INTEGRATED MANAGEMENT OF WATER HYACINTH IN SOUTH AFRICA:

Development of an integrated management plan for water hyacinth control, combining biological control, herbicidal control and nutrient control, tailored to the climatic regions of South Africa

Marcus Byrne, Martin Hill, Mark Robertson, Anthony King, Ashwini Jadhav, Naweji Katembo, John Wilson, Ryan Brudvig & Jolene Fisher

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This report emanates from a project titled *Integrated Management of Water Hyacinth in South Africa: Development of an integrated management plan for water hyacinth control, combining biological control, herbicidal control and nutrient control, tailored to the climatic regions of South Africa* (WRC Report No. K5/1487).

EXECUTIVE SUMMARY

Introduction

Water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) is South Africa's most damaging floating aquatic weed. Despite notable successes with the biological control of other floating aquatic weeds, and a concerted biological control effort against water hyacinth, its populations continue to reach newsworthy proportions on major rivers and dams. Hill and Olckers (2001) ascribed the variable success of the biological control programme on water hyacinth in South Africa to variable climatic conditions, eutrophication of aquatic ecosystems, interference from integrated control operations, the hydrology of infested systems and techniques for establishing biological control agents. The research presented in this report addresses the effect of temperature and nutrients on the growth of water hyacinth and some of its biological control agents and investigates the interaction of herbicide application with biological control. This has been done in light of discovering a sublethal dose of herbicide which will retain water hyacinth plants in a system to maintain populations of the agents. In addition, a management plan has been developed to guide water managers as what action should be taken in terms of combining biological control with herbicidal control under different climatic and nutrient conditions.

Objectives

- To understand the current status of water hyacinth and its biological control agents in South Africa, under different climatic and nutrient conditions.
- To determine if low temperatures hamper the biological control of water hyacinth in South Africa.
- To examine the impact of nutrients on the biological control of water hyacinth in South Africa.
- To examine the feasibility of combining herbicides with biological control of water hyacinth.
- To develop methods for management of water hyacinth infestations, with a particular emphasis on remote sensing of water hyacinth.
- To develop a simple management plan that can be applied by water managers, to develop expectations of what is possible in terms of water hyacinth control within a given set of environmental conditions at an infestation site.

Methods and Results

Fourteen field sites were selected around the country to encompass the ecoclimatic range of water hyacinth. These were monitored monthly for two years, measuring both plant and insect parameters to evaluate their growth behaviour over a long time period. Temperature and nutrients were also measured at each site. Biological control agent numbers were shown to be low and adversely affected by frost and high nutrients. Nevertheless, the water hyacinth weevils were present and persisted at all sites, and some measure of control, as evidenced by a reduction in biomass, was recorded at most sites. However, sites were generally unstable

having been disturbed by frost, flooding or herbicides. Most of the other biological control agents were generally absent from most sites. Although not tested in this project, this aspect concurs with Hill and Olckers' (2001) hypothesis that the lack of correct release procedures has contributed to the variable success of the water hyacinth biological control programme.

In an attempt to better integrated biological control with herbicide applications, trials were undertaken to determine a glyphosate dose which would stunt plant growth and reproduction, without harming three of the two weevils species, *Neochetina eichhorniae* and *N. bruchi* and the mirid, *Eccritotarsus catarinensis*. This dosage (0.8%) was then applied in the field, where measurements were taken of its effect on the plants, their nutrient status and populations of these three agent species. The herbicide varied between being benign to beneficial, to biological control insect populations, and promoted herbivory of the plants, while causing an increase in the carbon:nitrogen ratio of the plant tissue, which was expected to make the weed less palatable. High levels of nutrients did not reduce the stunting effect of the herbicide dose and did not adversely affect agent numbers. Non-target effects of the low dose of herbicide was investigated and it had no effect on the growth or survival of frog tadpoles, and water hyacinth alone was found to present a greater threat than any herbicide dose used in the experiment. It is recommended that herbicide be applied to recalcitrant water hyacinth sites as late in autumn as temperature will allow for that site, before the plants reproduce asexually by producing ramets; and again in spring, just as the new leaves are starting to develop and the plants add biomass by leaf elongation.

Satellite imagery of selected field sites was used to test the hypothesis that remote sensing could be used for monitoring water hyacinth growth, as a tool to help decide when and where herbicide intervention should take place. Water hyacinth mats could be detected on small water bodies using multispectral imagery with a 10 m or less resolution. As much of this imagery is free it has potential to be used if regular, at least twice yearly, images are available. Failing that it is recommended that hyperspectral images be commissioned, and to that end the technique should be developed for water hyacinth so that the physiological status of the plant can be assessed as well as its physical extent.

A site-specific management tool for controlling water hyacinth, an adaptive decision making framework was developed from local and international knowledge of the effects of the ecoclimatic conditions under which water hyacinth grows, and their effects on the efficacy of the biological control agents. Management decisions, such as the proportion of water surface that can be lost to water hyacinth, and the length of time available to allow biological control to take effect, are incorporated with herbicidal options to give a series of recommendations and expectations to water managers intending to control water hyacinth. Most importantly, biological control, maintained by active release of agents and their management by creation of refuges and use of low herbicide doses (generally accounted for by spray drift), is recommended for all levels of hyacinth infestation, except where complete eradication is required.

Conclusions

Water hyacinth remains a serious threat to South African freshwater bodies, but as a symptom of a larger problem of eutrophication, rather than a unique condition in itself. Biological control will be less effective and take longer under such circumstances, but will still provide a measure of control and is likely to eventually reduce weed biomass over a several year period. In the interim, glyphosate based herbicides can be used in an integrated manner with biological control, to maintain and encourage agent populations which will suppress the weed's growth in summer. To this end, populations of biological control agents must first be actively and aggressively established in large numbers, and secondly reintroduced if they are lost due to flooding or frost. They must be maintained by providing refuges of unsprayed water hyacinth plants, or water hyacinth that has received a sublethal herbicide dose through spray drift. Ultimately nutrient inflows must be curtailed to cure this problem before the next new weed discovers South African water.

Recommendations

- Water hyacinth infestations remain a symptom of nutrient enriched waters. Every effort should be made to ensure that South Africa aquatic ecosystems and in particular discharge water comply with the South African water quality guidelines.
- All available agents for water hyacinth should be correctly implemented at all sites of infestation.
- Herbicide should be applied to recalcitrant water hyacinth sites as late in autumn as temperature will allow for that site, before the plants reproduce asexually by producing ramets; and again in spring, just as the new leaves are starting to develop and the plants add biomass by leaf elongation.
- Hyperspectral images should be commissioned, and to that end the technique should be developed for water hyacinth so that the physiological status of the plant can be assessed as well as its physical extent.
- Hill and Coetzee (2008) developed a 10 point plan for the integrated control of water hyacinth in South Africa. These points are: identification of the weed; map the extent of the weed; identify the cause of the infestation; consult interested and affected parties; appointment of a lead agency or champion; ascertain an acceptable level of control; consider control options; implement control options; monitor control options; evaluate plan and adjust accordingly. This plan is applicable to all water hyacinth infestations in South Africa, but they should be implemented on a site specific basis and should be flexible.

STATEMENT OF THE PROBLEM

i Introduction

Water hyacinth remains South Africa's most damaging water weed. It blocks waterways, impeding navigation and blocking drainage, which contributes to flooding. The annual cost for water hyacinth management in the USA ranges from $500,000.00 in California, to $3 million in Florida (Center et al., 2002). In South Africa, the herbicide costs alone are almost R2000 per kilometre of river sprayed, resulting in over R482 000 being spent on water hyacinth control on the Crocodile River, Mpumalanga, in 2008. The total cost to the country is estimated at about R12m per year (Rael Hughes, Working for Water, personal communication). The economic damage is matched by widespread ecological effects which result in displacement of indigenous flora and fauna by habitat modification. Water hyacinth has been shown to reduce biodiversity in affected South African water bodies (Midgley et al., 2006).

South African infestations are considered to be most serious on water at high altitude, or water that is nutrient enriched (Hill and Olckers, 2001). Cold winters are seen as a serious impediment to the efficacy of biological control agents that have been used since 1974, because low temperatures slow the development of the biocontrol insects and frost removes the leaves of the plant which are the site for feeding and egg-laying of most of the biological control agents. In addition to biological control, a variety of other methods are used with mixed results. Herbicidal control is expensive (Jones, 2009) and will have environmental impacts on other organisms that use the water (Relyea, 2005, a,b,c), including humans, who often perceive herbicides as poisons (Hill and Coetzee, 2008). Herbicidal control usually conflicts with biological control by killing the weed, which results in extermination of the biocontrol agent (Cilliers, 1991) with subsequent resurgence of the weed from seeds or untreated plants. In many situations, management of the weed requires an integrated approach that can include careful herbicide application, with a minimal impact on the biocontrol agents and the environment (Center et al., 1999).

The integrated control approach to water hyacinth control has been touched on in South Africa (Ueckermann and Hill, 2001), and has been successfully employed by Roy Jones, Ezemvelo Wildlife Manager of Enseleni Reserve, since 1995 to control water hyacinth along 22 km of the Enselini River in KwaZulu-Natal. This method offers a promising insight into the combined use of biological control agents and herbicides for water hyacinth control, but Enseleni is a low-altitude subtropical site with low levels of nutrients in the water. The beneficial effects of elevated nutrients on water hyacinth growth are well known (Reddy et al., 1989, 1990), but it has also been shown that high levels of nitrogen and phosphorus allow the plant to outgrow the biological control agents (Coetzee, 2007b). Therefore, control of nutrient inputs will form an important part of any future weed management strategy for water bodies. However, little was known at the start of this project about the nutrient status of hyacinth-infested waters and its effect on the populations of biocontrol agents.

ii History of Water Hyacinth Control in South Africa

Water hyacinth is a floating aquatic plant of South American origin which was recorded on the South African continent in Egypt by the end of the nineteenth century and in South Africa by 1908 (Gopal 1987). Water hyacinth was recorded in KwaZulu-Natal around 1910 (Edwards and Musil, 1975), and is now found throughout the country, excluding the Karoo region (Henderson, 2001). It has been controlled by herbicides since the late 1970s, with the notorious infestation on Hartbeespoort Dam being cleared by aerial applications of the terbutryn herbicide, under the trade name Clarosan 500FW (Ashton et al., 1979). More recently, the glyphosate preparation "Mamba" (Dow Agro Sciences, South Africa), sprayed at a label-recommended dose of 2% to 4%, has generally been used by Working for Water.

Biological control of water hyacinth was initiated in the early 1970s and the South American weevil *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) was introduced into quarantine in 1973 and released in 1974 (Cilliers, 1991; Julien and Griffiths, 1998). It is notable that it was re-released in 1977 and re-introduced in 1985, which suggests that establishment had been poor and possibly the release effort had been inadequate. A second biocontrol agent, the mite *Orthogalumna terebrantis* Wallwork (Acari: Galumnidae), was discovered on the weed in South Africa in 1989, and is consequently considered to be an adventive release, having been inadvertently transported from South America by an unknown route. A year later in 1990, two more agents, a second weevil *N. bruchi* Hustache, and the moth *Niphograpta albiguttalis* Warren (Lepidoptera: Pyralidae) (formerly *Sameodes albiguttalis*), were released. *Neochetina bruchi* was re-released in 1996, which again raises the question as to why the two *Neochetina* weevils, which have been successfully used against water hyacinth in the USA, Australia, Uganda, Kenya, Tanzania, and Benin (Ochiel et al., 2001; Wilson et al., 2007; Ajuonu et al., 2003; Cilliers et al., 2003), failed to establish easily in South Africa. An additional agent, the mirid *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae) was also released in 1996, and has successfully established without further re-introductions (Julien & Griffiths, 1998; Hill and Cilliers, 1999).

Other biological control agents have been developed by the Plant Protection Research Institute (PPRI), including the grasshopper *Cornops aquaticum* Bruner (Orthoptera: Acrididae), which has been approved for limited release, but is being investigated with regard to its efficacy and effect on the other biocontrol agents before release. The suitability of several other natural enemies, including *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) (Sosa et al., 2004; Sosa et al., 2007), *Thrypticus spp.* (Dolichopodidae: Diptera) (Bickel and Hernández, 2004) and *Taosa inexacta* Walker (Dictyopharidae: Homoptera) from South America, are being considered for release in South Africa (Hill and Olckers, 2000). In addition two moths, *Xubida infusella* Walker (Lepidoptera: Pyralidae) and *Bellura densa* Walker (Lepidoptera: Noctuidae), and a scarab, *Brachinus spp.* were tested and rejected as biocontrol agents against water hyacinth (Cordo, 1999; M. Hill, personal communication). The pathogen *Cercospora paropi* (= *Cercospora rodmanii* Conway (Hyphomycetes)), first recorded in South Africa in 1987, is another adventive release.

Consequently, South Africa has the greatest number of biocontrol agents on water hyacinth of any country in the world, with five arthropods and one (possibly two) pathogens, yet the weed is not considered to be under satisfactory control.

iii New Approaches

Hill and Olckers (2001) reviewed the state of water hyacinth biocontrol in South Africa. Hill and Olckers concluded that low temperatures, high nutrients, misuse of herbicides and small water body hydrology could all be contributing to the notable lack of success in controlling water hyacinth in South Africa. However, these conclusions were made by inference and no hard data from South Africa could be presented to support or refute these conclusions. Therefore, in 2004 this project, K5/1487, "Integrated Management of Water Hyacinth in South Africa" was initiated to examine the factors constraining biological control of water hyacinth in South Africa as proposed by Hill and Olckers. In addition, because herbicides were seen as a factor contributing to failure of biocontrol and at one site, the Enseleni River in KwaZulu-Natal, water hyacinth was being successfully managed by a combination of herbicides and biological control, the project aimed to investigate the effects of herbicides on water hyacinth biocontrol agents; and subsequently to propose techniques by which the two methods could be integrated across South Africa.

iv Project Aims

This project started with the following aims:

iv.i Aim 1: Integration of Biocontrol and Herbicidal Control

Key output: How can biocontrol and herbicides be combined?

Aim 1a)

Assessment of the effects of sublethal doses of herbicide on water hyacinth, combined with the assessment of the effects of sublethal doses of herbicide on two weevil species *Neochetina bruchi* and *N. eichhorniae*, and the mirid *Eccritotarsus caterrinensis*.

Aim 1b)

Determination of herbicidal spray patterns, both spatial and temporal, that will suppress water hyacinth infestations without exterminating the biocontrol agents.

iv.ii Aim 2: Integrating Nutrient Control and Biocontrol

Key output: How do nutrients affect water hyacinth and its biocontrol agents?

Aim 2a)

Correlate water hyacinth vigour to water nutrient concentrations (nitrogen and phosphorus).

Aim 2b)

Correlate the performance (rate of development, body size, fecundity and longevity) of water hyacinth biological control agents to water nutrient concentrations (nitrogen and phosphate).

Aim 2c)
Nutrient profiling of infested water bodies to identify the source of nutrients (nitrogen and phosphate).

iv.iii Aim 3: Climatic Effects on Biocontrol of Water Hyacinth
Key output: How does climate affect water hyacinth and its biocontrol agents?

Aim 3a)
Correlation of climate (in particular temperature extremes), of water hyacinth infestation sites with indicators of the weed's vigour.

Aim 3b)
Measurement of the seasonal population structure of water hyacinth plants and the biological control agents at climatically different sites to determine at which stage either organism is most vulnerable to herbicide application.

Aim 3c)
Comparison of plant and insect survival at climatically different sites to determine what conditions will preclude biocontrol agents from certain (colder?) areas.

Aim 3d)
Determination of the cold tolerance of water hyacinth biocontrol agents.

Aim 3e)
Measurement of daily activity patterns of water hyacinth biocontrol agents in response to cold.

This aim has not been achieved as it became clear that this question was largely irrelevant to the integrated management plan.

Aim 3f)
Modelling of the population structure of the weed and the control agents during the year at climatically different sites.

This aim has not been achieved as it became clear that this was a task beyond the scope of the project. Instead, Remote Sensing was developed as a method which could be used for monitoring water hyacinth populations. Both water hyacinth modelling and remote sensing continue to be pursued outside of this project, for their potential contribution to water hyacinth management.

iv.iv Aim 4:Integrated Weed Management Plan for Water Hyacinth
Key output: A weed management plan for water hyacinth.

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CHAPTER ONE – METHODS: SITE SELECTION

1.1 Introduction

1.1.1 Climate

The behaviour and physiology of all organisms is largely determined by temperature, which influences the metabolic rate, nutrition, growth rate, fecundity and longevity of both insects and plants (Clarke, 1996). Consequently, their development occurs within a definite temperature range, which can be measured. Differential survival ability at temperature extremes is a critical determinant of insect and plant distribution limits, and can also be estimated from laboratory studies (Chown and Terblanche, 2007). Such studies can then serve as a basis from which models that estimate growth, development and reproduction can be formulated, and are useful in predicting field activity and phenology of both animals and plants.

1.1.2 Water Hyacinth and Climate

Water hyacinth infestations in South Africa are considered to be worst at high altitude, cold sites (Hill and Olckers, 2001), a conjecture which is supported by anecdotal observations, but lacks scientific evidence. Cold winters cause browning and death of emergent parts of the plant, which removes the habitat for all of the adult and most of the immature stages of introduced biocontrol agents. Therefore, one would predict that the control agents go through a severe winter population bottleneck, if not local extermination, which will be of importance to the rate at which they can recover in the following spring. Data to support this hypothesis are required, as well as data for the cold tolerance characteristics of water hyacinth and the rate at which it can recover in spring.

The growth rates of both water hyacinth and arthropod populations of biocontrol agents, at sites with widely differing climates, were used to test the above hypotheses and growth patterns in their respective populations. Therefore, selection of these sites was critical to the establishment of a meaningful monitoring programme.

1.1.3 Nutrients

Nutrient enrichment is often found in highly populated and developed areas where water-borne sewage systems and agriculture contribute to elevated loads of nitrogen and phosphorus, among other elements. South Africa is considered to have some of the most highly enriched surface waters in the world (Walmsley, 2000).

Nutrients dissolved in an aquatic system have an obvious and direct impact on plants within that system. Water hyacinth responds positively to nitrogen when phosphorus levels exceed 0.6 mg/*l* (Reddy, et al., 1990) and high nutrient levels are assumed to exacerbate water hyacinth infestations in South Africa (Hill and Olckers, 2001), but the supporting data are lacking. An interaction between the relative amounts of nitrogen and phosphorus in a water body would also be expected to have an effect on growth patterns of water hyacinth. Wilson (2002) has proposed a nitrogen-to-phosphorus ratio of 7:1 to be ideal for water hyacinth

growth. However, this ratio is based on a mean value derived from a literature survey, and remains unsubstantiated, both in the laboratory and the field.

1.1.4 Water Hyacinth Monitoring Sites

The Resource Quality Services (formerly Institute for Water Quality Studies), monitors water quality at over 3000 sites countrywide. From these sites, 14 were chosen for long-term monitoring that combined the climatic and nutrient range over which water hyacinth grows in South Africa. An excellent, but broad, picture of the South African climate is available from the South African Atlas of Agrohydrology and Climatology (Schulze et al., 1997). However, microclimate on a water body and within the water hyacinth mat differs substantially from the level at which it can be resolved from the climate atlas (McConnachie, 2004). Similarly, nutrient levels will fluctuate both spatially and temporally at scales that the Resource Quality Services' testing stations do not detect. Nevertheless, data from these sources can be used to sort water hyacinth sites into groups that represent the full climatic and nutrient range over which the weed grows in South Africa.

1.2 Methods

1.2.1 Production of a Map of Water Hyacinth Sites in South Africa

A water hyacinth distribution map was compiled using data from two sources: the Southern African Plant Invaders Atlas (SAPIA; Henderson, 1998), and release records of sites where biocontrol agents have been introduced to control water hyacinth. These data were compiled from records of the Weeds Division of the ARC – Plant Protection Research Institute, and from records obtained from the Working for Water Programme. Those sites lacking precise coordinates were either excluded or assigned coordinates from maps or gazetteers if the description was sufficiently detailed to allow this. In cases where only a quarter degree grid reference was available (especially in the case of the SAPIA data) the records were also excluded. Coordinates were recorded to the nearest minute. Maps were produced using a GIS package (ArcView, ESRI, 2002).

1.2.2 Characterizing Sites in Terms of Climate and Nutrient Status

Water hyacinth field sites were sorted using a combination of a Principal Components Analysis (PCA) (James and McCulloch, 1990; Manly, 1994) and local knowledge on the accessibility and security of sites, to select a sub-group that represented the full range of climatic conditions considered to be important to water hyacinth and its control. The map of water hyacinth occurrence created was used as the basis for characterizing infested sites in terms of their climate status and the PCA allowed us to visualize differences and variation in environmental characteristics of the water hyacinth sites.

1.2.2.1 Climate

Climate data were obtained from an atlas of long-term climatic records (Schulze et al., 1997). The coordinates of each water hyacinth site were used to retrieve climate data for a particular water hyacinth site from the atlas. Programmes to select climate data from the atlas were written in Mathlab (The Mathworks, 2000) and were used to process and display the data.

A PCA was conducted on values of eight environmental variables (Table 1.1) associated with water hyacinth sites. This multivariate statistical technique reduces the dimensions of a single group of data by producing a smaller number of abstract variables, which are called principal components (James and McCulloch, 1990). Each principal component is constructed so that it is uncorrelated with subsequent components. Most of the variation within a dataset can usually be summarized within the first few components. Each component is constructed from a weighted linear combination of the original variables and has an eigenvector and an eigenvalue associated with it. The eigenvector for a particular component indicates the weight of each of the original variables and the eigenvalue indicates the proportion of the total variation that the component summarizes. The PCA used here displays the climatic variation of the water hyacinth sites (represented by values from eight environmental variables), in two dimensions (represented by the first two components). Field sites were selected from each of the major climatic groups revealed by the analysis.

