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Mukarugwiro, Jeanne d' Arc

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University of the Witwatersrand, Johannesburg
School of Animal, Plant and Environmental Sciences

Title:

The effect of water nutrients on weevil herbivory and development on water hyacinth

A research report submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Science.

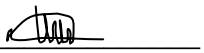
By

Jeanne d'Arc Mukarugwiro

Student number: 705282

DECLARATION

I, Jeanne d'Arc Mukarugwiro, declare that this research report is my own, unaided work. It is submitted for the Degree of Master of Science by coursework and research report to the University of the Witwatersrand. It has not been presented before for any degree or examination to any other University.



(Signature of candidate)

30th day of October, 2015 in Johannesburg

ABSTRACT

Because water hyacinth growth is highly dependent on nutrient availability, growth of the weevil *Neochetina eichhorniae*, which feeds only on water hyacinth, is closely linked to the plants' nutrient status. The aim of this research was to investigate whether the nutrient levels could influence the larval development and larval feeding on water hyacinth. To investigate this, water hyacinth plants were grown at three different nutrient concentrations (high (6 mg/l N; 0. 83 mg/l P), medium (2.8 mg/l N; 0.4 mg/l P) and low (0.7 mg/l N; 0.01 mg/l) chosen according to a range of nutrient conditions found in South African water bodies. Control plants, without larval inoculation and treatment plants, with two larvae per plant were used. Plant biomass and other plant growth parameters were measured every week. We predicted a higher larval feeding rate on plants grown at the low nutrient level and faster larval development and a higher larval survival rate at the higher nutrient levels.

Water nutrient levels had a great effect on water hyacinth growth, however the lower amount of nutrients negatively affected water hyacinth growth and these plants did not increase their biomass or other growth parameters compared to plants in high nutrient solutions. The larval feeding rate was influenced by nutrient availability. It was higher in the plants grown at the low nutrient levels because larvae consumed more to obtain the nutrients necessary to complete their development. These plants could not tolerate larval damage. Hence, they lost biomass and other plant growth parameters (number of leaves and petiole length) were reduced compared to plants grown in the high and medium nutrient treatments. *Neochetina eichhorniae* larvae were larger in the high nutrient treatment and their development was faster, followed by those from the medium nutrient then low nutrient levels. The larvae from the high nutrient plants reached the third instar and pupation stage when the larvae from the medium and the low nutrient plants were still in the second and first instar respectively and they took a long time to reach pupation stage. The larval survival rate was low for larvae reared on plants grown in the low nutrient treatment compared to those grown in the medium treatment while larval survival was high for those from the high nutrient level. The possible implications of this study are that *Neochetina eichhorniae* can perform better in high nutrient plants, but is not able to suppress water hyacinth growth to bring about control under eutrophic conditions. For that reason, it will be advisable to reduce the nutrient levels in water bodies before releasing of *Neochetina eichhorniae* weevils on water hyacinth infestation.

DEDICATION

I dedicate this work to my husband Innocent Harerimana, my son Maxime and my brother Jean Claude Izamuhaye for their unwavering moral support and help during my study

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CHAPTER I

1.1.Introduction

From the fifteenth century, there has been an increase of international trade and travel which has resulted in an increase of invasive alien species introductions (Van Devender *et al*., 2006). For example, 17 of the most serious invasive alien species including *Acacia saligna*, *Prosopis species, Pinus radiata* and *Pinus pinaster* were intentionally introduced into South Africa for agro-forestry sector (Zimmermann and Olckers, 2003). When they arrived in their new environments, they multiplied and proliferated as there were no natural enemies (insect herbivores or pathogens) to check and inhibit them (Mitchell and Power, 2003). Since then, they have caused ecological, environmental and social problems (Mark *et al*., 2000). This pushed many countries to look for ways to address concerns related to invasive species.

Water hyacinth (*Eichhornia crassipes*, Mart. Solms-Laub, Liliales; Pontederiaceae) is one of those invasive plant species and is capable of harming natural and agricultural environments involving water bodies. It is now considered a worldwide challenge (Hill, 2003; Byrne *et al*., 2010). Of the methods that are available to control water hyacinth, biological control which uses plant-feeding insect like *Neochetina eichhorniae* has been widely used to reduce water hyacinth infestations (Cilliers 1991; Charudattan *et al*., 1995;; Van Wilgen *et al*., 2001). Mechanical and chemical control methods have largely failed to control this invasive plant from water bodies in South Africa (Hill, 2003).

1.2. Problem statement

Water hyacinth is the worst invasive species of aquatic weed worldwide, as well as in South Africa. It affects social and economic activities by reducing water quality, modifying aquatic biodiversity and preventing activities in water bodies such as fishing, navigation and recreation (Ajounu *et al*., 2003; Hill, 2003; Byrne *et al*., 2010). Control measures, such as mechanical and chemical methods have been tried in order to mitigate the negative impacts of this invasive alien species. However they are not perceived as long-term control methods and are considered unsustainable to control water hyacinth invasions (Hill, 2003). The use of herbicides in water bodies affects non target species (Cilliers, 1991; Sodhi and Ehrlich, 2010). South Africa spends approximately R10 million per annum on herbicides for the reduction and mitigation of invasive

species damage including water hyacinth (Byrne *et al*., 2010). Biological control is perceived as the most successful and sustainable method to control and reduce water hyacinth populations in South African water bodies (Hill, 2003). It is affordable and environmentally friendly compared to the cost and consequences associated with herbicide use. *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae), one of the agents used in biological control, is a natural enemy of water hyacinth that feeds on its petioles and leaves (Ajounu *et al*, 2003). However, eutrophication and hypertrophication in water bodies inhibit the performance of *N. eichhorniae* as water hyacinth plants can grow and proliferate quicker than their natural enemies (Cilliers, 1991). Hence *N. eichhorniae* cannot control the plants, even if the growth and development of insect is assumed to depend on host plant nutrients (Wilson *et al*., 2006). Therefore, knowing the effect of nutrients on water hyacinth biomass removal by the weevils will allow the prediction of the impacts of biocontrol effects on the weed. Quantifying larval biomass removal from the plants will allow the impacts of *N. eichhorniae* and other weevils on water hyacinth to be modelled. Hence the modelling of the plant and weevil system will allow better management of the weed, tailored to different aquatic systems.

The main aim of this study was to investigate the effects of nutrient levels on the development and feeding of *N. eichhorniae* larvae on water hyacinth growth.

1.3.Objective

The objective was to monitor the development and feeding of *N. eichhorniae* larvae at high, medium, and low nutrient concentrations

1.4. Research questions

- ✓ What is the effect of nutrients on the developmental rate of *N. eichhorniae* larvae?
- ✓ What is the effect of nutrients on the feeding rate of *N. eichhorniae* larvae?
- ✓ What is the combined effect of nutrients and larval feeding of *N. eichhorniae* on the growth of water hyacinth?

CHAPTER II: LITERATURE REVIEW

2.1.Invasive species

Invasive alien species are non-indigenous species that when introduced to native ecosystems, multiply, proliferate, change and threaten indigenous species, causing their extinction and damage to the entire ecosystem (IUCN 2000). Invasive species (plants, animals or microorganisms) are now a major challenge worldwide and are considered as the second biggest threat to biodiversity and a threat to current and future human health (Mack *et al*., 2000). Even if invasion of certain species is a biological and therefore natural process, the spread of invasive species across the planet has been accelerated by human interventions through their socio-economic activities (Frenot *et al.* 2001). As described by Rejmanek (1996); Mark *et al*., (2000); McDowell (2002), invasive alien species are characterized by:

- Vegetative reproduction and a short time to reproduce
- Higher capacity to compensate tissue damaged by herbivores or other natural enemies.
- Pollination by wind
- Capacity to produce a lot of seed which may regenerate and grow in disturbed environmental conditions.
- Vegetative organs storing reserves of food that enhance species survival during bad conditions.
- Vegetative organs that have the capacity for regeneration after stressful environmental conditions
- High capacity to minimize the cost of carbon cost associated with photosynthesis

Invasive alien species lead to socio- economic problems as they negatively affect crops, fish production, as well as the production of livestock and other animals (Mack *et al*., 2000). Globally, invasive species occupy 3% of the total land surface area without considering the area under ice cover and areas designated to agricultural activities (Ricciardi, 2007). For example, *Melaleuca quinquenervia* (Myrtle, Myrtaceae), is an invasive plant in South Florida where it can multiply and occupy 20 ha per year (Schmitz *et al*., 1997). It causes extinction of native species such as cypress and sawgrass and induces intensification of fires and huge habitat loss (Schmitz *et al*., 1997). *Chromolaena odorata* (L) King and Robinson (Asterales; Asteraceae), is an

invasive species in humid tropical and subtropical regions including South Africa (Muniappan *et al*., 2009) and it often suppresses the regeneration of primary forest trees (Mack *et al*., 2000).

Among the major terrestrial invaders in South Africa, are *Acacia spp, Hakea sericea, Pinus spp, Eucalyptus spp, Jacaranda mimosifolia, Opuntia spps and Prosopis spp* (Richardson and van Wilgen, 2004). The principal aquatic invasive species are water lettuce (*Pistia stratiotes*), water hyacinth (*Eichhornia crassipes*); parrot's feather (*Myriophyllum aquaticum); Salvinia molesta*) and *Azolla filiculoides* (Richardson and van Wilgen, 2004).

Invasive plant species occupy 10 million ha in South Africa and cause a loss of water and biodiversity in general and negatively affect grazing lands. Species like *Prosopis* and *Opuntia* are known to impede the production of livestock and induce a decrease of herbaceous ground cover (Zimmermann, 1991). Every year, the loss of water is equal to R 5.8 billion and the loss of grazing resources lost is equal to R 300 million while the biodiversity loss is about R400 million (van Wilgen & De Lange, 2011). Aquatic invasive alien species induce the loss of 3.3 billion m^3 of water per year as they are able to increase evaporation rates, dilution capacity and induce a decrease river flows (Chamier *et al*., 2011). They invade entire fresh water ecosystems, dams and water reservoirs. Hence they decrease water quality and inhibit other activities in water bodies (van Wilgen & De Lange, 2011). Since invasive species cause transformation of ecosystems by using excessive amounts of resources, notably water, light and oxygen (Richardson & van Wilgen, 2004), the world's governments are trying to mobilize both human and financial resources to control weeds, by preventing their harmful impacts and restoring ecosystems already destroyed (Byers *et al*., 2001). Hence, effective strategies for management and control of invasive species including water hyacinth should be taken seriously and implemented in all countries where a water hyacinth invasion is presented to prevent a greater risk of ecosystem depletion.

2.2.Water hyacinth (*Eichhornia crassipes*)

Water hyacinth is a floating aquatic plant discovered by the naturalist C. Von Martius in 1823 (Jones, 2009). Water hyacinth represents a serious threat to fresh water bodies worldwide (Njoka, 2004). It is invasive alien species, native to South America (Gopal, 1987). It was spread all over the world by human agency (Shanab *et al*. 2010). Water hyacinth was introduced to South Africa as an ornamental plant in 1908 (Stent, 1913) and its spread and fast invasions on the surface of water systems was largely enhanced by the levels of eutrophication problems. Water hyacinth causes serious ecological and socio-economic problems by forming mats on water bodies. It blocks water flow and impairs socio-economic activities such as fishing, irrigation, drainage of water, recreation sports and boat navigation (Villamagna and Murphy, 2010; Hill 2003; Timmer and Weldon, 1966). For example in Zimbabwe, fisherman who were dependent on fishing activities, were forced to find other jobs due to a water hyacinth infestation in Lake Kariba between 1995 and 1996 (Chikwenhere *et al*., 1999). Accumulation of dead water hyacinth and its decomposition in water bodies reduce water quality, making it unsuitable for drinking and cooking (Patel, 2012). Water hyacinth impairs water flow and inhibits the growth of aquatic organisms by reducing oxygen in water (Jones 2009). It disrupts aquatic plants (eg. phytoplankton) by inhibiting the transfer of oxygen from air to water and by blocking the light necessary for photosynthesis (Villamagna and Murphy, 2010; Mironga, 2006). Due to reduced oxygen concentrations and increased carbon dioxide, aquatic animals do not get enough oxygen and they die from asphyxia (Timmer and Weldon, 1966).

