

Quantifying Ecosystem Recovery and
Restoration practise following the
Biological Control of Invasive Alien
Macrophytes in Southern Africa

THESIS

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Abstract

Invasive alien aquatic plants (IAAP) species are known to have deleterious effects on the freshwater ecosystems they invade. This includes both socio-economic and ecologically important ecosystem goods and services. Thus, IAAP species are declared a serious threat, second only to habitat modification for causing a loss of aquatic biodiversity. Three control methods have been widely applied to control IAAP species invasion globally; mechanical, chemical and biological control. Both mechanical and chemical control methods are considered short-term and expensive, whereas biological control methods are regarded an effective and long-term solution for IAAP species control at the landscape level. But, little is known of the ecological recovery following the biological control of IAAP species, with mechanical control known to have had mixed success and chemical control to have non-targeted effects on aquatic ecosystems, causing harm to wildlife and human well-being. Biological control practitioners measure the success of biological control based on: (1) the biological control agents' establishment and the negative impacts they impose on the targeted weed; and (2) the weeds biomass reduction and an increase in native macrophytes species. Arguably, measures of biological control success have been subjective and variable across the globe. Although some field studies have demonstrated biological control success to have positive socio-economic returns, there is little literature on ecological benefits. Furthermore, there is limited understanding on ecosystem recovery and possible restoration efforts following the biological control IAAP species, as compared to alien weeds in terrestrial and riparian ecosystems. Thus, this thesis aimed to quantify the ecological recovery i.e. aquatic biodiversity, ecosystem processes and trophic interactions following the management of *Salvinia molesta* in freshwater ecosystems. The research employed a suite of Before-After Control-Impact mesocosm and field studies to investigate the response of aquatic microalgae, macroinvertebrates and their interactions (food web structure and function) during *S. molesta* infestation and after mechanical and biological control.

The mesocosm experiment (Before invasion, During invasion & After control) showed that both aquatic microalgae and macroinvertebrate diversity indices were reliable biological indicators of *S. molesta* ecological impacts and recovery following control. The restored treatment (100% *S. molesta* cover + biological control agents), demonstrated complete aquatic microalgae and macroinvertebrate recovery following biological control, similar to the control treatment (open water), where the degraded/impacted treatment (100% *S. molesta* cover with

no biological control agents) showed a drastic decline in aquatic biodiversity and a complete shift in aquatic biota assemblage structure. Thus, the biological control effort by *Cyrtobagous salviniae*, the biological control agent for *S. molesta*, assisted in the recovery of aquatic biota following successful biological control.

The field study (four field sites, two sites controlled mechanically and two biologically) investigated water quality, aquatic biodiversity and community trophic interactions (aquatic food web) “before and after” *S. molesta* control. The study showed a drastic decline in aquatic biodiversity (with three sites showing no record of aquatic macroinvertebrates, thus no biotic interactions during infestation) and poor water quality due to the shade-effect (light barrier due to floating *S. molesta* mats on the water surface) during the “before” *S. molesta* control phase. However, following both mechanical and biological control (“after” *S. molesta* control phase), there was a significant shift in abiotic and biotic ecosystem characteristics as compared to the “before” *S. molesta* control phase. Thus, rapid ecosystem recovery was apparent as a result of aquatic microalgae and macroinvertebrates recolonisation. Sites showed a normal functioning ecosystem where improved water quality, increased biodiversity, productivity and trophic interactions, was indicative of the return of biologically and functionally important species which were lost during the “before” *S. molesta* phase. Although the clear water state showed positive outcomes at Westlake River, these were short lived when the system was dominated by a cosmopolitan submerged *Ceratophyllum demersum*, and later replaced by a floating-leaved emergent IAAP *Nymphaea mexicana*. Each state was responsible for a significant shift in both biotic and abiotic characteristics, affirming macrophyte abilities to influence aquatic environments structure and functions. Furthermore, this event showed a clear example of a secondary invasion. Thus, a holistic IAAP species management strategy is necessary to restore previously invaded ecosystems and prevent subsequent secondary invasion and ecosystem degradation.

In conclusion, the *S. molesta* shade-effect like any other free-floating IAAP species, was identified as the main degrading factor and responsible for water quality reduction, loss of aquatic diversity and shift in aquatic biota assemblage structure. Following *S. molesta* removal (or shade-effect elimination), there was a positive response to aquatic ecosystem species abundance, richness, diversity and community structure. Therefore, in combination, aquatic biota recolonisation rate and increases in biological and functional diversity were instrumental in the recovery of ecosystem structure and functions, following the control of *S. molesta*. Echoing existing literature, this thesis recommends: (1) IAAP species management programmes (mechanical and/or biological control) should not only aim to control the weed

but also focus on ecosystems recovery and possible restoration goals; (2) biological control should be used where appropriate to combat free-floating IAAP species in freshwater ecosystems, followed by active introduction of native macrophyte propagules since they are limited by anthropogenic activities; and (3) more freshwater case studies are needed to add to our understanding of IAAP species management and restoration effort incorporating long-term monitoring.

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Tshepong ya dilemong tse tlang,

Lefika le Tiileng.....

CHAPTER 1

General Introduction

1.1 Problem Statement

The presence of invasive alien aquatic plant (IAAP) species in their introduced range and the replacement of indigenous macrophyte species have deleterious effects on freshwater ecosystems, a situation that has been declared a global concern (Dudgeon et al., 2006; Hussner et al., 2017). According to the Millennium Ecosystem Assessment report (MEA, 2005), the establishment of IAAP species in freshwater ecosystems is regarded as the second major contributor to loss of aquatic biodiversity and related aquatic ecosystem services. Reversing the impact of IAAP species using various control methods, including biological control, would be of major benefit to aquatic ecosystems. Biological control is the use of imported co-evolved natural enemies of invasive species, particularly insects or pathogens, from their native range, for their control in novel environments. Subsequent host specificity testing of these phytophagous insects and/or pathogens ascertains their efficacy and whether they are safe for release in the introduced range (Moran et al., 2011). Following permission to release, these biological control agents are released in large numbers into invaded aquatic systems to weaken the fitness and reduce densities of the IAAP populations in the introduced range. In southern Africa, biological control is the most successful method for controlling free-floating IAAP species, and complete control can be achieved with no other interventions needed. Four of five South Africa's worst free-floating and mat-forming IAAP species including salvinia, *Salvinia molesta* D.S.Mitch (Salviniaceae); water lettuce, *Pistia stratiotes* L. (Araceae); parrot's feather, *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) and red water fern, *Azolla filiculoides* Lam. (Azollaceae) have been successfully controlled using a single biological control agent for each IAAP species (Coetzee et al., 2011).

Apart from reducing IAAP populations in their introduced range, successful application of biological control has shown positive socio-economic benefits, such as positive returns on investment and water saving benefits (McConnachie et al., 2003; Fraser et al., 2016; Arp et al., 2017). However, quantifying the ecological impacts of IAAP species and the benefits of successful biological control in aquatic ecosystems has been more challenging. Midgley et al. (2006) and Coetzee et al. (2014) demonstrated that the presence of free-floating IAAP species, like water hyacinth (*Pontederia crassipes* $\equiv$ *Eichhornia crassipes* (C.Mart) Solms (Pontederiaceae)), reduces aquatic macroinvertebrate diversity and alters community

composition. Similarly, a mesocosm study by Langa (2013) further showed autogenic recovery of aquatic macroinvertebrate diversity following successful biological control of water lettuce by the weevil *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae), but these studies were conducted at the taxon and community level, with no focus at an ecosystem level. On the other hand, a recent study by Hart and Matthews (2018) in Hartbeespoort Dam, one of South Africa's notorious hypertrophic system invaded by water hyacinth, attempted to quantify bio-remediation success based on reductions in chlorophyll-*a* and cyanobacteria content using real-time Medium Resolution Imaging Spectrometer satellite data. The study showed reductions in chlorophyll-*a* concentration and cyanobacteria since the implementation of the bio-remediation project, named Metsi-a-Me in 2008 (Hart and Matthews, 2018). Although the study showed positive recovery trends and innovative monitoring techniques, the remote sensing data might have overlooked the biological and functional interactions of the system that would have complemented the satellite data and provided useful ecological information for a better sustainable management practice. It is thus clear that IAAP invasions have deleterious impacts to aquatic environment and despite the implementation of control methods, including biological control, there is a limited understanding of aquatic ecosystem recovery following control at an ecosystem level. With IAAP species infesting many South African waterways, the long-term ecological benefits following successful biological control programmes of free-floating IAAP species have not yet been quantified, and this is the topic of this thesis.

1.2 Invasive alien aquatic plant species

Indigenous aquatic and riparian plant species play an important ecological role in the structure and functioning of freshwater ecosystems (Bakker et al., 2013; Hussner et al., 2017). The plants provide habitat structure for aquatic organisms, drive ecosystem productivity and provide energy resources through autochthonous and allochthonous inputs; provide phytoremediation services (i.e. assimilate contaminants, including excess nutrients and metals); and act as reliable biological indicators for external disturbances (Carpenter and Lodge, 1986). Free-floating and mat-forming IAAP species, i.e. water hyacinth, water lettuce, salvinia, parrot's feather and red water fern, native to South America, have completely altered aquatic ecosystems in southern Africa (Coetzee et al., 2011). These IAAP species were introduced around the world, including South Africa, as ornamental plants for aquaria and ponds, and in the case of water hyacinth and water lettuce, for horticultural purposes (Pieterse, 1990). In the absence of natural enemies, the weeds' rapid vegetative growth, and substantial seed banks (e.g. water hyacinth and water

lettuce), led to their proliferation and were all declared five of the world's worst aquatic weeds species (Hill, 2003).

Excessive IAAP species growth above the water surface threatens ecological functions by inhibiting light penetration into aquatic systems (i.e. shade effect), and in some cases, responsible for causing infrastructure damage to hydropower stations, affecting lake property value and threatening recreation in their introduced range (Zhang and Boyle, 2010). Not only does the invader biomass cause physical disturbances, but also emit chemical compounds that suppresses and outcompetes native macrophyte species (allelopathic) (Corbin and D'Antonio, 2011). These impacts have significant negative effects on native fauna and flora recovery, even long after the IAAP species have been controlled (legacy effects) (Callaway and Aschehoug, 2002). As a result, IAAP species are considered to have important ecological and socio-economic ramifications (Van Driesche et al., 2010).

Because natural standing freshwater bodies are limited in South Africa, the country does not have a high diversity of macrophytes, but rather high endemism and medium biodiversity (Chambers et al., 2008). However, the five IAAP species introduced into South Africa evolved in the Amazon basin, thus they take advantage of impounded (~75% of surface flowing water by volume) and nutrient-enriched South African freshwater bodies (Hart and Matthews, 2018). Therefore, reduced hydrological flow and anthropogenic inputs from the catchment have created favourable growing conditions for most free-floating IAAP species (Coetzee et al., 2011).

1.3 Impacts of free-floating invasive alien aquatic plant species

Invasive alien aquatic plant species are a significant environmental problem globally, because of the deleterious impacts they have on the socio-economic and ecological well-being of freshwater ecosystems (Table 1.1).

1.3.1 Habitat quality and modification

Van Driesche et al. (2010) describe the ecological impacts of free-floating IAAP species as having profound effects on the physical and chemical properties of the aquatic ecosystems they invade, negatively affecting aquatic biodiversity and related ecosystem services. Gutierrez (2017) highlights physical structure, consumable resources and abiotic conditions as three main aspects of habitat quality that are usually affected by non-native species. In the presence of free-floating IAAP mats, the assimilation (uptake) of light, water, nutrients, minerals and

dissimilation (release) of waste products processes are altered. The plants assimilate nutrients, minerals and water, and block sunlight penetration for submerged aquatic organisms. Lack of sunlight limits photosynthesis for autotrophic phytoplankton and submerged macrophytes, reduces dissolved oxygen (DO) concentrations, and increases carbon dioxide (CO₂) and hydrogen sulphide (H₂S) concentrations due to anaerobic decomposition of dead plant and animal matter in the water column (Schultz and Dibble, 2012). Increased CO₂, and H₂S concentrations and high biochemical oxygen demand (caused by low DO), collectively contribute to poor water quality, resulting to a tainted and foul odour from the waterbodies (Chamier et al., 2012). Thus, reduced water quality will change phytoplankton and periphyton development and affect aquatic food web basal resources (Cattaneo et al., 1998).

1.3.2 Impact of invasive alien aquatic plant species on water loss

Apart from altering abiotic properties and impairing water quality, free-floating IAAP species infestations have the ability to compromise and threaten freshwater hydrological services. In South Africa, IAAP species have invaded about 10 million hectares of land, threatening about 3.3 billion m³ of mean annual surface water runoff from catchment, riparian and wetland ecosystems (Culliney, 2005; Van Wilgen et al., 2012). Hill (2003) and Hussner et al. (2017) state that the presence of dense free-floating IAAP mats results in evapotranspiration rates that are higher than those of native macrophytes. However, while water loss to alien invasive riparian trees has been well documented in southern Africa (see Chamier et al., 2012; Le Maitre et al., 2016), little is known about the water loss caused by free-floating IAAP species, which could be as severe as seen in riparian invasion. Timmer and Weldon (1967) study on water loss caused by dense mats of free-floating IAAP species in Hadejia Nguru wetlands, North Eastern Nigeria estimated water loss to be between 1.02 and 12 times during water hyacinth invasion as compared to open water bodies, exacerbating freshwater demands in semi-arid and arid countries, including South Africa.

1.3.3 Loss of aquatic biodiversity

Physical, structural and functional properties of native macrophytes can be compromised by the presence of free-floating IAAP species. These ecosystem changes create unfavourable conditions for the majority of aquatic organisms, if sensitive and functionally important aquatic communities are replaced by tolerant and opportunistic aquatic communities of phytoplankton (Stiers et al., 2011; Stiers and Triest, 2017), aquatic macroinvertebrates (Midgley et al., 2006; Coetzee et al., 2014), and freshwater fish (Güereña et al., 2015). These community changes re-

organise internal ecosystem feedback mechanisms to create a resilient, degraded ecosystem, thus compromising ecosystem structure and functioning. To reverse the situation (or recover from a degraded state), free-floating IAAP species should be managed, not to historic conditions, but to an alternative state that allows ecosystem recovery (Kettenring and Adams, 2011).

Although some of the ecological impacts associated with free-floating IAAP species are understood in general, case studies quantifying biodiversity impacts are limited. However, IAAP species are recognised as competitors that have knock-on effects on native fauna and flora (Pyšek et al., 2017).

1.3.4 Impacts of invasive alien aquatic plant species on livelihoods

In developing countries, where rail and road transport are limited, riparian communities depend heavily on small boats for transportation, trading, fishing and accessing critical services such as the market place, schools, and health care facilities (Holm et al., 1969 ; Güereña et al., 2015). For example, on Lake Victoria, in Kisumu, Kenya, water hyacinth infestations, which blocked waterways and created difficulties for boat navigation, were responsible for the complete evacuation of riparian village residents. Similarly, in developed countries, free-floating aquatic weeds reduce recreation, fishing and the market value of lakefront estates, such as in the case for Eurasian watermilfoil, *Myriophyllum spicatum* L. (Haloragaceae) in Vermont, Rutland USA (Zhang and Boyle, 2010; Hussner et al., 2017)

Anopheles spp. and *Mansonia* spp. (Diptera: Culicidae) mosquitoes are vectors of medically important diseases, such as malaria, filariasis and encephalitis, which are prevalent in Afrotropical areas. Gangstad and Cardarelli (1990) report a positive relationship between aquatic weed infestations and the two mosquito species. Alien weeds invasions in aquatic ecosystems alter both abiotic and biotic ecosystem characteristics, thus only aquatic macroinvertebrates that are well adapted to survive low dissolved oxygen conditions for example, will dominate the system, and this include both the *Anopheles* and *Mansonia* mosquito species. Both species can survive in poor water quality and oxygen depleted environments, the *Anopheles* mosquito uses breathing tubes to acquire oxygen directly from the atmosphere, whereas *Mansonia* species derive their oxygen by puncturing air chambers in the roots and stems of emerged or free-floating aquatic macrophytes, for example *P. stratiotes* (Gangstad and Cardarelli, 1990). With the aquatic ecosystem community altered due to invasion and the absences in some cases of predators, such as odonates nymphs, air-breathing predacious aquatic beetles and water surface hemipterans. Consequently, the ecosystem role

for aquatic predator is compromised, resulting in mass emergence and outbreak of *Anopheles* and *Mansonia* species which exacerbate vector borne disease in developing countries (Gangstad and Cardarelli, 1990).

1.3.5 Impact of invasive alien aquatic plant species on economic development

Goal number 6 of the Sustainable Development Goals (SDG 2015) and the Convention on Biological Diversity (CBD 1992) state that signatories should provide clean water and proper sanitation facilities and facilitate environmental sustainability for the benefit of the society. Governmental departments are therefore responsible for ensuring a sustainable environment for the protection of biodiversity, and the sustainable use of its components for the benefit of the society. Currently, millions of dollars' worth of investments are used globally to restore natural ecosystems and control alien invasive aquatic and riparian plants.

According to Culliney (2005), economic losses due to alien invasive plants species in general outweigh those of agricultural pests. Economic losses associated with alien invasive plant species, combined with damage to the environment, and costs for mitigation and control, are estimated to have cost the equivalent of US\$39 billion in India, US\$34 billion in the United States of America, US\$17 billion in Brazil, US\$1.4 billion in the United Kingdom, US\$12 billion in South Africa, US\$3 billion in Australia and \$1 billion in New Zealand since the 1990s (Culliney, 2005).

In South Africa, Van Wyk and Van Wilgen (2002) reported a sum of ~ US\$6.5 million (at 1US\$ = R3.6 in 1995) as control costs for free-floating water hyacinth biological control at New Year's Dam (from 1991 - 2001), integrated control at the Nseleni River system (from 1985 - 2001) and herbicide application at Hartbeespoort Dam (from 1977 - 2001). In Nigeria, mechanical and chemical control of water hyacinth was estimated at US\$639 and US\$161 million respectively, and in Florida, chemical control of the submerged invasive aquatic weed hydrilla, *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) was estimated around US\$1235 for chemical application per hectare.

Table 1.1 A selection of globally important alien invasive macrophytes and related ecological impacts in freshwater systems. Information on alien invasive macrophytes species list, native, invaded range and ecological impacts compiled from Schultz and Dibble (2012) and Hussner et al. (2017).

Invasive alien aquatic plant species	Structure	Range	Impact on ecosystems	Reference
<i>Eichhornia crassipes</i>	Floating	Native to Amazon basin; invaded range is worldwide except for Europe	Outcompetes native vegetation, decreases dissolved oxygen levels, leads to shift in macroinvertebrate communities and fish diets, blocks hydropower; high evapotranspiration rate	Pietersen (1990), Masifwa et al., (2001), Coetzee et al., (2013), Villamagna & Murphy (2010), Frazer et al., (2016)
<i>Pistia stratiotes</i> , <i>Salvinia molesta</i> & <i>Azolla filiculoides</i>	Floating	Native to South America, invaded Africa, Asia, Europe, North America, Oceania	Outcompetes native vegetation, decreases dissolved oxygen levels, leads to shift in macroinvertebrate communities and fish diets, blocks hydropower	Van Driesche et al., (2010), Langa (2013)
<i>Myriophyllum aquaticum</i>	Submerged/Floating	Native to South America; invaded range includes the U.S., Europe, Africa, New Zealand and Australia	Outcompetes native plants for resources, decreases food source availability and quality for macroinvertebrates	Hussner, (2008), Stiers et al., (2014)
<i>Hydrocotyle ranunculoides</i>	Floating	Native to North and South America; invaded range includes Australia and Europe	Outcompetes native plants for resources, decreases food source availability and quality for macroinvertebrates	Hussner, (2008), Stiers et al., (2014)
<i>Ludwigia grandiflora</i>	Floating	Native to South America; invaded range includes the U.S. and Europe	Outcompetes native plants for resources, decreases food source availability and quality for aquatic macroinvertebrates	Hussner (2008), Stiers et al., (2014; 2017)
<i>Trapa natans</i>	Floating	Native to warm temperate Eurasia; invaded range is Australia, Burkina Faso, north-eastern U.S., and Canada	Decreases dissolved oxygen levels, can increase macroinvertebrate density	Cattaneo et al., (1998), Caraco & Cole (2002), Strayer et al. (2003), Kornijow et al. (2010)
<i>Cabomba caroliniana</i>	Submerged	Native to the southern U.S.; invaded range is northeast and northwest U.S, Asia, Europe and Canada	Increases habitat complexity	Hogsden et al. (2007), Kiraly et al. (2008), Jacobs & Macisaac (2009)
<i>Elodea canadensis</i>	Submerged	Native to North America; invaded range includes northern and western Europe	Increases habitat complexity, outcompetes native plants for resources, reduces feeding and growth of certain macroinvertebrates	Erhard (2005), Kelly & Hawes (2005), Kornijow et al. (2005)

Table 1.1 Continues

Invasive alien aquatic plant species	Structure	Range	Impact on ecosystems	Reference
<i>Elodea nuttallii</i>	Submerged	Native to North America, invaded range includes northern and western Europe	Increases habitat complexity, outcompetes native plants for resources, reduces feeding and growth of certain macroinvertebrates	Erhard (2005)
<i>Lagarosiphon major</i>	Submerged	Native to southern Africa; invaded range includes Australia, New Zealand, and Europe	Outcompetes native plants for resources, affects water sports, recreation lake estate property value	McGregor & Gourlay (2002)
<i>Egeria densa</i>	Submerged	Native to South America; invaded range includes Europe, Africa, the U.S., and Australasia	Increases habitat complexity, alters fish community composition	Parsons et al. (2009)
<i>Hydrilla verticillata</i>	Submerged	Native to Asia, specifically India and Korea; invaded range includes the Americas, Europe, Africa Australia, and New Zealand	Increases habitat complexity, outcompetes native plants for resources, decreases fish foraging efficiency at high densities, facilitates non-native fishes	Troutman et al. (2007), Barrientos & Allen (2008), Hershner & Havens (2008), Hoyer et al. (2008), Theel et al. (2008) Thomaz et al. (2009), Bianchini et al. (2010)
<i>Myriophyllum spicatum</i>	Submerged	Native to Europe, Asia and northern Africa; invaded range includes the U.S. and Canada	Increases habitat complexity, outcompetes native plants for resources, decreases food source availability and quality for macroinvertebrates, facilitates non-native fishes, alters fish diets and trophic dynamics	Ward & Newman (2006), Phillips (2008), Kovalenko et al. (2009), Collingsworth & Kohler (2010), Kovalenko & Dibble (2011)
<i>Egeria densa</i>	Submerged	Native to South America; invaded range includes Europe, Africa, the U.S., and Australasia	Increases habitat complexity, alters fish community composition	Parsons et al. (2009)
<i>Potamogeton crispus</i>	Submerged	Native to Eurasia; invaded range includes Canada and the U.S.	Increases habitat complexity, outcompetes native plants for resources	Croft & Chow-Fraser (2007), Kovalenko et al. (2009)

Table 1.1: Continues

Invasive alien aquatic plant species	Structure	Range	Impact on ecosystems	Reference
<i>Hymenachne amplexicaulis</i>	Emergent	Native to central and tropical America; invaded range includes Florida, Australia, Indonesia and Papua New Guinea	Reduces water flows, resulting in lower fish recruitment, decreases habitat complexity, facilitates non-native fishes	Kibbler & Bahnisch (1999), Houston & Duivenvoorden (2002)
<i>Phragmites australis</i>	Emergent	Found worldwide in temperate areas; is considered alien and/or invasive in the U.S., New Zealand and Burkina Faso	Outcompetes native vegetation, no adverse effects on fish populations, increases gastropod abundance	Aday (2007), Kulesza et al. (2009), Holomuzki & Klarer (2010)
<i>Alternanthera philoxeroides</i>	Emergent	Native to South America; invaded New Zealand, China, Europe, Oceania, South Eastern America, Thailand	Outcompetes native vegetation, changes water chemistry	Denslow & D'Antonio (2005)
<i>Sagittaria platyphylla</i>	Emergent	Native to US; invaded South Africa, Australia	Increases <i>Biomphalaria glabrata</i> abundance; carrier for <i>Schistosoma mansoni</i> (bilharzia); outcompetes native plants for resources and impairs water flow	Gangstad & Cardarelli (1990), Kwong et al. (2019)
<i>Iris pseudacoris</i>	Emergent	Native to Eurasia and North Africa; invaded South Africa and Brazil	Outcompetes native vegetation, and impairs water flow	Jaca & Mkhize (2015), Hill & Coetzee, (2017)

1.4 Management of invasive alien aquatic plant species

Prevention, eradication and control are the three major strategies employed globally to manage damaging alien invasive aquatic weeds. Prevention often involves using legislation to prohibit entry and restrict the movement of the IAAP species within a country. Eradication is part of an early detection and rapid response programme, which takes place soon after the IAAP species has been recorded, particularly when they have been predicted to cause ecological and socio-economic harm at high densities (Van Driesche et al., 2010). Hussner et al. (2017) recommend preventing the introduction of IAAP species from the outset as the best possible management method, and this includes monitoring the online plant trade and nurseries, together with international ports of entry. Hussner et al. (2017) emphasise and echo the recommendations of Olckers (2004), Culliney (2005) and Van Driesche et al. (2010) that, in the case of the entry of IAAP species, early detection and rapid response programmes using mechanical and chemical control will be beneficial. However, Hussner et al. (2017) further emphasise that early detection and eradication programmes are only considered effective for small scale and isolated alien invasive species infestations. Subsequent eradication of small, isolated infestations will minimise future costs and management efforts of the IAAP species and save the ecosystem from drastic alteration and ecosystem service losses. Olckers (2004), Van Driesche et al. (2010) and Hussner et al. (2017) further argue that of the three appropriate methods for managing IAAP species (i.e. chemical, mechanical and biological control), biological control is highly recommended for landscape and high-density infestations because it is efficient and self-sustaining.

1.4.1 Mechanical and chemical control for invasive alien aquatic plant species

Mechanical control of IAAP species has been favoured globally as a management strategy. However, mechanical control alone is insufficient for complete control of IAAP species, because small plant fragments are often left behind to re-sprout and lead to reinvasion (Hussner et al., 2017). Additionally, mechanical control requires follow-up manual intervention at regular intervals to completely eradicate remaining fragments and reduce re-sprouting, although this method may be insufficient for IAAP species with well-developed seed banks. Wade (1990) emphasises that mechanical control should only be applied if the reaction of the invasive aquatic weed to physical damage is well understood, and that the control method does not in any way damage the ecosystem. Such dynamics are poorly understood but vital in the management of IAAP species, particularly in determining the best management practice that

contributes to and enhances ecosystem restoration following IAAP species management. For example, mechanical control by dredging affects aquatic macroinvertebrate communities and ecosystem physical characteristics. This method removes hundreds of aquatic insects along with the macrophyte biomass, thus changing and/or damaging aquatic food web structure. Wade (1990) reported that only adults and good dispersers would be able to re-colonise and re-organise the ecosystem, leaving the larger proportion of aquatic macroinvertebrates immature life-stages behind, resulting in longer ecological recovery following mechanical control. For example, Masifwa et al. (2001) reported high diversity of aquatic macroinvertebrates on the roots of water hyacinth as compared to open water in Lake Victoria, Kenya. Thus, mass removal of free-floating IAAP species will result in a loss of aquatic biodiversity and longer recolonisation time.

In comparison, chemical control is notorious for its non-target effects, particularly when applied to aquatic systems (Stiers and Triest, 2017). Although herbicides are widely used, chemical control is considered ineffective in the case of IAAP species. Herbicide application indirect effects to the ecosystem where it changes the aquatic invertebrate and fish composition, aquatic microalgae (phytoplankton and periphyton) behaviour, and alter the chemical and biochemical processes taking place in the water column and sediments (Ashton et al., 1981; Murphy and Barrett 1990).

Integrated control which is the combination of herbicide, mechanical and biological control, if implemented correctly can show positive investment returns. The Rondegat River system in the Cederberg Mountains, Western Cape Province of South Africa is a good example of a multidisciplinary, large-scale, alien invasive species eradication (Weyl et al., 2016). Both the Department of Environmental Affairs: Natural Resources Management, Working for Water programme, and the Water Research Commission provided funding to eradicate the invasive riparian tree species, black wattle *Acacia mearnsii* De Wild. (Fabaceae) using mechanical control (Samways and Sharratt, 2010) and the largemouth brown bass, *Micropterus sp.* Lacepede (1802) (Centrarchidae) (Bellingan et al., 2019) using chemical control methods. A year later, the programme successfully saw the return of native biodiversity, including endemic aquatic macroinvertebrates and most notably the endangered freshwater Clanwilliam Redfin minnow, *Pseudobarbus calidus* Barnard (1938) (Cyprinidae) to the system which were threatened by alien riparian and fish invasions (Samways and Sharratt, 2010; Bellingan et al., 2019).

1.4.2 Biological control of invasive alien aquatic plant species

Biological control involves importing specific natural enemies, particularly phytophagous insects or pathogens, from the weed's native range. The process includes pre-release monitoring of the IAAP species, exploration for natural enemies and their identification in the native range, host-specificity testing, followed by release of host-specific biological control agents onto the invasive aquatic weed. The application and success of biological control is governed by the enemy release hypothesis, which states that, in the absence of natural enemies, non-native species proliferate and outcompete native flora (Keane and Crawley, 2002). Thus, the introduction of host-specific biological control agents aims to reduce invasive weed population densities using co-evolved natural enemies in their introduced range (McFadyen, 1998).

Free-floating IAAP species i.e. water hyacinth, water lettuce, salvinia, red water fern and parrot's feather are the most damaging aquatic weeds that have been targeted for biological control in southern African and beyond (Hill, 2003; Van Driesche et al., 2010; Coetzee et al., 2011). Currently, four of the five world's worst free-floating IAAP species are under complete control in South Africa, where no other interventions are needed. These weeds are water lettuce, salvinia, red water fern and parrot's feather, under control by the coleopteran weevils *Neohydronomus affinis* Hustache, *Cyrtobagous salviniae* Calder & Sands, *Stenopelmus rufinasus* Gyllenhal (all Curculionidae) and the leaf beetle *Lysathia* sp. (Chrysomelidae), respectively (Coetzee et al., 2011; Hill and Coetzee, 2017). The fifth, water hyacinth, is under substantial control with nine biological control agents released against it, including the fungal pathogen *Cercospora rodmanii* Conway (Mycosphaerellaceae), and the arthropods *Eccritotarsus catarinensis* Carvalho and *E. eichhorniae* Henry (Hemiptera: Miridae), *Neochetina bruchi* Warner and *N. eichhorniae* Hustache (Coleoptera: Eriirhinidae), *Niphograpta albiguttalis* Warren (Lepidoptera: Crambidae), *Orthogalumna terebrantis* Wallwork (Galumnidae), *Cornops aquaticum* Bruner (1906) (Orthoptera: Acrididae) and *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) (Hill and Coetzee, 2017). Despite the number of agents released to combat water hyacinth, eutrophication is still a problem in many cases and this hinders the success of the biological control. As such, other interventions including reducing the level of nutrients in water bodies are needed for the biological control of water hyacinths to be effective.

The biological control of free-floating and rooted invasive alien aquatic weeds has been a successful tool in many cases of IAAP species management for several decades. Although this method often requires augmentative releases of biological control agents, it is considered

cost effective, self-sustaining, environmentally friendly and effective under ambient environmental conditions in reducing free-floating IAAP species biomass (Coetzee et al., 2011). After the South African Government acknowledged that alien invasive plants species pose a threat to South African ecosystems, water supplies and natural biological diversity (Moran et al., 2011), the Department of Water Affairs and Forestry (now Department of Environment, Forestry and Fisheries), through the Natural Resources Management Programme, Working for Water generously funded alien invasive plant control and research in South Africa. Biological control research and its implementation success in southern Africa help combat IAAP species to provide water security and environmental sustainability, which is in line with the Sustainable Development Goals 16 (SDG 2015) and the Convention on Biological Diversity (CBD 1992) mandates.

1.5 Benefits of biological control of invasive alien aquatic plant species

1.5.1 Socio-economic benefits

The biological control programme for water hyacinth by the two *Neochetina* weevils in Lake Victoria, Kenya benefited almost 30 million riparian village residents, where fishing and boat transport navigation resumed following water hyacinth control (Güereña et al., 2015). With regards to surface water availability, Chamier et al. (2012) and Fraser et al. (2016) report that water loss in South Africa could have been approximately eight times greater if IAAP species were to occupy the full extent of their potential range. Van Wilgen and De Lange (2011) compare the costs of biological control research and implementation against the benefits of restored ecosystem services, and showed that biological control has been extremely beneficial in economic terms, where the estimated cost: benefit ratios ranged between be more than 1:8 and 1:3000 respectively. This low cost: benefit ratio was also observed with the biological control programme of red water fern in South Africa by (McConnachie et al., 2003), and salvinia in Sri-Lanka (Doeleman, 1989) where return on investment was recorded as 1:15 and 1:1675 respectively. Similarly, the biological control programme of water hyacinth in New Year's Dam, Eastern Cape, South Africa contributed to water saving of two million m³ (prevention of water loss through evapotranspiration, as opposed to evaporation) over 22 years (Fraser et al., 2016). The current spending on biological control is far lower than any other form of alien invasive control, despite significant returns on investment. The assessment from Van Wilgen and De Lange (2011) recommends continued investment in biological control research for attractive returns on investment.

1.5.2 Ecological benefits of invasive alien aquatic plant species management

Successful biological control of alien invasive plants is usually evaluated by the reduction of the targeted weed density, although Culliney (2005) and Denslow and D'Antonio (2005) argue that this does not always translate to improvements in ecosystem services. A seminal review by Reid et al. (2009), reports limited studies and knowledge on ecosystem recovery studies following alien invasive species management. For example, in a worldwide review of successful biological control projects, with noticeable reductions in cover and distribution of the target weed, Denslow and D'Antonio (2005) found limited quantitative information on the responses of the ecosystems following alien invasive plant control. In addition, of 95 research papers reviewed in Reid et al. (2009), the majority focused on terrestrial ecosystems, emphasising the lack of ecological studies (recovery and restoration) following IAAP species management in aquatic environments. Eighteen of the studies showed no plant community recovery post-alien invasive plant control, while others reported observations without data. King and Downey (2008) suggest that such inconsistency could be because qualitative assessments which include photo points observations are mostly done by land managers for evaluation. These observations are said to be inexpensive and not time consuming, but they are not appropriate for measuring the detailed response of native plant flora and fauna following a reduction in target weed biomass. Myers and Cory (2017) state that systematic and comprehensive monitoring assessments are necessary to test the positive contribution by biological control programmes to ecosystem services, similar to the negative environmental impacts documented against IAAP species.

However, there have been some attempts to try and quantify ecosystem recovery at a community level, using aquatic macroinvertebrates as biological indicators. A mesocosm study in South Africa by Langa (2013) illustrated that biological control of water lettuce can facilitate increase in aquatic macroinvertebrate diversity in freshwater ecosystems. Similarly, in their review Denslow and D'Antonio (2005) reported that most studies have showed reduced weed biomass, habitat range and spread, resulted in an increase in abundance of native species and economic productivity following the application of biological control to water hyacinth, water lettuce and alligator weed, *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae). Of the 49 projects reviewed by Van Driesche et al. (2010) targeting alien invasive plants species, 98% benefited biodiversity, 47% protected products harvested from the natural environment, and 25% preserved ecosystem services. Thus, Van Driesche et al. (2010) conclude that biological control programmes contributed successfully to the protection of native flora and

fauna of many natural ecosystems and is presently incorporated into many recovery and restoration efforts globally. The literature further emphasises and acknowledge that some of the benefits of biological control have not yet been fully quantified, thus more socio-economic and ecological cases-studies are needed.

Recent case-studies (Gaertner et al., 2011; Sher et al., 2018) and reviews in freshwater invasion and ecosystem recovery studies (Culliney, 2005; Hussner et al., 2017) clearly show that there is a limited ecological understanding of the benefits of biological control to ecosystem recovery and subsequent restoration, even though this phenomenon has been extensively studied in terrestrial and riparian ecosystems (Gaertner et al., 2014).

1.6 Difficulties associated with biodiversity recovery following invasive alien aquatic plant species management

The extent and rate of recovery for native macrophytes and aquatic organisms following alien invasive macrophyte control is highly dependent on the level of the degradation and the presence of native propagules (Bakker et al., 2013). The recovery of native species is difficult in cases where seed banks are depleted and there is a lack of ecological corridors to help in the movement and transfer of native vegetation propagules from the remaining natural fragments (Corbin and D'Antonio, 2011). Thus, reducing IAAP species may provide an opportunity for a secondary IAAP species to establish due to the absence of native propagules (D'Antonio and Meyerson, 2002; Buckley et al., 2007). Richardson et al. (2003), Reid et al. (2009) and Le Maitre et al. (2011) emphasise that, in situations where the abiotic and biotic characteristics of an ecosystem have been fundamentally changed due to a primary invader, the removal of the invader alone is highly unlikely to restore the ecosystem, but may benefit secondary invasion.

Lack of long-term funding, expertise or guidance on how to assess native community recovery may explain why we know less about the response of native communities following IAAP species control. In theory, controlling the alien invasive plant species should allow ecosystem recovery, according to Smith and Van der Bosch (1967), yet their results indicate that target invader suppression led instead to secondary invasion. Strange et al. (2018) report that although South Africa has a successful biological control programme for free-floating IAAP species, biological control could act as a catalyst for secondary submerged IAAP invasion. Strange et al. (2018) propose that the shift is driven mainly by the rapid decomposition of the free-floating IAAP species, and subsequent increase in availability of nutrients and light, giving rise to opportunistic submerged macrophytes. Pearson et al. (2016)

investigated the drivers of secondary invasion in terrestrial ecosystems, and like Strange et al. (2018), Pearson et al. (2016) consider alien invasive species management side effects, primary invader legacy effects, and shifting environmental conditions as possible factors responsible for secondary invasion. Thus, to address the re-introduction/secondary invasion requires management strategies that anticipate and suppress secondary invaders while rapidly restoring native communities to fill the space vacated by the target weed. In their conclusion, Pearson et al. (2016) emphasise that accomplishing the latter will require improved re-vegetation techniques; this could work for countries with abundance of standing water bodies and diverse macrophytes species to compete with IAAP species.

The view of Callaway and Aschehoug (2002) is that certain secondary alien invaders might be expected to benefit over natives in the presence of the primary invader legacies if the secondary invader shares the same evolutionary history as the primary invader. In conclusion, a major review of regime-shift research identified that shifts are more likely to occur when anthropogenic pressures have already reduced ecosystem resilience by actions such as removing or substituting functional species groups (Folke et al., 2004), thus these shifts can be beneficial to aliens over natives.

1.7 Ecological restoration practises

According to the Society of Ecological Restoration (SER), restoration efforts aim to recover the characteristics of an ecosystem, such as biological and functional biodiversity, and the supply of ecosystem services that have been degraded, damaged or destroyed, usually as a result of human activity (Ruiz-Jaen and Aide, 2005). Benayas et al. (2009) state that ecological restoration can have positive return towards native species and biodiversity recovery. A meta-analysis of 89 studies assessing the effects of restoration on a broad range of ecosystem types around the world found that ecological restoration projects increased biodiversity and ecosystem services levels by an average of 44% and 25% respectively. This was also the case in other ecological restoration meta-analyses in more specific ecosystem types such as forests (Felton et al., 2010), and wetlands (Meli et al., 2014).

Ecological restoration can be implemented following either passive or active strategies where passive restoration involves removing the degrading factors, followed by autogenic/natural regeneration of native communities; while active restoration (assisted re-generation) involves actions such as adding desired plant species, amending the soil and control fire regimes, which also drive secondary native succession (Holl and Aide, 2011). In aquatic

ecosystems, IAAP species management and restoration efforts mainly employ passive methods through herbicidal control, mechanical clearing and the application of biological control agents (Coetzee et al., 2011; Stiers et al., 2011). According to Gaertner et al. (2012), passive restoration can only facilitate autogenic recovery of native communities if the ecosystem or the invader effects did not compromise ecosystem resilience. Thus, this supported the study by Ruwanza et al. (2013), who recorded secondary invasion following passive restoration practise of an alien invasive riparian species in South Africa. Ruwanza et al. (2013) further stated that passive restoration alone can be very slow and ineffective to some extent in allowing natural regeneration of native communities.

Loch Leven in Scotland is a freshwater lake known to be highly eutrophic but showed a significant reduction in its nutrient status following catchment management strategies that limited nutrient-rich effluents. Thus, unlike many the system showed a autogenic regeneration (natural recovery) of native species following nutrient reduction in the system (Carvalho et al., 2011). However, most eutrophic European and Asian freshwater lake ecosystems require both passive (nutrient reduction input) and active restoration (biological manipulation) for successful ecosystem recovery (Liu et al., 2018). The dual approach to restoration has allowed the return of and re-establishment of native vegetation communities, followed by associated invertebrate and vertebrate recruitment, leading to the return of ecosystem structure and function. South Africa employs mainly passive restoration strategies for invasive alien riparian and aquatic weed management and it has been successful in doubling aquatic macroinvertebrate taxa and functional biodiversity returns in heavily invaded riparian ecosystems found in the headwaters of some river systems (Pryke and Samways, 2009; Magoba and Samways, 2010; Samways and Sharratt, 2010). However, recent field observations and mesocosm experiments show evidence of regime-shift where a free-floating IAAP *P. stratiotes* species was replaced by a submerged IAAP species *Egeria densa*, following successful biological control of the primary invader, in a mesocosm study (Strange et al., 2018).

1.8 Research aims

In order to increase our understanding of how IAAP species control contributes to freshwater ecosystem recovery and restoration, this thesis aimed to quantify ecological recovery in biodiversity following successful biological control of *Salvinia molesta* by the weevil *Cyrtobagous salviniae*, in freshwater ecosystems in southern Africa. This includes water and sediment chemistry; phytoplankton and periphyton development; aquatic macroinvertebrate

assemblage structure; and overall aquatic ecosystem food web structure and functioning, “before and after” the application of biological control. Recent ecosystem recovery studies focus mainly on the improvement of water chemistry and aquatic macroinvertebrate diversity and assemblage composition as biological indicators in the presence and absence of IAAP infestations (e.g. Midgley et al., 2006; Stiers et al., 2011; Coetzee et al., 2014). The research conducted in this thesis will provide evidence-based case studies that will assist in our understanding of the ecological impacts of free-floating IAAP species, and relating the biological and functional diversity of ecosystems and trophic level effects, “before and after” *S. molesta* management, will be beneficial for sustainable management and restoration efforts in aquatic ecosystems.

1.9 Thesis outline

Chapter 1 provides a general introduction to the research. **Chapter 2** describes the employment of a Before-After Control-Impact treatment design in a mesocosm study to investigate changes in water quality, and epiphyton and aquatic macroinvertebrate assemblage composition shifts, following the biological control of *S. molesta*. **Chapter 3** takes these findings to the field, and investigates ecosystem recovery (i.e. aquatic biodiversity, trophic interactions and food webs) following a “before-after” mechanical and biological control applications for *S. molesta*. **Chapter 4** then focuses on one of these field sites, and investigates biotic and abiotic responses following successional dominance of macrophytes; from a floating macrophyte state, to a clear water state, followed by a submerged cosmopolitan macrophyte state and finally to a floating-leaved rooted emergent macrophyte state, after mechanical control of *S. molesta*. **Chapter 5** consolidates the results and provides a discussion of significant findings and proposes recommendations for future freshwater management and restoration efforts for invaded freshwater ecosystems based on the insights gathered through the research.

CHAPTER 2

The use of epilithic algae and aquatic macroinvertebrate biodiversity indices as proxy for ecosystem recovery following *Salvinia molesta* biological control: a mesocosm study

2.1 Introduction

South Africa's worst free-floating and mat-forming IAAP species – *P. crassipes*, *P. stratiotes*, *S. molesta*, *M. aquaticum* and *A. filiculoides*, all native to South America, are responsible for altering invaded freshwater ecosystems in their introduced range (Hill, 2003). South Africa is a water-scarce country with limited natural standing freshwater bodies and as a result, major South African river systems are impounded to support the growing freshwater demands (Hart and Matthews, 2018). Due to the limited existence/presence of natural standing water bodies, South Africa lacks high diversity of indigenous macrophytes to occupy the now available niche created by impounding river systems. The limited diversity of free-floating macrophytes, together with constant anthropogenic inputs leading to eutrophication, create suitable conditions for non-native macrophytes to proliferate in the majority of South African freshwater systems. Upon infestation, dense mats alter physical and chemical freshwater properties, leading to loss of aquatic biodiversity, shifts in aquatic community structure, and reduced water quality and quantity (Coetzee et al., 2014; Arp et al., 2017). Having limited freshwater, impacted aquatic biodiversity, and altered freshwater ecosystems, South Africa has been faced with tough challenges due to alien plant invasion, robbing both society and the environment of this natural resource (Richardson and Van Wilgen, 2004).

Biological control programme of free-floating IAAP species, which employ the weeds' natural enemies imported from their native range, are regarded as highly successful on a global scale (Van Driesche et al., 2010; Hussner et al., 2017). The 'enemy release' and 'resource concentration' hypotheses are regarded as the main drivers of biological control efficacy and collectively predict that specialist insect herbivore abundance is directly proportional to the host plant abundance in homogenous stands rather than in heterogeneous stands (Maoela et al., 2016). Thus, with IAAP species forming monocultures in invaded systems, there is a better chance for host-specific natural enemies to successfully establish and control IAAP species. Biological control, if implemented correctly, has the potential to overcome environmental and demographic stochasticity, and thus suppress the IAAP populations through feeding (Grevstad, 1999).

As a result of increased nutrient availability in aquatic ecosystems due to anthropogenic activities, both nitrogen and phosphate concentrations in some freshwater systems are no longer limited but excessive, resulting in increased macrophyte reproduction, sometimes reducing the success of the biological control of IAAP species. Davis et al. (2000) echoed these findings and reported that the effectiveness of biological control can be restricted by nutrient enrichment in the aquatic environment. Under nutrient-rich environmental conditions, IAAP species are able to compensate for the damage inflicted by their natural enemies by investing either in plant reproduction or plant defence mechanisms, thus increasing IAAP species' tolerance to insect herbivory (Coetzee et al., 2007; Coetzee and Hill, 2012). Water hyacinth is one such species that has demonstrated this phenomenon, and thus requires a well-structured, integrated control approach to reduce its biomass below critical ecological thresholds (Coetzee et al., 2011).

Native, mostly submerged, macrophytes play a critical role in freshwater ecosystem structure and functions, and if replaced by IAAP species, ecosystem structure and functioning are altered, influencing freshwater ecosystem goods and services (Bakker et al., 2013). The status of ecosystem structure and functioning before IAAP infestation and after successful biological control is often unknown but critical to the management of IAAP species and freshwater ecosystem restoration. Biological control practitioners, together with aquatic ecologists and restoration practitioners, are challenged to manage, and then sustain, improved water quality and recovered aquatic biodiversity, and ecosystem function following successful IAAP species control. This is a global challenge with ample research opportunities to provide useful insight and understanding of ecosystem recovery dynamics that will aid in facilitating sustainable ecosystem management. While terrestrial and riparian ecosystem recovery dynamics and restoration following alien clearing have been extensively studied in South Africa (e.g. Pryke and Samways, 2009; Magoba and Samways, 2010; Gaertner et al., 2011; Ruwanza et al., 2013; Nsikani et al., 2018), comparatively little is known about IAAP species management and restoration.

In order to quantify ecosystem impacts by IAAP species and management benefits, the “before-after” experimental design, which can include a perceived impact at Control and Impacted locations, as proposed by Green (1979), is considered useful for investigating environmental impact studies. This experimental design requires both physicochemical and biological data-sets to compare “before and after” the environmental impact management. Studies conducted in terrestrial and riparian ecosystems have illustrated the potential of the Before-After Control-Impact (BACI) design to quantify restoration efforts in the recovery of native fauna and flora following invasive alien plant species management (Pryke and Samways,

2009; Magoba and Samways, 2010; Samways et al., 2011; Modiba et al., 2017), and can be used in aquatic ecosystems too.

Aquatic macroinvertebrate taxa are regarded as reliable biological indicators for freshwater ecosystem health, in that they are biologically and functionally diverse, they occupy critical and transitional trophic positions in aquatic food webs (between primary producers and tertiary consumers), they are fundamental in energy transfer and nutrient recycling, and thus play a critical role in ecosystem structure and functioning, while being highly responsive to changes in chemistry and physical properties of water (Covich et al., 1999; Kietzka et al., 2015). Similarly, aquatic microalgae contribute equally to aquatic ecosystem food web structure as primary producers, and show measurable responses to both bottom-up (nutrient-input) and top-down (herbivory) trophic effects, and changes in physical and chemical characteristics (Liboriussen and Jeppesen, 2006). Thus, aquatic microalgae and macroinvertebrates can provide a good understanding of IAAP species ecosystem impacts and recovery at an ecosystem level.

The ultimate goal of tackling IAAP infestations is to conserve freshwater ecosystems and re-establish native aquatic biodiversity and ecosystem functions (Hulme, 2006). Yet, without understanding how invasive species affect native ecosystem community structure and functions, it will be challenging to measure the return to a functioning ecosystem. To date, biological control success of IAAP species has been measured based on the desired clear water state following control, and rarely on aquatic community structure and the return of normal ecosystem functioning. This study uses an extension of the BACI experimental design to examine the impact of *S. molesta* and subsequent biological control by the weevil *C. salviniae* on the recovery of epilithic algae and aquatic macroinvertebrate communities and biodiversity. Coetzee et al. (2011) and Stiers et al. (2011) reported that non-native IAAP species like *S. molesta* sequester nutrients in invaded systems, but also reduce light penetration and dissolved oxygen concentrations. These changes reduce ecosystem productivity significantly, as well as abundance and diversity of aquatic microalgae and macroinvertebrates. I therefore hypothesise that: i) the presence of *S. molesta* will reduce chlorophyll-*a* concentration in water, as an indicator of ecosystem productivity, facilitate a shift in aquatic microalgae and macroinvertebrate assemblage structure, and reduce biodiversity, and ii) that the application of the biological control agent, *C. salviniae*, on *S. molesta* infestation will support the relative recovery of epilithic algae and aquatic macroinvertebrate biodiversity, serving as a proxy for ecosystem recovery, in a mesocosm setting.

2.2 Materials and methods

2.2.1 Experimental design

To quantify the impact of *S. molesta* “before and after” biological control by the weevil, *C. salviniae*, on epilithic algae and aquatic macroinvertebrate assemblage structure, a BACI design was employed. Twelve opaque mesocosm pools (diameter = 260 cm, height = 68 cm and a total volume of 3600 litres), were set up at the Department of Zoology and Entomology, Rhodes University, (-33° 30.9' 94.9" E, 26° 51.8' 70.6" S), Eastern Cape, South Africa. Each pool was filled with tap water to a total volume of ~3000 litres and allowed to settle for seven days. Mesocosms were divided into three treatments (i.e. Impacted: 100% *S. molesta* cover; Restored: 100% *S. molesta* cover & *C. salviniae* weevil; and Control: open water), forming a block design of four mesocosms per treatment, making up 12 sampling units (3 treatments × 4 replicates = 12 SU) (Figure 2.1). All treatments received ~40% natural shade from their surroundings throughout the day and were stationed within a 15-metre radius from a small stream on the periphery of Rhodes University campus to increase the aquatic macroinvertebrate colonisation rate and to sustain their population throughout the study, this was also seen in Coetzee et al. (2020). The experiment was allowed to run for 60 weeks with six-weekly sampling collection (defined below), starting from February 2018 to April 2019.

Epilithic algae and aquatic macroinvertebrate assemblage structure and biodiversity indices, together with changes in physicochemical variables between treatments and “before and after” *S. molesta* biological control, were used as proxy to estimate ecosystem recovery. Data collection consisted of three sampling phases: (1) the “before” *S. molesta* invasion, (2) the “during” *S. molesta* invasion, and (3) the “after” successful biological control of *S. molesta* (Figure 2.1). During the “before” invasion phase, all treatments were *S. molesta* free (open water), a pair of epilithic and aquatic macroinvertebrate artificial substrates were deployed and left for a period of six weeks before the baseline or pre-*S. molesta* invasion data including water chemistry, epilithic algae and aquatic macroinvertebrate composition were collected (see data collection below for details). Following the “before” invasion phase, fresh stock of *C. salviniae* weevil-free *S. molesta* plants (~400 kg) from the Centre for Biological Control, Waainek Mass Rearing Research Facility (CBC-WMRRF), Rhodes University were ~50 kg *S. molesta* introduced into each mesocosm of four, in the impacted treatment (100% *S. molesta*) and restored treatment (100% *S. molesta* & *C. salviniae* weevil), leaving treatment 3 as an open water control (Figure 2.1). Transplanted *S. molesta* in the impacted and restored was left to grow under a once-off Culterra Multisol ‘N’ fertiliser (5 mg N/L), commercial iron chelate

(100 mg Fe/L; 13% Fe, EDTA-FeNa-3H₂O) and continuous addition of Multicote crystal fertiliser (N:P:K 15:7:15; Haifa manufactures) slow-release diffuser supplements to allow rapid and healthy growth of *S. molesta* plants. After 60 days, the impacted and restored treatments achieved 100% *S. molesta* growth cover. The Multicote slow-release diffuser was then removed from the two treatments. Before the “during” invasion phase began, 1000 individual *C. salviniae* weevils collected from the CBC-WMRRF, were introduced into restored treatment (250 *C. salviniae* weevils were released per mesocosm). Thereafter, the “during” invasion phase was allowed to run for a period of 42 weeks (with seven ‘six weekly’ sampling occasions), incorporating establishment of *C. salviniae*, and successful control of *S. molesta* in the restored treatment (Figure 2.1). After successful biological control of *S. molesta* by *C. salviniae* (after 48 weeks), the “after” control phase was allowed to run for a period of 12 weeks to quantify the relative recovery of epilithic and aquatic macroinvertebrate biodiversity in comparison to the “during” invasion and the “before” invasion phase between three treatments.