Water hyacinth sites were also characterized by monthly median rainfall, mean daily maximum and minimum temperature for all months, and frost occurrence. These variables were then individually plotted to examine the range over which water hyacinth grows, and where within that range the field sites selected above for monitoring lay.

Table 1.1: Environmental variables used in a Principal Components Analysis (PCA) to group water hyacinth infestations into sites representative of the range of climate over which it grows in South Africa.

Variable	Abbreviation
January Median Rainfall	janrain
July Median Rainfall	julyrain
January Mean of Daily Maximum Temperature	janmaxt
July Mean of Daily Maximum Temperature	julymaxt
January Mean of Daily Minimum Temperature	janmint
July Mean of Daily Minimum Temperature	julymint
Average Number of Days with Frost	frost
Altitude	alt

1.2.2.2 Nutrients

Water quality data (nutrient data) were obtained from Resource Quality Services of the Department of Water Affairs and Forestry. Nutrient data were obtained for all stations in South Africa for which samples had been taken over the past five years. A number of summary statistics were obtained for each of the nutrients measured (e.g. total nitrogen concentration) at each testing station. Stations at sewage treatment works and mine effluent outlets were excluded, as were all stations that were more than 5 km away from a water hyacinth site (based on the water hyacinth sites map). Not all of the water hyacinth sites could be characterized in terms of their nutrient status because some of these sites did not have stations near to them. For those water hyacinth monitoring sites which did not have nutrient data available, three water samples were taken simultaneously and tested with a spectrophotometer (Hanna 106 Multiparameter Ion Specific Meter). Total nitrogen and phosphorus concentrations were measured and averaged for each site sampled and then used in the analysis.

1.2.2.3 Assessment of Site Selection

To test how successful the selection of field sites was in being representative of the full climate range in which water hyacinth occurs in South Africa, a number of climatically based 'stress indices' were created using thermal thresholds established for the weed and two of its biological control agents. These thermal thresholds are covered in more detail in Chapter 2, but for the purposes of this chapter can simply be described as the temperature at which certain biological processes such as growth or reproduction begin or end. Using very precise temperature measurements taken in the water hyacinth canopy every hour for two years (see Chapter 2 for details), temperature profiles were created for each of the 14 field sites, which were then analysed to characterise each field site by the amount of thermal stress which would accumulate for water hyacinth, as well as for the *Neochetina* weevils, and the water hyacinth mirid *E. catarinensis*. These stress indices account for the effects of prolonged exposure to unfavourable minimum temperature extremes, stochastic events such as frosting; as well as insect behaviour in terms of activity periods and their implications for feeding, reproduction and mortality; and were created as follows:

***Neochetina* weevils**.

Oviposition index: The number of nocturnal hours (Between 19H00 and 05H00) per annum with a mean hourly canopy temperature below their oviposition threshold (12.5°C);
Maintenance index: The maximum number of consecutive days each year with a mean daily canopy temperature below 9.6°C;
Feeding index: The number of days per annum with a mean nocturnal canopy temperature below 6.3°C;
Mortality index: The maximum number of consecutive days each year (mean of both years) with a daily minimum canopy temperature below their CTmin temperature (3.8°C).

***E. catarinensis* mirid**.

Oviposition index: The number of diurnal hours (Between 05H00 and 19H00) per annum with a mean hourly canopy temperature below their oviposition threshold (10.8°C);

Maintenance index: The maximum number of consecutive days each year with a mean daily canopy temperature below 10.3°C;
Feeding index: The number of days per annum with a mean diurnal canopy temperature below 10.8°C;
Mortality index: The maximum number of consecutive days each year with a daily minimum canopy temperature below its CTmin temperature (1.2°C).

***E. crassipes* water hyacinth**.

Growth index: The number of days per annum with a mean daily water temperature below 10°C;
Frost index: The number of days per annum with a daily minimum canopy temperature of 0°C or less.

Index values were averaged from both years of temperature data where available. Means were added together (Hourly counts divided by 365) to provide a relative overall score of thermal stress for each species at each site to allow comparisons between sites. Principal components analysis was used to group sites with similar stress magnitudes.

1.3 Results

1.3.1 Water Hyacinth Sites in South Africa

A map showing the water hyacinth infestation sites within South Africa was generated (Figure 1.1).

The Vaal River, where it separates the Free State from North West Province, has the greatest number of contiguous sites, followed by the Crocodile River in Mpumalanga, where it runs along the southern border of the Kruger National Park. The Crocodile River at Brits, North West Province, has several infestations, as does the Mgeni River where it flows into Durban.

1.3.2 Site Climate Characterization

The first two components of the PCA accounted for over 80% of the variation in the original dataset (Table 1.2). The component scores for water hyacinth sites and field sites proposed for monitoring are shown in Figure 1.2. Each of the field sites is labelled with a number that corresponds to the site numbers in Table 1.3. The wide distribution of the points representing the field monitoring sites relative to the water hyacinth sites indicates that the field sites adequately sample the variety of climatic environments inhabited by water hyacinth (Figure1.2).

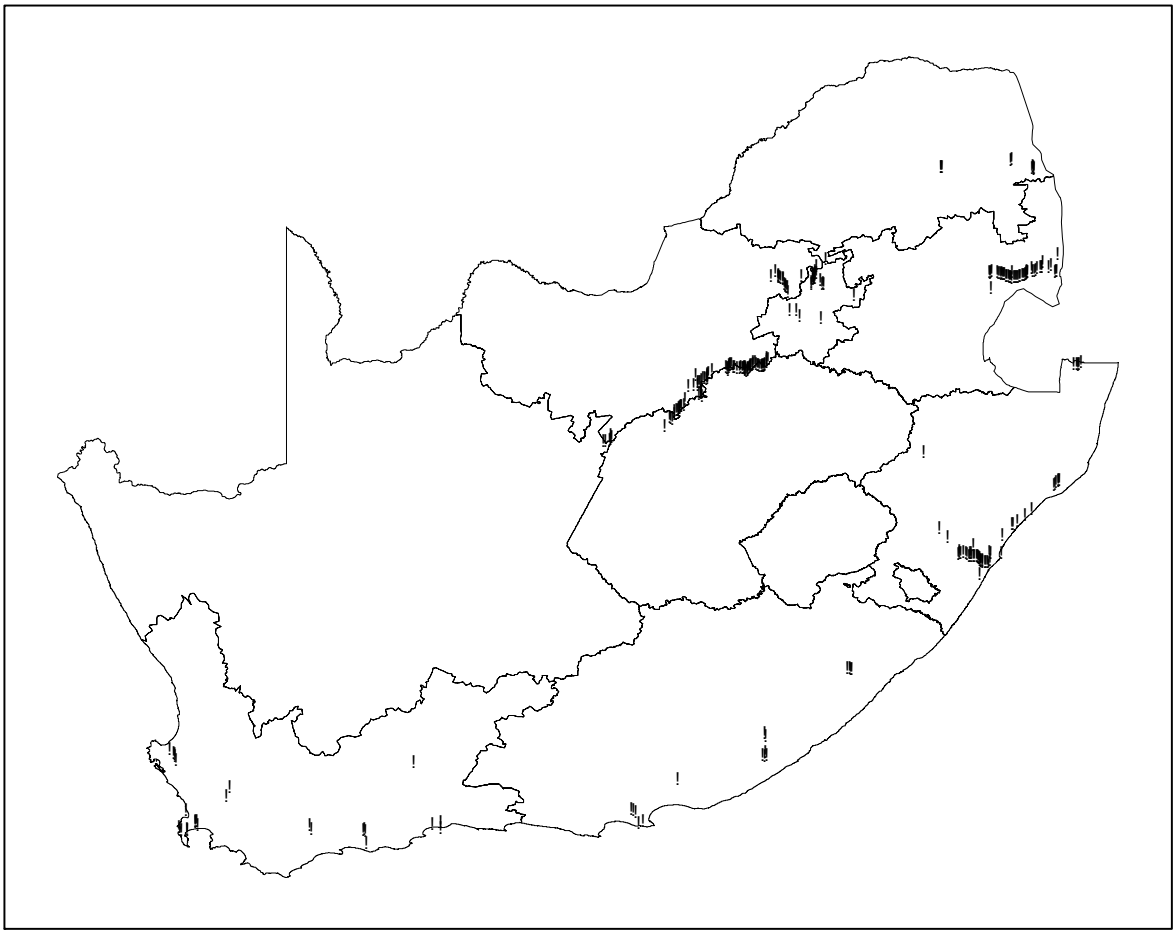


Figure 1.1: The recorded distribution of water hyacinth in South Africa (!) from SAPIA (Henderson, 1998) and agent release data (various sources, see text).

Table 1.2: Eigenvectors and eigenvalues for the first four principal components extracted from a PCA performed on the values of eight environmental variables (Table 1.1) for the water hyacinth infested sites. The proportion of the total variation in the original dataset accounted for by each principal component is indicated in the bottom row of the table (% of variation).

Variable	**z1**	**z2**	**z3**	**z4**
janrain	-0.024	0.458	0.665	-0.472
julyrain	-0.096	-0.572	-0.074	-0.781
janmaxt	-0.04	0.441	-0.708	-0.329
julymaxt	-0.426	0.306	0.016	0.036
janmint	-0.418	0.295	-0.11	-0.141
julymint	-0.473	-0.127	0.096	0.051
frost	0.456	0.146	-0.136	-0.127
alt	0.449	0.224	0.11	-0.136
Eigenvalues	4.197	2.319	0.986	0.318
% of variation	52.459	28.987	12.329	3.979

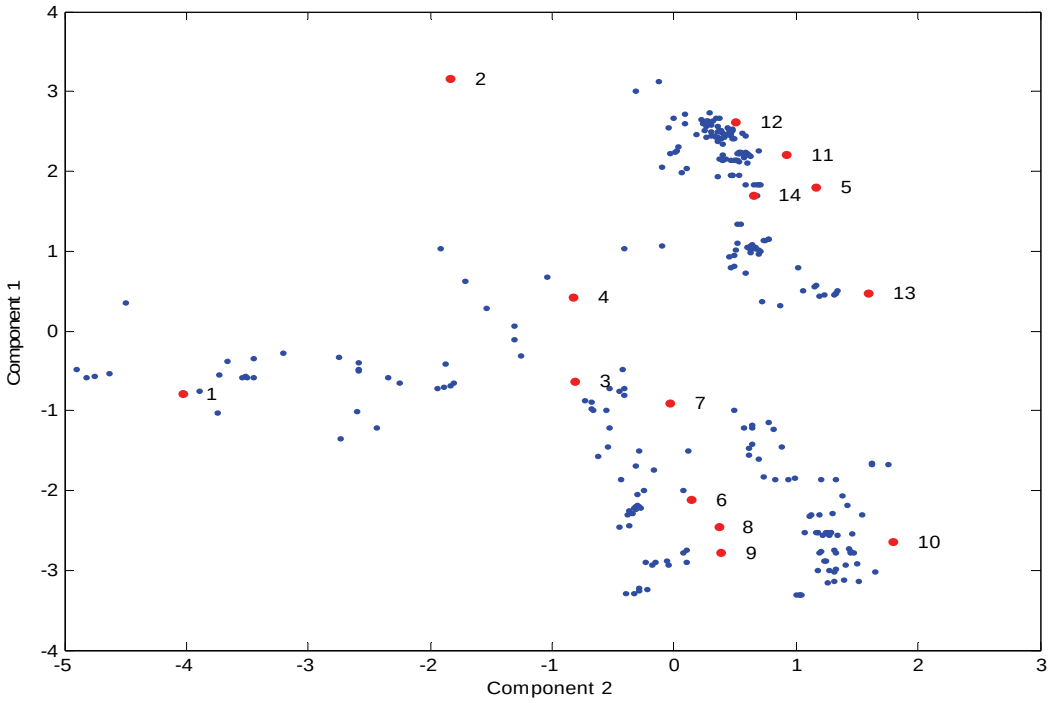


Figure 1.2: Plot of component 1 against component 2 of a PCA performed on values of eight environmental variables (Table 1.1) at sites where water hyacinth occurs. Water hyacinth sites (large numbered dots); Field sites selected for monitoring (small dots). Each of the field sites is labelled with a number that corresponds with the site numbers in Table 1.3.

Table 1.3: Field sites selected for long-term monitoring of water hyacinth populations and their biocontrol agents. These sites were selected from known water hyacinth infestation sites based on their climatic characteristics. Climates were summarized as: Rainfall: – Winter (W), Summer (S), All seasons (A). Frost: – High (H), Low (L), None (N). Temperature: – Temperate (T); Subtropical (St).

Site no.	Site name	Province	Latitude	Longitude	Climate
1	Rondevlei	Western Cape	34°03'S	18°30'E	W; L; T
2	Breede River	Western Cape	33°18'S	20°35'E	W; H; T
3	New Years Dam	Eastern Cape	33°17'S	26°07'E	A; L; T
4	Kubusi River	Eastern Cape	32°35'S	27°28'E	A; H; T
5	Vet River Hoopstad	Free State	27°50'S	25°55'E	S; H; T
6	Clairwood Quarry	KZN	29°54'S	30°57'E	S; N; St
7	Hammarsdale Dam	KZN	29°48'S	30°39'E	S; N; St
8	Mbozambo Swamp	KZN	29°21'S	31°18'E	S; N; St
9	Enseleni River	KZN	28°40'S	32°02'E	S; N; St
10	Mkadhzi Spruit	Limpopo	23°49'S	31°37'E	S; N; St
11	Vaal River Parys	Free State	26°54'S	27°27'E	S; H; T
12	Delta Park	Gauteng	26°07'S	28°00'E	S; H; T
13	Crocodile River Rennie	Northwest	25°39'S	27°47'E	S; H; T
14	Farm Dam Randburg	Gauteng	26°02'S	27°57'E	S; H; T

Water hyacinth monitoring sites, selected from sites of known infestation, were chosen to represent the spread of sites shown in the PCA plot (Figure 1.2), and moderated by the

practicalities of using particular sites around the country. These sites are illustrated in Figure 1.3, and their names and broad climatic classification are presented in Table 1.3.

Water hyacinth sites were also characterized in terms of rainfall and maximum and minimum temperature for each month of the year in (Figures 1.4, 5 and 6 respectively). In all three figures the dispersion of the monitoring sites within the infestation sites indicates that the monitoring sites are representative of the South Africa's major rainfall and temperature zones in which water hyacinth grows. The distribution of frost days at selected monitoring sites was plotted (Figure 1.7) and indicates that the sites are representative of the wide range of low temperatures experienced at known water hyacinth sites in South Africa.

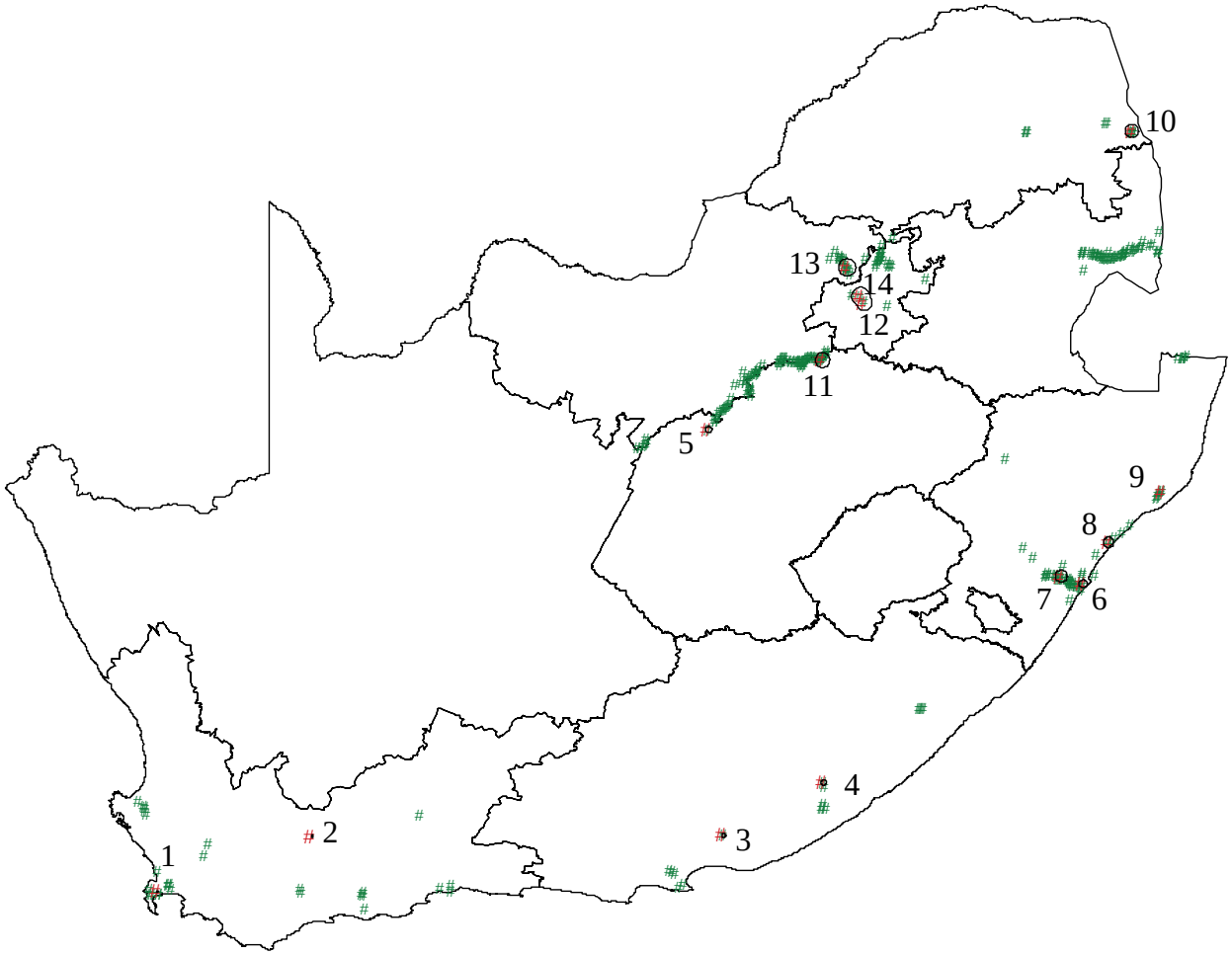


Figure 1.3: Geographical distribution of water hyacinth monitoring sites in South Africa (numbered) selected from known infestation sites (#). Names of monitoring sites are: 1. Rondevlei; 2. Breede River; 3. New Years Dam; 4. Kubusi River; 5. Vet River Hoopstad; 6. Clairwood Quarry; 7. Hammarsdale Dam; 8. Mobozambo Swamp; 9. Enseleni River; 10. Mkadhzi Spruit; 11. Vaal River Parys; 12. Delta Park; 13. Crocodile River, Malgas; 14. Farm Dam, Randburg.

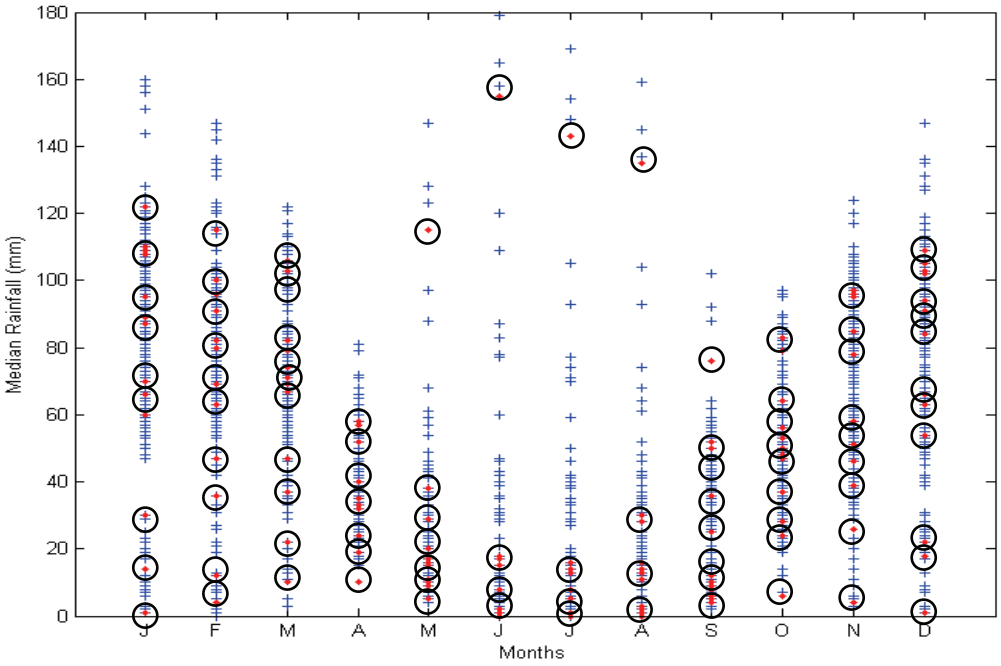


Figure 1.4: Median rainfall in all months of the year for known water hyacinth sites in South Africa (crosses). Sites selected for long-term monitoring (ringed crosses).

