoides*	*Pi. stratiotes*	*Po. crassipes*	Control	*M. aquaticum*
A. filiculoides	*Pi. stratiotes*	*Po. crassipes*	Control	*M. aquaticum*	*S. molesta*
Pi. stratiotes	*Po. crassipes*	Control	*M. aquaticum*	*S. molesta*	*A. filiculoides*
Po. crassipes	Control	*M. aquaticum*	*S. molesta*	*A. filiculoides*	*Pi. stratiotes*
Control	*M. aquaticum*	*S. molesta*	*A. filiculoides*	*Pi. stratiotes*	*Po. crassipes*

Figure 2. 2: Schematic representation of an aerial view of the mesocosm grid array. Six treatments comprising six replicates each of a control (black text) and 5 floating invasive alien aquatic plant species treatments (non-black text). Each mesocosm was spaced approximately 75 cm away from each surrounding mesocosm. *Po. crassipes*

= *Pontederia crassipes* (water hyacinth), *Pi. stratiotes* = *Pistia stratiotes* (water lettuce), *A. filiculoides* = *Azolla filiculoides* (red water fern), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather) and *S.* molesta = *Salvinia molesta* (giant salvinia).

2.2.2 Laboratory analyses

Once in the laboratory, nutrient samples were processed for phosphates (mg. L^{-1}) via the amino acid method, and ammonia (mg. L^{-1}) using the Nessler method. This was achieved through use of an Environmental Analysis Photometer (Hanna Instruments Model H183300) and associated test-kits. Chlorophyll-*a* water samples were filtered through a 0.7 µm Whatman glass fibre filter (Ø =47 mm), with the filtrate stored at -20 ℃ until chlorophyll-*a* extraction. Chlorophyll-*a* was extracted from the filtrate using 10ml of 0.9M acetone and placed in the dark at -4 ℃ for 24 hours, after which chlorophyll-*a* concentration was determined using the Turner 10 AU fluorometer and the Holm-Hansen method (Holm-Hansen & Riemann, 1978). Invertebrate samples were processed using an Olympus microscope with all insects isolated, identified to lowest taxonomic level and functional feeding group (FFG) using A Field Guide to Freshwater Macroinvertebrates of Southern Africa (Fry et al., 2021) and enumerated as catch per unit effort (CPUE) per sampling event.

2.2.3 Data analysis

Aquatic community characterization first involved the removal of any adult stages of semi-aquatic Chironomidae, Tanipodidae or Culicidae and terrestrial taxa from the dataset, given that the sampling strategy for invertebrates was not designed to quantitatively collect non-aquatic fauna or life-stages. Similarly, the snail *Pseudosuccine columella* was removed from the dataset as it was noted early in the study that individuals were likely transferred into the mesocosms as hitchhikers on plants given their presence on plants in the Waainek Facility from where plants were collected.

Generalized linear mixed effects models assuming a Gaussian distribution were used for assessing abiotic parameters across plant treatments over time. Response variables were log-transformed to improve model fits where needed. Models were fit with individual replicate (container) identities as a random effect to account for repeated measures. For characterization of the physico-chemical features across the 36 mesocosms over time, a Principle Component Analysis was performed in *Primer* version 6 (Clarke, 1993) on normalized physico-chemical data.

Generalised linear mixed models were used to assess the effects of plant treatment, time, and their interaction, on dominant invertebrate abundances, and were fit separately for *Culex pipiens*, *Rhantus* sp., *Tanypus* sp. and *Hydraena* sp. These models included individual replicate (container) identities as a random effect to control for repeated measures over time (Brooks et al., 2017). Day 0 was excluded from the invertebrate models as sampling for fauna only took place from Day 7 onward. For *Cx. pipiens* alone, the three other invertebrate taxa were also included as predictor variables, owing to our interest in them as potential natural enemies or competitors. All models were initially fit with a Poisson error distribution, but if residual overdispersion was detected, a negative binomial distribution was used instead. a parameter to account for residual zero inflation if that was detected was also included.

In all models, residual diagnostics were used to assess distribution assumptions via simulation analyses (Hartig, 2022). Post-hoc tests were then used to compare treatments and treatments over time where significant (Lenth, 2023).

To evaluate treatment and time effects on aquatic communities, multivariate approaches were employed to describe community assemblages over time. To do this, sample averages for each species across replicates (per temporal sampling event) were determined and considered for the analyses (Clarke & Gorley, 2006). Thus, thirtysix samples—six treatments and six time points—from four aquatic macroinvertebrate species made up the data matrix. First, using non-transformed data and a Bray-Curtis similarity index (Clarke, 1993), a 2-dimensional multi-dimensional scaling (MDS) plot was produced. To trace the way that time passes across the two-dimensional space and analyse the serial nature of change between treatments, a time trajectory was superimposed on the plot (per treatment). When taxa-specific dynamics are considered, longer trajectories indicate stronger community phenological shifts.

2.3 RESULTS

2.3.1 Physico-chemical

All physico-chemical and Chl-*a* levels were similar at the start of the experiment, removing potential confounds associated with experimental founding effects. Temperature, pH, and electrical conductivity (EC) exhibited consistent trends, with negligible differences among treatments. Temperature and electrical conductivity did not differ among treatments but were significantly different through time (Table 2.1). Temperature across all treatments dropped on day seven and then gradually increased until day 28, when it peaked, followed by a decline on days 35 and 42. pH started low on Day 0 and rose until Day 14. Electrical conductivity increased from days 0 to 14, then slightly dropped before rising again on day 21, reaching its highest level by day 42 across all treatments. pH was also not significantly affected by plant treatment but was significantly affected by time and by interactions between treatment and time (Table 2.1). The control treatment had a higher pH than the others from day 14 to day 42, while the pH of the other treatments decreased during this period.

Table 2. 1: Generalised linear mixed effects model coefficients assessing physicochemical parameters across plant treatments over time. Models were fit with a random effect to account for repeated measures. Coefficients are reported from a Type III analysis of deviance.

Parameter	Effect	Chi^2	Df	*P*-value
Temperature	Treatment	0.390	5	0.996
	Day	5.625	1	0.018
	Treatment × Day	0.488	5	0.993
Ph	Treatment	3.468	5	0.628
	Day	116.122	1	< 0.001
	Treatment × Day	14.103	5	0.015
Dissolved oxygen	Treatment	6.519	5	0.259
	Day	25.716	1	< 0.001
	Treatment × Day	16.360	5	0.006
Electrical conductivity	Treatment	0.519	5	0.991
	Day	2033.85	1	< 0.001
	Treatment × Day	6.916	5	0.227
Turbidity	Treatment	21.957	5	< 0.001
	Day	74.482	1	< 0.001
	Treatment × Day	31.585	5	< 0.001

In contrast, dissolved oxygen (DO) and turbidity showed significant variability across treatments, highlighting the influence of floating IAAPs and associated biotic effects (Table 2.1,). Dissolved Oxygen and turbidity were also significantly affected by time and by treatment × time interactions (Table 2.1). Dissolved oxygen concentrations for all treatments increased from days 0 to 7, followed by considerable variation among them (fig 2.3). The control treatment exhibited higher DO concentrations compared to others, followed by *A. filiculoides*, while *Pi. stratiotes* and *Po. crassipes* had the lowest levels. The control treatment had the highest turbidity from Day 0 to Day 28, after which it decreased, while *A. filiculoides* maintained the highest turbidity until Day 42 (fig 2.3). The turbidity for *M. aquaticum* increased from day 14 to day 28 before dropping until Day 42.

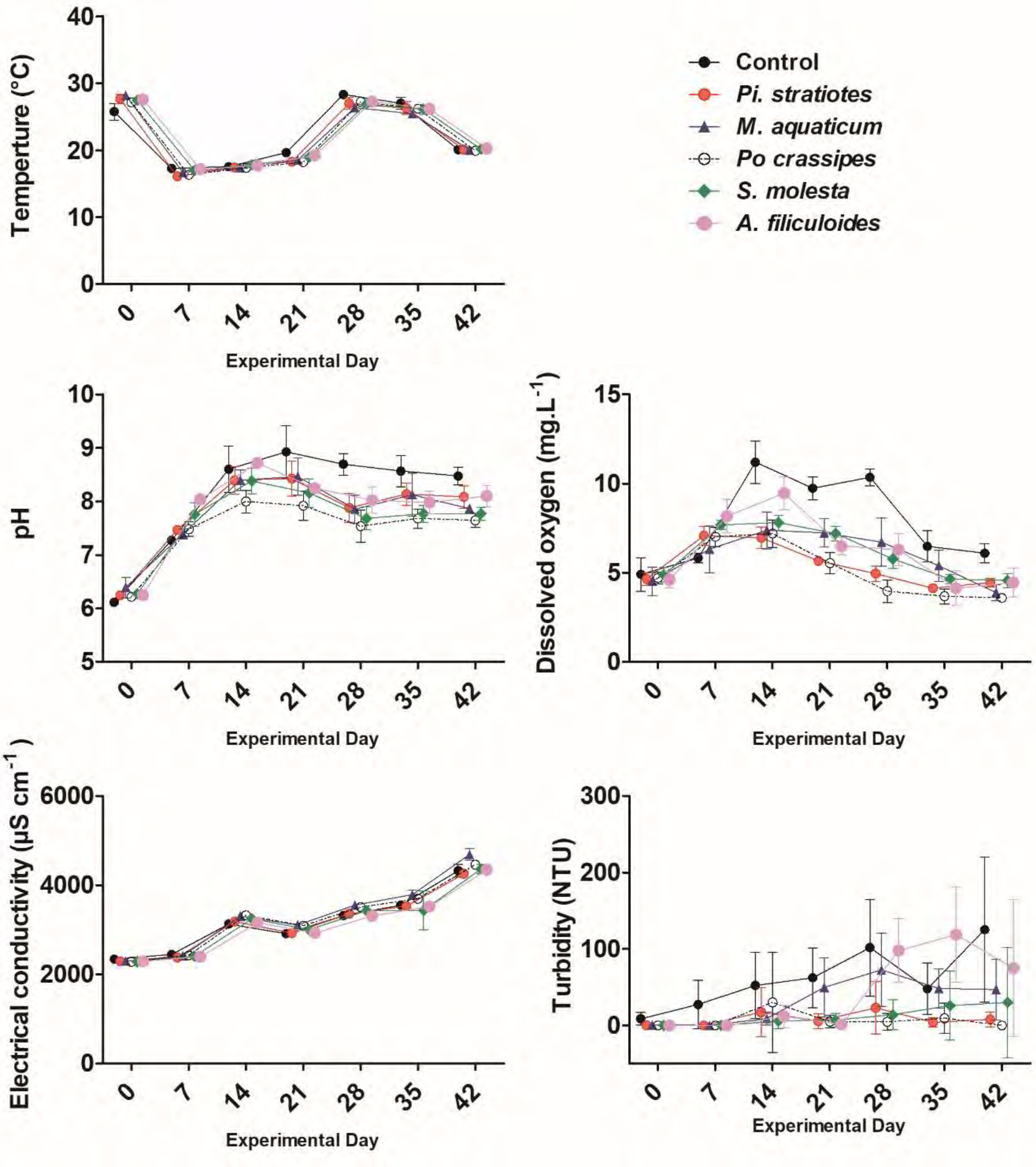


Figure 2. 3: Mean (± standard deviation) physico-chemical parameters measured over the 42-day experimental period across experimental treatments (control and 5 floating invasive alien aquatic plant species). *Po crassipes* = *Pontederia crassipes* (water hyacinth), *Pi. stratiotes* = *Pistia stratiotes* (water lettuce), *A. filiculoides* = *Azolla filiculoides* (red water fern), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather) and *S. molesta* = *Salvinia molesta* (giant salvinia).

2.3.2 Nutrients

Nitrate levels differed significantly over time, but not among treatments or treatment time interactions (Table 2.2). Nitrate levels exhibited an initial decrease in concentration from Day 0 to Day 7 (Fig 2,4), followed by minor fluctuations above the initial low peak that occurred on Day 7. *Pontederia crassipes* and *Pi. stratiotes* treatments had the lowest nitrate levels for the duration of the experiment, while the Control and *M. aquaticum* treatments had the highest nitrate levels. Ammonia showed significant treatment and time effects, while treatment × time effects were not significant (Table 2.2). *Pontederia crassipes* and *Pi. stratiotes* treatments had the lowest nitrate levels throughout the experiment. Control and *M. aquaticum* treatments had the highest levels over time, with the latter exhibiting the highest ammonia levels of all treatments by Day 42. Phosphate levels exhibited differences at the Treatment, Time and Treatment × Time interaction levels. Across all treatments, phosphate levels dropped from Day 0 to Day 7, after which there was limited variation among treatments over time, with the notable exceptions of *M. aquaticum* levels on days 14 and 28 (Fig 2.4).

Table 2. 2: Generalised linear mixed effects model coefficients assessing nutrient concentrations and Chlorophyll-a levels across plant treatments over time. Models were fit with a random effect to account for repeated measures. Coefficients are reported from a Type III analysis of deviance.

Parameter	Effect	Chi^2	Df	*P*-value
Nitrate	Treatment	29.462	5	< 0.001
	Day	3.055	1	0.080
	Treatment × Day	4.815	5	0.439
Ammonium	Treatment	58.404	5	< 0.001
	Day	4.143	1	0.042
	Treatment × Day	9.667	5	0.085
Phosphate	Treatment	22.867	5	< 0.001
	Day	32.183	1	< 0.001
	Treatment × Day	11.333	5	< 0.001
Chlorophyll-*a*	Treatment × Day	21.034	5	< 0.001
	Treatment	12.145	1	< 0.001
	Day	39.654	5	< 0.001

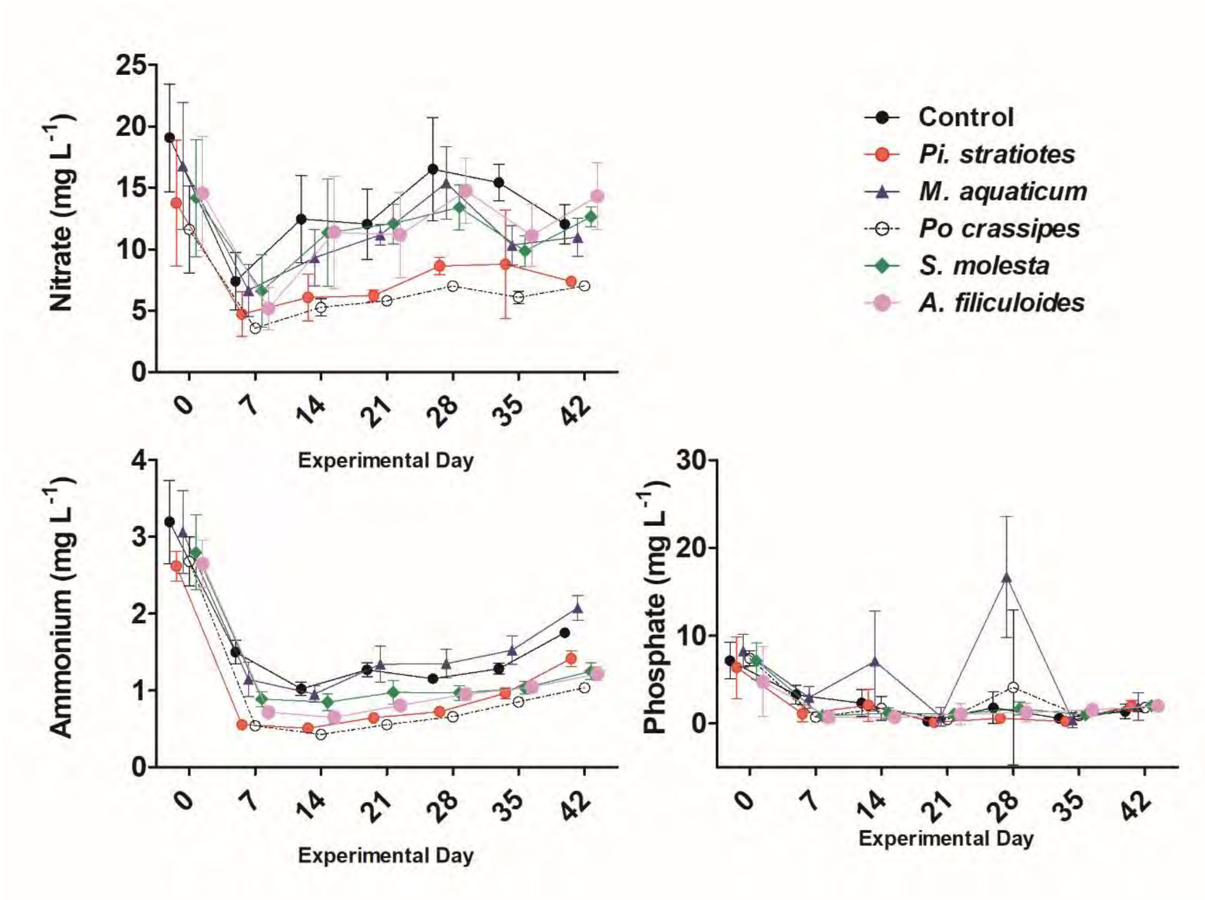


Figure 2. 4: Mean (± standard deviation) nutrient concentrations over the 42-day experimental period across experimental treatments (control and five floating invasive alien aquatic plant species). *Po crassipes* = *Pontederia crassipes* (water hyacinth), *Pi. stratiotes* = *Pistia stratiotes* (water lettuce), *A. filiculoides* = *Azolla filiculoides* (red water fern), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather) and *S. molesta* = *Salvinia molesta* (giant salvinia).

