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Evidence for a biological control-induced regime shift
between floating and submerged invasive plant
dominance in South Africa

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Abstract

South Africa has a long history battling the establishment and spread of invasive floating macrophytes. The negative consequences of these are costly both economically and ecologically. They form dense mats on the water's surface that deplete resources such as light and oxygen to the submerged community, which creates anoxic conditions, reduces biodiversity and limits access to freshwater. The past thirty years of South African invasive plant research and the implementation of nationwide biological control programmes has led to widespread control of these species in many degraded systems. Such initiatives are aimed at restoring access to potable freshwater and increasing native biodiversity. However, in recent years, where there has been a decline in floating invasive plant populations, an increase in the establishment and spread of submerged invasive plant species has been observed. Species such as Brazilian waterweed (*Egeria densa* (Planch.) (Hydrocharitaceae)) and Eurasian watermilfoil (*Myriophyllum spicatum* (L.) (Haloragaceae)) have been recorded in South African freshwater systems, posing significant threats to aquatic ecosystems.

This thesis proposes that the biological control of floating invasive plants, which occurs in numerous dams and rivers nationwide, is the catalyst of a regime shift from floating invasive to submerged invasive plant dominance. Regime shifts are large and often sudden changes in the key structure and functioning of ecosystems, and studies into their occurrence and driving mechanisms broadens understanding of community structures and can guide effective resource management. In order to explore the existence of this new regime shift, a multi-platform approach using controlled experiments and ecological modelling techniques was employed. A model system was created consisting of a floating invasive (*Pistia stratiotes* L. (Araceae)), a submerged invasive (*E. densa*) and an ecologically analogous submerged

native plant species (*Lagarosiphon major* (Ridl.) Moss (Hydrocharitaceae)). A suite of experiments was conducted to explore the interactions between the floating and submerged plants under varying regimes of floating plant biological control and levels of nutrient loading.

These experiments revealed a competitive advantage of the invasive *E. densa* over the native *L. major* that increased by 86% under heavy nutrient loading. The relative growth rate and accumulated biomass of *E. densa* was significantly higher for plants grown in the presence of biologically controlled *P. stratiotes* (compared to insect free plants). This demonstrates a high capacity for the invasive *E. densa* to capitalise on resources made newly available through the biological control of the floating plants. In contrast, the native *L. major* fared poorly when grown in the presence of the floating *P. stratiotes*, regardless of applied biological control measures. The experimental observations were then used to parameterise a mathematical model, built to provide a holistic understanding of the individually assessed interactions which work together as the driving mechanisms underpinning the newly identified regime shift.

This thesis utilised a multi-platform approach to build the first body of evidence in support of a newly recognised regime shift between floating invasive and submerged invasive plant dominance, as driven by biological control. The results indicate that a reduction in the nutrient loading of South Africa's freshwater systems will reduce negative impacts of submerged invasive macrophytes, whilst increasing system resilience against future invasion. The evidence presented has the potential to better inform management of South Africa's freshwater systems and highlights the importance of integrating multi-trophic interactions when considering future invasive plant management. This research also opens up a multitude of possibilities for studies into submerged plant invasion mechanisms and resilience of native macrophyte communities in South Africa, and further afield.

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Chapter 1

Introduction

1.1 Invasive species

The past century has witnessed exponential growth of the human population on a global scale. This has inevitably coincided with dramatic changes to natural environments as the need for resources and space has increased. For example, agriculture has undergone enormous expansion in the last 100 years, bringing changes to nutrient and water cycling. These disturbances to key ecosystem processes are also occurring at a time where advancing urbanization, trade and technology mean that opportunities for species across all taxa to spread worldwide is at an all-time high (Westphal *et al.* 2008; Finnoff and Tschirhart 2005).

The terminology surrounding invasive species can be variable and complex, with terms such as 'alien invasive', 'non-native', 'exotic' all commonly used. For the purpose of this study the term 'invasive species' is used in accordance with the definition presented by Richardson *et al.* (2000): Alien species that sustain self-replacing populations over multiple life cycles; produce reproductive off spring, often in large numbers at considerable distances from the parent and/or site of introduction; and have the potential to spread over long distances.

The establishment of invasive species is often credited as the second biggest threat to global biodiversity after habitat loss, and the negative ecological effects of invasions can be substantial and long lasting (Keane and Crawley 2002). These include hybridization, predation, competitive exclusion that leads to reductions in native species diversity and a

breakdown in ecosystem function, as well as changes to nutrient availability, disturbance regimes and species assemblages (Ayers *et al.* 2004; Kandori *et al.* 2009; Levine *et al.* 2003; Vila *et al.* 2011; Ehrenfeld 2003). Studying invasions can expand current knowledge of ecosystem functioning, extinctions and species' responses to climate change (Sax *et al.* 2007).

Furthering knowledge in this field can also help cut costs to the global economy. In South Africa alone, it has been estimated that invasive plants cost the economy approximately US\$646 million per year (de Lange & van Wilgen 2010).

1.1.1 Establishment

A commonly accepted driver behind many exotic plant invasions is termed the enemy release hypothesis (ERH) (Williams, 1954). ERH states that exotic species that arrive in new ranges are free from their natural enemies and this lack of regulation facilitates an increase in their distribution and abundance (Keane and Crawley 2002). Whilst logical, it has been proposed that the evidence for ERH in the field is not always as clear-cut as the theory suggests. A review published in 2012 (Jeschke *et al.* 2012) looks at six major hypotheses that attempt to explain and predict biological invasions, including ERH. They found that not only was the level of empirical support for ERH left wanting (54% of all ERH based studies), but that alternative theories of invasional meltdown and novel weapons were proportionally more supported (Fig.1.1). When broken down further by taxonomic groupings, only 50 percent of botanical based ERH studies substantiated the theory (Fig.1.2).

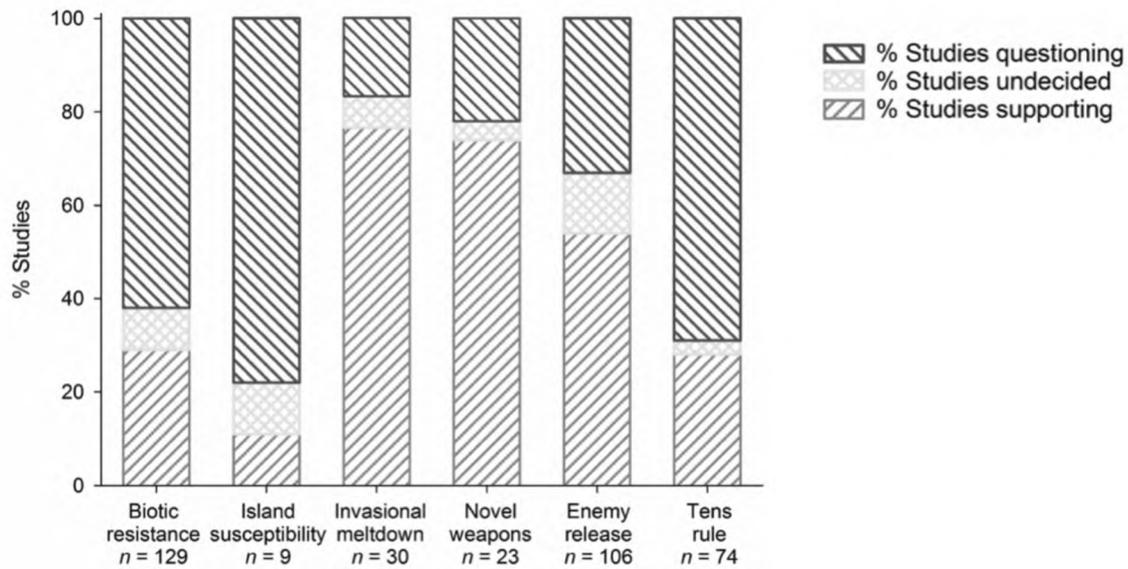


Fig. 1.1 Overall levels of empirical support for six major invasion biology hypotheses. Reproduced from Jeschke *et al.* (2012).

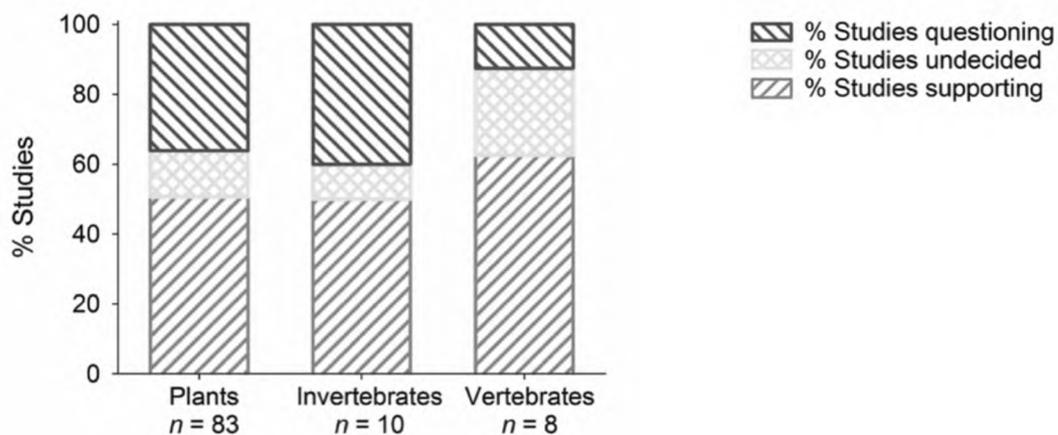


Fig. 1.2 Levels of empirical support for enemy release hypothesis sub-divided by taxonomic group. Reproduced from Jeschke *et al.* (2012).

As a further development of ERH Blossey and Nötzold (1995) proposed the evolution of increased competitive ability (EICA) theory. The theory states that in the absence of natural enemies, specifically herbivores, costly chemical defenses become reduced in some invasive plant species. Again, this theory has produced publications of mixed findings that both support and oppose it (Hull-Sanders *et al.* 2007).

Not all plants introduced to new ranges succeed in establishing, and it is a smaller fraction of those that cause severe negative impacts to their invaded ecosystems. Efforts to predict invasive success have led to the development of several theories on the subject. The 'Tens Rule' states that approximately ten percent of species successfully transition from being imported into a new region to becoming invasive (Williamson and Fitter 1996). This theory has been rebuked, with one study claiming it to be a dangerous assumption with little evidence that promotes the public image of invasive species being of little concern (Jarić and Cvijanović 2012). In agreement, the study into evidence for theories of invasion biology (Fig. 1.1, Jeschke *et al.* 2012) report less than a third of the 'Tens Rule' literature supports the theory.

Another theory used to explain the disparity between species introduced and species that become invasive is phenotypic plasticity, whereby species with genotypes that are able to express different phenotypes under varying environmental conditions are better equipped to adapt to novel environments, leading to an increased likelihood of establishment. This was first proposed by Baker (1965), and a review in 2006 (Richards *et al.* 2006) suggests that two primary hypotheses now underlie studies into successful establishment of invasive species; the first one is that successful invasive species are intrinsically more plastic than native ones, and the second is that invasive populations evolve higher plasticity once in their new range. It has also been suggested that whilst biological features such as phenotypic plasticity may indicate which species have relatively higher invasive potential, propagule pressure (the number of seeds introduced and the number of introduction opportunities) may ultimately override such features and be the most important factor in determining invasive plant success (Holle and Simberloff 2005; Colautti 2006; Berg *et al.* 2016). Whilst ERH and EICA play significant roles in the explanation behind some species' successes, the continuing

development of these additional theories highlights the complexity of invasive species establishment and encourages continued research in this field. A disparity between the theories we have a strong understanding of and the theories that are used as a basis for invasive species management increase chances of inefficiency and wasted resources (Suding *et al.* 2004; Dick *et al.* 2013).

1.1.2 Management

When faced with the management of invasive species, there are three strategies to consider: eradication, control, or allowing the species to become integrated within its new range without intervention (Hussner *et al.* 2017). In many cases, eradication is either impractical or only achievable through costly and/or labour intensive methods. However, leaving a species to establish can often lead to unpredictable ecological and economic problems in the future, and consequently the best approach is often that of control: maintaining low, manageable levels of invasive populations (Van Driesche *et al.* 2010).

Classical biological control (CBC) is the method of purposefully introducing co-evolved enemies, termed biological control agents, of an invasive species to reduce the vigour, reproductive capacity and density of invasive populations (DeLoach 1997). Biological control agents of invasive alien plants can include herbivorous vertebrates, diseases, fungal pathogens, but more commonly, insects and mites. The consequences of biological control agents on invasive plants can be direct, including feeding on plant tissue, plant fluid (sap suckers), defoliation, leaf mining, galling, predation of seeds, boring into the roots and shoots; or indirect, such as the weakening and decreasing of competitive capabilities within its environment (Lodge 1991; Wilson and McCaffery 1999).

CBC is a method that has often raised controversies, and as with most intervention-based ecological management options, there are both advantages and disadvantages (Palmer *et al.* 2010). From an advantageous perspective, the monetary costs and manual labour requirements associated with biological control are considerably lower than those of mechanical removal of plants and, unlike herbicides and pesticides, they are non-polluting and leave no toxic residues (Eisworth *et al.* 2002). Biological control agents can establish viable, self-sustaining populations that can spread throughout a target species' invasive range, reaching hard-to-access areas. The densities of agent populations also adjust in response to those of the target invaders, meaning continuous intervention of agent populations is minimal (Culliney 2005). Arguments against the implementation of CBC mostly stem from the risks associated with the introduction of more exotic species and fears they may harm the environment, economy and human health (Van Lenteren *et al.* 2006). Stringent host-specificity testing is routinely performed prior to release of biological control agents to ensure that they only damage the target invasive, and to minimise the risk to native species (McEvoy 1996). As research progresses, the levels of control over the agents used increases, and it is becoming a more successful tool worldwide. In the cases where it has been successful, the benefits have far outweighed the costs (Van Wilgen *et al.* 2001).

Within biological control research, there is a growing need for investigation into the complex system dynamics that are affected by its implementation. The majority of screenings and host specificity tests traditionally look at the direct interactions between a potential agent and its target species, and many plant interaction experiments focus on changes within a single trophic level (Van *et al.* 1998; James *et al.* 2006; Martin and Coetzee 2014). However, studying the indirect effects of the agents on the competitive interactions of the target species, as well as multi-trophic cascade effects of biological control, would paint a more

holistic picture of the impacts CBC can have on a system. Multi-trophic studies can also help elucidate the drivers that determine levels of success and failure in the establishment of invasive species, and their biological control agents (Harvey *et al.* 2010). For example, Coetzee *et al.* (2005) explored the effect that a particular biological control agent has on the competitive ability of the target plant, *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae). The results showed that despite a successful reduction in competitive fitness of the target plants, the effect was reduced under increased nutrient loading. Findings such as these can help to maximise success of biological control in the field and improve understanding of system dynamics.

Schultz and Dibble (2012) report a lack of research regarding invasive macrophytes impacts on multi-trophic system dynamics. In 2005, a study was carried out to test whether ecological variation at lower trophic levels (plants and herbivores) could be used to create a formula to estimate success of biological control against insect pests (Gross *et al.* 2005). Using historical data of recorded biological control attempts, and information on the test systems, they were able to construct a model that could accurately estimate historical successes of biological control. The findings reveal that consideration of multi-trophic parameters can be used to better understand the effects of biological control, and could even help determine levels of success in future research. Successful management is reliant on a thorough understanding of the affected ecosystem as a whole, and the most efficient management can be obtained by prioritizing the most at-risk systems.

1.2 Vulnerable systems

1.2.1 Aquatic invasions

Whilst establishment of invasive alien species is documented to be detrimental in a range of environments, it is believed that certain traits can increase a system's vulnerability

to colonization by invasive species. The ‘fluctuating resource theory’ proposed by Davis *et al.* (2000) explores this issue to explain why some systems are more vulnerable than others. The theory states that bouts of increased resource availability, created either by supply exceeding demand from native vegetation or resource demand declining due to disturbance, coinciding with high exposure to invasive propagules, will lead to the most susceptible systems. Davis *et al.* (2000) state that a vital aspect of this theory is the fluctuating, non-constant nature of increased resource availability. Stochastic floods, droughts and anthropogenic pollution can dramatically alter resource availability in aquatic systems (Dahm *et al.* 1998; Smith *et al.* 1999). These aspects, combined with their intrinsically high connectivity and opportunities of propagule spread via natural pathways and the extensive aquaria trade, mean that aquatic systems are highly susceptible to colonisation from invasive species (Padilla and Williams 2004; Moorhouse and McDonald 2015).

In 2013, the International Union for Conservation of Nature (IUCN) updated a list of the ‘100 worst invasive species’ to highlight awareness of the increasing problems associated with biological invasions (<http://www.iucngisd.org/>). This list includes two aquatic plant species that have caused significant problems globally: giant salvinia, *Salvinia molesta* D.S. Mitchell (Salviniaceae) and water hyacinth, *E. crassipes*, both originating from South America (Lowe *et al.* 2000; Courchamp *et al.* 2013). The release of the salvinia weevil (*Cyrtobagous salviniae* Calder & Sands (Coleoptera: Curculionidae)) on invasive *S. molesta* in South Africa has been so successful that a review published in 2011 (Coetzee *et al.* 2011b) stated that nationwide control had been achieved and that no alternative management besides CBC would be required in the future. CBC initiatives for *E. crassipes* have proven to be less successful, and research will continue exploring integrated management approaches using multiple agents (e.g. *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae), *N. bruchi*

Hustache (Coleoptera: Curculionidae), *Niphograptus albiguttalis* Warren (Lepidoptera: Pyralidae), and *Megamelus scutellaris* Berg (Hemiptera: Delphacidae). Because South Africa has been heavily invaded by invasive aquatic plants (there are currently 20 listed invasive aquatic plant species present in the country (NEMBA 2014)), there is a plethora of local literature on the topic, as well as a number of research centres such as the Plant Protection Research Institute (Agricultural Research Council), the Centre of Invasion Biology (Stellenbosch University), the Biological Control Research Group (Rhodes University) and several institutes that rear CBC agents for release.

1.2.2 South African freshwater systems

The natural topography and climate of South Africa has resulted in very few natural lakes, and in order to supply water to the rising population, large numbers of artificial dams and weirs have been constructed. These include approximately 500 large and 150,000 smaller reservoirs and farm dams that provide vital resources for employment, recreation, food and biodiversity (Basson *et al.* 1997). The artificial nature of such structures means there is a lack of evolutionary history in South Africa's native flora for species adapted to thrive in these slow moving or still waters. Nutrient pollution from increased urbanization, industry, mining, power generation, agricultural run-off, domestic and commercial sewage treatment, as well as previously poorly-regulated water management has led to the eutrophication of the majority of these water bodies (Oberholster and Ashton, 2008; van Ginkel 2011). The waters are heavily loaded with ammonium and nitrates from poorly managed sewage infrastructure and unregulated fertilizer usage, and in combination with a lack of naturally occurring native macrophytes, the freshwater ecosystems in South Africa are particularly vulnerable to invasive alien plant invasions (Hood and Naiman 2000; Odume *et al.* 2016).

South Africa has a long history of battling floating invasive macrophytes such as water hyacinth (*E. crassipes*), water lettuce (*Pistia stratiotes* L. (Araceae)), red water fern (*Azolla filiculoides* Lam. (Azollaceae)), parrots feather (*Myriophyllum aquaticum* Vell. (Haloragaceae)) and *Salvinia minima* Baker (Salviniaceae). These plants are able to reproduce quickly and form dense mats on the water's surface. These mats decrease water quality and quantity, increase siltation of rivers, dams and wetlands, reduce biodiversity and ecosystem functioning, drown livestock and cause problems for irrigation canals and pumps (Janse 1998; Scheffer *et al.* 2003; Caraco *et al.* 2006). Management strategies for such plants have involved mechanical removal, applications of herbicide and, more successfully, the release of multiple biological control agents. The invasions of these floating macrophytes have been well studied and managed and the majority are now regarded as being under some aspect of control (Hill 2003; Coetzee *et al.* 2011b). Whilst increased control of these floating plants is promising both ecologically and economically, the past decade has witnessed an increase in the establishment of submerged invasive macrophytes, with species such as *Myriophyllum spicatum* L. (Haloragaceae), *Elodea canadensis* Michx. (Hydrocharitaceae) and *Egeria densa* Planch. (Hydrocharitaceae) recorded as established across the country (Coetzee *et al.* 2011b). For example, a study on the Vaal River, South Africa's most economically important river system, between 2006-2010, showed that wherever the percentage cover of *E. crassipes* was reduced, there was an increase in the recorded percentage cover of submerged macrophytes (Fordham 2012; Fig.1.3).

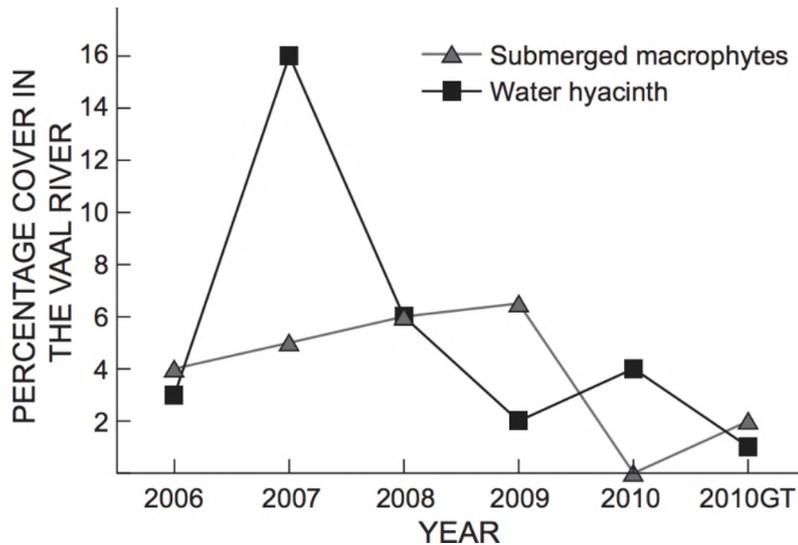


Fig.1.3. A study of changes in plant dominance over time in the Vaal River, South Africa. Reproduced from Fordham (2012).

Submerged plant invasions are often not identified until individual plants have reached the water's surface. This reduces chances of an early response regarding their control and management. Although the introduction of submerged plants initially increases levels of dissolved oxygen in the water column which improves water quality (Brix and Shierup 1989), when populations explode, the dense monoculture stands have negative implications which can be similar to those associated with floating invasive plants. These include damage to hydro-electrical equipment, decreased water quality and biodiversity, reduction in flow rate, limitations to water access, as well as altering nutrient regimes and sedimentation which may increase flood risk (Chen and Barko 1988; Barko *et al.* 1988; Vermaat *et al.* 2000; Bickel and Closs 2007; Yarrow *et al.* 2009; Stiers *et al.* 2011b).

The spread of invasive submerged plants into and across southern Africa has been driven by the ornamental and aquaria trade (Martin and Coetzee 2011). Efforts to curb such trade through criminalization often has little impact due to the logistics of monitoring and enforcement, particularly with regards to online trading (Kay and Hoyle 2001). Lack of wider

education of the physiology of such species can also inadvertently promote spread. For example, the invasive Brazilian waterweed, *E. densa* Planch autofragments and can reproduce vegetatively. Therefore if the machinery used to clear *E. densa* from sections of Cape Town rivers, for example, is not fully cleaned, it can transport plant fragments to other sites, promoting further establishment (Fig. 1.4) (Pers. Comm. City of Cape Town, Invasive Species Unit).



Fig. 1.4 *Egeria densa* being mechanically removed from the Liesbeek River, Cape Town, 2014 (Image: E. Strange)

To minimize the impacts of invasive submerged plants, aquatic weed research in South Africa must now focus on understanding the mechanisms facilitating these new invasions in order to form successful management strategies. Current understanding of the associated herbivores of submerged aquatic plants is limited in comparison to their floating counterparts, further research in this field will increase the likelihood of successful control programs for their management.

1.3 Regime shifts

1.3.1 Theory of regime shifts and hysteresis

Regime shifts, in an ecological context, are large and often sudden changes in the functioning and structure of an ecosystem (Scheffer 2003). It is proposed that these shifts are induced when a threshold of a limiting variable such as nutrients or light (a resource essential for growth or maintenance) in an ecosystem is crossed, either through a gradual change (e.g. long-term pollution) or through a sudden shock (e.g. hurricane or flood) (Fig. 1.5) (Biggs *et al.* 2011). The dominant system feedbacks are weakened or broken causing the trajectory or direction of the system to change towards a new regime. New feedback mechanisms are then created and maintained allowing the new regime to become stable (Scheffer and Carpenter 2003; Walker and Meyers 2004; Folke *et al.* 2004; Beisner 2012). Studies into the impacts of regime shifts on socio-ecological systems and their associated services have gained ground in recent years as a need was recognized for understanding the underlying processes to maximise efficient management and increase sustainability.

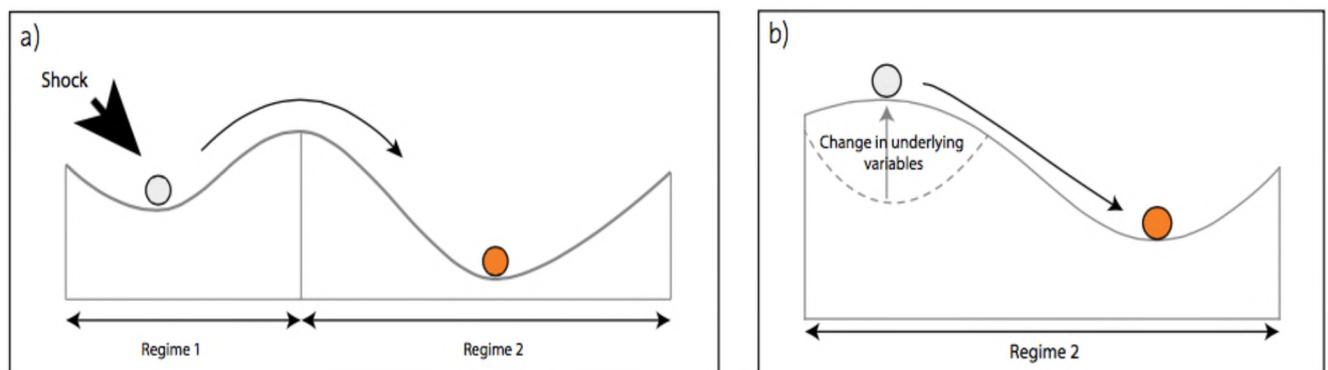


Fig. 1.5 The 'ball-in-cup' diagram is often used to demonstrate shifts of an ecosystem (ball) between two two stable regimes (valleys) as caused by a) a sudden shock to the system and/or b) a gradual change in a limiting variable (Reproduced from Biggs *et al.* 2012)

A classic and often cited example of an ecosystem experiencing a regime shift between two states is that of shallow eutrophic lakes (Blindow *et al.* 1993; Scheffer *et al.* 2001). Research has shown that the original stable regime of a healthy and productive aquatic ecosystem with clear waters and high biodiversity is maintained by internal positive feedback loops between plants and nutrients. External nutrient loading or the occurrence of a stochastic event (floods, storms etc.) can cause a key threshold to be crossed. This can result in the weakening or destruction of the positive feedback loop sustaining the system, prompting a shift into a state where persistently high nutrient levels lead to algal blooms, hypoxia, fish kills, toxicity and decreased biodiversity (Vitousek *et al.* 1997; Rabalais 2002; Green and Galatowitsch 2002).

Lake Apopka, in Florida, USA, was once in a stable macrophyte-dominated state (Bachmann *et al.* 1999). Organic waste from a nearby citrus processing plant and inadequately treated sewage effluent were discharged in the lake and over time it became saturated with phosphorous. It was assumed that this nutrient pollution was the cause of the lake's degradation into a stable turbid algal state, and what followed was one of the most expensive restoration projects in the US at that time (>\$US 100million). The objective was to reduce lake nutrient levels as a means of restoring the clear waters and to re-establish a macrophyte-dominated state. Bachmann *et al.* (1999) however, studied this lake within the context and consideration of regime shifts. Their results did not support the assumption that nutrient input was either the sole or reversible catalyst for the turbidity of Lake Apopka. They argued that the most plausible explanation was the occurrence of a regime shift, with the latest shift being induced by a local hurricane in 1947, which uprooted the majority of macrophytes, in turn decreasing sediment stability in the presence of wind-driven waves, creating perpetually turbid waters. Thirteen years after the restoration began, the system had still not been

restored, and they concluded that even a phosphorous reduction of 80% would not promote re-establishment of macrophytes, because nutrient loading was not the driving force of the degradation. This case highlights the importance of recognizing regime shifts and the broader issue of fully understanding the mechanisms of ecosystem structure in order to minimize the wasting of resources and maximize management success. In cases where nutrient input is the catalyst for stable state shifts in ecosystems, reduction of the nutrients in the water is often not enough to restore the previous stable eutrophic state. Instead, nutrient levels may need to be substantially lower than those at which the collapse of the system occurred (Scheffer *et al.* 2001).

The observation that the trajectories an ecosystem has followed when collapsing differs from those that are needed for recovery (and cannot simply be reversed) is termed hysteresis (Schröder 2005). Hysteresis is an important consideration when managing a system that may be exhibiting regime shifts, as ignoring this concept and the feedback patterns that developed to sustain alternate stable states could lead to undesirable and unpredictable results (Suding *et al.* 2004), creating more long-term ecological and economic damage. Being able to recognize regime shifts and understand what maintains them could allow for prediction of the direction in which the stable states are shifting and allow for more effective management (Beisner 2012).

Within the context of invasion biology, the theory of mechanisms that underpin regime shift research are transferable to the field. Ecosystems that are successfully colonised by non-native species often remain in long-term stable degraded states. Intervention aimed at restoring such systems has traditionally been based upon the premise of re-establishing historical abiotic conditions such as removing the invasive species (Dobson *et al.* 1997; Young *et al.* 2000; Prach *et al.* 2001). However, when we start to consider this restoration in the

context of regime shifts, hysteresis and resilience, it is clear there is a need for a much broader understanding of the system features such as community assemblage and nutrient cycling that are affected by invasive species and their subsequent management.

1.3.2 Resilience

For the purpose of clarification, in this study ecological resilience refers to Holling's definition (Hollings 1973) which describes it as the resistance of an ecosystem to change, particularly from one stable state into another (Holling 1973). Resilience is an important consideration in the management of invaded systems, as high resilience of a restored ecosystem lowers the risk of a shift back into the degraded state (Carpenter and Cottingham 1997). It is well documented that nutrient loading is a significant driver of invasions in aquatic systems; therefore, systems that are managed by reducing eutrophication will increase in resilience against future invasions (Davis *et al.* 2000; Daehler 2003). Scheffer *et al.* (2001) argue that the perception that many ecosystems respond smoothly to environmental change is not necessarily true, and management should focus on facilitating ecological resilience because this reduces the risk of stable state shifts.

A recent study examined long-term impacts of the invasive giant hogweed, *Heracleum mantegazzianum* Sommier and Levier (Apiaceae), in the Czech Republic (Dostal *et al.* 2013). They found that while native species richness and productivity were reduced by hogweed invasions, after long periods of time (~30 years), these features began to recover. This study highlights the plasticity of invaded and degraded ecosystems and it raises clear issues regarding the idea of a 'stable state': How do we define these states and are we considering their complexities when we do? Are the time frames we use to define 'stable' and 'unstable' valid in terms of ecological timescales? Scheffer and Carpenter (2003) profess that natural fluctuations should not be confused with regime shifts. There is a great need in invasion

ecology for research into the mechanisms behind invasions, including further exploration into regime shifts.

1.4 Competition

1.4.1 Competitive interactions

Within the context of regime shifts, the stability of a regime relies on the interactions and feedbacks within it. Competitive interactions play a key role in the structure and functioning of ecosystems. Competition is described as the interaction between separate taxa (interspecific), and separate individuals of the same taxa (intraspecific), sharing limited resources (Lacoul and Freedman 2006). It is one of the most important biotic interactions of invasion biology. Within aquatic systems, competitive fitness can determine the success of establishment of invaders and levels of an ecosystem's resilience to invasion. Some studies claim that interspecific competition could play a key role in the success of biological control of rooted submerged weeds (Cuda *et al.* 2008; Stiers and Njabuya 2011). Its role in structuring communities means that when exploring a potential regime shift between the dominance of different species, it is clear that competitive interactions remain at the core of the study (Menge *et al.* 1976; McCreary, 1991; Van *et al.* 1999). Within aquatic ecosystems, there are a multitude of factors that can affect competitive interactions, including nutrient concentrations of both the sediment and the water column, light intensity, dissolved oxygen, temperature, climate and species' growth forms (Titus and Adams 1979). Mony *et al.* (2006) explored the effects of season and sediment nutrient concentration on interspecific competition between two submerged macrophytes in North America, *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) and *E. densa*. Interspecific competition was found to be higher in spring than autumn. This study, along with several others (Haller and Sutton, 1975; Van *et al.* 1999; Spencer and Ksander, 2000) found a weakened competitive capacity of *H. verticillata*

under low nutrient concentrations when competing against native species with similar growth forms. Competition from *H. verticillata* also promoted resource allocation to the roots of *E. densa*, demonstrating the effects competition can have at both a community and species level. Top down mechanisms such as herbivory can also play a role in determining competitive fitness, as they affect plant growth and fecundity (Crawley 1989), alter community composition (Olf and Richie 1998), induce chemical and physical defences, as well as nutrient reallocation within individuals (Louda *et al.* 1990; Kessler and Baldwin 2001), and lead to compensatory plant re-growth (McNaughton 1983; Maron and Crone 2006).

Competitive ability is thus clearly a complex concept. Tipping *et al.* (2008) studied competition between two floating macrophytes (*Salvinia minima* Baker (Salviniaceae) and *Spirodela polyrhiza* (L.) Schleid (Araceae)) mediated by nutrient levels and herbivory in Florida, USA. They hypothesized that herbivory would increase the competitive capacity of *S. polyrhiza* by negating the apparent morphological advantage of *S. minima*. Their results did not support this and revealed a more complex dynamic between herbivory, nutrient levels and competition. They found that although *S. minima* may be more dominant in low nutrient environments and more influenced by herbivory, it had no effect on the competitive success of *S. polyrhiza*, which was dominant in eutrophic conditions. They also observed that these plant communities were vulnerable to wind, currents and passage of animals that create temporary gaps of open water, leading to plant assemblage reordering, which could all affect competitive interactions between plants. All these factors influence competitive interactions and highlight the need for a deeper understanding of the dynamics involved in competition.

As the management of invasive species is becoming more urgent, the use of biological control is increasing, leading to a global increase in the translocation of more biological control agents. In order to minimise risk and improve control, studies into the direct effects

these agents have on target invaders, as well as their effect on competitive interactions in a wider context are vital.

One particular species of submerged invasive plant that has recently experienced a rapid spread across multiple aquatic systems in South Africa is *E. densa*, commonly referred to as Brazilian waterweed. *Egeria densa* is the focal species for this thesis and in order to study its competitive fitness in a South African context, it will be tested against a native submerged plant with a similar growth form, *Lagarosiphon major* (Roxb.) (Ridley) Moss (Hydrocharitaceae), as the most intense competition within aquatic systems occurs between plants of similar growth forms occupying the same position in the water column (Gopal and Goel 1993).

1.5 *Egeria densa* Planch (Hydrocharitaceae)

1.5.1 Ecology, physiology and origins

Egeria densa (Fig. 1.6) is a submerged, dioecious, freshwater perennial originating from the subtropical regions of south-eastern Brazil, eastern Argentina and southern Uruguay (Cook *et al.* 1974). The stems can grow to over three metres long, sometimes forming dense monospecific stands that can cover large areas (Yarrow *et al.* 2009). The slender rooted stems are brittle, breaking easily and thus aiding fragmentation and spread. The leaves (15-30 mm long) occur in whorls of three in the lower parts of the stem, and whorls of four to eight in upper stem sections. The three-petalled white flowers, which range in size from 18 – 25 mm and extend 20 mm above the water's surface, are entomophilous (Cook *et al.* 2004). When submerged, the petals close, trapping air bubbles, thus keeping the stamens and stigmas dry (Cook and Urmi-König, 1984).