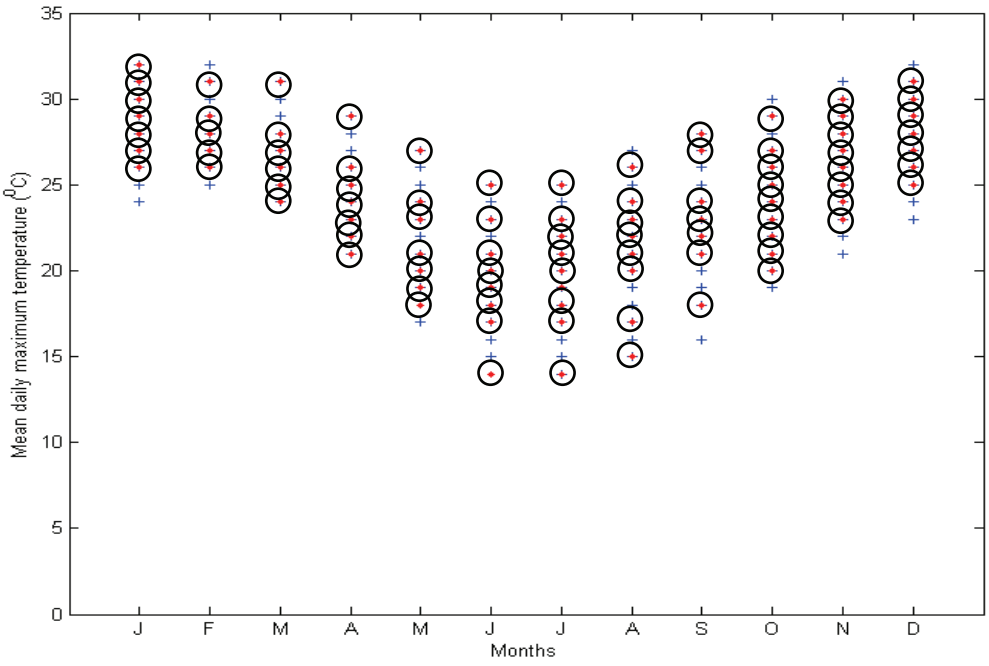


Figure 1.5: Mean daily maximum temperature in all months of the year for known water hyacinth sites in South Africa (crosses). Sites selected for long-term monitoring (ringed crosses).

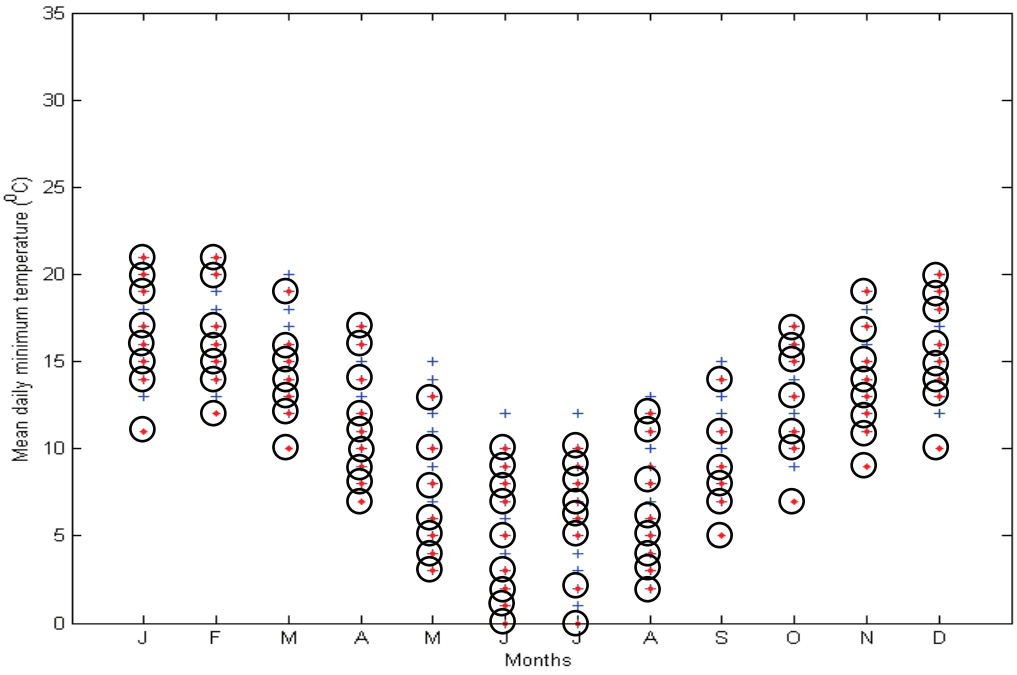


Figure 1.6: Mean daily minimum temperature in all months of the year for known water hyacinth sites in South Africa (crosses). Sites selected for long-term monitoring (ringed crosses).

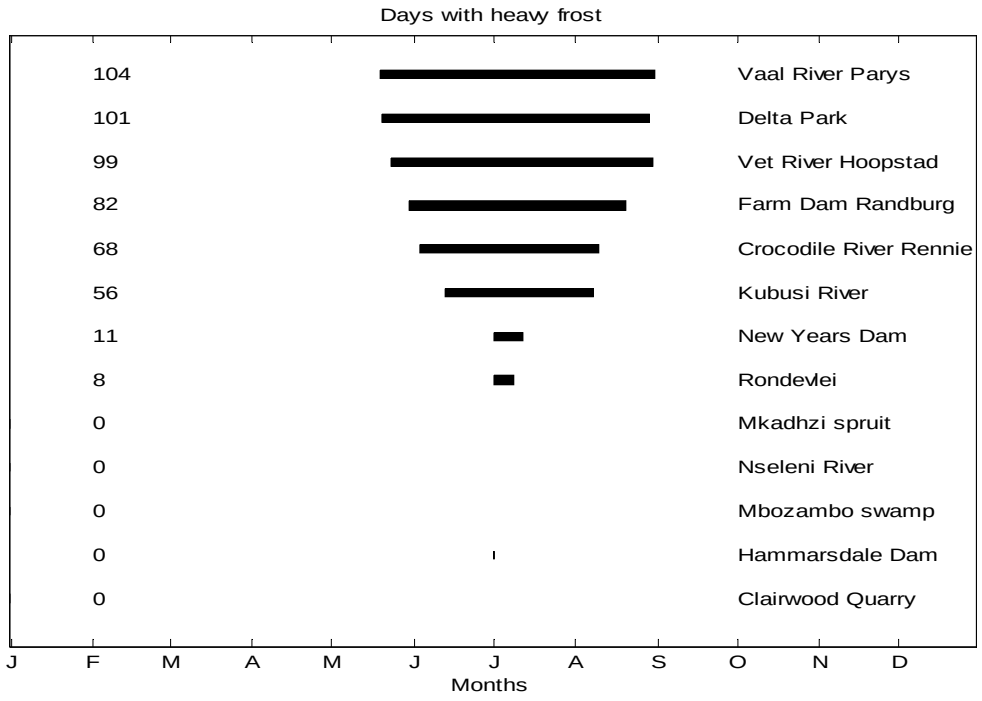


Figure 1.7: Number of days (left of plot) and timing (red bars) of heavy frost at selected water hyacinth monitoring sites.

1.3.3 Sites Characterized by Nutrient Status

Water hyacinth monitoring sites selected by their climatic variables were then characterized by their nitrogen and phosphorus profiles, which were correlated to reveal the range of nutrient habitats that would be encompassed by the long-term sampling. Figure 1.8 indicates that the monitoring sites are representative of a broad range of nutrient profiles where water hyacinth grows.

Because sites in the higher range of nitrogen or phosphorus concentrations lie off the main axis of the N:P correlation, ratios of total nitrogen to total phosphate concentration were calculated for water hyacinth monitoring sites where nutrient data was available (Table 1.4). These indicated that a wide range of N:P ratios will be covered by these particular monitoring sites. Water hyacinth sites which were not selected for long-term monitoring were identified (Figure 1.9), and characterized in terms of their N and P profiles (Table 1.5), to check that no important site had been omitted from those selected for monitoring.

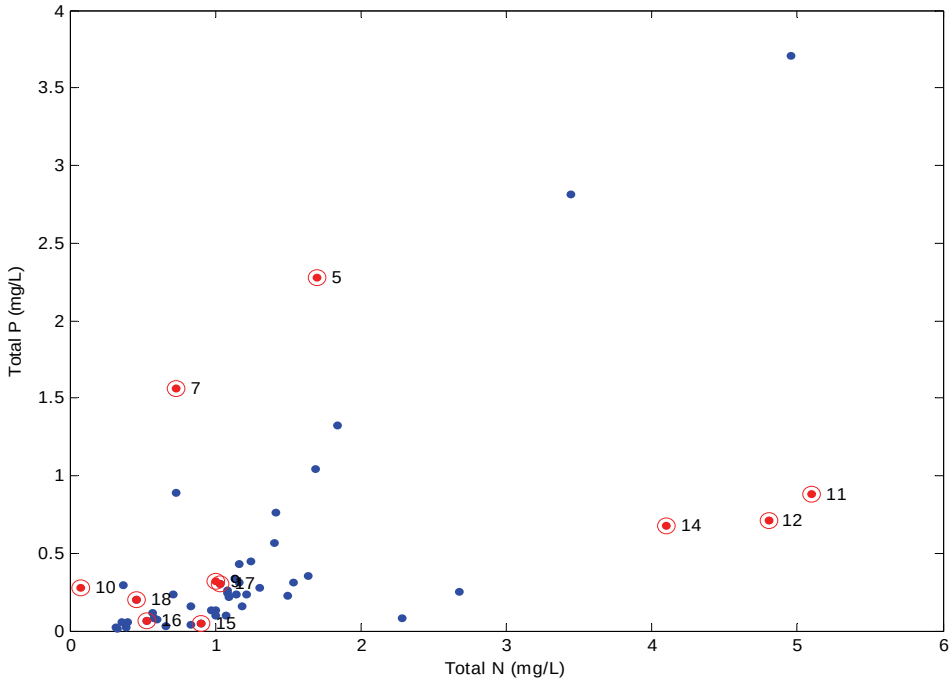


Figure 1.8: Plot of median values of total phosphorus concentration (Total P) against total nitrogen (Total N) concentration in water at recording stations within 5 km of water hyacinth infestation sites (dots). Sites proposed for long-term monitoring (ringed dots). Numbers correspond to site identifiers in Tables 1.3 and 1.4.

Table 1.4: Nutrient profiles of field sites selected for long-term monitoring of water hyacinth populations and their biocontrol agents. ID number corresponds with those used in Table 1.3 and Figure 1.8. Missing sites (1,2,3,4,6,8 & 13) do not have nutrient data available for them as yet.

ID no.	N:P ratio	Total N	Total P	Station name	Source
5	0.75	1.70	2.28	Vet River Hoopstad	Sample
7	0.47	0.73	1.56	Hammarsdale Dam	Sample
9	3.13	1.00	0.32	Enseleni River	Sample
10	0.29	0.08	0.28	Mkadhzi Spruit	Sample
11	5.80	5.10	0.88	Vaal River Parys	Sample
12	6.76	4.80	0.71	Delta Park	Sample
14	6.03	4.10	0.68	Farm Dam Randburg	Sample

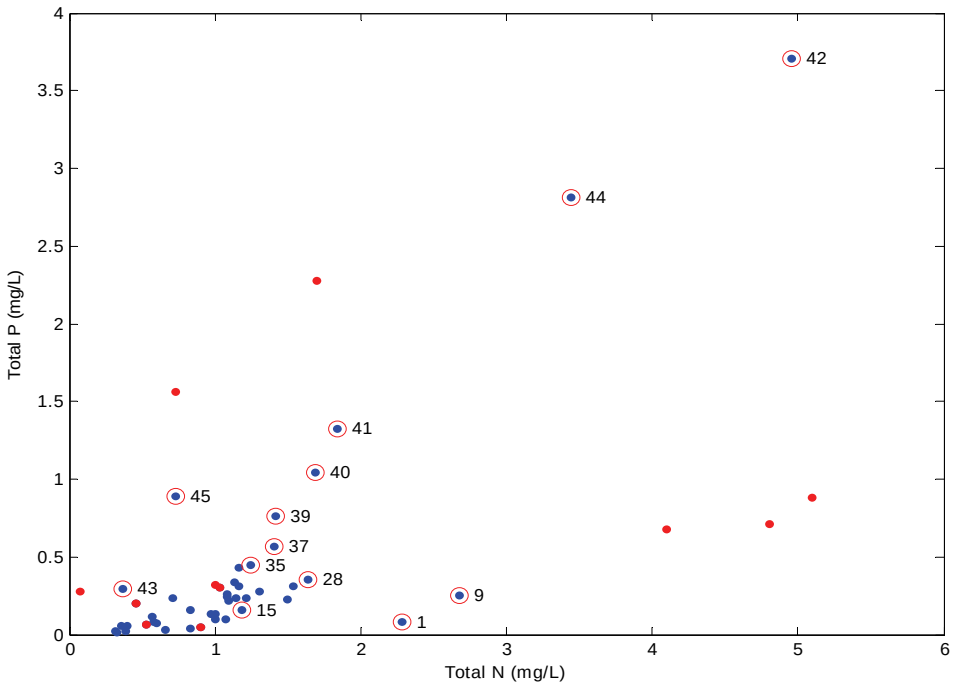


Figure 1.9: Plot of median values of total phosphorus concentration (Total P) against total nitrogen (Total N) concentration in water at recording stations within 5 km of water hyacinth infestation sites (dots). Sites proposed for long-term monitoring (ringed dots). Ringed sites correspond to site identifiers in Table 1.5.

1.3.4 Site Climate

The climate of each of the 14 monitoring sites is illustrated by means of climate and frost diagrams (Figures 1.10 to 16). The data used to produce these climate and frost diagrams were extracted from interpolated climate surfaces (Schulze et al., 1997).

Table 1.5: Water quality data for Resource Quality Services stations (Department of Water Affairs and Forestry) within 5 km of water hyacinth sites

ID no.	N:P ratio	Total N	Total P	station name
1	28.21	2.29	0.08	tol 1 tolwane upstream of klipgat sewage work
2	23.71	0.33	0.01	at komatipoort old road bridge on komati
3	21.79	0.83	0.04	isg 1 itsoseng tributary
4	19.56	0.67	0.03	gwatlhe river bridge
5	19.23	0.9	0.05	upstream of letaba rest camp 0n letaba
6	15.56	0.39	0.03	b8h050q01 tzaneen dam on great letaba river: downstream we
7	12.54	0.31	0.03	b8r005q01 tzaneen dam on great letaba river: near dam wall
8	11.27	1.07	0.1	a2r001q01 hartbeespoort dam on crocodile riv: near dam wall
9	10.75	2.68	0.25	isg2 itsoseng tributary
10	9.9	1.01	0.1	krokodil river at inlet to roodekopjes dam
11	8.48	0.53	0.06	b8h018q01 great letaba river at engelhardt dam/kruger nat p
12	8.31	0.53	0.06	at kameeldrift on hartbeesspruit
13	7.86	0.6	0.08	umtata dam on mtata river: near dam wall
14	7.75	1.01	0.13	hartbeespoort dam on crocodile riv: downstream w
15	7.48	1.18	0.16	nooitegedacht dam inlet
16	7.17	0.98	0.14	crocodile river at crocodile poort/thaba moya
17	7.07	0.58	0.08	at leeuwfontein on edendalspruit
18	6.62	1.5	0.23	bon accord dam on apies river: near dam wall
19	6.61	0.39	0.06	crocodile river at malelane bridge/kruger nat par
20	6.26	0.36	0.06	crocodile river at thankerton/kruger national par
21	5.31	0.83	0.16	laing dam on buffalo river: near dam wall
22	5.18	1.21	0.23	roodeplaat dam on pienaars river: near dam wall
23	5.06	0.57	0.11	a2h126q01 at franspoort road bridge on edendalspruit
24	5	1.54	0.31	a2r009q09 roodeplaat dam on pienaars river: point in dam
25	4.95	1.1	0.22	c2h061q01 vaal river at klipplaatdrift
26	4.86	1.15	0.24	a2r009q08 roodeplaat dam on pienaars river: point in dam
27	4.71	1.31	0.28	a2r009q02 roodeplaat dam on pienaars river: point in dam
28	4.61	1.64	0.36	a2r009q07 roodeplaat dam on pienaars river: point in dam
29	4.44	1.09	0.25	c2h018q01 vaal river at de vaal/schoemansdrift
30	4.19	1.08	0.26	c2h260q01 vaal river low water bridge at kromdraai
31	3.81	1.17	0.31	c2h250q01 vaalriver (bridge) at scandinawiedrif
32	3.41	1.03	0.3	c2h251q01 vaalriver at parys
33	3.35	1.13	0.34	r2h015q01 yellowwoods river at fort murray outspan
34	3.03	0.71	0.23	isg 3 itsoseng tributary confluence
35	2.82	1.25	0.44	kafferskraalspruit of confluence with sandriver
36	2.7	1.16	0.43	a2h102q01 roodeplaat dam on pienaars river: downstream wei
37	2.5	1.41	0.56	r2h010q01buffalo river at 135 kwtq u/s james mcintyre bridge
38	2.24	0.46	0.2	c4h005q01 vet river at floorsdrift/hoopstad
39	1.86	1.42	0.76	nooitegedacht dam outlet
40	1.62	1.69	1.05	sr5 sandriver before confluence with kafferskraalspruit
41	1.39	1.84	1.32	a2h027q01 pienaars river at baviaanspoort
42	1.34	4.95	3.7	c2h073q01 skoon spruit at goedgenoeg/orkney bridge
43	1.24	0.37	0.29	kafferskraalspruit before confluence with sandriver
44	1.23	3.45	2.81	pienaars river u/s roodeplaat dam/ zeekoegat(300 m d/s a2h
45	0.82	0.73	0.89	c2h085q01 mooi river at hoogekraal/kromdraai

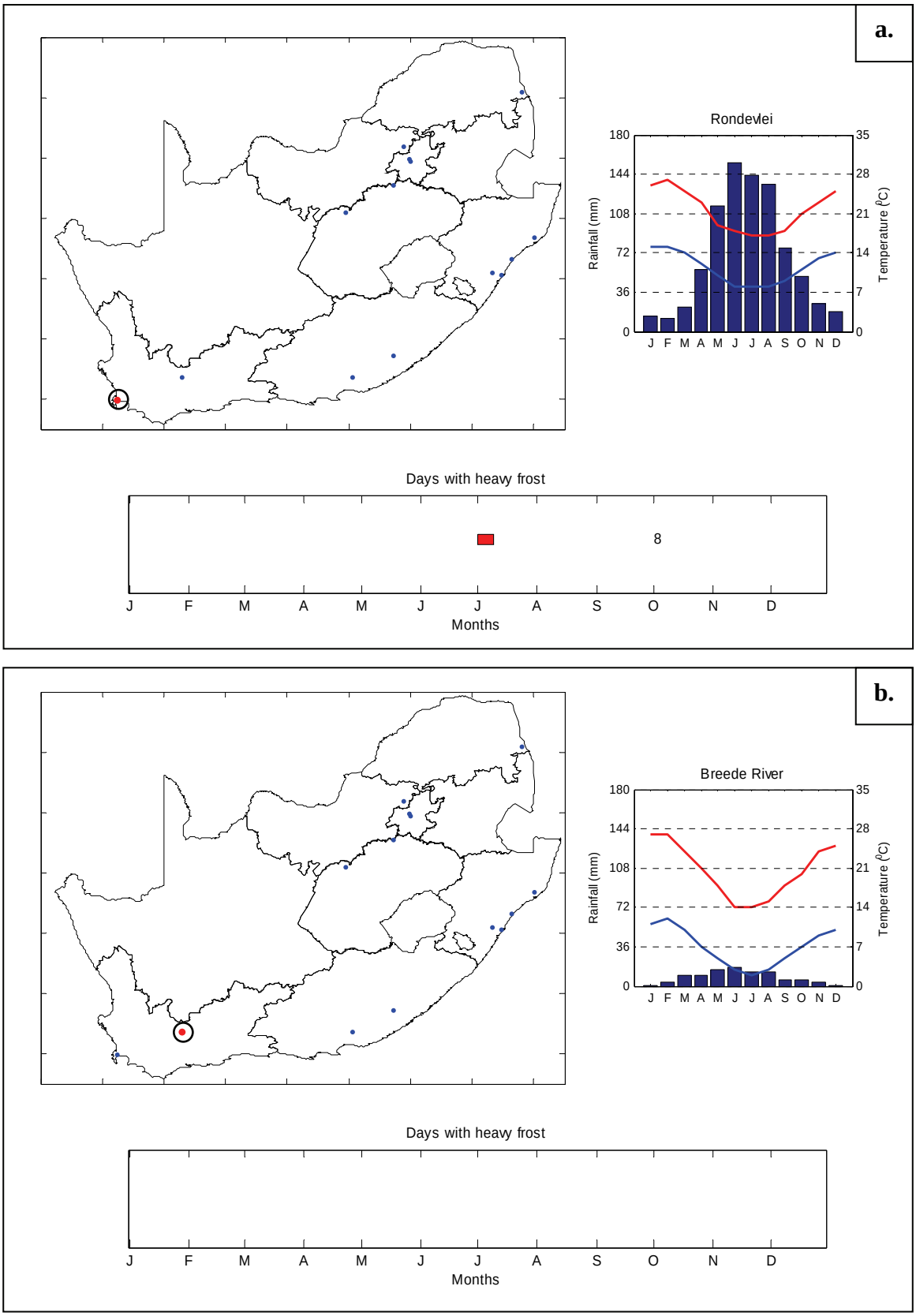


Figure 1.10: Climate and frost diagrams for field monitoring sites indicating median monthly rainfall, mean daily minimum and maximum temperature (by month), for the site (ringed dot on map). The frost diagram indicates duration and timing of heavy frost. The number of heavy frost days is indicated by a number to the right of the frost duration bar.

