# Drivers of Macrophyte Assemblages in South African Freshwater Systems

THESIS

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## Abstract

Potentially damaging submerged invasive freshwater macrophytes have been identified in South African freshwater systems, but have received less attention than their floating counterparts. To ascertain the changes and effects that these species may have on macrophyte ecology, an understanding of the drivers of macrophyte assemblages is essential. The aims of this thesis were to investigate select abiotic and biotic factors driving introduction, establishment and spread of submerged macrophytes in South Africa.

Surveys on the status of submerged plant species in South Africa were conducted to find out the distribution and diversity of the species present, imported to, and traded in South Africa. Numerous submerged indigenous and invasive macrophyte locality records were collected during field surveys, of which many were first time records. Pet stores and aquarist trading activities were identified as potential vectors for the spread of submerged macrophytes through online surveys and personal interviews. These results highlighted the potential these species have for continuing to enter, and spread within South African water bodies.

Maximum Entropy (MAXENT) is a general-purpose method used to predict or infer distributions from incomplete information, and was used here to predict areas suitable for the establishment of five of these invasive macrophytes. Many systems throughout South Africa, particularly those in the subtropical coastal regions, were found to be climatically suitable for the establishment of *Elodea canadensis* Michx., *Egeria densa* Planch., *Hydrilla verticillata* (L.f.) Royle (all Hydrocharitaceae), *Myriophyllum spicatum* L. (Haloragaceae), and *Cabomba caroliniana* Gray (Cabombaceae).

Despite the high probability of invasion, facilitated by vectors and suitable climate, South Africa's rich indigenous submerged aquatic flora may be preventing the establishment of these submerged invasive species. Studies on the competitive interactions between a common indigenous submerged macrophytes, *Lagarosiphon major* (Roxb.) (Hydrocharitaceae) and *M. spicatum*, an invasive native to Eurasia, were conducted to ascertain which conditions influence competitive superiority. High sediment nutrient conditions significantly increased the growth rate and competitive ability of both species, while clay sediments significantly increased the competitive ability of *L. major* over *M. spicatum*, but sandy sediments improved the competitive ability of *M. spicatum*. These results highlighted the dynamic changes in competition between submerged species driven by abiotic factors, but did not take into consideration the effect that herbivory, a biotic factor, could have on competition between the two species.

The effect of herbivory by phytophagous insects of submerged plant species has been regarded as negligible. To find out what this effect is, multiple field surveys were undertaken throughout South Africa to find natural enemies of indigenous *Lagarosiphon* species with the aim of identifying such species, and quantifying their influence on plant growth dynamics. Several new phytophagous species were recorded for the first time. An ephydrid fly, *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae) was ascertained to be the most ubiquitous and abundant species associated with *L. major* in South Africa. The influence of herbivory by this fly on the competitive ability of *L. major* in the presence of *M. spicatum* was investigated using an inverse linear model, which showed that herbivory by *H. lagarosiphon* reduced the competitive ability of *L. major* by approximately five times in favour of *M. spicatum*. This study served to highlight the importance of herbivory as a driver of submerged aquatic plant dynamics.

Abstract

Current ecological theory emphasises the importance of investigating beyond plant-herbivore interactions, by including multitrophic interactions in community dynamics. Therefore, the potential of parasitism by a parasitoid wasp, *Chaenusa luteostigma* sp. n. Achterberg (Hymenoptera: Braconidae: Alysiinae) on *H. lagarosiphon* to shift the competitive interactions between the two plant species was also examined. The addition of the parasitoid reduced the effect of herbivory by the fly on *L. major* by half, thereby shifting the competitive balance in favour of *L. major* over *M. spicatum*.

This study provides valuable insight into a selection of drivers of submerged macrophyte assemblages of South Africa. It highlights the precarious position of South African freshwater systems with regard to the potential invasion by damaging submerged invasive species. It also provides interesting insights into the effect of competition, herbivory and parasitism on the establishment and spread of species within submerged freshwater systems. Understanding the different influences could assist managers and policy makers to make validated decisions ensuring the integrity of South African freshwater systems.

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## Publications arising from this thesis

Baars, J-R., Coetzee, J.A., Martin, G.D., Hill, M.P. & Caffrey, J.M. 2010. Natural enemies from South Africa for biological control of *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae) in Europe. *Hydrobiologia* **656**: 149-158.

Martin, G.D. & Coetzee, J.A. 2011. Freshwater aquatic plant invasion risks posed by the aquarium trade, aquarists and the internet trade in South Africa. *WaterSA* **37:** 371-380.

Martin, G.D., Coetzee, J.A. & Baars, J-R. 2013. *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), a potential biological control agent for the submerged aquatic weed, *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae). *African Entomology* **21**:151-160.

Martin, G.D. & Coetzee, J.A. 201. Competition between two aquatic macrophytes, *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) and *Myriophyllum spicatum* L. (Haloragaceae) as influenced by substrate sediment and nutrients. *Aquatic Botany* in press.

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ХΧ

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## **Chapter 1: Introduction**

Historically, control efforts on aquatic weeds in South Africa have focused on floating weeds. As a result, there is a dearth of research into the invasion and control of submerged aquatic macrophytes (Coetzee *et al.* 2011a). With numerous submerged invasive species already established in South Africa, a thriving horticultural industry, highly affected water systems, and a limited knowledge of the drivers of invasive submerged macrophytes, South Africa is vulnerable to invasion by these plants.

#### 1.2 Invasion of freshwater aquatic ecosystems

Invasive species are the second largest cause of current biodiversity loss, after habitat destruction (Wilcove *et al.* 1998). Man has facilitated the spread of invasive species around the world, and globalisation, particularly inter-continental trade and travel, has resulted in immense changes in global biogeography and both accidental and intentional introductions are likely to increase (Cook 1985; Dunham *et al.* 2002; Keane & Crawley 2002; Larson 2007). Aquatic ecosystems are particularly susceptible to invasion. The homogeneous nature of the aquatic environment allows for easy establishment of aquatic species outside their native geographic range (Cook 1985; Santamaría 2002). Furthermore, water is an effective vector for species to be dispersed easily over long distances. For example, the invasive zebra mussel, *Dreissena polymorpha* Pallas (Bivalvia), which was introduced into North America from Russia in the late 1980s, spread rapidly across the eastern half of North America mostly via connected, navigable water bodies (Bossenbroek *et al.* 2007). Additionally, aquatic environments are more difficult to monitor than terrestrial environments, especially for submerged species, so early detection is seldom possible (Larson 2007).

Disturbance has also been highlighted by a number of authors as a major contributor to the invasion process (Hobbs 1989; Capers *et al.* 2007; Thomaz *et al.* 2009), especially when a disturbance opens niches devoid of competition from other species (Hobbs 1989). This once again may be more pronounced in aquatic systems. To meet increasing demands for the limited supply of freshwater, systems have been extensively altered through diversions and impoundments, resulting in more disturbed aquatic systems than terrestrial ecosystems (Vitousek *et al.* 1997). Thus aquatic ecosystems worldwide have been heavily invaded by numerous species, of which invasive aquatic plants are the most widespread and damaging, both environmentally and economically (Wilcove *et al.* 1998; Larson 2007).

## 1.2 Aquatic ecosystems in South Africa

Over the last 100 to 200 hundred years, South African freshwater ecosystems have been dramatically affected by anthropogenic disturbances such as the creation of impoundments, interbasin water transfers and land use changes, which have altered natural river flows and sediment regimes (Rowntree & Wadeson 1999; DEA 2010). An integrity assessment of South Africa's rivers showed that 48% of South African rivers are moderately modified, 26% are largely to critically modified, while only 26% are still considered intact (King *et al.* 2005). Additionally, South Africa has relied on agriculture, mining and industry for development. This, coupled with rapid increases in urbanization, has resulted in large amounts of pollutants flowing into freshwater systems (Oberholster & Ashton 2008; DEA 2010; Coetzee *et al.* 2011a). Eutrophication, especially nitrogen pollution, is often responsible for ecosystem degradation as well as the establishment of invasive aquatic weeds (Gerber *et al.* 2008; and 2011 show that more than 300 water systems are invaded with floating aquatic macrophytes, including some of the country's most important river systems

(M. Hill, Rhodes University, pers. comm. 2011). These invasive weeds often change the functioning of aquatic systems, resulting in adverse effects on the biotic assemblages (Mackay 2007), such as subsequent loss of species diversity, changes in relative dominance of native species, and establishment and spread of other invasive species (Cessford & Burke 2005; King *et al.* 2005). In a study at New Year's Dam, Eastern Cape Province, South Africa, it was found that invertebrate numbers were significantly lower under *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) (water hyacinth) mats than in *E. crassipes* free zones. Additionally, diversity of invertebrate communities was lower in areas where the plants were present than in open water (Midgley *et al.* 2006). A similar study on the Nseleni and Mposa River systems, KwaZulu-Natal, South Africa, showed that *E. crassipes* had a negative effect on biodiversity within the systems (Jones 2009). The Blaaukranz Nature Reserve, in the Albany district of the Eastern Cape, is one of the last remaining habitats of the Eastern Cape rocky (*Sandelia bainsii* Castelnau, Anabantidae), an endangered fish. The fish was nearly forced to extinction due to the rapid infestation of the water body by *Azolla filiculoides* Lam. (Azollaceae), a floating aquatic weed.

South Africa has seen the development of a number of control programmes aimed at conserving and rehabilitating ecosystems and their biodiversity. However there is a paucity of good data for decision making (King *et al.* 2005). For example, the Department of Environmental Affairs (DEA) has noted that data availability on river integrity on a national scale is a major limitation, and it is clear that there are some critical indicators for which they have no adequate data. Additionally they state "Although many taxa inhabit our rivers, the majority of conservation information is limited to fish" (DEA 2010), highlighting that there is very little information regarding aquatic plants and their assemblages in South Africa.

Since the early 1900s, South Africa's waters have been invaded by a number of floating aquatic macrophytes (Hill 2003). Species such as E. crassipes; Pistia stratiotes L. (Araceae) (water lettuce); Salvinia molesta D.S. Mitchell (Salviniaceae) (salvinia); Myriophyllum aquaticum (Vellozo Conceição) Verdcourt (parrot's feather), and A. filiculoides (red water fern) have been the most prominent and received the most attention (Hill 2003). Submerged invasive species such as Myriophyllum spicatum L. (Haloragaceae), Egeria densa Planch (Hydrocharitaceae), Elodea canadensis Michx (Hydrocharitaceae) and Echinodorus cordifolius (L.) Griseb (Alismataceae) have also been identified in South African water bodies (Cook 2004; Henderson & Cilliers 2002) but have received far less attention than their floating counterparts. Recently a few new submerged aquatic weeds have also been identified in South African waters, such as Hydrilla verticillata (L.f.) Royle (Hydrocharitaceae) and Cabomba caroliniana Grey (Cabombaceae). Very little is known about these weeds in South Africa, including their current and potential distributions (Coetzee et al. 2009; 2011a). To understand the changes and effects that invasive species may have on aquatic macrophytes and their community structures, an understanding of the drivers of macrophyte assemblages is essential. Several biotic and abiotic factors and their interactions shape macrophyte assemblages within a system, including the success and failure of both indigenous and invasive species to colonise, spread and become dominant. This thesis explores some of the drivers of aquatic communities in South Africa as well as the importance of investigating them holistically.

## 1.3 Introductory paths of aquatic macrophytes

Unlike terrestrial species, freshwater aquatic plants lack specialised adaptations for long distance dispersal such as adaptations for wind dispersal, low seed mass or seawater resistance (Cook 1985). Humans play a major role in the transportation of aquatic weeds over

both short and long ranges and are responsible for the majority of invasions around the world (di Castri 1989; Cook 1985). Despite the recent implementation of stringent import and export regulations worldwide (Kay & Hoyle 2001), movement of plant species still occurs extensively.

The majority of submerged invasive plants have been spread around the world, including South Africa, via the aquarium trade as ornamental and/or aquarium plants (Dehnen-Schmutz *et al.* 2007; Champion & Clayton 2000; Heywood & Brunel 2009). Genetic investigations into the origin of *H. verticillata* infestation in South Africa indicate that it was probably introduced via the aquarium trade as it was revealed that *H. verticillata* was most closely related to plants from Malaysia and Indonesia, the center of the South African aquarium plant import trade line (Madeira *et al.* 2007). Regardless of their status as declared invaders, aquatic plants continue to be sold in many countries (Kay & Hoyle 2001).

The lack of knowledge regarding invasive species results in less care given to the overflowing of ponds or the disposing of unwanted aquaria plants, which are often discarded into ponds, ditches, streams and rivers, sometimes resulting in species escape, establishment and spread. For example, *E. crassipes* escaped from an overflowing garden pond and established in the Nahoon River, Eastern Cape Province, South Africa (M. Hill, pers. comm. 2008). Once in a system the success of invasion is governed by numerous environmental drivers.

## 1.4 Drivers of aquatic plant assemblages

Many factors influence the macrophyte assemblage within a system, including the success and failure of invasive species to colonise, spread and become dominant. This is particularly important in South Africa where numerous new submerged macrophytes have recently been recorded. The factors determining distribution and spread of these macrophytes involve a variety of abiotic and biotic drivers, the most important of which are substrate, water and sediment nutrient concentrations, flow velocity and other hydrological conditions, substrate, pH, carbonate hardness, shading and anthropogenic effects (Szoszkiewicz et al. 2006). These drivers of macrophyte distributions and assemblages in South African water systems have received little or no attention, and require investigation to predict and understand these possible new invasions. The limited attention given to drivers of submerged aquatic plants assemblages in South Africa is in contrast to Australia, the U.S.A. and Europe where characterisation of the principle plant assemblage types, in terms of species composition and abundance, and the physio-chemical preferences of these assemblages, have been extensively described (Ali et al. 1999). This has allowed for their spatial and temporal variability and responses to anthropogenic disturbance to be understood (Szoszkiewicz et al. 2006; Mackay 2007). An understanding of the abiotic and biotic drivers, severally and in combination of these drivers, as well as anthropogenic changes to these drivers, would aid in predicting the factors determining spread, distribution and dominance of invasive species entering South African water systems.

#### 1.4.1. Abiotic Drivers

Aquatic macrophyte assemblages are driven by a number of abiotic factors (Szoszkiewicz *et al.* 2006) including substrate, sediment nutrients (Barko *et al.* 1991), surface irradiance which may limit some species of angiosperms (Allan 1995), water depth and translucency (Rooney & Kalff 2000), flow velocity and the hardness of water including calcium, alkalinity and pH (Allan 1995). These key drivers in macrophyte assemblage are especially susceptible to human induced-disturbances (Byers 2002; Barko *et al.* 1991; Szoszkiewicz *et al.* 2006). Changes in drivers such as nutrient levels are usually associated with increased growth of

selected species (Van *et al.* 1999), for example, accelerated eutrophication resulting from human activities has been strongly implicated as a major reason for the invasion of *H. verticillata* and other exotic species in the U.S.A. (di Castri 1989; Van *et al.* 1999) and the subsequent loss of native species (Santamaría 2002). Another effect on South African freshwater systems is the increase of riparian alien vegetation, which uses greater volumes of water than indigenous vegetation (Davies & Day 1998; Coetzee et al 2001a; Coetzee et al 2001a), resulting in decreased water flow in affected areas, allowing for invasion of submerged macrophytes in disturbed areas. These changes in abiotic drivers within systems influence freshwater systems' ability to provide fundamental goods and services (Scholes *et al.* 2005), such as good water quality, increased environmental heterogeneity, substrate composition and habitat for aquatic biota all of which is vital in South Africa where many people rely on these systems for their livelihoods.

#### 1.4.2. Biotic Drivers

Competitive interactions are commonly assumed to control community structure (McCreary *et al.* 1983). Several studies have documented changes in the composition of submerged macrophyte communities based on competitive ability and growth of species (McCreary & Carpenter 1987; Rattray *et al.* 1994; McCreary 1991; James *et al.* 1999; Van *et al.* 1999). Competition between macrophytes is influenced by environmental factors such as depth, water quality and substrate (Van *et al.* 1999), as well as by plant growth form (Titus & Adams1979). Intraspecific competition in submerged plant species can result in morphological changes, usually a decrease in individual plant biomass. An experiment evaluating intraspecific and interspecific competition between *Stuckenia pectinata* (= *Potamogeton pectinatus* L.) (Potamogetonaceae) and *Chara aspera* Deth. ex Willd (Characeae) under different light regimes showed a decrease in the individual plant mass of

both species (Van den Berg *et al.* 1998). Moen and Cohen (1989) conducted a similar experiment investigating interspecific competition where *S. pectinata* and *Myriophyllum exalbescens* Fern. (Haloragaceae) were grown in experimental aquaria in single- and mixed-species cultures. They showed that *M. exalbescens* did not affect *S. pectinata;* but *S. pectinata* was able to inhibit growth of *M. exalbescens* through the formation of a dense light-restricting canopy.

Invasive species such as Eg. densa, El. canadensis, Ceratophyllum demersum L. var. demersum (Ceratophyllaceae), Н. verticillata, Lagarosiphon major (Ridley) (Hydrocharitaceae) and *M. spicatum* have been shown to outcompete other species through their ability to spread rapidly from bud-bearing stem fragmentation and dormant stem apices (Hofstra et al. 1999; Van et al. 1999; Caffrey et al. 2010). Once fragments have entered a system, they continue to spread by stem fragmentation, giving rise to many new vertical shoots. As they develop and their numbers increase, they form dense closed canopies, which outcompete existing native vegetation (Hofstra et al. 1999). Plant growth form is important in determining competition for example, *M. spicatum*, which is able to form dense surface mats of leaves and stems, can replace Vallisneria americana L. (Hydrocharitaceae), which has a basal rosette of leaves that may extend to the surface, but does not form a canopy (Titus & Adams 1979).

The combination of herbivory and plant competition affects plant species composition (Wheeler & Center 1998). The reduction in plant vigour by herbivory and a plant's competitive ability is paramount in determining plant population dynamics, by affecting all aspects of plant growth and reproduction relative to their unattacked neighbours (Crawley 1989). Numerous studies have been conducted on the effect of individual drivers on the macrophyte assemblage structure, which may lead to the misconception or overestimation of

one driver (Mackay 2007). This highlights the need for a holistic understanding of the drivers of macrophyte distributions and establishment.

Macrophyte biomass, productivity, and species composition is often influenced by a variety of vertebrate and invertebrate grazers (Lodge 1991). Many herbivores destroy much more macrophyte tissue than they actually eat (Lodge 1991), and a small amount of tissue consumption may result in considerably greater plant tissue death from diseases (Crawley 1989; Lodge 1991; Newman 1991). The best examples can be found in the biological control of invasive species. Successful weed biological control agents do not necessarily cause direct mortality of the target plant, but the impact of a herbivore may be subtle and of a long-term nature, causing gradual reduction in the host plant vigour, thereby increasing its susceptibility to competition from other vegetation (Crawley 1989). This reduction in invasive species dominance and abundance may have dramatic effects on indigenous plant community structure (Crawley 1989).

As our knowledge of insect and plant interactions grows, we are increasingly faced with the question of how the network of interactions within communities occurs. Most studies of community dynamics remain narrowly focussed (Herrera & Pellmyr 2002; Harvey *et al.* 2010). The traditional way of looking at such interactions has been from a bitrophic viewpoint, focusing only on the plant and insect herbivores in the food web. However, Price *et al.* (1980), and more recently Harvey *et al.* (2010), suggest that understanding insect-plant interactions cannot progress realistically without consideration of the third trophic level. A closer look at the mechanisms of interactions reveals that many plants, directly and indirectly, positively and negatively, have an effect not only on herbivores but also on the enemies of herbivores. Hence several authors have started to investigate how plants mediate their herbivore populations by influencing parasitoid populations both indirectly, by affecting host

suitability, and directly, by providing the parasitoid with food and shelter, or by influencing their searching processes. Plants can influence the foraging process by attracting herbivore parasitoids through the release of attractant chemicals (Poppy 1997). However tritrophic relationships and parasitoid pressure on the competitive ability of plants is unresearched and may be a vital component in understanding multitrophic interactions, and the success of potential biological control agents (Hill & Hulley 1995; Paynter *et al.* 2010; Harvey *et al.* 2010). A component of this thesis investigates the role of multitrophic interactions as a driver of aquatic macrophyte invasion in South Africa.

In the last decade in South Africa, floating aquatic plant invasions have been controlled through a series of integrated control programmes with the emphasis on biological control (Coetzee *et al.* 2011b). Systems that have been completely covered by floating weeds are slowly returning or have already returned to open water. However these systems are now vulnerable to invasion by submerged aquatics. Systems such as the Vaal River that have historically been covered with *E. crassipes* are becoming infested with dense mats of the submerged species *M. spicatum*. Due to the poor and vulnerable condition of South African water systems, as well as the potential for the introduction and spread of submerged invasive species, knowledge of the drivers and potential distribution of invasive submerged macrophyte invasions and their associated risks is imperative to maintaining the integrity of these aquatic ecosystems.

#### 1.5 Aims

The aim of the thesis was to investigate drivers of submerged plant invasions in South Africa by investigating a few key abiotic and biotic factors influencing macrophyte assemblage structure and distribution. This study will enable practitioners to evaluate changes in macrophyte assemblage in relation to anthropogenic influences such as increased nutrient levels, change in sediment type, climate change and introductions of invasive species.

The first objective was to ascertain the magnitude of invasion by submerged macrophytes in South Africa. This was achieved by investigating botanical archives and distribution records of invasive plants in South Africa. Current distribution maps are available from the South African National Biodiversity Institute (SANBI). These data were supplemented with field surveys. Because this thesis focuses on a number of indigenous and invasive species which are common to many of the chapters, Chapter 2 provides a brief description of their biologies as well as their known distributions, and can be referred to throughout the thesis.

Essential to understanding the drivers of species within ecosystems is an understanding of the mechanism of their introduction. The aquarium trade is recognised as the primary mechanism for submerged plant introductions throughout the world, therefore a survey was conducted to find out the diversity of the species that are imported and traded in South Africa, and is presented in Chapter 3

Chapter 4 reports on the potential distributions of submerged invasive species in South Africa as influenced by climate. To date, only three submerged aquatic plant species are declared weeds or invader species in South Africa; *El. canadensis, Eg. densa* and *M. spicatum* (Henderson 2001). However, potentially damaging species such as *H. verticillata* are already established in South African but are as yet not under any controlling legislation. Their distributions around South African water bodies based on climatic variables are unknown. Thus distribution modelling with the program MAXENT (Phillips *et al.* 2006) was used to predict areas suitable for the establishment of these invasive macrophytes. This was achieved using all available distribution data from their introduced ranges, as well as data from their

native range. Knowledge of these species distributions is fundamental for the implementation of effective management strategies and eventual control.

Even though climate matching predicts areas in South Africa climatically suitable to a particular species, it is often an over-estimation, as the correlative modelling programs do not take into consideration the many biotic and abiotic influences specific to South African freshwater systems. South Africa has a typical but diverse submerged aquatic flora (Cook 2004), and this may be a possible biotic factor mitigating the establishment of invasive species into South African systems as they may competitively exclude invasive species. Chapter 5 considers biotic factors that may influence the establishment of these submerged species. Aspects such as competition were investigated to ascertain what effects interspecific and intraspecific competitions have on the vigour of invasive species. It examined the competition between the common indigenous submerged species *L. major* and the invasive *M. spicatum*, under different sediment and nutrient treatments, thereby investigating the combined effects of both abiotic and biotic drivers. These experiments however did not take into consideration the effect that herbivory could have on the competition between the two species.

Lagarosiphon major was chosen as a test species as it is a common submerged species in South Africa, has a wide distribution and is found in a variety of habitats. It has also been introduced and become invasive in many countries around the world (Baars *et al.* 2010). To find out the effect of herbivory as a driver, an understanding of phytophagous insects associated with *L. major* is required. Multiple field surveys were undertaken throughout South Africa to find natural enemies of *L. major*, the results of which are presented in Chapter 6. An ephydrid fly, *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), was shown to be the most ubiquitous and abundant phytophagous species associated with *L*. *major* in South Africa. The effect of the fly on *L. major* as well as its host specificity and distribution in the field was also investigated in Chapter 6.

There was a secondary advantage to investigating the natural enemies of *L. major*. *Lagarosiphon major* is indigenous to southern Africa (Cook 2004), has been introduced to Australia, New Zealand, United Kingdom, Ireland and mainland Europe (ISSG 2009). It is particularly problematic in Ireland where it has invaded Loch Corrib, the second largest lake in Ireland. *Lagarosiphon major* has proved particularly difficult to control using traditional mechanical and chemical methods, due to its ability to regenerate from stem fragments. Biological control using insect natural enemies appears to be a suitable alternative or complement to control methods. *Hydrellia lagarosiphon* may affect the competitive ability of *L. major*, but the manner in which it does so is unknown, and is therefore reported in Chapter 7. This study also gives invaluable insight into what can be expected in Ireland if the fly is released as a biological control agent against *L. major*.

During the survey for phytophagous species associated with *L. major*, a parasitoid wasp, *Chaenusa* Haliday sp. (Hymenoptera: Braconidae) of the fly, *H. lagarosiphon* was recorded. About ten species are known from this genus from the U.S.A., Europe and the Orient, and the known hosts of some of these include ephydrid flies. Considering the third trophic level (natural enemies of herbivorous insects) when studying the combined effect of herbivory and plant competitive interactions may enhance our understanding of them, thereby improving prospects for biological control. The effect of parasitism on biological control agent populations is a relatively unstudied field, and combining this with investigations into how parasitism changes the competitive ability of plant species is a completely unresearched field. The effect of the parasitoids on the impact of herbivory by the fly, *H. lagarosiphon* on *L. major*, and the resulting combined effect on the competitive ability of *L. major* against *M.* 

*spicatum* are reported in Chapter 7. Chapter 7 also further develops Spitters' (1983) addition series model to include trophic level interactions.

To date, submersed macrophyte competition studies have included manipulative studies, such as reciprocal replacement series, additive or removal perturbations, and character displacement studies (Spitters 1983; McCreary et al. 1987; Agami & Reddy 1990; McCreary 1991; Van et al. 1998; Coetzee et al. 2005). These studies can detect the possibility and intensity of competition among members of a community, yet usually are unable to determine the importance of competition relative to other mechanisms in providing structure to communities. In contrast to the manipulative studies are investigations into comparative resource utilization patterns, biomass allocation patterns, and change along environmental gradients which aim to detect non-random variation in community pattern and structure, yet are usually unable to specify causal mechanisms (Titus & Adams 1979; Madsen et al. 1991; Lacoul & Freedman 2006a). It has been argued that these studies only investigate competition and community from a single trophic (bi-trophic level). In order to obtain a better understanding of competitive interactions, a more trophic level view point needs to be considered (Harvey et al. 2010). The competition studies in this thesis (Chapter 5 and 7) investigated addition series, which allowed for the relative competitive abilities of each plant species in the experiment to be determined using reciprocal-yield models of mean plant dry mass under different sediment and nutrient characteristics. Additionally, Chapter 7 investigated the effect of different trophic levels (herbivory and parasitism) on plant competition. This is first time the effect of parasitism has been investigated as a driver of plant community structure in macrophyte communities (Chapter 7).

The final chapter, Chapter 8, discusses the results of these studies on the potential for invasion of South African water bodies by submerged weeds, and the drivers that either limit
or enable these species to establish and spread, highlighting the critical factors that may require further research.

## Chapter 2: Description of common invasive and indigenous submerged macrophytes in South Africa

## **2.1 Introduction**

Aquatic and wetland plants represent two percent of the total flora of South Africa, which is in line with the world average. There are two families, six genera and approximately 114 species endemic to South Africa (Cook 2004). Nine species are considered endangered and threatened by extinctions, while two species are vulnerable, as published by the South African plant red data lists (SABONET 2003). These numbers are extremely low and are probably an underestimation due to the lack of knowledge of aquatic and wetland plants in South Africa, rather than a representation of the actual status. This lack of knowledge is highlighted by the literature available. Only three books dealing with identification and information on aquatic species in South Africa have been published; "A guide to invasive aquatic plants in South Africa" (Henderson & Cilliers 2002), "An easy identification of aquatic plants handbook" (Gerber et al. 2004), which only presents a photographic guide to a limited number of species, and "Aquatic and Wetland Plants in South Africa" (Cook 2004), which provides illustrated descriptions of just less than 600 species with full biologies, ecological notes, and distributions (Cook 2004). This is primarily a taxonomic book which is useful in its own right, but does little to cater for the needs of practitioners without botanical training aiming to identify species (Ellery 2004). There is also limited accurate information about species' distributions in the region or the habitats in which they are likely to occur, particularly their distribution in relation to the hydrological regime (Ellery 2004). Thus it does help understand distributions within systems, macrophyte densities or assemblages. It is

frequently the combination of species morphology, distribution and habitat requirements that leads to species identification.

This chapter provides a brief description of the morphology, distribution and effects of five invasive submerged macrophytes of which four are established in South Africa, while the other is regularly found in the country's aquarium trade and is regarded as a high risk species (Henderson & Cilliers 2002). All five species are regularly discussed and used as examples in this thesis. This chapter also provides a description of three indigenous species commonly found throughout South Africa in impoundments and rivers. One of these species, *Lagarosiphon major*, is used commonly in the global aquarium trade and has become invasive in other countries around the world. Its invasive properties have attracted the attention of international research. All three species are also frequently used as examples in the thesis.

### 2.2 Invasive species

### 2.2.1 Egeria densa Planch (Hydrocharitaceae), dense waterweed

*Egeria densa* is a submerged aquatic plant that forms dense monoculture stands in both still and flowing waters (Fig. 2.1). Its rooted slender stems can grow up to 1.5m long, and are brittle and easily broken, which aids dispersal (Cook 2004). A distinguishing feature is that its leaves, which are 15-30mm long, occur in whorls of three in the lower sections of the stems, and four to eight in the upper sections, clustering towards the end of the branch. The leaves are also very finely serrated. It has white 3-petalled flowers measuring about 15mm across which extend 20 mm above the surface of the water (Cook 2004). It is a dioecious plant, but only female plants have been found in South Africa (Henderson & Cilliers 2002). *Egeria densa* is easily confused with a number of other species in the Hydrocharitaceae. Differences between these species are based on patterns of leaf distribution and leaf serrations.

*Egeria densa* is indigenous to South America, but has invaded numerous regions throughout the world, including Australia, Chile, Denmark, France, French Polynesia, Germany, Japan, New Zealand, Puerto Rico, the U.S.A. and South Africa. In southeast Brazil, *Eg. densa* causes great annual losses to the hydroelectric companies (ISSG 2010). It forms dense monospecific stands that restrict water movement, trap sediment, and cause fluctuations in water quality. Dense beds interfere with recreational uses of water bodies by interfering with navigation, fishing, swimming, and water skiing. In New Zealand, *Eg. densa* exhibited the ability to rapidly recolonise de-vegetated areas following floods (Tanner *et al.* 1990).



**Figure 2.1:** *Egeria densa.* (Line drawing provided by University of Florida/IFAS Center for Aquatic and Invasive Plants. Used with permission.).

*Egeria densa* was first recorded from South Africa in 1963, from the Durban area, and has since spread to flowing and still water systems throughout South Africa, favouring

Pietermaritzburg, Durban and Tongaat areas in KwaZulu-Natal (L. Henderson, South African National Biodiversity Institute, pers. comm. 2008). It has also been recorded in the Eastern Cape in the Nahoon River, East London (pers. obs.) and in the Bakaans and Swartkops rivers in Port Elizabeth, and in the Western Cape in the Berg River in Paarl and in systems near Stellenbosch (L. Henderson pers. comm. 2008) (Fig. 2.2).



**Figure 2.2:** Distribution records of *Egeria densa* in South Africa from South African National Biodiversity Institute (SANBI) and Rhodes University Surveys. Triangles indicate major towns. Open circles indicate sites not confirmed within the last five years, closed circles indicate sites where the plant's presence has been confirmed within the last five years in the provinces: Gauteng (GT), Mpumalanga (MP), Limpopo (NP), North West (NW), KwaZulu-Natal (KZ), Eastern Cape (EC), Western Cape (WC), Northern Cape (NC), and Free State (FS).

## 2.2.2 Elodea canadensis Michx (Hydrocharitaceae), Canadian waterweed

*Elodea canadensis* is a submerged aquatic plant very similar in morphology to *Eg. densa* (Fig. 2.3). It has minutely serrated leaves, 5-15 mm long, which are usually in whorls of three and four compared to four to six leaves per whorl of *Eg. densa*. Its flowers have equal perianth whorls, each with three membranous segments, 5 mm across, that floats on the water surface and form long thread-like stalks (Bowmer *et al.* 1995).

Initially, *El. canadensis* was documented and noted due to its rapid invasion of many of Europe's waterways in the 19<sup>th</sup> Century, invading waterways in Norway and the Czech Republic (ISSG 2011). In New Zealand, it has colonised many waterways, displacing plant communities (Ward & Talbot 1984), while in Australia, it negatively affects the use of irrigation, as it clogs pumps and drainage canals (Bowmer *et al.* 1995).

As an invasive *El. canadensis* in South Africa has been less significant than that of *Eg. densa,* and thus *El. canadensis* far has been recorded only in the Hennie Hugo Dam, Maraisburg Roodepoort (Henderson, 2002), and a few locations in KwaZulu-Natal (R. Glen 2008 ex South African National Biodiversity Institute, pers. comm.).



**Figure 2.3:** *Elodea canadensis* (Drawn by Gill Condy, first published in Henderson & Cilliers 2002, ARC-Plant Protection Research Institute, and Pretoria).

## 2.2.3 Hydrilla verticillata L. (Hydrocharitaceae), hydrilla

*Hydrilla verticillata* (also Hydrocharitaceae) is a submerged aquatic perennial with heavily branched stems towards the water surface (Fig. 2.4). Stems are slender and can grow to 9m long. Its leaves are 6-20mm long, 2-4mm wide, strap-shaped with pointed tips and saw-tooth edges, and grow in whorls of 4 to 8 around the stem. Leaf colour varies from green to brown (Cook 2004).

The main mode of reproduction of *H. verticillata* is vegetative through the production of turions (over-wintering dense vegetative buds) in the axils of leaves and tubers within the sediment (Balciunas *et al.* 2002). The plant sometimes produces flowers; female flowers are small white and found on long slender stems. Male flowers are small, green, free-floating and are inverted bell-shaped. The plant is usually rooted to the substrate but sometimes grows as floating mats at the surface.