Water hyacinth is capable of expanding quickly due its sexual and asexual vegetative reproduction; the latter is more important for rapid expansion and plant colonization (Jones, 2009). The growth of water hyacinth depends on nitrogen and phosphorus availability (Ready *et al*., 1989; 1990). It grows more rapidly under high water nutrient conditions (Heard and Winterton, 2000; Wilson *et al.,* 2006; Coetzee *et al*., 2007). Nitrogen and phosphorus in particular contribute to photosynthesis processes, growth and productivity of water hyacinth (Xie *et al*, 2004; Ripley *et al*., 2006). Byrne *et al*., (2010) found that nutrient concentrations in fresh water of South Africa ranged from 0.01 mg/l to 7 mg/l of nitrogen and 0.001 mg/l to 2.5 mg/l of phosphorus and enhance growth and proliferation of water hyacinth plants. The growth of water

hyacinth is also affected by temperature (Gutiérrez *et al*., 2001) and is limited by high and low temperatures. Water hyacinth does not grow above 40° C and below 8°C (Byrne *et al*., 2010).

Due to aggressiveness of water hyacinth invasion, every year enormous amounts of money and effort are expended to reduce its impact. The conventional way to bring invasion of water hyacinth plants under the control has often employed mechanical and chemical methods (Patel, 2012). However, the achievements of such management methods are often small, despite the enormous resources and efforts demanded (Zimmermann and Olckers, 2003). Mechanical method which consists of removal and harvesting of water hyacinth plants in water bodies has been used to control water hyacinth (Patel, 2012). However, its disadvantage is that the seeds and plant parts left behind during the removal of water hyacinth plants can grow and re-infest the water bodies again (Villamagna and Murphy, 2010). The second advantage is that it needs a manual labor work and time consuming. Thus it can be only applied in areas with a small scale not in large areas (Villamagna and Murphy, 2010). Herbicidal control provides immediate results but it can damage other water-living organisms and also induces water deoxygenation (Sodhi and Ehrlich, 2010). Hence to avoid the disadvantages of mechanical and chemical method, biological control has been adopted as a promising method to control water hyacinth infestations (Law, 2007).

2.3. **Biological control**

Biological control is a method aimed to use natural enemies including arthropods, parasites, predators and other pathogens to control in maintaining the invasion of invasive species' density at lower average than would occur in their absence (Culliney, 2005). It is a cost-effective and sustainable method (Charudattan *et al.*, 1995; Fowler *et al*., 2000a). This is because the natural enemies are specific on target weeds. They are able to destroy vital parts of the weed (leaves, petioles, roots and petioles), inhibit the weed's reproductive capacity, sometimes leading to plant death (Culliney, 2005; Low, 2007). They can indirectly destroy weeds by influencing the entry of pathogens, fungi and other saprophytic organism in weed tissues (Culliney, 2005). Moran (2004) found that the scars on the leaves and other wounds from the herbivores facilitate the pathogenic fungal infection on the plants. This was confirmed by Venter *et al* (2013), showing that the pathogens hosted on the weevils contribute to the decrease of photosynthetic capacity in

leaves of water hyacinth plants. The table below summarizes the advantages and disadvantages of biological control.

Table 1: Advantages and disadvantages of biological control of invasive plant species

Advantages	Disadvantages
Have a specific target	Require high cost at the beginning
Continuous action	Long time to show impacts on weeds
Long-term cost effective	Not easy to quantify and to predict their impacts on weed
Gradual in effect environmentally no intrusive	Uncertain non target effects ecosystem
Self dispersing even into difficult areas	Irreversible

Sources: Hajek, 2004; Culliney, 2005

The success of biological control in the control of invasive alien weed is categorised as complete or substantial or negligible (Hoffman, 1995). It is complete control when no further intervention of other control methods is required for reducing the weed population. It is a substantial control when further intervention of other control methods is required for controlling weed population. It is negligible where despite damage inflicted by biocontrol agents, the control of invasive alien species is still dependent on other control measures (Hoffmann, 1995). Globally, the success rate of the biocontrol method is 33% but considering analyses from each country, New Zealand uses biological control with an 83% success rate, followed by Mauritius with 80% success rate (Culliney, 2005). In South Africa, the success rate of using biological control is 61% (Culliney, 2005). However, the success of biological control can vary due to environmental abiotic factors including the low or high temperatures, excessive nutrients in water bodies, mismatching between plant and biocontrol agents and the rate of invasive species growth (Hill, 2003). Thus failing to establish biological control agents does not mean that biological control is not capable of controlling an invasion of an alien species. Syrett *et al*., (2000) found that eco-climatic mis-matching; phenological issues and biotic factors like predation are the principle factors that may cause biological control agents' failure to establish. Hence, it is necessary to understand how above factors can affect successful of biological control.

Water hyacinth has been controlled using biological control. However, in some countries they are still using mechanical and herbicide methods (Julien, 2001). Biological control has made a great contribution to the reduction of water hyacinth infestations worldwide. The release of natural enemies in infested water bodies, significantly contribute to the reduction of social, economic and ecological issues caused by water hyacinth (Njoka, 2004).

In 1973, biological control was initiated in South Africa and *Neochetina eichhorniae* (Hustache) one of natural enemies was established (Cilliers, 1991). Currently, biocontrol agents used to control water hyacinth are arthropods including two *Neochetina* species namely *Neochetina eichhorniae* and *Neochetina bruchi* (Warner) (Coleoptera: Curculionidae); a moth, *Niphograpta albiguttalis* (Warren) (Lepidoptera: Crambidae); the mirid *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae), a mite *Orthogalumna terebrantis* (Wallwork) (Acarina: Sarcoptiformes: Galumnidae); *Xubida infusellus* (Lepidoptera: Pyralidae) and fungal pathogen, *Cercospora piaropi* Tharp (Mycosphaerellales: Mycosphaerellaceae) (Julien *et al*., 2001; Coetzee *et al*., 2011). Moreover, the grasshopper, *Cornops aquaticum* (Orthoptera: Acrididae); *Taosa longula* (Remes Lenicov) (Hemiptera: Dictyopharidae); a moth, *Bellura densa* (Walker) (Lepidoptera: Noctuidae); the bug *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) and the fly *Thrypticus smaragdinus* (Diptera: Dolichopodidae), are also promising biological control agents of water hyacinth (Coetzee *et al*., 2011). Apart from South Africa, the two *Neochetina* species have controlled water hyacinth in many countries, including the Nile River in Sudan and water bodies in Zimbabwe, USA, Australia, India and Uganda (Beshir and Bennet, 1985; Haley, 1990; McFadyen, 2000).

2.3.1. Neochetina eichhorniae

Neochetina eichhorniae (Coleoptera: Curculionidae) is an insect native to South America (Deloach, 1975). It is a grey and brown weevil which feeds only on water hyacinth (Njoka, 2004). It lays eggs underneath the epidermis of the leaves (Deloach and Cordo, 1976) which hatch over seven to ten days (Julien, 2001). *Neochetina eichhorniae* is used as a biological control agent because it is an obligate feeder on water hyacinth and does not cause any damage to any other aquatic plants (Njoka, 2004). *Neochetina eichhorniae* is an important biological control agent of water hyacinth (Harley, 1990; Center, 1994) capable of limiting and destroying

the dispersal and growth of weed water hyacinth in established infestations (Forno, 1981). Both larva and adult *N. eichhorniae* contribute to the control of water hyacinth: The adult *N. eichhorniae* feeds on the plant leaf lamina and create characteristic scars while the larvae feed on the plant petioles (Ajounu *et al*., 2003). Larvae can create tunnels inside the plant petioles, destroying plant tissues (Deloach & Cordo; 1976Forno, 1981). Hence they allow the entry of pathogens into the plants which are able to cause necrosis infection in the plants. This infection is known to induce the decrease of photosynthetic rate (Ripley *et al*., 2008). With a disruption of photosynthesis, the water hyacinth plant does not grow well and suffers a decrease in biomass (Venter *et al.,* 2013). Although the use of *N. eichhorniae* can reduce water hyacinth infestations (Forno, 1981), it is ineffective in some areas of South Africa due to factors such as cold winters, eutrophication, flooding (Hill and Olckers, 2001).

2.3.2. **Factors affecting the effectiveness and development of *Neochetina eichhorniae***

The development and survival of *N. eichhorniae* is influenced by various factors such as predators, eutrophication, cold winters, flooding, drought, hydrology of small water bodies and the extensive use of herbicides (Byrne *et al*., 2010). Herbicide used in water ecosystems where *N. eichhorniae* have been introduced, destroy the weevil's food supply and interrupt its life cycle (Messersmith and Adkins, 1995). Herbicides cause high mortality rates of biological control agents including *N. eichhorniae* by destroying the waxy cuticle of their exoskeleton (Hill *et al*., 2012). During flooding, *N. eichhorniae* populations decrease because they are removed with water hyacinth. However the dormant seeds of water hyacinth plants under the water can germinate after environmental stress and give rise to new plants (Hill and Olckers, 2001). Heavy metals absorbed by water hyacinth plants have negative impacts on the *N. eichhorniae* (Newete *et al*., 2014). Accumulated heavy metals in water hyacinth plants reduce the weevils' fecundity and negatively impact larval development (Newete *et al*., 2014).

a) **Variability of climatic conditions**

The variability of climatic conditions in South Africa has a significant and negative effect on successful establishment of biocontrol agents including *Neochetina eichhorniae* (Byrne *et al*., 2010). Hill and Olckers, (2001) found that the variety of South African climate such as Temperate and Mediterranean climates, colder winters, summers and rainfall disturb *N.*

eichhorniae development by slowing down its reproductive processes and development of the eggs and larvae to adult. Normally the developmental rate and the survival of *N. eichhorniae* depend on the ambient temperature and this can be successful with moderate temperatures (Hill and Olkers, 2001). Low temperatures between 12°-15°C, affect negatively oviposition of *N. eichhorniae* as they stop laying eggs at such temperatures and below 12°C, *N. eichhorniae* larvae slow or stop their development (Byrne *et al*., 2010). The optimal temperature for the growth of *N. eichhorniae* larva and adult is 30°C (Julien, 2001) but temperatures above 30°C with low humidity induce a decrease in egg production. This leads to high decrease of *N. eichhorniae* populations (Julien, 2001).

b) **Eutrophication of water bodies**

Pollution of water bodies by nitrates and phosphates from agricultural activities facilitates the rapid growth of water hyacinth (Newman, 1998; Hill and Olckers, 2001). Consequently, with such plant growth vigour as a result of eutrophication, the impact of biocontrol agents on water hyacinth to curb the expanding weeds is insignificant. One such example is Hammarsadale Dam Kwa Zulu Natal where *N. eichhorniae* biontrol agents were unable to reduce water hyacinth invasion due to the levels of nitrates and phosphates in dam. This contributed to the rapid proliferation of water hyacinth in dam (Hill and Olckers, 2001). Although nitrogen and phosphorus has a great contribution on the development and survival of biocontrol agents (Wilson *et al*., 2006, Deloach and Cordo, 1976), their excessive concentrations in South African water bodies has contributed to the decrease of *N. eichhorniae* performance against invasions of water hyacinth (Coetzee and Hill, 2012).