Discrete clusters were evident in the PCA analyses, with cluster formation being largely driven by time effects (Figure 2.5b), rather than treatment effects (Figure 2.5a). Distinct clusters were observed on Day 0 and Day 7, with days 14 and 21 forming an additional cluster, followed by a cluster comprising Day 28, 35 and 42 (Figure 2.5b). The Day 0 cluster appeared to be driven primarily by phosphate and ammonia levels, while the Day 7 cluster was seemingly influenced by DO levels. Day 14 and 21 cluster appeared to be driven by both pH and DO. The cluster comprising the last 3 sampling events seemed to be consecutively driven primarily by nitrate levels and temperature (Day 28), Turbidity (Day 35) and E.C. (Day 42).

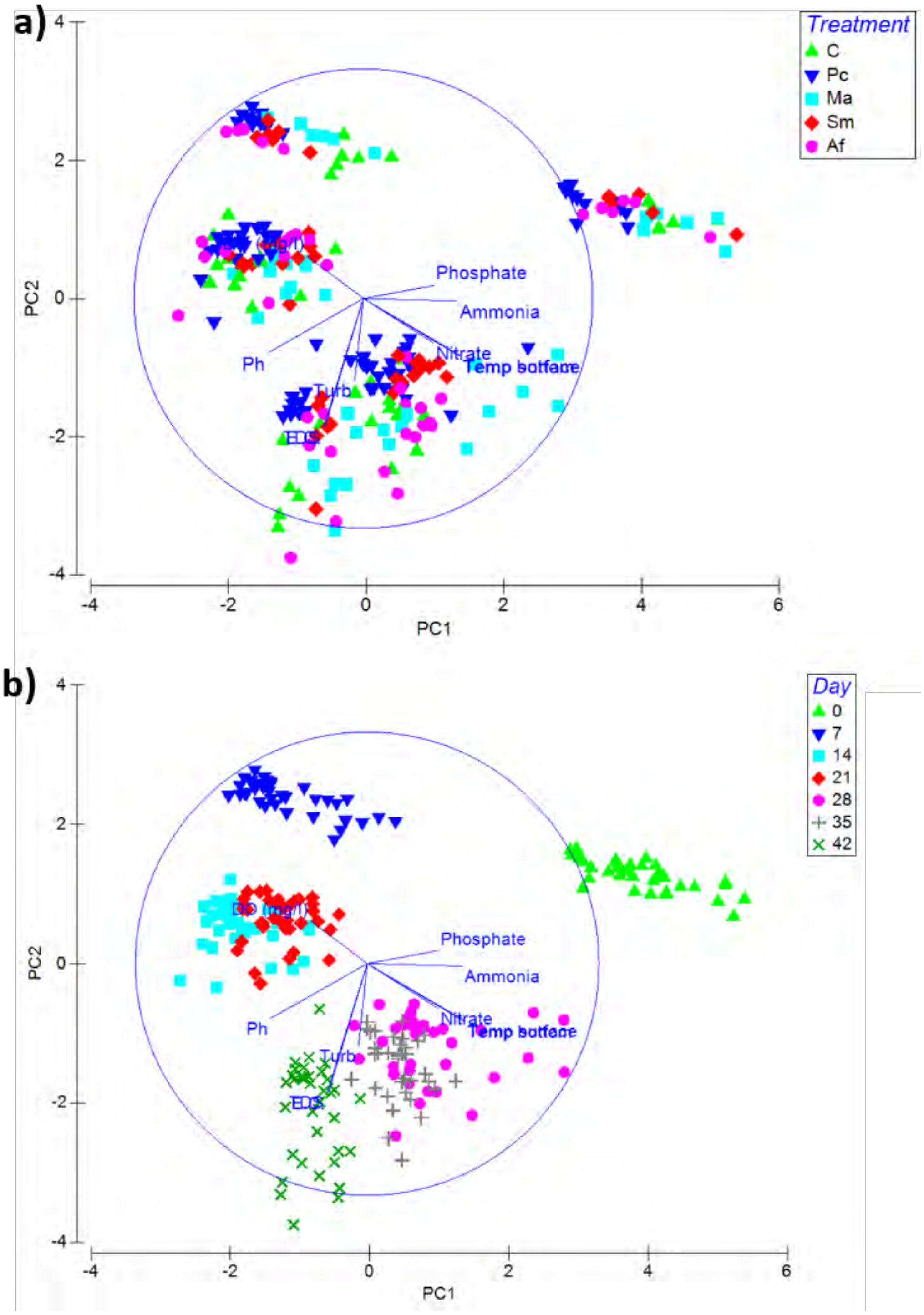


Figure 2. 5: Principle Component Analysis highlighting the physico-chemical characteristics associated with each of the 36 mesocosms across a) treatments and b) over time. C = control, Po = *Pontederia crassipes* (water hyacinth), Ps = *Pistia stratiotes* (water lettuce), Af = *Azolla filiculoides* (red water fern), Ma = *Myriophyllum aquaticum* (parrot's feather) and Sm = *Salvinia molesta* (giant salvinia.

2.3.3 Biotic components

Chlorophyll-*a* concentrations were the same at the commencement of the experiment, but thereafter exhibited significant variability across treatments, over time and at the Treatment × Time interaction level (Table 2.2). For the control treatment, Chl-*a* levels remained low for the first 7 days of the experiment, before increasing and peaking at Day 14 (Fig 2.6), although remaining relatively stable for the duration of the experiment. In the *Pi. stratiotes* mesocosms, Chl-*a* levels increased initially, peaking on Day 7, after which there was a general decline until Day 42. In the *M. aquaticum* treatment, Chl-*a* increased consistently over time from Day 0 to Day 28, then decreased to Day 35 (Fig 2.6), with Day 42 levels being similar to those measured in the penultimate sampling event. The *Po. crassipes* and *S. molesta* mesocosms had similar trends, with both exhibiting relatively low Chl-*a* levels throughout the experiment, peaking slightly on Day 14 before dropping consistently over time until Day 42. In the *A. filiculoides* mesocosms, Chl-*a* concentrations increased steadily from Day 0 to Day 14, then remained stable until Day 14, before increasing again to Day 21. The last two sampling events then saw a steady decrease in Chl-*a* levels in the *A. filiculoides* treatment.

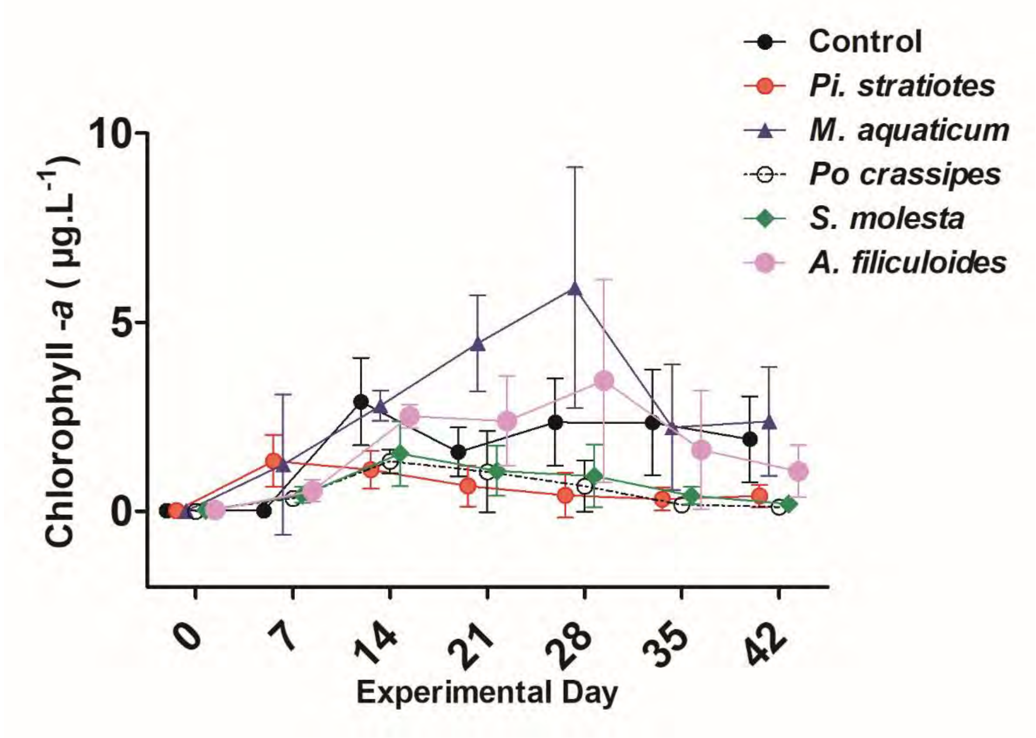


Figure 2. 6: Mean (± standard deviation) chlorophyll-a concentrations over the 42-day experimental period across experimental treatments (control and 5 floating invasive alien aquatic plant species). *Po crassipes* = *Pontederia crassipes* (water hyacinth), *Pi. stratiotes* = *Pistia stratiotes* (water lettuce), *A. filiculoides* = *Azolla filiculoides* (red water fern), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather) and *S. molesta* = *Salvinia molesta* (giant salvinia).

A total of 18032 aquatic invertebrate individuals were collected over the 42-day experiment across the 36 mesocosms (Table 2.3). Of these, four taxa comprised over 98% of invertebrates, namely; *Culex* sp. mosquitoes (52.4 %), *Rhantus* sp. Dyticidae (23.2 %), *Tanypus* sp. Chironomidae (15.0 %) and Hydraenidae coleopterans (8.0 %). All other taxa comprised less than 1 % of the overall contribution to the study with the majority restricted to less than 0.1% contribution. As such, further analyses were done only using the four dominant taxa. Identification of adult *Culex* sp. mosquitoes around the mesocosms, and collection of larval stages in other experiments in the same area revealed the presence of only *Cx pipiens* at the study site. As such, all sampled *Culex* sp. were regarded as *Cx. pipiens* for the remainder of the study.

Table 2. 3: Presence or absence of different aquatic invertebrate species collected over the 42-day experiment across six planting treatments in 36 mesocosms.

	Control	*Pistia stratiotes*	*Myriophyllum aquaticum*	*Pontederia crassipes*	*Salvinia molesta*	*Azolla filiculoides*
Rhantus sp.	+	+	+	+	+	+
Altica sp.	-	-	+	-	-	-
Acidocerinae sp.	+	-	-	-	+	-
Anacaena sp.	-	+	-	-	-	-
Laccobius sp.	-	-	+	-	-	-
Amphiops sp.	-	+	+	-	-	-
Hydraena sp.	+	+	+	+	+	+
Helophorus sp.	-	-	-	-	+	-
Pseudancyronyx sp.	-	-	+	-	-	-
Microvelia sp.	+	-	-	-	-	-
Paraplea sp.	-	+	-	-	-	-
Pseudosuccine columella	-	-	-	-	-	+
Dasyhelea sp.	-	-	+	-	+	+
Ochthera sp.	+	+	-	-	-	+
Brachydeutera sp.	-	+	-	+	-	-

Chironomini sp.	+	+	+	+	+	+
Tanypus sp.	+	+	+	+	+	+
Nymphalinae sp.	-	-	-	-	-	+
	+	+	+	+	+	+

Culex pipiens

+= present and **-** = absent

Significant differences in the abundances of aquatic macroinvertebrates were observed among treatments, across days, and in their interaction (Table 2.4). Overall, *Cx pipiens* abundances differed across treatments ($P < 0.001$; Table 2.4). Mosquito numbers were highest in the *Po. crassipes* treatment (426 ± 149), followed by the *M. aquaticum* treatment (217 ± 189), the control treatment (141 ± 92) and the *Pi. stratiotes* treatment (123 ± 83), with the *S. molesta* treatment (33 ± 40) and the *A. filiculoides* treatment producing exceptionally low numbers of mosquitoes (9 ± 12). Significant differences in temporal effects were also evident ($P < 0.001$; Table 2.4). In the control treatment, the abundance of *Cx pipiens* increased gradually from days 7 to 28. It reached its peak on Day 35 and slightly dropped on Day 42. In the *Pi. stratiotes* treatment, abundance increased from Day 7, reaching its peak on Day 14 and dropped from Day 21 to Day 42. For the *M. aquaticum* treatment, abundance rose from Days 7 to 21 reaching its peak on Day 28 then dropped on days 35 to 42. In the *Po. crassipes* treatment, abundance increased from Day 7 to Day 14 then slightly decreased on Day 21, peaking on Day 28, and then slightly decreased by Day 42. For the *S. molesta* treatment, the abundance increased between Day 7 and its peak on Day 14, before decreasing until day 42. In the *A. filiculoides* treatment, abundance remained at zero until Day 28 when it peaked and then dropped on Day 35 and slightly increased on Day 42. *Culex pipiens* abundances were significantly influenced by the interaction between treatment and day. Similarly, *Rhantus* sp. ($P < 0.001$), *Tanypus* sp. ($P < 0.001$) and *Hydraena* sp. ($P < 0.05$) significantly affected *Cx pipiens* abundance (Table 2.4).

Table 2. 4: Generalised linear mixed effects model coefficients assessing invertebrate abundances across plant treatments over time. Models were fit with a random effect to account for repeated measures. Coefficients are reported from a Type III analysis of deviance.

Taxon	Effect	Chi^2	Df	p-value
Culex pipiens	Treatment	37.222	5	< 0.001
	Day	16.419	1	< 0.001
	Treatment × Day	134.444	5	< 0.001
	Rhantus sp.	84.442	1	< 0.001
	Tanypus sp.	43.912	1	< 0.001
	Hydraena sp.	5.775	1	0.016
Rhantus sp.	Treatment	5.872	5	0.319
	Day	109.909	1	< 0.001
	Treatment × Day	4.806	5	0.439
Tanypus sp.	Treatment	131.787	5	< 0.001
	Day	5.915	1	0.015
	Treatment × Day	157.828	5	< 0.001
Hydraena sp.	Treatment	29.257	5	< 0.001
	Day	15.729	1	< 0.001
	Treatment × Day	18.637	5	0.002

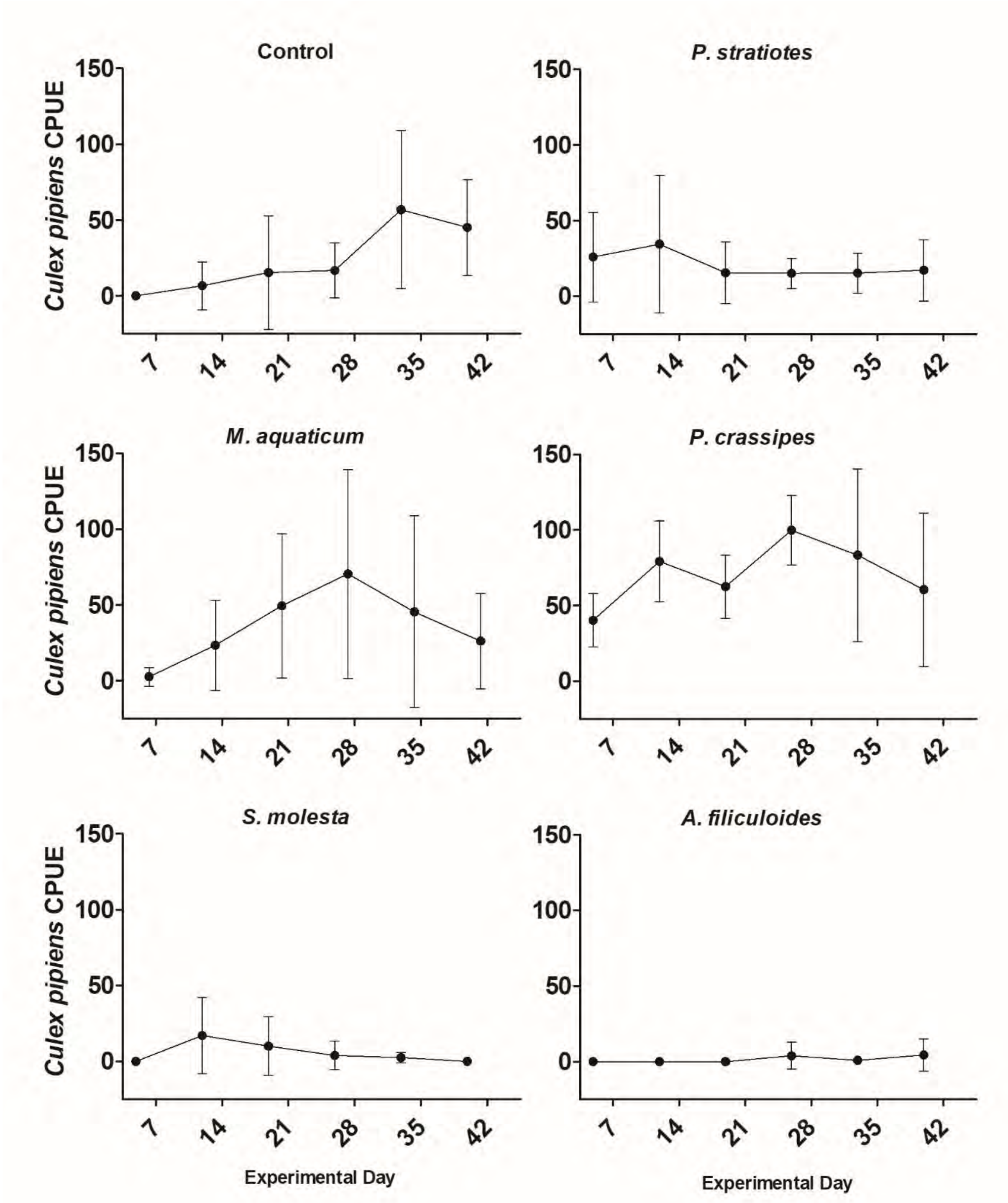


Figure 2. 7: Mean (± standard deviation) early life-history stage (larval and pupal) *Cx pipiens* abundances over the 42-day experimental period across experimental treatments (control and 5 floating invasive alien aquatic plant species). *Po crassipes* = *Pontederia crassipes* (water hyacinth), *Pi. stratiotes* = *Pistia stratiotes* (water lettuce), *A. filiculoides* = *Azolla filiculoides* (red water fern), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather) and *S. molesta* = *Salvinia molesta* (giant salvinia).