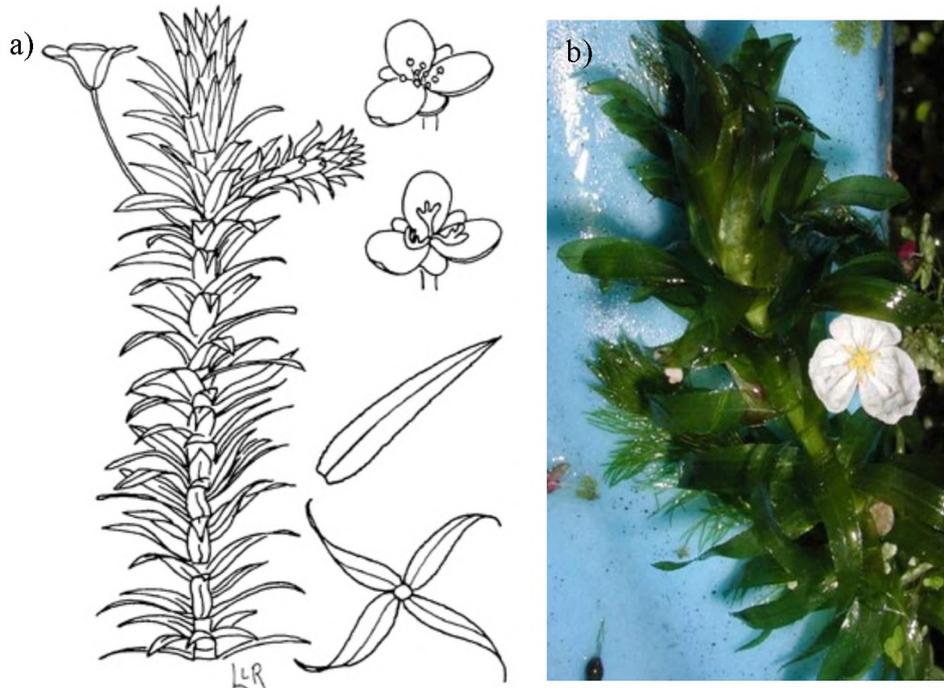


Fig. 1.6 a) Line drawing of *Egeria densa* (IFAS Centre for Aquatic and Invasive Plants), b) flowering *Egeria densa*.

1.5.2 Invasive distribution and negative impacts

Egeria densa has spread from its native South American range to many countries, including Australia, Chile, Denmark, France, French Polynesia, Germany, Japan, New Zealand, Puerto Rico, the USA and South Africa (Gassman *et al.* 2006), and its spread has been accelerated by the pond and aquarium trade (Martin and Coetzee 2011) (see Appendix C for South African distribution map). From an ecological perspective, *E. densa* removes nutrients from the water column, controlling the growth of phytoplankton and can alter sedimentation and water flow (Yarrow *et al.* 2009). This has led to the argument that *E. densa* is an 'ecosystem engineer' as its physical structure affects the light and nutrients available to other organisms (Jones *et al.* 1994). Historically, management of this plant has not included intensive mechanical removal, due to the expense and inefficiency, but more significantly, because it has brittle stems that can reproduce via fragmentation, thus mechanical interference could

ultimately promote growth. This combination of factors suggest that biological control is a more viable alternative (Cabrera Walsh *et al.* 2013). In South Africa host-specificity trials are currently being trialled for the release of the fly *Hydrellia egeriae* sp. nov. (Diptera: Ephydriidae) as a potential agent for *E. densa* (Pers. Comms. Rhodes University, SA).

1.6 *Lagarosiphon major* (Roxb.) (Ridley) Moss (Hydrocharitaceae)

Lagarosiphon major (Fig. 1.7) is one of the most common species of *Lagarosiphon* and is native to sub-Saharan Africa. This species is a perennial, submerged and rooted vascular plant found in lakes, rivers, streams and ponds within a wide range of trophic conditions. It is able to reproduce sexually and asexually (Cook 1974). Preferring cool waters of temperate regions, it is most often found in clear freshwater that is either still or slow moving. It can survive at relatively high pH levels and thrives at high light intensities (Schwarz and Howard-Williams 1993; ISSG 2008).

Lagarosiphon major has brittle, submersed stems measuring 3 mm in diameter and can reach 7 m in length with branches at every 10-12 nodes. When the water's surface is reached, thick mats are formed (Cook 2004). The densely crowded leaves are linear to linear-lanceolate, measuring 16 mm in length and 2 mm in width. The flowers are transparent to white or pale pink with female flowers on the water's surface tethered to the plant on thin, thread-like tubes (~25 cm long) and are pollinated by free-floating male flowers in the native range. Outside the native range, only female plants have been recorded and therefore reproduction only occurs via fragmentation.

This species has spread beyond its native range and is invasive in Ireland, the UK and New Zealand, where it has been recorded in canals, dams, drainage ditches and slow-moving rivers (Howard-Williams and Davies 1988; Caffrey *et al.* 2008). Establishment of this species

has resulted in the alteration of water flow and sedimentation, a reduction of light to lower levels of the water column and outcompeting native vegetation. Previous methods of control for this species have included mechanical removal, however, as with *E. densa*, this is not advised due to the brittle nature of the stems and its ability to reproduce via fragmentation, which can often promote overall growth. Chemical herbicides containing terbutryn or dichlobenil have been also used, both of which leave toxic residues that can harm other species present in the invaded water body (CEH, 2004). A potential biological control agent, *Hydrellia lagarosiphon* Deeming (Diptera: Ephydriidae) (native to South Africa) has been extensively researched in Ireland and is in the process of a draft application for release there and a final application for release has been submitted in New Zealand (Mangan and Baars 2013).

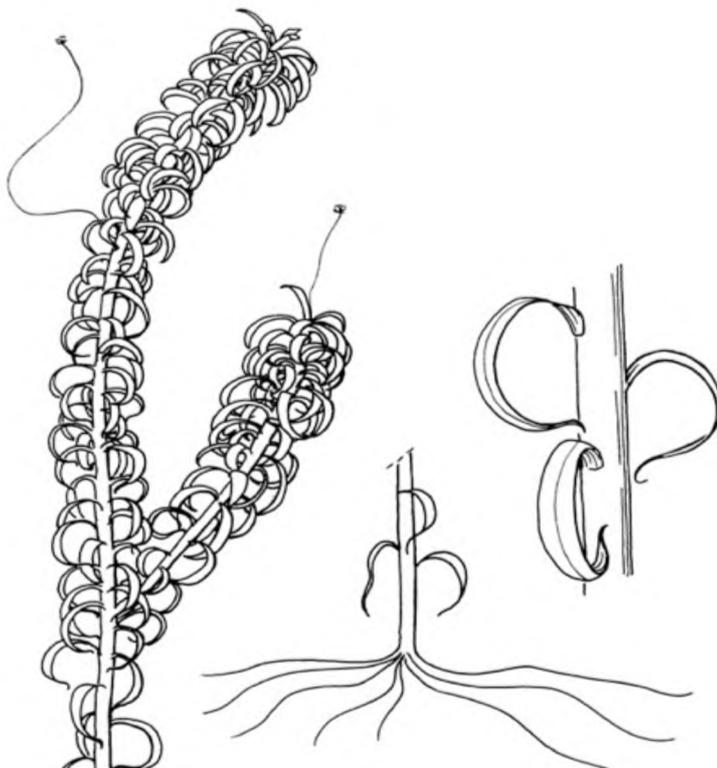


Fig. 1.7 Line drawing of flowering *Lagarosiphon major* (IFAS Centre for Aquatic and Invasive Plants)

1.7 *Pistia stratiotes* L. (Araceae) and its associated weevil, *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae)

Pistia stratiotes L. (Araceae), commonly known as water lettuce, is a Category 1b, free floating invasive aquatic plant in South Africa, of South American origin (National Environmental Management: Biodiversity Act (10/2004): Alien and Invasive Species List, 2014). Its attractive appearance and popularity as an ornamental species has led to its establishment across multiple subtropical rivers, dams and pans. Individual plants consist of a rosette of leaves, obovate to spatulate in shape, with longitudinal ribs, and its feathery roots can reach 50 cm long (Fig. 1.8 a). Both sides of the leaves are covered with short hairs that enable buoyancy. The flowers are inconspicuous (7–12.5 mm) and found at the centre of the rosette, growing on a stem (Neuenschwander *et al.* 2009).

In numerous South African systems, the plant thrives in nutrient rich conditions and can form dense floating mats, reducing water quality, damaging hydroelectric equipment and threatening biodiversity. Its ability to reproduce both vegetatively and through seed production made it a priority for control in the 1980s, which has now been achieved, solely through the implementation of CBC. The agent used, *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae) is a leaf mining and leaf feeding weevil, native to South America, that was released in South Africa in 1985 (Fig. 1.8 b & c). The population was originally from Brazil but was supplied to South Africa from Australia (Winston *et al.* 2014).

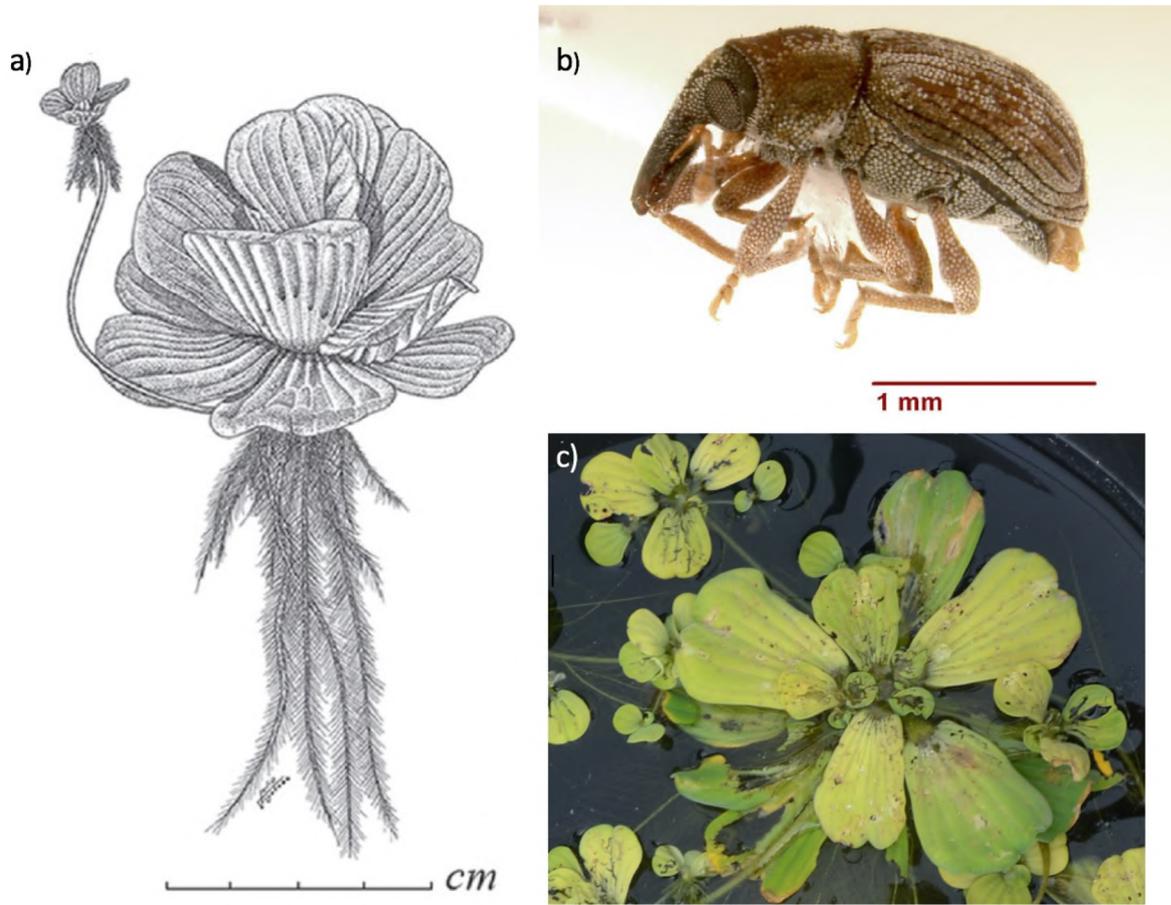


Fig.1.8 a) Line drawing of *Pistia stratiotes* (Evans 2013) b) Scaled photograph of *Neohydronomus affinis* (image: Katherine Parys, www.bugguide.net) and c) Feeding damage on *Pistia stratiotes* caused by *Neohydronomus affinis* (image: Emily Strange).

1.8 Hypotheses, Aims and Objectives

The wealth of research and knowledge that supports the implementation of CBC programmes has led to successes both in South Africa and further afield. However, the long-term effects that invasive species' management has upon the rest of the ecosystem remains largely under-researched. In order to change this, some of the fundamental questions of invasion ecology must be asked: What are the mechanisms driving and supporting invasions? What methods can be employed to better understand the consequences of invasions and their effects on community dynamics? And finally, how can we better predict invasions? For

example, very little work has been done regarding the theory of regime shifts in the context of invasions and the potential role that control methods for invasive species may play in their occurrence. However, in many cases, as the control of invasives involves the removal of a species that has been playing a dominant role in an ecosystem, it is very likely that these changes will influence many aspects, including stable state alterations.

The overall aim of this thesis was to explore the identification of a regime shift occurring in South African freshwater systems through a series of controlled experiments and ecological modelling tools. The shift was hypothesized between two stable regimes termed 'floating invasive plant dominance' and 'submerged invasive plant dominance'. This study further hypothesised that the biological control of the invasive floating plants is the driver of the regime shift. Once invasive floating plants become dominant, biological control agents are introduced to control them, with the hope of inducing a regime shift to the situation *ante*, with healthy biodiversity and good quality water. However, this thesis proposes that an alternate regime shift is occurring from the invasive floating degraded state into an equally problematic and stable state dominated by alien invasive submerged weeds. As the floating plants decompose due to herbivory from biological control agents, the system receives a sudden influx of freely available nutrients, and at the same time, light levels within the water column are restored (Chimney and Pietro 2006; Shilla *et al.* 2006; Longhi *et al.* 2008). As nutrients, light and space become available, submerged invasive plants are able to utilize the resources and proliferate (Reddy *et al.* 1982; Hammer and Knight 1992; James *et al.* 2006). Photosynthesis by submerged plants temporarily increases the levels of dissolved oxygen in the water column thus improving water quality (in the short-term) and allowing the submerged plants to become dominant (William *et al.* 1976). After this period of improvement, the water quality deteriorates as the plant biomass crosses a threshold after

which it begins to block out light, reduce biodiversity and alter sediment stability (Balciunas *et al.* 1996; Yarrow *et al.* 2009; Stiers *et al.* 2011b). External nutrient loading further facilitates their growth and helps to sustain the new stable regime of submerged invasive plant dominance (Duarte *et al.* 1995).

The first objective, discussed in Chapter Two, was to explore the competitive ability of the focal species of the study: the invasive submerged *E. densa*, with a native trophically analogous species, *L. major*. This provided important baseline data which were used in comparison with further experiments investigating submerged plant interactions. Both species were grown in a series of different planting densities and were left for three months to compete with each other for resources. After this period, the growth rate and end biomass were used to compare their competitive ability against each other in the presence of nutrient rich and nutrient poor conditions.

Chapter Three outlines the next objective, which was to compare the response of the native and invasive submerged plants within the context of the proposed regime shift. Both *E. densa* and *L. major* were independently grown in the presence of the floating invasive *P. stratiotes* under different herbivory regimes: one in which *P. stratiotes* was subjected to biological control by the host-specific agent *N. affinis*, and one in which *P. stratiotes* was not controlled. The growth rates of *E. densa* and *L. major* under these conditions demonstrated the effect biological control of floating plants has upon submerged plant communities, and whether or not the presence of biocontrol agents is a potential driver of the proposed regime shift.

The third objective, discussed in the fourth chapter, was to explore the idea that if regime shifts between floating invasive and submerged invasive plants are occurring due to human initiated CBC, there could be a possibility of inducing a third stable state: 'native

submerged plant dominance'. This was investigated by measuring the interspecific competition between *E. densa* and *L. major* and then incorporating the effect of the presence of controlled and non-controlled *P. stratiotes*.

Chapter Five aimed to combine the data obtained from these experiments to build and parameterise a mathematical model intended to test the theory of the proposed regime shift. The objective was to join theory, experimental techniques and ecological modelling together in a multifaceted study that would provide evidence for the existence of the regime shift, and expand current understanding of the mechanisms driving invasive plant establishment in a South African context.

Whilst the experiments and models presented in this study cannot reflect the complex, dynamic ecosystems upon which the theory is based, they can be the first step in a broader, systems-based approach to biological control methods. A major review into the evidence of regime shifts in aquatic and terrestrial environments (Folke *et al.* 2004) states that the first step to improve management of regime shifts is to strive to better understand the interactions between regime shifts, biodiversity and ecosystem resilience. In accordance with this viewpoint, this thesis will bring together different research techniques to highlight the importance of taking a critical look at how invaded systems are managed, and bring together the theoretical aspects of invasion biology with the applied side of biological control research.

Chapter 2

Competition between two Hydrocharitaceae: the native *Lagarosiphon major* (Ridl.) Moss and the invasive *Egeria densa* (Planch)

2.1. Introduction

The proposed regime shift at the centre of this thesis, from invasive floating to invasive submerged plant dominance as influenced by biological control, is underpinned by a key change in community structure. Rooted submerged macrophytes can define the structure and function of their ecosystems and provide a linking interface between the sediment with the water column (Duarte *et al.* 1995). Their root systems are able to influence sedimentation by increasing ground stability, and by trapping floating sediment particles, they can raise river beds, affecting flood risks and hydrological regimes (Vermaat *et al.* 2000; Yarrow *et al.* 2009). Different growth forms of submerged macrophytes can also determine the available habitats for other flora and fauna (Bickel and Closs 2007), alter macroinvertebrate biodiversity and reduce light accessibility to lower levels of the water column (Stiers *et al.* 2011b). As well as raising levels of dissolved oxygen, these plants can also alter nutrient cycling, which can have far-reaching consequences, ranging from individual species levels to community structuring, thus altering whole system functioning and key ecosystem services. The presence of invasive submerged macrophytes therefore poses significant threats to South African freshwater ecosystems.

Nutrient loading is a known driver of invasive species across a diverse range of ecosystems and taxa (Davis *et al.* 2000; Daehler 2003). Nutrient cycling plays a key role in the

spread, establishment and growth of rooted submerged plants, and the significant depletion of nitrogen and phosphorous from both the sediment and water column by submerged macrophytes has been well documented (Chen and Barko 1988; Barko *et al.* 1988; Mazzeo *et al.* 2003). Historically, this has meant that *Egeria densa* (Planch) (Hydrocharitaceae), *Potamogeton pectinatus* (L.) (Potamogetonaceae) and *Vallisneria americana* (Michx.) (Hydrocharitaceae) have been recommended as viable methods of wastewater treatment (Reddy *et al.* 1985; Hammer and Knight 1992). More recently, it has been documented that other submerged plant species can considerably increase the nutrient levels of the water column through the natural process of plant tissue decay (Shilla *et al.* 2006). Being able to alter the conditions of their surroundings can have knock-on effects for other submerged rooted macrophytes within the same habitat. For example, James *et al.* (2006) compared the species response of multiple invasive plants in the Hydrocharitaceae, which had been observed to displace one another in eutrophic inland waters in Europe; *Elodea canadensis* (Michx) was displaced by *Elodea nuttallii* (Planch), which in turn was succeeded by *Lagarosiphon major* (Ridl. Moss). They reported a negative relationship between relative growth rate and increased nutrient loading for all three species. *Elodea nuttallii* exhibited the strongest decline in root production and *E. canadensis* had the highest mean tissue nitrogen content, showing varied responses amongst related species. It is therefore apparent that the interactions between nutrients and submerged macrophytes can be complex, and studies aimed at elucidating the complexities can benefit the understanding and subsequent management of invasive submerged plants.

McCreary (1991) explore the importance of competition as a mechanism that drives submerged plant community structure. The study states that manipulation experiments that examine changes in biomass accumulation can determine the extent to which one species is

affected by a potential competitor and that community level interactions are an essential component of wider ecosystem-based approaches. Therefore, to create a holistic view of the proposed regime shift, an initial understanding of the competitive vigour of both the floating and submerged invasive species used in the suite of experiments is essential. There is a wealth of research on floating invasive macrophytes in a southern African context, exploring how their dominance is affected or driven by changes in biotic factors, such as the effects of nutrients and herbivory on the competitive performance (Coetzee *et al.* 2005) and biomass accumulation (Bownes *et al.* 2010) of *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae). In contrast, there are relatively few studies on submerged invasive macrophytes in a South African context (Coetzee *et al.* 2011a). However, some work has focussed on *E. densa* due to its successful and problematic invasion and establishment in regions outside its native South American range (Fig. 2.1) (Gassmann *et al.* 2006).



Fig. 2.1 International distribution of *Egeria densa* based on publications (www.q-bank.eu)

Global climate niche modelling has indicated a future increase in habitats ideally suited to the establishment of *E. densa*, where suitable climate is available (Kelly *et al.* 2014).

Furthermore, its thermal tolerance is quite wide, with reports of survival below ice, and with optimum temperatures for growth ranging from 16°C to 28°C, and with a maximum thermal limit of 35°C (Barko and Smart, 1981; Getsinger and Dillon 1984; Haramoto and Ikusima 1988; Di Tomaso & Healy 2003). Yarrow *et al.* (2009) report that *E. densa* exhibits adaptive physiological metabolic traits which contribute to its invasive success, with low CO₂ and high O₂ tolerances, promoting growth in suboptimal conditions. Other traits that promote invasive success include an ability to store energy in its basal stems and root crown, which allow survival through winter and enable rapid reinvasion (Pennington & Sytsma 2009). A competition study between *E. densa* and another species in the Hydrocharitaceae, *Hydrilla verticillata* (L.f.) (Royle) found the competition for nutrients to be higher than the competition for light (Mony *et al.* 2007).

Despite the recent rapid spread of *E. densa* across South Africa, there are no published studies exploring the potential local impacts of its establishment. The aim of this experiment was to quantify the competitive ability of *E. densa* in a South African context to provide initial baseline data for the proposed regime shift. *Lagarosiphon major* (Ridl.) Moss (Hydrocharitaceae) was chosen as a suitable native competitor due to its growth form and taxonomic similarities to *E. densa*, and its invasive successes in New Zealand (Howard-Williams and Davies 1988) and Ireland (Caffrey *et al.* 2008).

A review of pairwise competition experiments suggest that to accurately test whether an invasive species is more competitive than native ones, the best approach is to compare effects of closely related native and invasive species on each other (Vilà and Weiner 2004). A competition study between *L. major* and two other species in the Hydrocharitaceae (*E. canadensis* and *E. nutalli*) found *L. major* to have the highest photosynthetic rates even in stressful conditions of high pH and low CO₂ (Caffrey *et al.* 2009). Similar results were reported

in a competition study between *L. major* and *Ceratophyllum demersum* (L.) Ceratophyllaceae (Stiers *et al.* 2011a); while under specific substrate conditions in South Africa, *L. major* successfully outcompeted the invasive *Myriophyllum spicatum* (L.) Haloragaceae (Martin and Coetzee 2014). *Lagarosiphon major* thus has the potential to be highly competitive and could provide a useful comparison for the competitive abilities of *E. densa*.

Competitive strategies of invasive species are linked to the optimal resource theory (Tilman 1988). Nutrient cycling plays a key role in submerged aquatic plant life cycles and many of South Africa's freshwater systems are highly eutrophic (Oberholster and Ashton 2008). It is therefore essential to explore the role of nutrient loading on the interaction between *L. major* and *E. densa* to understand the mechanism of submerged plant invasions in a South African context. Thus, the aim of this experiment was to subject *E. densa* and *L. major* to direct competition for resources, at different planting density ratios, under two different nutrient regimes. Using different nutrient regimes will be useful for understanding the potential scale of invasion from *E. densa* in many of the mismanaged and eutrophic freshwater systems in South Africa. The results of this study will deepen current understanding of the potential threat of *E. densa* in a South African context. Not only will this benefit future management of this species, but it will also provide essential base line data on the competitive vigour of *E. densa* that represents submerged invasive plants in the overall model exploring the proposed regime shift.

2.2. Materials and methods

The experiment took place inside a greenhouse tunnel at the Waainek Research Facility at Rhodes University, Grahamstown and ran from 21 November 2014 until 16 March 2015. All *L. major* plants were collected from a quarry dam near Stutterheim, (-32.587336,

27.463443), and *E. densa* plants were collected from the Kouga River, near Patensie, (-33.748506, 24.635446), both in the Eastern Cape, South Africa. Following collection, the plants were washed and planted directly into the experiment and were left for a three-week acclimation period before the start of the experiment. The mean starting wet weight per shoot of *L. major* and *E. densa* planted in all treatments was 3.51g (± 0.09) and 0.88g (± 0.07) respectively. All plants were treated with an organophosphate insecticide, Malathion (Kombat, 0.01ml/10l), to kill any phytophagous insects associated with the plants collected from the field. Hoagland's solution (5.2mg/l) was added to each tank at the start of the experiment with the nitrogen sources omitted as this was controlled separately (Hoagland and Arnon 1938).

2.2.1 Experimental design

The experiment was conducted within two flow-through systems, each consisting of five connected tanks (measuring 120cm x 100cm x 70cm each) (Figs 2.2 and 2.3). Each tank was filled with spring water that was circulated, filtered and passed through an ultra violet light to prevent growth of planktonic algae. Each tank contained eight evenly-spaced plant pots (25cm diameter, 20cm height) filled with 5 kg of sediment collected from Jameson Dam, Eastern Cape, South Africa (-33.319073, 26.444206). The sediment was analyzed for nutrient composition by Bemlab (Pty) Ltd. Assay Laboratory (Stellenbosch, SA) and was reported to be loam sediment comprising of 0.11% nitrogen (for full analyses see Appendix D).

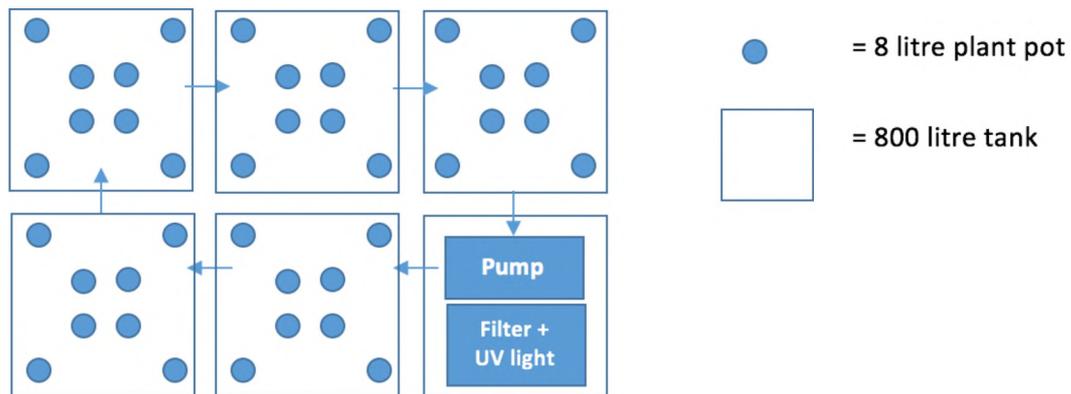


Fig. 2.2 Aerial schematic of one of the flow-through systems used to determine the competitive interactions between *Egeria densa* and *Lagarosiphon major*, as influenced by nutrients.



Fig. 2.3 One flow-through system with shade cloth (image: E. Strange)

The experimental design followed an addition series developed by Spitters (1983) that allows relative competitive abilities of two different species to be determined by using reciprocal yield models of the mean plant biomass at each planting density. *Lagarosiphon major* and *E. densa* were planted at eight different planting densities (including mixed and mono-cultures) of *L. major* : *E. densa*, which were 0:3, 3:3, 3:0, 3:9, 9:9, 9:3, 0:9 and 9:9. Each of the eight plant pots was randomly assigned one of the ratios. This method has been widely used to quantify competitive interactions between macrophytes ranging in growth forms and exposed to varying external pressures. Examples include the competition between *H.*

verticillata and *V. americana* as affected by soil fertility (Van *et al.* 1999) and herbivory (Van *et al.* 1998), as well as the effects of biological control on competition between water hyacinth, *E. crassipes* and water lettuce, *P. stratiotes* (Coetzee *et al.* 2005).

In this experiment, apical shoots of the relevant species, 20 cm in length with side shoots removed, were planted at the assigned ratios and a final 2 cm layer of silica sand was added to each pot to minimize algal growth and maintain water clarity. Each tank was randomly assigned a nutrient treatment of 'high' or 'low', with five replicates of each. The high nutrient treatment was achieved by adding 10 g (2 g/kg of sediment) of Multicote™ slow-release fertilizer with 17:7:14 N:P:K ratios mixed into the sediment of each plant pot (Haifa, Multicote™ 8; 17:7:14+micronutrients, 17% total nitrogen, formulated for a 5-6 month release rate at 30°C). The low nutrient tanks had no fertilizer added to the plant pot sediments. A single layer of shade cloth (50% density) was secured over all tanks to provide optimum conditions for submerged plant growth (Yarrow *et al.* 2009).

Thermachron iButtons (Climastats Environmental Monitoring software, Ver. 4) were added to each flow-through system to record temperature ranges, which may affect plant or insect survival. The surface water temperature was recorded at midday, daily, for the duration of the experiment. After 12 weeks, all of the above ground biomass of *L. major* and *E. densa* was collected, dried for 96 hours at 60°C and weighed. Below ground biomass was omitted from analyses as roots of both species were identical and fragile, thus those that broke away from their plant during collection could not be accurately identified.

2.2.2 Data Analysis

Relative growth rates (RGRs) of the shoots of each species were calculated using the standard formula described by Evans (1972):

$$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

where W_1 and W_2 are the dry weights at the start and end of the experiment respectively at times t_1 and t_2 (the start and end time in days). To calculate dry weights, 30 individuals taken from the same populations as those used in the experiments were weighed wet and then dried for 96 hours in a drying oven at 60°C. Linear regressions were then used on their wet and dry weights to obtain an equation used for wet to dry conversions:

$$dw_l = 0.0468 (ww_l) + 0.0188$$

$$dw_e = 0.0754 (ww_e) + 0.0105$$

where dw_l and dw_e are the dry weights of *L. major* and *E. densa*, and ww_l and ww_e are the wet weights, respectively.

Multiple regressions on the mean end dry biomass of each species at different planting densities and in different nutrient treatments were performed to determine the changes in competitive ability of the two species under the different treatments. The magnitude of the relationship was determined using the reciprocal-yield model as described by Spitters (1983) and Pantone *et al.* (1989). This involved multiple linear regressions with the inverse of the mean biomass yield for each species at the dependent variable, and the planting densities of *L. major* and *E. densa* as the independent variables. The equations for each plant follow the form of:

$$\frac{1}{W_e} = a_{e0} + a_{ee}d_e + a_{el}d_l$$

$$\frac{1}{W_l} = a_{l0} + a_{ll}d_l + a_{le}d_e$$

where W_e and W_l are the mean dry weights of *E. densa* and *L. major*, respectively. The planting densities for *E. densa* and *L. major* are expressed as d_e and d_l . The intercepts (a_{l0} and a_{e0}) measure the reciprocal of the maximum weight of individual plants. Intraspecific competition was estimated by the partial regression coefficients a_{ee} and a_{ll} . Interspecific competition was estimated by the coefficients a_{le} and a_{el} in terms of their effects on the reciprocal yield of biomass for *L. major* or *E. densa*, where each *L. major* plant has an effect of $1/W_l$ equal to a_e/a_l *E. densa* plants. Interspecific and intraspecific competition by one species on its own yield, as well as the yield of the other species, was measured using the ratio of the coefficients (a_{ll}/a_{le} and a_{ee}/a_{el}) (Pantone *et al.* 1989).

General linear model analyses of variance (GLM-ANOVAs), followed by Tukey HSD post-hoc tests, were performed to determine whether the mean end-yields and the RGRs of *L. major* and *E. densa* were significantly different between nutrient treatments and initial planting densities. All statistical analyses were conducted in the R environment (version 3.2.3; R Development Core Team, 2014; available at <http://cran.r-project.org>) using R Studio (version 0.98.1103).

2.3 Results

The mean daily surface temperature of the water was 26.4°C (\pm 0.2 S.E), with a minimum of 19.8°C and a maximum of 30.7°C. There were no significant differences between the temperatures of the two systems ($F_{1,150}=2.461$, $P=0.12$).

Egeria densa had significantly higher RGRs than *L. major* ($F_{1,66}=135.09$, $P\leq 0.000$), and had significantly higher RGRs when growing in low nutrient conditions compared to high ($F_{1,33}=10.103$, $P=0.003$) (Fig. 2.4). *Lagarosiphon major* responded poorly across all treatments,

producing negative RGRs, indicating varying degrees of plant mortality, although this was significantly reduced for plants in the low nutrient treatment ($F_{1,33}=16.760$, $P\leq 0.001$). The initial planting ratios significantly affected the RGR of *E. densa*, with higher growth rates at higher initial planting densities ($F_{1,33}=6.882$, $P\leq 0.001$). There was no effect of planting density on RGR of *L. major* ($F_{1,33}=0.791$, $P=0.56$). There were no significant differences between the RGRs of plants grown in mixed or mono-cultures for either *L. major* ($F_{1,41}=1.49$, $P=0.23$) or *E. densa* ($F_{1,41}=0.13$, $P=0.71$), and no significant interactions between the mixed/mono planting densities and nutrients for *L. major* ($F_{1,41}=0.8$, $P=0.37$) or *E. densa* ($F_{1,41}=0.28$, $P=0.6$).

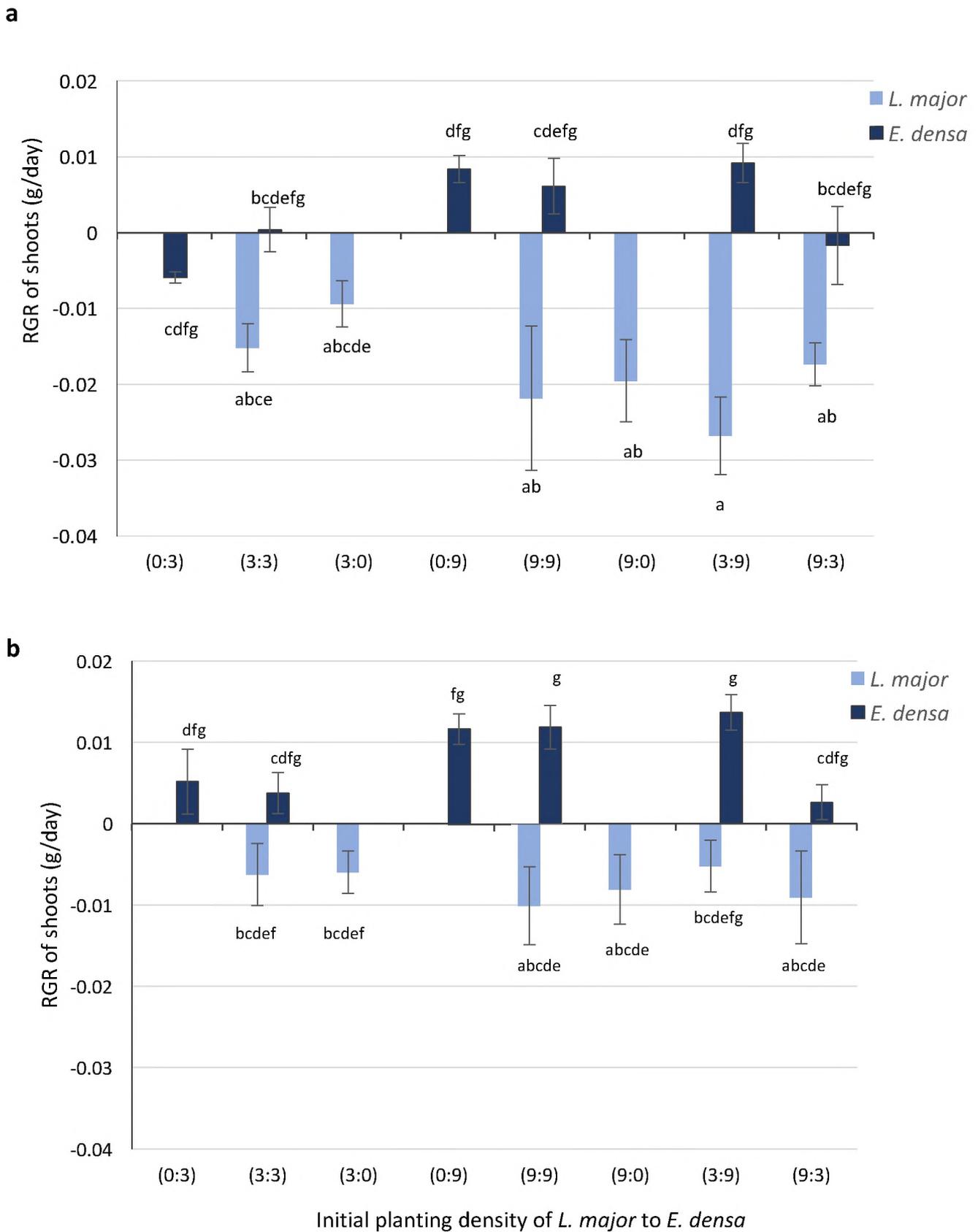


Fig. 2.4 The relative growth rate (mean \pm S.E.) of *Lagarosiphon major* and *Egeria densa* at each planting density (*L. major* : *E. densa*) grown at **(a)** high and **(b)** low nutrient conditions. Different letters denote significant differences between treatments. (Tukey HSD, $P < 0.05$).