Although native to Asia and Australia, *H. verticillata* is highly invasive in the U.S.A. (Balciunas *et al.* 2002). Here it colonises a wide variety of freshwater habitats, resulting in thick extensive mats that cause significant economic and ecological damage (Langeland 1996; Balciunas *et al.* 2002). In the south eastern U.S.A. dense *H. verticillata* infestations constitute the most severe aquatic problem (Center *et al.* 1997), affecting irrigation operations and hydroelectric power generation, while boat marinas and propeller driven boats are frequently hindered by the thick mats on the water's surface (Balciunas *et al.* 2002).

In 2006, the plant was found in Pongolapoort Dam, KwaZulu-Natal, South Africa and to date is restricted to this site (Coetzee 2005).



**Figure 2.4:** *Hydrilla verticillata.* (Line drawing provided by University of Florida/IFAS Center for Aquatic and Invasive Plants. Used with permission.).

## 2.2.4 Cabomba caroliniana Grey (Cabombaceae), cabomba, fanwort

*Cabomba caroliniana* is easily recognised by its finely dissected underwater leaves that are feathery or fan-like in appearance (Schooler *et al.* 2006) (Fig. 2.5). The leaves are predominantly olive green but may be reddish brown and are usually arranged oppositely or alternately. Flowers are 6-15mm in diameter, 6-12mm long, white, pale yellow or purplish and have three petals, and are emergent above the water surface (Ørgaard 1991).

*Cabomba caroliniana* is an extremely persistent and competitive plant. Under suitable environmental conditions it forms dense stands and crowds out previously well-established plants. Once established, it clogs drainage canals and freshwater streams interfering with recreational, agricultural, and aesthetic uses (Schooler *et al.* 2006). In Australia, *C. caroliniana* is regarded as a Weed of National Significance because of its invasiveness, potential for spread, and its negative economic and environmental effects (Mackey 1996; Mackey & Swarbrick 1997; CRC 2003).



**Figure 2.5:** *Cabomba caroliniana*. (Line drawing provided by University of Florida/IFAS Center for Aquatic and Invasive Plants. Used with permission.).

*Cabomba caroliniana* has yet to establish in South African freshwater systems or be found outside of cultivation but is available in many pet stores throughout the country (Chapter 3).

## 2.2.5 Myriophyllum spicatum L. (Haloragaceae) spiked water-milfoil

*Myriophyllum spicatum* is a submerged, perennial aquatic herb that typically grows in water 1-4m deep, but has been found in water up to 10m deep (Smith and Barko 1990; Buchan & Padilla 2000). Stems grow to 2-3 m with whorled, feathery leaves. It can be distinguished from other submerged species by its finely divided but unbranched leaves (Smith and Barko 1990; Sainty & Jacobs 1994) and its emergent spiked inflorescence 50- 100m long (Henderson & Cilliers 2002) (Fig. 2.6).



**Figure 2.6:** *Myriophyllum spicatum.* (Line drawing provided by University of Florida/IFAS Center for Aquatic and Invasive Plants. Used with permission.).

*Myriophyllum spicatum*, which is indigenous to Europe, Asia and North Africa, is the most important waterweed in U.S.A., requiring millions of dollars to be spent annually on its control (Couch and Nelson 1985). Like most submerged weeds, it negatively affects aquatic biodiversity (Madsen *et al.* 1991), and hinders recreational water use. *Myriophyllum spicatum* spreads between lakes and within lakes by fragmentation. Once established, it maintains large biomass throughout the winter, has rapid and early seasonal growth and easily outcompetes other macrophytes for both light and sediment nutrients (Sainty & Jacobs 1994). At high densities, it supports fewer aquatic insects which serve as a food resource for fish, large predatory fish lose foraging space and are less efficient at obtaining prey. Another effect is a reduction in oxygen levels in the water due to the decay of the large mats of the plant. The

dense mats impede water movement and interfere with recreational activities such as swimming, boating, fishing and water skiing (Smith and Barko 1990; ISSG 2010).

In southern Africa, *M. spicatum* already has a wide distribution with large infestations along the Vaal River from Warrenton down to Barkley West. Populations of the plant have also recently been confirmed in Lake Sibaya in KwaZulu-Natal, and the Hogsback region in the Eastern Cape. It has also been recorded in the Nyl, Crocodile, and Breede Rivers, and dams in the Bronkhorstspruit, Dullstroom, Kimberly and Underberg regions. (SANBI 2010) (Fig. 2.7).



**Figure 2.7:** Distribution records of *Myriophyllum spicatum* in South Africa from South African National Biodiversity Institute (SANBI) and Rhodes University Surveys. Open circles indicate sites not confirmed within the last five years, closed circles indicate sites where the plant's presence has been confirmed within the last five years in the provinces: Gauteng (GT), Mpumalanga (MP), Limpopo (NP), North West (NW), KwaZulu-Natal (KZ), Eastern Cape (EC), Western Cape (WC), Northern Cape (NC), Free State (FS).

## 2.3 Indigenous species

# 2.3.1 Lagarosiphon major (Roxb.) (Ridley) Moss (Hydrocharitaceae), African elodea, oxygen weed and Lagarosiphon muscoides Ridley (Hydrocharitaceae).

The genus *Lagarosiphon* is native to sub-Saharan Africa. Nine species are described with variable distribution ranges throughout the continent including Madagascar (Wager 1928;

Symoens & Triest 1983). In southern Africa, *Lagarosiphon muscoides* Ridley and *Lagarosiphon major* (Ridl.) (Hydrocharitaceae) are the most common species, with most of the records of *L. major* occurring south of Zambia (from the Western Cape Province to Zimbabwe). Both *L. major* and *L. muscoides* are considered noxious weeds in South Africa (Obermeyer 1964; 1966), and often proliferate in manmade impoundments (Anonymous 1980a; b). *Lagarosiphon major* is also found in several countries in Europe (Symoens & Triest 1983; Preston *et al.* 2002; Reynolds 2002; Stokes *et al.* 2004; van Valkenburg & Pot 2007) and New Zealand (McGregor & Gourlay 2002).

*Lagarosiphon major* and *L. muscoides* are perennial, submerged, rooted, vascular plants capable of sexual and asexual reproduction (Cook 2004).



**Figure 2.8:** *Lagarosiphon major* and *Lagarosiphon muscoides*. (Line drawing provided by University of Florida/IFAS Center for Aquatic and Invasive Plants. Used with permission.).

Both species of *Lagarosiphon* are very similar in appearance, but close inspection of their leaves and stems allows them to be distinguished from each other. The stems of *L. major* (3 mm in diameter) are more robust than those of *L. muscoides* (0.5-1.5 mm in diameter) (Cook 2004). The leaves of *L. major* are 6.5- 30 mm long, 2.5 mm wide, arranged alternately and possess the diagnostic characteristic of having translucent leaf margins consisting of two rows of fibre-like cells with more than 50-100 short blunt teeth on each side (Cook 2004). In contrast, the leaves of *L. muscoides* are 4.8-20 mm long, 0.5-1.4 mm wide and have 28-86 sharp teeth emerging from a translucent leaf margin consisting of 3-6 rows of fibre-like cells (Cook 2004). Generally the leaves are arranged in an alternate manner; however, they also form locally consecutive whorls, which is a diagnostic feature (Cook 2004).

*Lagarosiphon major* prefers clear, still or slow-moving freshwater with silty or sandy bottoms. It prefers the cooler waters of the temperate zone, with optimum temperatures of 20-23°C and a maximum temperature of around 25°C. It can live in high and low nutrient levels, grows best under conditions of high light intensity, and can tolerate relatively high pH (ISSG 2008).

The distribution of *L. major* in southern Africa ranges from the Western Cape Province to Zimbabwe (Symoens & Triest 1983). In South Africa, *L. major* is limited to the colder areas of the country, namely the Amatola Region of the Eastern Cape, Drakensberg regions of KwaZulu-Natal and the Eastern Cape, and the Mpumalanga Highlands.

*Lagarosiphon muscoides* is mainly distributed in the southern part of Africa but has been recorded from isolated localities in Zambia, Malawi, Tanzania, Sudan, Central African Republic, Chad and Mali (Symoens & Triest 1983). In South Africa, it has a wide distribution and is found in most water body types.

## 2.3.2 Stuckenia pectinata (= Potamogeton pectinatus L.) (Potamogetonaceae), sago pondweed

*Stuckenia pectinata* has thread-like stems 1 mm in diameter and repeatedly branched (Cook 2004) (Fig. 2.9). Its leaves are 10-70 mm long, 8-65 mm wide and taper to a fine point (Cook 2004). The leaf sheaths have stipules arising from them, which are wrapped around the stem. A primary diagnostic feature is the jointed appearance of the leaves (Cook 2004). *Stuckenia pectinata* has a wide distribution globally and is regarded as a cosmopolitan species in most regions (van Wijk 1989). In South Africa it is found in large mats throughout the country in lakes, dams and river systems.



**Figure 2.9:** *Stuckenia pectinata.* (Line drawing provided by Aquatic Weed Control Seattle, Washington).

## **2.4 Discussion**

The importance of wetlands globally and in South Africa is generally accepted (Cook 2004). Of concern for South Africa is the number of introduced aquatic plant species which are also naturalised, and the alarming rate at which newly introduced, as well as new localities of already introduced species are being detected.

The very nature of aquatic ecosystems in southern Africa contributes to the relative ease of invasion and spread of exotic aquatic macrophytes. Because of the climate and topography of South Africa, there are only about 180 natural lakes or pans that have open water, in the country (Noble and Hemens 1978, Rowntree 2000). The majority of these water systems are filled by a combination of precipitation, irregular stream flow and ground water which results in many of the smaller lakes drying out annually and the larger ones being regarded as semi-permanent (Ramsar Convention on Wetlands 2012). The lack of permanency of water has resulted in an absence of establishment and evolution of higher plant species (Ramsar Convention on Wetlands 2012), particularly floating species suited to still open waters, thus southern Africa has very few indigenous floating plants, but by developing artificial lakes, through the construction of dams and impoundments, ideal habitats for invasive floating plants have been created (Davis and Day 1998). This has resulted in South Africa having a long history of, free-floating aquatic weed invasions.

Invasive submerged aquatics have received very little attention in South Africa, perhaps because they are not as obvious as floating weeds, or they have been competitively excluded by the rich indigenous submerged flora or by floating weeds in disturbed systems. As an initial step into investigating submerged macrophytes in South Africa, the introduction and spread of potentially invasive species must be considered. Pet traders, aquarists, boating enthusiasts and fishermen play a significant role in the introduction and spread of submerged aquatic plants throughout the world, therefore Chapter 3 investigates the aquarium and pet trade as potential drivers of submerged species introductions into South Africa.

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## Chapter 3: Pet stores, aquarists and the internet trade as modes of introduction and spread of invasive macrophytes in South Africa<sup>1</sup>

## **3.1 Introduction**

The introduction of harmful invasive aquatic plants is recognised as a major environmental threat to many aquatic ecosystems throughout the world, including South Africa. Numerous pathways of introduction are responsible for the distribution and spread of many of these species, such as the horticultural and aquarium trade, dumping of ballast water, unintentional movement of propagules (i.e. hitchhikers) and, increasingly, the internet trade. Historically, invasive species such as water hyacinth (*E. crassipes*), flowering rush (*Butomus umbellatus* L. (Butomaceae)) and water poppy (*Hydrocleys nymphoides* (Willd.)) Bucherau (Limnocharitaceae)) have been transported and traded due to their aesthetic and horticultural value (Cook 1985). Other species have been traded for use in aquaria, such as fanwort (*C. caroliniana*), dense water weed (*Eg. densa*), Canadian water weed (*El. canadensis*), hydrilla (*H. verticillata*) and tape grass (*Vallisneria spiralis* L. (Hydrocharitaceae)) (Cook 1985). Despite the threat that these species pose, regulations to prevent unwanted species introductions from aquarium and ornamental sources are not enforced worldwide (Padilla & Williams 2004).

One third of the aquatic species on the International Union for the Conservation of Nature (IUCN) Invasive Species Specialist Group's list of the top 100 worst invasive species have spread via the aquarium trade or ornamental releases (Padilla & Williams 2004). It is not surprising then that the majority of submerged invasive plants in South Africa, the U.S.A. and

<sup>&</sup>lt;sup>1</sup> Martin, G.D. & Coetzee, J.A. 2011. Freshwater aquatic plant invasion risks posed by the aquarium trade, aquarists and the internet trade in South Africa. *WaterSA* **37**: 371-380.

New Zealand were introduced via the aquarium trade as ornamental and/or aquarium plants (Champion & Clayton 2000; Rixon 2005; Dehnen-Schmutz *et al.* 2007; Heywood & Brunel 2009). For example, genetic analysis of hydrilla, recently found to be invading a water body in South Africa, revealed it to be identical to samples from Malaysia and Indonesia, the centre of the South African aquarium plant import trade line (Madeira *et al.* 2007). Regardless of their status as declared invaders, aquatic plants continue to be sold in many countries (Kay & Hoyle 2001).

A growing aid to introductory pathways is the escalating use of internet and e-commerce, which has to a large extent been overlooked by researchers and policy makers alike (Derraik & Phillips 2010). Numerous listings of online nursery catalogues contain invasive aquatic or wetland plants (Kay & Hoyle 2001). The invasive seaweed *Caulerpa taxifolia* (Vahl) C. Agardh (Caulerpales) has had dramatic ecological and economic consequences worldwide (Padilla & Williams 2004). This species is readily available for purchase over the internet, and most, if not all, invasions of *C. taxifolia* worldwide can be traced back to aquaria releases or escapes (Padilla &Williams, 2004; Stam *et al.* 2006). An e-commerce search conducted in California for species of *Caulerpa* revealed that it could be purchase from 30 internet retailers and 60 internet sites from all over the U.S.A. and Great Britain (Stam *et al.* 2006). The aquarium trade appears to be the main driver behind the increasing risks to aquatic ecosystems associated with the internet trade (Derraik & Phillips 2010).

While most attention has been paid to the role of the international trade in species introductions, both deliberately introduced and passenger species (Dehnen-Schmutz *et al.* 2007), it is equally important to ascertain the rate of repeated local introductions of invasive species, not only through local outlets, but also through the general public discarding unwanted material (Kay & Hoyle 2001; Duggan 2010). There is a positive relationship

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between the number of propagules of a species released into systems and the chance of that species being able to establish (Cohen *et al.* 2007). In South Africa there are approximately 160 pet and aquarium traders registered with the South African Pet Traders Association (SAPTA) (SAPTA 2008), but there are also numerous unlisted vendors and traders. Both the general public and plant dealers often misidentify and/or do not know the ecological repercussions of the species they are dealing with. The lack of knowledge regarding invasive species results in less care given to the overflow of ponds or the disposal of plants, which are often discarded into ponds, ditches, streams and rivers (Duggan 2010).

Invasive submerged plants, arising from aquaria releases, pose a significant environmental and economic threat to South Africa, but thus far have been allowed to escape and spread with few or no control measures, as most attention has been paid to controlling more obvious floating aquatic plant invasions. Legislation exists in South Africa against the possession, importation, purchase, transportation and introduction of invasive species under the Agricultural Pests Act 1983 (Act No. 36 of 1983) and the Conservation of Agricultural Resources Act (CARA) 1983 (Act No. 43 of 1983, amended 2001). These regulations group species into one of three categories. Each category contains its own set of regulations and prohibitory measures; however, very few submerged aquatic plants are categorised as declared invaders under this Act (Table 3.1) (Henderson 2001). Subsequently, the CARA regulations on invasive species were revised and aligned with the draft regulations on invasive species under the National Environmental Management: Biodiversity Act (Act No. 10 of 2004) (NEMBA). However, delays in the promulgation of these regulations mean that no enforcement has been possible. There is also a list of prohibited species that may not be imported or propagated in South Africa under the Agricultural Pests Act (No. 36 of 1983), which has recently been amended by the South African Department of Agriculture, Forestry and Fisheries (previously Department of Agriculture) and the Directorate of Plant Health, yet this does not appear to be aligned with the NEMBA or CARA regulations.

To date, insufficient research has been conducted on the introduction and movement of aquatic plants, particularly submerged aquatic plants, in South Africa, and on their potential to establish and spread. Aquatic plants are bought and traded through various organisations and private companies throughout South Africa, and often these plants are incorrectly labelled and/or unidentified. Therefore, the aims of this study were to investigate:

- The role of pet stores and aquarists in the introduction, movement and trade of aquatic plants around South Africa
- The extent to which invasive aquatic plant species are imported and traded in South Africa
- Which aquatic plant species are already in South African pet stores and are being traded to the general public
- The knowledge of pet store owners and aquarists with regard to which species they trade in , as well as the associated legislation
- The potential contribution of the internet trade to the introduction and movement of harmful and invasive species into South Africa

## Table 3.1: Plants currently categorised as declared weeds or those that pose a threat to South African waterways, their conservation status and

their current establishment status in South Africa.

Family	Species	Common name	Natural distribution	Legal status	Current status in South Africa
Haloragaceae	Myriophyllum spicatum L.	Spiked water- milfoil	Eurasia	Declared Weed: Category 1: (CARA (Act No. 43 of 1983) <sup>1</sup> ) Importation prohibited (APA (Act 36 of 1983) <sup>2</sup> )	Established
Hydrocharitaceae	<i>Egeria densa</i> Planch.	Dense water weed	Brazil	Declared Weed: Category 1 (CARA (Act No. 43 of 1983) <sup>1</sup> ). Importation prohibited (APA (Act No. 36 of 1983) <sup>2</sup> )	Established
Hydrocharitaceae	<i>Hydrilla verticillata</i> (L. fill)	Hydrilla	Australia, Asia, Central Africa	Proposed Category 1 (prohibited) invader under revised CARA and draft NEMBA regulations <sup>3</sup> . Importation prohibited (APA (Act No. 36 of 1983))	Established (one location)
Hydrocharitaceae	<i>Elodea canadensis</i> Michx	Canadian water weed	North America	Declared Weed: Category 1 (CARA (Act No. 43 of 1983) <sup>1</sup> ) Importation prohibited (APA (Act No. 36 of 1983) <sup>2</sup> )	Established
Cabombaceae	Cabomba caroliniana Grey	Cabomba, fanwort	Native to temperate and subtropical America (Ørgaard, 1991)	Proposed Category 1 (prohibited) invader under revised CARA and draft NEMBA regulations <sup>3</sup> Importation prohibited (APA (Act No. 36 of 1983) <sup>2</sup> )	Cultivated, not yet established
Cabombaceae	<i>Cabomba frucata</i> Schult.	Red cabomba	South America (Ørgaard, 1991)	Importation prohibited (APA (Act No. 36 of 1983) <sup>2</sup> )	Cultivated, not yet established
Alismataceae	<i>Echinodorus</i> <i>cordifolius</i> (L.) Griseb	Spade-leaf sword	Wetlands of Mexico and North America	No legislation Potentially invasive (Henderson and Cilliers, 2002) Importation prohibited (APA (Act No. 36 of 1983) <sup>2</sup> )	Established (one location) (D. Bellstedt, Stellenbosch University, pers. comm.)

1. Conservation of Agricultural Resources Act (CARA), 1983 (Act No. 43 of 1983 amended 2001) (Complete list -Appendix 1)...

2. Agricultural Pests Act, 1983 (Act No. 36 of 1983) (Complete list -Appendix 1)..

3. National Environmental Management: Biodiversity Act (Act No. 10 of 2004) (NEMBA). The draft NEMBA regulations were published in *Government Gazette 32090* on 3 April 2009.

## 3.2 Methods & Materials

Two online surveys were conducted to find out if pet stores and aquarists are possible vectors of submerged aquatic plant invasions in South Africa. This was achieved by creating two separate online questionnaires (Appendix 2). Pet stores were also investigated for the sale of prohibited plant species. Pet store and aquarists surveys were analysed separately.

## 3.2.1 Survey development for pet stores and aquarists

The survey software, SurveyMonkey (SurveyMonkey 2009), was used to create simple, anonymous, easy to use, online questionnaires. The survey presented pet store owners and aquarists with ten questions regarding their knowledge of plants in which they traded (Appendix 2). The surveys were physically taken to pet stores around South Africa and distributed online for aquarists to complete.

## 3.2.2 Selection of test species

Only 12 plant species were included in the survey. A comprehensive list of all blacklisted and invasive plants, under the Conservation of Agricultural Resources Act (CARA), 1983 (ACT No. 43 of 1983, amended 2001) and the list of species prohibited for importation or propagation under the Agricultural Pests Act, 1983 (Act No. 36 of 1983) would have proved too cumbersome and time consuming for respondents. The 12 test species<sup>2</sup> were selected for the following reasons:

**Common aquarium, established out of cultivation in South Africa, non-native harmful species**: Canadian water weed, dense water weed, spiked water-milfoil, hydrilla and spade-leaf sword are regarded as dangerous invaders to South Africa and

<sup>&</sup>lt;sup>2</sup> Common names were used in this chapter because the chapter was published with common names and sent to pet stores and aquarists who prefer the use of common names

have established in South African waterways (Henderson & Cilliers 2002; Coetzee *et al.* 2011a; Bellstedt pers. comm. 2009) (Table 3.1).

**Common aquarium, potential non-native harmful species**: Red cabomba is often confused with fanwort, and both are potential invader species in South African waterways (Coetzee *et al.* 2011a; Henderson & Cilliers, 2002) (Table 3.1).

**Common aquarium species**: Tape grass (*V. spiralis*), a cosmopolitan species, and hornwort (*C. demersum*), which is indigenous to the Old World, are common aquarium plants but are not classified as invasive (Cook 2004).

**Indigenous alternative species**: South African oxygen weed (*L. major*), *Lagarosiphon muscoides* and curly pond weed (*Potamogeton crispus* L. (Potamogetonaceae)) are common species indigenous to South Africa that could easily be utilised as alternatives to invasive aquarium species (Cook 2004).

All common names, alternate names and scientific names were provided on the questionnaires. No pictures were provided.

Three aquatic plant dealers/pet store owners and three members of an aquatic plant society completed an initial version of the surveys to assess the difficulty of the survey. In response to their comments, a few minor changes to the questions were made. The complete questionnaires were then uploaded to the internet for completion by respondents.

The survey was also taken to approximately 125 pet stores trading in aquatic plants in South Africa. Locations visited included Johannesburg, Pretoria, Port Elizabeth, East London, Grahamstown, King Williams Town, Jeffrey's Bay, Bethlehem, Bloemfontein, Cape Town, George, Knysna, Riebeeck Kasteel, Stellenbosch, Somerset West, Durban, Pietermaritzburg, Ballito, Hillcrest, Pinetown and Richards Bay. Of the 125 pet stores visited, twenty stores failed to complete the survey, 35 refused to complete the survey, five pet store owners requested to return the survey via the postal service but the survey was never received, and one store prohibited entry onto to their premises. A total of 64 surveys were completed by pet store owners. Data from questionnaires were collected and tested for correlations between key questions.

In order to promote the survey to aquatic plant enthusiasts, it was advertised in the SAPTA newsletter (August, 2009), 20 000 copies of which were produced and distributed nationally. All 160 members of SAPTA were sent an e-mail message from the SAPTA secretary requesting their co-operation in the survey. The survey was advertised and distributed on popular aquarist blog pages, internet sites, by word-of-mouth and via e-mails within the aquarist communities. The survey was advertised for four months, and 23 surveys were completed by aquarists. The survey focused on informed aquarists that frequent aquatic blogs and aquatic plant internet sites.

An internet search for the sale and distribution of prohibited and/or invasive aquatic plants in South Africa was also conducted. Search engines such as *Google* and *Yahoo* were initially used, but business websites were also examined, as were forums on aquatic plant enthusiasts, clubs and societies. Common names and choice key words were utilised for the searches (e.g. cabomba, oxygen plant or aquarium plant). Once a list of aquarium or water garden plants for sale was located, the list was examined for invasive and harmful species. A vendor was classified as a business or person selling plants for a monetary income; a hobbyist discarding unwanted or surplus plants would not be classified as a vendor. A second determinant of a vendor was that they had to post or courier live plants to the customer. The search was conducted to give a broad perspective of the extent of the online aquatic plant trade in South Africa. The plants were not purchased to find out if they were correctly identified by vendors, nor ordered to see if they would be delivered via the postal service, as this was not the aim of the search. The search did provide insight into the trade in South Africa thereby bringing attention to the inherent and documented dangers of the online live organism trade.

## 3.3 Results

### 3.3.1 Pet stores

Sixty-four pet stores responded to the questionnaires. Of the pet store respondents, 34% could not identify a single test plant species presented on the survey. Less than 15% of respondents were able to identify spiked water milfoil, hydrilla and *L. muscoides* (Fig. 3.1). Tape grass (61 %), Canadian water weed (52%), spade-leaf sword (46%) and hornwort (61%) proved to be the most recognisable species to pet store respondents; 41% could identify both red cabomba and fanwort (Fig. 3.1). There was a strong correlation between the ability of a pet store respondent to identify specific plant species and whether they had the opportunity to acquire the plant species ( $R^2$ = 0.97, P < 0.5).



**Figure 3.1:** The percentage of pet store respondents able to successfully identify the test species provided.

Even though the percentage of pet store owners able to identify the test species was low, 50% of pet store respondents had the opportunity to acquire many of the test species (Fig. 3.2a). The least available species included hydrilla (12%), *L. muscoides* (12%) and spiked water-milfoil (15%), whereas tape grass (82%) and spade-leaf sword (74%) were the most common test species available to pet stores (Fig. 3.2a). There was a strong correlation between what species the pet store respondents were able to acquire and what species they had in stock for sale ( $R^2 = 0.92$ , *P* < 0.05). Test species that pet stores had offered for sale at some stage included tape grass (71%), spade-leaf sword (68%), oxygen weed (47%) and hornwort (49%), whereas hydrilla (3%), spiked water milfoil (7%), oxygen weed (21%) and *L. muscoides* (17%) were stocked the least. The prohibited species, Canadian water weed (38%), fanwort (43%), red cabomba (38%) and dense water weed (48%), were also stocked regularly by the pet stores (Fig. 3.2b).

Ninety-four percent of pet stores received their test species from suppliers rather than growing their own plants, while mail order or self-collections were infrequent modes of plant

acquisition. A few respondents described how they collected dense oxygen weed, hornwort, Kariba weed and water hyacinth from water bodies in their area to sell in their stores. With regard to selection of test species, 84% of pet stores selected their stock based on availability, followed by demand (25%) and aesthetic value (18%). Only a few stores had stock imported from abroad, including Malaysia, the Netherlands and Singapore.

а



Figure 3.2a: The percentage of pet store respondents able to acquire the test species. **b** The percentage of respondents who stocked the test species.

The majority of pet store owners were not aware of the CARA (63%) or NEMBA (72%) regulations. Respondents who had knowledge of the regulations did not personally agree with the regulations, with the common explanation that they were not consulted and/or informed of the regulations.

Pet stores' stock was investigated during the time of the survey; however, the species were not purchased to confirm their identification. Prohibited species as well as species not permitted for import into South Africa available at pet stores included: red water fern (A. *filiculoides*) water lettuce (*P. stratiotes*), Kariba weed, all prohibited under CARA, 2001; and Hygrophila sp. (Acanthaceae), Asian ambulia (Limnophylla sessiliflora (Vahl) Blume (Plantaginaceae)), and sagittaria (Sagittaria platyphylla (Engelm.) J.G. Sm (Alismataceae)), prohibited under the draft regulations on invasive species under the NEMBA (Act No. 10 of 2004). Furthermore, broadleaved Anubias (Anubias barteri Schott (Araceae)), dwarf Anubias (Anubias barteri var. nana (Engler) Crusio (Araceae)), water trumpet (Cryptocorvne sp. (Araceae)), Cryptocoryne wendtii de Wit (Araceae), water chestnut (Trapa natans L. (Lythraceae)), melon sword *Echinodorus osiris* (Alismataceae), Echinodorus sp. (Alismataceae), Ludwigia sp. (Onagraceae), Myriophyllum sp. (Haloragaceae), yellow waterlily (Nymphaea mexicana Zucc (Nymphaea)), Rotala macrandra Koehne (Lythraceae), and Vallisneria sp. (Hydrocharitaceae) all of which were also recorded. These species are prohibited for importation into or propagation in South Africa on the Department of Agriculture import permit (Pests Act, No. 36 of 1983), yet do not appear on any of the CARA or NEMBA regulations.

## 3.3.2 Aquarist respondents

Twenty-three aquarists responded to the questionnaire. In contrast to the pet store surveys, over 50% of aquarist respondents could successfully identify all of the species on the test list,

with tape grass (91.3%) and hornwort (91.3%) the most frequently positively identified. *Lagarosiphon muscoides* and curly pondweed species proved to be the most difficult test species for aquarist respondents to identify (Fig. 3.3).



**Figure 3.3:** The percentage of aquarists who claimed to be able to successfully identify test species.

All of the aquarist respondents had the opportunity to acquire the majority of the test species. Tape grass (87%), dense water weed (70%), Canadian water weed (70%), fanwort (79%), red cabomba (74%), hornwort (96%) and spade-leaf sword (78%) were the most prevalent species available to aquarists (Fig. 3.4a). Hydrilla (39%), oxygen weed (34%) and *L. muscoides* (30%) were the least common species available to aquarists (Fig. 3.4a). There was a strong correlation between the percentage of aquarists who had the opportunity to acquire the test species and the percentage of aquarists who had at some stage kept the test species in their aquarium or planted tank (R<sup>2</sup>=0.95, P < 0.05), with *L. muscoides* (4%) and curly pond weed (21%) being the least kept species. Tape grass (74%), dense water weed (70%), and

Canadian water weed (56%), fanwort (65%) hornwort (91%) and spade leaf sword (65%) were most commonly cultured by aquarists (Fig. 3.4b). Low numbers of the test species were recorded to be traded or passed between colleagues. Fewer than 30% of aquarists had ever traded or passed on any of the test species; however, some of the test species were still traded, with tape grass (26%), dense water weed (30 %) and fanwort (30%) being the most common (Fig. 3.4c). There was also a strong correlation between the percentage of test species that had at some stage been kept in the aquarist's aquarium or planted with the test species that had been traded or passed on between aquarists ( $R^2=0.82$ , *P*<0.05).

The aquarists surveyed appeared to have good knowledge regarding the threat that prohibited species pose to South African waterways, indicating, in their opinion, that hydrilla (65%), spiked water milfoil (65%), dense oxygen weed (65%) and fanwort (65%) pose the most significant threat to South African waterways. However, they also felt that spade-leaf sword (26%), a potential invader, poses a lesser threat to South African waterways than Hydrilla and spiked water milfoil. It was also noted that no aquarist respondent had ever released any prohibited species into any waterways within the country.



**Figure 3.4 a**: The percentage of aquarists who had the opportunity to acquire test species. **b** Percentage of aquarists who had at some stage kept the test species in their aquarium or planted tank. **c** Percentage of aquarists respondents who traded with the test species

In contrast to the pet store respondents, a high proportion of aquarists were familiar with the CARA (66%) as well as the NEMBA regulations (65%), and the majority of those

respondents did not personally agree with the regulations (87%). A variety of reasons were given for the apparent dissatisfaction by aquarists with the regulations. Common reasons included: firstly, that the lack of information and research regarding aquatic plants species in South Africa has resulted in decisions being made without a suitable knowledge base; secondly, the persons taking the decisions or making recommendations about imports had no experience in aquatic plants; thirdly, suitably knowledgeable aquarists or pet shop owners, of which there are quite a number, were not consulted or were wilfully excluded from decision making; and, finally, a number of respondents raised the point that the guidelines have been amended from Hawaiian and Singapore guidelines which has led to the banning of the importation of some plants that may not actually be invasive in South Africa.

## 3.3.3 Internet survey

Over 40 invasive species to South Africa or species not permitted for import into South Africa were found to be sold online by South African online vendors. However, these species were restricted to only 8 online vendors. Trading of species online for other plants, aquarium fish and sometimes money was common but very difficult to quantify.

Of the eight sites actively selling plants online, only a single site had a large variety of invasive and harmful species for sale. Most of the other sites were limited to a select few invasive or harmful species, namely: broadleaved Anubias (five), dense water weed (four), *Cryptocoryne wendtii* (three) red melon sword (*Echinodorus bathii* Muhlberg (Alismataceae )) (three) and dwarf hygrophila *Hygrophila polysperma* (Roxb.) Anders (Acanthaceae)) (three). *Anubias congensis* N.E. Brown (Araceae)), *Anubias nana*, fanwort, *Cryptocoryne ciliate* (Roxb.) (Araceae), water hyacinth, Canadian water weed, pennywort (*Hydrocotyle leucocephala* Cham. & Schlecht. (Apiaceae)), Ambulia (*Limnophyla sessiliflora* (Vahl)

Blume (Plantaginaceae)), spiked water-milfoil, water lettuce and Kariba weed were recorded for sale on only two South African internet sites.

## **3.4 Discussion**

Intentional release from aquarium into the environment is one of the top five avenues for introduction of non-native invasive aquatic species, but has received relatively little attention from both scientists and policy makers (Padilla & Williams 2004). This study shows that potentially invasive and prohibited plant species are being sold and traded by pet stores and aquarists in South Africa. Identifying and quantifying the effects and threats posed by this trade is invaluable, as prevention of introductions usually proves more cost-effective than post-introduction eradication or control (Leung *et al.* 2002; Coetzee *et al.* 2009). The results of this study are likely to have relevance for the non-native aquatic plant trade of other aquarium taxa in South Africa.

While the survey presents a sample of the potentially invasive and prohibited species in South Africa, it gives adequate representation of the variety and quantity of plants moving through pet stores, allowing for the opportunity to predict potential invasions before they arise. It also highlights the immediate need for the implementation and enforcement of the regulations to prevent the continued trade of invasive species to the general public. In the U.S.A. alone, the total cost of controlling just three escapees – purple loosestrife (*Lythrum salicaria* L. (Lythraceae)), spiked water milfoil, and water-chestnut – exceeds US\$800 million per annum (Pimentel *et al.* 2005). The current survey shows that in South African pet stores similar invasive and prohibited species are frequently sold, which could ultimately cost the country millions to control.

The introduction and establishment of non-native species in the natural environment via the aquarium trade relies on the likelihood that the more organisms that are sold to the general public, the greater the chances of escape, and, ultimately, the greater the chance of establishment (Cohen et al. 2007; Duggan 2010). Quantification of the number of invasive plants that reach the consumer is essential. However, quantification of the risk posed by the trade in South Africa is very difficult because a proportion of pet trader respondents were unable to identify the majority of species being sold, let alone the invasive aquatic species. In a survey in the U.S.A. it was also shown that misidentification in the live organism trade is common and that consumers can never be sure which species they receive (Keller & Lodge 2007). The test plants provided in this survey were chosen because they were deemed to be common aquarium species and should have been easier to identify than some of the other less frequently encountered aquarium species. Interestingly, the type of plant species sold to the public by the pet stores predominantly relied on the availability of plants to the pet store and not on the actual species, thus highlighting the need for education among the general public, as well as pet store owners, on the risks associated with the species. This also highlights the fact that suppliers of aquatic plants should be targeted as the first step in control. If the distributors do not provide invasive species in the first place, prohibited and potentially troublesome plants will not end up in the pet stores.