Unlike with mosquitoes, the overall abundances of the predatory *Rhantus* sp. did not differ across most of the treatments (*P* > 0.05; Table 2.4) with the Control (83 ± 33), *Po. crassipes* (77 ± 33), *Pi. stratiotes* (75 ± 20), *M. aquaticum* (71 ± 45) and *S. molesta* treatment (68 ± 26) producing very similar numbers. Like mosquito abundance, however, *Rhantus* sp. numbers were much lower in the *A. filiculoides* treatment (13 ± 9). Differences in *Rhantus* sp. dynamics over time were evident though (*P* < 0.001; Table 2.4). In the control treatment, the abundance of this species remained at zero from days 7 to 21, then increased on Day 28 reaching its highest abundance on Day 35 and slightly dropped on Day 42. In the *Pi. stratiotes* treatment, abundance increased from Day 21, reaching its peak on Day 35 and dropped on Day 42. For the *M. aquaticum* treatment, abundance rose gradually from Day 28 then dropped on Day 35, peaking on Day 42. In the *Po. crassipes* treatment, abundance increased from Day 21, peaked on Day 28, and then slightly decreased until Day 42. For the *S. molesta* treatment, the abundance started increasing from Day 21 reaching a peak on days 35 and 42. In the *A. filiculoides* treatment, abundance remained at zero until Day 35 and then dropped on Day 42. *Rhantus* sp. abundances were not significantly influenced by the interaction between treatment and day (*P* > 0.05; Table 2.4).

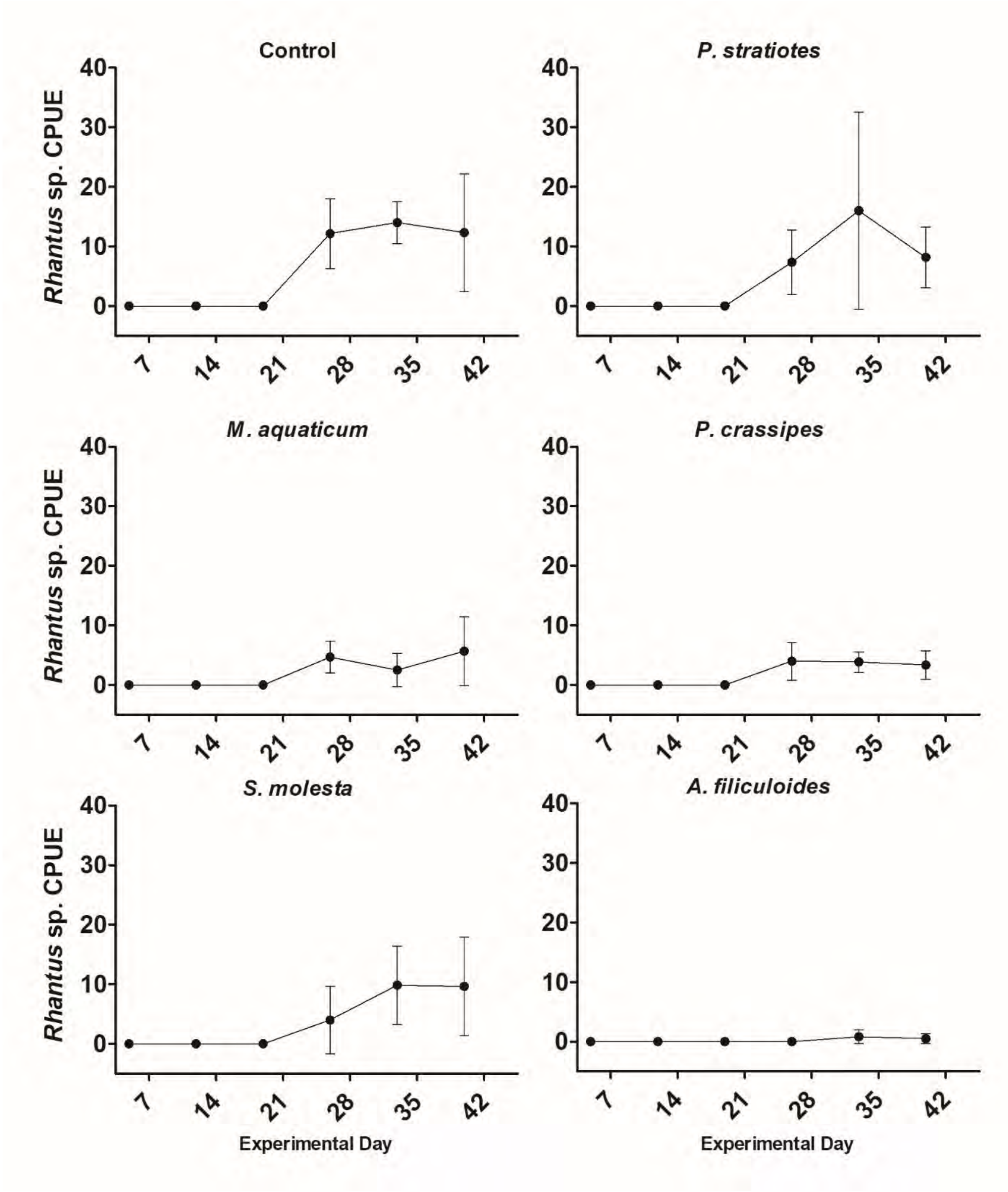


Figure 2. 8: Mean (± standard deviation) *Rhantus* sp. abundances over the 42-day experimental period across experimental treatments (control and 5 floating invasive alien aquatic plant species). *Po crassipes* = *Pontederia crassipes* (water hyacinth), *Pi. stratiotes* = *Pistia stratiotes* (water lettuce), *A. filiculoides* = *Azolla filiculoides* (red water fern), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather) and *S. molesta* = *Salvinia molesta* (giant salvinia).

The overall abundances of the competitor *Tanypus* sp. also differed across treatments (*P* < 0.001; Table 2.4) with the *M. aquaticum* treatment (103 ± 41) producing the highest numbers, followed by the *Po. crassipes* treatment (77 ± 31), the control treatment (43 ± 22) and the *A. filiculoides* treatment (32 ± 13). The *Pi. stratiotes* treatment (18 ± 11) and *S. molesta* treatment (18 ± 22) contained the lowest numbers of *Tanypus* sp. overall. Over time, differences between treatments in *Tanypus* sp. numbers were, however, not significant (*P* > 0.05; Table 2.4). In the control treatment, the abundance of this species remained at zero from days 7 to 28, then increased on days 35 and 42. In the *Pi. stratiotes* treatment, abundance gradually increased from days 7 to 21, peaked on Day 28, then dropped on day 35, reaching zero by Day 42. For the *M. aquaticum* treatment, abundance rose rapidly from days 7 to 14, peaking on Day 21, then dropped on Day 28, increased again on Day 35, and decreased once more on Day 42. In the *Po. crassipes* treatment, abundance increased on Day 14, peaked on Day 28, and then decreased until Day 42. For the *S. molesta* treatment, the highest abundance was observed on Day 14, followed by a decline from days 21 to 42. In the *A. filiculoides* treatment, abundance remained at zero until Day 21, then peaked on days 28 and 35 before dropping again on Day 42. Significant differences were, however, at the treatment×day interaction level (*P* < 0.001; Table 2.4).

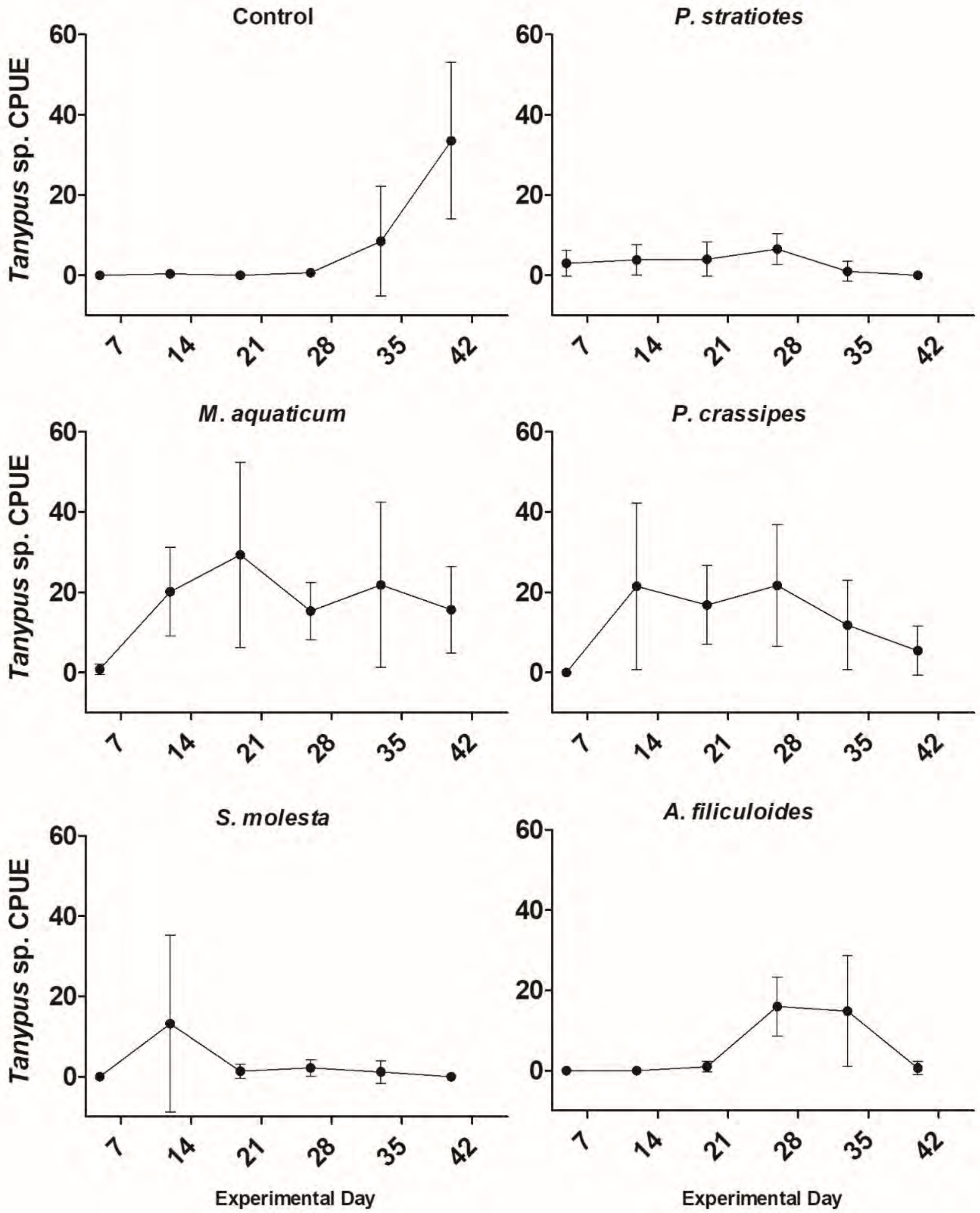


Figure 2. 9: Mean (± standard deviation) early life-history stage (larval and pupal) *Tanypus* sp. abundances over the 42-day experimental period across experimental treatments (control and 5 floating invasive alien aquatic plant species). *Po crassipes* = *Pontederia crassipes* (water hyacinth), *Pi. stratiotes* = *Pistia stratiotes* (water lettuce), *A. filiculoides* = *Azolla filiculoides* (red water fern), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather) and *S. molesta* = *Salvinia molesta* (giant salvinia).

The predatory *Hydraena* sp. numbers also differed across treatments (*P* < 0.001; Table 2.4). with overall abundances highest the Control treatment (39 ± 15), followed by the *M. aquaticum* treatment (13 ± 9). The *Po. crassipes*, *A. filiculoides*, *Pi. stratiotes* and *S. molesta* treatments all produced very low numbers of hydrangea sp. (1 ± 1 in each case). Over time, differences in *Hydraena* sp. numbers also differed across treatments (*P* < 0.001; Table 2.4). In the control treatment, the abundance of this species increased from days 7 to 28, then peaked on Day 35 then dropped on Day 42. In the *Pi. stratiotes* treatment, abundance increased from Day 7, reaching its peak on Day 14 and dropped on days 21 to 42. For the *M. aquaticum* treatment, abundance increased from days 7 to 21 peaking on Day 28 then dropped on days 35 and 42. In the *Po. crassipes* treatment, abundance increased from days 7 to 14 then decreased on Day 21, peaked on Day 28, and then slightly decreased until Day 42. For the *S. molesta* treatment, the abundance started increasing from Day 7 reaching the peak on Day 14 and decreased until Day 42. In the *A. filiculoides* treatment, abundance remained at zero until Day 28 where it was at its peak and then dropped on Day 42. *Hydraena* sp. abundances were also significantly influenced by the interaction between Treatment and Time (*P* < 0.05; Table 2.4).

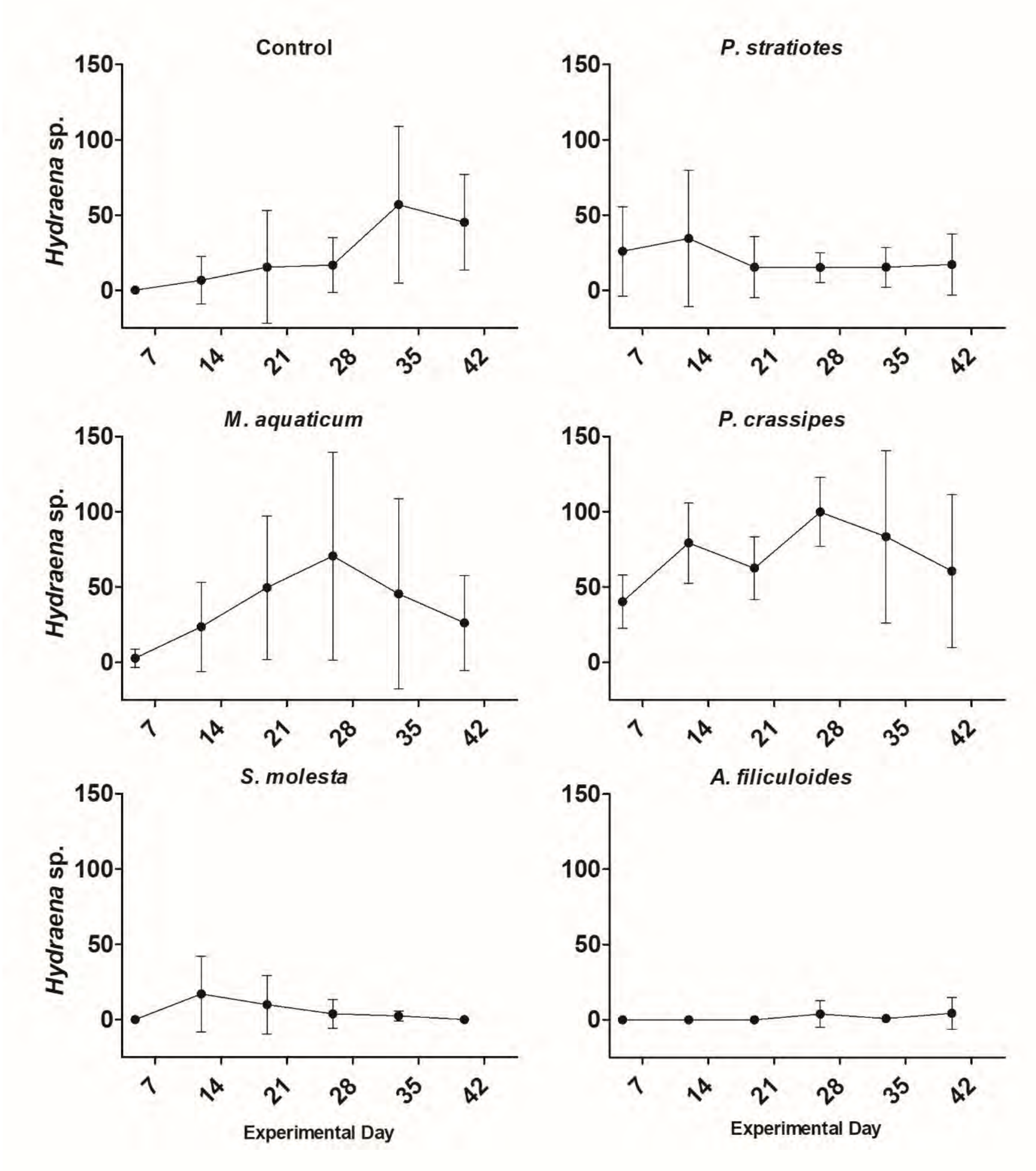


Figure 2. 10: Mean (± standard deviation) *Hydraena* sp. abundances over the 42-day experimental period across experimental treatments (control and 5 floating invasive alien aquatic plant species). *Po crassipes* = *Pontederia crassipes* (water hyacinth), *Pi. stratiotes* = *Pistia stratiotes* (water lettuce), *A. filiculoides* = *Azolla filiculoides* (red water fern), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather) and *S. molesta* = *Salvinia molesta* (giant salvinia).