2.4. **The effects of nutrients on insect development**

The growth and development of insect herbivores depends on the host plant's nutrients which are generally correlated with the nutrient levels of the medium in which the plants are growing (Awmack and Leather, 2002). Insects' nutritional requirements include proteins, amino-acids, carbohydrates, lipids and vitamins (Awmack and Leather, 2002; Nation, 2008). Nitrogen in particular is a critical nutritional element for insect herbivores and it has been suggested that its availability is the limiting factor in insect development (White, 1976; Mattson, 1980, Huberty and Denno, 2006). By manipulating the nutrient composition of an insect's food and then

measuring the insect's growth response to the nutrients, Sterner and Elser (2002) found that nitrogen and phosphorus are important for insect survival, development and reproduction. A decrease in phosphorus is the principle cause of cellular functioning problems (Sterner and Elser, 2002). A great amount of nitrogen in plant tissues is correlated with an increase of nitrogen in the plant's growth environment and can have a positive impact on insect survival and insect body size (Hogendorp *et al*., 2009). However, high nutrient levels in plants are generally positive factors in insect development (Mattson, 1980) and this may enhance the success of biological control agents against an invasive species. For example an increase in nutrients led to a greater number of *Cryptophagous salvaniae* (Coleoptera: Curculionidae) which were used to reduce *Salvania molesta* (D.S. Mitchell) (Salviniales, Salviniaceae) invasions (Room and Thomas, 1985).

The nutritional requirements for larvae, nymphs or adult insects are different. It is in this regard, therefore, that some Lepidopteran larvae need large amounts of nutrients. These are accumulated and stored for later use by pupae and adults that do not feed (Ojeda-Avila, 2003). Improved larval development may lead to higher insect fecundity and reproduction as the large larvae will pupate and give rise to a large female capable of laying more eggs (Honek, 1993). For instance, at high nutrient levels in *Eucalyptus blakyli* (Myrtale; Myrtaceae), larvae of *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae) developed quickly and increased their body size which resulted in the raising of the adults' fecundity (Ohmart *et al.,* 1985). In the presence of high nutrients the larvae develop quickly and their survival rate increases and this consequently leads to an increase of insect's populations (Hogendorp *et al*., 2006; Honek, (1993).

However, the quantities of nutrients required by female and male insects are different. Female insects need a greater amount of nutrients than their males counterpart especially proteins because the lower nutrients may impair the secretion of juvenile hormones, which influence the development of ovaries and eggs (Nation, 2008). For example, higher amounts of nitrogen and phosphorus enhanced weight gain in *Infusela xibida* (Lepidoptera: Pyralidae) females. Hence they lay a lots of large eggs compared to other females reared on host plant with low nutrients (Staneley *et al.,* 2007). Gotthard *et al* (1999) also found that female butterflies increased their weight with an increase in nutrient availability.

Increases of proteins, sterols and other host plant nutrients such as plant potassium, zinc and manganese have great importance in herbivore fecundity (Jamil and Jyothi, 1988). Sand and King (1961) showed that in the absence of sterol, necessary for *Drosophila melanogaster* (Priya) (Diptera: Drosophilidae) development, the fecundity rate was low which negatively affected the populations of *Drosophila*. While with a higher quantity of cholesterol the insect's fecundity increased. Other examples of insects whose development depends on availability of nutrients are discussed below.

a) Effects of nutrients on *Cornops aquaticum* (Brüner) (Orthoptera: Acrididae) development

The availability of nitrogen and phosphorus in water hyacinth affects the growth of *Cornops aquaticum*. The nutrient dose response of *C. aquaticum* nymphs was tested at three concentrations as high (7.6 mg/l N, 1.37mg/l P); medium (2.52 mg/l N 0.36 mg/l P); and low (0.032mg/l N, 0.020mg/l P) and it was found that at high nutrient level, insect developed rapidly and nymphs increased their body weight compared to medium and low nutrient levels (Bownes *et al.,* 2013; Bownes, 2009; Bownes, 2011).

An increase in dietary nitrogen increases the *C. aquaticum* nymphs' survival and female fecundity to 82% in high nitrogen and phosphorus plant nutrients; 71% in medium nutrient concentrations, while only 62% of the nymphs survived in low nutrient concentrations (Bownes, 2011). The development of *C. aquaticum* nymphs took 39 days at high nutrient concentration, 40 days at medium nutrient concentrations and 41 days at low nutrient concentrations (Bownes, 2009). Due to high nutrient nutrients, *C. aquaticum* females were able lay more eggs compared to those reared on plants poor in nutrients and they weighed more than males (Bownes, 2009).

b) Effects of nutrients on the Cinnabar moth (*Tyria jacobaeae*) (Lepidoptera: Erebidae) development

The growth and development of the Cinnabar moth depends on the quality of food consumed. By comparing moths reared on plants grown using urea fertilizers and those reared on plants grown without the fertilizers, researchers were able to establish that a high quantity of nitrogen has a positive influence on the moth larval growth and survival, while lower nutrients have negative

effects on the moth larval development (Myers and Post, 1981). However these authors gave no indication of the amount of urea that affects development (time) and survival of moth larvae.

c) Nutrient effects on *Paropsis atomaria* (Olivier) (Coleoptera: Chrysomelidae) larvae development

A lower amount of nutrients in its host plants negatively influences *Paropsis atomaria* larval development, as they were stunted at the first instar while larvae reared oh host plant rich in nutrients developed quickly (Fox and Macauley, 1977). The feeding rate of *P. atomaria* was higher at low concentrations of nitrogen than at high concentrations (Ohmart *et al.,* 1985; Fox and Macauley, 1977). These researchers gave no indication of the amount of nitrogen and phosphorus that was needed for the insects to grow fast. They also did not explain the effects of nutrients on larval body size (by measuring head width), developmental time of larvae or larval survival.

d) Nutrient effects on aphids, *Drepanosiphum platanoidis* Schrank (Hemiptera: Aphididae)

While the development of aphids is dependent on quality and quantity of nutrients, they differ from other insects as they can grow at low nitrogen concentrations (Awmack and Leather, 2002). Aphids are insects able to create symbiotic relationships with Buchnera bacteria and protozoa that provide essential amino acids necessary for aphid growth (Douglas, 1998). Buchnera contains the genes for the biosynthesis of amino acids essential for its aphid associate in its genome (Shigenobu *et al*., 2000). Thus *D. platanoidis* can grow at low nitrogen levels and the female aphid is able to gain weight and produce eggs which result in an increase in aphid population.

e) Nutrient effects on the water hyacinth mite *Orthogalumna terebrantis* development

The availability of nutrients plays a major role in the development, reproduction and survival of arthropods like the mite *O. terebrantis* (Schoonhoven *et al.,* 1998), whose development is faster at high nutrient level than at low nutrient levels. Higher nitrogen has a great influence on the rapid growth of *O. telebrantis* since the occurrence of their first generation approximately six weeks after *O. terebrantis* inoculation onto plants compared to medium and low nutrient levels.

Thus the population growth of *O. terebrantis* is high in environments rich in nutrients (Marlin *et al.*, 2013).

f) Effects of nutrients on *Neochetina eichhorniae* development

Neochetina eichhorniae weevils feed only on water hyacinth; hence they obtain all the required nutrients for development from this plant (Wilson *et al*., 2006; Wilson, 2000; Moran, 2006). *Neochetina eichhorniae* requires nitrogen (in the form of proteins) and phosphorus for its development (Deloach and Cordo, 1976; Wilson *et al*., 2006; Center and Dray, 2010). To determine the effects of nutrients on *N. eichhorniae* development, the recent researchers including Deloach and Cordo, (1976), grew the plants in different nutrients concentrations (low concentration: 0.4mg/l N and 0.052 mg/l P and high concentration: 4mg/l N and 0.57mg/l P, respectively). They found out that *N. eichhorniae* larvae reared on plants grown at high nitrogen and phosphorus concentrations developed faster than those fed on plants grown at low nutrient concentrations. The size of *N. eichhorniae* larvae was determined by measuring the head capsule. As they used ten larvae per plant, all larvae could not have the same size and there was a variation of their headwidth in the instars: 0.24-0.34mm, 0.36-0.5mm 0.52-0.95mm (at first, second and third instars respectively). Though these studies showed that nutrients contributed to *N. eichhorniae* larvae development, the nutrient concentrations used only represented those found in the tropical lakes which not rich in nutrients compared to South African lakes (ILEC and UNEP, 2001)

The present research uses nutrient concentrations that are different from those used in the above mentioned studies. The concentrations are in the range of the total nitrogen and phosphorus found in South African water bodies as determined by the Institute for Water Quality Service, and the following nutrient concentrations were used in this study: at low nutrient concentration 0.7 mg/l N; 0.1mg/l P; at medium nutrient concentration 2.8 mg/l N; 0.4 mg/l P and at high nutrient concentration 6 mg/l N; 0.83 mg/l P. Furthermore, in this study two larvae were inoculated in each plant while the above studies used ten larvae per plant. The other difference between this study and literature on the nutrient effect on *N. eichhorniae* is that the effect of medium nutrient level on the insect was tested while in literature they did not pay attention on it that in terms of insect development and feeding. The previous studies also did not determine the larval development within each larval instar at all nutrient levels (low, medium and high nutrient

levels). Therefore, this research investigated the effects of nutrients on the larval survival, larval development (time) and larval feeding rates on water hyacinth plants which previous studies did not consider

2.5. **Nutrient levels and plant compensatory growth as a response to insect herbivory**

Plants may be attacked and eaten by insect herbivores but they have means to cope or to tolerate insect damage (Strauss and Agrawal, 1999). They defend themselves using toxic secondary metabolites like allelochemicals capable of reducing the plant's digestibility. They can also use morphological defences such as hairs and spines (Dirzo, 1984) or they can tolerate herbivory, decreasing the negative impacts on their fitness levels through a process called compensation (Maschinski & Whitham, 1989). Plant compensation is an ability of plants to replace the tissues damaged by herbivory so that the plants can maintain their fitness through reproduction and proliferation after being damaged by insect herbivores (McNaughton, 1983; Belsky, 1986). Compensation is a phenomenon associated with plant photosynthetic capacity and carbon accumulation. Higher photosynthetic rates and increase of carbon acquisition capacity have a high contribution in inhibition of leaf defoliation which would otherwise lead to the reduction of stored reserves in the plants (Li *et al*., 2002). Plant compensation is influenced by environmental conditions such as nutrient availability, climate and light (Anten and Ackerly, 2001). In addition, (Boege, 2005) showed that plant ontogeny (evolution of plant developmental system) also can influence the capability of plant compensation. Through field and laboratory trials on how ontogenetic stages could influence the compensation of *Casearia nitida* (L.) Jacq. (Salicaceae) Boege (2005) found the changes in plant ontogeny decreased the capacity of *C. nitidia* to replace the damaged tissues. This is because the changes in plant ontogeny disrupted plant resource allocation, plant architecture and storage capacity of nutrients. This may prevented the regrowth and proliferation of plants after herbivore damage. This was confirmed by Barton and Koricheva (2010) saying that the change in evolutional plant development can reduce the ability of plant to replace the foliage lost due to herbivorous damage. Haukiaja *et al* (1998) showed that the plant compensatory response to herbivore damage enhances the way plants defend themselves especially in environment rich in nutrients which are necessary for plant growth and secondary metabolites that can be produced to prevent and inhibit the insect attack.