Multiple regression analysis of the biomass of both *E. densa* and *L. major* after 16 weeks showed a significant effect of nutrient treatment on their competitive abilities (Table 2.1). The coefficients that measure the relative competitive ability of *E. densa* compared to *L. major* (a_{ee}/a_{le}) increased by 86% in high nutrients where *E. densa* was 28 times more competitive than *L. major* ($P < 0.01$). In low nutrient conditions this ratio decreased to 3.92 times more competitive ($P < 0.001$). This shows that intraspecific competition is more important than interspecific competition from *L. major* on the growth of *E. densa* within mixed planting densities. In other words, in high nutrients it would take 28 *L. major* plants to have the same effect on *E. densa* growth as one *E. densa* plant. Under low nutrient treatments, *E. densa* had a slight competitive advantage over *L. major* at 3.9 times more competitive compared to the effect *L. major* had on *E. densa*; 3.3 times, a difference of 15%. This means that when nutrients are reduced, adding 3.9 *L. major* plants has the same impact on *E. densa* as one *E. densa* plant. The ratio of coefficients that measure the relative competitive ability of *L. major* (a_{ll}/a_{el}) implies that *L. major* became an even weaker competitor relative to *E. densa* when more nutrients were available; one *L. major* plant was competitively equivalent to just less than one (0.99) *E. densa* plant.

Table 2.1 Multiple regression analysis of competition between *Lagarosiphon major* and *Egeria densa* grown at varying planting densities, and two nutrient treatments.

Biomass	Nutrients	Intercept	Intraspecific competition ¹	Interspecific competition ²	Ratio of competition coefficients ³	R ²	F ₍₂₋₂₄₎	P
<i>L. major</i>	High	0.435	0.0239	0.024	0.995	0.086	1.122	0.342
<i>E. densa</i>	High	0.462	0.037	0.002	28	0.372	7.406	<0.01*
<i>L. major</i>	Low	0.214	0.011	0.003	3.333	0.054	0.776	0.47
<i>E. densa</i>	Low	0.229	0.019	0.005	3.918	0.412	9.448	<0.001*

¹ Intraspecific competition: represented by regression coefficients a_{egeg} for *E. densa* and a_{lmim} for *L. major*.

² Interspecific competition: represented by regression coefficients a_{eglm} for *E. densa* and a_{lmeg} for *L. major*.

³ The ratio of competition coefficients measures the effect of intraspecific competition by one species on its own mass relative to the effects of interspecific competition by the other species; $a_{\text{egeg}}/a_{\text{eglm}}$ for *E. densa* and $a_{\text{lmim}}/a_{\text{lmeg}}$ for *L. major*.

These results reveal that *E. densa* is a superior competitor for resources compared to *L. major*, and nutrient availability has a strong impact on their competitive fitness. The changes in this relationship at the two nutrient treatments were graphically analysed using three-dimensional graphs (Fig. 2.5, a-d) that illustrate the four reciprocal yield models (Table 2.1). The higher the values on the figure, the lower the actual yield because inverse weight is used; and the steeper the gradient of the slope, the larger the competitive coefficient. The graphs allow for easier comparisons between competitive interactions at both nutrient treatments. The slopes of the planes indicating the effect *E. densa* has on the inverse biomass of *L. major* (Fig. 2.5. a and c) depict the significant increase in competitive ability of *E. densa* over *L. major* in high nutrients compared to low.

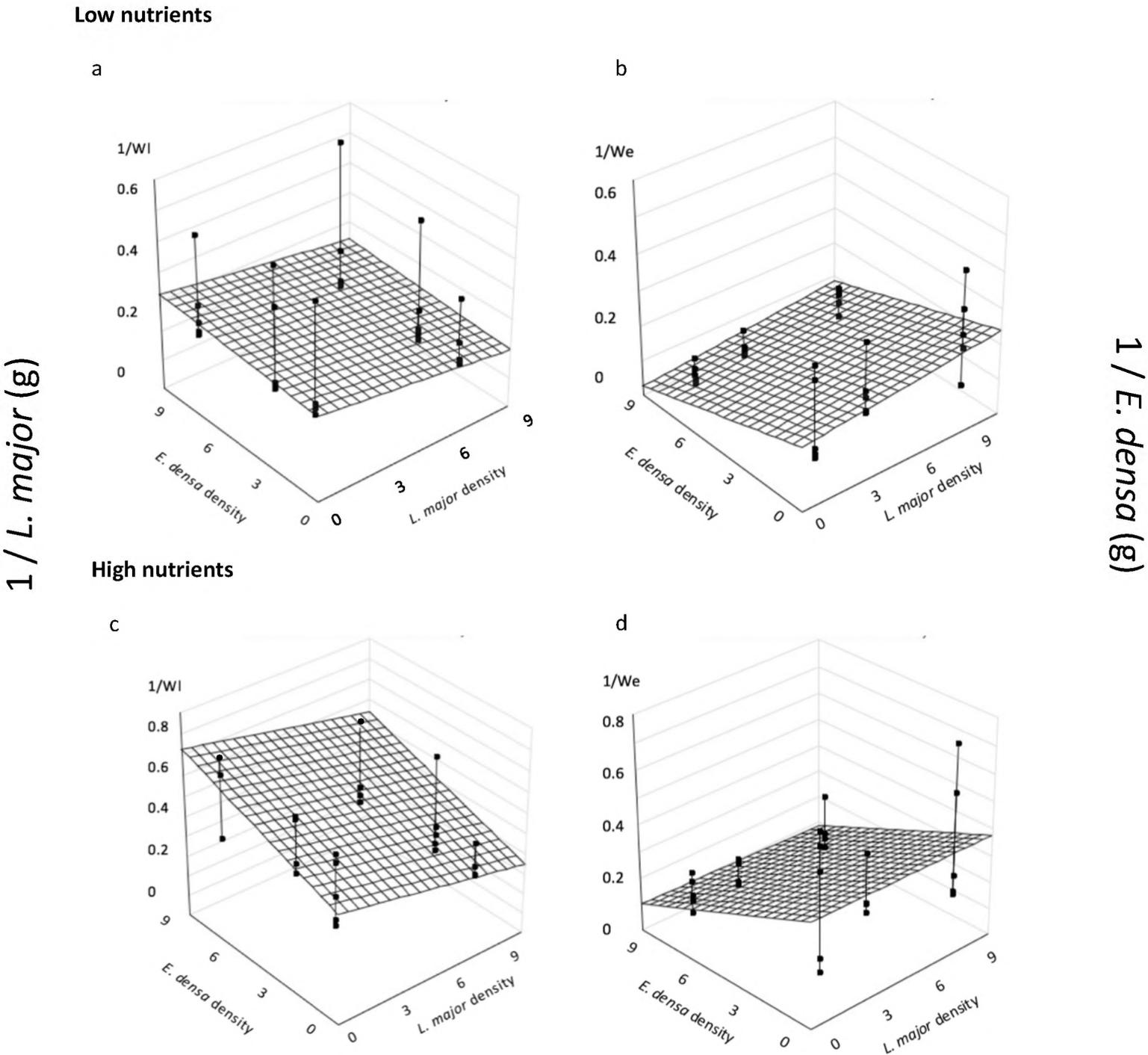


Fig. 2.5 Multiple regression planes demonstrate the combined effect of *Egeria densa* and *Lagarosiphon major* planting densities on the inverse mean dry biomass (1/g) of one *L. major* plant (1/Wl) and one *E. densa* plant (1/We) in low (a, b) and high (c, d) nutrients. Points indicate observations (n=5) and the vertical lines between data points represent the residuals. Values on X and Y axes represent *E. densa* and *L. major* planting densities at the outset of the experiment.

The results of this experiment reveal a competitive advantage of the invasive *E. densa* over the trophically analogous native *L. major*. This advantage is considerably greater where nutrient availability is increased.

2.4 Discussion

The low levels of *L. major* survival were surprising as this is a species considered highly invasive in New Zealand (Howard-Williams and Davies 1988) and Ireland (Caffrey *et al.* 2008), and it has been reported to have high tolerances to biotic factors such as fluctuating pH and CO₂ levels (Caffrey *et al.* 2008). In addition to the elevated temperatures, the high levels of plant mortality are likely also attributable in some part to competition from *E. densa*. This is supported by the observed elevated levels of *L. major* mortality in the high nutrient treatment, which also saw the competitive ability of *E. densa* increase seven-fold compared to the low nutrient treatments. Under all treatments, the invasive *E. densa* had a competitive advantage over *L. major*.

The increase in *E. densa*'s competitive ability was, rather counterintuitively, observed in the same system that saw a significant decrease in *E. densa* growth rate. Growth rate and competitive vigour have been previously found to be positively correlated (Dawson *et al.* 2011). In this instance however, the disparity between growth rate and competitive ability can be explained by the poor survival of *L. major* in the high nutrient treatment. As *L. major* suffered greater mortality, despite the drop in growth rate of *E. densa*, there remained a far wider gap between the end biomass of each species, thus resulting in a higher competition coefficient for *E. densa* in this treatment.

The competition coefficients for each of the submerged species were far more equal in the low nutrient treatment, suggesting that *E. densa* has a superior ability to take up available resources, which underpins the species' competitive advantage, adding further support to the well-documented association between nutrient loading and invasive plant establishment (Davis *et al.* 2000; Daehler 2003). However, in the wider literature that considers *E. densa*, there are conflicting findings. Feijoo *et al.* (1996) report positive correlations between the growth of *E. densa* and both the levels of ammonium in the water column and nitrogen in the sediment, whereas Mony *et al.* (2007) found no effect of increased substrate nutrients on *E. densa* biomass. These contrasting results could be the effects of another variable besides nutrient levels that was perhaps not controlled for, such as temperature. Alternatively they could be the result of resource allocation dependent on competition as discussed previously.

In contrast to the wider literature, the relative growth rate (RGR) of the shoots of *E. densa* were consistently and significantly higher at its highest initial planting densities. An earlier study into the RGR and doubling time of *E. densa* concluded that RGR was notably higher at lower densities (Pistori *et al.* 2004). Similarly, another study of floating macrophytes also report a negative relationship between growth rate and plant population densities (Henry-Silva *et al.* 2002), because as plants grow, they limit the access of other individuals to key resources (Silverston and Doust 1993). However, in this experiment, where fewer resources were available (the low nutrient treatment), there were significantly higher growth rates of the shoots. This trend was also observed in a study comparing growth rates of three Hydrocharitaceae (*Elodea nuttali*, *E. canadensis* and *L. major*), where all three species exhibited a decrease in root mass and root to shoot ratio in high nutrients, indicating a change in resource allocation away from root production when nutrients are in rich supply (James *et*

al. 2006). This could provide an explanation for the decrease in growth rates of *E. densa* under high nutrient conditions; a decrease in root production would lead to reduced nutrient uptake from the sediment resulting in slower plant growth. This is further supported by the fact that in this experiment, nutrients were provided via the substrate and not in the water column.

The RGRs of *L. major* were consistently negative for all planting densities in both nutrient treatments, and in contrast to the results for *E. densa*, there was no relationship between growth rate and planting density. This means that none of the plants grew, and the majority suffered some degree of mortality, potentially due to the temperatures within the experimental mesocosms. The optimum temperature range for *L. major* plant growth has previously been reported as 20°C to 23°C with a maximum tolerance of 25°C (Kasselman 1995). The average water temperature of this experiment was 26°C with a peak temperature of just over 30°C. In comparison, the optimum thermal range for *E. densa* falls within those observed in the experiment (16°C to 28°C), and has a recorded maximum tolerance of 35°C suggesting that *E. densa* is unlikely to have been negatively affected by temperature (Barko and Smart, 1981; Getsinger and Dillon 1984; Haramoto and Ikusima 1988; Di Tomaso and Healy 2003).

As *E. densa* has already established in multiple freshwater systems across South Africa, these results should be viewed as a cautionary indication of their potential to out-compete native species and create degraded resilient systems under the right environmental conditions. Whilst *E. densa* is competitively superior to *L. major*, the magnitude of that relationship can be significantly augmented by eutrophication. Research into top-down methods of controlling invasive submerged plants, such as biological control, continues to grow worldwide (McGregor and Gourlay 2002; Coetzee *et al.* 2011a; Cabrera Walsh *et al.* 2012). However, the monitoring and biological control of submerged invasive plants is less

reliable and more difficult than control programmes targeting terrestrial or floating plants (Coetzee *et al.* 2011a). Also, biological control success of aquatic macrophytes has been shown to be negatively correlated with nutrient loading (Heard and Winterton 2000). Considering the eutrophic status of many of South Africa's freshwater systems and the weakening effect of reduced nutrients on the competitive ability of *E. densa* observed in this experiment, there are clear benefits to adopting a more holistic approach to submerged invasive plant control in South Africa.

The results of this experiment provide a useful starting point for the exploration of the proposed regime shift. They showed that *E. densa* is a superior competitor for resources compared to *L. major*, and that the dominance of *E. densa* is greatly increased in eutrophic conditions. They provide baseline information regarding the competitive fitness of *E. densa* under ideal conditions, to which comparisons can be made against future experiments testing the competitive ability of the same species in the context of floating versus submerged plant competition, in a range of nutrient environments.

Under all treatments, the invasive *E. densa* had a competitive advantage over the trophically analogous native *L. major*. In contrast to the wider literature, the relative growth rate (RGR) of *E. densa* was consistently and significantly higher at its highest initial planting densities. An earlier study into the RGR and doubling time of *E. densa* concluded that RGR was notably higher at lower densities (Pistori *et al.* 2004). Similarly, another study of floating macrophytes also report a negative relationship between growth rate and plant population densities (Henry-Silva *et al.* 2002), because as plants grow, they limit the access of other individuals to key resources (Silverston and Doust 1993). However, in this experiment, where fewer resources were available (the low nutrient treatment), there were significantly higher growth rates. This trend was also observed in a study comparing growth rates of three

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the competitive ability of the same species in the context of floating versus submerged plant competition, in a range of nutrient environments. The next step is to assess the responses of each species, individually, to the conditions under which the regime shift is believed to occur. This is important to address as many of the artificially built water bodies in South Africa have low numbers of native submerged plants occupying them, meaning that when invasive plants such as *E. densa* colonise them they are free from native plant competition which could strengthen the feedback loops within the proposed regime shift.

Chapter 3

A mesocosm study exploring the shift between floating and submerged invasive plant dominance

3.1 Introduction

Ecosystems are dynamic structures defined by the interactions and internal processes that occur within them. These processes maintain an equilibrium that can be fluid in its response to external pressures and maintain varying degrees of stability. This means that when internal processes such as competition and nutrient cycling are altered by external pressures, such as nutrient loading or species removal, the result can be a shift from one stable state to another (Scheffer and Carpenter 2003; Scheffer 2009). It is not always possible to predict the occurrence of a regime shift or how the alternate regime will function (Folke *et al.* 2009). A shift can occur when the change in environmental conditions is small, but passes a critical threshold referred to as the bifurcation point (Scheffer and Carpenter 2003). The capacity for a system to withstand significant disturbance or pressure without experiencing a regime shift is termed resilience (Hollings 1973; Walker *et al.* 2004) and stability landscapes are a helpful method of visually demonstrating this dynamic (Fig. 3.1).

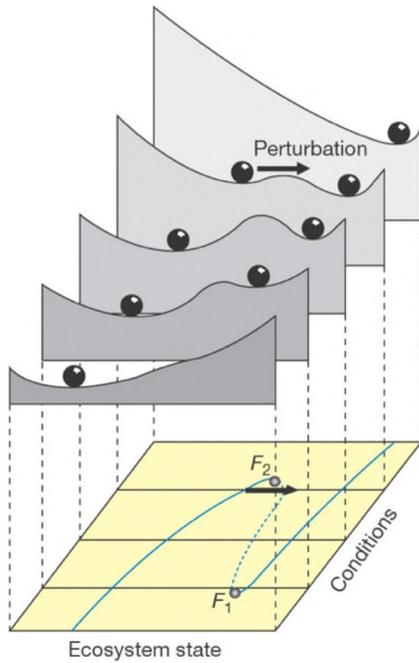


Fig. 3.1. Stability landscapes used to demonstrate regime shifts. In this example, the ball represents the system (e.g. lake, forest, grassland) as it responds to changes in environmental conditions (e.g. nutrient loading, species removal, fire). The steepness of the valleys corresponds to the system's stability; shallow valleys have low resilience and can be perturbed more easily. The bottom plane depicts the equilibrium curve; stable equilibria align with valleys (reproduced from Scheffer 2009).

The structure and functioning of an ecosystem can be determined by those species that dominate it; such species are often termed 'ecosystem engineers' (Wright and Jones 2006). Depending on environmental conditions, both submerged and floating macrophytes can be superior competitors for different resources, and it has therefore been suggested that alternate stable states of either submerged or floating plants can exist (Scheffer 2003). This is due to an asymmetry in competition for resources; submerged plants can access nutrients in the sediment whilst removing nutrients in the water column, limiting the growth of floating macrophytes, whereas floating plants have more access to light and are able to shade out submerged species (Scheffer 2009). Competition between floating and submerged aquatic

plants has previously been explored experimentally. A study on the submerged *Elodea nuttallii* John (Hydrocharitaceae St.) and the floating *Lemna gibba* L. (Araceae) found the competitive relationship to be highly nutrient dependent, with *E. nuttallii* only able to sustain dominance at low nutrient levels (Szabo *et al.* 2010). Similarly, Netten *et al.* (2010) quantified the role of nutrients, temperature and initial planting density on competition between *E. nuttallii* and the floating *Salvinia natans* (L.) All. (Salviniaceae) in a mesocosm environment. They also found nutrient conditions to be highly influential, but found the floating species outcompeted the submerged across all treatments, even at low nutrients. The growth forms of competing plants also affect interactions; larger surface area of floating species will affect shading ability, and substantial root structures will affect nutrient uptake (Weiner and Thomas 1986; Schwinning and Weiner 1998; Horvitz and Scemske 2002).

Regime shifts, driven by external nutrient loading (due to increased urbanisation and agricultural practices), changes in water depth (also from flooding) and turbidity (from sediment run off and algal growth), between submerged and floating plants are recognised in the literature (Rabalais 2002; Scheffer *et al.* 2003). Changes in water depth and turbidity reduce light availability in the lower levels of the water column, reducing photosynthetic opportunities for submerged plants and therefore increasing the competitive advantage of floating plants for light (Nolzen 2013). When nutrient levels are not too high, nutrients settle onto the sediment floor to be taken up by submerged plants, their growth then removes nutrients from the system, thereby maintaining low nutrient levels. However, if nutrient loading crosses a critical threshold, levels of nutrients in the water column increase and provide favourable conditions for floating plants to thrive (Oliver 1993; Coops and Doef 1996; Janse and Van Puijenbroeck 1998).

Undesireable stable states defined by the dominance of floating invasive plants, such as giant salvinia, (*Salvinia molesta* D.S. Mitch. (Salviniaceae)) and water hyacinth (*Eichhornia crassipes* (C. Mart.) Solms (Pontederiacaeae)) occur in systems worldwide (Mitchell 1985; Center *et al.* 1994; Scheffer *et al.* 2003; Gaertner *et al.* 2014). Once established, these plants can be difficult to eradicate as they form dense mats on the water's surface that promote anoxic conditions within the water column and negatively impact submerged biodiversity (Midgley *et al.* 2006; Hill 2003)

In South Africa, systems dominated by such species have been known to remain in that stable state for long periods of time. Eutrophication is a known driver of the initial establishment and growth of these species (Davis *et al.* 2000; Daehler 2003) and their superiority in competition for light can lead to the reduction and death of submerged plant populations (Janes *et al.* 1996). Nutrients previously trapped within the sediment and locked within submerged plants are then returned to the water column, augmenting nutrient concentrations in the water, thereby supporting floating plant growth and increasing system resilience (Ludwig *et al.* 1997; Scheffer *et al.* 2003).

A stable state defined by the dominance of submerged macrophytes, which are able to outcompete floating plants, as described by Scheffer (2003), is rarely seen in South African systems where many of the most problematic invasive floating macrophytes have established. However, the nationwide success of biological control programmes against floating invasive macrophytes in South Africa, has resulted in a weakening of the stable states dominated by these species, and opportunities for new species assemblages is being made apparent (Coetzee *et al.* 2011a).

South Africa's freshwater systems have been vulnerable to invasive macrophyte establishment due to an unfavourable combination of pre-existing conditions. The natural

topography of the region means that the majority of freshwater bodies are manmade (Basson *et al.* 1997), and historic mismanagement has resulted in their eutrophication (Ashton *et al.* 2008). It also means there is a relatively low diversity of native species for invasive plants to compete with for resources, resulting in the establishment of very resilient degraded states, dominated by floating invasive plants (Hill 2003). The past 45 years of invasive aquatic plant management in South Africa has primarily focussed on controlling five key species: the previously mentioned *S. molesta* and water hyacinth (*E. crassipes*), water lettuce (*Pistia stratiotes* L. (Araceae)), red water fern (*Azolla filiculoides* Lam. (Azollaceae)) and parrot's feather (*Myriophyllum aquaticum* Vell. Verde (Haloragaceae)) (Hill 2003; Coetzee *et al.* 2011b).

The overall aim of these biological control programmes has been to return the invaded water body into a functioning system with high native biodiversity and improved accessibility to freshwater, essentially inducing a regime shift. However, as noted by Coetzee *et al.* (2011a), the successful biological control of floating invasives often results in habitats that are highly vulnerable to invasion from submerged aquatic plants such as *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), Brazilian waterweed, *Egeria densa* Planch. (Hydrocharitaceae) and Eurasian watermilfoil, *Myriophyllum spicatum* L. (Haloragaceae), whose distribution and spread throughout South Africa has increased significantly over the last 10 years (Madeira *et al.* 2007; Fordham 2012).

The application of biological control agents on invasive floating plants rapidly diminishes their populations, and their decay results in a sudden influx of nutrients and increased light in the water column (Jewell 1971; Hill 1979; Shilla *et al.* 2006;; Chimney and Pietro 2006; Longhi *et al.* 2008). This study proposes that these attributes create a resource-rich and poorly occupied habitat that is vulnerable to submerged invasive plant colonisation.

Whilst biological control effectively reduces populations of floating invasive plants, the effect this has on the submerged plant community structure has not previously been explored. Thus, this study proposes that freshwater ecosystems may experience a regime shift, from floating invasive plant dominance to submerged invasive plant dominance, driven by the application of biological control agents on the floating plants. The equilibria maintaining the initial stable state are altered and a new degraded state, dominated by submerged invasives plants, is created.

As discussed in Chapter Two, both *Lagarosiphon major* (Ridl.) Moss (Hydrocharitaceae) and *E. densa* have been cause for ecological and economic concern in regions around the globe. Their ability to dominate systems by outcompeting other submerged macrophytes, lower biodiversity and degrade water quality have all well been documented (Feijoo *et al.* 1996; Hofstra *et al.* 1999; Mony *et al.* 2005; Caffery *et al.* 2011; Borgnis and Boyer 2015). Findings presented in the previous chapter showed that *E. densa* was a superior competitor to *L. major*, a trait enhanced in nutrient rich environments. In order to determine the possibility of a regime shift occurring, this chapter explored the competitive ability of each species against the floating *P. stratiotes*, and investigated how this relationship is affected by the biological control of the floating plant.

To explore this proposed regime shift, mesocosm experiments were conducted to compare how the growth of native submerged *L. major* and invasive submerged *E. densa* was (independently) affected by the biological control of the floating invasive macrophyte, *P. stratiotes*, in a range of nutrient conditions and planting densities. Whilst the mesocosms cannot replicate the highly complex web of interactions and processes that occur within whole, natural ecosystems, the competitive interactions between species are easier to

interpret in an experimental setting, and findings can be used to guide understanding of observations in the field.

3.2 Materials and methods

The potential for the biological control of the floating invasive *P. stratiotes* to induce a regime shift was investigated in two experiments. The first explored a shift to an invasive submerged state, and the second to an indigenous submerged state.

3.2.1 Experimental design

Both experiments were conducted inside greenhouse tunnels at the Waainek Research Facility at Rhodes University in Grahamstown, South Africa. It was not possible to run both experiments concurrently due to space constraints, therefore they were repeated at similar times in consecutive years to minimise differences in environmental conditions. The first experiment (*P. stratiotes* and *E. densa*) was completed between 8 April - 17 June 2015, while the second experiment (*P. stratiotes* and *L. major*) ran from 4 April - 20 May 2016. Individual *P. stratiotes* plants were sourced from insect-free stock plants maintained at the Waainek Research Facility. Adult *Neohydronomus affinis*, a weevil routinely used in the biological control of *P. stratiotes* (Cilliers 1991c), were supplied by the South African Sugarcane Research Institute (SASRI). Each of the submerged plants was collected six months before the start of their respective experiments and were cultivated at the Waainek Research Facility where they grew in connected tanks of spring water under 80% shade cloth under optimum nutrient conditions. The *E. densa* culture was collected from the Kouga River, near Patensie, Eastern Cape, (-33.748506, 24.635446), and the *L. major* culture was collected from a population in a quarry dam near Stutterheim, Eastern Cape, (-32.587336, 27.463443). Prior to the initiation of both experiments, the submerged plants were treated with Malathion

(Kombat), an organophosphate insecticide (0.01ml/10l) to kill any phytophagous insects associated with the submerged plants collected from the field.

Both experiments were conducted in mesocosms constructed from individual black plastic 70L bins (55cm diameter), each filled with 65L of spring water. In the bottom of each bin, a 10L planting container was filled with a 10cm layer of pond sediment topped with a 2cm layer of silica sand to prevent clouding of the water and limit algal growth. Sediment was collected from Jameson Dam, Eastern Cape, South Africa, (−33.319073 S; 26.444206 N). The sediment was analyzed for nutrient composition by Bemlab (Pty) Ltd. Assay Laboratory (Stellenbosch, SA) and was reported to be loam sediment comprising of 0.11% nitrogen (for full analyses see Appendix D).

Thirty apical shoots (20 cm in length) of the relevant submerged species were planted in each container, and left for three weeks to acclimatise and grow to a starting density of 100 percent cover. Digital thermometers (Thermachron iButtons used with Climastats Environmental Monitoring software, version 4) were placed in waterproof vials and floated on the surface of the water to record the temperature at midday throughout the experiment.

There were three initial planting density treatments, each a different ratio of floating to submerged plants: 90:10%, 50:50% and 10:90% respectively. After the acclimation phase, either 27, 15 or 3 shoots were removed from each container in order to represent the 10, 50 and 90% densities of the submerged plant, respectively. To achieve the planting densities of the floating *P. stratiotes* (90, 50 and 10%), either nine, five or one rosette(s) each of a similar size class (12-16cm diameter) were added to the mesocosms (Fig. 3.2).



Fig. 3.2. The three initial planting density ratios of floating to submerged plant percent cover (90:10 left, 50:50 centre and 10:90 right). (Photo: Emily Strange)

There were a total of 24 possible treatment combinations arranged in a full factorial design, replicated three times, resulting in a total of 72 mesocosms. The mesocosms were set up in a random block design (Fig. 3.3). The treatment combinations consisted of the three initial planting densities, in the presence or absence of the host specific biological control agent *N. affinis* on *P. stratiotes*, growing at four initial nitrogen concentrations of the water. For the biological control treatments, two mating weevil pairs per water lettuce rosette were used to ensure control would be reached (Diop *et al.* 2010). There were four nutrient treatments; very low, low, high and very high (0.0, 0.1, 1.0 and 10.0 mg N/l respectively). Nitrogen was added using a solution of ammonium nitrate and water. Phosphorous was added to each nutrient treatment as part of a standard Hoaglands solution (5.2mg/l, Hoagland and Arnon 1938) which ensures sufficient micronutrients for optimal plant growth, as wider literature concludes phosphorus is not limiting for *E. densa* (Feijoo *et al.* 1996) or *L. major* (Rattray 1991). Once the experiment was set up, fine white gauze was secured over each mesocosm to prevent *N. affinis* movement between containers, and both experiments ran for 10 weeks.



Fig. 3.3. The mesocosms were arranged in a full factorial, random block design. (Photo Emily Strange)

3.2.2 Measurements

The shoots of the submerged plants that were removed at the beginning of each experiment (to create the three planting densities) were dried, weighed and the average shoot weight was used to calculate the remaining above-ground biomass in each mesocosm at the start of the experiments. After ten weeks, the above and below-ground biomass for the submerged plants were harvested, dried in drying ovens at 96°C for 72 hours and weighed to calculate the relative growth rate (RGR) over the experimental period.

The RGRs were calculated using the standard formula (Evans, 1972):

$$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

where W_1 and W_2 are the start and end dry weights respectively at t_1 and t_2 , the start and end time in days.

The damage to *P. stratiotes* caused by *N. affinis* resulted in fragile plants that could not be handled, therefore only start and end weights were recorded. Aerial photographs of the mesocosms were taken weekly and digital image analysis software (imageJ version 1.49, National Institutes of Health, available at <http://imagej.nih.gov/ij>) was used to calculate the percentage cover of healthy *P. stratiotes* plant tissue over time. This was achieved by calculating the percentage cover of each tank that contained pixels of healthy green tissue, and omitting shades of yellow and brown that are characteristic of damaged or dead plant tissue.

Leaf tissue samples from *E. densa* and *P. stratiotes* were collected for analyses of C:N ratios from every mesocosm at the start and end of the experiment to determine changes in plant quality. *Lagarosiphon major* samples were collected but were not included in the analysis, as there were not enough viable samples at the end point of the experiment due to high plant mortality. Samples were oven dried for 72 hours at 60°C and ground to a fine powder. Leaf tissue analyses were performed on 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), housed at the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria. Precision of replicate determinations was 0.15 for nitrogen and 0.18 for carbon.

Multiple environmental parameters within the mesocosms were also measured. Weekly ammonium [NH_4^+] (mg/L) and nitrate [NO_3^-] (mg/L) levels were measured with Vernier Software and Technology™ ion specific electrodes (ISE) and a LabQuest® 2 interface.

These were measured to monitor temporal changes in nutrient concentrations. Every fortnight, dissolved oxygen was measured using a Sper Scientific 850045 DO pen, and pH was measured using a Eutech PCTEST35 multi-parameter pen. Light levels (PAR) were recorded five centimetres below water surface in the centre of each mesocosm using an Apogee MQ-200 Quantum Meter.

3.2.3 Data analyses

General linear model (GLM) full factorial analyses of variance (ANOVA), followed by Tukey HSD post-hoc tests were performed to investigate interactions between initial planting density, biological control and nutrient level on relative growth rates (RGR) and plant quality measurements across treatments. Linear regression models tested for significant correlations between both plant biomass and growth rates of each species and all environmental and plant quality variables within the mesocosms, as well as percentage cover of *P. stratiotes*. Homogeneity of slopes was analysed using an analyses of co-variance (ANCOVA), to determine interactions between *P. stratiotes* biomass/percent cover and biological control on submerged plant biomass. All statistical analyses were conducted in the R environment (version 3.2.3; R Development Core Team, 2014; available at <http://cran.r-project.org>) using R Studio (version 0.98.1103).

3.5 Results

Temperatures were not significantly different between the two experimental time periods and therefore can be ruled out as a significant variable ($F_{(1,33)}=0.551$, $P=0.46$). Neither pH nor light were significantly correlated with the growth of *E. densa* (light: $F_{(1,70)}=0.658$, $P=0.42$; pH: $F_{(1,70)}=1.795$, $P=0.185$) or *L. major* (light: $F_{(1,63)}=0.103$, $P=0.749$, pH: $F_{(1,53)}=0.922$, $P=0.341$). These variables were subsequently removed from further analyses.

Both the dry biomass of *L. major* or *E. densa* in relation to *P. stratiotes* dry biomass at the beginning of each experiment show a general clustering of the data into three groups, which was representative of the three initial planting ratios of the submerged and floating plants i.e. 10:90, 50:50 and 90:10 (%) floating to submerged (Fig. 3.4).

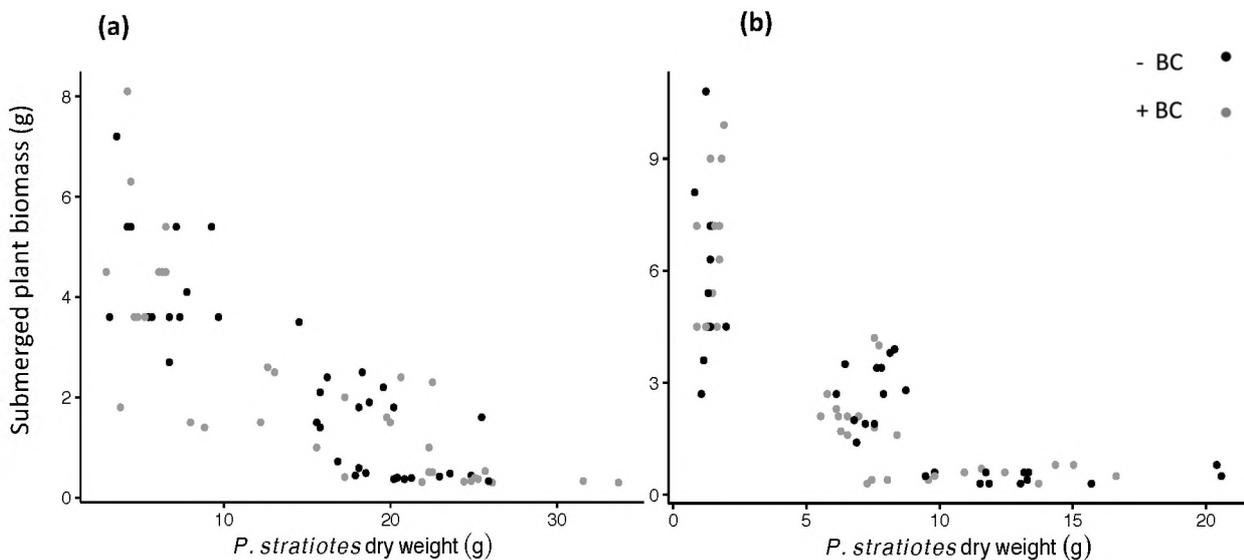


Fig. 3.4. The relationship between the starting biomass of *Pistia stratiotes* and the starting biomass of (a) *Lagarosiphon major* and (b) *Egeria densa*. Each marker represents a mesocosm, in the absence (- BC) or presence (+BC) of *Neohydronomus affinis*, the biological control agent of *P. stratiotes*.

At the end of the experiment, an increase in the biomass of *P. stratiotes* significantly reduced the biomass of *L. major* and *E. densa*, both in the presence and absence of biological control agents on *P. stratiotes* (Fig. 3.5). The relationships between the biomass of *L. major* and *P. stratiotes* in the presence and absence of biological control were significant ($F_{(1,60)} = 16.12, P=0.0002$). However an ANOVA used to test the homogeneity of slopes revealed that the slopes of each relationship did not differ ($F_{(59,60)} = 0.1836, P=0.6699$). In contrast, the biomass of *E. densa* was significantly affected by the biomass of *P. stratiotes* ($F_{(63,64)} = 7.6674, P=0.0074$), and in the presence of biocontrol agents, the slope was significantly steeper ($F_{(1,63)} = 4.184, P=0.0449$). This indicates that as *P. stratiotes* is controlled (and biomass is reduced), the biomass of *E. densa* plants increases; supporting the theory that *E. densa* can capitalise on the resources made available through the process of biological control.