The dominant community showed strong phenological shifts between Day 0 and Day 42 with treatment level effects evident. The control treatment trajectory shifted the least between Day 0 and Day 7, highlighting relatively limited early stage phenological dynamics in this treatment when compared to the floating IAAP species treatments (Figure 2.11a). Eventually, however, further phenological shifts resulted in Day 42 Control treatment communities being relatively similar to those of *M. aquaticum* and *Po. crassipes* treatments (Figure 2.11a, c, d). Phenological shifts were largely similarly between Day 0 and Day 7 in the *Pi. stratiotes* and *Po. crassipes* treatments, driven primarily by elevated abundances of *Cx pipiens* at this early stage (Figure 2.11c, d, Figure 7). Between Days 7 and 42, however, *Pi. stratiotes* and *Po. crassipes* treatment trajectories diverged, with the former trajectory shifting further away and being driven partly by a general decrease in *Cx pipiens* numbers, while the latter trajectory shifting little, driven by a relatively stable but high number of *Cx pipiens* abundances (Figure 2.11, Figure 2.7). Overall, *S. molesta* and *A. filiculoides* treatments were characterized by relatively atypical trajectories, with both early stage (Day 7) and late stage (days 35 and 42) deviations from other treatment community dynamics at these stages (Figure 2.11e, f). The *A. filiculoides* treatment was characterised by very low *Cx pipiens*, *Rhantus* sp. and *Hydraena* sp. numbers throughout the experiment (Figure 2.7, 2.8, 2.10), coupled with moderate numbers of *Tanypus* sp. on days 28 and 35 (Figure 2.9). The *S. molesta* treatment was similarly characterised by relatively low abundances of *Cx pipiens* and *Hydraena* sp. numbers, along with low *Tanypus* sp. numbers throughout the experiment and elevated *Rhantus* sp. numbers near the end of the study.

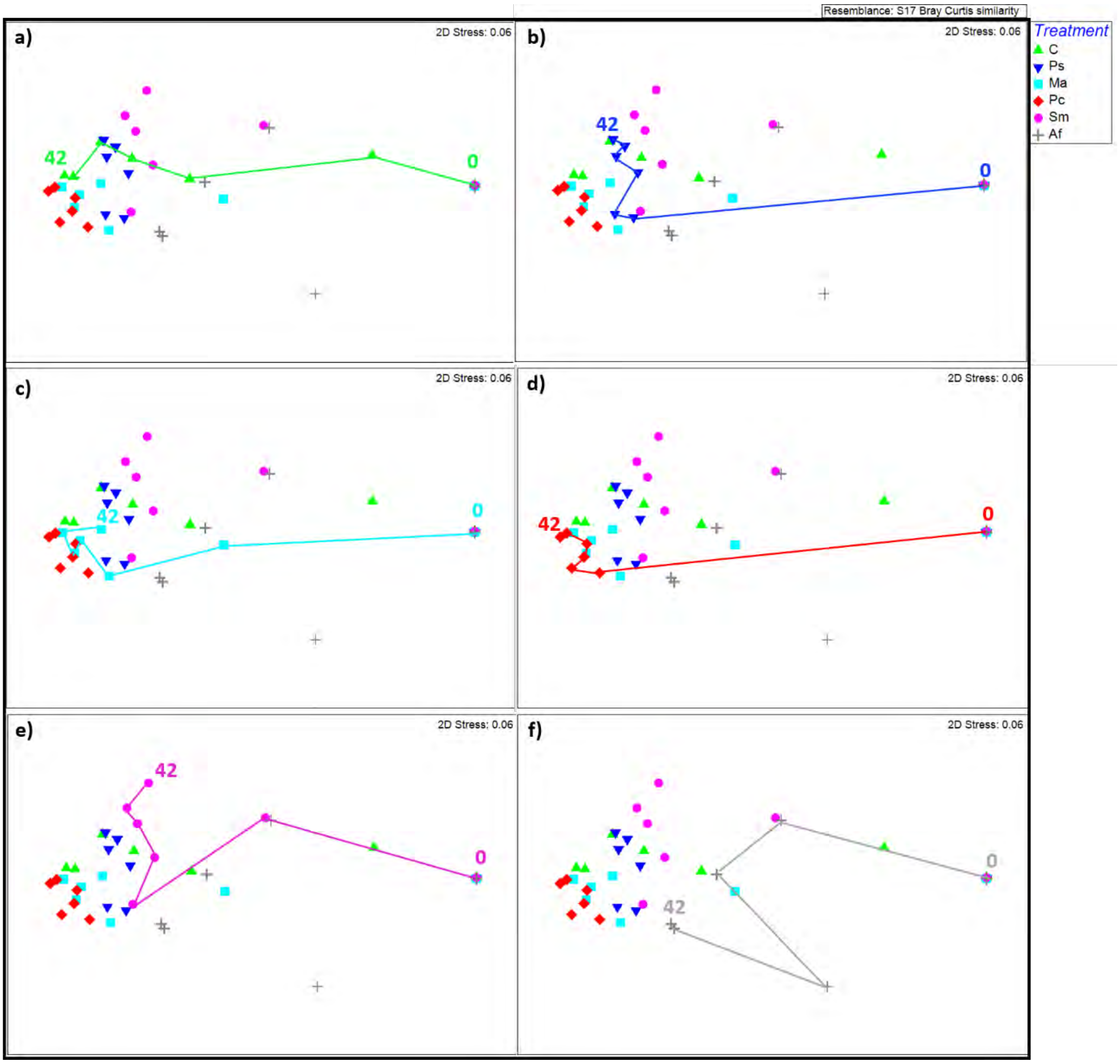


Figure 2. 11: 2-D Multi-Dimensional Scaling (MDS) plots, overlaid with time trajectories. The same plot is presented in a-f, with each graph highlighting the trajectory of the biological community in a specific treatment over time, between Day 0 and Day 42 across 6 treatments. a) = Control treatment, b) = *Pistia stratiotes* (water lettuce) treatment, c = *Myriophyllum aquaticum* (parrot's feather) treatment, d) = *Pontederia crassipes* (water hyacinth) treatment, e) = *Salvinia molesta* (giant salvinia) treatment and f) = *Azolla filiculoides* (red water fern) treatment.

2.4 DISCUSSION

Overall, the study highlights that floating IAAPs have implications for aquatic invertebrate community succession dynamics. While experimental and *ex situ* in nature, the study shows that despite relatively limited diversity in colonization dynamics, differences in population and community structure emerge among experiment treatments. Specifically, the study shows that while distinct communities at the alpha diversity level were not observed, when considering numbers per taxa, community distinctions over time were evident as predicted in the first hypothesis. It was further highlighted that mosquito abundances were key drivers of differences in community structure across treatments, as per the second hypothesis. The study represents the first of its kind, directly comparing the role various floating IAAPs play in facilitating optimal habitat preference for early-stage mosquitoes, using an experimental approach.

Numerous biotic and abiotic factors have been shown to have implications for oviposition dynamics and subsequent larval mosquito abundances (Blaustein & Chase 2007; Silver 2008). In this study, physico-chemical conditions were significantly similar at the commencement of the study, but temporal effects were highly evident, with time being the key overall driver in differences in measured abiotic conditions. Differences among treatments did, however, emerge, often interacting with temporal effects. The role of floating IAAP type in facilitating subtle habitat differences was, therefore, evident. However, it is highly likely that various differences were not detected given the limited suite of variables measured. For example, differences in light attenuation and structural complexity were not measured, with both providing important environmental differences with potential implications for colonization and phenological dynamics (Mormul et al., 2010). Of the measured physico-chemical variables that showed floating AIP induced effects, only turbidity showed treatment level effects, while all physico-chemical variables (excluding temperature and electrical conductivity) showed significant Treatment ×Time effects. Turbidity variations are likely a result of an interplay between nutrient and Chl-*a* levels (Rommens et al., 2003). Of the nutrient levels, significant time effects were evident for nitrates, phosphates and ammonium, suggesting that primary productivity and nutrient conversion effects strongly influenced all nutrient levels over time. Phosphate was the only nutrient showing differences across treatment at the treatment and Treatment ×Time levels. Chlorophyll-*a* levels were significantly affected by Time, Treatment and Treatment ×Time interactions. These results highlight how the different IAAPs used in the treatments differentially interacted with nutrients, with implications for nutrient uptake, cycling and phytoplankton primary productivity dynamics (Carpenter & Lodge, 1986; Chambers et al., 2008).

The colonizing communities in the present study almost completely comprised only four taxa. Mosquito larvae comprised over half of the sampled community across the study, dominating in most mesocosms with the exception of *S. molesta* and *A. filiculoides* treatments. Overall, these findings strongly suggest that *S. molesta* and *A. filiculoides* may provide suboptimal habitat for mosquito larvae, and that fecund females may be able to detect the presence of these plants and seek out alternative sites for oviposition. Indeed, studies have shown that *Cx pipiens* ovipositing females have the ability to respond to chemical cues as proxies for larval habitat suitability (Bentley & Day, 1989; Reiskind & Wilson, 2004(a); Khan et al., 2022). For example, Reiskind et al. (2009) showed that fecund females are capable of detecting lower levels of dissolved oxygen, which serve as an indication of food availability and larval survival chances. Fader & Juliano (2014) highlighted that *A. albopictus* and *A. aegypti* prefer to oviposit in habitats that are rich in detritus as the presence of detritus may provide higher levels of microbial food resources for larvae. *Culex pipiens*., in this study, preferentially utilized *M. aquaticum* and *Po. crassipes* treatments, likely because these plants provide conditions that are favourable for mosquito larvae development. These aquatic plants create dense mats that impact the surrounding ecosystems in ways that enhance the availability of food resources for larvae. The dense vegetative structures such as those formed by *M. aquaticum* and *Po. crassipes* trap detritus and organic matter which decomposes to produce microbial biofilms and bacteria (Merritt et al., 1996). Mosquito larvae rely heavily on microbial communities as a primary food source. As such, increased detrital build-up associated with these plants supports larger microbial populations, thus boosting food availability for developing larvae (Bond et al., 2005). Furthermore, research has shown that mosquitoes are often attracted to habitats with higher levels of organic matter and microbial density because these cues signal better survival prospects for their offspring (Bond et al., 2004; Bond et al., 2007).

In addition to the dominant *Cx pipiens*, one potential competitor and two potential predators were present. The competitor *Tanypus* sp. also seemed to prefer the *M. aquaticum* and *Po. crassipes* treatments but showed less aversion for the *S. molesta* and *A. filiculoides* treatments. The presence of *Tanypus* sp. seemed to have implications for mosquito abundance, given its significance in contributing to larval mosquito abundances in the study. It is, however, unclear if these interactions are driven by *Tanypus* sp., *Cx pipiens*, or a combination of both. Indeed, chironomids have also been found to be highly selective in oviposition sites, responding to the competitive and predatory pressures present in their environments (Pinder, 1986; Jansen & Aarts, 2008). The two predatory species also had implications for mosquito oviposition, with both independently contributing significant effects potentially driving mosquito numbers. Mosquitoes have been shown to avoid predators, which can lead to decreased oviposition rates (Fitzgerald & Sweeney, 2007).

The resultant overall assemblages over time were that of largely discrete community characteristics and phenology, despite the very low alpha diversity level. The present study highlights the considerable influence that aquatic plant species play in shaping the composition and abundance of certain ecological groups. Given the experimental nature of this study, it is likely that within-system dynamics may be more pronounced, whereby exploitation of water bodies will be characterised by a more diverse array of semi-aquatic fauna, coupled with the full aquatic compliment that may also be shaped by floating IAAPs. These findings highlight that managing vegetation in aquatic habitats is crucial for potential ecosystem disservice dynamics. In particular, floating IAAPs may play a central role in facilitating nuisance, pest and even disease vector species such as mosquitoes. This study could help prioritise where action should be directed, when needing to triage for management of various water bodies invaded by various floating IAAPs (Lowe et al., 2000).

CHAPTER 3 Floating aquatic alien invasive plant species have varying implications for mosquito predation risk

Plate 3. Predator behaviour experiment set up in a controlled environment room at the Rhodes University Department of Zoology and Entomology. Photo credit: Ryan J Wasserman, September 2024.

ABSTRACT

Floating invasive alien aquatic plants (IAAPs) have the potential to alter aquatic ecosystems through various direct and indirect means. The previous chapter highlighted that these plants can even facilitate the proliferation of mosquitoes, presumably through combinations of increased oviposition, and reduced predation and competition pressure. The relative roles of oviposition and biotic interaction contribution dynamics, however, remains unclear. With regard to predation specifically, once these habitats have been selected by mosquitoes, it is unknown whether the habitat features resulting from the various floating IAAPs have differing implications for predator prey dynamics. To test for potential predation-related effects of floating aquatic plants on mosquito larvae, two laboratory-based experiments were conducted. The first assessed predator-prey interaction outcomes between *Culex pipiens* (Diptera: Culicidae) larvae and the widespread predator, *Anisops debilis* (Hemiptera: Notonectidae), under 100% cover associated with various floating aquatic plant species. The second experiment assessed predator behaviour in relation to floating aquatic plant species, when given the option of utilising cover, edge or open-water habitats. The study found no difference between control and native (*Lemna minor*) floating plant predation effects, while the IAAPs *Azolla filiculoides*, *Myriophyllum aquaticum* and *Pontederia crassipes* treatments conferred prey risk reduction. Similarly, no differences in predator behaviour between control and native floating plant treatments were observed. However, *Po. crassipes, A. filiculoides* and *M. aquaticum* treatments exhibited high levels of floating plant avoidance. The results in combination, suggest that the various IAAPs reduce natural biological control of mosquito larvae, but through different ways. This chapter supplements the previous chapter offering further insight into how floating IAAPs facilitate ecosystem disservices.

Keywords: backswimmer, chemical cues, habitat complexity, mosquito larvae, predator-prey interactions.

3.1 INTRODUCTION

The impacts that floating aquatic alien invasive plants (IAAPs) have on freshwater ecosystems are profound and multifaceted, affecting numerous ecological processes in complex ways (Chamier, et al., 2012). In extreme cases, floating IAAPs can result in ecosystem state switches, such is their role in many aquatic ecosystems (Sheffer et al., 2003). These plants also alter habitat structure directly and indirectly, altering ecological states (Harvey & Fortuna, 2012). For instance, these invasive species often form dense mats or canopies on the water's surface, which significantly limits light penetration (Strange et al., 2019). This reduction in light can directly reduce photosynthesis in submerged aquatic plants and phytoplankton, crucial for aquatic primary production and dissolved oxygen levels (Stiers and Triest, 2017; Hassan and Nawchoo, 2020;; Shen et al., 2024). Additional aquatic microhabitats, facilitated by cover and structural complexity associated with floating IAAPs, can also indirectly impact faunal community structure, favouring certain species over others (Unsworth et al., 2007; Sellheim et al., 2010). The role floating IAAPs play in indirectly shaping aquatic communities through changes in habitat is, however, largely unknown (Menden-Deuer et al., 2023). Given that various semi-aquatic nuisance species and species of healthcare concern are potentially favoured by ecosystem switches associated with IAAPs (Portilla et al., 2021), there is value in gaining insight into the mechanistic understanding of how such processes unfold.

Predation is a fundamental ecological interaction that helps maintain a stable and diverse community by preventing single species from dominating the entire system (Beauchamp et al., 2007; Buxton et al., 2020). Predation aids in prey population regulation through top-down control, often even impacting multiple subsequent lower trophic levels (Salo et al., 2010; Weis, 2011). Predator-prey interactions can, therefore, regulate algal growth, prevent overgrazing of aquatic vegetation, support nutrient cycling by controlling herbivore populations and keep pest numbers low (Schmitz et al., 2010). In addition, predation drives evolutionary adaptations in both predators and prey which promotes biodiversity and resilience within aquatic systems (Schmitz et al., 2010). Effective predation helps with sustaining ecosystem services and functions such as habitat stability and water quality by maintaining balanced species interactions, preventing ecosystem degradation (Hammerschlag et al., 2019). There are several factors that affect predation efficiency including prey availability, prey behaviour and defence mechanisms, and predator adaptations such as specialized hunting strategies and physical traits that enhance their foraging success (Alexander et al., 2012). Given the importance of prey and predator behaviour in interactions outcomes, shifts in habitat characteristics can also result in shifts in interaction outcomes (Wasserman et al., 2016). Given the role floating IAAPs can play in reshaping habitat complexity dynamics in freshwater ecosystems, it is highly likely that they alter predator-prey dynamics, with implications for predation efficiency.