In general, plant may compensate for insect herbivory damage in three ways: (1) Overcompensation when the herbivore insect is beneficial, and the productivity of plant

increases. However, plants may also overcompensate due to the high nutrients in which they are growing and a low density of insect herbivores (McNaughton, 1983). (2) Equal compensation may occur where there is no benefit to the plants from insect feeding and the productivity of exposed plant to insect herbivory is equal to the productivity of unexposed plant. (3) Undercompensation occurs when herbivores are able to negatively influence plants; hence the plants lose more biomass and more leaves than an unexposed plant (Maschinski & Whitham, 1989).

Using meta-analysis techniques Hawkes & Sullivan (2001) found that plants were able to compensate more for herbivory damage under high nutrients while plants grown at low nutrient levels failed to compensate. However, plant recovery from insect herbivory damage depends on the type of plant (monocotyledon or dicotyledon). Hilbert *et al.,* (1981) found that dicotyledons and woody plants are able to overcompensate under low nutrient conditions due to their capacity to extend their growth for long period which can lead to the increased plant production. However monocotyledons overcompensate under high nutrients. This is because monocots and dicots have different meristematic tissues. Monocotyledon plants have a basal meristematic tissue able to limit damage caused by herbivory feeding; hence plants can regrow and proliferate. Dicotyledons and woody plants have apical meristems that enhance their plant recovery from herbivory damage under low nutrients (Hawkes & Sullivan, 2001).

Water hyacinth as monocotyledon plants are able to compensate for herbivory damage and this also depends on environmental conditions including nutrient levels. Soti and Volin (2010) found that water hyacinth grown at high nutrient levels compensated more than those grown at low nutrient levels. Their findings showed that leaf turnover (considered as the major determinant of plant growth) increased, which resulted in significantly higher area–based photosynthesis in the leaves.Hence final biomass and relative growth rates (RGR) considered as the consequences of water hyacinth overcompensation, were increased under high nutrient levels. Thus overcompensation is regarded as one of the major factor that influences the failure of biological control of water hyacinth invasion in eutrophic water bodies (Soti and Volin 2010).

The main focus of this research was to investigate the influence of water nutrient levels on *N. eichhorniae* larval development, and larval feeding rate on water hyacinth. The outcome from this study will contribute to an understanding of water hyacinth's responses to weevil herbivory

damage, a deeper understanding of biological control failures and assist in the prediction of effective strategies for the success of biological control agents on water hyacinth under different nutrient regimes.

CHAPTER III: METHODS

3.1. Plant and insect cultures

These experiments were conducted in the laboratory at University of Witwatersrand over a period of eight weeks. The water hyacinth plants used in the experiments were obtained from stock cultures maintained in plastic pools at the Witwatersrand University. The plants were acclimated in nutrient solutions at different concentrations (High, Medium and Low) for two weeks prior to the experiments. The nutrient solutions were changed weekly and the plants were sprayed with water and wiped every day to prevent infestation by other insects.

3.2. Materials

Plants were grown in growth rooms of 25ºC, a favourable temperature for water hyacinth growth. Photoperiod was 12:12. Water hyacinth plants were grown in 48 buckets of 15 litres each. Each bucket was filled with 10 litres of tap water, one healthy plant of water hyacinth with two larvae of *N. eichhorniae* for the treatment plants only while the control plants were not punctured for larval inoculation. Nitrogen and phosphorus in the form of Hoagland's solutions were added to each bucket. The ratio of nitrogen to phosphorus was approximately 7:1 (Wilson, 2002).

3.3. Oviposition

The larvae used in the experiments were obtained by placing the leaves of water hyacinth in a small plastic tub of 0.5 litres, with *N. eichhorniae* adults (15 females and 15 males). Three days later, the leaves were dissected under a stereo microscope to remove the eggs, using a sterilized forceps and scalpel. Eggs were placed on the sterilized wet filter paper in Petri-dishes and placed at 25º C. The eggs were examined daily for hatching and the eggs hatched after 11 days. The resulting larvae were placed into small punctures made in the petioles of experimental water hyacinth plants. We did not allow adult insect oviposition on experimental plants as the adult insects could damage them before their use and our intention was to examine only larval feeding, not adult insects.

3.4. Nutrient conditions

The larval developmental experiments were conducted at three different nutrient concentrations: low (0.7 mg/l N; 0.1 mg/l P, medium (2.8 mg/l N; 0.4 mg/l P) and high (6 mg/l N; 0. 83 mg/l P). These concentrations were chosen according to the range of total nitrogen and phosphorus found in South African water bodies (Table 2) (the Institute for Water Quality Service). Each of the three nutrient concentrations had eight treatment and eight control buckets making a total of 48 buckets. Every week, 5 mg/l of chelated iron was added to each bucket (treatment and control) to avoid iron deficiency. The nutrient solutions (treatment and control) were tested weekly using a Hach colorimeter (DR/870), in order to ensure that the concentrations of nitrogen and phosphorus were correct. The nutrient solutions were changed weekly to maintain the required nutrient supply to the plants. Since the growth of water hyacinth is positively correlated primarily with nitrogen and phosphorus (Reddy *et al*., 1990), these two elements were manipulated while the micronutrient elements supplied by Hoagland's solutions remained constant.

Table 2: Classification of water bodies according to nitrate levels in mg/L from the South African Water Quality Guidelines

Classification	Nitrate levels (mgL-1)
Eutrophic (high)	2.5 – 10
Mesotrophic (medium)	0.5 - 2.5
Oligotrophic (low)	<0.5

From Byrne *et al* (2010)

3.5. Larval inoculation and data collection

Weevil larvae were inoculated into the plants on the second and third leaf of each water hyacinth plant counting outwards from the center of the shoots. In each treatment, the plant was inoculated with two larvae while the controls were not punctured for larvae inoculation purpose. The buckets, in two growth rooms, were distributed according to a randomization table for six conditions in order to share the light equitably. The wet weight loss or gain was checked every week by weighing all plants (Ohaus Scout pro balance scale, model: SP601).

The length of the longest petiole was measured weekly. To check the rate of leaf production, new leaves were tagged each week. Hence, the number of the leaves produced or lost on each plant was counted weekly in all nutrient solutions. Thus leaf turnover was calculated by comparing number of leaves counted for each week with the initial number of leaves.

To determine the size of larvae, as the measure of larval development, the headwidth of larvae detected when dissecting plants, was measured weekly using an eyepiece micrometer. The rootstock of the plants was checked for the appearance of pupal cases. One plant from each nutrient level was randomly selected for dissection each week. All petioles and the roots of water hyacinth plants were carefully checked every week for the presence of larvae using dissecting microscope.

3.6. Data analysis

The data were captured using Microsoft Excel. Descriptive statistics were used in linear regression, scatter and bar charts; while inferential statistics were used to conduct statistical tests. Homogeneity of variance and equality of variances were checked prior to data analysis. Using Statistica software (version 12.6), a general linear model (GLM) was conducted to compare each plant growth parameters (biomass, leaves and length of petioles) in the control and treatment plants.

Estimated larval feeding rate per larva per week was calculated using the formula below

$$\text{Larval feeding rate} = \frac{total\ weight\ gains\ (all\ plants) - total\ weight\ losses}{no.of\ larvae\ surviving}$$

Mean larvae surviving was calculated using the number of larvae remaining in the experiment multiplied by average larval recovery (mean of larvae found during dissection over all weeks divided by number of inoculated larvae = 2 in each plant). Larval feeding means the consumption by larvae on water hyacinth plant materials.

Leaf turnover was calculated by comparing the number of the leaves counted for each week and initial number of the leaves per plant, according to Orbita and Mukai, (2009) and Hikosaka, (2005). One Way ANOVA was used to compare the means of water hyacinth wet biomass, number of the leaves and length of longest petioles between nutrient treatments. Linear regression was performed on the rate of change in plant growth parameters (wet biomass, number of the leaves and length of longest petioles) and to test the relationship between the plant growth parameters and nutrient levels. Standardised Major Axis Tests & Routines (SMATR)

Version 2.0 which based a Standardised Major Axis (SMA) technique (Warton *et al*., 2006) was conducted to detect the presence of heterogeneity among the slopes of the treatments and controls. Hence, a pair-wise comparison post-hoc test was conducted after detecting heterogeneity between the slopes.

CHAPTER IV: RESULTS

4.1. Plant growth

4.1.1. The effect of the combination of nutrients and *N. eichhorniae* larvae on water hyacinth biomass

The plants grown in the insect treatments lost weight, while the plants grown in the control group, without weevils, gained weight (Figure 4.1). The treatment plants grown at low nutrient level showed greater loss of biomass ($F_{2, 131}$= 4.351, P <0.015) than the plants grown at medium and high nutrient levels, while the control plants grown in the high nutrient gained more biomass than those grown at medium and low nutrient levels. There were statistical differences in plant biomass between the respective control and treatment groups at low nutrient level ($F_{1, 86}$= 95.423 P < 0.0000), at medium nutrient level ($F_{1, 86}$ = 37.25, P < 0.0001), and at high nutrient level ($F_{1, 86}$ = 13.34, P < 0.00051). Furthermore, the interaction of nutrients and weevils on plant biomass was also significant at the low nutrient level ($F_{8, 79}$ = 38.26, P < 0.002), at the medium nutrient level ($F_{8, 79}$ = 6.690, P < 0.0001) and at the high nutrient level ($F_{8, 79}$ = 4.3, P < 0.00032). A comparison of slopes using a Standardized Major Axis (SMA), showed that there was significant heterogeneity amongst the treatments and controls (Test stat = 57.848, P = 0.01). This indicates that rate of plant biomass increase was not the same in the controls and the rate of plant biomass loss was not the same in all the treatments. As control plants (unexposed to insects) should increase their biomass, the rate of plant biomass increase was greater at high nutrient levels than at medium and low nutrient levels. For plants exposed to insect, the rate of plant biomass loss was greater at the low nutrient level than at medium and high nutrient levels (Figure 4.2). Note that the high variance in biomass of treatment plants grown at the low nutrient level was caused by the mistake done in selecting plant at the binning of experiment.

Estimated larval feeding rates were calculated for each week using an average larval recovery rate of 0.5 larvae per plant for the plants grown at the low nutrient level, 0.6 larvae per plant for plants grown at the medium nutrient level and 0.8 larvae per plant for plants grown at the high nutrient level. Larval feeding rates were greatest for plants grown at the low nutrient level (with a mean of 9.60 g per larva) compared to plants grown at the medium nutrient level (with a mean of 8.79 g per larva) and plants from the high nutrient level (a mean of 8.05 g per larva) (Figure 4.3).

