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SUSCEPTIBILITY OF INDIGENOUS AQUATIC PLANTS TO ALIEN INVASIVES: COMPETITIVE INTERACTIONS AS INFLUENCED BY NUTRIENT LEVELS AND DENSITY

By

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FINAL CORRECTED REPORT

A research report submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the Degree of Master of Science: Environmental Science.

School of Animal, Plant and Environmental Science

Durban, August 2011

DECLARATION

I declare that this research report is my own, unaided work. This report was supervised by Prof. M.J. Byrne. It is being submitted for the Degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.



Kelly May Taylor (nee van Rooyen)

13th day of October 2011

ABSTRACT

This research investigated the susceptibility of South African indigenous aquatic plants to competition from invasive species, using the competitive interactions of two sets of aquatic plants as a potential indicator. These interactions were studied in two separate experiments: the submersed weeds, *Hydrilla verticillata* (L.F.) Royle (Hydrocharitaceae) and an indigenous species, *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) and; the floating weeds, *Azolla filiculoides* Lamarck (Azollaceae) and the indigenous *Spirodela polyrhiza* (L.) Schleid (Lemnaceae). Plants were grown under differing nutrient levels, and in an addition series of eight different densities, using the reciprocal yield model to estimate competitive ability. The invasive Hydrilla outcompeted Lagarosiphon in terms of mean length, dry mass, and survival. Major algal infestation in the high nutrient level of the Hydrilla/Lagarosiphon experiment altered light and nutrient conditions, which may have played a significant role in the lack of establishment of Lagarosiphon and the poor growth performance of Hydrilla. The invasive Azolla and indigenous Spirodela both performed well in terms of plant mass and increase in number. While Azolla was affected by intraspecific competition, it showed a steady increase in growth and multiplication with an increase in nutrients. The individual mass of Spirodela plants was highest in the low nutrient level, and multiplication rates were greatest in the high nutrient level. Results indicate that the susceptibility of indigenous plants may be increased in high-nutrient systems, and that a continuous monitoring programme of aquatic alien species is vital in protecting our indigenous plants from extinction. This research recommends that the method of investigating competitive interactions between alien and indigenous plants be repeated with a variety of aquatic plants, as a means of anticipating susceptibility to invasions.

DEDICATION

I dedicate this work to all of my family and friends who supported me during the course of my research, and particularly my husband James Taylor, for his endless encouragement, hours spent carrying heavy loads of sand and plants, and being there to help me through every struggle.

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NOMENCLATURE

- **Biotype**: Group of organisms having the same or almost the same genotype.
- **Dioecious:** Having the male and female reproductive organs occurring on separate individuals of the same species; sexually distinct.
- **Epiphyte:** A plant that grows on another plant and depends on it for support, obtaining moisture and nutrients from surrounding air or water.
- **Eutrophic:** Having waters rich in nutrients (e.g. phosphates, nitrates) which support a dense growth of algae and other organisms, and showing increasing signs of water quality problems.
- **Heterosporous:** Producing two types of spores which differ in size and sex – the male microspore and the female megaspore, which develop into male and female gametophytes.
- **Hypertrophic:** Having water with very high nutrient concentrations. Water quality problems are serious and can be continuous, limiting biological activity.
- **Interspecific competition:** Competition between different species
- **Intraspecific competition:** Competition between members of a single species
- **Macrophyte:** A plant large enough to be visible to the naked eye.
- **Mesotrophic:** Having waters with intermediate levels of nutrients, fairly productive in terms of aquatic fauna and flora, and showing emerging signs of water quality problems.
- **Monoculture:** A single, homogeneous culture without diversity.
- **Monoecious:** Having both male and female organs occurring in separate flowers on the same individual; hermaphroditic.
- **NPK ratio:** Ratio of Nitrogen:Phosphorus:Potassium
- **Oligotrophic:** Having waters with a low accumulation of dissolved nutrient salts (e.g. phosphates, nitrates), supporting a sparse growth of algae and other organisms, and a large amount of dissolved oxygen throughout.
- **Polymorphic:** The occurrence of different forms, stages, or types in individual organisms of the same species, independent of sexual variations.
- **PPRI:** Plant Protection Research Institute, Agricultural Research Council.

- **SE:** Standard error
- **Submersed:** Growing or remaining under water.
- **Tuber:** The thickened part of an underground stem of a plant, bearing buds from which new plant shoots grow.
- **Turion:** A small shoot from which a new plant can develop.

CHAPTER 1:
GENERAL INTRODUCTION

1.1 Introduction

The course of human history reflects a steady increase in the pressure that we have placed on our environment, and on the natural dynamics of species change. These human influences are moving some species towards extinction, and causing some to increase their natural ranges and become invasive. The specific causes of extinction or invasiveness are external, such as habitat destruction and climate change, while the ultimate causes are the ecological and life-history characteristics of species (Kotiaho *et al.*, 2005; van Kleunen & Richardson, 2007).

One of the most important research directions in conservation biology is the analysis of species characteristics associated with invasiveness (van Kleunen & Richardson 2007). This research is invaluable in determining important focus areas for conservation. A number of studies have compared invasive plant species with native or non-invasive alien plants, and show that there is some evidence of characters which predict plant invasiveness (Pyšek & Richardson, 2007; van Kleunen & Richardson, 2007). The invasive plant tends to displace native species rather than colonise empty niches – a trait which shows strong competitive ability (Krohne, 2001).

Seastedt (2009) looked at studies of common traits of plant invaders. These plants often have a smaller pathogen load in their new habitats than in their native ones, a trait which can translate into a competitive advantage (Mitchell & Power, 2003). This relates to the Enemy Release Hypothesis, which states that introduced plant species experience decreased control by herbivores and other natural enemies, resulting in their rapid proliferation and distribution (Keane & Crawley, 2002). This characteristic, combined with the ability to grow quickly in new environments, completely separates alien plants from native species in terms of their growth form and competitive strength (Blumenthal *et al.*, 2009). An example from South Africa is that of *Opuntia stricta* (Haw.) Haw. (Cactaceae), an invasive alien plant occurring in the Kruger National Park. A recent study

indicates that the distribution and abundance of *O. stricta* in the Park is not influenced or limited by environmental factors, and that the plant may completely colonise the area if effective management is not carried out (Foxcroft *et al.*, 2007). Predictors of rarity and invasiveness must, however, be considered in context (Alpert *et al.*, 2000; van Kleunen & Richardson, 2007). Invasion success must be seen as a result of various dynamic factors, and will always be dependent on the context of invasion (Catford *et al.*, 2009). The impacts of invasive species are based subjectively on what we observe, and ecological and economic impacts do not always correlate with each other (Catford *et al.*, 2009; Williamson, 1993).

Ecosystems around the world are threatened by increasing numbers of invasive alien plants as system disturbance, global trade and travel accelerate the spread of propagules (Richardson & van Wilgen, 2004; Richardson *et al.*, 2008). System disturbance is recognised as a key factor in the spread of invasive species, and research suggests that invasive plants are passengers of disturbance rather than drivers of ecological change (MacDougall & Turkington, 2005). Much research has shown that invasion by alien plants can have detrimental effects on aquatic systems, notably in terms of variation in light availability, temperature, water flow, soil properties, dissolved nutrients, biotic interactions of macrophytes, herbivory and detritivory (Carpenter & Lodge, 1986; Görgens & van Wilgen, 2004; Wilcove *et al.,* 1998). In addition to this, invasive species are considered to pose a major worldwide threat to biodiversity, and may deplete natural resources, impact indigenous species, change community dynamics, reduce ecosystem stability, and harm economic productivity (Catford *et al.,* 2009; Haynes, 1988; Richardson & van Wilgen, 2004). Richardson *et al* (2008) note that the damage caused by invasive species can be revealed in different ways, and is often difficult to measure, since some impacts are direct and easily recognisable, while others may be delayed and indirect. Many invasive species remain dormant for long periods of time, and only begin to invade and damage habitats under optimal conditions (Crooks, 2005; Kowarik, 1995; Richardson *et al.,* 2008).

1.2 Resource competition

One of the general characteristics of invasive species is the ability to compete in a new habitat. Competition is defined as: “Any interaction between two or more

species over a limiting resource that causes a decrease in population growth of one of the species (Krohne, 2001)". Plant competition is recognised as playing an important role in determining species distribution in aquatic ecosystems (Connell, 1980; Gopal & Goel, 1993; Van *et al.,* 1998). There are two main forms of competition: Interspecific (between different species) and intraspecific (between members of a single species). The way in which an invader tolerates inter- and intraspecific competition may either increase or decrease its invasiveness. Competition in plants is mainly for resources such as light, water, space and nutrients - the basic resources for survival.

Nutrient levels influence plant community structure, and high nutrient levels are often a determinant in the success or failure of alien plant invasions (Rattray *et al.,* 1991; Van *et al.,* 1999). The fast and efficient use of nutrients in water and soil may reduce the availability of nutrients to epiphytes and other macrophytes, thereby acting as a potential competitive strategy (James *et al.,* 2006). The distribution and growth of invasive plants may be predetermined by an assessment of hydrosoil fertility, therefore, if an invasive plant's performance under specific fertility conditions is known, one can predict and understand what the risks of spread are, and what kind of environments the plant is likely to perform well in (Steward, 1984).

Submersed plants have the ability to draw nutrients from both substrate and surrounding water (Denny, 1972; Gu, 2006; Tipping *et al.,* 2009), although indispensable nitrogen, phosphorus and micronutrients are usually present only in very small quantities in water (Rattray *et al.,* 1991). Eutrophication of water systems results in increased concentrations of these nutrients in the water, which can either hamper or support submersed plant growth, depending on their tolerance to nutrient levels. An important point to consider in studying competition between submersed plants is that both light and nutrients are determining factors of species distribution (Duarte *et al.,* 1986; Gu, 2006; Rattray *et al.,* 1991). Sunlight is an important resource in interspecific and intraspecific competitive interactions (Barrat-Segretain, 2005; Newman, 1973; Spencer & van Viersson, 1988). In nutrient-limited systems, competition for light is related to the size of individuals (Cahill, 1999). When sufficient nutrients are available for plant growth, competition is for available sunlight, after which the competitive performance of a

plant is dependent on: The rate of dry matter production; the maximum height and; the timing of canopy growth and shading-out (Aerts *et al.,* 1990).

The space to survive and grow is another ‘resource’ that plants compete for (Agami & Reddy, 1990; Krohne, 2001). The densities of plants in a habitat, and not only limiting resources, will influence their competitive interactions, bringing into play competition between and within species groups. For example, a low plant density increases the amount of light available to plants for photosynthesis. Self-thinning reveals the importance of intraspecific competition, whereby a higher initial density of seedlings leads to a higher mortality rate, resulting in a decrease in population density and an increase in size of the remaining individuals (Krohne, 2001). In studying competitive interactions between two species of *Elodea* (Hydrocharitaceae), Barrat-Segretain (2005) found that both spatial pattern and the developmental stage of an indigenous species may determine the result of competition with possible invading species. The arrangement of plant species in their habitat plays a vital role in competition. For example, if populations of plant species are grouped together intraspecifically, there can be a significant reduction in interspecific competition (Rejmánek, 2002).

1.3 Research aims and questions

Hydrilla verticillata (L.F.) Royle (Hydrocharitaceae), which is commonly referred to as Hydrilla, was considered as the typical invader species for the purposes of this research. It is a submersed freshwater macrophyte, and is one of the most dangerous and costly invasive plants in many parts of the world (Cook, 1988; Langeland, 1996). *Azolla filiculoides* Lamarck (Pteridophyta: Azollaceae), commonly named red water fern, is a floating freshwater fern native to warm temperate and subtropical America (Hussner, 2006; Stergianou & Fowler, 1990). It has invaded many aquatic habitats worldwide, and is recognized as a weed in many countries (McConnachie *et al.,* 2004). In addition to the study of *H. verticillata*, *A. filiculoides* was considered as a different form of invader in this research: The floating macrophyte.

1.3.1 Aims

The principle aim of this research was to gain an understanding of the susceptibility of indigenous aquatic plants to invasive species. The competitive interactions of two sets of aquatic plants were studied as an indication of how indigenous species might perform growing alongside alien invasives under differing nutrient levels and densities. The two sets of plants were studied in two separate experiments: The submersed weeds, *H. verticillata* and an indigenous species, *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) and; the floating weeds, *A. filiculoides* and the indigenous *Spirodela polyrhiza* (L.) Schleid (Lemnaceae).

1.3.2 Key Research Questions

- How will planting density affect the establishment and growth of the indigenous species in competition with the alien species?
- How will differing nutrient conditions affect the establishment and growth of the indigenous species in competition with the alien species?

1.3.3 Hypotheses

There is much evidence for the strong competitive abilities of alien plants in new habitats, but little or no research has yet been done regarding their interaction with indigenous species in South Africa. Little information is available as to whether similar indigenous plants will be able to successfully compete with aquatic invasives. This research hypothesised that the competitive performance of both alien and indigenous plants would be affected by planting density. Plant densities are likely to influence growth due to a change in available resources, such as light, space and nutrients. Therefore, the second hypothesis was that differing nutrient levels would have an influence on the establishment and growth of the alien and indigenous species. The predicted outcome was that the presence of the alien plants would have a negative effect on growth and competitive performance of the indigenous species.

1.3.4 Report structure

This research report is divided into four chapters. The first chapter is an introduction and literature review of the project topic. The second chapter details the experimental methods and materials. The third chapter deals with results and outcomes of the Hydrilla:Lagarosiphon and Azolla:Spirodela experiments. The fourth chapter serves as a summary and general discussion. It presents the final conclusions of the project and makes recommendations for possible future research in the field of invasive weeds.

1.4 Hydrilla verticillata

Hydrilla is native to the warmer regions of Asia, with widespread non-native populations in Europe, Australia, New Zealand, the Pacific Islands, Africa, and the Americas (Cook & Lüönd, 1982). Hydrilla is recognised as a major aquatic weed in the southern United States, and its growth is related to many water-use problems and freshwater ecological changes (Gu, 2006; Van *et al.,* 1999). Where Hydrilla infestations are dense, irrigation operations and hydroelectric power generation activities have been greatly affected, with the use of boat marinas and propeller-driven boats being hampered by Hydrilla's presence on the water's surface (Gu, 2006; Ramatsui, 2006). Although there are several benefits of Hydrilla infestations, such as provision of spawning habitats for various fishes and food for waterfowl, these are eclipsed by its harmful effects (Gu, 2006).

Hydrilla's presence in South Africa was positively confirmed in February 2006 by Lesley Henderson of South Africa's Plant Protection Research Institute (PPRI), when it was found in Pongolapoort Dam (27.3537 S, 31.9063 E), KwaZulu-Natal (Coetzee *et al.,* 2009). Pongolapoort Dam, which is the third largest dam in the country, is Hydrilla's only known location in South Africa although, considering its invasive nature in other countries, there is a great chance of further spread (Coetzee *et al.,* 2009).

Hydrilla is a polymorphic plant, with a growth form dependent on environmental conditions (Langeland, 1996). Biotypes can be either monoecious or dioecious, and the South African biotype has been identified as having the same DNA sequence as the monoecious Hydrilla biotype from Indonesia and Malaysia,

which suggests that this is the area of origin of the South African biotype (Madeira *et al.,* 2007). The plant is submersed, with roots below the substrate, although fragments can survive in water unrooted. There are many stems both above and below the substrate. Leaves are 2-4mm wide, 6-20mm long, and present in whorls of 3-8, with 11-39 teeth per cm along the leaf margins (Langeland, 1996). Turions are produced in leaf axils, and fall from the plant when mature, producing new plants. These turions are 5-8mm long, dark green in colour and appear spiny. Tubers are also produced terminally on rhizomes, and are 5-10mm long with a white/yellowish colour (Steward, 1969). Turions and tubers are vital components of Hydrilla's life history and reproductive strategy. While vegetative reproduction from plant fragments relies on environmental conditions (substrate quality, water quality, etc.), turions and tubers can remain dormant for long periods of time in the sediment. Turions are particularly hardy and are resistant to drying, cold temperatures, herbicides, ingestion and regurgitation (Gu, 2006; Langeland, 1996).

1.4.1 Characteristics for invasiveness

Hydrilla has been referred to as 'the perfect aquatic plant' and an 'aggressive and competitive coloniser of aquatic habitats' (Langeland, 1996). It functions by quickly establishing itself before displacing native vegetation. The family Hydrocharitaceae is entirely aquatic, with inflorescence evolution being linked with a shift from entomophily (insect pollination) to hydrophily (water pollination), and from terrestrial to marine and fresh-water habitats (Kaul, 1970). Hydrilla has several adaptive strategies for survival in aquatic ecosystems, which allow the plant to compete successfully with indigenous species (Gu, 2006; Langeland, 1996). Hydrilla is able to tolerate a wide range of environmental conditions, such as a broad pH range, different nutrient levels and salinity, and low light levels for photosynthesis (Cook & Lüönd, 1982; Steward & Van, 1987; Van *et al.,* 1976). Table 1.1 presents a summary of environmental conditions favouring the establishment of Hydrilla.

Table 1.1: Environmental conditions favouring the establishment of Hydrilla (Cook & Lüönd, 1982; Van *et al.* 1999).

Environmental variable	**Optimal growth conditions of Hydrilla**
Water bodies	Still, freshwater ecosystems
Water depth	Normally grows in shallow water
Light availability	Prefers high levels of light Can tolerate low light levels
Water quality	Very tolerant of: Oligotrophic to eutrophic water Acidic to strongly alkaline water Organic pollution and nutrient enrichment in water Performs best in high nutrient environments

Plants in this family have many adaptations for specialised pollination, and Hydrilla is no exception, with a unique pollination biology. The male Hydrilla flowers are released from the plant as buds when a build-up of gas causes the submerged spathe to open (Cook & Lüönd, 1982). Once the buds are released onto the water surface, the petals and sepals open horizontally, and the stamens spring into a vertical position. Pollen grains burst out into the air and fall straight down where they stick to the female stigmas (Cook, 1988). The plant uses four different methods of reproduction: Fragmentation, tubers (which can be very resistant in the soil), turions and seed (Basiouny *et al.,* 1978; Gu, 2006). The ability of a plant to reproduce through fragments is recognised as a trait of successful invaders, as these fragments can be spread quickly and easily in water currents (Barrat-Segretain, 2005).

Hydrilla can successfully compete for sunlight by lengthening quickly until it reaches the water surface, then branching copiously to form a canopy of plant material on the water surface to receive maximum sunlight (Balciunas *et al.,* 2002; Haller & Sutton, 1975). This means that the growth and competitive ability of other submersed plants beneath the canopy is inhibited, while Hydrilla monopolizes most of the light (Barko & Smart, 1981; Gu, 2006). Despite this ability to intercept maximum sunlight, a study by Van *et al* (1976) reveals that Hydrilla has a low light compensation point, meaning that it can tolerate low levels of light and may have a competitive advantage in such conditions. It often

grows at a depth of 3m in Florida lakes, however it has been found growing as deep as 15m (Langeland, 1996; Gu, 2006). Barko and Smart (1981) reveal that Hydrilla growing in maximum shade (with low light levels) was able to successfully increase shoot length, although there was minimum growth in biomass.

While terrestrial plants obtain their nutrients from roots alone, submersed plants can use nutrients from both the water and sediment (Gu, 2006). Hydrilla can grow in a range of sediment and water environments, from oligotrophic to eutrophic (Cook & Lüönd, 1982). Another biological trait favouring Hydrilla is its tolerance of a wide pH range and its ability to use both free carbon dioxide and bicarbonate ions for photosynthesis (Gu, 2006; Steward & Van, 1987). Hydrilla has a low carbon dioxide compensation point, meaning that when free carbon dioxide is depleted at high pH, Hydrilla can then use bicarbonate ions in the process of photosynthesis (Holaday & Bowes, 1980; Salvucci & Bowes, 1983).

