The role of nutrients in the biological control of water lettuce, *Pistia stratiotes* Lamarck (Araceae) by the leaf-feeding weevil, *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae) with particular reference to eutrophic conditions

Thesis

Submitted in fulfillment of the requirements for the degree of Master of Science at Rhodes University

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December 2005

Frontispiece

Fig. i. Thick water lettuce mat at Cape Recife before biological control was implemented (April 2003).

Fig. ii. Cape Recife after the *Neohydronomus affinis* weevils successfully caused the water lettuce weed mat to crash (September 2003).

Fig. iii. *Neohydronomus affinis* larva and pupa (photograph courtesy USDA).

Fig. iv. *Neohydronomus affinis* adults and their feeding shotholes on water lettuce leaf (photograph courtesy of T. Center, USDA).

Fig. v. *Neohydronomus affinis* larval and adult feeding damage.

Acknowledgements

I would very much like to thank the following people and institutions for supporting this thesis: Rhodes University for their sponsorship and funding of the project.

Professor Martin Hill for all his help, guidance and funding throughout the project.

Professor Tony Booth, Professor Martin Villet, and Jeremy Baxter for all their help on the statistical aspect of the project.

Rob Williams and Cape Recife Nature Reserve Nelson Mandela Metropolitan Water Reclamation Works for allowing me to sample their ponds.

Professor Ric Bernard for kindly providing his electronic balance for weighing the plants.

Lesley Henderson and Candy Roux for kindly providing the map of distribution of water lettuce in South Africa.

Jeanette de Leeuw for supplying nitrogen data of water quality at Cape Recife.

Garth Sampson for providing Port Elizabeth climate information.

Brad Ripley and Michelle Behenna for assistance in making nutrient concentrations.

Daniel Parker for support out in the field and for his input on the manuscript.

Table of Contents

Table of Figures

Index of Tables

Abstract

Water lettuce, *Pistia stratiotes* Lamarck (Araceae) is a South American plant that has the potential to be a very damaging and important aquatic weed in many tropical countries, including South Africa. It has the potential to rapidly multiply vegetatively and completely cover watercourses in a very short space of time outside of its natural range under ideal conditions and without its natural enemies. In such instances, the weed may cause hindrances to water transport and fishing, increasing chances of malaria, as well as affecting the natural ecology of the system. Water lettuce can also set seed, which may lay dormant for long periods, germinating when conditions are favourable. It is therefore very necessary to adopt control methods against the weed where it is a problem. However, water lettuce has also been effectively and completely controlled in many countries by the leaf-feeding weevil, *Neohydronomus affinis* Hustache. High nutrient levels in the form of nitrates and phosphates have been shown to have largely negative effects on biological control in several studies, with control being incomplete or taking longer than in similar areas with lower nutrient levels.

The effectiveness of *N. affinis* on the biological control of water lettuce was investigated in a laboratory study, growing *P. stratiotes* plants with and without insects at different nutrient concentrations. In these studies biological control of water lettuce with *N. affinis* was found to be complete under eutrophic nutrient conditions, although control took longer when higher nutrient levels were tested.

A field site study was conducted at a sewage settlement pond in Cape Recife Nature Reserve near Port Elizabeth, South Africa. This highly eutrophic system was used as a field example for the effectiveness of biocontrol of *P. stratiotes* by *N. affinis* under eutrophic conditions. The weevils at Cape Recife caused a massive and rapid crash in the percentage coverage of the weed, from 100% in May 2003, to approximately 0.5 % in September 2003. Plant growth parameters were also found to decrease considerably in size correspondingly with this crash from May 2003 until spring 2003. Plant size only again started to increase gradually but steadily through spring 2003 and into summer.

In the laboratory studies, the fecundity of weevils was shown to be much higher on plants grown under higher nutrient concentrations than on plants grown in lower nutrient concentrations. The results from the wing-muscle analysis under different nutrient concentrations were not easy to interpret, and there were few differences in wing muscle state between most of the concentrations.

From these findings it is suggested that nutrient concentration, particularly high levels of nitrates and phosphates is not a limiting factor in terms of effective biological control of *P. stratiotes* with *N. affinis*, but that under high nutrient conditions biological control might take longer.

Chapter 1

General introduction and literature review

1.1. Description of water lettuce

Pistia is a monotypic genus in the subfamily Aroideae (Grayum, 1990). There are at least 2 extinct species; *Pistia siberica* Dorofeev (Dorofeev, 1955, 1958, 1963 (in Russian)) and *Pistia corrugate* Lesquereux (Stockey *et al.*, 1997). The genus is also closely associated with the fossil genus *Limnophyllum* Krassilov, through which it is related to the Lemnaceae (Kvacek, 1995; Stockey *et al*., 1997). Water lettuce, *Pistia stratiotes* L. is the only extant species, in this genus. It is a free-floating stoloniferous, small aquatic perennial herb belonging to the aroid family (Araceae). Water lettuce is thought to have originated from South America, but it is now pan-tropical, and is considered a weed in many tropical countries. The leaves are grey-green, densely pubescent, and wedge-shaped (obovate-cuneate). Conspicuous parallel veins run down the leaves with leaf bases often having thick, spongy parenchymous tissue at the base. Leaves range from 2-35 cm long and vary in shape from being slightly broader (at the apex) than long to much longer than broad (Dray and Center, 2002).

Pistia roots are unbranched with many lateral rootlets (Sculthorpe, 1967). The flowers are relatively inconspicuous pale green spathes near the centre of the rosette. These spathes are constricted near the middle, with whorls of male flowers above and a single female flower below the constriction. The seeds are housed in green berries. Mature seeds are hard, wrinkled and golden brown in colour (Dray and Center, 2002).

1.2. Distribution of water lettuce

Pistia stratiotes is widely distributed through much of the tropics and subtropics. The free-floating plants are found in reservoirs, ponds, and marshes along the edges of large lakes where they thrive amidst the offshore vegetation and debris as well as slow-moving or stagnant water. The plants are cold sensitive and are usually restricted to areas between the tropics of Cancer and Capricorn, however it can survive as an annual in colder climates (T. Center, pers comm.). Water lettuce has a minimum growth temperature requirement of 15 º C, with an optimum growth temperature of 22-30 º C, and a maximum growth temperature tolerance of 35 º C (Kasselmann, 1995).

1.3. Origin of water lettuce

The origin of water lettuce is still uncertain, (Sculthorpe, 1967; Cordo *et al*., 1981). However, 11 host-specific weevil species have been found on the plant in South America, suggesting a neotropical origin for the plant (Bennett, 1975; Cordo *et al*., 1981). Grayum (1990) suggested that *Pistia* is an ancient genus with subtropical Laurasian origins, which later migrated into tropical West Gondwanaland. This view is supported by recoveries of fossil *Pistia* genus species in strata from the Upper Cretaceous Period (103-65 million years ago [MYA]) in the United States and southern France, and in strata from the Tertiary Period (65-2.5 MYA) in the southern United States and western Siberia (Stoddard, 1989). Stoddard (1989) argued that Florida served as a refugium for the genus *Pistia* during the Tertiary Period and that the genus is therefore native to the United States. However, July temperatures in the southeastern United States were on average 12 ° C colder in the Pleistocene than present times (Watts, 1980) and it is likely that the genus would have become extinct (Stuckey and Les, 1984). Support for this hypothesis is found in the lack of specialist herbivores found on water lettuce in Florida compared to other parts of the world (Dray *et al*., 1993). Ancient folk medicines using *P. stratiotes* are known from Africa and Asia (Stoddard, 1989), which would argue for the origin of the plant in these regions. However the lack of specialist herbivores in these two regions creates little support for these two theories. Considering this fact that most of the phytophagous insects found on water lettuce are to be found in South America would tend to suggest that the plant originated in Latin America, but likely dispersed widely from there many years ago.

1.4. Biology

Pistia stratiotes has short, depressed hairs on both surfaces which trap air, repel water, and thus prevent the epidermis from becoming wet (Sculthorpe 1967). All the leaves are succulent and some have conspicuous, ovoid swellings on the undersides filled with spongy parenchyma, which gives flotation to the plant. The bladder-like swellings with aerenchyma cells are several centimetres long and usually contain 70 % air. In *P. stratiotes,* transpiration takes place through apical hydathodes, which are located in a protected pocket. Beneath the pore is a cavity lined with thin-walled cells and into this chamber the tracheids of the vein endings open (Sculthorpe, 1967).

1.5. Reproduction

Water lettuce reproduces mainly by vegetative offshoots that are connected to the mother plant by stolons, which may be 60 cm in length. The vegetative buds that give rise to these extensions form in a lateral pocket, which is derived partly from the leaf sheath and partly from the axial tissue. In Africa, it is believed that the plant reproduces principally by seeds (Sculthorpe, 1967). However, vegetative propagation is very prevalent in Africa, and probably the most common form of reproduction with regards to water lettuce (personal observation).

Flowering, fruiting and seed production have been observed in Australia, Thailand, Brazil, India, the Philippines and a few African countries (Holm *et al.*, 1977; da Silva, 1981; Harley, 1990). Although, it is most likely that it flowers and sets seed throughout it's distribution, only that it has not been documented thoroughly. In India, flowering begins in the hot season and continues up to the rainy season: the fruits appear after the rainy season. Most plants produce three to eight flowers in a whorl at the centre. The flowers are 1-2 cm long, lack perianth, and have a unilocular ovary. When it separates, the spathe first exposes the pistil: then within a few hours the stamens and the flowers abort. The period from the appearance of the first flower buds until the flowers open is about 8 days. The flowers fall from the plant within 2 weeks. The seeds are small and float on the water for 2 days, after which they sink and germinate. Seedlings then generally float to the surface within 5 days (Sculthorpe, 1967).

The viability of water lettuce seeds is variable. Dray and Center (1989) found that about 80 % of mature seeds from fruits collected in February in Florida germinated. This was much higher than the 24 % reported by da Silva (1981), but compared favourably with seed viabilities reported in India by Datta and Biswas (1969) and Mitra (1966). For germination, mature seeds required an after-ripening period of 7-14 days. Those from the seed bank began germinating within a day. These seeds can remain dormant for months (Buangam and Mercado, 1975; Mercado-Noriel and Mercado, 1978), withstand freezing and drought (Pieterse *et al.*, 1981) and still germinate when favourable conditions prevail (Dray and Center, 1989). The ability of seeds to lie dormant, leads to *P. stratiotes* becoming a problem in areas with seasonal water, such as seasonal pans, where the seeds germinate at the beginning of new rains. This makes control difficult, because of a resurgence of new seedling plants.

1.6. Pest status of *Pistia stratiotes*

Water lettuce forms extensive mats; capable of blocking navigational channels, impeding water flow in irrigation and flood control canals, and can disrupt submersed animal and plant communities (Sculthorpe, 1967). Water lettuce is also recognized as being among the world's worst aquatic weeds (Holm *et al*., 1977) because of its invasive properties; i.e. very fast growing, reproducing and spreading. It has been placed on prohibited plant lists in many countries (Dray and Center, 2002). In the United States, it is 'state-listed' as a prohibited plant in Arizona, Florida, and South Carolina. However in other states of the U.S, it is available for sale as a pond-plant (http://www.aquat1ifas.ufl.edu/seagrant/pisstr2.html).

1.6.1. Economic damage and threat to human and animal health

Water lettuce is a serious weed of rice crops in several countries where it competes for space and nutrients with rice (Suasa-ard, 1976), but has not been reported as interfering with production in the United States. It may also interfere with hydroelectric operations (Napompeth, 1990) where the plants block turbines and pipes, leading to equipment damage, as well as a loss in efficiency of hydroelectric power production and labour costs. Direct losses can also be attributable to water lettuce clogging up and restricting water flow irrigation equipment and in flood control canals. The economic costs of such losses, however have not been quantified, but federal and state water lettuce control operations in Florida alone cost nearly $650 000 in 1994 (Center, 1994). The plants may also form mats, preventing livestock from drinking, and may be a threat in terms of drowning livestock or children, who may not discern the difference between water and land, because of the weed coverage. Degradation of water quality also occurs, from plants dying and sinking, creating anoxic conditions.

Indirect losses accrue when large floating mats interfere with recreational activities such as boating and fishing, but these have not been quantified. In tropical Africa, water lettuce has been linked to increased malarial infections, likely due to the plants providing refuge for the mosquito larvae, where they are safe from fish predation.

Several species of mosquitoes, responsible for causing malaria, encephalitis, and filariasis breed and thrive with water lettuce present (Dunn, 1934; Bennett, 1975; Lounibos and Dewald, 1989; Lounibos *et al*., 1990). Costs associated with these diseases are unknown, and portions of mosquito control operations directed toward water lettuce-borne mosquitoes have not been reported. Dense water lettuce mats also impede spraying operations and limit access to water sources by boats, interfering with the livelihood of resource-poor people in third world countries who rely on open water for fishing and transport across lakes and rivers.

1.6.2. Ecological damage

Few reports of deleterious ecological impacts associated with *P. stratiotes* infestations have been reported, and these studies have generally been limited in scope. Sculthorpe (1967) noted that the intertwined root systems of extensive infestations accelerate siltation rates as they slow water velocities in rivers and streams. The resultant degradation of benthic substrates under these infestations has not been studied, but accelerated siltation often renders the affected benthos unsuitable as nesting sites for various fish species (Beumer, 1980) and as macroinvertebrate habitat (Roback, 1974). The accumulation of water lettuce-generated detritus under large mats only adds to this problem and is likely to increase sediment and nutrient loadings much as it does under water hyacinth mats (Schmitz *et al*., 1993). Sridhar (1986) also reported that water lettuce can bioaccumulate considerable amounts of heavy metals, rendering the detritus under the mats toxic. These heavy metals could well have a negative effect on biocontrol agents feeding on the plants (Center *et al*., 2002).

Water under dense water lettuce mats becomes thermally stratified (Sculthorpe, 1967; Attionu, 1976), with much reduced dissolved oxygen levels and increased alkalinity (Yount, 1963; Attionu, 1976; Sridhar and Sharma, 1985) with increased mortality of fish (Ayles and Barica, 1977; Clady, 1977) and macroinvertebrates (Roback, 1974; Cole, 1979). Sharma, (1984) reported that the evapotranspiration rate over a water lettuce mat in one African lake was ten-fold greater than the evaporation rate over similar open water. However, the discussion in Allen *et al.*, 1997 would tend to suggest that this figure might not be a true reflection of the amount of evaporation. This could lead to premature drying up of pans and other temporary water-sources, further affecting natural cycles of native flora and fauna within them.

1.7. Water Lettuce in South Africa

In South Africa, water lettuce is declared a noxious weed in terms of the Conservation of Agricultural Resources Act, (Act 43 of 1983), however, it was not regarded as damaging as water hyacinth (Cilliers, 1987). However, it is still a very damaging weed, especially in areas where biological control agents are not present, such as remote pans. Water lettuce is often out-competed by water hyacinth where both occur, and therefore its full potential of damage is often not seen (personal observation). Water lettuce has occurred in the low-lying subtropical areas of the Transvaal (Gauteng) since 1953, when it was first recorded on the Pafuri River (Cilliers, 1987). In KwaZulu-Natal, the weed was first recorded as early as 1865 on the Umhlanga River; since 1981 it has only been recorded from 1 locality (Gonubie) in the Eastern Cape Province (National Herbarium, Botanical Research Institute, Pretoria). However, a recent infestation at Cape Recife in Port Elizabeth, suggests that its distribution may be wider than previously thought in the Eastern Cape Province.

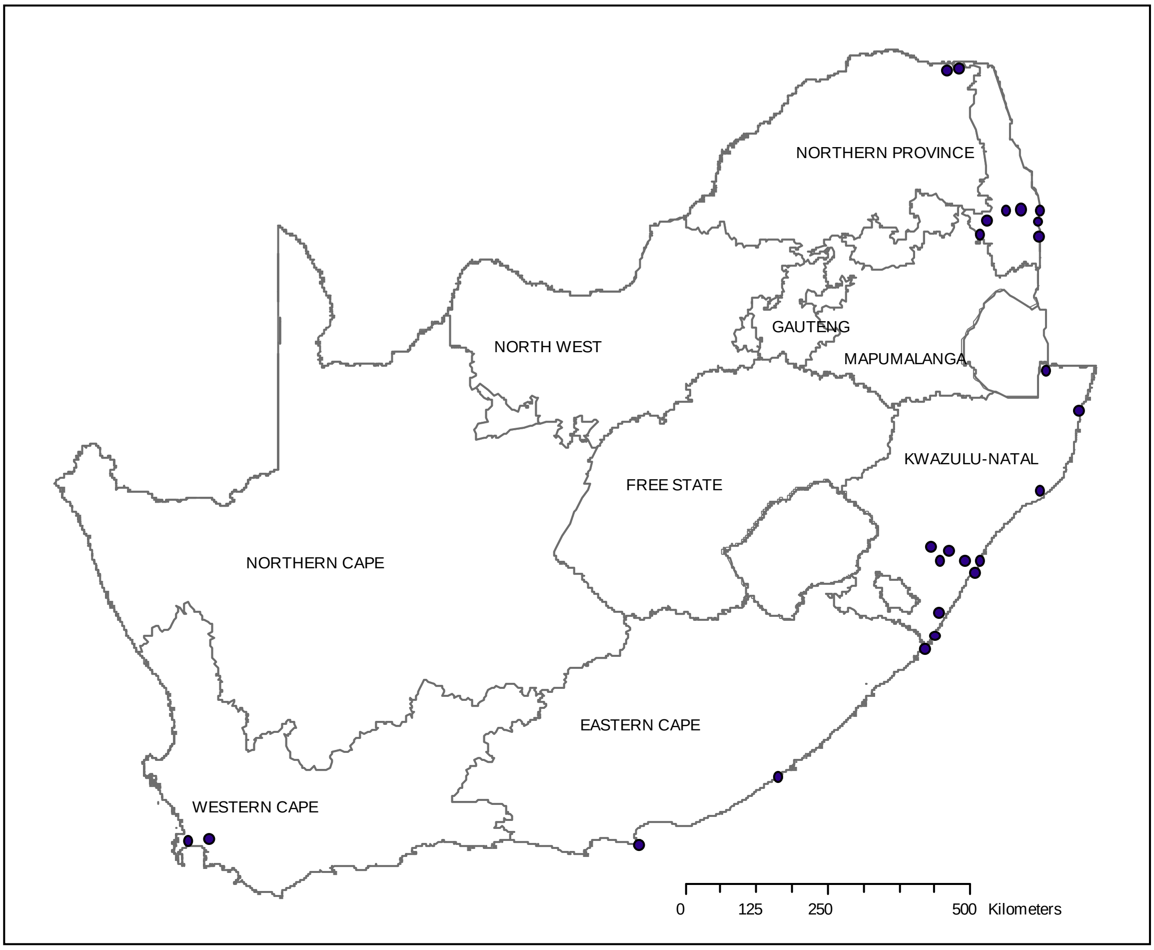

Figure 1.1. Distribution of water lettuce in South Africa (map provided by Lesley Henderson, Plant Protection Research Institute from the SAPIA databases). Black dots indicate recent records of reported water lettuce infestations.

Water lettuce is one of three important aquatic weeds in the Kruger National Park (KNP). It occurs in several areas within the park, including seasonal pans in the northern Pafuri area, on the Limpopo flood plain and in the southern area of the perennial Sabie River (Cilliers *et al*., 1996). The pans where water lettuce is a problem include Nhlangaluwe, Dakamila, Makwadsi and Mapimbi. These pans are seasonal but may contain water for several seasons depending on rainfall (Cilliers *et al*., 1996). The Sabie River runs through the southern part of the KNP where originally 12 km of the river was infested with water lettuce; a sparse infestation further downstream was followed by a dense infestation at lower Sabie over approximately 3 km (16-20 ha). Control of water lettuce in these pans was of concern, as they are not very accessible and infestations of the weed, threatening the indigenous flora and fauna (Chickwenhere and Forno, 1991; Deacon and Gagiano, 1992, Zeller, 1993; Cilliers, *et al*., 1996). Apart from the detailed report of control of water lettuce in the KNP there appear to be few accounts of the impacts of water lettuce in South Africa.

1.8. Control of water lettuce

1.8.1. Chemical control

Terbutryn is the only herbicide currently registered for control against *P. stratiotes* in South Africa (Grobler *et al*., 2000), although glyphosate and 2, 4 D-amine have also been used with some success. However, 2, 4 D was discontinued in both South Africa and Zimbabwe because of concerns about its effects on broad-leaved crops surrounding rivers. Terbutryn is usually applied as a 3 % mix with water either from a boat or from riverbanks using backpack spray units (Cilliers *et al*., 1996). In the United States, glyphosate, copper and diquat are registered for use against water lettuce (http://edis.ifas.ufl.edu/Topic_guide_aquatic_weed_management).

The most thorough account of herbicide control of water lettuce is from the KNP. In the KNP, chemical control of water lettuce on the Sabie River was conducted in 1987 and by the end of 1988; 6 km of river were under control (generally considered to be 10-20 % weed coverage, Hill, pers comm.). In 1989, these controls were continued, and were supplemented by an aerial application of Igran at 30 % by helicopter using a micronair system (giving 6 litres/ha). Twelve kilometers of river below Skukuza was cleared of water lettuce and maintained by 2 follow-up operations the same year. These follow-ups were implemented in 1990 using Igran (triazine), Roundup (glyphosate) or Arsenal (imazypur) and the plant was thought to have been eradicated from this section of the river (Zeller, 1993, unpub. report).

One of the problems with chemical control is that it is expensive, not sustainable, and re-applications of herbicides have to be administrated frequently, as seed-regeneration occurs with water lettuce as soon as light and temperature conditions are favourable. Many herbicides also have adverse effects on biocontrol agents, (Ueckermann and Hill, 2001) which is important when integrated control is considered. Unfortunately, very little literature is available for chemical control of water lettuce outside of South Africa and Zimbabwe.

1.8.2. Manual and mechanical control

Because of the rapid growth and reproduction potential of this plant species, manual and mechanical control, especially on a large-scale is not really suitable or sustainable and therefore is not recommended. Rapid regeneration of plants from vegetative reproduction or by seed would limit the success of these control methods. Manual control would involve physical labour, collecting plants by hand, where they are removed from the water and dried on the bank. Mechanical control would be when machines replace manual labour (Lindsey and Hirt, 2000). These could include harvesting machines, conveyors, draglines, mowing and dredging buckets and push boats.

These methods of control would have to be continuous and would be better limited to small infestations. There are other problems associated with mechanical control, such as finding suitable areas to dump the weeds where they will not re-infest the water source, which may involve transport costs, labour costs, with rotting weeds also producing unpleasant smells and health risks. Plant heaps are also aesthetically unpleasing and may harbour breeding sites for malarial mosquitoes, unless treated with an insecticide. The water lettuce could be used as compost as indeed water hyacinth (Lindsey and Hirt, 2000) and salvinia has been, however, there are no references to this. Mechanical removal may also hinder the usefulness of biological control, especially when there are only a few plants left with agents, it would be better to leave the weed to biological control in such instances.

1.8.3. Biological control

1.8.3.1. Introduction

Biological control of water lettuce has been highly successful in most areas around the world, where the weed is present, mainly due to the weevil *Neohydronomus affinis*, which has been introduced widely along with the weed. *Neohydronomus affinis* has been officially released in at least ten countries; Australia, Benin, Botswana, Ghana, Papua New Guinea, South Africa, Senegal, United States of America, Zambia, and Zimbabwe (Julian & Griffiths, 1998).

There are also 2 moth species that are very destructive to water lettuce, but one, *Samea multiplicalis*, Guenee (Lepidoptera: Pyralidae) is not entirely host-specific. In addition, the release and establishment of *Spodoptera pectinicornis* Hampson (Lepidoptera: Noctuidae) in North America has been unsuccessful, despite mass releases at numerous sites (Dray and Center, 1993). There are several other likely host-specific weevil species in South America; however, at present in South Africa, *N. affinis* appears to be highly destructive and adequate to successfully control water lettuce in most situations, without the need for further biocontrol agents (personal observation).

1.8.3.2. *Neohydronomus affinis* Hustache (Coleoptera:Curculionidae)

1.8.3.3. Biology of *Neohydronomus affinis*

Adult *Neohydronomus affinis* are small (3 mm long) and have a nearly straight rostrum that is strongly constricted ventrally at the base. *Neohydronomus affinis* ranges in colour from uniform bluish grey to reddish brown (depending on age) with a tan, lunate band across the elytra. The colour pattern is associated with scales and may be difficult to distinguish if they are wet, dirty, or missing (Center *et al*., 2002).