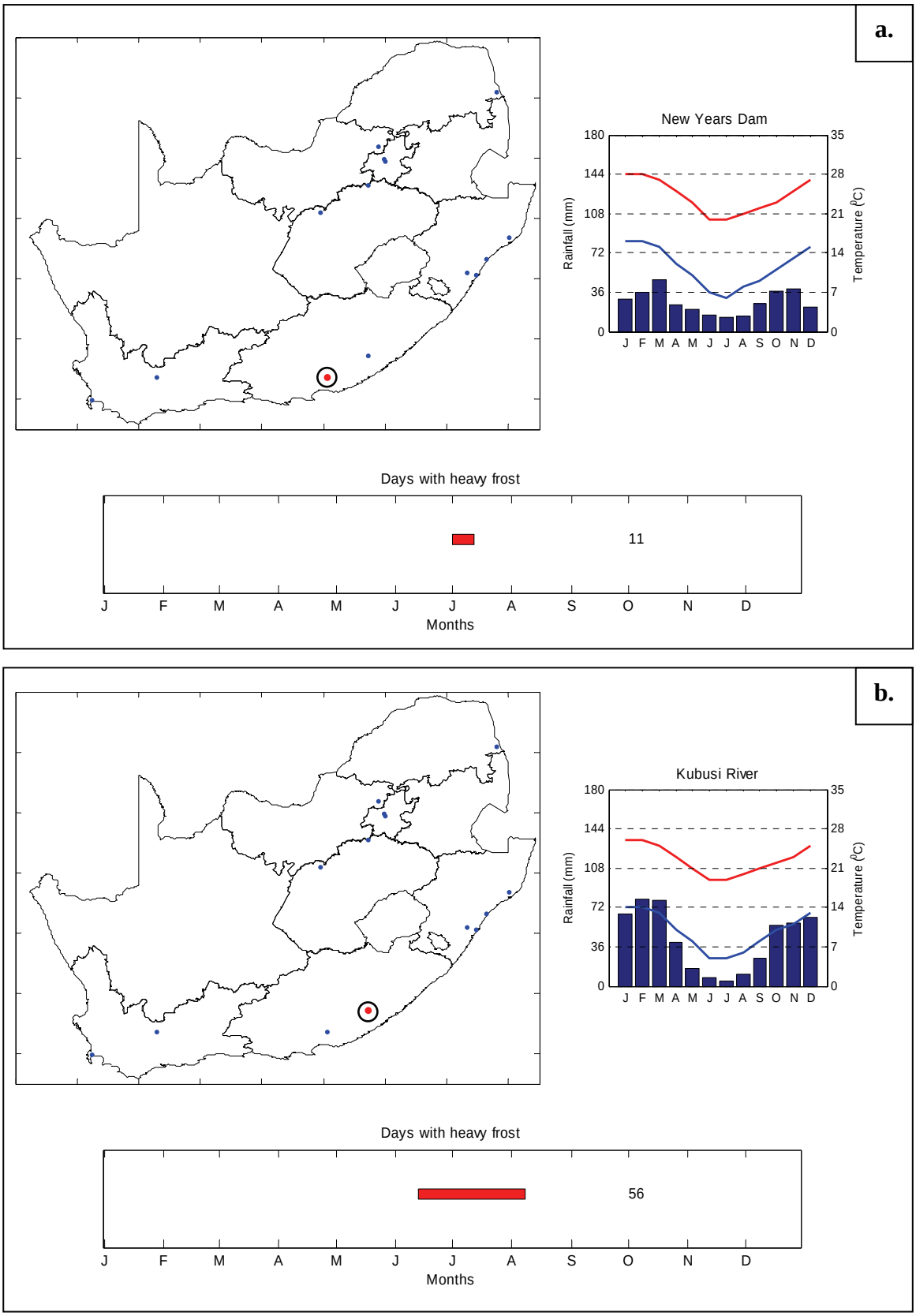


Figure 1.11: Climate and frost diagrams for field monitoring sites indicating median monthly rainfall, mean daily minimum and maximum temperature (by month), for the site (ringed dot on map). The frost diagram indicates duration and timing of heavy frost. The number of heavy frost days is indicated by a number to the right of the frost duration bar.

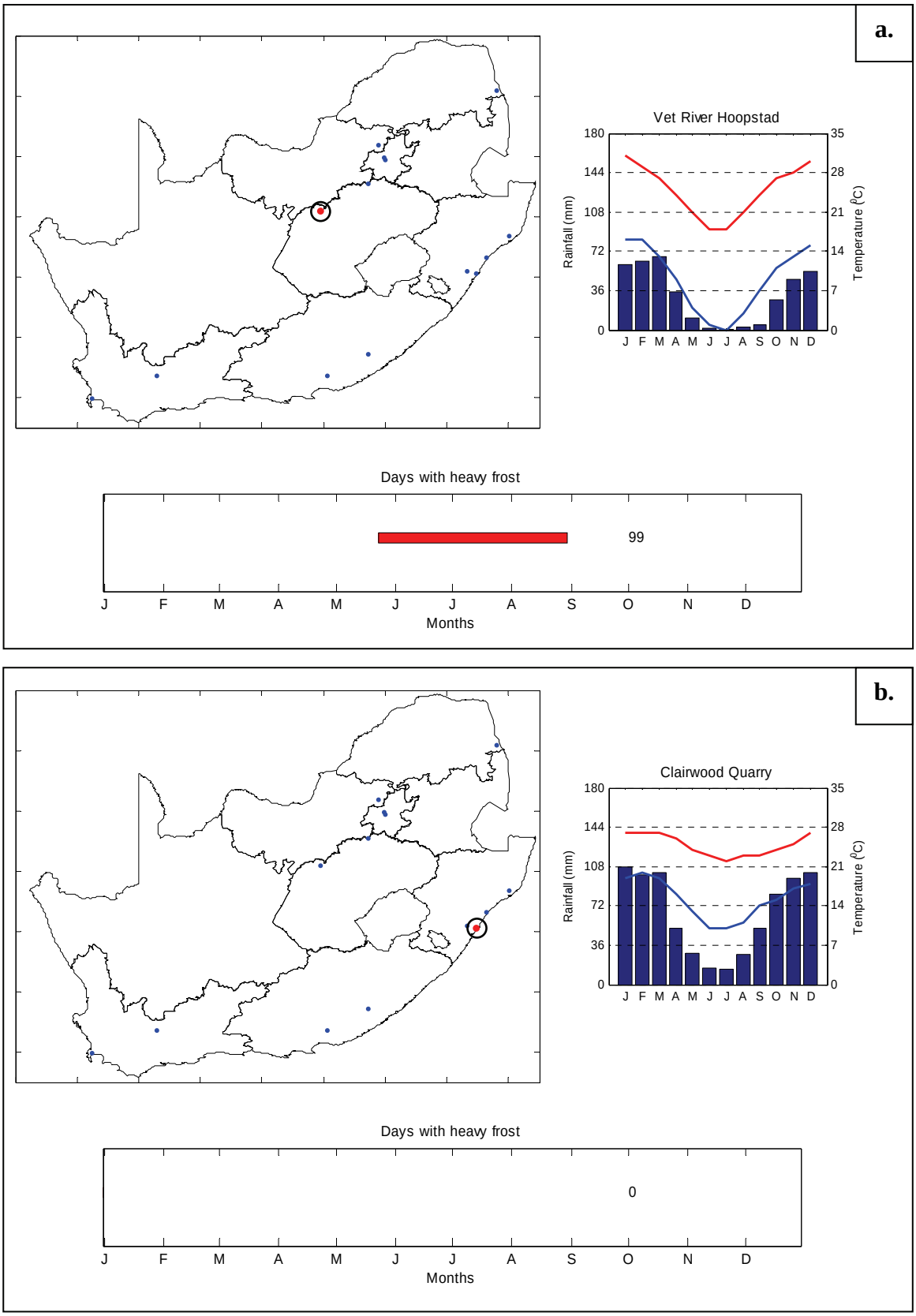


Figure 1.12: Climate and frost diagrams for field monitoring sites indicating median monthly rainfall, mean daily minimum and maximum temperature (by month), for the site (ringed dot on map). The frost diagram indicates duration and timing of heavy frost. The number of heavy frost days is indicated by a number to the right of the frost duration bar.

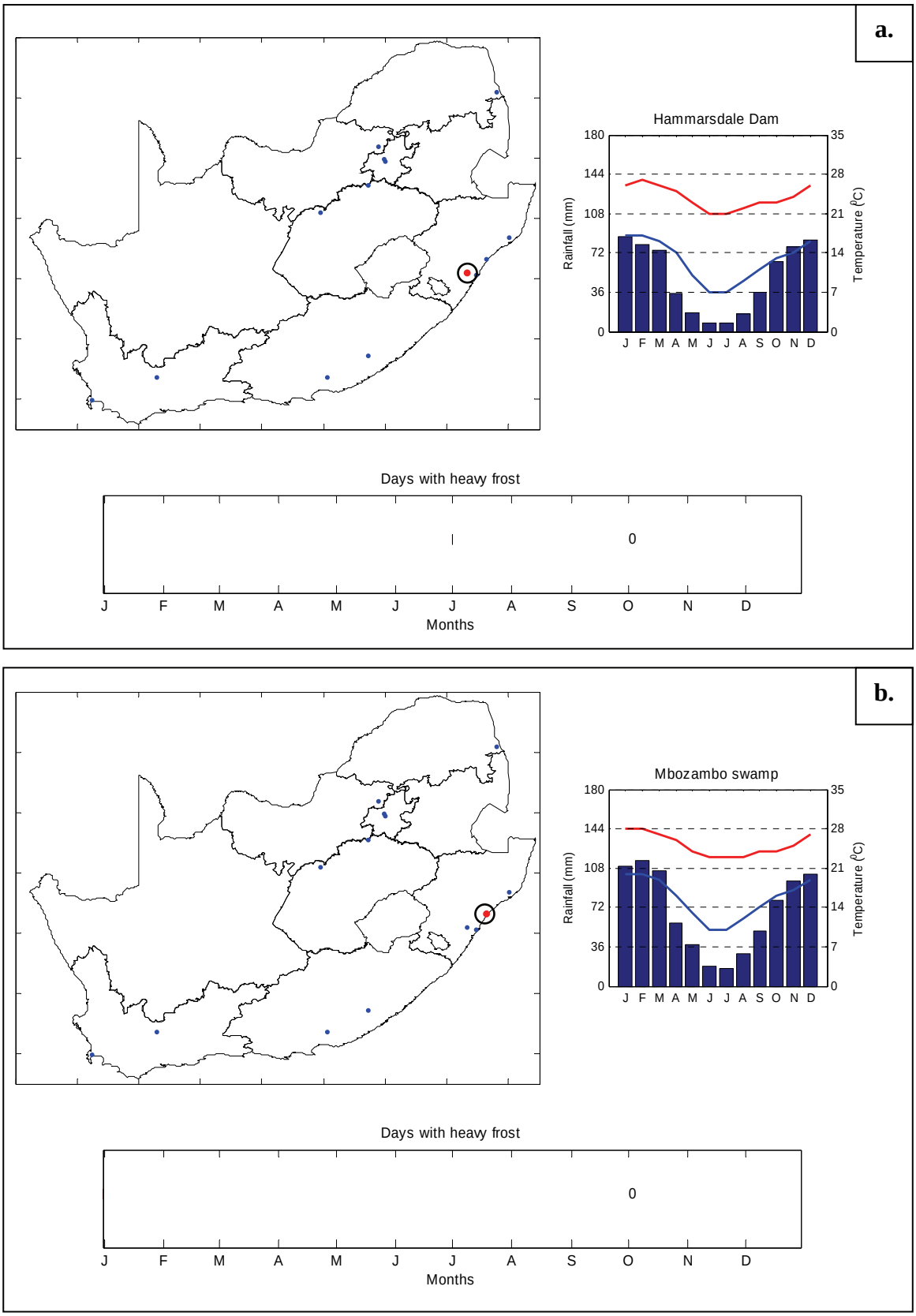


Figure 1.13: Climate and frost diagrams for field monitoring sites indicating median monthly rainfall, mean daily minimum and maximum temperature (by month), for the site (ringed dot on map). The frost diagram indicates duration and timing of heavy frost. The number of heavy frost days is indicated by a number to the right of the frost duration bar.

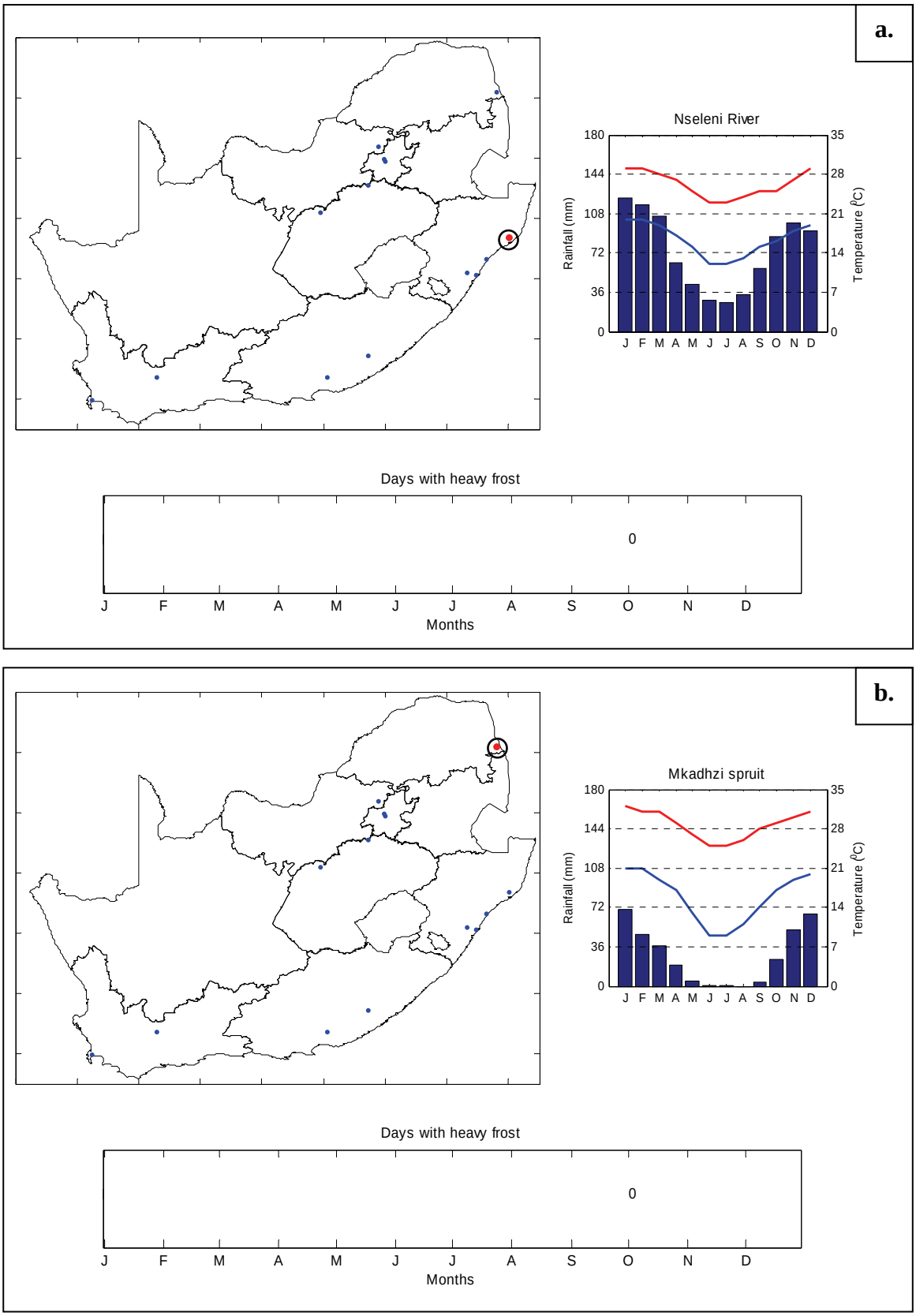


Figure 1.14: Climate and frost diagrams for field monitoring sites indicating median monthly rainfall, mean daily minimum and maximum temperature (by month), for the site (ringed dot on map). The frost diagram indicates duration and timing of heavy frost. The number of heavy frost days is indicated by a number to the right of the frost duration bar.

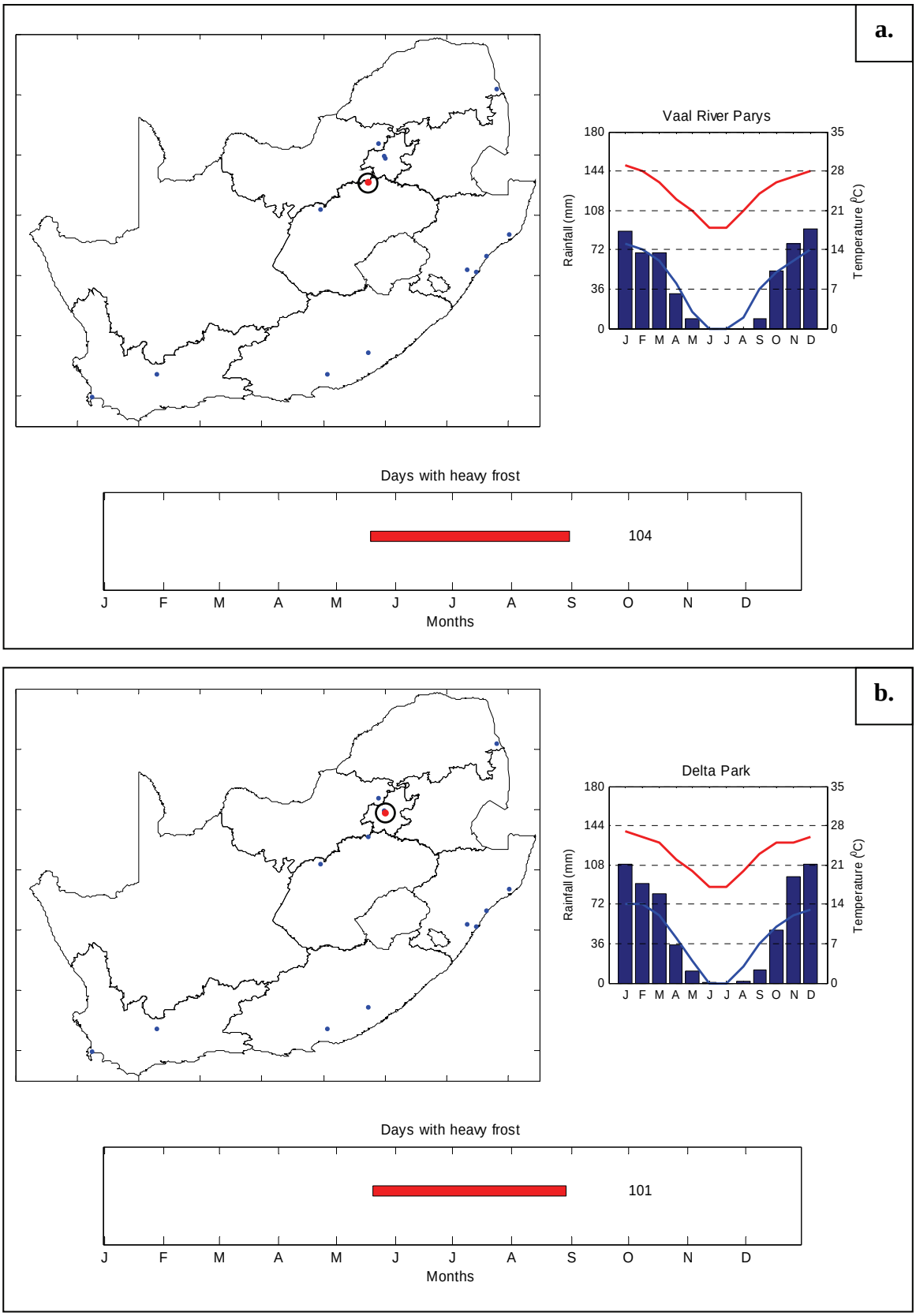


Figure 1.15: Climate and frost diagrams for field monitoring sites indicating median monthly rainfall, mean daily minimum and maximum temperature (by month), for the site (ringed dot on map). The frost diagram indicates duration and timing of heavy frost. The number of heavy frost days is indicated by a number to the right of the frost duration bar.