1.4.2 Control of Hydrilla

The confirmation of Hydrilla's presence in South Africa is of great concern, primarily due to its harmful nature in freshwater habitats, and the great difficulty encountered elsewhere in attempting to control its spread. There is no current legislation against this weed in South Africa, although steps are being taken to have Hydrilla declared a Category 1 weed (Ramatsui, 2006), which would cause it to be strictly prohibited and controlled or eradicated where possible. Langeland (1996) recognises several serious implications of possible Hydrilla infestations, including economic impacts, water use disturbances, the replacing of indigenous aquatic plants and negative impacts on freshwater habitats. A particular concern about the Hydrilla infestation in Pongolapoort is that the lucrative tourism industry of the dam may be threatened, and that recreational boating activities may lead to Hydrilla spreading to other South African dams (Coetzee *et al.,* 2009). The Invasive Alien Species Programme (IASP) of KwaZulu-Natal's Department of Agriculture, Environmental Affairs and Rural Development has launched a public awareness and boat/trailer cleaning campaign in an effort to both educate the public on the dangers of the spread of Hydrilla, and enlist the participation of the

public in ensuring that fragments of Hydrilla are removed from boats leaving the water (Madeira *et al.,* 2007).

The processes of controlling alien species, reducing or preventing harmful impacts, and repairing damaged ecosystems are time-consuming and expensive (Byers *et al.,* 2001; Richardson & van Wilgen, 2004). Concern has been growing over the sustainability of herbicide use due to increased resistance from plants, and pressure due to environmental degradation, which has resulted in the development of new strategies for weed management (Kropff & Lotz, 1992; Steward, 1969). Applying water-borne herbicides over a large area could also compromise the health of non-target species and lead to a mass die-off of vegetation, releasing substantial amounts of nutrients into the water system and compromising water quality (Gu, 2006). This may have serious long term impacts in South Africa, where many people rely directly on rivers for drinking water and irrigating their crops.

Various techniques for the control or eradication of Hydrilla have been developed. These include the use of herbicides, biological control (including the use of grass carp, *Ctenopharyngodon idella* (Valenciennes in Cuvier & Valenciennes, 1844) (Cyprinidae)) and mechanical removal. There are four host-specific insect biocontrol agents that have been introduced into North America for the purpose of controlling Hydrilla (Doyle *et al.,* 2002). These are two species of ephydrid (leaf-mining) flies, *Hydrellia pakistanae* Deonier and *H. balciunasi* Bock (Diptera: Ephydridae), and two weevil species, *Bagous affinis* Hustache and *B. hydrillae* O'Brien (Coleoptera: Curculionidae) (Cuda *et al.,* 2008). *Hydrellia pakistanae* is native to the tropical and temperate areas of Asia (Deonier, 1993), and was introduced successfully to the United States in 1987 after host specificity was confirmed both in Pakistan and the US (Doyle *et al.,* 2002). *Hydrellia balciunasi* is native to Australia, and was identified as a potential biocontrol agent for Hydrilla in the 1980s. It was brought into quarantine in the United States in 1988 and released in 1989 (Grodowitz *et al.,* 1997). The Plant Protection Research Institute (PPRI) of South Africa is currently investigating the suitability of the leaf-mining flies for biological control of Hydrilla in South Africa (A. Bownes 2007, pers. comm.; Coetzee, 2006). An important limitation with the application of biocontrol agents in South Africa is that the South African monoecious biotype of Hydrilla is

different to the dioecious biotype found in the United States (J. Coetzee 2007, pers. comm.). The flies might therefore be less effective on the South African biotype than on the US dioecious biotype (Madeira *et al.,* 2007). For this reason, *Hydrellia* sp., has been imported from Singapore, where it was collected from monoecious Hydrilla. This species is currently being tested by the PPRI for its suitability as a biocontrol agent (A. Bownes 2011, pers. comm.).

A new potential biocontrol agent for Hydrilla has recently been identified by the PPRI, The aquatic plant moth, *Parapoynx diminutalis* Snellen, 1880 (Lepidoptera: Crambidae), which is native to Asia and Australia, has been found on Hydrilla mats in Pongolapoort Dam and seems to be controlling its spread (Bownes, 2010). The long term effect of the moth on Hydrilla is currently being monitored by the PPRI.

1.4.3 Previous work on Hydrilla in competition

The competitive ability of Hydrilla has been studied in interactions with plants such as *Egeria densa* Planch. (Hydrocharitaceae), *Ceratophyllum demersum* L. (Ceratophyllaceae), *Myriophyllum spicatum* L. (Haloragaceae) and *Vallisneria americana* Michx. (Hydrocharitaceae) (Hofstra *et al.,* 1999; Mony *et al.,* 2007; Van *et al.,* 1999; Wang *et al.,* 2008). The results of previous research on Hydrilla in competition experiments are mixed, with Hydrilla reacting differently under varying environmental conditions. Several studies have investigated competitive interactions between Hydrilla and *V. americana*, a submersed aquatic native to the US, and have shown mixed results. Van *et al.* (1976) demonstrate that Hydrilla has the ability to utilize sunlight more efficiently than *C. demersum* and *M. spicatum*, growing quickly to the surface and forming a dense mat, excluding sunlight from other submersed plants. The experiment measured photosynthetic rates at different radiation levels, and determined that Hydrilla had the lowest light requirement of the three species studied, giving Hydrilla a competitive advantage. On the other hand, a reciprocal replacement series study shows that while Hydrilla had a competitive advantage over *V. americana* under high levels of light, *V. americana* dominated when light was limited (Smart & Barko, 1989). Hydrilla competed more successfully at a low level of sediment nutrients, while *V. americana* outcompeted at a high nutrient level. In a similar study (Barko & Smart,

1989), Hydrilla was outcompeted by *V. americana* in a high-nutrient environment, enabling *V. americana* to compensate for the ability of Hydrilla to form a shading canopy. In contrast to this, Van *et al.* (1999) found that the competitive ability of Hydrilla under nutrient-limiting conditions was depressed, while it competed strongly with *V. americana* when grown under high nutrient levels. Similarly, McCreary (1991) found that Hydrilla was significantly outcompeted by *Potamogeton americanus* Cham. & Schltdl. (Potamogetonaceae) in nitrogen-poor sediments. Research by Steward (1988) suggests that Hydrilla may not be as affected by competition from *V. americana* as it is self-limited by intraspecific competition, a possible explanation for these mixed results. An experiment by Gu (2006) demonstrates that in monocultures of Hydrilla, abundance of individuals increases with a rise in pH, alkalinity, total phosphorus and total nitrogen.

A study investigating above- and belowground competition between Hydrilla and *M. spicatum* reveals that the root and shoot biomass of *M. spicatum* was significantly higher with intraspecific competition. The root-to-shoot ratio of *M. spicatum* increased significantly with interspecific competition, while that of Hydrilla increased with intraspecific competition (Wang *et al.,* 2008). The study also shows that Hydrilla competed strongly for light by shading, while reducing the root biomass of its competitor under conditions of interspecific competition. Spencer and Ksander (2000) investigated interactions between monoecious Hydrilla and American pondweed (*Potamogeton nodosus* Poiret (Potamogetonaceae)), where Hydrilla grown from small propagules managed to coexist with American pondweed grown from larger propagules, pointing to Hydrilla's strong competitive ability. The competitive and invasive behaviour of Hydrilla is related to its biotic release in areas where it has no natural enemies (Spencer & Ksander, 2000). This increases the difficultly in controlling its spread.

The use of both insect herbivory and an established competitive indigenous plant species may prove to have the best long-term results in terms of controlling invasive species such as Hydrilla (Doyle *et al.,* 2002; Doyle *et al.,* 2007; Van & Center, 1994). Results from an experiment investigating competitive interactions between Hydrilla and *V. americana* as influenced by insect herbivory show that biological control agents can shift competition between plant species in favour of an indigenous species (Van *et al.,* 1998). A similar experiment shows that snail

herbivory can influence competition between two species of *Elodea* (Barrat-Segretain & Lemoine, 2007). The impacts of herbivory are often subtle and may not cause direct death of a plant, but the slow decrease in the plant's health makes it prone to competition from other plants (Barrat-Segretain & Lemoine, 2007; Van *et al.,* 1998; Van & Center, 1994).

Competitive interactions among submersed aquatic plants in South Africa have not been investigated in detail (Spencer & Ksander, 2000), and little or no research has been conducted in South Africa in terms of how competitive Hydrilla is against similar indigenous plants (J. Coetzee 2007, pers. comm.). This information would be important in determining the potential for similar alien species to invade existing aquatic plant communities. Although there have been a number of recent advances in the field of plant invasion biology, there are still many opportunities for gaining a greater understanding of interactions in plant communities (Rejmánek *et al.,* 2004; Richardson & Pyšek, 2006). Further research on the competitive abilities of exotic and native aquatic plants under a variety of conditions is necessary in order to understand the potential for biological control (Spencer & Ksander, 2000).

As McCreary (1991) postulates, although the characteristics of Hydrilla give it the ability to have a competitive advantage in many water systems, it may not have the upper hand when in competition with a similar canopy-forming species. Studies on the interactions between Hydrilla and such similar species may help to gain further understanding of Hydrilla's competitive strengths and community structure dynamics (McCreary, 1991). In studying competitive interactions of this sort, the use of both mixtures and monocultures of species in a variety of densities is necessary in order to clarify the effects of intra- and interspecific competition (McCreary, 1991).

1.5 Lagarosiphon major

The selection of *L. major* as an indigenous competitor species for the Hydrilla experiment was made in consultation with various researchers working with Hydrilla and the issue of its existence in Pongolapoort Dam: Dr Angela Bownes, Dr Julie Coetzee, and Mr Luke Schutz. Common names of *L. major* are African

elodea, curly waterweed, oxygen weed, hereafter referred to as Lagarosiphon (National Heritage Trust, 2007).

Very similar in morphology to Hydrilla, Lagarosiphon is an aquatic plant, native to southern Africa (James *et al.,* 1999), but now found in lakes, dams and rivers worldwide, forming thick mats that reduce light penetration and possibly reduce oxygen availability (Symoens & Triest, 1983). Lagarosiphon propagates quickly from easily broken stems, and it is this feature that has contributed to the plant being recognised as a serious pest in many parts of Europe and New Zealand (Caffrey & Acevedo, 2007; McGregor & Gourlay, 2002). In South Africa, it is often considered to be a 'weedy' species, and can be particularly invasive outside of its native range. The plant is limited in South Africa by natural enemies.[1] It can grow to a depth of 6.5m in clear water, and although it flourishes in shallow alkaline waters, it is able to tolerate a variety of conditions (Caffrey & Acevedo, 2007). Its optimal temperature range is between 20 and 23°C, and it tolerates both high and low nutrient levels (National Heritage Trust, 2007). Lagarosiphon has thread-like adventitious roots which grow from the stem, and horizontal stems (rhizomes) securing the plant to the substrate. The leaves are 5-20 mm in length and 2-3mm wide (Haynes, 1988). The most notable visual difference between Hydrilla and Lagarosiphon, and the best one for identification, is that the leaves of Lagarosiphon occur in alternate spirals along the stem, while those of Hydrilla are whorled, with 3-8 leaves per whorl.

1.6 Azolla filiculoides

Azolla filiculoides, hereafter referred to as Azolla, is a small (1–2.5 cm) heterosporous plant, which flourishes in ponds, water reservoirs, wetlands, channels and slow-flowing rivers (Hussner, 2006; Lumpkin & Plucknett, 1980). It is well known for its symbiotic association with the nitrogen-fixing blue-green alga *Anabaena azollae* Strasburger, 1884 (Cyanobacteria: Nostocaceae), which enables it to grow in nitrogen-poor waters (Hill & Cilliers, 1999; Hussner, 2006).

Azolla is distributed by waterfowl, ships or humans. Azolla was first documented as a naturalized species in South Africa in 1948, in the Oorlogspoort River, Northern Cape (Oosthuizen & Walters, 1961), and is a Category 1 declared weed

[1] Note: Information provided by the examiner during review of the Research Report.

in South Africa (Henderson & Cilliers, 2002). Possible reasons for its rapid spread include few natural enemies, dispersal by humans and nutrient-rich water systems (Hill, 1998). The presence of Azolla in water systems can restrict the flow of water, increase siltation rates and reduce water quality (McConnachie *et al.,* 2004). It has been used in Southeast Asia as a green manure on rice paddies for over 200 years (Hill & Cilliers, 1999), and is widely studied for its role as a bio-fertiliser, water and effluent purifier, and an animal feed (Forni *et al.,* 2001; *Wagner*, 1997; Zhao *et al.,* 1999). Azolla is also recognised for its ability to accumulate nutrients such as phosphorus and potassium, making them available to other plants when it decomposes (Hussner, 2006).

The fern is free-floating, and can be present as individual plants, or as dense mats on the water surface. The plant consists of a primary rhizome, which branches out into secondary rhizomes. The small leaves are two-lobed and alternately arranged. The adventitious roots grow from nodes on the ventral side of the rhizomes, and absorb nutrients from the surrounding water (Wagner, 1997). Symbiotic *A. azollae* live in the leaf cavities of the dorsal lobes (Lumpkin & Plucknett, 1980). Azolla can reproduce either sexually with the production of spores, or asexually, depending on environmental conditions. According to Wagner (1997), asexual reproduction occurs most often, with fragmentation of the fronds giving rise to new individuals.

Azolla has a rapid surface-area doubling time of seven to ten days under favourable conditions, with sporulation being regulated by factors such as light intensity, photoperiod, temperature, pH and availability of nutrients (Hussner, 2006; Janes, 1998). It grows best in half-shade, and at a temperature range of 15 to 20°C (Hussner, 2006), although the optimum temperature for nitrogen fixation and oxygen production is stated to be 25°C (Wong Fong Sang *et al.,* 1987). The optimal pH range of Azolla is 4.5 to 7, while the plant still survives in a range of 3.5 to 10 (Lumpkin & Plucknett, 1980; Wagner, 1997). According to Lumpkin and Plucknett (1980), phosphorus is the most important element for the growth of Azolla, and can often be a limiting nutrient. Azolla plants with insufficient phosphorus can become smaller, more fragile, red in colour and can develop long roots. Azolla also becomes red in high levels of sunlight or in cold weather, due to anthocyanin production. Much research has been conducted on the

nitrogen-fixing ability of the Azolla/Anabaena symbionts (Arora & Singh 2003; Kitoh *et al.,* 1993; Reddy, 1987; van Kempen *et al.,* 2010). However, research suggests that while Azolla does not require the presence of nitrogen in the surrounding water for its survival, an increased level of nitrogen has a positive effect on its growth rate (Wagner, 1997). In a study by Cary and Weerts (1992), Azolla attained maximum biomass at 20mgP/L and had maximum growth at 10mgN/L.

Biocontrol has been a feasible solution to the problem of Azolla in South Africa (Hill, 1998; Hussner, 2006; McConnachie *et al.,* 2004). In a field assessment of a frond-feeding weevil (*Stenopelmus rufinasus* Gyllenhal 1836 (Curculionoidea)) on Azolla in southern Africa, the biocontrol agent was found to be remarkably successful in systems where Azolla had covered dams and water reservoirs (McConnachie *et al.,* 2004).

1.7 Spirodela polyrhiza

Azolla has been found growing together with *Lemna minuta* Kunth (Araceae), *Lemna minor* L. (Araceae) and *S. polyrhiza* (Hussner, 2006). *S. polyrhiza* commonly referred to as greater duckweed and hereafter referred to as Spirodela, is a small floating aquatic plant. It is distributed widely in freshwater lakes, ponds and slow-flowing rivers in many parts of the world, and has a wide native range (Davidson & Simon, 1981). The selection of Azolla and Spirodela as competing species was based on their similar growth form and habitats.

Spirodela plants consist of 2-5 small leaves, which reach about 1cm in length and are arranged in a rosette. Adventitious roots hang down from this rosette of leaves. The plant flowers very seldom, with vegetative reproduction occurring by alternate budding of meristemic cells (Davidson & Simon, 1981). When environmental conditions are unfavourable, the plant is dormant in the form of turions (modified fronds), which are small, hard and dark. As with Azolla, Spirodela grows rapidly under favourable conditions and forms a mat on the water surface. It has been extensively studied for its use in waste water treatment, nutrient recovery and as an animal feed (Fasakin, 1999; Vermaat & Hanif, 1998; Xu & Shen, 2011). In South Africa, Spirodela is a cosmopolitan plant

which can become problematic under conditions of nutrient enrichment. The plant is limited in South Africa by natural enemies. [2]

1.8 Conclusion

The two alien invasive species, Hydrilla and Azolla, are currently problem plants in South Africa and may continue to proliferate and disperse throughout fresh water systems in the country. The above research examples have shown that they are hardy and competitive in nature, and have the potential to outcompete similar native aquatic species. The following research aims to gain an understanding of the susceptibility of South African aquatic plants to invasive species such as these.

[2] Note: Information provided by the examiner during a review of the Research Report.

CHAPTER 2: METHODS AND MATERIALS

2.1 Competitive interactions between Hydrilla and Lagarosiphon

2.1.1 Introduction

This research has been designed to be statistically comparable with previous research on competitive interactions between Hydrilla and two US native plants: *V. americana* and *P. nodosus*, in which reciprocal yield models were used to assess competitive ability (Spencer & Ksander, 2000; Van et al., 1998; Van et al., 1999). Reciprocal yield models are useful in studying competition, in that both inter- and intraspecific competition can be studied, and their effects separated quantitatively (Firbank & Watkinson, 1985; Spencer & Rejmánek, 1989). Spencer and Rejmánek (1989) state that the careful use of reciprocal yield models can increase understanding of the value of competition in aquatic plant community structure.

The reciprocal yield model of Spitters (1983) was used to estimate the competitive ability of Hydrilla and Lagarosiphon, whereby multiple linear regressions of the following equation were performed:

$$1/W_h = a_{h0} + a_{hh}N_h + a_{hL}N_L$$
$$1/W_L = a_{L0} + a_{LL}N_L + a_{Lh}N_h$$

where W_h and W_L (dependent variables) represent the mean dry mass per plant for Hydrilla and Lagarosiphon respectively. N_h and N_L (independent variables) are their respective planting densities, and the intercepts (or constants) a_{h0} and a_{L0} are estimates of the reciprocal of maximum plant weight. Intraspecific competition was estimated with the partial regression coefficients a_{hh} and a_{LL}, and interspecific competition was estimated with a_{hL} and a_{Lh}.

2.1.2 Experimental design

Both Lagarosiphon and Hydrilla plant specimens were obtained from the PPRI (Plant Protection Research Institute) and the University of the Witwatersrand.

Hydrilla specimens had previously been collected on site at Pongolapoort Dam, and Lagarosiphon was collected from Mearns Weir in Mooi River. The study was conducted at the PPRI, which is located in Pretoria, South Africa.

Hydrilla and Lagarosiphon segments (15cm long) were grown in plastic planting bags, submersed in large indoor water pools. Planting bags were 23cm diameter; 24cm height, with 20cm of river sand substrate in each bag. Various pond sediments were used in trial growth of Hydrilla, but were found to be problematic due to the difficultly in controlling nutrient levels with high-nutrient sediment. Plants were grown under two different nutrient levels using Multicote (Multigreen 4), a controlled release fertilizer: 2.0g Multicote (low fertility L1) and 25.0g Multicote (high fertility L2) per 15kg of sand. These nutrient levels were chosen based on the methods of a similar experiment by Van et al (1999), which investigated competition between Hydrilla and *V. americana* as influenced by soil fertility. The Multicote used is formulated for an eight-month release period in soil, with an NPK ratio of 23:5:23. Algae were controlled using a combination of mechanical removal and algaecide (AlgiMin, TetraAqua). The bags were weighted down in the pools by placing a stone in the bottom of each bag. Mean individual plant dry masses were determined at the start of the experiment, where 10 plant segments of each species were dried and weighed, resulting in an average dry mass for each species. These 10 plant segments were used to estimate the representative plant mass for segments planted in the experiment. The two species were planted in different densities in an addition series (Spitters, 1983; Van *et al.,* 1999), with three experimental replicates. Hydrilla:Lagarosiphon planting densities were 0:3, 0:9, 3:0, 3:3, 3:9, 9:0, 9:3 and 9:9 plant segments per bag. The ratio of 3:9 therefore indicates that this particular bag contained three Hydrilla segments and nine Lagarosiphon segments. This addition series was used for both nutrient levels, and three replicates of each treatment experiment were done. Bags were placed into the water pools in a random distribution. In total, the experiment therefore included two species, three replicates, 48 bags, 216 plants of each species, eight planting densities, and two nutrient levels. The experimental plan is shown in Figure 2.1.