The eggs are cream coloured and subspherical (0.33 mm by 0.40 mm). Females chew a hole of about 0.5 mm diameter in the water lettuce leaf (usually the upper surface near the leaf edge), deposit a single egg inside this puncture, and close the hole with frass. The eggs usually hatch within 4 days (at temperatures above 24 ° C). The young larvae, which are very small (head diameter of 0.2 mm), burrow under the epidermis and work their way toward the spongy portions of the leaf at a rate of about 1.5-2.0 cm/day (Center *et al*., 2002). Larval mines are often plainly visible in the outer third of the leaf where tissues are thin, but are less apparent in the central and basal portions of the leaf.

The first moult occurs when larvae are about 3 days old and the second, 3-4 days later. Second-instar larvae have heads 0.25-0.27 mm in diameter; third-instar larvae are 2.5-3.0 mm long and have heads 0.32-0.37 mm in diameter. The larval stages last 11-14 days in total (Center *et al*., 2002). Third instars are generally found excavating the spongy portions of the leaf where they moult to become naked pupae. Under optimum temperatures, 4-6 weeks are generally required for *N. affinis* to complete the transition from egg to adult. Adults chew holes (about 1.4 mm in diameter) in the leaf surface and burrow in the spongy tissues of the leaf. The characteristic round feeding holes are easily observed when weevil populations are large, but may be concentrated near leaf edges and more difficult to observe when weevil populations are small (Center *et al*., 2002).

1.9. Post-release evaluation of *Neohydronomus affinis* in South Africa

Neohydronomus affinis is the only biological control agent to have been released in South Africa on water lettuce, and since its introduction, it has established widely and has effectively controlled water lettuce in most parts of the country. The weevils were first introduced into South Africa in 1985, after control of the water lettuce had been obtained by *N. affinis* in Australia, and after which Harley *et al*. (1984) suggested that the weevil would probably also affect similar control in Africa (Cilliers *et al*., 1996). A starter colony of weevils was obtained and imported into South Africa from CSIRO, Brisbane, Australia in 1985. The beetle was first introduced onto a water lettuce infestation on Nhlangaluwe Pan in the KNP in December 1985 and the progress and effect on the plants was monitored (Cilliers, 1987). A population of 500 adults was first released on the Sabie River at Lower Sabie in September 1987. Four further releases of between 100 and 1000 adults and larvae totaling approximately 5000 weevils took place over the next five years (Cilliers *et al*., 1996).

Great success was achieved with biological control on seasonal pans in Nhlangaluwe and in Dakamila in the northern part of the KNP (Cilliers, 1987, 1991). On Nhlangaluwe Pan in the Pafuri area, biological control was achieved within 10 months (Cilliers, 1987). The pan then dried up and no water lettuce remained. On the Lower Sabie River both chemical and biological control programmes were followed within the KNP in 1987/88. The weevil population at this site remained low and only a year after initial release of the weevils, damage to the plants could be easily observed (Cilliers *et al*., 1996).

By November 1990, and January 1991, the number of weevil-damaged plants on the Lower Sabie River had reached 100 %. Between May 1991 and March 1992, weevil-damaged plants ranged from 54-100 % (Cilliers *et al*., 1996). This introduction of weevils to the Salitjie River therefore appeared to stop the infestation of weevil-free plants, making control downstream more effective.

By September 1992, plant coverage had been reduced to less than 10 %. Between early 1991 and the beginning of 1992 it was evident that *N. affinis* could control *P. stratiotes* on a flowing river (Cilliers *et al*., 1996). On the Sabie River at Lower Sabie, a cover of less than 10 % of the water surface is presently regarded as the residual plant population that can be tolerated. Biological control was and is very successful on the pans and on the Sabie River and remains the main form of control in the KNP (Cilliers *et al*., 1996).

1.10. Biological control at Sunset Dam

A repeating cycle of open water followed by total coverage of the weed seems to occur every few years. Increasing weevil populations, which cause the plant mats to collapse, drives the cycle. Low recourse availability causes weevil numbers to decline, which, in turn, enables the plants to recover, and the cycle to repeat.

There appears to be a cycle every few years of open water total coverage of the weed on this dam, where the weevils catch up with the plants, causing them to crash, but thereafter weevil numbers crash in turn, the plants escape for a while, and the cycle continues again. Recently Sunset Dam has been totally clear of weed for several months, even over winter, which it has never done before (L. Foxcroft personal communication). It may therefore be possible, that biological control at Sunset Dam has finally stabilized and can be considered to be complete. Complete biological control could be achieved when no other control measures are needed to reduce the weed to acceptable levels, at least in areas where the agents are established (Hoffmann, 1995).

1.11. Impact of the weevil, *Neohydronomus affinis* in the rest of the world

Neohydronomus affinis was also introduced into Zimbabwe onto *P. stratiotes* in the Manyame River in April 1988. By July 1988, the weevils were well established. Many plants were severely damaged, plant size was declining and other aquatic plants, namely water hyacinth and parrot's feather (*Myriophyllum aquaticum*) (Velloso Verde) had begun to invade the area. By October 1988, the weevils were active throughout a region, 9 km upstream and 5 km downstream of the release site. Population density averaged 5.6 adults/plant and many plants were rotting and sinking, due to insect-damage. By February 1989, water lettuce had been successfully controlled and was no longer a problem on the Manyame River (Chikwenhere and Forno, 1991).

In Benin, West Africa, *N. affinis* was first imported from Zimbabwe and reared at the International Institute of Tropical Agriculture (IITA) in Cotonou (Ajuonu and Neuenschwander, 2003). The weevils were first released in 1995, and 2 years later it had spread 90 km from the release site to the Oueme River, and by 2000 to Savalou, which is 250 km to the northwest (Ajuonu and Neuenschwander, 2003). The weevils were also introduced and released several times into the Republic of Congo from 1999-2002 (Mbati and Neuenschwander, 2005).

Neohydronomus affinis was also introduced into the south-eastern United States. *Neohydronomus affinis* was first released in North America for biological control of water lettuce, at Kreamer Island, Lake Okeechobee, Florida, on 29 April 1987 (Dray *et al.*, 1990). A further 6 releases were made at additional sites in southern Florida. Periodic observations at several of these sites indicated the weevils established and were dispersing. Plants in some of these areas showed symptoms of *N. affinis* attack typical of areas successfully controlled by the weevils (Dray *et al.* 1990). Although the weevil *N. affinis* has been used successfully in other countries, it has only had a limited effect on water lettuce in Florida (Dray and Center, 1992), possibly due to pollution (with eutrophication being of particular importance).

1.12. Aims

It would appear that water quality status might have a large role to play in the biological control of water lettuce and other aquatic weeds (Hill and Olckers, 2001). However, by the same token, it is an aspect that has been neglected in general. Thus the main focus of this study was to investigate whether nutrients are a limiting factor in terms of biological control of water lettuce with *N. affinis*.

1.13. Hypothesis

Ho: High levels of nutrients prevent effective biological control of water lettuce.
Ha. High levels of nutrients do not prevent effective biological control of water lettuce.

The above hypothesis forms the basis for all chapters. There are 2 ways of addressing the above hypothesis. One approach is to assess the effects of different nutrient levels (especially high nutrient levels) on biological control of water lettuce with *Neohydronomus affinis* in the laboratory by manipulating the nutrients. Another approach is to conduct a quantitative post-release evaluation of the weevil *N. affinis* in the field at Cape Recife, (which is classified as a eutrophic system). Both of these studies would be very useful as weed management tools, and may help explain why eutrophic systems are often difficult to control with biological control alone. However comparison to examples of water lettuce under different nutrient statuses would be useful if they were available.

1.14. Main questions

1.) Is there a eutrophication threshold above which biological control is ineffective and if so, where does this level occur?
2.) How important is eutrophication in terms of biological control of water lettuce?
3.) Can *N. affinis* control water lettuce in a hypertrophic system?

Chapter 2

Effect of differing nitrate and phosphate concentrations on the successful biological control of water lettuce.

2.1. Introduction

Water lettuce is no longer perceived as a problem weed in many areas of South Africa, and is normally brought under complete control by the biological control agent, *N. affinis*. However, in highly eutrophic water bodies, the weevils are thought to be less effective at controlling the weed below an economic or environmental threshold level. These threshold levels have not been quantified but the generally accepted level is 20 % cover of the weed (M.P. Hill pers comm.).

It is widely known that eutrophic waters result in excessive algal and macrophyte growth, which in turn cause more eutrophication and anoxic conditions when these plants die and sink. Macrophyte infestations also decrease light penetration into the water column, which results in little or no primary production below the macrophyte mats, causing massive disturbances to the natural ecology of the system (Ayles and Barica, 1977; Clady, 1977, Roback, 1974, and Cole 1979).

2.1.1. Plant nutrition

Many terrestrial studies have shown that limits set by temperature, water, and nitrogen are the most important abiotic factors affecting plant and herbivore dynamics (e.g. Scriber and Slansky 1981). In fairly closed systems, such as dams, the dominance of nitrogen should be expected to be even more important, as the nutrients are leached from catchment areas and tend to accumulate in pans, dams, and lakes, where water lettuce occurs, which could lead to eutrophic conditions.

Most aquatic systems experience fluxes in nitrogen availability, with the timing and magnitude dependent on rainfall and the nature of the catchment. An agricultural region of Australia that experienced heavy rain, followed by nutrient run off, resulted in rising levels of nitrogen in the lake as the water rose, which in turn raised levels of nitrogen in *Salvinia molesta* Mitchell (Salviniaceae), and increased the growth rate (Room and Thomas, 1985). In contrast, in a primary rainforest catchment in Papua New Guinea, high water levels in the rainy season were accompanied by reduced levels of nitrogen in the plants and reduced rates of growth (Room *et al.*, 1989).

Salvinia responds to nitrogen shortage by increased investment in roots and mobilization of nitrogen from senescing ramets. If nitrogen is abundant, it is stored and vegetative reproduction increases through extra branching and earlier fragmentation of colonies of ramets (Room, 1988). The weevil biocontrol agents of salvinia (*Cyrtobagous salviniae* and *C. singularis*) are adapted to variable nitrogen availability by having extremely long lived, almost sedentary adults (virtual K-strategists), with rapid fecundity responses but not much behavioural response to nitrogen levels (Forno and Bourne, 1988). In contrast, the moth agent, *Samea multiplicalis* adults are short-lived (r-strategists), vagile, and discriminate between ovipositional sites on the basis of nitrogen content of the host (Forno and Semple, 1987). K-selected populations are also called equilibrial populations, and can be defined as populations that are likely to be living at a density near the limit imposed by their resources (K, or carrying capacity, Campbell, 1995). On the other hand, r-selected populations, also called opportunistic populations are likely to be found in variable environments in which population densities fluctuate or in open habitats where individuals are likely to face little competition (Campbell, 1995). It would therefore seem that r-strategists rely more on food with high nitrogen content than K-strategists, where high fecundity and reproduction are not as critical. This means that some past biological control releases may have been unsuccessful because of inadequate fertilizer levels, with plants being nutritionally inadequate. Future releases of agents may in fact benefit from judicious use of fertilizer as was suggested by Harris (1981).

Future releases of agents may benefit from judicious use of fertilizer as was suggested by Harris (1981). Room and Thomas (1985), for example, found that increasing fertilizer treatments resulted in rapid increases in recruitment of *Cyrtobagous* sp. Weevils. The Moth *Niphograpta albigutallis* on water hyacinth, *Eichhornia crassipes*, provides another example.

Higher than ‘normal’ nutrient levels can generally exacerbate weed problems if natural herbivores are excluded, such as was seen at Cape Recife before *Neohydronomus affinis* weevils were introduced (personal observation). However, field-cage experiments have shown that although nitrogen enriched salvinia grew faster and suffered less damage per individual plant, they sustained more total damage

because the insects became more numerous due to numerical responses (Room *et al*., 1989). This means, that in very eutrophic/hypertrophic waters, although biocontrol agents may be causing large amounts of damage, this damage may be compensated for by the growth of plants due to unlimited nitrates and phosphates. Conversely, higher nitrates and phosphates may mean that the reproduction of the biocontrol agents increases and more damage accrues as their numbers increase.
Laboratory studies have found higher rates of development by *Cyrtobagous* sp. larvae when fed *S. molesta* containing higher nitrogen levels (Sands *et al.*, 1983). Taylor (1984, 1988) also demonstrated the importance of nitrogen concentrations for *Samea multiplicalis*, another herbivore of salvinia and water lettuce. The importance of plant nutritional factors, especially nitrogen (Mattson, 1980) and leaf toughness (Coley and Barone, 1996) for weed biocontrol has been shown for a number of other aquatic weeds, including alligator weed, *Alternanthera philoxeroides* (Mart.) Grieb. (Amaranthaeae) (Maddos and Rhyne 1975); salvinia, *Salvinia molesta* (Taylor 1984, 1988; Room 1990); water hyacinth, *Eichhornia crassipes* (Center 1994) and hydrilla, *Hydrilla verticillata* (L. f.) Royle (Hydrocharitaceae) (Wheeler and Center, 1996, 1997). Wheeler *et al*. (1998) also found that *Spodoptera pectinicornis* (Lepidoptera: Noctuidae) larvae compensated for low nitrogen-leaves by increasing their fresh weight consumption 3-fold.

Thus, in general, low nitrogen levels (oligotrophic conditions) appear to result in low plant growth and poor insect establishment; whereas high nitrogen levels result in high plant growth and generally better insect development and establishment. For *S. pectinicornis* at least, it would seem that food quality with regards to nitrogen is very important, and governs the quantity of food the insect consumes, i.e. lots of low quality food or a little high quality food, as mentioned in Wheeler and Halpern, (1999). However, excess nutrients (eutrophic and hypertrophic conditions) appear to interfere with the effectiveness of biological control in many cases. There would therefore seem to be a divide where nutrients aid biological control and where they interfere with it, although there is very little literature to support this.

2.1.2. Aims

The main objective of this part of the study was to determine whether nutrient levels (notably nitrates and phosphates) affect the efficacy of biological control with *N. affinis* on water lettuce, especially at high nutrient levels.

Ho: High levels of nutrients reduce the effective biological control of water lettuce with *N. affinis*.

Ha: High levels of nutrients do not reduce the effective control of water lettuce with *N. affinis*.

2.2. Materials and methods

Cultures of insect-free water lettuce plants were maintained in a paddling pool (diameter 1.5 m x 0.5 m high), and were used for the laboratory experiments. Sixty 10 L plastic containers were set up in a glasshouse, containing various nutrient concentrations (see Table 2.1). Each treatment container, consisted of a water lettuce plant of between 15-20 g with two mating pairs of adult *N. affinis* weevils (the weevils were pre-sexed before the experiments when found copula) per plant at the 10 different nutrient concentrations, while the control tubs contained plants without insects, at the same 10 different nutrient concentrations, under the same conditions. An original Long-Ashton nutrient solution (Hewitt, 1966) was used as a growth medium, with only the nitrates and phosphates modified according to the treatment (Table 2.2) the rest of the macro and micronutrients were kept constant throughout. Tap water was used for the medium and high nutrient treatments and de-ionised water for all the low nutrient concentrations. Deionised water was used for the low levels, because tap water in Grahamstown was found to contain 0.3 mg/L N (Analytical and advisory service report for Makana Municipality by A.M. Mancotywa, 02/06/2003), which is higher than the 0.2 mg/L N required for the experiments. There were 3 replicates for each treatment, i.e. either with or without insects at the 10 different concentrations.

Table 2.1. The ten different nutrient combination concentration treatments used in the laboratory experiments.

Concentration (abbreviations)	**Concentration (in full)**
LNLP	Low nitrogen, low phosphate
LNMP	Low nitrate, medium phosphate
LNHP	Low nitrate, high phosphate
MNLP	Medium nitrate, low phosphate
MNMP	Medium nitrate, medium phosphate
MNHP	Medium nitrate, high phosphate
HNLP	High nitrate, low phosphate
HNMP	High nitrate, medium phosphate
HNHP	High nitrate, high phosphate
VHNVHP	Very high nitrate, very high phosphate

Table 2.2. The Long-Ashton nutrient solution used in the laboratory experiments.

Macronutrients	**Micronutrients**
KN03	MnS04.4H20
K2SO4	CuS04.5H20
Ca(N03)2	ZnS04.7H20
CaCl2	H3B03
MgS04.7H20	Na2M04.2H20
NaH2P04.2H20	NaCl
	FeCl(.3H20)

Table 2.3. Nitrate and phosphate treatment concentrations used for the laboratory experiments (all units in mg/L).

Treatment	**Phosphate**	**Nitrate**
Low	0.005	0.2 (Oligo-Mesotrophic)
Medium	0.01	2 (Mesotrophic)
High	0.2	20 (Eutrohic-Hypertrophic)
Very High	20	200 (Highly Hypertrophic)

Table 2.4. South African water quality guidelines for nitrogen and phosphorus (from Coetzee unpub. 2003).

Water nutrient Classification	**Inorganic P (μg/L)**	**Inorganic N (mg/ L)**
Oligotrophic	<5	<0.05
Mesotrophic	5-25	0.5-2.5
Eutrophic	25-250	2.5-10
Hypertrophic	>250	>10

The low nutrient levels correspond to oligotrophic nutrient levels (which are rarely found in South African waters), whereas the medium levels were near the upper limit of the mesotrophic water nutrient levels, the high and very high nutrient levels were well within the hypertrophic nutrient zone (Table 2.4).

The tubs were stirred weekly to mix the nutrients and tubs were all scoured down and any algae removed from the sides of the containers before the next nutrient solution (every 2 weeks) was added. Temperature and light intensity within the glasshouse were constant for all treatments. All the tubs were covered with white gauze curtaining, with an elastic cord around them, to keep insects either in or out of the tubs.

Young plants of similar size and age were used for the experiments (between 5 and 20 g), and these were collected from a quarantined, previously insecticide-treated pool containing field-collected insect-free plants. A separate pool was used to mass-rear insects for the experiments, additional insects were kindly provided by the Plant Protection Research Institute (PPRI) in Pretoria. Two pairs of weevils were used per tub, with weevils being sexed in copula.

At the beginning of the experiments, a single plant was placed inside each container. These plants had been pre-weighed before any insects were introduced onto them, and plant growth rate parameters recorded before and after the experimental period.
The plants were left in the greenhouse for about 2 weeks to acclimatize to the gauze and nutrient conditions within tubs, before the experiments were initiated. Nutrient concentrations were changed every second week to maintain relatively constant nutrient levels. The experiments were run for 6 weeks in total (8 weeks for the VHNVHP treatments). In consequence of rapid and unexpected deterioration in the condition of the insect treatment plants, due to heavy feeding damage, the experiments were terminated much sooner than was hoped for. Many of the plants were starting to die, and it was decided that the experiments should be terminated before the insect treatment plants died.

2.2.1. Statistics

A factorial 3-way analysis of variance (ANOVA) was used to test for differences between the different treatments, i.e. plants with and without insects, between insect/control treatments at the 10 different concentrations. However, due to almost all the variables not being normally distributed, after running a Kolmogorov-Smirnov (K-S) normality test, nonparametric statistics were used. However, a simple square-root transformation of the data revealed the residuals to all be normally distributed with a K-S test, and therefore an ANOVA was deemed permissible, without violating any assumptions.

2.3. Results

2.3.0. Plant Growth

2.3.1. Effect of nutrient treatment on water lettuce plant growth

All plant growth variables revealed very similar trends between the start and end of the 6-week duration of this experiment (see Figures 2.1-2.10 starting over page). However, root length and plant height showed much variation. Only the very high concentrations, VHNVHP were significantly different to all the other concentrations with respect to the means of all the plant growth variables, including plant mass, plant height, root length, number of ramets and number of leaves. Some of the plants in the medium level treatments surprisingly grew better than some of the high treatment plants.

2.3.1.1. Mean water lettuce wet weight

The control and insect treatments at the ten different concentrations after the end of the 6-week duration of the experiments revealed some differences. The graph (Fig. 2.1) showed that when insects were absent from the plants, plant mass was almost always higher at the end of the experiments compared to the insect treatments. Wet weight and therefore growth was more vigorous at higher nutrient levels. The HPHN, HPMN and MPHN concentrations were all higher than the low (LPLN) and low-medium treatments, but not statistically so. The VHNVHP concentrations (treatment and control) were significantly higher than any of the other concentrations.

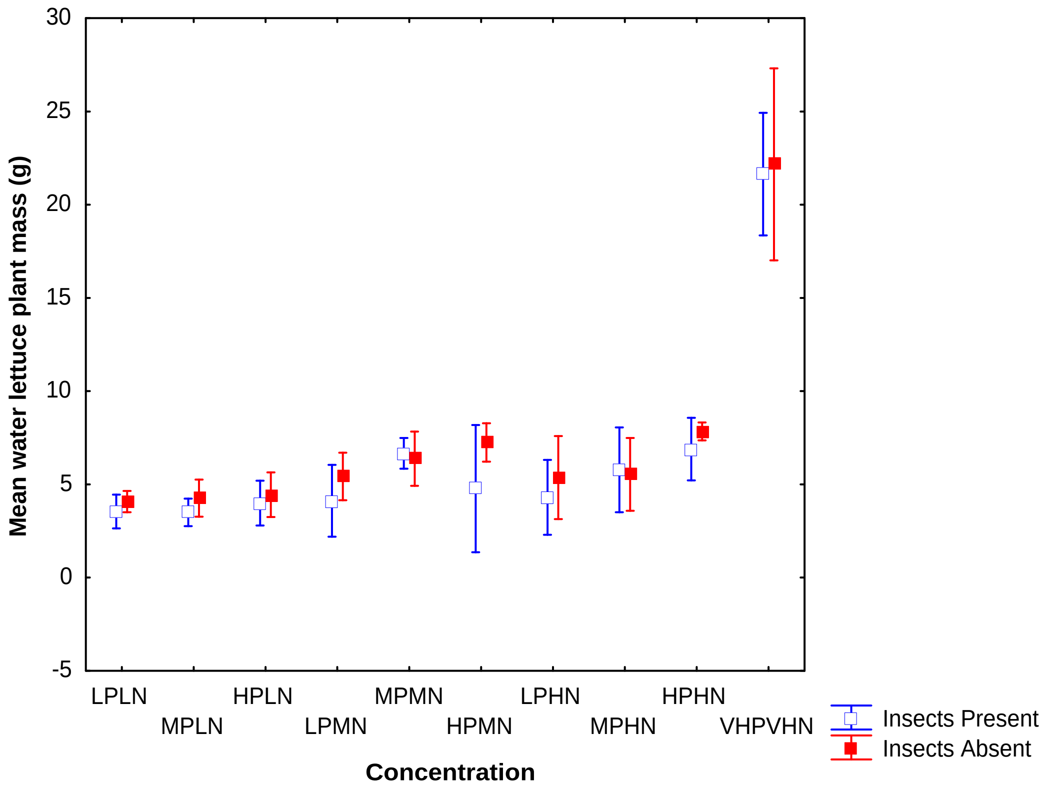

Figure 2.1. Control vs. insect treatments; square-root transformed graph of mean water lettuce plant masses at the end of the lab experiments with insects either present or absent at the 10 different nutrient concentrations (df = 9, F= .491, p = 0.877, error bars denote standard errors).

2.3.1.2. Mean water lettuce plant height

The control treatments grew taller than the insect treatments at all concentrations, but not significantly so. The ten different concentrations were all very similar with respect to plant height, with only the VHNVHP concentrations really standing out above the rest. Statistics revealed that there were no significant differences between the different nutrient concentrations. At six weeks, there were virtually no differences between the control and insect treatments at most concentrations.

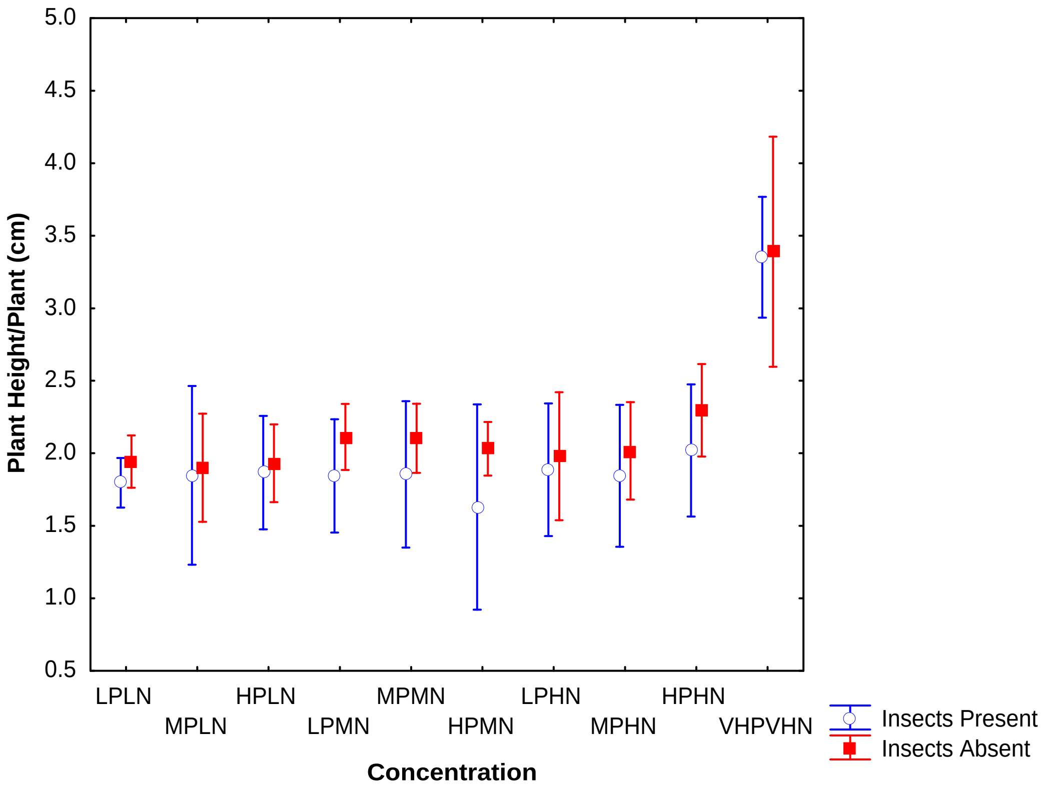

Figure 2.2. Control vs. insect treatment of square root transformed mean water lettuce plant heights at the 10 different nutrient concentrations with insects both present and absent. (df = 9, F = 0.255, p = 0.985, error bars denote standard errors).