Physical structure can modify certain aspects of an organism’s performance in its environment, as the features can alter key behaviours, such as, swimming trajectories and ability to hide (Heck & Crowder, 1991; James & Heck, 1994; Barrios-O’Neill et al., 2014). This may result in reduced predatory efficiency due to a physical structure acting as a barrier to predator movement (Beekey et al., 2004) or through the provision of refuge space for the prey (Sih et al., 1992; Barrios-O’Neill et al., 2015). Habitat complexity, or structural complexity, defined by plant density or configuration, is hypothesized to affect species interactions, population densities, and community composition in a variety of invertebrate-dominated systems (Warfe & Barmuta, 2004). Invasive alien aquatic plants greatly influence habitat complexity, with different plant types characterised by various levels of surface cover and submerged plant material architecture (Strange et al., 2019). One of the key ways in which IAAPs affect predation is by altering the physical habitat, often by increasing the density of vegetation (Villamagna & Murphy, 2010). This dense, homogenous plant growth can create barriers that impede the movement of both predators and prey (Hussner et al., 2017). For instance, thick mats of invasive species like *Pontederia crassipes* (water hyacinth) or *Hydrilla verticillata* Royle (Hydrocharitaceae) can provide refuge for prey species, making them less accessible to predators and thus reducing predation rates (Schmitz & Suttle, 2001). Moreover, the changes brought about by IAAPs can create environments unsuitable for certain predators. For instance, the alteration of water chemistry and oxygen levels due to the decomposition of invasive plant biomass can create hypoxic conditions, which many predatory fish species cannot tolerate (Carpenter & Lodge, 1986). Similarly, many open-water predatory species may not be able to optimally forage in vegetated habitat structure, often typically restricted to riparian zones (Greenberg et al., 1995; Michel & Adams, 2009). While some studies have assessed components of predator-prey dynamics associated with floating IAAPs (Beauchamp et al., 2007; Ingrum et al., 2010; Grutters et al., 2015), comparisons across key IAAP species are underexplored, as are implications for relevance within the context of mosquito as prey.

In Chapter 2, it was highlighted that floating IAAPs have the ability to favour mosquito proliferation. It was also highlighted that there was a significant interaction effect between *Cx pipiens* larvae numbers and predator numbers. However, the role IAAPs play in mediating predator-prey interaction outcomes is still unclear and as such, is explored in this chapter. Using a model insect predator species, widely distributed throughout southern Africa, including water bodies invaded by various floating IAAP species, this chapter aims to separately address predation efficiency on mosquitoes; and predator habitat utilization under various floating IAAP scenarios. This was achieved through the establishment of two separate laboratory-based experiments. The first experiment specifically assessed *Culex pipiens* mosquito larvae consumption by the predatory backswimmer, *Anisops debilis* Harris (Hemiptera: Notonectidae) under 100% cover across five floating plant treatment scenarios. The second experiment assessed how the water column in and around IAAP treatment scenarios was used by the predator *An. debilis* when given the choice of proximity to cover types.

It was hypothesised that (1) cover by plant species would hinder predation success, but that (2) the level of hindrance would differ among plant types, with invasive species hindering predation dynamics more than the native floating plant. It was further hypothesised that when given the choice, (3) predators would not avoid activity under native floating plants, but would (4) avoid key floating IAAP species. Understanding how IAAPs affect predator-prey dynamics is critical for developing effective management strategies to control these invasive species. By enhancing our knowledge of how invasive plants alter predator foraging efficiency, this study could provide insights into managing both aquatic plant populations and the trophic structures that depend on them, ultimately contributing to better ecosystem conservation practices.

3.2 METHODS AND MATERIALS

3.2.1 Animal collection

In March 2024, *Anisops debilis* adults and *Cx pipiens* larvae were collected from water bodies behind the Life Sciences building at Rhodes University and housed in a controlled environmental (CE) room at 21° C with a 12:12 light:dark cycle. *Anisops debilis* were collected from an abandoned aquaculture facility at Rhodes University, using a square framed 30cm × 30cm SASS net attached to a 1m long pole. The backswimmers were transferred to a 20L bucket and transported to the CE room in the Life Sciences building and placed in a 200L aquarium at a density of no more than 1 individual per litre, filled with borehole water. Predators were housed for a period of seven days prior to experimentation and were fed on *Cx pipiens* larvae ad libitum. *Culex pipiens* rafts were collected from 70-liter mesocosms (as used in Chapter 2) with each mesocosm filled with 50 liters of borehole water and 2.48g of nutrients (Multisol (N), Culterra Pty Ltd). *Culex pipiens* egg rafts were collected from these tubs and transferred to fish tanks (30 cm x 45 cm) in the same CE room, filled with water from the outdoor mesocosms. *Culex pipiens* eggs were allowed to hatch and the larvae were used for the predation experiment within the first 48 hours.

3.2.2 Predation experiment

Predation experiment trials were run in 10L black microcosm buckets, filled with 5L of borehole water. The microcosms treatments were 1) Control (no plant cover), or 100% plant cover by 2) native *Lemna minor* (common duckweed), 3) invasive *Azolla filiculoides* (red water fern), 4) invasive *Myriophyllum aquaticum (*parrot's feather) and 5) invasive *Pontederia crassipes* (water hyacinth) (Figure 3.1a). In each microcosm, environmental characteristics were recorded. Specifically, temperature (ºC), pH, dissolved oxygen (mg/L^{-1}) and turbidity (NTU) were measured using an Aquaread device (Aquaread Ltd., UK). Light levels were also recorded using an LT Lutron LX101 LUX meter, with the sensor placed at the bottom centre of the experimental chamber.

Two predatory *An. debilis* were then introduced into each microcosm at 16:00 and allowed to settle overnight until 8:00 am of the following morning. At 08:00 the next morning, 10 *Cx pipiens* larvae were added to each mesocosm, and the predators were allowed to feed undisturbed for six hours during light conditions. Each treatment was replicated 7 times (n = 7). To rule out mosquito loss associated with non-predatory considerations, the same treatments (n =3) were established and experiments run, but in the absence of the addition of predators (Figure 3.1(b)). Upon termination, plant material was gently removed and rinsed three times in a clean 10L bucket filled with filtered borehole water (Figure 3.2). This water, along with the water from the microcosm was then strained through a 30 cm Ø 200 µm mesh sieve. Predators were gently removed from the sieve and returned to their tanks. The contents of the sieve were then examined under a dissecting microscope, and any live mosquito larvae were counted and recorded. All treatments, including both predator and predator-free trials, were randomized in position within the CE room and over time to account for any potential temporal or spatial confounding factors.

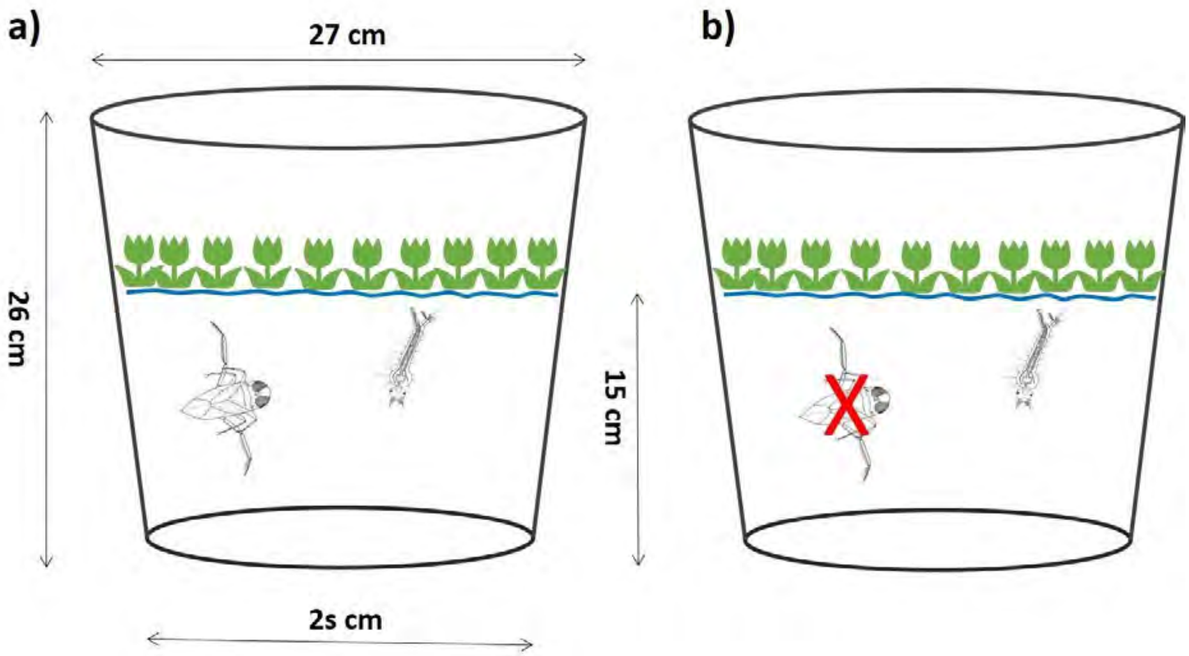


Figure 3. 1: Schematic representation of each chamber used for predation trials across experimental treatments, namely; Treatment 1 = no plant cover/Control, Treatment 2 = *Lemna minor* (Common duckweed), Treatment 3 = *Azolla filiculoides* (red water fern), Treatment 4 = *Myriophyllum aquaticum* (parrot's feather), Treatment 5 = *Pontederia crassipes* (water hyacinth). a) represents chambers where semiaquatic predator (*Anispos debilis*) and prey (*Culex pipiens*) were present, while b) represents environments that only contained *Culex pipiens* prey.

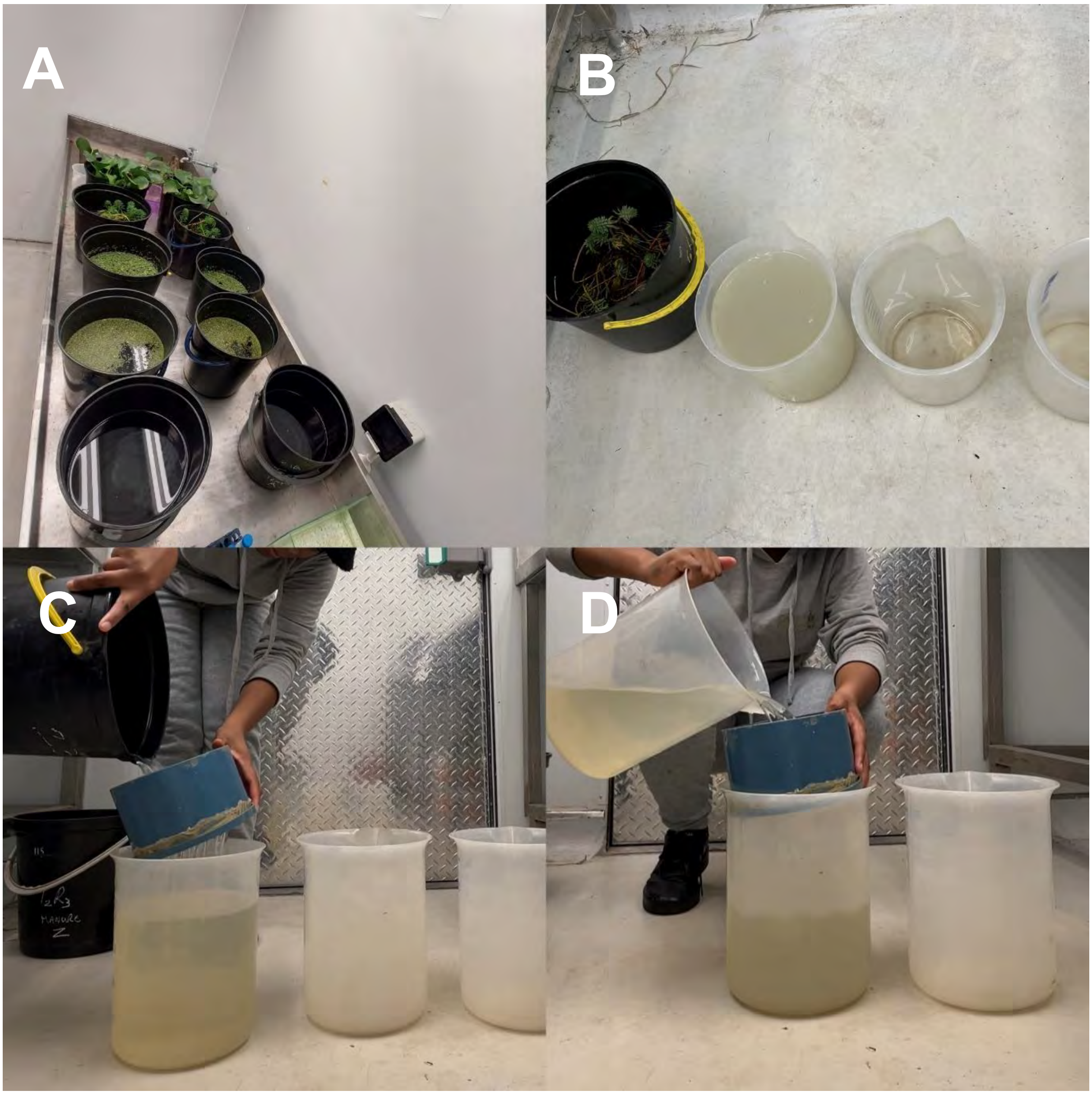


Figure 3. 2: Steps followed to assess the number of larvae consumed by the predator. A: plants before consumption rate was assessed. B: plants were placed in a bucket with clean water and then serially rinsed in an additional two buckets to dislodge any live mosquito larvae and *Anisops debilis* predators. C: water from the buckets, from which the plants were removed, was sieved to check for the presence of any remaining mosquito larvae. D: water from the three rinsing containers was sieved to ensure all animals were collected.

3.2.3 Predator behaviour experiment

Behavioural trials were run in 30cm (h) × 45cm (l) × 23cm (d) fish tanks (Figure 3.3). Each tank was filled to the 24 cm mark with borehole water. At the water level, 15 cm from the left edge, a PVC divider was positioned to facilitate the housing of one third of the surface area with floating aquatic plant material. In order to zone the tank, the front panel of the tank was divided into a 3 × 3 array (15 cm × 8 cm each) of 9 panels using a permanent marker. Horizontally, the panels A - C denoted the *Surface* zone, D - F the *Middle* zone and G - I the *Bottom* zone. Vertically, panels A, D and G denoted the *Vegetated* zone, B, E and H the *Edge* zone and C, F and I the *Open-water* zone. The treatments used in this experiment were the same as in the predation experiment, whereby 1) was a control with no plant cover, 2) the top left panel was 100% covered by the native *L. minor* (common duckweed), 3) the top left panel was 100% covered by invasive *A. filiculoides* (red water fern), 4) the top left panel was 100% covered by invasive *M. aquaticum (*parrot's feather) and 5) the top left panel was 100% covered by invasive *Po. crassipes* (water hyacinth) (Figure 3.3). For each trial, individual adult *An. debilis* were gently transferred to the observation tank and allowed to acclimate for thirty minutes, after which, they were continuously observed for thirty minutes. For the thirty minutes of observation, the time spent (seconds) in each panel (A – I) was recorded using a lap counter, with a new lap started whenever the backswimmer moved from one panel to another. From this dataset, total time per panel (A - I) and zone (Surface/ Middle/ Bottom and Vegetated/ Edge/ Open-water), as well as total number of crosses among panels (line-crosses) could be gleaned. In addition, surfacing events for replenishment of oxygen supply were enumerated over the thirtyminute period. Five replicates (n = 5) per treatment were run for this experiment.

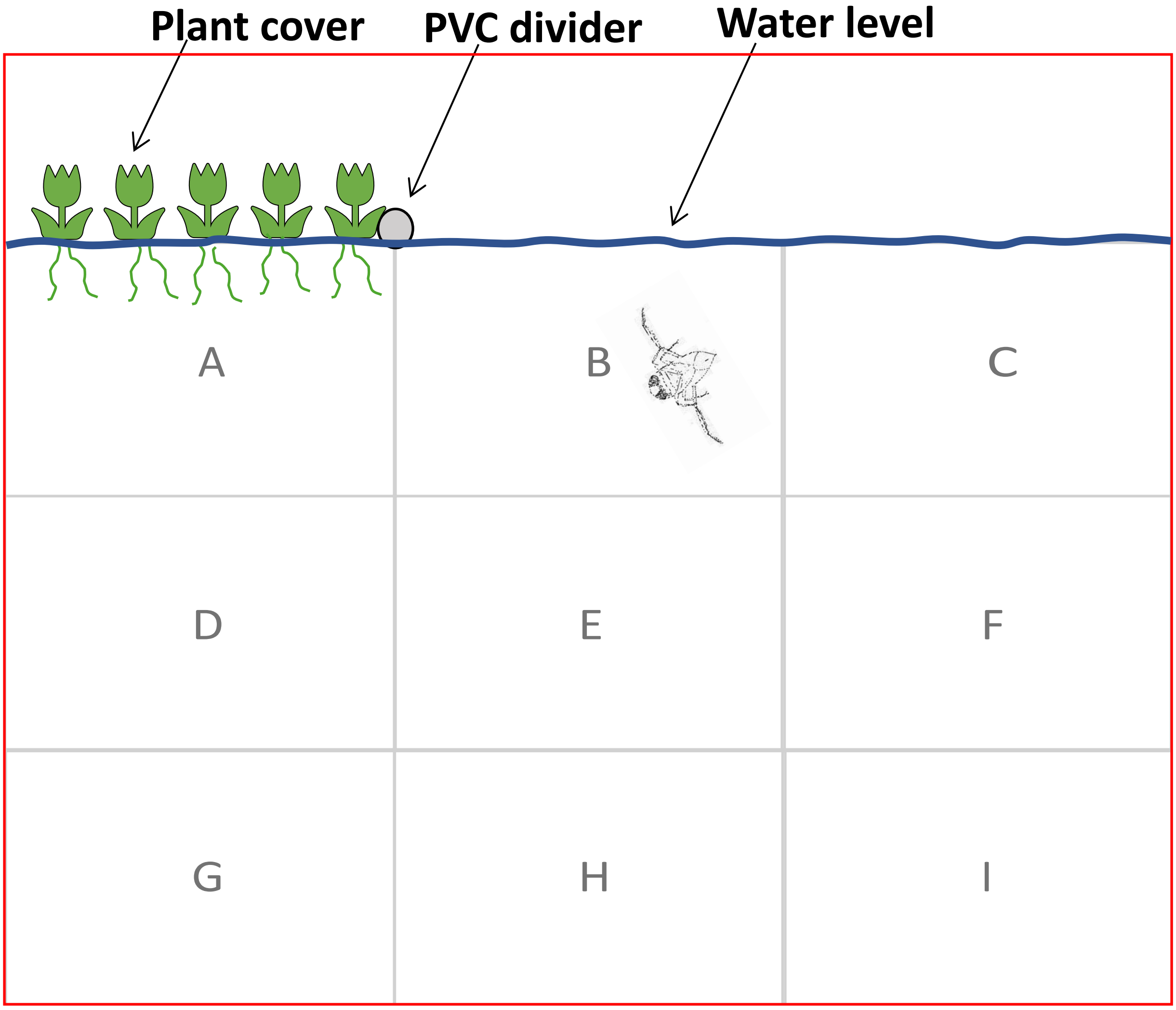


Figure 3. 3: Front view of 30cm (h) × 45cm (l) × 23cm (d) tank (red line), modified for behavioural observations of *Anisops debilis*. The tank was filled to the 24 cm level (blue line) with borehole water. A PVC divider was positioned 15 cm from the left glass panel to secure floating plants to the top left third of the tank. The portion of the tank submerged was divided into a 3 × 3 array of 9 panels (15 cm × 8 cm each), each denoting a position in relation to horizontal (Surface [A/B/C]/ Middle [D/E/F] / Bottom [G/H/I]) and Vertical (Vegetated [A/D/G]/ Edge [B/E/H]/ Open-water [C/F/I]) tank use. For illustrative purposes, *An. debilis* is located in panel B (Surface+Edge) in this figure.