Harvesting occurred after 10 weeks, when the two species were separated and dried at 70°C for 24 hours. Plant dry weights of above-ground shoots and below-

ground roots were measured separately for each plant. Plant lengths (cm) were measured from the base of the main stem to the apex of the main stem after harvesting. The allocation of shoots versus roots dry mass was determined as a percentage (shoot/root) for each density and nutrient level. The death of plants during the experiment was also recorded to determine a percentage survival for each replicate.

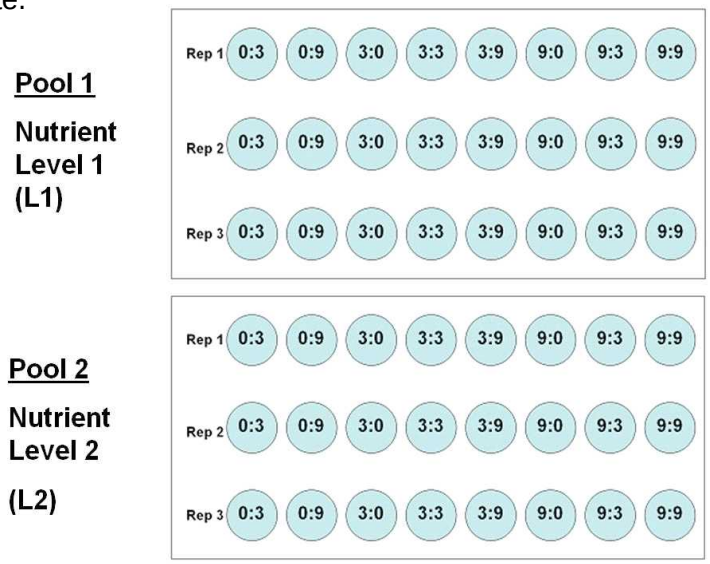


Figure 2.1: Experimental plan showing bag densities and replicates (Rep 1, 2 & 3) in each pool. Bags were placed in the pools in a random distribution.

2.1.3 Data analysis

Percentage survival of plants was calculated as a measure of competitive performance: The number of plants surviving in each bag as a percentage of the number of plants originally planted in the bag. The mean length of plants in each density ratio was calculated based on the mean lengths of surviving plants in each replicate (therefore, n = 3). The change in mean length was determined using individual plant length of surviving plants and the length of plants at planting (15cm). The mean dry mass of plants was calculated for each density ratio based on the dry mass of surviving plants. A correlation analysis was performed between the mean dry mass and length of plants.

Kruskal Wallis non-parametric ANOVAs (Analysis of Variance), which compare ranks and medians, were performed on all data (between species, nutrient levels and density ratios) using a significance level of 0.05 and confidence limits of 0.95.

If the computed results show a *p*-value smaller than the significance level, the null hypothesis can be rejected (which states that there is no significant difference between groups). Kruskal Wallis was chosen due to the small sample size (n) in this experiment, and was followed with the Kruskal Wallis post-hoc test which gives an indication of difference within groups.

As described above, multiple linear regressions were run as part of the reciprocal yield model (Spitters, 1983). Results of these regressions gave an indication of the direction of competition between species. These regressions and tests of significance (F-tests) were performed using the General Linear Model in Statistica v.8. F-tests determined the significance of the regression coefficients.

2.2 Competitive interactions between Azolla and Spirodela

2.2.1 Introduction

This investigation follows the same methods used in the Hydrilla:Lagarosiphon experiment, as a means of further testing the competitive interactions between alien and indigenous plants. As in the Hydrilla:Lagarosiphon experiment, the reciprocal yield model of Spitters (1983) was used to estimate the competitive abilities of Azolla and Spirodela, whereby multiple linear regressions of the following equation were performed:

$$1/W_A = a_{A0} + a_{AA}N_A + a_{AS}N_S$$
$$1/W_S = a_{S0} + a_{SS}N_S + a_{SA}N_A$$

where W_A and W_S (dependent variables) represent the mean individual wet mass of Azolla and Spirodela respectively. N_A and N_S (independent variables) are their respective planting densities, and the intercepts (or constants) a_{A0} and a_{S0} are estimates of the reciprocal of maximum plant weight. Intraspecific competition was estimated with the partial regression coefficients a_{AA} and a_{SS}, and interspecific competition was estimated with a_{AS} and a_{SA}. These regressions and tests of significance (F-tests) were performed using the General Linear Model in Statistica v.8. F-tests determined the significance of the regression coefficients.

2.2.2 Experimental design

Both Azolla and Spirodela were obtained from the University of the Witwatersrand, Johannesburg, where the study was conducted. Azolla and Spirodela plants were separated into individual plant fragments, of about 3 fronds each. At the start of the experiment, 60 plants of each species were weighed (wet mass) in order to get an average starting mass per plant. Dry mass was not used in this experiment due to the negligible dry mass of the minute Spirodela individuals.

Azolla and Spirodela plants were placed in 1 litre plastic tubs, 15cm diameter and 13cm height. No sediment was used in these tubs, as the plants obtain their nutrients directly from the water. Plants were grown in three different nutrient levels: Low (L1), medium (L2) and high (L3). While two nutrient levels were used in the Hydrilla:Lagarosiphon experiment, three nutrient levels were recommended for the Azolla:Spirodela experiment in order to understand competitive interactions over a gradient of nutrient levels. Recommended concentrations of N, P and K for these nutrient levels were obtained from consultation with staff of the University of the Witwatersrand (D. C. Drake 2010, pers. comm.). Cary and Weerts (1992) used similar nutrient levels in a study investigating the growth and nutrient composition of Azolla in varying environmental conditions. Potassium diphosphate (KH_2PO_4) and potassium nitrate (KNO_3) were mixed with de-ionised water as a source of available N, P and K. The amounts used in each nutrient level are presented in Table 2.1. Tubs were filled with approximately 780ml of the nutrient enriched water. A ring of polystyrene (about 7cm in diameter) was placed on top of the water surface in each tub, in order to keep the plants together so that they would compete for space.

Table 2.1: Concentrations of N, P and K (KH_2PO_4 and KNO_3) used in competition experiments for Azolla and Spirodela.

	KNO_3	*N*	*K*	**KH_2PO_4**	*P*
LOW (L1)	0.072mg/L	0.01mg/L	0.028mg/L	0.0044mg/L	0.001mg/L
	2.16mg/30L			0.1318mg/30L	
MEDIUM (L2)	7.218mg/L	1mg/L	2.79mg/L	0.4394mg/L	0.1mg/L
	216.54mg/30L			13.182mg/30L	
HIGH (L3)	72.181mg/L	10mg/L	27.914mg/L	4.3934mg/L	1mg/L
	2165.43mg/30L			131.802mg/30L	

The two species were planted in eight different densities in an addition series, as used in the Hydrilla:Lagarosiphon experiment. Therefore, the Azolla:Spirodela planting densities were 0:3, 0:9, 3:0, 3:3, 3:9, 9:0, 9:3 and 9:9 individuals per tub. The ratio of 3:9 therefore indicates that this particular tub contained three Azolla plants and nine Spirodela plants. This addition series was used for all three nutrient levels, and four replicates of each treatment experiment were done. Tubs were placed into a controlled phytotron, regulated at 25°C during the day, 15°C at night, and daylight light intensity. Tubs were placed on shelves in the phytotron in a random distribution. In total, the experiment therefore included two species, four replicates, 96 tubs, 324 plants of each species, eight planting densities, and three nutrient levels. During the seven week growth period, the tubs were moved outside for one week in order to suppress fungus present on Azolla plants, after which they were returned to the phytotron.

Harvesting occurred after seven weeks, where species were separated and weighed. While a ten week growth period was used in the Hydrilla:Lagarosiphon experiment, a seven week growth period was recommended for the Azolla:Spirodela experiment as harvesting could occur once the plants had multiplied considerably and tubs were full. The total wet mass of Azolla and Spirodela present in each tub was recorded, and the number of 'individual' plants was counted using an estimation of individual plant size (about 3 fronds each). A mean individual plant mass was calculated for each tub, based on the total wet mass and number of individual plants. The reason for this method was the fact that the plants grow together in a 'cluster', and separating them into the 'individual' sizes used at the start of the experiment would have been a lengthy and delicate process, especially considering the rapid multiplication rate of these species. Therefore, where 'total plant mass' is referred to, this indicates the 'cluster' mass of each species per tub.

2.2.3 Data analysis

Data analysis followed a very similar method as that used in the Hydrilla:Lagarosiphon experiment. Non-parametric ANOVAs (Kruskal Wallis) were performed on all data using a significance level of 0.05 and confidence limits of 0.95. Kruskal Wallis was chosen due to the small sample size (n) in this

experiment, and was followed with the Kruskal Wallis post-hoc test. Multiple linear regressions were run as part of the reciprocal yield model (Spitters, 1983).

Comparisons were not made between the mass of Azolla and Spirodela plants (total or individual mass), due to the variation in mass of the two species. The average starting mass of individual plants, determined at the beginning of the experiment, were: Azolla = 0.046g and Spirodela = 0.002g. For this reason, it would be difficult to interpret results by comparing plant mass of Azolla and Spirodela without the context of reproductive rates and plant numbers. The percentage change in plant numbers (per tub) was determined as a measure of the reproductive rate of Azolla and Spirodela. Significant changes in the individual wet mass of Azolla and Spirodela plants were determined using the Kruskal Wallis ANOVA, which compares ranks and medians.

CHAPTER 3: RESULTS

3.1 Competitive interactions between Hydrilla and Lagarosiphon

Hydrilla significantly outcompeted Lagarosiphon in both nutrient treatments in terms of the survival of plant segments. The percentage survival, mean length and dry mass of Hydrilla plants were significantly greater in L1 than in L2, while the establishment of Lagarosiphon was poor in both nutrient treatments. During the 10 week experiment, the L2 pool became infested with a large amount of algae which altered the conditions of the pool in terms of available light and nutrients. This is a possible explanation for the exceptionally poor performance of Lagarosiphon in L2. Algal contamination was the result of nutrient availability, especially in the case of the high nutrient treatment. During the 10 week growth period, algae were regularly removed from the pools in an effort to reduce infestations. Photographs of the pools at the start and end of the experiment are presented in Appendix A.

3.1.1 Survival

Table 3.1 shows the percentage survival of Hydrilla and Lagarosiphon plants in both L1 and L2. In both nutrient treatments, Hydrilla showed the lowest percentage survival in the density ratio of 9:0, with the overall lowest percentage survival in L2. While half of the density ratios showed a 100% survival of Hydrilla in L1 (3:9, 3:0 and 3:3), none showed a 100% survival in L2.

Table 3.1: Percentage survival of Hydrilla and Lagarosiphon plants in L1 & L2.

Mean (n=3) Percentage Survival over 10 weeks								
	3:0	**9:0**	**3:3**	**9:3**	**9:9**	**3:9**	**0:3**	**0:9**
Hydrilla - L1	100.00	85.19	100.00	92.59	92.59	100.00		
Hydrilla - L2	55.56	29.63	44.44	66.67	44.44	66.67		
Lagarosiphon - L1			0.00	0.00	11.11	11.11	0.00	3.70
Lagarosiphon - L2			0.00	0.00	0.00	0.00	0.00	3.70

Table 3.2 presents results of the Kruskal-Wallis ANOVA, indicating differences in percentage survival of Hydrilla and Lagarosiphon plants in L1 and L2. Percentage survival of Hydrilla plants was significantly higher than that of

Lagarosiphon in both L1 and L2. Hydrilla had a significantly higher percentage survival in L1 than in L2. The difference in survival of Lagarosiphon plants between the two nutrient treatments was not significant, since the final number of surviving plants, and therefore sample size, was so low. These results were obtained by pooling the data for each species in each nutrient level (i.e. for all density ratios). The pooling of data within nutrient levels was possible as no significant differences were observed between density ratios for Hydrilla or Lagarosiphon.

Table 3.2: Results of Kruskal Wallis non-parametric ANOVA, indicating differences in percentage survival of Hydrilla and Lagarosiphon plants in L1 & L2 (where * indicates significance).

Results of Non-parametric ANOVA: Percentage survival			
Dependent Variable	**Categorical Variable**	**H statistic**	***p* value**
% Survival in L1	Hydrilla vs. Lagarosiphon	$H_{1,48}$=38.88724	0.0000 *
% Survival in L2	Hydrilla vs. Lagarosiphon	$H_{1,48}$=39.23478	0.0000 *
% Survival of Hydrilla	L1 vs. L2	$H_{1,48}$=28.44348	0.0000 *
% Survival of Lagarosiphon	L1 vs. L2	$H_{1,48}$=1.631521	0.2015

3.1.2 Length

The length of Hydrilla plants was significantly greater in L1 than in L2, although the length of Lagarosiphon plants was not significantly different between nutrient levels, as presented in Table 3.3. There was no significant difference between the length of Hydrilla and Lagarosiphon plants, due to the small number of surviving Lagarosiphon plants. These results were obtained by pooling the data for each species in each nutrient level (i.e. for all density ratios). As in the case of percentage survival, pooling of data was justified as there were no significant differences between density ratios. Figures 3.1 and 3.2 present the differences between density ratios and within species, and indicate that there were no significant differences in length observed between density ratios for either Hydrilla or Lagarosiphon.

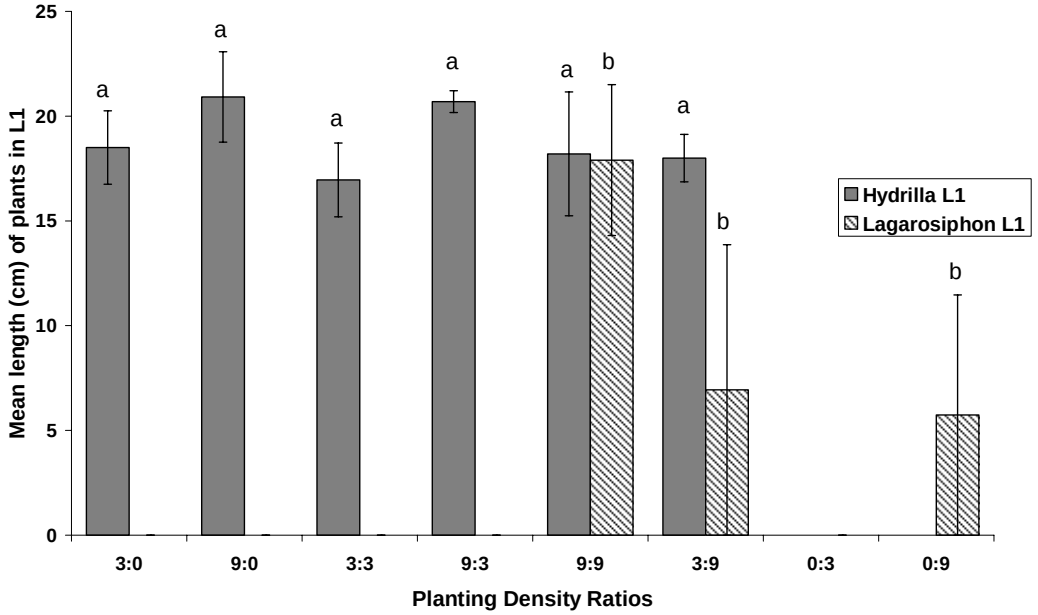


Figure 3.1: Mean length of Hydrilla and Lagarosiphon plants at different planting densities in L1. Error bars represent SE. Planting density ratios with letters in common within species are not significantly different (*p>0.05*).

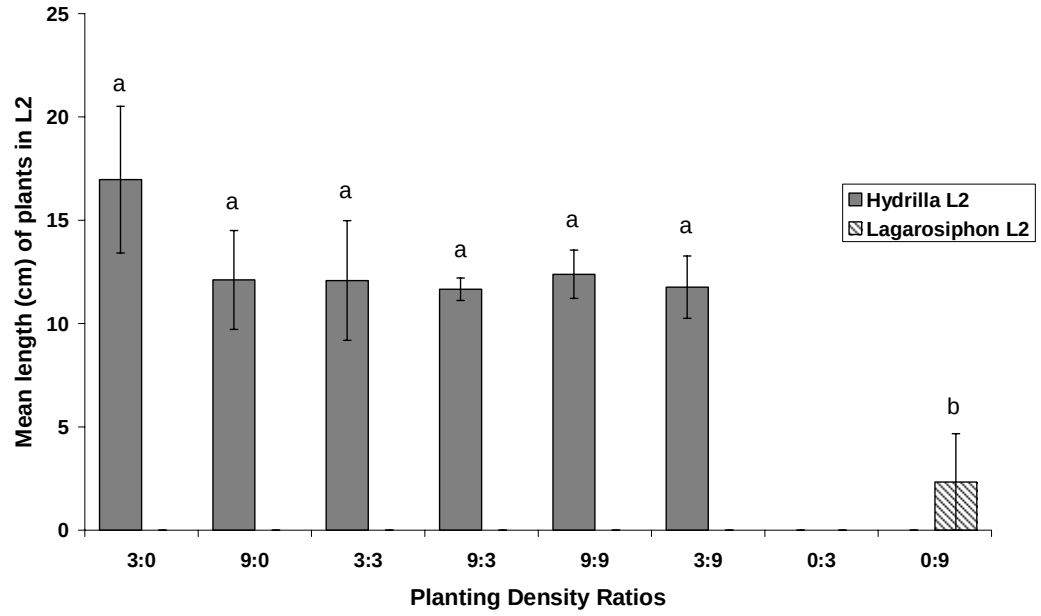


Figure 3.2: Mean length of Hydrilla and Lagarosiphon plants at different planting densities in L2. Error bars represent SE. Planting density ratios with letters in common within species are not significantly different (*p>0.05*).

Table 3.3: Results of Kruskal Wallis non-parametric ANOVA, indicating differences in length (cm) of Hydrilla and Lagarosiphon plants in L1 & L2 (where * indicates significance).

ANOVA Results - Length of plants (cm)			
Dependent Variable	**Categorical Variable**	**H statistic**	***p* value**
Length in L1	Hydrilla vs. Lagarosiphon	$H_{1,107}$=0.3004351	0.5836
Length in L2	Hydrilla vs. Lagarosiphon	$H_{1,54}$=2.474691	0.1157
Length of Hydrilla	L1 vs. L2	$H_{1,153}$=58.97154	0.0000 *
Length of Lagarosiphon	L1 vs. L2	$H_{1,8}$=1.190476	0.2752

Table 3.4 details results of the Kruskal Wallis ANOVA for groups in which a significant change in length was observed. Hydrilla plants in L1 did not show significant increases in length in 3:3 and 3:9, where Hydrilla was present in a low density and Lagarosiphon plants grew alongside. Hydrilla plants growing in L2 decreased in length in all but the 3:0 density ratio. However, only two density ratios showed a significant decrease in length: 9:9 and 9:3. Lagarosiphon had no significant differences in plant length in either nutrient level, due to the low number of surviving plants.

Table 3.4: Results of the Kruskal Wallis non-parametric ANOVA for change in length (cm) of Hydrilla and Lagarosiphon plants, indicating those density ratios where a significant change in plant length occurred.