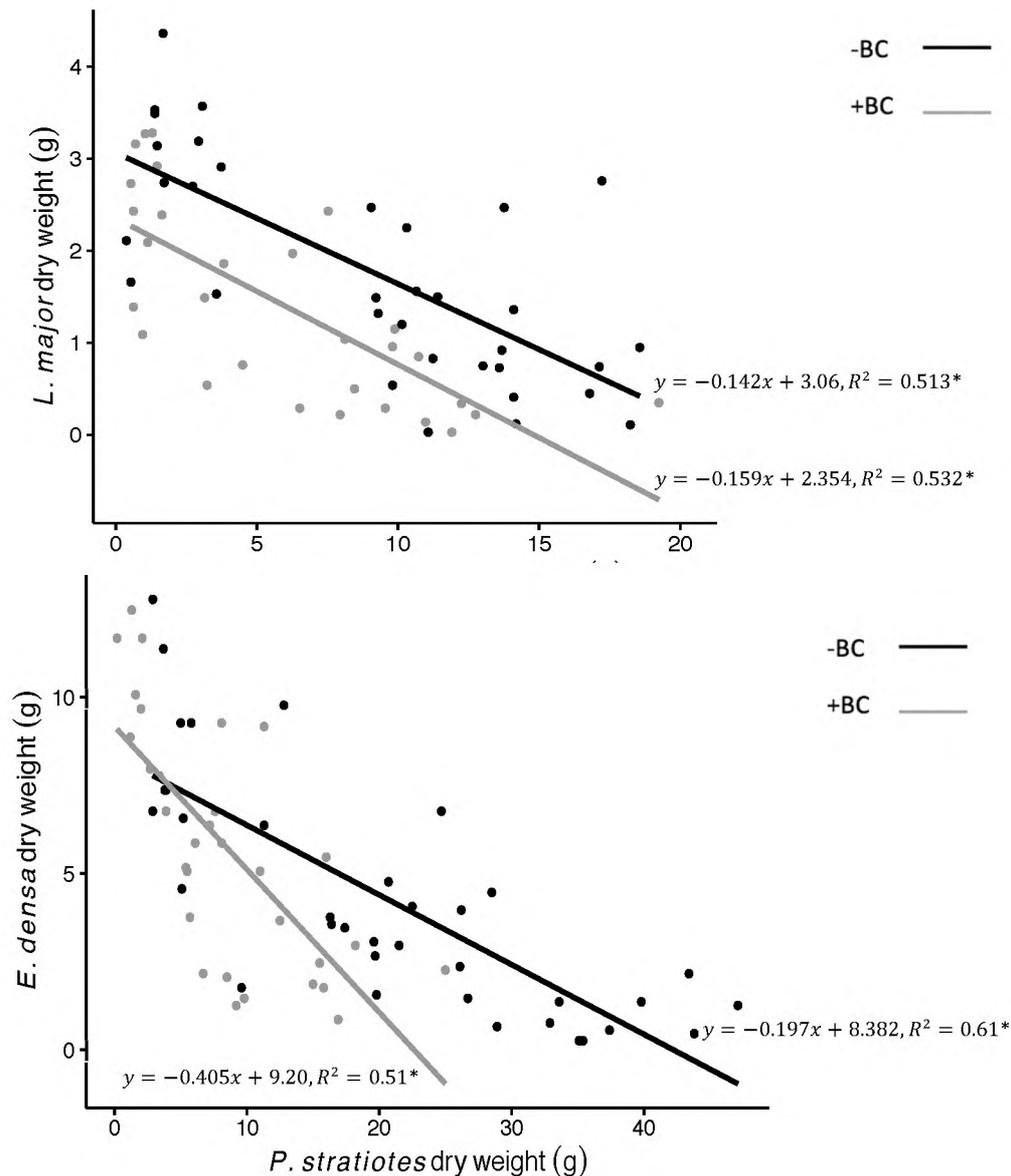


Fig. 3.5. The relationship between biomass of *Pistia stratiotes* and (a) *Lagarosiphon major* and (b) *Egeria densa* at the end of each experiment. Each marker represents a mesocosm, in the presence (+BC) or absence (-BC) of *Neohydronomus affinis*, the biological control agent of *P. stratiotes*. * indicates statistical significance.

Full factorial ANOVAs were conducted to analyse interspecific differences between the relative growth rates (RGRs) of *L. major* and *E. densa*, as well as the intraspecific differences between the various initial planting densities and biological control treatments (Fig. 3.6). The main effects of the four nutrient treatments, under all conditions were not significant, therefore this variable was removed from the model. Post-hoc Tukey HSD tests

were performed to explore the significant differences between each combination of treatments (denoted with letters on Fig. 3.6.).

Interspecific differences

The RGRs of *L. major* were significantly lower than those of *E. densa* in both the absence ($F_{(1,64)}=14.911, P\leq 0.001$) (Fig. 3.6 a), and presence ($F_{(1,61)}=73.905, P\leq 0.0001$) (Fig. 3.6 b) of biocontrol agents on *P. stratiotes*. In addition, the effect of initial planting density on RGR was significantly different between the two species ($F_{(2,61)}=3.771, P=0.0286$), with lower planting densities exhibiting increased growth rates.

Intraspecific differences

There was no significant highest order interaction between biological control treatments, initial planting density and nutrient levels for both *E. densa* ($F_{6,42}=0.0548, P=0.769$) and *L. major* ($F_{6,40}=2.043, P=0.082$) (Fig. 3.6). However, the biological control treatment of *P. stratiotes* significantly increased the RGRs of *E. densa* ($F_{(2,42)}=16.458, P\leq 0.001$) but not *L. major* ($F_{(1,40)}=2.988, P=0.0916$). Similarly, the initial planting density also significantly affected the RGRs of *E. densa* ($F_{(2,42)}=10.980, P\leq 0.0001$) and not *L. major* ($F_{(1,40)}=1.583, P=0.2179$). RGRs of *E. densa* were significantly higher at the lowest initial planting density compared to 90 and 50%.

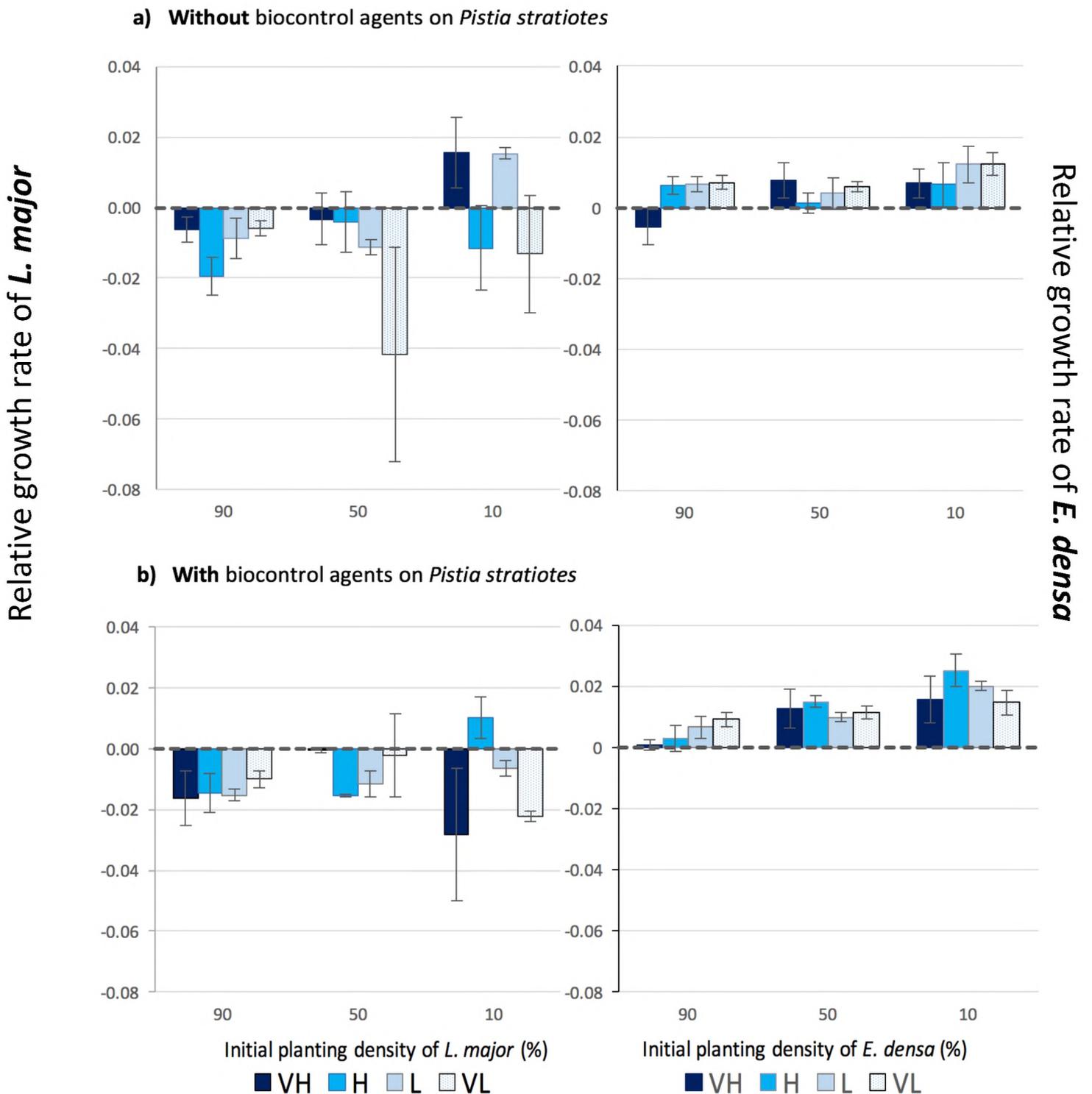


Fig. 3.6 The relative growth rate (mean \pm S.E) of *Lagarosiphon major* (left) and *Egeria densa* (right), at the three initial planting densities, **(a)** in the absence of biological control of *Pistia stratiotes* and **(b)** in the presence of the biological control agent *Neohydronomus affinis*. The four data series are the nutrient treatments of the mesocosms (VH=very high, H=high, L=low, VL=very low). There were no significant higher order interactions between variables.

The levels of dissolved oxygen recorded in the mesocosms was considerably lower than expected. After 10 weeks, the final biomass of both species significantly increased with higher levels of dissolved oxygen in the water column, although the relationship between biomass and dissolved oxygen was much weaker for *L. major* (Fig. 3.7). The correlation differed significantly between the two species ($F_{(2,134)} = 16.178, P \leq 0.0001$), with a significantly steeper slope for *E. densa* than for *L. major* ($F_{(1,133)} = 105.81, P \leq 0.0001$). This suggests an increased level of photosynthesis for *E. densa* compared to *L. major*.

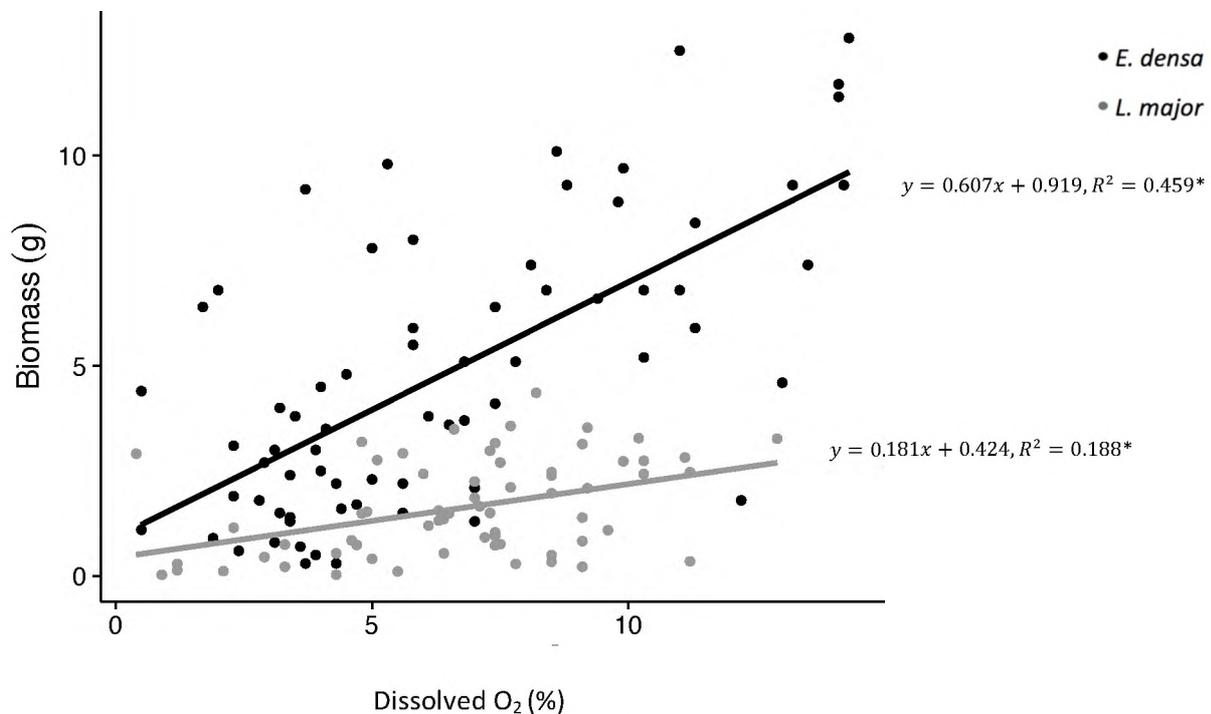


Fig. 3.7 Relationship between the biomass of *Egeria densa* and *Lagarosiphon major* after 10 weeks, with the dissolved oxygen (%) of the water in each mesocosm. * indicates significance.

There was a significant negative correlation between the biomass of *P. stratiotes* and the levels of dissolved oxygen in the water (Fig. 3.8). In the context of the previous results, this relationship makes sense ecologically; as biomass of *P. stratiotes* decreases, that of *E. densa* increases, therefore photosynthetic rates rise, leading to elevated dissolved oxygen in the water.

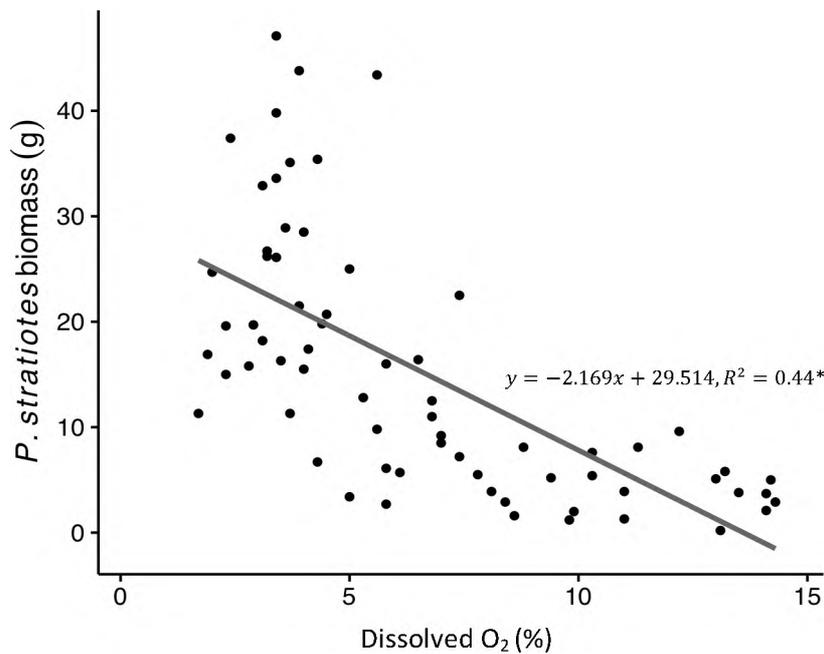


Fig. 3.8. The relationship between the dry weight (g) of *P. stratiotes* and the recorded levels of dissolved oxygen (%) in the water after 10 weeks. * indicates significance.

Significant differences were also found between the relationships of the two submerged species and the corresponding levels of nitrogen in the water ($F_{(2,132)} = 7.7815, P = 0.006$). Once again the relationship between the two variables was very weak for *L. major*, while the slope for *E. densa* was significantly steeper than that of *L. major* ($F_{(1,131)} = 59.584, P \leq 0.0001$) (Fig. 3.9). As levels of nitrogen (mg N/l) in the water column increased, the biomass of *E. densa* significantly increased. For total and weekly measurements of ammonium and nitrate recorded in the *E. densa* mesocosms see Appendices A and B. Contrastingly, the negative

relationship between biomass of *L.* and concentrations of nitrogen in the water were non-significant.

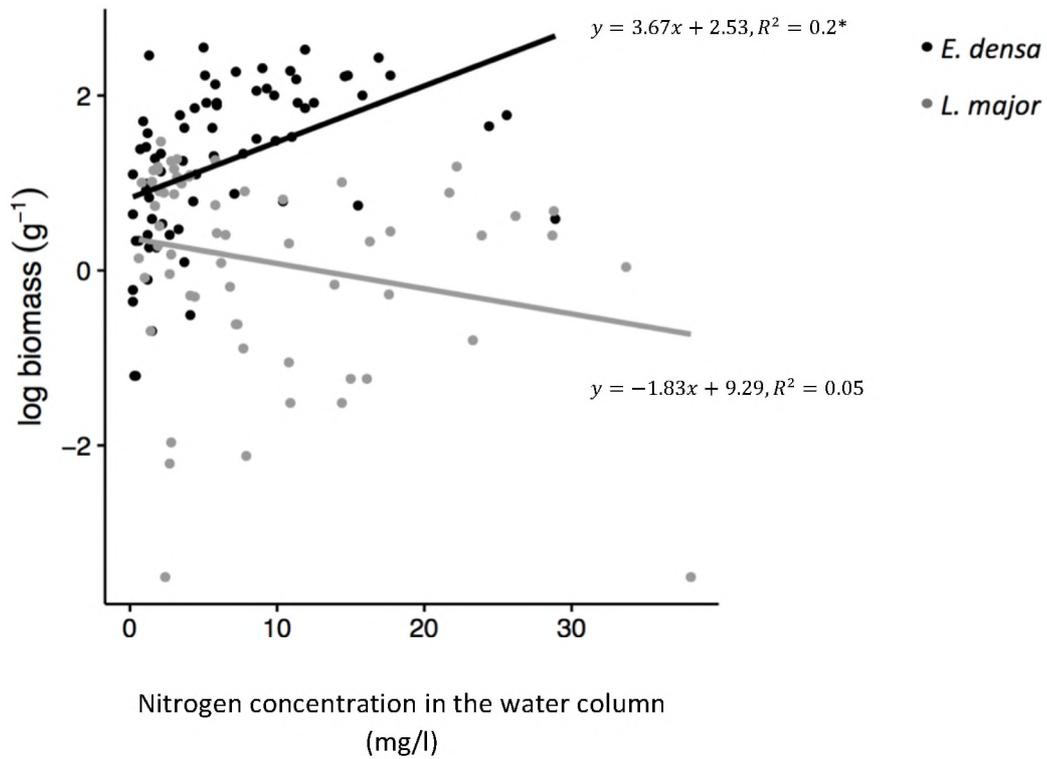


Fig. 3.9. The relationship between end dry biomass (log g⁻¹) of *Egeria densa* and *Lagarosiphon major* and the concentrations of nitrogen in the water (mg/l) of each mesocosm. * indicates significance.

The biomass of *P. stratiotes* decreased significantly as concentrations of nitrogen in the water increased (Fig. 3.10). This trend supports the hypothesis that as *P. stratiotes* is controlled, plant decomposition leads to increased nutrient release into the water.

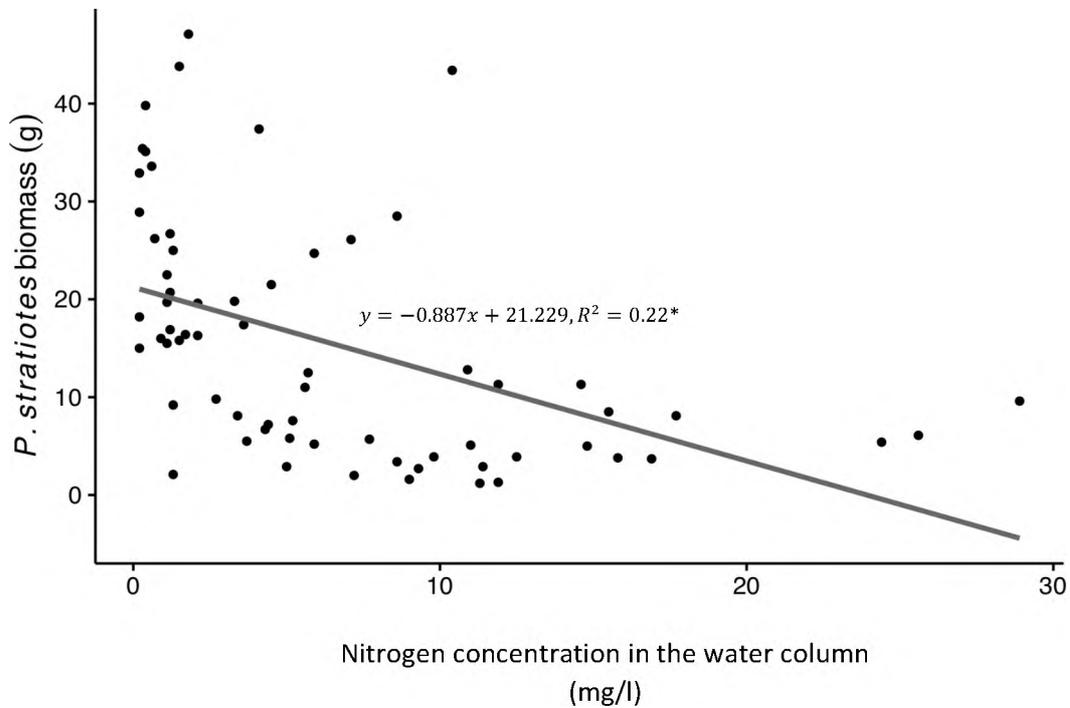


Fig. 3.10 The relationship between biomass (g) of *P. stratiotes* after ten weeks and the nitrogen concentration in the water (mg/l). (*) denotes significance.

As the surface cover of *P. stratiotes* increased, there was a significant decrease in the biomass of both *E. densa* and *L. major* (Fig. 3.11). The relationship between submerged plant biomass and the surface cover of *P. stratiotes* differed significantly between the two species ($F_{(1,133)} = 5.1947, P=0.0245$). The slope for *E. densa* was, again, significantly steeper than that of *L. major* ($F_{(1,133)} = 165.864, P \leq 0.0001$), indicating that as percentage cover of *P. stratiotes* decreases, *E. densa* capitalises on the newly available resources for growth, compared to *L. major*.

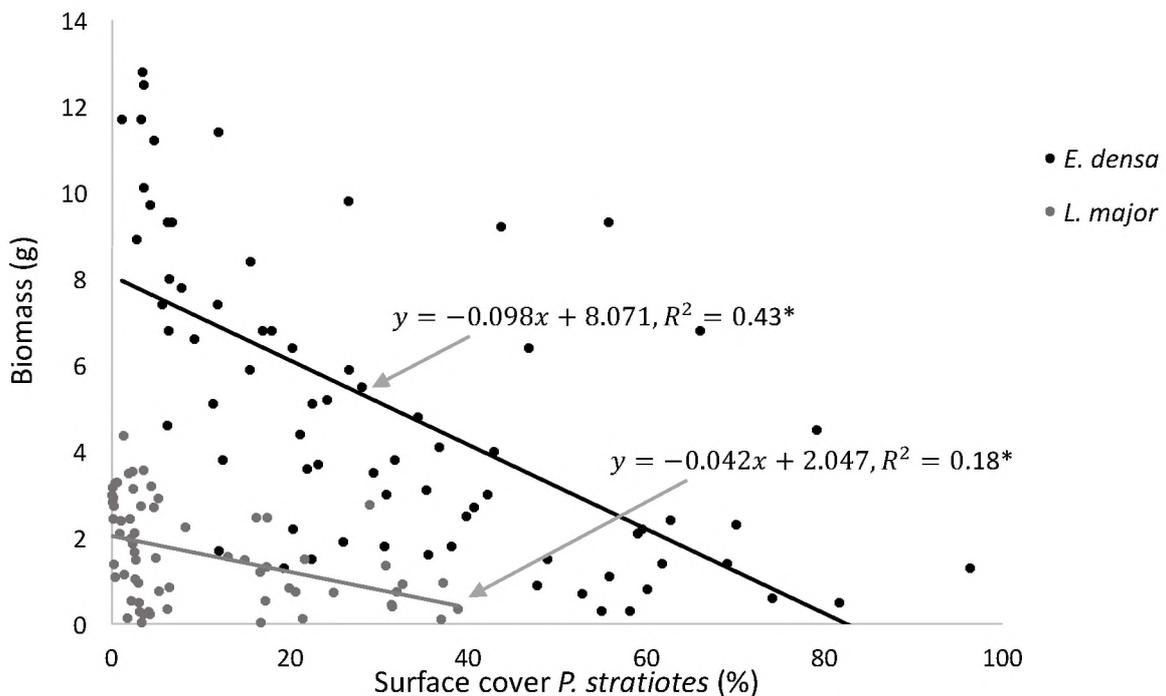


Fig. 3.11 The relationship between the dry weight (g) of *Egeria densa* and *Lagarosiphon major* with the surface cover (%) of *Pistia stratiotes* after ten weeks. (*) indicates significance.

At the beginning of the experiment, there were no significant differences between the carbon to nitrogen (C:N) ratios of *E. densa* across all treatments ($F_{(6,46)}=1.136$, $P=0.356$) (Fig. 3.12 a). After ten weeks, there was no significant higher order interaction between biological control treatments, initial planting density and nutrient levels for the C:N ratios of *E. densa* ($F_{(6,42)}=0.308$, $P=0.929$) or *P. stratiotes* ($F_{(6,40)}=2.043$, $P=0.082$) (Fig. 3.13). However, there was a significant effect of initial planting density for *E. densa* ($F_{(2,53)}=5.520$, $P=0.006$), as well as initial nutrient treatment of the water ($F_{(3,53)}=9.094$, $P\leq 0.0001$). The C:N ratios were significantly lower at the lowest initial planting density compared to the highest, and significantly lower at the very high nutrient treatment compared to all others (Fig. 3.12 b). These results indicate that *E. densa* growing at low initial densities and in high nutrient conditions produces higher quality plant tissue compared to those in high densities and with fewer available resources.

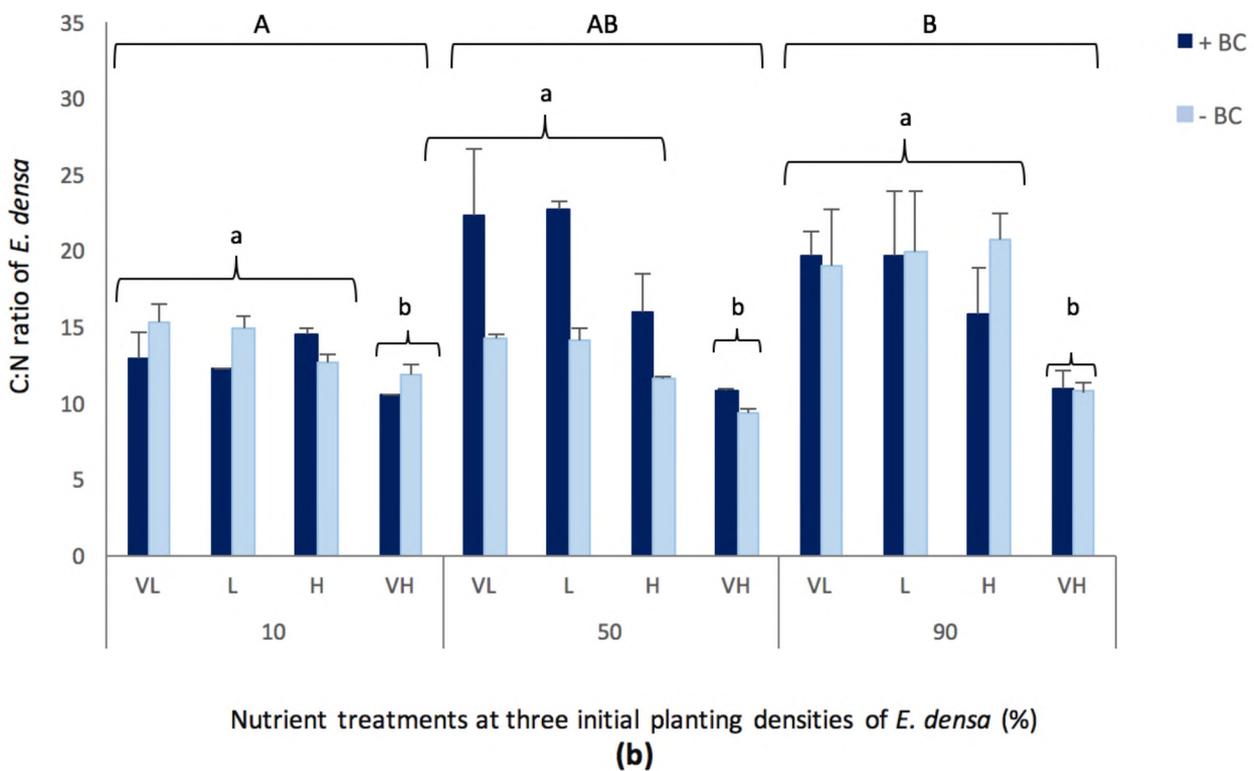
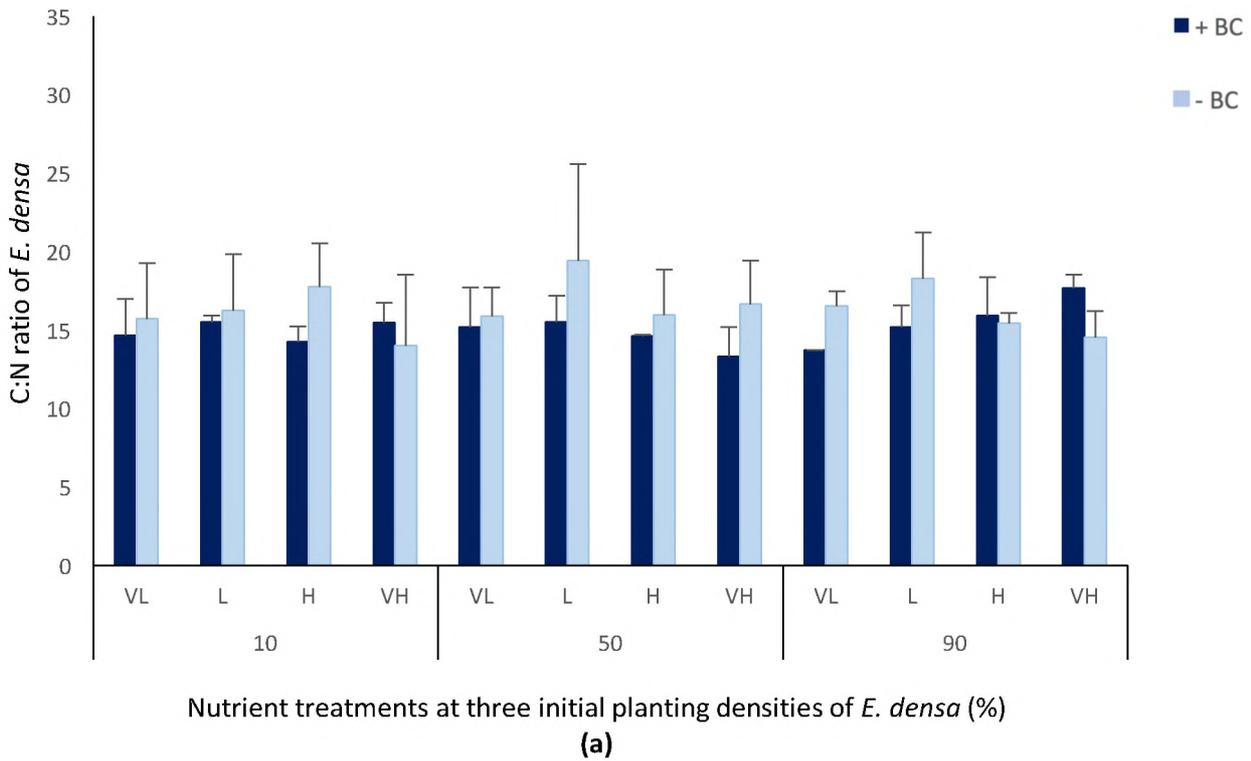


Fig. 3.12 The mean (\pm S.E) C:N ratio of *Egeria densa* for each initial treatment at the beginning (a) and the end (b) of the experiment. Capital letters denote sig. differences between planting densities, lower case letters denote significant differences between nutrient treatments. Nutrient treatments are denoted as per the following; VL= very low, L = low, H= high and VH = very high.

The relationships between the C:N ratios of *E. densa* and nitrogen concentration in the water were not significant, regardless of the biological control treatments of *P. stratiotes* (Fig. 3.13).

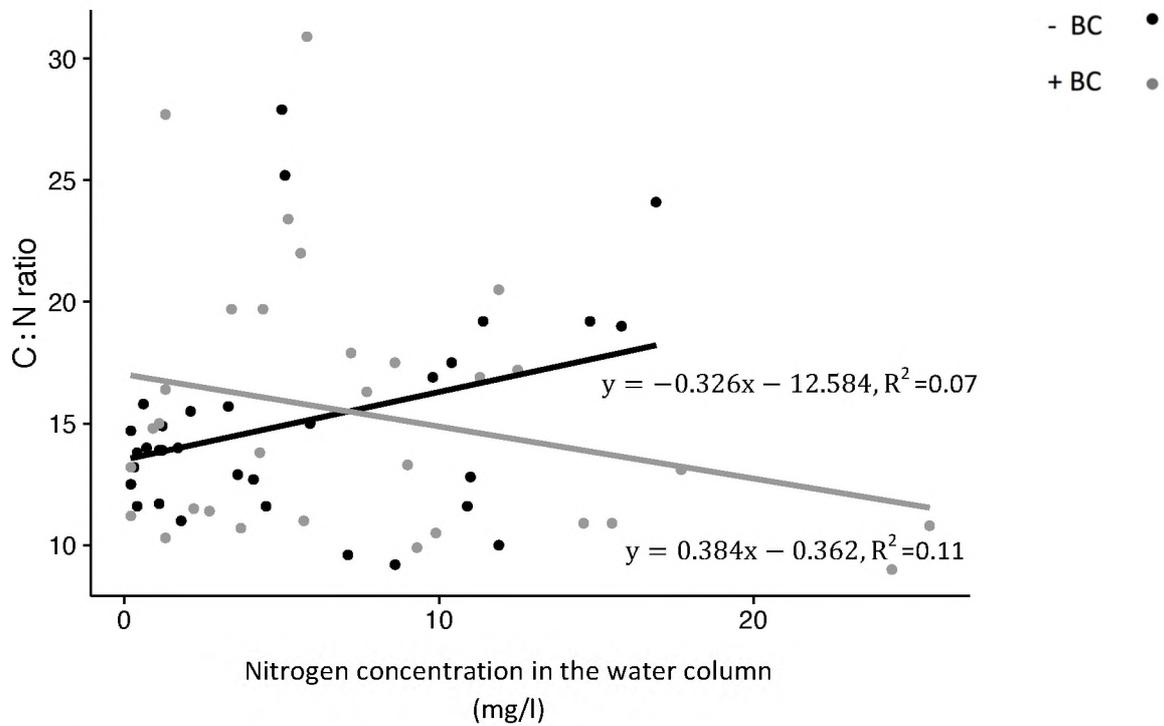


Fig. 3.13 The relationship between the C:N ratio of *Egeria densa* and the nitrogen concentration (mg/l) in the water. Each marker represents a mesocosm, in the presence (BC = 1) or absence (BC = 0) of *Neohydronomus affinis*, the biological control agent of *Pistia stratiotes*.

The C:N ratios of *P. stratiotes* at the end of the experiment (Fig. 3.14) were significantly affected by the presence or absence of biological control agents ($F_{(1,58)}=9.768$, $P=0.002$) and nutrient level ($F_{(3,58)}=19.891$, $P\leq 0.0001$), but initial planting density had no significant effect ($F_{(2,42)}=0.430$, $P=0.71$). The C:N ratios were significantly lower for plants growing in the very high nutrient treatment compared to all other nutrient levels, indicative of higher plant quality and health.