The lack of knowledge regarding regulations by pet store owners results in the continued importation and trade of invasive species. The most recent example in South Africa is the occurrence of 600 ha of *H. verticillata* in Pongolapoort Dam, which has been traced to Malaysian origins (Madeira *et al.* 2007). It seems that in many cases the culprits are ignorant or misinformed of the potential dangers, rather than intentionally attempting to breach the legislation. Additionally, the legislation itself proves confusing. For example, *Anubias* spp. has been in South Africa for the past 40 years and is quite common, yet it has been included

in the list of species that may not be imported or propagated in South Africa under the Agricultural Pests Act (No. 36 of 1983), in a category stating that it does not occur in the country and must never be allowed in (N Stallard, South African Pet Traders Association, pers. comm. 2010). Numerous other species found during this study are also on this list. This highlights the need for scientists, legislators and industry to have more input into decision making, which should not be conducted by one of these groups acting alone.

A challenge for enforcement is that, outside of the aquarium trade, numerous plants are moved through networks of aquarists who often stay in contact via the internet. Improved transport and packaging technology has made trade between countries and continents possible, making it very difficult for the enforcement of regulations. This study showed that aquarist respondents from this survey have a far greater knowledge than pet store owners of both indigenous and invasive plant species, and was also far more informed about invasive plant regulations. As a result, it is likely that informed aquarists pose a lesser threat to biosecurity than pet stores as no aquarist respondent claimed to have ever considered releasing or actually released a plant species into waterways. However, these data were obtained from informed aquarist respondents who would have knowledge of best practice, and do not represent the casual aquarist who would have more chance of releasing or discarding unwanted live organisms into storm drains or local waterways, the occurrence of which has been documented in multiple examples worldwide (Fuller 2003; Rixon *et al.* 2005; Duggan 2010). However, pet stores have no control over whom they sell plants to, thus uninformed aquarists may still pose a significant threat to biosecurity.

Aquatic plants are known to be dispatched around the world by mail order. The internet trade only makes the task of finding and purchasing mail order plants easier (Champion & Clayton, 2000). In US markets, federal noxious weeds are extensively sold online (Maki &
Galatowitsch 2004). In South Africa, approximately 40 different prohibited or potentially troublesome macrophytes are sold online. This mode of introduction is cause for considerable concern as it is very difficult to control and monitor (Maki and Galatowitsch, 2004).

This study has clearly shown that the movement and trade of submerged invasive species in the aquarium and water garden community poses a substantial risk for introductions into the natural environment. Four areas of risk were identified in this study. Firstly, and most importantly, a variety of invasive and/or prohibited plants are sold by pet stores. Secondly, there is a lack of knowledge regarding identification as well as regulation of submerged species, which may then result in the unintentional trade of potentially invasive species. It seems that in many cases pet stores are ignorant or misinformed of the potential dangers, rather than intentionally attempting to breach the legislation. Thirdly, aquarists own, trade and move plants in and around the country, making it very difficult to monitor which species are being moved around South Africa and to what extent. Finally, the internet is a pathway of potential concern, but it is difficult to quantify its contribution to the trade of invasive species in South Africa. This trade currently remains small but its development should be monitored.

To mitigate the potential negative effects of further aquatic plant invasions in South Africa, the pathways of aquarium plant movement need to be monitored and controlled with more rigour until the regulations and policies are developed, promulgated and agreed upon by the majority of vendors and aquarists involved. Once these regulations are in place, investment and effort has to be made in the education of pet store owners and aquarists regarding the dangers of invasive and harmful plants species.

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# **Chapter 4: Predicting the distributions of invasive submerged aquatic weeds in South Africa using distribution modelling**

# 4.1 Introduction

Invasive species pose a significant threat to both the ecology and economy of a country (Kareiva 1996; Kolar & Lodge 2001; Pimentel 2002; van Driesche *et al.* 2002; Peterson 2003) and are widely recognized as posing the second largest threat, after direct habitat destruction, to the health and biodiversity of native environments throughout the world (Vitousek *et al.* 1997; Wilcove 1998; Davis *et al.* 2000). The increased rate of invasion over the past century is largely the result of ever-increasing human numbers and activity (NAS 2002; Robinson 2008; Chapter 3). These species invasions have been facilitated by anthropogenic forms of transport such as air travel, shipping and recreational boating, making it difficult to predict when and where a species might invade (Johnstone *et al.* 1985; Floerl & Inglis 2005; Floer *et al.* 2005). Of the invasive species, submerged weeds are particularly problematic, not least because they affect ecosystems and are difficult to control using chemicals or machinery, but also because they develop into monospecific stands, causing disturbances to freshwater systems (see Chapter 2 for examples).

South Africa has a high diversity of habitat types making it susceptible to invasion by exotic species (Rutherford *et al.* 2006). This has resulted in the country having one of the most serious alien plant invasion problems of any country in the world (Richardson & van Wilgen 2004). Of the invasive species, several submerged weeds have become established in rivers and impoundments in the country (Schoonbee 1991, Coetzee *et al.* 2011a). To date, only three submerged aquatic plants are declared weeds or invader species in South Africa, and their control is subject to the Conservation of Agricultural resources Act (Act 43 of 1983), as amended in 2001 (CARA) see chapter 3 for discussion on the potentially invasive species.

These include El. canadensis, Eg. densa and M. spicatum (Henderson & Cilliers 2002). Thus far, there have been no effective long-term sustainable control measures implemented against them in South Africa, despite their declaration as invasive species. These weeds were included on the CARA regulation as precautionary measures, however to date no weed risk assessment has been conducted on any of these invasive species in South Africa. Several additional submerged aquatic weeds have also been identified in South African waters, such as *H. verticillata* regarded as the most economically significant submerged weed in the U.S.A. (Langeland 1996), and C. caroliniana, a weed that is rapidly invading Australia (Schooler et al. 2006). Both these species have been included in the new National Environmental Management: Biodiversity Act (Act No. 10 of 2004) (NEMBA) which is currently being promulgated. Again, these are precautionary measures as very little is known about these weeds in South Africa, including their current and potential distributions. Hydrilla verticillata is recorded from only one water body, Pongolapoort Dam in KwaZulu-Natal, while C. caroliniana was cultivated by an aquatic plant dealer in the same province until 2008 (R. Glen pers. comm. 2008). Through trade, particularly the aquarium trade (Martin & Coetzee 2011; Chapter 3), it is likely that these could become problematic invasive species in South Africa.

The majority of submerged invasive plants in South Africa, USA and New Zealand were introduced via the aquarium trade as ornamental aquarium plants (Schmitz *et al.* 1997). Despite their status as declared invaders in many countries, they continue to be sold, particularly via the internet (Kay & Hoyle 2001; Martin and Coetzee 2011). A recent study in South Africa showed that a variety of invasive and/or prohibited plants are sold by pet stores, and are owned, traded and moved by aquarists around the country (Martin & Coetzee 2011; Chapter 3), making it very difficult to monitor which species are being moved around South Africa and to what extent. Thus there is a need for technologies or techniques that can help

ascertain where efforts should be focussed to ensure that invasions by submerged species do not occur, and to monitor the species which have already established in South African freshwater systems. The modelling of species distributions using climate and distribution data is one such technology used to predict invasive species' potential distributions (Jimènez-Valverde *et al.* 2008; Webber *et al.* 2011).

With the improvement of computer technology, significant progress has been made with regard to distribution modelling, making the process more user-friendly and feasible. The modelling of species' distributions is now considered central to diverse applications within ecological, evolutionary and conservation science (Elith *et al.* 2006). It has also been extensively used to predict potential distributions of invasive species around the world (Tucker & Richardson 1995; Peterson *et al.* 2003; Peterson 2005; Thompson *et al.* 2011) including South Africa (Rouget *et al.* 2004; Thompson *et al.* 2011).

A technique such as distribution modelling is designed to provide some form of early warning of the potential for new invasions, as well as to be a valuable tool for invasive species risk assessments. Because information on the distribution of non-indigenous aquatic species is generally widely scattered and difficult to find, resource managers, scientists, policymakers, and even the public could benefit from access to information on distribution, status, and possible introductory pathways and habitat of non-indigenous species (Benson *et al.* 2004), as accessibility to information allows for improved decision-making for the management of invasive species. For example, water resource managers have traditionally responded reactively to invasions by non-native aquatic plants because of the major threat that they pose (Madsen 1999), yet knowledge of these threats prior to invasion could pre-empt these reactive responses.

This chapter aimed to firstly identify areas suitable for the establishment of five potentially invasive submerged aquatic species: *Eg. densa, H. verticillata, El. canadensis, C. caroliniana* and *M. spicatum* using the computer program MAXENT; secondly, to determine the suitability and accuracy of MAXENT in predicting the potential distributions of submerged freshwater aquatics in South Africa using easily accessible distribution and environmental data. Finally, it aimed to create a methodology to guide weed risk assessors, policy makers, and freshwater managers in determining potential distributions of submerged invasive plants in South African freshwater systems, with the ultimate goal of providing initial steps that could be used to create an early warning system for potential invaders into South Africa.

# 4.2 Methodology

There are two types of predictive modelling tools: correlative models, which predict the potential distribution of an organism by using positive (present) or negative (absent) locality records and a set of predictor variables, in the form of digital maps (Robertson 2004), and the more complex mechanistic models that simulate the actual processes that produce the apparent correlations (Beerling *et al.* 1995; Robertson *et al.* 2003). Mechanistic models are often robust in predicting whether a species could occur in a given environment, and one of the most accessible and user-friendly mechanistic modelling techniques available is the program CLIMEX (Maywald & Sutherst 1991; Sutherst & Maywald 1985). Although mechanistic models are desirable and arguably lead to the best estimates of potential distributions (Anderson & Raza 2010), these types of species distribution models (SDM) were not feasible for this study because the expertise and resources to paramatise the models, especially for submerged macrophytes, are limited. Mechanistic models predominantly use data that are laboratory-generated and give a representation of the species' fundamental niche, an area in space where a species can maintain viable populations under a given set of

environmental constraints. They do not however predict the realized niche, i.e. the actual areas where a species is located. The realized niche can be defined as space that an organism inhabits and the resources it can access as a result of limiting pressures from other species such as pollinators, competitors, predators and parasites (Ulrichs & Hopper 2008). Furthermore, physiological data are difficult to obtain for some species, especially the more cryptic unstudied aquatic species, and generating one's own physiological data is time consuming and expensive. Contrary to mechanistic models, the data required to fit correlative models are widely available and easy to obtain (Elith *et al.* 2010), and were used for this study.

Most of the SDM currently generated use a correlative approach (Webber *et al.* 2011) and can be divided into two groups based on the input data used to build them. These include group discrimination techniques, which make use of both presence and absence data, and profile techniques which make use of presence-only data (Caithness 1995). As absence data are typically expensive and time consuming to collect, models using presence-only data are more commonly used. When using presence-only data to make predictions, one can use either a standard profile technique, or opt for a group discrimination technique that makes use of artificially generated pseudo-absence data (Zaniewski *et al.* 2002). There are numerous different profile techniques that use a variety of algorithms, including the programs BIOCLIM (Busby 1991) and DOMAIN (Carpenter *et al.* 1993), and factor analysis (Hirzel *et al.* 2002) and Principal Components Analysis in the program Floramap (Jones & Gladkov 1999). There are several different types of group discrimination techniques, but the most popular of these are Generalized Additive Models (GAM) (Austin & Meyers 1996) and Generalized Linear Models (GLM) (Pearce & Ferrier 2000; Guisan & Zimmerman 2000). However the program MAXENT (Phillips *et al.* 2006) is regarded as one of the better

distribution modelling software packages available as it produces the most robust results (Elith *et al.* 2011; Thompson *et al.* 2011).

### 4.2.1 Maximum Entropy (MAXENT)

MAXENT is a general-purpose method used for making predictions or inferences from incomplete information (Phillips *et al.* 2006) and is regarded as one of the premier distribution-modelling software packages available (Elith *et al.* 2011; Thompson *et al.* 2011). MAXENT allows one to estimate (approximate) the probability distribution of a species (Phillips *et al.* 2006). The best approach is to ensure that the approximation has maximum entropy, where entropy is defined as how much 'choice' is involved in the selection of an event. Thus, maximum entropy refers to maximum choice. Maximum choice is available when there are few constraints (environmental layers); i.e. unnecessary environmental layers or occurrence data should be avoided as they only serve to clutter the model and reduce the choices available (Negga 2007).

The software uses a set of input layers, or environmental variables (such as temperature and precipitation) as well as a set of georeferenced occurrence locations or 'training data' (Phillips *et al.* 2006). The model then expresses the suitability of each grid cell as a function of the environmental variables at that grid cell. A high value of the function at a particular grid cell indicates that the grid cell is predicted to have suitable conditions for that species (Phillips *et al.* 2006). The computed model is then a probability distribution over all the grid cells. The distribution chosen is the one that has maximum entropy subject to some constraints: it must have the same set of characteristics for each feature (derived from the environmental layers) as the average over sample locations (Phillips *et al.* 2006). If an area in the study has similar distribution as the training data, then higher values are assigned, and accordingly, areas with different distribution are assigned lower values (Negga 2007).

#### 4.2.2 Bioclimatic variable selection

The distribution, productivity and species composition of submerged aquatic communities are affected by various environmental factors (Herb & Stefan 2003), but the abiotic factors such as light, water temperature, sediment composition and inorganic carbon availability are considered to be the most important (Barko & Smart 1981). These four factors shape the structure of submerged aquatic communities at localised and regional scales. Of these four factors, light and water temperature are generally considered most important in determining the distribution of species at both localised and regional scales compared to sediment composition or inorganic carbon availability.

Water temperature and light together have been shown to be key in submerged macrophyte species' growth and morphology (Barko *et al.* 1982), photosynthesis (Barko & Smart 1981), chlorophyll composition (Barko & Filbin 1983) and reproduction (Chambers *et al.* 1984). Under natural conditions, light plays an important role in seasonal changes in macrophyte dominance and in interspecific competition (Barko *et al.* 1986) and distribution at the community level (Spence 1982). The effect of temperature, although not having received as much attention as light, is considered important in determining the distribution of submerged aquatic species also at the community level (Masini & Manning 1997; Livingston *et al.* 1998; Rooney & Kalff 2000) and at a regional scale (Skulthorpe 1967). The characteristics of water are dependent on light as seasonal changes in photoperiod and incident solar radiation are known to promote corresponding changes in water temperature in most aquatic systems (Barko *et al.* 1986). Thus the influence of light on submerged macrophytes cannot be properly evaluated without also considering the influence of temperature.

# 4.2.3 Geographically defined background

In forming a model, many of the computer programs compare the environmental conditions of localities from where a species has been recorded to a random set of samples taken from the entire study area (background sample), or from all pixels lacking presence data (pseudoabsence sample). Despite the different definitions, in practice the two are very similar (Anderson & Raza 2010). When recorded absence data are unavailable, MAXENT creates pseudo-absence data drawn randomly from a geographically defined background, from pixels lacking presence records. Furthermore the size background from which pseudo-absence data are obtained can significantly influence model results (Anderson & Raza 2010), Thompson et al. 2011), therefore it is recommended that the background should be restricted to areas within which the species could reasonably be expected to occur (Elith et al. 2010). Thompson et al. (2011) add that when selecting background size, a balance needs to be found between good regional performance driven by climate variables and one that can perform reasonably at a continental scale by not being constrained by a reduced set of variables largely unrelated to the species in question. To do this, one would restrict the size of the background to areas where the plant is likely to occur. However, numerous aquatic plant species have extremely large ranges due to the uniformity of aquatic environments (Cook 1985).

Aquatic plant species tend to inhabit larger ranges than closely related terrestrial species (Santamaría 2002). Some even have broad, worldwide ranges, e.g. *C. demersum, Lemna minor* L. (Araceae), *S. pectinata; Typha latifolia* L. (Typhaceae) and *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae), which is probably the most cosmopolitan angiosperm on Earth (Santamaría 2002). Aquatic plants can extend their ranges via anthropogenic introductions, becoming serious pests in regions outside their native range. For example, the native range of *H. verticillata* includes parts of Asia, Australia and Africa, but it has been

introduced to almost every other continent except Antarctica (Langeland 1996). The genus *Myriophyllum* is also found on every continent except Antarctica (Smith & Barko 1990), while the distribution of *M. spicatum* ranges between areas of different climatic conditions. The distribution of the genus *Cabomba* covers three broad climate zones: equatorial; tropical with summer rains; and warm temperate climate (Ørgaard 1991). Jacobs and Wilson (1996) conclude that for aquatic plants, local speciation is of occasional importance but proliferation can occur easily in certain regions following arrival via long-distance dispersal.

It is recognized that potential distributions for some species, especially invasive species and pioneer species, may be underestimated due to the lack of opportunity for a species to be introduced into an area which may be climatically suitable rather than limiting climatic conditions (Rouget *et al.* 2004). Similarly, for those species where distribution is associated with human-induced disturbance more than environmental influences, such as climate, this may result in the potential distribution being over- or under-estimated (Rouget *et al.* 2004). For this study, it was also accepted that none of these programs was specifically created with the intention of predicting submerged species distributions as numerous climatic factors are buffered by water. Despite these potential limiting factors, the ability to model submerged macrophytes should be investigated as the use of ecological spatial modelling to forecast the spread of invaders is a frontier of biological invasions (Anderson & Raza 2010). The application of these SDMs over multiple scales promises to improve the understanding of the processes and driving factors of the spread of invasive species (Phillips *et al.* 2006).

# 4.3 Methods & Materials

#### 4.3.1 Distribution records

In order to have the most complete understanding of a species' known distribution, as many occurrence records as possible were sourced from the internet, available literature and through collaborating organisations for the five selected test species. The number of locality records is provided in Table 4.1. The records were scrutinised and 'cleaned' as follows: only records that were sufficiently accurate were retained, records that were older than 50 years were discarded, as misidentifications are more common in older specimens, and duplicates were removed. Maps of occurrence data were made in ArcMap 10 (ESRI 2012) and checked for outliers - i.e. points in the ocean or on dry-land; any outliers and points deemed to be erroneous were removed.

The amount of pseudo-replication in a model depends on the distance between sample points (i.e. a set of closely spaced localities (a single water body) effectively provides less information than the same number of observations more widely separated in space). Such spatial dependency is termed spatial autocorrelation (Parolo *et al.* 2008) and, although often overlooked, may bias model accuracy (Parolo *et al.* 2008). To avoid pseudo-replication the number of locality records was spatially reduced by selecting one record per 10'grid cell. This method, unlike random point removal, retains peripheral points that may be important to the model (Trethowan *et al.* 2010). Reducing the number of occurrence records to one locality per 10' cell reduces the sampling bias.

The majority of the species had high numbers of invaded range localities, with fewer indigenous range localities. The highest number of invaded range localities was 8319 for *El. canadensis* and 7529 for *H. verticillata* (Table 4.1). South African occurrence records were

sourced from the Southern African Plant Invaders 115 Atlas (SAPIA, Henderson 2009) and from data collected annually from nationwide surveys of South African water bodies conducted by Rhodes University.

Table 4.1: Source and number of occurrence data points used in species distribution models.

Species	Sauras Isaalitu data	Occurrence	Number of records used in SWD	South African
Species	Source locality data	data points	Iormat	localities
canadensis	Global Biodiversity			
indigenous range	Information Facility 2012	952		
indigenous runge		<i>,,,,</i>	-	
<i>Elodea</i> canadensis invaded range	Global Biodiversity Information Facility, 2012, South African National biodiversity institute 2012,	6095	7701	0
<i>Egeria densa</i> indigenous range	Global Biodiversity Information Facility, 2012	16		
Egeria densa invaded range Hydrilla verticillata	Global Biodiversity Information Facility, 2012,South African National Biodiversity Institute 2012, Rhodes University survey data	924	76	15
indigenous range	Australian National Herbarium	428		
Hydrilla verticillata invaded range	Global Biodiversity Information Facility, 2012 (http://www.gbif.org/), South African National Biodiversity Institute 2012, Rhodes University survey data	2642	1716	3
Cabomba				
caroliniana	Global Biodiversity	20		
Indigenous range Cabomba caroliniana invaded range	Global Biodiversity Information Facility, 2012	510	241	0
Myriophyllum				
spicatum	Global Biodiversity			
indigenous range	Information Facility, 2012	16159		
<i>Myriophyllum</i> <i>spicatum</i> invaded	Global Biodiversity Information Facility, 2012, South African National Biodiversity Institute 2012, Rhodes University survey data	2916	16254	14

### **4.3.2 Bioclimatic variables**

Suitable bioclimatic predictor variables associated with the distribution of submerged species were selected and downloaded from the WORLDCLIM database (Hijmans *et al.* 2005) (http://biogeo.berkeley.edu/worldclim/worldclim.htm). These data included an uncorrelated range of averages, outliers and seasonal variations (Hijmans *et al.* 2005). The predictor values were obtained from a number of 'bioclimatic variables' (BIO x), which included mean annual temperature (BIO 1); mean diurnal range (mean of monthly (maximum temperature - minimum temperature)) (BIO 2); isothermality (BIO 3); temperature seasonality (BIO 4); maximum temperature of warmest month (BIO 5); minimum temperature of coldest month (BIO 6); temperature annual range (BIO 7); mean temperature of warmest quarter (BIO 8);mean temperature of driest quarter (BIO 9); mean temperature of warmest quarter (BIO 10); and mean temperature of coldest quarter (BIO 11).

#### **4.3.3 MAXENT**

For the purpose of this study, default MAXENT parameters, with 500 iterations and 0.00001 convergence threshold were used when running the program. 'Logistic output' that creates a continuous, linear scaled map which allows fine distinctions to be made between the modelled probabilities of habitat suitability; 'create response curves'; and 'jackknife measures of variable importance' were also used in the models. Additionally, 'do clamping' which resets values that are outside the range found in the study area to match the upper or lower values found in the study area, and a regularisation value of 1, were applied, to avoid overfitting. The feature type was restricted to 'hinge features' to create smoother response curves to focus models on the 'strongest trends' in the data (Elith *et al.* 2010). This approach was recommended for introduced species because it produces models that are likely to be

more ecologically realistic (Elith *et al.* 2010; Thompson *et al.* 2011). A large background including all the continents, with the exception of Antarctica, was used.

Species ditribution models were made using the species native range distributions. This technique is probably an underestimation as areas where the species have become invasive outside the indigenous range were not included. In a separate model, models of distributions based on the invaded range for each species were created, excluding data from South Africa.

Then using the models from the native and the invasive rages, an 'R' (Hornik 2012) script was created (R version 2.15.0) using the package 'Sp' (Pebesma & Bivand 2005), which produces a file in SWD (''samples with data'') format which allows the inclusion of both presence and absence data. The 'training data set' used to run the final model comprised 100% of the native range data and 70% of the introduced range data. This split was recommended by Trethowen *et al.* (2011) to avoid sampling bias. The independent 'testing data set', used to test the accuracy of the model, comprised 30% of the introduced range data and 30% testing was done randomly. As there can be considerable variation in the performance of models when choosing a particular random selection of points for the training and testing sets, it is best to make several random selections (splits). This allowed for five models to be created for each species, so that the models could be compared for consistency.

## 4.3.4 Model evaluation

Model evaluation is an essential component of the model building process, but it is often neglected. Model evaluation allows the user to objectively assess the quality of the model's predictions. Without an objective assessment, the accuracy of the model is unknown. The best means of objectively evaluating model performance is to use an independent set of locality records and a quantitative accuracy measure.

The models were evaluated with Jackknife analyses and Area Under Curve (AUC) statistics for each replicate, generated by MAXENT. Response curves were also examined to check if models were plausible. Other measures of predictive success are available (Fielding & Bell 1997), but AUC has been found to be robust for uneven prevalence in observations of occurrence that can produce artefacts in other performance measures (McPherson *et al.* 2004), however even the AUC accuracy has been questioned (by Lobo *et al.* 2008), predominantly when models predict a potential species distribution from presence-only data, as the 'true' potential distribution is unknown (Pearson *et al.* 2007). Consequently, in evaluating presence-only models, the ability to give a realistic prediction of species occupancy potential in the study area is considered to be more important than model accuracy (Lobo *et al.* 2008). Detailed descriptions of these AUC curves and Jackknife analyses appear in Pearson *et al.* (2007). The Jackknife method is used to identify the most influential predictor variable(s).

The AUC values generated from the model can range between 0.5 and 1.0, where 0.5 is no better than a randomly generated model and 1 is excellent. It is generally accepted that an AUC of less than 0.8 is a poor model, between 0.8 and 0.9 is a fair model, between 0.9 and 0.995 a good model, and >0.995 an excellent model (Fielding & Bell 1997; Trethowan *et al.* 2011). The mean and standard deviation of the five replicate AUC values were calculated for each species, then used to determine the quality of the models.

An additional method of determining the accuracy of the model is to conduct a groundtruthing exercise after the model has been built. Since no records from South Africa were used in the creation of the models, ground-truthing could be conducted. Species with known localities in South Africa were visually checked, to see if locations where they have been recorded in South Africa were located within the areas of potential distribution. Additionally the points were used as 'test data' in the model to ascertain the accuracy of the models with a second independent data set (Table 4.1). These data were collected or confirmed from nationwide surveys conducted by Rhodes University or obtained from SAPIA (Henderson 2009).

Although MAXENT results can be seen in a picture format, greater visualization and control over the visualization is possible in ArcMap 10. The MAXENT images were transferred from ASCII files to Raster files in ArcMap, to allow for data manipulation. In ArcMap 10, under symbology, a "Minimum-Maximum" as the Stretch Type was selected to emphasize areas of risk. Maps for the five species were generated in ArcMap.

# 4.4 Results

*Elodea canadensis* and *C. caroliniana* have already been recorded in South Africa in the aquarium trade, but neither has had confirmed sightings of establishment outside cultivation. Conversely, *Eg. densa*, *M. spicatum* and *H. verticillata*, which are also cultivated by South African aquarists, are all established and widely distributed in South Africa (Chapter 2).

# 4.4.1 Environmental variables

The environmental variables identified as most suitable for the SDM were subjected to Jackknife analysis to find out the influence of variables, for all weed species. The Jackknife analysis indicated that the variables of least overall influence on submerged invasive species in South Africa were temperature annual range (BIO 7), followed by mean temperature of wettest quarter (BIO 8) and mean temperature of driest quarter (BIO 9) with respective ranks of 6.6, 7.2, and 8.2 out of 11 (Table 2). The Jackknife also indicated that overall, for all

species, annual mean temperature (BIO 1) with a mean rank of 2.2 out of 11 had the greatest contribution to the models (Table 4.2). Mean temperature of warmest quarter (BIO 10), and mean temperature of coldest quarter (BIO 11) also had significant contributions. Annual mean temperature had the most influence for three of the five taxa. (BIO 7), one of the lowest contributors to the other species, significantly influenced the distribution of *M. spicatum* which probably resulted in the poorly fitted models; the reasons for which are discussed later. The poor-fitting models of *M. spicatum* increased the standard deviation amongst the ranks of the other species; removing *M. spicatum* would have reduced the standard deviation.

**Table 4.2:** Jackknife analysis for individual climate variables used to predict the distributions of five submerged invasive plants. The predictor variables are arranged by mean rank across all species. Numbers in bold indicate variables of most influence in a particular species, while numbers in italics indicate the variable with the least influence.

Rank		_	Egeria densa	Hydrilla verticillata	Elodea canadensis	Cabomba caroliniana	Myriophyllum spicatum	
Mean	S.E.	Variable*	Percent contribution					
2.2	1.0	BIO 1	0.08	0.33	0.40	0.41	0.34	
6.6	1.3	BIO 2	0.00	0.01	0.26	0.03	0.00	
4.2	1.2	BIO 3	0.14	0.17	0.12	0.00	0.12	
4.4	1.3	BIO 4	0.40	0.05	0.03	0.20	0.03	
4.8	0.7	BIO 5	0.09	0.07	0.07	0.16	0.00	
5.6	0.8	BIO 6	0.08	0.03	0.07	0.09	0.00	
6.6	1.9	BIO 7	0.01	0.01	0.00	0.09	0.36	
7.2	1.2	BIO 8	0.00	0.02	0.04	0.00	0.14	
7.0	1.1	BIO 9	0.00	0.16	0.01	0.01	0.00	
5.6	1.1	BIO 10	0.20	0.11	0.01	0.01	0.00	
8.0	0.9	BIO 11	0.00	0.06	0.00	0.00	0.01	

\*(BIO 1); mean diurnal range (mean of monthly (maximum temperature - minimum temperature)) (BIO 2); isothermality (BIO 3); temperature seasonality (BIO 4); maximum temperature of warmest month (BIO 5); minimum temperature of coldest month (BIO 6); temperature annual range (BIO 7); mean temperature of wettest quarter (BIO 8); mean

temperature of driest quarter (BIO 9); mean temperature of warmest quarter (BIO 10); and mean temperature of coldest quarter (BIO 11)

### 4.4.2 Invaded range – South Africa

Five potential models for each test species were created in MAXENT; of the five models, the model with the highest Area Under Curve (AUC) value was selected and is shown below. The mean AUC was calculated for the five models.

#### Egeria densa

*Egeria densa* was first recorded in the Durban area, South Africa, in 1963 and has since spread to both flowing and still water systems throughout South Africa, favouring the Pietermaritzburg, Durban and Tongaat areas in KwaZulu-Natal (Coetzee and Martin 2011; Coetzee *et al.* 2011a) Rhodes University survey data 2012). It has also been recorded in the Eastern Cape, in the Nahoon River, East London (pers. obs.), in the Bakaans and Swartkops Rivers, Port Elizabeth, in the Western Cape in the Liesbeeck River in Cape Town, and Berg River in Paarl and in systems near Stellenbosch (Henderson 2009). Internet searches of herbarium records produced 1258 invaded range and 30 indigenous range locality records that could be used.

To calibrate the model, 1258 invaded range records and 16 indigenous records were used to produce a SWD format for training a MAXENT model. The mean AUC for *Eg. densa* was  $0.977 \pm 0.018$ , indicating an excellent model. The SDM indicated the potential for *Eg. densa* to spread to large parts of South Africa (Fig.4.1). Regions of highest suitability for *Eg. densa* included areas just inland from the east coast of South Africa, from the Mozambique border down the coast to Knysna, as well as areas around the Soutpansberg in Limpopo Province (Fig. 4.1). The model also predicted large areas of the Zimbabwe interior to be suitable for

*Eg. densa* (Fig. 4.1). The model was re-run with 15 known localities from South Africa to determine how accurate the model was at projecting the potential distribution of *Eg. densa* in South Africa (Fig. 4.2). The test AUC was  $0.951 \pm 0.009$ , indicating once again that it was an excellent model. All known distribution records of *Eg. densa* within South Africa fell within the predicted area of the SDM.



**Figure 4.1:** Areas suitable for *Egeria densa* in southern Africa, as predicted by MAXENT. Darker shading represents areas of higher suitability.



**Figure 4.2:** Distribution records of *Egeria densa* in South Africa from South African National Biodiversity Institute (SANBI) and Rhodes University Surveys. Triangles indicate major towns. Open circles indicate sites not confirmed within the last five years, closed circles indicate sites where the plant's presence has been confirmed within the last five years in the provinces: Gauteng (GT), Mpumalanga (MP), Limpopo (NP), North West (NW), KwaZulu-Natal (KZ), Eastern Cape (EC), Western Cape (WC), Northern Cape (NC), and Free State (FS).

# Hydrilla verticillata

The first record of *H. verticillata* in southern Africa was from the Nkomati River in Mozambique near Maputo in 1961 (SANBI 2012). In South Africa it was discovered at the Pongolapoort Dam in 2006. The dam is part of the Pongola catchment that originates in the northern part of KwaZulu-Natal and flows into the Usutu River, which flows into Mozambique. There is also an early record of *H. verticillata* in Tongaat in KwaZulu-Natal. The SDM predicted that *H. verticillata* would be restricted to the north-eastern coastal regions of South Africa, and up into Mozambique, Swaziland and the northern-most part of South Africa, bordering Zimbabwe (Fig. 4.3). The AUC for the five models was  $0.974 \pm 0.0024$ , once again indicating an excellent model. The SDM model was re-run using the three known southern African localities as the test samples. The test AUC was  $0.953 \pm 0.003$ . All of the known southern African distributions were found in the SDM prediction within areas of high potential invasion risk (Fig. 4.4).



**Figure 4.3:** Areas suitable for *Hydrilla verticillata* in southern Africa, as predicted by Maxent. Darker shading represents areas of higher suitability.



**Figure 4.4:** Distribution records of *Hydrilla verticillata, Elodea canadensis and Cabomba caroliniana,* in South Africa obtained from the South African National Biodiversity Institute (SANBI) and Rhodes University surveys. Triangles represent major towns. Records are represented by open squares (*H. verticillata*), open pentagons (*El. canadensis*) and open circles (*C. caroliniana*), in Gauteng (GT), Mpumalanga (MP), Limpopo (NP), North West (NW), KwaZulu-Natal (KZ), Eastern Cape (EC), Western Cape (WC), Northern Cape (NC) and Free State (FS) provinces.

# Elodea canadensis

*Elodea canadensis* has only been reported from one location in South Africa (Fig. 4.4) (SANBI 2012); however, neither the locality nor whether this species has persisted has been confirmed. The SDM for *El. canadensis* predicted that very little of South Africa has suitable climate to support its establishment, with a limited potential distribution within a small area in the Free State, Eastern Cape and Lesotho, all with low probability, and only one small area of medium probability around the Lake Gariep Dam and Vanderkloof Dam (Fig. 4.5). The AUC for the model, based on native and invasive range was AUC 0.9594  $\pm$  0.0032, indicating an excellent model.



**Figure 4.5:** Areas suitable for *Elodea canadensis* in southern Africa, as predicted by Maxent. Darker shading represents areas of higher suitability.