Kruskal Wallis ANOVA Results – Change in length (cm)				
Species	**Nutrient Level**	**Density Ratio**	**H statistic**	***p* value**
Hydrilla	L1	3:0	$H_{1,18}$=4.505300	0.0338 *
Hydrilla	L1	9:0	$H_{1,46}$=16.41236	0.0001 *
Hydrilla	L1	9:3	$H_{1,50}$=19.42737	0.0000 *
Hydrilla	L1	9:9	$H_{1,50}$=5.445341	0.0196 *
Hydrilla	L2	9:9	$H_{1,24}$=13.71088	0.0002 *
Hydrilla	L2	9:3	$H_{1,36}$=11.50121	0.0007 *

Figures 3.3 and 3.4 illustrate the percentage change in mean length (cm) of Hydrilla and Lagarosiphon in both L1 and L2, based on individual plant lengths of surviving plants. Although Lagarosiphon did not show any significant differences in length in either nutrient level, it can be seen from these figures that where Lagarosiphon plants did survive, they decreased in length. ANOVA codes in Figures 3.3 and 3.4 indicate that there were no significant differences observed between density ratios and within species.

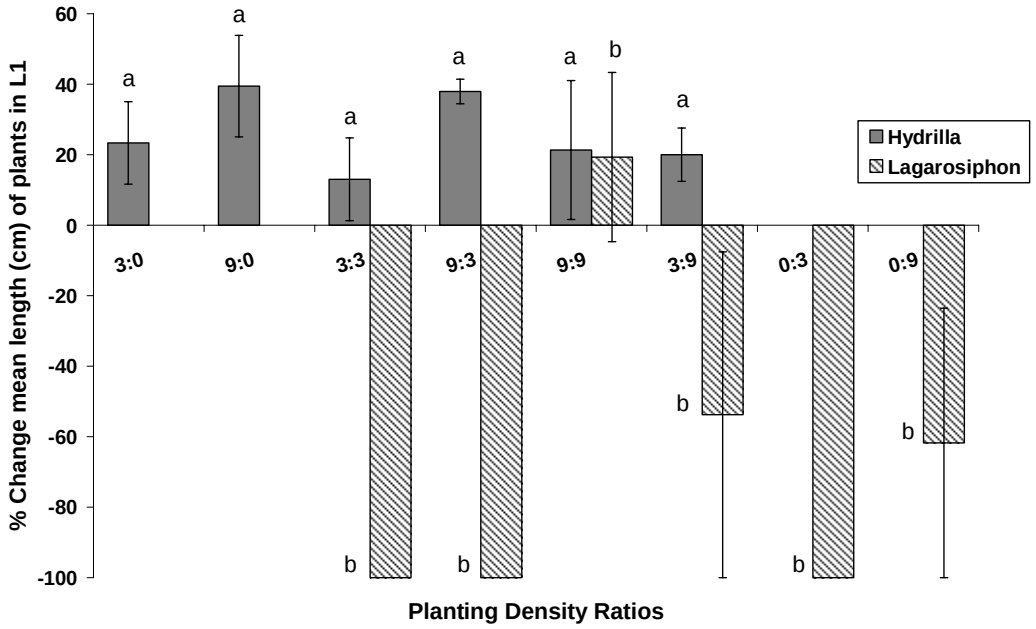


Figure 3.3: Percentage change in mean length (cm) of Hydrilla and Lagarosiphon plants in each planting density in L1. Error bars represent SE. Planting density ratios with letters in common within species are not significantly different (*p>0.05*).

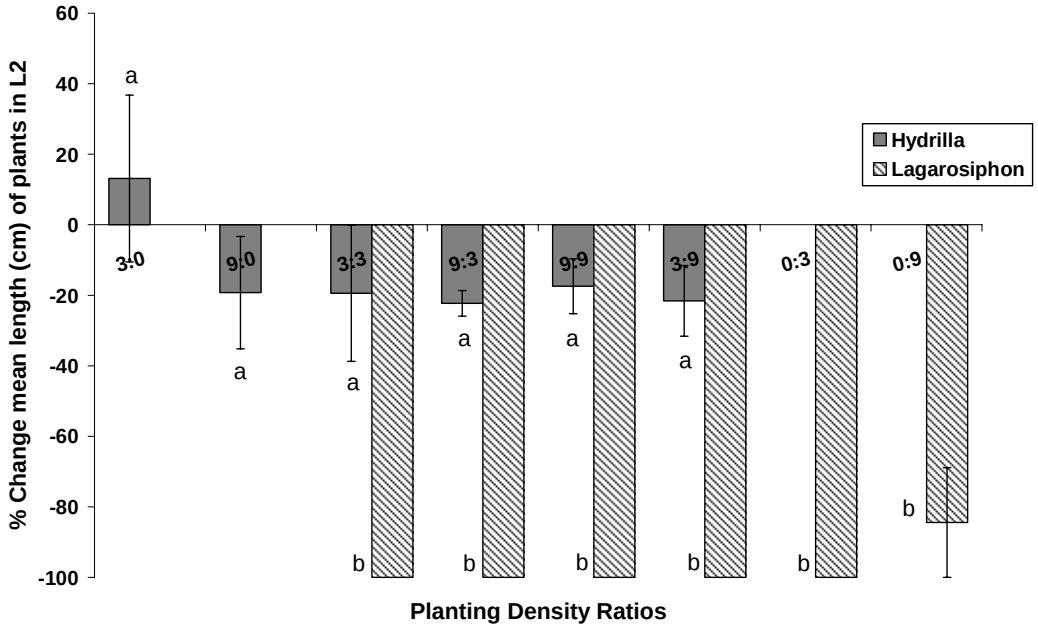


Figure 3.4: Percentage change in mean length (cm) of Hydrilla and Lagarosiphon plants in each planting density in L2. Error bars represent SE. Planting density ratios with letters in common within species are not significantly different (*p>0.05*).

3.1.3 Dry mass

Results of a Kruskal Wallis ANOVA which tested differences in dry mass of Hydrilla and Lagarosiphon are given in Table 3.5. This table indicates that the dry mass of Hydrilla plants was significantly greater in L1 than in L2, although Lagarosiphon dry mass did not differ significantly between nutrient levels (due to the small sample size). For the same reason, dry mass was not significantly different between Hydrilla and Lagarosiphon plants in either nutrient level.

Table 3.5: Results of Kruskal Wallis non-parametric ANOVA, indicating differences in dry mass (g) of Hydrilla and Lagarosiphon plants in L1 & L2 (where * indicates significance).

ANOVA Results - Dry Mass of plants (g)			
Dependent Variable	**Categorical Variable**	**H statistic**	**p value**
Dry mass in L1	Hydrilla vs. Lagarosiphon	$H_{1,107}$=2.211133	0.1370
Dry mass in L2	Hydrilla vs. Lagarosiphon	$H_{1,54}$=2.447785	0.1177
Dry mass of Hydrilla	L1 vs. L2	$H_{1,153}$=101.8154	0.0000 *
Dry mass of Lagarosiphon	L1 vs. L2	$H_{1,8}$=2.361446	0.1244

The mean dry mass of individual plants was calculated at the start of the experiment: Hydrilla = 0.0215g and Lagarosiphon = 0.0205g. Table 3.6 gives results of a Kruskal Wallis ANOVA which tested for significant changes in dry mass of Hydrilla and Lagarosiphon plants from the start to the end of the growth period. The table gives values for those density ratios in which the dry mass of Hydrilla or Lagarosiphon changed significantly. Hydrilla dry mass increased significantly in each of the L1 density ratios, but decreased significantly in the L2 density ratios of 3:3 and 9:9. Lagarosiphon dry mass decreased significantly in the L1 nutrient level, in the ratios of 3:9 and 9:9.

The mean dry mass of Hydrilla and Lagarosiphon plants in both L1 and L2 is indicated in Figures 3.5 and 3.6, and is based on the individual dry mass of surviving plants. The density ratio of 0:9 in L2 showed a negligible dry mass of surviving Lagarosiphon, marked as 0.00. ANOVA codes in these figures indicate that there were no significant differences observed between density ratios and within species.

Table 3.6: Results of the Kruskal Wallis non-parametric ANOVA for change in dry mass (g) of Hydrilla and Lagarosiphon plants, indicating those density ratios where a significant change in plant dry mass occurred.

Kruskal Wallis ANOVA Results – Change in dry mass (g)				
Species	**Nutrient Level**	**Density Ratio**	**H statistic**	***p* value**
Hydrilla	L1	3:0	$H_{1,18}$=14.63164	0.0001 *
Hydrilla	L1	9:0	$H_{1,46}$=38.59545	0.0000 *
Hydrilla	L1	3:3	$H_{1,18}$=14.59717	0.0001 *
Hydrilla	L1	9:3	$H_{1,50}$=42.01191	0.0000 *
Hydrilla	L1	9:9	$H_{1,50}$=42.00960	0.0000 *
Hydrilla	L1	3:9	$H_{1,18}$=14.59717	0.0001 *
Hydrilla	L2	3:3	$H_{1,8}$=6.054054	0.0139 *
Hydrilla	L2	9:9	$H_{1,24}$=8.840841	0.0029 *
Lagarosiphon	L1	3:9	$H_{1,6}$=4.354839	0.0369 *
Lagarosiphon	L1	9:9	$H_{1,6}$=4.354839	0.0369 *

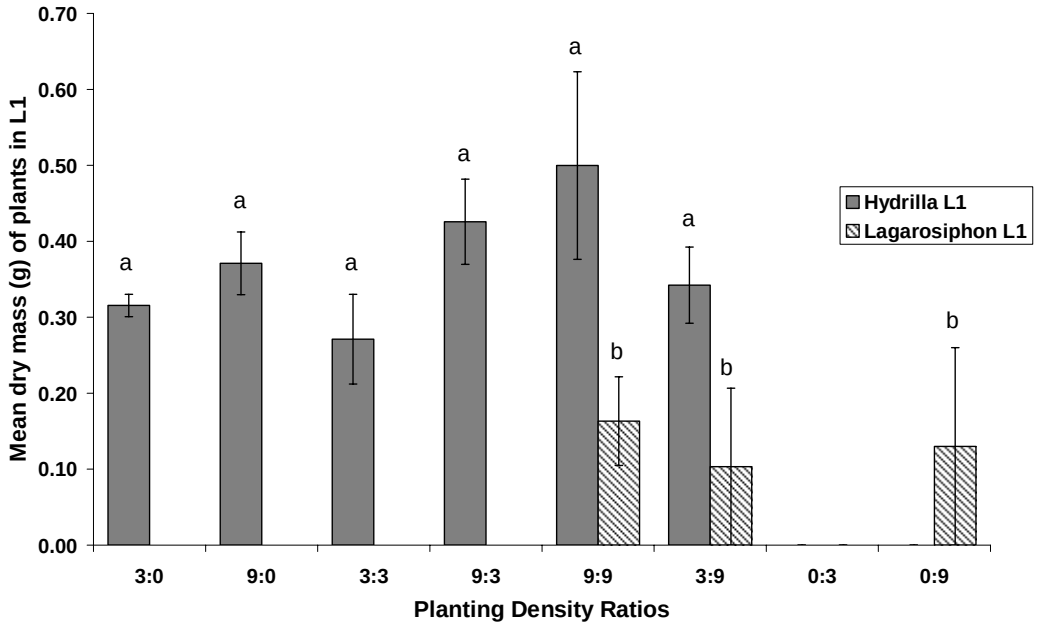


Figure 3.5: Mean dry mass of Hydrilla and Lagarosiphon plants at different planting densities in L1. Error bars represent SE. Planting density ratios with letters in common are not significantly different (*p>0.05*) within species.

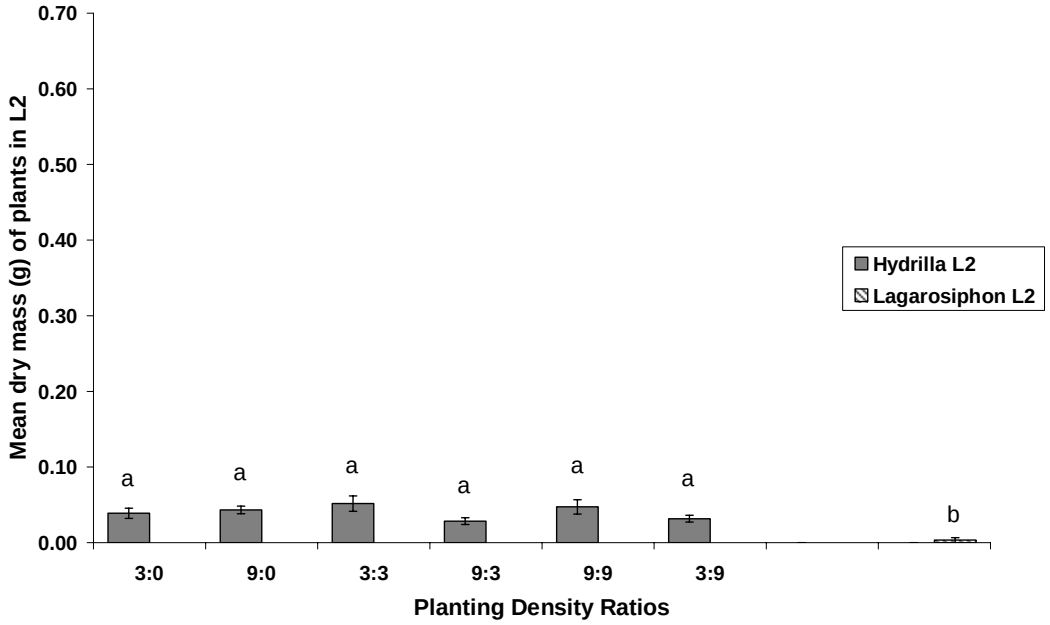


Figure 3.6: Mean dry mass of Hydrilla and Lagarosiphon plants at different planting densities in L2. Error bars represent SE. Planting density ratios with letters in common are not significantly different (*p>0.05*) within species

The correlation between mean dry mass and length of plants is represented in Figure 3.7. In the case of Hydrilla in L1, dry mass increased as length increased, while the mean dry mass of Hydrilla in L2 remained relatively low (all between 0 and 0.1g). Points in Figure 3.7 represent surviving plants. Therefore, in the group of Lagarosiphon L1, three points represent the three density ratios which contained Lagarosiphon survivors. Lagarosiphon in L2 is represented by a single point. The Hydrilla correlations do however have low R^2 values, indicating a low level of significance.

Figure 3.8 illustrates the mean shoot/root ratio of Hydrilla and Lagarosiphon plants in both nutrient treatments, with Hydrilla in L1 having the highest shoot/root ratio in the planting density of 3:0, and the lowest in 3:3. Hydrilla growing in L2 had the highest shoot/root ratio in 9:9, and the lowest in both 3:3 and 3:9. However, these differences between density ratios were not significant, as represented by the ANOVA codes in Figure 3.8. Lagarosiphon in L1 is represented twice here, in 3:9 and 9:9. The shoot/root ratios of Lagarosiphon plants in L2 were zero due to the negligible dry root mass in most cases.

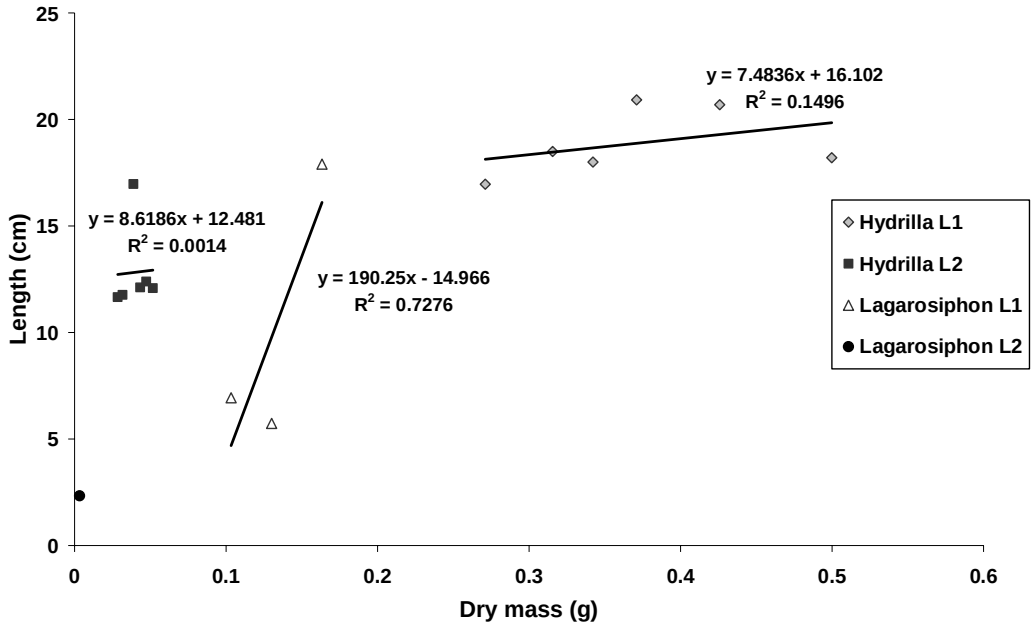


Figure 3.7: Correlation between mean dry mass (g) and mean length (cm) of Hydrilla and Lagarosiphon plants in L1 & L2. Linear trend lines with R squared values and equations are displayed for each group of data.

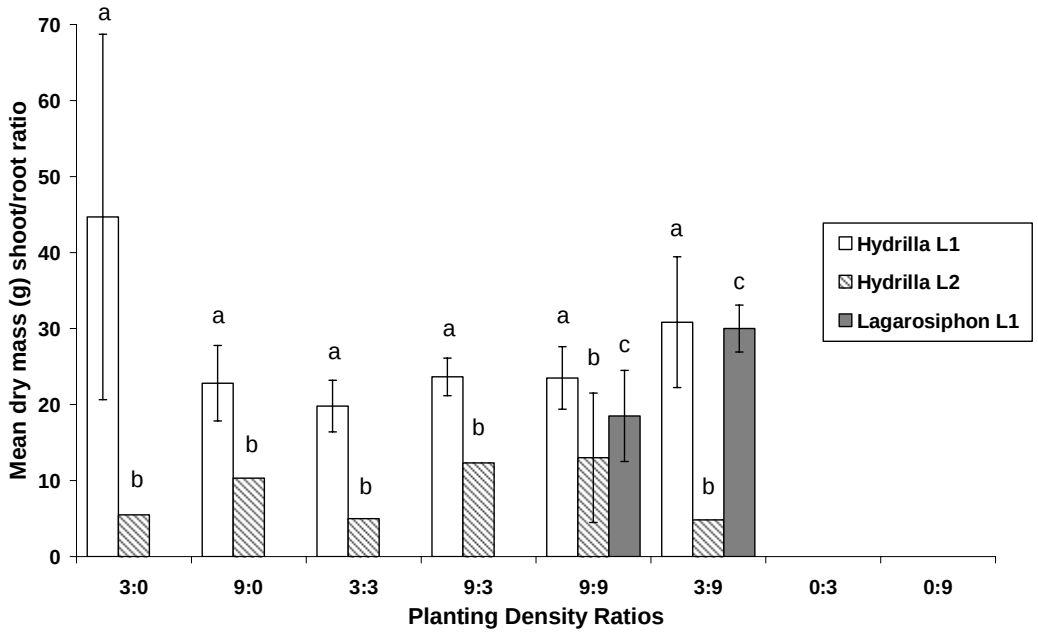


Figure 3.8: Mean dry mass shoot/root ratio (g) of Hydrilla and Lagarosiphon plants in L1 & L2. Errors bars represent SE. Shoot/root ratios with letters in common within species are not significantly different (*p>0.05*).

3.1.4 Multiple Regression Analysis

3.1.4.1 Low Nutrient Level

The following equations were obtained from multiple regression analysis of the mean plant dry mass in L1, using the reciprocal yield model (where * indicates significance of F-test results):

$$1/W_h = 3.95^* - 0.83^*N_h - 0.4N_L,\ R^2=0.85,\ F_{2,3}=8.70,\ p=0.056399,\ N=6 \quad (1)$$

$$1/W_L = -3.45 + 0.97^*N_L - 0.11N_h,\ R^2=0.95,\ F_{2,3}=26.88,\ p=0.012148^*,\ N=6 \quad (2)$$

The R^2 value explains how well the model fits the data. Therefore, the R^2 values of 0.85 for equation (1) and 0.95 for equation (2) indicate that we can account for 85% and 95% of the variability in this model, for equations (1) and (2) respectively. The intercept of equation (1) is significant at p=0.000948, as is the coefficient a_{hh}, with p=0.032776. The coefficient a_{hL} is not significant at p=0.170483. The intercept of equation (2) is not significant, with p=0.081177, as is the coefficient a_{Lh}, with p=0.477605. The coefficient a_{LL} is significant at p=0.005335.