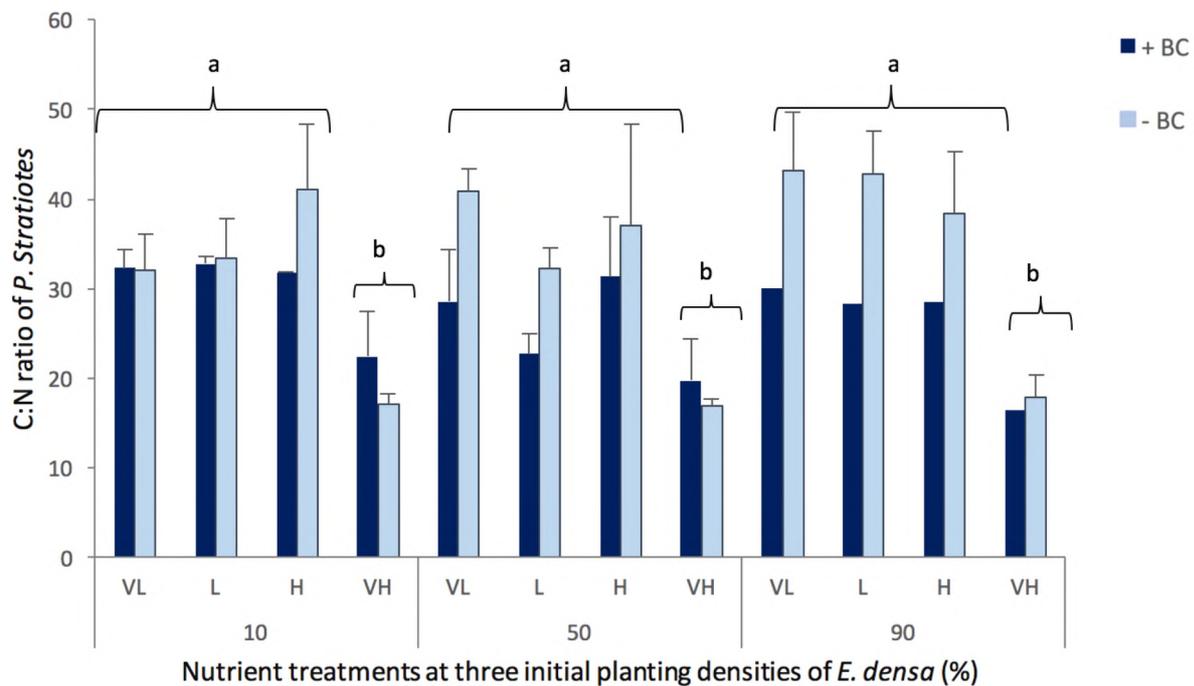


Fig. 3.14 The mean (\pm S.E) C:N ratio of *Pistia stratiotes* for each initial treatment after ten weeks in the presence and absence of biological control. Capital letters denote significant differences between planting densities, lower case letters denote significant differences between nutrient treatments. Nutrient treatments are denoted as per the following; VL= very low, L = low, H= high and VH = very high.

Due to high levels of plant mortality for *L. major*, the collection of sufficient samples to obtain C:N ratio analyses was not possible, therefore this analysis was not completed.

The results from these experiments show contrasting trends in the response of *L. major* and *E. densa* to the biological control of *P. stratiotes*. They suggest an increased capacity of *E. densa* to capitalise on resources made newly available through the biological control of *P. stratiotes* compared to *L. major*. The findings provide evidence for the proposed regime shift between floating invasive and submerged invasive plant dominance as driven by the biological control of the floating plants. The shift between floating and submerged plants did not occur between the invasive *P. stratiotes* and the native *L. major*.

3.4 Discussion

The experiments outlined in this chapter found that healthy *P. stratiotes* significantly lowered the biomass of both submerged species across all nutrient treatments and planting densities, supporting the findings of Netten *et al.* (2010). However, over time *E. densa* and *L. major* each responded differently to the presence of damaged (controlled) *P. stratiotes*. The biological control treatment did not alter the relationship between floating and submerged biomass for the native *L. major*. In contrast, the process of biological control did significantly alter the relationship between the biomass of *P. stratiotes* and the invasive *E. densa*. Mesocosms with controlled *P. stratiotes* had a significantly higher biomass of *E. densa* than those without control agents. As the biological control agents reduced the competitive vigour of *P. stratiotes*, *E. densa* was able to exploit the newly available resources to promote growth, indicated by a significant increase in RGR and biomass. This altered dynamic between controlled floating and submerged plants was not reflected in the *L. major* experiment, demonstrating the native species' reduced capacity to compete for newly available resources

compared to *E. densa*. A contributing factor to this outcome could be that *L. major* has evolved in the absence of floating macrophytes for which to compete for resources, whereas *E. densa* originates from South America that does have native floating macrophytes.

Studies have shown a strong positive association between a species' RGR and its competitive ability and invasiveness (Kolar and Lodge 2001; Grotkopp *et al.* 2002; Dawson *et al.* 2011). In this experiment, the RGRs of *L. major*, even when starting at a much higher initial density than that of *P. stratiotes*, were below zero, demonstrating plant mortality in almost all treatments. This trend occurred despite the biological control of *P. stratiotes*, once again supporting the previous conclusion that *L. major* cannot compete for resources in the presence of *P. stratiotes*, regardless of its condition. In contrast, the RGRs of *E. densa* demonstrated growth across all but one of the treatment combinations. When the initial planting density of *E. densa* was at just 10%, the highest RGRs were observed. This is in contrast to the findings reported in the previous chapter, but is supported by the wider literature, where a study by Pistori *et al.* (2004) on the RGR and doubling time of *E. densa* also found increased growth rates at lower plant densities, and a more general study into the growth rates of floating aquatic plants report a similar trend (Henry-Silva *et al.* 2002). It has been suggested that this occurs because as plant density increases, access to resources from individuals is reduced, thus following the conventions of intraspecific competition (Silverston and Doust 1993). Interestingly, some of the results from this experiment and those discussed in Chapter two found increased growth rates of *E. densa* in lower nutrient environments. This negative relationship between nutrient availability and growth rate was reported in a study previously referenced, exploring competition between three Hydrocharitaceae species (*Elodea canadensis* (Michx), *Elodea nuttallii* (Planch) and *L. major*) (James *et al.* 2006). The study suggests the relationship between nutrients and growth rate is due to increased root

production in high nutrients, so resources are partitioned away from producing above ground biomass, which could be an explanation for the results of this experiment.

The presence of biologically controlled *P. stratiotes* significantly increased the RGRs of *E. densa*; an expected outcome as more resources were made available through the breakdown of the damaged floating plants (Chimney and Pietro 2006; Shilla *et al.* 2006; Longhi *et al.* 2008). This conclusion is also supported by the plant tissue nutrient analysis of *E. densa*. Nitrogen content of plant tissue, reported as a ratio to carbon content (C:N), is used as an indicator of plant health. It has been used to gauge plant quality in numerous herbivory studies. Those with lower ratios are in better condition and therefore more palatable to potential herbivores (Byrant *et al.* 1983; White 1984; Price 2000). The increased intraspecific competition limiting the growth rates of *E. densa* at the 90% planting density can also explain the reduced condition of these plants as indicated by their elevated C:N ratios. With more plants competing for the same resources compared to the lower planting densities, each individual had fewer opportunities to capitalise on them. In keeping with this trend, the C:N ratios of *E. densa* at the lowest initial planting density (10%) demonstrated increased plant condition.

Interestingly, *E. densa* in mesocosms with controlled *P. stratiotes* that exhibited increased growth rates, did not produce plant tissue with lower C:N ratios when compared to mesocosms without biological control. Despite more nutrients being made available, and more biomass being produced, the quality of the plant tissue, overall, was diminished. This contradictory pattern could be a function of resource allocation, where submerged plants expend more resources on increasing biomass as opposed to producing high quality shoots when more nutrients are available, exhibiting a preference for quantity over quality. Resource

allocation studies have documented this occurrence in other submerged macrophytes such as *Myriophyllum spicatum* L. (Haloragaceae), and other members of the Hydrocharitaceae family: *E. canadensis* and *H. verticillata* (Madsen 1991; Nichols and Shaw 1986). Changes in light, temperature, herbivory, sediment type and nutrient environments have all been cited as triggers for changes in resource allocation (Xie *et al.* 2004; Xie *et al.* 2005; Pratt *et al.* 2005).

The results for the plant tissue analysis of *P. stratiotes* revealed that when subjected to biological control, plants had significantly lower C:N ratios compared to those undamaged, indicating increased plant health for controlled plants. Although this seems counterintuitive, this is most likely due to the age of leaves sampled; the youngest leaf of the middle rosette in each mesocosm was used for the C:N analysis. However, in non-controlled mesocosms, growth of *P. stratiotes* will have initially increased, then levelled out as resources depleted, resulting in reduced production of daughter plants as intraspecific competition increases (this is supported by studies that show *P. stratiotes* to be highly efficient at removing nutrients from the water column (Lu *et al.* 2010)). In contrast, plants subjected to biological control, and particularly those in very high nutrients which had significantly lower C:N ratios, became damaged and plant tissue broke down. This created fluctuations in resource availability over the course of the experiment as opposed to consistently decreasing levels of nutrients when healthy *P. stratiotes* were present. The decomposition released more nutrients into the system which the remaining plants used to rapidly produce new daughter plants. Compensatory regrowth as a product of altered nutrient cycling driven by herbivory pressure has been previously documented (McNaughton 1983; Holland *et al.* 1992). Therefore, at the end of the experimental period, the individual plant used for nutrient analysis is potentially younger in the controlled mesocosms as opposed to non-controlled.

Due to the high levels of plant mortality exhibited by *L. major* across all treatments, and the fact a regime shift was not observed, analysis of plant tissue was not performed. However, changes in biomass patterns, RGRs and plant health of *E. densa* in the presence of controlled *P. stratiotes* provide enough support to state that a regime shift occurred in the *E. densa* mesocosms.

It is clear that *E. densa* is capable of capitalising on resources made available through the biological control of *P. stratiotes*. The question remains: Which resource is driving *E. densa*'s success? The original theory of the proposed regime shift was that as biological control of *P. stratiotes* increases, two changes occur within the system. Firstly, light levels in the water column increase as surface area of *P. stratiotes* is reduced, thus promoting photosynthesis and growth of the submerged species. Secondly, the nutrients stored within *P. stratiotes* are returned to the water column as the plants degrade, and any remaining *P. stratiotes* are too damaged to compete for them. This follows logically from the previous experiments, whereby competitive ability of *E. densa* was an order of magnitude higher than *L. major* in nutrient rich conditions.

Changes in surface area cover of *P. stratiotes* revealed, as predicted, that as surface area decreased, the biomass of *E. densa* significantly increased. A positive relationship did not occur between measured light levels and the RGR or biomass of *E. densa*, whereas a strong correlation between biomass and nutrient levels in the water was apparent. Therefore, it can be concluded that whilst light still plays an important role in the success of *E. densa*, the shift in plant dominance was driven by the nutrients made available from the degradation of the floating plants. This outcome is supported by the numerous studies citing nutrient loading as a key factor impacting species assemblages and increasing invasive species establishment in

aquatic plant communities (Davis *et al.* 2000; Daehler 2003; Sharip *et al.* 2012). Observations made during the process of cultivating *E. densa* populations for experimental purposes also support this conclusion, where growth and plant quality noticeably increased when grown under 50% shade cloth compared to no shade cloth. In addition, field studies have recorded increased competitive advantage for *E. densa* in low-light conditions (Bini *et al.* 1999; Carrillo *et al.* 2006).

The results discussed here have demonstrated the proposed regime shift of invasive floating to invasive submerged plant dominance is possible, and that biological control of one species can have a significant impact on another species occupying a different habitat within the same system. They have also shown the shift to be predominantly nutrient driven. Potentially, the success of biological control of floating invasive plants could be undermined by inducing an equally problematic alternate state, leading to wasted time, money and resources.

The surprisingly low levels of dissolved oxygen recorded across all treatments could be a product of the experimental time frame. Reduced levels of dissolved oxygen are often recorded below mats of floating plants due to a reduction in gas exchange between the air and water, limited photosynthetic opportunities for submerged plants and increased microbial decomposition activity (Pokorny and Rejmankova 1983; Janes *et al.* 1996; Morris *et al.* 2004; Netten *et al.* 2010). Despite the low levels recorded there was still a positive relationship between growth of *E. densa* and dissolved oxygen levels that suggest if left for a longer period of time the levels of dissolved oxygen may have increased to more expected values. The overall trend between biomass and dissolved oxygen supports the assumption that as *E. densa* acquires more resources for growth, photosynthetic rates increase, which in

turn causes dissolved oxygen levels to rise (Cook and Urmi-König 1984). This is one of the reasons *E. densa* is promoted for use in aquariums and ponds which has led to its global spread, and also one of the contributing factors to the term ‘autogenic ecosystem engineer’ given to *E. densa* (Yarrow *et al.* 2009). The improvement in water quality further promotes the growth of *E. densa*, resulting in increased resilience. Another feedback mechanism published in a separate study found that as populations spread, roots of *E. densa* stabilize the sediment and reduce sedimentary re-suspension leading to clearer waters, thus maintaining their growth (Yarrow *et al.* 2009).

Whilst the regime shift was only observed for *E. densa*, the potential for the dynamic between *P. stratiotes* and *E. densa* to be altered by the presence of *L. major* cannot be ruled out. The next, and final, experiment aimed to explore this possible interaction effect. Increasing knowledge of the complex interactions between species can promote effective management towards resilient systems that support native flora and a rich biodiversity.

The next step is to understand how resilient the invasive submerged plant state would be. The strength or weakness of the key feedback mechanisms within a system determine the degree of resilience (Scheffer and Carpenter 2003; Walker and Meyers 2004; Folke *et al.* 2004; Beisner, 2012). Systems with lower resilience, or weakened feedback mechanisms, are more susceptible to shifts in overall structure and functioning (Hollings 1973; Scheffer 2003). Understanding these mechanisms, especially within unwanted stable states, is vital for maximising chances of successful and efficient management/restoration. Whilst mesocosm experiments do not reflect the multitude of complex interactions that occur in natural ecosystems, the trends in data can be a useful starting point for understanding key mechanisms affecting their resilience.

Chapter 4

Exploring resilience within the context of the regime shift

4.1 Introduction

A fundamental concept underpinning the theory of regime shifts is resilience. This is described as a system's ability to maintain its key structure and functioning under changing external pressures. Therefore, a system with low resilience is more vulnerable and requires a smaller perturbation to induce a shift into an alternate stable state (Holling *et al.* 1973; Scheffer *et al.* 2003). South Africa's natural topography has few large bodies of still or slow moving freshwater, which has resulted in very few indigenous plants adapted to occupy these spaces and a paucity in local plant evolutionary history. This characteristic, as well as the eutrophic status of the majority of South Africa's rivers and dams (Oberholster and Anderson 2008), increases their vulnerability to macrophyte invasion (and a regime shift to invasive plant dominance) and therefore these systems have inherently low resilience (Davis *et al.* 2000; Daehler 2003).

Once invasive floating macrophytes establish and their dominance becomes a stable state, they are able to alter the structure and functioning of the invaded system by decreasing light accessibility, reducing water quality and community biodiversity, changing nutrient cycling and creating anoxic conditions (Mitchell 1978; Vitousek 1986; Hill 2003; Scheffer *et al.* 2003; Midgley *et al.* 2006). Following establishment, controlling invasive floating macrophyte invasions has proven to be extremely difficult in many regions around the globe, indicating that these systems have very high resilience when in this stable state (Villamagna and Murphy 2009). For example, in South Africa, despite the overall success of many control programmes

for floating invasive plants, water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laub. (Pontederiaceae)) remains the most problematic aquatic invasive, despite the nationwide implementation of mechanical, chemical and biological control programmes against it (Hill 2003, Coetzee *et al.* 2011b). The first biological control agent for this plant was released in 1973 (Cilliers and Neser 1991), and since then, six more arthropods and one pathogen have been released, yet control has not been achieved in many regions (Coetzee *et al.* 2011b; Sutton *et al.* 2016). South Africa's high prevalence of eutrophic waters not only increases susceptibility to colonisation from invasive macrophytes, but also helps to maintain stability of these systems post-invasion (Hill and Cilliers 1999). In the case of *E. crassipes*, eutrophication has been found to be a key factor in situations where control of the species has been unattainable. Coetzee and Hill (2012) report that the only site where acceptable levels of control have been achieved in South Africa, at New Years Dam (Eastern Cape), can be wholly attributed to the levels of phosphorous within the water column being maintained below 0.1 mg/l. Therefore, the resilience of South Africa's eutrophic systems dominated by invasive floating plants is known to be relatively high.

The previous chapter presented experimental evidence in support of a potential switch between floating invasive (*Pistia stratiotes* L. (Araceae)) and submerged invasive (*Egeria densa* Planch. (Hydrocharitaceae)) plant dominance, triggered by the biological control of the floating plants. The next question becomes, when control of floating plants is achieved, can systems be managed in such a way to increase resilience and minimise the chance of a shift to submerged invasive plant dominance? Ecological restoration is often aimed at restoring a system to its original state, although systems do not always respond on a linear trajectory and, as previously discussed, restoring many of the man-made water bodies in South Africa to their 'original state' would mean restoring them to a state of low resilience

(Suding *et al.* 2009). The use of biotic resistance to increase system resilience and minimise invasive species success could be applied to the restoration of South African freshwater systems, which have been degraded by floating invasive species. Biotic resistance is the term given to a community's ability to limit the risk of invasion by non-native alien species, either from herbivory, disease or competitive pressures (Elton 1958). The theory is inherently linked with the enemy release hypothesis, which states that as species enter systems where they are free from such pressures, their chances of proliferation increase. Thus increasing local levels of these pressures can suppress invasive success (Maron and Vila 2001). Following this, if competition from submerged native species decreases the chance of successful colonisation by submerged invasive species, it may be possible to increase biotic resistance (and thus system resilience) by intentionally increasing richness and densities of native submerged plant populations.

The theory that biotic resistance can suppress invasive species success has sparked much debate over the years and produced publications that both support (Sax 2002; Kennedy *et al.* 2002) and argue (Crawley *et al.* 1999; Davis *et al.* 2000) its validity. Whilst Davis *et al.* (2000) disagree with the basic principal of Elton's hypothesis by stating there is no necessary relationship between plant community richness and invasibility, they do propose that invasibility will increase when there is an excess of unused resources. In a South African context, with many eutrophic systems carrying low native plant diversity, any increase in native species richness would reduce the availability of resources to invasive species, curbing the potential for invasion.

Pokorny *et al.* (2005) argue that rather than just increasing species richness, it is more beneficial to prevent invasive success by increasing functional group richness. Their study used manipulation experiments to explore how changes in functional group diversity affected

successful establishment of the forb, spotted knapweed (*Centaurea maculosa* Lam. (Asteraceae)). They concluded that native plants that perform similar ecological functions can help decrease successful establishment of invasive plant species. They also suggest studies reporting no association between species richness and invasive species establishment are often focussed purely on species diversity and do not consider species' functionality.

The previous two chapters explored firstly the interaction between the submerged native *Lagarosiphon major* (Ridl.) Moss (Hydrocharitaceae) and the submerged invasive *Egeria densa* Planch. (Hydrocharitaceae), when in direct competition for resources, and secondly the independent responses of these species to the biological control of the floating invasive *Pistia stratiotes* L. (Araceae). The aim of this chapter was thus to complete the suite of experiments by exploring how the interactions between the two submerged plant species are affected by the presence, and control of *P. stratiotes*. The findings of all the experiments will contribute to understanding the key interactions within the proposed regime shift. This experiment utilised large mesocosms of factorial combinations of submerged *L. major* and *E. densa* in the presence of floating *P. stratiotes*, under a range of nutrient and biological control regimes, to determine the outcome of competition between the submerged species as influenced by nutrient status and biological control of the floating species.

4.2 Materials and Methods

4.2.1 Plant and insect cultures

This experiment was conducted inside a greenhouse tunnel at the Waainek Research Facility at Rhodes University in Grahamstown, South Africa, from 8 January - 1 April 2016. The *E. densa* culture was collected from the Kouga River, near Patensie, Eastern Cape, (-33.748506, 24.635446), and the *L. major* culture was collected from a population in a quarry dam near Stutterheim, Eastern Cape, (-32.587336, 27.463443). Both species were collected six months before the start of the experiment. They were cultivated at the Waainek Research Facility in a flow through system, treated with optimal nutrients in spring water, under 80% shade cloth. They were planted in sediment collected from Jameson Dam, Eastern Cape, South Africa (-33.319073, 26.444206).

Prior to the start of the experiment, the submerged plants were treated with an organophosphate insecticide, Malathion (Kombat, 0.01ml/10l), to kill any phytophagous insects associated with the plants collected from the field. Hoagland's solution (5.2mg/l) was added to each tank at the start of the experiment, with the nitrogen sources omitted as this was controlled separately (Hoagland and Arnon, 1938). The mean starting wet weight per shoot of *L. major* and *E. densa* planted in all treatments was 3.5 g (\pm 0.09 S.E.) and 3.8 g (\pm 0.07 S.E.) respectively. All *P. stratiotes* plants were sourced from insect-free stock populations maintained at the Waainek Research Facility. Adult *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae), a weevil routinely used in the biological control of *P. stratiotes* (Cilliers 1991c), were supplied by the South African Sugarcane Research Institute (SASRI).

4.2.2 Experimental design

This experiment utilized 16 tanks across four flow-through systems (four tanks per system, measuring 120cm x 100cm x 70cm each), and each system was assigned a treatment allowing for four replicates (each tank is a replicate). Each tank used the same planting design for *L. major* and *E. densa* based on Spitters (1983) full factorial additive design, as described in Chapter Two. However, every tank also had 20 individuals of *P. stratiotes* floating on the surface to achieve 90% plant cover (Fig. 4.1).



Fig. 4.1 Example of submerged plants beneath a layer of floating *Pistia stratiotes*.

There were four combinations of nutrient and biological control treatments. Each system, 1-4, was assigned one of the treatment combinations. Two systems had 'high' nutrients in the water column (sediment nutrients were not used as they would only be accessible to the submerged plants) and two systems had 'low' nutrients. The high nutrient treatment was achieved by adding 10 mg N/l using dissolved ammonium nitrate (137 g NH_4NO_3 per system). No additional nutrients were added to the low treatment. Within each nutrient regime, one

system was inoculated with *N. affinis*, the biological control agent of *P. stratiotes*, and the other was not. A 50:50 sex ratio of the weevils was assumed and four adults (two mating pairs, 80 individuals per tank) were placed directly onto each plant rosette to ensure plant death (Diop *et al.* 2010). A layer of fine white gauze was secured over all tanks to prevent insect movement between tanks and the experiment ran for 12 weeks.

4.2.3 Measurements

Thermachron iButtons were added to two of the flow through systems (Climastats Environmental Monitoring software, Ver. 4) and measured surface temperature of the water at midday, everyday, for the duration of the experiment. This was done to monitor any fluctuations in temperature that may affect plant growth. Surface area photographs of each tank were taken at weeks 4, 8, 12 and were used in digital imaging software (imageJ version 1.49, National Institutes of Health, available at <http://imagej.nih.gov/ij>) to calculate percentage cover of healthy *P. stratiotes* leaf tissue. This was achieved by calculating the percentage cover of each tank that contained pixels of healthy green tissue, and omitting shades of yellow and brown that are characteristic of damaged or dead plant tissue.

After 12 weeks, the wet biomass of all *P. stratiotes* plants was recorded. General linear model analyses of variance (GLM-ANOVAs), followed by Tukey HSD post-hoc tests, were performed to determine significant effects of nutrient and biocontrol treatments on the biomass and percentage cover of *P. stratiotes*.

Ammonium [NH₄⁺] (mg/l) and nitrate [NO₃⁻] (mg/l) were measured monthly to monitor changes in nutrient availability within the water column and look for correlations with plant growth and success of biological control. They were measured with Vernier™ ion specific electrodes (ISE) using a LabQuest 2 interface.

4.2.4 Data analyses

Relative growth rates (RGRs) of each species were calculated using the standard formula described by Evans (1972):

$$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

where W_1 and W_2 are the dry weights at the start and end of the experiment respectively at times t_1 and t_2 (the start and end time in days). To calculate initial dry weights, 30 individuals taken from the same populations as those used in the experiments were weighed wet and then dried for 96 hours in a drying oven at 60°C. The relationship between wet and dry weight of each species was determined using linear regressions to obtain an equation for wet to dry conversions:

$$dw_l = 0.0468 (ww_l) + 0.0188$$

$$dw_e = 0.0754 (ww_e) + 0.0105$$

where dw_l and dw_e are the dry weights of *L. major* and *E. densa*, and ww_l and ww_e are the wet weights.

After 12 weeks, all of the above ground biomass of *L. major* and *E. densa* was collected, dried for 96 hours at 60°C and weighed. Below ground biomass was omitted from analyses as roots of both species were identical and fragile, thus those that broke away from shoots during collection could not be accurately identified. Percentages of *L. major* and *E. densa* that survived and that demonstrated positive growth rates were calculated for each treatment. Chi-square analyses of independence were performed on percentage survival of *L. major* and *E. densa* under each treatment. All statistical analyses were conducted in the R environment

(version 3.2.3; R Development Core Team, 2014; available at <http://cran.r-project.org>) using R Studio (version 0.98.1103).

Multiple regressions on the mean end dry biomass of *L. major* and *E. densa*, at different planting densities and in different nutrient and biological control treatments, were performed to determine changes in competitive ability of *L. major* and *E. densa* under each regime. The magnitude of the relationship was determined using the reciprocal-yield model (Spitters, 1983), as used in Chapter Two. This involved multiple linear regressions with the inverse of the mean biomass yield for each species as the dependent variable, and the planting densities of *L. major* and *E. densa* as the independent variables. The equations for each plant follow the form:

$$\frac{1}{W_e} = a_{e0} + a_{ee}d_e + a_{el}d_l$$

$$\frac{1}{W_l} = a_{l0} + a_{ll}d_l + a_{le}d_e$$

where W_e and W_l are the mean dry weights of *E. densa* and *L. major* respectively. The planting densities for *E. densa* and *L. major* are expressed as d_e and d_l . The intercepts (a_{l0} and a_{e0}) measure the reciprocal of the maximum weight of individual plants. Intraspecific competition is estimated by the partial regression coefficients a_{ee} and a_{ll} . Interspecific competition is estimated by the coefficients a_{le} and a_{el} in terms of their effects on the reciprocal yield of biomass for *L. major* or *E. densa*, where each *L. major* plant has an effect of $1/W_l$ equal to a_e/a_l *E. densa* plants. Interspecific and intraspecific competition by one species on its own yield, as well as the yield of the other species, is measured using the ratio of the coefficients (a_{ll}/a_{le} and a_{ee}/a_{el}) (Pantone *et al.* 1989).

4.3 Results

The mean daily surface temperature recorded in the system with high nutrients and biological control agents present on *P. stratiotes* was 30.61°C (S.E. 0.37), with a minimum of 23.09°C and a maximum of 38.07°C. The mean daily surface temperature recorded in the system with low nutrients and an absence of biological control agents was 28.23°C (S.E. 0.44), with a minimum of 19°C and a maximum of 37.82°C. There was a significant difference between the temperatures of the two systems ($F_{(1,64)}=1191, P<0.000$).

There was a significant effect of the interaction between nutrient treatment and the presence or absence of biological control agents (*N. affinis*) on the percentage cover of healthy *P. stratiotes* at the end of the experiment ($F_{(1,12)}=18.08, P<0.01$) (Fig. 4.2 and Fig. 4.3). Post-hoc Tukey HSD analyses revealed that in high nutrients, the percentage cover of *P. stratiotes* was significantly reduced in the presence of biological control agents. Under low nutrients, percentage cover of *P. stratiotes* was significantly lower than cover at high nutrient treatments, but there was no significant difference in the presence or absence of biological control agents.

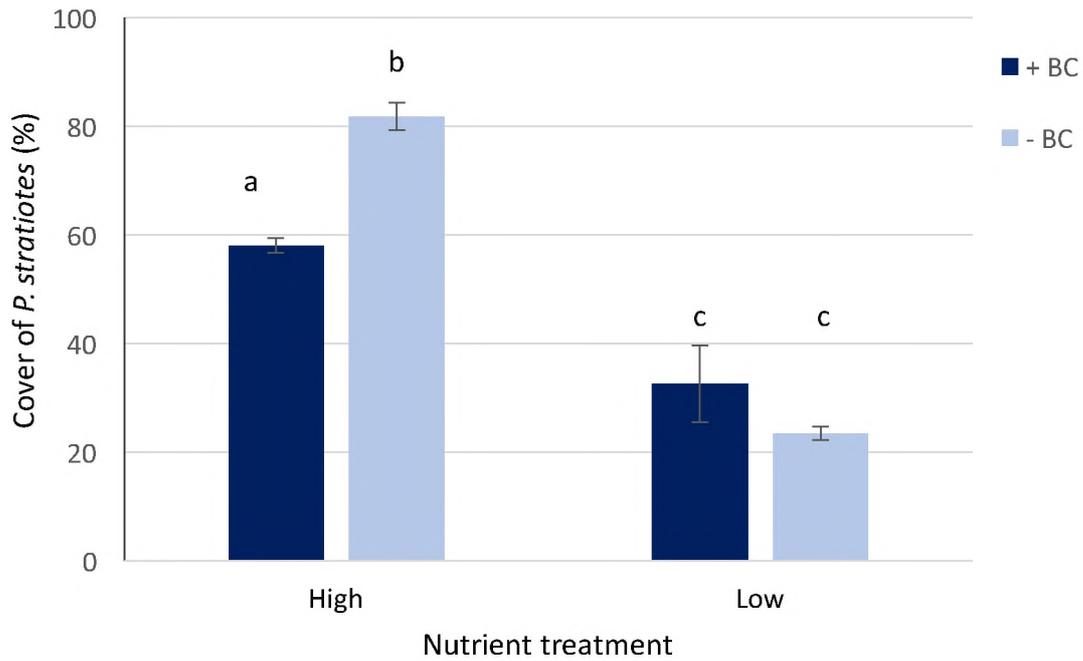


Fig. 4.2 The mean percentage cover (\pm S.E.) of healthy *Pistia stratiotes* (%) after 12 weeks at each combination of nutrient and biological control (BC) treatments. Different letters denote significant differences between treatments ($P < 0.05$).

High Nutrients



+ biological control

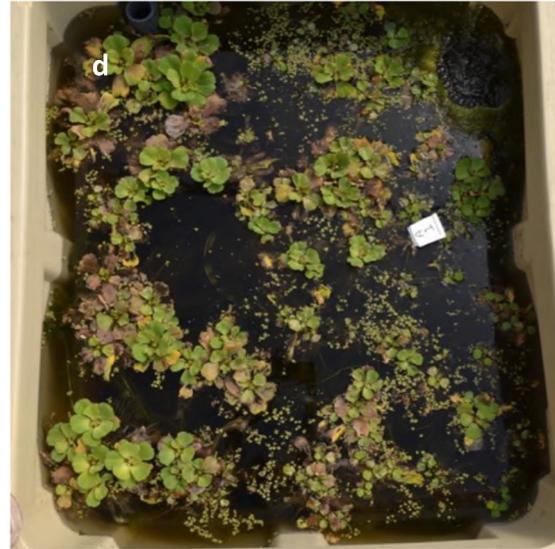


- biological control

Low Nutrients



+ biological control



- biological control

Fig. 4.3 Sample photographs of one tank in each system after 12 weeks of the experiment, indicating the effect of nutrient level and presence or absence of control agents on the surface area cover of *Pistia stratiotes*.

As expected, the results for the biomass of *P. stratiotes* at the end of the experiment reflect the trends observed in the percentage cover data. There was a significant interaction between nutrient treatment and the presence or absence of biological control agents (*N. affinis*) on the biomass of *P. stratiotes* at the end of the experiment (Fig. 4.4) ($F_{1,12}=22.559$, $P<0.001$). Under high nutrients, the presence of biological control agents significantly reduced the biomass of *P. stratiotes*, whereas there was no significant effect under low nutrients.

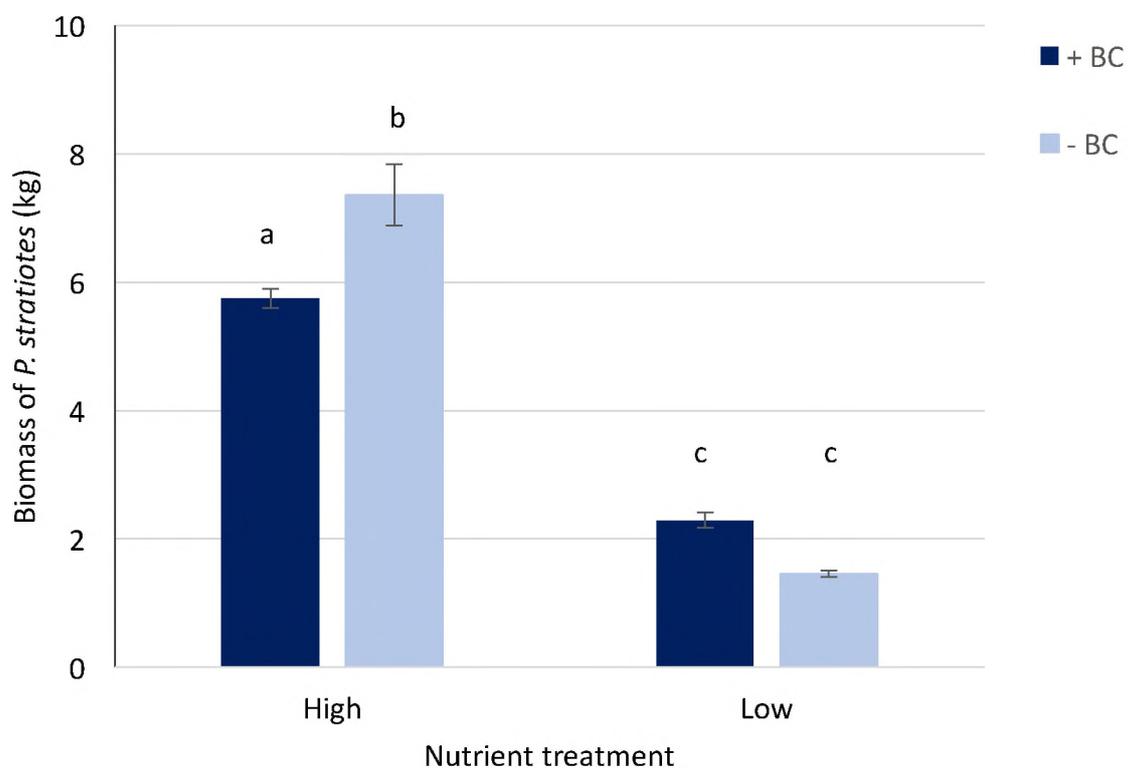


Fig. 4.4 Mean wet biomass (\pm S.E.) of *Pistia stratiotes* after 12 weeks at each combination of nutrient and biological control (BC) treatments. Different letters denote significant differences between treatments ($P<0.05$).

Total nitrogen concentrations in the water (mg/l) were unexpectedly high after four weeks of the experiment, particularly for the high nutrient treatment systems (Fig. 4.5). The nitrogen levels remained relatively consistent within the low nutrient treatments throughout

the 12 weeks. In contrast, there was a steep decline in both of the high nutrient systems. This steep decline occurred in systems with a high abundance of *P. stratiotes* (Fig. 4.2 and Fig. 4.3), and low survival of the submerged species, indicating a superior ability of *P. stratiotes* to take up available resources and thrive in highly eutrophic waters.