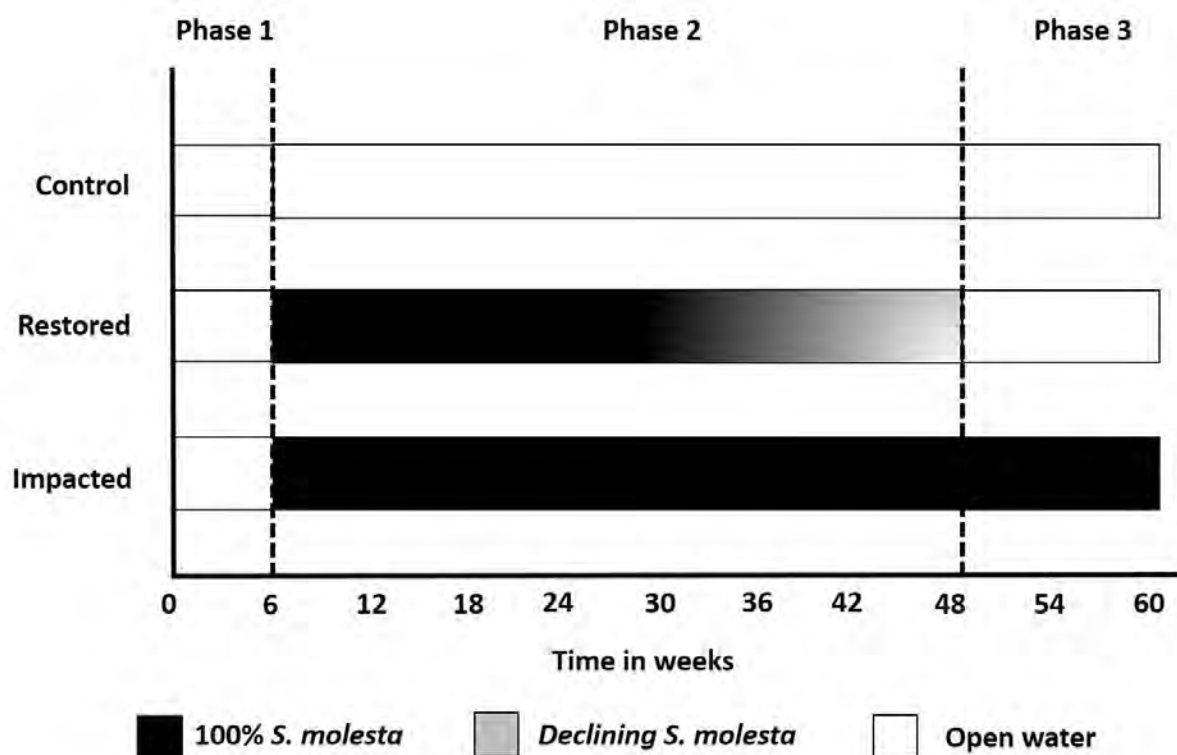


Figure 2.1: A mesocosm experiment design and timeline showing three sampling phases; (1) “before” invasion, (2) “during” invasion and (3) “after” control and three treatments (Impacted – 100% *Salvinia molesta*; Restored – 100% *Salvinia molesta* & *Cyrtobagous salviniae*; Control – open water).

2.2.2 Data collection

2.2.2.1 Water physicochemical variables

Physicochemical data were collected every 6 weeks, including: pH, conductivity (EC; $\mu\text{S}/\text{cm}$), total dissolved solids (TDS; ppm), salinity (ppm) and water temperature ($^{\circ}\text{C}$) using a Eutech multi-parameter testr 35 Series; and water clarity using a water clarity tube (cm) (manufactured by GroundTruth, South Africa). Dissolved oxygen (DO; mg/l) concentration was measured with a DO Pen Sper-Scientific (850045) meter. Additionally, water samples (500 ml) per mesocosm were taken to the laboratory to determine nitrate [NO_3^-] (Range: 1.0 to 14 000 mg/l or ppm; Accuracy after calibration: $\pm 10\%$ of full scale (calibrated 1 to 100 mg/l)) and ammonium [NH_4^+] using Ion Specific Electrodes (Range: 1.0 to 18 000 mg/l or ppm; Accuracy after calibration: $\pm 10\%$ of full scale (calibrated 1 to 100 mg/l)) (Vernier LabQuest®2) and phosphate [PO_4^{3-}] concentrations using a HI 83203 Multiparameter Bench Photometer for Aquaculture (Range: 0.0 to 30.0 mg/l; Resolution: 0.1 mg/l; Accuracy: $\pm 1 \text{ mg/l} \pm 4\%$).

2.2.2.2 Biological data

Epilithic algae assemblage

To quantify epilithic algal species assemblages and biodiversity between treatments and sampling phases, two artificial substrates ($22 \times 22 \text{ cm}$ stone tiles) were deployed at the bottom of each mesocosm and allowed to stand for a period of six weeks for complete epilithic algae colonisation (Kelly et al., 1998; Lane et al., 2003; Dalu et al., 2016). On each sampling occasion, both tiles were retrieved from the mesocosms, placed onto a white collecting tray with approximately 1500 ml filtered mesocosm water and the periphyton biofilm brushed off with a new toothbrush (Taylor et al., 2007a). The resulting $\sim 1500 \text{ ml}$ epilithic sample was divided into three 500 ml sub-samples where were transferred into one clear polyethylene sample container, and two opaque polyethylene containers for epilithic algae community analysis and for chlorophyll-*a* (Chl-*a*) concentration estimation respectively (described below). The epilithic algae community analysis sub-samples were thereafter preserved with 5 ml of Lugol's iodine solution (prepared by dissolving 100 g potassium iodide and 50 g iodine crystals in 2 litres of distilled water). Samples were then taken to the laboratory and allowed to sediment out following LeGresley and Mc Dermott (2010), on a stable flat bench surface for 72 hours. Thereafter, $\sim 450 \text{ ml}$ of the sample supernatant was discarded using a top-down siphoning system. Care was taken not to agitate the sample during the process. The remaining concentrated sample of $\pm 50 \text{ ml}$ was homogenised by moderately agitating the sample contents by hand for five seconds to evenly distribute epilithic algae cells. A Pasteur pipette was used

to remove about ~0.1 ml of the sample, which was placed onto a haemocytometer counting chamber (Neubauer improved; 0.1 mm depth, with a total grid area of 9 mm²), and covered with a cover slip (LeGresley and McDermott, 2010). Identification of epilithic algae to the lowest possible taxonomic level (e.g. genus or species) and counting were conducted using a combination of field guides and identification keys, including John et al. (2002), Van Vuuren et al. (2006) and Taylor et al. (2007b), under a light-phase microscope (Olympus CX21) at 400X magnification.

Cell counting was declared sufficient and to have satisfied statistical requirements when either a maximum of 400 cells or a total of four 9 mm² grid counting area was achieved (which was equivalent to $4 \times 9 \text{ mm}^2 = 36 \text{ mm}^2$ counting grid area) per sub-sample. Thereafter, to estimate the relative abundance of epilithic algae cells/ml, a modified LeGresley and McDermott (2010) equation was employed, taking the total sample volume, concentrated sample volume, the area counted and the chamber depth into account;

$$\text{Cells/ml} = \frac{\text{Cells counted} \times \text{Concentrated sample volume}}{\text{Area counted} \times 0.1 \text{ (chamber depth)}} \times \text{Total sample volume}$$

Phytoplankton and epilithic algae Chlorophyll-*a* concentration

To determine the production of the mesocosm ecosystem between treatments and sampling phases, phytoplankton and epilithic Chl-*a* concentration was used as a proxy to estimate the mesocosm net production. Using opaque polyethylene sample containers, 500 ml water samples (phytoplankton) were collected from each mesocosm, and together with the periphyton Chl-*a* concentration sub-samples (previously collected), were stored on ice until they reached the laboratory for further preparation. The storage method limited light penetration and kept the samples at a lower temperature (cooler with ice) to inhibit photosynthesis and other cellular processes that might lead to Chl-*a* concentration mis-interpretation. Prior to Chl-*a* concentration determination, samples were homogenised by moderately agitating the sample containers by hand for five seconds; thereafter a standard sample volume of 200 ml of phytoplankton and 100 ml periphyton samples, were filtered through Millipore nylon net filters (50 mm diameter, 20 µm mesh size) using a vacuum pump (Instruvac® Rocker 300) at 20 kPa. After filtering, any small unwanted animals (zooplankton and small invertebrates) and plant litter (*Wolffia*, *Lemna* species and debris) were removed from the nylon filter nets using forceps.

An acetone extraction method was used to determine Chl-*a* concentration fluorometrically, following methods described by Holm-Hansen and Riemann (1978). Each filtered nylon net (n=12 phytoplankton, and n=12 periphyton samples per sampling occasion) was folded in half, placed into a reaction tube with a screw, and 10 ml of 90% acetone solution was added. The reaction tubes were left for Chl-*a* extraction, in complete darkness at -20°C for a minimum of 48 hours. Thereafter, the Chl-*a* concentration was determined using a 10AU Field and Laboratory fluorometer (Turner Designs), noting the concentration before and after the sample was acidified by adding 2/3 drops of 0.1 M hydrochloric acid (Dalu et al., 2014a). The final Chl-*a* concentration was then calculated using the following formula modified from Lorenzen (1967) and Daemen (1986):

$$\text{Chl-}a \text{ (mg/m}^3\text{)} = \left(\frac{\text{Acetone volume}}{\text{Filtered sample volume}} \right) \times (\text{Reading before acidification} - \text{Reading after acidification}) \times 0.325$$

Aquatic macroinvertebrates

As with the epilithic algae, two artificial substrates – mesh bags (1 × 0.3 cm, mesh size) with 30 g of shredded plastic strips and 60 g of plant litter (a combination of leaves, twigs and stems) – were used to quantify and collect aquatic macroinvertebrates between treatments and sampling phases (Booth et al., 2013; Coetzee et al., 2014). Artificial substrates were suspended mid-height and placed on opposite sides of each mesocosm for a period of six weeks for complete aquatic macroinvertebrate colonisation (Thirion, 2000). After six weeks, artificial substrates were retrieved from each mesocosm using a hand-held aquatic net (30 cm × 30 cm square frame, 1 mm mesh size) to help prevent aquatic macroinvertebrates from escaping during collection. Substrates were then placed into a white sorting tray, and the contents of each mesh bag emptied and rinsed using tap water, to wash off and dislodge all aquatic macroinvertebrate samples into the tray. Samples were then left to settle for two minutes; thereafter, identification and counting to family was conducted following Day et al., (2002); Day and de Moor (2002); Gerber and Gabriel (2002a; b) and de Moor et al., (2003a; b) identification guides.

2.2.3 Data analysis

Physicochemical variables

A two-way analysis of variance (ANOVA) was performed to determine the variation in physicochemical variables (i.e. pH, EC, TDS, salinity, Water temperature, DO, NO₃⁻, NH₄⁺,

PO₄³⁻, water clarity, phytoplankton and periphyton Chl-*a* concentration) using treatment and sampling phase as factors. However, none of the physicochemical variables were found to be normally distributed (Shapiro-Wilks; $P < 0.001$ in all cases), nor were the variances homogeneous (Cochran's C-test, $P < 0.05$). Therefore, all variables were $\log(x+1)$ transformed to meet ANOVA assumptions for normality. Then, ANOVA ($P < 0.05$) was employed to test for the main effect on physicochemical variables between treatments and phases and their interaction. All statistical analysis except when specified, were conducted in R version 3.6.1 (R Core Development Team, 2016).

Epilithic algae and aquatic macroinvertebrate biodiversity patterns and response ratios

To estimate epilithic algae and aquatic macroinvertebrate biodiversity indices between treatments and sampling phases, relative taxa abundances (N), taxa richness (S), the Shannon diversity index: $H' = -\sum_{i=1}^s p_i \ln p_i$, (where p_i is the proportional abundance of taxa i in the sample given s taxa), and Pielou's evenness; $J' = \frac{H'}{\ln(S)}$ indices were used and computed per mesocosm in PRIMER version 6.1.16 and PERMANOVA⁺ version 1.0.6 using the DIVERSE function (PRIMER-E Ltd, Plymouth; Clarke and Gorley, 2006).

Furthermore, to investigate the relative epilithic algae and aquatic macroinvertebrate biodiversity recovery following successful biological control of *S. molesta* by *C. salviniae* between treatments, biodiversity indices mean response ratios were computed (Osenberg et al., 1997; Benayas et al., 2009). The impacted/degraded treatment (100% *S. molesta*) = $\ln\left(\frac{Degraded}{Reference}\right)$, and Reference treatment (open water) = $\ln\left(\frac{Restored}{Reference}\right)$ mean response ratios were compared to the restored systems (100% *S. molesta* & *C. salviniae*) mean response ratios to estimate the effect size or change in biodiversity indices following control by *C. salviniae*. Thereafter, the Wilcoxon signed rank test ($P < 0.05$) was used to test whether the response ratios were different from zero to ascertain whether the application of the biological control affected epilithic algae and aquatic macroinvertebrate biodiversity indices. The Kruskal-Wallis ANOVA ($P < 0.05$) test was then used to test for significant differences in mean response ratios between treatments.

To investigate epilithic algae and aquatic macroinvertebrate response “before” *S. molesta* invasion, “during” *S. molesta* invasion and “after” successful biological control of *S. molesta* invasion phase between the impacted, restored and control treatments, an extension of the BACI analysis (Before-After-During invasion, Control-Impacted and Restored experimental design) was performed. Prior to analysis, epilithic algae relative taxa abundance,

taxa richness and diversity data were $\ln(x+1)$ transformed to meet normality-distributed residuals and homoscedasticity. A Linear Mixed-Effects Model (lme4 package) and a post-hoc Tukey test (package multcomp) was used to test main effects and the interactions (Bates et al., 2015), where fixed effects were treatments (i.e. Control, Impacted and Restored, three levels) and phase (i.e. Before invasion, During invasion and After successful biological control), and mesocosm were treated as a random effect. The model fit was inspected using residuals and fitted values plots and were found to satisfy the assumptions of normality and heterogeneity (Zuur et al., 2009).

Epilithic algae and aquatic macroinvertebrate assemblage structure

To test the epilithic algae and aquatic macroinvertebrate assemblage structure and shifts between treatments and sampling phases, a permutational analysis of multivariate dispersions (PERMDISP; 9999 permutations) based on the mean distance to the centroid was performed on all epilithic algae and aquatic macroinvertebrate relative taxa abundance to test for homogeneity of variances (PRIMER-E Ltd, Plymouth; Clarke and Gorley, 2006). Additionally, a permutation analysis of variance (PERMANOVA) was further used to test if epilithic algae and aquatic macroinvertebrate assemblages were different between treatments and sampling phases. Unconstrained ordinations were completed using principal coordinate ordination (PCO) on Bray–Curtis similarity matrices to visualise both epilithic algae and aquatic macroinvertebrate data. The PCO ordination was followed by a constrained canonical analysis of principal coordinates (CAP) to emphasise and visualise epilithic algae and aquatic macroinvertebrate assemblage patterns between treatments and phases. Epilithic algae and aquatic macroinvertebrate relative taxa abundances were fourth-root transformed and correlated using the Pearson's correlation ($r > 0.5$) with the canonical axes of the CAP to identify taxa driving the differences in assemblage structure between treatments and phases (Anderson and Willis, 2003). Analyses were performed using PRIMER version 6.1.16 and PERMANOVA⁺ version 1.0.6 (PRIMER-E Ltd, Plymouth; Clarke and Gorley, 2006; Anderson et al., 2008).

Multiple linear regression analysis

Multiple linear regression analysis was used to examine which physicochemical variables affected epilithic algae and aquatic macroinvertebrate biodiversity indices. The initial model included the following variables: pH, EC, TDS, salinity, water temperature, DO, NO_3^- , NH_4^+ , PO_4^{3-} and water clarity. Prior to analysis, multi-collinear variables (e.g. EC, TDS and salinity),

were removed based on the variance inflation factor and ecological rationale, and thereafter the remaining physicochemical variables (pH, EC, water temperature, DO, NO_3^- , NH_4^+ , PO_4^{3-} and water clarity) were $\log(x+1)$ transformed (Mlambo et al., 2019). In addition, phytoplankton, periphyton Chl-*a* concentration and aquatic macroinvertebrate functional feeding guilds, including collector-filters, collector-gatherers, scrapers/herbivores, shredders and predators were assigned following Cummins and Klug (1979), Palmer et al. (1996), Merritt et al. (2008) and Hawking et al. (2013), were included as potential explanatory variables to influence aquatic macroinvertebrate and epilithic algae diversity indices, respectively. The StepAIC function from the package MASS (Venables and Ripley, 2002) was employed to perform forward-backward selection of the predictor variables and the best model, that is, the one with the lowest Akaike's information criterion (AIC) score, was selected.

2.3 Results

Water physicochemical variables

Dissolved oxygen concentration, water clarity and periphyton Chl-*a* concentration were significantly different between treatments, phases and between treatment $\times$ phase (Table 2.1), while pH was significantly different between phases and treatments $\times$ phases; water temperature only between phases and phytoplankton Chl-*a* concentration was significantly different between treatments and phases (Table 2.1). Water nutrient concentrations (i.e. NO_3^- , NH_4^+ and PO_4^{3-}) were only significantly different between treatments and not between phases throughout the study (Table 2.1).

Water clarity and periphyton Chl-*a* concentration were correlated, where high water clarity and high periphyton Chl-*a* concentration were recorded in the control treatment during the “after” biological control phase. Low water clarity and low periphyton Chl-*a* concentration were recorded in the impacted treatment also during the “after” biological control phase (Table S1). This shows that light penetration (i.e. water clarity) had a positive effect to periphyton Chl-*a* concentration. On the contrary, water clarity and phytoplankton Chl-*a* concentration were indirectly correlated, with the second highest water clarity and low phytoplankton Chl-*a* concentration being recorded in the impacted treatment during the “before” invasion phase and the lowest water clarity and the highest phytoplankton Chl-*a* concentration being recorded in the impacted treatment during the “after” biological control phase (Table S1). This indicates that high light penetration had a negative effect on phytoplankton Chl-*a* concentration, whereas *S. molesta* cover had a positive effect on phytoplankton Chl-*a* concentration.

Epilithic algae and aquatic macroinvertebrate biological diversity patterns

Linear Mixed-Effects Models showed that the effect of treatment and the interaction between treatment and phase were not significant for epilithic algae biodiversity indices throughout the study. However, epilithic relative taxon abundance and taxon richness were significant between sampling phases (Table 2.2). In contrast, aquatic macroinvertebrate relative taxa abundance, taxa richness, Pielou's evenness and the Shannon diversity index were significant between treatments and phases. The interactions between treatments and phases were significantly different for relative taxa abundance, taxa abundance, Pielou's evenness and the Shannon diversity index respectively (Table 2.2).

Table 2.1: Summary of ANOVA statistics of all physicochemical variables collected during the BACI study. Significant differences ($P < 0.05$) highlighted in bold.

Physicochemical variables	Treatments (Df=2, 111)		Phases (Df=2, 111)		Treatment $\times$ Phase (Df=4, 111)	
	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
pH	2.36	0.099	6.46	<0.01	6.14	<0.001
EC ($\mu\text{S}/\text{cm}$)	1.88	0.158	1.23	0.296	0.253	0.907
TDS (ppm)	0.87	0.423	1.36	0.262	0.12	0.979
Salinity (ppm)	2.67	0.074	0.81	0.499	0.16	0.957
DO (mg/l)	21.74	<0.001	10.39	<0.001	3.15	0.017
Water temperature ($^{\circ}\text{C}$)	2.44	0.092	31.27	<0.001	0.06	0.993
Water clarity (cm)	27.034	<0.001	3.05	0.05	4.88	0.001
NO_3^- (mg/l)	20.23	<0.001	2.06	0.133	2.19	0.074
NH_4^+ (mg/l)	3.49	0.03	0.97	0.383	0.32	0.863
PO_4^{3-} (m/l)	9.12	<0.001	1.32	0.271	1.05	0.383
Phytoplankton Chl- <i>a</i> (mg/m^3)	6.03	0.003	5.49	0.005	1.61	0.178
Periphyton Chl- <i>a</i> (mg/m^3)	36.52	<0.001	11.45	<0.001	12.27	<0.001

Table 2.2: Summary of ANOVA table of type III (Kenward-Roger approximation), *F* values and level of significance for fixed effects and interactions fitted with Linear Mixed-Effects Model (LMER). Significant differences ($P < 0.05$) highlighted in bold.

Factors	Relative taxa abundance		Taxa richness		Pielou's evenness		Shannon diversity index	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Epilithic algae								
Treatment	0.59	0.56	0.57	0.57	1.32	0.29	0.33	0.72
Phase	7.01	<0.01	2.90	0.05	0.08	0.91	1.01	0.37
Treatment × Phase	1.07	0.38	1.12	0.35	1.13	0.35	1.65	0.17
Aquatic macroinvertebrates								
Treatment	4.78	<0.05	24.24	<0.001	3.42	<0.05	22.11	<0.001
Phase	4.14	<0.05	5.92	<0.01	5.48	<0.01	7.02	0.001
Treatment × Phase	7.62	<0.001	8.44	<0.001	2.98	<0.05	4.47	<0.01

Relative epilithic algae abundance was highest during the “before” *S. molesta* invasion phase, followed by the “after” biological control of *S. molesta* phase, and was lowest “during” *S. molesta* invasion phase (Figure 2.2). Comparatively, taxa richness was highest “after” biological control of *S. molesta* phase, when compared to the “before” and “during” *S. molesta* invasion phases (Figure 2.2).

Aquatic macroinvertebrate biodiversity indices were significantly different between treatments and phases throughout the study. In all cases, the control treatment recorded the highest diversity scores, followed by the restored treatment, and the impacted treatment was the least diverse, although Pielou's evenness was equal between the restored and impacted treatments. Between sampling phases, aquatic macroinvertebrate relative taxa abundance and richness were higher “after” biological control of *S. molesta* phase when compared to the “before” *S. molesta* invasion phase (Figure 2.3). However, Pielou's evenness and the Shannon diversity index, was higher in the “before” *S. molesta* invasion phase, followed by the “after” biological control of *S. molesta* phase, with the “during” *S. molesta* invasion phase recording the lowest measures of biodiversity (Figure 2.3).

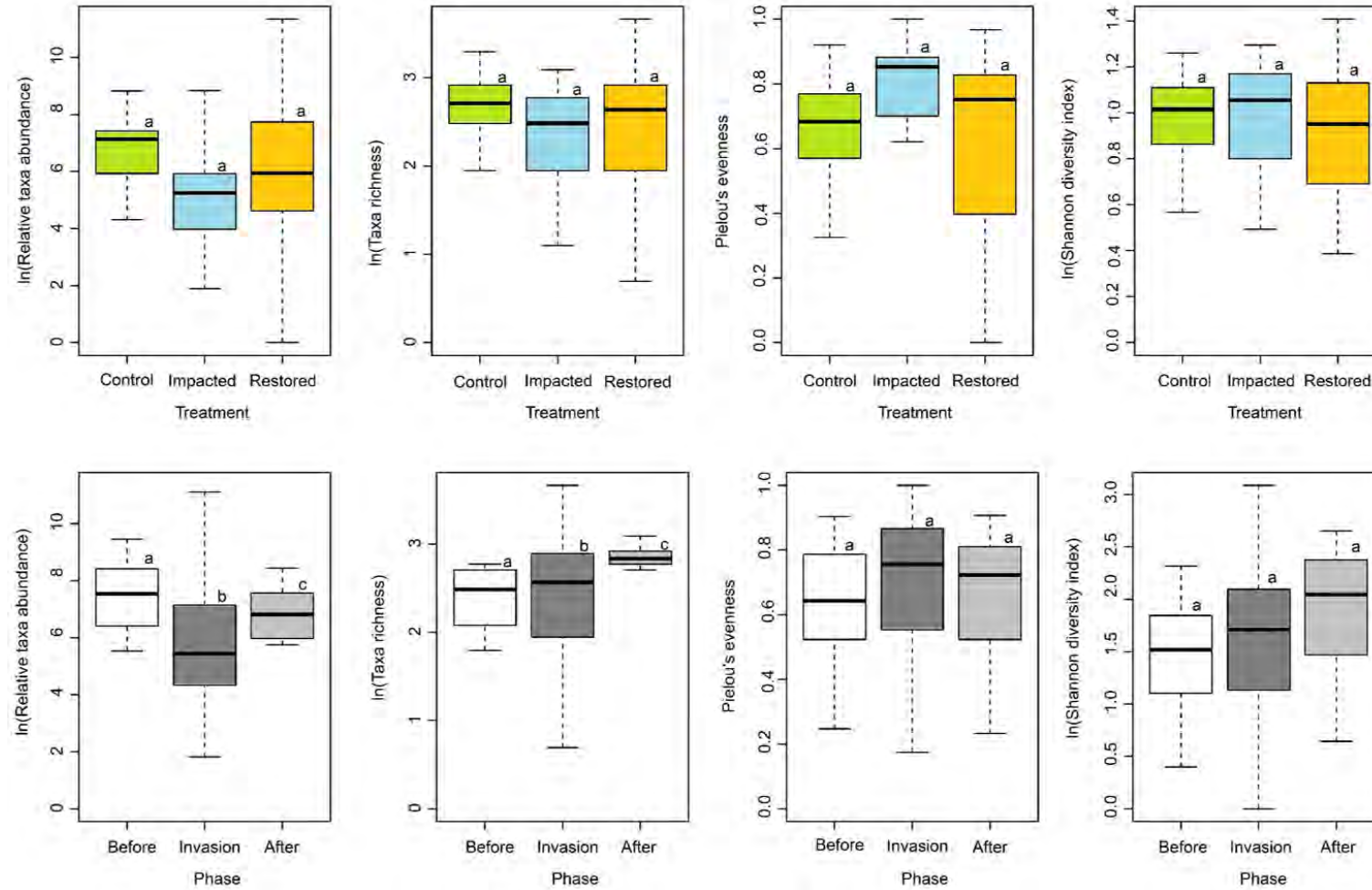


Figure 2.2: Epilithic algae biodiversity indices (average $\pm$ standard deviation) between treatments and sampling phases recorded throughout the study. Different lowercase letters represent significant differences. Control – open water; Impacted – 100% *Salvinia molesta*; and Restored – 100% *Salvinia molesta* & *Cyrtobagous salviniae* weevil treatments; Before – “before” *Salvinia molesta* invasion phase; Invasion – “during” *Salvinia molesta* invasion phase; After – “after” successful *Salvinia molesta* biological control phase. Epilithic algae biodiversity indices data (mean and standard deviation) per week are presented in Figure S1.

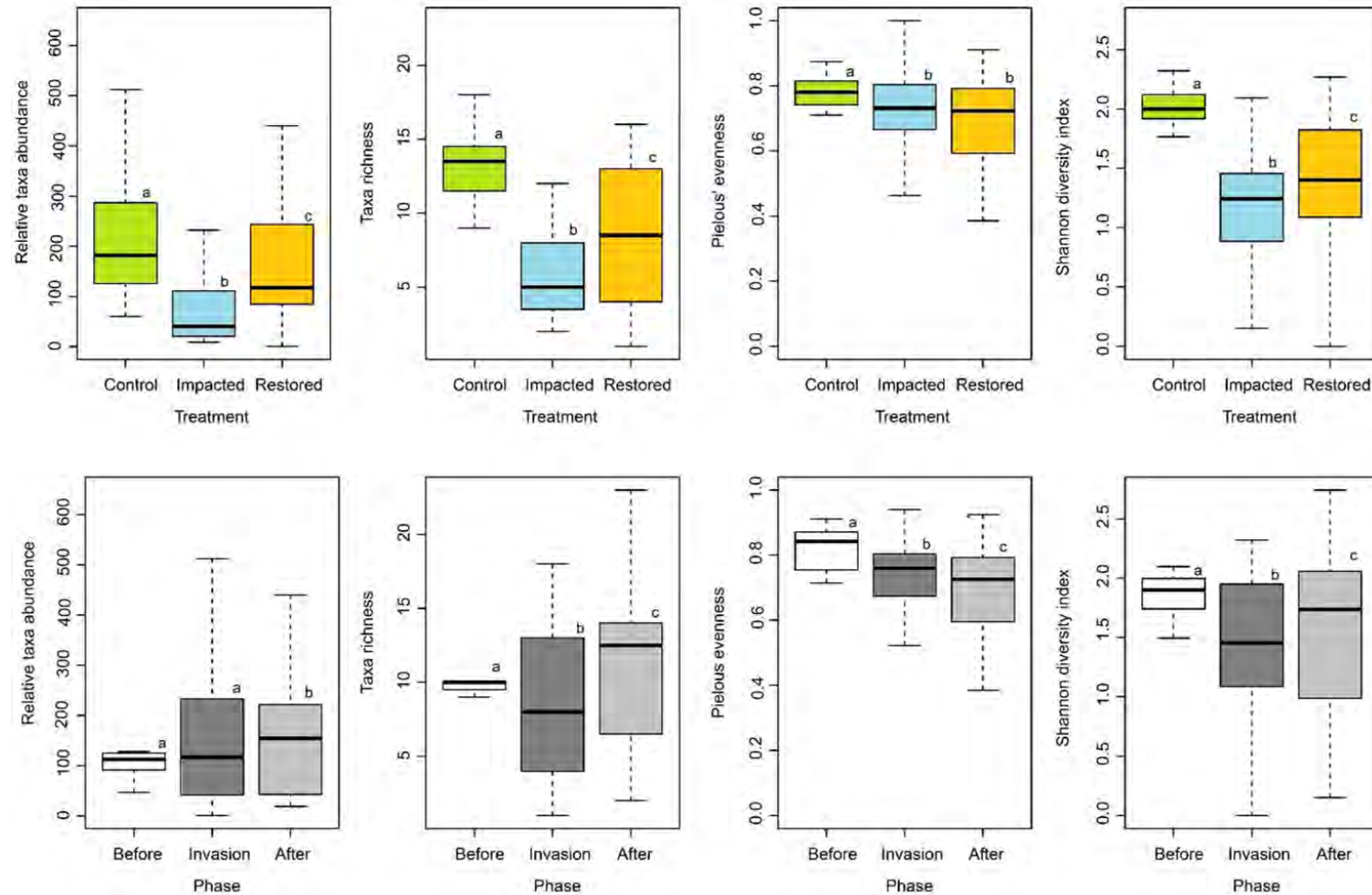


Figure 2.3: Aquatic macroinvertebrate biodiversity indices (average $\pm$ standard deviation) between treatments and sampling phases recorded throughout the study. Different lowercase letters represent significant differences. Control – open water; Impacted – 100% *Salvinia molesta*; and Restored – 100% *Salvinia molesta* & *Cyrtobagous salviniae* weevil treatments; Before – “before” *Salvinia molesta* invasion phase; Invasion – “during” *Salvinia molesta* invasion phase; After – “after” successful *Salvinia molesta* biological control phase. Epilithic algae biodiversity indices data (mean and standard deviation) per week are presented in Figure S2.

Biodiversity indices response ratios

The Wilcoxon test revealed that epilithic algae relative taxa abundance ($W=228$, $P<0.001$), taxa richness ($W=284.5$, $P<0.01$), and Pielou's evenness ($W=494$, $P=0.05$) mean response ratios were significantly different from zero, unlike the Shannon diversity index ($W=358$, $P>0.05$). Similarly, aquatic macroinvertebrate relative taxa abundance ($W=275$, $P<0.001$), taxa richness ($W=224$, $P<0.001$), and the Shannon diversity index ($W=186$, $P<0.001$) mean response ratios were significantly different from zero, but not Pielou's evenness ($W=500$, $P>0.05$). Thus, most of the epilithic algae and aquatic macroinvertebrate diversity indices were significantly improved as a result of biological control by *C. salviniae* in the restored treatment.

Epilithic algae and aquatic macroinvertebrate relative taxa abundance, taxa richness and the Shannon diversity index mean response ratios were higher (e.g. greater than 0) when the restored treatment was compared to the impacted treatment (Figure 2.4A & C), thus indicating a positive recovery following *S. molesta* biological control. However, this trend was not consistent for Pielou's evenness in all cases. In contrast, the biodiversity indices mean response ratios between the restored and reference treatments were less than zero (Figure 2.4B & D), indicating that epilithic algae and aquatic macroinvertebrate biodiversity recovery from restored treatments did not surpass that of the reference conditions.

Epilithic algae relative taxa abundance ($H=12.64$, $P<0.05$), taxa richness ($H=7.62$, $P<0.05$), and Pielou's evenness ($H=3.89$, $P=0.05$) were significantly different and slightly different between the restored and reference treatments. These results were consistent with aquatic macroinvertebrate relative taxa abundance ($H=17.65$, $P<0.05$), taxa richness ($H=22.77$, $P<0.05$), and the Shannon diversity index ($H=25.10$, $P<0.05$).

Relative taxa abundances for both epilithic algae and aquatic macroinvertebrate showed positive recovery following the biological control of *S. molesta* by the weevil *C. salviniae*, whereas taxa richness and the Shannon diversity index recovered the least (Figure 2.4A & Figure 2.4C).

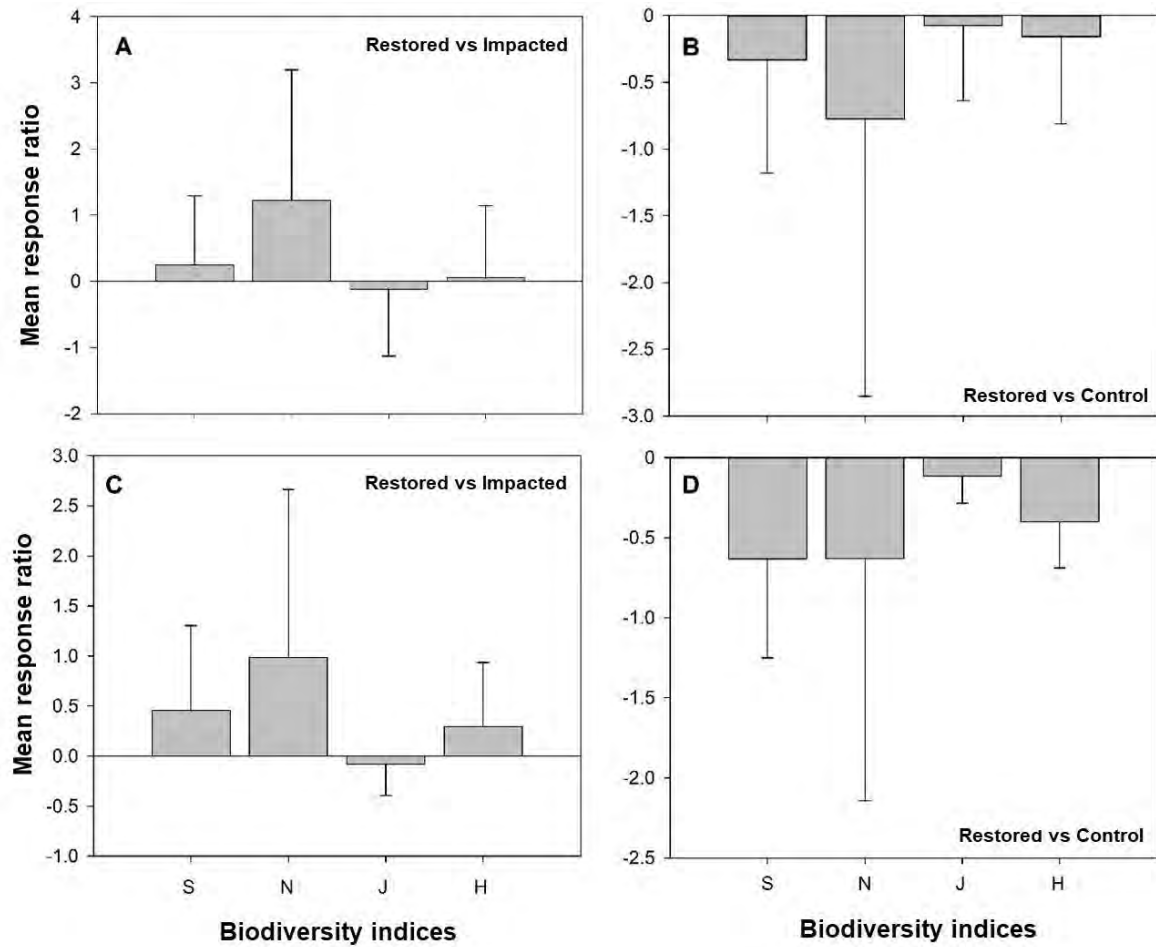


Figure 2.4: Response ratio (mean $\pm$ standard deviation) of biodiversity indices for epilithic algae from (A) Restored vs Impacted/Degraded, (B) Restored vs Reference, and for aquatic macroinvertebrates (C) Restored vs Impacted/Degraded, (D) Restored vs Reference; where S: taxa richness, N: relative taxa abundance, J: Pielou's evenness and H: Shannon diversity index.

Epilithic algae and aquatic macroinvertebrate assemblage structure

PERMANOVA results showed significant differences in assemblage patterns between treatments (epilithic algae pseudo- $F=2.77$, $P=0.0001$; aquatic macroinvertebrates pseudo- $F=6.61$, $P=0.0001$), phases (epilithic algae pseudo- $F=5.56$, $P=0.0001$; aquatic macroinvertebrates pseudo- $F=13.91$, $P=0.0001$) and the interaction between treatment $\times$ phase (epilithic algae pseudo- $F=1.56$, $P=0.0004$; aquatic macroinvertebrates pseudo- $F=3.50$, $P=0.0001$). Additionally, variances were heterogeneous in all cases for epilithic algae (PERMDISP, $P<0.05$) and aquatic macroinvertebrates (PERMDISP, $P<0.05$), and according to Anderson et al. (2008) PERMANOVA is not affected by heterogeneity in variances. This effect affirms that epilithic algae and aquatic macroinvertebrate assemblage structures were different between treatments and phases. Both epilithic algae and aquatic macroinvertebrate

assemblage structure were visually illustrated by CAP ordination (Figure 2.5A & B, Figure 2.6A & B). See supplementary material for both epilithic algae and aquatic macroinvertebrates taxa list (Table S2 and S3).

CAP ordination also illustrated differences in epilithic taxa assemblages between treatments, and this was supported by canonical correlation (CAP 1: $\delta^2=0.89$ and CAP 2: $\delta^2=0.40$), showing clear assemblage pattern differences (Figure 2.5A). *Cocconeis placentula*, *Gomphonema laticollum*, *G. affine*, *Nitzschia filiformis*, *N. linearis*, *Navicula zanonii*, *Monoraphidium irregular*, *Pseudanabaena* sp. and *Cocconeis englebrechtii* were correlated ($r>0.5$) to the control and the restored treatments (Figure 2.5A). Similarly, epilithic algae assemblages were significantly different between phases and showed complete assemblage pattern separation with a large canonical correlation (CAP 1: $\delta^2=0.91$ and CAP 2: $\delta^2=0.72$). *Scenedesmus dimorphus*, *Cosmarium subcostatum*, *M. irregular* and *M. contortum* were characteristic epilithic algae taxa during the “before” *S. molesta* invasion phase, compared to *G. laticollum* and *C. placentula* which were more frequently associated with the “after” *S. molesta* biological control phase (Figure 2.5B).

Aquatic macroinvertebrate treatment and phase CAP ordinations demonstrated three distinct assemblage patterns, where each cluster represents a different treatment and phase respectively (Figure 2.6A & B). Between treatments, the canonical correlation axis was $\delta^2 = 0.78$ (CAP1) and $\delta^2 = 0.71$ (CAP2), whereas between phases, it was $\delta^2 = 0.80$ (CAP1) and $\delta^2 = 0.67$ (CAP2). Hydrophilidae, Belostomatidae and Hirudinea showed a strong association (Pearson’s correlation, $r>0.5$) with the restored treatment and the control treatment (Figure 2.6A). The “before” *S. molesta* invasion phase showed a strong association with Caenidae, Baetidae and Notonectidae, whereas the “after” *S. molesta* biological control phase favoured both Hirudinea and Cypridoidae (Figure 2.6B).

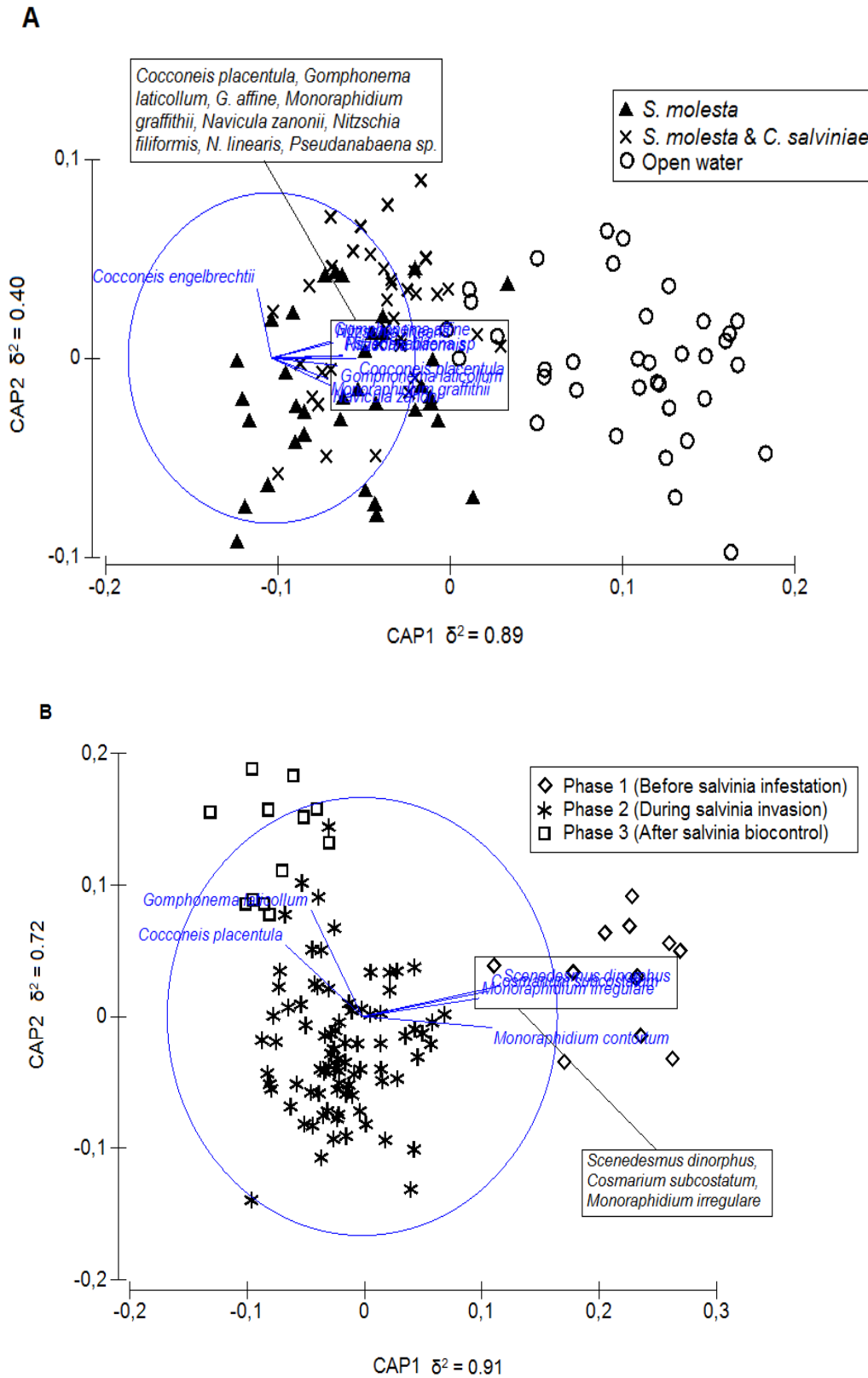


Figure 2.5: Canonical analysis of principal coordinate (CAP) ordination bi-plot of epilithic algae assemblages between (A) treatments, and (B) phases. δ^2 = indicating canonical correlation by axis.

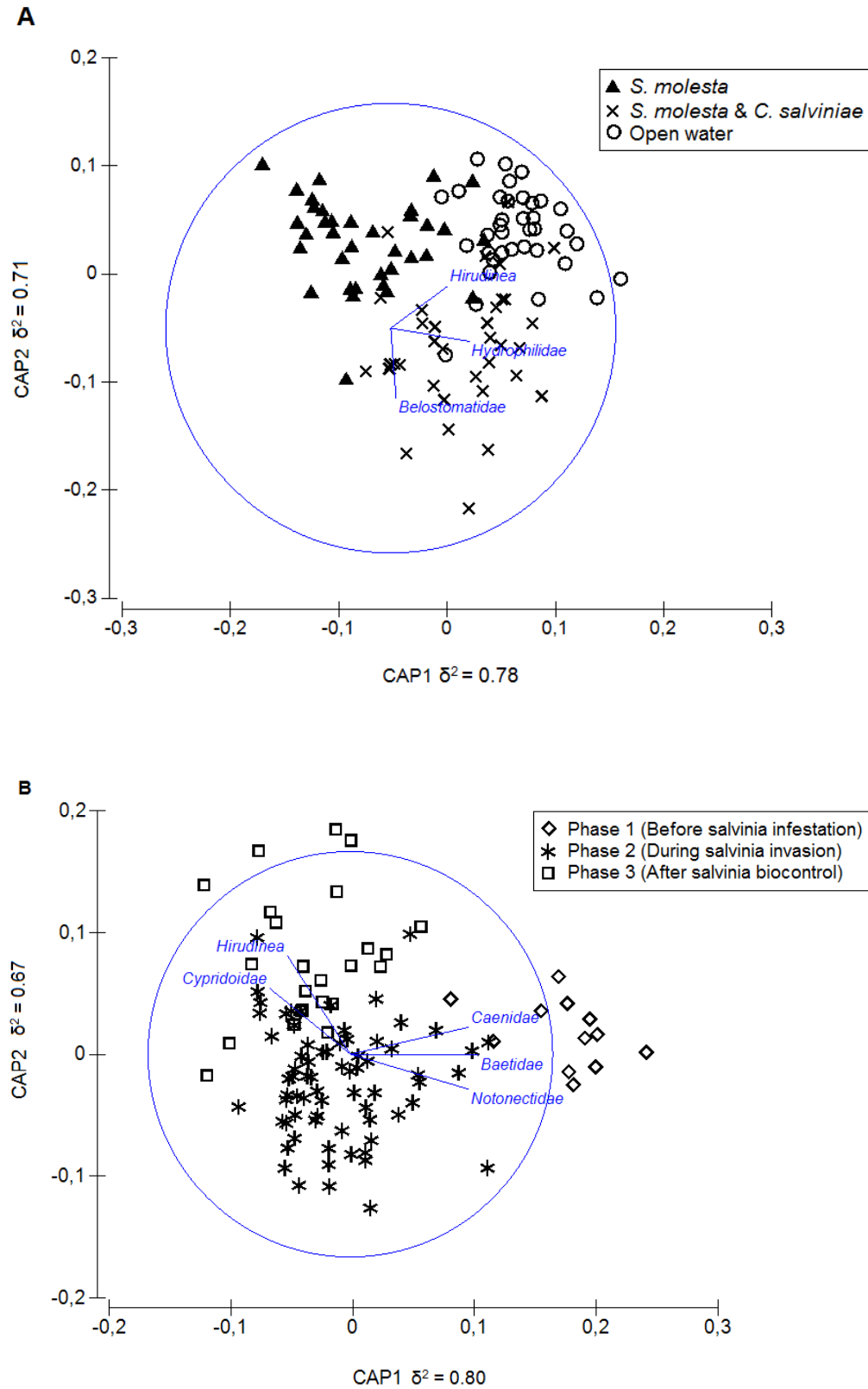


Figure 2.6: Canonical analysis of principal coordinate (CAP) ordination bi-plot of aquatic macroinvertebrate taxa between (A) treatments, and (B) phases. δ^2 = indicating canonical correlation by axis.

Multiple linear regression

Aquatic macroinvertebrate collector-gatherers, *S. molesta* cover, periphyton Chl-*a* concentration, pH, water temperature, DO and NH_4^+ as predictor variables explained 31% of epilithic algae relative taxa abundance. *Salvinia molesta* cover and pH negatively affected relative epilithic algae abundance, whereas periphyton Chl-*a* concentration positively affected relative epilithic algae abundance. Epilithic algae richness was affected by *S. molesta* cover, pH, water temperature, NH_4^+ and PO_4^{3-} , explaining 21% variation of epilithic algae richness. All variables negatively affected epilithic algae richness, but only *S. molesta* cover, pH and water temperature were significant. Although not significant, epilithic algae Pielou's evenness was affected by both *S. molesta* cover and NH_4^+ , and explained <1% variation. The Shannon diversity index, on the other hand, was affected by pH, NH_4^+ and PO_4^{3-} , and explained less than 1% of epilithic algae Shannon diversity index, where only NH_4^+ showed a significant and a negative correlation (Table 2.3).

Salvinia molesta cover, phytoplankton Chl-*a* concentration, pH and water temperature explained more than 40% variation in aquatic macroinvertebrate taxa richness and the Shannon diversity index during the study. In both cases, *S. molesta* cover was negatively correlated with aquatic macroinvertebrate taxa richness and the Shannon diversity index, whereas phytoplankton Chl-*a* concentration, pH, and water temperature positively affected both taxa richness and Shannon diversity. *Salvinia molesta* cover, phytoplankton Chl-*a* concentration, pH and DO explained 20% variation in aquatic macroinvertebrate relative taxa abundance, where only *S. molesta* cover and DO showed a negative correlation, in contrast to pH and phytoplankton Chl-*a* concentration. Aquatic macroinvertebrate Pielou's evenness was positively affected by water clarity, phytoplankton Chl-*a* concentration, and water temperature and collectively, only explained less than 1% variation. Water clarity and water temperature were the two significant variables that showed a positive correlation to aquatic macroinvertebrates Pielou's evenness (Table 2.3).

Table 2.3: Multiple linear regression analyses summary for epilithic algae and aquatic macroinvertebrate biodiversity indices, fitted using lm R function. The table shows *t*-statistics and *P*-value for regression coefficients, indicating if the value of the coefficient is significantly different from zero. Significant differences are in bold. S: taxa richness, N: relative taxa abundance, J: Pielou's evenness and H: Shannon diversity index. ln – indicating ln(x) transformed response variables. Where Cover – *Salvinia molesta* cover; Phytoplankton – Chl-*a* concentration; Periphyton – Chl-*a* concentration, Clarity – Water clarity.

Diversity indices	Predictors	Estimates	SE	<i>t</i>	<i>P</i>	AdjR ²	df	<i>F</i>	<i>P</i>
Epilithic algae									
lnN	Intercept	31.047	7.008	4.430	<0.0001	0.308	7, 100	7.801	<0.0001
	Collector-gatherers	0.260	0.140	1.852	0.067				
	Cover	-0.543	0.095	-5.731	<0.0001				
	Periphyton	0.288	0.174	1.661	0.01				
	pH	-10.114	3.311	-3.055	0.003				
	Water temperature	-1.990	1.076	-1.850	0.067				
	DO	1.354	0.896	1.512	0.134				
	NH ₄ ⁺	-9.915	6.857	-1.446	0.151				
lnS	Intercept	9.789	2.102	4.658	<0.0001	0.209	5, 102	6.646	<0.0001
	Cover	-0.125	0.028	-4.549	<0.0001				
	pH	-2.230	0.896	-2.490	0.014				
	Water temperature	-0.724	0.312	-2.320	0.022				
	NH ₄ ⁺	-4.679	2.054	-2.278	0.248				
	PO ₄ ³⁻	-0.151	0.106	-1.430	0.156				
J	Intercept	0.642	0.034	18.677	<0.0001	0.027	2, 105	2.529	0.085
	Cover	0.016	0.011	1.511	0.133				
	NH ₄ ⁺	-1.511	0.878	-1.721	0.088				
H	Intercept	4.806	2.086	2.304	0.023	0.044	3, 104	2.659	0.05
	pH	-1.404	0.959	-1.464	0.146				
	NH ₄ ⁺	-4.737	2.391	-1.981	0.050				
	PO ₄ ³⁻	-0.206	0.123	-1.671	0.098				

Table 2.3 cont. Multiple linear regression analyses summary for epilithic algae and aquatic macroinvertebrate biodiversity indices, fitted using lm R function. The table shows *t*-statistics and *P*-value for regression coefficients, indicating if the value of the coefficient is significantly different from zero. Significant differences are in bold. S: taxa richness, N: relative taxa abundance, J: Pielou's evenness and H: Shannon diversity index. ln – indicating ln(x) transformed response variables. Where Cover – *Salvinia molesta* cover; Phytoplankton – Chl-*a* concentration; Periphyton – Chl-*a* concentration; Clarity – Water clarity.

Diversity indices	Predictors	Estimates	SE	<i>t</i>	<i>P</i>	AdjR ²	df	<i>F</i>	<i>P</i>
Aquatic macroinvertebrates									
lnN	Intercept	-7.341	3.608	-2.035	0.044	0.204	4, 115	8.617	<0.0001
	Cover	-0.122	0.046	-2.667	0.009				
	Phytoplankton	0.226	0.115	1.963	0.052				
	pH	6.192	1.707	3.628	0.0004				
	DO	-0.881	0.433	-2.032	0.045				
S	Intercept	-39.085	13.11	-2.981	0.004	0.444	4, 115	24.74	<0.0001
	Cover	-1.030	0.157	-6.547	<0.0001				
	Phytoplankton	1.059	0.393	2.692	0.008				
	pH	16.194	5.518	2.935	0.004				
	Water temperature	4.957	1.745	2.841	0.005				
J	Intercept	0.068	0.224	0.301	0.764	0.060	3, 116	3.52	0.017
	Clarity	0.049	0.021	2.322	0.022				
	Phytoplankton	0.028	0.017	1.607	0.111				
	Water temperature	0.153	0.070	2.190	0.031				
H	Intercept	-3.902	1.547	-2.523	0.013	0.410	4, 115	21.64	<0.0001
	Cover	-0.111	0.019	-5.993	<0.0001				
	Phytoplankton	0.096	0.046	2.060	0.042				
	pH	1.708	0.651	2.624	0.01				
	Water temperature	0.629	0.209	3.102	0.002				

2.4 Discussion

The presence of *S. molesta*, like any other free-floating IAAP species, alter water chemistry (e.g. reduces DO concentration, light penetration and water clarity) and negatively affects aquatic biodiversity, as illustrated by Masifwa et al. (2001), Brendonck et al. (2003), Midgley et al. (2006), Chamier et al. (2012) and Coetzee et al. (2014). The results presented in this thesis agree with my hypothesis, in that the presence of *S. molesta* altered the water quality, and reduced ecosystem productivity and aquatic biodiversity in the mesocosm setting. However, the presence of *C. salviniae* lessened the impact of the *S. molesta* invasion, and facilitated water quality improvement, increase in ecosystem productivity, and the recovery of epilithic algae and aquatic macroinvertebrate diversity.