In terms of the Hydrilla equation (1), the ratio of coefficients (which compares intraspecific and interspecific competition) is a_{hh}/a_{hL} = 2.1. This means that intraspecific competition from other Hydrilla plants had a greater effect on Hydrilla than interspecific competition from Lagarosiphon. Therefore, the competitive effect that a single Hydrilla plant had on the mean dry mass of Hydrilla was equivalent to the presence of 2.1 Lagarosiphon plants in L1, i.e., the impact of Hydrilla on itself is double that of Lagarosiphon on Hydrilla. The direction of the relationship between variables is predicted by the signs of the *B* coefficients. If the coefficient is positive, the relationship of that variable with the dependent variable (dry mass) is positive. If the coefficient is negative, the relationship will be negative. Therefore, since both *B* coefficients in equation (1) are negative, relationships with the dependent variable are negative: As N_h decreases, $1/W_h$ increases; and as N_L decreases, $1/W_h$ increases. In other words, the lower the density of Hydrilla and Lagarosiphon, the lower the mean dry mass of Hydrilla.

The ratio of coefficients for equation (2) is a_{LL}/a_{Lh} = -8.94. In theory this means that intraspecific competition from other Lagarosiphon plants had a greater effect on Lagarosiphon than interspecific competition from Hydrilla. Therefore, the competitive effect that one Lagarosiphon plant had on the mean dry mass of Lagarosiphon was equivalent to the presence of 8.94 Hydrilla plants in L1. The *B* coefficient N_h is negative, meaning that the lower the density of Hydrilla, the lower the mean dry mass of Lagarosiphon. Conversely, the *B* coefficient N_L is positive, meaning that the greater the density of Lagarosiphon, the lower the mean dry mass of Lagarosiphon. These values cannot be interpreted as those in equation (1), due to the small number of surviving Lagarosiphon plants and consequently small statistical sample size (Lagarosiphon survived in only three density ratios in L1). In addition to this, Lagarosiphon's competitive ability was possibly reduced due to the presence of algae, and not necessary as a result of competition within the species or from Hydrilla.

3.1.4.2 High Nutrient Level

The following equations were obtained from multiple regression analysis of the mean plant dry mass in L2, using the reciprocal yield model (where * indicates significance):

$$1/W_h = 24.43 + 0.08N_h + 0.11N_L,\ R^2=0.02,\ F_{2,3}=0.03,\ p=0.972347,\ N=6 \quad (3)$$

$$1/W_L = 7.14 + 0.45N_L - 0.48N_h,\ R^2=0.43,\ F_{2,3}=1.13,\ p=0.431959,\ N=6 \quad (4)$$

The R^2 values of 0.02 for equation (3) and 0.43 for equation (4) indicate that we can only account for 2% and 43% of the variability in this model, for equations (3) and (4) respectively. This can be explained by the small sample size of mean dry mass values for Lagarosiphon. The intercept of equation (3) is not significant at p=0.055283, as are the coefficients a_{hh} (p=0.897375) and a_{hL} (p=0.859840). The intercept of equation (4) is not significant, with p=0.956617, as are the coefficients a_{LL} (p=0.380916) and a_{Lh} (p=0.353387).

3.1.5 Discussion

3.1.5.1 Competition under low nutrient conditions

The greatest survival of Hydrilla in interspecific competition occurred when Hydrilla was in low densities (3:3 and 3:9), linking with the 'self-thinning' behaviour in monocultures of Hydrilla. Even in high densities of Lagarosiphon, Hydrilla performed well. The effects of intra- and interspecific competition on Hydrilla resulted in survival decreasing with increasing Hydrilla density, while length and dry mass increased with increasing Hydrilla density. Hydrilla dry mass also decreased with decreasing Lagarosiphon density, although the survival of Hydrilla was not affected by Lagarosiphon density. This corresponds with the results of the multiple regression analysis, in that Hydrilla was not as affected by competition from Lagarosiphon as it was self-limiting, as in the case of competition with *V. americana* (Steward, 1988).

In terms of Lagarosiphon, the results showed that intraspecific competition had a greater effect on Lagarosiphon dry mass than interspecific competition with Hydrilla. In L1, Lagarosiphon plants survived only when present in high densities. All density ratios had no significant change in mean length of Lagarosiphon, due to the small sample size of surviving plants. Under interspecific competition, the length and dry mass of Lagarosiphon plants was greater in cases where Hydrilla was present in a high density (i.e. 9:9), despite a low survival rate (11.11%). This may be the result of a combination of inter- and intraspecific competition, or of a nurse plant effect (Holmgren & Scheffer, 1997; Ren *et al.,* 2008), whereby the establishment of young plants is ensured by the numbers of plants 'protecting' it from damage (such as the negative effects of algae hampering plant growth). Lagarosiphon dry mass was not as affected by Hydrilla density as it was by its own density, since there was no gradient of increase or decrease in Lagarosiphon dry mass with increasing Hydrilla density (Fig 3.5). However, as mentioned above, these results are difficult to interpret, as Lagarosiphon's competitive ability may have been reduced due to the presence of algae, and not necessary as a result of competition Hydrilla or other Lagarosiphon plants.

3.1.5.2 Competition under high nutrient conditions

Results of the multiple regression analysis were not significant, and there was no clear pattern of Hydrilla survival between density levels in the high nutrient treatment. There was a significant difference in the survival of Hydrilla and Lagarosiphon plants, with greater numbers of surviving Hydrilla plants in each density ratio. The mean lengths of Hydrilla showed that plants in fact became shorter (due to fragments breaking from the main stem) in all density levels except 3:0, with the density ratios 9:9 and 9:3 having a significant decrease in plant length. Most Lagarosiphon plants died out completely, and survived only when growing in monocultures of a high density, which indicated a nurse-plant effect (Holmgren & Scheffer, 1997; Ren *et al.,* 2008),

A possible reason for the poor growth performance of both species is the difficult conditions in L2, specifically due to the presence of algae, which increased in abundance over the 10 weeks of the experiment. The high nutrient content in L2 provided an opportunity for algae to establish in great quantities. Algae directly affect plant growth in terms of: Limiting available light; removing nutrients from the soil and water; changing water quality (high pH and low CO_2) and therefore affecting macrophyte photosynthesis; and hampering growth by entangling plant segments in fibrous material (Allen & Spence, 1981; Maberly, 1983; Phillips *et al.*, 1978; Simpson & Eaton, 1986)[3]. Findings from a study by Ozimek *et al.* (1991) show that algae decreased the growth of macrophyte shoots and accelerated the decay of old shoots. Assuming that the presence of algae was not the primary cause of death of Lagarosiphon plants, it could be concluded that Hydrilla's competitive ability is enhanced under high nutrient conditions.

The shoot/root ratio is lower in L2 than in L1 for both species, meaning that the plants allocated more biomass to roots in L2 than in L1. This is in contrast to results of Van *et al* (1999), where both species have a relatively lower amount of biomass allocated to roots in L2 than in L1. Root length is generally decreased with increasing nutrient availability, as found in a number of studies (Bradshaw, 1965; Chapin, 1980; Wilson, 1988). Results of the present study were not,

[3] Note: References on the effects of algae on submerged plants were suggested by the examiner during a review of the Research Report, including Ozimek *et al.*, 1991.

however, due to an increase in root production in L2, where the sediment had a high nutrient content, but due to the lower shoot dry mass in L2.

3.1.6 Conclusions

The mean plant lengths, dry masses and the low percentage survival of Lagarosiphon reveal a lack of establishment of this plant. There are several interacting reasons for this, one of which is the growth strategy of Lagarosiphon. From observation during the experiment, Lagarosiphon segments reacted very differently to Hydrilla after planting: Leaves fell from the original stem and a new and fragile branch stem was produced. The original stem did not continue to produce roots – rather, it seems that establishment then depended on the new, thin and delicate branch stem, which produced new thin roots. If establishment did not occur from this branch stem, the plant died very rapidly. Another possible reason for this lack of establishment is the presence of algae, especially in the high nutrient pool. As algae formed over the surface of the sediment, new roots from branch stems may have been unable to penetrate the algae and anchor themselves in the sediment. As stated above, the presence of algae can alter water quality by increasing pH and reducing available CO_2 (Allen & Spence, 1981; Ozimek *et al.,* 1991; Maberly, 1983; Phillips *et al.*, 1978; Simpson & Eaton, 1986). Water chemistry, and particularly CO_2 availability, plays an essential role in the growth of submerged plants. Lagarosiphon requires high concentrations of dissolved CO_2 for optimal growth, which would explain why Lagarosiphon plants were brittle and died rapidly under conditions of algal infestation[4].

The first key research question asked how the planting density would affect the establishment and growth of indigenous plants (Lagarosiphon) in competition with alien invaders (Hydrilla). The second asked how different nutrient conditions would affect the establishment and growth of Lagarosiphon in competition with Hydrilla. In answering the latter, the different nutrient conditions had a distinct influence on the establishment and growth of both Hydrilla and Lagarosiphon, although Lagarosiphon performed poorly in both nutrient levels. The percentage survival, mean lengths and mean dry mass of Hydrilla were all significantly greater in L1, and therefore it can be said that the establishment of Hydrilla was

[4] Note: As suggested by the examiner during a review of the Research Report.

limited by competition from algae in the high nutrient environment. Further, the nutrient treatments in this experiment dictated the direction of competition between and within species. In L1, the effect that Hydrilla had in competition with itself was twice the effect that Lagarosiphon had on Hydrilla. Sutton (1985) also reports high levels of Hydrilla intraspecific competition at these planting densities.

According to results of this research, the establishment and growth of Hydrilla growing in a low nutrient environment would be favoured if it was present in large populations (high density). Hydrilla significantly outperformed Lagarosiphon in both nutrient levels and all density ratios. The competitive performance of Lagarosiphon during this experiment indicates that it would be very susceptible to invasion by Hydrilla, especially in eutrophic and hypertrophic waters. It is important not to understate the role that algae may have played in these results, and the fact that Lagarosiphon was not well established. It must be noted, however, that Hydrilla was subjected to the same conditions of algae, but managed to overcome its effects and outcompete Lagarosiphon nevertheless.

It is difficult to extrapolate the results of this experiment to how these plants would behave in South African waters, due to the complications of algae infestation and poor performance of Lagarosiphon. It is also difficult to draw real conclusions as to how susceptible Lagarosiphon is to competition from Hydrilla. The second experiment was run using two floating aquatic plants, Azolla and Spirodela. The intention of this experiment was two-fold: First, to validate the method used in the first experiment and; second, to gain a better understanding of the interactions between indigenous and alien aquatic plants, and how susceptible our indigenous species are to invasions.

3.2 Competitive interactions between Azolla and Spirodela

After seven weeks, both Azolla and Spirodela plants had multiplied many times over, although the tubs did not reach full density. The total plant mass and individual mass of Azolla increased with increasing nutrients, with the total mass in L3 significantly greater than that in L1 (Table 3.7). The individual mass of Azolla plants was significantly greater in L2 than in L1 (Table 3.9). In terms of Spirodela, the total mass was significantly lower in L2 than in L1 and L3 (Table 3.7). Spirodela individual mass tended to decrease as nutrient levels increased, with L1 being significantly greater than L2 and L3 (Table 3.9). The plant numbers of Azolla and Spirodela were significantly greater in L3 than in L1 and L2 (Table 3.12). Results of the regression analysis suggest that Azolla was affected by intraspecific competition, while interspecific competition played a more important role for Spirodela. Photographs of the tubs of Azolla and Spirodela are presented in Appendix C.

3.2.1 Total Wet mass

Results of a Kruskal Wallis ANOVA which tested differences in total plant mass of Azolla and Spirodela in L1, L2 and L3 are presented in Table 3.7. The total wet mass of Azolla was significantly greater in L3 than in L1, while Spirodela total plant mass was significantly lower in L2 than in L1 and L3. The percentage change in total mass was dramatically higher in L3, with a minimum of 968% in 3:9. The total mass of Spirodela increased dramatically in L1, with a minimum percentage change of 590% (9:9), as per Figure 3.10. These results were obtained by pooling the data for each species in each nutrient level (i.e. for all density ratios).

Table 3.7: Results of Kruskal Wallis non-parametric post hoc test, indicating differences in total wet mass (g) of Azolla and Spirodela plants in L1, L2 & L3 (where * indicates significance).

ANOVA Results – Total Wet Mass of plants (g)			
Dependent Variable	**Categorical Variable**	**H statistic**	***p* value**
Total wet mass of Azolla	L1 vs. L2	$H_{2,72}$=8.695396	1.0000
	L1 vs. L3	$H_{2,72}$=8.695396	0.0135 *
	L2 vs. L3	$H_{2,72}$=8.695396	0.1063
Total wet mass of Spirodela	L1 vs. L2	$H_{2,72}$=36.31297	0.0000 *
	L1 vs. L3	$H_{2,72}$=36.31297	1.0000
	L2 vs. L3	$H_{2,72}$=36.31297	0.0000 *

Figures 3.9 and 3.10 illustrate the differences in total plant mass between density ratios and within nutrient levels, with ANOVA codes indicating significant differences between density ratios. Figure 3.9 shows the general trend of an increase in total plant mass with an increase in initial planting density. There were significant differences in Azolla total mass between 3:0 and 9:9 in both L1 (p=0.04050, $H_{1,24}$=14.1400) and L2 (p=0.03433, $H_{1,24}$=17.1700). The total mass of Azolla plants in each tub was greatest in L3 for all density ratios except 3:9, indicating that Azolla responds positively to an increase in nutrients. No significant differences were observed between density ratios for the total plant mass of Spirodela. Tests run between nutrient levels, however, show that the plant did respond significantly to a change in nutrient levels. In each of the planting densities, plant mass decreased from L1 to L2.

Post-hoc Kruskal Wallis tests gave results of significant differences between nutrient levels for each density ratio, as presented in Table 3.8. The table gives values for those density ratios in which the total wet mass of Azolla or Spirodela changed significantly. The planting densities 3:0, 9:0 and 9:9 show significant differences in Azolla total wet mass between low and high nutrient treatments. The planting density ratios of 3:3 and 0:3 showed significant differences in Spirodela total mass between L1 and L2. In L3 the Spirodela plant mass increased with increasing nutrients, with the 9:9 and 0:9 planting densities showing significant differences.

Table 3.8: Results of a Kruskal Wallis non-parametric post hoc test, indicating differences in the total wet mass of Azolla and Spirodela plants between nutrient levels and within density ratios (where * indicates significance).

ANOVA Results –Total Wet Mass of plants (g)			
Dependent Variable	**Categorical Variable**	**H statistic**	***p* value**
Azolla 3:0	L1 vs. L3	$H_{1,12}$=9.2692	0.00710 *
Azolla 9:0	L1 vs. L3	$H_{1,12}$=7.4231	0.04268 *
Azolla 9:9	L1 vs. L3	$H_{1,12}$=6.730769	0.04269 *
Spirodela 3:3	L1 vs. L2	$H_{1,12}$=8.0	0.01812 *
Spirodela 0:3	L1 vs. L2	$H_{1,12}$=7.4491	0.04268 *
Spirodela 9:9	L2 vs. L3	$H_{1,12}$=8.0281	0.01812 *
Spirodela 0:9	L2 vs. L3	$H_{1,12}$=7.7308	0.02432 *

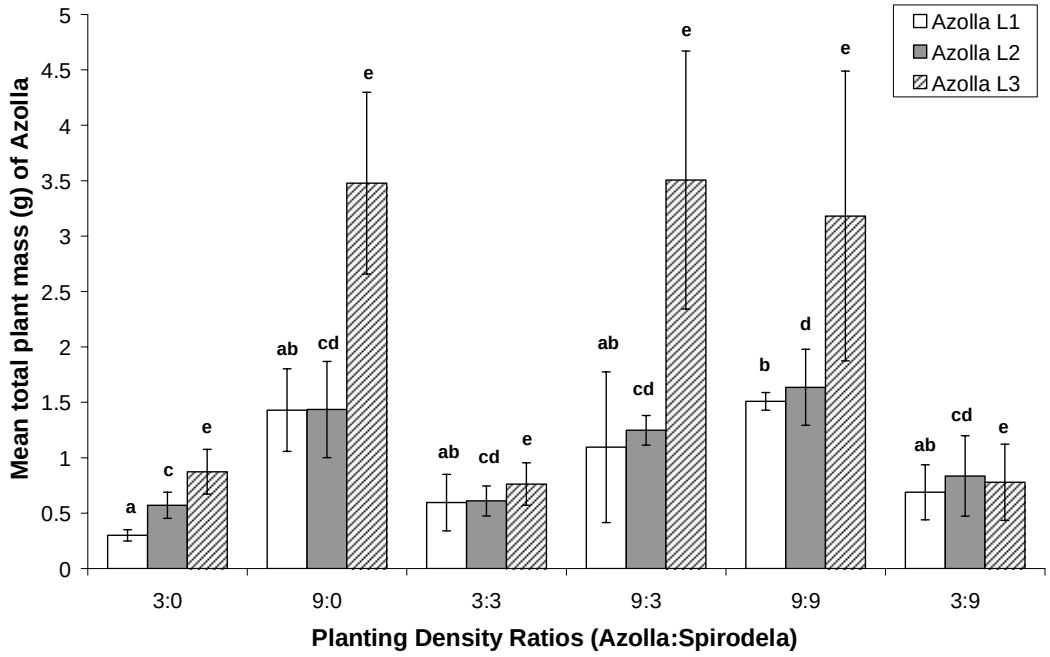


Figure 3.9: Mean total wet mass of Azolla plants at different planting densities in three nutrient levels. Error bars represent SE. Planting density ratios with letters in common within nutrient levels are not significantly different (*p>0.05*).

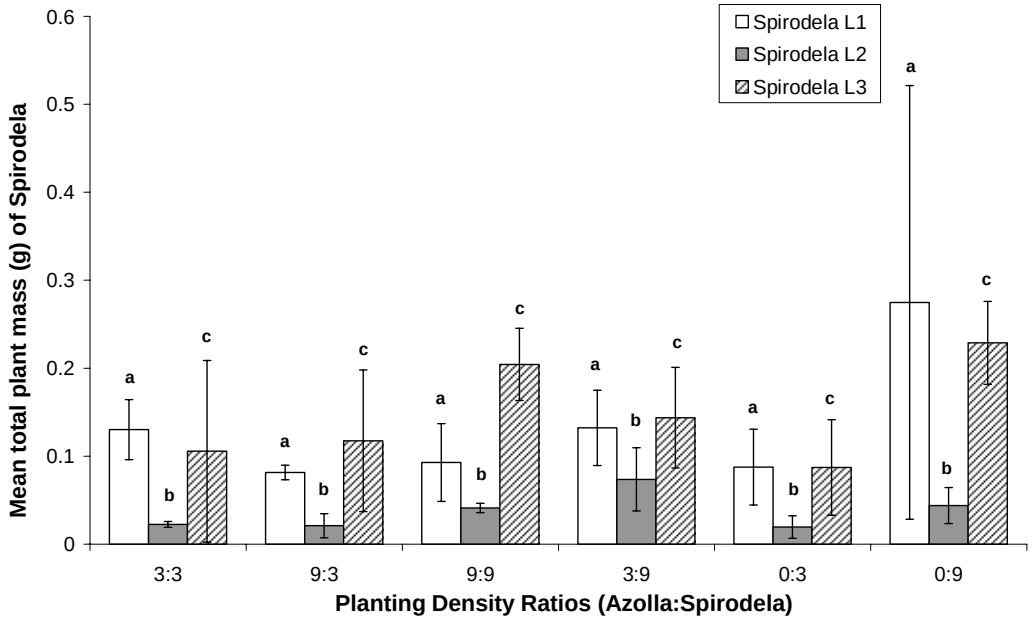


Figure 3.10: Mean total wet mass of Spirodela plants at different planting densities in three nutrient levels. Error bars represent SE. Planting density ratios with letters in common within nutrient levels are not significantly different (*p>0.05*).