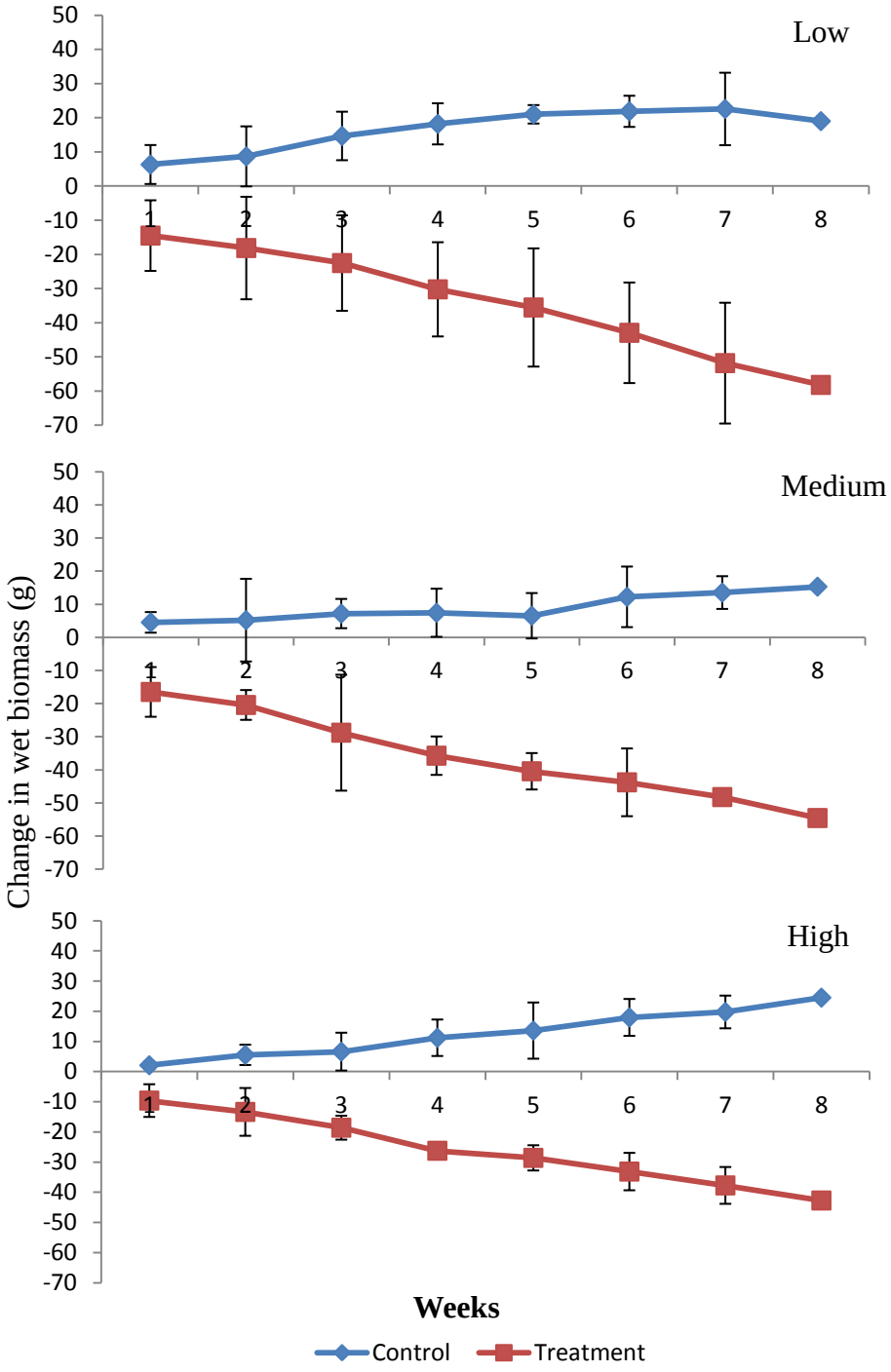

Figure 4.1: The effects of the combination of nutrients and weevils on the water hyacinth biomass in plants grown at different nutrient concentrations. Treatment = two *N. eichhorniae* larvae were inoculated in each plant at week 0, control = no *N. eichhorniae* larvae inoculation. Change in biomass was calculated as the initial biomass subtracted from the weekly biomass measure. Errors bars represent the standard errors of the mean. $P < 0.05$

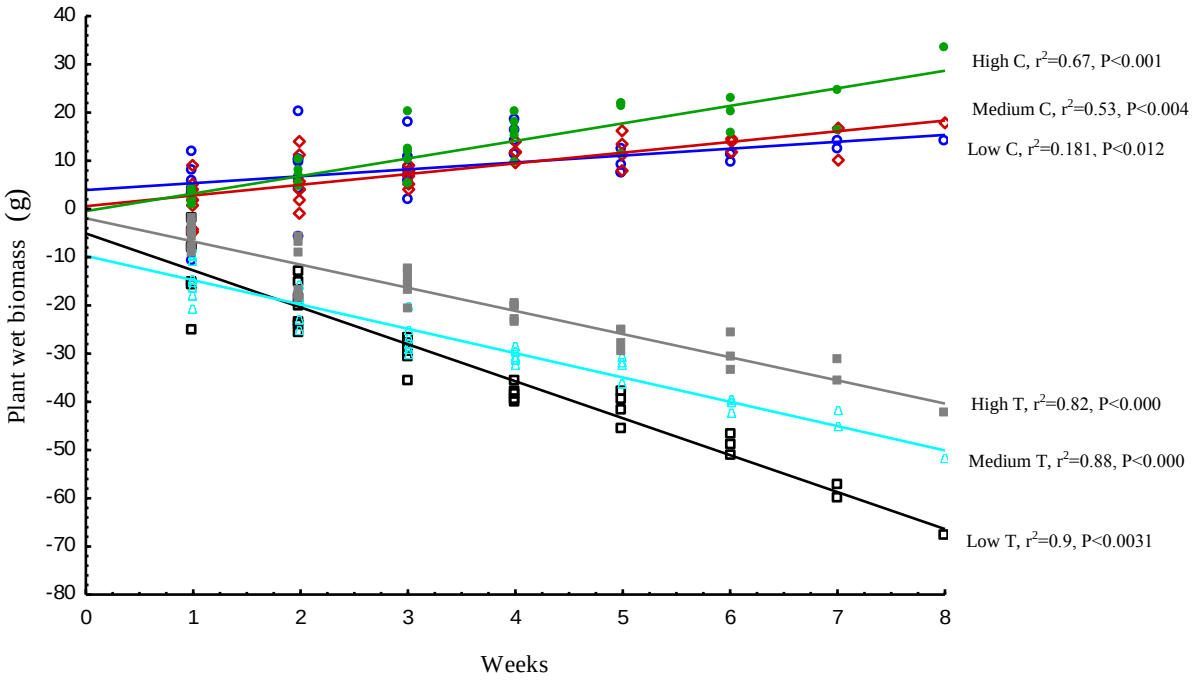

Figure 4.2: The effects of nutrients and *N. eichhorniae* larvae on the rate of plant biomass change across low, medium and high nutrient levels. C = control plants grown without inoculation of *N.eichhorniae* larvae, T = treatment plants in which *N. eichhorniae* larvae were inoculated (two larvae per plant). Significant of differences in slopes were set up at P<0.05

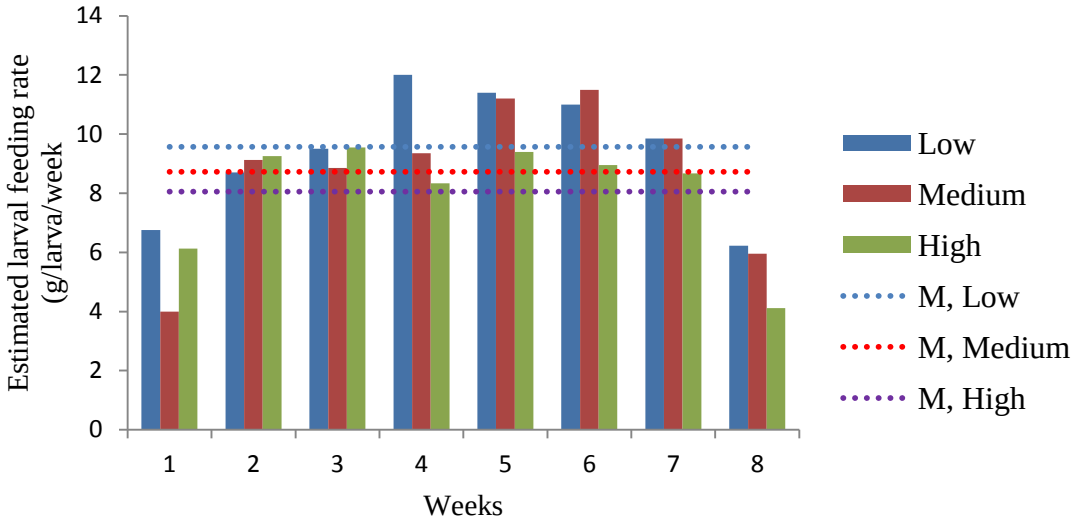

Figure 4.3: Estimated biomass removal by *N. eichhorniae* larvae over eight weeks. Water hyacinth plants were grown at low, medium and high nutrient concentrations. Dotted lines indicate the mean (M) of plant biomass removal per larva per week in each nutrient level (low, medium and high nutrient levels respectively.

4.1.2. The effect of the combination of nutrients and *N. eichhorniae* larvae on the number of water hyacinth leaves

Most plant growth parameters were significantly affected by the nutrient levels and by the interactions between nutrients and insect treatments.

The plants grown in the control groups produced significantly more leaves, while those grown in the treatments lost leaves, particularly plants grown at the low nutrient level (Figure 4.4). Leaf turnover between controls and treatments was significantly different in all nutrient levels ($p<0.05$). In addition, the interaction of nutrients and weevils on leaf turnover was significant at the low nutrient level ($F_{8, 79} = 18.44$, $P < 0.0005$), at the medium nutrient level ($F_{7, 56} = 17.94$, $P < 0.0064$) and at the high nutrient level ($F_{8, 69} = 19.16$, $P = 0.023$) indicating that the combination of nutrients and *N. eichhorniae* larvae had an effect on leaf turnover.

The results from SMA indicated that there was no common slope among, either treatment or control groups, indicating that the rate of increase or loss in number of the leaves per plant was significantly different across all nutrient levels (Test Stat = 27.399, P = 0.001). The rate of change in the number of leaves added was greater at high nutrient levels than at medium and low nutrient levels while the rate of change in the number of leaves lost was greater at the low nutrient levels (Figure 4.5).