Periphyton Chl-*a* concentration increased with water clarity, thus water clarity in the control treatment (open water) was highest, followed by the restored treatment (100% *S. molesta* & *C. salviniae* weevil), while the impacted treatment (100% *S. molesta*) was the least productive, resulting in poor water quality. However, the opposite was observed for phytoplankton Chl-*a* concentration, which was highest in the impacted treatment and the lowest in the control treatment. This phenomenon was not surprising, as periphyton Chl-*a* concentration and community diversity are positively influenced by light penetration and available growing substrate, which were only available in the control and later the restored treatments following control. In addition, decaying free-floating *S. molesta* plants increased suspended organic matter thus reducing the water clarity; this, in turn, positively influenced the phytoplankton Chl-*a* concentration. Cattaneo et al. (1998) and Chamier et al. (2012), report that under conditions of limited light penetration due to dense mats of IAAPs, the high Chl-*a* concentration of suspended material is mainly dominated by both organic debris (in this case from *S. molesta*) and bacteria.

Epilithic algae relative taxa abundance and richness were sensitive to *S. molesta* invasion and its biological control efforts. That is, epilithic algae colonisation and resident time (experiment period) influenced, positively and gradually, an increase in taxa richness in all experimental treatments. Epilithic algae in general, are a diverse group of aquatic organisms, widely represented in aquatic ecosystems and show ubiquitous measurable effects in aquatic ecosystems (Kock et al., 2019), so it is not surprising that biodiversity indices between treatments were not sensitive enough to demonstrate the impact of *S. molesta* during the present study. This may be due to the ability of epilithic algae to shift in composition, depending on environmental conditions e.g. light, and still maintain their high taxa richness when light is a

limiting factor (De Tezanos Pinto et al., 2007). It is clear that free-floating IAAP species provide habitat structure for some aquatic organisms, including epilithic algae, as evident in the present study. Epilithic algae richness was significantly different between treatments and phases and showed a gradual increase from the “before” *S. molesta* invasion phase, to “during” the *S. molesta* invasion phase, with the “after” *S. molesta* invasion biological control showing the highest taxa richness (Figure S1). These results clearly show that *S. molesta* cover and light limitation did not reduce epilithic algae taxa richness, but the residence time and habitat provided by *S. molesta* roots increased epilithic algae richness. However, these findings do not account for the potential sensitivity of periphyton assemblages and biodiversity indices as biological indicators in water pollution studies, as observed by Beyene et al. (2009).

Aquatic macroinvertebrates, on the other hand, were reliable biological indicators of the impact of *S. molesta*, and showed changes following successful biological control of *S. molesta*. This was also consistent with findings from Midgley et al. (2006), Coetzee et al. (2014) and Langa (2013) who reported higher aquatic macroinvertebrate diversity in open water than under water hyacinth mats and following biological control of *P. stratiotes*. In the present study, the biological control effort of *S. molesta* was able to facilitate recovery of the epilithic algae relative taxa abundance as well as aquatic macroinvertebrate relative taxa abundance, richness, and the Shannon diversity index. Estimated biodiversity recovery for epilithic algae and aquatic macroinvertebrate was largely similar to that of the reference treatment. Having said that, the two groups differentiated between the abiotic barriers manifested by the *S. molesta* infestation (i.e. artificial shading, water clarity and reduced DO concentration), as a result of improved physicochemical parameters following successful biological control of *S. molesta*.

Results of the present study and that of Midgley et al. (2006), Langa (2013) and Coetzee et al. (2014) differed from that of Carey et al. (2018) who reported that the presence of free-floating IAAP species, Indian waterfern in Australian arid zone provide habitat complexity for aquatic organisms, and thus contribute to an increase in aquatic biodiversity, particularly aquatic macroinvertebrates. A study by Masifwa et al. (2001) revealed increased aquatic macroinvertebrate diversity only at the interface of invaded and open water regions, whereas reduced aquatic macroinvertebrate diversity was recorded deeper in the water hyacinth mat on Lake Victoria, Uganda. Brendonck et al. (2003), on the other hand, reported no clear positive relationship between water hyacinth cover and aquatic macroinvertebrate diversity, except for certain zooplankton and fish species. Water hyacinth in these case studies was associated with

shelter for predators, as well as feeding grounds since the root structure recruits a high phytoplankton and periphyton Chl-*a* concentration.

According to Denslow and D'Antonio (2005), the primary objective for the development of a biological control programme is to reduce the IAAP species biomass in their introduced range. This objective is followed by the recovery of native species and ecosystem services. Denslow and D'Antonio (2005) acknowledge that, following the successful management of IAAP species, quantifying the benefits of the control methods has been challenging. Traditionally, community-based matrices have been used to assess or monitor environmental impacts in both terrestrial and aquatic environments. In the case of restoration ecology in particular, there is controversy over how the success of ecological restoration should be measured. According to Adams et al. (2002) and Muotka and Laasonen (2002), species abundance, diversity, presence and absence of key species, and other community attributes were useful to indicate changes but insufficient to account for the recovery of ecosystem functioning. Authors further argue that restoration of ecosystem processes and ecosystem self-reorganisation has received less attention than that of aquatic community responses. Thus, it is not yet clear which biological attributes are most useful for assessing ecological restoration. For example, Durigan and Sukanuma (2015)'s response to Reid (2015) on the exclusion of species composition as an assessment for riparian forests restoration, considers the number of species regenerating as a surrogate for biodiversity recovery and persistency, rather than species composition which seemed to be unpredictable over time, and did not match that of the reference sites in a reasonable time frame. Durigan and Sukanuma (2015) further added that ecological indicators need to capture the complexities of the ecosystem but remain simple enough to be easily and routinely applied to monitored systems under investigation. Ecosystems generally show a variable response during their recovery, that is, perturbed systems, and those in various states of recovery are generally characterised by higher levels of variability than stable or complex systems, because variability due to anthropogenic stressors is superimposed on the natural, inherent variability of ecosystems (Adams et al., 2002). This high degree of variability can complicate the accurate assessment of recovery in many instances, and because of this variability, additional knowledge of ecosystem states is required before and after disturbance in order to adequately characterise recovery status and potential. Thus, microcosms and mesocosms are suitable experimental platforms to enable ecologists to test global ecological problems and provide empirical data that can be conceptualised to simplify some of the complex analyses that are necessary in ecological studies (Benton et al., 2007; Strange et al., 2019). Adams et al. (2002) further emphasise that, since aquatic

ecosystems demonstrate this high variability in response to different restoration techniques, no single biological attribute will be adequate for assessing the recovery of an aquatic ecosystem. Other authors (e.g. Adams et al., 2002; Muotka and Laasonen, 2002; Reid et al., 2009) recommend a suite of both chemical and biological components and if possible, their interactions, “before and after” the environmental impacts to provide a useful and holistic understanding of an ecosystem recovery dynamics.

The present study echoes findings reported by Benayas et al. (2009) that restoration efforts assist degraded ecosystems by promoting characteristics to help return the ecosystem to a desirable functioning state. Biodiversity indices from the restored treatment were significantly higher compared to the impacted treatment and partially similar to that of the control treatment, thus showing that the restoration effort by *C. salviniae* was sufficient to restore the aquatic biota to desired state which is attribute by improved water quality, high biological and functional biodiversity, but not more than the reference condition. Unfortunately, this is often the fate of ecological restoration, where the return of a degraded ecosystem to historical conditions is impossible, but the restored system can assume a desirable state. At this state, the ecosystem can self-reorganise some of the fundamental ecological feedback mechanisms to help provide ecosystem goods and services, including good water quality. While the socio-economic and biodiversity effects of free-floating IAAP species have been well documented, IAAP species impacts on ecosystem functioning and ecological recovery following their management are less well understood (Villamagna and Murphy, 2010). Antonio and Meyerson (2002) and Magoba and Samways (2010) state that the impact of IAAP species and any other invasive alien species can be irreversible or sometime expensive to restore.. According to the comprehensive reviews of Prior et al. (2018) and Kettenring and Adams (2011), almost 30% of programme removing alien invasive species mechanically were unsuccessful (showed unexpected outputs) and more than 50% showed mixed results. Having said that, Benayas et al. (2009) emphasise that biodiversity-ecosystem functions studies have generally been laboratory based or have employed small field plots or mesocosms, as in the present study. It might be argued that findings from such small-scale studies have little relevance to the larger scale ecosystem level at which land management decisions are made. However, such small-scale studies are building blocks and provide empirical evidence to help understand systems, so that when large-scale studies are conducted, fundamental ecosystem process and feedback are well captured (Strange et al., 2018; Turunen et al., 2018; Richardson et al., 2019; Strange et al., 2019).

In conclusion, aquatic ecosystem recovery is complex, and limited case studies have attempted to address some of the inconsistencies. The present study provides useful empirical information on the impact of *S. molesta* and successful biological control of South Africa's worst free-floating IAAP species in a Before-During and After impact verses Control-Impacted and Restored in a mesocosm setting. Epilithic algae and aquatic macroinvertebrate community-based matrices were clearly reliable biological indicators of *S. molesta* impacts and successful indicators for ecosystem recovery following biological control by *C. salviniae*. The beyond BACI design by Green (1979) and Underwood (1992) applied by Muotka and Laasonen (2002), Kadye and Booth (2012) and Bellingan et al. (2019) can provide ecologists with useful empirical information when tackling global ecological, biodiversity, and restoration case studies. The present chapter highlights a useful ecological approach in restoration studies and provides empirical data on assessing complex experimental set-ups. The next chapters provide further high resolution in ecosystem recovery and restoration, looking at field case studies, which are recognised as challenging because of their variability and the unpredictable responses to restoration measures. These chapters will include a suite of biological and chemical attributes and their interactions to quantify ecosystem recovery following *S. molesta* infestation and successful control.

CHAPTER 3

Ecosystem recovery following *Salvinia molesta* control: Emphasis on aquatic biodiversity and ecosystem trophic functioning, a field study

3.1 Introduction

Invasive alien aquatic plant (IAAP) species within their introduced range are able to manipulate ecosystem internal feedback mechanisms and become part of the aquatic food web system (Bakker et al., 2013). In so doing, IAAP species compete for light and nutrients with phytoplankton, periphyton and other native macrophytes and fauna. As a result, IAAP species alter the energy flow and transfer from one trophic level to the other within an invaded ecosystem. The energy flow restrictions (or resource subsidy limitations) to higher trophic level organisms can cause a major decline in aquatic organism abundance and diversity, thus destabilising the aquatic food web and leading to major trophic cascades (Carpenter et al., 2011). It is for this reason that IAAP species are declared a major threat to aquatic ecosystem biodiversity and economically important ecosystem goods and services (Reid et al., 2019). Reversing the impact of IAAP species in freshwater ecosystems would be of major benefit to aquatic biodiversity recovery and to the re-establishment of desirable ecosystem, with good water quality and that is biologically and functionally diverse (Magoba and Samways, 2010). Magoba and Samways (2010) and Samways et al. (2011) reported a decline in aquatic macroinvertebrate taxa richness and diversity in sites heavily invaded by *Acacia* sp. in the Limpopo and Western Cape Provinces of South Africa. This was consistent with reports by Midgley et al. (2006), Masese et al. (2009), Langa (2013), and Coetzee et al. (2014) who also reported drastic declines in aquatic macroinvertebrate taxa abundance and diversity due to free-floating IAAP species invasions. It is evident that both invasive alien riparian and aquatic weed species alter community composition and reduce diversity of aquatic macroinvertebrates in invaded systems, causing biotic homogenisation and the loss of biological difference (or functional diversity) (Petsch, 2016). Biotic homogenisation refers to a complete shift in aquatic community composition where typically only tolerant and generalist taxa survive biotic and abiotic filters as a result of IAAP infestation as seen in Midgley et al. (2006), Masese et al. (2009), Coetzee et al. (2014) and Chapter 2. However, there is not much known about ecosystem trophic structure, “before and after” following IAAP species control in freshwater ecosystems. Unfortunately, this has implications for freshwater restoration and aquatic

biodiversity conservation. On the contrary, terrestrial and riparian alien invasion have been extensively studied in South Africa and abroad, with restoration dynamics being well understood (Pryke and Samways, 2009; Le Maitre et al., 2011; Gaertner et al., 2012). Thus, this chapter presents a novel approach that will contribute to our understanding of the ecosystem dynamics in the presence of free-floating IAAP species invasion and following their subsequent control, and provides a useful empirical data-set to propose effective restoration interventions on a global scale.

Ecological restoration assists the recovery of an ecosystem that has been degraded, damaged or destroyed due to human-mediated activities (Benayas et al., 2009). Restoration actions are increasingly being implemented globally, supported by the global policy commitment such as the Convention on Biological Diversity (1992). During the past two decades ecological restoration outcomes have shifted from the recovery of individual taxa to the recovery of functional groups (Montoya et al., 2012), although some authors still regard species richness as a good indicator of ecological restoration (Durigan and Suganuma, 2015). Montoya et al. (2012) argue that not all species equally contribute to ecosystem functioning, but rather some species are functionally more important, and despite species richness acting as a biological insurance, functionally important species should be prioritised for restoration programmes. Secondly, these authors report that trait-based research shows that community-based indices (i.e. species richness, evenness and diversity) are not as relevant as functional diversity when re-instating ecosystem functioning.

Having considered the inconsistencies and controversy around ecosystem recovery and restoration assessment attributes in freshwater ecosystems as described by Adams et al. (2002), Muotka and Laasonen (2002), Reid et al. (2009), Montoya et al. (2012) and Durigan and Suganuma (2015), the present chapter aims to quantify ecosystem recovery and shifts in trophic ecosystem structure and function “before and after” *S. molesta* control in four freshwater systems in southern Africa, as a proxy for ecological restoration. Traditionally, trophic interactions have been challenging to quantify, thus making it impossible to estimate ecological impacts of invasive alien species in freshwater ecosystems in general (Hill et al., 2015). However developments in trophic ecology have introduced a powerful whole-system approach to trace changes within an ecosystem trophic structure and energy flow using a quantifiable framework called the Stable Isotope Bayesian Ellipses in R (SIBER) (Layman et al., 2007). Nitrogen ratios, N^{15} to N^{14} (expressed as $\delta^{15}N$) provide a clear nitrogen enrichment with each energy transfer within an ecosystem, thus it is sensitive for estimating trophic position of organisms. Whereas carbon ratios (C^{13} to $C^{12} = \delta^{13}C$) provide an indication of energy resources

available to the ecosystems from a range of primary producers with different photosynthetic pathways (e.g. C3 vs. C4 plants), but does not change much with trophic transfers. As such, $\delta^{13}\text{C}$ are used to indicate different energy sources within the systems (Post 2002). Previously, food webs were constructed using $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ bi-plots, with species plotted based on their mean stable isotope signatures. Although the qualitative insights from $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ bi-plots were found informative in some instances, the community-wide metrics (or Layman’s metrics) provided a quantitative approach to measure trophic structure using stable isotope ratios. This approach differs from traditional food webs bi-plots, because it is based on a representation of realized trophic niches instead of presumed trophic roles derived from phenotypic characteristics. This framework not only contributes to our in-depth understanding of trophic interactions but it has showed a wide application in ecological studies particularly on how non-native species (i.e. alien species) can affect native species and the entire ecosystem (see Vander Zanden et al., 2003; Jackson et al., 2012; Jackson and Britton, 2014; Hill et al., 2015).

Additionally, epiphytic algae and aquatic macroinvertebrate community-based metrics were quantified here “before and after” *S. molesta* control, which are considered effective in measuring the degree of recovery in freshwater ecosystems but rather superficial in quantifying ecosystem trophic shifts. Thus, firstly I hypothesise that the presence of *S. molesta* will have negative effects on water quality, epiphyton and aquatic macroinvertebrate taxa richness and diversity and cause a significant shift in assemblage structure. Similar to Stiers et al. (2011) and Stiers and Triest (2017), who showed significant shifts in phytoplankton and aquatic macroinvertebrate assemblage structure in IAAP invaded ponds in Belgium. Secondly, I hypothesise that alteration to aquatic assemblage structure will further manifest knock-on effects on ecosystem trophic structure and function, thus limiting energy transfers to higher trophic levels and causing local biotic die-off and/or displacement. Comparatively, following the control of *S. molesta*, I expect a significant increase in epiphyton and aquatic macroinvertebrate taxa diversity to shift towards more functionally important aquatic organisms (of high functional diversity) that will assist in ecosystem recovery, self-reorganisation, resulting in normal ecosystem functioning.

3.2 Materials and methods

3.2.1 Study sites description

This study employed a Before-After Control-Impact (BACI) experimental design (Green 1979), to investigate aquatic ecosystem biodiversity recovery and trophic food web structure

and functions “before and after” successful control of *S. molesta*. Four field sites that were heavily infested with the free-floating *S. molesta* were identified. Three sites were situated in the Western Cape Province of South Africa (Figure 3.1A & B), within the Cape Floristic Region and include the Westlake River (S-33°08’39.6”, E018°46’19.9” near Lakeside), Silverhurst Impoundment (S-34°02’25.2”, E018°43’17.4” near Constantia) and Kogmanskloof River (S-33°94’49.2”, E020°07’92.1” near Robertson). The fourth site, Rosle Farm Reservoir (S-25°12’21.5”, E29°25’19.0” near Groblersdal) was located in the northern highlands within the Savanna-Mopani region of the Limpopo Province, South Africa.

3.2.1.1 Westlake River system

The Westlake River is an urban river system, draining the majority of the Westlake urban and formal settlements. Both the Westlake River and the Keyser River are important water sources to the Westlake wetland and the Zandvlei Estuary Nature Reserve near Muizenberg, which is the only functioning estuary within the False Bay coast line of the City of Cape Town (Jack, 2006). Westlake River and the adjacent wetland has been previously infested by multiple free-floating IAAP species including *P. crassipes*, *A. filiculoides*, *N. mexicana*, *P. stratiotes*, *M. aquaticum* and *S. molesta* (Archibald, 1998). In March 2017, *P. stratiotes* and *M. aquaticum* were recorded on-site, but *S. molesta* was the dominant invader (Figure 3.2A).

3.2.1.2 Silverhurst Impoundment

The Silverhurst Impoundment is a man-made pond situated on the upper reaches of the Keyser River, and consists of a modified in-flow and out-flow onto the Keyser Stream. The Silverhurst Impoundment is located in the foothills of the Noordhoek Mountains, and together with the Westlake River falls within the West Strandveld bioregion of the Western Cape Province of South Africa. The site is surrounded by Constantia, a residential area, and it is a well-known recreational park allowing activities such as cycling, horse riding, and dog walking. In March 2017, the impoundment was infested with *S. molesta*, the dominant IAAP species, and <10% infestation of *M. aquaticum* (Figure 3.2B).

3.2.1.3 Kogmanskloof River system

The Kogmanskloof River system is a tributary of the Breede River system and falls within the Breede River catchment area in the east coast Renosterveld bioregion. The Kogmanskloof River arises from the Langeberg-Wes mountain range. The river flows through wine lands from the upper Breede River Valley, through urban Montagu and down to Ashton where it joins the

Breede River 5 km downstream of Robertson. In March 2017, the river was mainly infested by *S. molesta* with dense stands of the giant reed *Arundo donax* L. (Poaceae) along the riparian zone. During events of high flow, *S. molesta* plants escape the Kogmanskloof River into the Breede River main stem and further wash down river. Both *S. molesta* and *P. crassipes* are recorded problematic weeds within the Breede River, where herbicide application is frequently used as a control method since the system is considered the main source for irrigation schemes for neighboring wine lands (Figure 3.2C).

3.2.1.4 Rosle Farm Reservoir

Rosle Farm Reservoir is a 40 000 m² (4 ha), man-made system used mainly for irrigation and recreation. The system is situated within the Olifant's River catchment, in the central bushveld bioregion and drains the majority of the southeastern region of Limpopo Province. In November 2017, *S. molesta* was the dominant non-native aquatic plant species within the reservoir, with patches of *A. donax* spread evenly on the riparian zone of the system. Previous records showed successful biological control of *S. molesta* on the reservoir by the biological control agents *C. salviniae* weevils (Peter Roets pers. comm.) (Figure 3.2D).

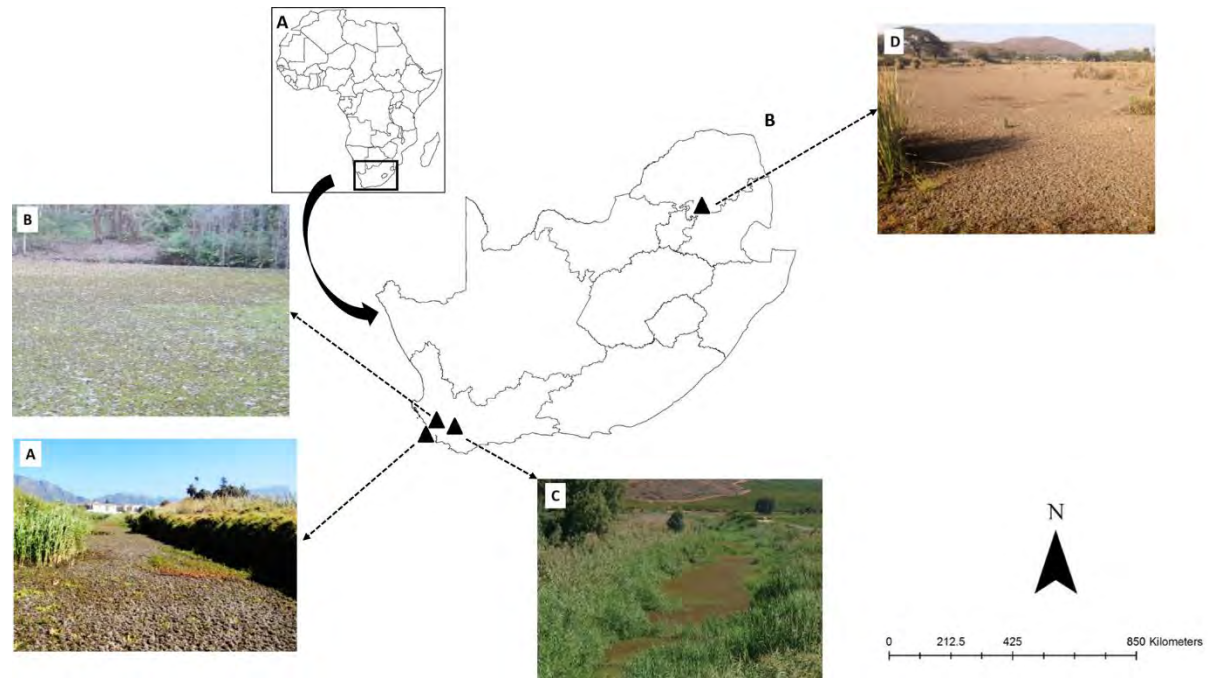


Figure 3.1: African continent (a) with (b) the South Africa insert indicating the four study sites infested with *Salvinia molesta* (black triangles). Three sites were situated within the Western Cape and one in the Limpopo Province of South Africa.

3.2.2 Experimental design

Similar to the mesocosm study (Chapter 2), the present field study was carried out in a series of phases. Phase 1 was conducted during *S. molesta* infestation (hereafter referred to as “before” *S. molesta* control), where full aquatic biodiversity and trophic ecology surveys were completed and samples including water chemical analysis, phytoplankton, periphyton, aquatic macroinvertebrates, and adult odonates species were collected. The Westlake River, Silverhurst Impoundment and Kogmanskloof River system were sampled during March 2017, whereas the Rosle Farm Reservoir was sampled in November 2017 (Figure 3.2, A-D). Following phase 1 sampling, ~1000 adult *C. salviniae* weevils from the Center for Biological Control and the South African Sugar Cane Research Institution: Biological control Mass Rearing Facilities, were released per site, and thereafter quarterly site monitoring and further *C. salviniae* weevil inoculation were completed every three months over a period of 12 months. Thereafter if no signs of weevil establishment and feeding were observed, more weevils were released as it was the case for the Rosle Farm Reservoir. Unfortunately, two months after phase 1 and weevil inoculation, on the Westlake River and the Silverhurst Impoundment, *S. molesta* was mechanically removed by the City of Cape Town, as part of the City’s zero tolerance approach to invasive alien aquatic weeds. Westlake River and the Silverhurst Impoundment were therefore assigned to a “before-after” mechanical control treatment (Figure 3.2, E-F). Comparatively, successful biological control was reported after 14 months at the Kogmanskloof River with 8850 weevils released, and 16 months at the Rosle Farm Reservoir with 10500 weevils released (Figure 3.2, G-H). Therefore, the Kogmanskloof River and Rosle Farm Reservoir were assigned to the “before-after” biological control treatment. As mechanical control is a quicker process (although often less successful long-term) than control via biological control agents, both the Westlake River and Silverhurst Impoundment were further monitored following mechanical control throughout the experimental time-line (24 months, Figure 3.3). Surprisingly, the Westlake River showed a series of macrophyte succession following *S. molesta* removal. Thus, for each ecological state observed (i.e. free-floating *S. molesta* state, to clear water state, submerged *Ceratophyllum demersum* state and the emergent *Nymphaea mexicana* state: Figure 3.3), consistent water chemistry, aquatic microalgae and macroinvertebrates biodiversity survey were conducted, and the data is presented in the next chapter (Chapter 4). The Silverhurst Impoundment on the other hand suffered a drought 8 months following *S. molesta* removal (Figure 3.3). This was a regional phenomenon where dams and wetlands within the Cape Floristic Region dried out for almost a year due to below average rainfall during the previous two years. Seven months later, the impoundment re-filled

and was invaded by a rooted emergent IAAP species, *M. aquaticum*, and in less than six months, the impoundment was dry once again. Following successful *S. molesta* control, phase 2, “after” *S. molesta* control began, where biodiversity and trophic ecology data-sets were consistently collected and compared with the “before” *S. molesta* sampling phase (Figure 3.3).

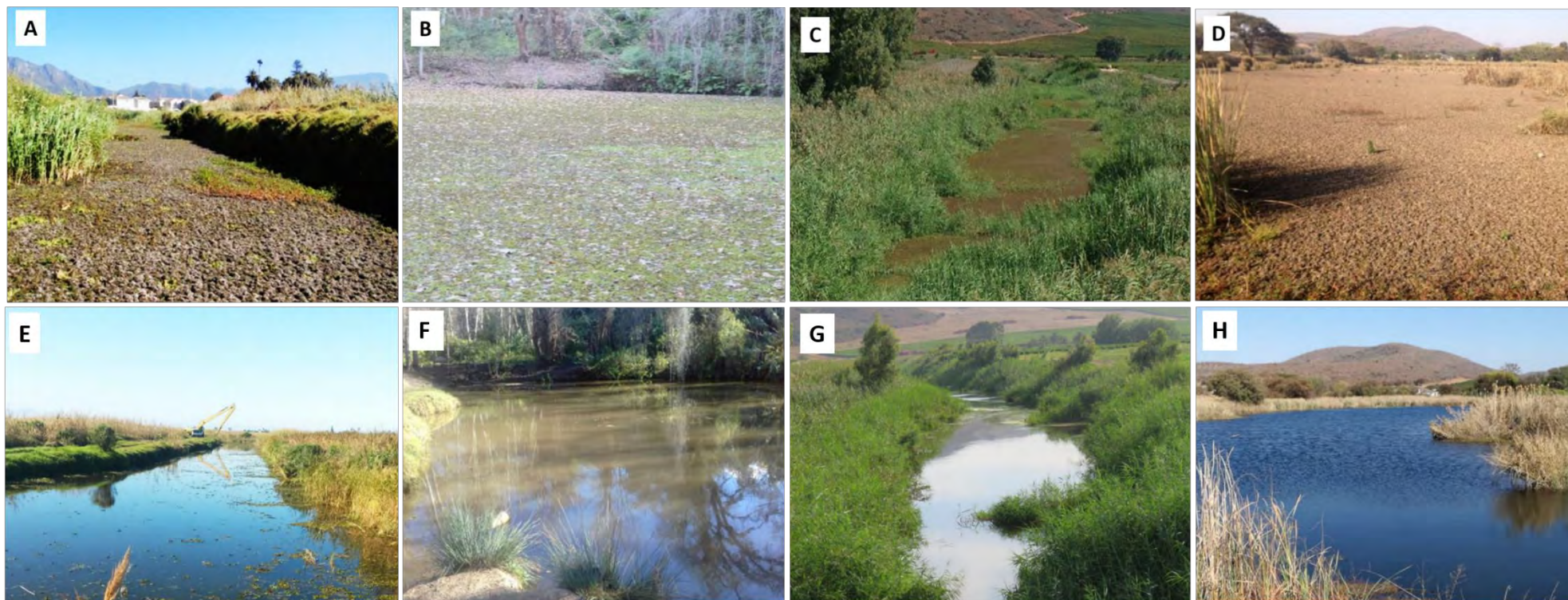


Figure 3.2: Photographs of four study sites infested by *Salvinia molesta* taken “before” control at (A) Westlake River near Lakeside in March 2017, (B) Silverhurst Impoundment near Constantia in March 2017, (C) Kogmanskloof River near Robertson in March 2017, and (D) Rosle Farm Reservoir near Groblersdal in November 2017; and “after” successful control of *S. molesta* at (E) Westlake River in May 2017, (F) the Silverhurst Impoundment in May 2017, (G) Kogmanskloof River in May 2018, and (H) the Rosle Farm Reservoir in April 2019, South Africa.

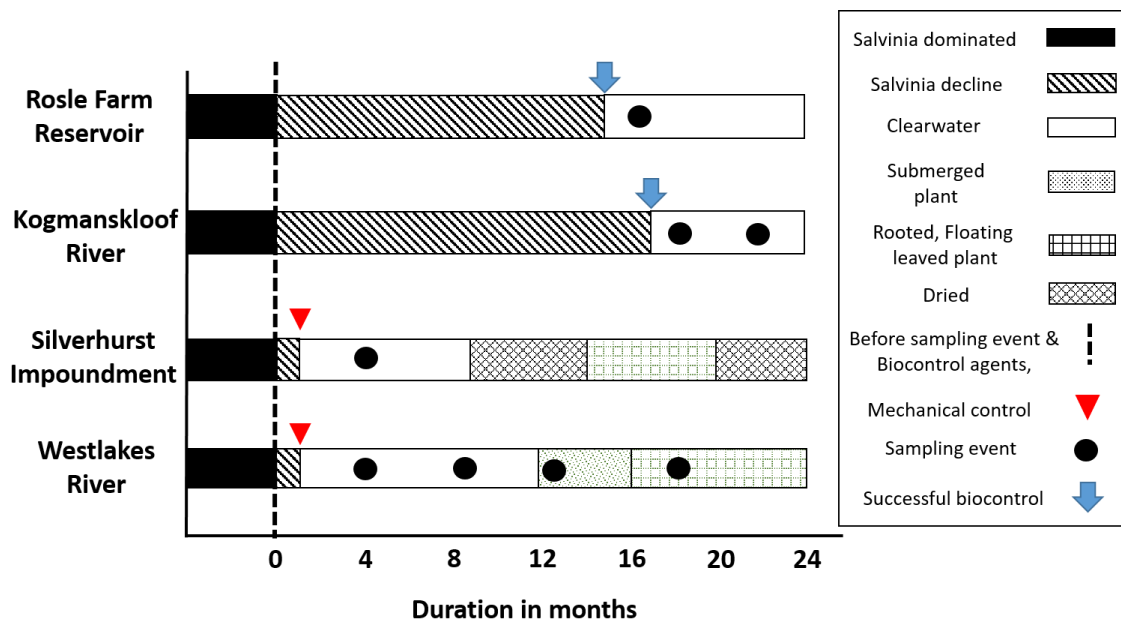


Figure 3.3: A Before-After experimental design time-line showing experimental events and ecological changes in four-field sites initially infested by *Salvinia molesta*. The assumption is that all sites were sampled at the same time, “before” *S. molesta* control.

3.2.3 Data collection

To ensure consistency in data collection during and between “before and after” events of *S. molesta* control, six randomly selected quadrats (2 m²), three in the littoral zone and three in the water column, were selected for sample collection. Quadrats were placed within a 50 meter infested river stretch, and distributed evenly (3-5 meters apart) between each other on both the Westlake and Kogmanskloof River systems. However, for the two infested standing water bodies i.e. Silverhurst Impoundment and Rosle Farm Reservoir, samples were collected from six permanently demarcated stations marked with floats. At each sampling event, physical and chemical parameters together with aquatic macroinvertebrates were collected from each station and only four stations (two in littoral and two in the water column) were sampled for phytoplankton and periphyton samples respectively and this was the case for the two river system.

3.2.3.1 Environmental variables

At each sampling event, physical and chemical water data were collected including water clarity (cm), water level (m), and photosynthetic active radiation (PAR; μMol) using a clarity tube (manufactured by GroundTruth®, South Africa), custom-made water depth measuring stick, and all-direction spherical underwater quantum sensor (LI-193) respectively. *Salvinia molesta* percentage cover was visually estimated, recording 100% *S. molesta* cover before control and 0% *S. molesta* cover following control. pH, conductivity (EC; $\mu\text{S/cm}$), total dissolved solids (TDS; ppm), salinity (ppm), water temperature ($^{\circ}\text{C}$) and dissolved oxygen (DO; mg/l) were recorded using Eutech multi-parameter testr 35 Series and DO Pen Sper-Scientific (850045) meters respectively. 500 ml water samples ($n=6$) were collected and taken to the laboratory to determine water nitrate [NO_3^-], ammonium [NH_4^+] and phosphate [PO_4^{3-}] as described in chapter 2 (page 25).

3.2.3.2 Biological data

Epiphyton

To compare aquatic microalgae assemblage composition and biodiversity indices “before and after” *S. molesta* control, epiphyton algae samples (microalgae found growing on aquatic macrophyte surfaces) were collected from a combination of free-floating (*S. molesta*, *P. stratiotes* roots), rooted floating (*N. mexicana* leaves), submerged (*C. demersum* and *Potamogeton* sp. leaves and stems) and emergent (*Cyperus* sp., *Typha* sp., *A. donax*, *M. aquaticum* and *N. mexicana* stems) macrophyte species. Epiphyton samples were collected following a modified procedure described in Taylor et al. (2007a), where microalgae growing on the roots, stems and leaves of macrophytes were completely scraped off, using a new toothbrush, onto a white collecting tray filled with approximately 2000 ml of filtered site water. This epiphyte sampling procedure was repeated three times to; (1) determine epiphyte algae species assemblages, (2) determine periphyton Chl-*a* concentrations, and (3) determine periphyton algae carbon and nitrogen isotopic values (see below for detailed methods).

Epiphytic algae samples for species assemblage analysis were divided into four subsamples ($n=4$, each 500 ml) and immediately preserved with 5 ml of Lugol’s iodine solution (prepared by dissolving 100 g Potassium Iodide and 50 g Iodine crystals in 2 litres of distilled water).

Further epiphyte assemblage analysis preparations, identification and enumeration followed the procedure described in Chapter 2 (page 26).

Phytoplankton and epiphyton Chl-*a* concentration

To estimate phytoplankton and periphyton Chl-*a* concentration as a proxy for ecosystem primary productivity “before and after” *S. molesta* control, four additional water sub-samples (n=4, 500 ml) were collected approximately 10–15 cm below the water surface at four different stations/quadrants per site. Together with four previously collected periphyton sub-samples (for Chl-*a* concentration determination), samples were used to estimate available ecosystem primary production, using Chl-*a* concentration as a proxy. Further, Chl-*a* samples preparation and determination followed the procedure described in chapter 2 (page 27).

Aquatic macroinvertebrates and adult Odonata species

To quantify aquatic macroinvertebrate biological and functional diversity together with aquatic ecosystem trophic interactions “before and after” *S. molesta* control, aquatic macroinvertebrates were collected using the kick sampling method described by Dickens and Graham (2002). A hand-held aquatic net (30 × 30 cm squared frame and 1 mm mesh size) was used to collect aquatic macroinvertebrate samples from six stations by vigorously disturbing and agitating all available habitats (marginal and riparian vegetation, gravel-mud and stones) at all six stations/quadrats collectively for a period of three minutes. Collected aquatic macroinvertebrates were transferred into a Ziploc™ bag and stored on ice until they reached the laboratory for sorting, counting and identification to family level using Day et al. (2002), Day and de Moor (2002), Gerber and Gabriel (2002a; b) and de Moor et al. (2003a; b) field guides and identification keys. Odonata are important biological indicators of habitat health and they are widely known of their active predatory behavior in aquatic ecosystems, thus they contribute to aquatic food webs dynamics and energy transfer to the terrestrial environment (Samways and Simaika, 2016; Chari et al., 2018). Adult Odonata species were collected using an active sweeping method around the riparian zone for 90 minutes (by two observers, each 45 minutes). Collected samples were preserved in an ethyl-acetate killing jar and taken to the laboratory for identification using relevant identification keys (e.g. Samways, 2008; Tarboton and Tarboton, 2015; Samways and Simaika, 2016).

3.2.3.3 Aquatic community trophic structure analysis

To investigate aquatic ecosystem trophic structure and function “before and after” *S. molesta* control, collected aquatic organisms were prepared for stable isotope analysis. A standard volume of 250 ml for phytoplankton and 100-150 ml for periphyton samples volume (n=3 each)

were vacuum-filtered through Whatman Glass microfibre filter (GFFs) papers (GFFs, 0.7 micron pore, 47 mm diameter), using a vacuum pump (Instruvac® Rocker 300) at 20 kilopascals (kPa). GFFs (n=6) were then immediately oven dried for 24 hours at 50 °C, and thereafter enclosed into an aluminium foil envelope.

Aquatic macroinvertebrate, adult Odonata species, freshwater fish (when present), dominant aquatic macrophytes, detritus and filamentous algae (if present) were all identified to the lowest taxonomic level possible (Skelton, 2001; Gerber et al., 2004; Griffiths et al., 2015). Three sub-samples of each taxa/species were transferred into an Eppendorf tube (2 ml) and also oven dried for 48 hours at 50 °C. Thereafter, samples were ground into a fine homogenous powder using a mortar and pestle. Three replicates of 0.5 – 0.6 mg ground animal tissues (i.e. aquatic invertebrates and vertebrates) and 1.0 - 1.2 mg of primary producers' tissues (macrophytes and filamentous algae) and detritus were weighed into separate aluminium tin capsules (8 × 5 mm).

All isotope samples, including phytoplankton and periphyton GFFs, aquatic invertebrates, fish and plants samples were then sent for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope analysis using a Flash EA 1112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (all equipment supplied by Thermo Fischer, Bremen, Germany), at the Stable Isotope Facility, University of Pretoria, South Africa. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values were reported as ‰ vs air, normalised to internal standards (Merck and DL-Valine) calibrated to the International Atomic Energy reference materials (IAEA-CH-3 and IAEA-CH-6 for $\delta^{13}\text{C}$, IAEA-N1 and IAEA-N2 for $\delta^{15}\text{N}$). Results were expressed in standard delta notation using per mil scale, $\delta X (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$, where $X = ^{15}\text{N}$ or ^{13}C , and R is the ratio of the heavy over the light isotope ($^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$), respectively. Average analytical precision for $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ was +0.08 and +0.07 respectively.

3.2.4 Data analysis

Environmental variables

To test for significant differences in physicochemical variables “before and after” *S. molesta* control, variables were firstly tested for normality and homogeneity of variances using the Shapiro-Wilks test and Levene's test respectively. All physicochemical variables were found to be not normally distributed (Shapiro-Wilks test, $P < 0.05$) and variances not homogeneous (Levene's test, $P > 0.05$), except for pH. Thus, a non-parametric Mann-Whitney U test (Wilcox

function) for paired data was employed to test for significance differences in physicochemical variables “before and after” *S. molesta* control.

Epiphytic algae and aquatic macroinvertebrate biological diversity indices

To investigate epiphytic algae and aquatic macroinvertebrate biological diversity recovery “before and after” *S. molesta* control, relative taxa abundance (N), taxa richness (S), Shannon diversity index, $H' = -\sum_{i=1}^S p_i \ln p_i$ (where p_i is the proportional abundance of taxa i in the sample given s taxa) and Pielou’s evenness, $J' = \frac{H'}{\ln(S)}$ indices were computed in PRIMER version 6.1.16 and PERMANOVA⁺ version 1.0.6 using the DIVERSE function (PRIMER-E Ltd, Plymouth: Clarke and Gorley, 2006). Epiphytic algae biodiversity indices were further tested for normality and homogeneity of variances, and in all cases, data was found to be normally distributed (Shapiro-Wilks test, $P>0.05$) and variances were homogenous (Levene’s test, $P<0.05$). Subsequent Student’s t-tests were employed to test for significance difference in epiphytic algae biodiversity indices “before and after” *S. molesta* control.

Additionally, to investigate functional diversity and community assemblage structure for epiphytic algae and aquatic macroinvertebrates (excluding adult Odonata data which were only used for trophic interactions), “before and after” *S. molesta* control, samples were categorised into algal phyla and invertebrate functional feeding groups (FFGs) respectively. Aquatic macroinvertebrate FFGs included collector-filters, collector-gatherers, scrapers/herbivores, shredders and predators, and were assigned according to Cummins and Klug (1979), Palmer et al. (1996), Merritt et al. (2008) and Hawking et al. (2013), respectively. Epiphytic algal phyla included Cyanophyta, Bacillariophyta, Euglenophyta, Dinophyta, Cryptophyta, Charophyta and Chlorophyta (John et al., 2002; Van Vuuren et al., 2006; Taylor et al., 2007b). To test for differences in FFGs and algal phyla composition (categorical data) “before and after” *S. molesta* control, a Chi-squared test was employed.

Epiphytic algae and aquatic macroinvertebrate community assemblage patterns

To illustrate significant shifts in biotic assemblages “before and after” *S. molesta* control, unconstrained ordination analysis was conducted. Epiphytic algae assemblage structure was assessed using a two-dimensional solution of Non-metric Multidimensional Scaling (NMDS) based on Bray-Curtis similarity coefficients. Differences in epiphytic algae community assemblages, “before vs. after” *S. molesta* control sampling periods were analysed by multi-response permutation procedures (MRPP), which are non-parametric methods for testing differences between 2 or more assemblage groups (Mielke et al., 1981). According to Mielke

et al. (1981), the statistic A in MRPP is a descriptor of within-group homogeneity compared to the random expectation, known as chance-corrected within-group agreement. When all items are identical within groups, then the observed delta = 0 and $A = 1$ (the highest possible value for A). If heterogeneity within groups equals expectation by chance, then $A = 0$. In comparison, if there is less agreement within groups than expected by chance, then $A < 0$. The null hypothesis of no difference in aquatic biota among sampling phases (e.g. before and after) was tested by a Monte Carlo procedure with 9999 permutations, $\alpha=0.05$. If MRPP returned a significant difference in assemblage groups between the “before and after” *S. molesta* sampling phases, indicator value analysis (*IndVal*; Dufrêne and Legendre, 1997) was used to identify epiphytic algae species that best discriminated among the two sampling phases (i.e. Before vs. After) per study sites. The indicator value of a species varies from 0 to 100, attaining its maximum value when all individuals of a species occur at all sites of a single group (González et al., 2013). The significance of the indicator value for each species was tested by a Monte Carlo randomisation test with 9999 permutations, $\alpha=0.05$. Both the MRPP and *IndVal* were carried out in PC-ORD version 5.10 (McCune and Mefford, 2006). MRPP used the Sorensen (Bray-Curtis) as a measure of distance and the weighting of the groups using $n/\text{sum}(n)$. Comparatively, unconstrained PCA ordination was used to demonstrate and visualise aquatic macroinvertebrate assemblage structure “before and after” *S. molesta* control between sites in PRIMER version 6.1.16 and PERMANOVA⁺ version 1.0.6 (PRIMER-E Ltd, Plymouth: Clarke and Gorley, 2006).

Ecosystem food webs

Long-lived aquatic invertebrate taxa, preferably primary consumers such as mussels and molluscs are generally used to baseline nitrogen and carbon isotopes values, thus making them comparable over time and space (Cabana and Rasmussen, 1996; Vander Zanden et al., 1997; Post 2002). Since these organisms feed mainly on plankton and detritus, their nitrogen and carbon ratios are time integrated, representing wider fluxes of primary productivity and are thus good indicators for trophic function over time (Cabana and Rasmussen, 1996; Post, 2002). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope values of all organisms collected “before and after” *S. molesta* control (Table S7) at each site were adjusted using baseline values based on the following equations:

$$(1) \text{ Trophic position} = \frac{\delta^{15}\text{N}_{\text{organism}} - \delta^{15}\text{N}_{\text{baseline}}}{\Delta^{15}\text{N}} + 2,$$

where 2 represents the trophic position of the baseline organism, $\delta^{15}\text{N}$ represents the fractionation factor calculated as 3.23‰ (Post, 2002), $\delta^{15}\text{N}_{organism}$ is the isotope ratio of the organism in question, $\delta^{15}\text{N}_{baseline}$ is the isotope ratio of the primary consumers used for the baseline (Post, 2002), and

$$(2) \quad \delta^{13}\text{C}_{corrected} = \frac{\delta^{13}\text{C}_{organism} - \delta^{13}\text{C}_{baseline}}{CR_{baseline}},$$

where $\delta^{13}\text{C}_{corrected}$ is the corrected carbon isotope ratio of the consumer, $\delta^{13}\text{C}_{organism}$ is the uncorrected isotope ratio of the organism in question, $\delta^{13}\text{C}_{baseline}$ is the mean primary consumer isotope ratio, and $CR_{baseline}$ is the primary consumer carbon range ($\delta^{13}\text{C}_{max} - \delta^{13}\text{C}_{min}$) (Olsson et al., 2009; Jackson and Britton, 2014).

Before and After ecosystem community trophic structure

To investigate changes in trophic food web structure, i.e. reduction in ecosystem basal resources, trophic diversity, trophic niche size and food chain length “before and after” *S. molesta* control per site, corrected $\delta^{15}\text{N}$ (trophic position) and $\delta^{13}\text{C}$ (corrected Carbon) values were investigated using the Stable Isotope Bayesian Ellipses (SIBER) package (Jackson et al., 2011) in R version 3.6.1 (R Development Core Team, 2016). Layman’s metrics (Layman et al., 2007) were used to describe trophic food web characteristics and included nitrogen range (NR_b), which describes trophic length (or food web length); carbon range (CR_b), which represents basal resources diversity; mean distance to the centroid (CD_b), which indicates trophic diversity and species spacing; mean nearest neighbour distance (MNND_b), which estimates density and clustering of species within the community and trophic redundancy; the standard deviation of nearest neighbour distance (SDNND_b), measuring evenness of spatial density and packing of species in the isotopic space, and standard ellipse area (SEAc), which provides a bivariate measure of mean core isotopic niche (Layman et al., 2007; Jackson et al., 2011). The calculation of SEAc further allows for a measure of niche overlap (%; with a maximum of 100% indicating complete overlap between trophic food web niche), which can then be used as a quantitative measure of trophic food web niche similarity between phases/ecological scenarios following Jackson et al. (2012) and Jackson and Britton (2014). Stable isotope ratios from all individual samples were used to estimate trophic interactions and changes “before and after” *S. molesta* control. However due to differences in samples sizes between the “before and after” sampling phases and for comparison purposes, all metrics were

bootstrapped (N = 10 000, indicated with a subscript ‘b’) as seen in Jackson et al. (2012), Jackson and Britton (2014), Hill et al. (2015) and Taylor et al. (2017).

All statistical tests except where specified were conducted in R version 3.6.1 (R Development Core Team, 2016).

3.3 Results

Environmental variables

Salvinia molesta percentage cover and EC were significantly different between the “before and after” *S. molesta* control at all study sites (Table 3.1). Water level, $[\text{NO}_3^-]$, $[\text{PO}_4^{3-}]$ and periphyton Chl-*a* concentration were only significantly different “before and after” the biological control of *S. molesta* at the Rosle Farm Reservoir. PAR was the only variable that varied significantly “before and after” *S. molesta* control in Kogmanskloof River, whereas water clarity was significantly different between “before and after” *S. molesta* control in the Rosle Farm Reservoir and Westlake River system. pH was significantly different “before and after” *S. molesta* control at Westlake River and the Rosle Farm Reservoir; water temperature in Westlake River, Silverhurst Impoundment, and Rosle Farm Reservoir, and $[\text{DO}]$ in the Silverhurst Impoundment, Kogmanskloof River and the Rosle Farm Reservoir. $[\text{NH}_4^+]$ was different “before and after” *S. molesta* control at the Silverhurst Impoundment and phytoplankton Chl-*a* concentration different at Westlake River and Rosle Farm Reservoir “before and after” *S. molesta* control (Table 3.1).

pH, EC, water clarity, PAR, $[\text{NO}_3^-]$, $[\text{PO}_4^{3-}]$, phytoplankton Chl-*a* concentration and *S. molesta* percentage cover were eight physicochemical variables that showed strong Pearson’s correlation ($r > 0.7$), and contributed to sites and treatment differences throughout the study (Figure 3.5). The PCA axes (PC1 and PC2) represented 58.0% total variation in physicochemical variables (in Euclidean distance) between sites and “before and after” *S. molesta* control treatments. There were clear and distinct dissimilarity patterns between sites and treatments “before” as compared to “after” *S. molesta* control (Figure 3.4). The Kogmanskloof River, Westlake River and the Rosle Farm Reservoir were significantly different from the Silverhurst Impoundment in water quality characteristics, with the Westlake River and Rosle Farm Reservoir sharing similar variables i.e. phytoplankton Chl-*a* concentration and *S. molesta* percentage cover. Kogmanskloof River showed high levels of conductivity and phytoplankton Chl-*a* concentration whereas the Silverhurst Impoundment had a higher water level compared to the Rosle Farm Reservoir and the Westlake River “before” *S.*

molesta control. “after” successful *S. molesta* control, there was a shift in physicochemical properties between sites and treatments as expected. Where the Rosle Farm Reservoir, the Silverhurst Impoundment and the Westlake River clustered together, indicative of shared and/or similarities in physicochemical characteristics. The sites showed a strong negative correlation to *S. molesta* percentage cover, conductivity, phytoplankton Chl-*a* concentration and a strong positive correlation to water clarity and PAR. In contrast, the Kogmanskloof River “before and after” treatments clustered separately, where the “before” *S. molesta* control phase was positively correlated to conductivity and following *S. molesta* control the site was more nutrient rich, with high levels of $[\text{PO}_4^{3-}]$, $[\text{NO}_3^-]$, pH and water clarity (Figure 3.4).

Table 3.1: Mean ($\pm$ standard deviation) of collected physicochemical variables from the four field sites, each sampled “before and after” *Salvinia molesta* control. Mann-Whitney U tests were used to test for significant differences in variables “before and after” *S. molesta* mechanical removal (Westlake River and Silverhurst Impoundment) and biological control (Kogmanskloof River and Rosle Farm Reservoir). Bold values indicate significance differences $P < 0.05$. “Cover” refers to *Salvinia molesta* percentage cover. *W*, is the Wilcoxon test statistic.