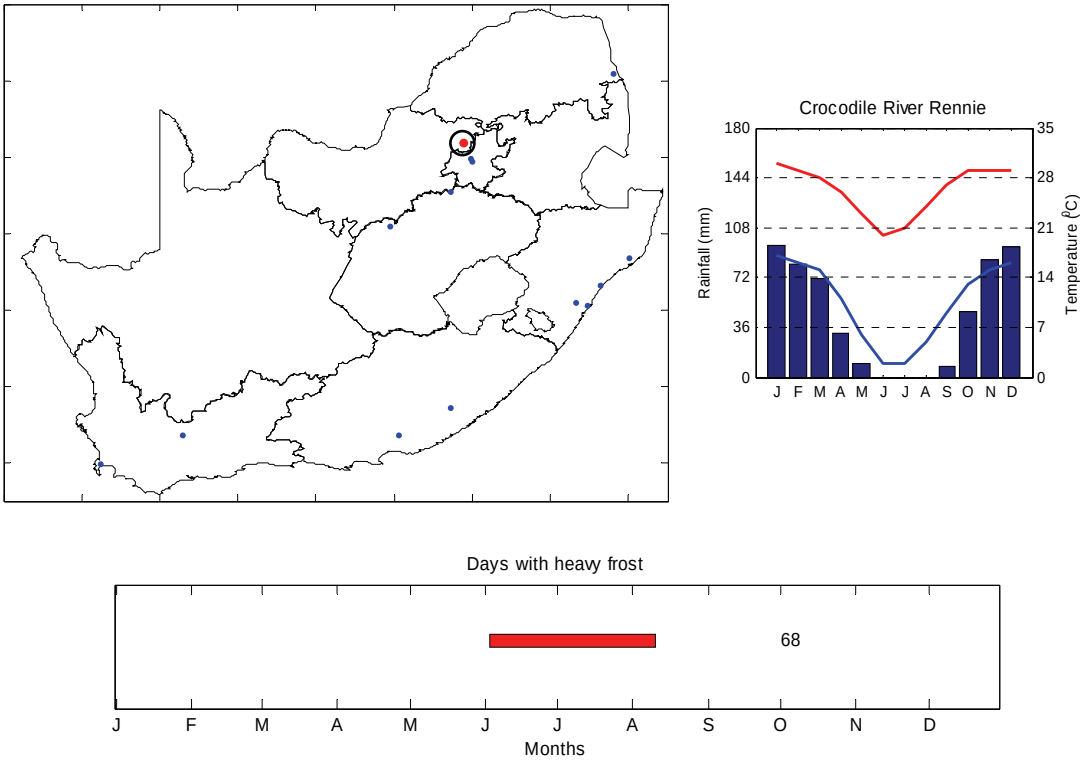


Figure 1.16: Climate and frost diagrams for field monitoring sites indicating median monthly rainfall, mean daily minimum and maximum temperature (by month), for the site (ringed dot on map). The frost diagram indicates duration and timing of heavy frost. The number of heavy frost days is indicated by a number to the right of the frost duration bar.

1.3.5 Stress Indices

The stress indices for water hyacinth and two of its biocontrol agents showed a broad range across the field sites selected for long term monitoring of the weed and its control agents. Principal components analysis allowed the field sites to be grouped based on the amount of climatic 'stress' experienced by each organism at a given site.

Whilst the sites were grouped similarly for both *E. catarinensis* and the *Neochetina* weevils when analysed separately, the relative contribution of each stress index in explaining the variance between sites differed for each species. When differentiating between sites based on the perceived stressors on the *Neochetina* weevils (Table 1.6), 88.9% of the variation among sites could be explained by the largest eigenvalue. The relative contribution of each of the four eigenvectors or stress indices to this component was roughly similar, with the feeding index (26.1%) making the highest, and oviposition index (23.3%) the lowest contribution. This suggests that *Neochetina* efficacy and possibly distribution are limited primarily by the number of cold nights per annum with a mean nocturnal temperature of less than 6.3°C. Although the second factor accounted for only 8.0% of the variance between sites, it was dominated by the oviposition index. Thus, the relative importance of the oviposition

threshold declined with decreasing temperature making differentiation with this index possible only between the warmer sites.

When differentiating between sites based on perceived *E. catarinensis* stress (Table 1.7), the analysis was again dominated by a single eigenvalue accounting for 81.0% of the variation. The variability inherent in this principal component, similar to the *Neochetina* analysis, was evenly spread between the four stress indices. The maintenance index was the most prominent eigenvector explaining 26.7% of the variation in the principal component while the feeding index (22.8%) was the least significant. Differentiating between sites based on the number of consecutive cold days, as described by the maintenance index, is especially important for short-lived species such as *E. catarinensis*

Table 1.6: Thermal stress indices for the *Neochetina* weevils at 14 field sites distributed around South Africa. (Oviposition Index: Mean nocturnal hours < 12.5ºC per day; Maintenance Index: Max consecutive days with mean < 9.6ºC pa; Feeding Index: Days with nocturnal mean < 6.3ºC pa; Mortality Index: Max consecutive days with daily minimum < 3.8ºC pa).

Site Name	Oviposition index (Hrs/day)	Maintenance index (Days/year)	Feeding Index (Days/year)	Mortality index (Days/year)
Breede River	2.3	4.0	2.0	0.5
Crocodile River	3.5	12.5	45.5	8.0
Delta Park	5.4	51.5	107.5	77.0
Farm Dam	4.8	55.5	94.0	44.5
Feesgronde	4.0	21	60	34
Hammarsdale Dam	3.0	6.5	12.0	2.5
Kubusi River	3.9	9.0	32.0	9.5
Mbozambo Swamp	1.1	0.0	1.5	1.5
Mkadhzi Spruit	0.7	0.0	0.0	0.0
New Years Dam	2.3	4.0	9.5	3.0
Enseleni River	0.1	0.0	0.0	0.0
Princess Vlei	0.7	0	0	0
Warrenton Weir	4.4	8	62	22
Wolseley	4.0	5.0	12.5	4.0

Table 1.7: Thermal stress indices for *Eccritotarsus catarinensis* at 14 field sites distributed around South Africa. (Oviposition Index: Mean diurnal hours < 10.8ºC per day; Maintenance Index: Max consecutive days with mean < 10.3ºC pa; Feeding Index: Days with diurnal mean < 10.8ºC pa; Mortality Index: Max consecutive days with daily minimum < 1.2ºC pa).

Site Name	Oviposition index (Hrs/day)	Maintenance index (Days/year)	Feeding Index (Days/year)	Mortality index (Days/year)
Breede River	1.2	4.5	17.0	0
Crocodile River	3.0	20.5	19.0	3.0
Delta Park	4.8	68.50	50.0	20.0
Farm Dam	4.4	72.50	64.5	15
Feesgronde	3.5	22	3	14
Hammarsdale Dam	4.4	7.5	7.5	0
Kubusi River	3.0	14.0	57.0	2.5
Mbozambo Swamp	0.7	0.0	0.0	0.0
Mkadhzi Spruit	0.4	0.0	0.0	0.0
New Years Dam	1.6	6.5	33.5	1
Enseleni River	0.02	0.0	0.0	0.0
Princess Vlei	0.1	0	3	0
Warrenton Weir	3.7	8	14	4
Wolseley	2.6	7.0	21.5	0.5

As the effects of cold on water hyacinth were seen to negatively influence both the weevils and *E. catarinensis*, field sites were also differentiated by incorporating factors affecting plant growth and quality (Table 1.8). Again the principal component analysis was dominated by a single factor accounting for 83.0% of the variation among the sites. No single eigenvector or stress index dominated the principle component but the *Neochetina* feeding (10.7%) and *E. catarinensis* maintenance (10.6%) indices were again prominent. Only five eigenvectors explained more than 10.0% of the variation between sites as described by the principal component. These indicated that consecutive days with consistently low mean temperatures (maintenance indices), cold nocturnal temperatures (*Neochetina* feeding index), and extreme temperature minima (mortality indices) were most useful for differentiating between field sites. As the dominant factor in the analysis accounted for 83.0% of the

variation, sites were grouped primarily by differences along the y-axis of Figure 1.17. Four distinct groups were isolated, the most inhospitable being made up of Delta Park and Farm Dam and the least stressful containing Mbozambo Swamp, Mkadhzi Spruit, Enseleni River and Princess Vlei. Despite being ranked as the coldest field site (Figure 1.18) with reference to the yearly accumulation of degree-days, the Kubusi River site was found to be far less 'stressful' on both the insects and water hyacinth. This conclusion was confirmed by the fact that *E. catarinensis* has persisted at both Kubusi and Crocodile River sites throughout the study but has never permanently established at either Farm Dam or Delta Park, despite being released several times at each site. Using the subjective stress measures generated here, water hyacinth would appear to be under less physiological pressure from its thermal environment at each site than its biological control agents, which might go some way to explain why the weed has largely escaped control by its insect herbivores.

Table 1.8: Climatic stress indices for *Eichhorniae crassipes* at 14 field sites around South Africa. (Growth Index: Days with mean water temperature < 10°C pa; Frost Index: Frost days per year).

Site Name	**Growth index (Days/year)**	**Frost index (Days/year)**
Breede River	0.0	0.0
Crocodile River	12.0	3.0
Delta Park	99.0	46.5
Farm Dam	78.0	19.5
Feesgronde	0	26
Hammarsdale Dam	4.0	0.0
Kubusi River	48.0	5.5
Mbozambo Swamp	0.0	0.0
Mkadhzi Spruit	0.0	0.0
New Years Dam	0.0	0.5
Enseleni River	0.0	0.0
Princess Vlei	0	0
Warrenton Weir	0	11
Wolseley	0.5	0.0

1.4 Discussion

The above analyses indicate that water hyacinth grows across a wide range of climatic and nutrient conditions in South Africa. Concentrations of the weed are associated with higher densities of people or water usage. This effect has been found for other invasive plants, where the best predictor of invasion is the number of people per square kilometre (Chytry et al., 2008). There has been a degree of compromise in selection of monitoring sites to accommodate the logistics of the relative placement of personnel and sites, and also security and accessibility, but the sites chosen for long-term monitoring of the weed and its associated biocontrol agents are broadly representative of a spread of ecological conditions and should reveal important patterns in the progression of their respective populations over time.

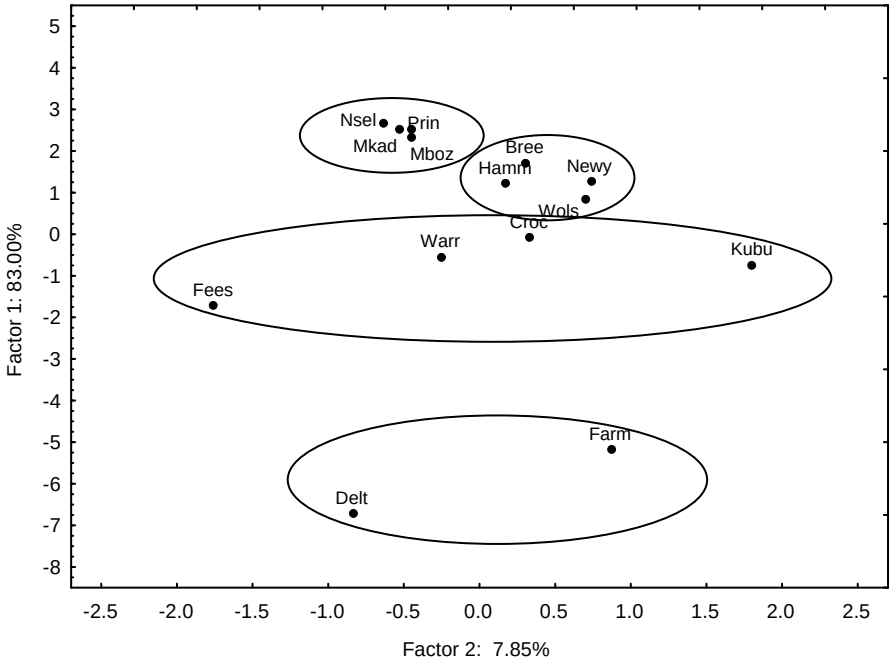


Figure 1.17: Grouping of field sites using Principal Components Analysis incorporating 10 thermal stress indices determined for the *Neochetina* weevils, *Eccritotarsus catarinensis* and water hyacinth.

1.4.1 Climate

From the distribution map and climate diagrams it is clear that water hyacinth grows across a wide range of temperature, rainfall and frost conditions in South Africa.

Water hyacinth in South Africa has been shown here to grow at mean temperature extremes from 33°C, down to 0°C, and at sites with up to 104 days of frost. The lower extreme of temperature at which the weed can survive (24hrs at -16° C; Owens and Madsen, 1995) is not reached at any site where the weed has been recorded in South Africa. Some of the water hyacinth biocontrol agents are constrained by minimum temperatures well above this (Coetzee et al., 2007a; 2009). However, the microclimate of each site differs from the coarse scale climate surface (Schulze et al., 1997) which gave rise to the average values in the

climate data used to select the field sites shown here; and most importantly, local extreme temperature events are largely lost from averaged data. Subsequent placement of temperature data loggers at each site allowed an increase in precision of the spatial and temporal quality of the temperature data used to select the sites, by recording temperatures in the water hyacinth canopy at each site. Analysis of these data in biological terms, as thermal thresholds of the weed and two of its biocontrol insects, shows the sites to be varied, and to a large degree, to have at least one replicate per climate group. This allows comparison of sites within groups and also between groups when considering the growth and behaviour of the weed and its biocontrol agents.

1.4.2 Nutrients

Water hyacinth grows at a wide range of both phosphorus (0.01-2.81 mg/*l*) and nitrogen (0.33-4.95 mg/*l*) levels in South Africa, and N:P ratios varied from 28.21 to 0.82) . The water hyacinth nutrient studies currently available in the literature (Heard and Winterton, 2000; Reddy et al., 1989, 1990; Wilson, 2002; Wilson et al., 2006; Xie et al., 2004) cannot be applied to many South African circumstances because nutrient levels at sites where the weed has been recorded do not approach the 220 mg/*l* nitrogen and 40 mg/*l* phosphorus used in some of those investigations (Walmsley, 2000; Harding, 2008). Nevertheless, a broad range of both nitrogen and phosphorus levels have been encompassed by the sampling programme.

1.4.3 Conclusion

A water hyacinth distribution map was generated, and then used to characterize the climatic and nutrient status of sites where water hyacinth occurs. Fourteen sampling site were chosen from around the country to encompass the full range of climate and nutrients under which water hyacinth grows. These 14 sites were sampled monthly for two years, collecting temperature, plant and insect data. The temperature data were used to calculate thermal stress indices for each site, which ranged from zero at coastal and low-altitude sites, to very high at inland Highveld sites. These indices indicate that the site selection method helped choose a broad range of sites from climatically benign to highly stressed, for both the weed and its biological control agents. Notably by the measures used here, the plant is generally less thermally stressed than its biological control agents (Figure 1.18). Finally, the data collected at these sites over two years is analysed in subsequent chapters to describe the population structure of water hyacinth and its biocontrol agents.

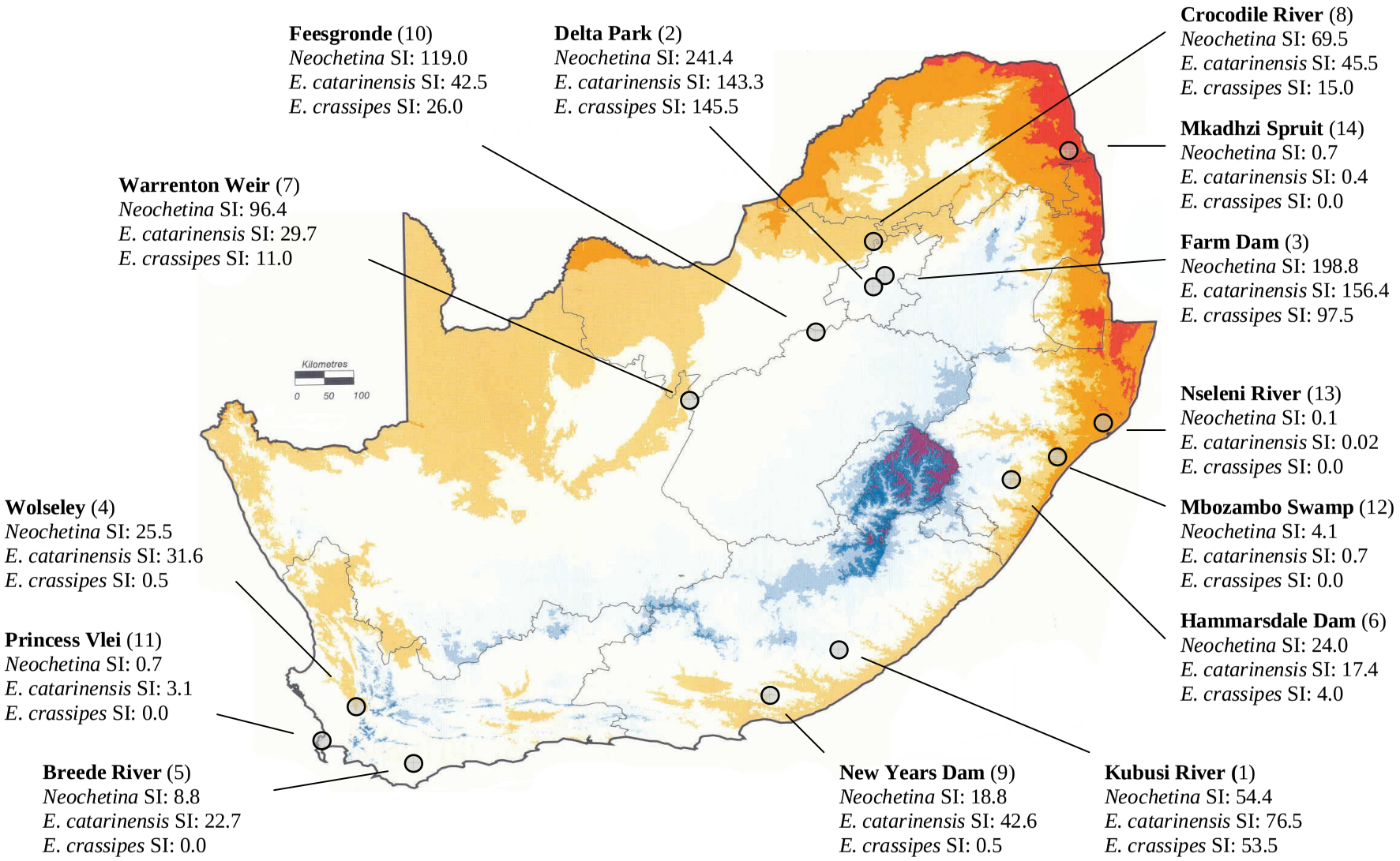


Figure 1.18: Relative stress indices for 14 climatically distinct water hyacinth infested field sites based on physiologically relevant thresholds for the *Neochetina* weevils, *Eccritotarsus catarinensis* and *Eichhornia crassipes*. Sites are ranked from coldest to warmest according to the yearly accumulation of degree-days. Map depicts the mean annual temperature for South Africa ranging from < 8°C (Purple) to > 22°C (Red) (Source: Schulze et al., 1997).

CHAPTER TWO –WATER HYACINTH AND TEMPERATURE: THE EFFECT OF TEMPERATURE ON WATER HYACINTH GROWTH, AND THE DEVELOPMENT OF ITS BIOLOGICAL CONTROL AGENTS

2.1 Introduction

2.1.1 The Impact of Low Temperature on Biological Control of Water Hyacinth

Of the factors limiting the impact from the biological control agents released against water hyacinth in South Africa, highly eutrophic waters and minimum temperature extremes are emphasised most often in the literature (Hill and Olckers, 2001; Julien 2001). Various authors speculate that waters enriched with nitrates and phosphates enable water hyacinth growth to outpace any detrimental effects inflicted by their natural enemies (Reddy et al., 1990). Additionally, the feeding rate of insects and the growth rate of the plants they feed on are differently affected by temperature (Van der Heide et al., 2006). Possible asynchrony of population growth of the agent and target, brought on by different responses of water hyacinth and its natural enemies to low temperatures, will compound these problems, further limiting control, especially in colder areas.

Cold climates are assumed to limit water hyacinth control in two ways: indirectly, as cooler average temperatures may hamper the efficacy of natural enemies by slowing development and consequently population growth rates; or directly, by causing mortality. Winter minimums in most regions of South Africa frequently drop below physiologically significant temperatures; for example, the temperatures at which development is arrested in both weevils and the mirid (Table 2.1). Secondly, because water hyacinth is adversely affected by low temperature extremes, natural enemy survival is thought to be further limited by their habitat destruction. Aerial parts of the plant brown and die back, and the crowns submerge when exposed to low temperatures and frost (Owens and Madsen, 1995). This loss of habitat and food supply is assumed to lead to an increase in mortality of the natural enemies overwintering on aerial parts of the plant. Frost has been shown to influence the geographical distribution of other insect species and has even been implicated in local extinctions (Inouye, 2000). Ehrlich et al., (1972) describe the local extinction of the butterfly *Glycopsyche lygdamus,* caused by the loss of host plants as a direct result of heavy frosting.

The low temperature limits of water hyacinth have been investigated by Owens and Madsen (1995). Regrowth experiments in field and laboratory conditions were conducted on the ability of water hyacinth to survive low air temperatures. Subjecting plants to low temperature extremes for short periods of time in the laboratory, it was found that exposure to -16ºC for less than 12 hours would not produce any significant stem base mortality. Placing this into a South African context, air temperatures will never get cold enough, for long enough, to cause stem base mortality in the field.

Longer-term experiments with mean minimums revealed that exposure to constant temperatures below 5ºC for at least two weeks are required to cause stem base mortality, an equally unlikely scenario in infested South African water bodies.

Table 2.1: Thermal tolerance data for the biological control agents *Neochetina eichhorniae, Neochetina bruchi* and *Eccritotarsus catarinensis* released against water hyacinth. Sources: 1. Coetzee, 2003; 2. Coetzee, unpub.; 3. DeLoach and Cordo, 1976; 4. Julien, 2001.