# Cabomba caroliniana

*Cabomba caroliniana* was introduced to South Africa via the aquarium trade and has since been recorded in the Berg River in the Western Cape and in the Nahoon River in the Eastern Cape (Fig.4.4), however presence at neither of these sites has been confirmed. It was confirmed at one site in Tinley Manor in KwaZulu-Natal but was subsequently removed (R. Glen pers. comm. 2008). The SDM predicted a limited range for *C. caroliniana* in South Africa, predominantly restricted to the northern coastal part of KwaZulu-Natal around Richards Bay, with areas of medium probability extending down to Durban, including Tinley Manor (Fig. 4.6). The SDM also predicted low probability along the east coast down to Cape Town. Amidst the area of low probability along the east coast were a few areas of medium probability, one on the Berg River in the Western Cape and a small locality near East London, which encompassed the Nahoon River (Fig. 6). The model had an AUC 0.977  $\pm$  0.0176, indicating an excellent model.



**Figure 4.6:** Areas suitable for *Cabomba caroliniana* in southern Africa, as predicted by Maxent. Darker shading represents areas of higher suitability.

# Myriophyllum spicatum

The SDM of *M. spicatum* predicted a very sparse distribution, with virtually the whole country being unsuitable for the establishment of the weed. The estimated areas suitable for establishment occur along the east and south coasts, between Durban in KwaZulu-Natal and Cape Town in the Western Cape, and a small area in the north-eastern region of the Eastern Cape Province and Lesotho (Fig. 4.7). The AUC of  $0.769 \pm 0.0042$  indicated that the model was poor. *Myriophyllum spicatum* has a long history in South Africa, with the first record from the Vaal River in the 1880s. Presently, dense mats occur along an 800 km stretch from Parys on the Free State border to Douglas in the Northern Cape. It has also been recorded in the Nyl, Crocodile, and Breede rivers, and dams in the Bronkhorstspruit, Dullstroom and Underberg regions, but these sites have not been confirmed recently (Fig. 4.8)(SANBI 20010). There are also confirmed records of *M. spicatum* in Lake Sibaya, in northern KwaZulu-Natal (SANBI 2010). The model failed to predict any of the South Africa as the test sample. The AUC for the test sample was  $0.382 \pm 0.009$ , indicating a poor model.



**Figure 4.7:** Areas suitable for *Myriophyllum spicatum* in southern Africa, as predicted by MAXENT. Darker shading represents areas of higher suitability.



**Figure 4.8:** Distribution records of *Myriophyllum spicatum* in South Africa from South African National Biodiversity Institute (SANBI) and Rhodes University Surveys. Open circles indicate sites not confirmed within the last five years, closed circles indicate sites where the plant's presence has been confirmed within the last five years in the provinces: Gauteng (GT), Mpumalanga (MP), Limpopo (NP), North West (NW), KwaZulu-Natal (KZ), Eastern Cape (EC), Western Cape (WC), Northern Cape (NC), Free State (FS).

#### 4.5 Discussion

The results provide interesting and valuable insights to freshwater-system managers and scientists involved in weed risk analysis of invasive species. The models were calibrated with an easy to use, tested method that is easily replicated and repeatable for numerous species (Anderson & Raza 2010). When good AUC statistics were obtained, the models were accurate and highlighted areas favourable for the establishment of potentially invasive submerged species in South Africa. As with all models, however, it is recognised that the quality of the tests requires careful assessment of their accuracy.

## 4.5.1 Model assessment and relevance

Correlative models can easily be applied to a large number of species, with species distribution data usually easily obtainable (Anderson and Raza 2010). MAXENT is regarded as one of the premier distribution modelling software packages available, as it performs well in comparison with other correlative approaches (Elith *et al.* 2011; Thompson *et al.* 2011) and is freely available online (Phillips *et al.* 2006). As with all correlative approaches, MAXENT is generally considered to provide an underestimation of a species' fundamental niche and potential distribution, primarily due to the limits in distribution or dispersal and the limiting effects of biotic interactions. In other words, species may not have had the opportunity to establish in both native and invasive ranges which may have suitable conditions (Soberon & Peterson 2005; Anderson & Raza 2010), limitations that one would expect to be exacerbated in modelling freshwater aquatic species distributions because their habitats resemble island-like habitats, reducing the opportunity of plants to spread between them. Despite their isolation, this does not seem to be the case as aquatic plant species tend to have broader

distributions than their terrestrial counterparts (Santamaría 2002). Nor does the apparent isolation of systems from each other seem to hamper their spread, as evidenced by the establishment of many submerged species around the world. The distribution records of the species modelled in this study ranged from the relatively restricted *C. caroliniana*, recorded in 16 countries with over 600 hundred localities, to the widespread *M. spicatum*, recorded in 58 countries with over 30 000 locality records. There was no lack of localities from where the species had become invasive and a wide range of distribution records was easily available, thus giving a good representation of potential spread for the species. Native records were less abundant but sufficient records were obtained to run the models.

The variables used by the model were related to various aspects of temperature, as this SDM attempted to ascertain the influence of climate on the distribution of submerged species, as climate is recognised as a major contributor to freshwater aquatic plant distribution (Santamaría 2002). The variable temperature refers to air temperature and not water temperature in environmental layers, which could affect the accuracy of the models for two main reasons. Firstly, it is often presumed that a general linear relationship exists between air and water temperatures, based on the assumption that the rate of change in heat storage in a water body, such as a river, can be related to air temperature change (Stefan & Preud'homme 1993). Since air temperature has typically been widely measured, the existence of such a relationship makes it convenient to use as a proxy for changes in water temperatures (Webb & Nobilis 1997), but this is not always the case under natural conditions because: 1) at high and low water temperatures, the generalised linear relationship may vary between river catchments because of factors such as slope, aspect and seasonality of flows, and between dams depending on their surface area, depth and level of turbidity (Essig 1998).

Secondly, water acts as a buffer which will reduce the effect of wide and rapid daily airtemperature fluctuations on the growth of aquatic plants. Unfortunately, despite knowing that these factors might affect the accuracy of the models in estimating the distribution of aquatic plant species, without a uniform relationship between air temperature and water temperature and a way to factor in the buffering effect of water, these errors cannot be accommodated, or adjustments made to avoid them.

The results show that the majority of the models performed well, with predictions of *Eg. densa*, *El. canadensis*, *H. verticillata* and *C. caroliniana* having excellent AUC statistics. It is recognized that there are limitations of the AUC approach (Lobo *et al.* 2008). However, from visual observations, models that had high AUC values also had accurate models.

When the models were tested using independent known distributions of *Eg. densa* and *H. verticillata* from South Africa, they showed a high level of accuracy, with the *Eg. densa* model having an AUC test statistic of 0.951 and the *H. verticillata* model having a test AUC of 0.953, placing both models in the "excellent" category. When the known localities were visually inspected against the potential distributions, the known localities were all within the areas considered to represent areas suitable for the two species. The potential distribution of *H. verticillata* within South Africa has been estimated using the mechanistic modelling tool CLIMEX 1.1, using the predefined physiological parameters of hydrilla obtained from the program. An Ecoclimatic Index was generated, using the 'compare locations' function of the program, for each weather station locality in South Africa (Coetzee *et al.* 2009). CLIMEX predicted a similar but larger distribution than the potential distribution generated by the MAXENT model. The MAXENT model failed to predict areas along the coast in the Western Cape. The models were tested twice - once with the 'testing data', where 30% of the known localities of the species were tested against the models, and again with a set of known

independent distributions of the species from South Africa. There are a number of reasons why *H. verticillata* may not occupy all suitable sites predicted by the mechanistic model (e.g. geographic barriers that limit dispersal, establishment opportunity, competition from other species and other biological interactions). Additionally the model may be an overestimation of the species distribution as the plant has not as yet been recorded in any areas predicted by the model, suggesting that MAXENT may provide a better model for the potential distribution of *H. verticillata*.

Unlike the predictions for the above mentioned species, MAXENT predicted that very few localities were suitable for the establishment of *M. spicatum* within South Africa, even though it is known to have a wide distribution in South Africa (Chapter 2). The model had the lowest AUC value and greatly underestimated the distribution of *M. spicatum* in South Africa. Although *M. spicatum* had the greatest number of distribution records, the localities were highly biased to the native range data from Europe and to North America.

A similar study that attempted to model the potential distribution of *M. spicatum* in South Africa using CLIMEX v.3.02, and Floramap (version 1.03, 2005) (Jones & Gladcove 2005); a modelling technique based on Principal Component Analysis (PCA), also had trouble creating accurate distributions models(S. Walton unpublished data, 2008). The models were run with distribution records from both the introduced and native range (GBIF 2008) and growth preference data derived from Smith & Barko (1990). Both modelling techniques underestimated the potential distribution of *M. spicatum* in South Africa. The Vaal River system was also not predicted to be suitable for *M. spicatum* by either program (S. Walton, unpublished data, 2008). It was hypothesised that the poor water quality of the Vaal River has facilitated the establishment of the weed within an area that would be unfavourable under natural conditions for the weed's establishment (S. Walton, unpublished data, 2008). The

CLIMEX prediction was much less disjunct than the MAXENT predictions and extended further inland and also included a suitable area in Mpumalanga, west of Swaziland, however the weed has not been found at these localities.

Analysis of North American *M. spicatum* has shown it to be genetically different from the *M. spicatum* currently found in South Africa (R. Thum, unpublished data, 2010). Because interspecific genetic variation has been identified as a potential influence on the distribution of invasive species (Thompson *et al.* 2011), the genetic variation within *M. spicatum* is currently being studied. Once populations have been identified that are more genetically similar to the species invading South Africa, the models can be rerun.

# 4.5.2 Implications for South Africa

*Egeria densa* is native to South America and is grown in aquaria and in outdoor ponds throughout South Africa (Martin & Coetzee 2011). It has become naturalised in numerous systems throughout South Africa. As a precautionary measure, it is a Category 1 declared weed (CARA (Act No. 43 of 1983), and importation is prohibited (APA Act No. 36 of 1983), yet this has not reduced its movement and popularity around South Africa in the aquarium and pet store trade (Martin & Coetzee 2011). The MAXENT model indicated that every province in South Africa is susceptible to invasion by *Eg. densa. Egeria densa* can have serious negative environmental and economic effects (Chapter 2), and should thus be given high priority in management to control its spread.

Coetzee *et al.* (2009) showed that there is considerable potential for *H. verticillata* to spread from Pongolapoort Dam via recreational boating and fishing to uninvaded systems around South Africa. The MAXENT model indicated that large areas of the South African east coast and Limpopo province are vulnerable to invasion by *H. verticillata*. In order to mitigate the potential detrimental effects that *H. verticillata* could have on water bodies in South Africa, management efforts should restrict the transportation of *H. verticillata* by focussing preventative measures on recreational boaters and anglers who frequent the Pongolapoort Dam.

*Elodea canadensis* is also a popular and extensively traded aquarium plant throughout South Africa, due to its aesthetic value and apparent hardiness under cultured conditions (Martin & Coetzee 2011). Even with *El. canadensis* prevalence in the aquarium and planted tank industry, and thus its potential for escape, it has not become established in South Africa. The apparent absence of *El. canadensis* outside cultivation may be explained by the SDM, which predicts that very little of South Africa is climatically suitable for *El. canadensis*, limiting its potential distribution to a small area in the Free State, Eastern Cape and Lesotho, all with low probability and only one small area of medium probability around Lake Gariep and Vanderkloof Dam. These dams are the largest and second largest water reservoirs in South Africa respectively, and are part of two important hydro-electric power stations and part of interbasin transfers. These dams should be carefully monitored to ensure that if establishment is recorded, it is rapidly controlled, but more attention should be given to avoid introductions into the two dams in the first place.

*Cabomba caroliniana* is rapidly invading Australia (Schooler 2006), where it is regarded as one of the worst weeds due to its invasive nature and its negative economic and environmental effects (Mackey 1996; Mackey & Swarbrick 1997; CRC 2003). Within Australia, it has been classified as a Weed of National Significance (CRC 2003). *Cabomba caroliniana* is a very popular aquarium plant and has been dispersed throughout the world via the aquarium trade (Ørgaard 1991; Martin & Coetzee 2011; Chapter 3). The weed is believed to have entered South Africa in this manner. It would be useful to know what threat the weed poses to South Africa since its dispersal into freshwater ecosystems is probably due to its presence in the pet trade. Within South Africa, MAXENT estimated the area suitable for establishment to be somewhat limited to medium probability of distribution to the east coast of South Africa and a high potential in the northern part of KwaZulu–Natal. This is concerning as it was cultivated by an aquatic plant dealer in the same area (R. Glen, pers. comm. 2008). Large areas of the interior are predicted to be climatically unsuitable for the establishment of *C. caroliniana* but despite the limited potential distribution predicted by the model, there is still cause for concern because the area covered is extensive and effective methods of managing *C. caroliniana*, infestations are yet to be identified. The threat that *C. caroliniana* poses to South Africa is great, therefore it is imperative that the public and aquarium dealers are made aware of this to help prevent an invasion.

*Myriophyllum spicatum* is considered one of the worst submerged aquatic weeds in North America due to its negative environmental and economic effects (Smith & Barko 1990). It is native to Europe, Asia and northern Africa (Couch & Nelson 1985), but within South Africa it is widely established within the Vaal River and Lake Sibaya historical records of its presence from a number of rivers and dams across the country are housed at National Herbaria (SANBI 2012).

MAXENT predicted very few localities to be suitable for the establishment of the weed in South Africa, thus until improved models are created, the weed should be given national priority to ensure that all areas are monitored to avoid further spread and establishment.

Estimating the potential distributions of invasive species in a new environment tests our most fundamental understanding of ecological systems, and can therefore be a daunting task. We should continue to strive for more accurate predictions, and in the meantime, make use of the technology available to prioritise threats on the basis of the predictions made, using the models available. This should allow us to eliminate at least some potentially invasive species on the basis of a single factor such as climate. Management strategies can be adopted proactively in the event that a particular species is discovered to pose a serious threat to the health of a country's aquatic ecosystems.

Modelling algorithms and software have been widely employed to model the potential geographic distribution of different organisms, thus providing important information about species ecology and contributing to more efficient species management and conservation strategies. This approach has not often been used with aquatics. This preset study is one of the first to attempt such a modelling process on submerged macrophytes. MAXENT has proven useful for modelling the distributions of aquatic plants within South Africa, using native and invaded range data. Of the five submerged species, *Eg. densa* is predicted to have the widest distribution, followed by *H. verticillata* and *C. caroliniana*. Continued improvement of prediction accuracy, when estimating the distribution of submerged aquatic species, will depend largely on a better understanding of the air-water temperature relationship and the inclusion of other factors within the models, such as sediment composition and inorganic carbon availability. For now, MAXENT has been shown to be valuable for estimating the distribution of these species at regional scales. The merit of being able to make such predictions using fairly limited data should not be overlooked or be underestimated.

Modelling potential distribution of species into South Africa based on climatic variables provides valuable information to custodians and researchers of freshwater systems. However this method of modelling does not take into consideration the numerous site specific influences determining species establishment and spread. Drivers such as sediment type, nutrients, anthropogenic influences, such as pollution, and competition from other species can

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also influence freshwater ecosystems. Chapter 5 investigates the influence of sediment type and nutrients on submerged macrophyte assemblages.

# Chapter 5: Competition between two aquatic macrophytes, Lagarosiphon major and Myriophyllum spicatum as influenced by substrate sediment and nutrients<sup>3</sup>.

# **5.1 Introduction**

The recent increase in the occurrence and spread of invasive submerged macrophytes in South Africa has focussed attention on the principle drivers of their invasion. Many factors influence macrophyte assemblages within a system, including the success or failure of invasive species to colonise, spread and/or to become dominant. The factors determining the distribution and spread of these macrophytes involve a variety of abiotic and biotic drivers, of which substrate and sediment nutrient concentrations are critical (Barko *et al.* 1991; James *et al.* 1999; Szoszkiewicz *et al.* 2006). Drivers in aquatic ecosystems are easily altered or affected by anthropogenic influences such as eutrophication and the creation of impoundments. For example, eutrophication resulting from human activities has been strongly implicated as a major cause of invasion by *H. verticillata* (and other exotic species in the U.S.A. (Belanger *et al.* 1989; Dye 1995 in Van *et al.* 1999). The drivers of submerged macrophyte distributions and assemblages in South African freshwater bodies have received insufficient attention and require investigation to predict and understand current and possible new invasions.

<sup>&</sup>lt;sup>3</sup> Martin, G.D. & Coetzee, J.A. 201. Competition between two aquatic macrophytes, *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) and *Myriophyllum spicatum* L. (Haloragaceae) as influenced by substrate sediment and nutrients. *Aquatic Botany* in press.
Since the early 1900s, South Africa's waters have been invaded by a number of floating aquatic macrophytes (Hill 2003). These species are known to form dense mats on rivers and dams throughout South Africa and have had detrimental economic and environmental effects (Hill 2003; Marais *et al.* 2004; Turpie 2004). Various control programmes have been implemented against these weeds and the majority are now regarded as under acceptable control (Hill 2003; Coetzee *et al.* 2011a). Submerged and rooted invasive species have also been identified in South African waterways (Henderson & Cilliers 2002; Cook 2004; Chapter 2) but have received far less attention than floating species despite their known environmental and economic effects. As of 2012, no biological control agents have been released against submerged aquatic plants in South Africa, but agents against *M. spicatum* and *H. verticillata* are currently under pre-release evaluation (Coetzee *et al.* 2011b). All herbicidal control measures are also still in preliminary testing phases.

One of the theories as to why submerged invasive macrophytes have not become as problematic as their floating counterparts is the notion that the suite of indigenous and cosmopolitan species already established in southern Africa may be superior competitors (Chapter 2). In Loch Corrib, Ireland, *L. major* has become invasive and successfully outcompeted native macrophytes including a variety of charophytes, *Myriophyllum* spp. including *M. spicatum*, and *Potamogeton* spp. (Caffrey *et al.* 2010). In New Zealand, native *Potamogeton* spp. and *Myriophyllum* spp. have also been outcompeted by *L. major* (Rattray *et al.* 1994), suggesting its potential to exclude new invading species in its native range, southern Africa.

Increasing research has been dedicated to understanding how co-occurring plant species compete with one another for limited resources (Connolly *et al.* 2001). A commonly accepted method of determining competitive ability between aquatic macrophytes is the Spitters

(1983) model. The model uses an addition series, which allows for the relative competitive abilities of each plant species in the experiment to be ascertained using reciprocal-yield models of mean plant dry mass. The method has been used to ascertain competition between a variety of aquatic species of different growth forms under different environmental conditions (Van et al. 1999; Coetzee et al. 2005; Mony et al. 2007). Van et al. (1999) used this method to investigate the influence of soil fertility on competitive interactions between an invasive dioecious H. verticillata and indigenous V. americana in the U.S.A. H. *verticillata* forms a dense surface mat of photosynthetic material can almost totally suppress penetration of light into the water column, whereas V. americana produces a basal rosette of leaves which elongates up to the surface. Van et al. (1999) found that in high nutrient concentrations, *H. verticillata* was 7.2 times stronger a competitor than *V. americana*, under lower nutrient conditions, V. americana was the dominant species. Comparatively, Mony et al. (2007), also using Spitter's model, investigated the competition between two plant species of similar growth characteristics, H. verticillata and Eg. densa as influenced by sediment fertility and season. Both species are invasive in the U.S.A. Competition from *H. verticillata* resulted in greater resource allocation to the roots of Eg. densa. Significant below-ground competition by Eg. densa on H. verticillata was also evident at low nutrient concentrations.

Lagarosiphon major and *M. spicatum* are submerged perennial species that share similar growth forms, similar invasive characteristics and are found within the same areas in the littoral zone (Chapter 2). Lagarosiphon major is endemic to southern Africa, where it is restricted to the colder, higher escarpment regions, such as the Drakensburg and Mpumalanga highlands. Outside its native range *L. major* has successfully outcompeted indigenous submerged species and causes significant detrimental economic effects (Rattray 1994; Csurhes & Edwards 1998). *Myriophyllum spicatum*, which is indigenous to Europe, Asia and North Africa, was first recorded in South Africa in the early 1800s and has since spread to

freshwater systems within South Africa. The plant has been declared a Category 1 Weed (Conservation of Agricultural Resources Act, 1983) as a precautionary measure based on the problems it causes in other parts of the world.

In South Africa, no co-occurring populations of *L. major* and *M. spicatum* have been recorded, but *M. spicatum* has the potential to spread though anthropogenic activities. Due to the similar growth forms and preferred habitat types, competition between the two species could be expected if *M. spicatum* were to establish in *L. major* localities. It is recognised that the composition of the bottom sediments, including physical properties within these systems, may affect the growth of both species (Barko & Smart 1986). Additionally, South Africa's rapid increase in urbanisation and agriculture has resulted in elevated pollution, especially nitrogen in many freshwater habitats (Coetzee *et al.* 2011a). Nutrient enrichment of aquatic and terrestrial environments is often linked with the invasion of alien plants and is an important factor in determining plant community composition (Byers 2002; Van *et al.* 1999). This study used addition series methods to examine the effects of increased nutrients as well as sediment types (sand vs. loam) on the growth and competitive abilities of *L. major* and *M. spicatum* to determine whether sediment types and nutrient levels may be important in influencing the establishment, dominance and distribution of these macrophytes within

## 5.2 Methods and Materials

## 5.2.1 Sediment selection

Competition studies between the two plant species were conducted at the Department of Zoology and Entomology, Rhodes University, South Africa. Two separate studies were conducted to assess how the competitive interactions between *M. spicatum* and *L. major* 

change as influenced by increased nutrients as well as substrate type (sand vs. loam). For the nutrient study, commercial building sand was used with either a high or low fertilizer regime. For the sediment study, sediments that provided favourable growing conditions of the two species were selected. These were ascertained by selecting sediments that represent similar physical and chemical characteristics to the 29 *L. major* and 11 *M. spicatum* sites sampled in South Africa (Appendix 4) (see method below). Literature regarding optimal *M. spicatum* sediments was also considered.

#### 5.2.2 Soil Analyses

Five hundred gram soil samples were collected using a 6 cm diameter auger at a depth of 15-30 cm at each sample site. Soil analyses of soil samples and from soils selected for use in the experiment, were sent to Bemlab Laboratory in Strand (2010-2011), Western Cape, South Africa, for physical and chemical analysis. Soil was analysed in the following methods:

#### pH, P, extractable cations & organic C

The soil was air dried, sieved through a 2 mm sieve and analysed for pH (1.0 M KCl), P (Bray II) and total extractable cations, namely K, Ca, Mg and Na (extracted at pH = 7 with 0.2 M ammonium acetate) and organic matter by means of the Walkley-Black method (The Non-affiliated Soil Analyses Work Committee, 1990). The extracted solutions were analysed with a Varian ICP-OES optical emission spectrometer.

## **Total P in soil**

Total P was extracted with a 1:1 mixture of 1N nitric acid and hydrochloric acid at 80°C for 30 minutes. The P concentration in the extract was then determined with a Varian ICP-OES optical emission spectrometer.

## Soil texture (% clay, silt & sand)

Chemical dispersion was determined using sodium hexametaphosphate (calgon) and the percentage of the three sand fractions (silt, clay and sand) were determined through sieving as described in The Non-affiliated Soil Analyses Work Committee (1990). % silt and clay were then determined using sedimentation rates at 20°C, using an ASTM E100 (152H-TP) hydrometer.

Soil characteristics from the field sites were plotted on a scatter plot using nonmetric multidimensional scaling (MDS) analysis. Sediments selected for use in the experiment were added to the plot to determine if they were characteristic of field sediment conditions. PAST: Paleontological Statistics package ver. 1.81 (Hammer *et al.* 2001) was used for the analysis. The two-dimensional (2D) MDS plot indicated the similarity of these samples to other samples in the plot. The accuracy of the 2D representations is indicated by the 'stress' value (Kruskal's stress formula). Stress values <0.05 indicate an excellent representation with no prospect of misinterpretation. A Bray-Curtis Cluster analysis was conducted to find out how closely related the sites were to each other based on their chemical characteristics. Differences between sediment properties taken from *L. major* and *M. spicatum* field sites were analysed using a Mann-Whitney U test, in STATISTICA ver. 8.0.

## 5.2.3 Competition studies experimental design

Two separate experiments were conducted in a greenhouse tunnel at Rhodes University. One investigated the effect of soil nutrient concentrations (high vs. low) and the second investigated sediment characteristics (sand vs. loam). Addition series methods were used to examine the effects of increased nutrients as well as natural sediment types (sand vs. loam) on the growth and competitive abilities of *L. major* and *M. spicatum*. The experimental

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designs followed an addition series developed by Spitters (1983), consisting of factorial combinations of different planting densities of the two competing plants. The setup for both experiments was the same. The planting densities of L. major : M. spicatum were 0:3, 0:9, 3:3, 3:9,3:0, 9:0, 9:3, 9:9 planted into 12-L plastic tubs (42cm diam. x 14cm deep), for both sediment types and nutrient levels being tested, giving a total of 16 tubs. The tubs were placed in a polypropylene pool (215cm diam. x 40cm deep, 1452 l), fitted with a steel frame filled with clean borehole water (Fig. 5.1). The experiment was replicated four times for each treatment. The planting medium was covered with a fine layer of silica sand to minimise algal growth and reduce the amount of nutrients leaving the sediment. Tubs were placed with enough space between them to avoid interaction between plants from different tubs. The tubs were placed in a pre-determined order based on planting densities and treatment, but the starting point of the order was randomly selected. Pools were regularly topped up. Thermachron iButtons (Climastats Environmental Monitoring software, Version 4) were used to detect significant changes in water and sediment temperatures during the experiment. The buttons were placed in a water-tight container and either floated on the water surface or positioned within the pond sediment. Temperature data (maximum, minimum and daily mean temperatures) were recorded every two hours. The mean daily temperatures and temperature differences between the surface and sediment were calculated. A Student's t-test determined if there were significant differences between surface and sediment water treatments. Light was not a factor in the experiment as it was conducted in shallow pools with clear water.

The studies were run for 14 weeks after planting, after which the plants were harvested, separated from each other when in combination, then washed and cleaned and divided into roots and shoots. The plants from each tub were placed in separate brown paper bags and dried in a Heraeus drying oven for 96 hours at 60 °C. Dry biomass (g) was measured using an Ohaus<sup>®</sup> Adventurer<sup>™</sup> balance.

## 5.2.4 The influence of nutrient availability on competition

To evaluate how competition between *L. major* and *M. spicatum* is influenced by sediment nutrient availability, a high and low fertilizer regime was used. In the high nutrient treatment, 10g of a controlled slow-release 15-7-15 N:P:K slow-release fertiliser (Haifa, Multicote 8; 15-7-15+2MgO+Micro-nutrients formulated for an 8-month release rate at 21°C or 5-6 months release at 30 °C) was thoroughly mixed into 5kg of building sand (Table 5.2). Both species preferentially assimilate nutrients through their roots (Barko and Smart 1980; Rattray *et al.* 1991, Barko 1993;). For the low nutrient substrate treatment, no fertilizer was added to the sand. The sand was placed in each of the 16 tubs (42cm diam. x 14cm deep), 8 high and 8 low, the sediment filled up most of the tub. The mean starting mass per shoot of *L. major* and *M. spicatum* planted in the nutrient treatment was 1.3g (+/- 0.20 S.E: n = 216) and 1.7g (+/- 0.22 S.E: n = 216) respectively.

## 5.2.5 The influence of sediment type on competition

Two different sediment treatments representative of *L. major* and *M. spicatum* field sites were selected. The sediments were termed 'loam' for the sediment type sharing chemical and physical properties more representative of the *L. major* field sites than the M. spicatum sites and 'sand' for the sediment type representative of *M. spicatum* field sites. Both sediments were collected from Jameson Dam, Eastern Cape, South Africa (-33.319073 S; 26.444206 N). Five kilograms of sediment per tub was used for both treatments, and once again the sediment filled the majority of the tub. The mean starting wet mass per shoot of *L. major* and *M. spicatum* was  $1.3g (\pm 0.20$  S.E. n = 216) and  $1.7 (\pm 0.22$  S.E. n = 216) g per respectively.



**Figure 5.1:** Experimental layout: grey circles indicate tubs with high nutrients (experiment 1) or 'loam' treatment (experiment 2); clear circles indicate low nutrient (experiment 1) or 'sand' treatment (experiment 2). Ratios indicate *Lagarosiphon major*: *Myriophyllum spicatum* planting densities.

## 5.2.6 Statistical analyses

Data were analysed using inverse linear models. Multiple regressions were conducted on the mean end dry masses of shoots, roots, and total biomass under each planting density. The magnitude of the relationship was analysed using the reciprocal-yield model (Spitters 1983). This model involves multiple linear regressions of the form:

 $1/W_l = a_{l0} + a_{ll}d_l + a_{lm}d_m$ 

 $1/W_m = a_{m0} + a_{mm} d_m + a_{ml} d_l$ 

Where  $1/W_1$  and  $1/W_m$  are the inverse dry biomass yields of individual *L. major* and *M. spicatum* respectively, while  $d_1$  and  $d_m$  represent the respective planting densities for *L. major* and *M. spicatum*. Intraspecific competition was estimated by the partial regression coefficients  $a_{ll}$  and  $a_{mm}$  and interspecific competition by the coefficients  $a_{lm}$  and  $a_{ml}$  in terms

of their effects on the reciprocal yield of *L. major* or *M. spicatum* masses. Competitive interactions were analysed for total dry mass of the roots, shoots. The intercepts ( $a_{10}$  and  $a_{m0}$ ) measure the reciprocal of the maximum mass of isolated plants. The magnitude of 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 ( $a_{11}$ /  $a_{1m}$  and  $a_{mm}$  /  $a_{m1}$ ). Two-way Analysis of Variance (ANOVA), followed by a Tukey Post-Hoc HSD test determined whether the mean end-yields of *L. major* and *M. spicatum* were significantly different between both treatments. All statistical analyses were conducted in STATISTICA ver. 8.0.

## 5.3 Results

## 5.3.1 Field site sediment characteristics

Analysis of soil from field sites indicated that sediments used in the competition experiments were representative of field growing conditions. The *L. major* field sites were predominantly of a loam soil type and were characterised by high clay, silt and fine sand percentages, and were of an intermediate texture consisting almost entirely of micropores. Soils of this type are very sticky and have high nutrient-holding capacity. Generally, medium-textured sediments that have high nutrient-holding capacity are most suitable for plant growth (Barko *et al.* 1991).

The *M. spicatum* field sites were characterised by sand type sediments, having higher medium sand percentage and thus having increased macropores, which provide space for roots and organisms to inhabit the soil, but results in the sediment having a lower cation-exchange, buffer capacity and nutrient retention. Similar fine textured inorganic sediments of intermediate density have been shown to favour the growth of *M. spicatum* (Barko & Smart

1983). Sediment from the *L. major* sites had significantly lower pH values, and higher, Soil Organic Carbon (SOC) and Fe content than to the *M. spicatum* sites (Table 5.1). *Myriophyllum spicatum* sites had higher P Bray II and phosphorus levels than the *L. major* sites (Table 5.1).

The loam treatment used in the sediment type experiment was characterised by high clay, silt and fine sand percentages and was of an intermediate texture. The SOC content of the loam treatment was approximately five times higher than the SOC content of the sand treatment (Table 5.2). The loam treatment had a higher ratio of exchangeable sodium, potassium, calcium, and magnesium than the other treatments. A high iron concentration was found in the loam treatment (Table 5.2). Macronutrients including potassium, sodium, calcium, and magnesium were highest in the loam treatment. The sand sediment was characterised by having a much higher medium sand percentage (Table 5.2). Phosphorus, another essential nutrient for plants, was highest in the sand treatment. The building sand used in the nutrient treatment had high, medium sand percentages and a low SOC content of 0.21 %. All three samples had low pH values (Table 5.2).

		Lagarosiphon major sites (Median, n=29)	Upper; Lower percentiles	<i>Myriophyllum</i> <i>spicatum</i> sites (Median, n=11)	Upper; Lower percentiles	U- value, * <i>P</i> < 0.05
pН	(KCl)	4.50	4.7; 4.5	7.40	7.4; 7	U=7*
$\mathrm{H}^{+}$	(cmol/k g)	1.33	1.8; 1.33	NA	NA	NA
P (Bray II)	mg/kg	8.50	13.75; 8.5	42.00	186.5; 34	U=14*
Κ	mg/kg	145.00	189.75; 145	121.00	150; 103.5	U=141
Exchangeable	Na	0.23	0.325; 0.225	0.39	0.41; 0.27	U=0.70
cations (cmol(+)/kg)	K	0.37	0.4825; 0.37	0.31	0.385; 0.26	U=140.5
	Ca	4.19	5.0625; 4.19	12.57	14.265; 8.61	U=38*
	Mg	1.99	2.7025; 1.99	3.29	3.99; 2.19	U=90*
Cu	mg/kg	3.99	6; 3.99	2.59	4.535; 1.76	U=121
Zn	mg/kg	1.90	3.25; 1.9	1.80	7; 1.2	U=135
Mn	mg/kg	124.30	198.75; 124.3	106.60	267.25; 76.75	U=138
В	mg/kg	0.24	0.2725; 0.24	0.42	1.415; 0.35	U=62*
Fe	mg/kg	1368.42	1702.67; 1368.42	326.22	997.145; 266.8	U= 58*
SOC	%	1.56	2.275; 1.56	0.94	1.05; 0.72	U=86
Na	%	3.42	4.8; 3.42	2.40	2.575; 2.23	U=107
Κ	%	4.58	6.33; 4.58	2.45	2.825; 2	U=45*
Ca	%	52.39	55.34; 52.39	74.78	76.485; 66.37	U=24*
Mg	%	28.06	32.325; 28.055	20.73	24.26; 19.31	U=94*

**Table 5.1:** Soil characteristics of sediments collected from Lagarosiphon major andMyriophyllum spicatum field sites.

Table	5.2:	Soil	characteristics	of 1	the	sediments	used	in	the	nutrient	(building	sand)	and
sedime	ent (lo	oam a	and sand) experi	men	nts								

Soil treatment		Loam	Sand	Building Sand*
Soil		Loam	Sand	Sand
pН	(KCl)	4.5	4.4	6.0
Resistance	(Ohm)	660	1930	2100
$\mathrm{H}^{+}$	(cmol/kg)	1.83	0.49	0.25
Stone	%	1	1	1
P Bray II	mg/kg	16	52	5
Κ		167	37	54
Exchangeable cations	Na	0.59	0.14	0.13
(cmol(+)/kg)	Κ	0.43	0.09	0.14
	Ca	4.37	0.84	1.28
	Mg	3.68	0.57	0.43
Cu	mg/kg	1.44	0.53	0.03
Zn	mg/kg	3	1	0.3
Mn	mg/kg	26.1	5.2	30.7
В	mg/kg	0.61	0.17	0.14
Fe	mg/kg	2416.27	306.6	91.75
SOC	%	2.15	0.35	0.21
Na	%	5.38	6.74	5.88
Κ	%	3.93	4.37	6.22
Ca	%	40.14	39.38	57.26
Mg	%	33.75	26.63	19.41
Clay	%	10.2	1.8	2.8
Silt	%	9	1	2
Fine Sand	%	72.9	66.72	65.6
Medium Sand	%	6.64	28.68	27.4
Coarse Sand	%	1.26	1.8	2.2
Classification		Sand Loam	Sand	Sand

\*2g/kg of slow release fertiliser was added to building sand for the high nutrient treatment after soil analysis

The loam, sand and building sand sediment types were plotted in a two-step analysis, using a cluster analysis to define groupings in Multidimensional scaling (MDS) with the sediments collected from the field sites (Fig. 5.2). The cluster analysis showed similarities between the *L. major* sites and the *M. spicatum* sites based on the physical and chemical analyses of

sediment types. The loam sediment type showed the greatest similarities to sediments collected from the *L. major* field sites. The sand and building sand were clustered with *M. spicatum* field sites, indicating their appropriate use in the experiments

## 5.3.2 Competition studies

The mean water temperature recorded in the experimental ponds during the study was  $26.5^{\circ}$ C (S.E. 0.5; minimum  $17.5^{\circ}$ C, maximum  $37.5^{\circ}$ C), while mean sediment temperature was  $26.5^{\circ}$ C (S.E.0.5; minimum  $18.5^{\circ}$ C, maximum  $37^{\circ}$ C). The mean daily temperature decreased throughout the experiment as it was conducted from the end of summer to the beginning of winter. There was no significant difference between the surface and the sediment temperatures ( $t_{(296)} = -1.15$ , P > 0.05).