Environmental variables	Westlake River			Silverhurst Impoundment			Kogmanskloof River			Rosle Farm Reservoir		
	Before	After	<i>W</i>	Before	After	<i>W</i>	Before	After	<i>W</i>	Before	After	<i>W</i>
Cover (%)	100	0	0	100	0	0	100	0	0	100	0	0
Water depth (cm)	45 (10)	41 (15.3)	11	88.3 (26.4)	75.5 (31.1)	5	151.6 (19.4)	137.5 (27.1)	6	180.8 (82.7)	198.7 (100.4)	16.5
PAR (μ Mol)	0	989.4 (605.1)	21	288.6 (382.6)	1070.4 (1289.2)	15	0	167.6 (69.9)	21	0	40.1 (9.8)	21
Water clarity (cm)	15.8 (6.6)	58.7 (19.9)	21	43.2 (17.6)	50 (11.3)	15	27.3 (8.4)	87.3 (21.9)	21	9.1 (1.7)	72.8 (12.1)	21
pH	7.3 (0.11)	6.9 (0.5)	0	7.17 (0.3)	7.3 (0.1)	17	8.0 (0.3)	8.1 (0.1)	13	7.02 (0.3)	7.6 (0.1)	21
EC (μ S/cm)	945.5 (110.7)	595.8 (10.5)	0	476.5 (8.2)	449 (3.5)	0	3800 (100)	2400 (10)	0	874.5 (57.8)	672.5 (11.3)	0
Water temperature ($^{\circ}$ C)	19.8 (0.96)	23.3 (0.65)	21	12.9 (0.4)	18.3 (0.7)	21	23.8 (1.6)	22.7 (0.4)	3	27 (1.1)	22.9 (1.0)	0
DO (mg/l)	6.0 (1.4)	4.2 (1.2)	3	10.6 (1.6)	4.6 (0.4)	0	5.2 (1.7)	3.4 (0.2)	0	2.2 (0.5)	4.1 (0.5)	21
NO ₃ ⁻ (mg/l)	3.7 (2.3)	7.2 (1.6)	20	10.6 (2.9)	5.8 (1.7)	1	23.2 (5.7)	21.1 (2.6)	9	2.4 (1.5)	2.4 (0.6)	10
NH ₄ ⁺ (mg/l)	0	0	NA	0.13 (0.2)	0	0	0	0.02 (0.04)	1	0	0	NA
PO ₄ ³⁻ (mg/l)	0.9 (0.4)	1.1 (0.6)	11.5	0.7 (0.2)	0.7 (0.6)	9	1.3 (0.6)	1.6 (0.3)	16	0.08 (0.2)	1.3 (0.3)	21
Periphyton Chl- <i>a</i> (mg/m ³)	120.5 (89.4)	132.5 (134)	11	42.7 (18.2)	39.2 (28.5)	9	82.7 (20.8)	44.7 (40.7)	1	29.5 (10.8)	72.1 (42.6)	18
Phytoplankton Chl- <i>a</i> (mg/m ³)	59.7 (26.4)	2.9 (1.4)	0	1.8 (2.1)	3.1 (2.0)	16	43.3 (30.2)	3.6 (4.2)	1	15.6 (5.4)	3.2 (2.1)	0

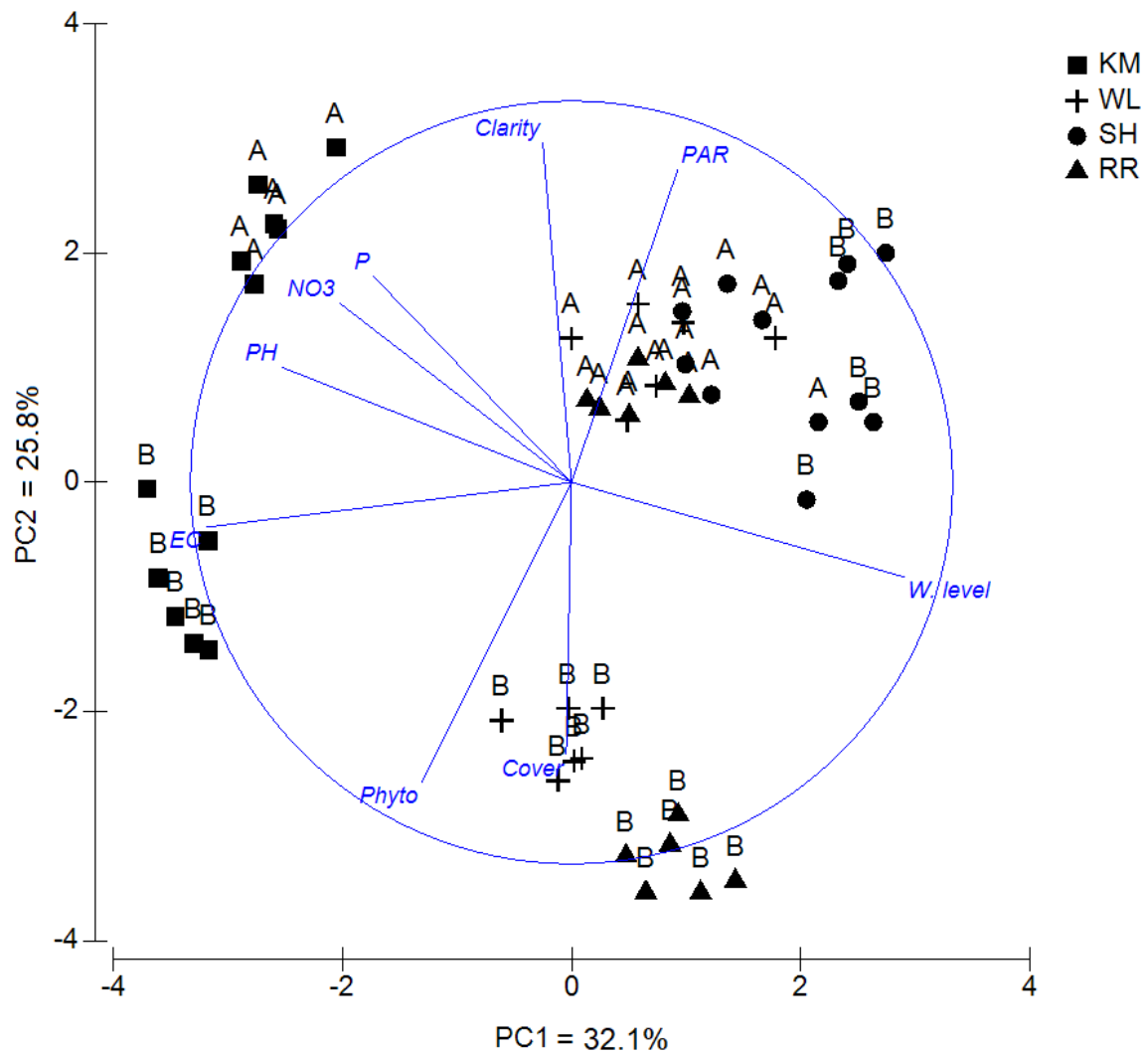


Figure 3.4: Principal Component Analysis (PCA) of physicochemical variables collected for each study site, “before” (B) and “after” (A) *Salvinia molesta* mechanical (WL & SH) and biological control (KM & RR). Only variables that showed a very strong correlation (Pearson’s correlation, $r > 0.7$) are highlighted on the plot. WL – Westlake River, SH – Silverhurst Impoundment, KM – Kogmanskloof River and RR – Rosle Farm Reservoir.

Epiphytic algae and aquatic macroinvertebrate biodiversity patterns and assemblage structure

Relative epiphytic algae abundance was not significantly different “before and after” *S. molesta* control at Westlake River ($t=0.05$, $df=3$, $P=0.96$), the Silverhurst Impoundment ($t=-0.68$, $df=3$, $P=0.54$) and at Kogmanskloof River ($t=0.49$, $df=3$, $P=0.66$), but was significantly different at the Rosle Farm Reservoir ($t=4.14$, $df=3$, $P=0.03$). Although not significant, Kogmanskloof River had the highest relative epiphytic algae abundance, followed by the Westlake River and the Silverhurst Impoundment with the least abundances during both “before and after” *S. molesta* control. Comparatively, the Rosle Farm Reservoir epiphytic algae relative abundance

was lowest during *S. molesta* infestation (“before” *S. molesta* control) and following *S. molesta* biological control, epiphytic algae relative abundance increased significantly (Figure 3.5A).

Epiphytic algae taxa richness on the other hand was significantly different at Westlake River ($t=3.25$, $df=3$, $P=0.04$), the Rosle Farm Reservoir ($t=4.14$, $df=3$, $P=0.02$), and not significantly different at the Silverhurst Impoundment ($t=1.93$, $df=3$, $P=0.15$) and the Kogmanskloof River ($t=2.20$, $df=3$, $P=0.12$), “before and after” *S. molesta* control. Following *S. molesta* control, Westlake River and the Rosle Farm Reservoir showed increased epiphytic algae taxa richness, followed by Kogmanskloof River and the Silverhurst Impoundment (Figure 3.5B). Unlike epiphytic taxa richness, Pielous’ evenness was significantly different “before and after” *S. molesta* control at the Silverhurst Impoundment ($t=3.22$, $df=3$, $P=0.04$) and Kogmanskloof River ($t=-3.26$, $df=3$, $P=0.04$), and not different in the Westlake River ($t=2.43$, $df=3$, $P=0.09$) and the Rosle Farm Reservoir ($t=2.25$, $df=3$, $P=0.11$). The Silverhurst Impoundment and Westlake River showed high epiphytic algae evenness, followed by the Rosle Farm Reservoir and the Kogmanskloof River “After” *S. molesta* control (Figure 3.5C). Epiphytic algae diversity (Shannon diversity index) was significantly different in the Westlake River ($t=4.18$, $df=3$, $P=0.03$), the Silverhurst Impoundment ($t=5.20$, $df=3$, $P=0.01$), and the Rosle Farm Reservoir ($t=4.69$, $df=3$, $P=0.02$), but was not different in the Kogmanskloof River ($t=-0.60$, $df=3$, $P=0.59$), “before and after” *S. molesta* control. A high Shannon diversity index was recorded in the Westlake River, followed by the Silverhurst Impoundment, the Rosle Farm Reservoir and the least in the Kogmanskloof River site “after” *S. molesta* control (Figure 3.5D). See supplementary information (Table S4) for epiphytic algal species list and relative abundances per site.

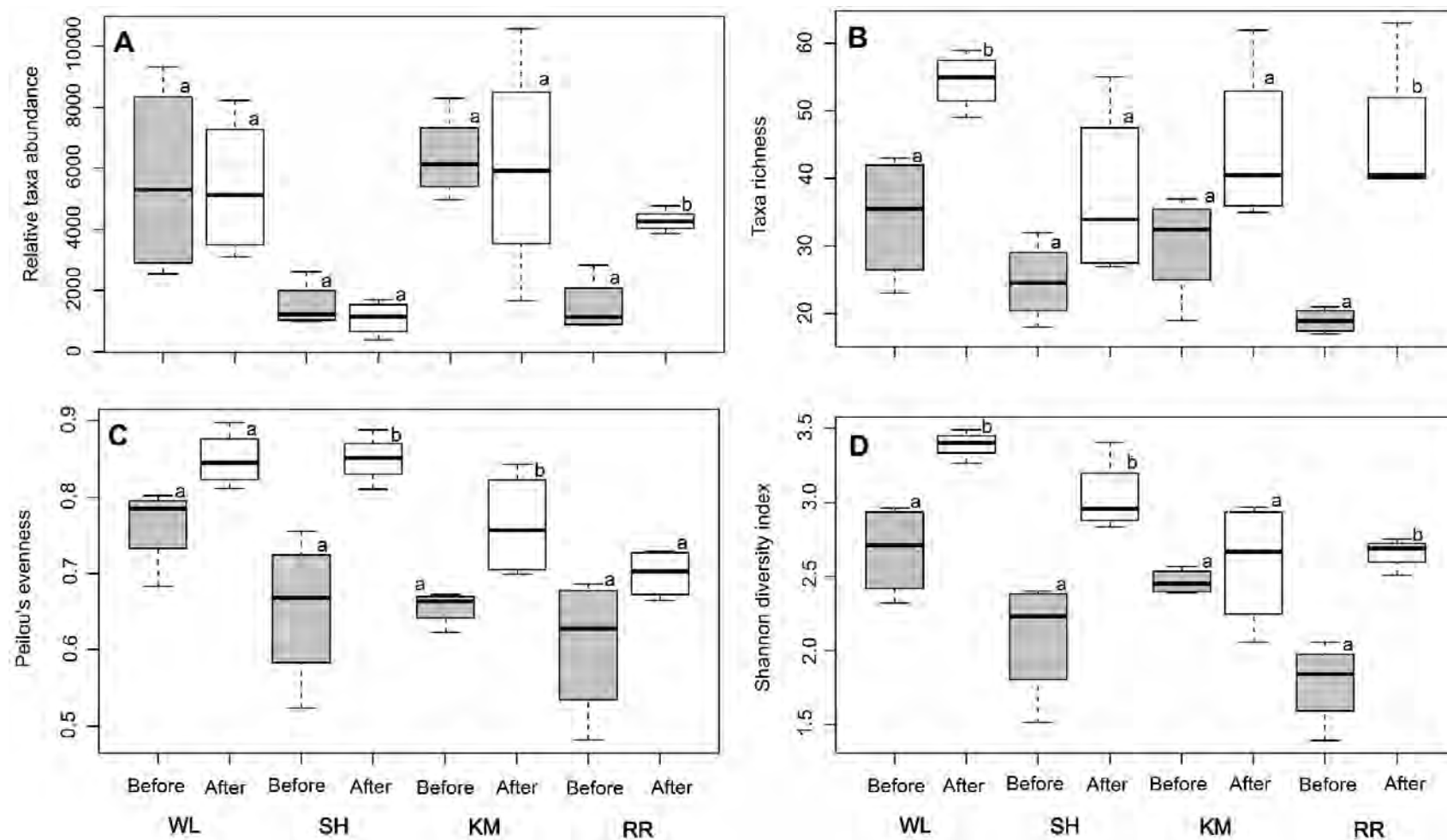


Figure 3.5: Epiphytic algae biodiversity indices; (A) relative taxa abundance, (B) taxa richness, (C) Pielou's evenness and (D) Shannon diversity indices from the four study sites "before and after" *Salvinia molesta* mechanical (WL & SH) and biological (KM & RR) control. Different lowercase letters denote homogenous groups (t -test, $P < 0.05$) "before and after" control. WL – Westlake River, SH – Silverhurst Impoundment, KM – Kogmanskloof River and RR – Rosle Farm Reservoir.

Epiphytic algae assemblage composition was significantly different between study sites and treatments (Cyanophyta: $\chi^2=596.76$, $df=63$, $P<0.01$; Bacillariophyta: $\chi^2=2314.6$, $df=63$, $P<0.01$; Cryptophyta: $\chi^2=205.83$, $df=63$, $P<0.01$; Dinophyta: $\chi^2=215.11$, $df=63$, $P<0.01$; Euglenophyta: $\chi^2=175.01$, $df=63$, $P<0.01$; Chlorophyta: $\chi^2=725.91$, $df=63$, $P<0.01$; Charophyta: $\chi^2=215.52$, $df=63$, $P<0.01$) except for Cryptophyta ($\chi^2=37.33$, $df=31$, $P>0.01$). Bacillariophyta was the common epiphytic algae group among sites and treatments throughout the study, with percentage contribution of more than 50% during each sampling phase. In terms of relative percentage composition, Bacillariophyta was followed by Cyanophyta, Chlorophyta, Euglenophyta and Cryptophyta (Figure 3.6). Dinophyta and Charophyta were the least abundant epiphytic algae groups. During the study, Bacillariophyta, Cyanophyta, Chlorophyta and Cryptophyta were the dominant groups “before” *S. molesta* biological control at Kogmanskloof River. However, following *S. molesta* biological control, Cryptophyta was absent, and both Cyanophyta and Chlorophyta percentage composition was reduced significantly with Bacillariophyta being the dominant group. The Silverhurst Impoundment “before and after” *S. molesta* mechanical control showed similar Bacillariophyta percentages, followed by Cyanophyta, with Euglenophyta only present “after” *S. molesta* control. In comparison to the Westlake River Bacillariophyta, Cyanophyta, Chlorophyta and Euglenophyta groups were present “before” *S. molesta* mechanical control, and “after” *S. molesta* control, while there was no change in Cyanophyta percentage, there was an increase in Chlorophyta and a reduction for Euglenophyta percentage abundance. Only the Rosle Farm Reservoir showed a consistent epiphytic algae assemblage composition with only small differences in percentages “before and after” *S. molesta* biological control. Chlorophyta percentage was similar “before and after”, whereas Bacillariophyta increased by ~10%, and Euglenophyta and Cyanophyta recording approximately 50% reduction “after” *S. molesta* biological control (Figure 3.6).

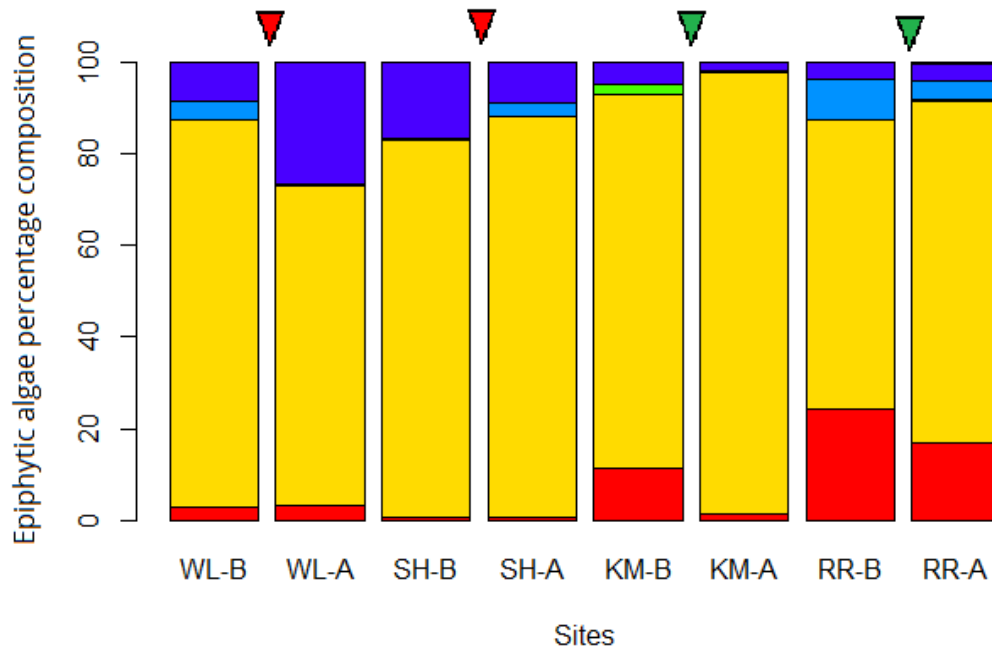


Figure 3.6: Epiphytic algae assemblage percentage composition between four study sites sampled, “before” (B) and “after” (A) *Salvinia molesta* mechanical (WL & SH) and biological (KM & RR) control, where; WL-Westlake River, SH-Silverhurst Impoundment, KM-Kogmansklouf River and RR-Rosle Farm Reservoir. Cyanophyta (Red), Bacillariophyta (Yellow), Euglenophyta (Light-blue), Chlorophyta (Blue), Cryptophyta (Green). Red and green arrows indicate mechanical and biological control methods respectively.

“Before” *S. molesta* control, aquatic macroinvertebrates were only recorded at Kogmansklouf River, whereas Westlake River, the Silverhurst Impoundment and Rosle Farm Reservoir sites had no aquatic macroinvertebrates during *S. molesta* infestation (Figure 3.7A). Aquatic macroinvertebrate taxa abundance, taxa richness, Pielou’s evenness and Shannon diversity index were low during *S. molesta* infestation (“before” *S. molesta* control phase) at Kogmansklouf River, however increased substantially “after” *S. molesta* biological control (Figure 3.7). The recovery of aquatic macroinvertebrates was seen 30 days following *S. molesta* mechanical control at Westlake River and Silverhurst Impoundment and only after 12 months following *S. molesta* biological control at Kogmansklouf River and Rosle Farm Reservoir. “after” *S. molesta* control, the Rosle Farm Reservoir showed increased relative aquatic macroinvertebrate abundance, followed by Westlake River, Kogmansklouf River and the Silverhurst Impoundment (Figure 3.7A). Taxa richness was high at Westlake River, followed by the Rosle Farm Reservoir, Kogmansklouf River, with the Silverhurst Impoundment having the lowest taxa richness (Figure 3.7B). Pielou’s evenness was high at the Rosle Farm Reservoir, followed by Kogmansklouf River, Westlake River and the lowest at the Silverhurst

Impoundment (Figure 3.7C). This trend was also consistent for the Shannon diversity index, where the Rosle Farm Reservoir had the highest diversity, followed by Kogmanskloof River, Westlake River, and the Silverhurst Impoundment, which was the least diverse site “after” *S. molesta* control (Figure 3.7D). See supplementary material (Table S5) for aquatic macroinvertebrate taxa list and relative abundances per site.

Aquatic macroinvertebrates were absent during the “before” *S. molesta* control phase, except in the Kogmanskloof River, but aquatic macroinvertebrate functional feeding groups were different between study sites following *S. molesta* control. Kogmanskloof River was the only site to have macroinvertebrate communities present “before” *S. molesta* control, where scrapers/herbivores were the most abundant group, followed by predators, and collector-filter feeders being the least. However, following *S. molesta* biological control, predators were more abundant followed by collector-filter feeders, scrapers/herbivores and shredders respectively (Figure 3.8). Following *S. molesta* mechanical control at Westlake River and the Silverhurst Impoundment, collector-filter feeders were the dominant aquatic macroinvertebrate FFG, followed by scrapers/herbivores, predators and shredders at Westlake River. At Silverhurst Impoundment collector-filter feeders were the dominant FFG >60%, followed by scrapers/herbivores ~20% with predators being the least dominant. The Rosle Farm Reservoir showed equivalent FFG composition where predators were slightly more abundant than collector-filter feeders, followed by scrapers/herbivores (>20%) and then collector-gatherers with ≤5% contribution (Figure 3.8).

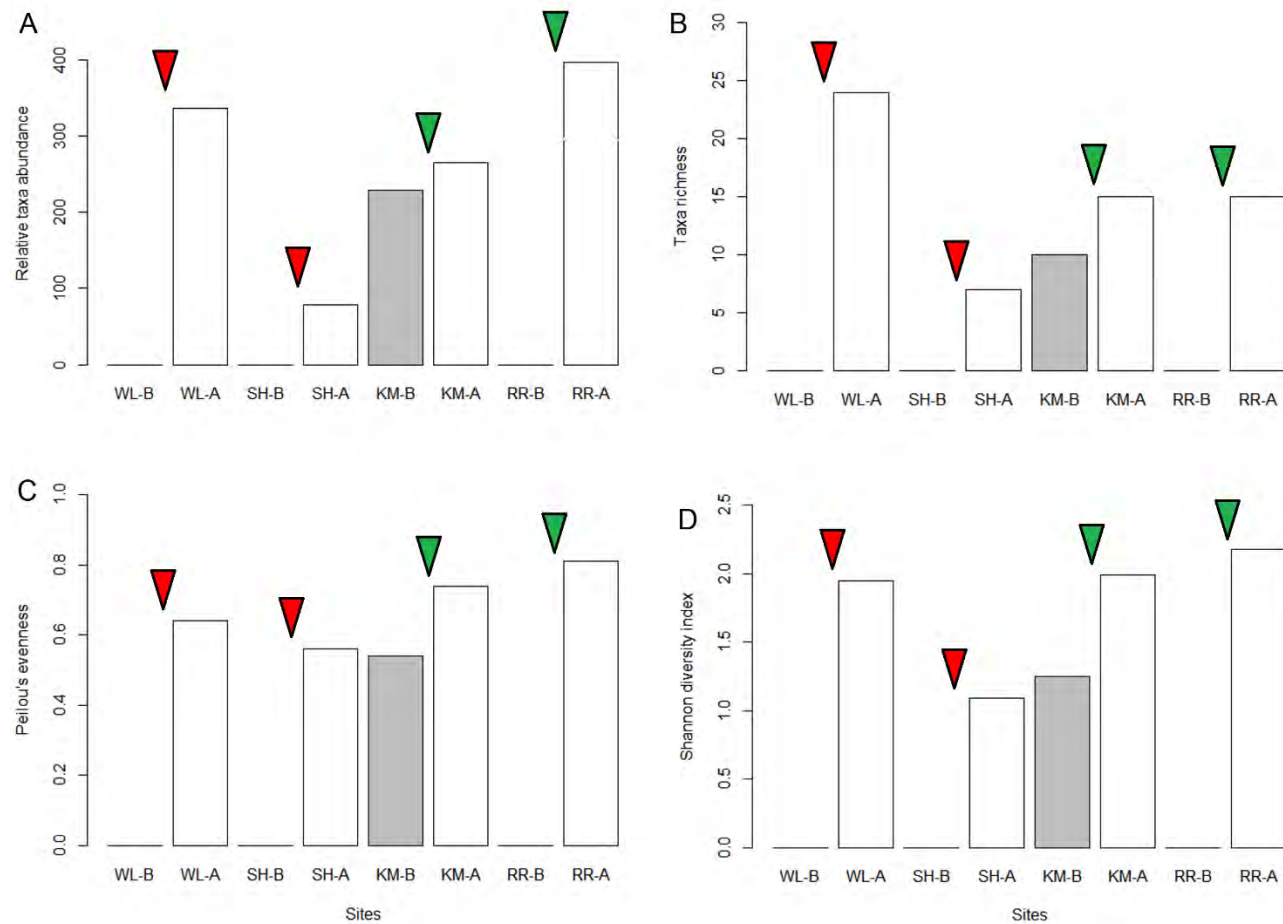


Figure 3.7: Aquatic macroinvertebrate biodiversity indices (A) relative taxa abundance, (B) taxa richness, (C) Pielou's evenness and (D) Shannon diversity index from four selected field sites sampled, "before" (B, grey bar) and "after" (A, white bar) *Salvinia molesta* mechanical (WL & SH) and biological (KM & RR) control. Where; WL-Westlake River, SH-Silverhurst Impoundment, KM-Kogmanskloof River and RR-Rosle Farm Reservoir. Red and green arrows indicate mechanical and biological control methods respectively.

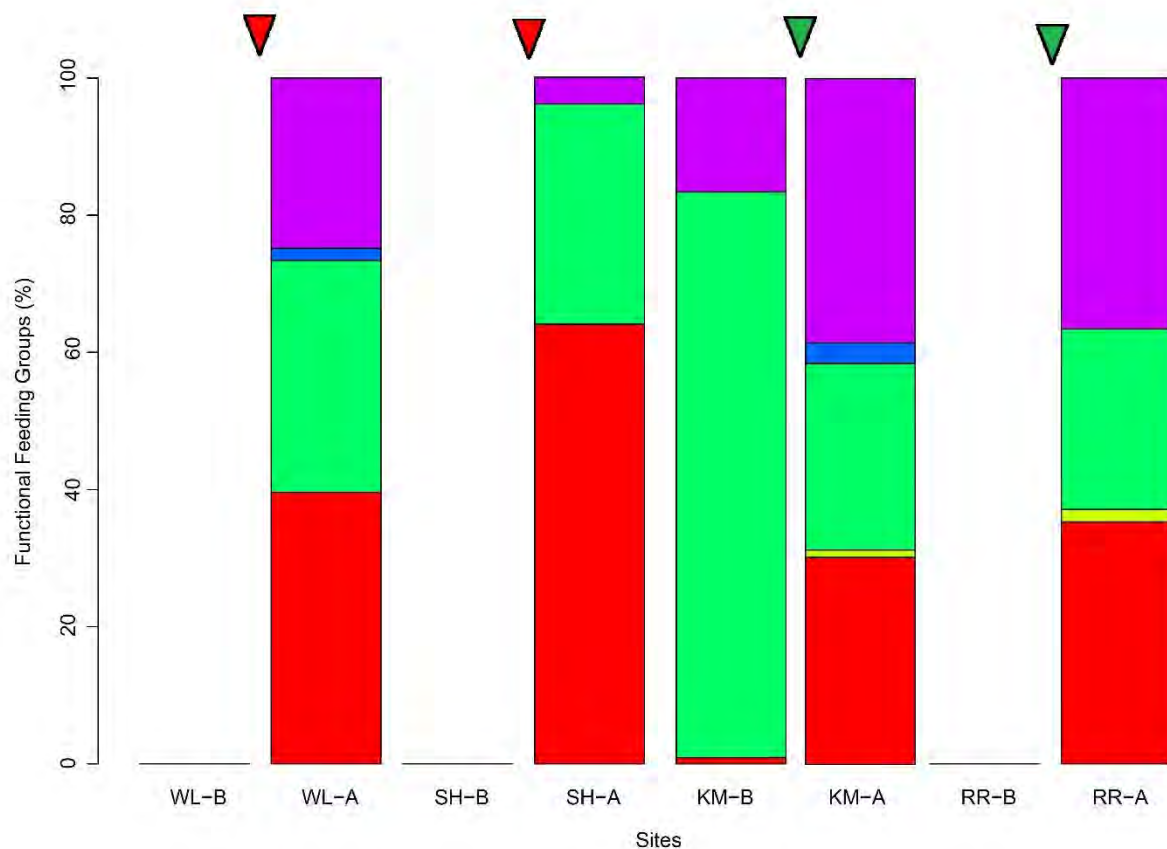


Figure 3.8: Aquatic macroinvertebrate Functional Feeding Groups (FFGs) percentage composition at four study sites, “before” (B) and “after” (A) *Salvinia molesta* mechanical (WL & SH) and biological (KM & RR) control. Collector-filters (Red), Collector-gatherers (Yellow), Scrapers/herbivores (Green), Shredders (Blue) and Predators (Purple). WL-Westlake River, SH-Silverhurst Impoundment, KM-Kogmansklouf River and RR-Rosle Farm Reservoir. Red and green arrows indicate mechanical and biological control methods respectively.

Epiphytic algae community structure at each site showed two distinct clusters in the NMDS ordination, where each cluster represented different epiphytic algae community composition forms “before and after” *S. molesta* control (Figure 3.9). When examined further, MRPP analysis returned a significant difference in epiphytic algae community assemblages between the “before and after” *S. molesta* control phases, at all study sites; Westlake River ($A=0.412$, $P<0.01$), the Silverhurst Impoundment ($A=0.366$, $P<0.01$), Kogmansklouf River ($A=0.356$, $P<0.01$) and Rosle Farm Reservoir ($A=0.444$, $P<0.01$) (Figure 3.9A - D). Indicator values species analysis identified five significant indicator species for Westlake River “before” *S. molesta* control and twelve indicator species “after” *S. molesta* control phase (Table 3.2). Five epiphytic algae indicator species were significantly associated with the Silverhurst Impoundment during *S. molesta* infestation, and following mechanical control, ten different

species were associated with the clear water state (Table 3.2). For both Kogmanskloof River and the Rosle Farm Reservoir, four species were fully (*IndVal* = 100%) associated with *S. molesta* infestation (“before” control), thirteen and nineteen epiphytic algae indicator species “after” *S. molesta* biological control were associated with Kogmanskloof River and the Rosle Farm Reservoir respectively (Table 3.3).

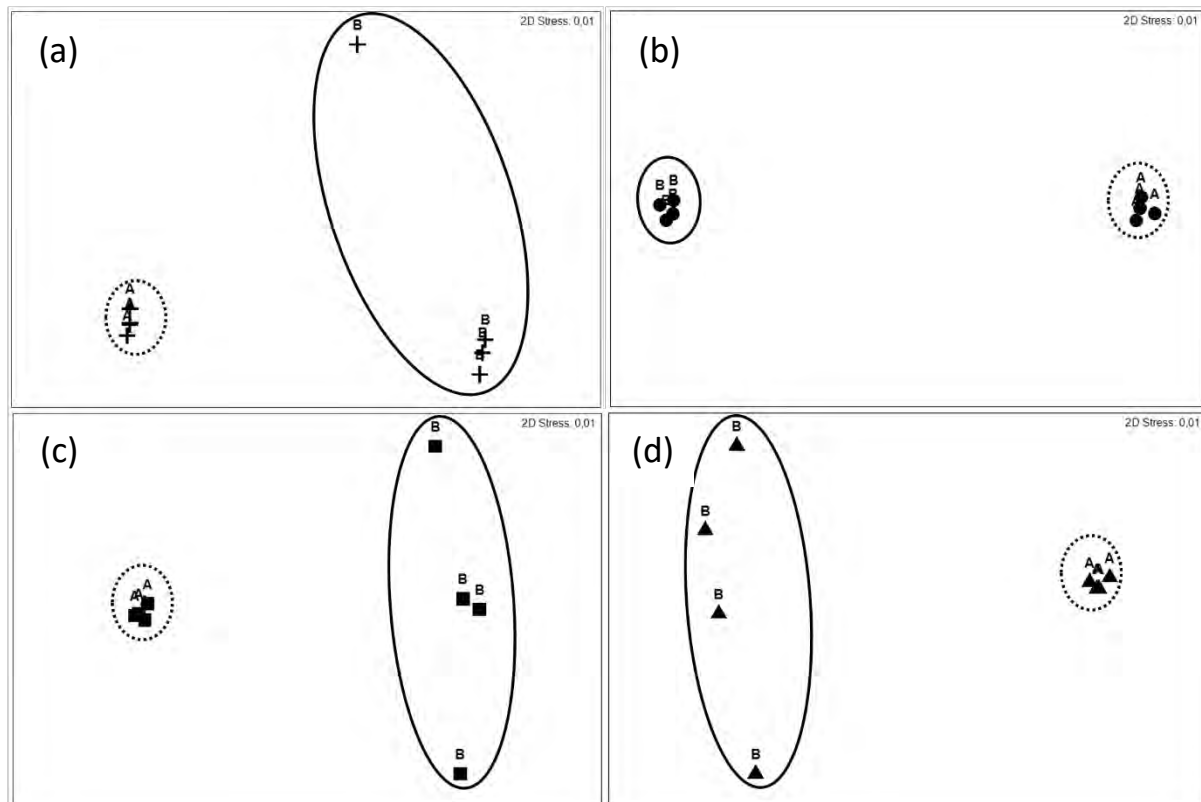


Figure 3.9: Non-metric multidimensional scaling (NMDS) ordination of epiphytic algae community structure at four selected field sites, “before” (B, solid line) and “after” (A, dotted line) mechanical control of *Salvinia molesta* at (a) Westlake River, (b) Silverhurst Impoundment, and biological control of *S. molesta* at (c) Kogmanskloof River, (d) the Rosle Farm Reservoir, South Africa.

Table 3.2: Significant Indicator values ($IndVal \geq 70\%$) for the most important epiphytic algae species discriminating between Westlake River and Silverhurst Impoundment, “before and after” mechanical control of *Salvinia molesta*.

Taxa	Observed $IndVal$ (%)		$IndVal$ from Monte Carlo tests		
	Before	After	Mean	SD	P
Westlake River					
<i>Oscillatoria tenuis</i>		100	46.6	17.48	0.027
<i>Pseudanabaena</i> sp.	100		44.7	16.54	0.027
<i>Craticula</i> sp.		100	43.7	16.87	0.027
<i>Cyclotella meneghiniana</i>		87.8	64.2	13.44	0.027
<i>Diadesmis</i> sp.	100		43.5	17.20	0.027
<i>Fragilaria ulna</i> var. <i>acus</i>		100	49.4	17.69	0.027
<i>Gomphonema affine</i>		95.8	51.1	16.37	0.027
<i>G. pseudoaugur</i>	84.7		59.2	11.11	0.027
<i>G. venusta</i>		100	45.6	17.44	0.027
<i>Lemnicola hungarica</i>		95.7	49.5	14.60	0.027
<i>Navicula riediana</i>		96	55.9	17.26	0.027
<i>N. veneta</i>		100	44.9	16.23	0.027
<i>Nitzschia intermedia</i>	100		43	16.79	0.027
<i>N. sublinearis</i>		100	45.5	16.72	0.027
<i>Planothidium rostratum</i>		93.5	49.5	13.41	0.027
<i>Sellaphora pupula</i>		98.5	50.7	15.79	0.027
<i>Zygnema</i> sp.	83.4		74.0	5.81	0.054
Silverhurst Impoundment					
<i>Amphora</i> sp.	78.1		57.0	9.23	0.026
<i>Eunotia bilunaris</i>		100	43	16.71	0.026
<i>E. minor</i>		100	44.3	16.27	0.026
<i>Fragilaria ulna</i> var. <i>acus</i>		98.6	51.2	16.44	0.026
<i>Gomphonema affine gracile</i>		97.5	51.3	16.48	0.026
<i>G. parvulum</i>		97.3	52.1	17.10	0.026
<i>Melosira varians</i>	86.9		55.3	14.89	0.026
<i>Navicula antonii</i>	81		58.1	10.19	0.026
<i>N. travialis</i>	100		43.6	16.65	0.026
<i>N. veneta</i>		100	46.2	16.94	0.026
<i>Nitzschia palea</i>	79.5		61.6	7.82	0.026
<i>N. sigma</i>		100	44.3	16.76	0.026
<i>Pinnularis subbrevistriata</i>		69.8	57.0	16.76	0.026
<i>Rhopalodia gibba</i>		100	45.2	16.77	0.026
<i>Scenedesmus dimorphus</i>		87.6	49.6	12.96	0.026

Table 3.3: Significant Indicator value ($IndVal \geq 70\%$) for the most important epiphytic algae species discriminating between Kogmanskloof River and the Rosle Farm Reservoir “before and after” biological control of *Salvinia molesta*.

Taxa	Observed $IndVal$ (%)		$IndVal$ from Monte Carlo tests		
	Before	After	Mean	SD	P
Kogmanskloof River					
<i>Lyngbya</i> sp.	100		48.8	17.95	0.021
<i>Amphora veneta</i>		100	45.3	16.62	0.021
<i>Cocconeis</i> sp.	100		47.2	17.17	0.021
<i>C. engelbrechtii</i>	100		45.8	16.21	0.021
<i>C. pediculus</i>		81.4	60.5	7.83	0.021
<i>C. placentula</i>	100		43.5	17.24	0.021
<i>Cyclotella meneghiniana</i>		98.2	60.8	13.62	0.021
<i>Entomoneis</i> sp.		100	44.6	16.45	0.021
<i>Fragilaria ulna</i> var. <i>acus</i>		74.5	61.2	7.02	0.021
<i>Frustulia</i> sp.		100	43.9	16.36	0.021
<i>Gomphonema venusta</i>		100	44.4	16.05	0.021
<i>Navicula</i> sp.		100	44.1	16.48	0.021
<i>Nitzschia filiformis</i>		100	46.5	16.43	0.021
<i>N. pusilla</i>		94	49.7	15.38	0.021
<i>N. sublinearis</i>		100	45.6	16.12	0.021
<i>Pinnularia subbrevistriata</i>		96.4	52	16.74	0.021
<i>Tryblionella apiculata</i>		98.1	51.5	16.02	0.021
Rosle Farm Reservoir					
<i>Lyngbya</i> sp.		100	43.3	16.69	0.025
<i>Merismopedia</i> sp.		100	42.2	17.06	0.025
<i>Microcystis aeruginosa</i>		100	42.2	17.06	0.025
<i>Oscillatoria limosa</i>		100	41.2	17.72	0.025
<i>Pleurocapsa minor</i>		100	42.7	16.79	0.025
<i>Fragilaria biceps</i>		100	43.2	16.58	0.025
<i>F. nanana</i>		100	42.4	16.54	0.025
<i>F. tenera</i>		100	43	16.64	0.025
<i>Frustulia</i> sp.		100	42.7	16.68	0.025
<i>Gomphonema affine gracile</i>		100	41.1	17.86	0.025
<i>Hippodonata capitata</i>	100		44.8	16.10	0.025
<i>Nitzschia amphibia</i>		100	43.2	16.58	0.025
<i>N. draviellensis</i>		100	43.2	16.75	0.025
<i>N. filiformis</i>		96.2	53.8	14.82	0.025
<i>N. littorea</i>	100		43.4	17.77	0.025
<i>N. reversa</i>		100	43.9	16.28	0.025
<i>N. umbonata</i>	100		42.9	16.55	0.025
<i>Placoneis</i> sp.		100	41.4	17.53	0.025
<i>Suriella angusta</i>		100	41.2	17.72	0.025
<i>Euglena texta</i>		100	45.2	16.22	0.025
<i>Phacus pleuronectes</i>		100	42.7	16.85	0.025
<i>Strombomonas eurystoma</i>	100		43.3	16.02	0.025
<i>Scenedesmus bernardii</i>		100	44	16.37	0.025

PCA (Figure 3.10) ordination showed aquatic macroinvertebrate community assemblage patterns to be significantly different between sites and treatments (i.e. “before and after” *S. molesta* control), with a cumulative explained percentage variation of 66.2%. Due to the presence of *S. molesta* which reduced aquatic macroinvertebrate diversity, Westlake River, the Silverhurst Impoundment and the Rosle Farm Reservoir clustered together, however the Kogmanskloof River was different, separating out during the “before” *S. molesta* control phase (Figure 3.10). Following *S. molesta* control, sites formed four separate points, and each point was indicative of a different aquatic macroinvertebrate assemblage group (Figure 3.10).

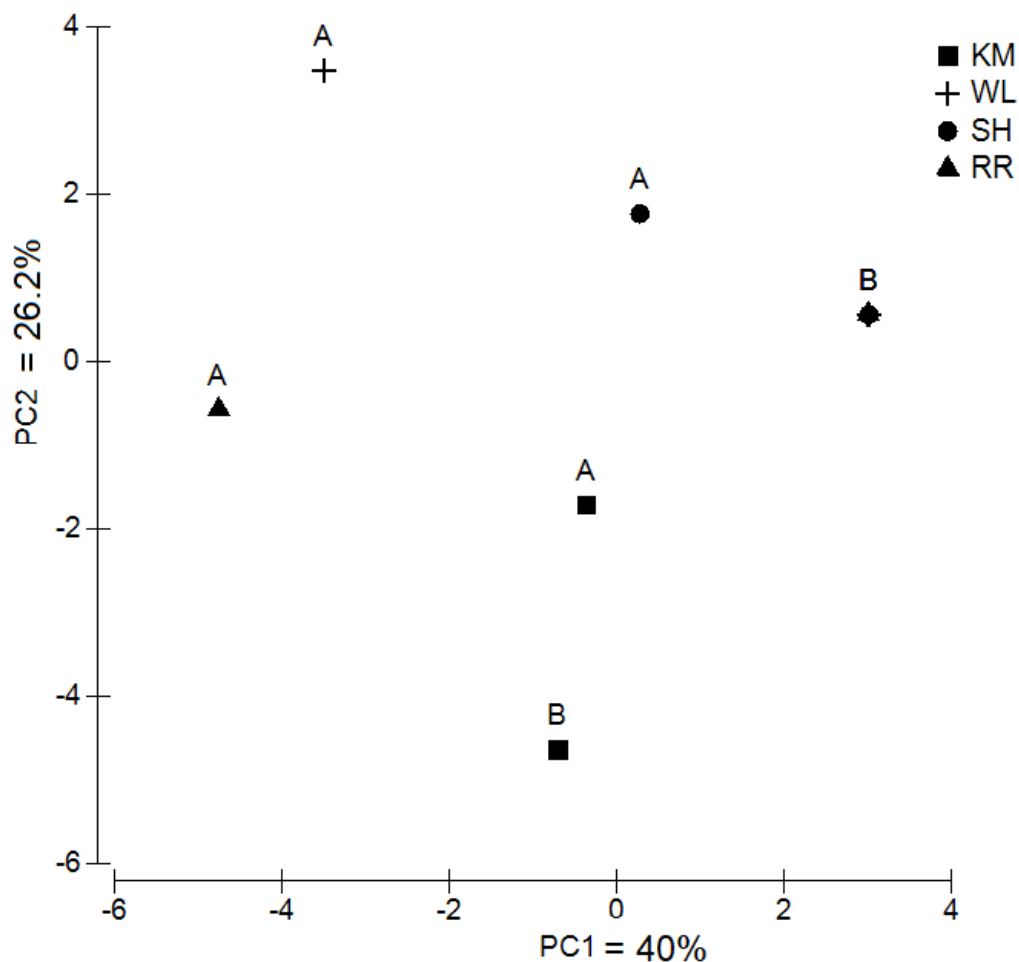


Figure 3.10: Principal Component Analysis (PCA), where each point represent aquatic macroinvertebrate assemblages, “before” (B) and “after” (A) *Salvinia molesta* mechanical (WL & SH) and biological (KM & RR) control. WL – Westlake River, SH – Silverhurst Impoundment, KM – Kogmanskloof River and RR – Rosle Farm Reservoir.

Ecosystem trophic structure “before and after” *S. molesta* control was significantly different (Table 3.4, Table S6). This indicated a shift in aquatic food web structure between the two phases respectively, where both biological and functional diversity differed “before and after” control of *S. molesta*, which was achieved either by mechanical or biological control. Due to a lack of aquatic macroinvertebrates at Westlake River, the Silverhurst Impoundment and the Rosle Farm Reservoir “before” *S. molesta* control, there were no Layman’s metrics generated to describe the trophic communities during the “before” phase (Table 3.4). However, Kogmanskloof River provided a good comparison of how biological control altered aquatic community trophic structure and function. “before” *S. molesta* control in the Kogmanskloof River, trophic community length (NR_b) and basal resource diversity (CR_b) collectively contributed to low trophic diversity (CD_b and TA_b). The community also had low Mean Nearest Neighbour values (MNND_b), indicative of taxa with similar trophic functions, which were more evenly distributed, indicated by low standard deviation of the nearest neighbour distance (SDNND_b). Following *S. molesta* control however, increases were seen in all Layman’s community metrics (NR_b, CR_b, CD_b, TA_b, MNND_b, and SDNND_b) for all study sites, describing positive changes in trophic structure and function (Table 3.4).

Because no aquatic macroinvertebrates were present during the “before” *S. molesta* control, Westlake River, the Silverhurst Impoundment and the Rosle Farm Reservoir, the development of aquatic macroinvertebrate communities “after” *S. molesta* control resulted in strong positive changes in standard ellipse areas (SEA_c) between the “before and after” *S. molesta* aquatic communities (Figure 3.11). Comparatively, the Kogmanskloof River aquatic community standard ellipses areas (SEA_c) were only slightly different “before and after” *S. molesta* biological control (Figure 3.11C). While the SEA_c of the Kogmanskloof River site did not differ substantially between “before and after” *S. molesta* biological control, the communities showed no overlap, thus each phase (i.e. “before and after”) occupied completely different positions in isotopic space (Figure 3.12C).

Table 3.4: Layman’s Bayesian community-wide metrics comparing aquatic organisms sampled from four study sites “before and after” *Salvinia molesta* mechanical (Westlake River & Silverhurst Impoundment) and biological (Kogmanskloof River & Rosle Farm Reservoir) control (97.5% CI). Abbreviations: NR_b – nitrogen range, CR_b – carbon range, TA_b – indication of total community niche area, CD_b – trophic diversity and species spacing, MNND_b – mean nearest neighbour values, SDNND_b – measure of evenness of spatial density and packing.

Layman’s matrix	Westlake River		Silverhurst Impoundment		Kogmanskloof River		Rosle farm reservoir	
	Before	After	Before	After	Before	After	Before	After
NR _b	0	1.32 (1.30-1.33)	0	2.13 (2.10-2.16)	1.37 (1.34-1.39)	1.72 (1.68-1.75)	0	2.36 (2.32-2.40)
CR _b	0	0.99 (0.98-1.0)	0	2.46 (2.43-2.49)	2.76 (2.74-2.78)	6.75 (6.72-6.78)	0	4.50 (4.54-4.45)
TA _b	0	0.60 (0.58-0.61)	0	2.29 (2.22-2.35)	1.84 (1.80-1.88)	5.60 (5.49-5.72)	0	4.41 (4.50-4.33)
CD _b	0	0.55 (0.54-0.55)	0	1.21 (1.20-1.23)	1.12 (1.11-1.13)	2.34 (2.33-2.35)	0	1.87 (1.88-1.85)
MNND _b	0	0.48 (0.48-0.49)	0	1.25 (1.24-1.27)	0.73 (0.72-0.73)	1.59 (1.58-1.60)	0	1.90 (1.91-1.87)
SDNND _b	0	0.27 (0.26-0.27)	0	0.48 (0.46-0.49)	0.38 (0.37-0.39)	0.74 (0.72-0.74)	0	0.62 (0.64-0.60)

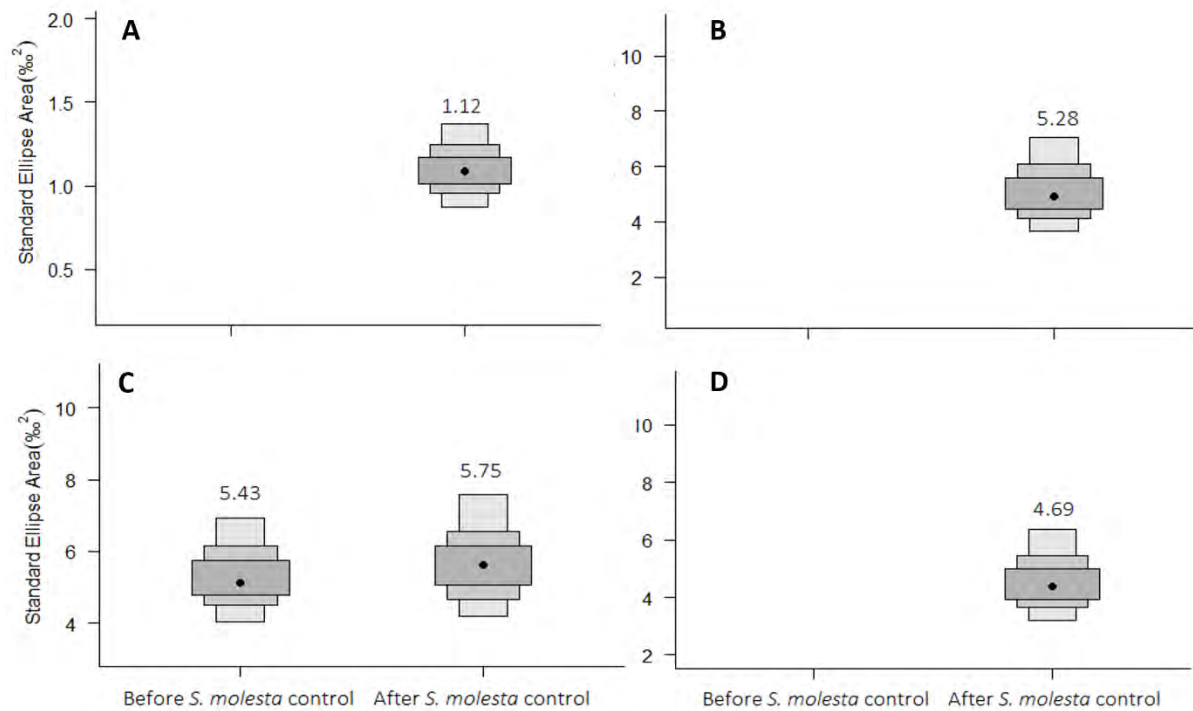


Figure 3.11: Density plots showing the confidence interval of the standard ellipses areas (SEA_c) for "before and after" control efforts for *Salvinia molesta* for all sampled sites (A) Westlake River; (B) Silverhurst Impoundment; (C) Kogmanskloof River; (D) Rosle Farm Reservoir). No Before SEA_c are presented for Westlake River, Silverhurst Impoundment, or Rosle Farm Reservoir as no aquatic macroinvertebrate communities were present during infestation. The black points correspond to the mean standard ellipse area for each community "before and after" *S. molesta* control. The grey to light grey boxed areas reflect the 95, 75 and 50% confidence interval for the overall aquatic community niche area respectively.

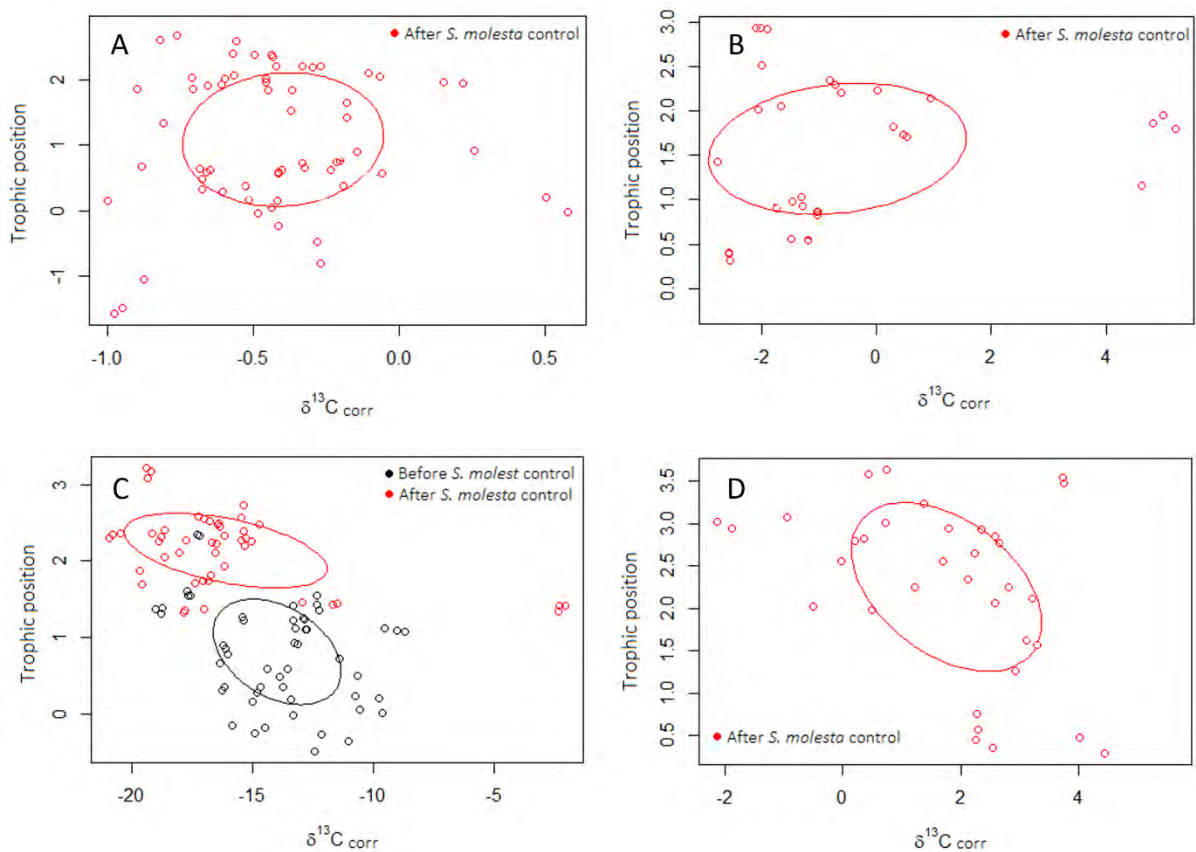


Figure 3.12: Core niche width of aquatic communities based on trophic position (TP) and corrected $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{corr}}$) “before” (red) and “after” (black) mechanical (Westlake River & Silverhurst Impoundment) and biological (Kogmanskloof River & Rosle Farm Reservoir) control efforts for *Salvinia molesta* for all sampled sites; (A) Westlake River; (B) Silverhurst Impoundment; (C) Kogmanskloof River; (D) Rosle Farm Reservoir. No Before ellipses are presented for Westlake River, Silverhurst Impoundment or Rosle Farm Reservoir as no aquatic macroinvertebrate communities were present during infestation.

Interestingly when the trophic positioning of all the aquatic communities were compared “after” successful control of *S. molesta* by either mechanical (Westlake River and Silverhurst Impoundment) or biological control (Kogmanskloof River and Rosle Farm Reservoir), the trophic ecosystem niche width for Kogmanskloof River was the largest and was completely different from the other sites with no trophic similarities/overlap (Figure 3.13). Furthermore, while it had a reduced trophic range compared to all other sites, Kogmanskloof River demonstrated the largest diversity in food resources (e.g. carbon range). Rosle Farm Reservoir (biological control) was clearly different from the Kogmanskloof River community trophic ecology, and only showed 46.7% overlap to the Silverhurst Impoundment site. The two mechanical control sites, Westlake River and the Silverhurst Impoundment shared 71.6% overlap in isotopic niche width “after” *S. molesta* control, and Westlake River in particular had

extremely low resource diversity, but a large trophic range. Thus, the community trophic structure within isotopic space between the biological control study sites was significantly different from the mechanical control study sites following *S. molesta* control (Figure 3.13).