	Neochetina bruchi	*Neochetina eichhorniae*	*Eccritotarsus catarinensis*
Lower LT_{50}	-	-7.4°C [2]	-3.5°C [1]
Upper LT_{50}	41.57°C [2]	41.7°C [2]	37.0°C [1]
CT_{min}	3.3°C [2]	4.3°C [2]	1.2 ± 1.17°C [1]
CT_{max}	48.84°C [2]	51.0°C [2]	49.6 ± 3.37°C [1]
Lower oviposition threshold	-	10°C [3]	-
Lower developmental threshold	≈15°C [4]	-	10.3°C [1]
Degree-day requirements (egg to adult)	-	-	342°D [1]
Optimum temperature for feeding/ oviposition	30°C [4]	30°C [4]	-

These findings are useful in that unlike its biological control agents, water hyacinth survival is unlikely to be restricted by temperature anywhere in South Africa. However, Owens and Madsen's (1995) treatment plants had all aerial parts removed, leaving only stem bases with roots attached. This is unfortunate as the results give no indication of the temperature range at which leaf mortality occurs, or when plant vigour is reduced. As leaf mortality is linked to natural enemy survival and, because of frost, is prevalent throughout much of the interior regions of South Africa (Van Wyk and Van Wilgen, 2002), this temperature range would indicate periods of plant dormancy and reduced growth rate and could serve as a valuable predictor of potential establishment of the mirid and mite, which persist and feed predominantly on aerial parts of the plant. However, predictions of the biological control agents' developmental rates based on temperature records can nevertheless be made.

2.1.2 Estimating Rates of Insect Development

In most temperature-dependent development studies, the relationship between insect growth and temperature is linear between an upper and lower temperature threshold. Insect development can then be expressed in terms of degree-days. The linear intercept method proposed by Campbell et al., (1974) is approximated by the line:

$$y = a + bT$$

where y is the rate of development (1/Days), and T is the temperature (°C) at which the insect was reared. This remains one of the most widely-used methods for approximating insect development due to its simplicity and relative accuracy. However, this method is less effective when considering smaller data sets that may include developmental rates at extreme temperatures that would certainly lie off the linear portion of the developmental line. Ikemoto and Takai (2000) describe three drawbacks associated with this method. Firstly, as there is an optimum range of temperatures for which an insect's development is roughly linear, and which is bounded by specific upper and lower temperatures, a regression line should only be applied to this portion of development. The detection of these critical upper and lower temperatures, outside of which development deviates from the linear trend, is difficult and open to bias when using the regression method, resulting in unreliable estimations of the developmental threshold (t) and the degree-day requirements or thermal constant (K). Secondly, a simple regression assumes constant variances on the 1/Days scale at all temperatures resulting in the disproportionate weighting of data points in the upper and lower sections of the line, being more exaggerated at the lower temperature range. Thirdly, the regression line ignores variation in temperature. The second and third problems would result in an underestimation of t and an overestimation of K. To overcome these problems Ikemoto and Takai (2000) propose a different line-fitting method.

The reduced major axis regression method (Ikemoto and Takai, 2000) has been shown to give greater precision in estimating an insect's lower developmental threshold and thermal constant, as these are not estimated from the linear equation. Their equation represents a straight line defined by the formula:

$$(DT) = K + tD$$

where D is the duration of development recorded in the laboratory and DT is the product of this development and its corresponding temperature (°C). Using this method, the optimum temperature range for development can be easily identified allowing for the exclusion of data that deviates from the linear tendency created by points along this optimum range. This provides more reliable estimates of t and K.

In order to construct accurate, predictive phenological models, relevant physiological thresholds are needed for both the host plant and its associated insects. In this study the developmental thresholds for both *N. eichhorniae* and *N. bruchi* and the mirid *E. catarinensis* were estimated using data derived from existing literature. Degree-day requirements from egg to adult were then calculated and incorporated into degree-day based phenological model to calculate growth rates of these insects.

A long term evaluation of the effects of low temperature and frost on the growth of water hyacinth was also undertaken here. Leaf turnover rates and leaf mortality in response to temperature was addressed for three high-altitude highveld sites prone to frequent winter frost for an 18-week period moving into winter 2006.

Relevant physiologically important temperatures, such as insect developmental thresholds, or small temperature ranges, which could for instance trigger plant dormancy, are essential for underpinning and establishing boundaries or biofix temperatures for phenological models. Whilst the vast majority of these temperatures are derived from constant temperature experimentation, much literature exists cautioning its use. Liu et al., (1995) recognise that the developmental times of insects can, often quite considerably, differ between constant and varying temperature regimes with the same mean temperature. Although evaluating the developmental times of the weevils and the mirid, which are based on constant temperature experimentation, against those derived from fluctuating temperatures was not within the scope of this study, controlled fluctuating temperature regimes were used to evaluate other processes linked to natural enemy efficacy, such as feeding, mortality and oviposition rates. Results from these experiments are applicable to field situations and are especially relevant for nocturnal insects, such as the weevils, where night-time temperatures can drop substantially and therefore constant temperatures can lead to a false impression of efficacy. Water hyacinth as well as both weevil species and the mirid were subjected to long-term exposure to low, yet ecologically meaningful temperatures in order to evaluate their performance in both favourable and unfavourable conditions.

2.2 Methods and Materials

2.2.1 Developmental Rates of *Neochetina bruchi* and *N. eichhorniae*

Thermal tolerance data for *N. bruchi*, *N. eichhorniae* and *E. catarinensis* were collated from the literature. These data included upper and lower lethal temperatures (LT_{50}'s), thermal maxima (CT_{Max}) and minima (CT_{Min}), oviposition temperature thresholds, and developmental duration from egg to adult where available (Table 2.1). In the case of *N. bruchi* and *N. eichhorniae*, mean developmental times for eggs, larvae and pupae at different constant temperatures were collected from a variety of published and unpublished sources. Data points for *N. bruchi* pupal development were used for the corresponding *N. eichhorniae* regression as no development times for the latter species could be found in the literature. These data allowed the rates of development, the

developmental thresholds, and subsequent egg to adult degree-day requirements to be calculated for both weevil species by regressing developmental duration against temperature.

As per the method described by Campbell et al., (1974), for each stage of development, the lower developmental threshold (*t*) is equal to the *x*-intercept of the extrapolated regression line. The thermal constant (K) for each life stage was calculated as the reciprocal of this line. Degree-day requirements from egg to adult were calculated as the sum of these thermal constants and the developmental threshold for each species was calculated as the mean *t* estimated for each stage of development. The standard error (S.E.) of *t* and K is approximated by the equations:

$$SE_t = \frac{\bar{y}}{b}\sqrt{\frac{S^2}{N\bar{y}^2} + \left[\frac{SEb}{b}\right]^2}$$

and:

$$SE_K = \frac{SE_b}{b}$$

where $\bar{y}$ is the sample mean, S^2 is the residual mean square and *N* is the number of paired observations.

In light of the problems associated with the linear intercept method, the reduced major axis regression proposed by Ikemoto and Takai (2000) was also used in addition to that of Campbell et al., (1974). This allowed the developmental parameters calculated from each method to be compared and the most realistic estimates to be used for further calculations. Upon fitting the straight line x = D on y = DT to the data, the optimum temperature range was determined by excluding some data points at higher and lower extreme temperatures which appeared to deviate from the linear tendency created by the points in the optimum temperature range. A reduced major axis regression was then applied to this optimum temperature range in order to estimate the parameters *t* and K, determined from the line equation.

Due to the limited developmental dataset available from existing literature, some data points for *N. eichhorniae* pupal development were missing. Given that the immature stages of the weevils are indistinguishable in the field, data for both weevil species was combined and reanalysed using both the linear intercept and reduced major axis regression methods. Although not species-specific, these estimates of *t* and K are more applicable to field scenarios.

2.2.2 The Effects of Low Temperature and Frost on Water Hyacinth

Field observations were done over an 18 week period from 28/03/2006 to 31/07/2006 at three highveld sites that receive regular frosting during winter. These consisted of a 4.9 m^2 plastic-lined, wire-mesh pool at the University of the Witwatersrand and two approximately 3 000 m^2 dams at Delta Park, Johannesburg, the lower of which, due to the park's topography, is more heavily frosted (Geoff Lockwood, personal communication). The ratio of alive to dead leaves per plant, leaf production, number of ramets, longest petiole length and the severity of frost damage on 10 tagged plants per week at each site was recorded. Frost damage was categorised by ranking the amount of dead leaf tissue into one of six categories: 0%; <5%; 5-25%; 25-50%, 50-75% and 75-100%. These measures were correlated to 'continuous' (every 30 minutes) microclimate monitoring facilitated by Thermachron iButtons (Temperature range: -40°C to 85°C in 1°C increments with an accuracy of ±1°C). Water temperature 5 cm below the surface, air temperature within the water hyacinth canopy and ambient air temperature 1.2 m above the water surface was recorded. Canopy temperatures were adjusted to minimise errors associated with radiative heating of the temperature buttons, due to the sun striking the probe housing at certain times of the day. The rate of leaf production per week was then regressed against the corresponding mean weekly water temperature. Grouping this data from the three sites enabled the construction of a standard curve describing the rate of leaf production in response to water temperature.

Asexual reproduction of water hyacinth was recorded at each field site during the course of monitoring by counting the ramets found on 10 randomly selected plants. These data were plotted for each site with water temperature to reveal what effect temperature was having on ramet production.

2.2.3 Insect Performance and Plant Productivity

Experiments were run in environmental chambers with controlled air temperature, humidity and photophase, to determine feeding, mortality and oviposition rates of *N. bruchi*, *N. eichhorniae*, and *E. catarinensis* relative to daily fluctuating temperatures. Each of the three natural enemies was exposed to two separate temperature regimes for a period of approximately eight weeks. The cooler of these regimes (Table 2.2) simulated winter conditions, encompassing the mean daily minimum and maximum air temperature, mean daily relative humidity and photophase of a release site where each of the respective agents have failed to establish or where their impact has been negligible. The second temperature regime acted as a control and was maintained at temperatures approximating winter conditions at a site where successful management of the weed has been achieved through biological control alone.

Each environmental chamber housed a pool containing 30 water hyacinth plants on which two pairs of adult weevils per plant, and 12 plants on which 10 adult mirids per plant were released respectively for both treatment and control. Water nutrient

concentrations approximated intermediate levels observed in waters around South Africa (2 mg N l^{-1}; 0.29 mg P l^{-1}) and were standard throughout all treatments, for both temperature regimes, and for all agents. Plants and insects were left to acclimate for a week before any observations were made. For the duration of the weevil experiment, two plants were removed weekly from each temperature regime and destructively sampled.

Table 2.2: Environmental chamber regimes simulating winter conditions at field sites where biological control has failed or had negligible results (Treatment) and where it has been successful (Control). Sites are approximated using the mean daily minimum and maximum temperatures from June and July 2002 and 2003.

	Site approximated	**Mean daily min/max temperature**	**Mean daily relative humidity**	**Photophase (L:D)**
TREATMENT:				
Neochetina eichhorniae and *Neochetina bruchi*	Farm Dam	1-17ºC	58%	8:16
Eccritotarsus catarinensis	Delta Park	1-16ºC	58%	8:16
CONTROL:				
Neochetina eichhorniae and *Neochetina bruchi*	New Years Dam	9-21ºC	65%	12:12
Eccritotarsus catarinensis	Enseleni River	11-23ºC	69%	12:12

Temperature and Humidity data supplied by the South African Weather Service.

As the weevils prefer to feed on younger leaves, the number of feeding scars on the second youngest leaf (DeLoach and Cordo, 1976) and the number of eggs laid on the third youngest leaf were recorded. Observations also recorded the number of larvae, pupae and adults present per plant removed. Adult insects found in excess of what was initially released per plant were released back onto their respective pools to ensure constant population densities.

Non-destructive sampling was performed weekly for the duration of the mirid experiment on three randomly selected plants, re-sampling of which was done only once all initial 12 plants had been sampled. Recorded parameters included the number of adults and nymphs per plant, and the leaf area damaged by mirid feeding on the first, second and third youngest leaves. Feeding was categorised by ranking the area of leaf

tissue damaged into one of six categories: 0%; <5%; 5-25%; 25-50%, 50-75% and 75-100%. These measures were correlated to 'continuous' temperatures facilitated by Thermachron iButtons. Water temperature 5 cm below the surface, air temperature within the water hyacinth canopy and ambient air temperature 1.2 m above the water surface were also recorded.

At the onset of weekly measures, plant productivity was gauged via counts of ramets, flowers and leaves per plant. Growth parameters recorded the plants' phenostage, the longest petiole, length of the second youngest petiole, maximum root length, and the area of the second youngest leaf.

2.3 Results

2.3.1 Developmental Rates of *Neochetina bruchi* and *N. eichhorniae*

Large discrepancies were found in the estimates for *t* and K between the different regression methods of Campbell et al., (1974) and Ikemoto and Takai (2000) (Table 2.3). The linear intercept method (Figure 2.1) returned unrealistically low developmental thresholds for both weevil species of 2.87°C and 3.31°C for *N. bruchi* and *N. eichhorniae* respectively. As predicted by Ikemoto and Takai (2000), this method also returned very large estimates of the thermal constants for both weevils, up to 1000 degree-days higher than expected when compared with other weevils living on aquatic plants. The reduced major axis regression technique returned more realistic values for *t*, 9.84°C and 9.01°C for *N. bruchi* and *N. eichhorniae* respectively (Figure 2.2). Thermal constants calculated by that method were higher than anticipated, but coupled with the developmental thresholds, yielded approximations of generational turnover similar to those observed in the field (Stark and Goyer, 1983; DeLoach and Cordo, 1976).

Due to the lack of larval and pupal developmental data used for the linear intercept regressions, the optimum temperature range or linear portion of the relationship between temperature and rate of development could not be determined with any confidence for either weevil species. Therefore, data points that may lie outside this temperature range might have been included in the linear array resulting in unreliable estimates of *t* and K. Fitting the straight line x = D on y = DT to the same data allowed data points that deviated significantly from the linear tendency to be removed from the final regression. For *N. bruchi* egg development, two points above 31°C were omitted from the final regression indicating an optimum temperature range of 13°C to 30°C. Larval development had one point at 26°C omitted limiting the optimum temperature range to between 16°C and 25°C. However, due to a lack of data points below 13°C and 16°C, it is uncertain whether this range could extend beyond these lower limits. No points were removed from the pupal development regression due to limited data. For *N. eichhorniae* egg development, one point at 35°C was omitted indicating an optimum temperature range between 20°C and 30°C, although the true developmental range undoubtedly

extends below this lower limit. No points were removed from the larval development regression due to the low number of data points.

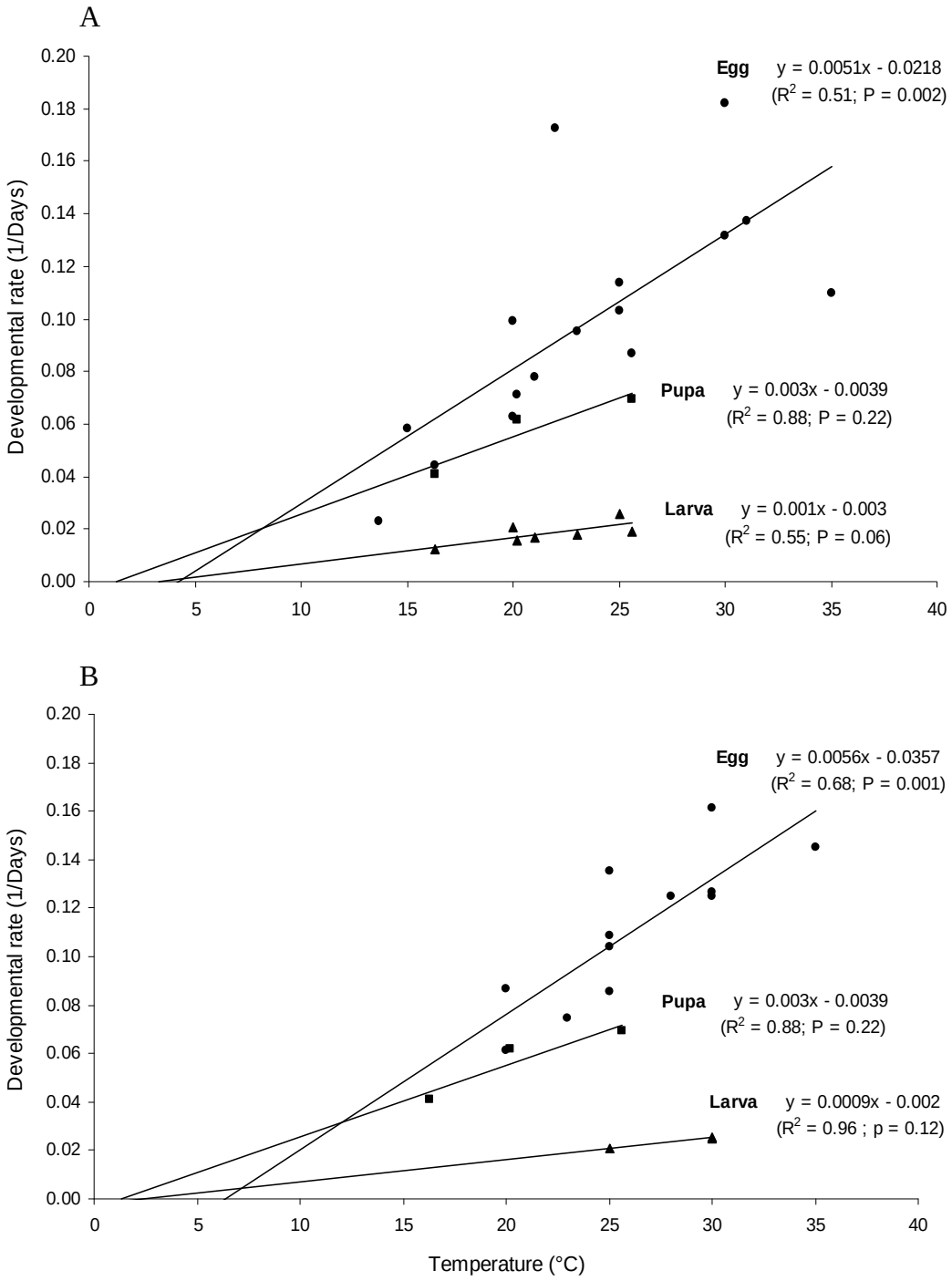


Figure 2.1: Temperature dependent development of A. *Neochetina bruchi* and B. *Neochetina eichhorniae* egg, larval and pupal stages using the linear intercept method (Cambell et al., 1974).

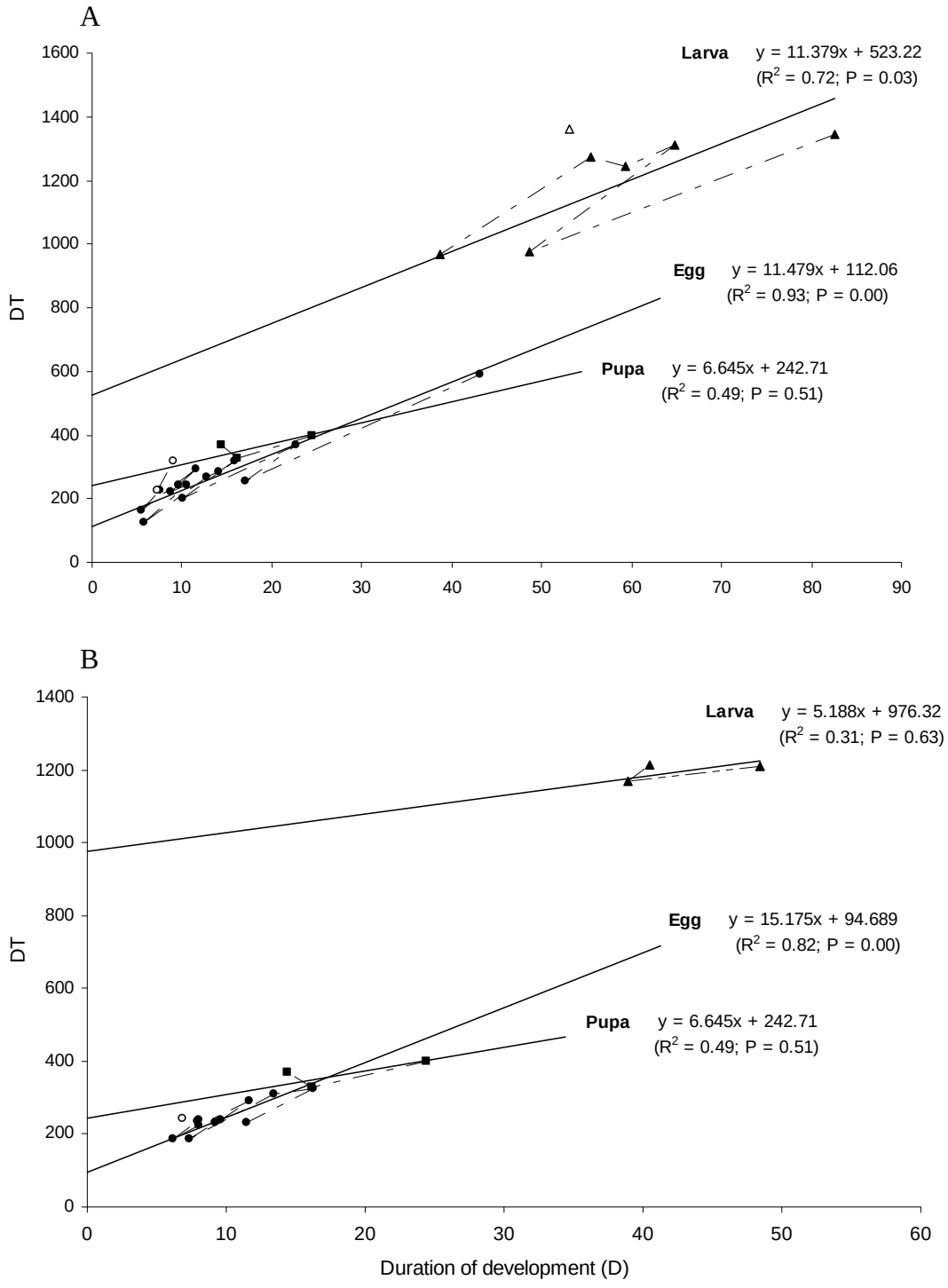


Figure 2.2: Temperature dependent development of A. *Neochetina bruchi* and B. *N. eichhorniae* egg, larval and pupal stages using the reduced major axis regression technique (Ikemoto and Takai, 2000). Unshaded points were omitted from the regression.