**Figure 5.2:** Multidimensional scaling (MDS) and cluster analysis of Bray–Curtis similarity indexes of field soil samples, and loam, sand, and building sand treatments used in the experiments. The MDS plot gives a 2D representation of relative similarities. Hence, samples that are close together are more similar to each other (Stress value < 0.2). L.m – *Lagarosiphon major* sites, M.s – *Myriophyllum spicatum* sites, Loam- Loam sediment, SAND- sandy sediment, B-SAND- building sand sediment

## 5.3.3 Influence of nutrients on competition

Analysis of the effect of increased nutrients on competition between *L. major* and *M. spicatum* showed that sediment nutrient levels significantly affected the competitive ability of both plant species (Table 5.3). The competition coefficients  $a_{II}/a_{Im}$  for *L. major* showed it to be 2.5 times more competitive than *M. spicatum* at high nutrients levels i.e. competition from one *L. major* plant on itself was equal to competition from 2.5 *M. spicatum* plants (Table 5.3). In the high nutrient treatment, the regression co-efficient  $a_{II}$  indicating intraspecific competition on the total yield of *L. major* was higher (0.02) than the low treatment (0.003) (Table 5.3). Further, in the low nutrient treatment the regression co-efficient  $a_{Im}$  indicating interspecific competition from *M. spicatum* was 0.09, higher than in the high treatment where it was 0.08 (Table 5.3). Under both the high and low nutrient concentrations, the overall competitive ability of *M. spicatum*, indicated by the ratio of coefficients  $a_{mm}/a_{ml}$ , remained at approximately 0.3 for both treatments, higher than *L. major* in the low treatment (Table 5.3).

The change in competitive relationships between interspecific and intraspecific competition was graphically analysed (Fig.5.3). These figures present reciprocal yield planes corresponding to the competition coefficients for total biomass (Table 5.3). The higher the values on the figure, the lower the actual yield; and the greater the slope, the larger the competitive coefficient. By placing the figures with equivalent dependent values next to each other, comparisons of differences can easily be made. In the case of the high nutrient treatment, intraspecific competition at high nutrient levels is the dominant factor reducing total mean yield of *L. major* and *M. spicatum* (Fig. 5.3 a and b), and for *M. spicatum* at low nutrient levels (Fig. 5.3 c). At low nutrient levels, interspecific competition of *M. spicatum* on *L. major* seems to be the major factor reducing the overall mean yield of *L. major* (Fig. 5.3 d).

Significantly more shoots were produced by *L. major* in the high nutrient treatment, than in the low nutrient treatment (F<sub>(5, 36)</sub> =3.01; *P* < 0.05) (Fig. 5.4 a). *Myriophyllum spicatum* did not produce significantly more stems under high nutrient levels F<sub>(5, 36)</sub>=1.4902, *P* = 0.21726 (Fig. 5.4 b). Under high nutrients, both species had a lower root: shoot ratio, but they were not statistically significant for either *L. major* (F<sub>(5, 36)=</sub>.36275, *P*=0.87057) or *M. spicatum* (F<sub>(5, 36)</sub>=0.95287, *P*=0.45929) (Fig. 5.5a and b).

The total mean dry biomass of *L. major* and *M. spicatum* in the high nutrient treatment were significantly higher than in the low nutrient treatment, under both the three and nine planting densities (*L. major*  $F_{(1, 44)}$ =6.6369, *P*=.01342 (*M. spicatum*  $F_{(1, 44)}$ =10.617, *P*=.00216) (Fig. 5.6 a and b). The mean dry total biomass of *M. spicatum* under a low nutrient treatment and at a planting density of nine was not different from the dry mass of *M. spicatum* grown at the low nutrient treatment and at a planting density of three (Fig. 5.6 b).

**Table 5.3:** Multiple regression analysis of total competition, above ground and below ground

 competition between *Myriophyllum spicatum* and *Lagarosiphon major* grown under different

 nutrient and sediment treatments.

		Regress	ion coefficients		
	Intercept	Intraspecific competition <sup>1</sup>	Interspecific competition <sup>2</sup>	Ratio of competition coefficients <sup>3</sup>	<b>R</b> <sup>2</sup> ; <i>P</i>
Total biomass					
Nutrient treatment					
L. major (high)	0.1074	0.0202	0.0082	2.463415	0.3; 0.03
<i>L. major</i> (low)	1.1520	-0.0037	0.0901	-0.04107	0.08; 0.4
M. spicatum (high)	0.1093	0.0057	0.0185	0.308108	0.5; 0.0001
<i>M. spicatum</i> (low)	0.5878	0.0214	0.0689	0.310595	0.22; 0.07
Above-ground comp	etition				
Nutrient treatment					
L. major (high)	0.1153	0.0203	0.0081	2.5062	0.29; 0.032
<i>L. major</i> (low)	1.2421	-0.0079	0.0960	-0.0823	0.075; 0.44
M. spicatum (high)	0.1331	0.0083	0.0248	0.3347	0.71; 0.0006
M. spicatum (low)	0.8249	0.0277	0.1629	0.1700	0.38; 0.19
Below-ground compe	etition				
Nutrient treatment					
L. major (high)	0.6076	1.2825	1.2977	0.988287	0.15; 0.20
<i>L. major</i> (low)	20.358	0.9522	3.0406	0.313162	0.29;0.03
M. spicatum (high)	0.5671	0.0145	0.0788	0.18401	0.38;0.006
M. spicatum (low)	1.6879	0.0575	0.1471	0.390891	0.24;0.05

<sup>1</sup>Intraspecific competition is represented by the regression coefficients all for *L. major* and a<sub>mm</sub> for *M. spicatum* 

<sup>2</sup>Interspecific competition is represented by the regression coefficient  $a_{lm}$  for *L. major* and  $a_{ml}$  for *M. spicatum*.

<sup>3</sup>The ratio of the 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,  $a_{ll}/a_{lm}$  for *L. major* and  $a_{mm}/a_{ml}$  for *M. spicatum*.



**Figure 5.3:** Multiple regression planes indicate the combined effect of *Lagarosiphon major* and *Myriophyllum spicatum* on the reciprocal of the mean dry mass (1/g) of one *L. major* plant (a- high nutrient treatment; b- low nutrient treatment), and the combined effect of *L. major* and *M. spicatum* on the reciprocal of the mean total dry mass (1/g) of one *M. spicatum* plant (c-high nutrients; d-low nutrients) (i.e. higher values represent lower yields). Points indicate observations (n = 24) and vertical lines between data points represent the residuals. Values on X and Y axes represent *L. major* and *M. spicatum* planting densities at the start of the experiment.



**Figure 5.4:** Mean number of *Lagarosiphon major shoots* (**a**) and *Myriophyllum spicatum* shoots (**b**) under different planting densities, grown at high and low nutrient levels. Error bars represent S.E., means followed by the same letter are not significantly different.



**Figure 5.5:** Mean *Lagarosiphon major* root: shoot ratio (**a**) and *Myriophyllum spicatum* root: shoot ratio (**b**) in (**g**) grown under different planting densities and nutrient levels. Error bars represent S.E., means followed by the same letter are not significantly different.



**Figure 5.6:** Mean end final dry mass (g) of *Lagarosiphon major* (a), and *Myriophyllum spicatum* (b) at the original planting densities of three and nine stems, under different nutrient levels. Error bars represent S.E., means followed by the same letter are not significantly different.

## 5.3.4 Influence of sediment type on competition

Plant species respond differently to the substrate they are grown in, therefore the outcome of competition between *L. major* and *M. spicatum* changed according to sediment type. The ratio of competition coefficients in the form  $a_{II}/a_{Im}$ , under the loam treatment indicated that *L. major* had a significant competitive advantage over *M. spicatum*, proving to be 10 times more competitive, whereas the effect of *M. spicatum*  $a_{mm}/a_{ml}$  on *L. major* was only 1.3 times (Table 5.4). Intraspecific competition shown by the regression coefficient  $a_{II}$  for *L. major* and  $a_{mm}$  for *M. spicatum* indicated a reduced below-ground yield of both species in the sand treatment, but in the loam treatment, below-ground biomass of *M. spicatum* was influenced by interspecific competition (Table 5.4). In the sand treatment, the ratio of competition coefficients  $a_{mm}/a_{ml}$  indicated that *M. spicatum* was 2.3 times more competitive in reducing total yield of *L. major*, compared to 1.3 in the loam treatment (Table 5.4).

The change in competitive relationships between interspecific and intraspecific competition was graphically analysed (Fig. 5.7). Under the loam treatment (Fig. 5.7 a and b), competition of *M. spicatum* on *L. major* was negligible, but there was significant interspecific  $a_{ml}$  and intraspecific  $a_{mm}$  competition on *M. spicatum*. Under the sand treatment (Fig. 5.7 b and d), the steep slopes of the regression planes are associated with increasing density of both plants, thus interspecific and intraspecific competition were substantial causes of yield reduction in both species.

Under the loam treatment, *L. major* produced significantly more branches than under the sand treatment (F<sub>(5, 36)</sub> = 3.62; *P* = 0.05) (Fig 5.8 a). No difference in root: shoot ratio was found between loam and sand treatment for either species (*L. major* (F<sub>(5, 36)</sub> = 1.0918, *P*=0.38) and *M. spicatum* (F<sub>(5, 36)</sub> = 0.64236, *P*=0.67) (Fig. 5.9).

**Table 5.4:** Multiple regression analysis of total competition, above-ground and below-ground

 competition between *Myriophyllum spicatum* and *Lagarosiphon major* grown under different

 nutrient and sediment treatments.

		Regress	ion coefficients		
	Intercept	Intraspecific competition <sup>1</sup>	Interspecific competition <sup>2</sup>	Ratio of competition coefficients <sup>3</sup>	<b>R</b> <sup>2</sup> ; <i>P</i>
Total biomass					
Sediment treatment					
L. major (sand)	0.8926	0.0462	0.0503	0.918489	0.23; 0.06
L. major (loam)	0.2574	0.0327	0.0032	10.21875	0.37; 0.008
M. spicatum (sand)	1.0711	0.0618	0.0294	2.102041	0.22; 0.07
M. spicatum(loam)	0.5958	0.0355	0.0266	1.334586	0.3; 0.02
Above-ground compe	etition				
Sediment treatment					
L. major (sand)	1.0948	0.0276	0.0723	0.3817	0.24; 0.05
L. major (loam)	0.2900	0.0326	0.0024	13.5833	0.27; 0.04
M. spicatum (sand)	1.7248	0.0761	0.0642	1.1854	0.27; 0.03
M. spicatum (loam)	0.9345	0.0688	0.0350	1.9657	0.25; 0.05
Below-ground compe	tition				
Sediment treatment					
L. major (sand)	6.8901	2.4407	0.4472	5.457737	0.24; 0.05
L. major (loam)	3.4136	1.0086	0.2377	4.243164	0.21; 0.09
M. spicatum (sand)	4.4731	0.5263	-0.2542	-2.07042	0.15; 0.17
M. spicatum (loam)	1.8343	0.0572	0.0808	0.707921	0.1; 0.3

<sup>1</sup>Intraspecific competition is represented by the regression coefficients all for *L. major* and a<sub>mm</sub> for *M. spicatum* 

<sup>2</sup>Interspecific competition is represented by the regression coefficient a<sub>lm</sub> for *L. major* and a<sub>ml</sub> for *M. spicatum*.

<sup>3</sup>The ratio of the 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,  $a_{ll}/a_{lm}$  for *L. major* and  $a_{mm}/a_{ml}$  for *M. spicatum*.



**Figure 5.7:** Multiple regression planes indicate the combined effect of *Lagarosiphon major* and *Myriophyllum spicatum* on the reciprocal of the total mean dry mass (1/g) of one *L. major* plant (a-loam treatment, b-sand treatment) and the combined effect of *L. major* and *M. spicatum* on the reciprocal of the total mean dry mass (1/g) of one *M. spicatum plant* (c-loam treatment d= sand treatment) (i.e. higher values represent lower yields). Points indicate observations (n= 24) and vertical lines between data points represent the residuals. Values on X and Y axes represent *L. major* and *M. spicatum* planting densities at the start of the experiment.



**Figure 5.8:** Mean number of *Lagarosiphon major* shoots (a) and *Myriophyllum spicatum* shoots (b) under different planting densities grown under 2 sediment types. Error bars represent S.E., means followed by the same letter are not significantly different.



**Figure 5.9:** Difference in *Lagarosiphon major* root: shoot ratio (a) and *Myriophyllum spicatum* root: shoot ratio (b) in (g) under different planting densities and sediment types. Error bars represent S.E., means followed by the same letter are not significantly different.

The mean end dry biomass of *L. major* and *M. spicatum* grown in the loam sediment was significantly higher than the mean dry biomass of *L. major* and *M. spicatum* grown in sand at both the three and nine planting densities (*L. major*:  $F_{(1, 44)}$ = 4.1109, *P*= 0.049) (*M. spicatum*:  $F_{(1, 44)}$ =1.5033, *P*=0.2) (Fig 5.10 a and b), however the mean dry biomass of *M. spicatum* 

grown in the sand treatment at a planting density of nine was significantly higher than the dry mass of *M. spicatum* grown in loam under a planting density of three (Fig. 5.10 b).

The results of this study show that when grown in competition with each other under high sediment nutrient availability, *L. major* was more competitive than *M. spicatum*. However under low nutrient availability and in sand, *M. spicatum* increases its competitive advantage against *L. major*.

а



**Figure 5.10:** Mean final masses (g) of *Lagarosiphon major* (a), and *Myriophyllum spicatum* (b) at the original planting densities three and nine stems, under different sediment substrate types. Error bars represent S.E., means followed by the same letter are not significantly different.

## 5.4 Discussion

*Lagarosiphon major* and *M. spicatum* have both been shown to actively outcompete other submerged macrophytes (Titus *et al.* 1975; Agami & Waisel 1985; James *et al.* 1999; Hofstra *et al.* 1999; Caffrey & Acevedo 2007). Competition involving these species shows that nutrient levels and sediment types affect the growth and competitive ability of both plant species differently, influencing the outcome of the competition between the two species when grown in mixed cultures under different planting densities.

Sediment nutrient availability has been identified as highly important for the growth of many submerged macrophyte species (Denny 1980; Barko & Smart 1986) as sediment is the primary source for the uptake of N, P, Fe, Mg and micronutrients (Smart & Barko 1985; Barko *et al.* 1991; Rattray et al 1991; Xie *et al.* 2007). Alternatively, the physical properties of sediments, rather than their chemical composition, have been suggested as a primary sediment attribute influencing growth of aquatic macrophytes (Denny 1980; Smart & Barko 1985). However, it is probably a combination of nutrients and physical properties which influence macrophyte establishment and growth. Fertile, finely grained sediments, such as the loam treatment in this study, contain high organic matter content which often equates to anaerobic conditions, which is suitable for *L. major* growth (Barko and Smart 1983). The same does not apply for all macrophytes as different macrophytes vary in their responses to sediment conditions (Barko & Smart 1980; 1983). Infertile sandy sediment, such as the sand treatment used here, has low organic matter content with aerobic conditions, which increased the competitive ability of *M. spicatum* over *L. major*.

In Loch Corrib, Ireland, *L. major* outcompetes other species, including *M. spicatum*, by spreading rapidly from stem fragmentation; the fragments grow rapidly and may form a dense canopy (Caffrey & Acevedo 2007). *Lagarosiphon major* may have a competitive advantage in Ireland as it grows during the winter months when most indigenous species die back (Caffrey *et al.* 2010). Morphologically, *L. major* has a competitive advantage over other species by tolerating a raised pH and dissolved O<sub>2</sub> and lowered free CO<sub>2</sub>, often created during photosynthesis in submersed aquatic plant stands (James *et al.* 1999). In New Zealand lakes, *L. major* has successfully outcompeted all native species by being able to produce roots faster, and growing faster, both in length and biomass, than the native species (Rattray *et al.* 1994). Thus in the absence of herbivory, *L. major* proves to be a superior competitor to most indigenous submerged macrophyte species as well as some invasive submerged macrophyte species. This is supported by the results of the present study in high nutrient or finer sediments.

When sediment nutrients were limited, both *L. major* and *M. spicatum* allocated more resources to the growth of roots, which is consistent with allocation patterns observed in most herbaceous terrestrial plants and other aquatic macrophytes (Barko & Smart 1986; Wang *et al.* 2008). This in turn resulted in significant below-ground competition between the two species. *Myriophyllum spicatum* however had higher root: shoot ratios at low soil nutrients, than *L. major*. Morphologically, *L. major* outcompetes other submerged macrophytes through rapid shoot production and above ground biomass accumulation (at the expense of root growth), allowing *M. spicatum* to establish increased root reserves within the sediments. The results suggest that under low sediment nutrients, *M. spicatum* was more competitive than *L. major* probably because it allocated more roots under low and thus can outcompete. In a similar study, high sediment fertility favoured the lesser competitor *V. americana* over *H.* 

*verticillata,* while *H. verticillata* was a more effective competitor when sediment nutrients were in short supply (McCreary 1991).

When the two species in this study were grown in combination under different sediment types, *L. major* had a significant competitive advantage over *M. spicatum* in the loam treatment, but in the sand treatment, *M. spicatum* had a competitive advantage over *L. major*. It is recognized that sediment composition exerts an important influence on macrophyte productivity and species composition (Barko *et al.* 1991). *Myriophyllum spicatum* grows optimally on fine-textured, inorganic sediments and relatively poorly on highly organic sediments (Smith & Barko 1990). As *M. spicatum* plants grow upward, lower leaves are shaded, resulting in a canopy-like cover near the water surface, allowing for successful competition for both light and sediment nutrients (Titus *et al.* 1975). Thus by being able to grow faster than *L. major*, which had a low root to shoot ratio, in the sand treatment, *M. spicatum* was able to gain a competitive advantage in this study. Leu *et al.* (2002) showed that lipophilic extracts of *M. spicatum* inhibit photosynthetic electron transport of cyanobacteria and other macrophytes, aiding its competitive advantage over other species. This was not investigated in the present study but may have had an inhibitory effect on the competitive ability of *L. major*.

The general increase in eutrophication in aquatic ecosystems throughout the world facilitates the growth of plant species that are better competitors at high nutrient levels (Byers 2002), such as *H. verticillata*, which is becoming invasive around the world (Langeland 1996). Therefore it is not likely that *L. major* may gain a competitive advantage over *M. spicatum* and other submerged species under the right conditions. This study has shown that the competitive ability of *L. major* may be the reason why so few submerged invasive species have invaded South Africa water systems, and why *L. major* is becoming such an aggressive

invasive species in other parts of the world. However in South Africa, in the Vaal River, *M. spicatum* is replacing indigenous submerged species (Coetzee *et al.* 2011a). A similar superior competitive ability of *M. spicatum* over *L. major* may occur in the indigenous range, due to a suite of natural enemies of *L. major* affecting its competitive ability (see Chapter 7). In the light of this, it then follows that in countries where *L. major* has become invasive, the introduction of herbivore pressure could reduce the competitive advantage of *L. major*, giving indigenous species the opportunity to re-establish.

Numerous biotic and abiotic drivers affect the establishment and growth of submerged invasive species. These findings show that some of the indigenous aquatic vegetation in South Africa may prevent the establishment of invasive species in certain environments through competition. However, mechanisms such as increased nutrients and sedimentation may influence the growth and competitive strategies of certain species, and could thus create environments suitable for invasive species. It is also recognised that aquatic environments are dynamic systems, and other influences such as herbivory should also be considered when trying to understand the drivers of establishment and aquatic macrophytes within systems and it is thus important to quantify the effects of phytophagous insects on *L. major* to ascertain the biotic influence (Chapter 6).

# Chapter 6: Biology of *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae) and its impact *Lagarosiphon major* <sup>4</sup>

# 6.1: Introduction

Abiotic drivers such as light, water temperature, sediment composition and inorganic carbon availability are considered fundamental drivers of macrophyte communities (Barko & Smart 1981; Chapters 4 and 5). Additionally, both inter- and intraspecific competition significantly influences the establishment and distribution of species within a system (McCreary & Carpenter 1987; Chapter 5). Chapter 5 investigated the influence of sediment type and nutrient levels on the competitive ability of *M. spicatum* and *L. major*, in the absence of herbivory but in order to assess how these relationships may occur in natural ecosystems, the influence of herbivory needs to be considered.

Herbivory, including insect herbivory, is an important biotic driver of plant community composition through its influence on the competitive ability, abundance and distribution of plants (Carpenter & Lodge 1986; Carson & Root 2000: Speight *et al.* 2008; Price *et al.* 2011). In comparison with terrestrial environments where phytophagous insects constitute nearly 45% of the insect community, only 20% of the insects associated with aquatic systems feed on actively growing macrophytes and the occurrence of many of these species is regarded as rare (Newman 1991). Of this 20%, more than 75% are from terrestrial orders such as Coleoptera, Diptera, Lepidoptera, and Orthoptera (Newman 1991). A limited number of studies on herbivores associated with submerged macrophytes have been published and most

<sup>&</sup>lt;sup>4</sup> Martin, G.D., Coetzee, J.A. & Baars, J-R. 2012. *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), a potential biological control agent for the submerged aquatic weed, *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae). *African Entomology* in press.

of those are surveys for potential biological control agents where the plants have become weeds (Newman 1991). These biological control studies give insight into the diversity of phytophagous insects associated with common submerged macrophytes. Surveys on *H. verticillata* found more than 25 species of herbivorous insects, from five orders, while intensive surveys in the native range of *M. spicatum* revealed a total of 10 herbivorous families and 44 phytophagous species associated with this plant (Cock *et al.* 2008). Surveys conducted on insect fauna associated with *C. caroliniana* in its native range in South America identified several phytophagous species, including Coleoptera and Lepidoptera (Palmer *et al.* 2010). The numbers associated with submerged species are significantly lower than terrestrial weeds such as *Lantana* spp. (Verbenaceae) (Palmer & Pullen 1995), *Melaleuca quinquenervia* (Cav.) S. T. Blake (Myrtaceae) (Balciunas *et al.* 1995), and *Sida acuta* N. L. Burman (Malvaceae) (Gillet *et al.* 1991), where more than 40 phytophagous species are associated with each.

The submerged aquatic *L. major* has an extensive native distribution in southern Africa where it is often dominant among the submerged flora and has become invasive in many countries where it is considered problematic. The plant has recently received more attention due to increasing records of occurrence, and its detrimental effects on freshwater systems (Rattray *et al.* 1994; McGregor & Gourlay 2002; Cook 2004; Baars *et al.* 2010; Chapter 2), and is now the subject of a biological control programme in Ireland (Baars *et al.* 2010). Two studies have investigated phytophagous species associated with *L. major*, but neither has quantified the influence of herbivory on the plant. Schutz (2007) investigated the insect fauna associated with three South African macrophytes *L. major*, *L. muscoides* and *S. pectinata*, at two sites in the Mooi River area, South Africa, in order to fill the knowledge gap regarding insect assemblages associated with submerged species. The study identified several species

associated with *L. major* of which four species were deemed to be phytophagous, including two species of caddisfly; *Athripsodes harrisoni* Barnard and *Leptocerus* sp. (both Trichoptera: Leptoceridae); a snout moth *Parapoynx fluctuosalis* Meyrick (Lepidoptera: Pyralidae) and an unidentified Chironomid species (Diptera: Chironomidae).

As an initial step in the biological control of *L. major* in Ireland, Baars *et al.* (2010) surveyed 33 *L. major* sites throughout the plant's distribution in South Africa. Seven phytophagous species associated with *L. major* were recorded, including a broadly distributed and abundant leaf-mining fly, *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae); a stem-mining fly, *Hydrellia* sp. (Diptera: Ephydridae) from a single site; leaf-feeding Lepidoptera, *Synclita obliteralis* Walker and *Parapoynx* sp. (both Crambidae: Nymphulinae), which had been recorded by Schutz (2007); a shoot-tip mining midge, *Polypedilum (Pentapedilum* n. sp. (Chironomidae); and two curculionid *Bagous* sp. (Coleoptera: Curculionidae, Bagoini) were encountered at separate single sites (Baars *et al.* 2010).

Field observations showed the ephydrid fly, *H. lagarosiphon*, to have a wide distribution and to cause significant leaf damage, and Baars *et al.* (2010) consider it to hold the best potential as a biological control agent for *L. major* where it is a significant weed in Ireland. In order to understand the influence of the fly on *L. major*, a greater knowledge of the species' biology and its impact on the plant was required. This study describes the results of field surveys and laboratory studies on the distribution, biology and impact of *H. lagarosiphon* to understand how this herbivory might influence *L. major*, thereby elucidating the importance of herbivory as a driver of macrophyte assemblages.

# 6.2 Methods and Materials

# 6.2.1.1 Field surveys

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Botanical records from the South African National Biodiversity Institute (SANBI) and Rhodes University were used to ascertain the distribution of *L. major* in South Africa (Baars *et al.* 2010). Surveys for potential biological control agents across the distribution of *L. major* were undertaken in November 2008 (Baars *et al.* 2010), December 2009 and May 2010 (Table 6.1). Two sites (Site 1 & 2) in the Eastern Cape Province were regularly surveyed between 2008 and 2011 to determine seasonal variations in insect and plant populations.

	7 0 1	5		
Site	Location	Province	Latitude	Longitude
1	Featherstone Farm Dam	EC	32.3325	27.3009
2	Wriggleswade Dam	EC	32.5862	27.4642
3	Road side North of Cala	EC	31.4182	27.7842
4	Bushman's Nek Farm Dam	EC	29.7825	29.4395
5	Moshesh's Ford	EC	27.8945	31.1513
6	Farm Dam South of Little Pot Rv.	EC	30.9908	28.2114
7	David Aucamp Dam (nr Maclear)	EC	31.0722	28.3280
8	Cedarberg	EC	30.4261	29.0275
9	Cedarberg	EC	30.4336	29.0185
10	Cedarberg	EC	30.4099	29.0077
11	2nd Dam on Bushman's Nek Rd.	KZN	29.8305	29.3479
12	Small Dam Nr Underburg	KZN	29.8469	29.3007
13	LargeDam on Sani Pass Rd.	KZN	29.6841	29.4895
14	Stillwater Dam (Nr Rosetta)	KZN	29.3049	29.9736
15	The junction Dam	KZN	29.3576	29.9974
16	Rawdons Dam	KZN	29.3712	30.0133
17	Mearns Dam (Nr Station House)	KZN	29.1532	29.5748
18	Mooi River (Nr Rosetta)	KZN	29.3012	29.9630
19	Ntabamhlope (Dam nr T junction)	KZN	29.1773	29.7824
20	Dam in Reitz	FS	27.8049	28.4358
21	Farm Dam on Rd. to Volksrust	MP	27.3879	29.6734
22	Volksrust	MP	27.3624	29.8691
23	Vrede rd	MP	27.3874	29.6677
24	Chrissiesmeer	MP	26.2848	30.2137
25	Hendrina	MP	26.1366	29.8570
26	Rd from Chrissiesmeer	MP	26.4979	30.2535
27	Lydenberg Fisheries (Top Dam)	MP	25.1103	30.4768
28	Misty Mt. Lodge (W of Sabie)	MP	25.1035	30.3936

 Table 6.1: Surveyed Lagarosiphon major sites in South Africa

	Malaga/Elands Rv (Irrigation			
29	Canal)	MP	25.5970	30.4681

Where *H. lagarosiphon* was found, 100 *L. major* shoots, approximately 15-20cm long, were examined for larvae and puparia of *H. lagarosiphon*. One hundred shoots of indigenous macrophyte species present within the immediate vicinity of the *L. major* stand were also examined for any sign of damage from *H. lagarosiphon*. All damaged material was returned to the laboratory and placed in emergence chambers. Any adults emerging from the material were identified. Distribution records of *H. lagarosiphon* arising from the field surveys were overlaid onto a map incorporating mean daily minimum air temperature data during the coldest months in South Africa (Schulze 1997), using ArcView v.9. (ESRI 2012) to estimate temperatures typically experienced by fly larvae populations in the field during the coldest months of the year.

The holotype (specimen number TYPH01958) of *H. lagarosiphon*, housed at the National Collection of Insects, Biosystematics Division, Agricultural Research Council-Plant Protection Research Institute, South Africa, was collected from Featherstone Farm Dam, near Stutterheim in the Eastern Cape (Site 1) (Table 6.1). Before being submitted to the National Museum of Wales, Cardiff, for description, a culture of the fly collected from Featherstone Farm was established at University College Dublin, Ireland. To ensure that the same species of *Hydrellia* was feeding on *L. major* throughout South Africa, *Hydrellia* spp. adults were collected from the water surface above *L. major* mats at selected sites in South Africa, reared to F3 generation on *L. major*, and then sent for comparison with the holotype specimen (Deeming 2012).

## 6.2.1.2 Parasitism of Hydrellia lagarosiphon in the field

Parasitoid pressure on *H. lagarosiphon* was determined by monitoring parasitoid populations at two field sites in the Amatola region of the Eastern Cape: Site 1 (Table 6.1), Featherstone

Farm Dam, a small, shallow, sheltered farm dam; and Site 2 (Table 6.1), Wriggleswade Dam, with two sampling stations (32.58618 S, 27.46415 E and 32. 36531 S, 27. 33223 E), a comparatively large and deep impoundment. Both dams had large healthy beds of *L. major* with both *H. lagarosiphon* and parasitoids present. At each site, a  $1m^2$  quadrat was randomly thrown onto the *L. major* mat and all plant material within the quadrat was collected. Three quadrats were sampled monthly at each site for 8 months (March – October 2010). The *L. major* material from each quadrat was then sorted into stems with larval damage. All leaves containing larvae or puparia were removed from the stems and placed individually into 5ml Epindorph<sup>TM</sup> tubes half-filled with clean water and sufficient undamaged leaves of *L. major*, which were replaced when required. The tubes were left under a growth light and checked daily for fly pupariation and eclosion or parasitoid emergence. Percent parasitism could then be calculated.

#### 6.2.2 Biology of Hydrellia lagarosiphon in the laboratory

The initial laboratory colony of *H. lagarosiphon* on which these studies were conducted was started from approximately 200 adults collected from Featherstone Farm Dam near Stutterheim, Eastern Cape (Site 1) (Table 6.1) in 2009.

Insect-free *L. major* was grown in polypropylene pools (267cm x 65 cm, 3300 litres), fitted with a steel frame for support in a greenhouse tunnel at Rhodes University. When needed, additional plant material was collected from the field to replenish the laboratory stock. The laboratory population of *H. lagarosiphon* was reared in a greenhouse in a similar polypropylene pool (see above), covered with fine gauze mesh to prevent the flies from escaping. The pool was stocked with fresh *L. major* and the flies were allowed to complete their life cycle within the pool. The adults were supplemented with a combination of yeast hydrolysate and sugar (4g: 7g) to promote oviposition (Buckingham & Okrah 1993). Larvae
and adults were harvested from the pool when required for experiments. Larvae were dissected from infested shoots under a stereomicroscope while adults were collected from the water surface using aspirators.

#### 6.2.2.1 Lifespan and fecundity of Hydrellia lagarosiphon

Seven to eight newly eclosed flies were confined in polystyrene containers (12cm X 8cm, 500ml), containing 100ml of water. Water was provided to maintain humidity. A yeast hydrolysate and sugar mixture was provided as food on a plastic float (2 x 2cm). The flies were allowed to mate for 24 hours and the females were separated and placed individually in a petri-dish with moist filter paper, yeast hydrolysate/sugar mixture and a shoot of *L. major*. The females were confined and allowed to oviposit until death. This study was conducted under controlled temperatures of  $21 \pm 1.5^{\circ}$ C and fluorescent plant growth lights (85W OSRAM Plant lighting) at 12:12 day: night regimes. Lifespan of the flies as well as the fecundity of the females were calculated.

# 6.2.2.2 Larval and pupal development of Hydrellia lagarosiphon

Sixty apical shoots (10cm length) of insect-free *L. major* were exposed to recently mated flies. Each apical shoot was kept in an individual polystyrene container (12cm X 8cm, 500ml) and exposed to 12 flies with an (approximate) sex ratio of 1:1 for 12 hours at 21°C, to ensure oviposition on the shoots. The sex ratio was confirmed by sexing all adults after death. Once the flies had been removed, the apical shoots were checked for oviposition, indicated by the presence of clusters of eggs on the leaves. The eggs were then checked daily to ascertain incubation time. Larvae were also monitored daily to determine when moulting and pupariation occurred. Once the larvae had pupated within the *L. major* leaves, the leaves were removed from the main stem and placed in 5ml Epindorphs<sup>TM</sup> containing 2ml water. The number of days until adult eclosion was then monitored.

Measurements of eggs, larvae and puparia were made at 50X or 25X magnification with an ocular micrometer in a stereomicroscope. Egg length was determined as the distance between the posterior and anterior ends of the eggs, and the width as the maximum transverse extent of the egg. Larval length was measured from the anterior edge of the head lobe to the posterior end of the spiracular peritremes, with the larvae outstretched, and the width as the maximum transverse extent, measured in the dorsal view. Puparia were measured similarly and were weighed on a CAHN C-31 microbalance. Means  $\pm$  S.E. were calculated for each parameter.