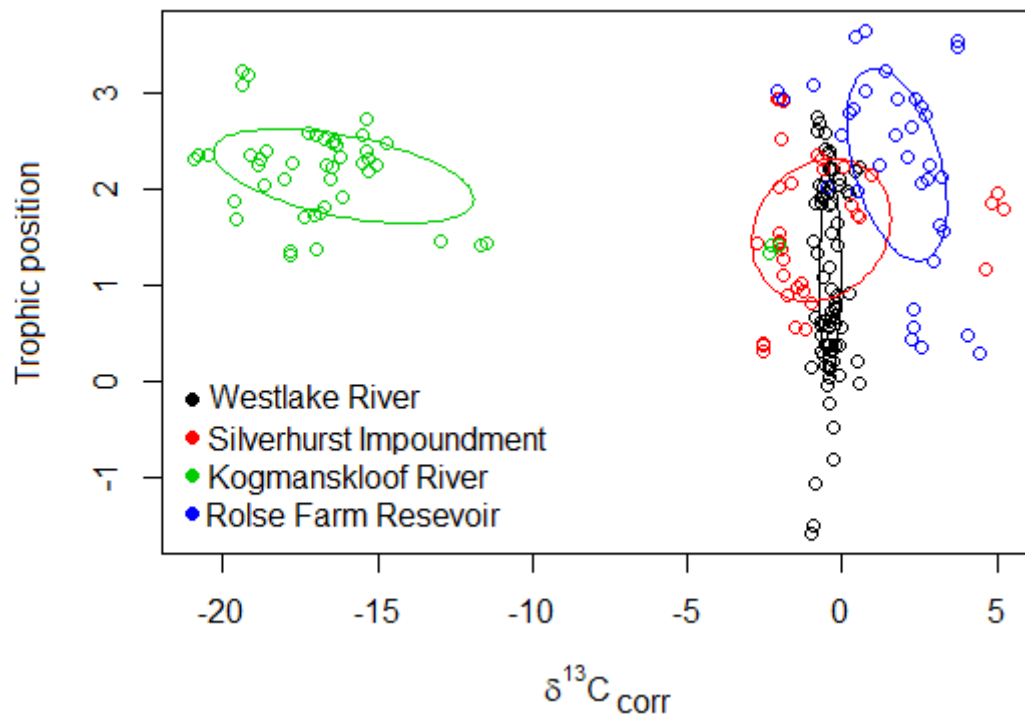


Figure 3.13: Core niche width of aquatic communities based on trophic position (TP) and corrected $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{corr}}$), “after” control efforts for *Salvinia molesta* at all sampled sites; Westlake River (black); Silverhurst Impoundment (Red); Kogmansklouf River (Green); Rolse Farm Reservoir (Blue).

3.4 Discussion

Findings from this chapter were consistent with the predicted hypothesis that *S. molesta* invasion affect ecosystem structure and functions and also in agreement with the conclusion from De Tezanos Pinto et al. (2007), Villamagma and Murphy (2010), Stiers et al. (2011), and Stiers and Triest (2017), that IAAP species alter water quality, aquatic biodiversity, ecosystem processes and trophic structure. As expected, during *S. molesta* infestation, there was a direct correlation between *S. molesta* percentage cover and water clarity, as well as PAR, but the high phytoplankton Chl-*a* concentration was surprising. Free-floating IAAP mats provide physical obstruction by creating artificial shade for underwater life, thus reducing light penetration, photosynthesis and increasing biochemical oxygen demand (De Tezanos Pinto et al., 2007; Jouanneau et al., 2014). As a result of high biochemical oxygen demand, dissolved oxygen concentration will be reduced, providing insufficient dissolved oxygen to support the aerobic processes microorganisms used to break down organic materials (Beyene et al., 2009; Dasgupta and Yildiz, 2016). Although this was not fully investigated in the present study, it is highly likely that reduced light penetration is a result of high amounts of suspended organic matter and subsequent reduction in water clarity during *S. molesta* infestation (Cattaneo et al., 1998). Due to limited dissolved oxygen, aquatic microorganisms were unable to decompose the suspended organic material exacerbated by *S. molesta* decaying plants (and anaerobic decomposers usually take over), thus suspended organic matter, bacteria, slow growing and shade-tolerant phytoplankton could have contributed to increases in phytoplankton Chl-*a* concentration, as also seen in Cattaneo et al. (1998) and in Chapter 2.

Additionally, DO was surprisingly high during *S. molesta* infestation compared to “after” *S. molesta* control, and while this was consistent with findings from Nyananyo et al. (2010), it was opposite to the present study’s hypothesis. Nyananyo et al. (2010) showed elevated pH and DO concentrations within water hyacinth mats, as compared to the open water, in the river Nun in Niger. Some authors (e.g. Villamagma and Murphy, 2010; Stiers and Triest, 2017), have reported deteriorating DO concentrations in the presence of IAAP infestation, which they attributed to IAAP species shade-effect and increased anoxic conditions. This is opposite to the present chapter findings and to that observed in the mesocosm study in Chapter 2 during the impacted treatment (i.e. 100% *S. molesta* cover)). Since DO concentration can be affected by the time of the day, and is thus highly variable, it is likely that measurements of DO concentration in the present study were not sensitive enough to detect internal ecosystem processes or alterations. For further and more in-depth investigations, including measurement

of both biochemical oxygen demand and chemical oxygen demand would be useful for the interpretation of changes in ecosystem processes during *S. molesta* infestation and “after” control.

In the present study, following the control of *S. molesta*, there was a significant improvement in water chemistry parameters brought about by the removal of the dense *S. molesta* biomass. The clear water state was reflected in an increase in water clarity, light penetration, and a reduction in total dissolved solids. Thus, there was sufficient light and nutrients available for both phytoplankton and periphyton development and the incorporation of organic material into energy resources, which could then be transferred higher up the food web (Dasgupta and Yildiz, 2016). As both periphyton and phytoplankton communities are considered important primary components of aquatic ecosystem trophic food web (Cattaneo et al., 1998), it was expected that there would be a positive change in food web structure, with more efficient and direct energy transfers between trophic levels.

Although there are some inconsistencies and controversies around how ecosystem recovery is estimated, aquatic taxa and biodiversity recovery following disturbance are among the most commonly used indicators (Durigan and Suganuma, 2015). From a community level assessment, both epiphytic algae and aquatic macroinvertebrate diversity were negatively affected by the presence of *S. molesta* in the present study (Chapter 2). This trend was consistent with the hypothesis and that of previous studies (e.g. Stiers et al., 2011; Schultz and Dibble, 2012; Coetzee et al., 2014), that reported an inversely proportional relationship between aquatic biodiversity and free-floating IAAP species cover. At low diversity scores, epiphytic algae were present during *S. molesta* infestation and substantially increased in abundance following the control of *S. molesta*. On the other hand, aquatic macroinvertebrates were completely absent from the system during *S. molesta* infestation (with exception to one site), and likewise showed a positive recovery “after” *S. molesta* control. The absence of an aquatic macroinvertebrate community during infestation was in contrast to Brendonck et al. (2003) and Masese et al. (2009)’s findings, who reported an increase in aquatic macroinvertebrate diversity under free-floating mats of water hyacinth. The increase in aquatic taxa is attributable to an increasingly complex habitat structure provided by the extensive root structure of water hyacinth. In the present study, aquatic macroinvertebrates were completely absent from systems in three study sites during *S. molesta* infestation. If we consider that Hill et al. (2015) reported that due to ecological processes being severely altered by alien invasive species, the transfer of energy (or resource subsidies) to higher trophic levels becomes limited, perhaps this is not surprising.

Abiotic filters introduced by *S. molesta* infestation, i.e. poor water quality, affected negative aquatic macroinvertebrate taxa and to some degree, cause local die-off. This type of response was also seen in Chapter 2 and in Stiers et al. (2011), Uwadiae et al. (2011), Schultz and Dibble (2012) and Copatti et al. (2013), where authors reported drastic declines in aquatic macroinvertebrates diversity and caused a significant shift in assemblage structure due to dense IAAP mats and associated water chemistry deterioration. Although this was not the case for Kogmanskloof River, there is some evidence to suggest complete homogenisation of aquatic macroinvertebrate communities as seen in Coetzee et al. (2020), who showed aquatic macroinvertebrates shift to favour taxa that are more tolerant to reduced DO, shade and poor water chemistry due to introduced abiotic filters as a result of IAAP invasion, *P. stratiotes*. Biotic homogenisation reduced the diversity scores, and altered aquatic biotic assemblage structure replacing functionally important species with generalists and disturbance tolerant species (Simberloff, 2006). In comparison, the presence of *S. molesta* did not obstruct epiphytic algae growth, in fact, *S. molesta* roots could have provided epiphytic algae with an alternative substrate, but with limited light and nutrients, epiphytic algae diversity scores were low. According to De Tezanos Pinto et al. (2007), light limitation affected phytoplankton strategies and dynamics by promoting pigmentation shifts and favouring the growth of phytoplankton species able to grow at low light intensities. These authors further added that, in this way, phytoplankton species are able to thrive under non-optimal light conditions, due to their ability to adjust to a changing environment by displaying different mechanisms that allow them to maintain an efficient light harvest.

Similar to findings in Chapter 2, and echoing Adams et al. (2002), Muotka and Laasonen (2002), Ruiz-Jaen and Aide (2005) and Durigan and Suganuma (2015), I conclude that both epiphytic and aquatic macroinvertebrate diversity scores (i.e. relative abundance, taxa richness and diversity) were sensitive to *S. molesta* invasion and subsequently showed recovery following control, acting as reliable biological indicators of ecosystem health and ecosystem recovery following disturbance (Beyene et al., 2009; Siddig et al., 2016).

“After” *S. molesta* mechanical control at Westlake River and the Silverhurst Impoundment, both sites recorded rapid recovery of epiphytic algal and aquatic macroinvertebrate assemblages. This trend affirms the ability and potential of impacted freshwater systems to recover shortly after partial-passive restoration effort. Due to an over reliance on traditional recovery, ecologists still find it challenging to quantify the functional recovery of an ecosystem (see below). Contrary to the mechanical control sites, the biological control sites, Kogmanskloof River and Rosle Farm Reservoir, showed similar ecosystem

recovery following *S. molesta* biological control, although aquatic biodiversity returns were only achieved 12 months later. There was no noticeable difference in epiphytic algae and aquatic macroinvertebrate biodiversity recovery trajectories between the two control methods, except that mechanical removal at Westlake River further invited invasion by an opportunistic cosmopolitan submerged macrophyte species, *C. demersum*, which was later substituted by the floating-leaved *N. mexicana*, an invasive alien aquatic weed species new in southern Africa (ecological effects of the changing macrophyte dominance to be discussed in the next chapter). Overall, the Silverhurst Impoundment experienced the least recovery, this could possibly be due to its man-made nature, and was unable to support more native organisms and ecosystem functions.

The present study showed distinct epiphytic algae and aquatic macroinvertebrate community assemblages in both “before and after” *S. molesta* control phase. Although Stiers and Triest (2017) showed similar aquatic microalgae diversity trends as described in the present study, their phytoplankton community assemblages were not different from each other, where major taxa overlapped between the invaded and uninvaded freshwater ponds in Belgium. This was contrary to De Tezanos Pinto et al. (2007) and the present study, which both clearly showed different aquatic microalgae assemblage structures between the free-floating macrophyte and open water states or following free-floating IAAP control. In Chapter 2, aquatic microalgae and macroinvertebrate community structure were entirely different between the impacted (*S. molesta* 100% cover) restored (100% *S. molesta* & biological control agents) and control (open water), and this was consistent in the present study, where both epiphytic algae and aquatic macroinvertebrate community structures were significantly different between the two phases. Epiphytic taxa including *Lyngbya* sp., *Pseudanabaena* sp., *Amphora* sp., *Cocconeis* sp., *Cocconeis englebrechtii*, *C. placentula*, *Diademis* sp., *Hippodonata capitata*, *Gomphonema pseudoaugur*, *Nitzschia littorea*, *N. umbonata*, *N. intermedia*, *N. palea*, *N. sigma*, *Melosira varians*, *Strombomonas* sp. and *Zygnema* sp. were identified as discriminatory species (*IndVal*) between the “before and after” *S. molesta* control, showing a strong association with *S. molesta* infestation. According to Reynolds et al. (2002) and Padisák et al. (2009) northern hemisphere aquatic microalgae functional group classification using species traits, classified *Pseudanabaena* sp. (functional group: Codon S1), *Lyngbya* sp. (Codon MP), *Nitzschia* spp. (Codon D) and *Strombomonas* sp. (Codon W2), as species with high preference for turbid, shaded and meso-eutrophic freshwater ecosystems. Beyene et al. (2009) also identified *Nitzschia palea* as a good indicator for nutrient enrichment in Ethiopian river systems. Additionally, De Tezanos Pinto et al. (2007) and Stiers and Triest (2017) reported several

shade-adapted and high tolerance to low oxygen level cyanobacteria species to be more abundant in free-floating macrophyte treatments and non-native-macrophyte-dominated sites. In the present study, this was consistent with the *IndVal* analysis and the community composition differences observed throughout the study. Thus, epiphytic algae were instrumental in indicating environmental changes in ecosystem processes, trophic structure and functional groupings as a result of *S. molesta* invasion and subsequent control. To mention a few, *Cyclotella meneghiniana* (Codon A), *Scenedemus* sp. (Codon J), *Euglena texta* (Codon W1) and *Microcystis* sp. (Codon L_M) were associated with the “after” *S. molesta* control phase, with taxa indicative of clear water, being sensitive to low light and to high grazing pressure (Reynolds et al., 2002).

Advancements in ecological restoration studies have now focused on ecosystem processes (Muotka and Laasonen, 2002) and ecosystem trophic structure and functioning as indicators of ecosystem recovery, thus unifying studies on biodiversity and ecosystem functioning (Thompson et al., 2012). It is clear that the human-mediated introduction of non-native species has severely altered natural ecosystems, and the impact has been well summarised by the Invasion Meltdown Model proposed by Simberloff (2006). The Invasion Meltdown Model describes how non-native invasive species facilitate other establishment (secondary invasion, see Chapter 4), thus maximising their socio-economic and ecological impacts on the invaded ecosystems. Using the application of stable isotope analysis to estimate trophic interactions, change in trophic level and niche width within ecosystems (Jackson et al., 2011; 2012) can be attempted to better understand the disruptive ability of environmental disturbances, and in this case invasive species to ecosystem structure and function, after alien invasive species establishments (Jackson and Britton, 2014) and after their control. The above-mentioned examples (i.e. Jackson and Britton, 2014) highlight the impacts of alien invasive fish species within freshwater ecosystems, but much less is known about IAAP species.

During *S. molesta* infestation, no aquatic macroinvertebrate communities were present in the Westlake River, the Silverhurst Impoundment or the Rosle Farm Reservoir, thus *S. molesta* invasion clearly reduced energy subsidies to higher trophic levels, severely affecting the abundance of aquatic macroinvertebrates and other taxa, as well as their associated ecosystem functions. The only ecosystem which had aquatic macroinvertebrate communities during *S. molesta* infestation was the Kogmanskloof River system. The descriptive metrics for this community indicated low diversity of basal resource subsidies (CR_b), a small trophic community length (NR_b), a lack of trophic diversity (CD_b), with more ecological redundancy (MNND_b), indicating simplistic and poorly developed food webs. Findings of this chapter were

in agreement with the hypothesis that *S. molesta* infestations will have deleterious effects on ecosystem structure and function during invasion, and following *S. molesta* control, ecosystem trophic interactions will assume a normal functioning role, including the transfer of energy to higher trophic levels. As expected, following successful biological control of *S. molesta* at Kogmanskloof River, there were increases in basal resource diversity, trophic community length (NR_b), an increase in trophic diversity (CD_b), with more niche specialisation (MNND_b, SDNND_b), indicating better developed and more complex food webs. These “before and after” communities also occupied completely different positions in isotopic space, indicating the development of new energy transfers and the utilisation of new basal food resource subsidies. Following *S. molesta* control, sites recovered and according to the trophic ecosystem structure analysis, aquatic communities “after” *S. molesta* biological control were completely different as compared to the mechanical control sites.

It is likely that taxa present during *S. molesta* infestation at Kogmanskloof River were highly tolerant of the physical light obstruction, deteriorating water chemistry and habitat alteration. Furthermore, a lack of competition for resources likely aided their survival since sensitive and specialised taxa were eliminated, or alternatively taxa were exploiting resources made available by *S. molesta* species that other species did not find palatable (Hill et al., 2015). These findings highlight the significance of IAAP species as an ecologically important freshwater threat globally. The absence of aquatic macroinvertebrate communities at three sites indicate that both ecosystem processes and functions were compromised, impacting ecosystem goods and services (Covich et al., 1999; Vilamagma and Murphy, 2010). Hill et al. (2015) further emphasised that this could be a result of alien invasive species’ abilities to alter food webs in invaded systems, thus limiting energy transfers to higher trophic levels and causing a decline in aquatic invertebrates (and other invertebrate-dependent organisms) that largely benefited from the energy transfers. From the present Chapter, the presence of *S. molesta* completely shifted aquatic macroinvertebrate assemblages (aquatic macroinvertebrate homogenisation) and in some cases caused a complete collapse of the trophic processes and structures due to absence of functionally important aquatic taxa (Covich et al., 1999). Shifts to clear-water states with more light penetration resulted in the re-establishment of some ecosystem processes, which were then followed by substantial increases in epiphytic algae, and aquatic macroinvertebrate re-colonisation, which was expected. Results show that there was significant ecosystem recovery and structural re-organisation following the removal and biological control of *S. molesta*, considering the fact that three sites were completely degraded by the presence of the weed. This included an increase in basal resource diversity, trophic food

web length, diversity of trophic levels and ecosystem niche width following control. The potential of ecosystem recovery following control of IAAP species are promising, but further long-term monitoring and assessments are needed to fully document and map ecosystem shifts in time to inform effective restoration programmes at a global scale.

In the case of secondary invasion following primary invader control, the following Chapter 4, provides an evidence-based case study looking at how ecosystem physicochemical, biological and trophic ecology characteristics respond to changing macrophyte dominance, thus providing insights that can be used for active intervention to aid in efforts to prevent secondary invasions and restore invaded ecosystems.

CHAPTER 4

Biotic and abiotic responses following multiple macrophyte succession, a case study

4.1 Introduction

Biotic and abiotic interactions within the aquatic environment play a vital role for ecosystem structure and functioning, and can provide useful interpretation when investigating environmental changes, particularly in the case IAAP species invasions (Cattaneo et al., 1998). David et al. (2017) emphasise that the interactions between biotic and abiotic factors provide a clear understanding of the impact of IAAP species in aquatic environments. According to Rejmánková et al. (2018), macrophytes have three fundamental roles in aquatic environments: (1) a physical and chemical role, relating to water and sediment quality; (2) a functional role, including algal development, organic matter production, nutrient cycling, and; (3) a structural role, such as habitat structure and representation of aquatic organisms. Therefore macrophyte traits and functional groups (i.e. emergent, submerged, and free-floating) directly and indirectly influence ecological processes within aquatic ecosystems (Bakker et al., 2013; Hilt et al., 2018). Consequently the impact of IAAP species displacing native aquatic macrophytes are expected to have ecological implications, and in some cases compromising ecosystem goods and services (Rejmánková et al., 2018). As primary producers, aquatic macrophytes provide aquatic food webs with basal resources (Bakker et al., 2016), such that any slight change in aquatic macrophyte trait or morphology will affect trophic interactions within a system, altering phytoplankton and periphyton development, and so facilitating a shift in aquatic community composition (Cattaneo et al., 1998; Schultz and Dibble, 2012; David et al., 2017). Robust and resilient aquatic ecosystems are characterised by diverse primary resources and trophic levels, dominated by specialised aquatic organisms, resulting in large trophic niche widths and complex food webs, indicative of a mature, stable and resilient ecosystem (Jackson et al., 2011; Su et al., 2019).

However, constant anthropogenic activities that lead to eutrophication and subsequent alien invasion largely alter the biotic and abiotic characteristics of aquatic ecosystems, compromising their resilience (Carpenter et al., 2011). Consequently, for an ecosystem to recover, the return of desirable conditions alone (i.e. native species compositions, improved biodiversity and/or community structure and the re-establishment of desired ecosystem functions) is not sufficient, or possible (Suding, 2011). Le Maitre et al. (2011) and Gaertner et

al. (2014) emphasise that once a disturbance has progressed beyond the critical ecological threshold (e.g. drastic changes in biotic and abiotic characteristics), the system experiences a reorganised internal feedback mechanism which is often unpredictable. As a result, restoration practitioners are challenged by these degraded systems which do not respond as predicted, largely because the efforts focus mainly on restoring the historic abiotic conditions of the system (Suding et al., 2004). However, restoration practitioners must not ignore changes that have developed in the degraded state or new interactions between biotic and abiotic factors. These feedbacks can make a degraded system persistent and resilient; and may then become an alternative state requiring a unique recovery pathway that might be very difficult to achieve (Suding, 2011). The internal re-organisation mechanism suggests that some degraded systems have shifted to a new state that cannot be restored to their historic conditions by simply eliminating abiotic filters (Suding et al., 2004). Gaertner et al. (2014) point out that only resilient systems can recover autogenically, a recovery which often involves re-establishing the ecological processes that will enable the rest of the system to self-organise, with little or no further management intervention. Loch Leven in Scotland is one such example, which showed autogenic recovery following reduction in nutrient input into the lake, which surprisingly saw a biological recovery of native aquatic invertebrates and macrophytes species (Carvalho et al., 2011).

Carpenter and Kitchell (1992) describe top-down (introduction of an aggressive aquatic predator) and bottom-up (increase in water nutrients) trophic effects as classical examples of aquatic ecosystem internal feedback mechanism and re-organisation, which have the potential to trigger a large ecological shift in the ecosystem structure and functioning. Biological control is the introduction of natural enemies (e.g. phytophagous insects) to control IAAP species in freshwater systems, thus a top-down interaction. Strange et al. (2018) reported that biological control methods can cause partial trophic cascades in freshwater ecosystems following control of free-floating IAAP species. Although not fully quantified, IAAP species have shown strong biotic and abiotic effects on invaded ecosystems, thus compromising the ecosystem's resilience and its ability to recover autogenically following IAAP species management (Le Maitre et al., 2011; Gaertner et al., 2014). However, South Africa has successfully managed free-floating IAAP species using biological control programme for decades (Hill and Coetzee, 2017), with promising socio-economic and, to some extent, ecological returns (Coetzee et al., 2014; Fraser et al., 2016). Recent field and mesocosm studies have shown strong evidence for regime-shift scenarios following successful biological control of free-floating IAAP species, where free-floating IAAP species are replaced by submerged IAAP species (Strange et al., 2018). Hill and

Coetzee (2017) and Strange et al. (2018) suggest that such ecological shifts are largely driven by bottom-up trophic effects, where nutrient availability, light and space following free-floating IAAP species biological control benefit secondary submerged aquatic weed invaders. This shift in IAAP species has been observed in a number of South African freshwater systems, and this may largely be due to limited native macrophyte diversity in southern Africa, to compete with a suite of invasive aquatic weeds (Strange et al., 2018). The depauperate nature of macrophyte species in South Africa and a lack natural open water ecosystem combined with severely disturbed freshwater ecosystems, has given invaders an advantage, enabling them to proliferate and re-invade most slow-moving waters in southern Africa and beyond, thus impacting restoration practices.

Recommendations put forward by previous authors (i.e. Villamagma and Murphy, 2010; Kettenring and Adams, 2011; Schultz and Dibble, 2012; Prior et al., 2018; Strange et al., 2018), confirm that little is known about the long-term ecological effects following IAAP species management, particularly in the case of secondary invasion and the implication for freshwater restoration. Thus, this chapter aims to investigate biotic and abiotic factors and the interactions following multiple states (between non-native vegetated and clear-water states) in a freshwater ecosystem. I hypothesise a species-specific shift in water and sediment chemistry, biological diversity and trophic ecosystem structure and function between each macrophyte species dominance.

4.2 Material and methods

4.2.1 Study site and design

The study was conducted in the Westlake River system (S-33°08'39.6", E018°46'19.9"), located near Lakeside in the Western Cape, South Africa (see Chapter 3: Figure 3.1 for study site map and description). The site was predominantly infested by free-floating *S. molesta* in March 2017.

This field study consisted of four stochastic events that took place on site and each event (hereafter, state) was treated as a sampling occasion, from March 2017 to September 2018. In March 2017 the river was dominated by the non-native free-floating *S. molesta* (Figure 4.1A), which was cleared mechanically in April 2017. The removal of *S. molesta* resulted in a clear-water state, which was surveyed in June 2017 and in October 2017 (Figure 4.1B). Five months later, in April 2018, a cosmopolitan submerged macrophyte, *C. demersum*-dominated state was recorded on-site (Figure 4.1C). In September 2018, the submerged *C. demersum* was

replaced by the non-native floating-leaved emergent *N. mexicana*-dominated state, however at the time of sampling the system was 70% covered by *N. mexicana* (Figure 4.1D), and in March 2019 the site was 100% covered by *N. mexicana* (Julie Coetzee pers. comm.; 15 March 2019, Centre for Biological Control, Rhodes University). By visual inspection, the mechanical removal of *S. molesta* at Westlake River did not promote any native macrophyte dominance, except *Typha* sp. and *Pennisetum clandestinum* Hochst. Ex Chiov. (Kikuyu grass), which were observed throughout the study. There were, however, sporadic but inconsistent patches of *Stuckenia pectinata* (L.) Boerner (Potamogetonaceae) (pondweed), *M. aquaticum*, *P. stratiotes* and *S. molesta*. At each state, sampling methods included: (1) water and sediment chemistry; (2) epiphytic algae and aquatic macroinvertebrates diversity and assemblage structure analysis, and; (3) trophic food web analysis (see below for details).

4.2.2 Data collection

4.2.2.1 Environmental variables

During each state, a combination of water physical and chemical variables were measured as described in Chapter 3 (page 58).



Figure 4.1: Physical changes and ecological scenarios observed at the Westlake River system near Lakeside, Western Cape, South Africa, where (A) free-floating IAAP *Salvinia molesta*-dominated state (100% cover: March 2017), (B) clear-water state (June and October 2017) after mechanical control of *S. molesta*, (C) submerged cosmopolitan *C. demersum*-dominated state (80% cover: April 2018), and (D) floating-leaved IAAP *N. mexicana*-dominated state (70% cover: September 2018).

4.2.2.2 Sediment analysis

To investigate sediment chemistry between different states, an integrated sediment sample (~1kg, n=1) collected (5-10 cm depth) along the river channel and littoral zone using a plastic shovel. Sediment samples (n=1, per sampling occasion) were stored in 2 kg Ziploc™ bags, placed in a cooler with ice until they reached the laboratory. Prior to analysis, sediment samples were divided into two sub-samples of ~500 g each, for (1) sediment chemistry analysis (n=2, 250 g) including; pH, conductivity (EC), Potassium (K), Sodium (Na), Calcium (Ca), Magnesium (Mg), Manganese (Mn), Boron (B), Iron (Fe), Resistance, Chlorine (Cl), Sulphur (S), Carbon (C%), Copper (Cu), Zinc (Zn), Phosphate (P), Nitrogen (NO₃-N), Ammonium

(NH₄-N) and Lead (Pb) at Bemlabs (Pty Ltd), Somerset West, South Africa, and (2) percentage sediment organic matter (SOM%), and percentage sediment organic carbon (SOC%) analysis (n=3, 150 g) using a modified Walkley–Black method (Shen et al., 2016; Nel et al., 2018), as follows, 150 g of freshly collected sediment were transferred into aluminium containers and oven dried for 48 hours at 50 °C. The resulting oven-dried sediment was weighed and recorded, after which the samples were further combusted for six hours at 450 °C using a Labcon furnace (L-1200). The resulting combusted sediment weight was recorded, where the difference in the mass (oven-dried mass and combusted mass) was used to determine sediment organic matter and sediment organic carbon percentages using the following formula respectively:

$$\text{SOM (\%)} = \left(\frac{\text{Oven dried weight} - \text{Combusted weight}}{\text{Oven dried weight}} \right) \times 100$$

$$\text{SOC (\%)} = \left(\frac{\text{SOM\%}}{1.724} \right)$$

4.2.2.3 Biological data

Epiphytic algae

To compare epiphytic algae species assemblages, periphyton Chl-*a* concentration and ecosystem basal resources diversity (using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope ratios) between different states, epiphytic algae samples (aquatic microalgae found growing on macrophyte surfaces) were collected from a combination of randomly selected free-floating (*S. molesta*, *P. stratiotes* roots), floating-leaved (*N. mexicana* leaves), submerged (*C. demersum* stems and leaves) and emergent (*Cyperus* sp., *Typha* sp., *M. aquaticum* and *N. mexicana* stems) macrophyte species. Epiphyton samples were collected following a modified procedure described in Taylor et al. (2007a), where aquatic microalgae growing on macrophytes roots, stems and leaves, were completely scraped off, using a new toothbrush, onto a white collecting tray filled with approximately 2000 ml of filtered site water. This epiphyte sampling procedure was repeated three times for: (1) epiphyte algae species assemblages; (2) periphyton Chl-*a*, and (3) epiphyte algae carbon and nitrogen isotopic values during each sampling state (see below for detailed methods).

Epiphytic algae samples for species assemblage analysis was divided into four subsamples (n=4, each 500 ml) and immediately preserved with 5 ml of Lugol's iodine solution. Further sample preparation, cell identification and enumeration followed the procedure described in Chapter 2 (page 26).

Phytoplankton and periphyton chlorophyll-*a* concentration

Ecosystem production (estimated through phytoplankton and periphyton Chl-*a* concentration) at each sampling state was determined as described in Chapter 2 (page 27).

Aquatic macroinvertebrates and adult Odonata species

To quantify aquatic macroinvertebrates biological and functional diversity together with trophic ecosystem interactions at each sampling state, aquatic macroinvertebrates and adult Odonata were collected as described in Chapter 3 (page 59).

4.2.2.4 Aquatic community trophic structure analysis

To estimate community level ecosystem trophic structure and interactions, biological samples (i.e. primary, invertebrates and vertebrates' samples) for each state, were prepared as described in Chapter 3 (page 60) and sent for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope analysis at the Stable Isotope Facility, University of Pretoria, South Africa. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values were reported as ‰ vs air, normalised to internal standards (Merck and DL-Valine) calibrated to the International Atomic Energy reference materials (IAEA-CH-3 and IAEA-CH-6 for $\delta^{13}\text{C}$, IAEA-N1 and IAEA-N2 for $\delta^{15}\text{N}$). Results are expressed in standard delta notation using per mil scale, δX (‰) = $\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$, where $X = ^{15}\text{N}$ or ^{13}C , and R is the ratio of the heavy over the light isotope ($^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$), respectively. Average analytical precision for $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ was ± 0.03 and ± 0.02 respectively.

4.2.3 Data analysis

Environmental variables

To test for significant changes in physicochemical variables (i.e. pH, EC, TDS, salinity, water temperature, DO, NO_3^- , NH_4^+ , PO_4^{3-} , phytoplankton and periphyton Chl-*a* concentration, water level, water clarity and PAR) and sediments chemistry (i.e. pH, EC, K, Na, Ca, Mg, Mn, B, Fe, Resistance, Cl, S, C%, Cu, Zn, P, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and Pb), between different states, a one-way analysis of variance (ANOVA) with Tukey's HSD post-hoc tests were used to test for significance differences between environmental variables of water and sediment chemistry, with states as factors: *S. molesta*-dominated state, clear-water state (June and October), *C. demersum*-dominated state, and *N. mexicana*-dominated state. Prior to analysis, data were normally distributed (Shapiro-Wilks test, $P > 0.05$) and the variances were homogenous (Levene's test, $P < 0.05$), except for DO, NO_3^- , phytoplankton and periphyton Chl-*a*

concentration and sediment NO₃-N, B, Mg, Ca, and Fe, these variables were subsequently log(x+1) transformed to meet ANOVA assumptions.

Biological diversity indices

To investigate biological and functional diversity and composition changes in epiphytic algae and aquatic macroinvertebrate taxa between the different states, relative abundance (N), taxa/species richness (S), Shannon diversity index, $H' = -\sum_{i=1}^s p_i \ln p_i$ (where p_i is the proportional abundance of taxa i in the sample given s taxa) and Pielou's evenness, $J' = \frac{H'}{\ln(S)}$ indices were computed in PRIMER version 6.1.16 and PERMANOVA⁺ version 1.0.6 using the DIVERSE function (PRIMER-E Ltd, Plymouth: Clarke and Gorley, 2006). Epiphytic algae and aquatic macroinvertebrate taxa were further categorised into phyla and functional feeding groups (FFGs) respectively. Aquatic macroinvertebrates were assigned to different FFGs including: collector-filter feeders, collector-gatherers, scraper/herbivores, shredders and predators, according to Cummins and Klug (1979), Palmer et al. (1996), Merritt et al. (2008) and Hawking et al. (2013). Epiphytic algae were assigned to phyla including Cyanophyta, Bacillariophyta, Euglenophyta, Dinophyta, Cryptophyta and Chlorophyta (John et al., 2002; Van Vuuren et al., 2006; Taylor et al., 2007b).

Similarly, data used to derive epiphytic algae diversity indices met both normality and homogenous variances, thus a one-way ANOVA was used to test for significant differences in epiphytic algae diversity indices between states, whereas epiphytic algae and aquatic macroinvertebrates composition data (categorical data) were tested for significance between states using Chi-squared tests.

Epiphytic algae and aquatic macroinvertebrate community assemblage structure

To visualise differences in both physicochemical and biological characteristics between states, unconstrained ordinations were completed using the principal component analysis (PCA), and a constrained ordination using the canonical analysis of principal coordinate (CAP) ordination to visualise the multivariate patterns of environmental variables (in Euclidean distance) and biological assemblages (Bray-Curtis similarities) (Clarke and Gorley, 2006). Additionally, epiphytic algae assemblage structure similarities between different states were further analysed using a one-way analysis of similarity (ANOSIM: $0.75 < R < 1$ = high assemblage difference; $0.5 < R < 0.75$ = different assemblage; $0.25 < R < 0.5$ = differences in assemblage with some

overlap; $0.1 < R < 0.25$ = some differences, but high overlap; $R < 0.1$ = assemblages similar), where the H_0 = no assemblage differences between states and H_1 = there are clear assemblage differences between states. If the ANOSIM was significant, the indicator species analysis (*IndVal*; Dufrêne and Legendre, 1997) was used to identify discriminatory species that resulted in differences in species assemblages between states. The indicator values range from zero (no indication) to 100 (perfect indication). Thus, only epiphytic algae species with significant indicator values (Monte Carlo test; permutation = 999, $P < 0.05$) were considered characteristic indicator species.

Aquatic macroinvertebrate assemblage structure was assessed using cluster analysis (dendrogram). ANOSIM, ordination and cluster analysis were computed in PRIMER version 6.1.16 and PERMANOVA⁺ version 1.0.6 (PRIMER-E Ltd, Plymouth: Clarke and Gorley, 2006), whereas *IndVal* analysis was computed in PC-ORD version 5.10 (McCune and Mefford, 2006).

Ecosystem trophic food webs

Collected biological samples, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope ratios were treated and ecosystem community trophic metrics analysed as outlined in Chapter 3 (page 64).

All statistical tests except where specified were conducted in R version 3.6.1 (R Development Core Team, 2016).

4.3 Results

Environmental variables associated with different states

All environmental variables (water and sediment variables collectively) showed significant variations between different states, except for two water chemistry variables, PO_4^{3-} ($F_{3, 26} = 1.07$, $P > 0.05$) and periphyton Chl-*a* concentration ($F_{3, 26} = 1.43$, $P > 0.05$), and six sediment chemistry variables which included: percentage SOM ($F_{3, 4} = 0.51$, $P > 0.05$), percentage SOC ($F_{3, 4} = 0.51$, $P > 0.05$), $\text{NH}_4\text{-N}$ ($F_{3, 4} = 3.47$, $P > 0.05$), Mn ($F_{3, 4} = 3.28$, $P > 0.05$), B ($F_{3, 4} = 3.35$, $P > 0.05$), and Fe ($F_{3, 4} = 2.83$, $P > 0.05$) (Table 4.1).

During the *S. molesta*-dominated state, PAR, water clarity, Zn, Ca and Mg were significantly different from the rest of the macrophyte states. The *S. molesta*-dominated state had the lowest PAR and water clarity, together with minimum sediment chemistry of P, $\text{NO}_3\text{-N}$, Cu, Zn concentration and C%. However, the *S. molesta*-dominated state had very high

concentrations of phytoplankton Chl-*a* concentration and sediment Pb, Mg and K. In comparison to the *S. molesta*-dominated state, the clear-water state in June, recorded high water level, PAR, EC, TDS, salinity, DO and sediment Cl, S, Cu (also similar during the *N. mexicana*-dominated state) and Zn concentrations. However, the clear-water state in June recorded the lowest concentration of sediment Pb and K (also similar during the *N. mexicana*-dominated state), and phytoplankton Chl-*a* concentration. During the clear-water state in October, sediments chemistry was not collected. The *C. demersum*-dominated state showed elevated concentrations of water pH, sediment C%, Resistance, NO₃-N concentration and the lowest water EC, TDS, salinity, and sediment Cl, S, Ca, Mg concentrations respectively. The *N. mexicana*-dominated state showed the high water clarity, NO₃-N and sediment pH and the lowest DO concentration of all the phases (Table 4.1).

Table 4.1: Mean ($\pm$ standard deviation) of environmental variables recorded at each state, in the Westlake River, South Africa. Different superscript letters indicate values that were significantly different between states (Tukey HSD post-hoc tests). Abbreviation, ND – not detected. Sediment chemistry was not collected during the clear-water state in October. Lakatos (1989) is a trophic classification system based on phytoplankton and periphyton composition.

Environmental variables		Multiple stable states				Statistics summary		
		<i>S. molesta</i> state	Clear-water state June	Clear-water state October	<i>C. demersum</i> state			<i>N. mexicana</i> state
Physical Parameters						<i>F</i> -values (df 3, 26)	ANOVA	
Water depth (cm)		45 (±10.00) ^a	74.17 (±16.56) ^b	41 (±15.27) ^a	37.17 (±9.13) ^a	25 (±8.17) ^a	5.31	<i>P</i> <0.05
PAR (μMol)		0 ^a	1433.08 (±78.42) ^b	989.43 (±605.07) ^b	989.43 (±605.07) ^b	1211.26 (±300.95) ^b	12.14	<i>P</i> <0.05
Water clarity (cm)		15.83 (±6.55) ^a	51 (±20.61) ^b	58.67 (±18.92) ^b	52.83 (±15.92) ^b	66.83 (±7.81) ^b	13.28	<i>P</i> <0.05
Water Chemistry								
pH		7.28 (±0.12) ^a	7.39 (±0.28) ^a	6.88 (±0.25) ^b	7.90 (±0.21) ^c	7.57 (±0.14) ^{ac}	11.64	<i>P</i> <0.05
Conductivity (μS/cm)		945.50 (±110.64) ^a	991 (±98.53) ^a	595.83 (±10.50) ^b	392 (±9.76) ^c	731.33 (±5.47) ^d	15.14	<i>P</i> <0.05
Water Temperature (°C)		19.77 (±0.97) ^a	16.42 (±1.10) ^b	23.25 (±0.65) ^c	19.27 (±0.94) ^a	18.4 (±1.06) ^a	40.61	<i>P</i> <0.05
Dissolved oxygen (mg/l)		5.97 (±1.38) ^a	8.48 (±0.51) ^b	4.23 (±1.21) ^{ac}	3.52 (±1.87) ^c	2.62 (±0.16) ^{cd}	7.21	<i>P</i> <0.05
NO ₃ ⁻ (mg/l)		3.73 (±2.25) ^a	8.17 (±0.51) ^b	7.18 (±1.63) ^b	3.30 (±1.42) ^a	13.95 (±3.80) ^c	33.39	<i>P</i> <0.05
NH ₄ ⁺ (mg/l)		0	0	0	0	0		-
PO ₄ ³⁻ (mg/l)		0.93 (±0.43) ^a	0.89 (±0.21) ^a	1.1 (±0.59) ^a	0.53 (±0.52) ^a	0.75 (±0.63) ^a	1.21	<i>P</i> >0.05
Phytoplankton (mg/m ³)	Chl- <i>a</i>	59.73 (±26.41) ^a	5.42 (±4.96) ^a	2.85 (±1.37) ^a	4.28 (±2.40) ^a	8.11 (±11.19) ^a	1.47	<i>P</i> >0.05
Periphyton (mg/m ³)	Chl- <i>a</i>	120.47 (±89.42) ^a	128.24 (±84.21) ^a	132.46 (±134.00) ^a	720.17 (±393.20) ^b	26.10 (±7.83) ^a	28.58	<i>P</i> <0.05
Lakatos classification	(1989)	Heterotrophic	Heterotrophic	Heterotrophic	Heterotrophic	Heterotrophic		
Sediment Chemistry						<i>F</i> -values (df 3, 4)	ANOVA	
Sediment organic matter (SOM%)		23.47 (±1.71) ^a	1.89 (±0.06) ^a	-	1.90 (±0.32) ^a	2.99 (±0.56) ^a	0.51	<i>P</i> >0.05
Sediment organic carbon (SOC%)		2.01 (±0.99) ^a	1.10 (±0.04) ^a	-	1.10 (±0.18) ^a	1.74 (±0.33) ^a	0.51	<i>P</i> >0.05

Carbon (C%)	0.56 (± 0.02) ^a	3.55 (± 0.84) ^b	-	9.16 (± 0.31) ^c	3.65(± 0.23) ^{cd}	119.9	<i>P</i><0.05
pH	5.90 (0) ^a	5.95 (± 0.71) ^a	-	7.10 (± 0.14) ^{ab}	7.70 (± 0.14) ^b	12	<i>P</i><0.05
Resistance (Ohm)	600 (± 99.00) ^{abc}	495 (± 35.36) ^b	-	845 (± 21.21) ^c	530 (± 127.28) ^{abc}	7.19	<i>P</i><0.05
Chlorine (Cl mg/kg)	119.83 (± 39.10) ^{ab}	417.36 (± 138.35) ^b	-	0.03 (± 0.01) ^{ac}	ND	13.44	<i>P</i><0.05
Phosphate (P mg/l)	95 (± 4.24) ^a	125 (± 21.21) ^{ab}	-	195 (± 35.36) ^b	90 (± 28.28) ^a	7.43	<i>P</i><0.05
Nitrate (NO ₃ -N mg/kg)	0.23 (± 0.01) ^a	30.50 (± 6.36) ^b	-	150 (± 145.66) ^b	8.00 (± 4.24) ^a	33.93	<i>P</i><0.05
Ammonium (NH ₄ -N mg/kg)	4.24 (± 0.02) ^a	184 (± 111.72) ^a	-	90.50 (± 2.12) ^a	60.50 (± 23.34) ^a	3.47	<i>P</i> >0.05
Manganese (Mn mg/kg)	1.95 (± 0.07) ^a	62.50 (± 43.13) ^a	-	49 (0) ^a	18.50 (± 3.54) ^a	3.28	<i>P</i> >0.05
Boron (B mg/kg)	0.28 (± 0.02) ^a	3 (± 2.83) ^a	-	4 (0) ^a	4.50 (± 0.71) ^a	3.35	<i>P</i> >0.05
Sulphur (S mg/kg)	73.79 (± 1.75) ^{abc}	97.29 (± 2.44) ^{ac}	-	38.23 (± 4.20) ^b	82.99 (± 21.81) ^c	10.08	<i>P</i><0.05
Iron (Fe mg/kg)	164.50 (± 2.12) ^a	15702 (± 11196.33) ^a	-	7189 (± 1128.54) ^a	3422.50 (± 333.05) ^a	2.83	<i>P</i> >0.05
Copper (Cu mg/kg)	0.55 (± 0.07) ^a	7.50 (± 0.71) ^b	-	7.50 (± 0.71) ^b	4 (0) ^c	88.23	<i>P</i><0.05
Zinc (Zn mg/kg)	5.25 (± 0.07) ^a	38.50 (± 7.78) ^b	-	33.50 (± 2.12) ^b	26.50 (± 7.78) ^b	58.31	<i>P</i><0.05
Lead (Pb mg/kg)	6.42 (± 1.40) ^a	1.89 (± 0.29) ^b	-	2.96 (± 0.66) ^b	5.22 (± 0.66) ^{ab}	11.7	<i>P</i><0.05
Calcium (Ca%)	49.85 (± 1.41) ^a	0.14 (± 0.08) ^b	-	0.08 (± 0.007) ^a	0.45 (± 0.04) ^a	124.5	<i>P</i><0.05
Magnesium (Mg%)	30.43 (± 0.11) ^a	0.04 (± 0.03) ^b	-	0.02 (0) ^b	0.03 (± 0.007) ^b	135.4	<i>P</i><0.05
Potassium (K%)	0.96 (± 0.02) ^a	0.03 (± 0.03) ^{ab}	-	0.04 (± 0.01) ^b	0.03 (± 0.007) ^{ab}	7.12	<i>P</i><0.05

The PCA ordination of Euclidean distance reduced 14 physicochemical variables to five significant, highly correlative variables using the Pearson correlation, ($r=0.7$, $P<0.05$): phytoplankton Chl-*a* concentration, EC, NO_3^- , PAR, and water clarity. These variables showed an overall strong association, contributing to different states clustering separately (Figure 4.2). PAR and water clarity were two variables that were shared between the clear-water state and *N. mexicana*-dominated state. The *S. molesta*-dominated state showed a strong correlation with phytoplankton Chl-*a* concentration, which set it apart from the rest of the states. The *S. molesta*-dominated state was negatively correlated to PAR, water clarity and NO_3^- . The *C. demersum*-dominated state clustered separately from the rest and showed a negative correlation to EC (Figure 4.2).

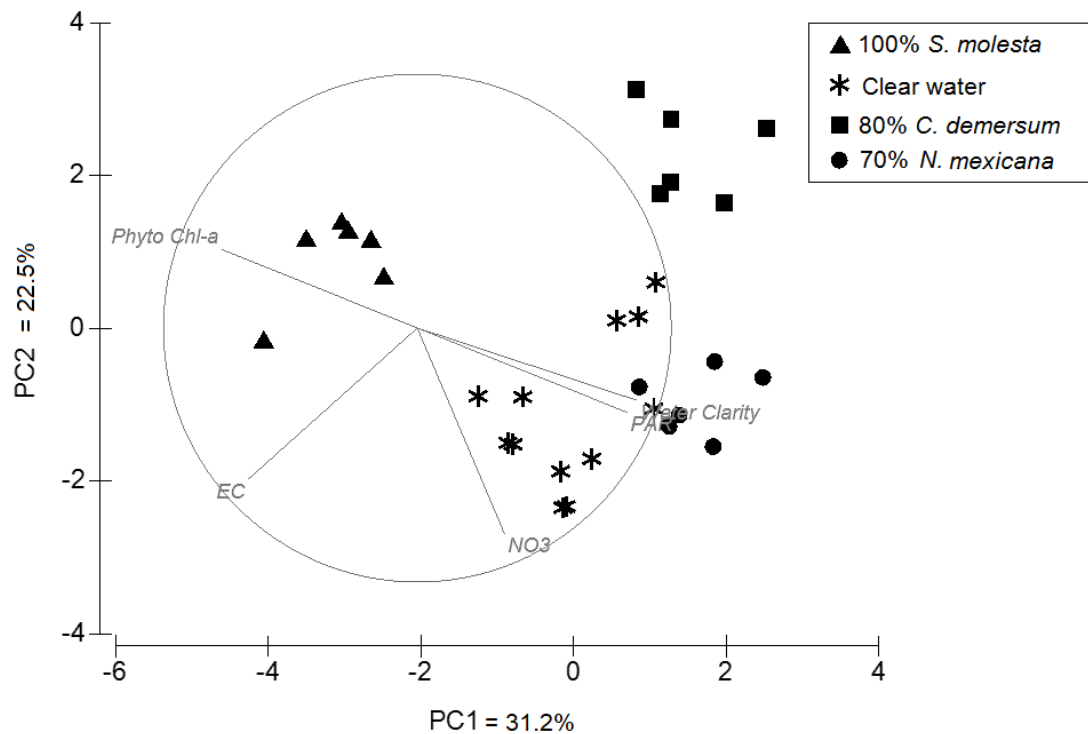


Figure 4.2: Principal component analysis (PCA) ordination for physical and water quality variables (Euclidean distance of similarities) from 30 sampling units of four states (with the two clear water states analysed together) at Westlake River system, South Africa.

Epiphytic algae diversity and assemblage composition

A total of 104,790 epiphytic algal individuals from 181 genera and species, belonging to six Phyla (Cyanophyta, Bacillariophyta, Dinophyta, Cryptophyta, Euglenophyta and Chlorophyta) were identified during the study (Table S7). Bacillariophyta was the most abundant Phylum

represented by *Cocconeis placentula*, *Cyclotella meneghiniana*, *Nitzschia palea*, *Cocconeis englebrechtii* and *Hippodonta capitata* which were most abundant taxa respectively; followed by Chlorophyta represented by *Zygnema* sp., *Scenedesmus dimorphus*, *Scenedesmus* sp. and *Scenedesmus communis*; Cyanophyta represented by *Anabaena* sp., *Oscillatoria tenuis*, *Calothrix parientina* and *Pseudanabaena* sp.; Euglenophyta represented by *Phacus pleuronectes*, *Phacus cochleatus*, *Phacus caudatus* and *Trachelomonas* sp.; Cryptophyta represented by *Cryptomonas erosa* and *Cryptomonas ovata*, with Dinophyta being the least abundant Phylum, represented only by *Chroomonas baltica*.

Relative epiphyte algae abundance ($F_{4,15}=0.18$, $P<0.05$) was not significantly different between the states throughout the study. Comparatively, epiphyte taxa richness ($F_{4,15}=7.45$, $P<0.05$), Pielou's evenness ($F_{4,15}=8.61$, $P<0.05$) and Shannon diversity index ($F_{4,15}=15.27$, $P<0.05$) were significantly different between states (Figure 4.3). The clear-water state in June produced high species richness, Pielou's evenness, and Shannon diversity index compared to other states. However, a gradual increase was seen in epiphyte algae species evenness and diversity from the clear-water state in October 2017 to the *C. demersum*-dominated state in April 2018, and the *N. mexicana*-dominated state in September 2018, however, this was not true for relative abundance and taxa richness (Figure 4.3A - D).

Epiphytic algae phyla percentage composition showed a significant overall difference between states (Chi-squared, $P<0.001$) (Figure 4.4). Cyanophyta relative abundance was high during the *C. demersum*-dominated state and the lowest in October clear-water state. Bacillariophyta was the most represented group, reaching more than 90% relative contribution during the *N. mexicana*-dominated state and recording the lowest, but still high, percentage of 66.9% during *S. molesta*-dominated state. Dinophyta and Cryptophyta had the lowest relative abundance of less than 0.5% during the clear-water state in June and *C. demersum*-dominated states. Euglenophyta and Chlorophyta showed the highest percentage during the clear-water state in June and *S. molesta*-dominated state (Figure 4.4).

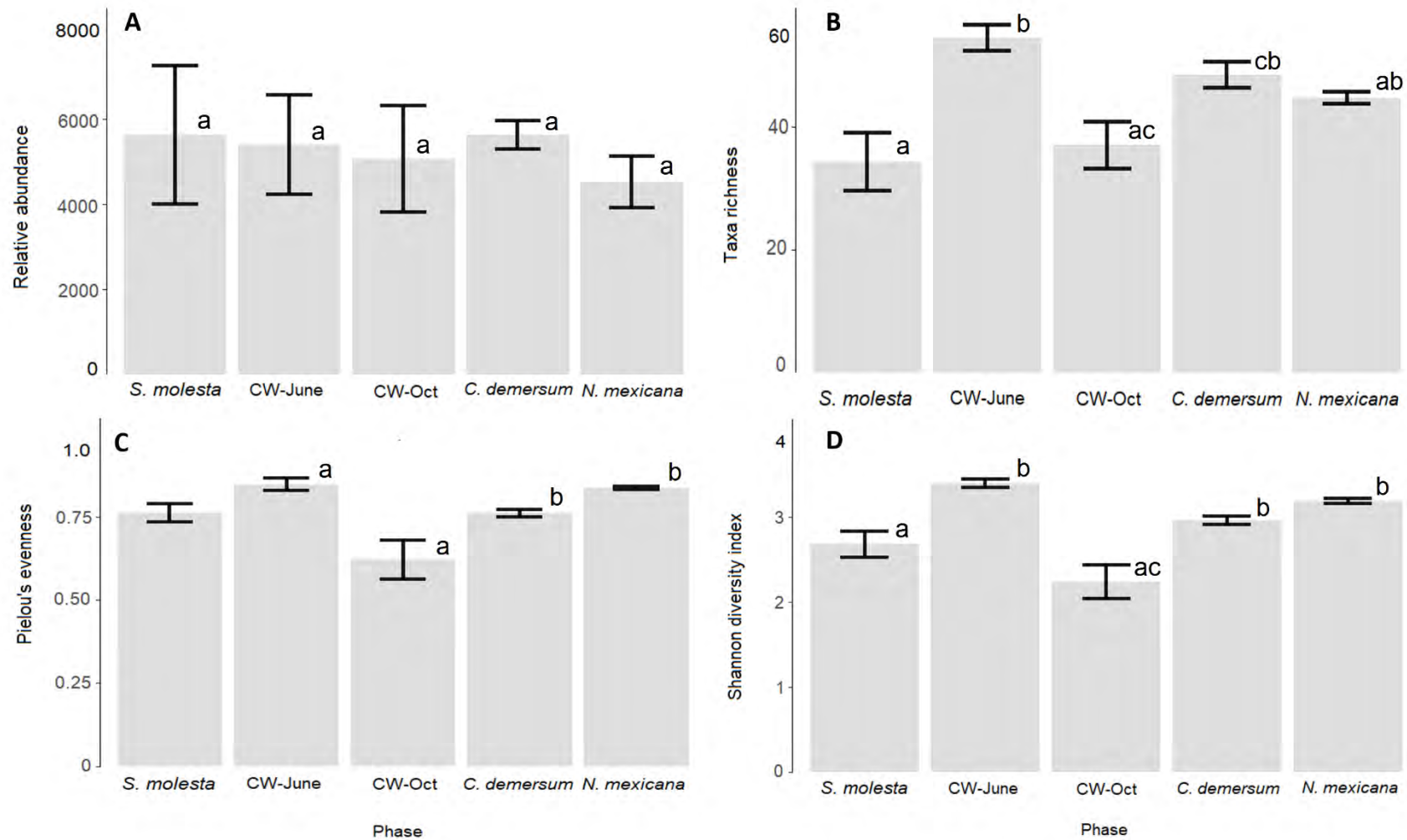


Figure 4.3: Mean epiphytic algae diversity indices ($\pm$ standard error), for (A) relative abundance, (B) taxa richness, (C) Pielou's evenness and (D) Shannon diversity index during each state, at Westlake River system, South Africa. CW Jun: clear-water in June; CW Oct: clear-water state in October. Different superscript letters indicate state that were significantly different.