3.2.4 Data analyses

For the predation experiment component, as well as differences among treatments in overall movement (line crossing events) and surfacing events in the behavioural experiment, the data were tested for normality using Shapiro-Wilk tests. If data conformed to parametric test assumptions, an Analysis of Variance (ANOVA) approach was employed, along with a Tukey post-hoc test where significant differences were detected. Where data did not conform to parametric assumptions, a Kruskal-Wallis test was used, followed by the Dunns pairwise tests where significant differences ensued.

For the behaviour experiment component, generalised linear models GLMs), with a Gamma Log-Link function were employed to test for differences between Treatment, Zone and Treatment × Zone (vertical/horizontal) interactions. Separate GLMs were conducted for analysis of behavioural differences at the vertical and horizontal levels. In each case, when differences were observed, Post hoc comparisons were run to assess for homogenous groups. All data analysis was conducted in R.

3.3 RESULTS

3.3.1 Predation experiment

No significant differences ($P > 0.05$) for any of the aquatic water quality physicochemical variables measured were evident among treatments or between predator and predator-free trials in each case, highlighting that water quality effects on predation outcomes were unlikely and that any observed effects were a result of predation (Figure 3.4). Lux levels, however, were significantly different across treatments (X^2= 31.3, D.F. = 4, $P < 0.01$). The greatest Lux levels observed in the control treatment (mean = 383), followed by the *L. minor* treatment (mean = 81.6) and the *A. filiculoides* treatment (mean = 76.4), which formed a significantly homogenous group ($P < 0.05$). The *M. aquaticum* treatment (mean = 31.4) and the *Po. crassipes* treatment (mean =15.0) had the lowest Lux levels and also formed a significantly homogenous group ($P < 0.05$). There were, similarly, no differences in Lux levels between predator and predator=free trials ($P > 0.05$ in each case).

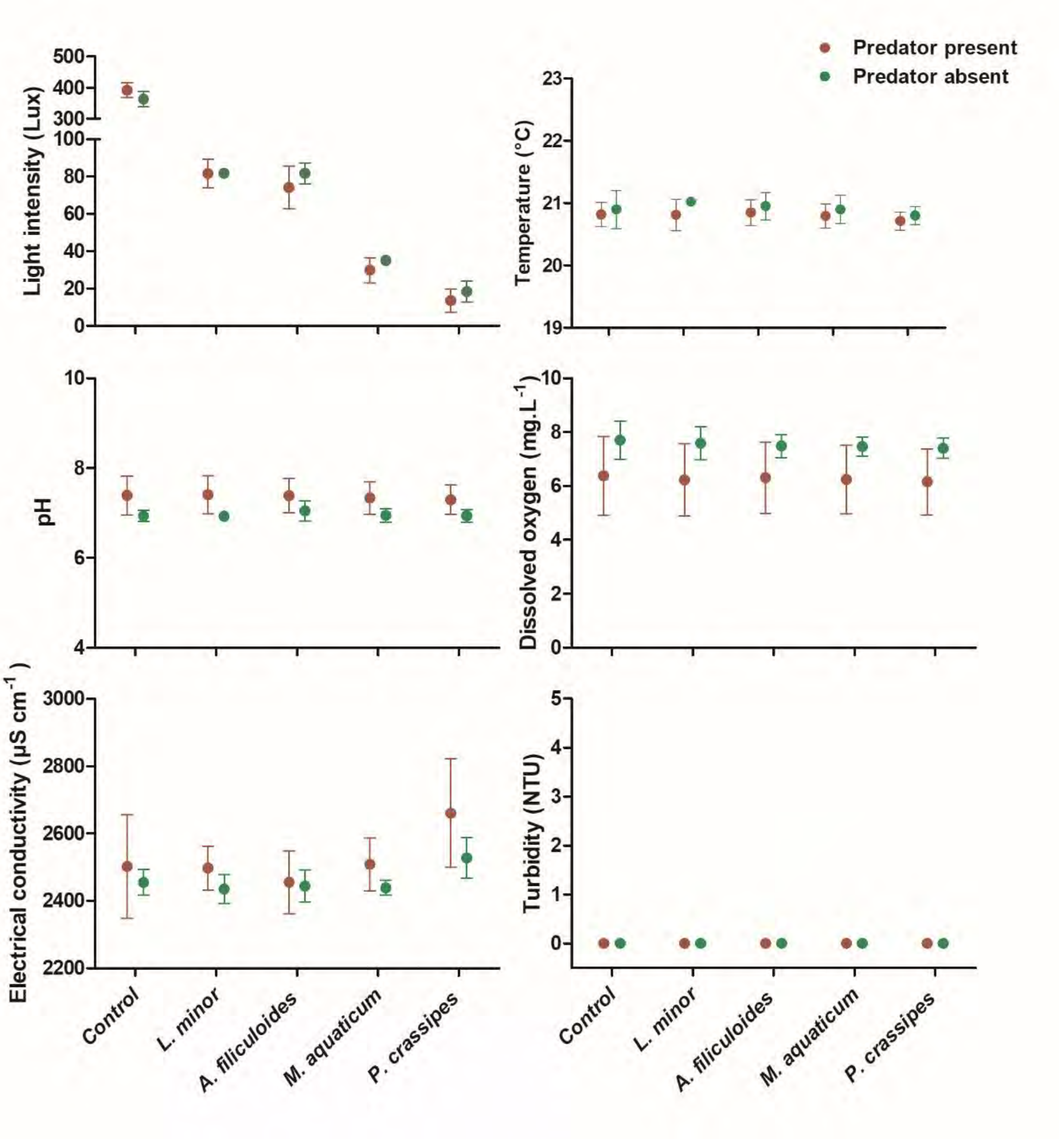


Figure 3. 4: Mean (± standard deviation) physico-chemical parameters measured from each chamber under predator (Hemiptera: *Anisops debilis*) and predator-free scenarios. Control = no plant cover, *L. minor* = *Lemna minor* (common duckweed)*, A. filiculoides* = *Azolla filiculoides* (red water fern)*, M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather)*, Po. crassipes* = *Pontederia crassipes* (water hyacinth).

All *Cx pipiens* larvae were accounted for alive in the predator-free trials (Figure 3.5), ruling out mortality effects associated with environmental variables. In the presence of predators, differences in mosquito consumption across treatments were evident ($F_{1,4}$=63.0, *P* = 0.0000001). Tukey post-hoc comparisons highlighted that all treatments were significantly different from each other (P < 0.05 in each case). Consumption was greatest in the *L. minor* treatment, followed by that of the Control and then the *A. filiculoides* treatments. The *M. aquaticum* treatment had the lowest *C. pipiens* larvae consumption.

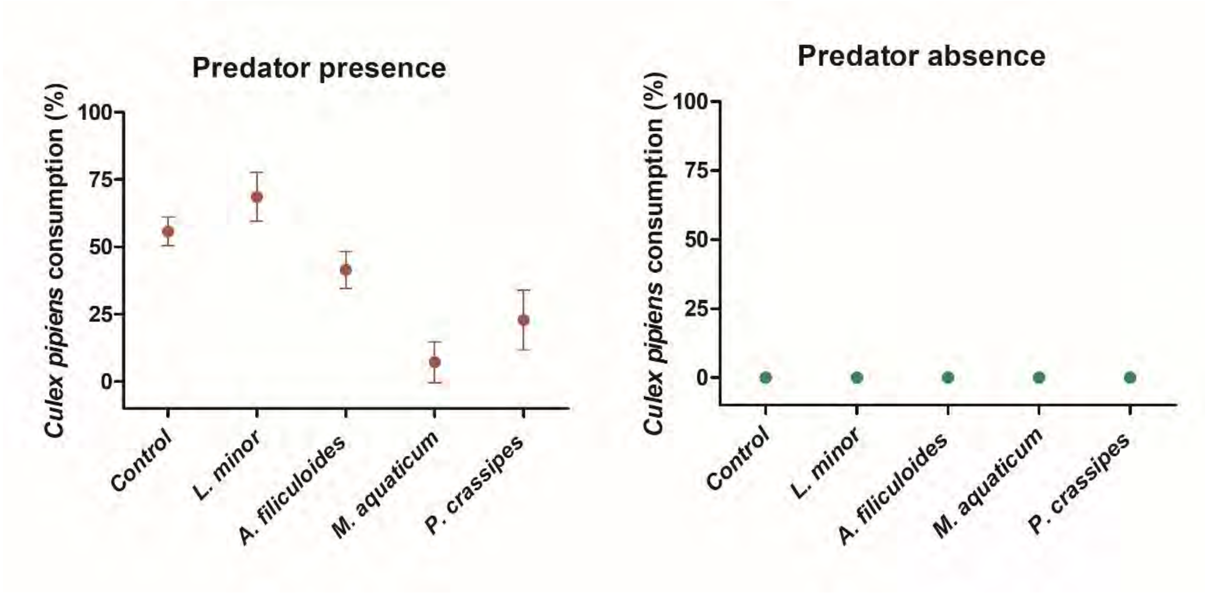


Figure 3. 5: Mean (± standard deviation) proportional consumption of *Culex pipiens* larvae prey from each chamber under predator (Hemiptera: *Anisops debilis*) and predator-free scenarios. Control = no plant cover, *L. minor* = *Lemna minor* (common duckweed), *A. filiculoides* = *Azolla filiculoides* (red water fern), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather), *Po. crassipes* = *Pontederia crassipes* (water hyacinth).

3.3.2 Predator behaviour experiment

Overall, differences in vertical use of the water column were evident at the Zone and Treatment × Zone levels, but not at the Treatment level (Table 3.1; Figure 3.6). In the control treatment, no differences in time spent by *An. debilis* across any of the vertical zones were evident (Figure 3.6). In the *L. minor* treatment significant differences in vertical zone use ensued, but specifically with the predator spending significantly more time in the vegetated zone than in the Open-water zone (Figure 3.6). In the *A. filiculoides* treatment differences in vertical zone use were also evident, with differences between vegetated vs open zones and between Edge and Open zones observed (Figure 3.6). *Anisops debilis* spent significantly more time in the Open-water zone and similar (and lower) amounts of time in the Edge and Vegetated zones although spending the least amount of time in the latter. In the *Po. crassipes* treatment there was a significant gradient of use between the Open-water and Vegetated sites, with *An. debilis* spending significantly more time in the former and less in the latter (Figure 3.6). In the *M. aquaticum* treatment differences in vertical use were also evident. Specifically, there was the highest level of avoidance in the vegetated zone compared to the Open zone, with *An. debilis* spending significantly more of its time in the Open water zone away from the Vegetated and Edge zones (Figure 3.6).

Table 3. 1: Generalised linear model coefficients assessing differences in zone use (vertical and horizontal). Models were fit with a Gamma Log-Link function.

	DF	Wald Stat	p-value
Horizontal zone			
Intercept	1	6829.476	0.000000*
Treatment	4	2.924	0.570597
Zone	2	83.885	0.000000*
Treatment × Zone	8	33.617	0.000048*
Vertical zone			
Intercept	1	6829.476	0.000000*
Treatment	4	2.924	0.570597
Zone	2	83.885	0.000000*
Treatment × Zone	8	33.617	0.000048*

Time spent in the vertical zones was significantly affected by the higher order interaction of plant treatment and zone (Wald X^2_8 = 7586, $P<0.001$)In the control treatment, no significant differences in time spent by *An. debilis* across any of the vertical zones were evident (Figure 3.6). In the *L. minor* treatment, significant differences in vertical zone use ensued, but specifically with the predator spending significantly more time in the vegetated zone than in the Open-water zone ($P < 0.05$). In the *A. filiculoides* treatment differences in vertical zone use were also evident ($P < 0.05$, D.F. = 2, F= 22.24), with differences between vegetated vs open zones ($P < 0.05$) and between Edge and Open zones ($P < 0.05$) observed. *Anisops debilis* spent significantly more time in the Open-water zone and similar (and lower) amounts of time in the Edge and Vegetated zones although spending the least amount of time in the latter. In the *Po. crassipes* treatment there was a significant gradient of use ($P < 0.005$, D.F. = 2, X^2= 9.92), between the Open-water and Vegetated sites ($P < 0.001$), with *An. debilis* spending significantly more time in the former and less in the latter. In the *M. aquaticum* treatment differences in vertical use were also evident ($P < 0.005$, D.F. = 2, X^2= 22.24). Specifically, there was the highest level of avoidance of the vegetated zone compared to the Open zone ($P < 0.005$), with *An. debilis* spending significantly more of its time in the Open water zone away from the Vegetated and Edge zones.

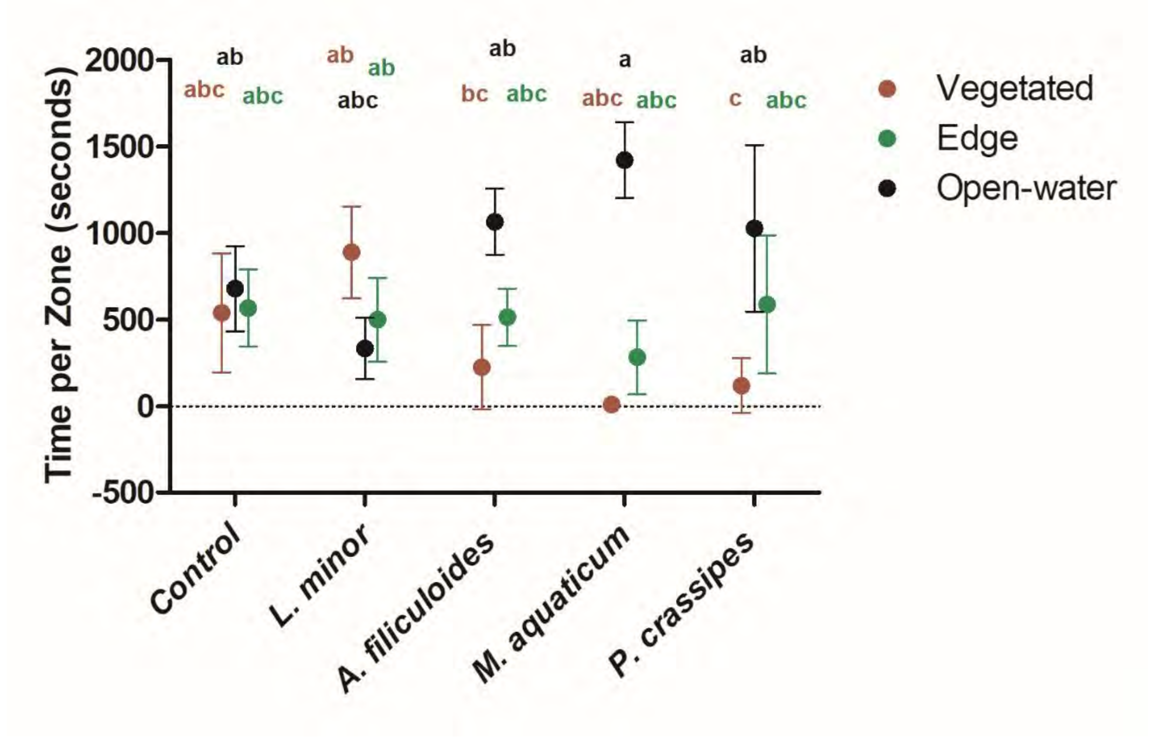


Figure 3. 6: Mean (± standard deviation) of time in seconds that the predator (Hemiptera: Anisops debilis) spent in each vertical zone in the tank. Vegetated zone denotes panels A + D + G use, Edge zone denotes panels B + E +H use, Open-water zone denotes panels C + F + I use (see Figure 3 for panel details). Control = no plant cover, *A. filiculoides* = *Azolla filiculoides* (red water fern), *L. minor* = *Lemna minor* (common duckweed), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather), *Po. crassipes* = *Pontederia crassipes* (water hyacinth). Letters denote significant homogenous groups as identified by the GLM Pos hoc comparisons, colour coordinated per zone within each treatment.