#### 6.2.3 Effect of herbivory by Hydrellia lagarosiphon on Lagarosiphon major

To determine the effect of feeding by *H. lagarosiphon* on *L. major*, 60 apical shoots, 20cm long, were placed individually in clear plastic containers (7.5cm x 25cm, 900ml), filled with tap water and allowed to grow for two weeks under controlled temperatures of  $25^{\circ} \pm 2^{\circ}$  C, and a 12:12 day: night regime provided by fluorescent lights (85W OSRAM Plant lighting). After two weeks, eggs laid on the same day by recently mated flies, were placed on the individual *L. major* shoots in a random block design at densities of 0 (control), 1, 2, 4, and 8 eggs per plant. The emerging larvae were then allowed to feed and develop until pupariation. Each egg density treatment was replicated 12 times. Once puparial shoot length, number of branches, number of damaged leaves and the number of puparia were recorded. The influence of egg density on plant parameters was analysed using Kruskal-Wallis ANOVAs and posthoc multiple comparison of mean rank tests. All statistical analyses were conducted in STATISTICA ver. 8.0.

## 6.3 Results

## 6.3.1 Field surveys

Water bodies where *H. lagarosiphon* was recorded included impoundments, natural lakes, perennial streams and rivers. Stands of *L. major* infested with *H. lagarosiphon* ranged from small clumps amongst beds of other submerged macrophytes along the edges of dams to large beds occupying the entire water column of small dams and natural lakes. *Hydrellia lagarosiphon* was found at all 29 sites sampled around South Africa. Repeated visits to dams in the Eastern Cape Province showed that flies and larvae were always present. Some 1-100% of damaged shoots produced at least one fly or parasitoid, or when dissected was found to contain at least one live larva or puparium. In the field, the highest number of larvae recorded per 20cm of stem was 10. Larvae were also not restricted to growth tips near the surface and were sometimes found throughout the water column. Damage to the plant was clearly visible where high densities of *H. lagarosiphon* were present. Other submerged species investigated at these sites were infrequently damaged by *Hydrellia* spp.; some puparia were found on adjacent plant species, such as *S. pectinata* and *L. muscoides*. Unfortunately all these puparia were parasitised. As a result no adults were reared through for identification from these non-target plants.

Sites where *L. major* and *H. lagarosiphon* were recorded were limited to the colder areas of South Africa, namely the Amatola Region of the Eastern Cape, Drakensberg regions of KwaZulu-Natal and the Eastern Cape, and the Mpumalanga Highlands. Only one site was found outside these areas at Reitz, in the Free State (Fig. 6.1). Minimum winter ambient temperatures at these sites ranges from -2 to  $+ 4^{\circ}$ C (Schulze 1997) (Fig. 6.1). The mean minimum temperature in these areas can drop to well below 0°C (Schulze 1997). Even at the coldest sites, *H. lagarosiphon* adults and larvae were found in abundance.

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### 6.3.1.2 Parasitism of Hydrellia lagarosiphon in the field

Three different braconid parasitoids were recovered from *H. lagarosiphon* collected in the field as well as from stock cultures. Adult parasitoids were observed in the field searching and probing under water for larvae or pupae; this was achieved by holding a bubble of air in the wings and walking over the plant material while searching for larvae or pupae (Baars *et al.* 2010). Investigations at Featherstone Farm Dam showed that over the eight months studied,  $32\% \pm 14$  (S.E.) of *H. lagarosiphon* larvae were parasitised by the wasps. The highest mean % parasitism in summer was  $28.9\% \pm 19.8$  S.E. at Wriggleswade Dam (Site 2), while the highest mean % parasitism in winter was  $52.8\% \pm 2.3$  (S.E.) at Featherstone Farm Dam (Site 1) (Table 6.3). Specimens reared from the three study sites were identified as *Ademon lagarosiphonae* sp. n. (Opiinae), *Chaenusa luteostigma* sp. n. and *C. nigristigma* sp. n. (Alysiinae: Dacnusini) (van Achterberg & Prinsloo 2012).



**Figure 6.1:** Distribution of *Lagarosiphon major* sites and *Hydrellia lagarosiphon* occurrence in South Africa, in relation to the mean daily minimum temperatures during July (winter). The plant and the fly were limited to the colder higher regions of the Eastern Cape, KwaZulu-Natal and Mpumalanga. Data generated from the South African Atlas of Agrohydrology and Climatology (Schulze, 1997). GT – Gauteng, MP – Mpumalanga, NW – Northwest, KZN – KwaZulu-Natal, EC – Eastern Cape, WC – Western Cape, NC – Northern Cape, FS - Free State.

**Table 6.2:** The number of *Lagarosiphon major* shoots infested with *Hydrellia lagarosiphon* 

 larvae or puparia found at sites as well as the number of *H. lagarosiphon* larvae or puparia

 found on associated species

Site	Number of Lagarosiphon major shoots containing H. lagarosiphon per 100 shoots	Associated species (Species name)	Family	Number of associated species containing <i>H</i> . <i>lagarosiphon</i> per 100 shoots
1	1	-	-	-
2	50	Stuckenia pectinata*	Potamogetonaceae	1
-		Persicaria decipiens	Polygonaceae	0
		Poaceae sp. 1	Poaceae	0
		Eichhornia crassipes	Pontederiaceae	0
4	16	-	-	-
5	7	Stuckenia pectinata*	Potamogetonaceae	0
-		Potamogeton thunbergii	Potamogetonaceae	1
8	100	Stuckenia pectinata*	Potamogetonaceae	1
-		Poaceae sp. 2.	Poaceae	0
9	35	Potamogeton thunbergii	Potamogetonaceae	-
10	2	-	-	_
11	62	<i>Hydrocotyle</i> sp.	Araliaceae	0
12	3	Stuckenia pectinata*	Potamogetonaceae	0
13	23	Stuckenia pectinata*	Potamogetonaceae	0
14	21	Lagarosiphon muscoides	Hydrocharitaceae	0
15	98	<i>Wolffia</i> sp.	Lemnaceae	0
16	16	-	-	-
17	100	Lagarosiphon muscoides	Hydrocharitaceae	3
20	100	-	-	-
21	75	Stuckenia pectinata*	Potamogetonaceae	0
		Utricularia stellaris	Lentibulariceae	0
22	100	Lagarosiphon muscoides	Hydrocharitaceae	0
24	85	Nymphoides sp.	Nymphaeaceae	0
		Lagarosiphon muscoides	Hydrocharitaceae	3
25	100	Nymphoides sp.	Nymphaeaceae	0
26	100	Potamogeton thunbergii	Potamogetonaceae	0
27	76	Pennisetum clandestinum	Poaceae	0
28	41	Lagarosiphon muscoides	Hydrocharitaceae	0
29	90	Potamogeton schweinfurthii	Potamogetonaceae	0
	~ ~	Utricularia stellaris	Lentibulariaceae	0

\* Stuckenia pectinata (= Potamogeton pectinatus L.) (Potamogetonaceae).

Site	Mean (± S.E.) Parasitism (%)			
	Annual	Summer	Winter	
Featherstone Farm	$32 \pm 14.4$	$18 \pm 14.8$	$52 \pm 2.3$	
Dam				
Wriggleswade Dam	$17 \pm 16.7$	$28 \pm 19.8$	0	
Site 1				
Wriggleswade Dam	$8 \pm 8.3$	$13 \pm 10.2$	0	
Site 2				

**Table 6.3:** Mean percentage of *Hydrellia lagarosiphon* larvae parasitised by wasps at three sites for the entire sampling period and during summer and winter.

#### 6.3.2 Biology of Hydrellia lagarosiphon in the laboratory

# Eggs

Females laid up to 25 eggs (mean=  $18.8 \pm 1.9$  (S.E.), n=18 females) over their life span. Eggs were 0.68 mm  $\pm$  0.01 (S.E.) long and 0.20 mm  $\pm$  0.003 (S.E.) wide (n=22), white, and had longitudinal ridges running along their length. Eggs were mostly deposited one day after mating and hatched two days post oviposition. In the laboratory, egg-laying depended upon the plant structures available for oviposition; when shoots of *L. major* were available, the eggs were deposited on parts that protruded from the water such as exposed leaves or growth tips. When leaves protruded, eggs were often laid on the abaxial sides of the recurved leaves. The eggs were laid singly or in clusters of up to 15. Even when fresh shoots were available, eggs were found floating on the surface of the water as well as on any other available structures, particularly slight indentations or ridges, such as the rim of the lid of the holding containers. Similar observations were made on *H. pakistanae*, which feeds on *H. verticillata* (Buckingham & Okrah 1993). It has been shown in other *Hydrellia* spp. that newly hatched larvae are highly mobile and are able to leave the egg site to search for the host plant, and thus the oviposition substrate is not necessarily important to larval survival (Buckingham & Okrah 1993).

# Larvae

The larvae were translucent yellow/white and were usually visible within the leaf by their conspicuously dark feeding apparatus, the cephalopharyngeal skeleton. Neonates were found between the epidermal layers of the leaves. Most early instars were found within the youngest leaves of the growth tips. There was very little apparent difference between the three larval instars other than the change in shape of the feeding apparatus and slight darkening of the larval spiracular peritremes. The spiracular peritremes became reduced in size and changed colour as the larvae developed from first to third instar. Besides this, the three instars differed morphologically in size only (Table 6.4). First instars were 0.48 mm  $\pm$  0.02 (S.E.) long by 0.1 mm  $\pm$  0.08 (S.E.) wide. Third instars were 3.3 mm  $\pm$  0.1 (S.E.) long by 0.7  $\pm$  0.03 (S.E.) wide just before pupation. The larval stage lasted around 26 days  $\pm$  0.7 (S.E.) (n=16) (Table 6.4).

**Table 6.4:** Mean larval developmental time at  $21^{\circ}$ C and mean body lengths and widths of the three larval instars of *Hydrellia lagarosiphon* (n = 25 for each instar).

	Time (days)	Length (mm)	Width (mm)
1st instar	1	$0.48 \pm 0.02$ (S.E.)	$0.1 \pm 0.004$ (S.E.)
2nd instar	5	$1.0 \pm 0.08$ (S.E.)	$0.2 \pm 0.02$ (S.E.)
3rd instar	11	$1.6 \pm 0.10$ (S.E.)	$0.3 \pm 0.02$ (S.E.)
Pre-pupa	26	$3.3 \pm 0.10$ (S.E.)	$0.7 \pm 0.03$ (S.E.)

Neonates moved quite freely after hatching in search of fresh young leaves in the growth tips. The larvae mined between the epidermal layers of the leaf and removed the leaf mesophyll tissue. Larvae damaged on average  $19.2 \pm 1.1$  (S.E.) leaves, ranging from 6-37 leaves; the majority of the leaf contents were usually consumed before the larva moved on (n=38).

Larvae predominantly moved down the stem, from the tip towards the roots, in search of new leaves and were able to move below the stem epidermis to reach new leaves.

## Puparia

Puparia were usually located within the epidermal layers of the tunnelled leaf, predominantly 1-5cm below the growth tip, or occasionally within the growth tip. On average, they were  $3.42 \text{ mm} \pm 0.03$  (S.E.) long and  $1.07 \text{ mm} \pm 0.02$  (S.E.) wide, and weighed  $0.31g \pm 0.01$  (S.E.) (n=50). They were initially yellow to white in colour but gradually turned dark brown to black as the flies developed. Pupae eclosed in 14 days  $\pm 0.2$  (S.E.) (n=17).

### Adults

*Hydrellia* spp. adults sent for identification were confirmed as *H. lagarosiphon* (Deeming 2012). Adults were dark, with a lighter shiny face. The knob of the haltere was yellow. All hairs and bristles were black apart from some on the dorsal surface which were greyish. The wings too were greyish. The morphology and colouration of *H. lagarosiphon* closely resembled that of other African *Hydrellia* spp., such as *H. bicolorithorax* Giordani Soika, from Rwanda, and *H. varipes* Lamb, from the Seychelles (Deeming 2012). The sexes were easily distinguishable by their genitalia. The females had a uniformly flat abdomen with clearly visible sternum segments separated by an intersegmental membrane. The males had a very apparent cavity in the abdomen covered with small dark bristles; this cavity is protected by the cercus which pulls open to expose the inner copulatory organ during mating (Deeming 2012).

# 6.3.3 Impact studies of Hydrellia lagarosiphon on Lagarosiphon major

Laboratory experiments showed that all shoots infested with *H. lagarosiphon*, independent of density, were significantly longer than uninfested shoots (H  $_{(4, 44)}$  =14.38; *P* <0.05). This was

an unexpected result, since one would have expected more growth from the control shoots. There was no significant difference in shoot length at different egg densities (Fig. 6.2a). Infested shoots grew to over 30 cm, but averaged 27cm  $\pm$  0.6 (S.E.), whereas shoots with no *H. lagarosiphon* eggs rarely grew over 22cm  $\pm$  0.9 (S.E.). Uninfested shoots produced significantly more branches than those infested with *H. lagarosiphon* (H <sub>(4, 44)</sub> =24.32; *P*< 0.05) (Fig. 6.2c); however, the number of branches produced was not influenced by egg density, i.e. there were no differences between shoots exposed to 1, 2, 4 or 8 eggs (Fig. 6.2c). Up to four branches were produced on uninfected *L. major* shoots, whereas significantly fewer branches were found on *L. major* infested with *H. lagarosiphon* (Fig 6.2c). There were significant differences in damage between shoots exposed to 1 and 4 eggs, and between those exposed to 1-2 eggs and 8 eggs (Fig. 6.2b). Larvae from 8 eggs per shoot caused significantly more damage than 1 or 2 eggs per shoot (H <sub>(4, 44)</sub> =39.6; *P* < 0.05) (Fig. 6.2c).

Failure of eggs to hatch and of larvae to pupate did occur. There was 100% survival at the single egg stocking density, whereas at densities of 2 or 4 eggs, approximately 60% of eggs produced larvae that reached pupation. At the highest egg density (8 eggs), there was an increase in mortality and a reduction in larval survival to pupation (42%), probably due to larval competition. There was no significant difference in survival between 1 and 2 eggs; however, there was significantly greater survival at a density of 1 than of 4 and 8 eggs. There was no significant difference in survival between 2, 4 and 8 eggs (H <sub>(3, 34)</sub>=13.15, P < 0.05) (Fig. 6.2d).



**Figure 6.2a:** Impact of *Hydrellia lagarosiphon* on *Lagarosiphon major* in relation to the number of *H. lagarosiphon* eggs placed on the shoots. **a.** Differences in *L. major* shoot length (cm) (H ( $_{4, 44}$ ) =4.72 P>0.05). **b.** Number of damaged *L. major* leaves per shoot (H ( $_{4,44}$ ) =39.6, *P* >0.05). **c.** Number of *L. major* branches produced per shoot (H  $_{4,44}$ , =24.3; *P* >0.05). **d.** Number of *H. lagarosiphon* pupae that survived per number of eggs placed on shoot (H ( $_{4, 44}$ , =40 P>0.05). Error bars represent S.E.; means followed by the same letter are not significantly different.

*Hydrellia lagarosiphon* did not affect the number of roots produced by the shoots as there was no significant difference between the control and the different egg densities (0, 1, 2, 4, and 8 eggs per shoot) (H  $_{(4, 49)}$  = 39.59, *P* < 0.05). On average, *L. major* produced 17 ± 0.1 (S.E.) roots per plant.

## 6.4 Discussion

Dedicated surveys on submerged macrophytes have revealed a complex of phytophagous natural enemies associated with submerged plant species; surveys on *L. major* found similar results. Schutz (2007) compared the diversity of insects found on *L. major* to insect communities found on *Potamogeton chessemanii* A. Benn (Potamogetonaceae), *Myriophyllum propinquum* A. cumm (Haloragaceae) and Characeae spp. in New Zealand, and found similar assemblages but fewer species than those found on *L. major*. The complex of herbivores found on *L. major* is notably similar to that discovered on *H. verticillata* on the Asian and Australia continent.

The genus *Hydrellia* is highly specialised and predominantly specific to aquatic plant species (Deonier 1971). Of 46 *Hydrellia* spp. found in the Holarctic region, where the genus has received most attention, 32 have host plants in the Hydrocharitaceae, Alismataceae, or Potamogetonaceae (Buckingham *et al.* 1989). Twenty-one of the *Hydrellia* species listed by Deonier (1971) were reported to have only one host plant, while 10 species were recorded from one plant genus, one species from one plant family, 11species from two plant families, and two species from three plant families. Only *H. griseola* (Fallkn) was reported from more than three plant families (Buckingham *et al.* 1989). Both Buckingham & Okrah (1993) and Deonier (1971) reported that late instar larvae of *Hydrellia* spp. may move to adjacent non-host plants for pupariation, but this does not cause significant damage to the new host.

Abiotic factors could influence the abundance and lifespan of *H. lagarosiphon*. Possible mitigating abiotic factors may be similar to those experienced by *H. pakistanae* on *H. verticillata* in the U.S.A., namely cold winter temperatures which reduce both the fly's activity and the presence of its host plant (Wheeler & Center 2001), while wind, wave action, temperature and humidity tolerance restrict oviposition to sheltered habitats (Deonier 1971).

However, field observations suggest that cold temperatures do not appear to limit the distribution of *H. lagarosiphon* in its indigenous range. All large infestations of *L. major* were restricted to areas of South Africa experiencing winter temperatures between - 2 and + 4°C. Thus *H. lagarosiphon* should be able to survive in the more temperate climates of Europe and New Zealand where *L. major* has become a considerable problem in different freshwater habitats and where biological control might be considered a viable control option (Rattray *et al.* 1994; Caffrey *et al.* 2011).

*Hydrellia lagarosiphon* has a similar biology to the Australian species, *H. balciunasi*, and the Asian species, *H. pakistanae*, found on *H. verticillata* (Wheeler & Center 2001), and *Hydrellia* sp. n. which feeds on *Eg. densa* (Cabrera Walsh *et al.* 2013). These species and *H. lagarosiphon* lay eggs predominantly on protruding vegetation and shoot tips, and the neonate larvae prefer to move to the crown of the plant to feed initially on the softer younger shoots, and then move down the plant in search of new leaves. The larvae feed in-between the upper and lower leaf epidermal tissue layers, effectively reducing the plant's ability to photosynthesise (Wheeler & Center 2001). Pupariation occurs between the epidermal layers of the leaf, and adults live on the surface of the water and water body edge, moving by walking and short hopping flights.

Experiments in tanks investigating the relationship between the invasive *H. verticillata* and the fly *H. pakistanae* showed that larval feeding damage to 10-30 % of leaves on a stem reduced the maximum rate of light-saturated photosynthesis of the plant by almost 40%. When leaf damage reached 70% of leaves on a stem, photosynthetic rates were reduced by up to 60% (Doyle *et al.* 2002). For the plant's daily respiratory requirements to no longer be met, leaf damage would have to reach densities of 70-90% (Doyle *et al.* 2002). However, Wheeler & Center (2001) indicated that it would take approximately 4000 fly larvae/m<sup>2</sup>

during a single generation to damage 60–70% of the whorls of the *H. verticillata* plant. *Hydrellia pakistanae* was released in the U.S.A. in 1990 and recent surveys have shown that numbers never reach such densities, even under optimal conditions. The highest field densities recorded for *H. pakistanae* translated to approximately 15% of the whorls damaged (Wheeler & Center 2001). In contrast, in the indigenous range of *H. lagarosiphon*, on average, 58% of stems contained *H. lagarosiphon*, while sites with 100% of stems infected were not uncommon. These comparatively higher densities suggest that field populations of the fly could significantly reduce photosynthesis of *L. major*, but this needs to be tested. The newest plant growth and growth tips were generally the most susceptible to larval damage. Similar damage has been recorded with other *Hydrellia* spp. and their host plants (Deonier 1971). In these laboratory experiments with *H. lagarosiphon*, only high larval densities (8 larvae per shoot) seemed to have a significant impact on the plant, although shoots were only exposed to a single generation of flies. Accumulative damage over several generations at low fly densities may cause significant levels of damage.

Hydrocharitaceae species such as *L. major* form dense mats and canopies, limiting competition from other species (Howard-Williams & Davies 1988; Van *et al.* 1999; James *et al.* 1999; Caffrey & Acevedo 2007; Caffrey *et al.* 2010). *Hydrellia lagarosiphon* was shown to reduce branching by *L. major*, thus potentially restricting the plant's ability to form a dense canopy. Laboratory competition studies in the U.S.A. revealed that *H. verticillata* is a much stronger competitor than *V. americana* at high nutrient levels, forming a dense surface canopy that competitively excludes *V. americana* (Van *et al.* 1999). When *H. pakistanae* was introduced, larval damage reduced the *H. verticillata* canopy in the top 30cm of the water column. As a result, there was a significant shift in the competitive balance between *H. verticillata* and *V. americana* (Van *et al.* 1999). The effect of an herbivore may be subtle, leading

to a gradual reduction in the host plant's health, thereby increasing its susceptibility to competition from other vegetation (Pantone *et al.* 1989; Wheeler & Center 2001; Coetzee *et al.* 2005). Thus, even at densities lower than recorded in its indigenous range, *H. lagarosiphon* may be able to reduce the biomass of *L. major*, thereby opening areas for native plant re-growth, similar to the reduction recorded in *H. verticillata* when grown under the influence of herbivory and in competition with native plant species (Grodowitz *et al.* 2007).

While the third trophic level is seldom considered to be important in weed biological control programmes (Harvey *et al.* 2010), parasitoids of *Hydrellia* spp. have been recorded from around the world (Deonier 1971; Hesler 1995; Wheeler & Center 2001; Diaz *et al.* 2009; Kula 2009). Thus, parasitism of *H. lagarosiphon* was expected in its native range and may possibly occur in its introduced range. The effect of parasitism on herbivorous insects is a relatively unstudied field, and warrants more investigation. At field sites in South Africa where parasitism levels were relatively high, *H. lagarosiphon* numbers remained high despite parasitism.

*Hydrellia lagarosiphon* is the most ubiquitous and common herbivore species associated with *L. major* within its native range. Its biology is well understood and it has proved easy to rear under laboratory conditions, thereby showing great potential as a candidate control agent for *L. major*. However further damage assessments and host-specificity testing would be required by any country intending to use the fly as a biological control agent.

This chapter investigated the known literature regarding the phytophagous communities associated with *L. major* and found interesting similarities to other submerged species. The results of the impact studies show that *H. lagarosiphon* mines the photosynthetic material of the plant and reduces the formation of side shoots. However it has been suggested that in order to understand the effect of herbivory on a plant species focus should be given to

understand how herbivory may influence plant competition, and that the effect on plant performance should be assessed (Van *et al.* 1998; Coetzee *et al.* 2005). Chapter 7 examines this further by investigating the effect of herbivory by *H. lagarosiphon* on the competitive ability of *L. major* and considers the next trophic level parasitism, and its combined effect on competition.

# Chapter 7: Multitrophic interactions in freshwater ecosystems: evaluating shifts in the competitive ability of *Lagarosiphon major* as influenced by herbivory and parasitism

# 7.1 Introduction

Aquatic plants are important components of aquatic ecosystems (Carpenter & Lodge 1986; Lacoul & Freedman 2006b; Larson 2007), whose introduction, distribution and abundance within ecosystems are influenced by numerous abiotic (bottom-up) (chapter 4 and 5) and biotic (top-down) (chapter 5 & 6) factors (Lacoul & Freedman 2006b). Trying to assess the relative importance of these top-down and bottom-up regulatory influences has been the focus of much debate and research in ecology (Speight et al. 2008). The abiotic factors driving aquatic macrophyte assemblages in particular are usually associated with the limnology of the water body, including geomorphology, climate and hydrology (Titus & Adams 1979; Smart & Barko 1985; Dawson 1988; Barko et al. 1991; Champion & Tanner 2000; Lacoul & Freedman 2006b; Mackey 2007; Loo et al. 2009), whereas the biotic factors include herbivory, competition and disease (Hofstra et al. 1999; Van et al. 1999; Lacoul & Freedman 2006b). Harvey et al. (2010) argue that plant community structure and function are also influenced by the biotic effect of natural enemies of herbivores; however, this interaction has received far less attention in ecological studies. A multitrophic approach could also aid in understanding the drivers underlying the success or failure of exotic plant species to spread and become dominant in new ranges. In response to the appeal by Harvey et al. (2010), this study investigated the interactions between a parasitoid wasp Chaenusa luteostigma. Achterberg (Hymenoptera: Braconidae: Alysiinae) and its host, a leaf-mining fly, H. lagarosiphon, and their effect on the competitive ability and yield of an invasive aquatic macrophyte, L. major. This study investigated select top-down regulating factors influencing aquatic systems, whereas the previous chapters have predominantly investigated the bottomup regulatory factors.

Competition, one of the important factors driving species distribution and plant structure (Van et al. 1999; Chapter 5), is described as the interaction between different taxa (interspecific) and between individuals of the same taxa (intraspecific), searching for limiting resources (Lacoul and Freedman 2006). In many freshwater systems, intraspecific competition has a greater influence on plant biomass and plant phenology than interspecific competition (Van et al. 1999; Coetzee et al. 2005). For example, E. crassipes (water hyacinth), forms more upright stands with elongated petioles in high plant densities when than in low plant densities, effectively allowing older, taller plants to outcompete younger plants for light through a process of self-thinning (Center & Spencer 1981; Agami & Reddy 1990). This is also evident in number of submerged aquatic species, where an experiment comparing the competitive ability between S. pectinata and C. aspera, showed that S. pectinata suppressed the biomass of C. aspera under high light conditions. However, the greatest result was shown in the individual biomass of S. pectinata, where at initial high planting densities, intraspecific competition reduced biomass by 70% but was not affected by interspecific competition (van den Berg et al. 1998). In a comparable experiment, Moen and Cohen (1989) showed that when S. pectinata and M. exalbescens were grown in experimental aquaria in single- and mixed-species cultures at low and high densities, intraspecific competition reduced growth rate, biomass of roots, shoots and tubers of S. pectinata, but was not affected by competition from *M. exalbescens* (Moen and Cohen 1989). Lagarosiphon major, the subject of this study, is also influenced by intraspecific competition, as evidenced by an addition series competition experiment conducted with mixed plantings of L. major and *M. spicatum* L. (Haloragaceae) grown under two sediment treatments (clay vs. sand) and two soil nutrient concentrations (high vs. low). Here, the influence of L. major on itself was far

more important to the final overall yield than interspecific competition from *M. spicatum* under both sediment and nutrient conditions (see Chapter 5).

The competition between different taxa for limiting resources, or interspecific competition, can also greatly influence the structure and phenology of aquatic macrophytes. Interspecific competition is most pronounced between biologically and ecologically similar taxa (Gopal & Goel 1993; Stiers *et al.* 2011), for example, aquatic species that occupy the same niche in the littoral zone (Van *et al.* 1999; Mony *et al.* 2007). Grace & Wetzel (1981) showed that *T. latifolia* and *T. angustifolia* L. (Typhaceae), two species that commonly co-occur in marshes and ponds, are strongly segregated along a gradient of increasing water depth. *Typha latifolia* is a stronger competitor than *T. angustifolia* in shallower water due to the higher cost of producing broader leaves; however, the greater leaf surface area allows it to compete for light in shallow water.

The best examples of interspecific competition are provided by invasive species which are capable of outcompeting and excluding native species from their habitats. This is possible because in their invaded range, they are free of their indigenous controlling herbivores and diseases (Keane & Crawley 2002). In its adventive range, *L. major* actively outcompetes other submerged macrophytes, including *M. spicatum, M. triphyllum* Orchard. (both Haloragaceae), *El. canadensis* and *Eg. densa* (Chapman *et al.* 1974; Titus *et al.* 1975; Agami & Waisel 1985; Rattray *et al.* 1994; James *et al.* 1999; Hofstra *et al.* 1999; Caffrey & Acevedo 2007). *Lagarosiphon major's* ability to grow rapidly and form a dense canopy, effectively outcompetes other species for both nutrients and light (Rattray *et al.* 1994; Caffrey *et al.* 2010; Chapter 5). However in the presence of its indigenous insect fauna, *L. major* may have reduced competitive ability.

Herbivores directly influence numerous plant species. In agricultural crops herbivores have been shown to effect roots, leaf growth, flower formation and seed yield, ultimately affecting plant productivity, performance and reproduction (Price et al. 2011). One of the best applied models that evaluates the negative or controlling effect of herbivores on plant composition is in the field of biological control of invasive plant species using insect herbivores. There is an abundance of literature evaluating the success of invasive species outside their indigenous range, and the resulting impact of introducing closely associated herbivores to control them (Macfadyan 1998; Van Driesche et al. 2010). For example, in the wetlands of North America, the prolific seed production, phenotypic plasticity and lack of natural enemies of L. salicaria collectively provided it with a competitive advantage over many native wetland species (Weihe & Neely 1997). Gaudet and Keddy (1988) showed L. salicaria to be competitively superior to 44 wetland species when grown in pair-wise combinations. However the introduction of the biological controls agents Galerucella calmariensis L. and Galerucella pusilla Duft. (Coleoptera: Chrysomelidae) has reduced the plant stature and seed productivity and ultimately its competitive advantage, allowing indigenous wetland plants to return to areas where they had previously been outcompeted by L. salicaria (Katovich et al. 1999).

Even the sublethal and long-term subtle effects of herbivory may have significant effects on a plant's response to herbivory. Species of *Hydrellia* flies influence the competitive ability and overall biomass of a variety of aquatic plant species. In the eastern United States, *H. verticillata* was shown to be more competitive than the indigenous *V. americana* in the absence of its indigenous herbivores. However the addition of the leaf-mining fly, *H. pakistanae*, a herbivore associated with *H. verticillata*, reduced the plant's competitive ability to nearly equal that of *V. americana* (Van *et al.* 1999).

The influence of competition and herbivory affects ecosystem assemblages, both independently and in combination (Van et al. 1999; Coetzee et al. 2005). Inter-trophic relationships can have widespread effects on herbivores, and therefore the interaction between herbivores and their predators and parasitoids needs to be considered (Speight et al. 2008). In an ecological sense, predators influence the abundance of herbivores which will have cascading effects on plant distribution and abundance (Price et al. 2011). Thus it is important to understand the process of parasitism and predation on species interactions, and population and community dynamics. There is strong empirical evidence that predators and parasites often keep insect herbivores from causing major damage to their host plants in terrestrial communities (Carson & Root 2000). Good examples of enemy effects on prev populations come from insect biological control. One such example is the control of cottony cushion scale, Icerva purchasi Maskell (Coccoidea: Monophlebidae), a citrus pest introduced into California, around 1868 from Australia, that negatively affected the California citrus industry. In response to the cottony cushion scale, two biological control agents, a parasitic fly, Cryptochetum icervae Williston (Diptera: Agromyzidae), and later the coccinellid, Rodolia cardinalis Molsant (Coleoptera: Coccinellidae), were imported into California from Australia to control the scale insect. By the end of 1889, the introduced insects had almost completely suppressed the scale and ultimately saved the Californian citrus industry (Caltagirone & Doutt 1989).

More recently, in greenhouses across the world, augmentative releases of commercial *Diglyphus* species (Hymenoptera: Eulophidae) have been used for controlling damaging leaf miners (Li & Seal 2010). Bazzocchi *et al.* (2003) demonstrated that *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) parasitized at least 18 different agromyzid species, and is the most effective commercial biological control product for controlling a variety of American pest leaf-miners.

Herbivore suppression by predators and parasitoids has been highlighted as a potentially important ecosystem service of biodiversity. However very few experiments have tried to quantify the effect of predators and parasitoids on plant yields (Carson & Root 1999). One of the few examples shows the effect of three of the most important natural enemies of aphids on the overall yield of alfalfa, *Medicago sativa* L. (Fabales: Fabaceae), namely the coccinellid beetle *Harmonia axyridis* Pallis (Coleoptera: Coccinellidae), the damsel bug *Nabis* species (Hemiptera: Nabidae), and the parasitic wasp *Aphidius ervi* Haliday (Hymenoptera: Braconidae) of pea aphids *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) (Cardinale *et al.* 2003). Only when all three enemy species occur together do they negatively effect on the population density of the pea aphid, which improves the crop yield of alfalfa. However where presented singly, the predators/parasitoid have a slight, but not significant influence on the yield of alfalfa (Cardinale *et al.* 2003).

Experimental manipulation provides some of the strongest support for the role that natural enemies can play in the dynamics of prey populations (Speight *et al.* 2008). There is no literature regarding the influence of parasitoids on the structure and function of submerged macrophytes. However it has increasingly been recognised that there needs to be greater understanding of the forces regulating community structure and function, which should include natural enemies of herbivores (Harvey *et al.* 2010). Freshwater systems provide an opportunity to investigate these relationships.

Lagarosiphon major is indigenous to South Africa, however in its invaded range, dense infestations of the plant readily outcompete indigenous submerged species, altering the ecology of freshwater systems. *Hydrellia lagarosiphon* is the most wide spread and abundant herbivore *L. major* (Chapter 6; Baars *et al.* 2010). Impact studies showed that *H. lagarosiphon* larvae destroy approximately 20 leaves of *L. major* before pupation and restrict

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the formation of side branches (Baars *et al.* 2010; Chapter 6). During the surveys for natural enemies, a parasitoid wasp, *Chaenusa luteostigma* was found on *H. lagarosiphon*. Adult parasitoids were observed in the field, searching underwater and probing the fly larvae (Baars *et al.* 2010; Chapter 6). The adult wasps parasitize late instar larvae and puparia. Field surveys indicated that up to  $28.89\% \pm 4.1$  S.E. of *H. lagarosiphon* larvae could be parasitized in summer and up to  $52.78\% \pm 0.49$  S.E. in winter (Martin & Coetzee in press; Chapter 6)

The aim of this study was to ascertain the shifts in competitive ability of *L. major* in relation to *M. spicatum* in the absence and presence of its herbivore and parasitoid. These interactions will provide a greater understanding of the drivers determining distribution and abundance of aquatic macrophytes in natural systems.

## 7.2 Methods & Materials

This study was conducted in a greenhouse at Rhodes University, Grahamstown, South Africa. The experiment comprised four treatments: an uncovered control, consisting of uncovered ponds with no insects; an insect-free covered control, consisting of covered ponds with no insects; a herbivory treatment, consisting of covered ponds with the leaf-mining fly *H. lagarosiphon*; and a parasitoid treatment, consisting of covered ponds with *H. lagarosiphon* and the braconid parasitoid, *C. luteostigma*.