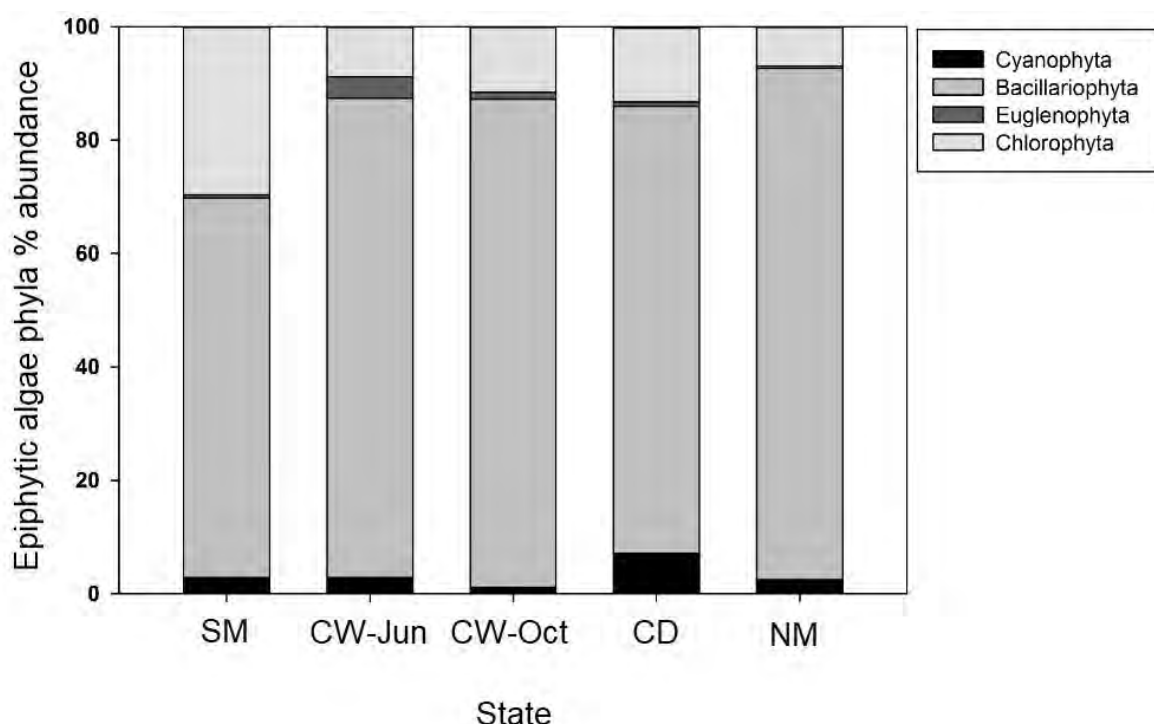


Figure 4.4: Epiphytic algae phyla composition during each state at Westlake River system, South Africa. SM: *Salvinia molesta*-dominated state; CW Jun: clear-water in June; CW Oct: clear-water state in October; CD: *Ceratophyllum demersum*-dominated state, and NM: *Nymphaea mexicana*-dominated state.

CAP ordination revealed four distinct clusters based on Bray-Curtis epiphytic algae assemblage composition similarity, and each cluster represented a different state, with the two periods of clear water analysed together (Figure 4.5). Epiphytic algae community assemblages between different states were significantly different (ANOSIM, $R=0.76$, $P<0.01$). ANOSIM pairwise comparisons further showed strong epiphytic algae assemblage differences, indicative of high separation or difference in assemblage structure between *S. molesta*-dominated state versus clear-water states ($R=0.96$, $P<0.05$); *S. molesta*-dominated state versus *C. demersum*-dominated state ($R=0.94$, $P<0.05$); *S. molesta*-dominated state versus *N. mexicana*-dominated state ($R=0.97$, $P<0.05$); clear-water versus *C. demersum*-dominated state ($R=0.84$, $P<0.05$); *C. demersum*-dominated state versus *N. mexicana*-dominated state ($R=0.99$, $P<0.05$). However, the clear-water state and *N. mexicana*-dominated state showed similarities in epiphytic algae assemblage ($R=0.16$, $P>0.05$) which suggests there were no statistically differences in epiphytic algae composition (Figure 4.5).

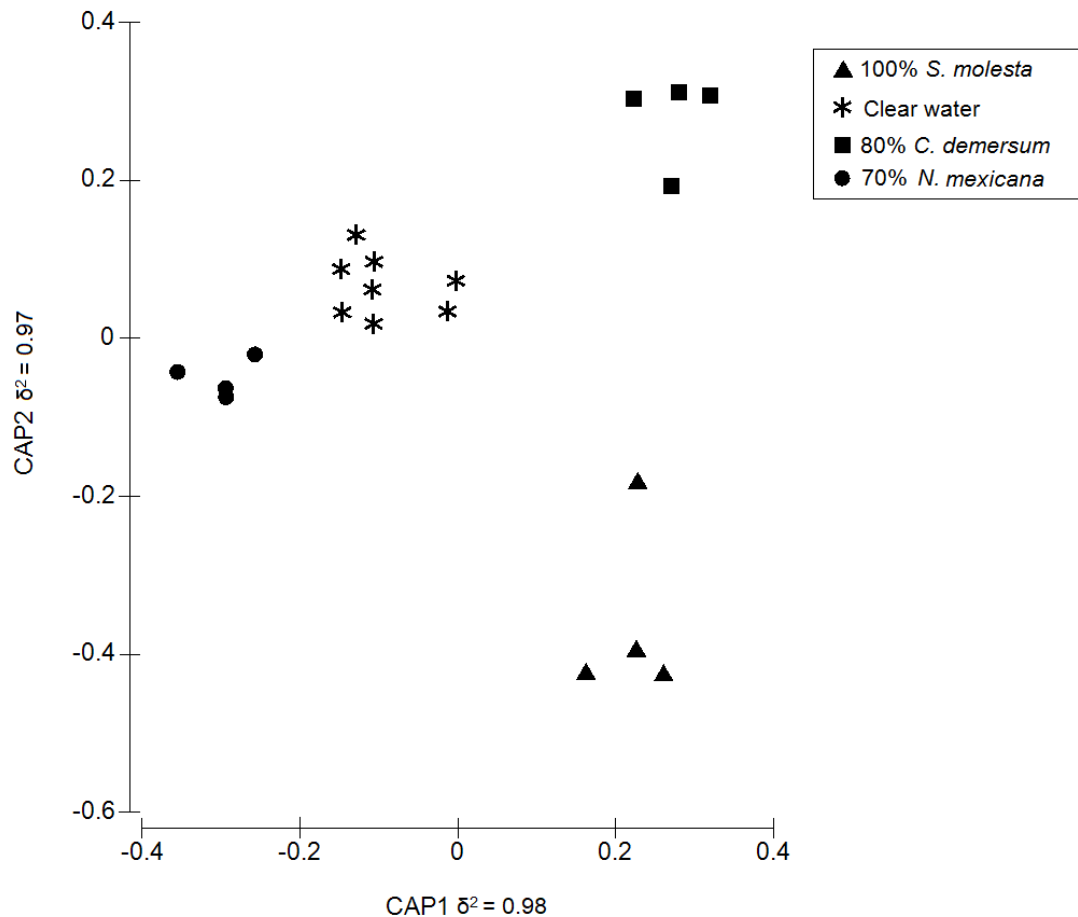


Figure 4.5: Canonical analysis of principal coordinate (CAP) ordination of epiphytic algae community composition (Bray-Curtis similarity) from 20 sampling units of four states (with the clear water state analysed together) at Westlake River system, South Africa.

Epiphytic algae as biological indicator species

Twenty-two epiphytic algae species met the indicator species criteria ($IndVal \geq 50\%$, $P < 0.05$). Twelve of these were indicative of the *S. molesta*-dominated state, four for the clear-water state (both June and October), 12 for the *C. demersum*-dominated state, and nine for the *N. mexicana*-dominated state (Table 4.2). *Pseudanabaena* sp. and *Diademesmis* sp. showed complete indication ($IndVal = 100\%$, $P < 0.05$), followed by *Cocconeis* sp., *Cyclotella* sp., *Gomphonema italicum*, *Gyrosigma rautenbachiae*, *Navicula recens*, *Amphora montana*, *Gomphonema laticollum*, *Nitzschia intermedia*, *Pleurosigma elongatum*, and *Zygnema* sp. which were all regarded as characteristic species for the *S. molesta*-dominated state (Table 4.2). *Sellaphora pupula*, *Gomphonema pseudoaugur*, *Planothidium rostratum*, and *Phacus pleuronectes* were the only indicator species for the clear-water state (Table 4.2). *Nitzschia reversa*, *Anabaena* sp., *Pediastrum tetras*, *Nitzschia palea*, *Nitzschia sublinearis*, *Calothrix parientina*, *Cosmarium*

subcostatum, *Seminavis strigose*, *Cocconeis englebrechtii*, *Fragilaria ulna* var. *acus*, *Navicula antonii*, and *Monoraphidium graffithii* were characteristic species for the *C. demersum*-dominated state (Table 4.2). The floating-leaved *N. mexicana*-dominated state was characterised by *Encyonopsis leei* var. *sinensis*, *Eunotia minor*, *Navicula riediana*, *Gomphonema parvulum*, *Nitzschia* sp., *Gomphonema affine*, *Oscillatoria tenuis*, *Scenedesmus opolienensis* var. *mononensis*, and *Gomphonema venusta* (Table 4.2).

Aquatic macroinvertebrate diversity and assemblage composition

A total of 95 individual aquatic macroinvertebrates taxa, were collected and identified (Table S8). Hemiptera (26%) was the most abundant taxon, followed by Diptera (22%), Annelida (15%), Gastropoda (11%), Ephemeroptera (8%), Odonata (4%), Coleoptera (3%); the least represented was Crustacea (1%). 65 adult male Odonata from three species were recorded during the study, most of them being *Ischnura senegalensis* (Zygoptera: Coenagrionidae), *Trithemis arteriosa* (Anisoptera: Libellulidae) and *Anax imperatus* (Anisoptera: Aeshnidae).

The *S. molesta*-dominated state supported no aquatic macroinvertebrate taxa during the study, whereas the clear-water state in October showed the highest relative abundance of aquatic macroinvertebrates. The clear-water state in October and the *N. mexicana*-dominated state showed similar aquatic macroinvertebrate abundance and the clear-water state in June the lowest abundance (Figure 4.6A). The *C. demersum*-dominated state supported the greatest taxa richness of all states, followed by the clear-water state in June, and then in October while the *N. mexicana*-dominated state showed the lowest taxa richness (Figure 4.6B). Aquatic macroinvertebrate Peilou's evenness showed less variability between states, with both the clear-water state in June and the *C. demersum*-dominated state showing similar evenness, the same was observed for clear-water state in October and *N. mexicana*-dominated state (Figure 4.6C). Shannon diversity index was high during the *C. demersum*-dominated state, followed by clear-water state in June, with the clear-water state in October and *N. mexicana*-dominated state showing equal diversity (Figure 4.6D).

Table 4.2: Indicator value species analysis ($IndVal \geq 50\%$, $P < 0.05$) for important epiphytic algae discriminatory between the states (the 2 clear-water state are considered together) in the Westlake River system, South Africa.

Species/Genera	<i>S. molesta</i> state	Clear-water state (Jun + Oct)	<i>C. demersum</i> state	<i>N. mexicana</i> state
<i>Anabaena</i> sp.			89.90	
<i>Oscillatoria tenuis</i>				65.60
<i>Calothrix parientina</i>			75.00	
<i>Pseudanabaena</i> sp.	100.00			
<i>Amphora montana</i>	68.10			
<i>Cocconeis</i> sp.	75.00			
<i>Cocconeis englebrechtii</i>			67.30	
<i>Cyclotella</i> sp.	75.00			
<i>Diadesmis</i> sp.	100.00			
<i>Encyonopsis leei</i> var. <i>sinensis</i>				97.50
<i>Eunotia minor</i>				92.70
<i>Fragilaria ulna</i> var. <i>acus</i>			66.20	
<i>Gomphonema affine</i>				73.60
<i>Gomphonema italicum</i>	75.00			
<i>Gomphonema laticollum</i>	60.90			
<i>Gomphonema parvulum</i>				81.50
<i>Gomphonema pseudoaugur</i>		56.80		
<i>Gomphonema venusta</i>				53.80
<i>Gyrosigma rautenbachiae</i>	75.00			
<i>Navicula antonii</i>			63.40	
<i>Navicula recens</i>	75.00			
<i>Navicula riediana</i>				84.60
<i>Nitzschia</i> sp.				75.50
<i>Nitzschia intermedia</i>	64.70			
<i>Nitzschia palea</i>			79.20	
<i>Nitzschia reversa</i>			96.30	
<i>Nitzschia sublinearis</i>			76.90	
<i>Planothidium rostratum</i>		59.40		
<i>Pleurosigma elongatum</i>	62.40			
<i>Sellaphora pupula</i>		75.00		
<i>Seminavis strigosa</i>			73.70	
<i>Phacus pleuronectes</i>		58.90		
<i>Cosmarium subcostatum</i>			75.00	
<i>Monoraphidium graffithii</i>			57.60	
<i>Pediastrum tetras</i>			88.70	
<i>Scenedesmus opoliensis</i> var. <i>mononensis</i>				58.00
<i>Zygnema</i> sp.	61.90			

Aquatic macroinvertebrates functional feeding groups (FFGs)

Collector-filter feeders made up more than 50% of the FFGs during the *N. mexicana*-dominated state, followed by the clear-water state in October (44.5%), the clear-water state in June (8.8%) and the *C. demersum*-dominated state (1.5%) which was the lowest. Collector-gatherers were only recorded during the *C. demersum*-dominated state with only 6.7%. Scrapers/herbivores were fairly well represented throughout different states, with the highest percentage of 36.5% recorded during the clear-water state in June, followed by the *N. mexicana*-dominated state with 28.9%, the *C. demersum*-dominated state with 19.7% and the clear-water state in October with 10% percentage abundance. The shredders percentage contribution varied between different states and was only present during the *C. demersum*-dominated state, the clear-water state in June and October with contributions of 6.3%, 3.7% and 0.7%, respectively. Predators, on the other hand, were the dominant group, contributing a relative percentage abundance of more than 50% of all functional feeding groups during the clear-water states in June (55.1%), October (44.8%), and the *C. demersum*-dominated state (65.8%), and about 15% during the *N. mexicana*-dominated state (Figure 4.7). The *C. demersum*-dominated state was more functionally represented with five functional feeding groups, followed by both the clear-water states in June and October, with four groups, and the *N. mexicana*-dominated state with only three groups (Figure 4.7).

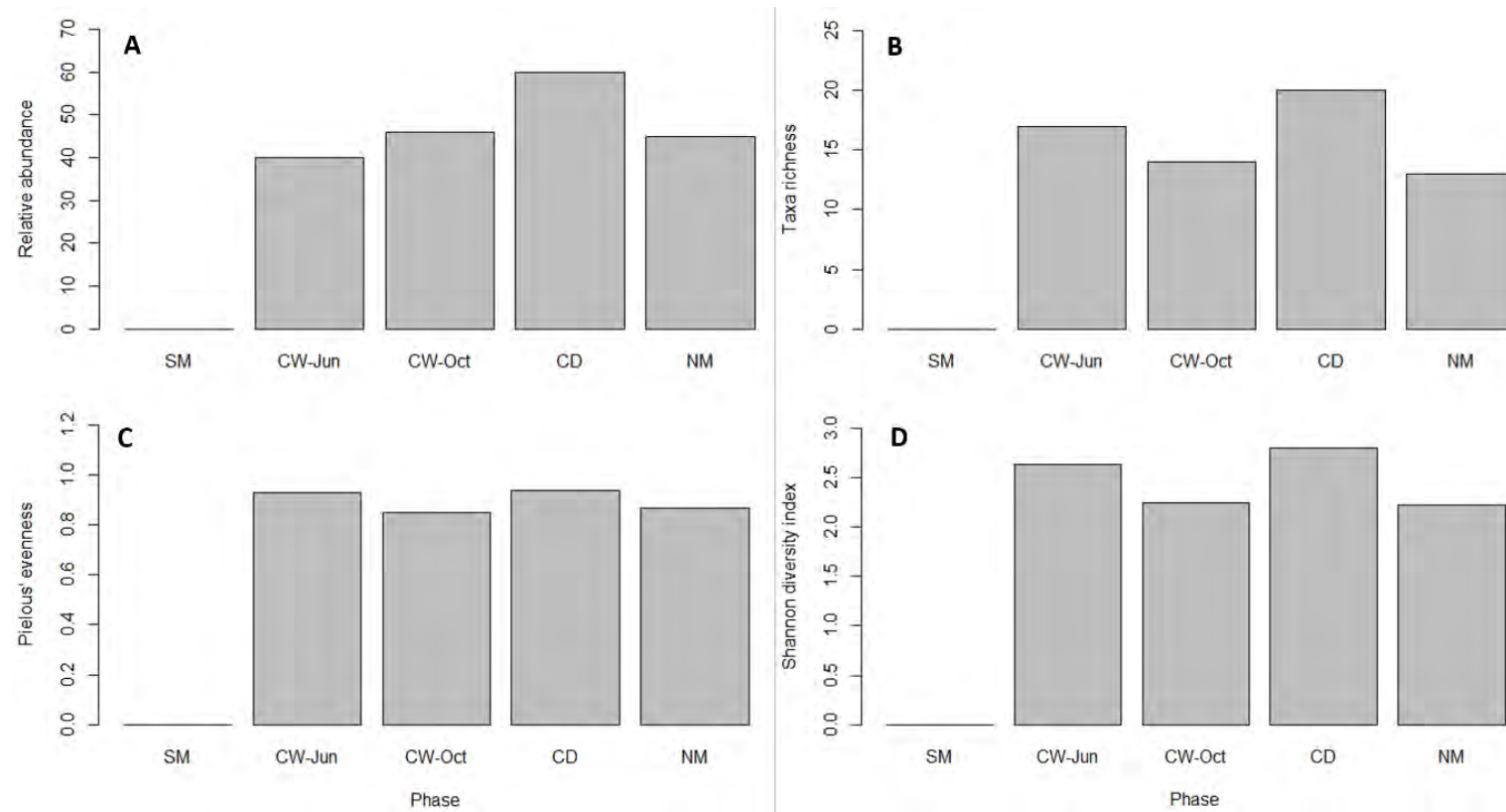


Figure 4.6: Aquatic macroinvertebrate biodiversity indices showing (A) relative abundance, (B) taxa richness, (C) Pielou's evenness and, (D) Shannon diversity during each state at Westlake River system, South Africa. SM: *Salvinia molesta*-dominated state; CW Jun: clear-water in June; CW Oct: clear-water state in October; CD: *Ceratophyllum demersum*-dominated state, and NM: *Nymphaea mexicana*-dominated state.

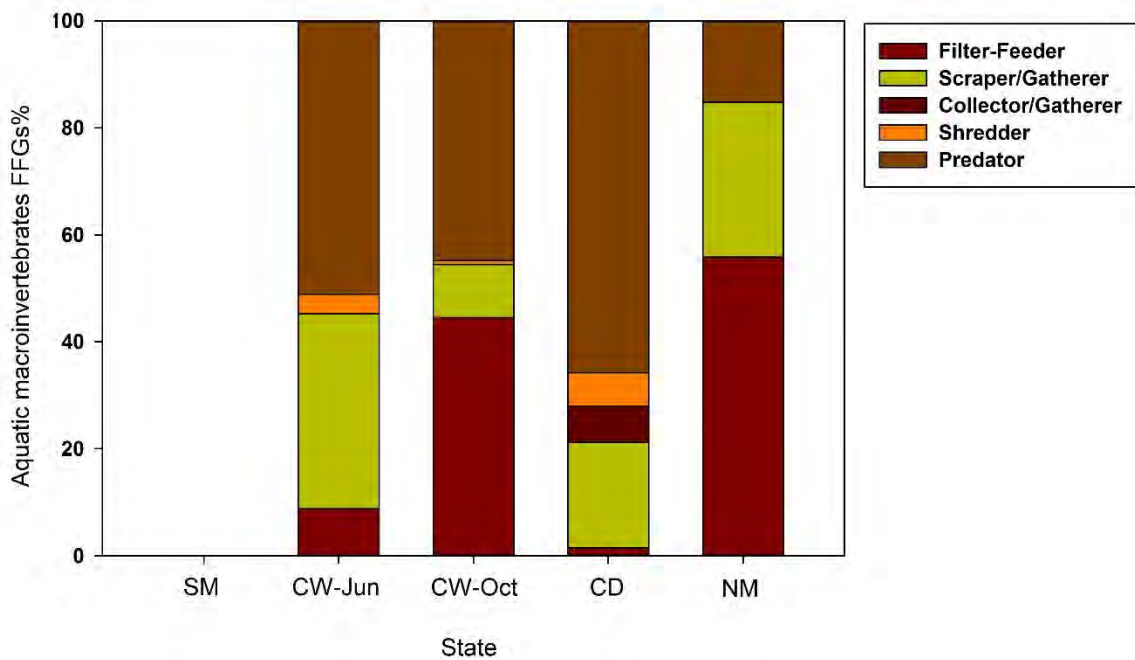


Figure 4.7: Aquatic macroinvertebrates Functional Feeding Groups (FFGs) during different state at Westlake River system, South Africa. SM: *Salvinia molesta*-dominated state; CW Jun: clear-water in June; CW Oct: clear-water state in October; CD: *Ceratophyllum demersum*-dominated state, and NM: *Nymphaea mexicana*-dominated state.

Aquatic macroinvertebrate assemblage structure

The hierarchical analysis of Bray-Curtis similarities illustrated three distinct clusters (Figure 4.8). Cluster one, the *S. molesta*-dominated state, was completely different and showed no aquatic macroinvertebrate assemblages, thus was completely different from the other states. In comparison, the clear-water states (June and October), the *C. demersum*-dominated state and *N. mexicana*-dominated state showed 52% similarity in aquatic macroinvertebrate assemblage (Figure 4.8). Furthermore, the clear-water state in June and the *C. demersum*-dominated state were 58% similar in aquatic macroinvertebrate assemblage structure, while the clear-water state in October and *N. mexicana*-dominated state demonstrated 68% aquatic macroinvertebrate similarity, thus forming cluster two and three respectively (Figure 4.8).

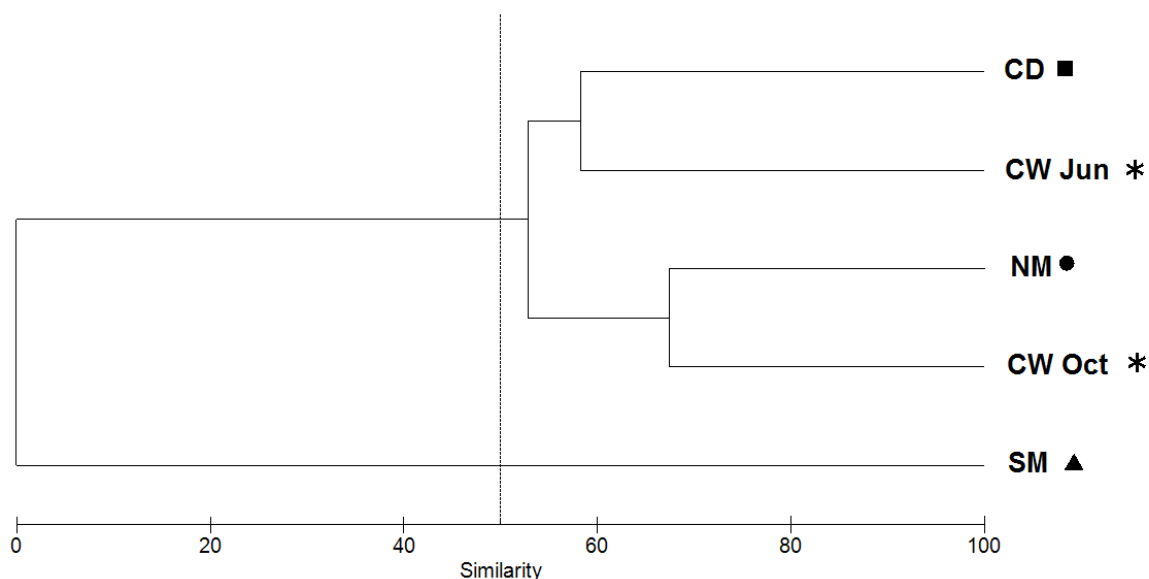


Figure 4.8: Cluster graph based on aquatic macroinvertebrate community assemblage (Bray-Curtis similarity) across five states at Westlake River system, South Africa. SM: *Salvinia molesta*-dominated state; CW Jun: clear-water state in June; CW Oct: clear-water state in October; CD: *Ceratophyllum demersum*-dominated state, and NM: *Nymphaea mexicana*-dominated state.

Westlake ecosystem community structure and functioning

The presence of free-floating *S. molesta* reduced the water clarity and light penetration, thereby altering the energy transfer to higher trophic levels leading to a total loss of aquatic macroinvertebrates and submerged macrophytes. *Salvinia molesta* sequestered water nutrients, leading to an increase in mat cover and thickness, and this contributed to increased suspended organic matter, phytoplankton Chl-*a* concentration (as also seen in Chapter 3) and sediment organic matter due to *S. molesta* decaying organic material (Figure 4.9A). Phytoplankton and periphyton Chl-*a* concentration were surprisingly high during the *S. molesta*-dominated state (Figure 4.9A). Following the mechanical removal of the dense *S. molesta* mat, the clear-water state (June and October collectively) experienced increased light penetration and water clarity which might have led to normal ecosystem processes e.g. photosynthesis and aerobic respiration (Table 4.1). More light and available water nutrients supported phytoplankton and periphyton development, and thus a shift in microalgae assemblage structure (Table 4.2). Increased aquatic biota re-colonisation including aquatic macroinvertebrates and freshwater fish, mainly the *Gambusia affinis* (Poeciliidae: mosquito fish) and *Oreochromis mossambicus* (Cichlidae: tilapia) were also observed during the clear-water state, thus increasing aquatic diversity. During the clear-water state, the presence and diversity of aquatic organisms

contributed to an increase in trophic interactions e.g. top-down effect, phytoplankton and periphyton herbivory. This interaction led to an increase in available water nutrients, while some were reallocated into the sediments (Figure 4.9B).

The *C. demersum*-dominated state followed the clear-water state, and this state was supported by increased light penetration and available water nutrients. Phytoplankton was competing for both light and nutrients with the submerged *C. demersum*, resulting in reduced phytoplankton Chl-*a* concentration, whereas the periphyton benefited through shelter provided by the submerged macrophyte (increased biomass) (Figure 4.10A). Similarly, the *C. demersum*-dominated state further supported aquatic macroinvertebrate biological and functional diversity. Although the state supported high periphyton Chl-*a* concentration and aquatic biota, periphyton herbivory by aquatic biota scrapers/herbivores was minimal. The submerged *C. demersum* utilised available nutrients with some being further reallocated into the sediment (Figure 4.9A). With high sediment nutrients and reduced water nutrients, a floating-leaved rooted emergent IAAP, *N. mexicana*, was able to substitute the submerged *C. demersum*-dominated state, through sediment nutrient assimilation and thus, outgrow the submerged species (Figure 4.10B). *Nymphaea mexicana* provided different strata for periphyton growth compared to the other vegetated state. Phytoplankton was out-competed by *N. mexicana* for light and nutrients, as seen in other vegetated states, periphyton also benefited from the substrate diversity provided by the floating-leaved emergent non-native macrophyte. Aquatic microalgae herbivory was highly effective, leading to a reduction in periphyton Chl-*a* concentration compared to other vegetated states (Figure 4.10B).

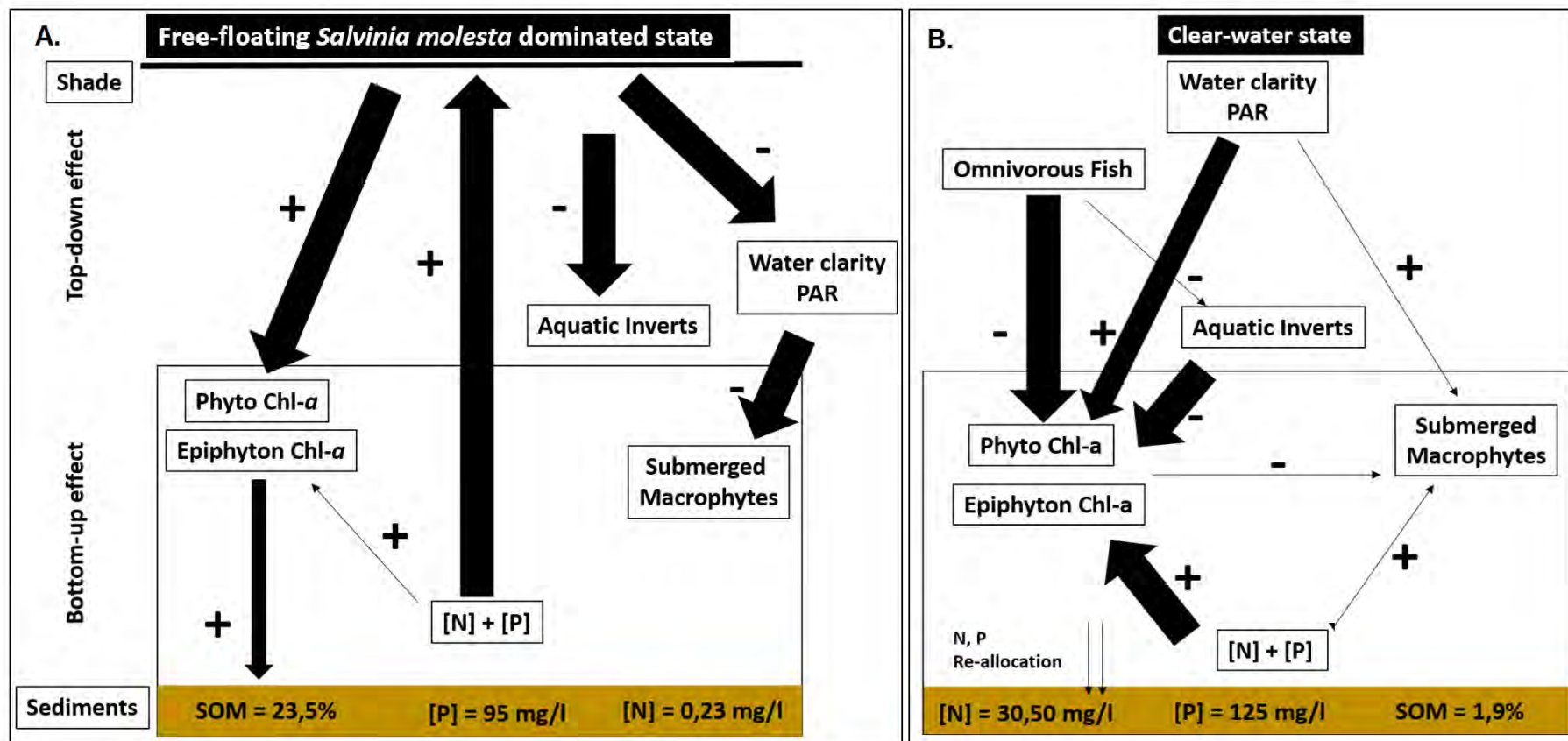


Figure 4.9: Major trophic interactions in Westlake River system, South Africa. (A) represents a summary of interactions in the *Salvinia molesta*-dominated state and, (B) the clear-water state (June and October). Arrows indicate the direction and magnitude of the effect (+ positive or - negative). The bottom half on each diagram focuses on bottom-up trophic effects related to nutrients and the top half shows the top-down trophic effects related to herbivory and predation.

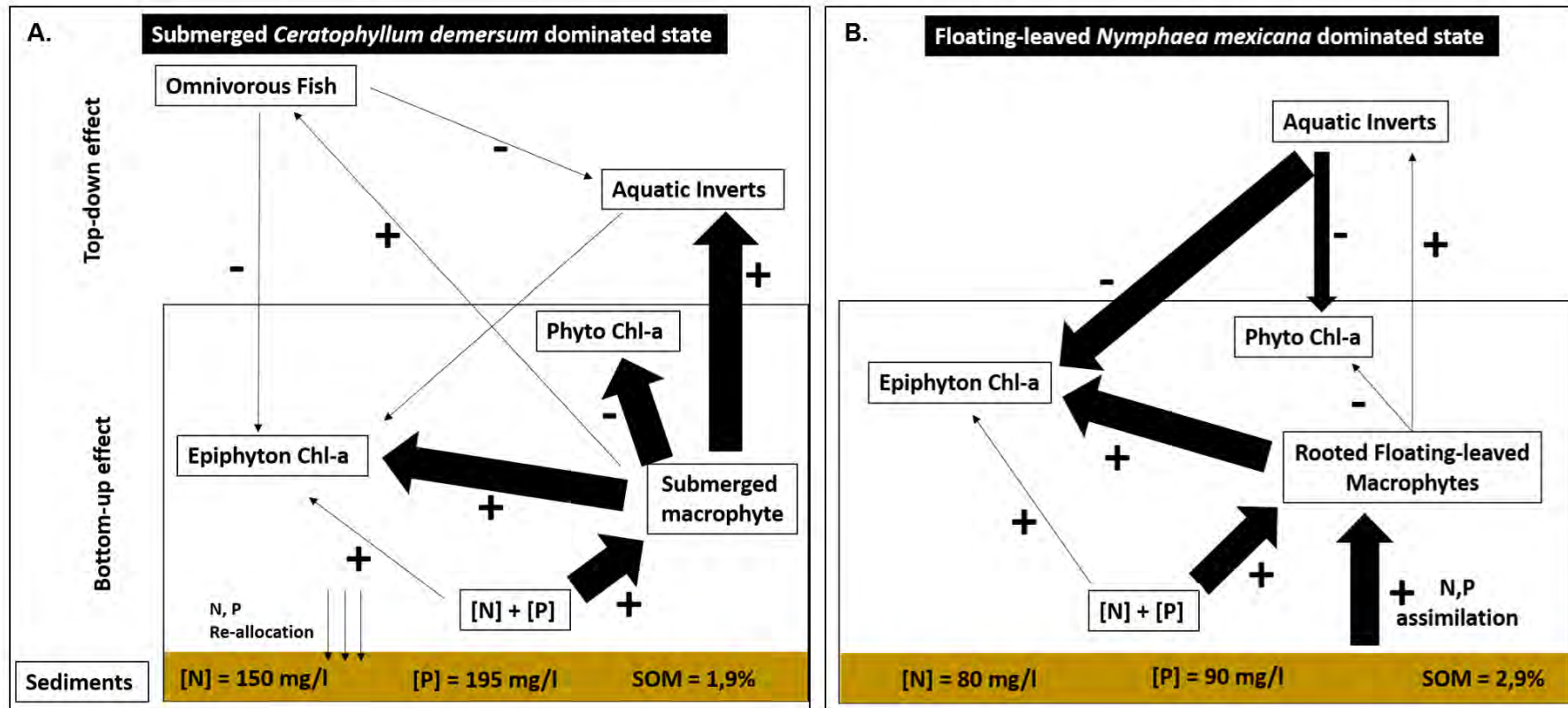


Figure 4.10: Major trophic interactions in Westlake River system, South Africa. (A) represents a summary of interactions in *Ceratophyllum demersum*-dominated state and, (B) the *Nymphaea mexicana*-dominated state. Arrows indicate the direction and magnitude of the effect (+ positive or - negative). The bottom half on each diagram focuses on bottom-up trophic effects related to nutrients and the top half shows the top-down trophic effects related to herbivory and predation.

Trophic food web structure

The *S. molesta*-dominated state supported no aquatic organisms except epiphytic algae, thus no trophic interactions were recorded during the state (Table 4.3, Figure 4.11 & 4.12). The clear-water state in October showed the largest nitrogen range (NR_b), indicative of a long trophic length, and the longest food chain. The *N. mexicana*-dominated state showed moderate trophic length; the *C. demersum*-dominated state and clear-water state in June recorded the shortest trophic length (low NR_b) (Table 4.3, Figure 4.11). Similarly, the clear-water state in October recorded high carbon range (CR_b) and the *N. mexicana*-dominated state showed the lowest, suggesting diverse primary/basal resources and narrower primary resource utilisation, respectively (Table 4.3, Figure 4.11). In combination, the high CD_b and TA_b (trophic diversity: Table 4.3, Figure 4.11) and high $SEAc$ (niche width: Figure 4.12) recorded during the clear-water state in October supported by high CR_b and NR_b ranges, suggest that during the clear-water state in October, the system was dominated by diverse trophic aquatic interactions: a variety of primary resources and specialist feeders, indicating a mature, complex, stable and functioning ecosystem (Table 4.3, Figure 4.8B). However, this was the opposite for the clear-water state in June and the *C. demersum*-dominated state (Table 4.3, Figure 4.11). The clear-water state in October and the *N. mexicana*-dominated state had the highest Mean Nearest Neighbour Distance values ($MNND_b$), showing the greatest divergence within the trophic level with the least trophic redundancy (Table 4.3, Figure 4.11), as compared to the *C. demersum*-dominated state and clear-water state in June that illustrated low $MNND_b$, indicative of increased trophic redundancy and less divergence (Table 4.3, Figure 4.11). The clear-water state in June and the *C. demersum*-dominated state had the lowest measure of evenness of spatial density ($SDNND_b$); the *N. mexicana*-dominated state was moderate and the clear-water state in October was high. These findings indicate an even distribution of trophic level organisms in isotopic space during the clear-water state in June compared with the clear-water state in October (Figure 4.11, Table 4.3).

Table 4.3: Layman's Bayesian community-wide metrics comparing aquatic organisms from the Westlake River system between five sampling phases (97.5% CI). Abbreviations: NR_b – nitrogen range, CR_b – carbon range, TA_b – indication of total community niche area, CD_b – trophic diversity and species spacing, MNND_b – mean nearest neighbour values, SDNND_b – measure of evenness of spatial density and packing.

Layman's matrix	<i>S. molesta</i> state	Clear-water state June	Clear-water state October	<i>C. demersum</i> state	<i>N. mexicana</i> state
NR _b	0	0.96 (0.97-0.944)	2.39 (2.41-2.36)	0.98 (0.99-0.96)	1.52 (1.53-1.50)
CR _b	0	0.74 (0.76-0.73)	1.09 (1.12-1.07)	0.84(0.86-0.83)	0.57 (0.59-0.56)
TA _b	0	0.29 (0.30-0.29)	1.17 (1.19-1.12)	0.31 (0.32-0.30)	0.25 (0.26-0.24)
CD _b	0	0.45 (0.45-0.44)	0.95 (0.96-0.94)	0.47 (0.48-0.47)	0.65 (0.66-0.64)
MNND _b	0	0.46 (0.46-0.45)	0.93 (0.94-0.92)	0.47 (0.48-0.46)	0.81 (0.82-0.80)
SDNND _b	0	0.21 (0.21-0.20)	0.52 (0.53-0.50)	0.25 (0.26-0.5)	0.31 (0.31-0.30)

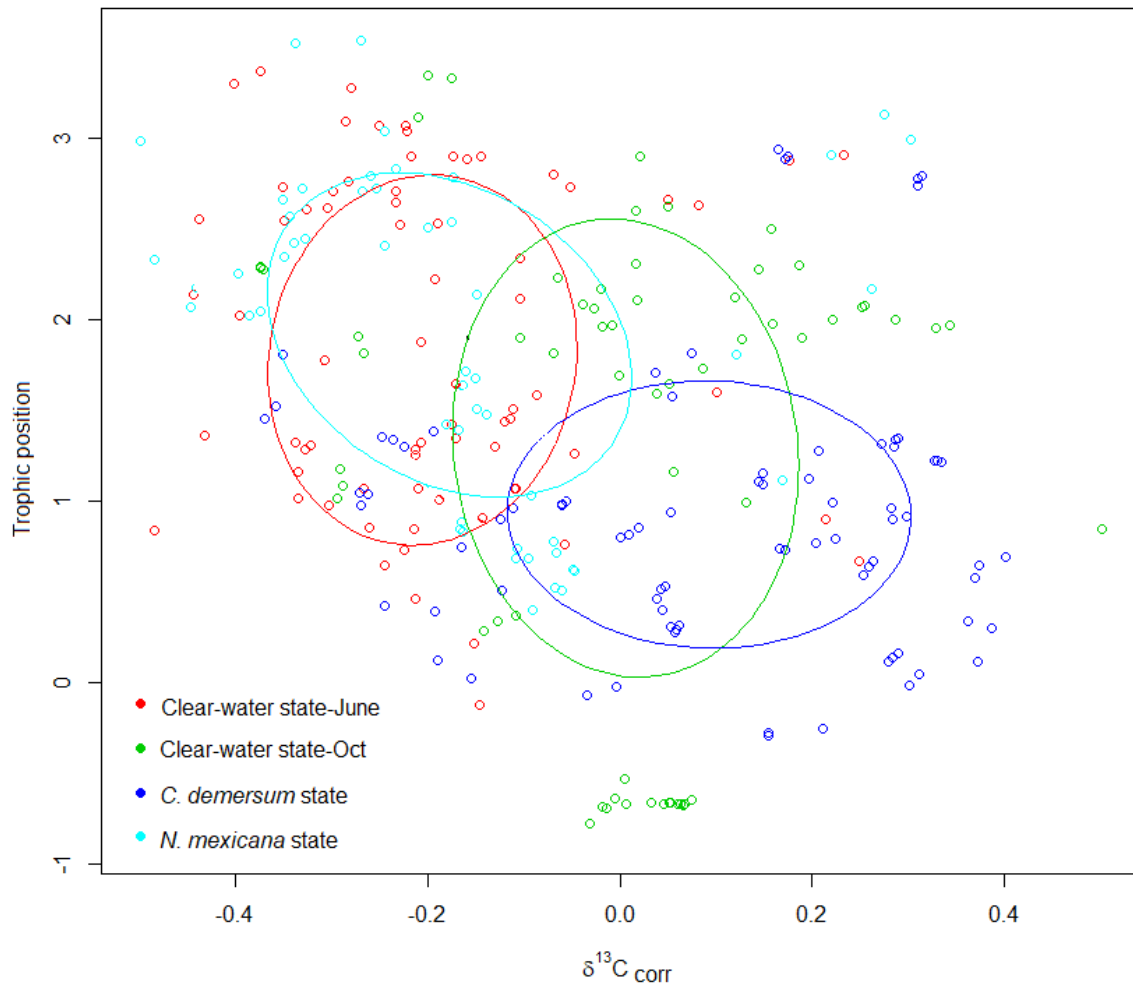


Figure 4.11: Core niche width of aquatic communities based on trophic position (TP) and corrected $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{corr}}$) for each state at Westlake River system, South Africa.

The clear-water state in October illustrated the largest standard ellipse area (SEAc), trophic food web length and resource utilisation, followed by the clear-water state in June (Figure 4.12). These results supported the Layman's metrics results (Table 4.3, Figure 4.11). Interestingly the core niche area of the June clear-water state was closer to that of the *N. mexicana*-dominated state than the October clear-water state. The clear-water state community niche area in June showed 14.5% overlap with the clear-water state community niche area in October; 0.9% with the *C. demersum*-dominated state community niche area; and 42.9% with the *N. mexicana*-dominated state community niche area (Table 4.3, Figure 4.11). The clear-water state in October showed 85.5% trophic level and structure similarity to the clear-water state in June; 35.8% to the *C. demersum*-dominated state; and 20.7% to the *N. mexicana*-dominated state (Table 4.3, Figure 4.11). The *C. demersum*-dominated state was 99.1%, 64.2%

and 3.7% similar to the clear-water state in June, clear-water state in October and *N. mexicana*-dominated state respectively (Table 4.3, Figure 4.11). The *N. mexicana*-dominated state was similar to the clear-water state in June (57.3%), clear-water state in October (79.3%) and *C. demersum*-dominated state (96.3%) (Table 4.3, Figure 4.11).

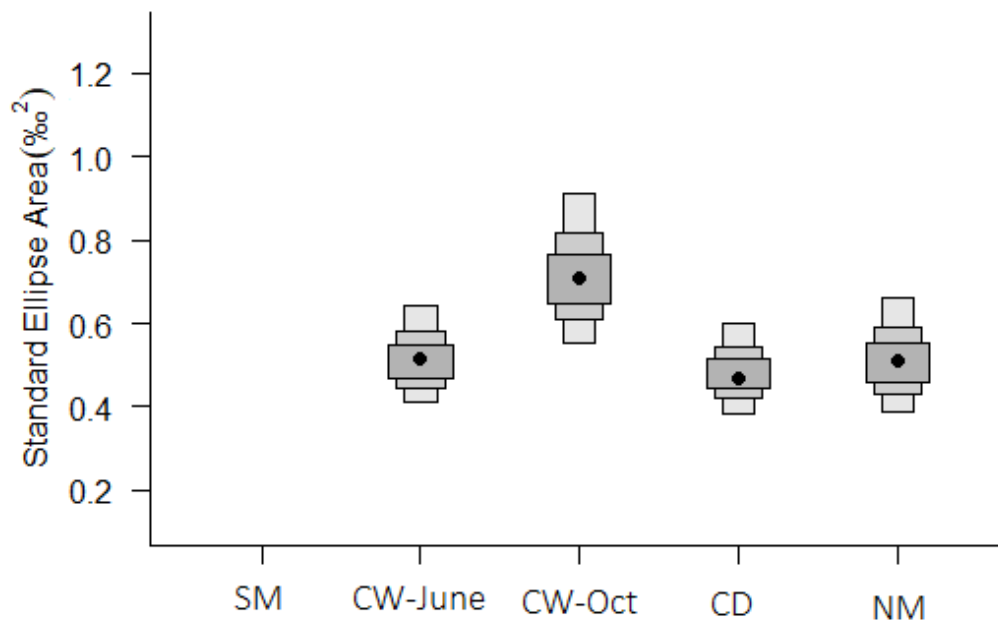


Figure 4.12: Density plots showing the confidence interval of the standard ellipses areas (SEA_c) for the dominant ecological state at Westlake River. The black points correspond to the mean standard ellipse area for each community following the dominant state. The grey to light grey-boxed areas reflect the 95, 75 and 50% confidence interval for the overall aquatic community niche area respectively.

Table 4.4: The core isotopic niche area (SEAc) and percent overlap between five different states at Westlake River. Columns indicate the state niche area being overlapped, e.g. 14.5% of the clear-water state June community niche is overlapped by the clear-water state in October, while 85.5% of the clear-water state community niche is overlapped by the clear-water state community niche. The *Salvinia molesta*-dominated state did not support any aquatic macroinvertebrates, thus no community interaction and community niche area.

State	SEAc	<i>S. molesta</i>	Clear-water Jun	Clear-water Oct	<i>C. demersum</i>	<i>N. mexicana</i>
<i>S. molesta</i>	-	-	-	-	-	-
Clear water Jun	0.55	-	-	85.5	99.1	57.3
Clear water Oct	0.72	-	14.5	-	64.2	79.3
<i>C. demersum</i>	0.45	-	0.9	35.8	-	96.3
<i>N. mexicana</i>	0.53	-	42.6	20.7	3.7	-

4.4 Discussion

Macrophyte form and functional traits vary in their contribution to aquatic ecosystem structure and processes. This phenomenon is well demonstrated by Cattaneo et al. (1998), who reported different aquatic communities between a submerged and floating-leaved macrophyte species in a freshwater lake in Italy; while Phiri et al. (2007) and Phiri et al. (2011a; b) reported that characteristic epiphytic algae and aquatic macroinvertebrate taxa respond positively to different macrophyte forms in a freshwater lake in Zimbabwe. This was in agreement with the present study's hypothesis, that each macrophyte state will demonstrate species-specific abiotic and biotic characteristics, and trophic interactions. Additionally, the introduction of an IAAP species with different growth forms and traits compared to those of native macrophytes species can facilitate stochastic events that may have serious and irreversible consequences for aquatic ecosystems (Rejmánková et al., 2018), and this was true for the present study, where the removal of *S. molesta* resulted in a regime-shift that led eventually to a secondary invasion of a new IAAP species, *N. mexicana*.

Photosynthetically active radiation, nitrate concentrations, conductivity and phytoplankton Chl-*a* concentration were identified as important environmental variables, which changed significantly among states. The *S. molesta*-dominated state, like other free-floating IAAP species, was responsible for reduced light penetration and water clarity (Stiers et al., 2011; Stiers and Triest, 2017), and elevated phytoplankton Chl-*a* concentrations, also observed in Chapter 3. Following the Lakatos (1989) aquatic microalgae trophic classification index, <0.10% of the observed phytoplankton and periphyton Chl-*a* concentrations during the *S. molesta* infestation were composed of different microalgae assemblages, indicating that composition was instead dominated by detritus and bacteria, which was similar to the findings of Cattaneo et al. (1998), and the findings described in Chapter 3.

The clear-water state (June and October) and *N. mexicana*-dominated state were characterized by high water clarity and increased light penetration and comparatively different from the *S. molesta*-dominated state and the *C. demersum*-dominated state, resulting in different aquatic microalgae community composition. After the removal of *S. molesta*, there was an increase in nitrate concentration, water clarity, and subsequent light penetration (PAR). These environmental variables promoted the establishment of *C. demersum*, which used up available nutrients, outcompeted phytoplankton, and subsequently provided suitable substrates for epiphytic algae. The *C. demersum* leaf architecture were able to effectively trap available suspended particulate organic matter left behind by the *S. molesta* removal. Submerged

macrophytes are known to stabilise and promote clear-water states of some shallow lake ecosystems by suppressing phytoplankton states (Hilt et al., 2018). In line with the present study, Song et al. (2019) also reported that submerged macrophytes are more effective in reducing phytoplankton Chl-*a* concentration and providing periphyton substrate as compared to other macrophytes forms, thus increasing the available ecosystem net productivity in terms of periphyton Chl-*a* concentration. The clear-water state (June and October) and the emergent *N. mexicana*-dominated state presented ideal and good water quality characteristics. Positive changes following the removal of *S. molesta* i.e. increased water clarity, PAR and DO concentration, are regarded as important factors for freshwater ecosystem recovery as observed in Chapter 3 and also for the restoration of temperate shallow lakes, among others (Bakker et al., 2013). The present study showed that the clear-water states, following the removal of *S. molesta* infestations, were sufficient to facilitate ecosystem self-reorganisation in terms of structure and function (although results were not seen until October, see discussion below on trophic ecology), during which time active introduction of native macrophyte propagules could be useful to promote ecosystem recovery. However, although extremely high, both the June clear-water state and the *N. mexicana*-dominated state were associated with partial increases in sediments ammonium and sulphur, which according to Bakker et al. (2013), under certain circumstances can be toxic to aquatic macrophyte species, if that is the case sediment rehabilitation may be required prior to active restoration interventions. During the *N. mexicana*-dominated state (in September 2017), the observed invasive plant cover was ~70%, showing similar physicochemical characteristics to that of the clear-water state in June. Following routine monitoring on the site in March 2019, the Westlake River system was reported to be 100% covered with IAAP species *N. mexicana* (Julie Coetzee pers. com.), therefore it is anticipated that ecological conditions similar to the original *S. molesta* infestation (e.g. reduced light penetration, water clarity and biodiversity of aquatic macroinvertebrates) now prevail, resulting in an IAAP dominated state.

Phytoplankton competes with macrophytes for nutrients and light, and in the present study, phytoplankton Chl-*a* concentration was lower during macrophyte-dominated states. This interaction was also described by Zimmer et al. (2003), Sánchez et al. (2010), Phillips et al. (2016), and Hilt et al. (2018) where submerged macrophytes outcompeted phytoplankton for light and nutrients. Although submerged macrophytes can be restricted by phytoplankton due to shading, macrophytes and periphyton can sequester nutrients from the water column, particularly phosphorus, more effectively than phytoplankton, while phytoplankton have the competitive advantage when it comes to light. Even with a limited understanding of

phytoplankton, periphyton and macrophyte interactions (Sánchez et al., 2010), the present study provides useful insight into the expected interactions during different macrophyte states, including IAAP species. Only during the *S. molesta*-dominated state was phytoplankton Chl-*a* concentration high; thereafter phytoplankton diversity was reduced through competition with *C. demersum*, and then *N. mexicana*, and by aquatic macroinvertebrates and fish grazing (*O. mossambicus*) during the clear-water states.