2.3.1.3. Mean root length

No apparent trend could be seen for root length at the 10 different nutrient concentrations. Even the VHNVHP concentrations did not show any differences from lower concentrations (see Fig. 2.3). Root length was apparently very variable, as can be seen by the high standard deviations at most concentrations and treatments. The control treatments had longer roots than the insect treatments, but not significantly so.

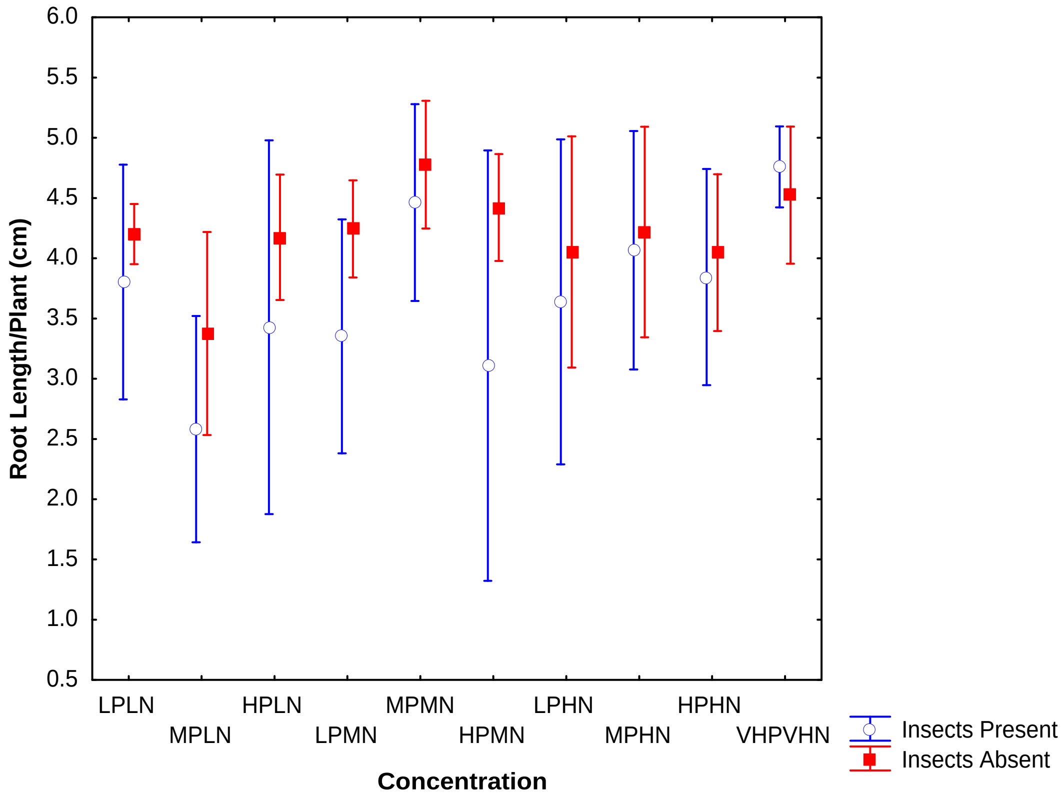

Figure 2.3. Control vs. insect treatments of mean square root transformed root lengths of water lettuce plants at the end of the experiments at the 10 different nutrient concentrations, with insects both present and absent (df = 9, F = 0.767, p = 0.647, error bars denote standard errors).

2.3.1.4. Mean number of ramets

For the mean number of ramets, the low nutrient treatments contained more ramets than the medium and medium high treatments, but not significantly so. The HPHN treatment plants contained a higher number of ramets than the low and medium-high treatments, but not significantly so. The VHNVHP treatment was much higher than the rest of the concentrations, but not significantly so. There were no real difference between the insect and control treatments with regards to the number of ramets, they were also not statistically significantly different either.

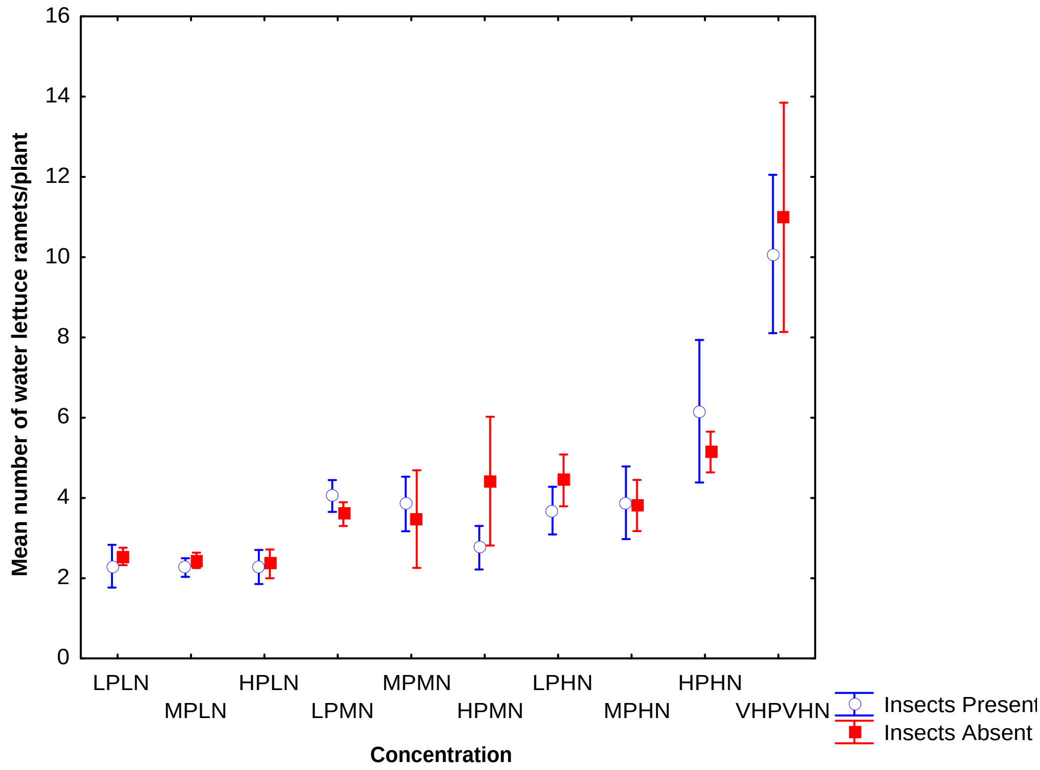

Figure 2.4. Control vs. insect treatments showing mean square root transformed number of water lettuce ramets at the 10 different nutrient concentrations at the end of the lab experiments with insects both present and absent (df = 9, F = 1.668, p = 0.106, error bars denote standard errors).

2.3.1.5. Mean number of water lettuce leaves

A very similar trend to previous graphs can be seen in Fig 2.5, whereby low treatments and medium low treatments were lower than the high treatment, HPHN. The LPHN treatment seemed to have more leaves considering it was restricted to low phosphorus treatment, suggesting that nitrates may have been the limiting nutrient. The VHNVHP treatments appeared to be significantly higher than all other treatments, but overall, there were no significant differences between control and insect treatments, p = 0.140. With respect to the insect and control treatments, the control treatments had more leaves than the insect treatment plants, but not significantly so. In such instances, the leaves may have been small and many, leading to confounding conclusions.

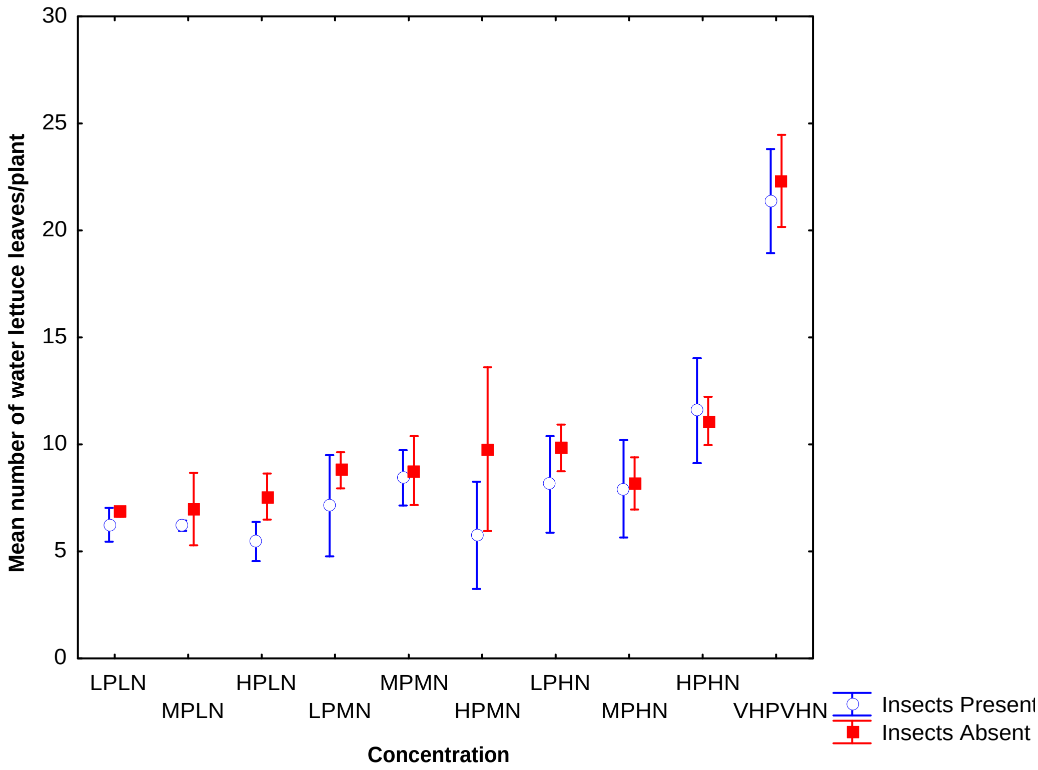

Figure 2.5. Control vs. insect treatments of mean square root transformed number of water lettuce leaves per plant at the end of the experiments at the 10 different nutrient concentrations with both insects present and absent (df = 9, F = 1.554, p = 0.140, error bars denote standard errors).

2.3.1.6. Mean wet weight vs. concentration vs. before and after

The following 5 figures show how the insect and control treatments differed before and after the six week duration of the nutrient tests at different nutrient concentrations, comparing plant growth parameters before to after the experiments. In Fig. 2.6, mean wet weight before the experiments was higher, (but not significantly) than after the experiments with the insect treatment. The same pattern can be seen for the control treatment plants, however, the difference between before and after are smaller in general than the insect treatment plants. Overall, a significant difference was detected between before an after vs. nutrient concentration and mass, p = 0.00289. This significant difference was mainly due specifically to the VHNVHP treatments.

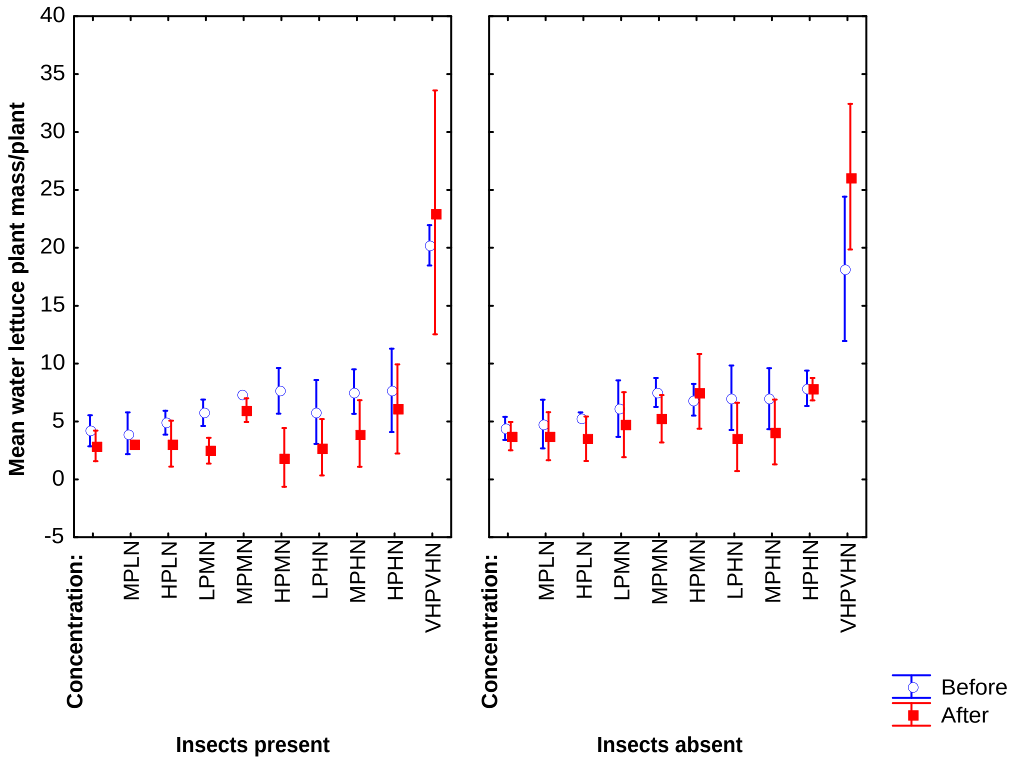

Figure 2.6. Before vs. after mean square root transformed water lettuce plant masses (g) for both control and insect treatments at the 10 nutrient concentrations (df = (9, 80), F = 3.119, p = 0.00289, error bars denote standard errors).

2.3.1.7. Mean plant height vs. before and after, vs. concentration

The same trends apply for this graph as the previous graph, except that the differences between before and after are much more pronounced this time with the insect treatment, showing that the insects were certainly having an impact on the plants. Plant height for the VHNVHP treatment was higher than all other concentrations, but not significantly so. In the VHNVHP control and treatment, at six weeks, the plants were taller after as opposed to before (as opposed to the rest of the concentrations), suggesting that the plants had not stabilized when the experiments were started, or they were perhaps growing vertically due to lack of space.

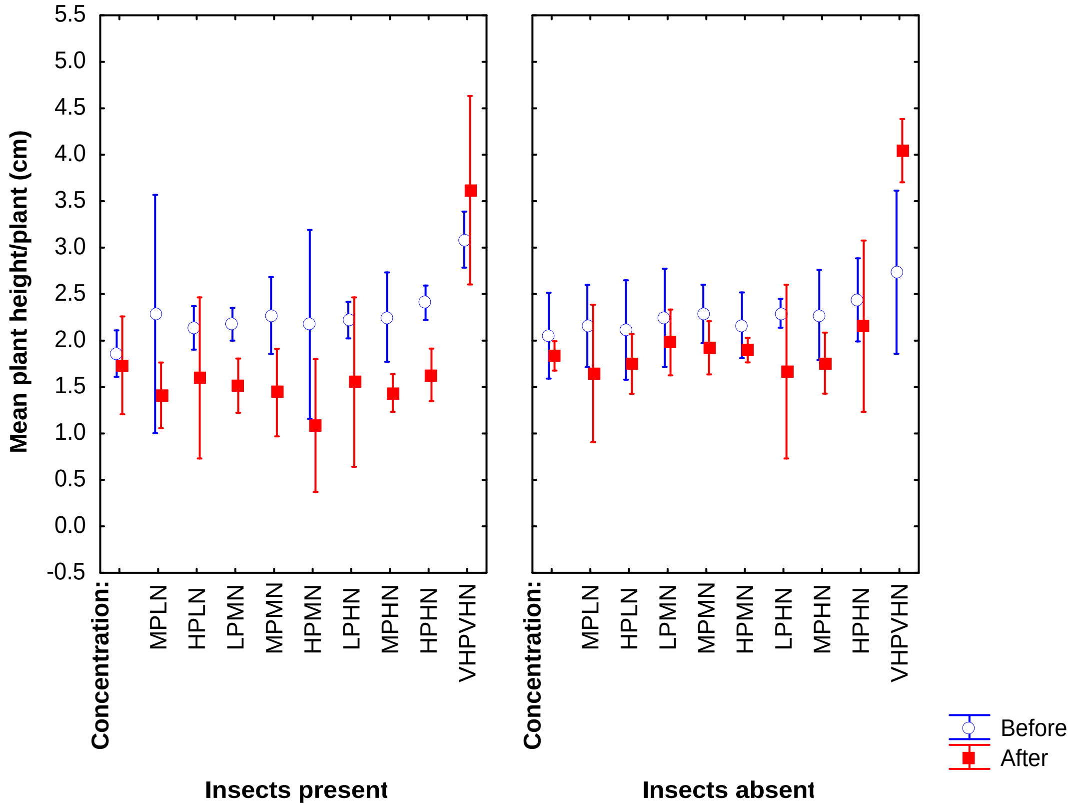

Figure 2.7. Before vs. after mean square root transformed water lettuce plant heights for both insect and control treatments at the 10 different nutrient concentrations before and after the lab experiments (df = (9, 80), F = 1.2368, p = 0.28486, error bars denote standard errors).

2.3.1.8. Mean root length vs. before vs. after, vs. concentration

Root length was highly variable even within the same treatments and concentrations, as shown by the large error bars (Fig. 2.8). Large differences were apparent between before and after with the insect treatment tubs as opposed to the controls. The big drop in root length for the treatment concentration after as opposed to before the experiments, highlights the damage that the weevils were causing to the plants compared to the control tubs, however this difference was not statistically significant.

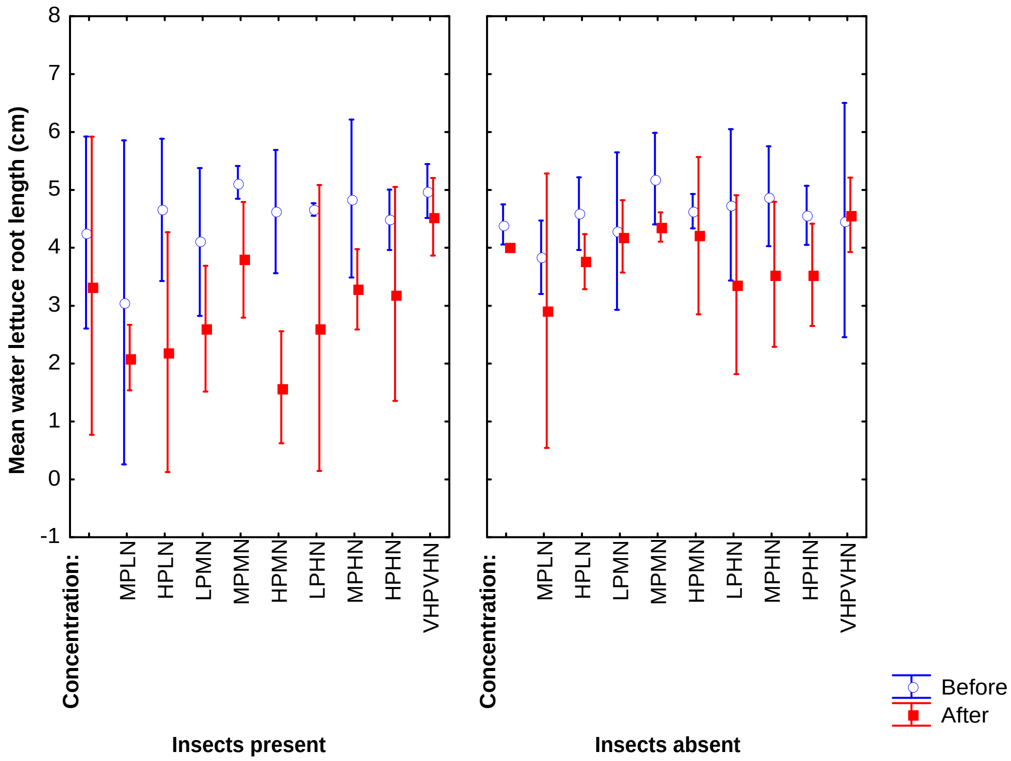

Figure 2.8. Before vs. after mean square root transformed water lettuce root lengths of both control and insect treatments at the 10 different nutrient concentrations (df = (9, 80), F = 1.8241, p = 0.07647, error bars denote standard errors).

2.3.1.9. Mean number of ramets vs. before vs. after vs. concentration

There was very little difference between before and after with the control treatment, except the VHNVHP control treatment. The overall p-value suggests that there were very significant differences, but these were mainly due to the VHNVHP treatments. Generally the insect treatment tubs contained less ramets than the control tubs (but not significantly). However, the HPHN treatment does not appear to make sense, since the numbers of ramets were higher than the control. The plants producing smaller plants as a result of insect feeding stress could have caused this.

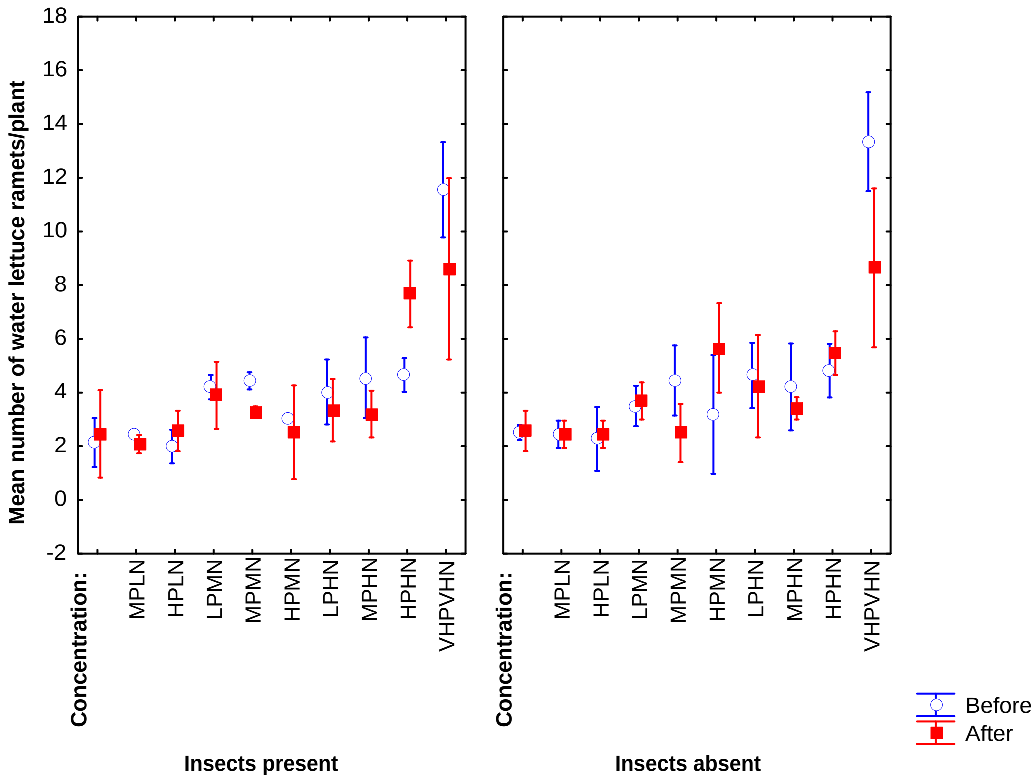

Figure 2.9. Before vs. after mean square root transformed number of water lettuce ramets for both control and insect treatments at the 10 different nutrient concentrations (df = (9, 80), F = 5.7510, p = 0.00000, error bars denote standard errors).

2.3.1.10. Mean number of leaves vs. before vs. after vs. concentration

A similar trend was seen with Fig. 2.10 as compared to the previous figure. Again the HPHN insect treatment revealed more leaves after as opposed to the start of the experiments, which is contradictory to the rest of the results, it is possible again that this was due to a result of feeding damage.