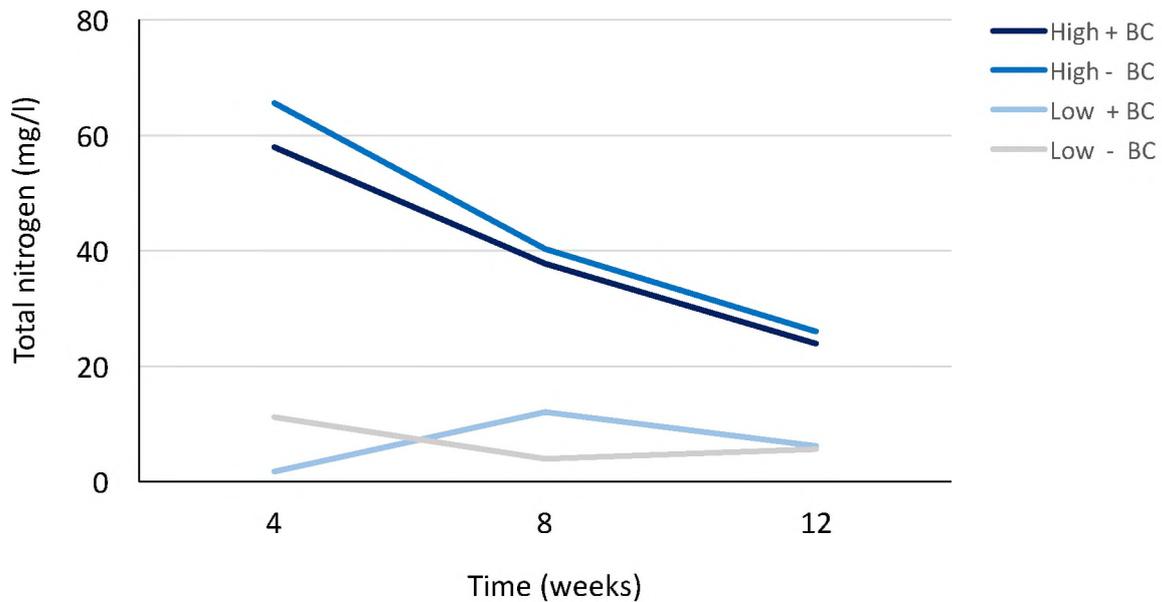


Fig. 4.5 Total dissolved nitrogen in the water column of each flow-through system, with different combinations of nutrient (high/low) and biological control (+/- BC) treatments, recorded at monthly intervals during the experiment.

Egeria densa had significantly higher RGRs than *L. major* overall ($F_{(1,98)}=128.367$, $P \leq 0.000$) (Fig. 4.6 a-d). At high nutrient treatments, and in the presence of biological control agents on *P. stratiotes*, there was high plant mortality of both submerged species, and therefore insufficient data to perform an ANOVA on the whole model (Fig. 4.6 d).

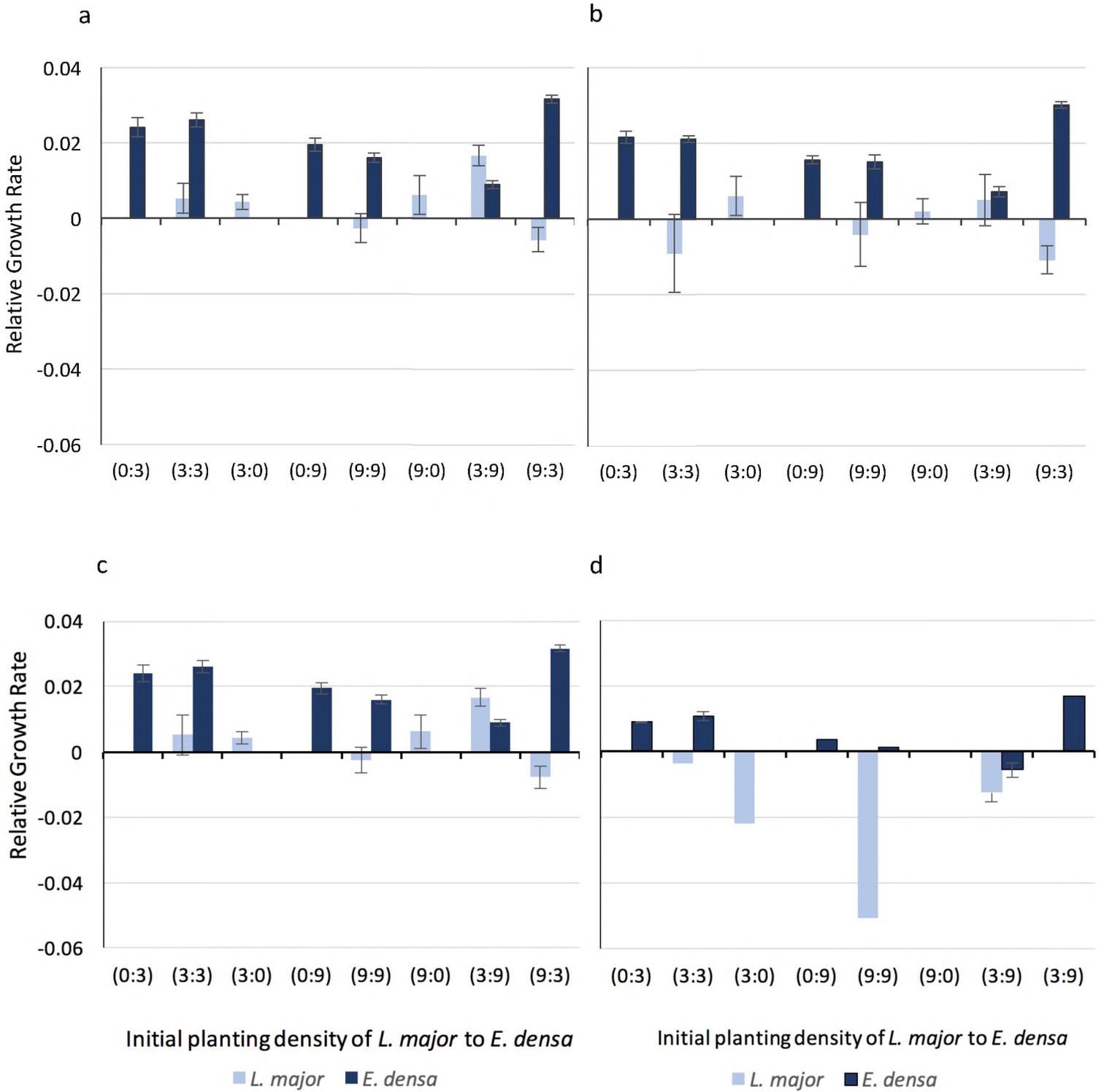


Fig. 4.6 The relative growth rate (mean \pm S.E.) of *Lagarosiphon major* and *Egeria densa* at each initial planting density under low nutrients (top row) and high nutrients (bottom row) in **(a and c)** the absence of biological control agents on *Pistia stratiotes* and **(b and d)** the presence of biological control agents on *Pistia stratiotes*.

Survival of *E. densa* was significantly higher than survival of *L. major* across all combinations of nutrient and biological control treatments ($X^2_{(3)} = 9.06, P < 0.05$) (Fig. 4.7). *Egeria densa* had 100% survival rates in all treatments except for the system with high nutrients and with biological control of *P. stratiotes*, where fewer than half the plants survived (42%). This was unexpected as these were the conditions in which a regime shift to *E. densa* dominance was observed in the mesocosm experiment discussed in Chapter Three. *Lagarosiphon major* also had the lowest level of survival in this treatment at 20%, although 100% *L. major* survival did not occur under any of the treatments. Survival of *L. major* was higher in low nutrients compared to high nutrients, and survival of *L. major* was reduced in systems with biological control agents present on *P. stratiotes*, indicating either a preference for lower nutrient conditions, or that in high nutrients, *L. major* is far more affected by competition from *E. densa*. The latter possibility is further supported by the observation that *L. major* had reduced survival in the presence of controlled *P. stratiotes*, conditions that have previously been found to promote *E. densa* growth. Furthermore, while survival of both species was unexpectedly low in the system with high nutrients and with controlled *P. stratiotes*, survival of *E. densa* was still double that of *L. major*.

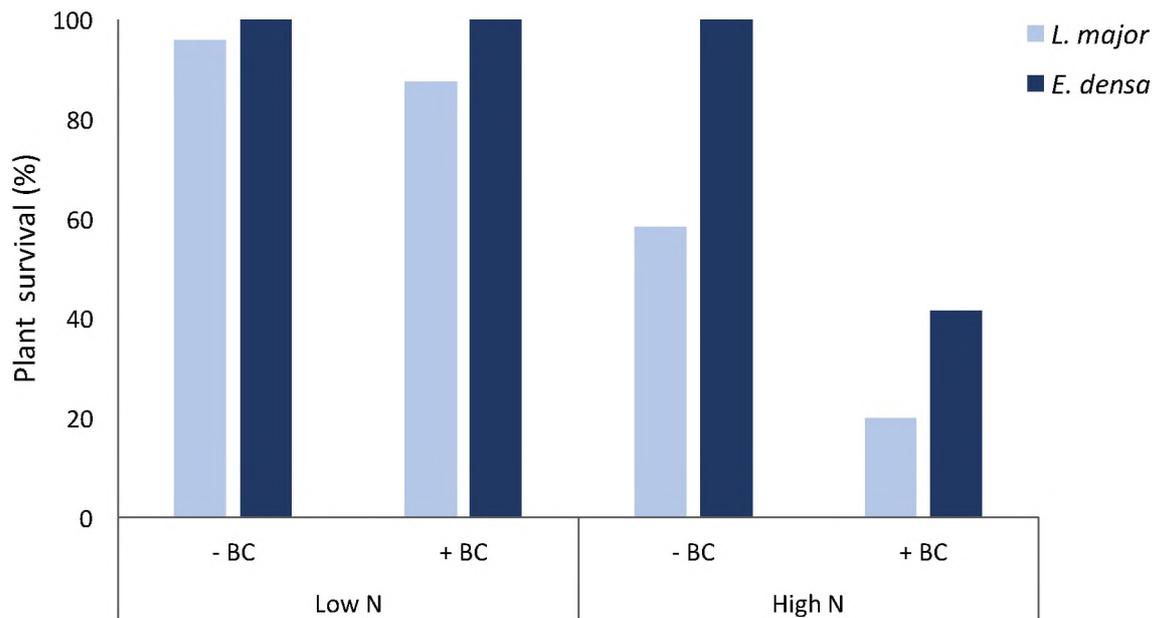


Fig. 4.7 Survival (%) of *Lagarosiphon major* and *Egeria densa* at each combination of nutrient (N) and biological control (BC) treatments after twelve weeks.

Following this, percentages of plants that both survived and demonstrated positive growth rates were determined (Fig. 4.8). These results show a much greater disparity between the submerged species, with *E. densa* exhibiting significantly higher percentages of positive growth in surviving plants compared to *L. major* ($X^2_{(3)} = 44.03$, $P < 0.0001$). This indicates a much higher competitive ability of *E. densa* even under conditions that might be less than optimal, as indicated by the reduced survival rates.

At low nutrients, all *E. densa* plants both survived and grew, which supports findings in the previous chapters of increased growth rates in conditions where competition for resources is higher. Under high nutrients where survival for *E. densa* was 100% in the absence of control agents on *P. stratiotes*, but just 41% in the presence of control agents, the percentage of plants that exhibited positive growth rates remained very high (minimum 80%). The highest percentage of surviving *L. major* plants with positive growth rates was much lower at 63%, which was recorded in the low nutrient treatments, with control-free *P.*

stratiotes. In the high nutrient treatment, although there was a 20% survival rate for *L. major* in the system with biological control agents, no plants demonstrated positive growth rates, indicating that all *L. major* plants in this treatment suffered some degree of mortality.

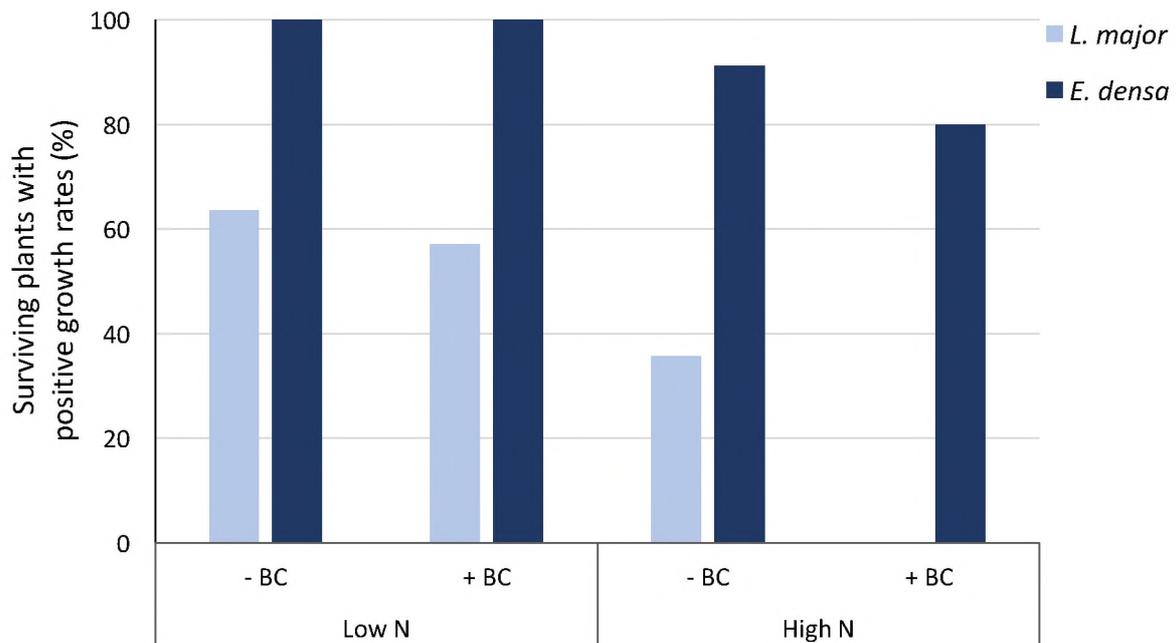


Fig. 4.8 Percentage of *Lagarosiphon major* and *Egeria densa* at each nutrient (N) and biological control (BC) treatment combination that demonstrated positive growth rates over the twelve-week experimental period.

Regression analysis on the mean end yields of *E. densa* and *L. major* revealed contrasting effects of nutrients and biological control of *P. stratiotes* on the outcome of competition between the two submerged species (Table 4.1). In low nutrients, the coefficients that measure relative competitive ability of *L. major* against *E. densa* (a_{ll}/a_{le}) were 1.12 (compared to 0.67 for *E. densa*) and 0.39 (compared to 0.16 for *E. densa*) when biological control agents were present and absent, respectively. In other words, under low

nutrients and in the presence of controlled *P. stratiotes*, it would take 1.12 *E. densa* plants to have the same effect on *L. major* growth as adding one *L. major* plant. This means that *L. major* had a competitive advantage over *E. densa* in low nutrients, and this increased by 65% in the presence of controlled *P. stratiotes*.

In contrast, under high nutrients, *E. densa* had the competitive advantage over *L. major* as expressed by the competition coefficients (a_{ee}/a_{el}), which were 2.1 (compared to 1.66 for *L. major*) in the absence of control agents on *P. stratiotes* and 0.67 in their presence (compared to 0.41 for *L. major*). These results indicate that the addition of biological control agents on *P. stratiotes* decreased the competitive ability of *E. densa* by 68% in high nutrients. However, these results must be interpreted with caution as this system (high nutrients, with biological control) suffered mass submerged plant mortality, which could have an effect on the validity of the results. The low R^2 values in the regression model outputs suggests that the high mortality of submerged plants may be a limitation in the experimental analysis. This is further confounded by the fact that only one of the eight models was statistically significant, and that was for the biomass of *E. densa* in low nutrients in the presence of biological control agents on *P. stratiotes* (Table 4.1).

Table 4.1 Multiple regression analysis of competition between *Lagarosiphon major* and *Egeria densa* grown in high and low nutrients, with and without biological control on *Pistia stratiotes*. (*) denotes statistical significance.

Biomass	Biological Control	Nutrient	Intercept	Intraspecific competition ¹	Interspecific competition ²	Ratio of competition coefficients ³	R ²	F ₍₃₋₂₀₎	P
<i>L. major</i>	+	High	0.64	0.2967	0.7253	0.41	0.11	0.82	0.50
<i>L. major</i>	-	High	5.17	0.1924	0.116	1.66	0.04	0.23	0.88
<i>E. densa</i>	+	High	0.27	0.0133	0.0199	0.67	0.14	1.12	0.37
<i>E. densa</i>	-	High	0.43	0.0321	0.0153	2.10	0.17	1.36	0.29
<i>L. major</i>	+	Low	2.50	0.4625	0.0414	1.12	0.12	0.88	0.46
<i>L. major</i>	-	Low	0.81	0.009	0.0227	0.39	0.24	2.16	0.13
<i>E. densa</i>	+	Low	0.16	0.0069	0.0103	0.67	0.53	7.53	0.00*
<i>E. densa</i>	-	Low	0.12	0.0007	0.0042	0.16	0.31	2.94	0.06

¹ Intraspecific competition: represented by regression coefficients a_{egeg} for *E. densa* and $a_{\text{lm lm}}$ for *L. major*.

² Interspecific competition: represented by regression coefficients a_{eglm} for *E. densa* and a_{lmeg} for *L. major*.

³ The ratio of competition coefficients measures the effect of intraspecific competition by one species on its own mass relative to the effects of interspecific competition by the other species: $a_{\text{egeg}}/a_{\text{eglm}}$ for *E. densa* and $a_{\text{lm lm}}/a_{\text{lmeg}}$ for *L. major*.

The changes in the competitive relationships between *L. major* and *E. densa* at the two nutrient and biological control treatments were graphically analysed using three-dimensional graphs (Figs 4.9 a-d and 4.10 a-d) that illustrate the eight reciprocal yield models (Table 4.1). The higher the values on the figure, the lower the actual yield; and the steeper the gradient of the slope, the larger the competitive coefficient. The graphs allow for easy comparisons between the competitive interactions of *E. densa* and *L. major*, both in the presence and absence of biological control agents on *P. stratiotes* in low (Fig. 4.9) and high (Fig. 4.10) nutrients.

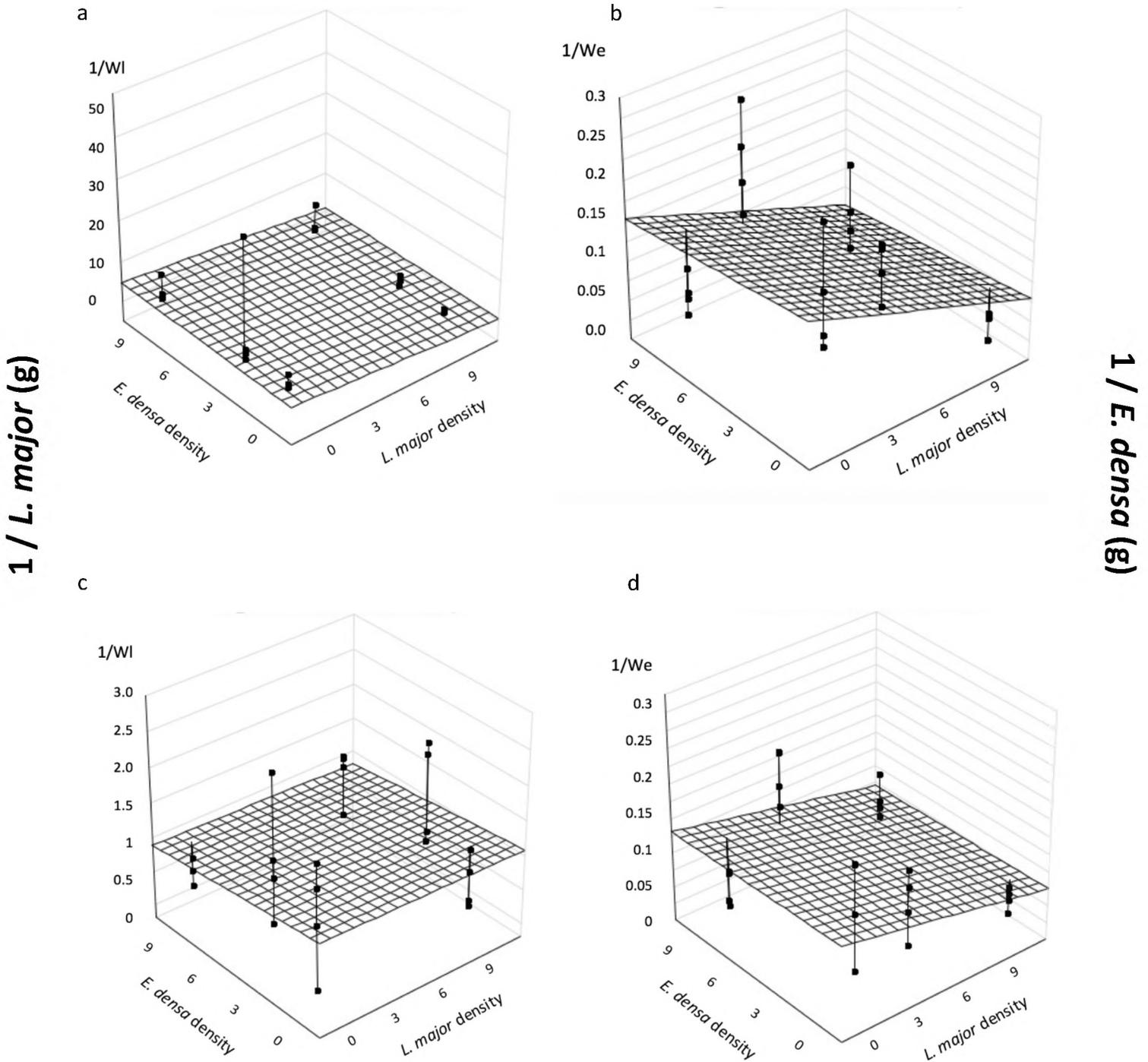


Fig. 4.9 Multiple regression planes demonstrate the combined effect of *Lagarosiphon major* and *Egeria densa* planting densities on the inverse mean dry biomass ($1/g$) of one *L. major* plant ($1/W_l$) in the presence (a) and absence (c) of biological control agents on *Pistia stratiotes*, and one *E. densa* plant ($1/W_e$) in the presence (b) and absence (d) of biological control, at low nutrients.

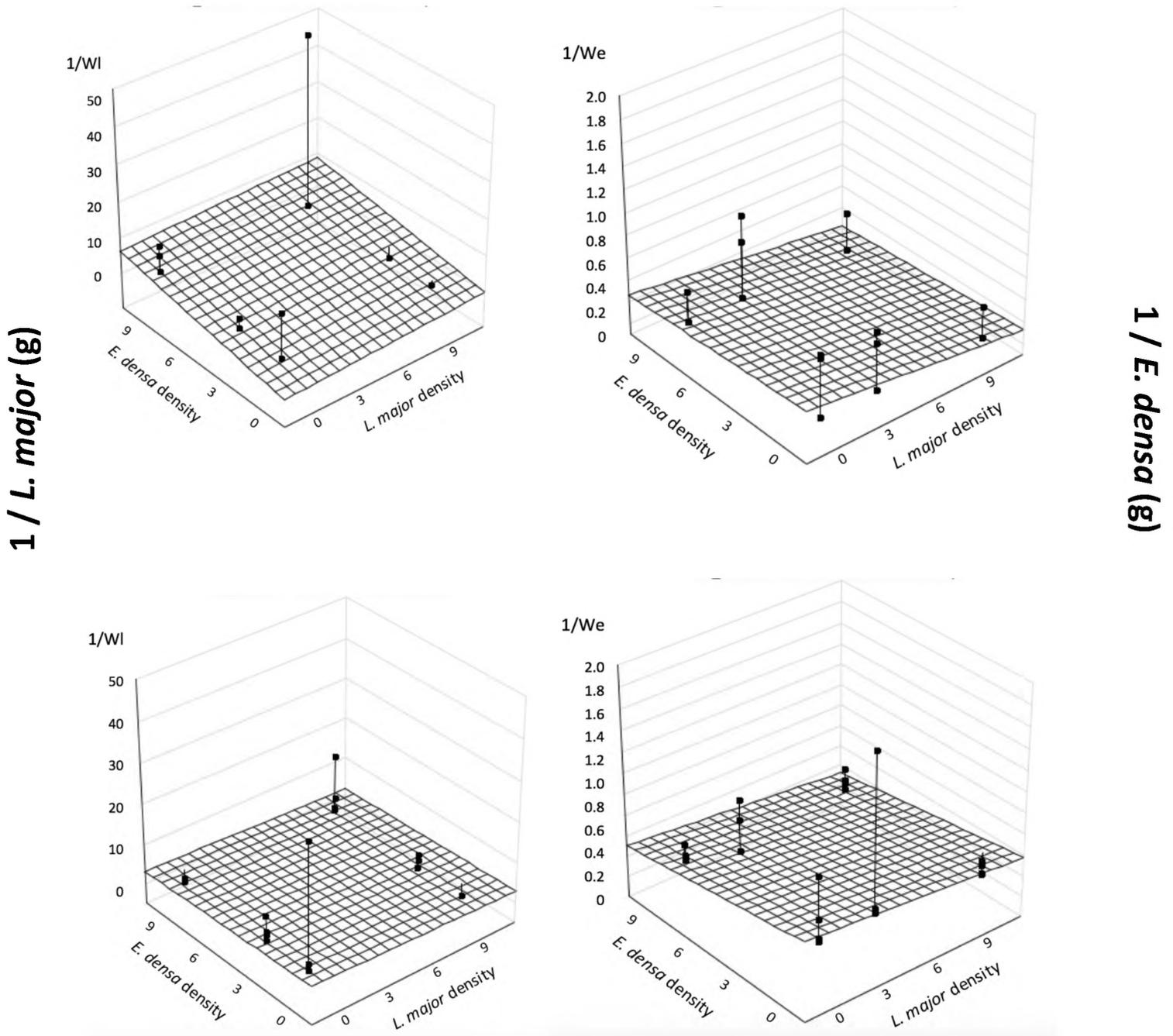


Fig. 4.10 Multiple regression planes demonstrate the combined effect of *Lagarosiphon major* and *Egeria densa* planting densities on the inverse mean dry biomass (1/g) of one *L. major* plant ($1/W_i$) in the presence (a) and absence (c) of biological control agents on *Pistia stratiotes*, and one *E. densa* plant ($1/W_e$) in the presence (b) and absence (d) of biological control, at high nutrients.

4.4 Discussion

The results from Chapter Two revealed that when in direct competition with only one another for resources, *E. densa* was competitively superior to *L. major*, and under high nutrients, this relationship increased seven-fold. The results from this chapter demonstrate that *E. densa* remained a superior competitor against *L. major* under high nutrients, but in the presence of biological control of *P. stratiotes*, the scale of the competitive advantage was reduced by 68% when compared to plants growing in the presence of non-controlled *P. stratiotes*. In addition, in the first experiment, *E. densa* was also a superior competitor to *L. major* under low nutrients, but with only a 15% difference between the two species. In contrast, when competing under low nutrients and in the presence of *P. stratiotes*, *L. major* had the competitive advantage over *E. densa*. These findings support the previous conclusion that in an environment of reduced nutrient loading, there is a significantly higher chance of native species being able to reduce the competitive advantage of submerged invasive species, which would in turn increase system resilience. The results also support several studies that propose an association between increasing species and functional richness with decreasing invasive establishment success (Elton 1958; Case 1990; Law and Morten 1996; Sax 2002; Kennedy *et al.* 2002; Pokorny 2005; Pinto and Ortega 2016).

A shift between the dominance of floating *P. stratiotes* to submerged (invasive or native) plant dominance did not occur in this experiment. The results suggest that the lack of regime shift in this experiment was because control of *P. stratiotes* by *N. affinis* did not cross the critical threshold required to induce the shift, further supporting the notion that systems dominated by floating invasive plants are highly resilient, particularly in the presence of nutrient loading (Gossett and Norris 1971; Reddy *et al.* 1989; Heard and Winterton 2000). The

required threshold of damage may not have been crossed due to the high densities of *P. stratiotes* used in the tanks (significantly larger than the mesocosms used in Chapter Three). In the high nutrient treatment, mean percent cover of *P. stratiotes* never fell below 55% despite herbivory damage, while in the low nutrient treatment, there was no difference in mean percent cover of *P. stratiotes* between biological control treatments, which did not exceed 23% cover. The plant to insect ratio used in this experiment was chosen based on Diop *et al.* (2010) who concluded two pairs of mating weevils per plant of *P. stratiotes* was sufficient to cause plant death. Also, this same ratio was used in the experiments discussed in Chapters Two and Three, with successful control of *P. stratiotes* achieved. Therefore, biological control agent density was not a factor limiting control. Temperature was also unlikely to reduce control, as *N. affinis* has been recorded to be highly effective at controlling *P. stratiotes* in tropical regions with significantly higher temperatures compared to the location from which they were sourced, indicating high thermal tolerance (Harley *et al.* 1990). An unexpected observation within the experiment was the much higher than anticipated levels of total nitrogen recorded in the water column, which may help explain the lack of control of *P. stratiotes*. In the high nutrient level systems, an initial solution of ammonium nitrate was added that would provide 10mg/l of total nitrogen to the water column. This concentration was chosen to represent eutrophic systems and is the concentration that Moore and Hill *et al.* (2012) recorded at an invaded South African system where percentage cover of *P. stratiotes* was reduced from 100% to 1% through biological control in three months. However, after four weeks of the experiment discussed here, nitrogen concentrations between 55 – 65 mg/l were recorded. The spike may have been caused by rapid decay of the submerged plants, particularly *L. major*, that exhibited high plant mortality, as decaying plant tissue of submerged macrophytes has been shown to considerably increase

water nutrient levels (Shilla *et al.* 2006). Over the twelve weeks, there was a steep decline in the total dissolved nitrogen concentrations within the high nutrient systems. This is due to assimilation of the available nutrients by *P. stratiotes*, and is a product of larger healthier *P. stratiotes* plants that sustained dominance (and where control was least successful) in these systems. These findings are supported by a study that reports a capacity for *P. stratiotes* to reduce inorganic nitrogen concentrations in eutrophic ponds by 50% compared to ponds without *P. stratiotes* (Lu *et al.* 2010). In the low nutrient system with biological control agents present on *P. stratiotes*, total nitrogen levels in the water were higher at the end of the experiment, supporting both previous findings, and the hypothesis that with increased biological control, more nutrients are made available within the water column.

Under increased nutrient loading, leaf turnover in aquatic floating plants has been shown to outpace rates of damage caused by herbivory, which in turn prevents successful control. This has been documented to occur in populations of *E. crassipes* growing in nutrient rich conditions (Ripley *et al.* 2006). While leaf turnover rates have not specifically been studied for *P. stratiotes*, the species has been documented to have a high capacity to rapidly build up biomass (Reddy and De Busk 1984). Even though successful control of *P. stratiotes* has occurred in two highly eutrophic systems in South Africa: Sunset Dam (Kruger National Park) and a Port Elizabeth waste water treatment plant (Cape Recife), the nitrogen levels recorded in the high nutrient treatment systems in this experiment were higher than any recorded in the field, and in the case of Sunset Dam, control did take up to five years to achieve (Neuenschwander *et al.* 2009).

Once again, in accordance with the trends observed in the first competition experiment (Chapter Two), *E. densa* exhibited higher growth rates compared to *L. major*. The submerged plant survival results reveal high mortality rates for *L. major* under high nutrients, and the

high nutrient system with biological control agents present on *P. stratiotes* saw a mortality rate of 58% for *E. densa*. Initially it was hypothesised that in high nutrients, *P. stratiotes* demonstrated superior competitive ability for the abundant resources and completely outcompeted both submerged species. However, if this were the case, then the same results would be expected for the system that contained healthier *P. stratiotes* not damaged by biological control agents, a trend that was not observed in the system with no biological control agents present. The mortality could be a product of the high temperatures recorded in the systems. Chapter Two discussed an average temperature of 26°C as a contributing factor for the *L. major* mortality in the first experiment, as the species has an optimum temperature range between 20-23°C, with a maximum tolerance of 25°C (Kasselman 1995). In this experiment, the system with high nutrients and with biological control agents on *P. stratiotes*, had an average surface temperature of 30°C reaching a maximum of 38°C. It therefore seems highly likely to be a contributing factor to the mortality of *L. major*. *Egeria densa* also exhibited some mortality and reduced growth rates compared to *E. densa* growing in the other systems. The thermal tolerance of *E. densa* is far wider than *L. major*, ranging from 16°C to 28°C, with a maximum tolerance of 35°C, but temperatures in the experiment were still beyond these (Barko and Smart, 1981; Getsinger and Dillon 1984; Haramoto and Ikusima 1988; Di Tomaso and Healy 2003).

Although a regime shift to submerged plant dominance did not occur in this experiment, the relationship within the submerged plant community within the model system was altered by the presence and control of *P. stratiotes*. This demonstrates the importance of research into the effects that management of floating invasive plants can have on non-target species communities and on sustainable restoration of valuable freshwater resources. The results suggest that bottom-up manipulations, such as reduced nutrient loading can improve top-

down restoration efforts such as biological control, and the implementation of a holistic approach can increase resilience and prevent future degradation. Integrated management practices that incorporate simultaneous top down and bottom up control are often required to achieve restoration of highly degraded systems (Center and Dray 2010; D'Antonio and Chambers 2013).

Combining the results from this experiment with the results discussed in the previous two chapters, an ecological model based on experimental data can be built to explain the interactions between key variables within the proposed regime shift. The subsequent model can be used to test the validity of the regime shift theory and help elucidate the mechanisms that underpin it.

Chapter 5

Dynamic modelling of the regime shift

5.1 Introduction

The field of regime shift research is not a new one but has recently experienced rapid growth; with a 12-fold increase in related publications between 1991 and 2006 (Anderson *et al.* 2009), and more recently, the development of the international Regime Shift Data Base, an initiative led by the Stockholm Resilience Centre (www.regimehifts.org, Stockholm University). Research in this field is often from the socio-ecological perspective, where anthropogenic activities have contributed to a shift between states that negatively impact human wellbeing. Examples include eutrophication of shallow lakes, bush encroachment, collapse of fisheries and soil salinization (Biggs *et al.* 2012)

The literature is regularly based on the parameter perturbation framework, where each of the alternative regimes is stable but corresponds to different parameter values, therefore associated dynamics such as population fluctuations differ (Beisner *et al.* 2003). Traditionally, research of this nature has leaned heavily on theoretical modelling, because quantitative field data as evidence of regime shifts is often difficult to obtain. This can be attributed to the logistical problems of collecting field data from systems pre, during and post regime shifts (i.e. a shift is often only recognized post occurrence); and the difficulty of projecting assumptions on multiple ecosystem types, based on observed trends from individual case-studies.

The use of controlled experiments to study alternative regimes are few and far between (Chase 2003; Anderson *et al.* 2009) and can be perceived as an over-simplification

of the complex natural systems they aim to explore (Dunham and Beaupre 1998). However, Scheffer and Carpenter (2003) refute this and suggest that there is a great importance in using controlled experiments in order to prove the existence of a systems' alternative attractors, in this case differing states of plant dominance, to bridge the gap between ecosystem dynamics (field observations) and abstract theory (modelling). Data obtained from controlled experiments can then provide the basic trends and interactions needed to build models which can be a considerably more powerful tool compared to qualitative reasoning for proving the existence of regime shifts (Scheffer and Carpenter 2003). Models can help untangle the individual mechanisms that lead to overall system changes, and by using the patterns extrapolated from experimental data, they can be parameterized, which increases their robustness and credibility (Scheller 2010). Modelling ecological interactions dates back to the pioneering efforts of Lotka and Volterra in the 1920s, who mathematically modelled predator-prey dynamics (Lotka 1924). Modelling has advanced in recent years with technological improvements, widening accessibility from not only mathematicians but to ecologists, managers and policy makers (McCallum 2008; Chatzinikolaou 2012). An increase in the use of models for management of high profile socio-ecological systems such as fisheries, dams and national parks means continued review of their uses and limitations is vital (Plagányi 2007).

The previous three chapters have each discussed the findings of four experiments, all designed to quantify the interactions involved in the proposed shift between two stable regimes; which have been termed 'floating invasive plant dominance' and 'submerged invasive plant dominance'. The proposed shift hypothesizes that as the floating invasive plants decompose due to damage from biological control agents, nutrients, light and space become available and submerged plants successfully capitalize on this new abundance of

resources and proliferate (Reddy *et al.* 1982; Hammer and Knight 1992; Shilla *et al.* 2006; Chimney and Pietro 2006; James *et al.* 2006; Longhi *et al.* 2008). However, the relative paucity of native submerged plant species, as a result of few natural freshwater systems in the South African landscape, combined with high levels of external nutrient loading means that invasive submerged plants are more likely to establish and outcompete native ones (Fig. 5.1). In other words, the system has two basins of attraction, one dominated by floating invasive plants and the other by submerged invasive plants - where biological control induces the regime shift.