This study detected changes in epiphytic algae assemblage structure, and although macrophyte complexity and architecture was not evaluated in this study, it is well understood to be a driving factor which influences epiphyton functional and biological diversity (Cattaneo et al., 1998; Phiri et al., 2007a; Phiri et al., 2011b). The clear-water state in June supported high epiphytic algae species richness, Pielou's evenness and Shannon diversity index compared to the free-floating *S. molesta*-dominated state, the cosmopolitan submerged *C. demersum*-dominated state and the floating-leaved emergent *N. mexicana*-dominated state throughout the study and the clear-water state in October being the least. Increased water transparency, available water nutrient and sunlight penetration during the clear-water state in June provided optimum physicochemical conditions for algal recruitment and diversity (De Tezanos Pinto et al., 2007). Comparisons of epiphytic biological diversity between vegetated states showed the *N. mexicana*-dominated state had high epiphyte diversity, similar to the findings of Cattaneo et al. (1998), where higher epiphyte diversity was observed on floating-leaved *Trapa natans* than on those of submerged *C. demersum*. The similar macrophyte form (submerged stems and floating leaves) in the present study (*N. mexicana*-dominated state) and that of Cattaneo et al. (1998) (*T. natans*) provided a diverse substrate with different morphology and different exposures to light, favouring a co-dominance of several epiphyte species. Epiphyte taxonomic composition was different between states and was not limited by plant architecture, but rather by physical characteristics. Bacillariophyta were dominant throughout the study, with significant reductions during the *S. molesta*-dominated and *C. demersum*-dominated states. Euglenophyta were only dominant during the clear-water state. Cyanophyta on the other hand are known for their preference for low dissolved oxygen and shade conditions, and this was illustrated by their association with the submerged *C. demersum*-dominated state (Padisák et al., 2009). Of the nine blue-green algae species (Cyanophyta) recorded during the study, seven species (i.e. *Anabaena* sp., *Anabaena spiroides*, *Merismopedia glauca*, *Oscillatoria limosa*, *Oscillatoria tenuis*, *Pseudanabaena* sp. and *Spirulina maior*) are known to produce cyanotoxins. Apart from their wide diversity, blue-green algae are undesirable from a water quality perspective because they can produce potent toxins known to be health hazards for

aquatic life, and both human and wildlife (Dalu and Wasserman, 2018). Most of these species were dominant during the *C. demersum*-dominated state, likely attributable to epiphyte trapping by *C. demersum* leaf architecture, which can also limit herbivory by both aquatic macroinvertebrates and freshwater fish due to the whorl leaf orientation of *C. demersum* compared to the flat and smooth grazing surface of *N. mexicana* species (Cattaneo et al., 1998). Herbivore limitation is a common phenomenon in aquatic ecology: closely packed, whorl-set leaves similar to those of *C. demersum* have been observed to limit epiphyte herbivory potential (Cattaneo et al., 1998; Schultz and Dibble, 2012). Dalu and Wasserman (2018) showed that environmental variables, such as high pH, low nitrate concentrations and water transparency, favoured and promoted growth of blue-green algae. Together with macrophyte dominance and herbivory limitations, this study showed similar blue-green algal responses. Therefore, dense mats of *C. demersum* likely provided habitat complexity, which led to increases in aquatic macroinvertebrate diversity, but limited aquatic macroinvertebrate herbivory.

Epiphytes are also considered reliable biological indicators of water quality, and this was true in the present study, where not only was plant architecture important, but also water chemistry. Chamier et al. (2012) identified the dominance of blue-green algae in aquatic ecosystems as an early warning indicator of nutrient enrichment. During the study, epiphytes provided more time-integrated water quality information compared to the water chemistry spot analysis (as did Phiri et al., 2007). While green algae were present only when nitrate concentrations were low, blue-green algae were present throughout the study, but were more abundant with low phosphate concentrations.

Habitat structural complexity plays an important role in biotic community structure (Cattaneo and Kalff, 1980; Palmer et al., 2010; Schultz and Dibble, 2012) while this was true for aquatic macroinvertebrates, it was not the case for epiphyton biodiversity, however. Aquatic macroinvertebrate biological and functional diversity were higher in the *C. demersum*-dominated state, likely correlated with an increase in habitat complexity and structure provided by the submerged macrophytes. The submerged macrophyte, *C. demersum* provided a highly structured habitat, and thus supported high aquatic macroinvertebrate taxa richness and diversity relative to the clear-water states (June and October), *N. mexicana*-dominated and *S. molesta*-dominated states. Similar results have been documented for other submerged macrophytes, for example, *Hydrilla verticillata* dominated areas such as parts of Lake Tanganyika (Copeland et al., 2012) and Lake Tutira, New Zealand (Schultz and Dibble, 2012) and in artificial ponds in Zimbabwe (Phiri et al., 2011). Comparatively, free-floating macrophytes such as duckweed or aquatic ferns (*Azolla* sp. and *Salvinia* sp.) generally provide

less favourable habitats because of the much lower complexity of their simple root structures (Fontanarrosa et al., 2013). This was also seen in the present study where the composition of aquatic macroinvertebrate FFGs corresponded to different states. There was a steady increase in collector-filter feeders due to available particulate organic matter during the clear-water states in June to October. The increase in collector-filter feeders was accompanied by a significant decline in shredders and scrapers in the absence of palatable indigenous plant litter following the removal of the invasive *S. molesta* species and limited epiphyton stock. Subsequently, there was a slight decline in predator percentage abundance. However, the eventual replacement of *C. demersum* by the rooted emergent floating-leaved *N. mexicana* state did not provide structure to hide from predators, resulting in their drastic decline. Scrapers and collector-filter feeders were the two main players during the *N. mexicana*-dominated state, which consisted of open water patches for collector-filter feeders and smooth macrophytes stems and leaves enhancing herbivory on epiphyte growth. Thus, the positive relationship between epiphyton and aquatic macroinvertebrate dynamics echoes a vital role played by periphyton as basal resources and an important component for aquatic food webs.

The presence *S. molesta* did not have negative effects on epiphytic algae species diversity; however, aquatic macroinvertebrate assemblages were completely absent. Even though some IAAP species contribute to structural complexity of the system through their root structure such as water hyacinth for example, studies by Masifwa et al. (2001), Midgley et al. (2006), Schultz and Dibble (2012), Coetzee et al. (2014) and Gezie et al. (2018) reported a significant decline in aquatic macroinvertebrate diversity in the presence of water hyacinth. Loss of aquatic macroinvertebrates in the present study was most likely related to *S. molesta* residence time and *S. molesta* mat which was estimated to be 20 cm thick. This was also the case in the mesocosm study (Chapter 2), where the *S. molesta* residence time of 54 weeks drastically reduced the relative abundance, taxa richness, and diversity of aquatic macroinvertebrates. Overall, the present study acknowledges that ecological recovery followed immediately (~30 days) after the removal of *S. molesta* species, where a rapid improvement in water quality was seen in the clear-water states, and diverse biological and functional characteristics of healthy ecosystems were eventually met by October, in the second clear-water state.

Macrophytes play an important structuring role in aquatic ecosystems, maintaining a clear-water state and in retaining nutrients (Bakker et al., 2013). Clear-water and nutrient retention are desirable abiotic conditions that underpin healthy levels of aquatic biodiversity and ecosystem structure and functioning, thus sustaining ecosystem goods and services.

However, in the presence of IAAP species, there is a high chance of re-invasion and secondary invasion following the management of the primary invader, (e.g. Kettenring and Adams, 2011; Prior et al., 2018) reported that more than 30% of their reviewed studies on IAAP removal and ecosystem recovery resulted in unintended outcomes, including secondary invasion. Additionally Strange et al. (2018) reported that the biological control of free-floating IAAP species can contribute to secondary invasion by submerged IAAP species. As seen in the present study, ecosystem regime-shifts driven by IAAP invasion can alter the aquatic basal resources available and consequently aquatic food web structure, which complicates restoration efforts in previously invaded ecosystems (see also Strange et al., 2018; 2019). If we consider the food web structure as a whole, this study showed that the clear-water state in October was more robust, stable and had a more complex ecosystem structure, supported by diverse trophic levels and basal resources, with large food web and community trophic niche widths, and with diverse trophic levels compared to non-native-vegetated dominated states and the cosmopolitan *C. demersum*-dominated states. It is likely that we are seeing some sort of trophic ecosystem succession, with the community niche space shifting in isotopic space, reflecting a change in resource utilisation as different aquatic macroinvertebrate communities establish. Isotopic values of carbon resources were very different in the *C. demersum*-dominated state relative to the June clear-water state and the *N. mexicana*-dominated state, with little to no overlap. Different again were the isotopic values of the trophic community of the October clear-water state, which overlapped more with the *C. demersum*-dominated state than anything else. It is possible then that the June clear-water state was too early in the recovery process to significantly alter trophic community interactions and thus its size and positioning in isotopic space closely resembled that of the *N. mexicana*-dominated state (effectively another floating aquatic invader state). This was followed by a more established recovery in the October clear-water state, where more resources are being used and a larger food web has developed. However, the establishment of the *C. demersum*-dominated state interrupted recovery, and while the scope of utilised resources appears to have increased, the food web has been substantially compressed, suggested fewer steps in the food chain and a reduction in energy transfers. To help disentangle these differences, the clear-water state in June was mostly dominated by generalist and opportunistic aquatic organisms likely supported by the sudden availability of resources and newly developed food webs, which attracted opportunistic and generalist species (high abundance of alien freshwater fish e.g. tilapia and mosquito fishes). The rapid recolonisation by aquatic macroinvertebrates seen in this study (30 days after removal of *S. molesta*) is likely driven primarily by active dispersal of adult fish and aquatic

macroinvertebrate stages, and through drifting for immature life stages (Wallace, 1990). In October clear-water state, the food web structure was more complex, stable and showed fewer trophic level redundancies, likely representing a more developed trophic community which supported specialist aquatic biota (Chapter 4, Layman's metrics). This is a well-known phenomenon in aquatic ecology, explained by Wallace (1990), who emphasise habitat conditions after disturbance control, the clear-water state and elimination of the barriers (shade-effect), nearby sources and hydrological connection which was also true in the present study as contributors to rapid aquatic macroinvertebrates recolonisation. Interestingly, the *C. demersum*-dominated state supported taxa-rich and diverse aquatic macroinvertebrates compared to the clear-water states, suggesting the *C. demersum*-dominated state was convincingly more robust and functionally diverse. However, the additional community-wide metrics analysis highlighted that the clear-water in October reached the most promising ecosystem structure and functioning compared to other states. The *N. mexicana*-dominated state at ~70% cover and clear-water state in October shared relatively similar environmental conditions and aquatic biological and functional diversity based on the Layman's metrics. However, we anticipate negative impacts to both biological and functional diversity at 100% *N. mexicana* cover observed in March 2019.

In conclusion, the clear-water state, in October particularly, represented an ideal, well-structured, functional ecosystem until it was replaced by the submerged *C. demersum*-dominated and later, the rooted emergent *N. mexicana*-dominated state. The mechanical removal of *S. molesta* did bring about temporary ecological recovery and ecosystem re-organisation, but it proved not to be a long-term solution due to macrophyte succession that took place. Thus, a biological control programme followed by active restoration practices (e.g. the introduction of native macrophytes propagules) is recommended, with further investigation on trophic structure and function, IAAP species legacy, including allelopathic effects. This will provide a holistic IAAP species management strategy for ecosystem recovery and restoration of invaded aquatic ecosystems in southern Africa and globally.

CHAPTER 5

General discussion

5.1 Introduction

The studies conducted in this thesis were aimed at quantifying ecosystem recovery and trophic dynamics following the control of a free-floating IAAP species, *S. molesta* and the implications for ecological restoration of freshwater ecosystems. Findings from this thesis clearly supported my hypothesis and that of Masifwa et al. (2001), Midgley et al. (2006) and Coetzee et al. (2014), that the presence of free-floating IAAP species have destructive effects on freshwater ecosystem aquatic biodiversity and assemblage structure. This was evident from results of both the mesocosm study (Chapter 2) and the field study (Chapter 3). This research illustrated substantial ecological recovery following both the mechanical removal and the biological control of *S. molesta*. Aquatic microalgae and macroinvertebrate communities were reliable biological indicators of *S. molesta* impacts and subsequent control, and this was consistent with Coetzee et al. (2020)'s findings which also reported improvement in aquatic macroinvertebrate functional diversity following the biological control of *P. stratiotes*. Furthermore, this research provided evidence-based quantitative assessments and recommendations for improving the application of IAAP species management for ecological restoration, which has been highlighted to be a fundamental requirement, yet lacking in quantitative data by Suding et al. (2004), Kettenring and Adams (2011) and Prior et al. (2018).

The mesocosm study (Chapter 2) highlighted ecosystem recovery following the biological control of *S. molesta*. According to the Society of Ecological Restoration (SER) Primer guidelines to assess ecological restoration success described by Ruiz-Jaen and Aide (2005), the biological control of *S. molesta* successfully assisted in ecosystem recovery, where the restored site/treatment resembled similar aquatic macroinvertebrate community structure and diversity to that of the reference site/treatment. Similar to the mesocosm study, the field study (Chapter 3) demonstrated positive ecosystem recovery trends after successful *S. molesta* control including improved water quality, followed by an increase in aquatic biological and functional diversity and normal ecosystem functioning. Due to the unpredictable nature of IAAP species and South Africa's severely altered freshwater ecosystems, pre-invasion and/or reference site data-sets (with comparative positions within the catchment and physical characteristics to invaded sites) were challenging to obtain. Thus, the mesocosm experiments

have value in illustrating ecological recovery success following *S. molesta* control, with the assumption that the pre-invasion/reference site shared similar landscape characteristics. Additionally, the stable isotope data-set (Chapter 3 & 4) further provided useful insights on trophic interactions during both *S. molesta* invasion and subsequent mechanical and biological control. Trophic interactions included ecosystem structure and functioning, which were estimated quantitatively using Layman et al.'s (2007) guidelines, which further supported the hypothesis that ecosystem structure and functions were directly affected by *S. molesta* infestation. This was expressed through the reduction in ecosystem productivity, and likely exacerbated by limited light penetration due to the shade-effect (abiotic barrier) by the presence of the *S. molesta* mat. *Salvinia molesta* also demonstrated bottom-up trophic effects, by sequestering available water nutrients and this, along with reducing ecosystem primary production, interrupted the energy transfer to higher trophic level organisms, particularly aquatic macroinvertebrates, which are considered a fundamental and transitional aquatic community contributing the largest biomass in aquatic environments (Covich et al., 1999). The energy transfer limitations significantly reduced aquatic macroinvertebrate abundances, diversity and caused biotic homogenisation, reducing functional diversity and aquatic macroinvertebrates resilience, which resulted in local die-off in some cases.

Surprisingly, the mechanical removal of *S. molesta* at the Westlake River site revealed an interesting ecological succession from a free-floating *S. molesta*-dominated state, to a clear-water state (following *S. molesta* removal), followed by a submerged *C. demersum*-dominated state which was later replaced by the floating-leaved, emergent *N. mexicana*-dominated state (Chapter 4). The multiple macrophyte states were responsible for a complete shift in abiotic and biotic characteristics, thus adding to the ecosystem restoration challenges. Findings from this chapter and that of Bakker et al. (2013), suggest that only during the clear-water state did the ecosystem demonstrate desirable biotic and abiotic characteristics for ecological restoration of previously degraded and/or disturbed ecosystems. Thus, the Chapter 4 findings provide evidence that: (1) following IAAP species management (the clear-water state), ecosystem recovery was apparent, but it is at this state that potential secondary invaders can establish; (2) trophic interactions (e.g. ecosystem structure and processes) are key drivers and indicators for ecosystem functional recovery and; (3) the clear-water state provided a good platform for active intervention that will support and sustain the recovered biotic and abiotic characteristics to increase the ecosystem resilience and suppress potential invaders. Furthermore, the multiple macrophyte states affirmed the new era in IAAP invasion, which is secondary invasion in the form of ecological succession in freshwater systems.

5.2 Quantifying the ecological benefits of invasive alien aquatic plant species control

Traditionally, biological control success has been measured through the reduction of the non-native weed biomass or some other plant demographic measure (Coetzee et al., 2011). Cuda et al. (2008) regard biological control success of alien invasive weeds as the establishment of the biological control agents and the impact it has on the weed population. It is evident that the assessment of biological control success is subjective, highly variable and often project-specific. Moreover, recent attempts to quantify biological control success have incorporated socio-economic aspects, where a positive correlation was shown between biological control and water saving following successfully biological control of water hyacinth and red water fern in southern Africa (McConnachie et al., 2003; Fraser et al., 2016; Arp et al., 2017). Güereña et al. (2015) also reported a significant improvement in villagers' livelihoods in East Africa following the control of water hyacinth in Lake Victoria, where these villagers were able to access critical services through boat navigation e.g. health care and markets, following successful weed control. In South Africa, the long-term (>10 years) post-release evaluation of the *S. molesta* biological control programme used reduction in surface area invaded as the measure of success (Martin et al., 2018). Although the study provided a comprehensive review for *S. molesta* biological control, there are very limited case studies to quantify the ecological benefits of the biological control of IAAP species globally, which was the main aim of this thesis. Kettenring and Adams (2011) and Prior et al. (2018) provided quantitative measures of ecosystem recovery following alien weed mechanical removal methods, but not for biological control. In both reviews, the trophic interactions (e.g. ecosystem structure and function) "before and after" IAAP species control were not quantified. Kettenring and Adams (2011) reported that the majority of case studies they reviewed had resulted in mixed success, particularly in the context of ecological restoration. Furthermore, of 151 studies reviewed by Prior et al. (2018), 31% reported no recovery, or worse, negative and unintended outcomes. Thus, this affirms the limited efforts in understanding IAAP ecosystem recovery following management, which should be prioritised in integrative conservation and restoration strategies for freshwater ecosystems (Prior et al., 2018). Instead of stopping after invader removal and allowing for passive native species recovery, biological control practitioners, environmental managers and restoration practitioners should anticipate that in many cases, additional management activities e.g. long-term monitoring and active introduction of native macrophyte propagules, are necessary to ensure viable results in ecosystem recovery.

International environmental laws clearly stipulate that introduction of IAAP species should be prevented, and if prevention fails, an effective control programme should be implemented with the aim of minimising the economic, ecological, and human health effects (Hussner et al., 2017). However, as Kettenring and Adams (2011) state, it is necessary to provide holistic ecosystem level IAAP species management to address the ultimate goals of alleviating the impacts. Although financial implications were not investigated in this thesis, Westlake Conservancy and the City of Cape Town Municipality spent an estimated R150 000 on mechanical removal of *S. molesta* at the Westlake River site (Westlake Conservancy and City of Cape town Municipality per. coms). This amount was 100 times more than what was required to initiate biological control programme on both the Kogmanskloof River and Rosle Farm Reservoir together, which resulted in equal success and a more stable recovery of biodiversity and ecosystem trophic structure.

5.3 Ecosystem recovery dynamics following invasive alien aquatic plant species control

Free-floating IAAP invasion causes abiotic barriers in invaded ecosystems, these barriers (e.g. limited light penetration), alter the aquatic community structure and cause biotic homogenisation of aquatic communities, where only tolerant and generalist species proliferate, displacing specialised and functionally important aquatic species. Secondly, these abiotic barriers negatively affect water quality (*viz.* increase suspended organic matter, decrease water clarity and dissolved oxygen and increase carbon dioxide concentrations causing anoxic conditions), as well as the quality and quantity of ecosystem basal resources for aquatic organisms (De Tezanos Pinto et al., 2007). De Tezanos Pinto et al. (2007), in a lake mesocosm study, showed strong negative feedback between free-floating macrophytes and phytoplankton interactions, and describe the shade-effect by free-floating macrophytes as the major contributor to aquatic ecosystem structure and function alterations. Following the control of the free-floating IAAP species (removal of abiotic barrier) as seen in Chapter 3 and 4, and during the De Tezanos Pinto et al. (2007) study, positive ecosystem recovery occurred, where both phytoplankton, periphyton and aquatic macroinvertebrate abundance and diversity responded with a significant shift in community composition.

Alien invasive species can either act as drivers or passengers of environmental change, and when invaders are not drivers, their removal will not alleviate impacts (Prior et al., 2018). Hill and Coetzee (2017) describe free-floating IAAP species in southern Africa as back-seat drivers of environmental change and suggest that they require initial environmental disturbance

to establish and proliferate freshwater ecosystems. However, following free-floating IAAP species control, aquatic ecosystem recovery was autogenic (Chapter 2, 3, 4), thus illustrating the potential of aquatic ecosystem recovery even under severe environmental stress. However, additional active management is necessary to sustain the recovered ecosystem functions and diversity, in order to prevent secondary invasion following IAAP species control (as seen in Westlake River, Chapter 4). Some studies (i.e. Zavaleta et al., 2001; Kettenring and Adams, 2011) highlight that heavily impacted ecosystems will not respond in the same way as less disturbed ecosystems, thus more effort should be given to less disturbed ecosystems to yield a better return of ecosystem goods and services.

Following control of free-floating IAAP species with the aim of restoring ecosystem functions, aquatic organism recolonisation is necessary and should be well supported by the restored site's environmental conditions. Aquatic microalgae recovery dynamics depend entirely on a combination of physical and chemical parameters, where desirable habitat conditions including; increased water clarity and high light penetration, increased water nutrient availability and high substrate diversity, positively influenced aquatic microalgae development, biomass and diversity, as seen in Chapter 4 and in Cattaneo et al. (1998), De Tezanos Pinto et al. (2007) and Grutters et al. (2017). It was expected that following the removal of IAAP species, there would be a significant shift in physicochemical characteristics, influencing aquatic microalgae assemblage composition and biodiversity recovery. Furthermore, aquatic microalgae species' traits, which include assemblage structure and functional groups as described by Reynolds et al. (2002), Padisak et al. (2009) and Stiers et al. (2017), are considered useful biological indicators for environmental change. This is contrary to Durigan and Sukanuma (2015) who suggest that species richness is a better indicator for ecological recovery. In Chapter 3 and 4, aquatic microalgae abundances and diversity were relatively higher under IAAP species infestation and this was attributed to aquatic microalgae growth strategies and the ability to shift in composition in relation to limiting environmental factors and still maintain high species abundance, richness and diversity. In this case, aquatic microalgae functional classification based on species-specific traits, revealed promising outcomes as indicators of environmental change, providing useful information for ecological restoration following IAAP species control. During *S. molesta* invasion, species associated with turbid ecosystems were abundant, thus indicative of limited light conditions, and this was the opposite after *S. molesta* control.

Aquatic macroinvertebrate recovery dynamics were also influenced by water quality parameters, available substrates (habitat heterogeneity), and most importantly, the ability of

aquatic macroinvertebrate communities to recolonise restored environments (e.g. aerial adult dispersal and immature drifting). Rapid aquatic macroinvertebrate recovery was observed in the present study, and according to a review by Yount and Niemi (1990), aquatic macroinvertebrate rapid recovery is attributed to: (1) aquatic macroinvertebrate life histories, which allow them to explore new habitats through their ability to disperse and high rate of reproduction; (2) the restored site's hydrological connectivity to unaffected upstream and downstream sites, which serve as a native species pool, thus contributing to rapid ecosystem recovery, and; (3) aquatic species' plasticity to adapt to changing aquatic environments. The majority of aquatic macroinvertebrate studies reported long-term recovery, however following IAAP species management, aquatic microalgae and aquatic macroinvertebrate biodiversity recovery responded within 30 days to 12 months following *S. molesta* mechanical and biological control methods, respectively. Both aquatic microalgae and macroinvertebrate community recovery supported 'the field of dreams hypothesis' (Palmer et al., 1997). The hypothesis states that 'if you build it, they will come', where aquatic species will recolonise restored ecosystems autogenically. However, Hughes (2007) and Li et al. (2016) emphasise that the autogenic recovery of aquatic microalgae and macroinvertebrate is linked to adult aerial dispersal, and immature aquatic species' drifting, swimming and crawling abilities from neighbouring unaffected sites that are hydrologically connected to the restored sites, and will contribute positively to rapid ecological recovery, thus supporting Yount and Nieme (1990)'s study.

The complexity and the highly dynamic nature of aquatic ecosystems suggest multiple biotic measures are necessary to assess aquatic ecosystem recovery (Adams et al., 2002). In agreement with Adams et al. (2002), the SER proposed a list of attributes/standards as guidelines to measure ecological restoration success, and they emphasize a combination of attributes including biological diversity, vegetation/habitat structure diversity and ecosystem processes as the main ecosystem characteristics to be investigated (Ruiz-Jaen and Aide, 2005). Furthermore, the proposed guidelines explicitly emphasise that the restored ecosystem should illustrate similar diversity and community structure to that of the reference site/treatment; should enhance the presence of indigenous species, and functional groups; exhibit desirable physicochemical variables to sustain the recovered aquatic species biological and functional diversity; illustrate normal ecosystem functions; and be more resilient to natural disturbances and self-sustainable (Ruiz-Jaen and Aide, 2005).

Rapid ecological assessments are commonplace for freshwater environmental impact studies, and incorporating some of the SER standards to these biological assessments tools,

aquatic organisms including phytoplankton, periphyton, macroinvertebrates and to some extent freshwater fish have been used as biological indicators (Dickens and Graham, 2002; Taylor et al., 2007b; Dalu et al., 2014a; Hart and Matthews, 2018). This includes studies by Cattaneo et al. (1998), Masifwa et al. (2001), Adams et al. (2002), Miller et al. (2010), Stiers et al. (2011), Schultz and Dibble (2012), Coetzee et al. (2014), Grutters et al. (2015), Kail et al. (2015), Stiers and Triest (2017), Carey et al. (2018) and Bellingan et al., (2019), that employed both aquatic microalgae and/or aquatic macroinvertebrates to assess alien invasive species ecological impacts in freshwater systems and subsequent ecosystem recovery following alien invasive species removal, as proxy for ecological restoration. A number of these studies including that of Midgley et al. (2006), Masifwa et al. (2001), Stiers et al. (2011), Copatti et al. (2013), Coetzee et al. (2014) and Coetzee et al. (2020) underline the reliability and sensitivity of aquatic macroinvertebrates in detecting ecological changes in freshwater ecosystems invaded by IAAP species, even at the highest taxonomic level (class and family identification). In combination, the biological and chemical analyses have, for a while, provided an in-depth interpretation of ecosystem disturbance, and this has been common to a number of freshwater studies (Adams et al., 2002). Even though they are still widely used, advancement in ecological studies have demonstrated that species richness cannot be used in isolation as an estimator for ecosystem recovery, but that the recovered species contribute to ecosystem functioning is probably more important.

In the case where native endemic species have returned following disturbance mitigation, according to the SER restoration Primer guidelines, restoration can be regarded as successful. However, if the site did not record any endemic fauna or flora before disturbance, restoration/ecosystem recovery should result in a normal functioning ecosystem with increased species richness, biological and functional diverse aquatic communities. Studies reviewed by Prior et al. (2018), recorded ecosystem recovery based solely on the community-level where the return of native species increased following the removal of alien invasive species, and the community structure was similar to that of pre-invaded state, thus the removal of alien invasive species in this case contributed to ecological restoration, as seen in this study. From the literature, species diversity is still regarded as a reliable indicator of environmental change and provides a rapid assessment of ecosystem stress (Suding, 2011). With that being said, there is still some controversy around biological monitoring and whether functional diversity or species richness is a better indicator for ecosystem degradation and recovery. De Tezanos Pinto et al. (2007) and Montoya et al. (2012) have shown that functional diversity and species traits are also equally important in biological monitoring, and prefer the former since it is less time

consuming and does not require prior taxonomic knowledge. Both aquatic microalgae and macroinvertebrates are known to be fundamental components of ecosystem food webs and have a specific functional role in ecosystem (Covich et al., 1999). However, aquatic ecologists have been warned of their over-reliance on superficial taxonomic resolution (family level identification) particularly for aquatic macroinvertebrates, as it is argued that family level identification underestimates aquatic species diversity, sensitivity scores and ecology when using family level sensitivity score. Thus, Barber-James and Pereira-da-Conceicao (2016) suggest that biological monitoring assessments are important for long-term monitoring particularly in the case of environmental impact and subsequent recovery to trace the degree of change in time. Yet, biological monitoring which is regarded as rapid assessment, should not replace traditional biodiversity studies, where species richness and diversity are important and have implications for conservation. Thus, biological monitoring (family level) as a rapid assessment and indicator for environmental changes has and still provide good environmental detection, as seen in the present study. On the other hand, aquatic microalgae are undoubtedly reliable indicators of freshwater ecosystem disturbance, and due to their wide and diverse distribution, it is only at genus and species level that they can provide environmental impact detection (Taylor et al., 2007b; Beyene et al., 2009; Padisák et al., 2009).

Techniques such as stable isotope community-wide trophic metrics proposed by Layman et al. (2007), have provided aquatic ecologists with promising techniques to quantify and interpret trophic ecosystem structure and function at an ecosystem level. This technique has gained favour in ecological impacts studies and it is widely applied in alien invasive species impacts studies (Jackson et al., 2012; Jackson and Britton, 2014; Hill et al., 2015). The technique is designed to describe trophic interactions, providing estimates of trophic species diversity and redundancy, ecosystem energy resources and transfer, and ecosystem food web length as a proxy for improved ecosystem processes, structure and functioning, following alien species control (as seen in Chapter 3 & 4). From the works of Sondergaard et al. (1990), Meijer et al. (1994), Jeppesen et al. (1997), and Hilt et al. (2018) on trophic interactions (bottom-up/top-down), further application of Layman's metrics have added tremendously to European lake ecosystem trophic dynamics and should be more routinely used.

5.4 Invasive alien aquatic plant species control and implications for ecological restoration

Many restoration programmes adopt a passive approach of restoring invaded ecosystems with the aim of removing the existing invader and preventing their regeneration and/or secondary

invasion (Ruwanza et al., 2013). However, this approach often fails to achieve the desired ecological outcomes of a normal functioning ecosystem dominated by native species. Removing invasive species to allow passive recovery of aquatic biota, can only operate if the ecological communities are resilient to the invader, such that removal will allow the ecosystems to recover (Gaertner et al., 2012). However, because most alien invasive species are backseat drivers, their removal will not facilitate complete passive ecosystem recovery that is similar to that of the pre-invasion state (as shown at the Westlake site), without active intervention and long-term assessments (Ruwanza et al., 2013). Also Suding (2011) warned that due to the ever changing environment e.g. climate, land use and general anthropogenic activities, it is challenging to reset the endpoint of ecological restoration, particularly for freshwater ecosystems in developing countries, to that of the pre-invasion state.

As a result, restoration success is based on the recovery of ecosystem processes and the normal functioning of an ecosystem, that will yield ecosystem goods and services for society and wildlife (Suding, 2011). In some cases, this is true, where the removal of IAAP species led to ecological recovery success in a short period of time as seen in Chapter 3. Thus, IAAP species control still remains a necessary step in restoration of invaded ecosystems. Complete ecosystem recovery was achieved in Chapter 2 and 3, whereas Kettenring and Adams (2011), Suding (2011), and Prior et al. (2018) reported over 30% of their reviewed studies showing no degree of recovery following alien invasive species control, and these authors associated their failure to hidden ecological barriers such as legacy effects and/or limited understanding of the control method. Alternatively, Zavaleta et al. (2001) also argue that there are cases where ecosystems do not recover or recover along an alternative trajectory when alien invasive species are removed. This could be attributed to the fact that degraded ecosystems form resilient internal feedback mechanisms that will counter the traditional restoration approach. Therefore, echoing Prior et al. (2018), invasive alien species management should be considered as one component of a holistic management programme to manage alien invasion.

It is evident that aquatic ecosystems recover more rapidly compared to terrestrial and riparian invaded ecosystems following IAAP species management. However, there are still a limited number of case studies that monitor the long-term effects of IAAP species management, making it difficult to conclusively determine the usefulness of a particular management approach and the ecological endpoint of IAAP species control in freshwater ecosystems. In fact, many studies (similar to that seen in Chapter 4) have revealed unexpected restoration outcomes based on the IAAP species management decisions. For example, non-native species

removal may not only increase native abundance but may also potentially introduce or facilitate new invaders into the system further compromising ecosystem recovery (Zavaleta et al., 2001; Antonio and Meyerson, 2002; Suding et al., 2004). Although these sorts of unintended consequences can be expected in the context of complex ecological systems, their exact nature and long-term impacts are difficult to identify or predict, and as seen in Chapter 4, they create suitable conditions for secondary invaders, which, from a resource managers' perspective with limited budget and time, will result in incomplete restoration success or failure (Cordell et al., 2016).

Native macrophyte propagule limitation is another factor contributing to failure of ecological restoration, where in the absence of native propagules following IAAP species control, native macrophyte establishment is poor (Hughes, 2007). In this case, active native revegetation may be necessary and deserves more investigation (Kettenring and Adams, 2011). Riparian invasion control in South Africa provides an excellent example of passive and active restoration practises. The Department of Environmental Affairs, Forestry and Fisheries, through the Working for Water programme, implemented a large-scale terrestrial and riparian invasive alien clearing programme, after which ecosystem recovery studies revealed complete recovery of introduced native seedlings at some sites (Ruwanza et al., 2013; Nsikani et al., 2019). Active restoration, can be costly, but it is justifiable for areas/regions of high conservation value i.e. high priority catchment areas for freshwater resources, biodiversity hotspots, and threatened/endangered biomes (Gaertner et al., 2011). As for IAAP species invasion, the use of biological control has been effective in the control of IAAP biomass and has contributed to water saving and return in investment, with ecosystem recovery following control (as seen in Fraser et al., 2016; Martin et al., 2018 and in Chapter 2, 3).

5.5 Invasive alien aquatic plants management and restoration: recommendations and future research opportunities

Ecological systems are complex, not always resilient, and increasingly altered by environmental change (Suding, 2011). This simply means that systems are not likely to recover in a straightforward manner following the alleviation of the environmental disturbance (Zavaleta et al., 2001; Suding et al., 2004). Research conducted in this thesis illustrated clear ecological benefits and ecosystem recovery following both mechanical and biological control of IAAP species in just under 30 days post control in both the mechanical and biological control sites. However, due to the lack of baseline data-set for the pre-invasion state and/or reference

site which are believed to be tempered by the changing environmental within landscapes, it is challenging to compare the recovered ecosystem to that of the reference condition (Suding, 2011). If this is the case, the SER recommends a biologically and functionally diverse ecosystem, that supports normal ecosystem functioning and that is self-sustaining (Ruiz-Jaen and Aide, 2005).

The Masifwa et al. (2001), Midgley et al. (2006), Langa (2013) and Coetzee et al. (2014) are some of the studies that illustrated the effect of free-floating IAAP species on aquatic macroinvertebrate abundance and diversity. These authors reported dense mats of a free-floating IAAP species e.g. water hyacinth and water lettuce, to have deleterious effects on aquatic macroinvertebrates. De Tezanos Pinto et al. (2007) further showed that not only do free-floating IAAP species affect aquatic macroinvertebrates, but also aquatic microalgae species community assemblages. Therefore, the artificial-shade effect is the main factor that negatively influences water quality, aquatic biodiversity and manifests a complete shift in ecosystem processes and trophic structure in the invaded ecosystems (Midgley et al., 2006; Langa, 2013; Coetzee et al., 2014). Thus, in order to recover normal ecosystem functions and processes, IAAP species control (abiotic filters elimination) is the first step. However, IAAP species control methods need to be well investigated and should not cause harm to freshwater ecosystem fauna and flora and should be socio-economically and ecologically viable. Having identified and eliminated abiotic filters in moderately disturbed ecosystems, biotic recovery is rapid, although further ecosystem active management might be necessary. An active management approach will create suitable environmental conditions i.e. increased water clarity and dissolved oxygen, resulting in increased ecosystem productivity and energy resources for the ecosystem, thus promoting autogenic recovery of aquatic organisms. This intervention should be able to sustain the recovered aquatic biodiversity, and increase the ecosystem resilience to natural disturbances (Ruiz-Jaen and Aide, 2005). Submerged macrophytes can provide habitat heterogeneity for aquatic organisms and in return enhance aquatic biodiversity and contribute to ecosystem trophic level diversity (Yu et al., 2016). This was highlighted in the Miller et al. (2010) seminal review, which showed that habitat heterogeneity provided by the submerged macrophyte to have significant and positive effects on aquatic macroinvertebrate richness. It is not known which other macrophytes will interact positively to contribute to aquatic diversity and manage phytoplankton Chl-*a* concentration, and at the same time provide habitat for periphyton, aquatic invertebrates and fish species (Cattaneo et al., 1998; Bakker et al., 2013; Su et al., 2019). Thus, this calls for a thorough investigation of

freshwater biotic interactions to improve our understanding in South Africa and, elsewhere, to aid in freshwater aquatic biodiversity conservation and restoration.

IAAP species are known for the legacy effects they impose on invaded ecosystems (Corbin and D'Antonio, 2011; Schultz and Dibble, 2012), these legacy effects are abiotic or biotic barriers left behind by IAAP species long after control (Corbin and D'Antonio, 2011). There is some evidence that IAAP species alter ecosystem sediment chemistry and change the fundamental microbial composition as a way to limit native macrophyte success, which clearly has strong implications for freshwater restoration (Elgersma et al., 2011; Vilà et al., 2011). Corbin and D'Antonio (2011) emphasise that even though local eradication of alien invasive plants can be achieved, the outcomes are unlikely to allow restoration of broader community or ecosystem characteristics. Thus, case studies of soil sediment chemistry and microbial composition comparing “before and after” the control of IAAP species should be given future attention, since they are directly associated with the re-establishment and success of native macrophytes. Techniques such as soil biological engineering have been proposed to restore microbial communities and to benefit native macrophyte recovery following IAAP control, but to date very few case studies have investigated this possibility.

The review by Prior et al. (2018) showed that of 151 reviewed ecosystem recovery studies following alien invasive species removal, 61% recovered successfully and this was because the sites were largely undisturbed as compared to severely impacted sites, thus yielding positive ecological recovery. These findings support Gaertner et al. (2014), in that only resilient ecosystems will show positive and rapid autogenic ecological recovery following the control of alien invasive plants. The field sites investigated in the present thesis were moderately disturbed, and hence responded positively to IAAP species management, with the exception of the Silverhurst Impoundment, which showed the least recovery. The Silverhurst Impoundment was located on the upper reaches of Keyzers River and was hydrologically connected to the stream with an artificial in-and-out flow, thus in terms of aquatic macroinvertebrate recovery, the site had limited connectivity to other similar lentic water bodies within the surrounding environment. Thus, major aquatic macroinvertebrate species (i.e. those more adapted to flowing river systems) did not establish well in the man-made standing water body in Silverhurst Impoundment. Anthropogenic activities often play a significant role and have the ability to compromise ecological recovery for freshwater ecosystems. Additionally, landscape developments leading to the conversion of natural systems to urban and agriculture lands, contribute to natural habitat fragmentation, disconnect ecosystems and limit native gene pool flow within landscapes (Kietzka et al., 2015). For the purpose of ecological restoration, such

disconnections have contributed to the failure of some restoration projects, where following IAAP species control, native macrophyte propagule flow and establishment was insufficient, indicating a call for active management and the introduction of native macrophyte propagules (Kettenring and Adams, 2011).

Landscape management is arguably necessary to assist in the ecological restoration of impacted ecosystems and needs to be integrated in the management of natural resources. The enhancement of ecological ‘corridors’ or networks in landscape and biodiversity studies have enormously contributed to alpha and beta diversity within regions, and showed consistent transfer of native propagules and pollen through native arthropods (Pryke and Samways, 2009; Kietzka et al., 2015). Thus, with ever changing environments, the remaining undisturbed habitat depends on ecological networks to assist in the movement of native propagules and the recovery of urban ecosystems. Additionally, keystone and pioneer species if identified and introduced can further assist in ecosystem recovery.

More studies and reviews on ecological restoration acknowledge the success and failures associated with restoring degraded ecosystems, however, long-term active management of such systems can provide some evidence to better understand the shortcomings of these approaches, the complexity variables. In line with the proposed recommendations, several authors (e.g. Zavaleta et al., 2001; Corbin and D’Antonio, 2011; Kettenring and Adams, 2011; Suding, 2011; Prior et al., 2018) support long-term post-IAAP species management and restoration monitoring to provide useful trajectories on restoration effort within aquatic environments. Therefore it is necessary to conduct more IAAP species recovery studies in Africa after biological control as the majority of meta-analyses and reviews investigate restoration projects on river channelisation, urbanisation, deforestation and IAAP species control through mechanical removal and are concentrated in North America and Europe (Miller et al., 2010; Kettenring and Adams, 2011; Kail et al., 2015; Prior et al., 2018). Having said that, Kettenring and Adams (2011) state that for IAAP species control and restoration efforts, there is no evidence of which control method will lead to native ecosystem recovery.

The research herein provides evidence-based case studies using a combination of ecological indices and metrics, and illustrates successful ecological recovery following both mechanical and biological control methods. This research recommends biological control methods for free-floating IAAP species, which is cost effective and provide a more stable ecosystem recovery trajectory (Hill and Coetzee, 2017). This method is highly recommended at a landscape scale where both mechanical and chemical control are deemed expensive and not effective (Hussner et al., 2017). Following control, as a part of a broader integrated

freshwater ecosystem conservation, an active restoration programme can be justified for further active intervention and management to assist in complete ecosystem recovery.

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Supplementary material

Table S1. Mean $\pm$ standard deviation of physicochemical variables between the Impacted, Restored and Control treatment during the “before”, “during” and “after” biological control of *Salvinia molesta*. Where EC – conductivity; TDS – total dissolved solids; Water temp. – water temperature; DO – dissolved oxygen; NO_3^- – nitrate; NH_4^+ – ammonium; PO_4^{3-} – phosphate.

Physicochemical variables	Impacted			Restored			Control		
	Before	During	After	Before	During	After	Before	During	After
pH	7.7 $\pm$ 0.04	7.1 $\pm$ 0.4	7.04 $\pm$ 0.14	8.1 $\pm$ 0.3	7.5 $\pm$ 0.4	7.8 $\pm$ 0.4	7.8 $\pm$ 0.04	8.2 $\pm$ 0.5	8.7 $\pm$ 0.6
EC ($\mu\text{S}/\text{cm}$)	843.5 $\pm$ 38.7	867.6 $\pm$ 311.6	1071.9 $\pm$ 422	850.5 $\pm$ 25.3	772.5 $\pm$ 291.3	821.5 $\pm$ 260.6	901 $\pm$ 68.6	871.8 $\pm$ 269.1	1039.1 $\pm$ 439.8
TDS (ppm)	598.3 $\pm$ 27.4	602.8 $\pm$ 223.6	762.1 $\pm$ 297.7	603 $\pm$ 17.6	547.9 $\pm$ 206.8	582.3 $\pm$ 185.7	642 $\pm$ 53.3	641.2 $\pm$ 159.2	726.5 $\pm$ 294.8
Salinity (ppm)	429 $\pm$ 19.8	456.3 $\pm$ 140.9	540.4 $\pm$ 211.7	433.8 $\pm$ 13.2	390.3 $\pm$ 150.9	413 $\pm$ 132.8	462.3 $\pm$ 39.5	457 $\pm$ 116.9	512.6 $\pm$ 206.7
Water temp. ($^{\circ}\text{C}$)	22.5 $\pm$ 0.5	16.8 $\pm$ 3.10	19.8 $\pm$ 3.4	24.6 $\pm$ 0.3	17.8 $\pm$ 2.4	21.8 $\pm$ 2.5	23.4 $\pm$ 0.46	17.1 $\pm$ 2.9	20.9 $\pm$ 2.9
DO (mg/l)	5.4 $\pm$ 0.3	3.51 $\pm$ 0.53	6.9 $\pm$ 1.4	5.1 $\pm$ 0.4	3.5 $\pm$ 1.3	3.3 $\pm$ 0.5	4.9 $\pm$ 0.5	5.2 $\pm$ 1.4	3.9 $\pm$ 0.3
NO_3^- (mg/l)	16.6 $\pm$ 5.3	5.7 $\pm$ 3.2	3.3 $\pm$ 1.9	2.6 $\pm$ 0.6	4.9 $\pm$ 2.9	3.3 $\pm$ 2.6	6.9 $\pm$ 2.9	20.8 $\pm$ 19.4	11.2 $\pm$ 8.2
NH_4^+ (mg/l)	0.03 $\pm$ 0.05	0.01 $\pm$ 0.03	0.03 $\pm$ 0.05	0.03 $\pm$ 0.05	0.01 $\pm$ 0.03	0.01 $\pm$ 0.04	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
PO_4^{3-} (mg/l)	1 $\pm$ 0.4	0.84 $\pm$ 0.81	0.81 $\pm$ 1.32	0.8 $\pm$ 0.5	3.04 $\pm$ 5.9	1.09 $\pm$ 1.02	0.93 $\pm$ 0.40	0.6 $\pm$ 0.67	0.4 $\pm$ 0.5
Water clarity (cm)	71.8 $\pm$ 10.7	32.6 $\pm$ 23	19.6 $\pm$ 16.4	43.8 $\pm$ 16.7	43.1 $\pm$ 31.8	67.6 $\pm$ 22.8	61.3 $\pm$ 1.85	66.8 $\pm$ 18	92.9 $\pm$ 3.5
Periphyton Chl- <i>a</i> (mg/m ³)	15.3 $\pm$ 13.9	17.8 $\pm$ 23.8	9.4 $\pm$ 7.5	17.6 $\pm$ 14.9	21.6 $\pm$ 27.7	60.5 $\pm$ 29.3	23.4 $\pm$ 19.2	23.6 $\pm$ 20.8	63 $\pm$ 54.9
Phytoplankton Chl- <i>a</i> (mg/m ³)	0.09 $\pm$ 0.06	4.3 $\pm$ 4.8	19.7 $\pm$ 16.8	0.13 $\pm$ 0.08	4.1 $\pm$ 4.5	0.63 $\pm$ 0.7	0.10 $\pm$ 0.07	0.40 $\pm$ 0.52	0.3 $\pm$ 0.2

Table S2. Mean $\pm$ standard deviation of dominant (>1000 cells per liter) epilithic algae species recorded between the Impacted, Restored and Control treatment during the “before”, “during” and “after” biological control of *Salvinia molesta*.

Species	Impacted			Restored			Control		
	Before	During	After	Before	During	After	Before	During	After
<i>Anabaena</i> sp.	1268.8	82.3 $\pm$ 88.2		162.5 $\pm$ 211.9	48.7 $\pm$ 51.9	1114.3 $\pm$ 1260.1	12.2 $\pm$ 0.69	334.7 $\pm$ 706	
<i>Pseudanabaena</i> sp.		163.4 $\pm$ 375.6	40.8 $\pm$ 26.7		227.9 $\pm$ 621.9	11.9		307.4 $\pm$ 654.3	
<i>Amphora veneta</i>		164.4 $\pm$ 237.4	102.4 $\pm$ 89		9.9 $\pm$ 5.3	51.7 $\pm$ 43.9		77.1 $\pm$ 100	8.4
<i>Cocconeis placentula</i>		49.4 $\pm$ 98.8	42.2 $\pm$ 19.3		19 $\pm$ 19.8			85.3 $\pm$ 190.6	29.4 $\pm$ 29.5
<i>Cocconeis placentula</i>			1.3 $\pm$ 2.6					150.3 $\pm$ 387.6	
<i>Craticula bruderi</i>		88.5			24.3 $\pm$ 35.7			2994.1 $\pm$ 6669.9	5.6
<i>Diploneis oploneis</i>								243.9 $\pm$ 231.3	
<i>Epithemia adnata</i>		13.3 $\pm$ 16.4			24.3 $\pm$ 35.7	6.2		791.4 $\pm$ 2016	314.3 $\pm$ 312.9
<i>Fragilaria ulna</i> var. <i>acus</i>		10.9 $\pm$ 16.4	13.2 $\pm$ 7.2		73.5	118.5	7.3	820.5 $\pm$ 1620.4	4.9
<i>Nitzschia filiformis</i>		34.9 $\pm$ 42.8	57.3 $\pm$ 43.5		50.5 $\pm$ 67.5	80.9 $\pm$ 120.1		1557.8 $\pm$ 3099.3	
<i>Nitzschia linearis</i>		68.9 $\pm$ 91.2	29.1 $\pm$ 21.2		32.6 $\pm$ 55.1	50.9 $\pm$ 62.4		1641.4 $\pm$ 3559.8	10.6 $\pm$ 9
<i>Nitzschia palea</i>		30.3 $\pm$ 60.3	10.3 $\pm$ 7.7		90 $\pm$ 158	56.2 $\pm$ 89.6		324.2 $\pm$ 488.1	
<i>Rhopalodia gibba</i>	21.9	4.9 $\pm$ 3.6	48.8 $\pm$ 60.9		7.4 $\pm$ 2.3		13.9	65.1 $\pm$ 142.4	5.7 $\pm$ 0.13
<i>Peridinium umbonatum</i>		1.9 $\pm$ 3.4			29.9 $\pm$ 27.8	34.8 $\pm$ 34.6	11.7	605.2 $\pm$ 2014.4	56 $\pm$ 64.8
<i>Characiopsis turgida</i>						866.4 $\pm$ 1167			
<i>Cryptomonas erosa</i>		988.5 $\pm$ 1690.1			970.9 $\pm$ 1654.9			2.8 $\pm$ 4.8	
<i>Cryptomonas marssonii</i>					466.6 $\pm$ 747.8			3.2 $\pm$ 5.6	
<i>Cryptomonas ovalata</i>		10.7	24.5		1699.4 $\pm$ 2943.5			56.4 $\pm$ 79.3	
<i>Phacus applanatus</i>					535.2 $\pm$ 857				
<i>Coelastrum sphaericum</i>					16.6			336.1 $\pm$ 646.4	45.9
<i>Oocystis</i> sp.		25.3			17.8 $\pm$ 10.2			471.6 $\pm$ 811.8	
<i>Oocystis marssonii</i>								127.2 $\pm$ 136.6	390.2 $\pm$ 495.9
<i>Characiopsis longipes</i>						565.2 $\pm$ 637.6			
<i>Closteriopsis acicularis</i>		11.9 $\pm$ 9.9			7735.8 $\pm$ 21498.5		23.3	176.6 $\pm$ 295.6	

<i>Closteriopsis longissima</i>	1609.4 ± 419.9	9.5		62.7 ± 72.2	962.3 ± 1892.6			
<i>Monoraphidium irregulare</i>	831.3 ± 810.9	2.1 ± 3.6		1180 ± 2246.7	84.3 ± 133.2	34.3 ± 17.5	127.2 ± 389.5	19.8 ± 19.7
<i>Monoraphidium contortum</i>	3003.7 ± 3131.7	7.9 ± 4.8		1613.5 ± 2696	423.9 ± 926.5	205 ± 317.5	211.8 ± 472.1	
<i>Monoraphidium graffithii</i>		6.3	5.2	173.9 ± 311	252.7 ± 433.1	99.9 ± 171	59.3 ± 101.5	
<i>Scenedesmus communis</i>		8.9 ± 13.7			205.6 ± 490.4	209.1 ± 332.6	138.5 ± 250	
<i>Scenedesmus dinorplus</i>	196.1 ± 162.9	18.2 ± 24.3	6.1	102.6 ± 59	41.1 ± 68.7	11.9	558.5 ± 584.3	5.9 ± 4.9
<i>Micospora floccosa</i>		5.1					1443.4 ± 1907.3	
<i>Zygnema</i> sp.		5.8	5.9 ± 0.5		438.8 ± 995.9	22.6	916.9 ± 833.9	
<i>Cosmarium cyclicum</i>		78.5 ± 134.7	5.2		35.9 ± 70.8	22.6 ± 18.4	230.2 ± 617.2	139.6 ± 90.9
<i>Cosmarium formosulum</i>							91.4 ± 112.4	6.6 2.8
<i>Cosmarium subcostatum</i>				153.3 ± 195.7		447.3 ± 499.1	7.2 ± 9.3	

Table S3. Mean $\pm$ standard deviation of aquatic macroinvertebrates taxa recorded between the Impacted, Restored and Control treatment during the “before”, “during” and “after” biological control of *Salvinia molesta*.