The experimental design followed an addition series developed by Spitters (1983), as used in Chapter 5, which used factorial combinations of different planting densities of the two competing plants. The setup for all four treatments was the same. The planting densities of *L. major*: *M. spicatum* were 0:3, 0:9, 3:3, 3:9, 9:0, 9:3, 9:9, 3:0 and were planted into 12-L plastic tubs (42cm diam. x 14cm deep), including a control for each treatment, giving a total of 16 tubs. Similar planting densities have shown to be effective in similar competition experiments (Van *et al.* 1999). The mean mass per shoot of *L. major* and *M. spicatum* planted was  $1.3g (\pm 0.20 \text{ S.E.: } n = 1152)$  and  $1.7g (\pm 0.24 \text{ S.E: } n = 1152)$  respectively.

Sediment shared characteristics with both the *L. major* and the *M. spicatum* sites, was selected for the experiment (see Chapter 5). The pond sediment was collected from Jameson Dam, Eastern Cape, South Africa (-33.319073 S; 26.444206 N). The treatments were replicated four times. The insect-free controls, herbivory treatments and parasitoid treatments were enclosed with a net canopy (0.8 mm  $\times$  0.5 mm mesh size). These covers reduced the fluence rate of the photosynthetically active radiation beneath by 63% (Recorded by a Li-cor LI 1 88B Integrating Quantum Radiometer).

Thermachron iButtons (Climastats Environmental Monitoring software, Version 4) were used to detect significant changes in water and sediment temperatures during the experiment. The buttons were placed in a water tight container and either floated on the water surface or were positioned within the pond sediment. Temperature data were recorded every two hours, maximum, minimum and daily mean were recorded. The mean daily temperatures were calculated and the differences between treatments and between the water and sediment treatments were calculated using One-way Analysis of Variance (ANOVA), followed by a Tukey Post-Hoc HSD test.

After five weeks of growing, half of the plants from all the treatments were harvested to determine plant growth, community structure and competition that may have occurred prior to insect release. The two plant species were separated and washed to removes excess sediment. The plants, from each tub were placed in separate brown paper bags and dried in a Heraeus drying oven for 96 hours at 60 °C. The dry masses (g) were measured using an Ohaus<sup>®</sup> Adventure<sup>™</sup> balance. Roots and shoots were weighed together giving a total biomass.

*Hydrellia lagarosiphon* eggs were released into the herbivore and parasitoid treatments five weeks after planting, once *L. major* and *M. spicatum* plants had grown to the water surface. Approximately 900 *H. lagarosiphon* adults were collected from a small impoundment near Rosetta, Kwa-Zulu Natal, South Africa  $(29^018'18"S; 29^058'28"E)$  between 28 and 30 January 2011. The flies were divided into groups of 15 and placed in 25 x 20 x 15 cm, 2L containers sealed with a gauze lid to ensure oviposition. A yeast hydrolysate and sugar mixture (4g yeast hydrolysate: 7g sugar) was provided as a food source for the flies (Freedman *et al.* 2001), while *L. major* stems were also included for oviposition structure. The flies were allowed to oviposit for 24 hours, and were then removed from the containers so that the eggs could be collected. This process was repeated every 24 hours until 800 eggs were collected.

The water level in the ponds was decreased to expose *L. major* stems. The *H. lagarosiphon* eggs were placed on the exposed tips of *L. major* using a fine paintbrush, at a stocking density of 327 eggs per m<sup>2</sup>. Similar studies used higher fly densities (Van *et al.* 1999), to maximise damage, while this study aimed to replicate field conditions (Chapter 6). During the experiment, a similar yeast hydrolysate and sugar mixture was provided as a food source on two floating Styrofoam feeding stations in the ponds.

Adult *C. luteostigma* were collected from the same field site as the *H. lagarosiphon* adults, between 13 and 14 March 2011. Five parasitoids were released into the parasitoids treatment ponds six weeks after the initial release of *H. lagarosiphon*, once the fly populations had had time to establish, ensuring there would be late instar fly larvae and pupae for the parasitoids.

#### 7.2.1 Plant/ insect harvest

Plants and insects were harvested 26 weeks after planting. The two plant species were separated from each other when they were in combination, washed and cleaned. The plants

were placed in brown paper bags and dried in a Heraeus drying oven for 96 hours at 60 °C. The dry masses (g) were measured using an Ohaus Adventurer<sup>tm</sup> balance. Total biomass, and biomass of roots and shoots were recorded.

Adult flies and parasitoids were collected from the water surface and net canopy using an aspirator. Late instar larvae and puparia were collected from all sprigs where they were visible and were allowed to eclose or emerge. The insect densities may have been an underestimation of actual number, as one cannot be certain that every puparium and larva was collected. *Hydrellia lagarosiphon* puparia that were exposed, either in stems with wider spaced nodes or where the surrounding covering leaves had died and fallen off, were easier to locate for the researcher as well as the parasitoids. Thus parasitoid numbers may have been less of an underestimation than *H. lagarosiphon* numbers.

# 7.2.2 Statistical analysis

As in Chapter 5 data were analysed using inverse linear models. Multiple regressions were conducted on the mean dry masses of the total biomass under each planting density. The magnitude of the relationship was analysed using the reciprocal-yield model (Spitters 1983). This model involves multiple linear regressions of the form:

 $1/W_l = a_{l0} + a_{ll}d_l + a_{lm}d_m$ 

 $1/W_m = a_{m0} + a_{mm} d_m + a_{ml} d_l$ 

Here  $1/W_1$  and  $1/W_m$  are the inverse dry biomass yields of individual *L. major* and *M. spicatum* respectively, while  $d_m$  and  $d_l$  represent the respective planting densities for *M. spicatum* and *L. major*. Intraspecific competition was estimated by the partial regression coefficients  $a_{mm}$  and  $a_{ll}$ , and interspecific competition by the coefficients  $a_{ml}$  and  $a_{lm}$ , in terms

of their effects on the reciprocal yield of *L. major* or *M. spicatum* total biomass. Competitive interactions were analysed for total dry weight. The intercepts  $(a_{m0} \text{ and } a_{l0})$  measure the reciprocal of the maximum mass of isolated 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  $(a_{mm} / a_{ml} \text{ and } a_{ll} / a_{lm})$ . F-tests determined whether competition coefficients were significantly different as a result of different treatments. Two-way Analysis of Variance (ANOVA), followed by a Tukey Post-Hoc HSD test determined whether the mean end-yields of *L. major* and *M. spicatum* were significantly different between the four treatments.

Differences between fly and parasitoid numbers between treatments were calculated using a Mann-Whitney U test. All statistical analyses were conducted in STATISTICA ver. 8.0.

# 7.3 Results

## 7.3.1 Temperature

Temperature was recorded to ascertain the influence of net covers in the controls and treatments measuring the influence of herbivory and parasitism on the competitive ability of *L. major*. In the uncovered control, the mean water temperatures were: surface 22  $^{\circ}$ C (S.E. 0.6; maximum 40 $^{\circ}$ C; minimum 11.5C $^{\circ}$ ) sediment 22.5C (S.E. 0.5; maximum 34  $^{\circ}$ C minimum 14C $^{\circ}$ ). Comparatively, the mean temperatures in the covered treatments were: surface 23.6 (S.E. 0.64; Maximum 39; minimum 12.5) Sediment 23.1  $^{\circ}$ C (S.E. 0.6; maximum 35.5; minimum 12.5) (Fig. 7.1). The temperature in the uncovered control was significantly lower than in the covered treatments (F <sub>(3, 444)</sub> = 3.7393, *P*=0.01) (Fig. 7.2). There were no significant differences in temperature between the surface and sediment temperatures in the uncovered treatments may be the surface and sediment temperatures in the uncovered treatments, nor was there a significant difference between water surface

temperature of the covered and uncovered treatments. There was a significant difference between sediment temperature in the covered treatment and the surface and sediment temperatures in the uncovered treatment (Fig. 7.2). However the temperature variance in both the daily maxima and minima, and daily mean between the treatments was negligible (Fig. 7.1). The mean daily temperature decreased throughout the duration of the experiment it was conducted from the end of summer (22 January 2011) to the beginning of winter (14 April 2011).

The high temperature recorded early on in the experiment may be an area of concern as the Upper lethal Limit (ULL) of the fly or the wasp is not yet known. However the flies and wasps were only introduced after the initial high temperature.



**Figure 7.1:** Mean daily water surface and sediment temperatures of the uncovered control, (water surface and sediment) and covered control and treatments (<sup>0</sup>C) over the study period.



**Figure 7.2:** Mean daily temperature of treatments ( $\pm$  S.E.), means followed by the same letter are not significantly different ( $F_{(3, 444)}$ =3.7393; *P* = 0.01).

#### 7.3.2 Competition prior to insect release

Before the insects were introduced into the treatments, plant growth, community structure and competition within the ponds was assessed. There was no significant difference between the yields of the plant species under each planting density between the treatments, therefore the treatments were grouped together (*L. major*: F <sub>(3, 88)</sub> =0.54, *P*=0.66) (Fig. 7.3); *M. spicatum*: (F <sub>(3, 88)</sub> = 0.34, *P* = 0.80 (Fig. 7.4)). Five weeks after planting, before the leafminer *H. lagarosiphon* had been introduced into the treatments, *L. major* had already established a competitive advantage over *M. spicatum*. The co-efficients measuring the relative competitive ability of *L. major* and *M. spicatum* ( $a_{ml} / a_{mm}$ ), showed *L. major* to be 9.8 times more competitive than *M. spicatum* (Table 7.1). The coefficients measuring the relative competitive ability of *M. spicatum* and *L. major* ( $a_{lm} / a_{ll}$ ) showed *M. spicatum* to be only 0.19 times as competitive as *L. major* (Table 7.1).

#### 7.3.3 Competition in the absence of herbivory

In the uncovered control, the Spitters model showed that *L. major* was the stronger competitor. In respect of *L. major*, the ratio of coefficients  $(a_{ml} / a_{mm})$  comparing intraspecific to interspecific competition was approximately 8.18. In this case, adding a single *L. major* plant had the same impact on the mean *L. major* plant mass as adding 8.18 *M. spicatum* plants (Table 7.1). The ratio of coefficients  $(a_{lm} / a_{ll})$  comparing *M. spicatum* to *L. major* was 1.11 (Table 7.1), implying that *L. major* had very little effect on the dry mass of *M. spicatum*. Neither inter- nor intra-specific competition influenced *M. spicatum* yield.

In the covered control treatment, *L. major* was once more the superior competitor. The ratio of coefficients  $(a_{ml} / a_{mm})$  showed *L. major* to be 15.5 times more competitive than *M. spicatum* (Table 7.1). Thus under a net canopy in slightly reduced light conditions and increased water temperature, *L. major* grew faster, formed a denser surface canopy than *M. spicatum* and therefore exerted a greater influence on itself than *M. spicatum* had on it. *Myriophyllum spicatum* was highly influenced by interspecific competition from *L. major* as it was only 0.11 times as competitive as *L. major* (Table 7.1).

The data were graphically analysed as three-dimensional response planes where the slope in one direction represents the effect of the density of *L. major* upon its yield of *L. major*, and the slope in the other direction represents the effects of *M. spicatum* density on yield of *L. major* (Figs. 7. 5a and c). The steep slope of the *L. major* regression planes indicates that increased intraspecific competition significantly reduced the overall yield of *L. major* in both the insect-free control and uncovered controls (Figs. 7.5 a and c).

The flat slope of the *M. spicatum* density regression planes in both the insect-free control and the uncovered control indicates that interspecific competition from *M. spicatum* was

negligible (Figs. 7.5 b and d). Competition from *M. spicatum* had little effect on the overall dry mass yield in both the insect-free control and the uncovered control (Figs. 7.5b and d). In both controls, planting densities of *L. major* and *M. spicatum* significantly decreased the overall dry mass yield of *M. spicatum*, as indicated by the increased gradients in both directions (Figs. 7.5 b and d). Therefore both inter- and intraspecific competition influenced the overall yield of *M. spicatum* in the absence of selective herbivory

**Table 7.1:** Multiple regression analysis of the impact of insect herbivory, parasitism and plant density on the reciprocals of the mean plant masses of *Lagarosiphon major* and *Myriophyllum spicatum* plant masses.

Regression coefficients							
	Intercept	Intraspecific competition <sup>1</sup>	Interspecific competition <sup>2</sup>	Ratio of competition coefficients	R²;P		
Uncovered control-Pre-release							
L. major M. spicatum	0.1408	0.0936	0.0095	9.85263158	0.47016; 0.001 0.133371;		
M. spicaium	1.3324	Uncover	od control	0.1904/039	0.001		
L. major	0.2358	0.2176	0.0266	8.18045	0.62737; 0.00024 0.48242:		
M. spicatum	0.2606	0.2635	0.2372	1.11	0.00280		
Insect-free control							
L. major	0.6934	0.2965	0.0191	15.5235602	0.50797 ;0.001 0.47851		
M. spicatum	1.3934	0.0224	0.1959	0.11434405	;0.001		
Herbivory treatment							
L. major	1.1657	0.2904	0.0929	3.12	0.33747; 0.01326		
M. spicatum	0.8566	0.0725	0.0611	1.19	;0.20869		
Parasitoid treatment							
L. major	0.4149	0.2207	0.0348	6.34	0.29605; 0.02507		
M. spicatum	0.9365	0.0975	0.0611	1.6	0.32334; 0.01655		

<sup>1</sup>Intraspecific competition represents the regression coefficients all for *L. major* and amm for *M. spicatum*.

<sup>2</sup>Interspecific competition represents the regression coefficient a<sub>im</sub> for *L. major* and a<sub>ml</sub> for *M. spicatum*.

<sup>3</sup>The ratio of the 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,  $a_{mm}/a_{ml}$  for *M. spicatum* and  $a_{ll}/a_{lm}$  for *L. major*.



**Figure 7.3:** Mean final masses (g) of *Lagarosiphon major*, at the original planting densities of three and nine stems, before insects were added to the experiment. Error bars represent S.E., means followed by the same letter are not significantly different.



**Figure 7.4:** Mean final dry masses (g) of *Myriophyllum spicatum*, at the original planting densities of three and nine stems, before insects were added to the experiment. Error bars represent S.E., means followed by the same letter are not significantly different.



**Figure 7.5:** Multiple regression planes indicating the combined effect of *Lagarosiphon major* and *Myriophyllum spicatum* on the reciprocal of the mean dry mass (1/g) of one *L. major* plant (a and c uncovered control experiment); and the combined effect of *L. major* and *M. spicatum* on the reciprocal of the mean dry mass (1/g) of one *M. spicatum* plant (b and d insect-free control) (higher values represent lower yields). Points indicate observations (n = 24) and vertical lines between data points represent the residuals. Values on X and Y axes represent *L. major* and *M. spicatum* planting densities at the start of the experiment.

### 7.3.4 Competition as influenced by herbivory

Once introduced, *H. lagarosiphon* larvae mined the *L. major* leaves, leaving the epidermal tissue intact, affecting the plants' ability to photosynthesise. *Hydrellia lagarosiphon* did not feed on *M. spicatum*. Observations when harvesting showed that the majority of *L. major* stems had *H. lagarosiphon* damage within the top 30cm of the water column. Populations of *H. lagarosiphon* were still present at the completion of the experiment (Fig. 7.7). *Hydrellia lagarosiphon* significantly reduced *L. major* dry mass in comparison to the covered control, under planting densities of both three and nine (F  $_{(3, 88)} = 4.03$ , P = 0.01) (Fig. 7.6). The dry mass yield at planting densities of three and nine of *L. major* in the insect-free control was significantly higher than both the herbivory treatment and parasitoid treatment. At a planting density of nine, the mean dry mass yield of *L. major* in the insect-free control was significantly higher than the herbivory treatment but higher but not significantly than the parasitoid treatment (Fig. 7.6).



Figure 7.6: Mean final masses (g) of Lagarosiphon major, at the original planting densities.

Error bars represent S.E., means followed by the same letter are not significantly different.



**Figure 7.7 a:** Number of adults flies collected from the water surface from the herbivory and parasitoid treatments (U<sub>1</sub>=0.5; n1=n2, P = 0.042). **b**. Number of *Hydrellia lagarosiphon* pupae collected from *Lagarosiphon major* sprigs from both herbivory and parasitoid treatments (U<sub>1</sub>=0.0001; P = 0.03). Error bars represent the median quartiles.

The competitive advantage of *L. major* in the insect-free control was 15.5, while the introduction of selective feeding by *H. lagarosiphon* in the herbivory treatment reduced this
competitive ability to 3.12 (Table 7.1). The ratio of coefficients  $(a_{mm} / a_{ml})$  evaluating interspecific and intraspecific competition of *M. spicatum* in relation to *L. major* was 1.19 (Table 7.1). Even under the influence of herbivory, interspecific competition was more important than intraspecific competition on the yield of *M. spicatum* in other words, *M. spicatum* was suppressed by competition with *L. major*.

The combined effects of *L. major* and *M. spicatum* on the reciprocal yield of *L. major* under herbivory are illustrated in the linear three-dimensional surface plot (Fig. 7.8a). The flat slope associated with *M. spicatum* had little influence on the mean dry mass per plant for *L. major*, compared to the very steep slope associated with increased *L. major* densities, indicating that intraspecific competition had a far greater effect on the mean dry mass per plant for *L. major* than interspecific competition even in the presence of herbivory. The three-dimensional surface plot for the overall yield of *M. spicatum* (Fig. 7.8b) showed an increased slope with regard to *M. spicatum* compared to the insect-free control, indicating that *M. spicatum* had an increased negative effect on the mean dry mass per plant of its self in the presence of herbivory on *L. major* (Fig. 7.8).



**Figure 7.8:** Multiple regression planes indicating the combined effect of *Lagarosiphon major* and *Myriophyllum spicatum* on the reciprocal of the mean dry mass (1/g) of one *L. major* plant (a.) and one *M. spicatum* plant (b.) respectively, in the presence of herbivory by *Hydrellia lagarosiphon*. Higher values represent lower yields. Points indicate observations (n = 24) and vertical lines between data points represent the residuals. Values on X and Y axes represent *L. major* and *M. spicatum* planting densities at the start of the experiment.

#### 7.3.5 Competition as influenced by parasitism

The addition of *C. luteostigma* decreased the *H. lagarosiphon* population within the parasitoid experiment. On average,  $16 \pm 0.62$  S.E. adult flies and  $14 \pm 0.85$  S.E. puparia were collected from the herbivory treatments (Fig.7.7). The number of flies within the herbivory treatment ( $16 \pm 0.62$  S.E.) was significantly higher than in the parasitoid treatment ( $10 \pm 0.9$  S.E.) ( $U_1$ =0.5; P = 0.042) (Fig. 7.7). In the parasitoid treatment, there were significantly fewer puparia containing flies than in the herbivory treatment, a reduction from 14 to 8 ( $U_1$ =0.0001; P = 0.03) (Fig. 7.7). A mean of 5 ± 0.63 S.E. adult parasitoids were collected

and  $9 \pm 0.23$  S.E. eclosed from collected pupae, resulting in a total parasitism percentage of 51 % ± 3.23 S.E.

This reduction in *H. lagarosiphon* densities increased the intraspecific competition by *L. major* on itself from three times in the herbivory treatment, to nearly six times in the parasitoid treatment (Table 7.1). The effect of *M. spicatum* on *L. major* changed with the introduction of the parasitoid from 1.19 to 1.6 (Table 7.1). The increased slope of the *L. major* regression plane in the parasitoid treatment compared to the herbivory treatment indicates that the parasitoid treatment increased the competitive advantage of *L. major* over *M. spicatum*. However the slope associated with *M. spicatum* on *L. major* remained relatively similar, indicating that the presence of the parasitoid had not yet affected the competitive ability of *M. spicatum* in relation to *L. major* (Fig. 7.9). The influence on the final end dry mass yield of *M. spicatum* was more related to interspecific competition from *L. major* than from intraspecific competition on itself (Fig. 7.9).

There was no significant difference between the overall dry mass of *M. spicatum* between the different planting densities and the four treatments (F  $_{(3, 88)}$  =0.96, *P*=0 .41). At a planting density of three both singularly and in combination, the herbivory treatment was significantly greater than both the uncovered and insect-free controls (Fig. 7.10), whereas in the parasitoid treatment and the two control treatments there was no significant difference. At a planting density of nine both singularly and in combination, *M spicatum* dry yield in the parasitoid treatment was not significantly different to the uncovered control, but the herbivory treatment was significantly higher (Fig. 7.10).



**Figure 7.9:** Multiple regression planes indicate the combined effect of *Lagarosiphon major* and *Myriophyllum spicatum* on the reciprocal of the mean dry mass (1/g) of one *L. major* plant and *M. spicatum* plant under the influence of herbivory and parasitism. Higher values represent lower yields. Points indicate observations (n = 24) and vertical lines between data points represent the residuals. Values on X and Y axes represent *L. major* and *M. spicatum* planting densities at the start of the experiment.



**Figure 7.10:** Mean final masses (g) of *M. spicatum*, at the original planting densities. Error bars represent S.E., means followed by the same letter are not significantly different.

## 7.4 Discussion

Numerous plant-insect ecological studies examine trophic structures from the viewpoint of a predominantly bi-trophic framework. Indeed, this study demonstrated that herbivory by *H. lagarosiphon* reduced the competitive ability of *L. major* by approximately 5 times in favour of *M. spicatum*. Yet by investigating the third trophic level, the importance of parasitism on plant competition dynamics was highlighted. The parasitoid wasp reduced the impact of herbivory by the fly on *L. major* by half, thereby shifting the competitive balance in favour of *L. major* at the expense of *M. spicatum*.

A multitrophic perspective should be considered in plant insect interactions, but there are very few examples of where this has been achieved or even attempted (Harvey *et al.* 2010). This study is unique as it has combined three biotic interrelations: plant competition, herbivory, and parasitism, within an aquatic system and illustrates the impact a parasitoid can have, not only on host population density, but more specifically on the overall yield of the host insect's host plant. Most importantly it highlights the need to investigate ecosystems and insect populations from numerous trophic levels in order to understand the drivers of aquatic ecosystems.

The superior competitive ability and the mechanisms of achieving competitive superiority of *L. major* over other submerged aquatic species has been reported previously, both in the field and in laboratory experiments (Chapter 7; Rattray 1994; James *et al.* 1999; Caffrey et el. 2010). Physiologically, *L. major* outcompetes other submerged macrophytes through rapid shoot production and biomass accumulation (at the expense of root growth), effectively creating a dense, light-excluding canopy layer on the water surface that allows it to outcompete other aquatic macrophytes for light (Rattray *et al.* 1994). In Ireland, where *L. major* is a problematic invasive species, it outcompetes indigenous *M. spicatum* in Loch

Corrib (Caffrey *et al.* 2010; Chapter 2). In a common garden experiment, James *et al.* (1999) showed that *L. major* may gain a competitive advantage over other submerged species at raised pH and dissolved  $O_2$  levels and lowered free  $CO_2$  levels. In this study, in the absence of herbivory, *L. major* proved to be superior to *M. spicatum* in both the uncovered control and insect-free control. In the uncovered control, the competitive ability of *L. major* relative to *M. spicatum* relative to *L. major* was only one. In the insect-free control, the competitive advantage of *L. major* rose to nearly 15 and that of *M. spicatum* relative to *L. major* reduced to nearly 0.1.

The change in competitive ratios between the two treatments may be explained by the reduced solar radiation in the covered control, and treatments favouring *L. major*. Barko and Smart (1981) showed that under decreased light, *M. spicatum* invested less in shoot elongation and new shoot production, and more in root formation. Comparatively, Rattray *et al.* (1994) showed *L. major* to rapidly increase shoot length (at the expense of root development) using stored reserves to increase length in lower light conditions. Even though it is known that different species possess different and discreet ranges of thermal tolerances which may affect a species' competitive ability (Barko & Smart 1981), the temperature variation between the treatments was not significant enough to have influenced the competitive strategies of the two species.

The impact of herbivory by *H. lagarosiphon* on the competitive ability of *L. major* is similar to previous studies comparing the impact of insect herbivores on the competitive ability of terrestrial plants (Bentley & Whittaker 1979; Brown & Gange 1992; Carson & Root 2000) and also of invasive floating aquatic species such as *E. crassipes* (Coetzee *et al.* 2005), and the submerged species *H. verticillata* (Van *et al.* 1998; Cabrera Walsh *et al.* 2007). In the absence of insect parasitoids and predators, herbivory by the leaf-mining fly, *H.* 

*lagarosiphon*, influenced the competitive advantage of *L. major* relative to *M. spicatum*. *Hydrellia lagarosiphon* reduced the competitive co-efficient  $(a_{III} / a_{Im})$  of *L. major* in relation to *M. spicatum* from 15 to three, while the relative competitive co-efficient  $(a_{mm} / a_{ml})$  of *M. spicatum* increased significantly in the presence of *H. lagarosiphon*. In a similar competition experiment, Van *et al.* (1998) observed a comparable change in competition between *H. verticillata* and *V. americana*, in the presence of *H. pakistanae*. *Hydrellia pakistanae* reduced the competitive superiority of *H. verticillata* in relation to *V. americana* from 8.3 to 1.3. In Van *et al.* (1998) and this study, under the influence of selective insect herbivory, the competitive ability of a dominant competitor was compromised, allowing for succession by the other species in the system. In the field, the effect of herbivory is likely to slow the spread of *L. major* and to give other species the opportunity to compete for limiting resources. This supports the basic philosophy of using host specific phytophagous species to control introduced weeds. Thus *H. lagarosiphon* is considered a valuable component in the management of *L. major* in localities where it has become problematic.

The direct influence of *C. luteostigma* on *H. lagarosiphon* population levels is also consistent with other studies on the influence of predators on prey population-levels, decreasing the abundance of herbivores impacting on host plants (Spiller & Schoener 1990; Marquis & Wheelan 1994; Schmitz *et al.* 1997; Cardinale 2003; Costamagna & Landis 2006). The parasitism of *H. lagarosiphon* by the braconid *C. luteostigma* is not a unique interaction. Many species of Hymenoptera are known to parasitize other *Hydrellia* species, principally during the larval and pupal stages (Deonier 1971). However, the knock-on influence of the parasitoids on the competitive ability of plant species in aquatic systems has not been investigated, despite the significant economic and ecological consequences that parasitism of *Hydrellia* sp. can have. For example, braconids are known to parasitize *Hydrellia griseola* Fallén (Diptera: Ephydridae), a significant pest of numerous hydrophyte species including

rice (Oryza sativa L.), and several crop species including wheat (Triticum aestivum L.), barley (Hordeum vulgare L.), oats (Avenasativa L.), Timothy (Phleum pratense L.), and onions (Allium spp.) (Hesler 1995). However the beneficial influence of parasitoids on crop yield is unknown. Two species of Hydrellia, H. pakistanae and H. balciunasi Bock, have been shown to suppress dioecious hydrilla, a significant weed in the U.S.A., by reducing photosynthesis, thereby impacting biomass production, tuber formation, fragment viability, and hydrilla's ability to compete effectively with beneficial native vegetation (Doyle et al. 2002; 2007; Grodowitz et al. 2003; Owens et al. 2006; 2008). The flies are parasitized by indigenous parasitoids (M. Grodowitz, U.S. Army Corps of Engineers, pers. comm. 2011), but the impact that the parasitism may have on the success of the flies as viable biological control agents has yet to be investigated. My study has shown that the parasitism of H. lagarosiphon by C. luteostigma significantly influenced the competitive ability of L. major in relation to *M. spicatum*. The competitive ability of *L. major* increased from three to six times more competitive than *M. spicatum*. The level of parasitism within the treatment is similar to what was often found in the field (Chapter 7). Parasitic hymenoptera undoubtedly exert considerable control on population densities of Hydrellia species, which in turn influences the level of herbivory on the host plant, ultimately reducing colonisation and competition from other submerged indigenous macrophytes.

The actual interactions between *H. lagarosiphon* and *C. luteostigma* and the mutual interactions between the *Ademon* parasitoids are largely unknown. Thus, the level and influence of parasitism within the parasitoid treatment may not give the exact effect of parasitism in the field as two other parasitoids were also found to parasitize *H. lagarosiphon* in the field, but were not included in the study. These species would interact with *H. lagarosiphon* as well as with *C. luteostigma*. This is important to consider as the interaction between parasitoids may decrease the searching efficiency of *C. luteostigma* (Hassell 1971;

Hassell *et al.* 1976). Additionally, encounters with already parasitized hosts may have a similar effect of decreased searching efficiency. Hyperparasitism has also not been investigated within this interaction. However this study provides valuable insight into the host-parasitoid interaction.

This study has provided evidence to Harvey's *et al.* (2010) call for research into the factors influencing ecological communities, especially from multitrophic perspectives, by showing how the subtle biotic effects of competition, herbivory and predation can change macrophyte community dynamics. It highlights the importance of understanding the interconnectedness between trophic levels when making decisions on an ecological scale such as introducing potential biological control agents. The results should serve to better understand the importance of parasitoids in crop management, as well as understanding how the ecology of a system may affect the success or failure of potential biological control agents.

# **Chapter 8: General Discussion**

This thesis illustrates how combined efforts of seemingly contrasting approaches are essential to the understanding of submersed macrophyte community dynamics. The research highlighted the need to investigate community ecology from a holistic approach and to utilise information from a wide variety of aspects. It is not as simple as doing once-off observational or manipulative, deductive or mechanistic, laboratory (or greenhouse) or *in situ* experimentation; instead, to understand macrophyte community ecology and mechanisms underlying observed patterns, one needs to obtain information from all available sources.

The drivers affecting the dynamics of submerged freshwater macrophytes in South African water systems were previously not well understood. To date, studies have focussed on morphological species descriptions and distribution (e.g. Codd *et al.* 1966; Symoens & Triest 1983; Cook 2004), select invasive species (e.g. Henderson & Cilliers 2002; Hill 2003; Coetzee *et al.* 2009; Coetzee *et al.* 2011a.b), and a few brief speculations into the biotic and abiotic influences of macrophyte assemblages (e.g. Rowntree 2000; Schutz 2007; Coetzee *et al.* 2011a). Neither abiotic nor biotic drivers influencing their distribution and abundance have been investigated sufficiently. This thesis, using a holistic approach, investigated some of the drivers of submerged aquatic macrophytes in South Africa, with a specific focus on *M. spicatum* and *L. major*, two common submerged freshwater species important to South Africa freshwater macrophyte assemblages and distribution and abundance in South African freshwater systems. It has also highlighted numerous factors that should be taken into account when considering management and control options, which are discussed later.

## 8.1 Environmental drivers of aquatic plants in freshwater systems

Lacoul and Freedman (2006b) proposed a conceptual model of the variables affecting the distribution and abundance of aquatic plants at different scales. The model gives a helpful framework to understand and analyse the conditions of South African freshwater systems and the various factors that may be driving and influencing the introduction, establishment and spread of submerged invasive species in South Africa.

The model suggests that the factors affecting freshwater aquatic plants can be considered at three spatial scales - regional, catchment and local, and three major complexes of environmental influences -biogeography, climate and geomorphology. Numerous abiotic and biotic interactions and drivers link these scales and environmental influences.

The regional scale is coarse and considers a system's geography (e.g. continental, or biome in terms of boreal, temperate or tropical distributions) or environmental conditions (e.g. hard or soft water, acidic or alkaline, turbidity, saline or freshwater). The catchment level, or medium scale, relates the hydrology of ecosystems (e.g. watershed or entire water body) and the physical conditions prevailing there (e.g. nutrients, pH, turbidity, bicarbonates). Finally, the local, or fine grain, scale relates the particular habitats and communities to factors operating at this level (e.g. competition, herbivory and sediment characteristics) (Lacoul & Freedman 2006b).

Environmental factors affecting the distribution and abundance of aquatic plant species are varied and interacting, but can be grouped into three key vectors that integrate all of the scales: biogeography, climate and geomorphology (Lacoul & Freedman 2006b). Biogeography refers to the latitudinal and altitudinal distribution of species (Lacoul & Freedman 2006b). At a large scale, biogeography refers to the distribution of species. In local

scales, it influences the nature of biological interactions. Climate relates to hydrology, temperature, length of growing season, and related factors, all of which can all vary at the three different scales. Geomorphology is relevant to physical dimensions related to geography, such as basin attributes and chemical qualities. This relates to regional topography, bedrock and soil, but also to the smaller regional scales (local soil qualities, and sedimentation) (Lacoul & Freedman 2006b).

The majority of studies that aim to understand the drivers of aquatic plant assemblages are either conducted through the testing of null models to distinguish non-random organisation in communities from that generated randomly, or the reductionist-mechanistic approach of exploring observed patterns by inferring drivers as the mechanism generating those patterns (McCreary 1991). Both methods have their strengths but fail to give a holistic or a quantitative interpretation of the drivers within a system. The importance of determining the contribution within a larger framework is fundamental in understanding what may or may not be contributing to the species distribution and abundance.

## 8.1.1 Climate

Climate is perhaps the single most important driver determining the amount, distribution and to some extent, the availability of water in the environment (Barry & Chorley 1992). Climate variables include temperature, ice cover, wind, hydrology and the inherent effects of altitude and latitude all fundamental to the distribution and abundance of aquatic plants (Boylen & Sheldon 1976; Barko & Smart 1981; Barko *et al.* 1986; Lacoul & Freedman, 2006a, 2006b). According to Barko *et al.* (1986), light and temperature of water and sediments determine the distribution of freshwater aquatic plants by affecting their physiology, seed germination, seasonal growth and onset of dormancy in cold winter regions. Barko *et al.* (1986) adds that the thermal optimum for many submersed macrophytes may be rather high - between 28 and

32 °C - and plant growth is often restricted by reduced light and temperatures. Consequently, the biomass and density of many species of aquatic plants are reduced or the plants enter a state of dormancy in low temperatures, especially during European and North American winters (Nichols & Shaw 1986).

However, South Africa's climatic conditions generally range from Mediterranean in the south-western corner of South Africa, to temperate in the interior plateau, and subtropical in the northeast (Schulze 1997; Vogel 2000). Frost is a frequent occurrence in the winter, and snow is common at altitudes above 1 500 meters, which is important as cold temperatures and altitude have been shown to significantly influence the diversity and abundance of numerous aquatic species (Lacoul & Freedman 2004a); for example, field surveys revealed that L. *major* was restricted to the colder, high altitude regions of the interior of the country (Chapter 7). The variation in climatic conditions of South Africa allows for a diversity of aquatic macrophytes; aquatic and wetland plants represent two percent of the total flora of South Africa, with two endemic families, six endemic genera and approximately 114 endemic species (Cook 2004; Chapter 2), a very high diversity in comparison to other regions of the world. The temperate climate also allows for the establishment of invasive species from similar climate regions as well as colder regions. Chapter four highlighted regions suitable for the establishment of five potentially invasive species in South Africa based on climate modelling. The chapter shows that a significant percentage of South Africa is suitable for the establishment of Eg. densa, a species that occurs in a similar climatic zone in South America. Cabomba caroliniana, also from South America was predicted to have a narrow coastal distribution in South Africa. El. canadensis and H. verticillata, from North America and Europe respectively, were also shown to find favourable conditions in South Africa. Myriophyllum spicatum was predicted to have a limited distribution in South Africa even though it is known to have a broad distribution in South Africa. It is hypothesised that genetic

differences found in South African *M. spicatum* compared to other populations may allow the South African varieties to grow in a broader climatic range.