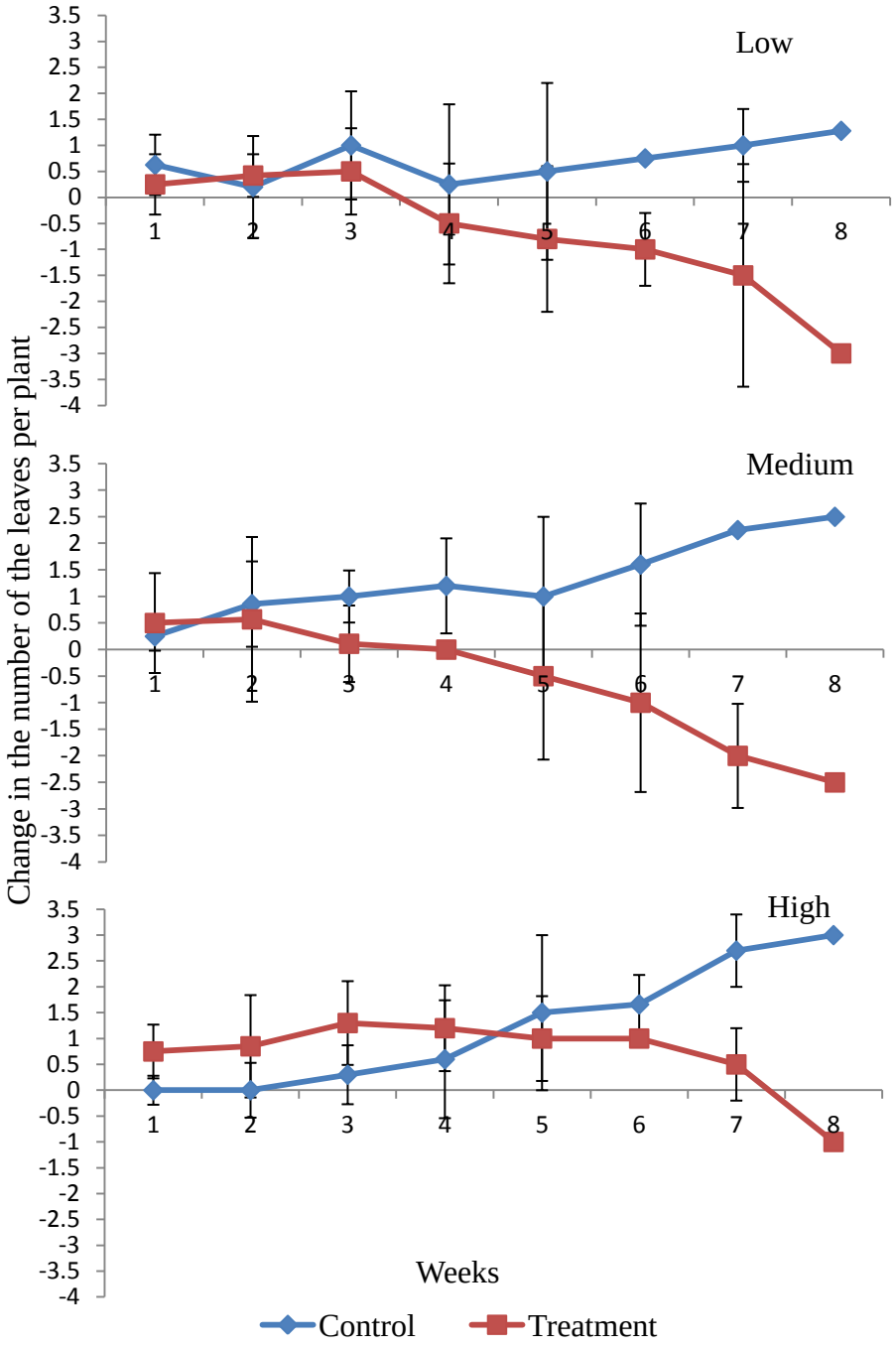

Figure 4.4: The effect of the combination of nutrients and weevils on leaf turnover for water hyacinth plants grown in different nutrient levels. Treatment = two *N. eichhorniae* larvae inoculated into each plant at week zero, control = no *N. eichhorniae* inoculated. The number of the leaves added or lost, each week was compared to initial number of the leaves. Error bars represent the standard error of the mean.

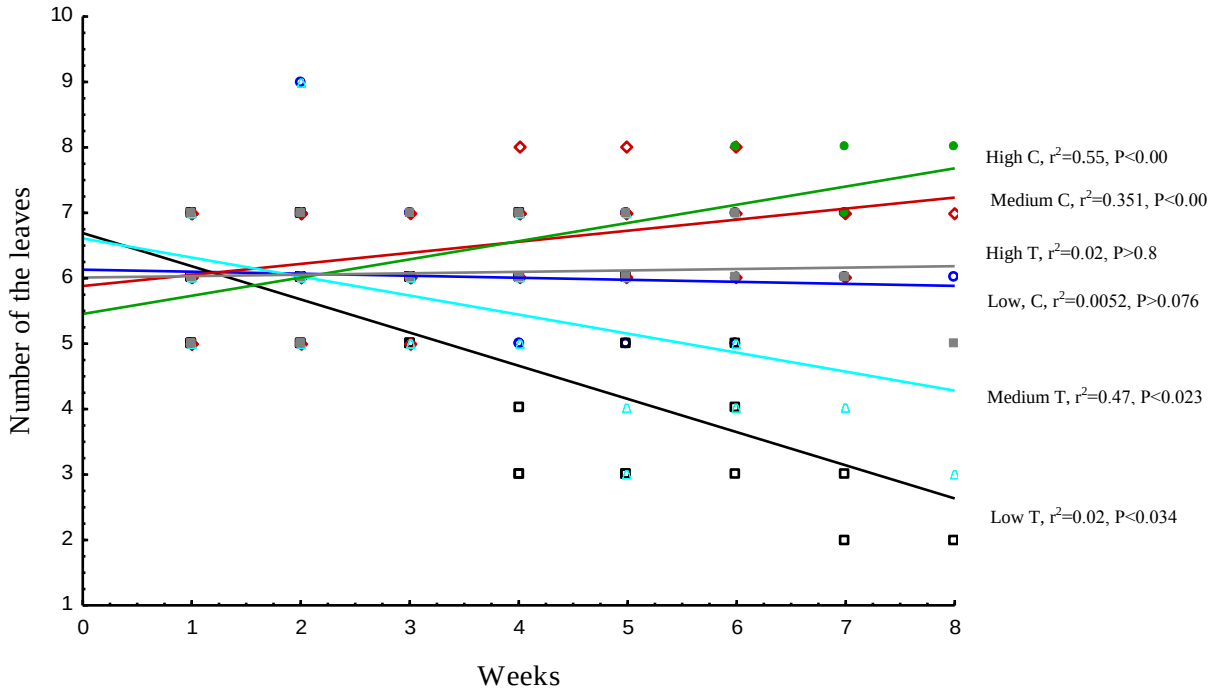

Figure 4.5: The effect of the combination of nutrients and *N. eichhorniae* larvae on the rate change in number of the leaves added or lost across low, medium and high nutrient levels. C = control plants grown without inoculation of *N.eichhorniae* larvae, T = treatment plants in which *N. eichhorniae* larvae were inoculated (two larvae per plant). Significant differences in slopes were set up at $P < 0.05$

4.1.3. The effect of the combination of nutrients and *N. eichhorniae* larvae on the length of the longest petioles of water hyacinth

It was found that the length of the longest petiole increased slightly in all the controls and decreased slightly in all treatments (Figure 4.6). There was significant difference in length of longest petiole between control and treatment groups at the low nutrient level ($F_{1, 86} = 3.78$, $P < 0.034$), at medium nutrient level ($F_{1, 86} = 1.525$, $P < 0.045$) but there was no significant difference between control and treatment at the high nutrient level ($F_{1, 86} = 0.089$, $P > 0.765$). The interaction of nutrients and weevils did not have an effect on the length of the longest petiole at the low nutrient level ($F_{8, 70} = 8.79$, $P > 0.951$), at the medium the level (F 7, 56=1.245, $P > 0.851$) or at the high nutrient level ($F_{8, 70} = 0.0064$, $P > 0.97$. A comparison of slopes using Standardized Major Axis (SMA) showed that there was no common slope either amongst treatments or amongst controls (Test Stat = 17.329, P = 0.004) indicating that the rate of length

of longest petiole change was significantly different across controls and treatment groups (Figure 4.7). In summary water nutrients had a great positive on water hyacinth growth. However their effects were confounded by weevils. The interaction of nutrients and weevils on plants explain how the insect and nutrients had an effect on water hyacinth growth.

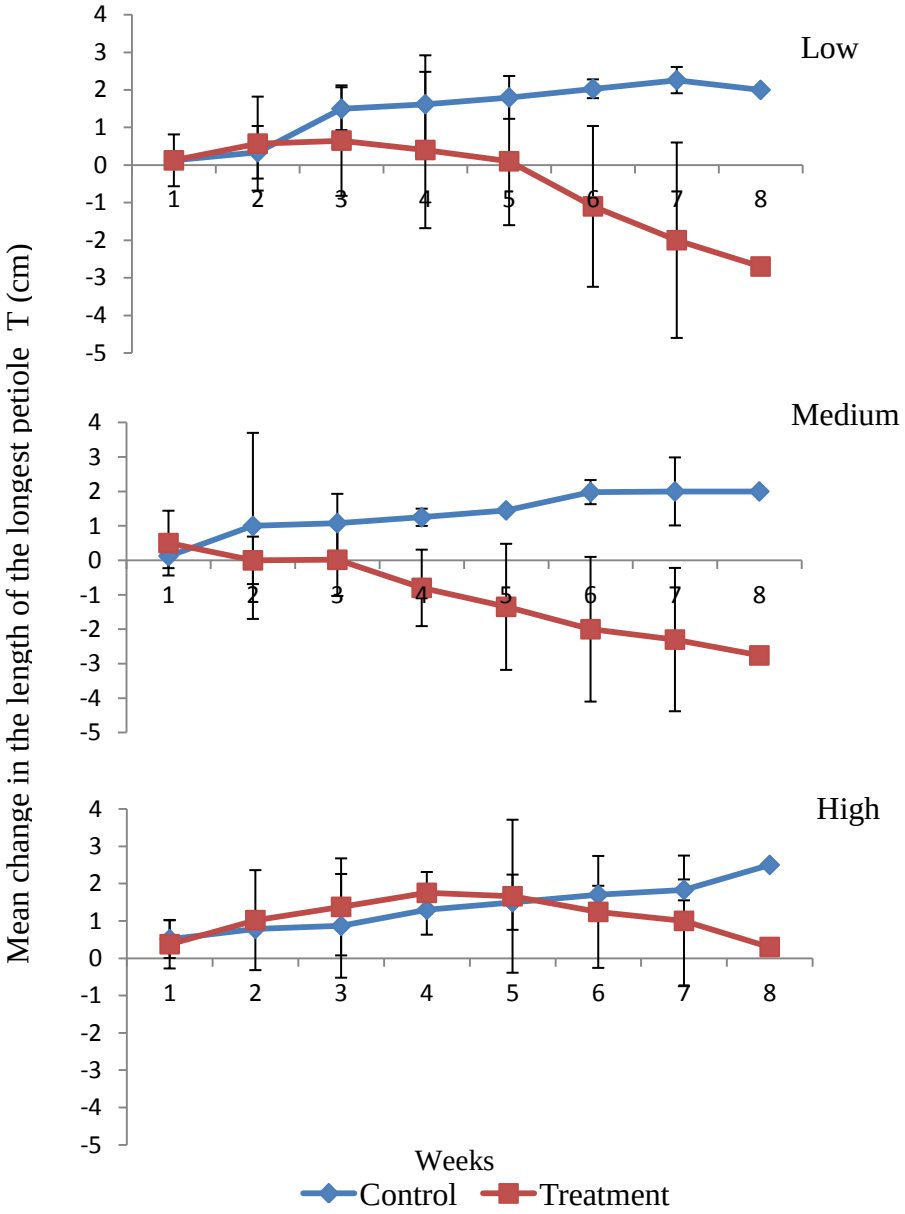

Figure 4.6: The effect of the combination of nutrients and *N.eichhorniae* larvae on the length of the longest petiole of water hyacinth plants grown in low, medium and high nutrient levels Treatment = two *N. eichhorniae* larvae inoculated into each plant at week zero, control = no *N. eichhorniae* inoculated. Change in the length of the longest petiole was calculated as the initial length of longest petiole subtracted from weekly length of longest petioles measure. Error bars represent the standard error of the mean.