3.2.2 Individual Wet mass

The differences in individual plant mass of Azolla and Spirodela were tested for significance using the Kruskal Wallis ANOVA (Table 3.9). These results show that the individual mass of Azolla increased significantly from L1 to L2. The individual mass of Spirodela plants was, however, significantly lower in L2 and L3 than in L1. The lowest individual mass of Spirodela plants was found in L2. These results were obtained by pooling the data for each species in each nutrient level (i.e. for all density ratios).

Table 3.9: Results of Kruskal Wallis non-parametric post hoc test, indicating differences in the individual wet mass of Azolla and Spirodela plants in L1, L2 & L3 (where * indicates significance).

ANOVA Results – Individual Wet Mass of plants (g)			
Dependent Variable	**Categorical Variable**	**H statistic**	***p* value**
Individual wet mass of Azolla	L1 vs. L2	$H_{2,72}$=7.298516	0.0369 *
	L1 vs. L3	$H_{2,72}$=7.298516	0.0993
	L2 vs. L3	$H_{2,72}$=7.298516	1.0000
Individual wet mass of Spirodela	L1 vs. L2	$H_{2,72}$=35.57325	0.0000 *
	L1 vs. L3	$H_{2,72}$=35.57325	0.0003 *
	L2 vs. L3	$H_{2,72}$=35.57325	0.1433

Figures 3.11 and 3.12 present the mean individual wet mass of Azolla and Spirodela plants in each planting density. No significant differences between planting densities were observed using the Kruskal-Wallis post-hoc test for either Azolla or Spirodela individual plant mass. In other words, there was no significant change in individual mass as planting density changed. Figure 3.11 indicates that the individual mass of Azolla plants tends to increase with increasing Azolla density, and tends to increase with higher nutrient levels. Results of post-hoc tests between nutrient levels, as given in Table 3.10, indicate that the only significant difference in individual mass of Azolla occurred in the 3:0 density ratio between L1 and L2. The individual mass of Spirodela plants was significantly different between L1 and L2 for the following density ratios: 3:3; 9:9 and; 0:9. Table 3.11 gives values for those density ratios in which the individual wet mass of Azolla or Spirodela changed significantly.

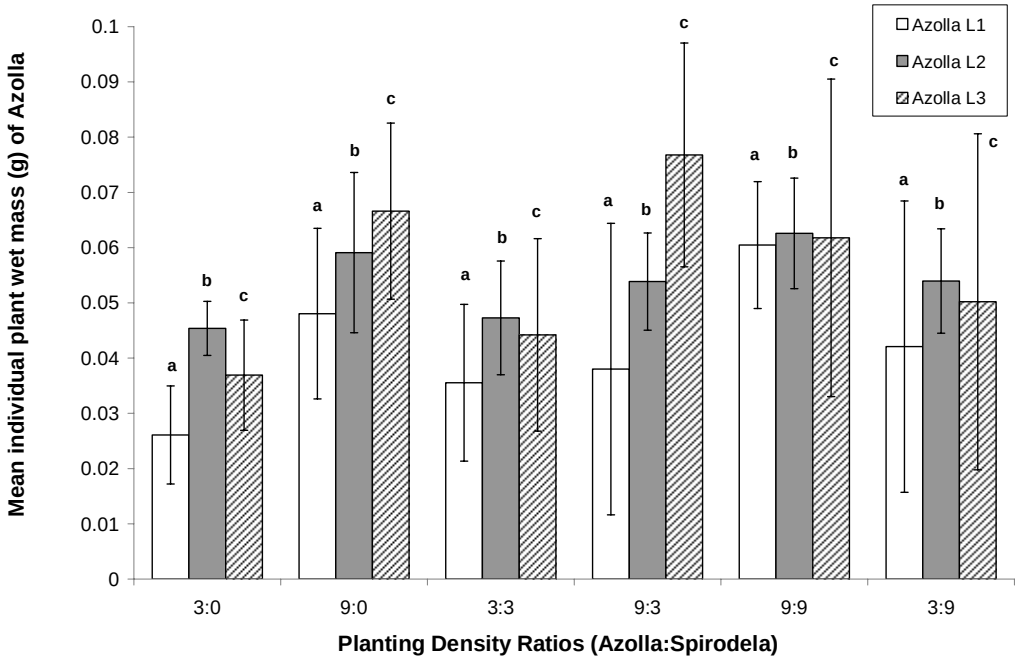


Figure 3.11: Mean individual wet mass of Azolla plants at different planting densities in three nutrient levels. Error bars represent SE. Planting density ratios with letters in common are not significantly different (*p>0.05*) within nutrient levels.

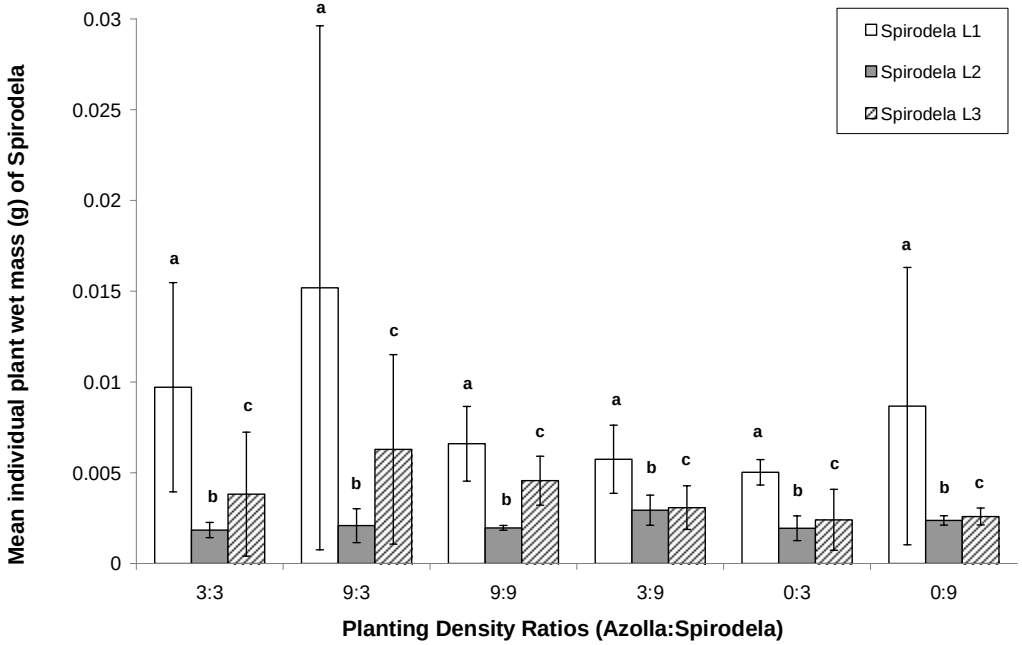


Figure 3.12: Mean individual wet mass of Spirodela plants at different planting densities in three nutrient levels. Error bars represent SE. Planting density ratios with letters in common are not significantly different (*p*>0.05) within nutrient levels.

Table 3.10: Results of a Kruskal Wallis non-parametric post hoc test, indicating differences in the individual wet mass of Azolla and Spirodela plants between nutrient levels and within density ratios (where * indicates significance).

ANOVA Results –Individual Wet Mass of plants (g)			
Dependent Variable	**Categorical Variable**	**H statistic**	***p* value**
Azolla 3:0	L1 vs. L2	$H_{1,12}$=6.5	0.03236 *
Spirodela 3:3	L1 vs. L2	$H_{1,12}$=6.6154	0.03236 *
Spirodela 9:9	L1 vs. L2	$H_{1,12}$=8.3462	0.01338 *
Spirodela 0:9	L1 vs. L2	$H_{1,12}$=7.5385	0.03236 *

Table 3.11 details results of the Kruskal Wallis ANOVA for density ratios in which a significant change in individual mass was observed (from planting to harvesting). The average starting mass of individual plants, determined at the beginning of the experiment was 0.046g and 0.002g for Azolla and Spirodela respectively. The individual mass of Azolla plants in L1 decreased significantly during the growth period in the 3:0 density ratio, and increased significantly in the 9:9 ratio. In L2, plants in the 9:9 ratio increased significantly in individual mass. Azolla growing in L3 increased its mass significantly in 9:0 and 9:3, while it decreased significantly in 3:0. Spirodela showed significant increases in individual mass in each of its L1 density ratios. Spirodela individual mass increased significantly in 3:9 and 0:9 of L2, and in 9:9, 3:9 and 0:9 of L3. Spirodela plants in the ratio of 9:9 (L2) decreased significantly in individual mass.

Table 3.11: Results of the Kruskal Wallis non-parametric ANOVA for change in individual wet mass (g) of Azolla and Spirodela plants, indicating those density ratios where a significant change in individual wet mass occurred.

Kruskal Wallis ANOVA Results – Change in individual wet mass (g)				
Species	**Nutrient Level**	**Density Ratio**	**H statistic**	**p value**
Azolla	L1	3:0	$H_{1,8}$=6.054054	0.0139 *
Azolla	L1	9:9	$H_{1,8}$=6.054054	0.0139 *
Azolla	L2	9:9	$H_{1,8}$=6.054054	0.0139 *
Azolla	L3	3:0	$H_{1,8}$=6.054054	0.0139 *
Azolla	L3	9:0	$H_{1,8}$=6.054054	0.0139 *
Azolla	L3	9:3	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L1	3:3	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L1	9:3	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L1	9:9	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L1	3:9	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L1	0:3	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L1	0:9	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L2	9:9	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L2	3:9	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L2	0:9	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L3	9:9	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L3	3:9	$H_{1,8}$=6.054054	0.0139 *
Spirodela	L3	0:9	$H_{1,8}$=6.054054	0.0139 *

3.2.3 Plant numbers

The differences in plant numbers of Azolla and Spirodela between nutrient levels were tested for significance using the Kruskal Wallis ANOVA (Table 3.12). Both Azolla and Spirodela plant numbers were significantly greater in L3 than in L1 and L2. The lowest plant numbers of Azolla were present in L2. These results were obtained by pooling the data for each species in each nutrient level (i.e. for all density ratios).

The percentage change in plant numbers for each of the nutrient levels is given in Figures 3.13, 3.14 and 3.15. In all nutrient levels, both Azolla and Spirodela tended to multiply to a greater extent when they were present in low densities. In the low nutrient treatment (Fig 3.13), the change in Azolla plant numbers differed significantly between 9:9 and 3:9 (p=0.04395, $H_{1,24}$=14.4414). From Figure 3.13 it can be seen that the reproduction rate of Azolla plants was highest where Azolla was present in low densities. No significant differences in the change in Spirodela

plant numbers were observed between density ratios in either L1 or L2. In L3 (Fig 3.15), the following Spirodela density ratios showed significant differences in plant numbers: 9:9 and 0:3 (p=0.01107, $H_{1,24}$=17.5878) and; 3:9 and 0:3 (p=0.01890, $H_{1,24}$=17.5878). The change in Azolla plant numbers did not show significant differences between density ratios in the medium and high nutrient treatments.

Table 3.12: Results of Kruskal Wallis non-parametric post hoc test, indicating differences in the number of Azolla and Spirodela plants in L1, L2 & L3 (where * indicates significance).

ANOVA Results – Plant numbers			
Dependent Variable	**Categorical Variable**	**H statistic**	***p* value**
Plant numbers of Azolla	L1 and L2	$H_{2,72}$=12.65823	0.9222
	L1 and L3	$H_{2,72}$=12.65823	0.0443 *
	L2 and L3	$H_{2,72}$=12.65823	0.0016 *
Plant numbers of Spirodela	L1 and L2	$H_{2,72}$=30.37264	0.9670
	L1 and L3	$H_{2,72}$=30.37264	0.0001 *
	L2 and L3	$H_{2,72}$=30.37264	0.0000 *

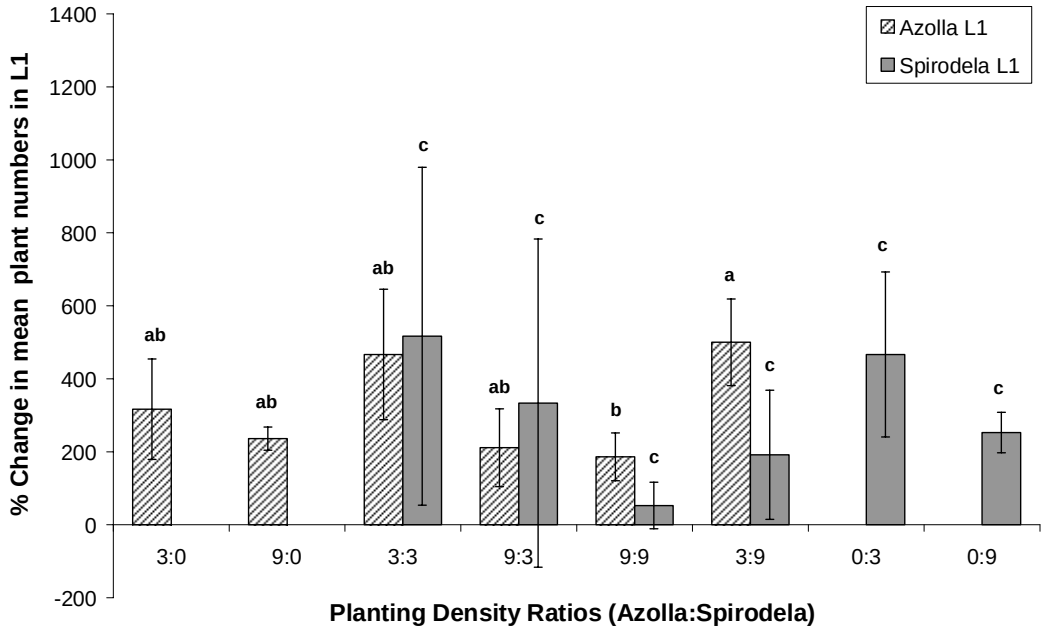


Figure 3.13: Percentage change in mean plant numbers of Azolla and Spirodela plants at different planting densities in the low nutrient level. Error bars represent SE. Planting density ratios with letters in common are not significantly different ($p>0.05$) within species.

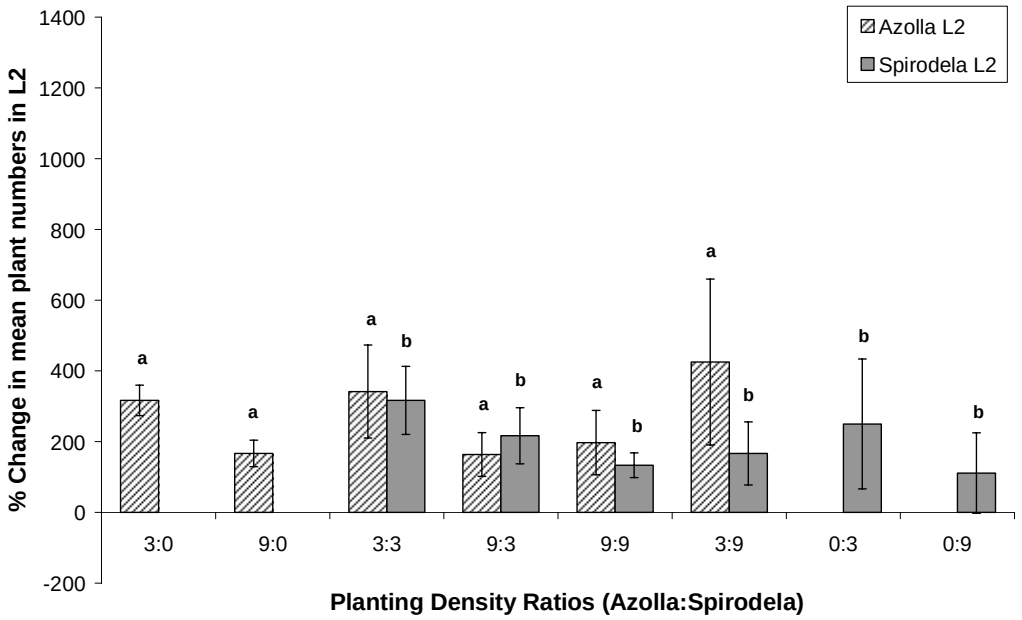


Figure 3.14: Percentage change in mean plant numbers of Azolla and Spirodela plants at different planting densities in the medium nutrient level. Error bars represent SE. Planting density ratios with letters in common are not significantly different (*p>0.05*) within species.

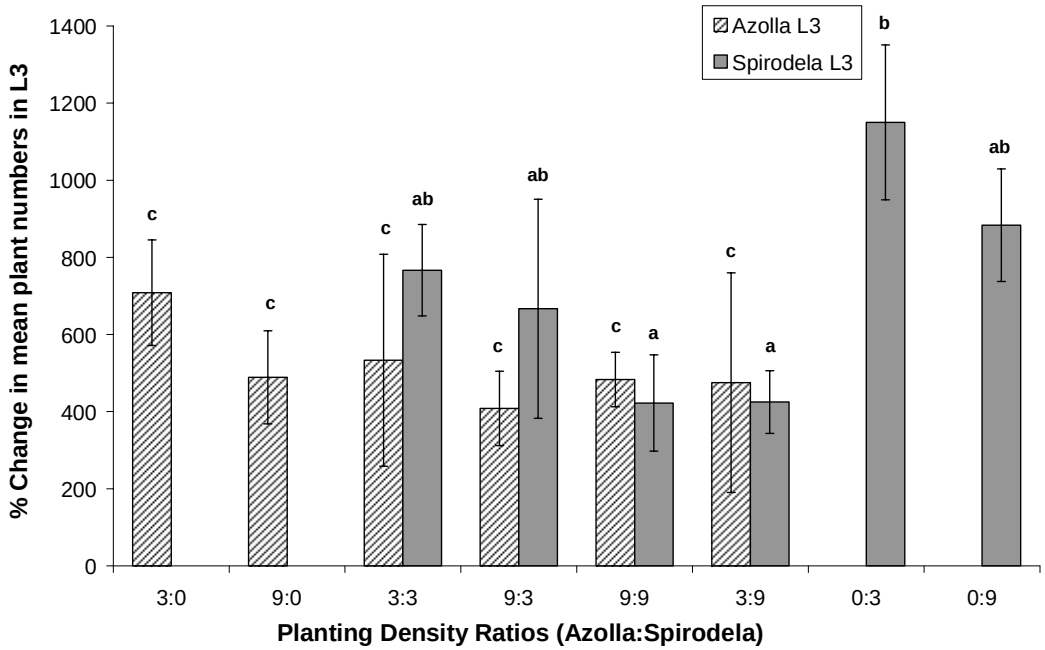


Figure 3.15: Percentage change in mean plant numbers of Azolla and Spirodela plants at different planting densities in the high nutrient level. Error bars represent SE. Planting density ratios with letters in common are not significantly different (*p>0.05*) within species.

Post-hoc tests were performed to investigate differences between nutrient levels in terms of the number of Azolla and Spirodela plants in each density ratio, as presented in Table 3.13. The table gives values for those density ratios in which Azolla or Spirodela plant numbers changed significantly. Azolla plant numbers were significantly greater in L2 than in L1 for the density ratios of 9:0 and 9:3. A significant difference in Azolla plant numbers was observed between L1 and L3 in 9:9. In the case of Spirodela, significant differences in plant numbers were observed between L1 and L3 in the 9:9 ratio, while differences between L2 and L3 were found in 0:3 and 0:9.

Table 3.13: Results of a Kruskal Wallis non-parametric post hoc test, indicating differences in Azolla and Spirodela plant numbers between nutrient levels and within density ratios (where * indicates significance).