Overall, differences in horizontal use of the water column were evident at the Zone, Treatment and Treatment × Zone levels (Table 3.1; Figure 3.7). In all treatments except the *Po. crassipes* treatment, *An. debilis* spent most of their time in the Surface zone, although not always significantly so (Figure 3.7). In the Control treatment, the backswimmers spend significantly more time in the Surface zone compared to the Middle and Bottom zones in which they spent a similar amount of time (Figure 3.7). In the *L. minor* treatment, the backswimmers spent significantly more time in the surface than the bottom zones, although no differences between Middle and Surface or Middle and Bottom were evident (Figure 3.7). In the *A. filiculoides* treatment Surface and Middle zone use was significantly similar, with Bottom zone use being significantly less than the Surface, but not less than the Middle zone (Figure 3.7). Patterns in the *M. aquaticum* treatment were similar to the *L. minor* treatment, although even more (significantly so) time was spent in the surface than in the bottom zones, with the Middle zone use being dissimilar to both the Surface and Bottom zones. The *Po.*

crassipes treatment was the only treatment where backswimmers spent significantly more time in the Middle zone than in the Surface zone and Bottom zones, while use of the Surface and Bottom zones were lower and similar (Figure 3.7).

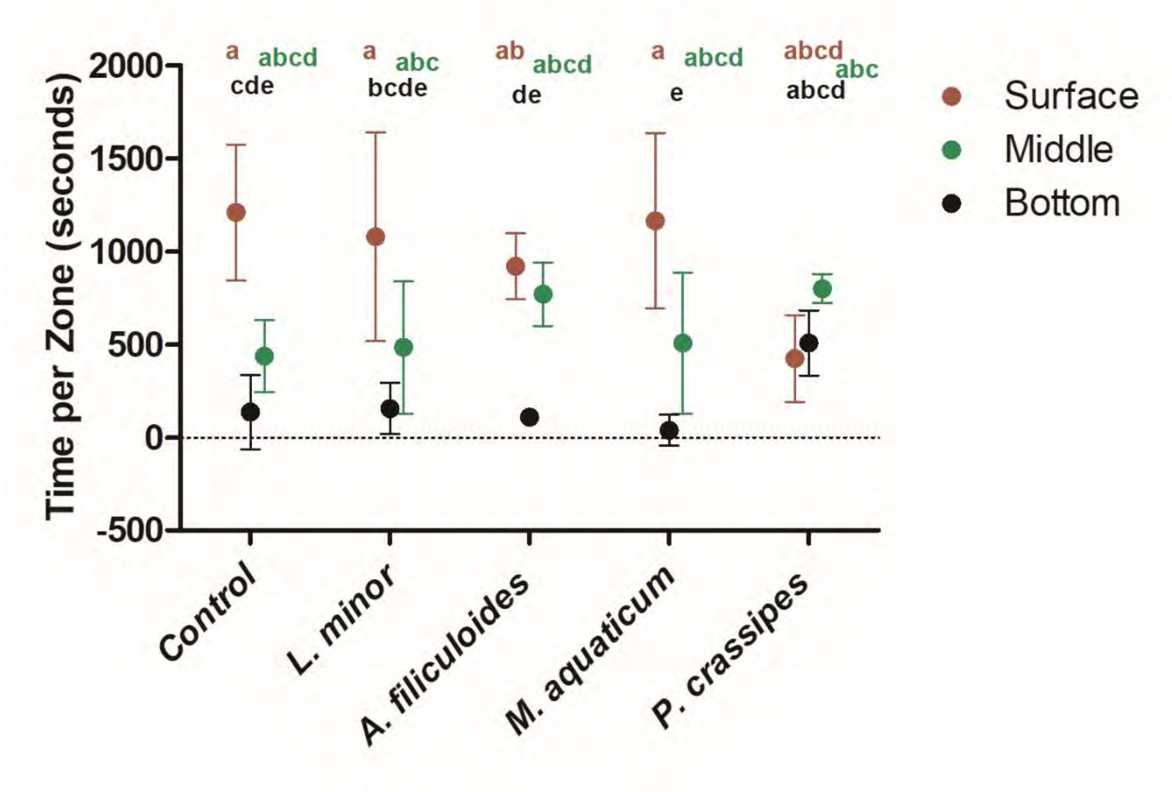


Figure 3. 7: Mean (± standard deviation) of time in seconds that the predator (*Hemiptera: Anisops debilis*) spent in each horizontal zone the tank. Surface zone denotes panels A + B + C use, Middle zone denotes panels D + E + F use, Bottom zone denotes panels G+ H+ I use (see Figure 3 for panel details). Control = no plant cover, *A. filiculoides* = *Azolla filiculoides* (red water fern), *L. minor* = *Lemna minor* (common duckweed), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather), *Po. crassipes* = *Pontederia crassipes* (water hyacinth). Letters denote significant homogenous groups as identified by the GLM Pos hoc comparisons, colour coordinated per zone within each treatment.

In terms of total movement (number of line/panel crosses), significant differences among treatments were evident ($F_{1,4}$= 2.53, $P < 0.05$) (Figure 3.7a). Post hoc comparisons revealed that the only difference in total movement observed was between the *Po. crassipes* and *M. aquaticum* treatments, with all other comparisons insignificant ($P > 0.05$ in all cases). Of the number of surfacing events, significant treatment effects were evident ($X^2 = 12.35$, D.F. = 4, $P < 0.05$). The only difference between specific treatments, however, was also between those of the *Po. crassipes* and *M. aquaticum* treatments (*P* < 0.05), with all other pairwise treatment comparisons revealing no significant differences (*P* > 0.05 in all cases).

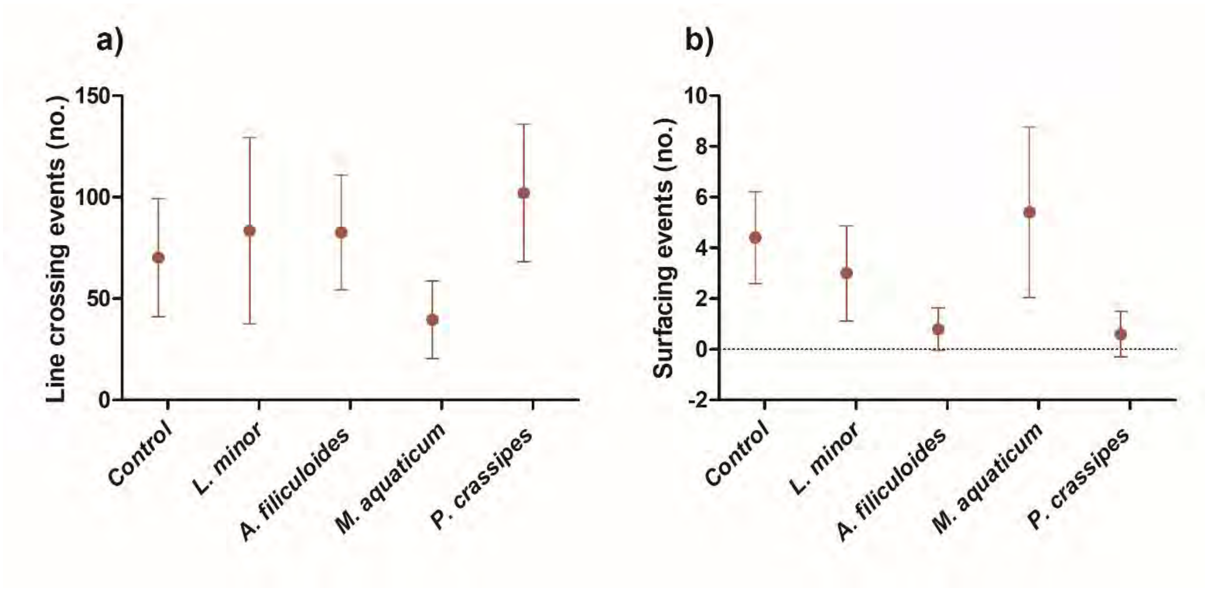


Figure 3. 8: Mean (± standard deviation) number of a) line/panel crosses and b) surfacing events by the Hemipteran predator *Anisops debilis* Control = no plant cover, *A. filiculoides* = *Azolla filiculoides* (red water fern), *L.* minor = *Lemna minor* (common duckweed), *M. aquaticum* = *Myriophyllum aquaticum* (parrot's feather), *Po. crassipes* = *Pontederia crassipes* (water hyacinth).

3.4 DISCUSSION

The findings of this chapter highlight that floating aquatic plants did not necessarily compromise predator-prey interaction outcomes, in contrast to the first hypothesis. Specifically, native floating plants induced no significant difference in larval mosquito predation when compared to the control treatment. Differences were, however, evident among *M. aquaticum* and *Po. crassipes*, both significantly hindering predation success, in line with the second hypothesis. Similar to the findings in the predation experiment, little difference in predator behaviour was evident between the control and the native floating plant species, as predicted in hypothesis 3. However, at the treatment levels there were differences in water column use. In particular, the *A. filiculoides* and *M. aquaticum* treatments exhibited high levels of floating IAAP avoidance by *An. debilis*. These findings build on those of Chapter 2, further highlighting the role floating IAAPs play in facilitating mosquito proliferation. These findings are locally relevant, adding to our understanding on ecosystem disservices associated with floating IAAPs in South Africa. However, the study is also of global relevance given that the problematic plant species and associated ecosystem services are widespread (Hussner, 2012).

Structural habitat complexity in aquatic ecosystems is a critical factor influencing predator-prey interactions. The physical structure provided by IAAPs can affect light availability and provide refuge for prey, thereby altering the foraging efficiency of predators (Ingrum et al., 2010). The study revealed that predator foraging efficiency varied significantly across different aquatic plant treatments. Specifically, the consumption of *Cx. pipiens* larvae by the backswimmer *An. debilis* was consistently above 50% in treatments with common duckweed (*L. minor*), red water fern (*A. filiculoides*), and parrot's feather (*M. aquaticum*). In contrast, predator foraging efficiency was significantly reduced to below 25% in the presence of water hyacinth. These results suggest that the physical structure of water hyacinth plays a pivotal role in impeding predator effectiveness, likely due to its impact on light penetration and the availability of hiding spaces for prey.

The study found that light intensity varied considerably between different plant treatments , with water hyacinth and parrot's feather having the lowest light levels. In contrast, the control treatment, which lacked dense vegetation, exhibited the highest light intensity . This variation in light intensity can be attributed to the differences in plant morphology. Water hyacinth, with its large, broad leaves and dense root systems, creates a thick mat on the water surface, significantly reducing the amount of light that penetrates the water column. Parrot's feather, while not as dense as water hyacinth, also has a substantial impact on light availability due to its feathery leaves that create shade beneath the water surface. These results therefore suggest that one of the key mechanisms by which IAAPs influence predator-prey interactions is through their impact on light penetration. Light is essential for visual predators, such as *An. debilis*, which rely on visual cues to detect and capture prey. Studies have shown that light availability is essential for visual predators, as it enhances their ability to detect prey (Crowder & Cooper, 1982). When light penetration is reduced, as in the case of dense aquatic vegetation, predators may struggle to locate prey, leading to a decrease in predation efficiency. This is evident in this study where the predator had a preference for well-lit areas, avoiding shaded vegetated areas. The reduced light intensity observed in the water hyacinth and parrot's feather treatments likely impaired the predators' ability to effectively locate and capture mosquito larvae . Furthermore, reductions in light intensity may also influence the behaviour of mosquito larvae, making them less visible or more difficult to detect in these environments.

In addition to limiting light penetration, the structural complexity provided by IAAPs can offer refuge to prey, further reducing the effectiveness of predators. To increase their likelihood of survival, animals can use cover as refuge and to escape from a predatory encounter (Johnsson et al., 2004). Various authors (Lazarus and Symonds, 1992;

Vasquez et al., 2002) found that individuals tend to increase their foraging effort where there are fewer protective covers and decrease it where there are more obstacles or protective covers (Butler et al., 2005; Ebensperger & Hurtado, 2005). This was evident in the predator behaviour experiment where the backswimmer was more active and moved around all the quadrants in the no cover treatment. Water hyacinth is known for its complex and extensive root system, which can create a dense network of hiding spaces for mosquito larvae. This structural complexity not only provides physical barriers that protect prey from predators but also disrupts the predators' ability to manoeuvre and capture prey efficiently. The complex root systems and large leaves of water hyacinth create a highly structured environment that not only obstructs light but also provides ample hiding spaces for prey (Barker et al., 2014). Such environments can reduce the encounter rate between predators and prey, further diminishing predation success. In the context of this study, this likely explains the significantly lower consumption rate of mosquito larvae in the water hyacinth treatment , as the predators were less able to effectively hunt and capture their prey. This is consistent with the findings of other studies that have documented how complex habitats can reduce predation rates by providing prey with better opportunities to hide (Werner & Hall, 1979; Bartholomew et al., 2000; Chang & Todd, 2023).

The implications of these findings extend beyond the immediate context of mosquito control. The ability of aquatic plants like water hyacinth to alter predator-prey dynamics has broader ecological consequences. In ecosystems where water hyacinth is prevalent, the reduced predation efficiency of natural mosquito predators could lead to higher mosquito populations. This, in turn, could exacerbate the spread of mosquito borne diseases, posing significant public health risks. Furthermore, the alteration of predator-prey interactions can have cascading effects on the entire aquatic ecosystem, potentially disrupting community compositions and affecting biodiversity (Leroux & Loreau, 2009). The role of invasive alien aquatic plants in shaping predator-prey dynamics also highlights the importance of managing these species in aquatic ecosystems. Effective management of water hyacinth and other invasive plants is crucial not only for maintaining ecological balance but also for supporting natural mosquito control efforts. By reducing the density of invasive plants, it may be possible to enhance the effectiveness of natural predators like *An. debilis*, thereby contributing to the control of mosquito populations which are a public health concern.

In conclusion, this study aimed to investigate the effects different aquatic plants, one native, and three IAAPs, have on the predation efficiency of the backswimmer *An. debilis* on larvae of the mosquito, *Cx pipiens*. The findings highlight the significant influence of aquatic plant structure on predator-prey interactions. Dense vegetation, such as that provided by water hyacinth, can hinder predation efficiency and potentially lead to higher mosquito populations which support the hypotheses of the study. This has important implications for natural mosquito control efforts and underscores the need for targeted management of invasive aquatic plants to support effective predator-prey dynamic. Future research should investigate the effects of varying densities of invasive plants on predation efficiency and explore how different environmental conditions might influence these interactions in both laboratory and field settings. Additionally, studies could examine the long-term impacts of these plants on mosquito population dynamics and ecosystem health.

CHAPTER 4

General discussion

Plate 4: Mesocosm containing water hyacinth with its flowers blooming. Rhodes University Department of Zoology and Entomology. Photo credit: Thoriso V. Masalesa, November 2024.

4.1 Overview

Invasive alien aquatic plants (IAAPs) have been studied extensively for the impacts they have on ecosystems, including their ability to outcompete native species, alter water quality, and modify habitats. However, a less explored aspect of this ecological disruption is the relationship between IAAPs and mosquitoes, particularly at the larval stage. Mosquitoes, which have aquatic larvae, interact with aquatic plants in complex ways that may influence their population dynamics. Interactions can occur at multiple levels, involving both ovipositing adults and the larvae themselves, with plants providing either suitable breeding sites or refuge from predators (Stone et al., 2018). This study aimed to address this knowledge gap by investigating key ecological interactions between floating IAAPs and locally prevalent mosquitoes using experimental approaches. Specifically, two key components were explored. First, the study examined whether alien IAAPs attract mosquitoes for oviposition and subsequent growth, through creation of discrete habitat features. The second component focussed on how IAAPs potentially alter predation risk for mosquitoes. This component comprised two sub-components dealing with a) actual predator-prey interaction outcomes in the form of prey consumption dynamics and b) behavioural observations of predators in relation to floating IAAP species, respectively.

4.2 Floating IAAPs and mosquito abundances

The effect of IAAPs on mosquito oviposition and larval development has received some attention due to the critical role that vegetation plays in creating suitable habitats for mosquito breeding. Numerous studies have shown that aquatic plants, both macrophytes and charophytes, can influence mosquito reproduction and larval survival, but the outcomes are often species-specific and context-dependent, sometimes leading to contradictory findings. For instance, certain macrophytes like *Po. crassipes* are known to create ideal oviposition sites by providing shelter and reducing predation, which can enhance mosquito breeding and larval survival (Portilla et al., 2021). The dense root systems of such plants offer refuge for larvae, shielding them from predators like fish and backswimmers, while also stabilizing water conditions by reducing turbulence and temperature fluctuations (Warfe & Barmuta 2004; Rejmankova et al., 2013). On the other hand, some studies report that particular aquatic plants may inhibit mosquito reproduction and larval development (Turnipseed et al., 2018; Portilla et al., 2021). These plants can create environments that are less suitable for mosquitoes due to factors such as reduced oxygen levels, limited access to sunlight, and changes in water chemistry (Juliano & Lounibos, 2005). Dense plant mats can block sunlight and alter water temperature, creating unfavourable conditions for larval development.

Additionally, the decomposition of such invasive plants may deplete oxygen levels, leading to hypoxic conditions that are detrimental to larvae (Carpenter & Lodge, 1986; Scheffer et al., 2003).