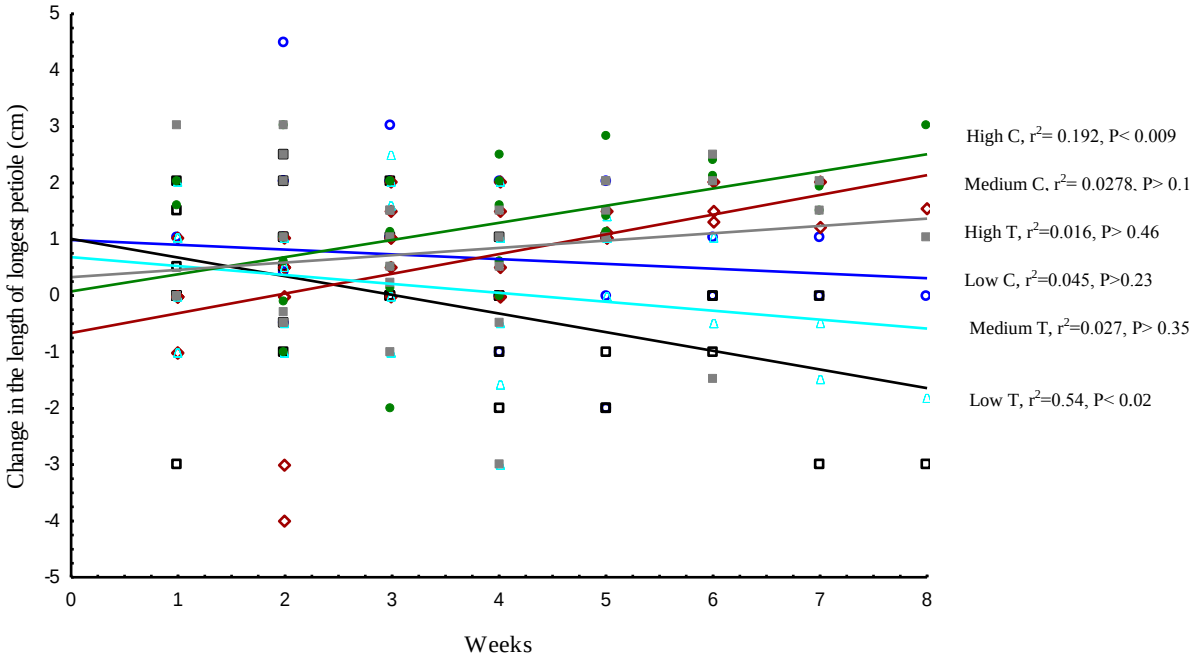

Figure 4. 7: Effects of the combination of nutrient and *N. eichhorniae* larvae on the rate of change in the length of the longest petiole for water hyacinth plants grown at low, medium and high nutrient levels. C = control plants grown without inoculation of *N.eichhorniae* larvae, T = treatment plants in which *N. eichhorniae* larvae were inoculated (two larvae per plant). Significant difference in slopes was set up at $P < 0.05$.

4.2. Insect development

4.2.1. The effects of nutrients on *N. eichhorniae* larval development

The nutrient treatments had a significant effect on *Neochetina eichhorniae* larval development. The size of the larvae increased over the eight weeks of the experiment, especially for the larvae reared on water hyacinth plants grown in the high nutrient levels when compared to larvae from medium and low nutrient levels (Figure 4.8). However, no test for significance could be applied as the larval development data set was too small due to the low number of replicates.

The larvae reached the pupal stage at different times in each nutrient treatment during the experiment. In the first week of my experiment, the larvae in all nutrient levels were in the first instar. However the larvae from the high nutrient levels grew faster than the others and reached the second and third instars before those from the medium and the low nutrient levels (Figure 4.8). Thus the larvae from the high nutrient levels were in the third instar on week 4 while those reared on water hyacinth plants from the medium nutrients were in the second instar and those

from the low nutrients were still in the first instar. In week five (34th day) of the experiment, a pupal case appeared on a plant grown in the high nutrient treatment. The larvae in the low nutrient treatment grew slowly and only reached pupation in week seven (48th day) while the appearance of a pupal case on plants from the medium nutrient level occurred in week six (41st day) of the experiment.

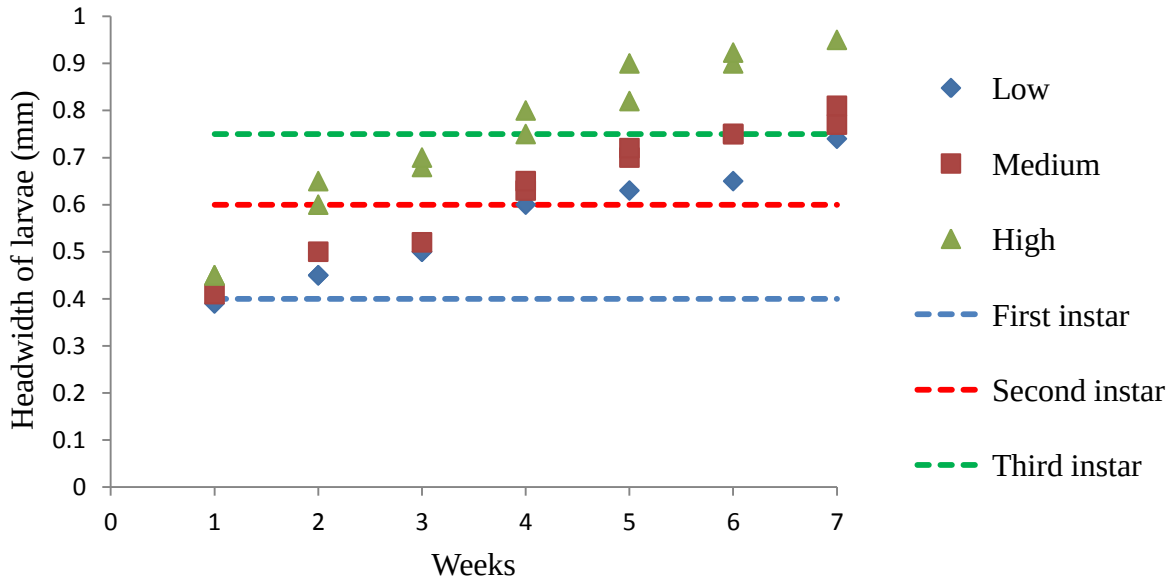

Figure 4.8: The effect of nutrients on *N. eichhorniae* larval development and progress through each developmental stage of larvae. The dotted lines show the instars of larvae according to their headwidth measurements at each nutrient level.

During the first and second instars, the larvae in all nutrients were found in the petioles into which they had been inoculated, but during the third instar the most larvae were found in the roots where they pupated. There was high mortality rate for the larvae reared on water hyacinth plants grown at the low nutrient level compared to the mortality rate of larvae reared on plants grown at the medium and high nutrient level (Table 3). However, larvae mortality was not tested due to small sample size.

Table 3: The effect of nutrients on *N. eichhorniae* larval survival. Larval mortality was calculated according to the number of larvae found or not found during the dissection of plants. Missing larvae were assumed dead.

Nutrient level	Number of larvae found	Number of larvae missing	%mortality
Low	9	7	43.75%
Medium	11	5	31.25%
High	13	3	18.75%

In summary, within each instar, larvae from high nutrient level have large size compared to the size of larvae from medium and low nutrient levels. The development of *N. eichhorniae* larvae was also faster in the high nutrient treatment and the larval survival rate was also greater at the high nutrient level compared to medium and low nutrient levels.

CHAPTER V: DISCUSSION

5.1. The effect of nutrients on plant growth and *N. eichhorniae* larval feeding

5.1.1. The effects of nutrients of plant growth

The influence of nutrient concentrations on the growth of water hyacinth and *N. eichhorniae* larvae feeding was shown by this study. The plants responded to different nutrient levels from since the day they were acclimated in nutrient solutions until the last week of the experiment. In the absence of weevils, elevated nitrogen and phosphorus resulted in healthy water hyacinth. Plants gained more weight and generally produced more leaves at high nutrient levels compared to those grown at the medium and low nutrient levels. Similarly, by manipulating nutrients like nitrate and phosphate concentrations (Table 4), Reddy *et al*., (1989, 1990), Xie *et al*., (2004), Ripley *et al.*, (2006) and Coetzee *et al*., (2007) demonstrated water hyacinth responded to increasing nutrients, growing rapidly with an increasing biomass, number of ramets, number of leaves and length of petioles. This is because, as determined by Ripley *et al* (2006), low amounts of nitrogen and phosphorus in water cause a decrease of chlorophyll content in the leaves, and a reduction of carboxylation efficiency in the plant, which negatively affects photosynthetic capacity and carbon acquisition. By measuring the size of the leaves and plant photosynthetic capacity (not measured in the present study), Lambers *et al* (2000) found that plants of water hyacinth grown at high nutrient levels had leaves with large surface area and the number of the leaves was high compared to water hyacinth plants from low nutrients. This resulted in high photosynthetic rate. Hence plants grew rapidly by gaining weight and producing ramets. Photosynthesis is important in plant growth, and when it is disturbed, the plants cannot grow well (Nishio, 2000). Therefore, the higher quantity of nitrogen in South African water bodies is one factor causing an increase in plant biomass and other plant growth parameters, which results in rapid proliferation of water hyacinth plants (Coetzee & Hill, 2012).

5.1.2. The effect of nutrients on *N. eichhorniae* larval feeding and water hyacinth growth

The present study showed that nutrients significantly influenced the weevils' feeding on water hyacinth growth and *N. eichhorniae* larvae significantly decreased the biomass of water hyacinth

and its growth parameters at all nutrients levels. However, the larval feeding was higher for the plants grown at the low nutrient level. Using different nutrient concentrations (Table 4), (Heard & Winterton (2000), Xie *et al*., (2004) and Ripley *et al*., (2006) showed that insect herbivores induced a significant decrease in water hyacinth growth parameters (short petioles, loss of weight, and loss of leaves) particularly when grown at low nutrient levels compared to those grown at high nutrient levels. The larvae are known to tunnel through plant petioles, and thereby consume the nutrients stored in the petioles (Wilson, 2002). When this is happening in plants poor in nutrients, the plants lose more tissue material, as determined by this study, because the nutrients from the roots are consumed by the larvae instead of going to the leaves where they contribute to the photosynthesis and carbon accumulation and these processes are biologically important for plant growth (Ripley *et al*., 2006). At the low nutrient levels, larvae tunnelled the petioles and destroyed more plant tissues, trying to gain the nutrients necessary to complete their development. Larvae tunnelling the petioles also contributed to a significant decrease of water hyacinth growth because the tunnels made in the petioles facilitate the entrance of pathogens like bacteria and fungi which can inhibit the growth of plant (Ripley *et al*., 2008). These pathogens are known to cause necrosis infection which contributes to a reduction in photosynthetic rates, translocation of water and nutrients and higher permeability of plasma membranes (Moran, 2005).This prevents the production of leaves, causes the dying of leaves and decrease of plant weight (Lambers *et al*., 2008) as plants cannot grow properly when the above physiological processes are disturbed. Through laboratory trials, Venter *et al*., (2013) showed that the pathogens carried by adults of *Neochetina* weevil also had a great effect on the decrease of plant growth. This is because the pathogens are to decrease the leaf surface area and thus induce the reduction of photosynthetic productivity. In general, those pathogens were able to induce 37% of plant weight loss (Venter *et al*., 2013).

The quantification and the understanding of larval biomass removal from water hyacinth plants at each nutrient level can inform models which can be used to manage water hyacinth invasion (Hauptfleisch, 2015).

Since such high larval feeding was most noticeable in plants grown at the low nutrient levels, the low feeding rates at high nutrient levels suggest that it is necessary to consider the combination of *N. eichhorniae* and pathogens in infested water bodies rich in nutrients as this combination has

been shown to be promising and add to biological control of water hyacinth invasion in such water bodies (Moran, 2005).