Many regions of South Africa experience higher summer temperatures as well as milder winters than the invasive species' native ranges, thus the predicted distributions based on suitable climates may be an underestimation of the potential South African distribution of the species. The severity of aquatic alien plant invasions cannot be underestimated, particularly in a water-scarce country such as South Africa, especially considering that so many systems were predicted, in Chapter four, to be climatically suited to the establishment of one or more potentially threatening species. Global climate change may also significantly influence the distribution of aquatic macrophytes.

The models used in chapter 4 prove to be a valuable tool in predicting potential distributions of submerged invasive species and should be used as a valuable component in early detection and management of potentially invasive species. However it is important to remember that other factors may contribute to species establishment, distribution and spread thus as many factors as possible should be considered when trying to manage freshwater systems.

## 8.2 Geomorphology

#### 8.2.1 Habitat area

In general, the number of species present in an environment increases with the area of suitable habitat and decreases with the isolation of habitat "islands" (Lacoul & Freedman 2006b). Changes in environmental conditions often influence the distribution and abundance of aquatic plants. Such plants are primarily influenced by factors inherent in the limnology of the water bodies, including those related to physical attributes such as geomorphology (geology and topography) (Chambers & Kalff 1985; Duarte & Kalf 1986; Santamaría, 2002).

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As a result of the climate and topography of South Africa, there are only about 180 natural lakes or pans that have open water (Nobel & Hemens 1978; Rowntree 2000). The majority of these water systems are filled by a combination of precipitation and irregular stream flow, resulting in many of the smaller lakes drying up annually and the larger ones being regarded as semi-permanent (Ramsar Convention on Wetlands 2011). The lack of permanency of water has resulted in a lack of establishment and evolution of higher plant species (Ramsar Convention on Wetlands 2011), particularly floating species suited to still, open waters.

South Africa has very few indigenous floating plants, limited to rooted floating aquatics; such as water lilies (Nymphaeaceae) and floating hearts (Menyanthaceae) and free floating aquatics; duckweeds (Menyanthaceae) and Azolla (Azollaceae). By developing artificial lakes, through the construction of dams and impoundments, ideal habitats for invasive floating plants have been created (Davies & Day 1998). South African records indicate that there were approximately 500 000 small farm dams in 1986 (DWAF 1986) primarily associated with agriculture and livestock farming. This number has probably increased significantly since 1986, but has not been recently estimated (Mantel et al. 2010). These dams pose a significant problem for management of troublesome weed species in South Africa. The great number of dams and their close proximity to one another facilitates movement of macrophytes between them, either by waterfowl and other animal or human activity (Linton & Goulder 2003; Floerl & Inglis 2005; Coetzee et al. 2009). The majority of invasive macrophytes that have invaded southern African waters are floating plants originating from the Amazon Basin (Coetzee et al. 2011b). These plants are capable of taking advantage of the similar climates and permanent and predictable waters provided by impoundments in South Africa, without any competition from floating indigenous flora (Hill 2003). For example, A. filiculoides was first recorded in South Africa in 1948 in the Oorlogspoort River, Colesberg, Northern Cape Province, where it was introduced as an

ornamental fishpond plant in 1947 (McConnachie *et al.* 2003). The combination of a lack of natural enemies, dispersal between water bodies by humans and possibly waterfowl, and eutrophic waters facilitated an increase in its distribution and establishment (McConnachie *et al.* 2003). By 1998 the weed was recorded at 152 sites throughout South Africa (Henderson 2009), which was probably an underestimation as recorded sites were significantly biased to road observations (M. Hill, pers. comm. 2012). This study shows the ease and speed with which species can move between freshwater systems within South Africa

#### 8.2.2 Substrate characteristics

Macrophyte species vary in their responses to different sediment conditions (Barko & Smart 1980; 1983), which often influence the species composition. Sediments are an anchoring substrate and source of valuable nutrients (Smart & Dick 1999). The local substrate is affected by various factors including geomorphology, land use, hydrology and vegetation cover (Smart & Barko 1985; Lacoul & Freedman 2006b). Based on an extensive investigation involving 40 sediments from 17 widespread North American lakes, Barko and Smart (1986) found broad variations in the growth of *H. verticillata* and *M. spicatum* on sediments of different texture and organic matter content.

Two species found throughout South Africa, *L. major* and *M. spicatum*, have been shown to outcompete other submerged macrophytes (Titus *et al.* 1975; Agami & Waisel, 1985; James *et al.* 1999; Hofstra *et al.* 1999; Caffrey & Acevedo, 2007). Chapter 5 showed that nutrient levels and sediment types affect the growth and competitive strategy of both plant species differently, influencing the outcome of the competition between the two when grown in mixed cultures under different planting densities. Chapter 5 showed that the fertile, finely grained sediments of the loam treatment contained high organic matter and probably anaerobic conditions suitable for *L. major* growth. The same does not apply for all

macrophytes as different macrophytes vary in their responses to sediment conditions (Barko & Smart, 1980; 1983). Infertile sandy sediments, such as the sand treatment, contained low organic matter content with aerobic conditions, which increased the competitive ability of *M. spicatum* over *L. major* (Chapter 5).

In South Africa, where soil erosion is high, dams as well as river systems play a role in trapping sediment. Although the exact timing of sediment yield increases and the amount of sediment deposited in these small reservoirs varies in relation to local factors (Rooseboom 1992; Boardman *et al.* 2009), this increase of nutrients sediments and the high solar radiation in many small impoundments may favour the establishment of certain submerged aquatic plant species, including *M. spicatum*. High fertilizer runoff from agriculture and golf estates, as well as increased nutrients from expanding urbanisation, also favour the rapid growth of certain species.

## 8.2.3 Nutrients, acidity and alkalinity

Various factors related to nutrients, acidity and alkalinity, affect the establishment and growth of aquatic macrophytes (Barko *et al.* 1991). In general, nitrates and phosphates facilitate increased productivity, but numerous other macro- and micro-nutrients also affect plant growth (Lacoul & Freedman 2006b). The rapid increases in urbanization in southern Africa have resulted in extensive pollution of freshwater systems from a variety of sources, including waste from agriculture, industry, and sewage (Oberholster & Ashton 2008). Elevated levels of pollution, especially nitrogen pollution, are often responsible for ecosystem degradation, animal sickness and death, as well as human disease (Camargo & Alonso 2006). These changes in ecosystem health have facilitated the rapid establishment and explosive growth of many of the invasive aquatic plants in local waterways that restrict the

use and availability of water. Effects of eutrophication are discussed further under anthropogenic influences.

Chapter 5 showed that an increase in nutrients, as could be expected from agricultural runoff, will facilitate the rapid growth of *L. major*. The study showed that increased nutrients in the system improved *L. major* competitive ability by 2.5 times, compared to when sediment nutrients were limited. At low nutrient levels, both *L. major* and *M. spicatum* allocated more resources to the growth of roots, which is consistent with allocation patterns observed in most herbaceous, terrestrial plants and other aquatic macrophytes (Barko & Smart 1986; Wang *et al.* 2008). The results from Chapter 5 suggest that under low sediment nutrients, *M. spicatum* was more competitive than *L. major*, however the introduction of nutrients will greatly enhance the growth and spread of *L. major* and other species sharing similar competitive traits. It is therefore expected that in mesotrophic systems *L. major* and *M. spicatum* abundance will be high, with decreases in abundance in nutrient-poor, oligotrophic systems (Lacoul and Freedman 2006a). It has also been shown, however, that in seriously eutrophic systems, not uncommon in South Africa, aquatic vegetation may be absent (Morris 2003).

# 8.3 Biological interactions and macrophyte communities

#### 8.3.1 Competition

The predominant theory regarding competition is that productivity will increase until one or more resources become limiting, at this stage the species that is better adapted to the conditions will outcompete, and even eliminate, a species from a community (Lacoul & Freedman 2006b). Studies aimed at understanding and predicting the outcome of competition between aquatic macrophytes generally involve one of two basic approaches, each reflecting an underlying body of theory on the appropriate way to view communities (McCreary 1991). The hypothetico-deductive approach aims to distinguish non-random organisation in communities from that generated randomly. In contrast, the reductionist-mechanistic approach attempts to explain observed patterns by inferring competition as the mechanism generating those patterns (McCreary 1991).

It is generally accepted that the intensity and outcome of competition is related to the biomass of the competitors and their density. Invasive species usually form dense biomasses and are highly successful competitors. South Africa has a large variety of habitats that are increasingly being threatened by invasive species (Chapter 2). Chapter 5 tested the competitive strategy between *M. spicatum* and *L. major* as influenced by sediment types. It was found that high nutrient treatments significantly influenced the growth rates of both species. However, *L. major* was better able to utilise the resources in high nutrient and clay type sediments compared to *M. spicatum*, effectively giving it a competitive advantage.

The competitive outcomes, under natural conditions, cannot necessarily be predicted on the basis of controlled experiments. For example, Valley and Newman (1998) found that when grown in outdoor experimental tanks in single- and mixed-species cultures, *Myriophyllum sibiricum* Kom. (Haloragaceae) suppressed the biomass accumulation of *M. spicatum* stems, findings that contrast with long-term field observations, whereby *M. spicatum* displaced northern *M. sibiricum* (Valley & Newman 1998). It may not just be the anatomical or physiological attributes investigated that determine the competitive ability of a plant. Other attributes may also be important and depending on the conditions and environment, may determine whether or not they become invasive. Certain species of Hydrocharitaceae have been shown to proliferate in difficult conditions, particularly low light conditions, additionally, some species may have a facultative C4 ability that allows them to access inorganic carbon under limiting conditions in eutrophic habitats (Bowes *et al.* 2002).

Competitive outcomes between species are influenced by the environment in which the species occur, including the multitude of environmental effects such as herbivory and disease.

# 8.3.2 Herbivory

Aquatic plants are grazed by a number of aquatic organisms, including fish, turtles, waterfowl and mammals, and a number of the world's worst invasive species, which include aquatic plants, have been successfully controlled around the world, including South Africa, using biological control (Macfadyen 1998; Charudattan 2001; Hill 2003; McConnachie *et al.* 2004).

Initially, it was considered that submerged macrophytes contributed nothing to food webs through herbivory by insects (Shelford 1918), but increasingly more studies have shown the importance of macrophytes as a nutritive source for invertebrates (Nachtrieb *et al.* 2011). Biological control, using insects, offers an interesting insight into the importance of insect herbivory in the structure and function of aquatic macrophyte communities. Chapters 6 and 7 investigated the effect of herbivory by a leaf-mining fly, *H. lagarosiphon*, on *L. major*. *Hydrellia lagarosiphon* larvae mined the *L. major* leaves, leaving the epidermal tissue intact, but affecting the plant's ability to photosynthesise (Chapter 6). Chapter 7 showed how selective feeding by the fly significantly reduced *L. major* dry mass compared to the insect-free controls. Perhaps more importantly though, herbivory by the fly reduced the competitive ability of *L. major*, which has important consequences for succession and dominance in aquatic communities.

## 8.3.3 Parasitism

Parasitism is not recognised in Lacoul and Freedman's (2006b) conceptual model, as very little literature exists on the effect of parasitism on plant ecology. It should however be considered as it can fundamentally affect the structure and distribution within macrophyte communities. While the influence of competition and herbivory affects ecosystem assemblages, both independently and in combination, by influencing species distributions and abundances (Van et al. 1998; Coetzee et al. 2005), inter-trophic relationships can have widespread effects on herbivores, and therefore the interaction between herbivores and their predators and parasitoids needs to be considered (Speight et al. 2008). In an ecological sense, predators or parasitoids influence the abundance of herbivores, which will have cascading effects on plant populations (Price et al. 2011). Chapter 7 elucidates how the addition of C. luteostigma, a braconid parasitoid of H. lagarosiphon, decreased the fly population, in turn increasing the intraspecific competition by L. major on itself and boosted the interspecific competitive ability of L. major over M. spicatum compared with the control and herbivory treatments. This should affect the structure of a macrophyte community as it influences both plant competition and the number of herbivores within a system, an important consideration for the introduction of biological control agents into systems.

This thesis has elucidated to the influence that parasitoides can have in aquatic systems as well as contributing to methods that could be used to further understand this process. The competition experiments conducted in this thesis allow for the determination of differences between plant populations grown in the presence of an interspecific competitor. By examining biomass accumulation in the plants, it was possible to determine the extent to which an individual plant is affected by abiotic and biotic influences, highlighting the influence that select drivers can have on macrophyte assemblages in fresh water systems. McCreary (1991) showed surprise regarding how infrequently aquatic macrophytes are used in manipulative competition experiments compared to their terrestrial counterparts as they are excellent for this kind of study. This study supports her notion of how important and relatively easy these kinds of studies are to better understand plant competition and ecosystems dynamics.

The competition experiments used methods that were initially developed in agricultural systems (Spitters 1983) but have been modified and used with great success in submerged aquatics competition experiments (Van *et al.* 1998; Mony *et al.* 2007), and then developed further to determine the subtle effect of herbivory on plant competition outcomes (Coetzee *et al.* 2005, Van *et al.* 1997). Chapters 5 and 7 developed the method and provided valuable insight into future studies by including more trophic levels in to the model. For example, an interesting addition would be to determine the effect of adding the insect herbivores associated of *M. spicatum* into the experiment. The experiments also open the door to a variety of similar experiments that would provide information into understanding the influence of herbivores and their parasitoids on plant competition.

Although attention has been paid to organisms in the third trophic level (Price *et al.* 1980; Harvey *et al.* 2003; Harvey *et al.* 2006), it has been limited to the effect that host plants play in mediating a suite of behavioural and physiological interactions amongst the herbivores feeding on them and natural enemies of the herbivores (Harvey *et al.* 2010); for example, how morphological traits such as hairs, trichomes, or adhesive glands on the leaf surface may inhibit herbivore colonization or movement whilst simultaneously impeding the searching efficiency of predators or parasitoids(Harvey *et al.* 2003). Recently attention has focused on the effect of allelochemicals on attracting herbivores and parasitoids (Harvey *et al.* 2010). The competition experiment used in this thesis investigated the interaction from a different point of view. It investigated how plant inter- and intraspecific competition were affected by herbivores and parasitoids, and not how the plant affected the parasitoids, giving interesting insight into the complexity of food webs. Neither morphological nor alleopathic effects of plants on herbivores or parasitoids have been investigated in submerged communities and may provide some very interesting questions.

The influence of different trophic levels does not stop at the third or fourth trophic level. Many parasitoids (in the third trophic level) are attacked by one or more species of obligate hyperparasitoids (in the fourth trophic level) which may in turn be attacked by hyperparasitoids (Price *et al.* 1980; Harvey *et al.* 2003; Price *et al.* 2011). The dynamics of tritrophic interactions involving plants, herbivores and parasitoids may be profoundly affected by hyperparasitoids. They may exert a significant negative effect on plant fitness by removing parasitoids or predators of the herbivores (top-down regulation), or plant allelochemicals may be transferred vertically through herbivores feeding on plants to the third trophic level and perhaps higher (bottom-up regulation) Harvey *et al.* 2003. However, thus far there is no evidence that aquatic plants may be able to affect the fourth trophic level in the latter way (Harvey *et al.* 2003, Harvey *et al.* 2010). Therefore, by using a simple manipulative laboratory experiment, we could increase our understanding of trophic levels as well as inter- and intra-specific completion in communities.

# 8.4 Anthropogenic influences

# 8.4.1 Climate Change

Climate change is likely to affect the habitats available and the abundance of macrophytes in South Africa. Global climate change and the subsequent regional and sub-regional effects are believed to be incited by anthropogenic emissions of greenhouse gases. Climate change is projected to cause significant alterations to aquatic biogeochemical processes, (including carbon dynamics), aquatic food web structure, biodiversity, and the distribution and structure of freshwater macrophytes (Wrona *et al.* 2006; Heino *et al.* 2009; Alahuhta *et al.* 2011). More specifically climate change should affect freshwater macrophyte species richness in colder regions, favouring species that form dense canopies, while some cold water species may become extinct (Alahuhta *et al.* 2011).

There is growing evidence of changes in temperature, precipitation and stream flow over many parts of South Africa. Potential evaporation appears to have increased over much of the interior of southern Africa in recent decades (Davis 2011). These temperature changes will have profound effects, both direct and indirect, on hydrology and ultimately macrophyte assemblages. It has already been shown that a number of species have expanded towards higher latitudes and altitudes (Hijmans & Graham 2006), thus it can be expected that the predicted models in Chapter 4 may in the future be under estimations of the potential suitable climate for the species. It is suggested that future climatic conditions should be considered when trying to infer species potential distributions.

# 8.4.2 Eutrophication

Eutrophication is a problem in many South African freshwater systems and may contribute to the growth of submerged macrophytes. Eutrophication is a concern because it has numerous negative effects. The higher the nutrient loading in an ecosystem, the greater the potential ecological effects. Increased productivity in an aquatic system can sometimes be beneficial. Fish and other desirable species may grow faster, providing a potential food source for humans and other animals (though this is not a common situation in South Africa) (DWAF 2012). However, detrimental ecological effects can in turn have other adverse repercussions, which vary from aesthetic and recreational to human health and economic impacts. The Green Drop report, compiled by the South African Department of Water Affairs from 2008 -2009 showed that only 45% (449 out of 852) of waste-water systems assessed on whether they met international standards, scored above 50% (only 7.4 % achieved a status roughly equivalent of international standards). This means that sewage is not being adequately treated in most of South Africa's 850 treatment works and millions of litres of insufficiently treated water/sewage is discharged into local river systems. These high levels of pollution, especially nitrogen pollution from these sanitation plants, are responsible for ecosystem degradation, animal urbanisation, sickness and death, as well as human disease (Camargo & Alonso 2006). These changes, coupled with the known effect of increased nutrients on *L. major* and *M. spicatum* (Chapter 5), could facilitated the rapid establishment and explosive growth of these species and potentially other invasive aquatic plants in local waterways (Coetzee *et al.* 2011a).

## 8.4.3 Dispersal

Humans are important dispersal vectors for some species, and are responsible for the intentional and unintentional introduction of species (Hulme *et al.* 2008). There are numerous anthropogenic-associated pathways of freshwater macrophytes including the horticultural and aquarium trade, dumping of ballast water, boating, aquatic recreation, unintentional hitchhikers and increasingly, the internet trade (Hulme *et al.* 2007; Martin & Coetzee 2011). Chapter 3 showed how pet stores, aquarists and the internet-mediated trade facilitate the movement of invasive, as well as indigenous, submerged plant species in South Africa. The chapter highlighted the lack of legislation in governing the trade and how this lack of controlling legislation has allowed the importation, spread and propagation of numerous invasive species in South Africa, ultimately increasing the chances of introduction. The threats of future introductions or continued introductions are severe. This study has shown

that in order to mitigate the potential negative effects of further aquatic plant invasions in South Africa, the pathways of aquarium plant movement need to be monitored and controlled with more rigour.

## **8.5** Conclusions

With numerous submerged invasive species already established in South Africa, thriving horticultural and aquarium industries and highly impacted water systems, South African is destined for submerged macrophyte-associated problems. This study highlighted the need to investigate freshwater macrophyte communities from a more holistic approach and to use information from a wide variety of approaches in order to understand the forces driving freshwater macrophyte establishment and spread. If the drivers and relationships between drivers are well known and viewed within a framework, they can be used by managers of aquatic ecosystems and by ecologists to predict the effects of change; anthropogenic or natural, in order to make educated management decisions.

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## Appendices

## Appendix 1

List of prohibited families and species who's importation and vegetative propagation are prohibited **under** Agricultural Pests Act, 1983 (Act No. 36 of 1983) and list of species prohibited by Conservation of Agricultural Resources Act (CARA), 1983 (Act No. 43 of 1983 amended 2001).

Prohibited families	Prohibited species	Prohibited species			
Agricultural Pests Act, 1983 (Act No. 36 of 1983).	Agricultural Pests Act, 1983 (Act No. 36 of 1983).	Conservation of Agricultural Resources Act (CARA), 1983 (Act No. 43 of 1983 amended 2001).			
Azollaceae	Altemanthera philoxemides	<i>Azolla filiculoides</i> <i>Salvinia molesta</i> and other species of the Family			
Cabombaceae	Ammania mulutifolia	Salviniaceae			
Gunneraceae	Anubias barteri	Egeria densa			
Haloragidaceae,	Anubias cofferolia	Eichhornia crassipes			
Hydrocharitaceae	Anubias congensis	Elodea canadensis			
Lemnaceae	Anubias lanceolata	Myriophyllum aquaticum			
Pontederiaceae	Anubias minima	Myriophyllum spicatum			
Salviniaceae	Anubias nana	Pistia stratiotes			
	Arundo donax	Pontederia cordata			
	Azolla filiculoides	Rorippa nasturtium-aquaticum			
	Azalia pinnata subsp. Asiatica				
	Blyxa echinosperma				
	Blyxa japonica				
	Cabomba. Caroliniania				
	Canna indica				
	Cardamine Iyrala				
	Cenchrus helmsii				
	Cryptocoryne ciliate				
	Cryptocoryne wendtii				
	Echinodorus argentinensis				
	Echinodorus bathii				

Echinodorus cordifolius Echinodorus madalengensis Echinodorus muricatus Echinodorus Osiris Echinodorus lenellus Egeria densa Eichhomia spp. except Echhomia natans (Indigenous to South Africa) Elodea Canadensis Eustralis stellata Egeria densa Eichhornia azurea *Eichhornia crassipes Eleocharis acicularis* Eleocharis parvulus Elodea Canadensis Fallopia japonica Gymnocoronis spilanfhoides Hemigraphis repanda Houttuvnia cordata var. chameleon *Hydrocotyle leucocephala Hydrocotyle sibthorpioides* Hydrocotyle verticillata Hydrilla verticillata. Hydrocleys nymphoides Hygrophila angustifolia Hygrophifa difformis *Hygrophila polysperma* Hygrophila salicifolia *Hymenachne amplexicaulis* Ipomoea carnea subsp. Fistulosa *Iris pseudacorus* Limnocharis flava Limnophila indica Limnophifa sessiliflora Ludwigia natans Ludwigia peruviana Ludwigia peploides subsp. Montevidensis Lythrum hyssopifolium Lythrum salicaria Marsilea mutica Melaleuca quinquenervia Menyanthes trifoliate

Monochoria hastate Monochoria vaginalis *Myriophyllum aquaticum Myriophyllum spicatum* Nechamandra altemifolia Nuphar lutea Nymphaea Mexicana Nymphoides geminate Nymphoides peltata Oryza rufipogon Ottelia alismoides Pistia sirafiotas Pontaderia rotundifolia Pontaderia cordata *Rannuculus scleratus Riccia fluitans Rotala indica* Rotala macrandra Sagittaria montevidensis Sagittaria platyphylla Sagittaria pygmaea Sagittaria sagittifofia Salvinis molesta Salvinia spp. except Salvinia hastala (Indigenous to South Africa) Solanum tampicense Sparganium erectum Spartina spp. except Sparlina maritima (Indigenous to *South Africa) Stratiotes aloides* Vallisneria gigantean Zizania latifolia

## Appendix 2

1.Pet store Questionnaire						
1. Could you successfully identify? $0 = NO$ 5		=100% certain				
Hydrilla verticillata (Hydrilla)	0	1	2	3	4	5
Vallisneria spiralis (Tape Grass)	0	1	2	3	4	5
Lagarosiphon major (South African oxygen weed)	0	1	2	3	4	5
Lagarosiphon muscoides	0	1	2	3	4	5
Egeria densa (Dense water weed)	0	1	2	3	4	5
Potamogeton crispus (Curly pondweed)	0	1	2	3	4	5
Elodea canadensis (Canadian water weed)	0	1	2	3	4	5
<i>Myriophyllum spicatum</i> (Spiked water-milfoil, Eurasia water-milfoil)	n 0	1	2	3	4	5
Cabomba caroliniana (Fan wort)		1	2	3	4	5
Cabomba furcata (Red cabomba)		1	2	3	4	5
Ceratophyllum demersum (hornwort)		1	2	3	4	5
<i>Echinodorus cordifolius</i> (Spade-leaf sword, radicans sword )	0	1	2	3	4	5

2. Have you ever had the opportunity to acquire this species for your business?

<i>Hydrilla verticillata</i> (Hydrilla)	Yes	No
Vallisneria spiralis (Tape grass)	Yes	No
Lagarosiphon major (South African oxygen weed)	Yes	No
Lagarosiphon muscoides	Yes	No
Egeria densa (Dense water weed)	Yes	No
Potamogeton crispus (Curly pondweed)	Yes	No
Elodea canadensis (Canadian water weed)	Yes	No
Myriophyllum spicatum (Spiked water-milfoil, Eurasian water-milfoil)	Yes	No
Cabomba caroliniana (Fan wort)	Yes	No
Cabomba furcata (Red cabomba)	Yes	No
Ceratophyllum demersum (hornwort)	Yes	No
Echinodorus cordifolius (Spade-leaf sword, radicans sword)	Yes	No
3. Do you have this species in stock?		
<i>Hydrilla verticillata</i> (Hydrilla)	Yes	No
Vallisneria spiralis (Tape grass)	Yes	No
Lagarosiphon major (South African oxygen weed)	Yes	No
Lagarosiphon muscoides	Yes	No
Egeria densa (Dense water weed)	Yes	No

Hydrilla verticillata (Hydrilla)	Yes	No
Potamogeton crispus (Curly pondweed)		No
Elodea canadensis (Canadian water weed)		No
Myriophyllum spicatum (Spiked water-milfoil, Eurasian water-milfoil)		No
Cabomba caroliniana (Fan wort)		No
Cabomba furcata (Red cabomba)		No
Ceratophyllum demersum (hornwort)		No
Echinodorus cordifolius (Spade-leaf sword, radicans sword)		No
4. How do you acquire your aquatic plants?		
Mail order		
Delivery from companies		
Self collection		
Self grown		
Other		
5. How do you select the plants you sell?		
Aesthetic value		
Availability		
Demand		
Hardiness

Catalogue

Other

- 6. Have you ever imported aquatic plants from abroad?
- 7. Are you aware of the Conservation of Agricultural Resources Act regarding aquatic plants?
- 8. Are you aware of the National Environmental Management Amendment Act (NEMBA)?
- 9. Do you agree with these regulatory measures and any other comments?
- 10. Optional, where is your business located?

City/Town:

State/Province:

## **Appendix 3. Aquarist Questionnaire**

1. Could you successfully identify?		
Hydrilla verticillata (hydrilla)	Yes	No
Vallisneria spiralis (Tape grass )	Yes	No
Lagarosiphon major (African elodea, curly waterweed, oxygen weed, South African oxygen weed )	Yes	No
Lagarosiphon muscoides	Yes	No
Egeria densa (Dense water weed)	Yes	No

Hydrilla verticillata (hydrilla)	Yes	No
Potamogeton crispus (Curly pondweed)	Yes	No
Elodea canadensis (Canadian water weed)	Yes	No
Myriophyllum spicatum (Spiked water-milfoil, Eurasian water-milfoil	l) Yes	No
Cabomba caroliniana (Fan wort)	Yes	No
Cabomba furcata (Red cabomba)	Yes	No
Ceratophyllum demersum (hornwort)	Yes	No
Echinodorus cordifolius (Spade-leaf sword, radicans sword)	Yes	No
2. Is it indigenous and/or invasive		
Hydrilla verticillata (Hydrilla)	Indigenous	Invasive
Vallisneria spiralis (Tape grass)	Indigenous	Invasive
Lagarosiphon major (African elodea, curly waterweed, oxygen weed, South African oxygen weed)	Indigenous	Invasive
Lagarosiphon muscoides	Indigenous	Invasive
Egeria densa (Dense water weed)	Indigenous	Invasive
Potamogeton crispus (Curly pondweed)	Indigenous	Invasive
Elodea canadensis (Canadian water weed)	Indigenous	Invasive
<i>Myriophyllum spicatum</i> (Spiked water-milfoil, Eurasian water- milfoil)	Indigenous	Invasive

Hydrilla verticillata (Hydrilla)	Indigenous	Invasive
Cabomba caroliniana (Fan wort)	Indigenous	Invasive
Cabomba furcata (Red cabomba)	Indigenous	Invasive
Ceratophyllum demersum (hornwort)	Indigenous	Invasive
Echinodorus cordifolius (Spade-leaf sword, radicans sword)	Indigenous	Invasive
3. Have you ever had the opportunity to acquire this species?		
Hydrilla verticillata (Hydrilla)	Yes	No
Vallisneria spiralis (Tape grass)	Yes	No
Lagarosiphon major (African elodea, curly waterweed, oxygen weed African oxygen weed)	l, South Yes	No
Lagarosiphon muscoides	Yes	No
Egeria densa (Dense water weed)	Yes	No
Potamogeton crispus (Curly pondweed)	Yes	No
Elodea canadensis (Canadian water weed)	Yes	No
Myriophyllum spicatum (Spiked water-milfoil, Eurasian water-milfoi	l) Yes	No
Cabomba caroliniana (Fan wort)	Yes	No
Cabomba furcata (Red cabomba)	Yes	No
Ceratophyllum demersum (hornwort)	Yes	No

<i>Hydrilla verticillata</i> (Hydrilla)	Yes	No
Echinodorus cordifolius (Spade-leaf sword, radicans sword)	Yes	No
4. Do you or have you ever had this species in you aquarium/tank?		
<i>Hydrilla verticillata</i> (Hydrilla)	Yes	No
Vallisneria spiralis (Tape grass)	Yes	No
Lagarosiphon major (African elodea, curly waterweed, oxygen weed, South African oxygen weed)	Yes	No
Lagarosiphon muscoides	Yes	No
Egeria densa (Dense water weed)	Yes	No
Potamogeton crispus (Curly pondweed)	Yes	No
Elodea canadensis (Canadian water weed)	Yes	No
Myriophyllum spicatum (Spiked water-milfoil, Eurasian water-milfoil)	Yes	No
Cabomba caroliniana (Fan wort)	Yes	No
Cabomba furcata (Red cabomba)	Yes	No
Ceratophyllum demersum (hornwort)	Yes	No
Echinodorus cordifolius (Spade-leaf sword, radicans sword)	Yes	No
5. Do you or have you ever passed this species on to colleague or friend?		
Hydrilla verticillata (Hydrilla)	Yes	No

Hydrilla verticillata (Hydrilla)	Yes	No
Vallisneria spiralis (Tape grass)	Yes	No
Lagarosiphon major (African elodea, curly waterweed, oxygen weed, South African oxygen weed)	Yes	No
Lagarosiphon muscoides	Yes	No
Egeria densa (Dense water weed)	Yes	No
Potamogeton crispus (Curly pondweed)	Yes	No
Elodea canadensis (Canadian water weed)	Yes	No
Myriophyllum spicatum (Spiked water-milfoil, Eurasian water-milfoil)	Yes	No
Cabomba caroliniana (Fan wort)	Yes	No
Cabomba furcata (Red cabomba)	Yes	No
Ceratophyllum demersum (hornwort)	Yes	No
Echinodorus cordifolius (Spade-leaf sword, radicans sword)	Yes	No
6. In your opinion does this species pose a threat to our water systems?		
<i>Hydrilla verticillata</i> (Hydrilla)	Yes	No
Vallisneria spiralis (Tape grass)	Yes	No
<i>Lagarosiphon major</i> (African elodea, curly waterweed, oxygen weed, South African oxygen weed)	Yes	No
Lagarosiphon muscoides	Yes	No

Hydrilla verticillata (Hydrilla)	Yes	No
Egeria densa (Dense water weed)	Yes	No
Potamogeton crispus (Curly pondweed)	Yes	No
Elodea canadensis (Canadian water weed)	Yes	No
Myriophyllum spicatum (Spiked water-milfoil, Eurasian water-milfoil)	Yes	No
Cabomba caroliniana (Fan wort)	Yes	No
Cabomba furcata (Red cabomba)	Yes	No
Ceratophyllum demersum (hornwort)	Yes	No
Echinodorus cordifolius (Spade-leaf sword, radicans sword)	Yes	No
7. Are you aware of the Conservation of Agricultural Resources Act regard	ing aqua	tic plants?

8. Are you aware of the National Environmental Management Amendment Act (NEMBA)?

9. Do you agree with these regulatory measures?

10. Any other comments regarding aquatic plants and /or their regulation in South Africa

## Appendix 4

Sites from where Lagarosiphon major and Myriophyllum spicatum soil samples were taken

Lagarosiphon major		Myriophyllum spicatum			
Site	Latitude	Longitude	Site	Latitude	Longitude
Site 1	-32.43983	27.29897	Site 1	-26.7621	27.6923
Site 2	-32.33246	27.30089	Site 2	-26.8822	27.476
Site 3	-31.41817	27.78415	Site 3	-26.9701	27.476
Site 4	-31.41817	27.78415	Site 4	-27.6601	26.6988
Site 5	-31.15127	27.89450	Site 5	-27.3868	26.5126
Site 6	-31.07221	28.32797	Site 6	-27.6606	25.6357
Site 7	-30.98641	28.20271	Site 7	-28.1136	24.9778
Site 8	-30.43358	29.01850	Site 8	-28.3338	24.7233
Site 9	-30.42608	29.02749	Site 9	-28.7021	24.0707
Site 10	-30.40985	29.00770	Site 10	-29.0121	23.8868
Site 11	-30.24063	29.24296	Site 11	-27.9204	25.1685
Site 12	-29.83050	29.34789			
Site 13	-29.74894	29.42598			
Site 14	-29.68407	29.48949			
Site 15	-29.30491	29.97362			
Site 16	-29.30118	29.96303			
Site 17	-29.24865	29.97366			
Site 18	-29.18759	29.91517			
Site 19	-27.80487	28.45358			
Site 20	-27.38735	29.66767			
Site 21	-27.36237	29.86907			
Site 22	-27.24412	29.75176			
Site 23	-26.56663	30.22893			
Site 24	-26.53847	30.24601			
Site 25	-26.49790	30.25354			
Site 26	-26.28477	30.21368			
Site 27	-26.13655	29.85702			
Site 28	-26.11557	29.69939			
Site 29	-25.60162	30.04664			