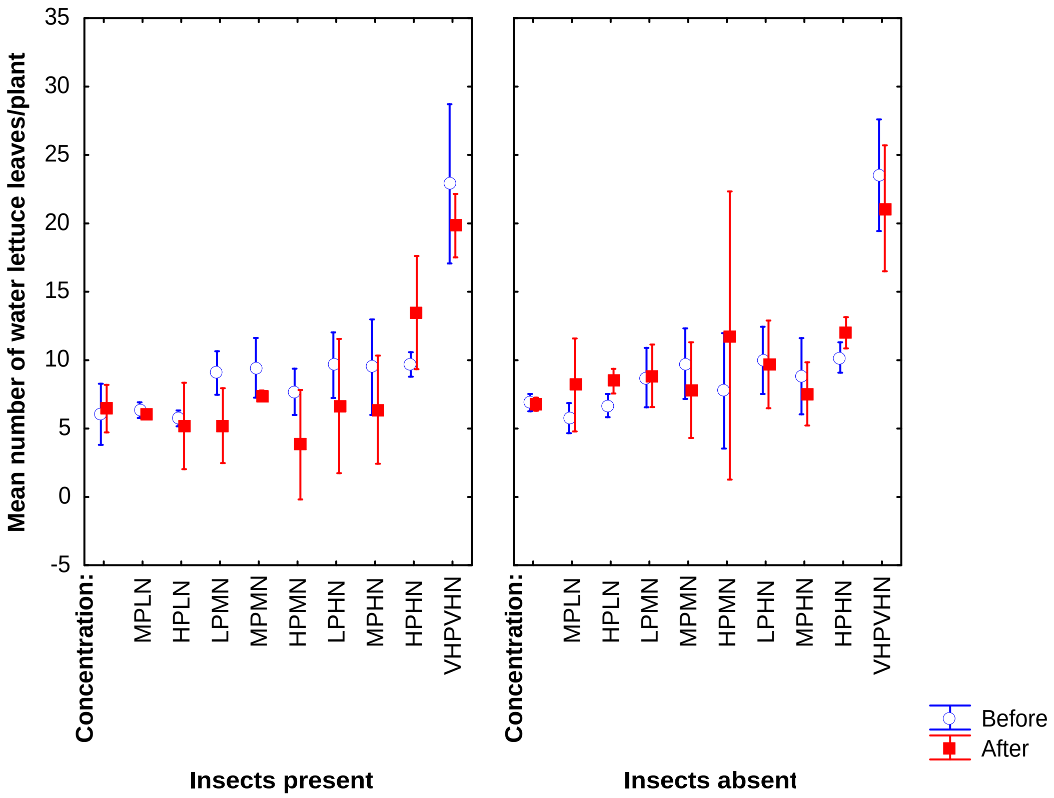

Figure 2.10. Before vs. after mean square root transformed number of water lettuce leaves for the control and insect treatments at the 10 different nutrient concentrations before (df = (9, 80), F = 3.4007, p = 0.00140, error bars denote standard errors).

2.3.1.11. Plant growth at the different nitrate and phosphate levels

The experiments were only run for 6-8 weeks, although even over this short period, extensive damage and collapse of many of the plants was evident, suggesting that the weevils are highly effective and destructive to water lettuce irrespective of nutrient status. Larval feeding and damage however appeared to be much lower with the lower concentrations as opposed to the higher nutrient concentrations, where reproduction and damage were much more obvious. However, there is very little quantification for this as the larval and adult damage ratings at the different concentrations were all found to be rather similar (and generally low) at the different nutrient concentrations (see Figures 2.14 and 2.15).

The VHNVHP concentrations were also very much stronger (an order of magnitude larger) than the HNHP concentrations, and the plant growth responses to the extra nutrients was also very apparent, with growth being far more vigorous under the VHNVHP compared to HNHP concentrations, which has implications for eutrophic waters and weed problems. The VHNVHP concentrations were left for another 2 weeks after the rest of the plants at the other concentrations were harvested, just to see what would happen to the plants (see Figures 2.16-2.18). In the 2 weeks, the plants had deteriorated rapidly, with one of the three plants virtually totally collapsing and the plants were showing very serious weevil damage, suggesting that at high nutrient levels biological control will take longer.

The 10 different nutrient concentration treatments used in the experiments were not found to show a large amount of variation between any of the treatments (insect and control treatments), except for the very high concentrations, both before and after the experiments. The general trend was that the plants actually lost mass after the end of the experiments, and the condition of many of the plants towards the end of sampling was generally very poor in the insect treatments, which was to be expected, considering the feeding damage.

Table 2.4. Results of a 3-Way ANOVA for square-root transformed data, for before/after vs. with/without insects vs. concentration vs. different water lettuce plant growth rate variables (stars denote significant differences at alpha = 0.95 level of significance).

Variable	**df**	**SS**	**MS**	**F**	**p**
Mass	9	32542	3615.8	2.2278	0.028327*
Plant Height	9	16.208	1.801	1.770	0.086972
Root Length	9	14166.4	1574	2.5792	0.011609*
Number of Ramets	9	2490	276.67	5.258	0.000013*
Number of Leaves	9	14875	1653	1.462	0.176577

The table above shows that there were only two plant variables that showed no significant differences between nutrient concentrations, vs. treatment vs. before/after. The multiple comparisons are not shown, due to the fact that they would have been too large to show. However the significant differences can largely be attributed to the VHNVHP concentrations.

Table 2.5. Kolmogorov-Smirnov normality test run for all the untransformed plant and insect variables for the lab experiments, showing that the data was not normally distributed and why a log-transformation was necessary.

Variable	**Statistical significance**	**d-value**	**p-value**
# Leaves before	Not significant	0.09691	P>0.20
# Leaves after	Significant difference	0.27296	P<0.01
Mass before	Significant difference	0.39820	P<0.01
Mass after	Significant difference	0.41423	P<0.01
Plant height before	Significant difference	0.40702	P<0.01
Plant height after	Significant difference	0.33979	P<0.01
Root length before	Significant difference	0.38730	P<0.01
Root length after	No significant difference	0.10148	p>0.2
# ramets before	Significant difference	0.17812	P<0.01
# ramets after	Significant difference	0.27862	P<0.01
# adult weevils after	Significant difference	0.34508	P<0.01
# larvae after	Significant difference	0.53594	P<0.01
# pupae after	Significant difference	0.53514	P<0.01
Larval damage after	Significant difference	0.35836	P<0.01
Adult damage after	Significant difference	0.35733	P<0.01

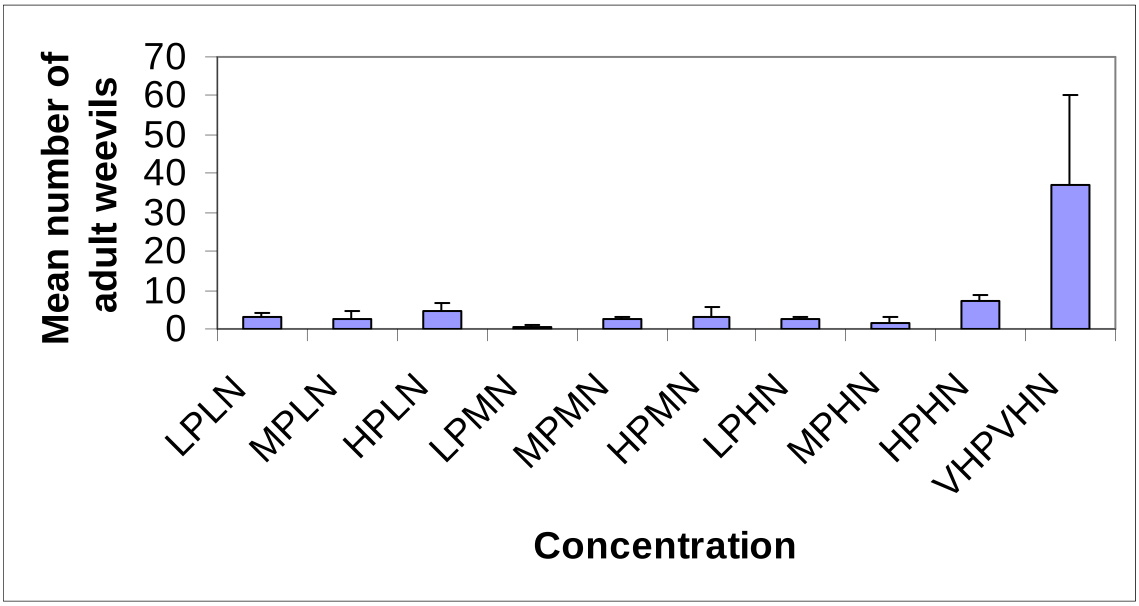

Figure 2.11. Mean number of adult *Neohydronomus affinis* weevils per tub after 6 weeks at the different nutrient concentrations (Kruskal-Wallis ANOVA H (9, N = 30) = 21.17891, p = 0.0119. The tubs were inoculated with two pairs of weevils, error bars denote standard deviations).

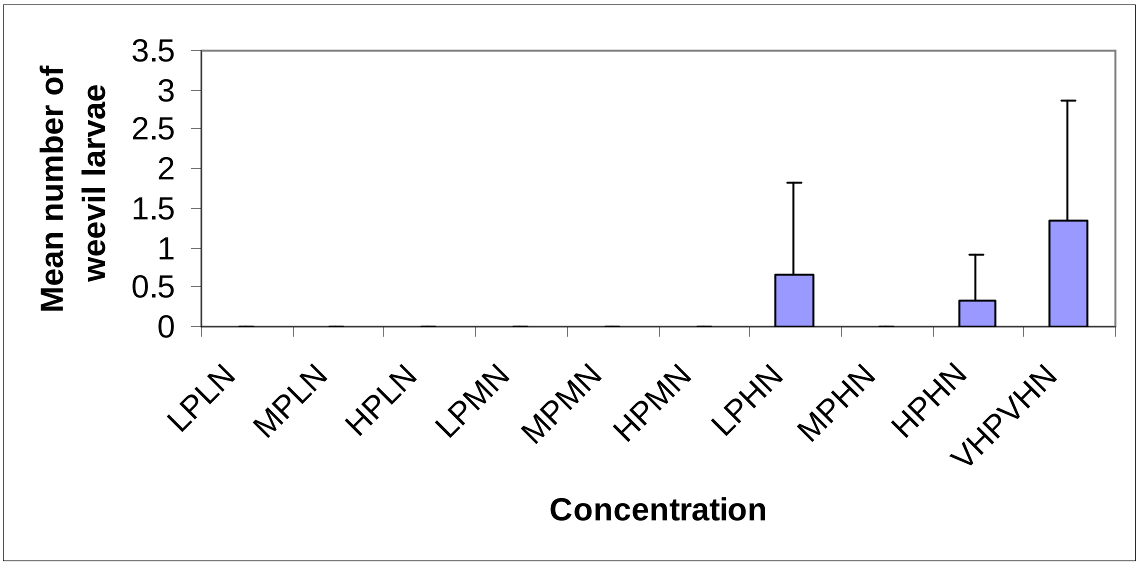

Figure 2.12. Mean number of *Neohydronomus affinis* larvae per tub after 6 weeks at the10 different nutrient concentrations (Kruskal-Wallis ANOVA H (9, N = 27) = 10.86970, p = 0.2848 (the tubs were inoculated with two pairs of weevils, error bars denote standard deviations).

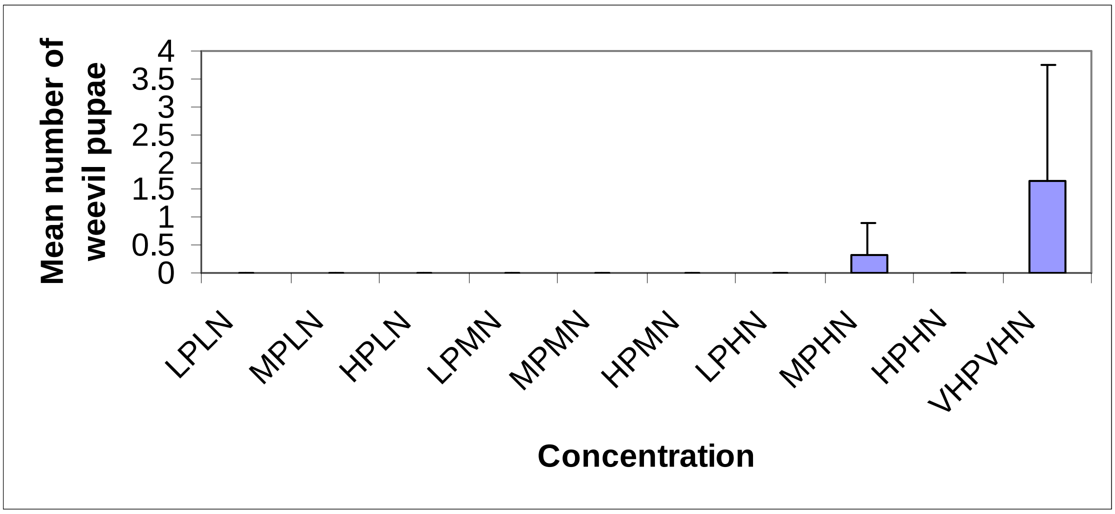

Figure 2.13. Mean number of *Neohydronomus affinis* pupae per tub after 6 weeks at the different nutrient concentrations (Mann-Whitney U-test, U = 2.5, Z = -0.8729, p = 0.38273. Tubs were inoculated with two pairs of weevils, error bars denote standard deviations).

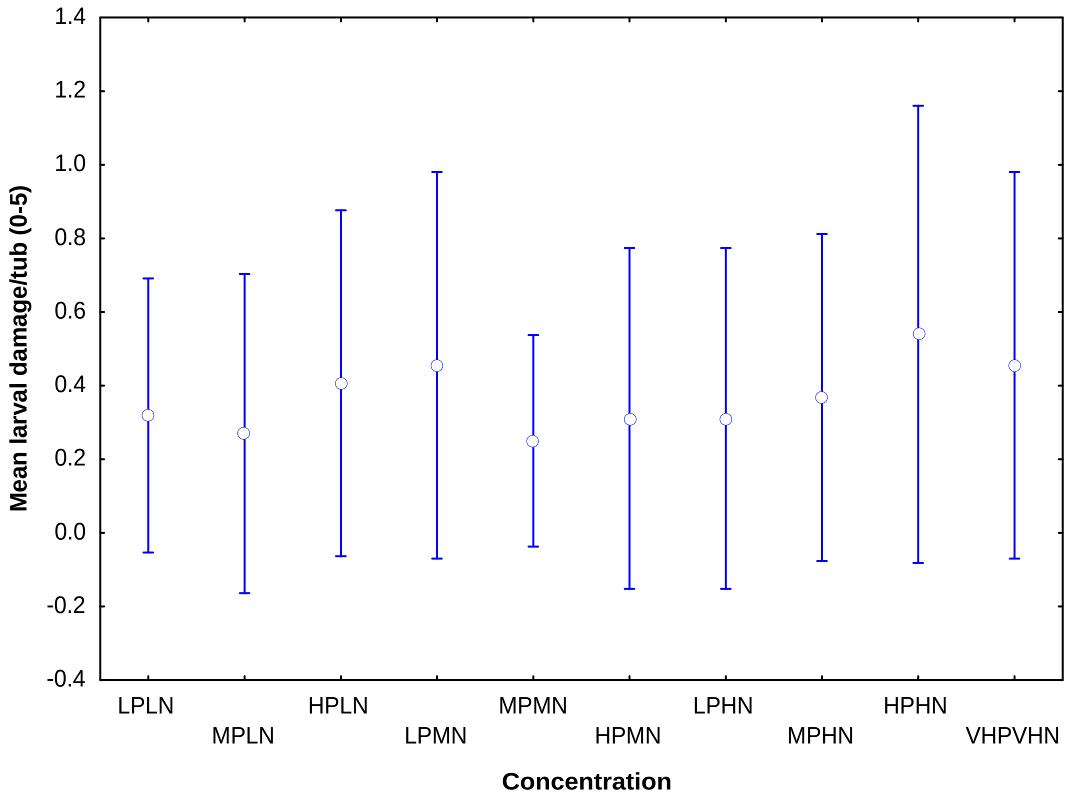

Figure 2.14. Mean square-root transformed larval damage rating scores (0-5) for the water lettuce plants at the ten different nutrient treatments after the 6-week duration of the experiments (F = 0.19286, p = 0.99453, error bars denote standard errors).

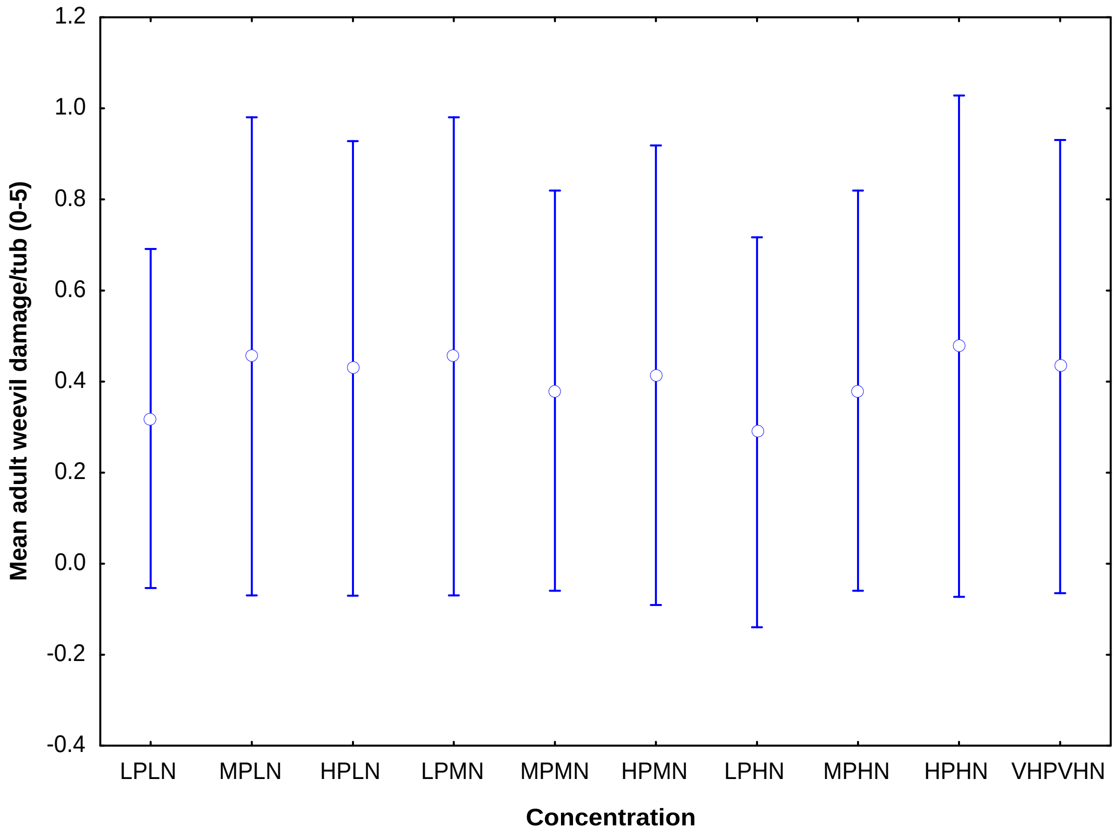

Figure 2.15. Mean adult *N. affinis* square-root transformed damage rating scores (0-5) for the water lettuce plants at the ten different nutrient treatments after the 6-week duration of the experiments ($F = 0.0786$, $p = 0.9999$, error bars denote standard errors).

2.3.1.12. Weevil damage to plants

The mean square-root larval and adult damage ratings assigned to each tub were found to be very similar for all the different concentrations, and as could be seen by the standard deviations, there were no significant differences for both the mean larval and adult damage estimates. Overall, the mean damage estimates were low for both larval and adult damage.

Figure 2.16. Comparison of the VHNVHP nutrient concentrations with respect to mean water lettuce plant masses at 6 and 8-week intervals after the start of the experiments.

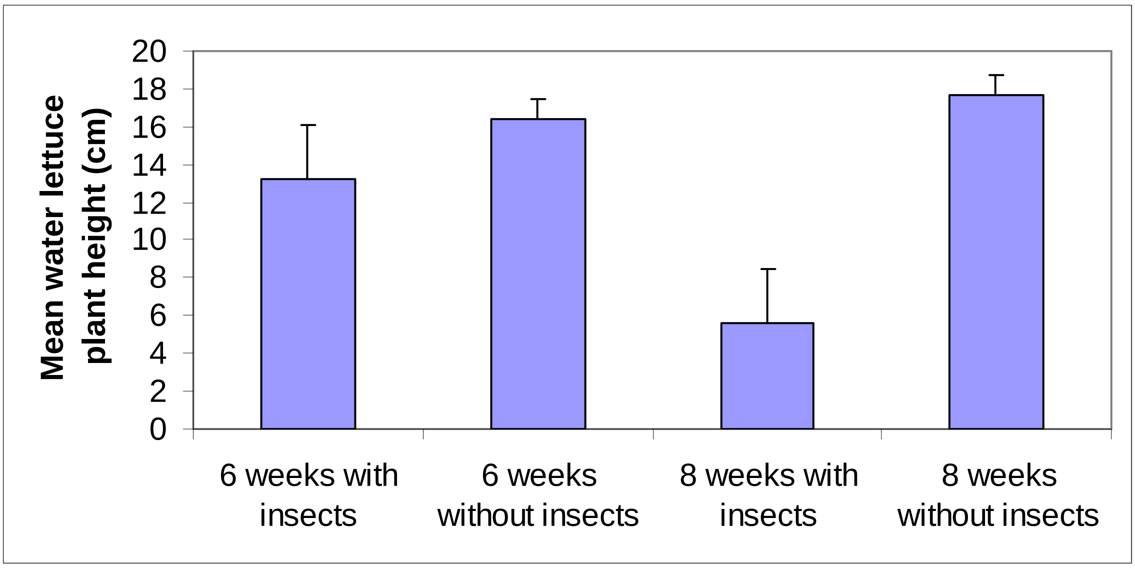

Figure 2.17. Mean water lettuce plant height for the VHNVHP control and insect treatments at 6 and 8-week intervals after the start of the experiments.

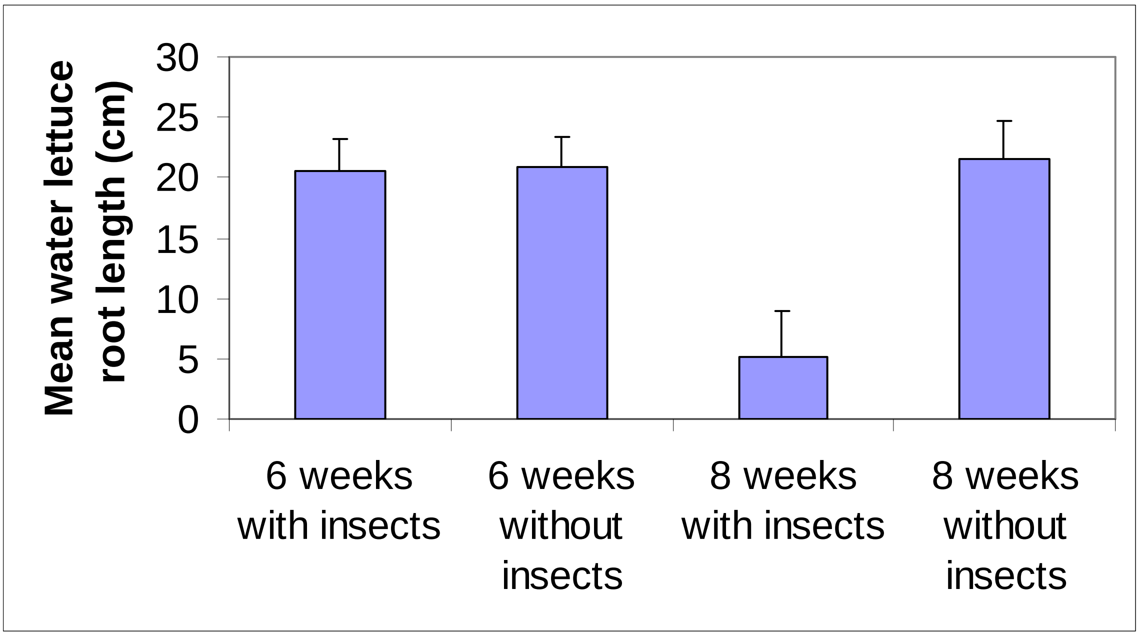

Figure 2.18. Mean water lettuce root length at the VHNVHP concentration 6 and 8-weeks after the start of the experiments.

The deterioration of the VHNVHP insect treatments was very dramatic between 6 and 8 weeks, (Figures 2.16-2.18). The VHNVHP treatments were left for another 2 weeks after 6 weeks, as they were still very healthy looking, although feeding damage was relatively intense, while all the remaining insect treatment concentrations had been terminated, due to severe weevil damage. The control treatments were still very healthy and much larger in general compared to the insect treatments.