The contradictory results observed across studies may stem from differences in plant morphology, habitat structure, and local environmental conditions. For example, plants with long, submerged root systems may offer more ideal conditions for larvae by providing additional microhabitats, while floating plants might inhibit larval growth due to reduced light penetration or water quality changes (Rejmankova et al., 2013; Portilla et al., 2021). Moreover, the specific interactions between mosquitoes and plants may depend on factors such as plant density, nutrient levels, and predator presence, all of which can vary across ecosystems (Silver, 2008). Differences in mosquito species habitat preferences likely also contribute to these variations in findings. As such, regionally specific studies are necessary to gain insight into local mosquito facilitation dynamics, such as the present study. Here, it was found that *Po. crassipes*, *Pi. stratiotes* and M*. aquaticum* harboured more larval mosquitoes than *S. molesta* and *A. filiculoides* (Chapter 2) suggesting that some aquatic IAAPs attract mosquitoes for oviposition while others repel them. In addition, the various floating IAAP species had discrete effects on biotic resistance potential, through influence on predator and competitor numbers. As such, it is likely that the mosquito facilitation is a result of multiple drivers, including abiotic and biotic components. While a mechanistic understanding of these drivers remains elusive, the results are clear, highlighting the clear link between floating IAAPs and mosquito success. The role various physico-chemical components play in facilitating the various considerations are outlined below.

Temperature can regulate invertebrate development, most critically larval mosquito growth (Silver, 2008). Floating IAAPs can significantly affect water temperature in ecosystems through various mechanisms. These effects arise from their growth patterns, coverage of water surfaces, and the way they interact with sunlight and water movement. Floating IAAPs, especially those with dense surface coverage such as *S. molesta* and *Po. crassipes* can reduce the amount of sunlight reaching the water. This shading effect lowers the overall water temperature in the areas directly beneath the plants by limiting solar radiation, which is critical for warming the water (Ding et al., 2020). This can lead to cooler surface waters and reduced thermal stratification, where temperature layers form within the water column.

Dense mats of invasive plants can decrease water evaporation by acting as a barrier between the water surface and the atmosphere. Reduced evaporation means that heat, which would normally dissipate from the water, is trapped, potentially leading to localized warming of the water surface (Barrett et al., 2010). However, this effect may vary depending on the plant type and the extent of coverage. Some invasive aquatic plants, such as *M. aquaticum* can slow down water flow in rivers, streams, or lakes by physically obstructing movement. Slower water movement reduces mixing, allowing surface waters to either warm or cool more rapidly depending on the amount of sunlight and shading present (Ehrenfeld, 2010). In this study, temperature did not vary significantly across the different treatments (Chapter 2), suggesting that temperature was not a major factor influencing larval mosquito distribution.

Additionally, IAAPs disrupt water chemistry by altering dissolved oxygen levels, nutrient cycling, and pH, often creating stagnant, low-oxygen environments. In this study, water pH also showed minimal variation between treatments (Chapter 2). Throughout the sampling period, the control treatment exhibited the highest pH levels, while the water hyacinth treatment had the lowest pH. The pH values for the other treatments fell between these two extremes, with only slight fluctuations. It is important to note that pH levels in all treatments remained within the tolerance ranges for larval mosquitoes, indicating that pH was not a limiting factor for mosquito development in this study (Silver, 2008). Studies have shown that mosquito larvae can tolerate a wide pH range, with optimal growth occurring at neutral to slightly alkaline conditions (Clark et al., 2004). Therefore, the slight pH differences between treatments are unlikely to have impacted mosquito oviposition or larval survival.

Dissolved oxygen (DO) levels varied more notably between treatments and across the sampling period (Chapter 2). DO concentration is an important factor in aquatic ecosystems, influencing both biological and chemical processes. However, since larval mosquitoes breathe atmospheric air through specialized siphons, changes in DO concentrations are not expected to directly affect mosquito larvae (Silver, 2008). Instead, variations in DO can impact the aquatic predators and competitors of mosquito larvae, such as fish and predatory insects, which rely on dissolved oxygen for respiration. For instance, low DO levels can create hypoxic conditions that reduce predator abundance and activity, potentially enhancing mosquito survival (Reiskind et al., 2009). In this study, the control treatment consistently had the highest DO concentration levels, while the *Po. crassipes* treatment experienced a sharp decrease in DO from day 21 onward, becoming the treatment with the lowest concentration (Chapter 2). This decline in DO under dense *Po. crassipes* coverage is likely due to reduced water circulation and increased organic matter decomposition, which are common effects of invasive plant species in aquatic habitats (Ribaudo et al., 2018).

Electrical conductivity (EC), which measures the water's ability to conduct electrical current and is related to ion concentration, also followed a similar trend to temperature, suggesting that neither EC nor temperature were significantly affected by the different treatments over time (Chapter 2). Both variables remained relatively stable and did not exhibit notable deviations, implying that they are unlikely to have had a major impact on mosquito larvae. Other studies (Nikookar et al., 2017; Musonda & Sichilima, 2019) have shown that there is a positive correlation between EC and mosquito larval abundance, but this was not observed in this study. Turbidity, on the other hand, exhibited greater variation across treatments (Chapter 2). This variation could be influenced by factors such as sediment disturbance, organic matter content, or differences in plant density between treatments. High turbidity levels are often associated with suspended particles in the water column, which can alter light penetration and influence both the aquatic food web and habitat structure (Vanni, 2002). In this study, there were no significant disturbances in the mesocosms during the sampling of physicochemical parameters, suggesting that turbidity changes were likely driven by plant-related factors, such as root structure or leaf decomposition. Further analysis of these variations in turbidity could help clarify their potential ecological implications, particularly concerning predator-prey interactions in aquatic environments with invasive aquatic plants.

The availability of nutrients in the water, such as nitrates and phosphates, plays a crucial role in larval survival, as these nutrients influence algal growth and other food resources for mosquito larvae (Muturi et al., 2008). High concentrations of nitrates and phosphates can stimulate the proliferation of phytoplankton and periphyton, providing abundant food for larval mosquitoes, particularly in genera such as *Culex* and *Aedes* (Navideh et al., 2015). This increase in algal biomass enhances the overall productivity of the aquatic ecosystem, leading to improved growth and survival rates of mosquito larvae due to the availability of essential nutrients found in algae, including proteins and lipids (Ahmad et al., 2001; Kaufman et al., 2006). However, while nutrient enrichment can benefit larval survival through increased food availability, it can also lead to harmful algal blooms that may create hypoxic conditions, adversely affecting not only larval populations but also the broader aquatic community (Paerl et al., 2018).

Therefore, the dynamics of nutrient availability and its effects on algal growth are pivotal in shaping the survival and development of mosquito larvae in freshwater ecosystems (Kaufman et al., 2006; Dubey & Dutta, 2019). The data in Chapter 2 show that nitrate and ammonia levels remained relatively stable with minimal variation observed between the treatments. This stability suggests that the different mesocosm treatments did not significantly impact the levels of these nutrients, indicating a possible equilibrium in the nitrogen cycle within the mesocosms. However, treatment type had a significant impact on nutrients (Chapter 2). In contrast, phosphate and chlorophyll-a concentrations exhibited considerable variability across the treatments. Notably, the treatment containing *M. aquaticum* had the highest levels of both phosphates and chlorophyll-a. This significant increase in phosphate levels could be attributed to the plant's ability to uptake and release phosphates into the water column (Madsen et al., 2001; Tanner et al., 1998). *Myriophyllum aquaticum*, known for its rapid growth and nutrient absorption capabilities, likely enhances the availability of phosphates, which in turn supports higher primary productivity as indicated by elevated chlorophyll-a levels. The elevated phosphate levels and corresponding increase in chlorophyll-a suggest that *M. aquaticum* creates a nutrient-rich environment. Elevated levels of chlorophyll-a indicate increased algal biomass, which can provide food and shelter for mosquito larvae, potentially enhancing their survival and growth (Sunahara et al., 2002). This environment can be particularly conducive to mosquito breeding, as algae can serve as both a direct food source for larval mosquitoes and as a habitat that offers protection from predators. The variability in phosphate and chlorophyll-a concentrations across treatments highlights the significant impact that *M. aquaticum* can have on aquatic ecosystems.

By altering nutrient dynamics, this invasive plant species can shift the ecological balance, promoting conditions that favour mosquito proliferation. This shift can have broader ecological implications, potentially affecting the diversity and abundance of other aquatic organisms. In turn treatments containing *Po. crassipes* and *Pi. stratiotes* had the lowest nutrient concentration levels. These plant species are known for their ability to take up nutrients. They have rapid growth rates and extensive root system which increases their surface area and causes them to absorb more nutrients (Sudiarto et al., 2019). There are many abiotic and biotic factors that affect larval mosquitoes in their environment (Merritt et al., 1992). This study investigated some of the water quality parameters which were the physico-chemical parameters and nutrients associated with different IAAPs. There are other factors not investigated in this study that could affect mosquito proliferation, including the interactions between larval mosquitoes and their competitors, and the volatile chemical cues associated with different IAAPs (Blaustein & Chase, 2007; Silver ,2008; Rejmankova et al., 2013).

4.3 Floating IAAPs and interactions between mosquitoes and predators.

The mesocosm experiment (Chapter 2) provided insight into the implications of floating IAAP species for mosquito success. However, the mechanistic process through which this occurs remains elusive. While this study could not address each mechanistic component, an attempt was made to augment Chapter 2 results with a component that dealt specifically with a biotic interaction aspect. In particular, the role floating IAAPs play in mediating predator-prey interactions was of interest. Indeed, predator numbers, in addition to mosquito numbers, varied across treatments in Chapter 2. As such, mosquito abundances were likely a result of interactions between differences in oviposition and differences predation pressure, each independently resulting in varied mosquito larvae abundances at the treatment level. For this experimental component, the predatory *Rhantus* and *Hydraenid* species which colonised treatments in Chapter 2 could not be sourced in large numbers. As such, the use of a wide-ranging aquatic predator (*Anispos debilis*) that also migrates and colonises water bodies alongside mosquitoes and various floating IAAPs in the region was used. The study highlights that when given the option to select for microhabitat, there seemed to be avoidance of certain floating IAAP cover by *An. debilis*, which was not observed with native floating plant cover (Chapter 3). In addition, forced plant species-level cover had implications for predator-prey interaction outcomes, with certain floating IAAPs reducing mosquito prey risk.

Results from these two predation-related components were complimentary and both suggested that *Po. crassipes* and *M. aquaticum* had the greatest implications for predation efficiency reduction (Chapter 3). It is likely that the mechanisms that drove similarities between these two treatments were, however, different with *M. aquaticum* facilitating aversion by the predator while *Po. crassipes* induced prey refuge through structural complexity considerations. The presence of invasive alien aquatic plants significantly influences predation efficiency within freshwater ecosystems by altering habitat structure, visibility, and prey availability. Invasive species, such as *Po. crassipes* and *Salvinia molesta* (giant salvinia), often create dense mats that provide complex habitats, which can benefit some predator species while simultaneously hindering others (Warfe & Barmuta, 2004; Portilla et al., 2021). For instance, while these structures may offer refuge for certain predators, they can also obstruct predator access to prey, reducing the effectiveness of predation on mosquito larvae and other aquatic organisms (Grutters et al., 2015). Moreover, the shading effect of invasive plants can drastically decrease light penetration, reducing primary productivity and altering the abundance and distribution of prey species (Zavaleta et al., 2001). In the study, it was observed that water hyacinth significantly hindered the backswimmer's ability to efficiently hunt or forage. As a result, treatments dominated by water hyacinth resulted in significantly fewer *Cx pipiens* being consumed. This illustrates how invasive aquatic plants can disrupt traditional food web dynamics, potentially leading to population outbreaks of prey species and shifting community structures within aquatic ecosystems.

4.3 Control of floating IAAPs

Invasive species have detrimental effects on ecosystems, economies, and societies, making their control a critical priority. This study highlights the ecosystem disservices caused by invasive aquatic alien plants (IAAPs), particularly in terms of public health. The findings underscore the role of IAAPs in harbouring mosquitoes, which are vectors of many diseases, emphasising their implications for human well-being. Specifically, this study demonstrates that *Po. crassipes* (water hyacinth) and *M. aquaticum* (parrot’s feather) facilitate mosquito proliferation. These species should therefore be prioritized in IAAP control efforts, not only for ecological reasons but also to mitigate the associated public health risks.

There are numerous methods that can be used to control invasions which include biological, chemical, and mechanical control methods. Biological control is one method that can be used to control invasive aquatic plants, it can be alone or combined with other control measures (Coetzee, et al., 2019). Biological control is the introduction and release of exotic insects, mites, or pathogens to give permanent control of invasive species (Hastings, et al., 2006). It has been successful in controlling most invasive aquatic species suppressing their populations (Morgan, et al., 2005). Chemical control of invasive species includes the use of chemicals such as herbicides or pesticides which are sprayed on the invasive species causing them to die. This is an effective way to eradicate invaders but the impact it has on the environment is harmful as spraying chemicals releases toxic substances to the environment, and it can also get rid of the other native species. Mechanical control includes physically removing the invaders from the environments that they have invaded. This method is costly as a lot of labour is required and it does not get rid of every single invader, allowing them to grow again.

The control of invasive aquatic plants is also dependent on the type of plant and rate of infestation. Certain invasive alien aquatic plants are more effectively controlled by biological control whereas some are more effectively controlled mechanically. The infestation rate also determines which control method will be the most effective in a certain waterbody. Waterbodies with small infestations can easily be controlled through mechanical removal as there is not a lot of plants to get rid of and it will not require a lot of labour (Coetzee, et al., 2018). *Myriophyllum aquaticum*, for example, is easier to control through biological control as opposed to the other methods. This is because it can easily break through physical removal. Those broken fragments will then develop and reproduce as the plant can reproduce vegetatively (Moreira, et al., 1999). Biological control is the most effective and environmentally safe way to eradicate invaders in areas which have high infestation rates. The biocontrol of these floating invasive alien aquatic plants has a high number of successful outcomes all over the world more especially in the warmer regions. Examples of floating aquatic plants that have been successfully controlled through biocontrol include *M. aquaticum*, *Pi stratiotes, S. molesta, A. filiculoides,* and to a lesser degree, *Po. crassipes* (Coetzee, et al., 2018). So biological control of these invasive aquatic plants will allow the environment to rehabilitate and regenerate, resulting in restoration of the of the environment, with the benefit of controlling mosquito populations. Competitors and predators would also be reintroduced to the environment controlling the mosquito populations

4.4 Conclusion and future recommendations.

In summary, this study highlights the complex interplay between invasive aquatic plants (IAAPs), mosquito populations, and predator-prey dynamics in freshwater ecosystems. The findings indicate that IAAPs like *Pi. stratiotes*, *M. aquaticum*, and *Po. crassipes* can create favourable conditions for mosquito oviposition and larval survival by providing refuge and enhancing food availability, while *S. molesta* and *A. filiculoides* tend to repel larvae due to their less suitable habitat characteristics. The study also revealed that IAAPs significantly alter predator behaviour and efficiency, particularly impacting species such as the backswimmer *An. debilis*. By increasing habitat complexity, these invasive plants hinder predator access to prey, potentially leading to increased mosquito populations and elevated risks of vector-borne diseases. The results underscore the importance of considering both the ecological benefits and detriments of IAAPs when assessing their impact on freshwater ecosystems. Furthermore, this research contributes to a better understanding of how invasive species influence aquatic community dynamics, which is crucial for developing effective management strategies.

Future studies should investigate the long-term effects of IAAPs on ecosystem health and resilience and explore potential interventions that can mitigate the negative impacts of these invasive species on native biodiversity and public health. Future research should also focus on several key areas to further elucidate the complex interactions between invasive aquatic plants, mosquito populations, and their predators in freshwater ecosystems. First, more studies are needed to identify the key factors driving the observed variations in how IAAPs either facilitate or repel mosquito proliferation. While this study highlights the potential roles of physico-chemical parameters, nutrient levels, and predator-prey interactions, the precise mechanisms underlying these responses remain unclear. Second, long-term studies examining seasonal variations and their impact on these dynamics would provide valuable insights, as the relationships between IAAPs and mosquito populations may change over time due to environmental factors and plant life cycles. Additionally, exploring the role of other mosquito species, and potential mosquito predators in IAAP-dominated habitats would enhance our understanding of food web interactions and the ecological consequences of these invasives. Furthermore, conducting comparative studies across diverse ecosystems can help determine how local environmental conditions influence the effects of IAAPs on mosquito populations and their predators. Investigating the impact of specific nutrient management practices in IAAP-infested areas could also reveal strategies to mitigate their negative effects while promoting native biodiversity. Another aspect completely understudied is the relationship between IAAP flowers and mosquitoes with regard to pollination and nectar use. The role mosquitoes may play in facilitating IAAP proliferation with augmenting pollination dynamics, and the role IAAPs play in providing nectar and sap for adult moquitoes is completely unexplored. For many of these investigations, combinations of field surveys, along with more controlled experiments (as conducted here) are required to fully understand the interactions between IAAPs and mosquitoes. Lastly, integrating molecular techniques to assess microbial communities in IAAP-dominated habitats may provide insights into how these invasives influence water quality and nutrient cycling, ultimately informing management strategies aimed at controlling IAAPs and their ecological consequences. Overall, these recommendations highlight the need for a multifaceted approach to studying the ecological implications of invasive aquatic plants on freshwater ecosystems.

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Plate 5: *Culex pipiens* larvae in the laboratory. Photo credit: Ryan J. Wasserman, September 2024.

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