Table 2.3: Lower developmental thresholds (*t*) and thermal constants (K) calculated for immature stages of *Neochetina bruchi* and *N. eichhorniae*, using two different line-fitting methods, 1 and 2. Developmental data compiled from Abjar and Bashir (1984); Coetzee, unpublished; Chikwenhere (2000); DeLoach and Cordo (1976); Stark and Goyer (1983); Shih et al. (1994); Wilson (2002).

1. LINEAR INTERCEPT:

Species	**n**	***t* ± SE (°C)**	**K ± SE (°D)**
Neochetina bruchi			
Egg	16	4.24 ± 0.87	194.44 ± 50.74
Larva	7	3.07 ± 0.91	1015.46 ± 413.49
Pupa	3	1.31 ± 0.90	337.92 ± 123.78
All	-	2.87	1547.82
Neochetina eichhorniae			
Egg	12	6.39 ± 0.86	178.73 ± 38.72
Larva	3	2.24 ± 0.88	1101.81 ± 213.66
Pupa *	3	1.31 ± 0.90	337.92 ± 123.78
All	-	3.31	1618.46

2. REDUCED MAJOR AXIS REGRESSION:

Species	**n**	***t* ± SE (°C)**	**K ± SE (°D)**
Neochetina bruchi			
Egg	14	11.48 ± 0.87	112.06 ± 14.49
Larva	6	11.38 ± 3.00	523.22 ± 179.32
Pupa	3	6.65 ± 4.74	242.71 ± 89.277
All	-	9.84	877.99
Neochetina eichhorniae			
Egg	11	15.18 ± 2.15	94.69 ± 22.20
Larva	3	5.19 ± 4.32	976.32 ± 184.93
Pupa *	3	6.65 ± 4.74	242.71 ± 89.277
All	-	9.01	1313.72

* Data substituted from *Neochetina bruchi*.

No data for *N. eichhorniae* pupal development exists, so data for *N. bruchi* was substituted for this purpose. The reduced major axis regression technique allowed for easier estimation of the temperature range which best approximates the linear section of the development curve. This provided better fitting of the regression line, higher R^2 values, and thus more reliable parameter estimates.

Combining developmental data for both weevil species provided a more robust and inclusive dataset by improving the regression fits relative to those produced for *N. eichhorniae* alone. As before, although the regression fits were better for *N. eichhorniae* (Figure 2.3A), the linear intercept method returned an unrealistically low developmental threshold and long duration of development. The reduced major axis regression method (Figure 2.3B) provided both suitable fits and good estimates for both *t* and K (Table 2.4).

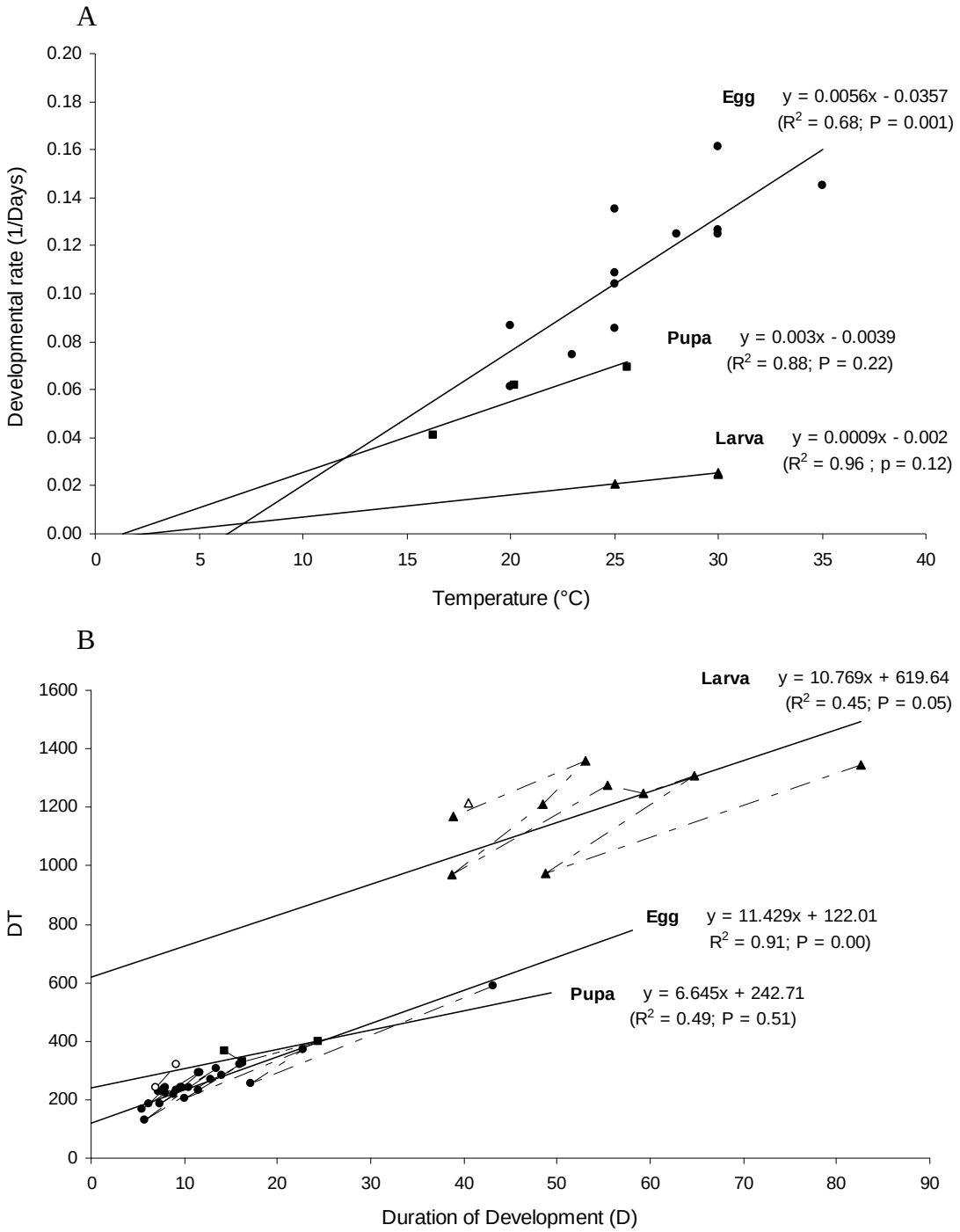


Figure 2.3: Temperature dependent development of *Neochetina bruchi* and *Neochetina eichhorniae*, egg to adult combined, using A. The linear intercept (Cambell et al., 1974) and B. Reduced major axis regression (Ikemoto and Takai, 2000) methods. Unshaded points were omitted from the regression.

Table 2.4: Lower developmental thresholds (*t*) and thermal constants (K) for immature stages of the *Neochetina* weevils using two line-fitting methods. Data for both species was combined making estimates of *t* and K more applicable to field scenarios.

	LINEAR INTERCEPT		REDUCED MAJOR AXIS REGRESSION	
Stage	***t* ± SE (°C)**	**K ± SE (°D)**	***t* ± SE (°C)**	**K ± SE (°D)**
Egg	4.67 ± 0.84	191.91 ± 32.78	11.43 ± 0.72	122.01 ± 10.1
Larva	1.04 ± 0.88	1135.72 ± 248.94	10.77 ± 3.02	619.64 ± 168.73
Pupa	1.31 ± 0.90	337.92 ± 123.78	6.65 ± 4.74	242.71 ± 89.277
All	2.34	1665.55	9.62	984.36

2.3.2 The Effects of Low Temperature and Frost on Water Hyacinth

During the 18-week winter observation period, distinct frost events were observed which were easily distinguishable at each site. Temperatures declined consistently for the first 10 to 11 weeks at all sites and remained consistently low for the remainder of the experiment (Figures 2.4-6). Water temperature best described plant growth trends whilst canopy temperature allowed the timing of individual frost events to be determined. Air temperature followed the same diurnal pattern exhibited by the canopy profile but with a higher variability in daily maximum and minimum temperatures and thus was largely omitted from the analyses. Of the three monitored sites, the lower dam at Delta Park was found to be significantly colder ($F_{2, 375} = 35.38$, $p < 0.001$) with a mean daily water temperature of 10.1 ±0.34°C and extremes of water, canopy and air temperature dropping as low as 5.5°C, -4.3°C and -3.0°C respectively. Mean daily water temperature at the upper dam (12.4 ±0.25°C) and at the pool on the university campus (13.3 ±0.24°C) were not found to be significantly different. The upper dam was the least extreme site with water, canopy and air temperatures only dropping as low as 7.0°C, 0.5°C and 2.5°C respectively.

Water temperature in the campus pool dropped to 6.5°C, with canopy and air temperature dropping as low as 0°C and 1°C respectively. These profiles fluctuated more extensively due to the relatively shallow water depth, translating into higher mean water temperature making the campus pool the warmest of the sites (Figure 2.6).

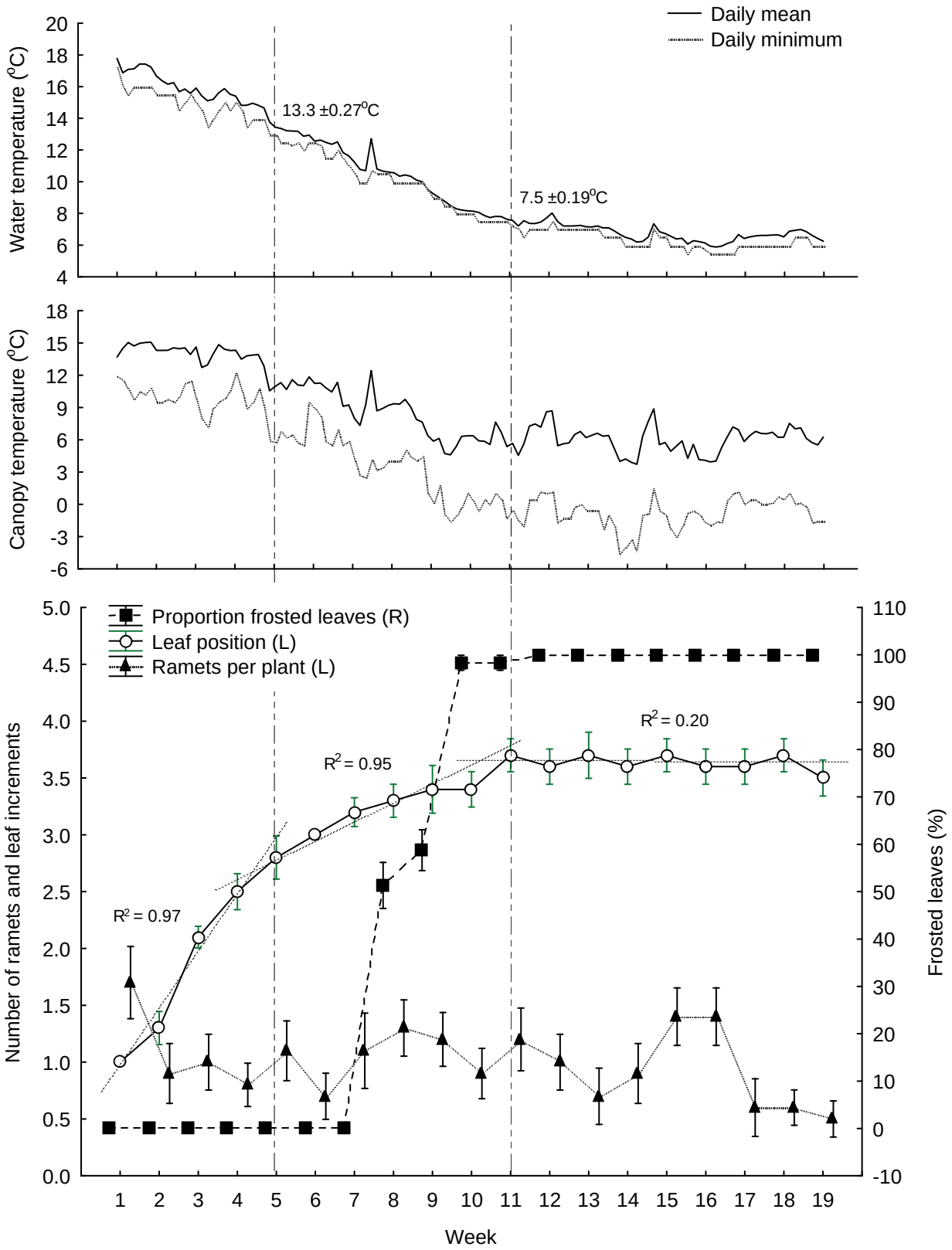


Figure 2.4: Water hyacinth growth and ramet production (Mean of 10 plants ±SE) at Delta Park lower dam, in response to frost and daily mean and minimum water and plant canopy temperature. Vertical lines demarcate distinct rates of leaf production.

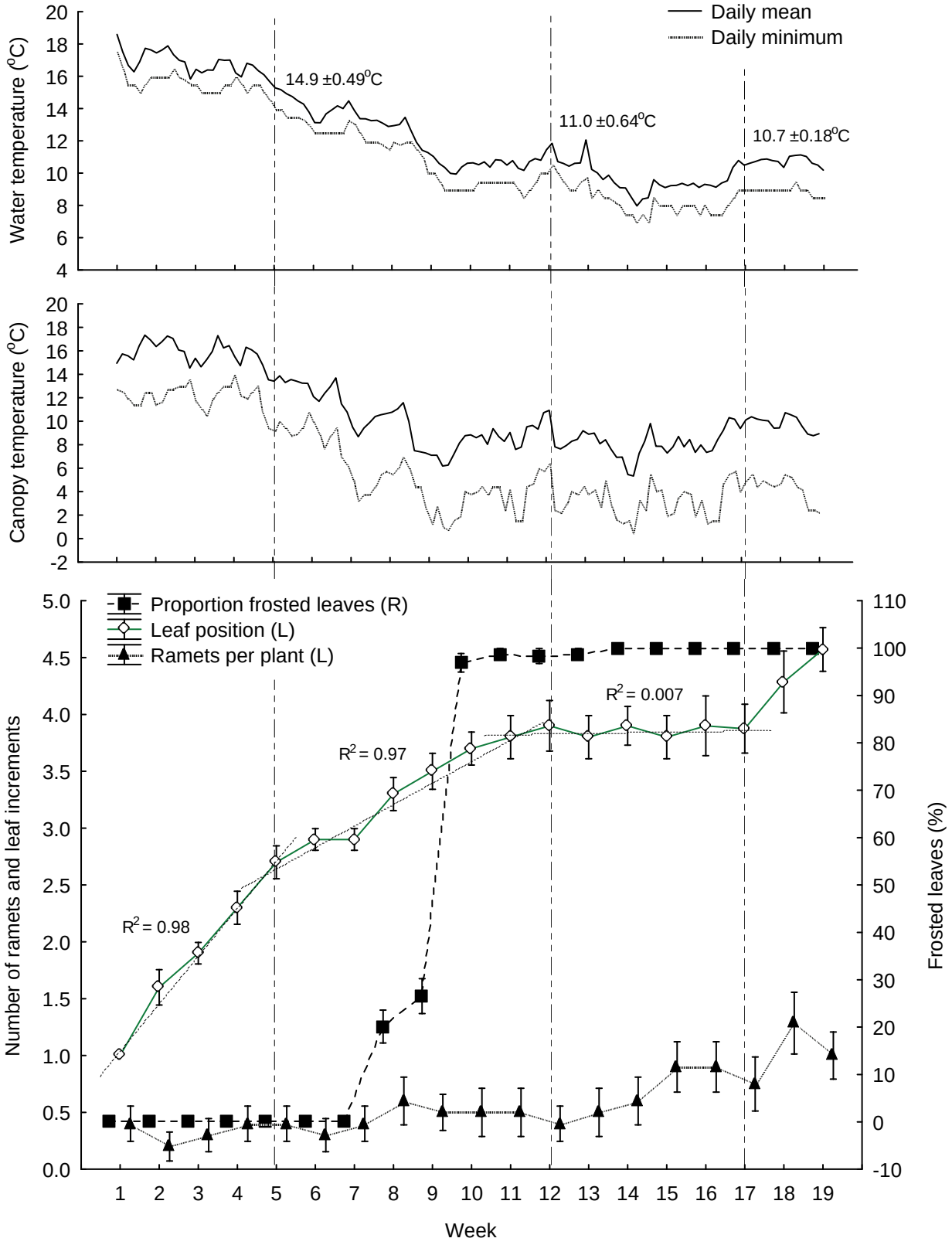


Figure 2.5: Water hyacinth growth and ramet production (Mean of 10 plants ±SE) at Delta Park upper dam, in response to frost and daily mean and minimum water and plant canopy temperature. Vertical lines demarcate distinct rates of leaf production.

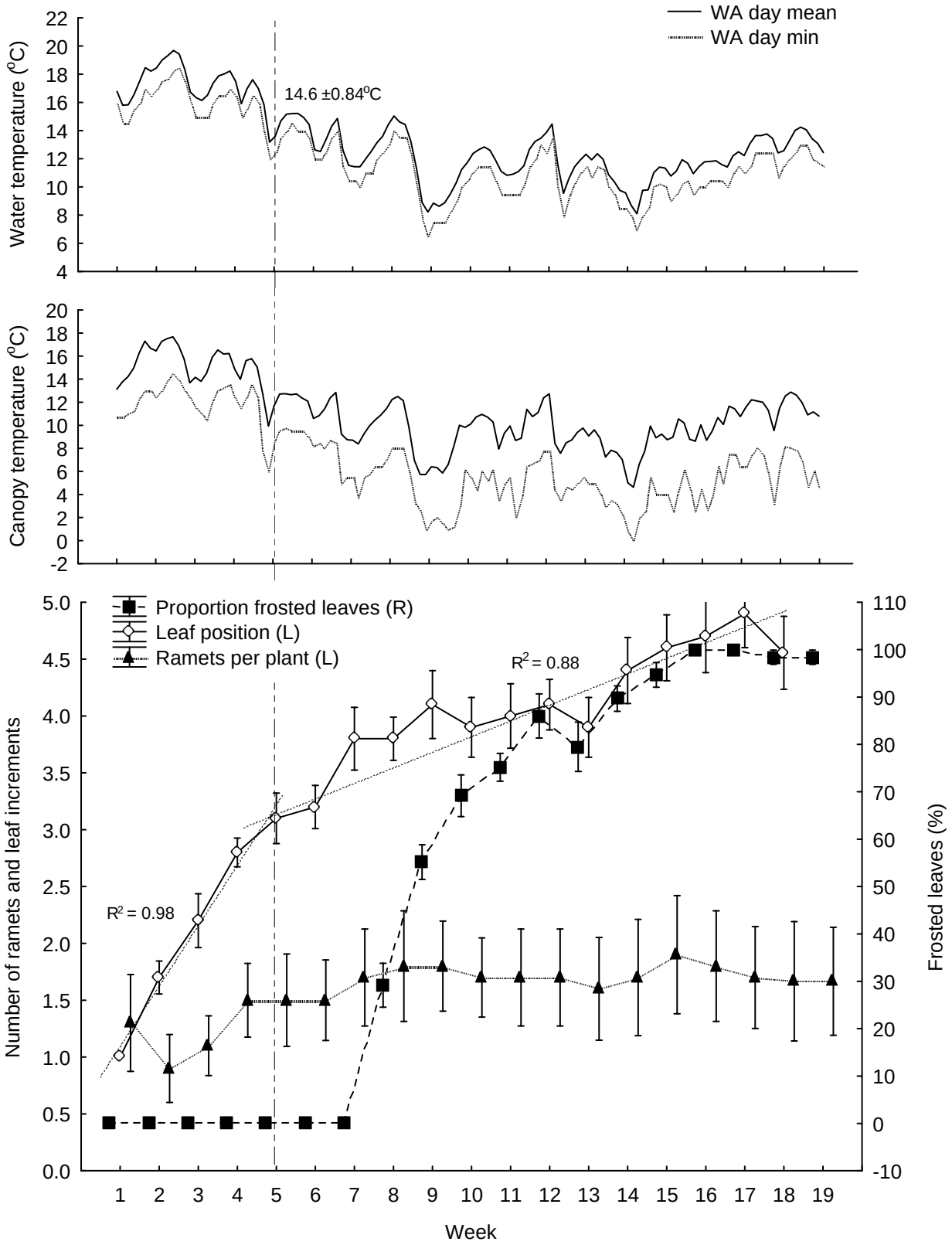


Figure 2.6: Water hyacinth growth and ramet production (Mean of 10 plants ±SE) on a 4.9 m^2 plastic lined wire mesh pool at the University of the Witwatersrand campus in response to frost and daily mean and minimum water and plant canopy temperature. Vertical lines demarcate distinct rates of leaf production.

Plotting the average position of tagged leaves per week allowed distinct periods or rates of leaf production to be noted at each site (Indicated by vertical lines, Figures 2.4-6). Regression lines were fitted to each of these distinct sections allowing the rates of leaf production to be estimated from the line equations. Transition temperatures between these periods were calculated as the mean of the subsequent week's water temperature. Three distinct growth periods were observed at the lower dam (Figure 2.4), the most rapid of which, between weeks one and five, occurred at mean water temperatures above 13.3°C with 0.48 leaves produced per week. The second growth period was observed between weeks five and 11 where lower water temperatures ranging between 13.3°C and 7.5°C resulted in a lower leaf production rate of 0.13 leaves per week. No leaves were produced once water temperatures dropped below 7.5°C after week 11.