2.3.1.13. Before/after vs. with/without insects vs. nutrient concentration

A 3-way ANOVA comparing before/after vs. with/without insects, vs. concentration for mean plant mass, plant height, root length, number of ramets, and number of leaves revealed the results shown in Table 2.4. Only the variables plant mass, root length and number of ramets per plant showed any statistical significant differences and for these, it was mainly only the VHNVHP that was significantly different from all the other concentrations and treatments.

2.3.1.14. Insect responses

The mean number of weevils per tub at the different concentrations was found to be much higher in the HPHN and VHNVHP nutrient tubs compared to the lower nutrient concentration tubs. The VHNVHP tubs contained a considerably higher number of weevils than the other concentrations (see Fig. 2.11). The mean numbers of larvae per tub were high in the high nitrogen tubs, in fact larvae were only found in the high nitrogen tubs (see Fig 2.12). Pupae were relatively scarce throughout the study, and were only found in the MPHN and VHNVHP tubs (see Fig. 2.13). Larval damage in all the insect treatments was very high in all the insect treatment tubs. However, in the very low nutrient tubs, larval damage appeared to be much visibly lower on the low nutrient concentrations compared to the medium and high ones. The damage on the VHNVHP plants after 6 weeks was very high, although the plants appeared much healthier than the mean and low nutrient concentrations, which were clearly dying from the intense adult weevil and larvae damage. Figures 2.16-2.18.

Fig. 2.19a. Water lettuce LPLN control after 6 weeks.

Fig. 2.19b. Water lettuce LPLN treatment after 6 weeks.

Fig. 2.20a. Water lettuce MPMN control after 6 weeks.

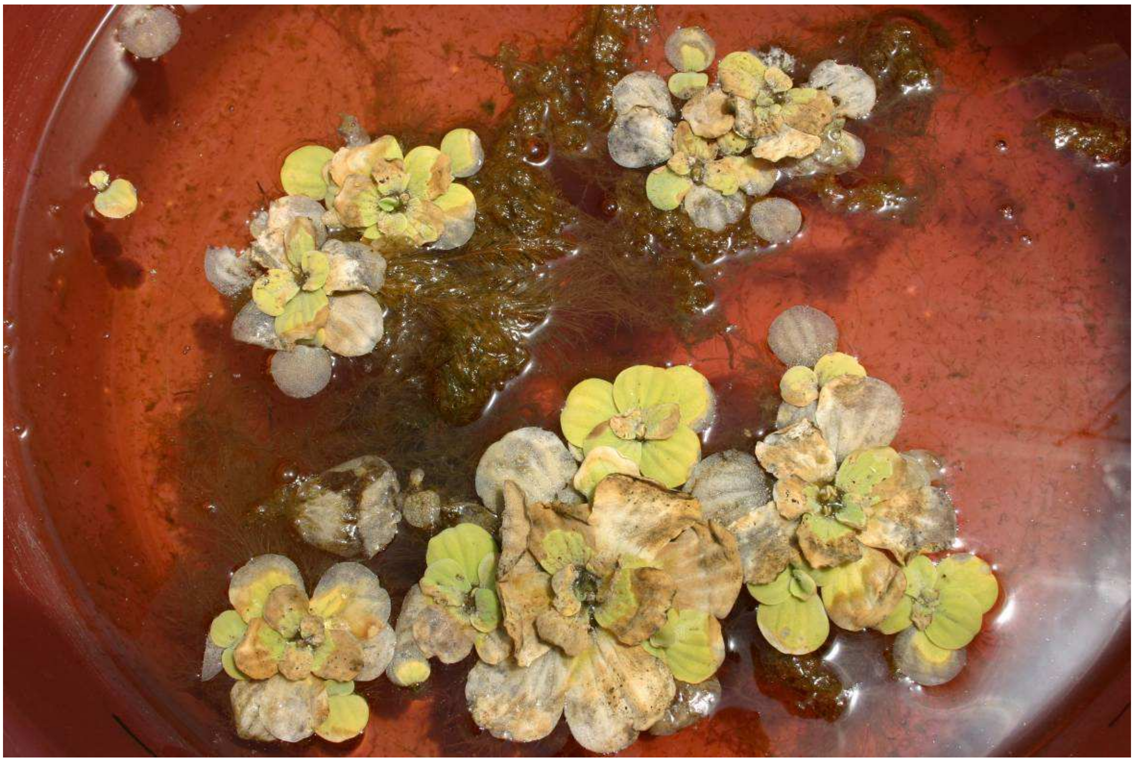

Fig. 2.20b. Water lettuce MPMN treatment after 6 weeks.

Fig. 2.21a. Water lettuce HPMN control after 6 weeks.

Fig. 2.21b. Water lettuce HPMN treatment after 6 weeks.

Fig. 2.22a. Water lettuce HPHN control after 6 weeks.

Fig. 2.22b. Water lettuce HPHN treatment after 6 weeks.

Fig. 2.23a. Water lettuce VHPVHN control after 6 weeks.

Fig. 2.23b. Water lettuce VHNVHP treatment after 6 weeks.

2.4. Discussion

2.4.1. Biological control at different nutrient levels

Biological control at the different nutrient levels revealed some exciting information, especially at the very high nutrient levels, it would appear that up to and possibly beyond 200 mg/L N, there is a continuous and likely linear growth of water lettuce, without any limits to growth. It was initially thought that the 200 mg/L N VHNVHP concentrations would kill or at least stress or poison the plants, however this was not the case, in fact quite the opposite, and the plants grew incredibly vigorously, without any signs of nutrient stress. By the same token, it was also suspected that the low 0.2 mg/L N concentration of the LNLP concentrations would also eventually lead to the death of the plants; however, this did not occur, although the plants did show very apparent signs of severe nutrient stress. In terms of the insects, the same was noted at the different concentrations, at the low nutrient levels there was little recruitment, while at high concentrations, weevil recruitment was very prominent (Moore and Hill unpub.). This would therefore seem to reinforce the notion that food quality governs fecundity in insects. Larval damage was visibly less in the very low concentrations compared to the medium and high concentrations, and this may have potentially been due to reduced ovarian development.

Surprisingly, some of the medium nutrient concentrations resulted in better growth than the higher nutrient concentrations. The reason for this was uncertain. It is possible that because some of the medium and medium-high concentrations were showing some signs of nutrient stress, it may have been other nutrients besides N or P that were causing the stress, which was likely the case, as the plants were showing burnt leaf tips and were light green, suggesting that at least one form of nutrients may have been limiting growth. Photosynthesis may also have been limited to some extent in all the treatments due to the gauze covering the tubs. With the low concentrations, the roots did not grow longer, indicative that the experiments may have been too short in duration to detect this.

What was apparent from the study was that there were few statistical differences between the different concentrations, except for the VHNVHP concentrations, especially with regards to damage between the insect treatments and the control treatments. Even though some of the plants were basically dead, they weighed roughly the same as the live control plants, which is what lead to the erroneous conclusion that there were no differences between the two treatments, which was apparently false, or at least visually so (see Figures 2.19a-2.23b), treatment and control plates). It was not the data, which was wrong, but likely the methods that were used to analyze them that were inappropriate. These visual differences should have been mirrored in the statistical analysis, but they were not. The methods that we used were not sufficient or suitable to show differences between live and dead plants.

The most exciting aspect about this study, was how rapidly 2 pairs of weevils per tub caused severe damage to most of the plants, with many plants tending to collapse, just 6 weeks after introduction of the weevils. With the VHNVHP concentrations, after 6 weeks the plants showed much damage, but the plants were still relatively healthy, however, just 2 weeks later, the weevils caused a complete collapse of most of these plants, under extremely hypertrophic nutrient levels (200mg/LN)!

2.4.2. Growth medium

Chadwick and Obeid (1966) studied the effects of water pH and nutrition on the growth of *P. stratiotes* and *Eichhornia crassipes*, plants which have tendencies to compete for the same sites. *Eichhornia crassipes* yielded the greatest dry weight yield at pH 7; whereas *Pistia stratiotes* performed best at pH 4, and would not grow at pH 3, losing vigour rapidly at any pH over the optimum. Water lettuce's pH tolerance range appears much narrower than that of water hyacinth. This means that the pH of most river water is thus likely to be more favourable for the growth of *E. crassipes* and this could be an additional advantage in its ability to crowd out *P. stratiotes* plants (Chadwick & Obeid, 1966). When grown together, *E. crassipes* plants have been shown to grow taller than and soon shade-out and out-compete the much shorter, *P. stratiotes* plants (Agami and Reddy, 1990; Coetzee *et al.*, 2005).

Pieterse *et al*., (1981) found that on a 1/5th strength Long Ashton medium as well as in mixtures of tap water and half tap water rain water with Long Ashton medium, there was a growth optimum for *P. stratiotes* at pH 7, whereas growth at a pH of 4 was strongly inhibited. These findings contradict the observations of Chadwick and Obeid (1966), who reported that *P. stratiotes* had a growth optimum at pH 4 on 1/5th strength Long Ashton medium as with tap water (Pieterse *et al*., 1981). Therefore, it seems possible that either water lettuce may have a wider pH tolerance range than previously expected.

2.4.3. Insect responses

The duration of the experiments limited the amount of time for the number of progeny that could have been produced, however, if one considered the number of adult weevils on the very high nutrient levels, the weevils had reproduced substantially within the short time frame. This was likely due to very warm temperatures within the green house, which would have been optimal for weevil growth and reproduction, never dropping below 22 º C. The damage sustained to many of the plants, was also very impressive, and substantial larval damage was noted on some plants within a week, which shows just how fast the weevils can reproduce, and why they are so damaging to water lettuce plants.

What was apparent from the results was that when nutrient concentration was increased, reproductive output seemed to increase correspondingly. This can be seen by the number of adult weevils being much higher than any other treatments in the HPHN and VHPVHN treatments, almost exponentially so in the VHPVHN treatments (see Fig. 2.11). The fact that the number of weevils remained low in the lower nutrient treatments would strongly suggest that the amount of N needed for successful development and reproduction might not have been available, as it would have been at the higher nutrient concentrations. Wheeler *et al*., (1997) found that fecundity of *S. pectinicornis* was dependent on pupal biomass, which was dependent on food quality. The adult *N. affinis* weevils introduced in the lower nutrient level treatments appeared to survive the experiments, however, their numbers did not appear to increase, suggesting that ovary development and reproduction may have been inhibited due to the low nitrogen levels. Wheeler *et al*., (1998) also found that *S. pectinicornis* adults preferred to oviposit on plants of higher nutritional quality, and that larval development took longer on lower quality food, which could have predation implications, as insects on lower quality food would have to forage for longer, increasing their chances of being predated or parasitised. The *S. pectinicornis* larvae compensated for low food quality by increasing fresh weigh food consumption, (more than a 3-fold increase compared to nitrogen-rich food). This information suggests that plant food quality is very important to insect growth and development.

2.5. Conclusion

The weevil *Neohydronomus affinis* would appear to be an extremely effective biological control agent against water lettuce. The question that we searched to answer in the beginning, as to whether nutrient levels, especially high nutrient levels are a limiting factor in terms of biological control with water lettuce would appear to have been answered. However, one can't really extrapolate from a short study done in the lab to actual field conditions. In this experiment, high nutrient levels did not prove to be a barrier to effective biological control with the weevil, although the amount of time taken before control was slightly longer. Plant nutrient status had a definite effect on weevil performance, with higher N and P concentrations resulting in significantly more weevils than lower concentrations.

Biological control was found to be complete, even under extremely high nutrient levels, far higher than could be found in any South African waters (South African Water Research Council). It would appear that control is possible irrespective of the nutrient level. The plants compensated for damage by more growth under higher nutrient conditions, but by the same token, the insects also grow and reproduced faster on plants with higher nitrogen content, and due to the devastating damage that the weevils cause in high numbers, collapse and death of the plants was inevitable once a threshold population of weevils was reached.

Chapter 3

Quantitative post-release evaluation of biological control of water lettuce, *Pistia stratiotes* L. (Araceae) with the weevil *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae) at Cape Recife Nature Reserve in the Eastern Cape Province of South Africa.

3.1. Introduction

Long-term post-release evaluations in weed biological control are important because they are the ultimate test of the success of any project. They are also important in the decision whether or not to release additional agent(s). Numerous studies (cited in Hill and Julien 2004) have demonstrated that biological control programmes against aquatic weeds have been highly successful. However, Hill and Olckers (2001) listed several factors that have mitigated against the success of the biological control programme of water hyacinth in South Africa. One of the most important was the nutrient status of the water body. Under conditions of high nutrients (nitrates and phosphates) the biological control of water hyacinth was less effective or took longer to achieve (Hill and Olckers, 2001). It is also thought that the seasonal recurrence of water lettuce on Sunset Dam in the Kruger National Park (Chapter 1) could also be ascribed to high levels of nutrients in the water, although this has not been tested (L. Foxcroft pers comm.).

The experiments conducted in Chapter 2 showed that the weevil (*N. affinis*) was highly successful at controlling water lettuce even at high levels of nutrients in the water. However as these experiments were conducted under laboratory conditions it was uncertain whether these results would persist under field conditions. The eutrophic nutrient levels at Cape Recife near Port Elizabeth are likely to have lead to water lettuce becoming a problem weed. Water lettuce was first noticed on the upper pond in March 2002, and within 2 months it had totally covered the upper pond (Algoa Sun, 20/03/2003). In August 2002, 240 *N. affinis* weevils were released on the upper wastewater treatment settlement pond at Cape Recife. This provided the ideal opportunity to investigate the impact of the weevil on the weed under eutrophic field conditions.

3.1.1. Hypothesis

Ho: *Neohydronomus affinis* does not effectively control water lettuce in the field under eutrophic conditions.

Ha: *Neohydronomus affinis* can effectively control water lettuce in a eutrophic environment.

3.2. Study site

Cape Recife Nature Reserve (34°01'11.9" S 25°41'18.7"E), is situated just a few kilometers outside the city of Port Elizabeth. It is 366 ha in size and houses two wastewater reclamation settlement ponds, the largest of which is about 1.5 ha in size. Port Elizabeth airport, the closest weather station receives a mean annual rainfall of 624mm (South African Weather Services). Mean summer maximum temperatures (January) are 25.4 º C; while maximum daily temperatures during winter (July) reach 19.5 º C. Extremes range between 2.8 º C and 41.3 º C and mean daily sunshine is 7.5 hrs. Sewage enters the sewage works at Cape Recife, where it is fully treated over a 24 hr period after which it enters the settlement ponds, where it is used to irrigate the grounds of the Nelson Mandela Metropolitan University (NMMU) and Humewood Golf Club. Any surplus water after this is eventually discharged into the Indian Ocean. The water reclamation ponds at Cape Recife are popular amongst tourists, especially birders. There is also an attractive 9 km nature trail, which is popular, and runs past both ponds.

3.3. Materials and methods

Water lettuce was sampled monthly on the upper pond between May 2003 and May 2004. During each sampling event, ten 0.5 x 0.5 m quadrats were thrown around the perimeter of the dam, about 3 m from the shore. Sampling was done close to the shore because of the limitation of wading depth, and due to the thick water lettuce mat, which precluded the use of a boat.

The positions of these quadrats remained constant on each sampling occasion, so that plants and insects in quadrats could be compared between months. However, in times when no weed was present within these fixed quadrats, a sample of plants was taken from the vicinity of where quadrats would normally have been. This was simply achieved by randomly taking handfuls of plants from a weed mat or pocket and placing them inside labeled plastic bags. However, these plants were not considered as part of the quadrats. If this were not done, no weeds would have been collected for several months, as the ponds became clear of weed for a substantial period of time. The monthly sample sizes were still large (Table 3.10) so it was possible to compare the condition of plants and insect damage within the upper pond, even though the quadrats were empty. The percentage coverage of the pond was estimated visually during each sampling event.

Plants from within quadrats or in the vicinity were placed in large waterproof bags and taken back to the laboratory. Plant parameters measured included fresh weight, plant height; root length and number of daughter plants (ramets). The number of fruit and seeds per plant were also measured. The numbers of plants per quadrat were noted, when there was weed present within the quadrats. Weevil damage was measured for each plant according to a damage score of 0-5 (Table 3.2). Adult shothole and larval mining-damage ratings were given a mean per plant, e.g. some plants had 1 or 2 leaves which showed heavy feeding damage, but the rest of the leaves were untouched, which would have lowered the total damage-rating score for that plant.

Table 3.1.0. Sample sizes of water lettuce plants sampled at Cape Recife from May 2003 to May 2004.

Month and Year	**Plant Sample Size**
May 03	268
June 03	404
July 03	263
August 03	136
September 03	406
October 03	533
November 03	461
December 03	110
January 04	496
February 04	191
March 04	118
April 04	32
May 04	357

3.3.1. Statistics

A normality test was run to test whether the data was normally distributed. A Kolmogorov-Smirnov (K-S) test was conducted on all the variables, which showed that the data were not normally distributed. This meant that non-parametric statistics (Kruskal-Wallis ANOVA) had to be used instead of a conventional ANOVA, (which uses parametric assumptions). The data were also not suitable for transformation (according to a Box-Cox transformation), so non-parametric statistics had to be used to analyze the data. The results of the Kruskal-Wallis ANOVA and tables of multiple comparisons, with p-values are shown in Appendix 3.

The median plant variables varied much less so than the mean plant variables per plant per month. Since the data was not normally distributed, the median was the centre of the data distribution, not the mean. The statistics also followed the medians and not the means, which is also why I only used the medians for analysis. This is why the statistics made sense if one looked at the median data, but not the values for the means, and hence the means were not used in analysis. The effect of many outliers and extremes caused the means in general to be less reliable than the median as a measure of the centre of the distribution.

Table 3.2. Larval and adult damage rating scores given for each plant.

Damage Category	**Percentage Leaf Area Damaged**
0	0
1	1-15
2	16-35
3	36-55
4	56-75
5	+75

When leaves were rated as “5”, they were covered with small circular holes to the extent that only a skeleton of the leaf remained. A “5” was scored for larval damage if the leaves looked as though they had been completely and totally scribbled on with a white pen, leaving lines all over the leaf. In such cases, the leaves were hanging on by threads, as the larval mines very often penetrate right through the leaf, usually only leaving the one-cell thick leaf membrane behind. To assess weevil populations, the numbers of adult weevils were noted for each plant, as well as larvae and pupae.

3.4. Results

3.4.1. Cape Recife nutrient data

NH3-nitrogen in the settlement ponds averaged 8.3 mg/L (± 7.3 mg/L, min. 0.1 mg/L, max. 57 mg/L) for 275 samples taken over a period of 2 years. Mean N03-nitrogen levels were 2.0 mg/L (± 2.4 mg/L, min. –0.4 mg/L, max. 12 mg/L), ($n = 275$) (from Nelson Mandela Metro database). This mean value of 8.3 mg/L N is well within the eutrophic classification zone of South African water sources (Table 2.3). However, the ponds frequently reach nutrient levels well above the minimum hypertrophic value of 10 mg/L N, but values fluctuated. No phosphate data was available for the ponds at Cape Recife, due to the lack of permission to access of files by the Nelson Mandela

Metro database, although the phosphate concentrations are measured for the raw sewage that enters the ponds.

3.4.2. Percentage coverage of water lettuce at Cape Recife

The percentage coverage of water lettuce at Cape Recife was found to decrease rapidly from 100 % coverage in May 2003 onwards, and remain relatively clear of weed over most of summer, slowly increasing again in percentage coverage towards the end of summer going into winter (Fig. 3.1). Fewer plants were sampled during May 2003 than during May 2004, although the plants present were much smaller during the latter period.

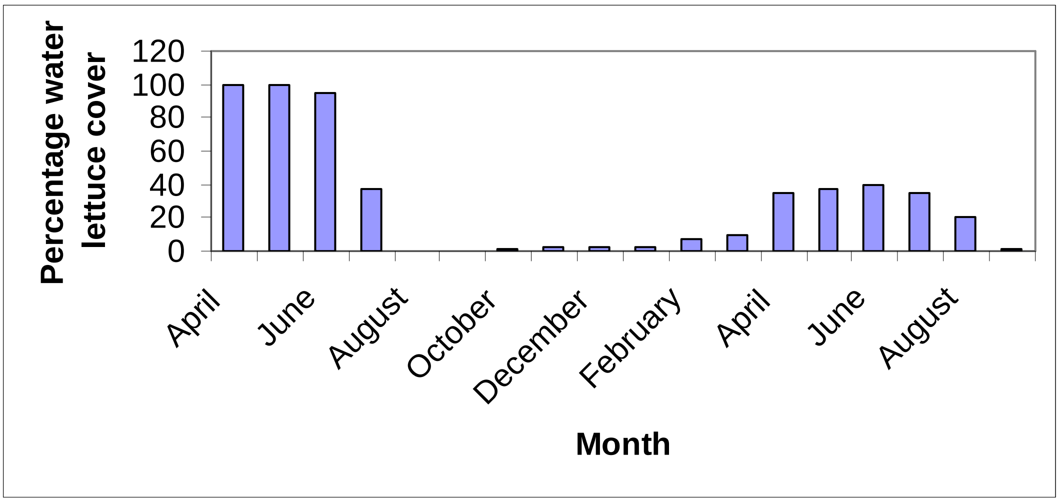

Fig. 3.1. Percentage coverage of water lettuce on the upper pond of Cape Recife for every month from April 2003 (seven months after the introduction of the weevils) to September 2004.

3.4.3. Number of plants per quadrat

The mean number of plants per quadrat showed that there were many gaps in the data, when there was no data available, because of the fact that the quadrats were free over many months (Fig. 3.2). The mean number of plants sampled in May 2003 appears to be much smaller than for May 2004, which is true, although the plants for May 2003 were much larger (mean 191.97 g ± 247.5938 g) than the plants for May 2004 (mean 3.79 g ± 8.2300 g), (Fig. 3.3).

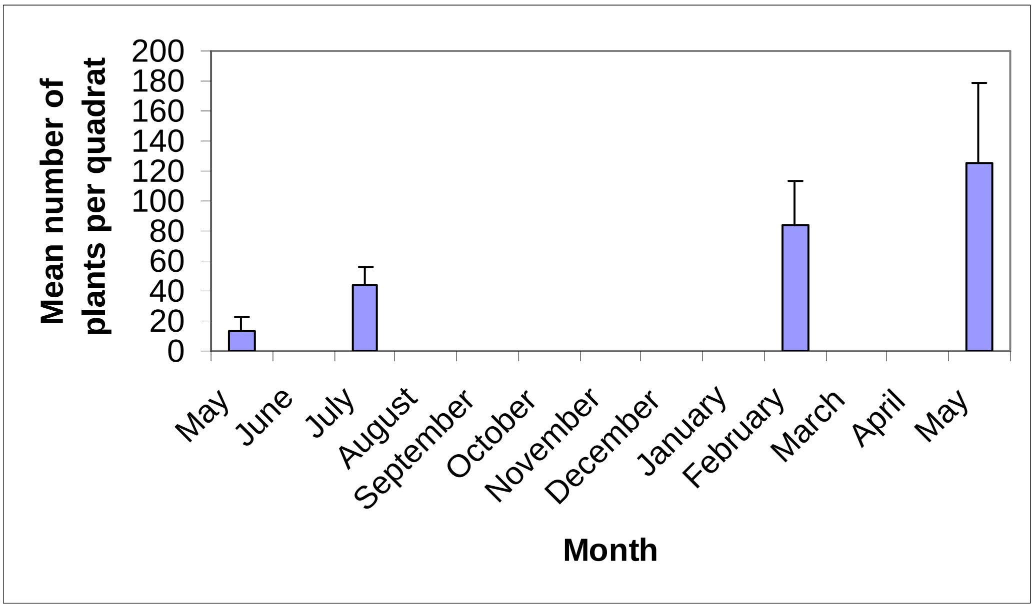

Fig. 3.2. Mean number of plants per quadrat for plants sampled at Cape Recife from May 2003 to May 2004.

3.4.4. Method difficulties

There were a few complications with using the fixed quadrat method to sample water lettuce. Firstly, when using fixed quadrats to measure waterweeds, wind often blows mats of weed around. Plants can be blown completely from one shore to another in a matter of minutes should the wind direction change. This was one consideration, which we did not take into account, as we did not expect the weed-mat to collapse completely within such a short space of time. Had the weed-mat not collapsed; fixed quadrat sampling would have been an ideal method of sampling the weed. However, once started, it was a good idea to continue with the same sampling technique, so fixed quadrat sampling was continued throughout the project. There were many zero values in the quadrat data, which were largely due to the fact that there were just no water lettuce plants within the quadrats, which made the usefulness of graphs taking into account for example number of plants per quadrat much less meaningful.