ANOVA Results – Plant Numbers			
Dependent Variable	**Categorical Variable**	**H statistic**	***p* value**
Azolla 9:0	L1 vs. L2	$H_{1,12}$=9.401	0.00710 *
Azolla 9:3	L1 vs. L2	$H_{1,12}$=7.7377	0.02432 *
Azolla 9:9	L1 vs. L3	$H_{1,12}$=7.5504	0.03721 *
Spirodela 9:9	L1 vs. L3	$H_{1,12}$=9.0412	0.00835 *
Spirodela 0:3	L2 vs. L3	$H_{1,12}$=8	0.01812 *
Spirodela 0:9	L2 vs. L3	$H_{1,12}$=8.3462	0.01338 *

3.2.4 Correlation between mean total mass and plant numbers

The correlations between mean total wet mass and plant numbers are represented in Figures 3.16 and 3.17. These graphs give an indication of the reproductive strategy of each species under competition and in different nutrient levels. In terms of the Azolla correlations (Fig 3.16), linear trend lines follow a similar pattern, with total plant mass steadily rising as the plant number increased. Azolla in L3 attained both high biomass and plant numbers, indicating the positive response of Azolla to an increase in nutrients. The correlation graph of Spirodela (Fig 3.17) shows that plants in L1 attained a fairly high biomass without a great increase in plant numbers. Spirodela in L2 had both a low total plant mass and low plant numbers. In the high nutrient level, Spirodela had a high rate of reproduction and a somewhat slower rate of biomass accumulation (as represented by the linear trend line of L3 in Figure 3.17).

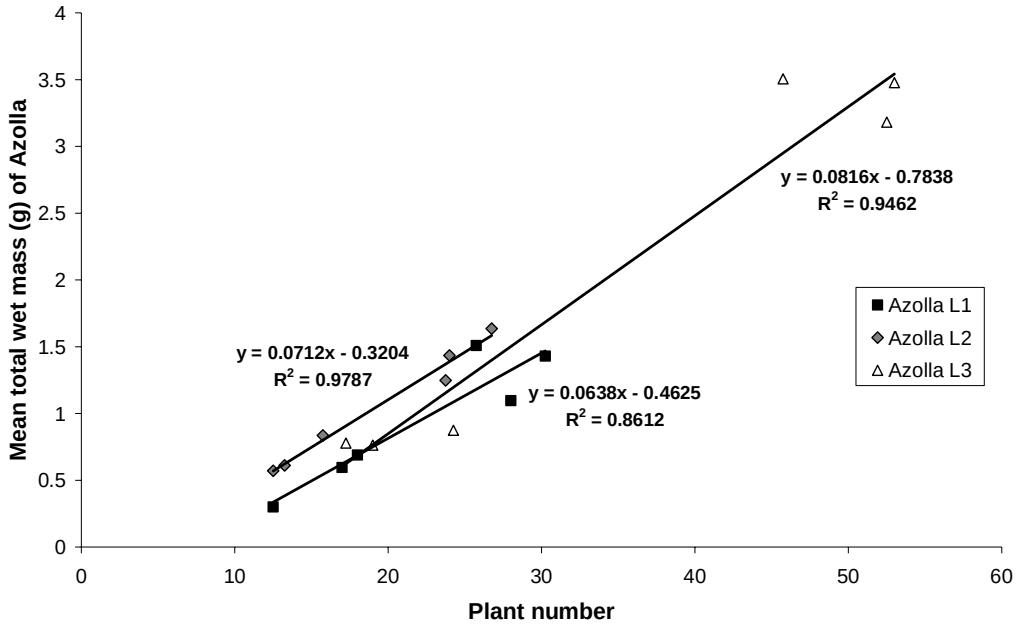


Figure 3.16: Correlation between mean total wet mass (g) per tub of Azolla and plant numbers in low, medium and high nutrient levels. Linear trend lines with R squared values and equations are displayed for each group of data.

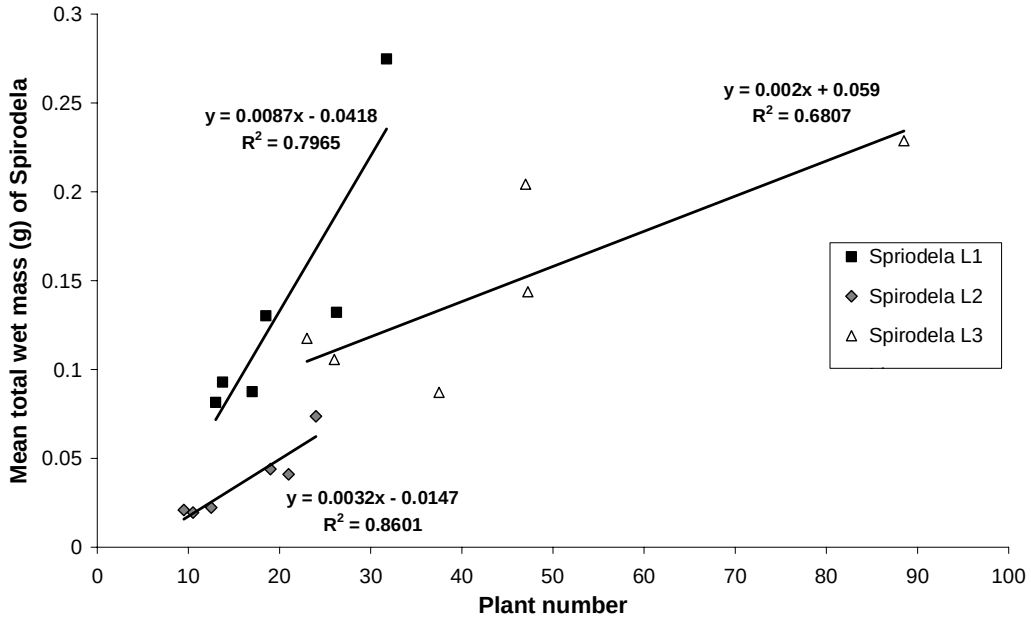


Figure 3.17: Correlation between mean total wet mass (g) per tub of Spirodela and plant numbers in low, medium and high nutrient levels. Linear trend lines with R squared values and equations are displayed for each group of data.

3.2.5 Multiple Regression Analysis

3.2.5.1 Low Nutrient Level

The following equations were obtained from multiple regression analysis of the mean individual plant wet mass in L1, using the reciprocal yield model (where * indicates significance):

$$1/W_A = 38.78^* - 0.65N_A - 0.59N_S,\ R^2=0.77,\ F_{2,3}= 4.97,\ p=0.111612,\ N=6 \quad (5)$$

$$1/W_S = 138.39 + 0.095N_S - 0.63N_A,\ R^2=0.41,\ F_{2,3}=1.05,\ p=0.450922,\ N=6 \quad (6)$$

The R^2 values of 0.77 for equation (5) and 0.41 for equation (6) indicate that we can account for 77% and 41% of the variability in this model, for equations (5) and (6) respectively. The intercept of equation (5) is significant at p=0.0037. The coefficients a_{AA} and a_{AS} are not significant at p=0.100916 and p=0.125421 respectively. The intercept of equation (6) is not significant, with p=0.1248, as are the coefficients a_{SS} and $a_{SA,}$ with p=0.844101 and p=0.247082 respectively.

In terms of the Azolla equation (5), the ratio of coefficients is a_{AA}/a_{AS} = 1.1. This means that the effects of intraspecific and interspecific competition on Azolla were fairly equal. In other words, the effect of one Azolla plant on another Azolla plant was the same as competition from one Spirodela plant. As in the Hydrilla/Lagarosiphon experiment, the direction of the relationship between variables is predicted by the signs of the *B* coefficients. Therefore, since both *B* coefficients in equation (5) are negative, relationships with the dependent variable are negative: As N_A decreases, $1/W_A$ increases; and as N_S decreases, $1/W_A$ increases. In other words, the lower the density of Azolla and Spirodela, the lower the mean individual wet mass of Azolla. This result corresponds with the data presented in Figure 3.11.

The Spirodela equation (6) has a coefficients ratio of a_{SS}/a_{SA} = -0.15. This means that interspecific competition from Azolla had a greater effect on the wet mass of Spirodela plants than competition from other Spirodela plants. The *B* coefficient N_A is negative, meaning that the lower the density of Azolla, the lower the mean wet mass of Spirodela. Therefore, as Azolla increased in density, Spirodela increased in mass. Conversely, the *B* coefficient N_S is positive, meaning that the

greater the density of Spirodela, the lower the mean individual wet mass of Spirodela. These results correspond with those observed in Figure 3.12, and indicate that Spirodela responded to competition from Azolla by increasing in mass, but was limited by its own density.

3.2.5.2 Medium Nutrient Level

The following equations were obtained from multiple regression analysis of the mean individual plant wet mass in L2 (where * indicates significance):

$$1/W_A = 23.38^* - 0.80^*N_A - 0.48N_S,\ R^2=0.86,\ F_{2,3}= 9.47,\ p=0.050540,\ N=6 \quad (7)$$

$$1/W_S = 541.30^* - 0.65N_S + 0.208N_A,\ R^2=0.47,\ F_{2,3}=1.30,\ p=0.391503,\ N=6 \quad (8)$$

The R^2 values of 0.86 for equation (7) and 0.47 for equation (8) indicate that we can account for 86% and 47% of the variability in this model, for equations (7) and (8) respectively. The intercept of equations (7) and (8) are both significant at p=0.0003 and p=0.0048 respectively. The coefficient a_{AA} is significant at p=0.033468, while the coefficient a_{AS} is not significant at p=0.111388. In equation (8), the coefficients a_{SS} and $a_{SA,}$ are not significant, with p=0.221862 and p=0.655873 respectively.

In terms of the Azolla equation (7), the ratio of coefficients is a_{AA}/a_{AS} = 1.67. This means that intraspecific competition had a greater impact on Azolla mass than interspecific competition. Since both *B* coefficients in equation (7) are negative, relationships with the dependent variable are negative: As N_A decreases, $1/W_A$ increases; and as N_S decreases, $1/W_A$ increases. Therefore, the lower the planting density of Azolla and Spirodela, the lower the mean individual mass of Azolla. This corresponds with results presented in Figure 3.11, and suggests that individual plants responded to both intra- and interspecific competition by increasing in mass.

The Spirodela equation (8) has a coefficients ratio of a_{SS}/a_{SA} = -3.13. This means that intraspecific competition had a greater effect on the wet mass of Spirodela plants than interspecific competition with Azolla. The *B* coefficient N_A is positive, meaning that the greater the density of Azolla, the lower the wet mass of Spirodela. On the other hand, the *B* coefficient N_S is negative, meaning that

Spirodela mass decreased with decreasing Spirodela density. This is shown in Figure 3.12 and Table 3.11, where Spirodela individual mass increased significantly in 3:9 and 0:9, where Spirodela was present in a high density and Azolla in low density.

3.2.5.3 High Nutrient Level

The following equations were obtained from multiple regression analysis of the mean individual plant wet mass in L3 (where * indicates significance):

$1/W_A = 28.57^* - 0.88^*N_A - 0.22N_S$, $R^2=0.82$, $F_{2,3}= 6.93$, $p=0.075087$, N=6 (9)

$1/W_S = 354.06^* + 0.172N_S - 0.94^*N_A$, $R^2=0.91$, $F_{2,3}=14.67$, $p=0.028266^*$, N=6 (10)

The R^2 values for equations (9) and (10) indicate that we can account for 82% and 91% of the variability in this model, for equations (9) and (10) respectively. The intercept of both equations are significant at $p=0.0022$ (9) and $p=0.0029$ (10). The coefficient a_{AA} of equation (9) is significant at $p=0.036416$, while the coefficient a_{AS} is not ($p=0.437290$). In equation (10), coefficient a_{SA} is significant ($p=0.012928$), and a_{SS} is not ($p=0.400579$).

In the Azolla equation (9), the ratio of coefficients is $a_{AA}/a_{AS} = 4$. This means that intraspecific competition had more of an effect on the wet mass of Azolla plants in L3 than interspecific competition. One Azolla plant had the same competitive effect on another Azolla plant as the presence of four Spirodela plants. Since both *B* coefficients in equation (9) are negative, relationships with the dependent variable are negative: As N_A (Azolla planting density) and N_S (Spirodela planting density) decreased, the individual wet mass of Azolla decreased. These results are equal to those presented in Figure 3.11, where the general pattern was for Azolla individual mass to increase as density increases.

The Spirodela equation (10) has a coefficients ratio of $a_{SS}/a_{SA} = - 0.18$. This means that interspecific competition from Azolla had a greater impact on the wet mass of Spirodela plants in L3. The *B* coefficient N_A is negative, meaning that the lower the density of Azolla, the lower the mean wet mass of Spirodela. As the coefficient N_S is positive, Spirodela mass decreased as it increased in density. Figure 3.12 and Table 3.11 show that the individual mass of Spirodela increased

significantly in 9:9, 3:9 and 0:9, and that Spirodela individual mass was greater where the planting density of Azolla was high.

3.2.6 Discussion

The performance of Spirodela in the low nutrient level was limited by its own density and the presence of Azolla. Spirodela attained the highest individual mass in this nutrient level, but reproduced at a slower rate than in L3, resulting in bigger, fewer plants (Fig 3.12 & 3.17). In the medium nutrient level, Spirodela was limited by Azolla, but seemed to stimulate its own growth. It is important to note the difference in how Azolla and Spirodela responded to this nutrient level. The individual mass of Azolla plants behaved in a steadily increasing manner with increasing nutrients. A possible reason for this is that as nutrients increased, the reproductive strategy of Spirodela shifted to producing a greater quantity of smaller individuals (Lemon *et al.,* 2001).

The presence of Spirodela could possibly have had a greater effect on Azolla in the high nutrient level than in the others. The results indicate that, even while Azolla was competing with itself, the individual mass was affected by Spirodela density (as in the ratio 9:9). Spirodela seemed to be self-limiting in the high nutrient level, while growth was stimulated by the presence of Azolla. As nutrients increased, plant numbers increased, but produced smaller individual plants. A possible explanation for this is that reproduction was stimulated by an increase in available nutrients.

3.2.7 Conclusions

The first key research question asked how the planting density would affect the establishment and growth of indigenous plants (Spirodela) in competition with alien invaders (Azolla). According to results of this research, both Azolla and Spirodela plants established and grew well in all planting densities. The density of Azolla affected the growth of Spirodela plants in L1 and L3 such that a low density of Azolla resulted in ‘positive’ competition or facilitation (Callaway, 1995), with Spirodela increasing in mass. The percentage change in Azolla plant numbers decreased with increasing initial planting density of Azolla, while

individual mass of Azolla tended to increase. This suggests fewer, bigger individuals as the density of Azolla increased.

The second research question asked how different nutrient conditions would affect the establishment and growth of Spirodela in competition with Azolla. In a study investigating the growth and nutrient composition of Azolla in varying environmental conditions (Cary & Weerts, 1992), biomass yield increased significantly when the nitrogen content was raised from 0.01 to 1mg/L, and again increased when the nitrogen content was raised to 10mg/L. Biomass yields were more than doubled when the phosphorus content was increased from 5 to 20mg/L. In the present study, Azolla performed best in terms of plant numbers and total mass when growing in a high nutrient level. These results suggest that hypertrophic and eutrophic waters would be hospitable environments for the growth and reproduction of Azolla.

In the low nutrient level, Spirodela's growth strategy was to reproduce at a slower rate and increase individual plant mass. The plant was more affected by interspecific competition in this nutrient level. In L2, however, the mean total mass of Spirodela was significantly lower than in the other two nutrient levels. Under high nutrient conditions, Spirodela was affected by interspecific competition from Azolla, and the individual mass of plants was significantly lower than in L1. However, Spirodela plant numbers were significantly greater in the high nutrient level than in L1 and L2. Therefore, Spirodela plants in L3 multiplied rapidly, but produced small individuals. In an experiment investigating vegetative reproduction in three species of Lemnaceae (Lemon *et al.,* 2001), Spirodela plants exhibited a slow multiplication rate but formed large propagules, although the nutrient levels used in the study are not mentioned. Spirodela might have different growth strategies depending on the costs and benefits associated with environmental conditions (Lemon *et al.,* 2001). The results of Lemon *et al* (2001) indicated that the slow reproductive rate with larger propagules might contribute towards competitive ability, while rapid reproductive rates would be part of an opportunistic strategy in situations with abundant resources and minimal competition. In this experiment, however, competition was not minimal as Spirodela plants growing in the high nutrient level were affected by interspecific competition.

Results of this experiment indicate that while neither plant completely dominated the other, Azolla was the more effective competitor. Azolla was more limited by its own presence than it was by the presence of Spirodela, while Spirodela was under the influence of interspecific competition. It can be inferred from these results that an increased reproductive rate in Spirodela is stimulated by an increase in nutrients, a factor which, in nutrient-rich South African water systems, could potentially increase its resistance to competition from invasive species. Considering Spirodela's performance here, its susceptibility to an invasion by Azolla is low. Azolla would, however, probably establish in most systems with ease, especially considering its growth success in the high nutrient level in this experiment and its ability to fix nitrogen in low nutrient levels. A potential limiting nutrient in the growth of Azolla would be phosphorus content in the water (Lumpkin & Plucknett, 1980). Azolla currently has a very effective biocontrol agent in South Africa (McConnachie *et al.,* 2004), which further reduces the risk to similar indigenous species.

CHAPTER 4: CONCLUSIONS

4.1 Competitive performance of Hydrilla and Lagarosiphon

There are a number of conclusions to be drawn from this research in terms of the interactions between Hydrilla and Lagarosiphon under a variety of conditions. Hydrilla tolerated different levels of nutrients and space, and outcompeted Lagarosiphon in high and low fertility in terms of its percentage survival. Hydrilla's ability to tolerate different conditions in the present study is in accordance with previous research (Cook & Lüönd, 1982; Steward & Van, 1987; Van *et al.,* 1976). Lagarosiphon did not establish in great abundance in either of these nutrient treatments, despite its documented ability to tolerate different nutrient levels (Caffrey & Acevedo, 2007). The poor performance of Lagarosiphon was largely due to poor growth conditions.

Previous research has shown a mixture of results in terms of Hydrilla's competitive abilities in different nutrient levels. For example, some show Hydrilla outcompeting *V. americana* in low fertility (Barko & Smart, 1989; Smart & Barko, 1989), while Van *et al* (1999) showed that Hydrilla competed poorly with *V. americana* in low fertility. Van *et al* (1999) used 2g and 25g of Sierra fertiliser (N:P:K ratio=18:6:12) [5] per 15kg of sand for low and high nutrient levels respectively. In a study examining the relationship between Hydrilla and sediment nutrient availability, reduced nitrogen availability resulted in a 30% reduction in the growth of Hydrilla (Barko *et al.,* 1988). Although the present experiment demonstrated that the establishment of Hydrilla was favoured in a low nutrient environment, the algal colonization in L2 created an unfavourable environment for both species, and one cannot accurately assess how the plants would compete intra- and interspecifically if this disturbance had not occurred. It is possible that, due to competition from algae, Hydrilla was affected by competition from Lagarosiphon to a greater degree than it would have been in a favourable environment. As discussed in Chapter 3.1, algae can directly affect plant growth and macrophyte photosynthesis by reducing available nutrients and

[5] Note: The Multicote used in the Hydrilla:Lagarosiphon experiment has been formulated for an eight-month release period in soil, with an NPK ratio of 23:5:23.

changing water quality (Allen & Spence, 1981; Maberly, 1983; Phillips *et al.*, 1978; Simpson & Eaton, 1986)[6].

The correlation between dry mass and length of plants indicates the importance of the branching effect and growth strategy of plants. A plant may be short in length but have many branches, or be great in length with few branches. Branching and densely packed leaves indicate the degree of etiolation of a plant, which occurs when a plant growing in low levels of light lengthens its stem and leaves in order to receive maximum sunlight for photosynthesis (Potter & Lovett-Doust, 2001). Some plants, such as *V. americana*, increase in shoot length at low intensities of light (Barko *et al.,* 1991; Twilley & Barko 1990). Titus and Stephens (1983) found that the density of surrounding plants influenced the growth of *V. americana* through light competition by shading, resulting in *V. americana* plants growing taller (Potter & Lovett-Doust, 2001).