The process of building a model starts with the development of a visual diagram that allows the structure, and the key interactions involved in the model, to be visually interpreted. One such method is the development of causal loop diagrams, these are used to display cause-effect interactions between key system variables, the strength of which is depicted by the thickness of the arrow connecting the variables. Variables positively related are denoted with a plus sign (+), and a negative cause-effect is denoted with a minus sign (-) (Chang *et al.* 2008). Sendzimir *et al.* (2007) use causal loop diagrams in the first step of modeling biocomplexity of the Tisza River basin, and conclude that they are an integral step in the process of refining conceptual models for exploring the relative effects of different interactions on ecosystem processes. Causal loop diagrams can also be used to communicate complex theories in a graphical language that is understood by interested parties from a diverse range of perspectives such as mathematicians, ecologists, stakeholders and policy makers (Sendzimir *et al.* 2007).

The initial hypothesis of the proposed regime shift in this thesis was graphically interpreted into a causal loop diagram in the Ventana Simulation Environment (Vensim® PLE version 6.3, Ventana systems Inc.) (Fig. 5.1). The diagram postulates that as the population of biological control agents increases, there is a strong negative effect on the population of

invasive floating plants. As the biomass is reduced through damage, there is an increase in nutrients in the water column, which is re-enforced by external nutrient loading, this has a positive effect on the populations of submerged plants. Invasive submerged plants are able to reduce the nutrients in the water column, and their superior competitive ability means they also reduce submerged native plants populations.

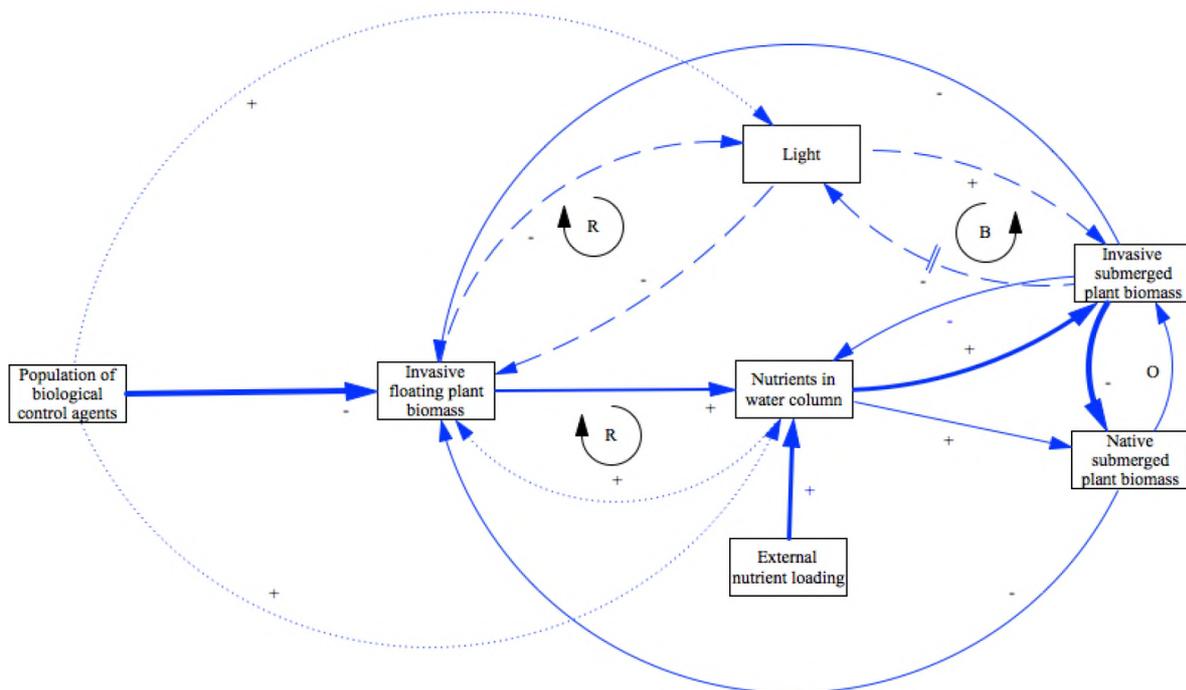


Fig. 5.1 Causal relationships between key factors and processes of the regime shift, and the internal feedback mechanisms that reinforce (R) and balance (B) the system. Created in Vensim PLE (ver. 6.3).

Floating invasive plant dominance is a well-documented stable state occurring in numerous systems world-wide, a seminal paper published in 2003 features a mathematical model of floating plant dominance as a stable state (Scheffer *et al.* 2003). The model, although contextually broad, explores the asymmetry between floating and submerged macrophytes with regards to their competition for resources; submerged plants are able to access nutrients in the sediment not available to the floating plants but they are less able to compete for light.

In this study, this model is used in combination with field observations, experimental data and data obtained from the wider literature. The combined evidence approach offers a strong argument for the existence of alternative attractors determined by competition between floating and submerged plants. Scheffer *et al.* (2003) conclude that nutrient loading reduces the resilience of the regime characterized by submerged rooted plant dominance. As levels of nutrients in the system surpass the level at which submerged plants can utilise them, floating plants are able to establish and as they can better compete for light, they become dominant in the system. This model is not specific to invasive plants; either submerged or floating. Thus, the aim of this chapter was to create a mathematical model that is more applicable to the context of South African invasive plant management. The first step was to create a mechanistic diagram of the model as a whole that conveys the dynamic interactions within the proposed regime shift, completed in the form of a causal loop diagram (Fig. 5.1). Subsequently, equations were built to form the base of the model. These were informed using the mechanisms observed in the previous experiments that explored the interactions of the regime shifts by studying the dynamics of a floating invasive (*Pistia stratiotes* (L.) Araceae), submerged invasive (*Egeria densa* (Planch) Hydrocharitaceae) and submerged native (*Lagarosiphon major* (Ridl.) Moss. Hydrocharitaceae) species. Results of the experiments were then used to parameterize the model as fully as possible, with support from the wider literature. The final model can then be used to see whether the individual interactions observed experimentally, when combined, produce support for the hypothesized regime shift. The intention is to bring together the theoretical, holistic aspects of regime shift research with the applied elements of biological control research and to better explain the mechanisms underpinning the proposed regime shift.

5.2 Methods

5.2.1 Model building

The results of the experiments discussed in the previous chapters of this thesis showed that light was not a significant driver of the proposed regime shift, and was therefore removed from the model. In addition, *L. major* exhibited high plant mortality regardless of the presence of *E. densa* or biological control agents on *P. stratiotes*, resulting in a lack of comparable data between treatments therefore even qualitative trends could not be reliably used to parameterise the model. This meant that the native submerged plant population variable needed to also be removed from the model, and a new, more streamlined model was developed to best describe the interactions and results quantified in Chapters Two to Four. This was graphically interpreted by developing a secondary causal loop diagram in the Ventana Simulation Environment (Vensim[®] PLE version 6.3, Ventana systems Inc.).

The new streamlined model, combined with the observations in the previous chapters, were used to inform and build a mathematical model to explore the implications of biological control on the interaction between invasive floating plants, invasive submerged plants and the availability of nutrients. The model consisted of three equations: explaining temporal population dynamics for densities of floating invasive and submerged invasive plants and temporal changes in the level of available nutrients.

5.2.2 STELLA model overview

The equations describing the regime shift were then put into a dynamic model created within the STELLA[®] Professional software environment (iSEE systems Inc., Version 1.0.3) (Fig. 5.2). STELLA models use stocks, flows and converters to produce time-series simulations. The model outputs trace the temporal changes in populations (stocks), as effected by the external

pressures (converters), according to the equations which are used to describe their interactions (flows).

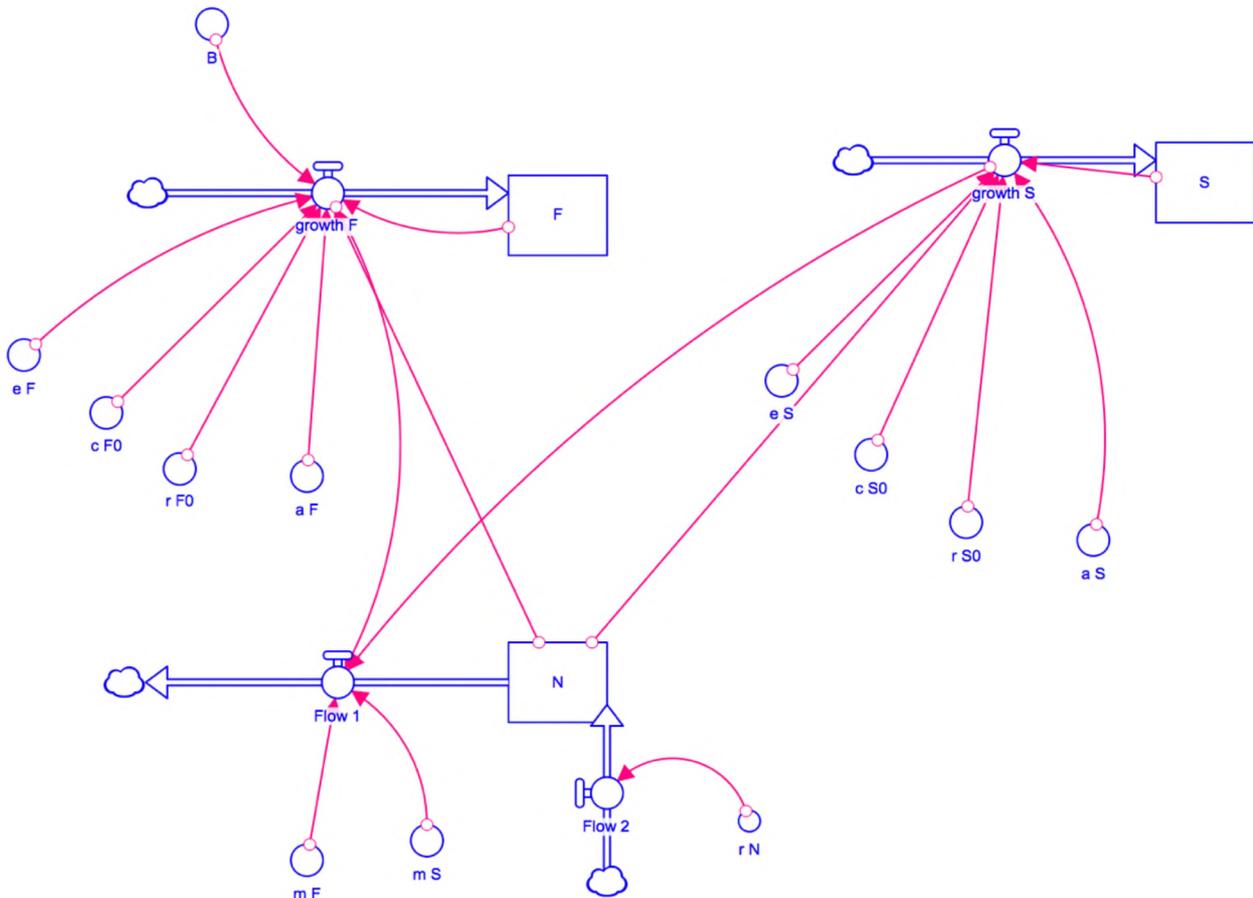


Fig. 5.2 Map diagram of the STELLA model incorporating the interactions between stock densities of floating invasive plant populations (F), submerged invasive plant populations (S) and temporal changes in nutrient availability (N).

5.2.3 Model parameters

Values were assigned to each stock density (F, S and N), which provides the initial populations, and to each converter. The flows contain only the equations that model interactions and therefore have no numeric values. The parameters for the model (Table 5.1) were estimated using a combination of the trends observed in the previous chapters and from the literature (Scheffer *et al.* 2003). Each plant population began with the same initial density (1). For the purposes of the model, mortality rates were assumed to also be equal (1) as there

are no specific data indicating otherwise. The floating plants were given a higher value for intraspecific competition (1) compared to the submerged plants (0.5), as reflected in the results of the previous experiments where healthy *P. stratiotes* remained dominant. The submerged plants were assigned a higher value for conversion of nutrients to biomass (0.1) compared to the floating plants (0.01), imitating their ability to access nutrients in both the sediment and water column. This was supported by the previous experiments where *E. densa* exhibited nutrient driven growth rates when nutrients were added to the sediment (Chapter Two) and the water column (Chapters Three and Four), as well as the literature based evidence (Chen and Barko 1988; Barko *et al.* 1988; Rattray *et al.* 1991; Mazzeo *et al.* 2003). The rate of nutrient uptake was set to be higher for floating plants (1) than submerged (0.5) as floating plants are better competitors for light, which increases plant growth and affects the size of roots, providing more surface area for nutrient uptake.

Table 5.1 Summary of model parameter estimations, their definitions and units where applicable.

Parameter	Description	Value	Unit
F	Density of floating invasive plant	1	g/m^2
S	Density of submerged invasive plant	1	g/m^2
N	Available nutrients	See fig. 5.4	mg/m^2
B	Density of biological control agents	See fig. 5.4	1/day
e_F	Relationship of nutrients and competition (floating plants)	1	m^2/mg
e_S	Relationship of nutrients and competition (submerged plants)	1	m^2/mg
c_F	Intraspecific competition (floating plants)	1	$\text{m}^2/(\text{g per day})$
c_S	Intraspecific competition (submerged plants)	0.5	$\text{m}^2/(\text{g per day})$
r_F	Natural mortality rate (floating plant)	1	1/day
r_S	Natural mortality rate (submerged plant)	1	1/day
a_F	Rate of nutrient uptake (floating plants)	1	$\text{m}^2/(\text{mg per day})$
a_S	Rate of nutrient uptake (submerged plants)	0.5	$\text{m}^2/(\text{mg per day})$
m_F	Conversion of nutrients to biomass (floating plant)	0.01	-
m_S	Conversion of nutrients to biomass (submerged plant)	0.1	-

5.3 Results

The more stream-lined model (Fig. 5.3) was formed based on observations and results from the experiments discussed in previous chapters. As the population of biological control agent's increases there is a negative effect on the population of invasive floating plants, which has a positive effect on the level of nutrients available in the water column. As an increase in nutrients also helps growth of the floating plants there is a positive feedback mechanism that helps to retain this system, indicating high resilience. As nutrients in the system become more available, there is a positive effect on the submerged invasive plant population, that remove nutrients from the water column limiting availability to the floating invasive plants which increases invasive submerged plant population resilience.

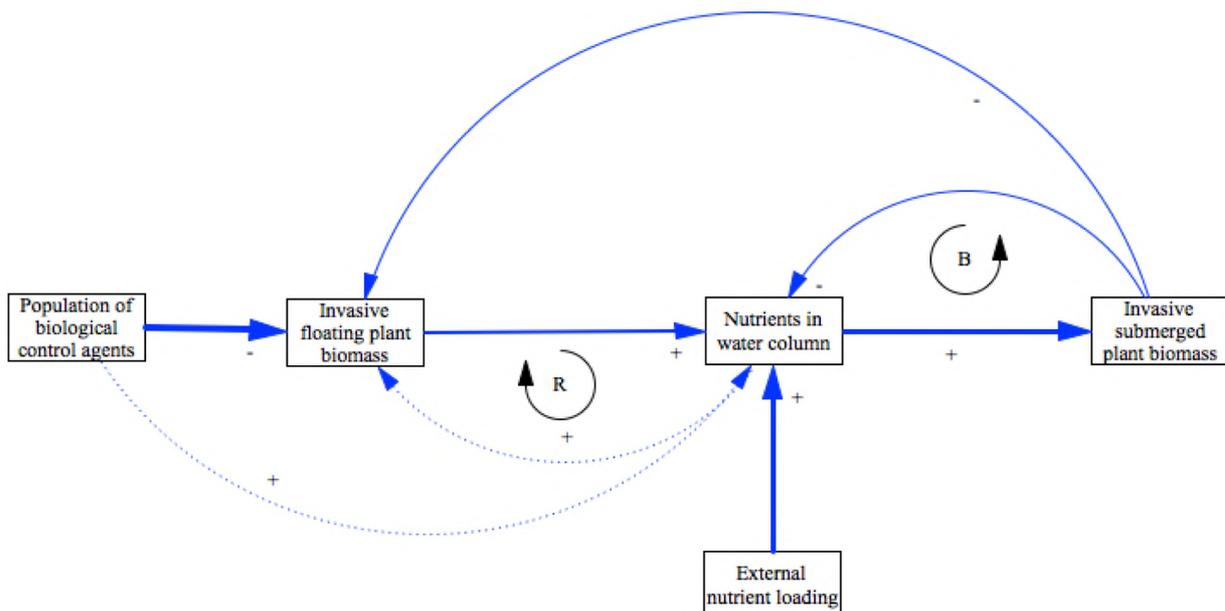


Fig. 5.3 Revised model of regime shift based on the interactions of the experiments supported by viable results. Causal relationships between key factors and processes of the regime shift, and the internal feedback mechanisms that reinforce (R) and balance (B) the system. Created in Vensim PLE (ver. 6.3). The strength of interactions is depicted by the thickness of the arrows connecting the variables that are positively related (+) or negatively related (-).

The following equations describe the interactions between the key variables of the regime shift:

$$\frac{dF}{dt} = -r_F F + a_F N F - c_F(N) F^2 - B F$$

$$\frac{dS}{dt} = -r_S S + a_S N S - c_S(N) S^2$$

where the changes over time (t) of the biomass of invasive floating plants (F) and invasive submerged plants (S) are modelled as a function of their mortality rates, r_F and r_S , modified by the rates of nutrient uptake, a_F and a_S , and their intra-specific competitive ability, c_F and c_S . The effect of biological control, B , was incorporated into the model for floating plants. The available nutrients in the water column, N , are assumed to change according to floating and submerged plant biomass dynamics:

$$\frac{dN}{dt} = -m_F \frac{dF}{dt} - m_S \frac{dS}{dt}$$

where m is the conversion of nutrients to plant biomass which changes over time.

The competition coefficients, c_F and c_S , are modelled using the following equations:

$$c_F(N) = c_{0F} \exp(-e_F N)$$

$$c_S(N) = c_{0S} \exp(-e_S N)$$

where intraspecific competition changes according to available nutrients in the water column and e represents the slope of this relationship.

Model development and predictions support the proposed regime shift between floating invasive and submerged invasive plant dominance under certain conditions of biological control (Fig. 5.4). The simulation outputs show temporal changes in the densities of floating and submerged plant populations, with increased densities indicating plant dominance, as well as changes in the levels of nutrients within the water column.

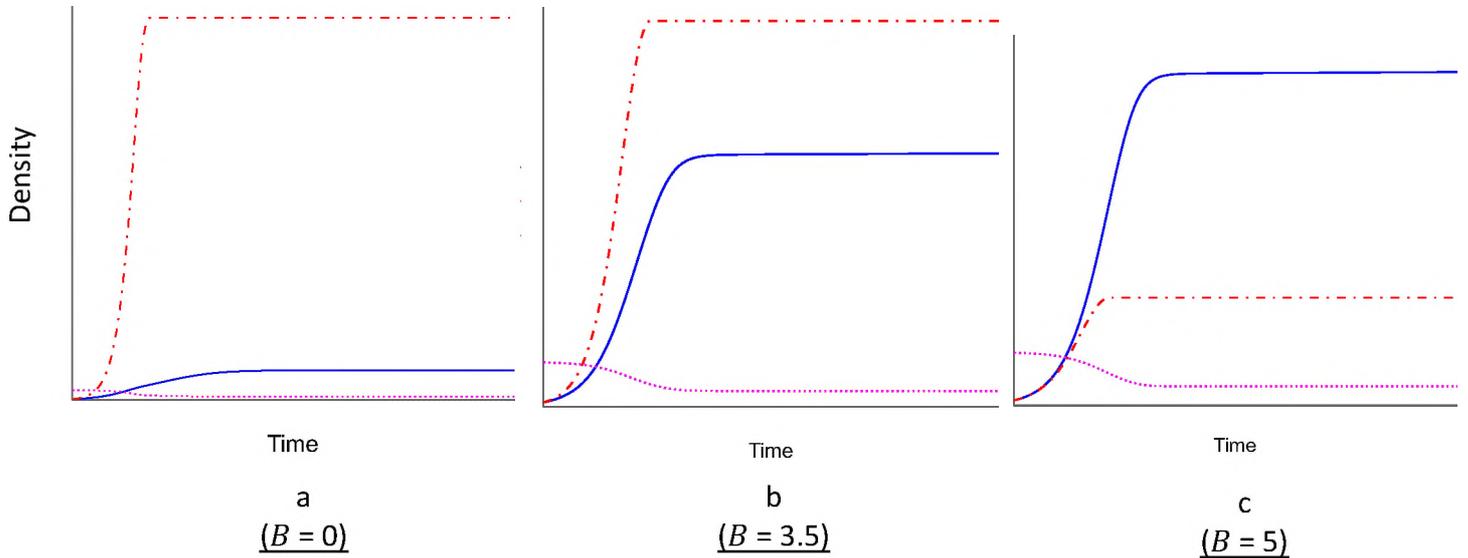
For the first series of simulations, the initial starting level of nutrients was set to a high value (10) (Fig. 5.4 a-c). The first output from this simulation (Fig. 5.4 a), where the initial density of biological control agents of the floating plants is set to zero, shows a sharp initial increase followed by a plateau in population density of the floating plants. Whilst the submerged plants also initially increase in density, the growth is slower and caps a short while after the point in which the floating plant population peaks. This indicates the floating plants become dominant and are able to maintain dominance in the system. The second output of this simulation (Fig. 5.4 b), where nutrients remain high but the biological control population density is increased (to 3.5), reveals the same overall trend of floating plant dominance but the relationship between the two plant densities has changed. There is a longer initial period of increased nutrients in the system (a product of the biological control of the floating plants and subsequent senescent plant material), and the submerged plant population demonstrates a much sharper increase before levelling off. The final output of this simulation (Fig. 5.4 c) shows a complete switch between the plant densities; as the biological control slows the growth of the floating populations and increases the levels of nutrients in the system, there is an initial phase of co-existence between the plant populations. Eventually a point is reached where the floating plants are completely limited by the biological control, and the submerged plant population is able to grow to a point that surpasses the floating plant densities and maintain dominance.

The second series of model simulations (Fig. 5.4 d-f) shows outputs for systems where the initial level of nutrients in the system is set to a lower value (5). The three outputs are once again set to a sequential increase in the initial population density of biological control agents on the floating plants. The results show the same overall relationship between the floating and submerged plant populations, with a switch in dominance once a critical level of biological control agents has been crossed. However, there are some differences in the interactions. Firstly, in the reduced nutrient setting of these simulations there are reduced gradients of slopes for both plant population densities. Secondly, the levels of biological control agents required to induce the shift are lower (3.5) than those required in the higher nutrient setting (5). Lastly, there is a reduced disparity between the two plant densities when the biological control agent densities are at the lowest (0) and highest setting (3.5) compared to the high nutrients.

Population density of biological control agents



HIGH N ($N = 10$)



LOW N ($N = 5$)

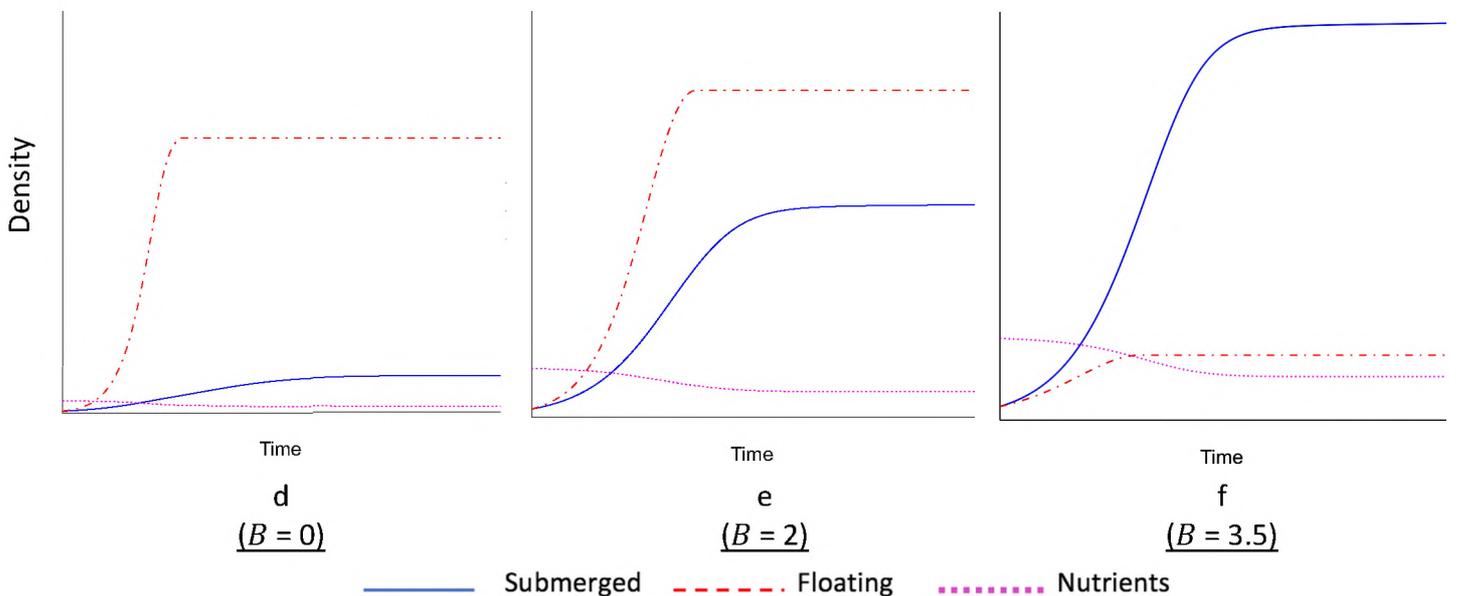


Fig. 5.4 Stella simulation outputs showing temporal changes in floating (F) and submerged (S) plant densities and nutrient (N) levels in scenarios with (a-c) high initial nutrients and (d-f) low initial nutrients. Within each nutrient condition, the populations of biocontrol agents (B) were altered, as indicated beneath each graph. Scales are not fixed.

5.4. Discussion

The model presented in this chapter, estimates the effects that current species-level management of floating invasive plants have upon wider community-level interactions, for the first time, in a South African context. The model supports the original hypothesis that the switch between the two stable states of floating invasive and submerged invasive plant dominance can be driven by the biological control of the floating plants. The model also shows that the top-down effects (that affect ecosystems on a wider scale than the intended control agent and target plant level), can be significantly altered by bottom-up changes to the system (nutrient loading). Gross *et al.* (2005) aimed to use ecological and biological factors at different trophic levels to create a formula estimating the probability of biological control success against insect pests. They conclude that consideration of lower trophic level variables can help predict outcomes of biological control.

The level of biological control required to induce the shift was reduced in systems where initial nutrient loading was lower. This is supported by evidence in the wider literature where success in biological control programmes increases with reduced nutrient loading; both Heard and Winterton (2000) and Coetzee and Hill (2012) found reducing nutrient loading significantly increased success of the biological control of water hyacinth (*E. crassipes*). This occurs because when less nutrients are available, macrophytes are not able to recover as quickly from herbivory damage (McNaughton *et al.* 1983), therefore plant mortality can be achieved at a lower density of biological control agents.

Originally, the model incorporated a positive relationship between increased light availability and the growth of submerged plants, acting as a key driver of the regime shift. Ecologically this makes sense, as light is an important factor for the growth of submerged

aquatic plants (Duarte 1995). However, in the experiments discussed thus far, there were no significant quantifiable interactions between light and the growth or biomass of *E. densa*. Tavecchio *et al.* (2003) reported similar findings, where growth of *E. densa* had a low light requirement, and is supported in the wider literature where growth of *E. densa* has been documented in turbid waters and studies have also shown a high competitive ability under a range of light regimes indicating that light is not a limiting factor (Wells and Clayton 1991; Bini and Thomaz, 2005; Yarrow *et al.* 2009). Therefore, the removal of the light variable from the model was justified. A further modification was made by removing the native submerged plant variable, because *L. major*, the species representing the population of submerged native plants, fared poorly in all treatments and provided few comparative responses to changes in other variables of the regime shift. Inclusion of this variable, based on the experimental data would therefore have reduced the accuracy of the overall model as it was not possible to determine which factors were driving native plant mortality.

The remaining key drivers of the regime shift between floating and submerged invasive macrophytes were nutrient availability and the population density of biological control agents on the floating plants. Nutrient loading as a driver of invasive plant establishment and growth was supported by the results discussed in Chapters Two to Four and is a well documented ecological occurrence (Davis *et al.* 2000; Daehler 2003). Damage caused to *P. stratiotes*, the species representing the floating invasive plant population, by its co-evolved biological control agent, *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae), were demonstrated in Chapters Three and Four, as well as in the wider literature regarding *P. stratiotes* control (Cilliers 1991c; Cilliers *et al.* 1996; Diop *et al.* 2010)

Mathematical models of ecological systems cannot always incorporate the full scope of natural processes, and compromises are often made regarding spatial or temporal

elements, but they are still very useful for ecosystem management and can guide future research (Bulling *et al.* 2006; Chatzinikolaou 2012). At this stage, the model in this chapter is qualitative, with the parameters modelling relative changes between key variables resulting in alternate attractors. However, further experiments could provide quantitative data for parameters such as species-specific rates of nutrient uptake, mortality and damage from biological control agents. This would open up the possibility to tailor the model in such a way that might benefit management of South African systems degraded by floating invasive macrophytes and allow the model to be used in a predictive capacity, potentially applicable at an individual ecosystem level.

Neither experimental evidence or mathematical models alone can prove or disprove the existence of regime shifts in natural systems. However, when combined as a multi-faceted study they provide substantial evidence for the regime shift. They demonstrate the potentially far wider-reaching impacts that biological control of floating plants can have on ecosystem species assemblage, and highlight the importance of a holistic approach to biological control research.

Chapter 6

General discussion

The findings presented in this thesis support the hypothesis of the proposed regime shift between floating invasive and submerged invasive plant dominance instigated by the biological control of floating plants, and driven by nutrient loading. Regime shifts are not a new concept, and have been applied to a vast array of processes affecting human populations; from stock market crashes to climate change, and within scales ranging from individual cells to whole planets (Scheffer 2009). Ecologically, such shifts can have devastating consequences to biodiversity and the provision of ecosystem services that human populations depend upon, yet acceptance of such shifts within both scientific and wider communities has often faced challenges, which can hinder their application to ecological management. One such example is bush encroachment, which negatively impacts southern African agricultural sectors and ecosystems. Savanna landscapes that have been historically grazed by wild herbivores or nomadic pastoralists are increasingly used by commercial cattle grazing, with often higher and more fixed levels of stock, and either intentional or unintentional fire suppression. The result can be a reduction in grass competition and a rapid encroachment of woody plant cover with high numbers of shrubs and trees. This transition is not easily reversed to grazing-suitable grasslands and results in altered fire regimes (Walker 1993; Scheffer *et al.* 2001; Scholes 2003; Rocha *et al.* 2015). Not only is the agricultural sector affected economically through reduced cattle productivity (Scholes 2003), but changes in landscape can also bring changes to local species composition, biodiversity and cultural landscape uses (Foley *et al.* 2005).

Being able to prove the existence, and perhaps predict the occurrence, of such shifts would clearly be highly beneficial to ecosystems and society alike. However, there is a missing link, which exists between ecologists researching regime shifts and those able to implement findings into policy and management: tangible evidence. Reviews of marine regime shift research report that such barriers even exist within the scientific community, not only between those who accept and reject regime shift theories, but between those who think the shifts are driven by top-down (over fishing) or bottom-up (climate change) pressures (De Young *et al.* 2008; Conversi *et al.* 2015).

Much of the evidence for large scale shifts, such as those that occur in marine environments or savanna landscapes, is presented in the form of ecological models (Noy-Meir *et al.* 1982; Anderies *et al.* 2002; De Young *et al.* 2008; D’Odorico *et al.* 2012). Whilst there have been some controlled field experiments, they are limited by spatial and temporal logistics, particularly because shifts can occur over varying timescales of multiple decades, and across vast landscapes (Scheffer *et al.* 2001; Folke *et al.* 2004; www.regimeshiftdatabase.org). Regarding bush encroachment, it has been reported that despite extensive research into the drivers of the regime shift, and solid evidence of commercial losses, translation of the findings into practical management aimed at avoiding such shifts is a rare occurrence (Rocha *et al.* 2015). The difficulties of translating regime shift research into management practices arise when attempting to link abstract mathematical theory with real-life situations and observations in the field. This is particularly an issue when much of the evidence is presented in the form of mathematical models. As stated decades ago, “all models are wrong, but some are useful” (Box 1987), there are inherent limitations to ecological models and although the complex, stochastic and vast interactions that maintain

natural systems cannot be accurately imitated, advances in the field over recent years has improved their stability and complexity (Chatzinikolaou 2012).

Within the field of biological control, where concerns over introducing more exotic species to combat current invasive aliens have often caused obstacles for implementation, models are more commonly being used to help tailor biological control strategies in order to maximise success and minimise risk. Jeger and Xu (2015) developed a general model for biological control of flower pathogens that can help determine which combination of biological control mechanisms will be the most effective, depending on target plant physiology. Raghu *et al.* (2007) present a dynamic model designed to help predict both the risks and benefits of introducing a biological control agent (*Charidotis auroguttata* (Boheman) (Coleoptera: Chrysomelidae)) to manage an invasive vine in Australia (*Macfadyena unguis-cati* (L.) (Bignoniaceae)), the aim of which is to assist both scientists and policy makers and help translate information between stakeholders.

As models become more accessible to non-mathematicians (i.e. ecologists and policy makers) through more user-friendly interfaces, their role in applied decision making will inevitably become more prominent (Plagányi 2007; McCallum 2008; Chatzinikolaou 2012). Garcia-Llorente *et al.* (2008) suggest that different stakeholder groups can hold very different perceptions regarding the impacts of invasive aquatic plant species, highlighting the importance of developing effective tools of communication that addresses an audience beyond researchers. Rightfully, there remain reservations regarding the application of modelling outputs to real life scenarios, and this is where the importance of controlled experiments that can describe the finer mechanisms of systems and increase model validity lie.

A review of the direct evidence for alternate stable states (Schröder *et al.* 2005) found an overall bias towards laboratory experiments compared to field experiments when attempting to demonstrate a system's capacity for bi-stability (i.e. when a system exhibits stability in more than one state), which can be partially explained by the logistical difficulties associated with field experiments. Schröder *et al.* (2005) conclude that future research in the field should primarily focus on the specific mechanisms behind switches in ecological states. Manipulation experiments may be bound by spatial and temporal constraints, but small-scale experiments can be crucial to help explain large-scale patterns, and can be a powerful way to show that a system has alternate attractors (Peterson 1984; Scheffer and Carpenter 2003). Although Schröder *et al.* (2005) report a bias towards laboratory experiments, there are still relatively low numbers of them, and they have been labelled as an over-looked asset in the exploration of regime shifts (Chase 2003; Anderson *et al.* 2009).

Regime shifts regarding changes in vegetation dominance within shallow lakes have been well studied, and are generally accepted within the scientific community. A seminal paper published by Scheffer *et al.* (2003) combines field observations, controlled experiments and a mathematical model to explain the shift. The field observations utilise a large historical data set of vegetation composition from 641 drainage ditches. They found negative correlations between floating plant cover and submerged plant abundance, and positive correlations between floating plant cover and nutrient levels in the water column. These observations fed the construction of a mathematical model to explain the shift. A key component of the model is light availability, where critical thresholds of light determine whether submerged macrophytes or algae dominate the system. However, in 2003, an experiment was carried out to test the effects of nutrient loading on plant species assemblages within mesocosms, monitoring changes in densities of submerged *Vallisneria*

americana Mich. (Hydrocharitaceae) (Morris *et al.* 2003). They report that subsequent to heavy nutrient loading, after four months there was a total loss of *V. americana* in mesocosms that became fully covered by a floating macrophyte, *Azolla pinnata* (R.Br.) (Azollaceae). Although the results support the model of bi-stability depending on submerged or floating plant dominance, Scheffer *et al.*'s (2003) model featured light as the key driving variable determining plant assemblage, although Morris *et al.* (2003) suggest their observed switch was predominantly driven by changes in dissolved oxygen concentrations and resulting anoxic conditions. Whilst these variables are associated with light availability, they conclude it is the indirect effects of light limitation that leads to submerged plant loss. As with *Egeria densa* (Planch.) (Hydrocharitaceae) in the experiments presented in this thesis, Morris *et al.* (2003) report that the submerged plant species of their study (*V. americana*) has been known to exhibit high tolerance for low light levels, supporting the conclusion that it is not the direct effect of light fluctuations altering the submerged plant community. Thus, the experiments presented by Morris *et al.* (2003) are able to better explain the mechanisms underpinning the original model and demonstrate the importance of incorporating species and system specific parameters into models for more valid application to real life scenarios. Controlled experiments can bridge some of the gaps in models that are based on generalisations or assumptions.