3.4.5. Plant Growth Rate Parameters

The plant variables measured in this study included fresh biomass (g), plant height (cm) along with counts of ramets, leaves, fruits and seeds per plant (Figs. 3.3-3.9). All the plant growth variables exhibited similar trends, with the plants large by May 2003 but becoming smaller during winter. Plant size was smallest during September because most were seedlings. Plants then gradually became larger until the end of sampling during May 2004.

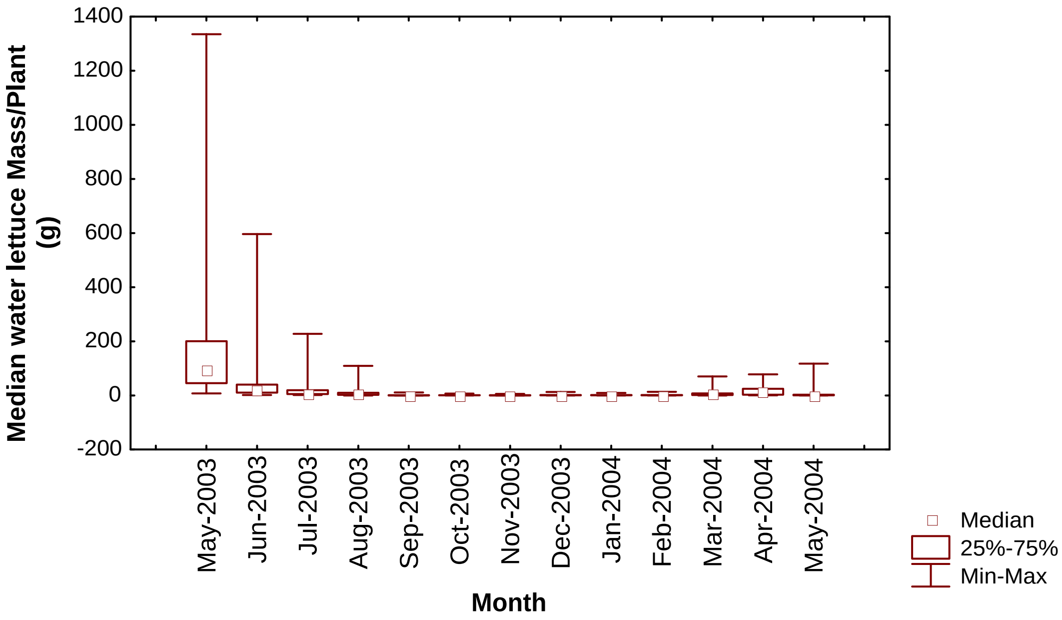

Figure 3.3. Median water lettuce plant masses per plant per month from a year of monthly sampling at Cape Recife (note the large range in plant mass for May 2003; see Appendix 3 for results of statistical analyses).

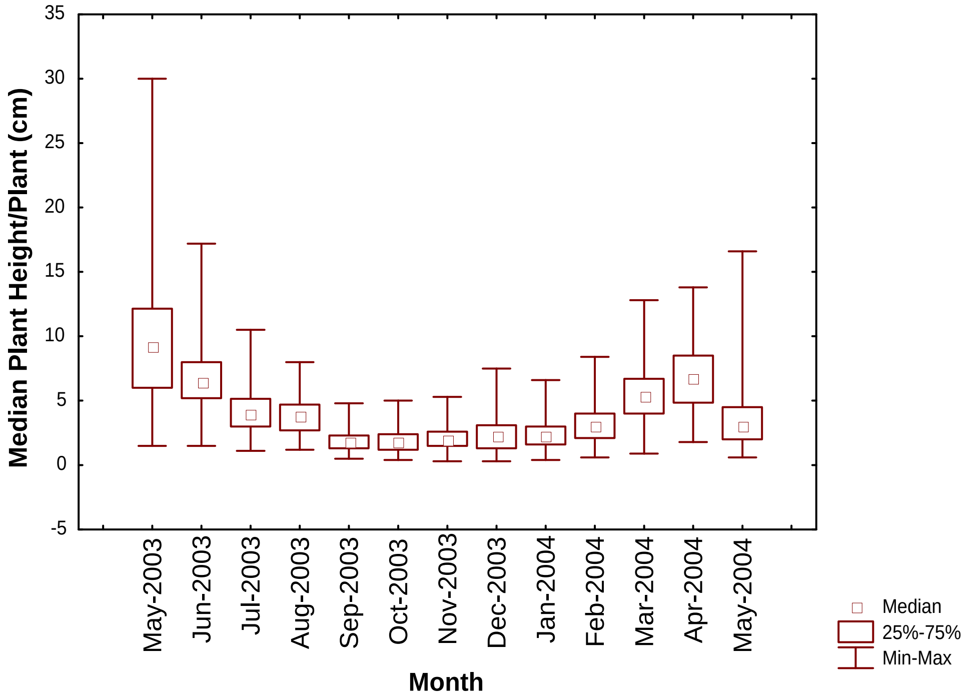

Figure 3.4. Boxplot of median water lettuce plant heights over a year of monthly sampling at Cape Recife from May 2003 to May 2004.

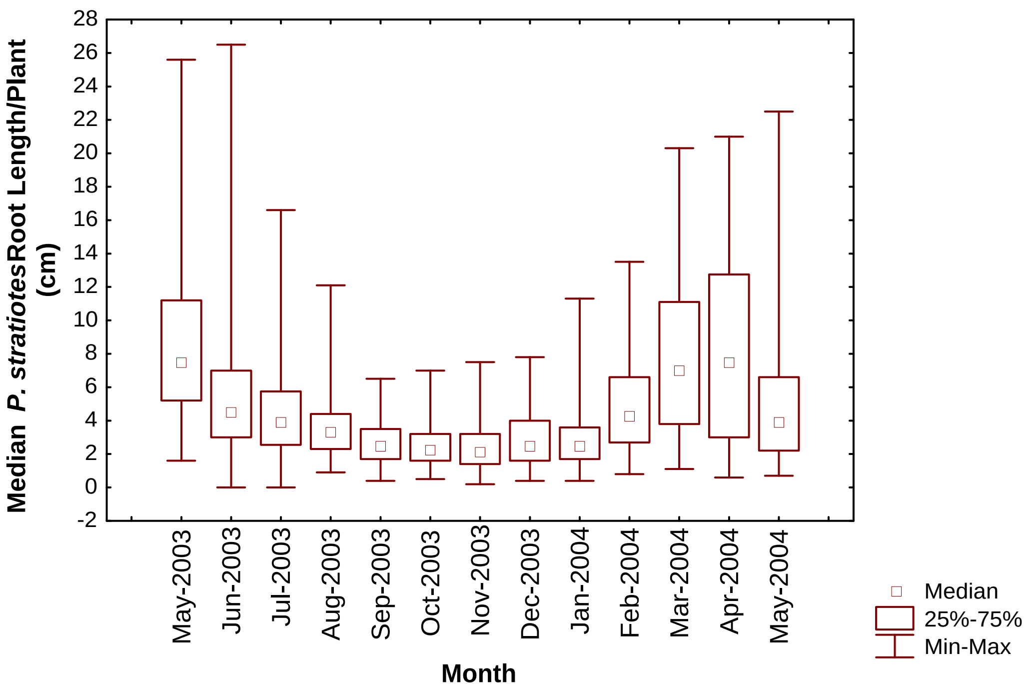

Figure 3.5. Box-plot of median water lettuce root lengths sampled at Cape Recife over a period of a year from May 2003 to May 2004.

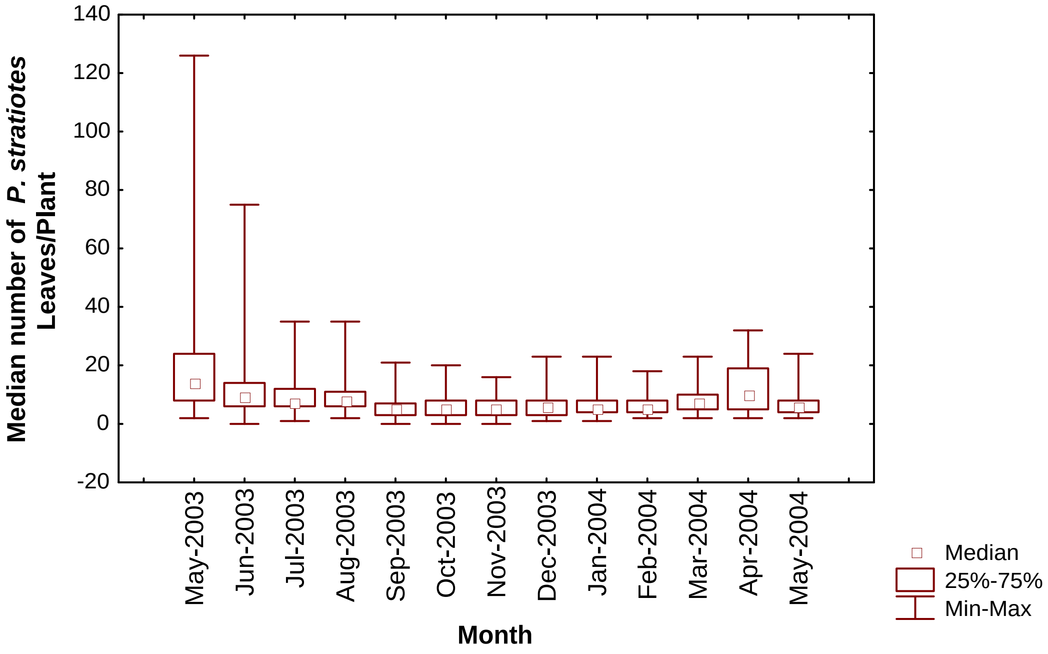

Figure 3.6. Median number of water lettuce plant leaves per plant per month for plants sampled monthly at Cape Recife over a period of a year.

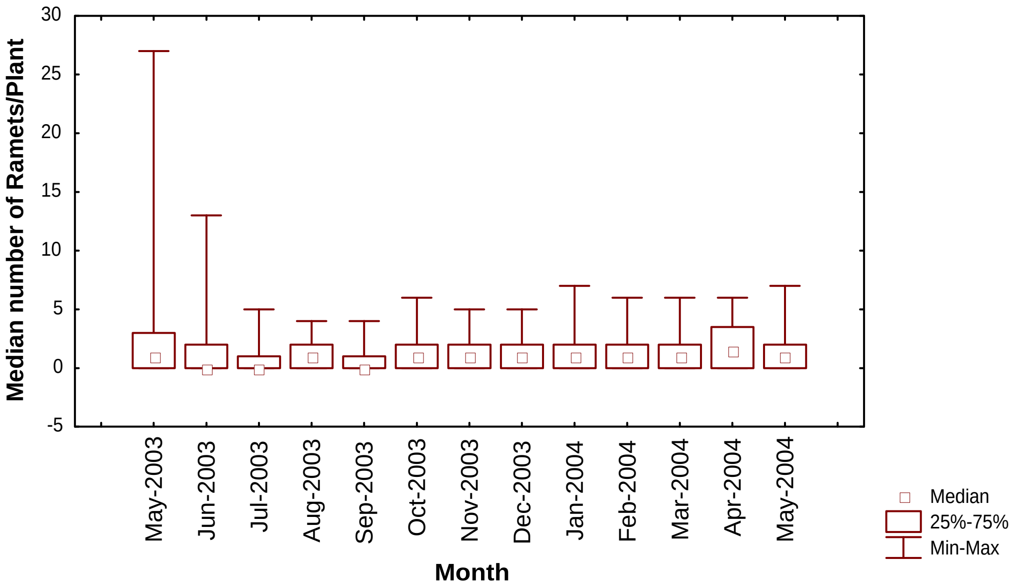

Figure 3.7. Median number of water lettuce ramets per plant per month after a years worth of sampling at Cape Recife.

3.4.5. Plant mass, height, root length and number of ramets

A sharp decrease was noted in all water lettuce plant growth variables after May 2003, going into winter, generally reaching their lowest values in September 2003, after which they all started to slowly increase again.

August 2003 and April 2004 may have stood out from the rest of the months in Fig. 3.4, possibly because these were relatively small sample sizes. In the August 2003 sample, the sampling was biased, as the pond was completely clear of weed, with only a few plants to be found and sampled in amongst the reeds and bulrushes, which on average housed larger plants than open water.

3.4.6. Number of fruit and seed

Figure 3.8 shows that there were virtually no fruits or seeds after May 2003. The numbers of fruits decreased drastically going into winter, with none to be found thereafter. The plants that appeared in spring were all seedlings. The plants remained very small during most of the summer.

Over most of summer, the plants remained very small in size, which is likely a reflection of stunting, most likely from heavy weevil feeding damage. All the energy resources would have been channeled into growth and vegetative reproduction as opposed to flower and seed formation (sexual reproduction), which are more energy costly. The plants may also have not flowered because a critical plant size was not reached before flowering could occur, because of heavy weevil-feeding damage.

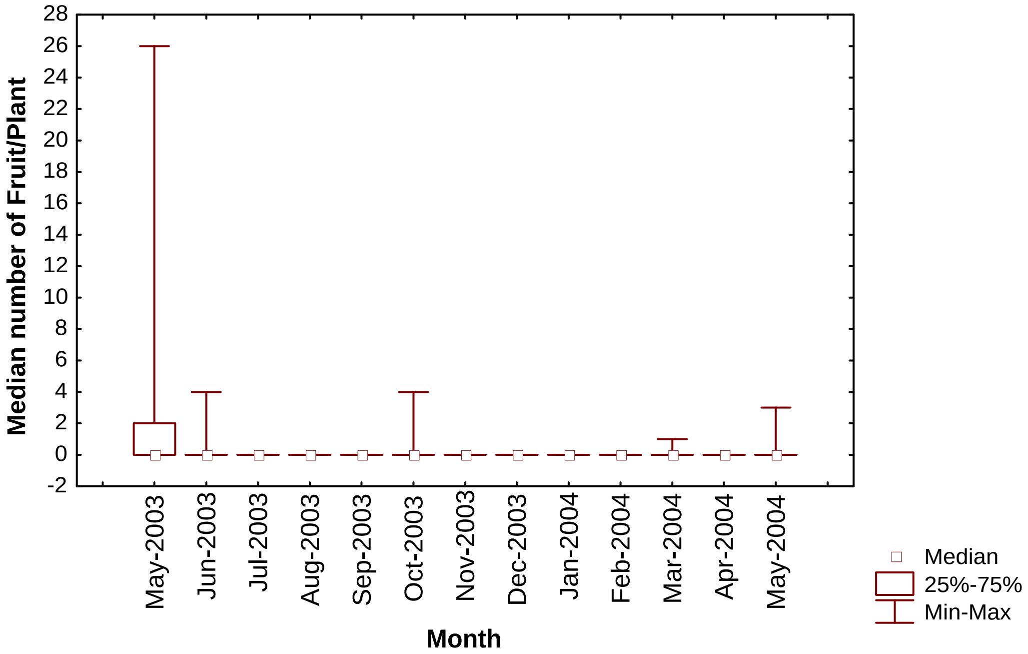

Figure 3.8. Median number of fruit per plant per month for water lettuce plants sampled at Cape Recife over a year of sampling.

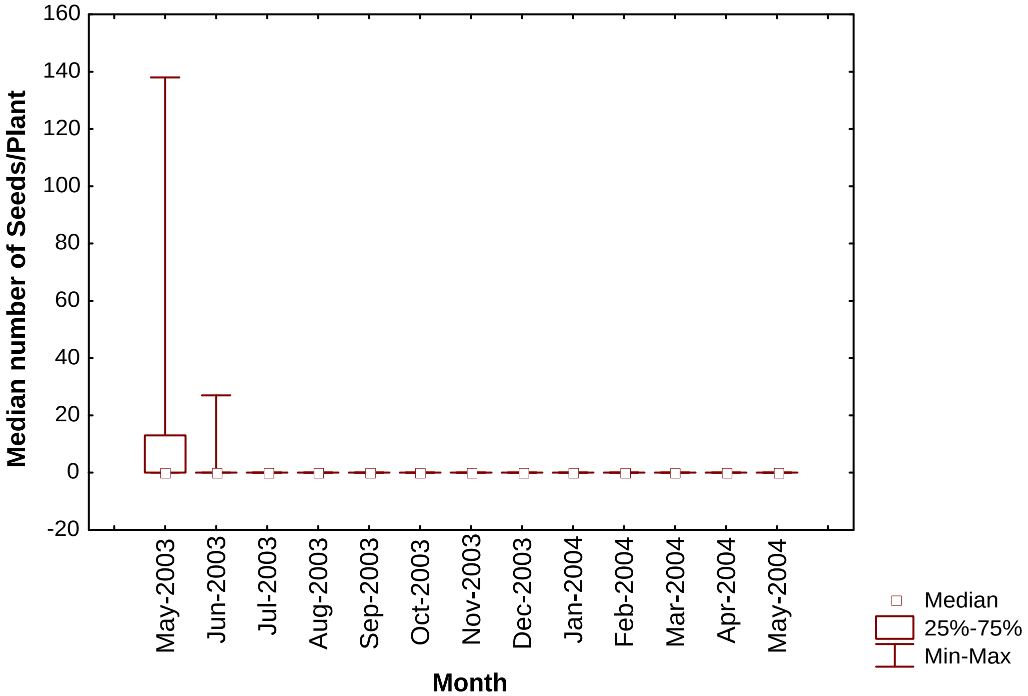

Figure 3.9. Median number of water lettuce seeds per plant sampled over a year at Cape Recife.

3.4.7. Weevil populations and their damage assessment at Cape Recife

During May 2003 weevil numbers were very high, as were larvae and pupae, when compared to other times of the year (Figs. 3.10-3.12). Weevil damage was simultaneously very prominent.

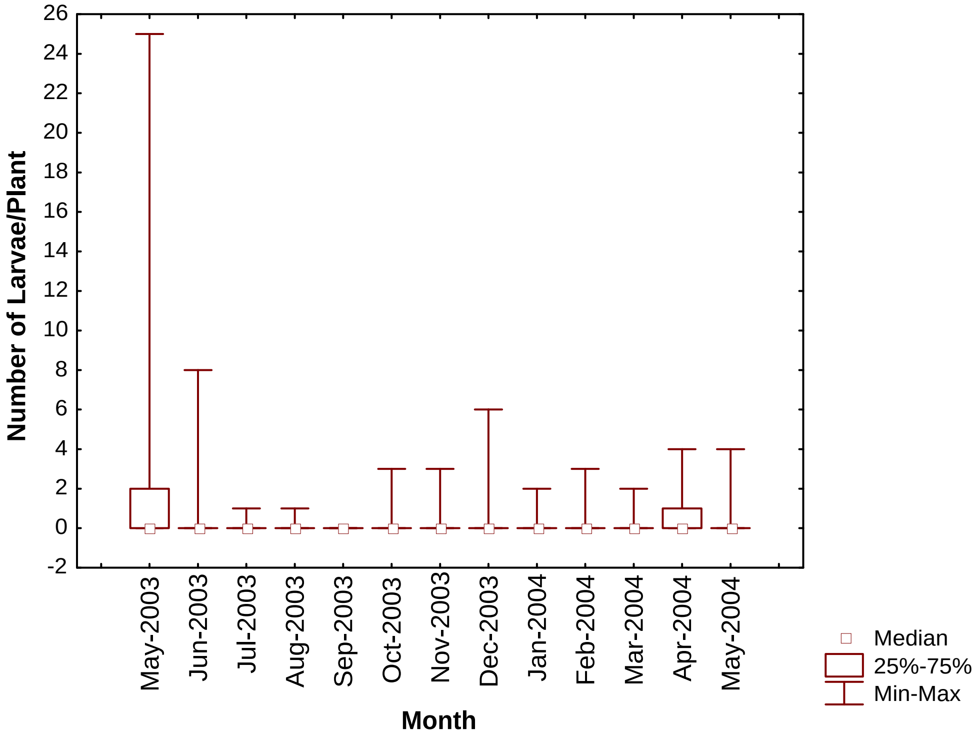

Figure 3.10. Median number of *N. affinis* larvae per plant collected at Cape Recife over a year of sampling.

3.4.8. Number of larvae

Counts of larvae (Fig. 3.10) showed a seasonal trend wherein numbers decreased rapidly from May 2003 onwards up until the end of the sampling period.

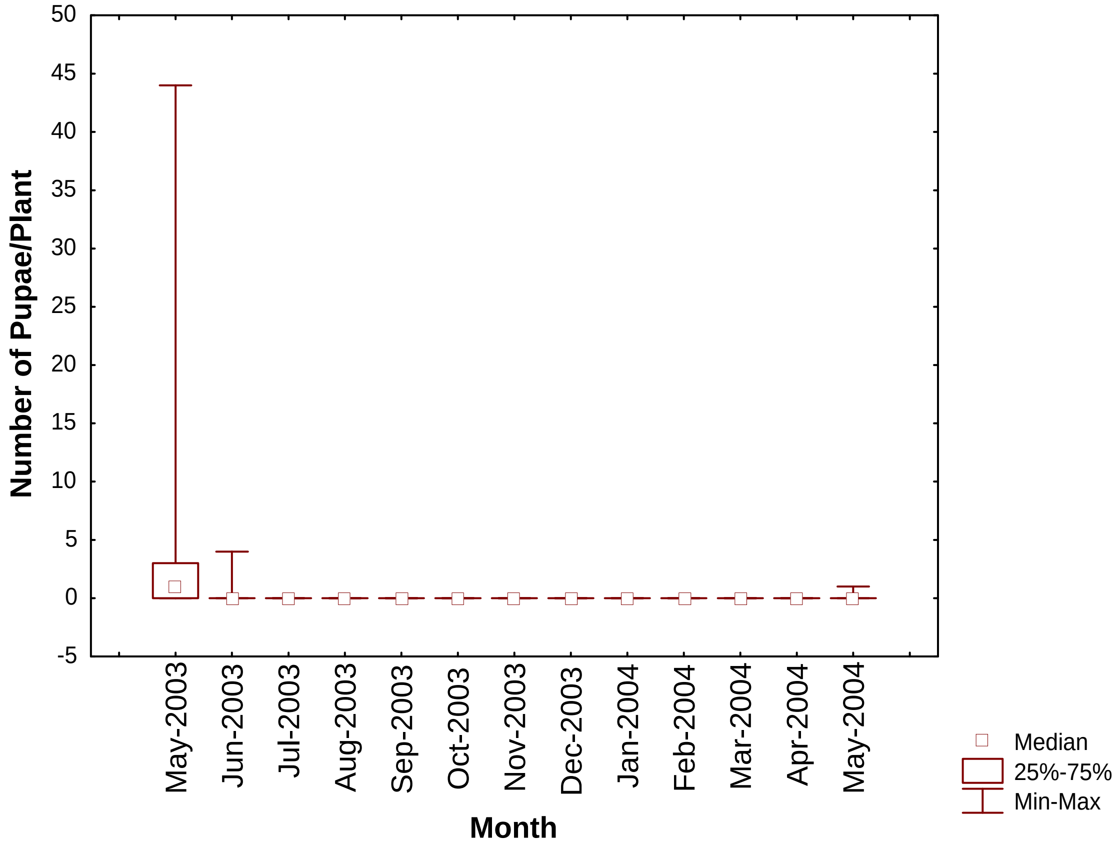

Figure 3.11. Median number of *N. affinis* pupae per plant over a year of monthly sampling at Cape Recife.

3.4.9. Number of Pupae

Most of the pupae died (Fig. 3.11) with the winter crash of plants. There was little evidence of pupae during mid to late summer thereafter, going into winter again in 2004.

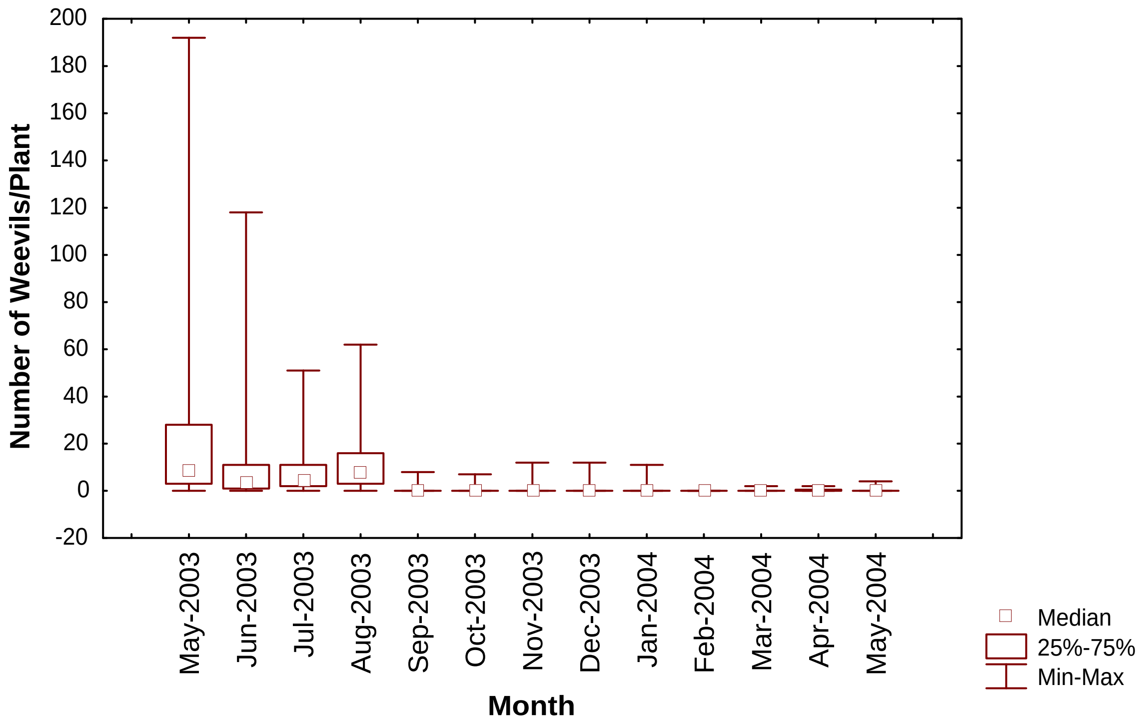

Figure 3.12. Median number of adult *N. affinis* weevils per plant sampled at Cape Recife.