In general, the larval feeding rates at all the nutrient levels declined during the last weeks of the experiment (week 7 and 8, Figure 4.3) where larvae in all nutrient levels were in the third instar and prepared for pupation. As determined by Scriber and Slansky (1981), in general the early stages larvae have a higher nutrient consumption than older stages of larvae because a great amount of nutrients are needed to accelerate the metabolic rates and other physiological process including the increase of larval size, increase in larva body weight, formation of other parts of the body and these are known to occur during early larval instars not in the later instars and pupation stage. *N. eichhorniae* larvae damaged more plant materials in first and second instar than third instar as they needed more nutrients to complete all physiological processes supposed to occur during early larval instars. However such decline of feeding rate in the third larval instar and zero consumption by pupae may have negative effects on the successful biological control of water hyacinth, because during these periods water hyacinth plants can regrow and reinfest water bodies. The biological control practioner here need to consider Integrated Pest Management (IPM) which involves the combination of herbicides and weevils on water hyacinth (Katembo *et al.,* 2013).The chemicals such as retardant dose of glyphosate are able to reduce and inhibit water hyacinth growth, not harming *Neochetina* larvae development or pupal metamorphosis. It has been found that larvae and pupae survived better on water hyacinth treated with a retardant dose of glyphosate and this herbicide can decrease water hyacinth growth (Jadhav *et al.,* 2008).

When plants are attacked by herbivory insects, they have their own way to tolerate insect damage as they have to try to retain their fitness by maintaining or increasing weight, number of the leaves or other growth parameters but it depends on nutrient conditions in which plants are growing (Trumble *et al*., 1993). Hawkes & Sullivan (2001) found that monocotyledon plants grown at low nutrient levels lost more biomass and leaves because they could not compensate for insect damage compared to plants rich in nutrients. However, our results showed that plants subjected to *Neochetina eichhorniae* larvae in general experienced undercompensation. Normally undercompensation occurs when insect herbivory is detrimental and the productivity of the plant exposed to herbivory is less than unexposed plant. At all nutrient levels, the plants

exposed to the insects lost biomass and leaves as they could not compensate for the tissues lost due to larval feeding but the loss was less for the plants grown at high nutrient levels. Hence, the inability of water hyacinth to compensate with *Neochetina eichhorniae* larval feeding induced the disruption of leaf turnover (Figure 4.4) at all nutrient treatments as the plants were unable to equal-compensate or overcompensate for the leaf loss which resulted in biomass loss even if the loss was different according to nutrient levels. As determined by Bownes (2009), at the low nutrient levels, plants may undercompensate because of two reasons: (1) Lower plant growth and leaf turnover rates at the low nutrient level, negatively influence the carbon acquisition and photosynthetic capacity. (2) Plants poor in nutrients have low levels of nitrogen based compounds which induces a low level of foliar nitrogen, thus insect herbivory significantly damages water hyacinth in lower nutrient levels. However, when comparing plants grown at high nutrient with insects and those grown at low nutrient without insects (Figure 4.5 and Figure 4.7), where number of the leaves and length of longest petiole are equal, we conclude that there was an equal compensation, which occurs when plants subjected to herbivory have the same productivity with plants not subjected to herbivory. These results are consistent with Heard &Winterton (2000) who showed that plants from a high nutrient treatment group and those from the control group had the same number of added leaves (Table 4).

The significance of these findings is that *N. eichhorniae* significantly can contribute to water hyacinth control and this can be successful when controlling water hyacinth plants from water bodies poor in nutrients (Byrne *et al*., 2010) where plants cannot compensate or undertake equal-compensation for the weevil's damage. However in water bodies rich in nutrients where water hyacinth plants can equal-compensate, and lose less biomass, leaves and other plant growth parameters, Integrated Pest Management (IPM) is the best option to control water hyacinth invasion. Jadhav *et al.* (2008), through laboratory trials and Katembo *et al*, (2013) field trials, showed that a combination of a sub-lethal dose of glyphosate and *Neochetina* weevils significantly reduced water hyacinth invasion and that herbicides did not impact the growth and development of weevils. Jadhav *et al.,* (2008) found that the high feeding rate of weevils was observed on sprayed plants compared to non sprayed plants. Thus the synergistic effect of the sub lethal dose of glyphosate on plant growth and feeding of weevils will have a great contribution to control water hyacinth invasion.

Table 4: Concentrations of nitrogen and phosphorus used in previous studies and the present study to investigate their effects on the growth of water hyacinth plants and the effects biological agents on plant growth parameters at different nutrient levels.

Authors	Organism/Plant	N (mg/l)		P(mg/l)		Plant growth (C Plants)				Plant growth (T plants)			
						Biomass (g)		# of the leaves		Biomass (g)		# of leaves	
		High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
Reddy et al., 1989;1990	Water hyacinth	50.5	0.5	10.06	0.06	3820	38	-	-	-	-	-	-
Ripley *et al*., 2006	*E. catarinensis* +Water hyacinth	200	0.2	20	0.01	-	-	-	-	6	3.9	-	-
Coetzee et al., 2007	*E. catarinensis* +water hyacinth	50.5	0.5	2.56	0.05	175	110	32	16	169	100	28	10
Center &Dray, 2010	Water hyacinth	8.5	0.5	0.03	0.04	-	-	-	-	-	-	-	-
Present study	*N. eichhorniae* +water hyacinth	6	0.7	0.83	0.01	20	7	8	6	-30	-60	6	3
Heard &Winterton, 2000	*Neochetina* spp+ water hyacinth	1.6	0.4	1	0.02	10	7.5	8	7	5.5	7	6	3

N = Nitrogen

P = Phosphorus

- = No measurements taken

C = Control plants (plants not exposed to insects)

T = Treatment plants (plants exposed to insects)

= number of leaves

5.2. **The effects on nutrients on *N. eichhorniae* larval development**

Since nutrients are the major factors that influence the growth of herbivores and other living organisms (Mattson. 1980), they therefore have a great effect on the weevils' development (Heard and Winterton, 2000). The larvae reared on water hyacinth plants with higher amounts of nutrients, had a large size, survived better, developed more quickly and reached the pupation stage earlier than the larvae reared on plants with low level of nutrients. Deloach and Cordo (1976); Chikwenhere (2000); Wilson *et al***.,** (2006) and Bownes (2009), using different nutrient concentrations (Table 5) showed that larvae developed quickly under high nutrient levels and the survival rate was also high for larvae reared on plants grown at the high nutrient levels (Table 5). *Planococcus citri* Risso (Homoptera: Pseudococcidae) larvae reared on plants grown at high nutrient level developed in a shorter time and their body size increased compared to *P. citris* larvae reared on plants grown at low nutrient levels (Hogendorp *et al*., 2006). The larvae of *Paropsis atomaria* (Coleoptera: Chrysomelidae) increased their body size when nutrient availability increased (Ohmart *et al.,* 1985). *Agromyza nigripe* (Fallen) (Diptera, Agromyzidae) larvae reared on plants grown at high nutrient levels also developed quickly compared to those grown in low nutrient level and which resulted in an increase of *Agromyza nigripe* population (De Bruyn *et al*., 2002). However at shortage of nutrients, insect larvae have three known strategies that they can use to complete their development. (1) They can pupate at smaller than usual size and carry the cost of that as adults, for example females will not be able to lay many eggs and male adults will be less competitive (Colasurdo *et al*., 2009). For example, caterpillars of *Malacosoma disstria* (Hübner**) (**Lepidoptera: Lasiocampidae). (2) They can undertake a feeding compensation process through which larvae damage more plant tissues until they find sufficient nutrients to complete their development (Ojeda-Avila *et al*., 2003; Huberty and Denno, 2006). For example, when moth larvae do not have sufficient food, they usually increase their consumption on the plant host to get the required amount of nutrients to complete growth (Colasurdo *et al*., 2009). (3) Some insect larvae can create symbiotic relationship with bacteria, protozoa or yeasts which can provide the nutrients by converting non essential nutrients to amino acids considered as the source of nitrogen necessary to complete the larval development (Chown and Nicolson (2004). For example *Drosophila melanogaster* (Diptera: Drosophilidae) larvae feed on yeast during nutrient shortages to get nutrients necessary to complete their development (Ja *et al*., 2007).

Our study revealed that *N.eichhorniae* larvae reared on plants poor in nutrients undertook the second strategy (2), whereby larvae experienced feeding compensation by damaging more water hyacinth tissues to get nutrients required for their full development. However, this development took a longer time. Similarly, Slansky (1981) also found that at the low nutrient level, the total consumption of plants was high when attacked by herbivory insects. The significance of this is that at the low nutrient level, *N. eichhorniae* weevils are expected to remove a greater amount of water hyacinth biomass because of its increased consumption on plant materials. This shows that *N. eichhorniae* has a high potential role in controlling water hyacinth infestations and this helps to predict the successful biological control on water hyacinth. This is the reason why water managers should aim to decrease nutrient levels and limit the increase of nitrogen and phosphorus in water bodies.

Table 5: Nitrogen and phosphorus concentrations used in previous studies and the present study to investigate larval development under different nutrient regimes on water hyacinth (from the lowest to the highest)

Authors	Organism/Insect	N (mg/l)		P (mg/l)		Larval development					
						Headwidth (mm)		Survival rate (%)		Developmental time (days)	
		High	Low	High	Low	High	Low	High	Low	High	Low
Bownes, 2009:	*C. aquaticum*	6	0.034	1.37	0.024	-	-	82	64	39	42
Present study:	*N. eichhorniae*	6	0.7	0.83	0.01	0.95	0.70	81.2	56.25	35	48
Chikwenhere, 2000	*N. bruchi*	6	2	1.6	0.2	-	-	43.7	33	-	-
Wilson *et al.*, 2006	*Neochetina spp*	4	0.4	0.57	0.057	0.76	0.70	82	64	-	0.4
Stanley *et al.*, 2007	*Xubida infusela*	1.6	0.1	1	0.02	-	-	57	45	-	-

N: Nitrogen

P: Phosphorus

-= No measurements taken

CONCLUSION

This study aimed to determine if water nutrients influence larval development and the feeding rate of *N. eichhorniae* larvae on water hyacinth plants, in order to predict the effects of biological control on water hyacinth under different nutrient regimes. Nutrients significantly influenced water hyacinth growth, and the plants exposed to *N. eichhorniae* larvae significantly lost weight compared to unexposed plants (control). Hence, water hyacinth plants grown in low nutrients showed a significant decrease of their growth parameters (weight, leaves and length of longest petioles) because they were not able to recover the damaged tissues. The larval feeding rate was higher in plants grown in the low nutrient levels than those grown in the medium and high nutrient levels. Larvae in low nutrient levels consumed more plant materials to get the nitrogen and phosphorus necessary to complete their development which took more days compared to larvae from high nutrient levels. Since larval development was prolonged, the pupation stage occurred later compared to larvae from high and medium nutrient.

Implications of this study are that *N. eichhorniae* can reduce water hyacinth growth in oligotrophic water bodies. However, in eutrophic environments where plants can undertake equal-compensation, *N. eichhorniae* cannot reduce water hyacinth growth. Therefore the Integrated Pest Management which involves the combination of herbicides and weevils needs to be considered in the eutrophic water bodies. Here herbicides will be applied as a strip of lethal, spray down to the center of water body, declining to a sub-lethal dose at the margins of spray drift.

Since nutrient levels have a great importance in biological control, it is necessary to know the exact number of weevils need to collapse one plant of water hyacinth at each nutrient level as it can help biocontrol practioner to know how many weevils.

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