Taxa	Impacted			Restored			Control		
	Before	During	After	Before	During	After	Before	During	After
Tubellaria		1	1.8 $\pm$ 0.9		4.2 $\pm$ 3.8	2		1.5 $\pm$ 0.7	27 $\pm$ 39.9
Oligochaeta		20 $\pm$ 28.1	2.5 $\pm$ 0.7		29.3 $\pm$ 26.9	23.4 $\pm$ 22.4		7.6 $\pm$ 2.6	6 $\pm$ 4.4
Hirudinea		3.5 $\pm$ 3.1	9.3 $\pm$ 5.5		24.6 $\pm$ 28.5	123.5 $\pm$ 149.1		4.4 $\pm$ 3.4	10.5 $\pm$ 7.3
Copepoidae	12 $\pm$ 8.5	9.9 $\pm$ 14.6	2.9 $\pm$ 1.6		11.4 $\pm$ 18.6	16 $\pm$ 12.4	5	13.9 $\pm$ 14.2	7 $\pm$ 8.1
Cypridoidae		11.2 $\pm$ 7	22.6 $\pm$ 1.2		16.3 $\pm$ 14.9	41.6 $\pm$ 34.6	19.5 $\pm$ 2.1	38.5 $\pm$ 55.6	39.3 $\pm$ 42.3
Daphnia					16.2 $\pm$ 20	6.2 $\pm$ 8.9			2.3 $\pm$ 1.5
Hydracarina	3.3 $\pm$ 3.2			6 $\pm$ 5	2.2 $\pm$ 1.3	8.3 $\pm$ 9.3	2.8 $\pm$ 2.4	4.1 $\pm$ 3.2	4 $\pm$ 4.8
Baetidae	25.8 $\pm$ 20.9	4.3 $\pm$ 5.2		11.3 $\pm$ 3.9	5.6 $\pm$ 7.1	2 $\pm$ 0.8	21.5 $\pm$ 6.9	11.2 $\pm$ 7.4	4.4 $\pm$ 5.4
Caenidae	23 $\pm$ 21.6	9.3 $\pm$ 4.7	1	6.8 $\pm$ 4.2	25 $\pm$ 15.6	2 $\pm$ 1.4	18.8 $\pm$ 0.9	7.2 $\pm$ 8.6	8.1 $\pm$ 7.9
Coenagrionidae		1							
Aeshnidae	1			1.5 $\pm$ 0.5			1		
Gomphidae					1.5 $\pm$ 0.7	2			
Libellulidae	8.7 $\pm$ 7.0	16.1 $\pm$ 17.2	2	6 $\pm$ 3.7	8 $\pm$ 15.3	6 $\pm$ 4.8	20 $\pm$ 17.9	25.7 $\pm$ 23.5	16.5 $\pm$ 12.4
Belostomatidae				8	1			3.1 $\pm$ 3.3	1
Corixidae	1				1	7 $\pm$ 8.9			2 $\pm$ 1.7
Gerridae	5.5 $\pm$ 5.1	1		8.8 $\pm$ 5.1	4 $\pm$ 2.8	3.3 $\pm$ 4	12.3 $\pm$ 9.3	11.6 $\pm$ 17.6	1
Hydrometridae					1.5 $\pm$ 0.7	2		1	4.5 $\pm$ 4.9
Notonectidae	8 $\pm$ 4.4			12.5 $\pm$ 9.2	1		4.7 $\pm$ 2.3	4.8 $\pm$ 2.9	
Pleidae			1	1.5 $\pm$ 0.5	29.9 $\pm$ 47.9	1 $\pm$ 4	1	8.2 $\pm$ 13	2.5 $\pm$ 1
Dytiscidae	41.2 $\pm$ 53.1	10.5 $\pm$ 13.4		5.3 $\pm$ 1.5	52.3 $\pm$ 84.8	48.8 $\pm$ 25	6.5 $\pm$ 6.3	24 $\pm$ 18.6	25 $\pm$ 35
Elmidae								1	
Hydraenidae					3.5 $\pm$ 2.1	1		3 $\pm$ 2.1	1
Hydrophilidae	1		1	1	5.8 $\pm$ 3.7	3.1 $\pm$ 1.6	1	6.6 $\pm$ 4.8	3.6 $\pm$ 2.6
Ceratopogonidae	8 $\pm$ 2.8	1.2 $\pm$ 0.5		3	4.1 $\pm$ 5.2	1.8 $\pm$ 0.9	2	1.9 $\pm$ 0.9	1.3 $\pm$ 0.6
Chironomidae	20.8 $\pm$ 7.8	12.7 $\pm$ 22.4	1.5 $\pm$ 0.7	20.3 $\pm$ 8.6	34.3 $\pm$ 48.8	34 $\pm$ 32.3	19.5 $\pm$ 5.3	53.5 $\pm$ 48.4	20.3 $\pm$ 12.3
Culicidae	3.5 $\pm$ 2.1	1		10				1.7 $\pm$ 1.2	
Muscidae		1						1	1
Simuliidae		1							
Psychodidae									1

Lymnaeidae			1		1.3 ± 0.6	9 ± 1		7.5 ± 12.4	3 ± 2.5
Physidae	2.5 ± 2.1	18 ± 19.4	4.1 ± 3.2	1	34.9 ± 45.5	43.7 ± 51.2	22	38.4 ± 24.1	28.9 ± 26.4
Chaoboridae		4.3 ± 3.2			1			1.6 ± 0.9	1.3 ± 0.5

Table S4 Epiphytic algae species list and relative abundances collected from four field sites; Westlake River, Silverhurst Impoundment, Kogmanskloof River and Rosle Farm Reservoir “before and after” *Salvinia molesta* control, South Africa.

Taxa	Westlake		Silverhurst		Kogmanskloof		Rosle Farm	
	Before	After	Before	After	Before	After	Before	After
Cyanophyta								
<i>Anabaena</i> sp.	69	59	11	11	0	147	1684	555
<i>Anabaena spiroides</i>	163	0	0	0	0	1099	0	0
<i>Chroococcus minutus</i>	0	0	0	0	0	0	0	14
<i>Gomphosphaeria</i> <i>aponima</i>	0	0	0	0	0	0	0	17
<i>Cylindrospermopsis</i> sp.	0	0	0	0	0	50	0	0
<i>Hyella balani</i>	0	0	0	0	0	0	0	17
<i>Lyngbya</i> sp.	0	0	0	0	0	604	84	0
<i>Lyngbya martensiana</i>	0	0	0	0	53	0	0	159
<i>Lyngbya subbrevis</i>	0	0	0	0	49	0	0	0
<i>Merismopedia</i> sp.	0	0	0	0	0	0	0	315
<i>Merismopedia glauca</i>	0	78	11	0	0	4	0	47
<i>Microcystis aeruginosa</i>	0	0	0	0	0	0	0	315
<i>Oscillatoria species</i>	15	0	11	0	0	17	0	0
<i>Oscillatoria limosa</i>	0	208	0	0	0	0	0	233
<i>Oscillatoria subbrevis</i>	0	0	0	0	0	0	0	45
<i>Oscillatoria tenuis</i>	0	259	0	0	0	0	0	66
<i>Pleurocapsa minor</i>	0	0	0	0	0	0	0	964
<i>Psuedanabaena</i> sp.	397	0	0	0	160	127	0	142
<i>Spirulina maior</i>	0	15	0	11	0	0	24	0
Bacillariophyta								
<i>Achnanthes standeri</i>	0	0	0	32	92	0	0	0
<i>Achnanthidium</i> <i>eutrophilum</i>	0	0	0	0	22	0	0	0
<i>Achnanthidium exiguum</i>	0	70	0	0	0	0	0	0
<i>Amphora</i> sp.	145	0	95	27	0	208	0	47
<i>Amphora coffeaeformis</i>	1199	634	0	0	289	127	23	0
<i>Amphora copulata</i>	18	0	0	11	0	0	8	0
<i>Amphora montana</i>	303	18	0	0	0	83	0	0

<i>Amphora pediculus</i>	0	0	0	0	119	0	0	89
<i>Amphora ovalis</i>	198	9	0	0	22	0	0	17
<i>Amphora veneta</i>	138	313	0	0	319	0	37	0
<i>Aulacoseira</i> sp.	0	18	0	0	0	0	0	0
<i>Aulacoseira subarctica</i> f. <i>suborealis</i>	0	0	0	11	0	0	0	0
<i>Caloneis</i> sp.	74	0	0	0	0	0	0	0
<i>Cocconeis</i> sp.	985	0	0	0	0	2823	0	0
<i>Cocconeis engelbrechtii</i>	462	432	6	28	0	3308	0	0
<i>Cocconeis pediculus</i>	143	379	8	18	9746	2222	0	0
<i>Cocconeis placentula</i>	0	0	0	0	0	838	0	0
<i>Craticula</i> sp.	0	386	6	11	170	0	0	0
<i>Craticula bruderi</i>	15	0	0	0	0	0	0	0
<i>Craticula cuspidata</i>	66	0	0	0	0	0	0	0
<i>Craticula halophila</i>	16	0	0	0	0	0	0	0
<i>Ctenophora pulchella</i>	0	9	17	27	0	0	0	0
<i>Cyclotella</i> sp.	2173	0	142	32	0	673	0	153
<i>Cyclotella meneghiniana</i>	319	2292	160	31	2623	47	0	0
<i>Cymbella</i> sp.	9	0	280	11	0	0	0	0
<i>Cymbella neocistula</i>	0	0	0	32	0	0	0	0
<i>Cymbella tumida</i>	0	15	0	11	0	0	0	0
<i>Cymbella turgidula</i>	0	0	0	11	0	0	0	0
<i>Diademsis</i> sp.	112	0	0	0	0	4	0	0
<i>Diademsis contentata</i>	0	70	0	0	0	0	0	48
<i>Diademsis confervacea</i>	7	0	0	0	35	0	0	0
<i>Diatoma vulgare</i>	0	0	8	11	0	0	0	0
<i>Encyonopsis leei</i> var. <i>sinensis</i>	0	27	0	0	0	0	0	0
<i>Entomoneis</i> sp.	0	0	0	0	148	0	0	16
<i>Eolimna minima</i>	0	0	0	0	132	0	0	0
<i>Epithemia adnata</i>	0	245	11	0	0	0	0	0
<i>Epithemia sorex</i>	0	0	0	21	0	0	0	0
<i>Eunotia bilunaris</i>	0	0	0	67	0	0	0	0
<i>Eunotia formica</i>	0	0	77	27	0	0	0	0
<i>Eunotia flexuosa</i>	0	0	0	14	0	0	0	0

<i>Eunotia incisa</i>	0	9	0	0	0	0	0	0
<i>Eunotia minor</i>	0	30	0	189	85	8	0	16
<i>Eunotia rhomboidea</i>	0	0	33	0	0	0	0	0
<i>Fallacia</i> sp.	0	0	0	0	0	0	0	0
<i>Fallacia pygmaea</i>	0	27	0	0	0	0	0	0
<i>Fragilaria biceps</i>	15	0	0	0	0	68	0	93
<i>Fragilaria capucina</i>	0	0	0	0	0	0	0	16
<i>Fragilaria nanana</i>	0	58	0	0	0	0	0	127
<i>Fragilaria tenera</i>	0	150	52	23	16	0	0	399
<i>Fragilaria ulna</i>	446	1275	5	377	1735	593	0	0
<i>Fragilaria ulna</i> var. <i>acus</i>	0	2031	651	217	453	0	0	0
<i>Frustulia</i> sp.	0	0	0	0	16	0	0	1518
<i>Frustulia vulgaris</i>	16	0	6	0	0	0	8	0
<i>Gomphonema</i> <i>accuminatum</i>	22	61	111	217	0	17	24	87
<i>Gomphonema affine</i>	7	167	5	210	452	1760	0	0
<i>Gomphonema affine.</i> <i>gracile</i>	0	0	0	0	0	0	0	2481
<i>Gomphonema capitatum</i>	0	0	6	0	0	0	0	0
<i>Gomphonema gracile</i>	7	0	0	0	0	20	0	0
<i>Gomphonema insigne</i>	0	0	0	0	16	0	0	0
<i>Gomphonema italicum</i>	557	0	5	0	0	33	0	81
<i>Gomphonema laticollum</i>	808	146	5	192	632	1448	0	0
<i>Gomphonema parvulum</i>	0	339	0	0	0	0	0	0
<i>Gomphonema</i> <i>pseudoaugur</i>	7	196	0	11	384	0	0	0
<i>Gomphonema venusta</i>	0	192	11	97	0	0	0	0
<i>Gyrosigma</i> sp.	0	15	6	0	0	0	0	0
<i>Gyrosigma</i> <i>rautenbachiae</i>	98	0	0	11	51	2378	0	0
<i>Hantzschia amphioxys</i>	60	88	31	18	220	0	0	0
<i>Hantzschia</i> <i>distinctepunctata</i>	0	0	0	11	0	0	0	0
<i>Hippodonata capitata</i>	726	575	6	0	0	0	2522	0
<i>Lemnicola hungarica</i>	7	165	0	0	242	0	0	108

<i>Luticola acidoclinata</i>	0	53	0	0	0	0	0	0
<i>Mastogloia smithii</i>	0	0	72	11	0	0	0	0
<i>Melosira varians</i>	0	223	0	0	321	0	0	0
<i>Navicula</i> sp.	497	912	181	42	151	1403	8	0
<i>Navicula antonii</i>	0	180	0	0	0	28	0	0
<i>Navicula capitata</i>	0	90	0	0	0	0	0	0
<i>Navicula cryptotonella</i>	74	0	0	11	100	0	0	0
<i>Navicula cryptotenelloides</i>	33	0	0	0	0	0	0	0
<i>Navicula erifuga</i>	0	41	0	0	0	0	0	0
<i>Navicula gregaria</i>	0	104	0	0	110	1244	0	0
<i>Navicula menisculus</i>	0	0	0	0	0	17	0	0
<i>Navicula notha</i>	15	0	0	0	0	0	0	0
<i>Navicula recens</i>	733	0	0	0	0	111	0	0
<i>Navicula riediana</i>	7	179	0	0	70	0	0	0
<i>Navicula riechardtiana</i>	0	0	0	0	66	0	0	0
<i>Navicula rostellata</i>	0	35	130	0	0	379	0	0
<i>Navicula trivialis</i>	0	1094	0	161	0	0	0	0
<i>Navicula veneta</i>	74	529	5	0	0	24	0	0
<i>Navicula zanoni</i>	88	53	0	0	0	0	0	33
<i>Nitzschia</i> sp.	54	82	0	48	0	83	48	0
<i>Nitzschia amphibia</i>	0	0	0	0	0	0	0	93
<i>Nitzschia aurariae</i>	0	0	0	0	0	0	0	364
<i>Nitzschia capitellata</i>	16	234	0	0	0	0	0	0
<i>Nitzschia closterium</i>	0	0	0	0	127	0	0	0
<i>Nitzschia dissipata</i>	0	18	0	0	0	0	0	0
<i>Nitzschia draviellensis</i>	0	68	0	11	241	0	0	226
<i>Nitzschia filiformis</i>	506	517	54	97	198	26	30	762
<i>Nitzschia frustulum</i>	7	18	0	0	0	0	0	0
<i>Nitzschia gracilis</i>	0	15	0	0	0	0	0	0
<i>Nitzschia intermedia</i>	747	0	181	222	0	72	0	0
<i>Nitzschia linearis</i>	1120	297	1872	483	124	223	8	0
<i>Nitzschia palea</i>	358	117	0	0	884	57	16	59
<i>Nitzschia pusilla</i>	0	0	17	0	0	0	0	0

<i>Nitzschia linearis</i> var. <i>subtilis</i>	0	0	123	0	0	0	0	16
<i>Nitzschia littorea</i>	0	0	0	0	0	0	62	0
<i>Nitzschia recta</i>	0	0	0	32	0	0	0	0
<i>Nitzschia reversa</i>	0	18	0	0	577	0	0	388
<i>Nitzschia sublinearis</i>	0	215	0	319	0	0	0	0
<i>Nitzschia sigma</i>	0	12	0	11	0	0	0	0
<i>Nitzschia umbonata</i>	88	12	37	0	38	87	659	0
<i>Pinnularia borealis</i>	15	0	0	0	0	0	0	0
<i>Pinnularia viridiformis</i>	543	524	11	332	218	8	15	0
<i>Pinnularia</i> <i>subbrevistriata</i>	0	0	0	0	40	0	0	0
<i>Placoneis</i> sp.	0	35	0	0	0	0	0	5456
<i>Placoneis placentula</i>	0	18	0	0	0	0	0	0
<i>Planothidium rostratum</i>	22	319	0	0	0	0	0	0
<i>Pleurosigma elongatum</i>	104	18	0	0	65	92	0	0
<i>Pleurosigma salinarum</i>	0	9	0	0	0	0	0	0
<i>Rhoicosphenia</i> <i>abbreviata</i>	0	0	0	31	0	0	0	0
<i>Rhopalodia gibba</i>	143	91	5	0	47	0	0	0
<i>Sellaphora pupula</i>	15	968	0	0	0	0	0	0
<i>Seminavis strigosa</i>	0	9	0	0	0	0	0	0
<i>Stephanodiscus</i> <i>agassizensis</i>	9	0	0	0	0	0	0	0
<i>Straurosira elliptica</i>	0	29	0	0	16	0	0	0
<i>Suriella angusta</i>	0	12	0	0	0	0	0	47
<i>Tabularia fasciculata</i>	539	583	0	0	2874	57	0	14
<i>Tryblionella apiculata</i>	0	12	11	0	44	0	0	16
<i>Tryblionella gracilis</i>	88	0	0	0	352	12	0	0
<i>Tryblionella stranderi</i>	0	0	0	0	53	0	0	0
Dinophyta								
<i>Peridinium</i> sp.	0	12	0	0	0	0	0	31
Cryptophyta								
<i>Chroomonas baltica</i>	0	0	0	0	0	0	0	0
<i>Chroomonas</i> sp.	0	0	0	0	0	615	0	14

<i>Cryptomonas curvata</i>	0	0	0	0	0	0	0	16
<i>Cryptomonas erosa</i>	0	0	0	0	0	0	0	0
<i>Closterium acutum</i> var. <i>variabile</i>	0	0	0	0	0	0	0	17
<i>Closterium incurvum</i>	0	15	0	0	0	0	0	0
<i>Closterium leibleni</i>	0	0	0	0	18	0	0	0
<i>Coelastrum</i> sp.	0	0	0	0	0	0	0	14
<i>Coelastrum proboscidem</i>	0	0	0	0	0	0	0	0
<i>Coelastrum sphaericum</i>	0	0	0	0	16	0	0	0
<i>Crucigenia</i> sp.	7	0	0	0	0	0	0	0
Euglenophyta								
<i>Euglen</i> sp.	0	45	6	0	22	14	38	0
<i>Euglena chlamydophora</i>	0	9	0	0	0	0	0	0
<i>Euglena contabrica</i>	0	0	0	0	0	0	105	0
<i>Euglena granulata</i>	0	61	0	0	0	0	0	0
<i>Euglena proxima</i>	0	115	0	0	0	0	0	0
<i>Euglena repulsans</i>	0	69	0	0	0	0	0	0
<i>Euglena texta</i>	0	53	0	0	0	0	0	94
<i>Phacus caudatus</i>	0	44	0	0	0	0	0	0
<i>Phacus cochleatus</i>	0	70	0	0	0	0	0	0
<i>Phacius elegans</i>	0	0	0	27	0	0	0	0
<i>Phacus moniltus</i>	0	0	0	0	0	0	7	0
<i>Phacus nordstedtii</i>	0	53	0	14	0	0	0	132
<i>Phacus orbiculus</i>	0	35	0	0	0	0	0	100
<i>Phacus pleuronectes</i>	0	235	0	0	38	0	0	282
<i>Phacus triqueter</i>	0	0	0	0	0	0	7	0
<i>Strombomonas</i> sp.	15	0	0	0	0	0	16	0
<i>Strombomonas</i> <i>eurystoma</i>	0	0	6	0	0	0	156	0
<i>Trachelomonas</i> sp.	81	15	0	0	0	18	56	0
<i>Trachelomonas abrupta</i>	0	0	0	0	0	0	0	64
<i>Trachelomonas globular</i>	0	0	0	27	0	0	8	0
<i>Trachelomonas</i> <i>granulata</i>	0	0	0	0	0	0	0	17

<i>Trachelomonas granulosa</i>	0	0	0	0	31	0	0	0
<i>Trachelomonas intermedia</i>	0	0	0	0	0	0	0	45
Chlorophyta								
<i>Ankistrodesmus fusiformis</i>	0	0	0	0	16	0	0	14
<i>Coelastrum</i> sp.	0	0	0	0	0	116	0	0
<i>Cosmarium</i> sp.	0	0	0	0	0	17	8	0
<i>Characium sieboldii</i>	0	42	0	0	16	0	28	0
<i>Characium ornithocephalum</i> var <i>harpochytriforme</i>	0	0	0	0	0	0	0	17
<i>Dictyosphaerium</i> sp.	0	0	0	0	0	18	7	0
<i>Golenkinia</i> sp.	0	0	0	0	0	46	0	0
<i>Kirchneriella lunaris</i>	0	0	0	0	0	0	0	45
<i>Microspora floccosa</i>	0	0	0	11	0	0	0	0
<i>Monoraphidium contortum</i>	0	0	0	11	35	0	0	0
<i>Monoraphidium graffithii</i>	0	0	0	0	0	0	0	82
<i>Monoraphidium irregulare</i>	0	0	34	0	16	0	0	0
<i>Mougeotia</i> sp.	0	0	0	0	0	96	0	0
<i>Oocystis</i> sp.	7	0	1350	0	0	0	24	0
<i>Pediastrum</i> sp.	29	0	0	0	0	0	0	179
<i>Pediastrum kawraiskyi</i>	0	15	0	0	0	0	0	0
<i>Pediastrum simplex</i>	0	15	0	0	0	0	0	0
<i>Pediastrum tetras</i>	0	50	6	0	0	0	0	0
<i>Scenedesmus</i> sp.	260	0	5	38	0	18	0	0
<i>Scenedesmus bernaadii</i>	0	9	0	0	0	0	0	75
<i>Scenedesmus communis</i>	0	188	8	0	16	0	0	0
<i>Scenedesmus costatis</i>	0	0	0	0	0	0	0	32
<i>Scenedesmus dinorplus</i>	0	233	0	0	31	0	0	14
<i>Scenedesmus opoliensis</i>	0	0	55	0	0	0	0	0

<i>Scenedesmus opoliensis</i>	0	12	51	0	0	0	0	0
<i>var. mononensis</i>								
<i>Scenedesmus opoliensis</i>	0	66	0	0	0	0	0	0
<i>var. carinatus</i>								
<i>Stephanodiscus</i>	0	0	0	0	0	25	0	0
<i>agassizensis</i>								
<i>Stichococcus contortus</i>	0	0	0	113	0	0	0	0
<i>Straurastum</i> sp.	0	0	0	0	0	17	0	0
<i>Tetraedron minimum</i>	0	0	0	0	0	0	0	0
<i>Tetrastrum</i> sp.	0	0	16	0	0	4	69	158
<i>Treubaria</i>	0	0	0	0	0	0	7	0
<i>triappendiculata</i>								
<i>Volvox globator</i>	0	0	0	310	0	0	0	0
<i>Zygnema</i> sp.	6385	1268	6	0	213	409	8	0

Table S5 Aquatic organisms' taxa and relative abundances collected from four field sites; Westlake River, Silverhurst Impoundment, Kogmanskloof River and Rolse Farm Reservoir "before and after" *Salvinia molesta* control, South Africa.

Taxa	Westlake		Silverhurst		Kogmanskloof		Rosle Farm	
	Before	After	Before	After	Before	After	Before	After
Freshwater Fish								
<i>Gambusia affinis</i>	-	2	-	-	-	-	-	-
<i>Oreochromis mossambicus</i>	-	5	-	-	-	-	-	-
Aquatic Macroinvertebrates								
Turbellaria	-	-	-	-	-	1	-	-
Hirudinea	-	69	-	3	-	-	-	-
Copepoidae	-	-	-	-	-	7	-	-
Cypridoidae	-	-	-	-	-	73	-	-
Hydracarina	-	-	-	-	-	-	-	43
Baetidae	-	25	-	1	-	10	-	17
Coenagrionidae	-	2	-	-	24	29	-	28
Aeshnidae	-	1	-	-	2	2	-	1
Gomphidae	-	2	-	-	-	-	-	-
Libellulidae	-	-	-	-	1	5	-	17
Crambidae	-	1	-	-	-	-	-	-
Belostomatidae	-	1	-	-	-	-	-	21
Corixidae	-	51	-	-	3	-	-	-
Gerridae	-	1	-	-	-	-	-	25
Pleidae	-	-	-	-	4	59	-	56
Veliidae	-	-	-	-	-	-	-	7
Dytiscidae	-	3	-	-	-	-	-	8
Hydraenidae	-	1	-	-	-	3	-	-
Hydrophilidae	-	6	-	-	1	19	-	-
Ceratopogonidae	-	-	-	-	-	3	-	-
Chironomidae	-	129	-	50	2	2	-	154
Culicidae	-	2	-	-	-	-	-	3

Muscidae	-	-	-	-	1	2	-	-
Psychodidae	-	-	-	-	-	-	-	8
Lymnaeidae	-	4	-	6	-	-	-	-
Physidae	-	19	-	17	118	59	-	39
Planorbinae	-	4	-	1	-	-	-	18
Ancylidae	-	-	-	-	-	68	-	-
Thiaridae	-	2	-	-	-	-	-	-
Viviparidae	-	6	-	-	-	-	-	-
Adult Odonata								
species								
<i>Pseudagrion massaicus</i>	-	-	-	-	-	-	-	15
<i>Ischnura senegalensis</i>	34	5	-	1	13	-	-	-
<i>Anax imperator</i>	-	2	-	-	3	2	-	4
<i>Anax speratus</i>	-	-	-	-	-	-	-	9
<i>Ictinogomphus ferox</i>	-	-	-	-	-	-	-	8
<i>Crocothemis erythraea</i>	-	-	-	-	-	-	1	-
<i>Brachythemis leucosticta</i>	-	-	-	-	-	-	2	-
<i>Palpopeura lucia</i>	-	-	-	-	-	-	-	2
<i>Trithemis arteriosa</i>	-	3	-	-	11	10	-	7
<i>Orthetrum</i> sp.	-	-	-	-	3	2	-	-
<i>Pantala flavescens</i>	-	-	-	-	-	-	-	6
<i>Urothemis edwardsii</i>	-	-	-	-	-	-	2	-

Table S6 Carbon and nitrogen stable isotope values (mean and standard deviation) of basal resources and aquatic consumer groups sampled and the taxonomic/feeding group to which species were assigned for broad food web analysis, collected from the Westlake River, Silverhurst Impoundment, Kogmanskloof River and Rosle Farm Reservoir “before and after” *Salvinia molesta* control.

System	Period	Species/Taxa	Functional Feeding Groups/Taxonomic group	N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Westlake River	Before	Phytoplankton	Basal resources	6	-26.04 (1.28)	10.69 (0.63)
		Periphyton	Basal resources	6	-25.90 (1.67)	10.10 (0.73)
		<i>Ceratophyllum demersum</i>	Basal resources	3	-31.16 (0.12)	18.35 (0.06)
		<i>Myriophyllum aquaticum</i>	Basal resources	3	-31.58 (0.10)	14.14 (0.12)
		<i>Pistia stratiotes</i>	Basal resources	3	-28.19 (0.18)	12.85 (0.14)
		Organic matter	Basal resources	3	-28.59 (0.13)	13.37 (0.16)
		<i>Cyperus</i> sp.	Basal resources	3	-29.28 (0.13)	10.47 (0.19)
		<i>Typha</i> sp.	Basal resources	3	-27.73 (0.10)	12.34 (0.13)
		<i>Salvinia molesta</i>	Basal resources	3	-29.08 (0.26)	12.15 (0.09)
	After	Phytoplankton	Basal resources	10	-28.82 (1.10)	10.10 (1.10)
		Periphyton	Basal resources	4	-26.39 (0.67)	9.63 (1.97)
		<i>Myriophyllum aquatic</i>	Basal resources	3	-31.01 (0.21)	18.11 (0.22)
		<i>Typha</i> sp.	Basal resources	3	-28.35 (0.05)	11.39 (0.11)
		<i>Salvinia molesta</i>	Basal resources	3	-31.26 (1.40)	10.66 (0.89)
		Floating algae	Basal resources	3	-26.76 (0.22)	12.13 (0.21)
		Organic matter	Basal resources	3	-29.96 (0.16)	11.94 (0.90)
		Lymnaeidae	Scraper	2	-22.16 (0.59)	16.58 (0.07)
		Baetidae	Scraper	3	-30.30 (0.21)	15.72 (0.31)
		Aeshnidae	Predator	3	-28.82 (0.52)	10.65 (0.55)
		Coenagrionidae	Predator	3	-29.26 (0.27)	17.40 (0.36)
		Hydraenidae	Scraper	3	-22.41 (1.16)	10.65 (0.55)
		Hydrophilidae	Shredder	3	-29.27 (2.51)	12.90 (1.84)
		Dytiscidae	Predator	3	-31.98 (0.36)	5.05 (0.88)
		Corixidae	Herbivore	1	-28.30	13.29
		Gerridae	Predator	1	-26.24	16.27

Silverhurst Impoundment	Before	Belostomatidae	Predator	3	-27.77 (0.05)	11.99 (0.49)
		Chironomidae	Collector-gatherer	1	-28.08	14.42
		<i>Oreochromis mossambicus</i>	Omnivore	6	-26.63 (2.10)	15.70 (0.26)
		<i>Gambusia affinis</i>	Omnivore	6	-28.68 (0.36)	9.85 (0.22)
		Phytoplankton	Basal resources	6	-27.21 (0.46)	5.54 (1.92)
	After	Periphyton	Basal resources	9	-27.29 (0.23)	7.23 (0.37)
		Organic matter	Basal resources	3	-30.14 (0.10)	6.08 (0.09)
		<i>Salvinia molesta</i>	Basal resources	3	-30.77 (0.30)	10.65 (1.11)
		Phytoplankton	Basal resources	6	-28.24 (0.40)	5.92 (0.68)
		Periphyton	Basal resources	4	-28.60 (2.22)	7.52 (1.13)
		Organic matter	Basal resources	3	-29.28 (0.22)	6.82 (0.58)
		Common grass	Basal resources	3	-13.59 (0.45)	9.34 (0.24)
		<i>Myriophyllum aquaticum</i>	Basal resources	3	-30.89 (0.03)	4.49 (0.16)
		Physidae	Scraper	3	-26.45 (3.39)	10.83 (0.60)
		Lymnaeidae	Scraper	6	-25.04 (1.24)	9.76 (0.88)
		Planorbidae	Scraper	1	-14.51	7.06
		Baetidae	Scraper	1	-31.35	7.93
		Hirudinea	Predator	3	-29.62 (0.22)	12.75 (0.02)
		Chironomidae	Collector-gatherer	3	-29.37 (0.47)	9.16 (1.21)
		Phytoplankton	Basal resources	6	-23.81 (1.21)	9.54 (1.03)
Kogmanskloof River	Before	Periphyton	Basal resources	6	-24.37 (1.78)	9.59 (1.11)
		<i>Ceratophyllum demersum</i>	Basal resources	3	-26.97 (0.04)	16.53 (0.04)
		Organic matter	Basal resources	3	-27.23 (1.49)	12.55 (0.92)
		<i>Salvinia molesta</i>	Basal resources	3	-27.24 (0.06)	14.07 (0.12)
		Crustacean	Collector-filter	3	-18.85 (0.41)	12.31 (0.07)
		Physidae	Scraper	3	-24.82 (0.59)	12.31 (1.25)
		Coenagrionidae	Predator	3	-22.42 (0.43)	13.43 (0.39)
		Libellulidae	Predator	3	-22.87 (0.21)	12.72 (0.21)
		Hydraenidae	Scraper	1	-19.40	9.04

		Hydrophilidae larvae	Predator	3	-20.21 (0.63)	8.87 (0.95)
		Hydrophilidae adult	Shredder	3	-21.16 (0.76)	10.02 (1.67)
		Pleidae	Predator	3	-22.80 (0.28)	12.16 (0.35)
After		Phytoplankton	Basal resources	6	-25.81 (0.59)	10.58 (0.85)
		Periphyton	Basal resources	6	-28.55 (0.99)	11.25 (1.01)
		<i>Ceratophyllum demersum</i>	Basal resources	3	-29.06 (0.08)	19.20 (0.25)
		Floating algae	Basal resources	3	-26.24 (0.29)	15.29 (0.48)
		<i>Arundo donax</i>	Basal resources	3	-11.99 (0.15)	13.48 (0.13)
		Organic matter	Basal resources	3	-27.30 (0.47)	13.35 (0.08)
		<i>Salvinia molesta</i>	Basal resources	3	-29.12 (0.45)	15.26 (0.93)
		Ostracoda	Collector-filter	3	-21.79 (0.80)	13.66 (0.06)
		Physidae	Scraper	3	-25.82 (0.98)	16.45 (0.51)
		Lymnaeidae	Scraper	3	-26.25 (0.17)	16.45 (0.40)
		Limpet	Scraper	3	-24.79 (0.47)	16.86 (0.20)
		Baetidae	Scraper	3	-30.48 (0.23)	16.54 (0.09)
		Coenagrionidae	Predator	3	-26.57 (0.11)	17.15 (0.17)
		Libellulidae	Predator	3	-25.12 (0.11)	16.29 (0.18)
		Aeshnidae	Predator	3	-25.49 (0.56)	17.38 (0.40)
		Hydrophilidae	Shredder	3	-26.84 (0.28)	14.58 (0.06)
		Belostomatidae	Predator	3	-28.61 (0.25)	16.62 (0.14)
		Chironomidae	Collector-gatherer	3	-27.89 (0.46)	15.92 (0.37)
Rosle Farm Reservoir	Before	Phytoplankton	Basal resources	6	-25.40 (0.86)	5.59 (0.36)
		Periphyton	Basal resources	6	-25.73 (0.38)	6.28 (0.47)
		<i>Stukina</i> sp.	Basal resources	3	-31.91 (0.07)	10.08 (0.20)
		<i>Salvinia molesta</i>	Basal resources	3	-30.19 (0.87)	11.08 (1.78)
		Adult Odonata	Predator	3	-24.56 (0.72)	12.07 (0.43)
After		Phytoplankton	Basal resources	6	-27.50 (0.32)	7.52 (0.59)
		Periphyton	Basal resources	6	-26.99 (0.42)	8.02 (0.39)
		<i>Stukina</i> sp.	Basal resources	3	-28.96 (0.46)	8.58 (0.24)
		<i>Salvinia molesta</i>	Basal resources	3	-30.21 (0.05)	3.51 (0.48)

Organic matter	Basal resources	3	-28.84 (0.71)	7.26 (0.89)
Baetidae	Scraper	3	-34.55 (1.35)	8.07 (0.11)
Coenagrionidae	Predator	3	-30.49 (0.55)	10.77 (0.54)
Libellulidae	Predator	3	-32.95 (1.17)	10.29 (0.95)
Aeshnidae	Predator	3	-32.92 (0.92)	12.86 (0.70)
Dytiscidae	Predator	3	-27.41 (0.01)	12.96 (0.14)
Pleidae	Predator	1	-28.96	5.67
Belostomatidae	Predator	3	-29.86 (0.54)	10.17 (0.89)
Chironomidae	Collector-gatherer	3	-36.92 (1.17)	10.29 (0.95)

Table S7 Epiphytic algae species list and relative abundance collected at Westlake River following macrophyte multiple succession phases, South Africa.

Taxa	<i>Salvinia molesta</i>	Clear- water June	Clear- water Oct	<i>Ceratophyllum demersum</i>	<i>Nymphaea mexicana</i>
<i>Anabaena</i> sp.	69	59	13	933	0
<i>Anabaena spiroides</i>	163	0	0	0	0
<i>Calothrix parietina</i>	0	0	0	512	0
<i>Lyngbya</i> sp.	0	0	0	29	0
<i>Lyngbya martensiana</i>	0	0	0	37	0
<i>Merismopedia glauca</i>	0	78	171	12	82
<i>Oscillatoria species</i>	15	0	0	0	0
<i>Oscillatoria limosa</i>	0	208	0	0	0
<i>Oscillatoria tenuis</i>	0	259	0	19	284
<i>Psuedanabaena</i> sp.	397	0	0	0	0
<i>Spirulina maior</i>	0	15	39	49	76
<i>Achnanthes standeri</i>	0	0	0	19	0
<i>Achnanthidium eutrophilum</i>	0	0	26	0	62
<i>Achnanthidium exiguum</i>	0	70	13	0	133
<i>Amphora</i> sp.	145	0	0	0	0
<i>Amphora coffeaeformis</i>	1199	634	34	265	75
<i>Amphora copulata</i>	18	0	0	0	0
<i>Amphora montana</i>	303	18	0	22	0
<i>Amphora pediculus</i>	0	0	0	153	0
<i>Amphora ovalis</i>	198	9	0	24	0
<i>Amphora veneta</i>	138	313	0	369	144
<i>Aulacoseira</i> sp.	0	18	0	0	0
<i>Aulacoseira subarctica</i> <i>f. subborealis</i>	0	0	0	10	0
<i>Caloneis</i> sp.	74	0	0	0	0
<i>Cocconeis</i> sp.	985	0	0	0	0
<i>Cocconeis engelbrechtii</i>	462	432	419	2071	118
<i>Cocconeis pediculus</i>	143	379	118	119	80
<i>Cocconeis placentula</i>	0	0	9833	0	1456
<i>Craticula</i> sp.	0	386	169	90	211

<i>Craticula bruderi</i>	15	0	0	0	0
<i>Craticula cuspidata</i>	66	0	0	37	0
<i>Craticula halophila</i>	16	0	0	0	0
<i>Ctenephora pulchella</i>	0	9	0	0	0
<i>Cyclotella</i> sp.	2173	0	0	0	0
<i>Cyclotella meneghiniana</i>	319	2292	1518	930	2178
<i>Cymbella tumida</i>	0	15	0	10	33
<i>Cymbella</i> sp.	9	0	0	26	0
<i>Diadismus</i> sp.	112	0	0	0	0
<i>Diadismus contentata</i>	0	70	0	0	0
<i>Diadismus confervacea</i>	7	0	44	0	0
<i>Diploneis</i> sp.	0	0	0	0	17
<i>Diploneis oploneis</i>	0	0	0	0	13
<i>Epithemia adnata</i>	0	245	0	48	10
<i>Epithemia sorex</i>	0	0	0	10	0
<i>Encyonopsis leei</i> var. <i>sinensis</i>	0	27	0	0	520
<i>Eunotia bilunaris</i>	0	0	0	0	24
<i>Eunotia incisa</i>	0	9	0	0	0
<i>Eunotia minor</i>	0	30	0	0	190
<i>Fallacia pygmaea</i>	0	27	0	0	0
<i>Fragilaria biceps</i>	15	0	0	99	0
<i>Fragilaria capucina</i>	0	0	0	0	10
<i>Fragilaria nanana</i>	0	58	0	38	0
<i>Fragilaria tenera</i>	0	150	0	115	19
<i>Fragilaria ulna</i>	446	1275	44	937	136
<i>Fragilaria ulna</i> var. <i>acus</i>	0	2031	0	2268	142
<i>Frustulia vulgaris</i>	16	0	0	0	0
<i>Gomphonema accuminatum</i>	22	61	49	32	90
<i>Gomphonema affine</i>	7	167	49	44	444
<i>Gomphonema affine</i> . <i>gracile</i>	0	0	0	0	21

<i>Gomphonema gracile</i>	7	0	0	0	0
<i>Gomphonema italicum</i>	557	0	0	0	0
<i>Gomphonema laticollum</i>	808	146	0	88	358
<i>Gomphonema parvulum</i>	0	339	617	10	2151
<i>Gomphonema parvulum</i> <i>var. rigidum</i>	0	0	0	0	7
<i>Gomphonema pseudoaugur</i>	7	196	214	10	49
<i>Gomphonema venusta</i>	0	192	260	0	264
<i>Gyrosigma sp.</i>	0	15	0	0	0
<i>Gyrosigma rautenbachiae</i>	98	0	0	0	0
<i>Hantzschia amphioxys</i>	60	88	0	29	0
<i>Hippodonata capitata</i>	726	575	1790	158	673
<i>Lemnicola hungarica</i>	7	165	147	94	144
<i>Luticola acidoclinata</i>	0	53	0	0	0
<i>Mastogloia smithii</i>	0	0	0	10	0
<i>Melosira varians</i>	0	223	529	12	382
<i>Navicula sp.</i>	497	912	364	86	1131
<i>Navicula antonii</i>	0	180	0	156	0
<i>Navicula capitata</i>	0	90	0	0	0
<i>Navicula cryptotonella</i>	74	0	0	0	0
<i>Navicula cryptotenelloides</i>	33	0	0	0	0
<i>Navicula erifuga</i>	0	41	0	19	0
<i>Navicula gregaria</i>	0	104	0	0	69
<i>Navicula notha</i>	15	0	0	0	0
<i>Navicula recens</i>	733	0	0	0	0
<i>Navicula radiosa</i>	0	0	0	26	0
<i>Navicula riediana</i>	7	179	26	90	1098
<i>Navicula rostellata</i>	0	35	0	10	0
<i>Navicula trivialis</i>	0	1094	144	1118	676
<i>Navicula veneta</i>	74	529	78	10	327
<i>Navicula zanoni</i>	88	53	0	671	0

<i>Nitzschia</i> sp.	54	82	83	49	574
<i>Nitzschia amphibia</i>	0	0	0	39	0
<i>Nitzschia aurariae</i>	0	0	0	13	0
<i>Nitzschia capitellata</i>	16	234	52	608	50
<i>Nitzschia dissipata</i>	0	18	0	0	0
<i>Nitzschia draviellensis</i>	0	68	26	57	14
<i>Nitzschia filiformis</i>	506	517	0	673	37
<i>Nitzschia frustulum</i>	7	18	0	0	0
<i>Nitzschia gracilis</i>	0	15	0	12	0
<i>Nitzschia intermedia</i>	747	0	0	408	0
<i>Nitzschia linearis</i>	1120	297	0	1075	190
<i>Nitzschia palea</i>	358	117	26	3103	387
<i>Nitzschia linearis</i> var. <i>subtilis</i>	0	0	0	12	0
<i>Nitzschia littorea</i>	0	0	0	13	17
<i>Nitzschia radicula</i>	0	0	0	12	0
<i>Nitzschia recta</i>	0	0	0	19	0
<i>Nitzschia reversa</i>	0	18	0	226	0
<i>Nitzschia sublinearis</i>	0	215	0	358	0
<i>Nitzschia sigma</i>	0	12	0	0	0
<i>Nitzschia umbonata</i>	88	12	26	83	75
<i>Pinnularia borealis</i>	15	0	0	0	13
<i>Pinnularia viridiformis</i>	543	524	54	150	516
<i>Placoneis</i> sp.	0	35	0	0	0
<i>Placoneis placentula</i>	0	18	0	0	0
<i>Planothidium rostratum</i>	22	319	295	0	187
<i>Pleurosigma elongatum</i>	104	18	0	12	0
<i>Pleurosigma salinarum</i>	0	9	0	0	0
<i>Rhoicosphenia</i> <i>abbreviata</i>	0	0	0	13	0
<i>Rhopalodia gibba</i>	143	91	0	19	0
<i>Rhopalodia gibberula</i>	0	0	0	10	23
<i>Rhopalodia musculus</i>	0	0	0	0	13
<i>Rhopalodia operculata</i>	0	0	0	0	13
<i>Sellaphora pupula</i>	15	968	273	10	182

<i>Seminavis strigosa</i>	0	9	0	253	0
<i>Stephanodiscus</i> <i>agassizensis</i>	9	0	0	0	0
<i>Straurosira elliptica</i>	0	29	26	63	0
<i>Suriella angusta</i>	0	12	0	0	7
<i>Tabellaria flocculosa</i>	0	0	0	12	92
<i>Tabularia fasciculata</i>	539	583	56	0	451
<i>Tryblionella apiculata</i>	0	12	0	0	0
<i>Tryblionella gracilis</i>	88	0	0	0	0
<i>Tryblionella littoralis</i>	0	0	0	48	0
<i>Peridinium</i> sp.	0	12	0	0	0
<i>Chroomonas baltica</i>	0	0	0	19	0
<i>Cryptomonas erosa</i>	0	0	0	19	0
<i>Cryptomonas ovata</i>	0	0	0	37	0
<i>Closterium acicularis</i>	0	0	0	10	0
<i>Closterium incurvum</i>	0	15	0	0	0
<i>Closterium leibleni</i>	0	0	0	19	0
<i>Closterium monoliferum</i>	0	0	0	25	0
<i>Coelastrum</i> <i>proboscidem</i>	0	0	26	0	0
<i>Crucigenia</i> sp.	7	0	0	0	0
<i>Euglena</i> sp.	0	45	0	0	0
<i>Euglena chlamydophora</i>	0	9	0	0	0
<i>Euglena granulata</i>	0	61	0	0	0
<i>Euglena oblonga</i>	0	0	0	0	13
<i>Euglena proxima</i>	0	70	74	0	33
<i>Euglena repulsans</i>	0	69	0	0	0
<i>Euglena proxima</i>	0	45	0	0	0
<i>Euglena texta</i>	0	53	0	25	0
<i>Oocystis</i> sp.	7	0	0	0	0
<i>Phacus</i> sp.	0	0	0	10	0
<i>Phacus caudatus</i>	0	44	52	13	0
<i>Phacus cochleatus</i>	0	70	19	0	0
<i>Phacus nordstedtii</i>	0	53	0	0	0
<i>Phacus orbiculus</i>	0	35	0	0	0

<i>Phacus pleuronectes</i>	0	235	49	26	13
<i>Strombomonas</i>	15	0	0	0	0
<i>Trachelomonas</i> sp.	81	15	0	0	17
<i>Trachelomonas abrupta</i>	0	0	19	0	0
<i>Trachelomonas granulata</i>	0	0	0	38	0
<i>Ankistrodesmus fusiformis</i>	0	0	0	13	0
<i>Cosmarium debaryi</i>	0	0	0	12	0
<i>Cosmarium subcostatum</i>	0	0	0	47	0
<i>Characium sieboldii</i>	0	42	0	0	0
<i>Franceia breviseta</i>	0	0	0	34	0
<i>Monoraphidium graffithii</i>	0	0	73	121	0
<i>Pediastrum</i> sp.	29	0	0	0	0
<i>Pediastrum kawraiskyi</i>	0	15	0	0	0
<i>Pediastrum simplex</i>	0	15	19	0	0
<i>Pediastrum tetras</i>	0	50	0	196	0
<i>Scenedesmus</i> sp.	260	0	0	156	10
<i>Scenedesmus bernatdii</i>	0	9	0	0	10
<i>Scenedesmus communis</i>	0	188	222	105	58
<i>Scenedesmus costatis</i>	0	0	0	10	0
<i>Scenedesmus dinorphus</i>	0	233	299	39	270
<i>Scenedesmus opoliensis</i> var. <i>mononensis</i>	0	12	142	0	78
<i>Scenedesmus opoliensis</i> var. <i>carinatus</i>	0	66	107	0	294
<i>Stichococcus conturtus</i>	0	0	0	50	0
<i>Tetraedron minimum</i>	0	0	0	107	0
<i>Treubaria triappendiculata</i>	0	0	0	10	0
<i>Zygnema</i> sp.	6385	1268	1484	2018	539

Table S8 Aquatic organisms collected at Westlake River following macrophyte multiple succession phases, South Africa.

Taxa	<i>Salvinia molesta</i>	Clear- water Jun	Clear- water Oct	<i>Ceratophyllum demersum</i>	<i>Nymphaea mexicana</i>
Freshwater Fish					
<i>Gambusia affinis</i>	-	53	2	6	1
<i>Oreochromis mossambicus</i>	-	5	-	-	-
Aquatic macroinvertebrates					
Oligochaeta	-	-	-	12	-
Hirudinea	-	-	69	23	24
Potamonautidae	-	-	-	7	-
Baetidae	-	25	-	30	-
Coenagrionidae	-	6	2	12	12
Aeshnidae	-	1	-	4	-
Gomphidae	-	-	2	-	-
Libellulidae	-	-	-	2	2
Crambidae	-	-	1	-	-
Belostomatidae	-	1	1	5	2
Corixidae	-	1	51	-	1
Gerridae	-	1	-	5	-
Notonectidae	-	-	-	1	-
Pleidae	-	-	-	115	-
Dytiscidae	-	2	3	1	1
Hydraenidae	-	1	-	-	-
Hydrophilidae	-	5	1	10	-
Ceratopogonidae	-	-	-	6	-
Chironomidae	-	10	129	4	158
Culicidae	-	2	-	-	-
Lymnaeidae	-	3	4	1	2
Physidae	-	10	19	6	43

Planorbinae	-	5	4	16	22
Ancylidae	-	-	-	-	11
Thiaridae	-	-	2	-	-
Viviparidae	-	6	-	-	4
Adult Odonata					
species					
<i>Ischnura</i>	34	-	5	17	-
<i>senegalensis</i>					
<i>Anax imperator</i>	-	-	2	-	2
<i>Trithemis arteriosa</i>	-	-	3	4	-

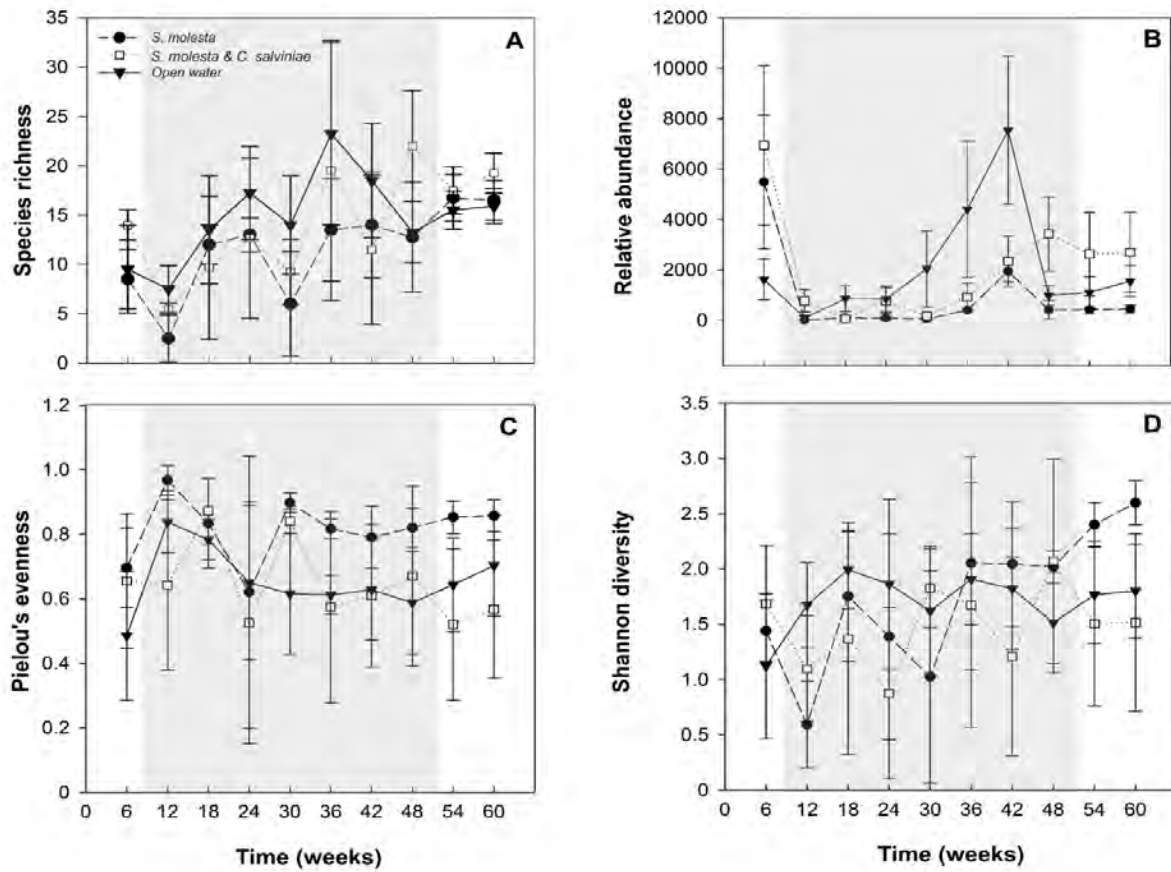


Figure S1. Epilithic algae biodiversity indices (mean and standard deviation); taxa richness (A), relative taxa abundance (B), Peilou's evenness (C) and Shannon diversity (D), between treatments and invasion phase per week for 60 weeks. Where *S. molesta* – Impacted treatment; *S. molesta* & *C. salviniae* – Restored treatment; Open water – Control treatment.

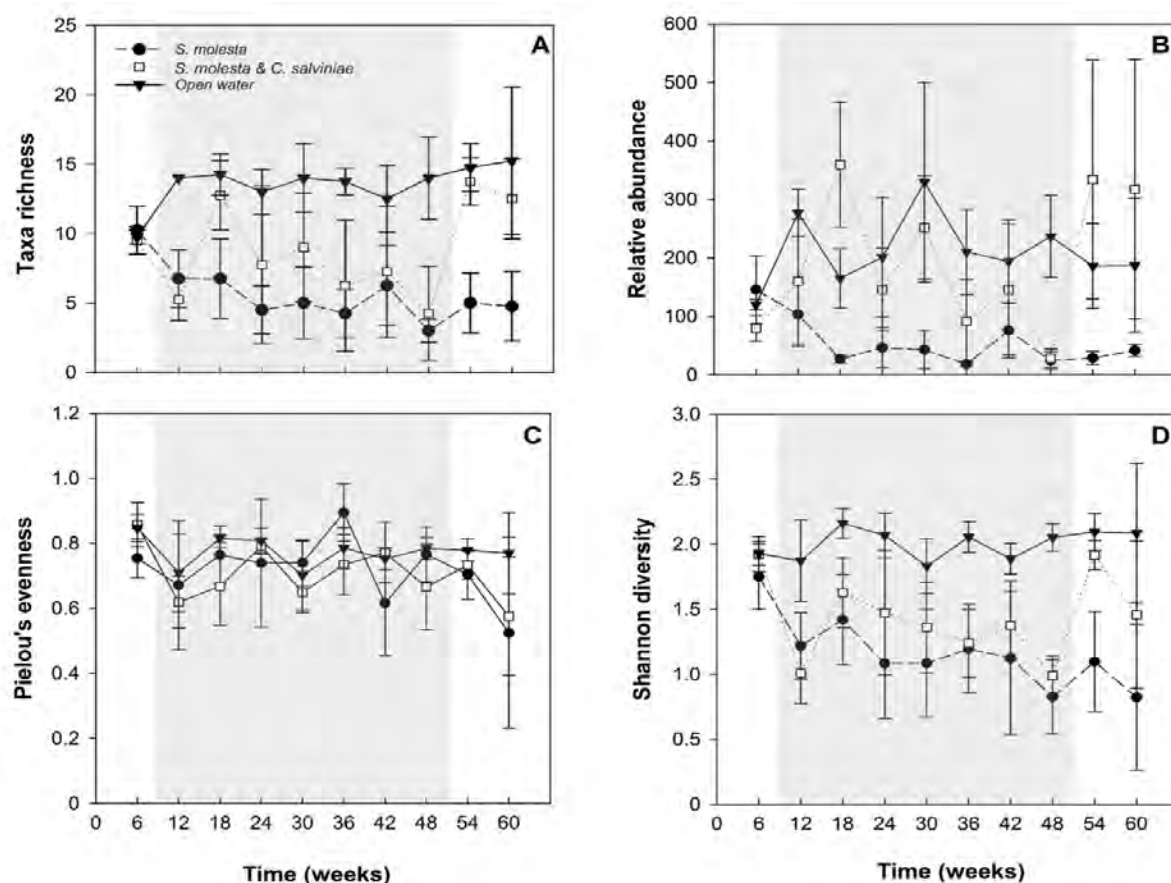


Figure S2. Aquatic macroinvertebrates biodiversity indices (mean and standard deviation); taxa richness (A), relative taxa abundance (B), Peilou's evenness (C) and Shannon diversity (D), between treatments and invasion phase per week for 60 weeks. Where *S. molesta* – Impacted treatment; *S. molesta* & *C. salviniae* – Restored treatment; Open water – Control treatment.