3.4.10. Adult weevils

The median number of adult weevils (Fig. 3.12) dropped steeply from May-June 2003, with very few adult weevils/plant to be found thereafter.

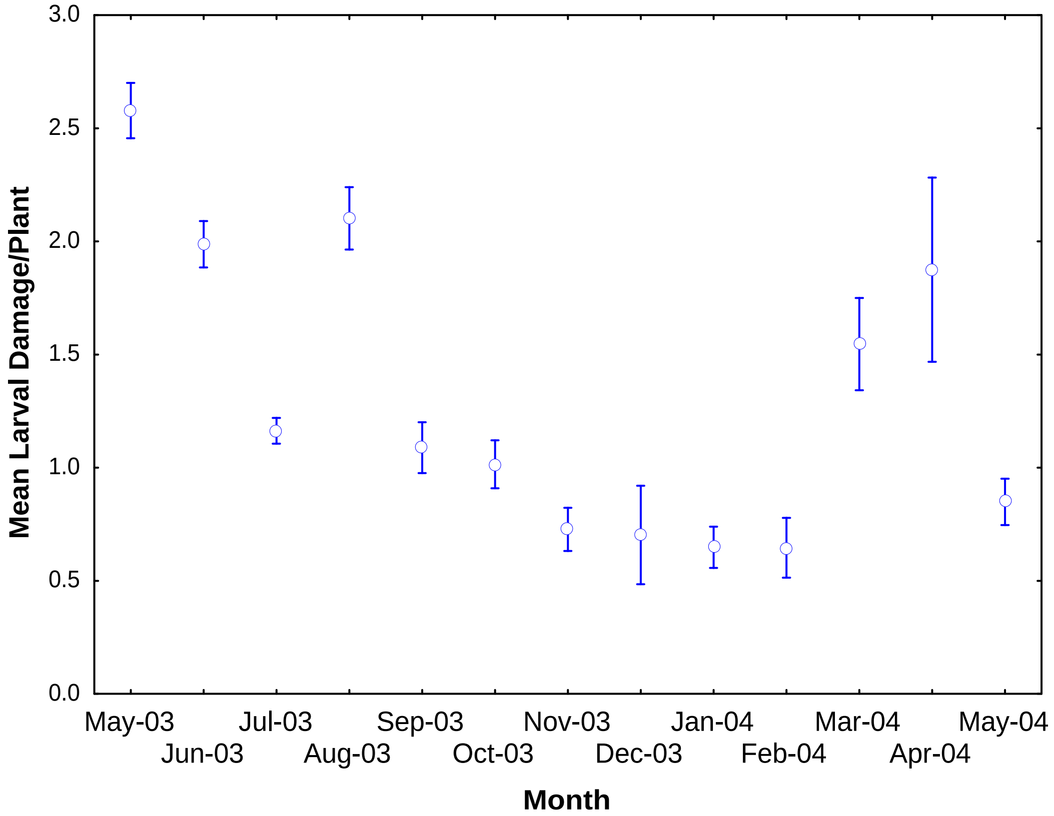

Figure 3.13. Mean *larval N. affinis* damage scores (from 0-5) per plant per month for plants collected over a year of monthly sampling at Cape Recife (error bars denote standard errors).

3.4.11. Larval Damage

Mean larval *N. affinis* damage scores (Fig. 3.13.) decreased steeply from a maximum in May 2003 till June 2003. From here it increased sharply for a month, decreasing sharply again going into September 2003. From here it decreased gradually to November 2003. From here larval damage remained very low, but constant up until February 2004. From February 2004 to April 2004 larval damage increased dramatically, finally decreasing again going into May 2004.

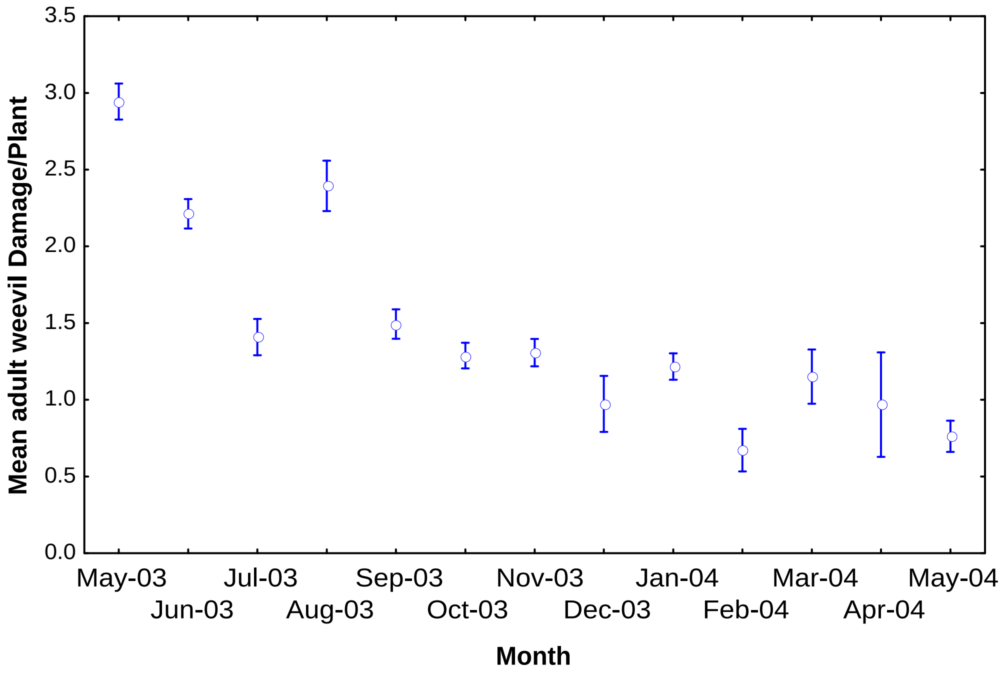

Figure 3.14. Mean adult *N. affinis* shothole damage scores (rated 0-5) for plants sampled at Cape Recife over a year of sampling (error bars denote standard errors).

3.4.12. Adult weevil damage

Adult weevil shothole damage scores followed a very similar pattern with the same trends as the larval damage scores, except the curve was smoother, (see Fig. 3.14).

3.5. Discussion

3.5.1. Plant growth

The winter 2003 decline by water lettuce at Cape Recife was due to insect damage although cold damage was undoubtedly an ancillary factor. Many plants showed cold damage and had rotting leaves during winter, even those with negligible weevil damage. However, cold alone was not enough to kill the plants during the winter of 2002 in the coastal environment of Cape Recife. Therefore the effects of the insects would seem to be far more important than the effect of cold in as much as plants did not crash during previous winters while insect numbers were low. Thus, insect and cold damage combined to increase the mortality of the plants.

The small size of plants throughout most of summer thereafter is indicative that weevils were largely responsible for keeping the water lettuce plants small. This trend continued through most of summer and it is highly likely that the plants would have totally covered the upper pond within a few months had the weevils not been present. The fact is that the weed has not completely covered the upper pond since the May 2003 crash, and this can only be attributed to the weevils. It is unlikely that the plants will again cover the entire ponds. However, it cannot be totally ruled out, and there will likely be fluctuations in numbers of weevils and weed-coverage until a stable balance is reached between weed and weevil. This balance will likely take several years, Sunset Dam, has taken about 6 years to stabilize (L. Foxcroft pers. com).

The size of water lettuce plants also creates some difficulties if one is to interpret the number of plants per quadrat. As can be seen in Fig. 3.2, it would appear that there were fewer plants in May 2003, which there were, however, these plants were much larger than the plants sampled in May 2004 (191.97 g ± 247.5938 g in May 2003 compared to 3.79 g ± 8.2300 g in May 2004), and this is one consideration which has to be taken into account when considering number of plants per quadrat alone and possibly a better measure would have been biomass per quadrat.

August 2003 and April 2004 may have stood out from the rest of the months in Fig. 3.4, possibly because these were relatively small sample sizes. In the August 2003 sample, the sampling was biased, as the pond was completely clear of weed, with only a few plants to be found and sampled in amongst the reeds and bulrushes, which on average housed larger plants than open water. The increase in mean plant height towards the end of summer going into Autumn was likely due to cold temperatures, where the plants had an edge over the weevils, which appear to be cold-sensitive, and hence the plants escaped a little around this point.

3.5.2. Number of fruit and seed

There were virtually no fruit or seeds after May 2003, with the number of fruit crashing drastically going into winter, with virtually no fruit or seed to be found thereafter. The resultant plants, which appeared in spring, were all seedlings and therefore would have taken time to mature and seed again.

3.5.3. Water lettuce weevil populations and damage at Cape Recife

The water lettuce weevil population at Cape Recife appears to be affected by seasonality to some degree and their populations follow a very similar trend to the weed population dynamics. Seasonality would appear to shape the weevil population, with cold winter temperatures appearing to have large negative effects on the weevil population numbers in all stages of their lifecycle, but more so for the larvae and pupae. However, weevil populations also crash when the plants rot, sink and die, as they did over this same cold winter period.

This same effect of seasonality has been found with other studies, e.g. Cilliers *et al*. (1996) found that water lettuce in the KNP was controlled most effectively in summer, with control being less effective going into winter. However in the absence of weevil damage the plants appear to tolerate cold much more so than they do when weevils were present, therefore cold and insect damage severely stress the plants, almost always resulting in a complete crash of the weed mat. Colder winter temperatures kill plants when ambient temperatures approach zero, this is evident from plants kept several times in tanks exposed to the elements of weather in Grahamstown over winter, (which has lower winter temperatures than Cape Recife) all plants died completely from cold-damage with and without any insects present (water lettuce growth actually ceases around 15°C (Kasselmann, 1995)).

3.5.4. Number of larvae

The large decrease in mean number of larvae from May 2003 onwards, in (Fig 3.10) could have been caused by the rapid drop in temperatures after this point, causing a crash in the weed mat, likely killing all larvae, which would have drowned and sunk with the dying plants.

3.5.5. Number of pupae

In early to mid-summer, there was little evidence of pupae as well as toward the end of summer, when one would have expected to have seen many pupae. This may have been due to the fact that the plants were small and did not provide easy pupation sites, which are usually found at the bases of thick leaves on larger plants. Alternatively, and more likely there were fewer observed pupae because of the small weevil population over this period, pupae were likely present, but were simply over-looked.

3.5.6. Biological control efficacy at Cape Recife

Biological control appears to have been highly successful at Cape Recife so far, even if it resulted in coverage of the ponds for some part of the year in 2003 and 2004. A stable equilibrium between plant and weevil (where the plant covers less than 20 % of the pond) would appear to exist through most of summer, but this wave of stability tends to break towards winter, when control becomes less effective, probably due to the reduction in insect damage under colder conditions. A minimum of 5-10 years study would be needed to show a more reliable picture than a years' worth of the Eastern Cape's unpredictable weather/climate. Alternatively a series of exclusion experiments would give a clearer picture of the insect/weevil dynamics. It is highly likely that over a few years, a cycle of opening and closing of the ponds by the weed might be found, similar to that that experienced at Sunset Dam. After more than 6 years it seems that biological control at Sunset Dam may have finally stabilized, and is currently offering total control of water lettuce there (L. Foxcroft pers. comm., Kruger National Park). It is well known that successful biological control does not always occur within a short time frame of one or two years, it may take several years to stabilize and become successful, and sometimes even up to 10 years.

Over a year of sampling at Cape Recife, we can deduce that adult and larval weevil feeding-damage can be devastating in terms of controlling water lettuce, despite high nutrient conditions. Both the weed and the weevil appear to be sensitive to relatively cold Eastern Cape winters. The combined damaging effects of the weevils appear to cause a total crash and clearing of the weed within a few months in this climate, once their numbers have built up. Relatively cool coastal Eastern Cape winters alone also do not appear to be enough to cause the weed to die back totally. Most of the differences in weed coverage and dynamics have been very pronounced and sudden. The biological significance under these circumstances should be perceived to be much more important than any statistical differences.

3.5.7. The cost savings of biological control to Cape Recife

Huge amounts of spraying have been avoided on the Cape Recife ponds, because of the release of the weevils. The cost had they not been introduced, would have been very large, as the area relies on open water for access to water birds, which supply tourism for the park. Herbicide applications would have had to have been applied at least three times a year, because of regeneration of plants from seeds. The cost of spraying water lettuce on Cape Recife would have been R 2632/ha (in 2000 Rands). This cost would have included equipment and labour costs. (US Dollar costs ± R 8 = 1 USD (van Wyk and van Wilgen 2002). As soon as open patches appeared waterfowl returned to the ponds, this would have attracted birders and therefore tourism to the reserve. The actual cost of biological control was likely very little. The weevils were provided and released free of charge by Abbie Heunis of PPRI, and my research at the ponds was also provided free of charge.

3.5.8. Further research

Water lettuce seeds would appear to play a large role in allowing the plants to re-infest and therefore this aspect should be looked at in more detail. The effect of the *N. affinis* weevils in reducing seed output also needs to be researched. There appears to be a seed bank at Cape Recife, but it is not known how long the seeds remain viable for and how long it will take for this seed bank to be depleted.

3.6. Conclusion

This study on the post-release evaluation of *N. affinis* at Cape Recife is still in its infancy, and it is still too soon to tell whether biocontrol of water lettuce with *Neohydronomus affinis* at Cape Recife will be totally effective. The very high levels of nutrients may mean that biocontrol will never be completely effective. However, from a recent trip to Cape Recife (May 2005), and personal communication with the water reclamation works officer, control at Cape Recife would seem to be complete, and has more or less stabilized, even under eutrophic/hypertrophic nutrient conditions. This information is very exciting and together with this thesis data (Chapters 2 and 3), it is suggested that *N. affinis* is capable of controlling water lettuce, under high nutrient levels in the field. The laboratory experiment results compliment the results from the field, and both suggest that biological control of water lettuce under eutrophic conditions is indeed possible. However, in the field, a time frame of several years needs to be given for the weevils to stabilize. Fluctuations in weed density will most likely occur, however, these fluctuations should dissipate over time as the weevil and weed populations stabilize further.

Chapter 4

Wing-muscle development in *Neohydronomus affinis*

4.1 Introduction

Wing-muscle development and wing polymorphism in many insects has been linked to food quality and dispersal (Young, 1965; Kisimoto, 1965; Tanaka, 1993; Zera and Mole, 1994; Mole and Zera, 1994; Denno, 1994). There are 2 main classes of insect dispersal polymorphisms; namely wing polymorphism, and wing-muscle polymorphism. Wing polymorphism occurs when there are variations in the size of wings and flight muscles. In wing polymorphic species, there are commonly macropterous or alate (fully developed wings and flight capable) and brachyperous or apterous (both incapable of flight) forms of the species. The flight incapable forms may be brachypterous (possessing reduced wings) or apterous (lacking wings). Species that possess wing polymorphisms are commonly found in aphids and plant-hoppers (Zera and Denno, 1997). Dispersal polymorphism has been noted to occur in the Orthoptera, Psocoptera, Thysanoptera, Homoptera, Heteroptera, Coleoptera, Diptera, Lepidoptera and Hymenoptera (Roff, 1990; Denno *et al*., 1991; Zera and Mole, 1994). However, dispersal and wing polymorphism was last studied by Hardie and Lees, (1985) and Pener, (1985).

With wing-muscle polymorphism, or flight polymorphism, wings are present; however there can be differences within the degree of development of the flight muscles, and this would result in insects that are either capable or incapable of flight. With wing-muscle polymorphism, there are often trade-offs between the development of flight muscles for dispersal and ovariole development for reproduction. In fact, ovarian development and wing-muscle development from studies done thus far have proven to be mutually exclusive. This trade-off is known as the 'flight-oogenesis syndrome' (Johnson, 1966; Mole and Zera, 1993; Roff, 1986; Zera and Mole, 1994). Insects reared on poorer quality or older plants are generally known to be smaller, have more developed wing-muscles, take longer to develop, and to have delayed reproduction compared to insects reared on higher quality food. All of these qualities would likely be more suited for insects that are migrating or about to disperse (Zera and Denno, 1997). This behaviour makes the macropter responsible for colonizing new habitats (Denno *et al*., 1991). Some macropters have the ability to histolyze their wing-muscles (Dixon and Howard, 1986; Fairbairn and Desranleau, 1987, Kaitala, 1988). Flight-muscle histolysis can also be caused by wing-shedding (de-alation) (Tanaka, 1993, 1994). In insect species that have both alate and flightless morphs, flightless morphs have been found to have larger ovaries, earlier ovarian development and reduced flight fuels, compared to the alate forms (Zera *et al.*, 1994). Dispersal by the macropter is often orders of magnitude larger than the brachypter, because the brachypter can only disperse by walking or hopping (Denno *et al*., 1980).

Many weevil species have been found to undertake wing-muscle polymorphism. A notable study of this phenomenon was produced by Muda *et al*., (1981), who described the generation and degeneration of flight muscles the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae). In the water hyacinth weevils, *Neochetina eichhorniae* and *N. bruchi* in northern Florida, flight muscle development appeared to be stimulated by increased temperature, and at the same time, while there was flight muscle development, there were corresponding degeneration in any ovarian and egg development (Buckingham and Passoa, 1984). These authors also found that overwintering *N. eichhorniae* and *N. bruchi* weevils in northern Florida were found to have undeveloped flight muscles.

4.1.1 Aims

Wing muscle development gives insight into the dispersal ability of many insects, most likely including *Neohydronomus affinis*. Therefore the aim of studying wing muscle development was to demonstrate at what state the *N. affinis* weevils were in at Cape Recife at any time, and hopefully get a better understanding about dispersal in this species. The effect of different nutrient concentrations on wing muscle development in *N. affinis* weevils would also show whether nutrients have an effect on wing muscle development and therefore dispersal.

4.1.2 Hypothesis

Ho: As food quality declines, *N. affinis* wing muscle development increases.
Ha: As food quality declines, *N. affinis* wing muscle development does not increase.

4.2. Methods

Neohydronomus affinis weevils were collected from all the plants sampled monthly from the field study site at Cape Recife. These weevils were placed inside glass vials, in 70 % ethanol, to be dissected later. Another set of weevils maintained for six weeks on water lettuce at different nutrient concentrations (Chapter 2) were collected and stored in alcohol after the laboratory experiments. The weevils were first removed from 70 % alcohol and dried on blotting paper. The insects were then fixed rostrum-down on a drop of super-glue on a glass slide. The elytras and wings were then removed with a pair of fine forceps, as well as the thin abdominal cuticle membrane, just behind the thorax, covering the wing-muscles. When this was done effectively, the dorso-ventral and median dorsal longitudinal muscles could clearly be seen lying along the tergum. The median dorsal longitudinal (MDL) muscles were chosen to categorize the wing-muscle development (Table 4.1). Three categories were developed, according to different stages of wing-muscle development; poor, moderate and well developed.

4.3. Results

Overall, wing-muscles were mainly found to belong to the category 1 state of development for most of the weevils sampled. Unfortunately sample sizes for each of the months sampled were not equal, with some months containing many more weevils than other months. For October 2003 and February 2004, no weevils, were sampled (4.3 and 4.4).

Table 4.1. Different categories of wing-muscle development assigned to *N. affinis* weevils sampled at Cape Recife over a year of sampling.

Category	**Comments**
1	Poorly developed MDL wing-muscles, thin and pale in colour, often appearing thread-like.
2	Medium developed MDL wing-muscles, larger than category 1, but smaller than category 3, with muscles darker in colour.
3	Well-developed MDL wing-muscles, with thick bands of muscle present, considerably thicker than category 2, and also dark in colour.

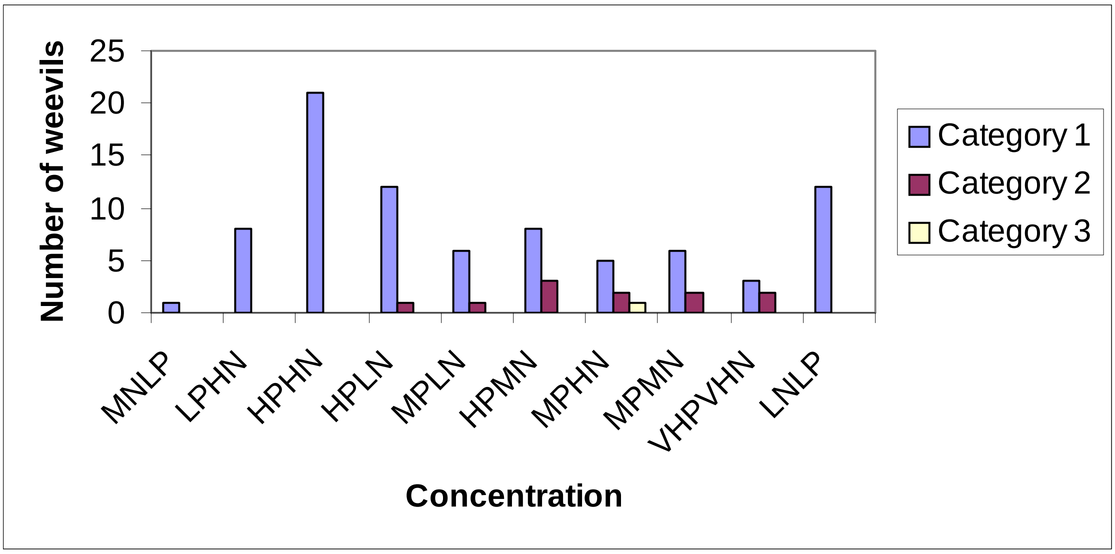

Figure 4.1. Number of weevils categorized from 1-3 wing-muscle state of development from the laboratory experiments conducted in Chapter 2 (see Table 4.1 above for reference to wing-muscle development categorization).

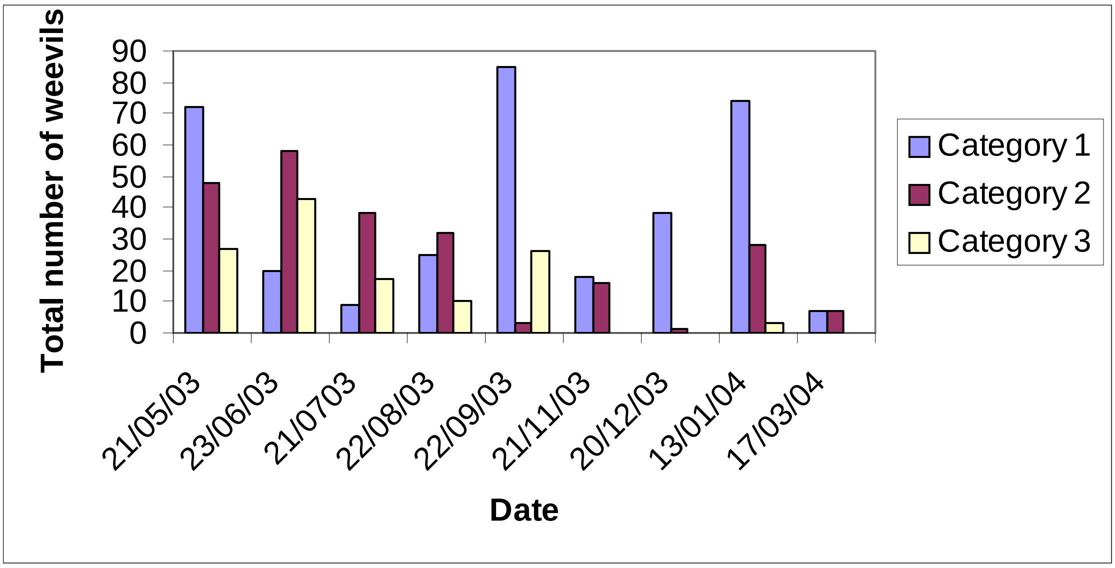

Figure 4.2. Categorization of wing-muscles according to 3 different sates (see Table 4.1 for the description of the different categories) of development of *N. affinis* weevils collected monthly at Cape Recife over a period of a year.