Branching in submersed plants shows a low level of etiolation – the plant is increasing in mass, receives sufficient light for photosynthesis, and has little need for extending in length. Observations made during the present study showed that branching did occur at the stem and base of plants. In the case of Lagarosiphon in 3:9 (L1) and Hydrilla in L2, the plants increased in dry mass but decreased in length, which may indicate branching. Branching in this case, however, may not be indicative of a 'healthy' plant, as Hydrilla and Lagarosiphon have the growth form of lengthening rapidly to reach the water surface. Although much published research has documented the ability of Hydrilla to tolerate low light levels, Hydrilla was not able to increase its shoot length in the high nutrient level of this experiment, where the presence of algae reduced the amount of available light. The algae growing in L2 may have damaged Hydrilla and Lagarosiphon plants significantly enough for the stems to break, reducing their ability to reach the water surface. This research recommends a more detailed measurement of branching in future studies, in order to further understand the interactions between branching, shading and shoot length in submersed plants such as Hydrilla and Lagarosiphon.

[6] Note: References on the effects of algae on submerged plants were suggested by the examiner during a review of the Research Report.

4.2 Competitive performance of Azolla and Spirodela

The interactions between Azolla and Spirodela in a range of different conditions demonstrated that Azolla is a strong competitor, and is mainly limited by its own density. As with the Hydrilla:Lagarosiphon experiment, the alien species is not much influenced by the presence of the indigenous plant, but tends to compete intraspecifically for space, light or nutrients, as shown by results of the multiple regression analysis. Spirodela performed well in terms of growth and reproduction, despite being affected by interspecific competition.

The results of this study show that the multiplication of Spirodela plants was stimulated by an increase in nutrients. This opportunistic growth strategy could potentially boost its resistance to Azolla invasions in eutrophic South African water systems. The development of Lemnaceae under different nutrient conditions showed that the multiplication rate of Spirodela increased with increasing nitrogen and phosphorus availability, while the root length decreased (Hürlimann-Lüönd, 1990). Tipping *et al* (2009) investigated competitive interactions between Spirodela and *Salvinia minima* Baker (Salviniaceae), with Spirodela performing best in high fertility (where low fertility = 0.5mg N/L and high fertility = 5mg N/L)[7]. They suggest that, since Spirodela biomass was primarily controlled by nutrients and not competition, there is the possibility of habitat sharing.

It should be noted that, while Azolla grows in the form of a spreading mat of fronds, Spirodela can form a number of layers within its mat. This may contribute towards habitat sharing between the two plants, since even though Azolla may be competing for space, Spirodela at a high multiplication rate can still attain a high total biomass. Considering the N-fixing ability of Azolla, there is the potential for this plant to coexist with other freshwater species in South Africa, and even aid in providing nutrients under low fertility conditions. On the other hand, the ability of Azolla to form a mat on the water surface and cause shading out is likely to negatively impact submersed species.

[7] Note: Nutrient concentrations used in this study (Azolla / Spirodela) are: Low (N=0.01mg/L); Medium (N=1mg/L) and; High (N=10mg/L).

4.3 Competition for sunlight and the influence of shading

Many studies have underlined the significance of canopy formation and shading as a means of interspecific competition for light as a resource (Aerts *et al.,* 1990; Barrat-Segretain, 2005; Cui & Caldwell, 1997; Jackson & Caldwell, 1992; Witkowski, 1991). When growing in light-limited systems, submersed species may assign more resources (reflected in dry mass of shoots) towards an increase in length until they reach sufficient sunlight (Cronin & Lodge, 2003; Larson, 2007). This form of lengthening for a light advantage has been studied in a number of experiments on submersed plant growth (Agami & Reddy, 1988; Barko & Smart, 1981; Janes *et al.,* 1996; Wang *et al.,* 2008). Therefore, a plant growing in conditions of low light may have both a nutritional and light advantage, as it grows taller and shades out its competitor (McCreary, 1991; Pronk *et al.,* 2007; Schwinning & Weiner, 1998). In an experiment investigating above- and belowground competition between Hydrilla and *M. spicatum* (Wang *et al.,* 2008), Hydrilla grew quickly to the surface and formed a canopy over that of *M. spicatum*. The shoot/root ratio of Hydrilla plants decreased with intra-specific competition, corresponding with the self-limiting behaviour of Hydrilla in this experiment.

Floating aquatic plants have a substantial impact on light availability on smaller floating plants and submersed species. Macrophytes with floating leaves have the competitive advantage of capturing light which cannot reach submersed plants below (Barrat-Segretain, 1996; Larson, 2007). Gaudet & Keddy (1988) studied the competitive abilities of 44 species of wetland plants, and found that the larger species had a competitive advantage through shading out the smaller species. Many water systems in South Africa are considered to be overrun by floating aquatic weeds such as water hyacinth (*Eichhornia crassipes* (Mart.) Solms (Pontederiaceae)). While it is possible that an invasion of plants such as Hydrilla or Azolla would be made easier in an already disturbed system, we do not know how the growth of such invaders would be affected by the presence of other weeds which may shade it out. In a study investigating competition for space, *E. crassipes* outcompeted *Pistia stratoites* L. (Araceae), whereby its prolific growth and high plasticity allowed it to grow above *P. stratoites*, shading it and reducing its light availability (Agami & Reddy, 1990). Cary and Weerts (1992) found that Azolla growing under 100% shading had restricted growth rates.

The chances of successful invasion may be considerably reduced by the formation of a closed canopy of indigenous species, which shades out the invader (Barrat-Segretain, 2005). For that reason, further studies on the interactions between alien aquatic plants and similar indigenous species as influenced by light availability are recommended in order to understand (and potentially minimise) establishment of the invaders in South African waters. Plants may react differently under low or high light levels, causing competitive interactions to shift. For example, in a reciprocal replacement series study, *V. americana* had a competitive advantage in low light levels, while Hydrilla outcompeted *V. americana* at high light levels (Smart & Barko, 1989).

4.4 Competitive behaviour in South African water systems

It is essential to relate experimental conditions to the 'real life' situation in South African waters. While alien and indigenous species might behave a certain way under controlled laboratory conditions with standardised nutrient levels, this may not give an adequate representation of which species would dominate in our water systems. South Africa's freshwater systems are some of the most nutrient enriched in the world due to human activity, dysfunctional sewage works, unsewered human settlements and effluent discharge (de Villierts & Thiart, 2007; Hohls *et al.,* 2002; Noble & Hemens, 1978). Most often, these systems occur in areas that are densely populated, or on rivers that are highly exploited, such as the Crocodile West, Vaal, and Umgeni rivers (Hohls *et al.,* 2002). According to a study of the nutrient status of South African rivers (de Villiers & Thiart, 2007), the majority of catchments had nutrient levels exceeding the recommended water quality guidelines for sustaining plant life, with elevated dissolved inorganic nitrogen levels ([NO_x] >400 μg N/L) occurring at least episodically in all but one of the catchments (Keurbooms River). The dissolved-phosphorus levels also exceeded the recommended concentrations for sustaining aquatic animal life (100 μg P/L), at least episodically, in all but six of the evaluated catchments. There was a significantly upward trend in the concentration of dissolved PO_4^{3-} in about 60% of the rivers investigated. While unpolluted conditions (<50 μg N/L and <10 μg P/L) occurred periodically in all of the river systems (except the Swartkops River), conditions favourable to the development of eutrophication

were present for the majority of the year in the Berg, Swartkops, Komati and Great Fish river catchments.

Accelerated eutrophication and nutrient enrichment from human activities has been recognised as a major driver of Hydrilla invasion (Swarbrick *et al.,* 1982; Van *et al.,* 1999). The results of the present experiments indicate that Lagarosiphon would be susceptible to an invasion by Hydrilla in South Africa's waters in most nutrient conditions, although a eutrophic or hypertrophic system would almost certainly increase the risk of competition from Hydrilla.

As Hydrilla is very restricted to one part of Pongolapoort Dam, it is possible that the weed is constrained by light conditions or nutrient levels. There is often a mild algal bloom in the dam (A. Bownes 2010, pers. comm.), although there is no reported evidence of it covering the surface of the water. It is therefore unlikely that shading out from algae would be restricting the spread of Hydrilla in this dam. A water sample taken from Pongolapoort in July 2009 gave nutrient levels of Nitrates=0.07, Ammonia=0.04 and Phosphates=0.3 mg/L (A. Bownes 2010, pers. comm.), although these levels are likely to be higher during the wet summer season, as stormwater and runoff from agricultural land flows into the dam. Turbidity varies with season, but the majority of the water body remains fairly clear all year round (A. Bownes 2010, pers. comm.). While this eutrophic water might increase levels of turbidity and restrict its spread through shading, Hydrilla is shown to thrive in high nutrient environments (Swarbrick *et al.,* 1982; Van *et al.,* 1999).

The research conducted here suggests that Azolla performs well in terms of plant growth and multiplication in a range of nutrient levels, especially considering its ability to fix nitrogen. Its growth performance in this study indicates that accelerated eutrophication in South Africa's water systems has provided an ideal environment for the historical rapid expansion of Azolla in South Africa (Hill & Cilliers, 1999). As indicated above, Spirodela would do well in most nutrient conditions, but would have different growth strategies: Larger propagules in low fertility and; an increased multiplication rate in high fertility (as in the case of South African eutrophic water systems, where Spirodela has had rapid growth

rates[8]). Results of this experiment indicate that Spirodela growing in a eutrophic water system would be affected by interspecific competition to a greater degree than in a low nutrient environment.

4.5 Implications for future research

4.5.1 Experimental disturbance

Environmental factors in the form of disturbance can play a major role in how competitive interactions affect community structure (McCreary, 1991; Wang *et al.,* 2008). In the case of this experiment, disturbance in the form of algae may have been instrumental in producing conditions which favoured the competitive performance of Hydrilla. However, due to the complex nature of light and nutrient interactions, more research is necessary in order to alleviate (as much as possible) the role of disturbance. The use of natural sediments as a source of nutrients has been suggested as a possible method for preventing excessive growth of algae (Smart & Barko, 1985).

4.5.2 Morphology of the South African Hydrilla biotype

In an experiment investigating the growth and morphology of both the dioecious and monoecious U.S. Hydrilla biotypes, the monoecious biotype was found to grow densely near the sediment, producing many horizontal stems (Van, 1989). This was in contrast to the dioecious biotype, which grew very quickly to the water surface. It is recommended that future research combine plant responses such as branching with those of height and dry mass, as these would provide better measures of establishment of the monoecious Hydrilla than height and dry mass alone.

The fact that the South African biotype is monoecious is vital to take into account in infestation surveys. By the time Hydrilla can be observed on the water surface, there may already be large amounts of Hydrilla biomass at sediment level, and it is possible that further infestations may exist in South Africa that have not yet been detected. Further research into the morphology of the South African

[8] Note: Information provided by the examiner during a review of the First Submission of the Research Report.

biotype is necessary, as well as accurate surveying methods to determine exactly which water bodies have been infested.

4.5.3 Combining different control methods

Herbivory has great potential to influence competitive interactions of plants by suppressing one of the two competitors (Crawley, 1989; Tipping *et al.,* 2009), and this effect has been documented in various studies (Barrat-Segretain & Lemoine, 2007; Van & Center, 1994; Van *et al.,* 1998). Doyle *et al* (2007) studied the effects of herbivory by *H. pakistanae* and competition from *V. americana* on the growth, expansion and tuber formation of Hydrilla. Results show that under the experimental conditions, herbivory and competition acted independently, and that a combination of these two factors should lead to effective management of Hydrilla. Shabana *et al* (2003) indicate that a combination of a plant pathogenic fungus (*Fusarium culmorum* (Wm.G. Sm.) Sacc. 1895 (Nectriaceae)) and the leaf mining fly, *H. pakistanae*, increases damage to Hydrilla, but suggest that further research be done into the efficacy of this combination for controlling infestations in natural lakes. Pathogen load is a factor which can prevent the establishment of a plant species in a new habitat, and valuable research could be done on the pathogen load of alien aquatics in South African water systems in terms of how this load affects their competitive abilities.

A survey of phytophagous insects on submerged aquatic plants found seven phytophagous morphospecies occurring on Lagarosiphon, from the following insect families: Corixidae, Chironomidae, Leptoceridae and Pyralidae (Schutz, 2007). Since both Lagarosiphon and Hydrilla are in the Hydrocharitaceae family, Schutz (2007) aimed to establish whether any of these insects would be damaging to Hydrilla and useful in its control. Results showed that Leptoceridae, Corixidae and Chironomidae are likely to move onto Hydrilla, but have not yet been tested for their potential to control it. While these insects may eat the plant, they might not be effective in controlling its growth and establishment. Lagarosiphon and Hydrilla might behave very differently in competition when under greater herbivore pressure, and might give clues as to how Hydrilla is in fact influenced by native herbivores. Future studies could also investigate herbivores present on these plants in competition. Examples of such studies are those undertaken as part of the PPRI's biocontrol programme against Hydrilla in

South Africa, as well as research investigating competitive interactions between Hydrilla and *V. americana* as influenced by insect herbivory (Van *et al.,* 1998).

4.5.4 Choosing a possible competitor

Larson (2007) identifies the need to study competition between aquatic plants of different life-forms. The growth form of a chosen plant competitor may determine its competitive success. Morphology must be considered when choosing a competitor for species such as Hydrilla or Azolla, since even high growth rates or the ability to take up nutrients may be insufficient if a plant cannot lengthen to avoid being shaded out (Tipping *et al.,* 2009). Other factors which should be regarded in plant competition, in that they may determine competitive strengths, are: Time of establishment (Hofstra *et al.,* 1999); biomass accumulation (Witkowski, 1991) and; reproductive allocation (McCreary, 1991).

According to Henderson (2006), Lagarosiphon occupies the same habitats as Hydrilla, but there is no evidence of the plant growing alongside Hydrilla in Pongolapoort Dam. Future research of possible competitors could investigate which plants are growing in the same area as the alien species, and if they have similar growth forms in the same conditions.

4.6 Final recommendations

Regardless of how much money is poured into invasive plant control every year, the elimination of invasive plants can be extremely difficult once they have established (Middleton, 2008). Invasion by alien plants can have detrimental effects on aquatic systems in terms of impacting indigenous species, changing community dynamics, reducing ecosystem stability, and harming economic productivity (Catford *et al.,* 2009; Haynes, 1988; Richardson & van Wilgen, 2004). Consequently, the key to protect indigenous plants and prevent their extinction is to avoid the establishment of such invaders. Studies on the competitive abilities of alien and indigenous aquatic plants under a wide spectrum of conditions would provide essential information in the field of aquatic macrophyte ecology (Spencer & Ksander, 2000). Further research over longer periods of time (complete growing seasons), and on established plants rather than transplanted fragments would be more representative of natural growing conditions, and would aid in

determining the susceptibility of indigenous plants to invasions (Doyle *et al.,* 2007).

This study has shown that Hydrilla performed well in a low nutrient environment, and that establishment and growth are favoured when growing in a high density. Azolla performed best in a high nutrient environment, and in high densities. Both Lagarosiphon and Spirodela were more influenced by interspecific competition under high nutrient conditions.

While alien plants such as Hydrilla and Azolla tolerate a broad spectrum of environmental conditions, and may theoretically spread to many freshwater systems in the country, their self-thinning behaviour and poorer performance in low densities may be an important constraining factor. As the susceptibility of indigenous plants to competition from invasives may be increased in high-nutrient systems, a continuous monitoring programme of aquatic alien species is vital in protecting our indigenous plants from extinction. Furthermore, this research recommends that the method of investigating competitive interactions between alien and indigenous plants be repeated with a variety of aquatic plants present in South Africa, as a means of anticipating susceptibility to invasions.

APPENDIX A
PHOTOGRAPHIC PLATE OF HYDRILLA AND LAGAROSIPHON



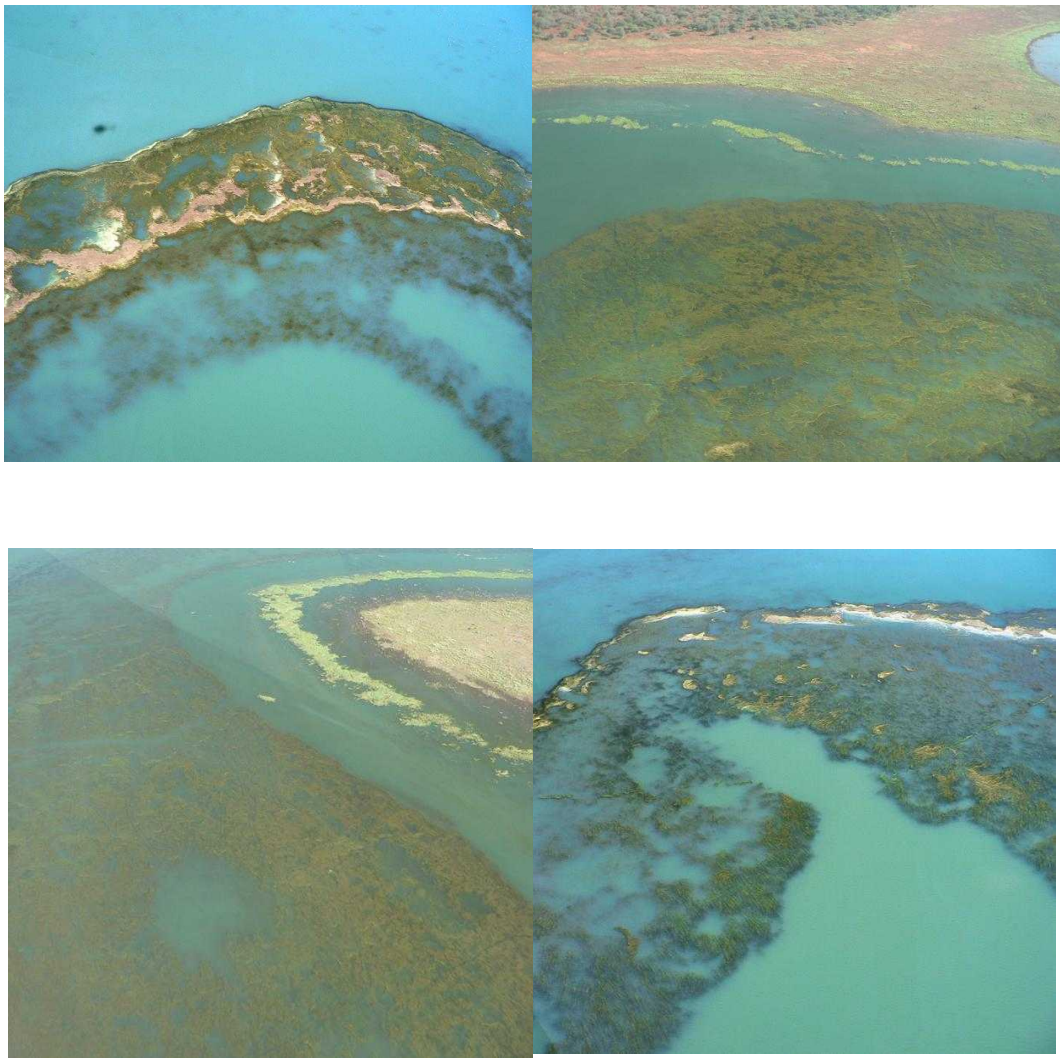






Clockwise from left:
Figure A.1: L1 at week 1
Figure A.2: L2 at week 1
Figure A.3: L2 at week 10
Figure A.4: L1 at week 10

APPENDIX B
PHOTOGRAPHIC PLATE



Figures B.1 - 4: Aerial photographs taken on 8 June 2006, showing sections of the Hydrilla infestation at Pongolapoort Dam. Reprinted with permission from Julie Coetzee.

APPENDIX C
PHOTOGRAPHIC PLATE OF AZOLLA AND SPIRODELA



Figures C.1 - 4: Photographs taken during week 2 of the Azolla/Spirodela experiment, showing experimental set up. Azolla are the darker, larger plants, while Spirodela are small and light green in colour.

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