Clearly modelling and experimental methods have advantages as well as disadvantages, but both carry merit in regime shift research. Although bound by different constraints, the combined approach of this thesis, employing both experimental and modelling techniques, lays the initial foundations for a solid evidence-based exploration into the proposed regime shift. The shift between floating invasive and submerged invasive plant dominance is defined by changes to species assemblage across different habitats within the

system, as well as different trophic levels: the plants (producers) and control agents (consumers). It has been claimed that there is a paucity in multi-trophic experiments within the field of invasive plant research (Harvey et al. 2010; Schultz and Dibble 2012), yet they are essential for understanding internal ecosystem processes and thus for guiding management.

The system used to explore the proposed regime shift at the centre of this thesis consisted of two submerged Hydrocharitaceae species, the native *Lagarosiphon major* (Ridl.) Moss and the invasive *E. densa*, and the floating invasive species, *Pistia stratiotes* (L.) (Araceae). The system was either subjected to herbivory to *P. stratiotes* by the biological control agent *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae), or not, and also to varying regimes of nutrient loading. Although the system chosen to model the regime shift is relatively simple, consisting of two species types and two external pressures, in a review of evidence for alternate stable states (Schröder et al. 2005), increased complexity of systems did not correlate with rates of success in proving bi-stability. The model is dependent on competitive interactions under a range of varying resource availability between the three focus species. Competition experiments have been widely used to compare aquatic plants species occupying the same habitat within systems to elucidate mechanisms of invasions (Van et al. 1999; Hofstra et al. 1999; James et al. 2006; Mony et al. 2006), and it has been found that comparing native and invasive species closely related to each other maximises the validity of the findings (Vilà and Weiner 2004). Competition experiments have also been used to explore interactions between macrophytes occupying different habitats within a shared system in order to define the mechanisms of regime shifts. For example, Netten et al. (2010) assess the effect of temperature and nutrients on the competition between floating *Salvinia natans* (L.) (Salviniaceae) and submerged *Elodea nuttallii* Planch. St. John (Hydrocharitaceae),

with the aim of exploring the mechanisms of floating invasive plant dominance driven by global warming.

The first experiment in this study, exploring the effect of nutrients on the competition between the two Hydrocharitaceae species, *E. densa* and *L. major*, provided the baseline information for the competitive ability of invasive *E. densa*. *Egeria densa* was competitively superior to *L. major*, a relationship that was amplified under heavy nutrient loading. This adheres to other reports of increased competitive ability of submerged plants in increased nutrient loading; *Myriophyllum aquaticum* Vell. (Verdc.) (Haloragaceae) was found to only become problematic under heavy nutrient loading (Wersal and Madsen 2011), and Kennedy *et al.* (2009) report a 2.8 times increase in biomass of *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) under high aquatic concentrations of nitrates compared to low.

Quantifying the competitive relationship between the native and invasive submerged species is vital for understanding the core interactions occurring within that layer of the model. The results presented in this thesis not only revealed the competitive advantage of *E. densa*, but also the strength of the effect of nutrient loading within the system. As the model crosses habitat boundaries, the next building block required was to understand how *L. major* and *E. densa* responded, individually, to the presence of another plant occupying a different habitat within the same system: floating *P. stratiotes*.

A major review of regime shift research identified that shifts are more likely to occur when anthropogenic pressures have reduced resilience by actions such as removing whole functional groups of species (Folke *et al.* 2004). The biological control programmes targeting floating invasive species in South Africa aim to do exactly that; remove a functional species. Even though the 'function' may not be a desired one, the ability of species such as *Salvinia molesta* D.S. Mitchell (Salviniaceae), water hyacinth (*Eichhornia crassipes* (Mart.) Solms.

(Pontederiaceae)), water lettuce (*Pistia stratiotes* L. (Araceae)), and red water fern (*Azolla filiculoides* Lam. (Azollaceae)) to dominate and alter their environments indicates that just as their presence has ecological impacts (Hill 2003; Midgley *et al.* 2006; Tellez *et al.* 2008), so too will their removal.

The results from the mesocosm experiments comparing the growth of each submerged species separately, in scenarios where *P. stratiotes* is able to thrive (not subjected to control) and is partially removed from the system (subjected to biological control from *N. affinis*), found that in instances of controlled *P. stratiotes*, *E. densa* was able to capitalise on the newly available resources, and exhibited significantly increased growth rates compared to plants growing in the presence of non-controlled *P. stratiotes*. The findings provide quantifiable evidence not only of the effect controlling floating species can have on submerged plant communities, but also in support of the proposed regime shift occurring in mesocosms where *P. stratiotes* was subject to biological control. In addition, when *E. densa* populations started at the lowest planting density of 10% (compared to 90% *P. stratiotes* cover), the largest increase in plant growth (relative growth rate) and production of plants with highest quality shoots (inferred from C:N ratios) was demonstrated. These results, combined with the knowledge that *E. densa* autofragments and reproduces vegetatively (Cabrera Walsh *et al.* 2012), suggest that immediately post-establishment, this species has the ability to very quickly become problematic. Ideally, future management should therefore be aimed at minimising exposure of systems to *E. densa* by halting its spread across the region. Unfortunately, this is difficult due to naturally high connectivity of many aquatic systems, the continued ornamental and aquaria trade (Martin and Coetzee 2011), and accidental spread of plant fragments. For example, machinery employed to clear large stands of *E. densa* in Cape Town was not thoroughly cleaned after usage and it is believed that

fragments caught on the machinery were then responsible for further spread of the plant to multiple sites across the city (*Pers. Comm.* City of Cape Town, Invasive Species Unit). Thus, as the introduction of *E. densa* into systems is already occurring, the next step should be to ensure that post-introduction the chances of successful establishment are as reduced as possible.

As stated by Suding *et al.* (2004), restoration of degraded systems, such as those dominated by floating invasive macrophytes, can be successional but it is not always linear. In a South African context, this means it is important to understand the full implications of current management options for floating plants on ecosystems and what that means for the sustainability and provision of South Africa's freshwater ecosystem services. The results obtained from the suite of experiments in this thesis indicate that current biological control programmes may not restore the degraded systems dominated by invasive floating macrophytes to the system quality required. This problem is amplified by the very nature of the systems; many of which are artificial and therefore the restoration of these systems is rarely to return them to their 'natural' state. Thus, the aim of the biological control initiatives against floating invasive plants is to diminish their populations and restore access to vital ecosystem resources such as potable water. However, the results suggest they may induce a system dominated by submerged invasive plants that reduce water quality and biodiversity.

Funk *et al.* (2008) explored restoration of systems through community reassembly. The authors take the concept of biotic resistance, a community's ability to limit the risk of invasion through competitive pressures (Elton 1958), and put it in the context of restoration. Funk *et al.* (2008) present evidence that selecting native species determined by resource-use traits to occupy vulnerable systems can increase resistance of a community to successful invasion. Indeed, several native aquatic plant species in North America have been found to

suppress the growth of the invasive *H. verticillata* by outcompeting the species for resources, Doyle *et al.* (2007) found that competition from *V. americana* reduced biomass of *H. verticillata* by 30-40% by limiting the spread of the plant into nearby containers compared to those grown with no competition from *V. americana*. Van *et al.* (1999) report that dominance from *H. verticillata* to *V. americana* can be induced by creating nutrient-limiting conditions

The degree to which biotic resistance is able to suppress invasive success has been a point of debate. Historically, ecologists have suggested that biotic resistance is such a powerful process that systems with high resistance are able to prevent invasions altogether, concluding that is the reason some habitats remain free of invasive species (Elton 1958; Moulton and Pimm 1983; Kennedy *et al.* 2002). However, whilst a meta analysis of all biotic resistance studies in plant communities agreed that competition played a major role in successful biotic resistance (Levine *et al.* 2004), they failed to find enough evidence to support the theory that it could provide a complete barrier to successful plant invasions, and recognised the influence of other factors such as harsh environmental conditions to explain some systems' lack of invasive species.

Biotic resistance is potentially of great importance to South African invasive plant management, raising the possibility of intentionally managing systems to become less vulnerable to the negative impacts of submerged invasive plant establishment by purposely populating systems with native submerged plant species. All the experiments in this study found that nutrient loading plays a key role in the biotic resistance of the submerged species communities within the model system. The final experiment explored the potential effect of native *L. major* upon the relationship between floating *P. stratiotes* and submerged *E. densa*. In this scenario, and in the initial experiment that compared competitive ability of both submerged species without floating plants, in the absence of nutrient loading, the

competitive advantage of *E. densa* was considerably reduced. The results suggest that a combination of reduced nutrient loading and increasing native submerged plant populations can increase resilience of systems post-biological control of floating macrophytes. Reduction in nutrient loading is also associated with increased control of invasive floating macrophytes, therefore decreasing the resilience of stable states dominated by their presence (Heard and Winterton 2000; Coetzee *et al.* 2007; Coetzee and Hill 2012).

Within all of the experiments, the plants were subjected to two different nutrient regimes. The initial experiment used sediment-based nutrient loading (soil fertiliser), as nutrients in the sediment have been shown to positively affect growth rates of submerged plants (Barko and Smart 1986), and it has been suggested that nutrients in the soil are more important for submerged plant growth compared to nutrients in the water column (Xie *et al.* 2005). However, in all subsequent experiments where *P. stratiotes* was present, nutrients were added to the water column so that availability for resources was open to both the submerged and floating plants. Also, the importance of understanding the relationship between invasive macrophyte growth and fluctuations in nitrogen concentrations of the water column are recognised, especially when the context of the research is regarding systems polluted with dissolved nutrients such as poor sewage treatment and agricultural chemicals (Shardendu-Ambasht 1991; Kennedy *et al.* 2009). This is of particular importance to South Africa as these are the two predominant sources of nutrient loading that occur in the region (Oberholster and Ashton 2008; van Ginkel 2011). The results from the four experiments in this study show a competitive advantage of *E. densa* in high nutrients, regardless of the nutrient source.

Coetzee *et al.* (2011a) report a recent increase in the numbers of observations of invasive submerged macrophytes in South African fresh water systems, such as *H. verticillata*,

E. densa and Eurasian watermilfoil, *Myriophyllum spicatum* L. (Haloragaceae). The same ecological attributes of these systems that have historically been credited with enabling successful floating invasive plant establishment (eutrophic waters and a paucity of native macrophytes), have not since changed and now increase vulnerability to submerged invasive macrophytes. The evidence presented in this thesis reveals that a switch between floating invasive and submerged invasive plant dominance can occur and it is now crucial to identify whether or not the switch is occurring in the field.

Demonstrating the occurrence of a regime shift in the field presents options for future research. The Rhodes University Biological Control Research Group (RUBC) undertakes annual nationwide surveys that document the presence and spread of invasive species in South African freshwater systems, as well as the efficacy of biological control programmes against floating aquatic macrophytes. Using these observations, in conjunction with historical data, the success of the biological control programmes can be assessed. These surveys offer an additional opportunity for detailed and regular assessment of changes in the compositions of submerged and floating plant communities which would provide excellent field data to test the hypothesis of the proposed regime shift in natural settings. This approach has been used to determine changes in plant composition in one South African system, the Vaal River, between 2006 and 2010 (Fordham 2012). A combination of field based sampling and Geographic Information Systems (GIS) remote sensing was used to monitor spatial and temporal fluctuations in plant dominance. The study found that floating *E. crassipes* and submerged *M. spicatum* could not co-exist for extended periods of time, and changes in dominance between the two species was correlated with fluctuations in nitrate concentrations recorded within the water column, and the occurrence of localised flooding.

The use of field surveys has also been used to demonstrate the existence of other regime shifts. For example, Beaugrand (2004) used the monitoring and assessment of species composition and diversity, correlated with climatic data, to offer retrospective evidence of a hydro-climatic driven regime shift occurring in the North Sea between 1982 and 1988 with consequences for abundances of commercially harvested fish and shrimps.

Long-term field-based experiments of invaded South African freshwater systems could also provide valuable data to better parameterise the model developed here: rates of invasive plant and control agents establishment in the field, rates of control of floating plants at certain densities of biological control agents, and the differences within these interactions depending on localised water quality would all be viable areas for further investigation. Long term monitoring of plant community assemblages, along with environmental conditions such as changes in water quality may also present opportunities for developing early warning signals to detect a system's approach to a tipping point before a regime shift occurs. Carpenter *et al.* (2003) test the use of statistical early warning indicators, via monitoring of system perturbation return rates, for the advanced prediction of regime shifts within a lake ecosystem. They predict that prior to a predator-driven regime shift, from planktivorous fish domination to largemouth bass domination, a detectable sequence of non-linear changes to communities will occur. Through statistical analyses of variability, autocorrelation and recovery time, they were able to detect non-linear cascading trophic changes and concluded that such findings can be translated as early warning shift indicators.

If the biological control driven regime shift is found to occur in the field in South African systems, there are several implications for the future of South African freshwater ecosystem management. Reflecting on the past thirty years of research and implementation dedicated to invasive aquatic plant management in this region, control of four of five of the

worst offenders has been relatively straightforward with high success rates (Coetzee *et al.* 2011b). For example, in the 1980s, *S. molesta* was deemed one of the most problematic aquatic weeds in South Africa. The first biological control agent against *S. molesta* was released in 1985 (Cilliers 1991b), and by the late 1990s, control was achieved (Coetzee *et al.* 2011b). In 2008, field surveys by the RUBC concluded that despite successful biological control, *S. molesta* infestations occurred at more sites than anticipated, but the appropriate measures were taken to increase releases of the biological control agent, and by 2011, it was no longer deemed a problematic aquatic weed for South Africa (Coetzee *et al.* 2011b). Although *P. stratiotes* was not considered as problematic as *S. molesta*, a biological control initiative programme began in 1985 with the release of *N. affinis* (Cilliers 1991b). Post-release evaluations in 2008 report high levels of control, with *P. stratiotes* only present in ~7% of surveyed water bodies (Hill and Coetzee 2017).

Perhaps the most successful biological control programme in South Africa to date is the initiative against red water fern (*A. filiculoides*). The weevil *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) was released in 1997, and within just five years, control was labelled sufficient to no longer consider red water fern a problematic species in South Africa (McConnachie *et al.* 2004). By 2008, complete control was evident at every site where the agent was released (Coetzee *et al.* 2011b).

Unfortunately, these success stories on floating weeds do not guarantee that controlling invasive submerged species will be as straightforward. Schmitz and Schartd (2015) claim that all of the “clearly successful” biological control programmes are associated with floating or emergent macrophytes and similar control of submerged plant species has proved more challenging. Looking at other regions of the globe for indications, control of submerged invasive species has presented several barriers to success. *Hydrilla verticillata*, which belongs

to the same family as *E. densa* (Hydrocharitaceae), has established in every continent except Antarctica (Cook and Luond 1982). Between the period of 1980 and 2005, the state of Florida, USA, spent \$174 million on *H. verticillata* control (Schmitz 2007). The first biological control agent was released in the USA in 1988 and over a quarter of a century later, it is still considered the most problematic aquatic plant in the USA (Gu 2006; True-Meadows *et al.* 2016). Control through mechanical and herbicidal methods have also had little success in limiting the threat *H. verticillata* has to potable water supplies, biodiversity and flood risks (Wheeler and Center 2001).

Australia has battled to control the South American native *Cobomba caroliniana* Gray (Cabombaceae), a fast growing species that grows well in slow-moving water bodies and is listed as an Australian Weed of National Significance (Schooler *et al.* 2006). Following rapid spread via vegetative reproduction, infestations of the plant have negatively affected populations of Australia's native species of fish, platypus and water-rats. As with *H. verticillata*, herbicides offer limited control and mechanical removal is costly and made harder by the preference of the species to grow in deep waters (Mackey and Swarbick 1997; Schooler 2008). A search for potential biological control agents began in 2003, and by 2010, just one agent was deemed to be potentially host specific; *Hydrotimetes natans* Kolbe (Coleoptera: Curculionidae). A study of the biology of the agent concluded the weevil has a narrower thermal tolerance range than the target plant, suggesting control to be limited to areas of ideal climate (Cabrera-Walsh *et al.* 2011). Other examples of limited control of submerged invasive species include the programmes against *M. spicatum* in the USA, and *L. major* in New Zealand and Republic of Ireland (Ratray *et al.* 1994; Madsen 2002; Caffery 2008).

The difficulties associated with the control of submerged species is cause for concern to South African invasive plant management. *Hydrilla verticillata* was first recorded from the

Pongolapoort Dam on the Pongola River in KwaZulu-Natal in 2006 (Coetzee 2006). *Cabomba caroliniana* has been previously earmarked as a potential threat to South Africa, and although not recorded in any freshwater systems to date, the species is available through the country's aquarium trade (Martin and Coetzee 2011). Also, the plant thrives in slow moving waters with a wide thermal tolerance range, increasing South Africa's susceptibility (Coetzee *et al.* 2011a; Schooler 2008; Martin and Coetzee 2011).

These examples highlight the difficulties associated with the control of invasive submerged plants that suggest, once established, systems dominated by such species are highly resilient. Scheffer *et al.* (2003) explain that in the absence of floating plants to compete for resources, submerged macrophytes are able to generate positive feedbacks sustaining their stability. Examples of mechanisms include an ability to outcompete algae for nutrients, outcompete phytoplankton for light, provide habitat for algal-grazing zooplankton, stabilise sediments and the production of allelopathic compounds inhibiting growth of algae. Highly resilient systems mean that even if suitable biological control agents can be identified for use in South Africa against submerged invasive species, restoring systems dominated by these plants may be challenging.

A further point of consideration is that South Africa is in the relatively early stages of research into biological control of submerged macrophytes. Reflecting on the progress of biological control research to manage floating invasive plants, sourcing suitable agents and completing full host specificity trials can be a lengthy process. This further supports the claim that if systems where a switch to submerged invasive plant dominance is likely to occur can be identified, a priority for management should be to explore strategies that minimise chances of successful invasive submerged plant establishment. The results presented in this thesis demonstrate the highly competitive capability of *E. densa*. However, they also reveal

the capacity for management techniques, such as bottom-up water quality restoration, to reduce that trait, and these findings can be extrapolated in to the landscape of South African invasive plant management. Continued experimental and observational research, in both controlled laboratory and natural field settings, that consider wider temporal, spatial and trophic scales, can improve resilience of systems. The model presented in Chapter Five allows a wider perspective on the potential interactions between bottom-up and top-down management strategies on the sustainability of vulnerable systems. Further trait-based experiments on the invasive species established and potentially threatening to South Africa will allow improvements and tailoring of the model to guide prioritisation of most at-risk systems. There is a paucity of research into the effects of invasive macrophytes on trophic dynamics, and such studies are essential to define system level processes (Schultz and Dibble 2012). Large-scale multi-system comparisons have been suggested as a useful tool in regime shift research, especially for systems where field experiments are logistically compromised (Conversi *et al.* 2015).

To conclude, this thesis has successfully achieved the original aim under which it was written: to use a multi-platform approach to build the first body of evidence in support of a newly recognised regime shift between floating invasive and submerged invasive plant dominance, as driven by biological control. The results suggest the proposed regime shift can occur, but further field testing is required to establish whether or not it is occurring following successful nationwide biological control. The results also indicate that a reduction in the nutrient loading of South Africa's freshwater systems will reduce negative impacts of submerged invasive macrophytes, and will increase system resilience against future colonisations. Finally, the results encourage further studies into the potential impacts of artificially increasing native vegetation diversity and abundance, with the aim of suppressing

invasive macrophyte success. The evidence presented has the potential to better inform management of South Africa's freshwater systems. It was intended to be the first step of a wider scheme and the results highlight the benefit of continuing multi-trophic considerations for future invasive plant management, as well as opening up a multitude of possibilities for research into the mechanisms of submerged plant invasions and resilience of native macrophyte communities in South Africa, and beyond.

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Appendices

APPENDIX A

Mean (\pm S.E.) recorded levels of Nitrate (NO₃) and Ammonium (NH₄) in the water of each mesocosm containing *Egeria densa* and *Pistia stratiotes*.

10% <i>E. densa</i>	Nutrient and BC treatment							
	Very low		Low		High		Very High	
	+ BC	-BC	+ BC	-BC	+ BC	-BC	+ BC	-BC
Mean (\pm S.E.) NITRATE (mg/l)	2.56 (\pm 0.66)	3.47 (\pm 0.83)	2.61 (\pm 0.49)	1.05 (\pm 0.26)	2.61 (\pm 0.49)	1.05 (\pm 0.26)	2.82 (\pm 0.77)	0.75 (\pm 0.24)
Mean (\pm S.E.) AMMONIUM (mg/l)	0.1 (\pm 0.03)	0.06 (\pm 0.02)	0.10 (\pm 0.04)	0.007 (\pm 0.03)	0.10 (\pm 0.04)	0.007 (\pm 0.03)	0.21 (\pm 0.11)	0.009 (\pm 0.03)

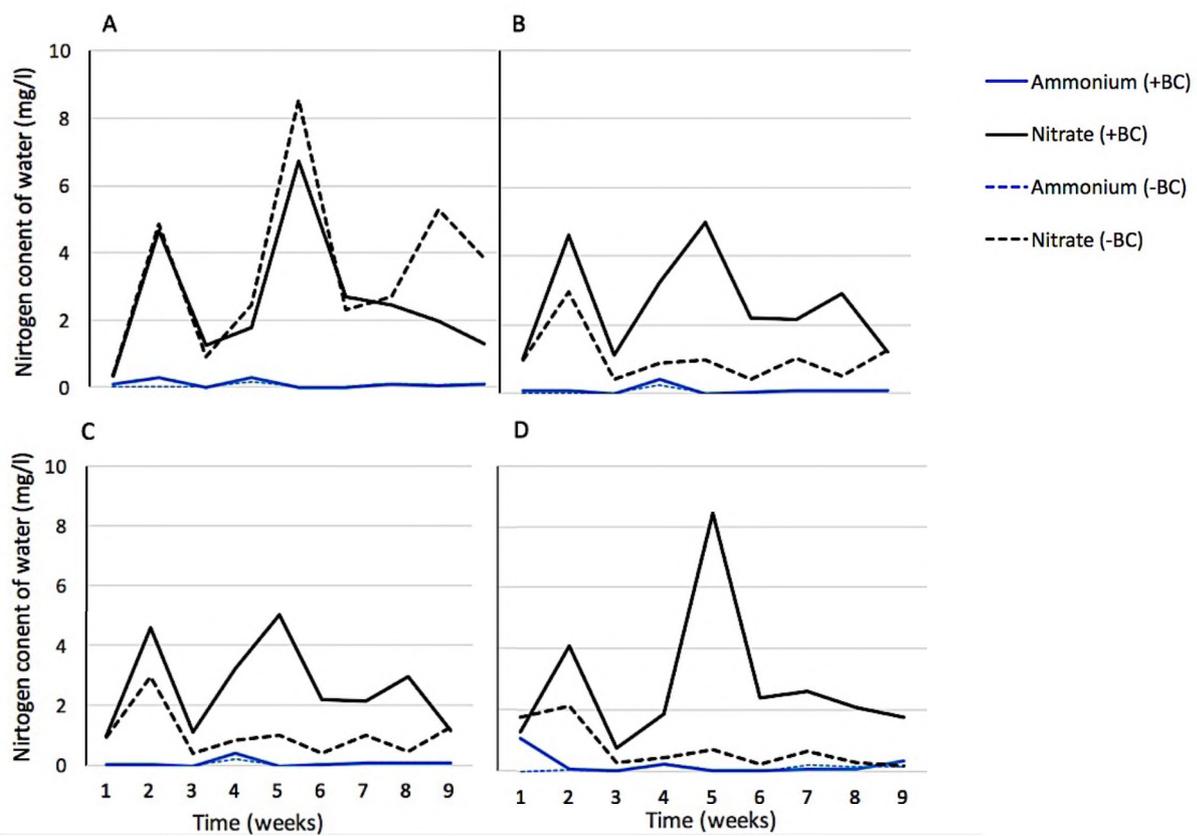
50% <i>E. densa</i>	Nutrient and BC treatment							
	Very low		Low		High		Very High	
	+ BC	-BC	+ BC	-BC	+ BC	-BC	+ BC	-BC
Mean (\pm S.E.) NITRATE (mg/l)	7.02 (\pm 2.12)	1.27 (\pm 0.28)	7.94 (\pm 3.00)	1.37 (\pm 0.28)	5.35 (\pm 1.16)	1.64 (\pm 0.28)	11.64 (\pm 1.86)	5.07 (\pm 1.67)
Mean (\pm S.E.) AMMONIUM (mg/l)	0.14 (\pm 0.07)	0.06 (\pm 0.03)	0.07 (\pm 0.03)	0.06 (\pm 0.02)	0.06 (\pm 0.02)	0.07 (\pm 0.02)	0.38 (\pm 0.09)	0.55 (\pm 0.16)

90% <i>E. densa</i>	Nutrient and BC treatment							
	Very low		Low		High		Very High	
	+ BC	-BC	+ BC	-BC	+ BC	-BC	+ BC	-BC
Mean (\pm S.E.) NITRATE (mg/l)	24.73 (\pm 4.56)	11.95 (\pm 2.38)	27.14 (\pm 5.97)	9.79 (\pm 2.20)	10.96 (\pm 1.93)	14.05 (\pm 1.92)	21.60 (\pm 1.08)	17.70 (\pm 2.30)
Mean (\pm S.E.) AMMONIUM (mg/l)	0.04 (\pm 0.02)	0.20 (\pm 0.13)	0.05 (\pm 0.02)	0.09 (\pm 0.05)	0.06 (\pm 0.02)	0.03 (\pm 0.02)	0.31 (\pm 0.12)	0.32 (\pm 0.11)

APPENDIX B.

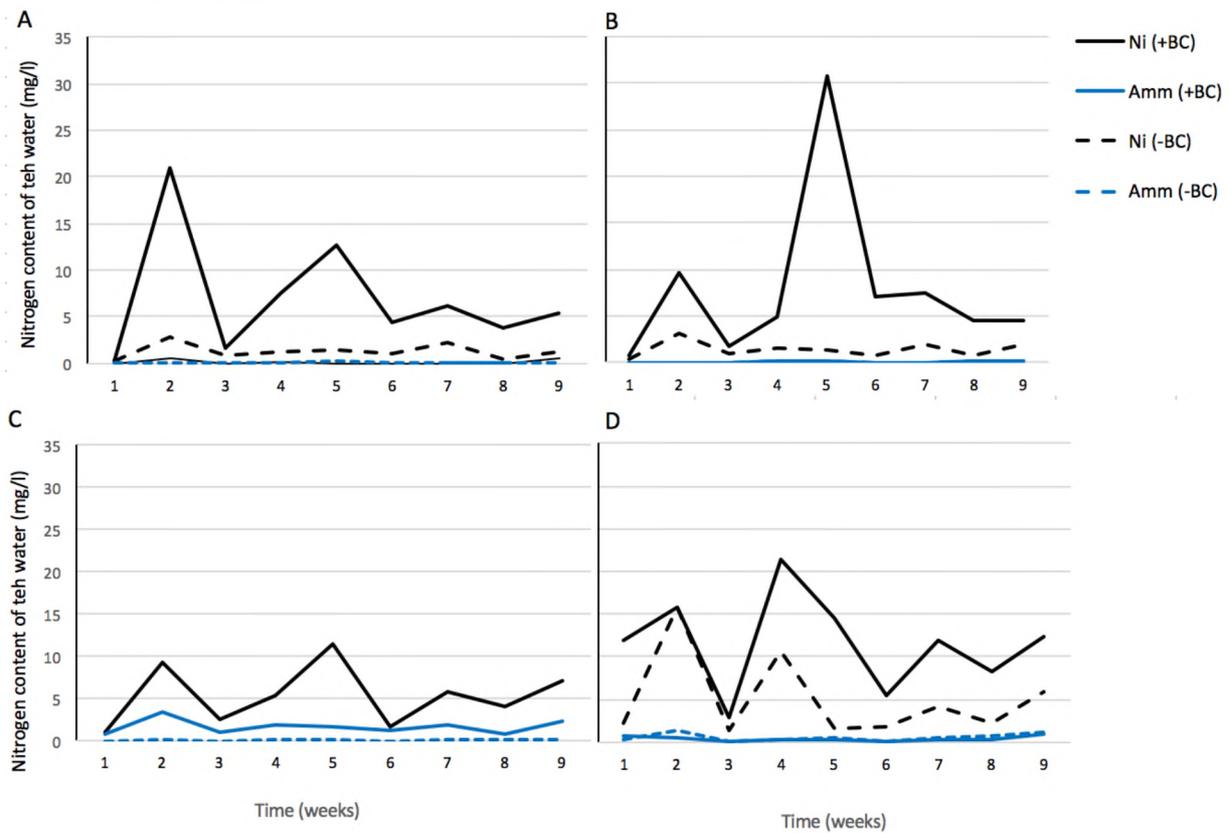
i)

Weekly levels of ammonium and nitrate recorded in mesocosms containing 10% *Egeria densa* and 90% *Pistia stratiotes* under four different initial nutrient treatments (A = 'very low', B = 'low', C = 'high', D = 'very high'), and subjected to presence (+BC) and absence (-BC) of the biological control agent, *Neohydronomus affinis*, on *P. stratiotes*.



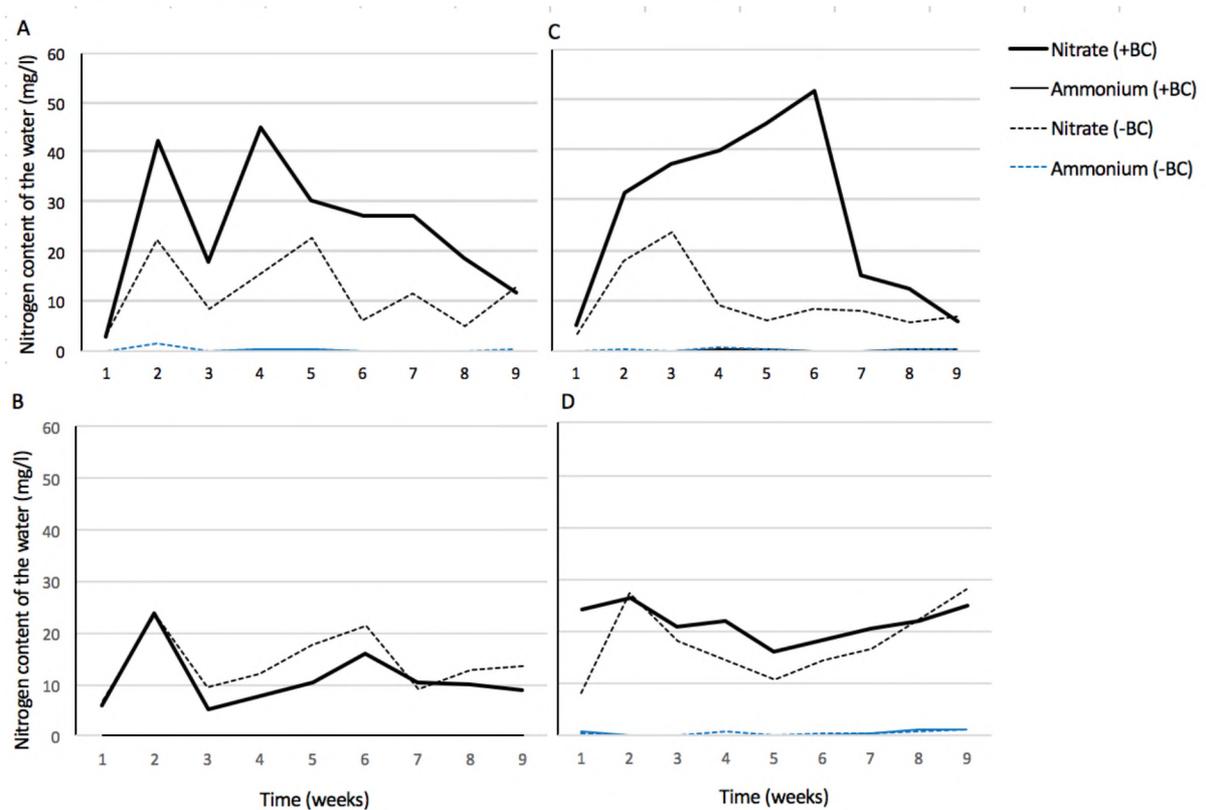
ii)

Weekly levels of ammonium (Amm) and nitrate (Ni) recorded in mesocosms containing 50% *Egeria densa* and 50% *Pistia stratiotes* under four different initial nutrient treatments (A = 'very low', B = 'low', C = 'high', D = 'very high'), and subjected to presence (+BC) and absence (-BC) of the biological control agent, *Neohydronomus affinis*, on *P. stratiotes*.

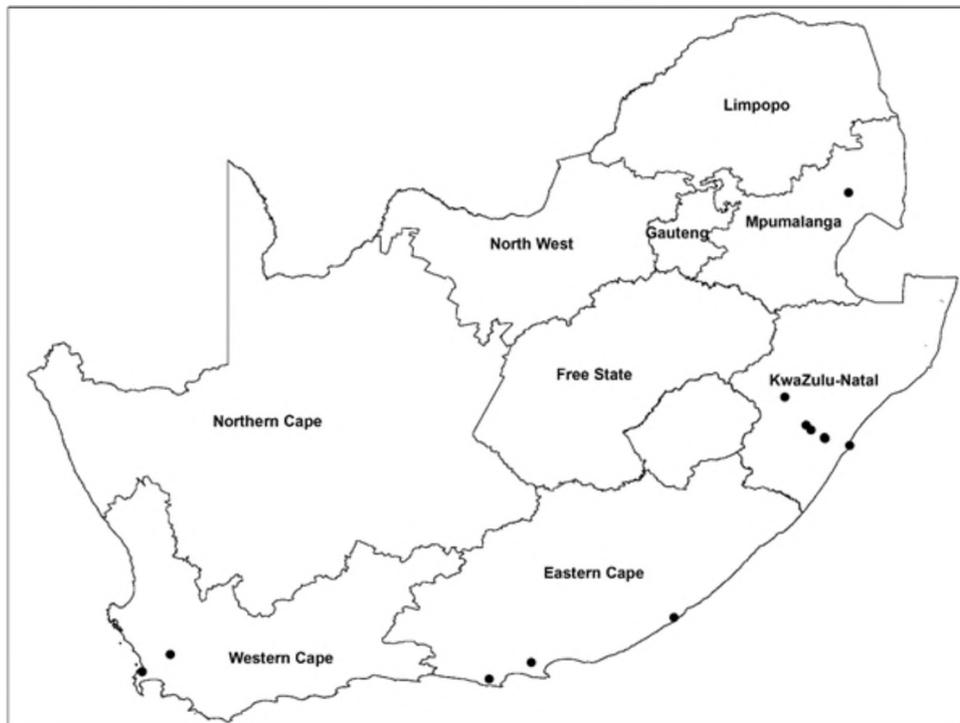


iii)

Weekly levels of ammonium (NH₄) and nitrate (NO₃) recorded in mesocosms containing 90% *Egeria densa* and 50% *Pistia stratiotes* under four different initial nutrient treatments (A = 'very low', B = 'low', C = 'high', D = 'very high'), and subjected to presence (+BC) and absence (-BC) of the biological control agent, *Neohydronomus affinis*, on *P. stratiotes*.



Appendix C – distribution map of *Egeria densa* in South Africa (Smith 2017)



Appendix D. Sediment analyses for Jameson Dam, EC, South Africa.

Measurement:	pH	Na	K	Ca	Mg	C	N
Unit:	KCl	Cmol/kg	Cmol/kg	Cmol/kg	Cmol/kg	%	%
Value recorded:	3.9	0.25	0.09	1.99	1.38	1.61	0.11