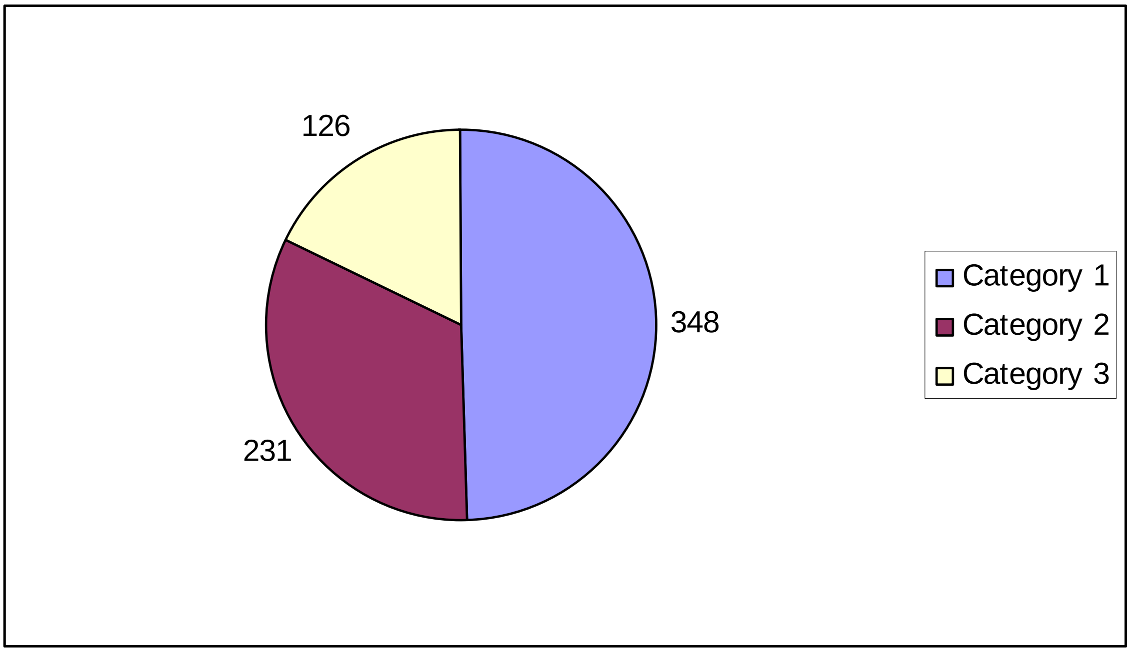

Figure 4.3. Total percentage of wing muscle development according to the 3 different categories of wing-muscle development of *N. affinis* weevils at Cape Recife over a year of sampling (numbers outside the pie-chart indicate sample sizes).

The different proportions of the different weevil wing-muscle categories sampled at Cape Recife can be seen in Fig. 4.2. From September 2003 onwards, no category 3 wing-muscles were noted, except for a small proportion in January 2004.

Excluding May and September 2003, the most abundant proportion of wing-muscles were made up of category 2, or moderately developed wing muscles, which would have suggested that most of the weevils were approaching a state of active dispersal. From September 2003 onwards, the major proportions of weevils were found to be in an inactive state of wing-muscle development, with category 1 or poorly developed wing muscles predominating.

In Fig. 4.1, the sample sizes were usually very small, but a trend can still be seen, with most of the weevils in an inactive state of wing-muscle development. However, quite a few concentrations were shown to reveal a small number of weevils in category 2 development, with a moderate state of wing-muscle development. Only one nutrient concentration, medium phosphate, medium nitrogen (MPMN), showed any signs of category 3 state of wing-muscle development.

4.4. Discussion

Wing-muscle development in the weevil *Neohydronomus affinis* would appear from the field collected weevils at Cape Recife to be related to food quality and dispersal, as found with many other insects. However, the time of year and state of wing-muscle development was found to be completely the opposite of that found for *Neochetina* weevils in northern Florida (Buckingham and Passoa, 1984). The reason for this is unclear, as one would have expected the biology of *Neochetina* weevils to be fairly similar to *Neohydronomus* weevils. However, if one looked at the time of year at Cape Recife and how it corresponded to food quality, one definitely discerned a trend in dispersal over winter with *N. affinis* as food quality deteriorated significantly leading into winter. Cold might represent a significant dispersal barrier, unless dispersal occurred over warm window-periods (cold weather interspersed with short warm periods), which is likely. The ability to disperse could be perceived to be very important with insects, especially when food quality declines substantially and if insects have the option of wing or wing-muscle polymorphism, selection would seem to favour such individuals which could potentially fly off to new areas of better quality food. However, the wing-muscle development in the laboratory study was inconclusive, probably due to the short duration of the study and the relatively small sample sizes encountered. Therefore the implications for biological control are that as the weevils disperse over autumn/winter where seedling recruitment occurs in spring there are fewer insects to combat the resurgence.

Most of the weevils were in a category 1 or 2 state of wing muscle development, which would have suggested that most of the weevils sampled at Cape Recife were not in an active state of dispersal. The most important facts to be interpreted from figure 4.2 are the high incidences of category 3 or strongly developed wing-muscles from May 2003 to September 2003, which corresponds to most of the winter period to early spring, the same time when the crash of the weed occurred.

Chapter 5

General Discussion and Conclusion

5.1. Introduction

Water nutrient status, in particular high levels of nitrates and phosphates have been shown to exacerbate waterweed problems in South Africa and around the world and have negatively impacted biological control efforts (Hill, 2003). A number of studies have raised the concerns that biological control of aquatic weeds might not be achievable with extremely high nutrient levels (Hill and Olckers 2001; Center *et al.*, 2003).

In this study, the laboratory studies showed clearly that *N. affinis* is capable of controlling water lettuce at extremely high nitrate and phosphate levels (Chapter 2). As these levels far exceed the ambient levels in South African aquatic ecosystems (Coetzee 2003). It was concluded that the weevil should be able to control the weed no matter what the nutrient status of the water. This was tested at a field site, Cape Recife that was classed as eutrophic according to the South African water quality guidelines (Coetzee, 2003). In spite of high levels of nitrates and phosphates, and despite a small founder colony the weevils successfully controlled the weed at this site (Chapter 3).

As the field study was only quantified over a 12-month period, subsequent observations indicate that although the plant populations do “bounce back” at Cape Recife, this is short-lived and the weevils are able to effect excellent control (<5 % cover). Whether this is due to the resident weevil population or due to weevils dispersing onto the expanding mat from elsewhere is uncertain, as the results from the wing muscle experiments (Chapter 4) were largely inconclusive.

5.2. Implications for biological control of water lettuce in South Africa

The results obtained during this study replicated what has happened in many other parts of South Africa and the world (Julian and Griffiths 1998) in that successful control of water lettuce is achieved through the introduction of *N. affinis*. More specifically this study is similar to that of Cilliers *et al.* (1996) on Sunset Dam in the Kruger National Park. Both at Cape Recife and Sunset Dam the control achieved was significant and rapid but followed by a resurgence of the weed, most commonly during autumn or winter at Sunset Dam and late winter at Cape Recife. Under these situations (small, highly eutrophic water bodies) several options are available.

5.2.1. Additional Agents

It is probably not necessary to release any more water lettuce biological control agents into South Africa, however there are several other potential biological control agents. In the United States of America (U.S.A.), the noctuid moth, *Spodoptera pectinicornis* was introduced from South-East Asia and following testing released (Dray *et al*., 2001). Despite large releases, this agent failed to establish possibly due to host incompatibility and severe larval and egg predation (Dray *et al*, 2001). It is unlikely that such an agent would be considered for release in South Africa after failure to establish in the U.S.A.

The oligophagous pyralid moth, *Samea multiplicalis* has also been considered for the control of water lettuce and was introduced to Australia for the control of *S. molesta* (Center *et al*., 2002). This insect includes a number of different species of aquatic plants within its host-range and should not be considered for release.

There are at least 11 different weevil species associated with water lettuce in Argentina (Cordo and Sosa 2000, Cordo *et al.*, 1981). However, these weevils, notably the genera *Argentinorhynchus* and *Ochetina* require a dry period for eclosion from pupation and would not be suitable for release in South Africa where water lettuce invades permanent water bodies. In conclusion, although there are several options for additional agents, these don't appear to be applicable to the South African scenario.

5.2.2. Augmentative Releases

From this study and that on Sunset Dam (Cilliers *et al*., 1996) it appears as though the insect controls the plant very effectively during the summer months, but the plant populations return in late winter in the absence of the agents. It has been suggested that augmentative releases of the insect could be made during this time to prevent this resurgence (Cilliers pers com.). However this would require the mass rearing of the weevils during the winter months, which is impractical, due to constraints such as money and space, however, augmentative releases should not be ruled out and demand further study.

One of reasons given for the sporadic resurgence of the weed is that the weevils are so effective in the absence of their natural enemies that they can cause a complete crash and eradication of the weed. This results in a crash of weevil populations and recruitment of the weed from seedlings in the absence of the weevil (Neser, pers com.). This implies that the weevil populations “overshoot” the weed population. A possible method to attenuate this “boom-bust” curve could be to introduce a parasitoid for the weevil. Once again this is impractical as firstly; no parasitoids have been recorded from *N. affinis* in South America. Secondly, the notion of introducing a “mild” parasitoid for a weed biological control agent would require a paradigm shift in the science, which is not recommended at all, especially since such parasitoids are often generalists.

Another possible method for preserving *N. affinis* populations could be to treat sections of the mat with a short residual contact insecticide, thus preserving refugia for the insect populations. Once again this is impractical given the small dams infected by the weed and the management effort required to implement such a method. However biological control at Cape Recife should eventually balance and eliminate the need for any other control measures.

5.2.3. Integrated Control

There is very little literature on integrated control for water lettuce, probably due to the success of the biological control agent *N. affinis*. However, it is likely that integrated control could be very useful in certain areas where the weevil is not effecting complete control, although care would have to be taken to leave refugia areas for the weevils and only insect-friendly herbicides used.

5.2.4. Recommendations

The biological control program on water lettuce is in its infancy at Cape Recife. To date the biocontrol effort has been very effective and it remains to be seen over the next five years how effective this will be in the long-term. Sunset Dam took six years before an acceptable level of control was achieved (Cilliers *et al.*, 2003). It is therefore recommended that no other management intervention be considered for Cape Recife and that the biological control program, as it stands, be afforded the opportunity to become another successful aquatic weed biological control example.

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Appendix

Table 3.1.1. Results of multiple comparisons of Kruskal-Wallis test of water lettuce plant mass collected at Cape Recife: H (12, N = 2360.75, p = 0.000). Yes = significant difference at the 0.95 level of significance, No = no significant difference.

Dependent variable: Mass	**May 2003**	**June 2003**	**July 2003**	**August 2003**	**September 2003**	**October 2003**	**November 2003**	**December 2003**	**January 2004**	**February 2004**	**March 2004**	**April 2004**	**May 2004**
May 2003		**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.007**	**0.000**
June 2003	**0.001**		0.389	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	**0.000**
July 2003	**0.000**	0.389		0.196	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.006**	1.000	**0.000**
August 2003	**0.000**	**0.000**	0.196		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000	**0.000**
September 2003	**0.000**	**0.000**	**0.000**	**0.000**		1.000	1.000	1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
October 2003	**0.000**	**0.000**	**0.000**	**0.000**	1.000		1.000	1.000	**0.021**	**0.003**	**0.000**	**0.000**	**0.000**
November 2003	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000		1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
December	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000	1.000		1.000	1.000	**0.000**	**0.000**	**0.023**

2003													
January 2004	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.021**	**0.001**	1.000		1.000	**0.000**	**0.000**	**0.013**
February 2004	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.004**	**0.000**	1.000	1.000		**0.000**	**0.000**	1.000
March 2004	**0.000**	**0.000**	**0.006**	1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		1.000	**0.000**
April 2004	**0.010**	1.000	1.000	1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000		**0.000**
May 2004	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.023**	**0.023**	1.000	**0.000**	**0.000**	

Table 3.1.2. Results of multiple comparisons of a Kruskal-Wallis test, on water lettuce plant heights recorded at Cape Recife over a period of a year. $H(12, N = 3786) = 2020.43$ $p = 0.000$.

Dependent variable: Plant height	**May 2003**	**June 2003**	**July 2003**	**August 2003**	**September 2003**	**October 2003**	**November 2003**	**December 2003**	**January 2004**	**February 2004**	**March 2004**	**April 2004**	**May 2004**
May 2003		1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	**0.000**

June 2003	1.000		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.346	1.000	**0.000**
July 2003	**0.000**	**0.000**		1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.701	0.712	**0.000**
August 2003	**0.000**	**0.000**	1.000		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.425	**0.016**	0.054	0.121
September 2003	**0.000**	**0.000**	**0.000**	**0.000**		1.000	1.000	0.248	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
October 2003	**0.000**	**0.000**	**0.000**	**0.000**	1.000		0.254	0.100	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
November 2003	**0.000**	**0.000**	**0.000**	**0.000**	1.000	0.254		1.000	0.190	**0.000**	**0.000**	**0.000**	**0.000**
December 2003	**0.000**	**0.000**	**0.000**	**0.000**	0.248	0.100	0.100		1.000	**0.000**	**0.000**	**0.000**	**0.000**
January 2004	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.190	1.000		**0.002**	**0.000**	**0.000**	**0.000**
February 2004	**0.000**	**0.000**	**0.000**	0.425	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		**0.000**	**0.000**	1.000
March 2004	**0.001**	0.346	0.701	**0.016**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		**0.000**	**0.000**
April 2004	1.000	1.000	0.712	0.054	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		**0.000**

May 2004	**0.000**	**0.000**	**0.000**	0.121	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	**0.000**	**0.000**	

Table 3.1.3. Results of multiple comparisons Kruskal-Wallis ANOVA for root lengths of plants collected at Cape Recife over a year of sampling. H (12, N = 3785) = 1125.2 p = 0.000.

Dependent variable: Root length	**May 2003**	**June 2003**	**July 2003**	**August 2003**	**September 2003**	**October 2003**	**November 2003**	**December 2003**	**January 2004**	**February 2004**	**March 2004**	**April 2004**	**May 2004**
May 2003		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000	**0.000**
June 2003	**0.000**		1.000	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	**0.004**	1.000	0.224
July 2003	**0.000**	1.000		1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	**0.000**	1.000	1.000
August 2003	**0.000**	**0.001**	1.000		**0.000**	**0.000**	**0.000**	**0.026**	**0.001**	0.220	**0.000**	**0.046**	1.000
September 2003	**0.000**	**0.000**	**0.000**	**0.000**		1.000	1.000	1.000	1.000	**0.000**	**0.000**	**0.000**	**0.000**
October 2003	1.000	**0.000**	**0.000**	**0.000**	**0.000**		1.000	1.000	0.161	**0.000**	**0.000**	**0.000**	**0.000**
November	1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		1.000	**0.042**	**0.000**	**0.000**	**0.000**	**0.000**

2003													
December 2003	**0.000**	**0.000**	**0.000**	**0.026**	1.000	1.000	1.000		1.000	**0.000**	**0.000**	**0.000**	**0.000**
January 2004	**0.000**	**0.000**	**0.000**	**0.001**	1.000	0.161	**0.042**	1.000		**0.000**	**0.000**	**0.000**	**0.000**
February 2004	**0.000**	1.000	1.000	0.220	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		**0.001**	1.000	1.000
March 2004	**0.000**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**		1.000	**0.000**
April 2004	**0.000**	1.000	1.000	**0.046**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000		1.000
May 2004	**0.000**	0.224	1.000	1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	**0.000**	1.000	

Table 3.1.4. Results of multiple comparisons of a Kruskal-Wallis ANOVA test, testing for differences between months in median number of water lettuce leaves. H (12, N = 3785) = 753.7 p = 0.000.

Dependent variable: Number	**May 2003**	**June 2003**	**July 2003**	**August 2003**	**September 2003**	**October 2003**	**November 2003**	**December 2003**	**January 2004**	**February 2004**	**March 2004**	**April 2004**	**May 2004**

of leaves													
May 2003		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	**0.000**
June 2003	**0.000**		**0.030**	1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.068	1.000	**0.000**
July 2003	**0.000**	**0.030**		1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000	**0.000**
August 2003	**0.000**	1.000	1.000		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000	**0.000**
September 2003	**0.000**	**0.000**	**0.000**	**0.000**		1.000	1.000	1.000	**0.006**	1.000	**0.000**	**0.000**	**0.004**
October 2003	**0.000**	**0.000**	**0.000**	**0.000**	1.000		1.000	1.000	1.000	1.000	**0.000**	**0.000**	1.000
November 2003	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000		1.000	1.000	1.000	**0.000**	**0.000**	0.853
December 2003	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000	1.000		1.000	1.000	**0.027**	**0.007**	1.000
January 2004	**0.000**	**0.000**	**0.000**	**0.000**	**0.006**	1.000	1.000	1.000		1.000	**0.000**	**0.001**	1.000
February 2004	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000	1.000	1.000	1.000		**0.001**	**0.001**	1.000
March	**0.000**	0.068	1.000	1.000	**0.000**	**0.000**	**0.000**	**0.027**	**0.000**	**0.000**		1.000	**0.002**

2004													
April 2004	1.000	1.000	1.000	1.000	**0.000**	**0.000**	**0.000**	**0.007**	**0.002**	**0.001**	1.000		**0.004**
May 2004	**0.000**	**0.000**	**0.000**	**0.000**	**0.004**	1.000	0.853	1.000	1.000	1.000	**0.002**	**0.004**	

Table 3.1.5. Results of multiple comparisons for a Kruskal-Wallis ANOVA H (12 N = 3786) = 101.5775, p = 0.0000 test done on the median number of water lettuce ramets per plant per month. Significant differences between the months are represented by either Yes or No at α = 0.95 level of significance.

Dependent variable: Number of ramets	**May 2003**	**June 2003**	**July 2003**	**August 2003**	**September 2003**	**October 2003**	**November 2003**	**December 2003**	**January 2004**	**February 2004**	**March 2004**	**April 2004**	**May 2004**
May 2003		**0.000**	**0.000**	0.244	**0.000**	**0.001**	**0.000**	1.000	1.000	0.211	1.000	1.000	**0.007**
June 2003	**0.000**		1.000	1.000	1.000	1.000	1.000	1.000	**0.000**	1.000	0.836	0.070	1.000
July 2003	**0.000**	1.000		1.000	1.000	0.188	0.471	0.177	**0.000**	0.284	0.104	**0.0143**	0.206
August 2003	2.444	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
September	**0.000**	1.000	1.000	1.000		0.445	1.000	0.413	**0.000**	0.685	0.244	**0.030**	0.500

2003													
October 2003	**0.001**	1.000	0.188	1.000	0.445		1.000	1.000	0.834	1.000	1.000	0.740	1.000
November 2003	**0.000**	1.000	0.471	1.000	1.000	1.000		1.000	0.517	1.000	1.000	0.587	1.000
December 2003	1.000	1.000	0.180	1.000	0.413	1.000	1.000		1.000	1.000	1.000	1.000	1.000
January 2004	1.000	**0.001**	**0.000**	1.000	**0.000**	0.840	0.517	1.000		1.000	1.000	1.000	1.000
February 2004	0.211	1.000	0.284	1.000	0.685	1.000	1.000	1.000	1.000		1.000	1.000	1.000
March 2004	1.000	0.836	0.104	1.000	0.244	1.000	1.000	1.000	1.000	1.000		1.000	1.000
April 2004	1.000	**0.070**	**0.014**	1.000	**0.030**	0.740	0.587	1.000	1.000	1.000	1.000		1.000
May 2004	**0.001**	1.000	0.206	1.000	0.500	1.000	1.000	1.000	1.000	1.000	1.000	1.000	

Table 3.1.6. Results of multiple comparisons for a Kruskal-Wallis test, testing for significant differences between different months in median number of fruit per plant per month. H (12, N = 3786) = 1248.2 p = 0.000.

Dependent variable: Number of fruit	**May 2003**	**June 2003**	**July 2003**	**August 2003**	**September 2003**	**October 2003**	**November 2003**	**December 2003**	**January 2004**	**February 2004**	**March 2004**	**April 2004**	**May 2004**
May 2003		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**
June 2003	**0.000**		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
July 2003	**0.000**	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
August 2003	**0.000**	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
September 2003	**0.000**	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
October 2003	**0.000**	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000
November 2003	**0.000**	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000
December	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000

2003													
January 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000
February 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000
March 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000
April 2004	**0.001**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000
May 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	

Table 3.1.7. Results of Kruskal-Wallis multiple-comparisons ANOVA, testing for significant differences between the different months in median number of water lettuce seeds per plant per month over a year of sampling at Cape Recife. H (12, N = 3786) = 1065.5 p = 0.000.

Dependent variable: Number of seeds	**May 2003**	**June 2003**	**July 2003**	**August 2003**	**September 2003**	**October 2003**	**November 2003**	**December 2003**	**January 2004**	**February 2004**	**March 2004**	**April 2004**	**May 2004**
May 2003		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.143	**0.000**

June 2003	**0.000**		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
July 2003	**0.000**	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
August 2003	**0.000**	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
September 2003	**0.000**	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
October 2003	**0.000**	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000
November 2003	**0.000**	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000
December 2003	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000
January 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000
February 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000
March 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000
April 2004	0.143	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000

May 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	

Table 3.1.8. Results of multiple-comparisons of a Kruskal-Wallis ANOVA, testing for significant differences between the different months in median number of adult *N. affinis* weevils per plant per month. H (12, N = 3786) = 2403.3 p = 0.000.

Dependent variable: Number of adult weevils	**May 2003**	**June 2003**	**July 2003**	**August 2003**	**September 2003**	**October 2003**	**November 2003**	**December 2003**	**January 2004**	**February 2004**	**March 2004**	**April 2004**	**May 2004**
May 2003		**0.020**	0.410	1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
June 2003	**0.020**		1.000	0.413	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
July 2003	0.410	1.000		1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
August 2003	1.000	0.413	1.000			**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
September 2003	**0.000**	**0.000**	**0.000**	**0.000**		0.405	1.000	1.000	1.000	0.273	1.000	1.000	0.470
October	**0.000**	**0.000**	**0.000**	**0.000**	0.405		1.000	1.000	0.108	1.000	1.000	1.000	1.000

2003													
November 2003	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000
December 2003	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000
January 2004	**0.000**	**0.000**	**0.000**	**0.000**	1.000	0.108	1.000	1.000		0.111	1.000	1.000	0.154
February 2004	**0.000**	**0.000**	**0.000**	**0.000**	0.273	1.000	1.000	1.000	1.111		1.000	1.000	1.000
March 2004	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000
April 2004	**0.000**	**0.000**	**0.000**	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000
May 2004	**0.000**	**0.000**	**0.000**	**0.000**	0.470	1.000	1.000	1.000	1.154	0.000	1.000	1.000	

Table 3.1.9. Results of a Kruskal-Wallis multiple-comparison's table, showing which months were statistically significant from each other, with respect to the median number of adult *N. affinis* larvae per plant per month H (12, 3786) = 722.8872, p = 0.000.

Dependent variable: Adult weevils	**May 2003**	**June 2003**	**July 2003**	**August 2003**	**Sept 2003**	**October 2003**	**November 2003**	**Dec 2003**	**January 2004**	**Feb 2004**	**March 2004**	**April 2004**	**May 2004**
May 2003		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
June 2003	**0.000**			1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.430	1.000
July 2003	**0.000**	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.100	1.000
August 2003	**0.000**	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.264	1.000
September 2003	**0.000**	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	**0.032**	1.000
October 2003	**0.000**	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	**0.045**	1.000
November 2003	**0.000**	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	0.265	1.000
December	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000

2003													
January 2004	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	0.082	1.000
February 2004	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	0.258	1.000
March 2004	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000
April 2004	1.000	0.430	0.100	0.264	**0.032**	**0.045**	0.265	1.000	0.082	0.258	1.000		0.318
May 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.318	

Table 3.1.10. Results of table of multiple-comparisons of Kruskal-Wallis ANOVA, testing for significant differences between different months in median number of pupae per plant H (12, 3786) = 1795.701, p = 0.000.

Dependent variable: Number of weevil pupae	**May 2003**	**June 2003**	**July 2003**	**August 2003**	**September 2003**	**October 2003**	**November 2003**	**December 2003**	**January 2004**	**February 2004**	**March 2004**	**April 2004**	**May 2004**
May 2003		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
June 2003	**0.000**		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
July 2003													
August 2003	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
September 2003	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
October 2003	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
November 2003	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

December 2003	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
January 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
February 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
March 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
April 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
May 2004	**0.000**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000