At the upper dam (Figure 2.5), four distinct growth periods were observed. Plants in water warmer than 14.9°C produced 0.41 leaves per week. As at the lower dam, leaf production at the upper dam slowed as water temperature dropped, producing only 0.19 leaves per week between weeks five and 12 at temperatures ranging between 14.9°C and 11.0°C. No leaves were produced as temperatures continued to decline between weeks 12 and 17, but once water temperature rose above 10.7°C after week 17, new leaves were observed.

The campus pool loosely followed the trends evident at the Delta Park dams. A leaf production rate of 0.53 leaves per week was recorded during the first distinct growth period lasting till week five above water temperatures of 14.6°C. Plant growth did not cease at any point at the campus pool but persisted through winter producing 0.12 leaves per week. Water temperatures were far more variable owing to the relatively small volume of water in the pool. From week five till the end of the experiment water temperatures varied around a mean of 13.9°C, with the coldest weeks, nine and 14, only dropping to a mean of 11.5°C.

Water hyacinth growth was more strongly correlated to water temperature ($r = 0.68$) than to canopy temperature ($r = 0.24$). A positive correlation was found between the rate of leaf production from all sites with increasing water temperature (Figure 2.7). Assuming a linear relationship allowed for the fitting of a standard curve ($y = -0.2836 + 0.0411x$) to describe leaf production in response to water temperature. This line, however, indicates a threshold temperature for plant growth of 6.9°C, 3°C lower than expected from the literature and own observations.

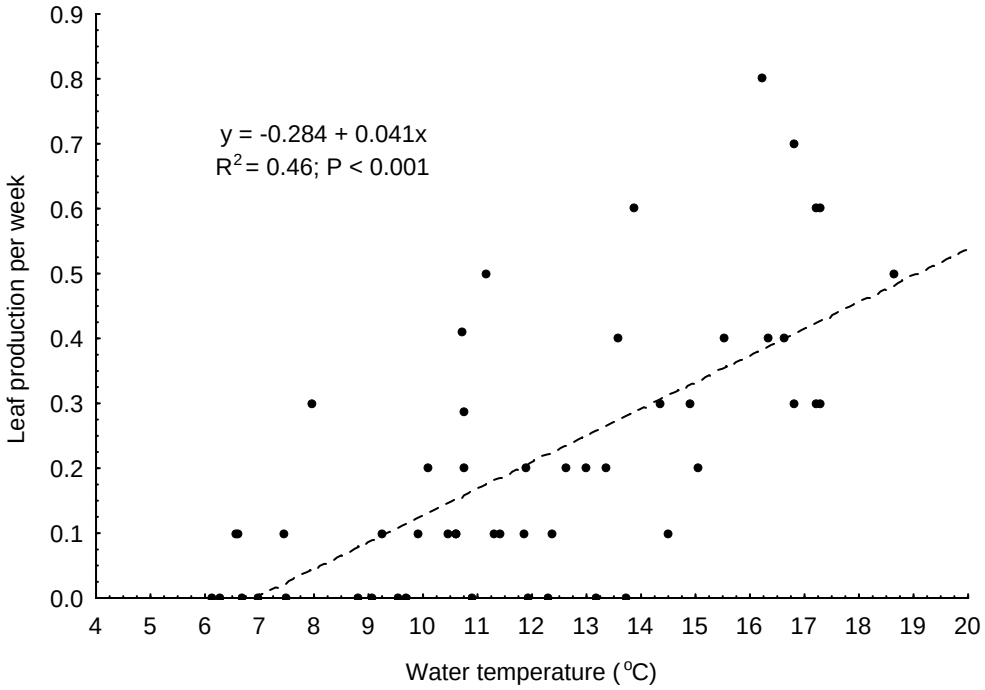


Figure 2.7: Rate of water hyacinth leaf production in response to water temperature.

Higher temperatures at the campus pool produced consistently high numbers of ramets per plant relative to the other sites. Ramet production continued throughout winter at all sites, picking up slightly after week 13 in conjunction with slow rises of mean canopy and water temperature. The numbers of ramets produced per plant did not correlate significantly with water or canopy temperature at any site. However plant density and frost-induced leaf mortality appeared to have a stronger influence on ramet production than temperature. This remains to be tested. The number of ramets produced per plant at the upper dam was lower than that seen at the other two sites ($F_{2, 507}$=50.30, p<0.001), which had significantly longer petiole lengths ($F_{2, 237}$ = 39.16, p< 0.001) up until the time of first frost.

Frost-damaged leaves were first recorded after week seven at all sites. Canopy temperatures dropped as low as -3°C with nightly lows of less than 0°C every night between weeks seven and eight at the lower dam. This initial cold spell resulted in half of the laminas per plant showing frost damage, the majority of which had between 50 and 100% of the lamina area damaged. Canopy temperatures were slightly higher between weeks eight and nine, only dropping as low as 0°C on three consecutive nights. This resulted in only a small increase of frost-damaged leaves. By the following week however, with only two nightly lows below 0°C between weeks nine and 10, 98% of laminas were damaged by frost and of those affected between 75 and 100% of the lamina area had browned and died.

Canopy temperatures were not as extreme at the upper dam as those recorded at the lower dam. This was most likely a consequence of the park's topography or the

temperature recording device being slightly buffered from extremes due to the longer petioles at the site. Nevertheless, frost damage was observed following daily lows of 3°C on three consecutive nights during week seven but with less than 5% of the lamina area being affected. By week nine, frost damage was only evident on 30% of the observed laminas but by week 10 this figure jumped to 97%. Between weeks nine and 10, six consecutive nights with lows of less than 3°C and an extreme low of 0.75°C resulted in between 75 and 100% of the lamina areas of almost all the leaves observed being killed. Frost damage was far less abrupt at the campus pool with daily canopy lows of between 1°C and 7°C from week seven to week 10 resulting in 25 to 75% of the lamina areas of 70% of observed leaves being damaged. Frost damage was evident on 100% of leaves observed by week 16 by which time between 50 and 100% of the affected lamina areas were dead.

Water temperature had little effect on asexual reproduction as most plants produced ramets throughout the coldest months of winter. Figures for all the sites are given in Chapter 5.

2.3.3 Insect Performance and Plant Productivity

Water temperatures in the control chamber, simulating moderate winter conditions, oscillated around a mean of 14.0°C with a daily fluctuation from 12.4°C to 15.5°C. Temperatures within the plant canopy were slightly buffered relative to air temperatures, having a daily range of 10.0°C to 17.7°C around a mean of 14.1°C. Air temperatures were more extreme having daily range of 9.3°C to 19.3°C around a mean of 14.6°C for the eight-week duration of the experiment. Temperatures within the treatment chamber simulating low winter conditions were significantly colder ($F_{2, 12270}$=13.66; p<0.001) than those in the control chamber. Daily water temperatures there fluctuated from 7.1°C to 12.2°C around a mean of 9.5°C. Air temperatures fluctuated daily between 2.8°C and 19.9°C with a mean of 10.9°C. Similar to the control chamber, canopy temperatures were slightly buffered from extremes having a warmer daily range of between 4.6°C and 18.3°C but with a mean identical to that of the air temperature profile.

2.3.3.1 Weevil Performance

Distinct differences were found in weevil performance between the treatment and control chambers, in terms of weevil reproduction and survival. No eggs were found on any sampled plants in the treatment chambers of either weevil species for the duration of the experiment. By contrast, one egg was found at week five in the *N. bruchi* control chamber and another two at week seven. More eggs were found on plants in the *N. eichhorniae* control chamber, with four being recorded at week six.

Higher levels of weevil mortality were evident at the colder temperatures (Figure 2.8). For *N. bruchi*, significantly fewer weevils survived the treatment chamber than the

warmer control chamber (mortality was 70% and 41% respectively {Chi-square = 20.66; d.f. = 1; $p< 0.001$}). Mortality was higher for *N. eichhorniae,* (86%) in the treatment chamber compared to only 50% in the control chamber (Chi-square = 35.36; d.f. = 1; $p< 0.001$).

The total amount of adult feeding differed significantly between temperature regimes (Figure 2.9). For both *N. bruchi* ($F_{1, 80}$=23.18; $p<0.001$) and *N. eichhorniae* ($F_{1, 80}$ = 30.86; $p < 0.001$), significantly more feeding scars were recorded in the control relative to the treatment chambers over the eight-week period. However, as the numbers of feeding scars recorded per week was a cumulative measure, the accumulation of scars per week relative to each temperature regime was also compared. Considering the interaction between temperature regime and week number, the overall accumulation of feeding scars per week between the control and treatment chambers was not significantly different for *N. bruchi* ($F_{7, 80}$ = 1.57; p= 0.16) but did differ significantly for *N. eichhorniae* ($F_{7, 80}$ = 2.98; p= 0.01) between chambers. Tukey post hoc testing showed that *N. bruchi* feeding scar accumulation per week did not differ significantly between chambers on any corresponding week although feeding in the control chamber during week eight was significantly higher than that in the treatment chamber during week seven. For *N. eichhorniae* on the other hand, post hoc testing showed a significantly higher weekly accumulation of feeding scars at week seven in the control compared with the corresponding week in the treatment chamber.

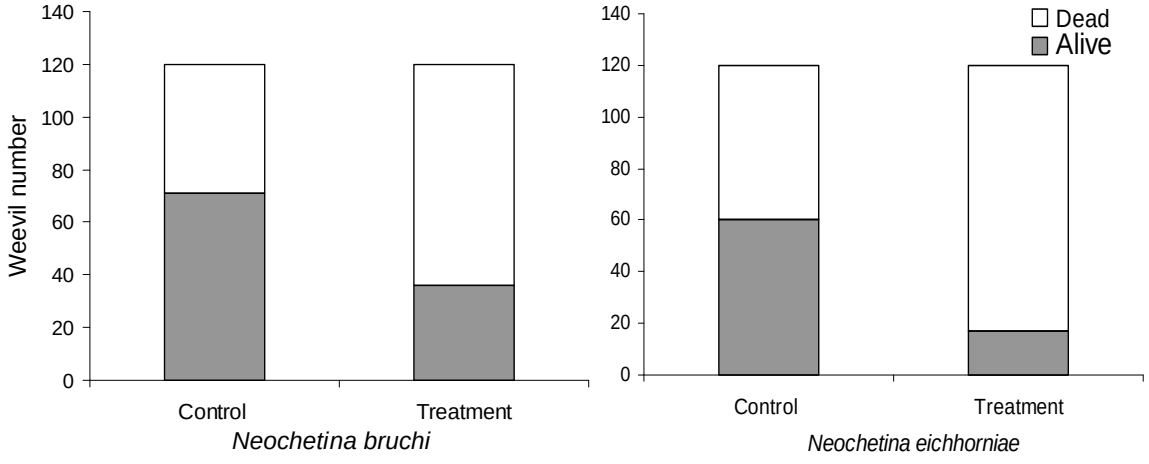


Figure 2.8: Relative mortality of *Neochetina bruchi* and *N. eichhorniae* in response to low (Treatment) and moderate (Control) winter temperature regimes.

2.3.3.2 Mirid Performance

Distinct differences were found in the levels of feeding damage recorded at each temperature regime (Z = 6.08; $p< 0.001$) (Figure 2.10). In the treatment chamber the extent of lamina area damaged by mirid feeding was highly skewed towards the lower categories with 59% of the observed leaves having none, or less than 5%, of their respective areas damaged. By contrast, leaves in the control chamber exhibited more

feeding damage, with 44% of the observed leaves having between 50 and 100% of their respective areas affected. These drastically higher levels of feeding damage were undoubtedly a consequence of the large nymphal population on the plants in the control chamber.

No nymphs were produced at air temperatures ranging between 3.2°C and 18.4°C around a mean of 10.3°C in the treatment chamber for the duration of the experiment. By contrast, a daily air temperature fluctuation of between 11.0°C and 22.5°C around a mean of 16.8°C in the control chamber, produced small numbers of nymphs by week 5 (Figure 2.11). At these temperatures, eggs presumably laid at week one took four weeks to hatch after which the population of nymphs grew roughly exponentially until the experiment was terminated.

Adult population numbers declined steadily in both of the temperature regimes over the duration of the experiment. However, significantly more individuals were recorded per week from the control chamber ($F_{1, 28} = 11.93$; $p < 0.001$) indicating that adult mortality was significantly higher at colder temperatures.

2.3.4 Plant Productivity

Plant growth and quality differed between the two test temperature regimes in both the weevil and mirid experiments. During the mirid trial, significantly more ramets ($F_{1, 28} = 16.00$; $p < 0.001$) and leaves ($F_{1, 28} = 40.50$; $p < 0.001$) were produced per plant in the control chamber despite significantly higher levels of mirid feeding damage. This trend was mirrored during the weevil trial although it was somewhat accentuated by very low temperatures during the first two weeks of the experiment. Problems with the treatment chamber for this initial period caused water temperatures to drop as low as 3.8°C with a daily mean and maximum of 6.0°C and 8.8°C respectively. Canopy temperatures fluctuated around a mean of 6.4°C, with a daily range of between 1.4°C and 13.6°C. Canopy extremes were slightly buffered relative to air temperatures which ranged between 0.6°C and 17.2°C around a daily mean of 6.7°C. These lower temperatures were analogous to minor frosting resulting in the browning of between 0 and 10% of leaf areas but did not cause any significant rise in leaf senescence or any plant mortality.

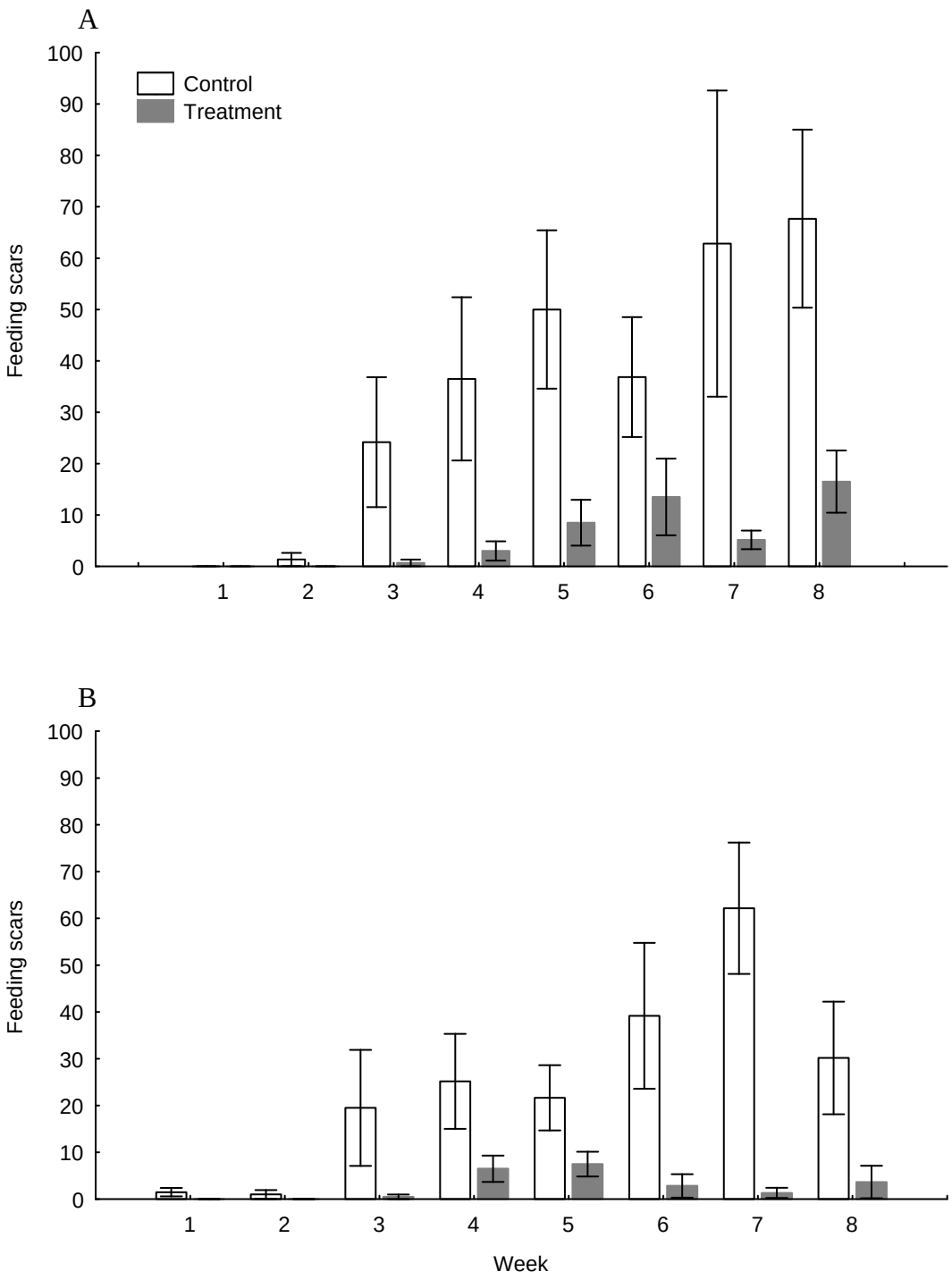


Figure 2.9: The number of adult feeding scars (Mean of the three youngest leaves per plant ±SE) in response to low (Treatment) and moderate (Control) winter temperature regimes for A) *Neochetina bruchi* and B) *N.eichhorniae*.

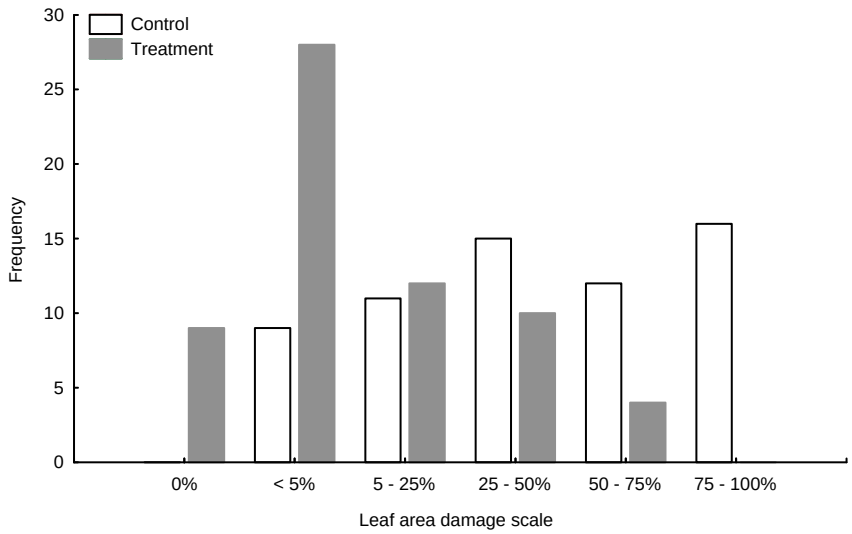


Figure 2.10: Categorised *Eccritotarsus catarinensis* feeding damage over an eight week period in response to low (Treatment) and moderate (Control) winter temperature regimes.

Over the duration of the experiment (Figure 2.12), the number of leaves per plant was significantly lower in the treatment chamber ($F_{1, 48} = 55.34$; $p < 0.001$) as was the number of ramets produced ($F_{1, 48} = 8.44$; $p = 0.01$). Plant quality within the treatment chamber declined steadily throughout the experiment leading to hardening of lamina and petiole tissue, a decrease in effective leaf area due to browning of the lamina margins, and 40% mortality of plants not sampled. No plant mortality was recorded in the control chamber and plants remained healthy and actively growing throughout the experiment.

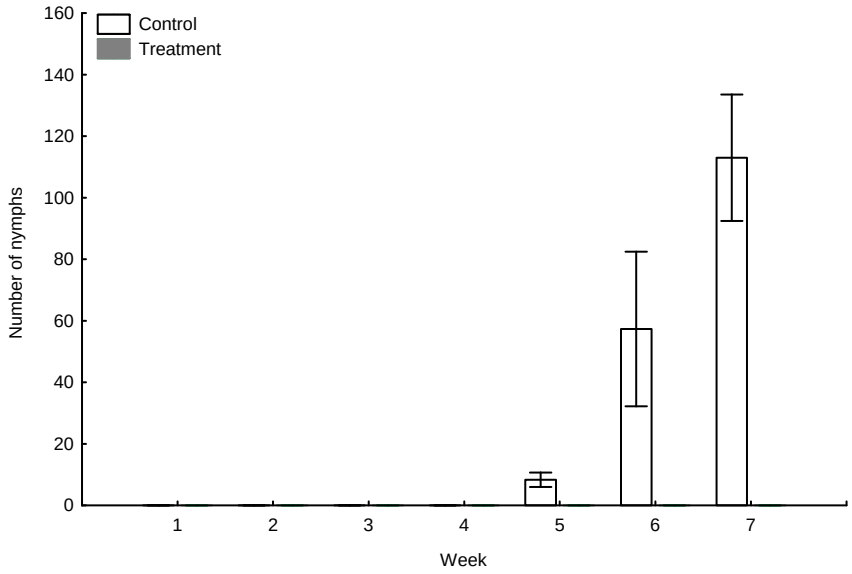


Figure 2.11: *Eccritotarsus catarinensis* weekly reproduction (Mean of three plants ±SE) for a seven week period in response to low (Treatment) and moderate (Control) winter temperature regimes.

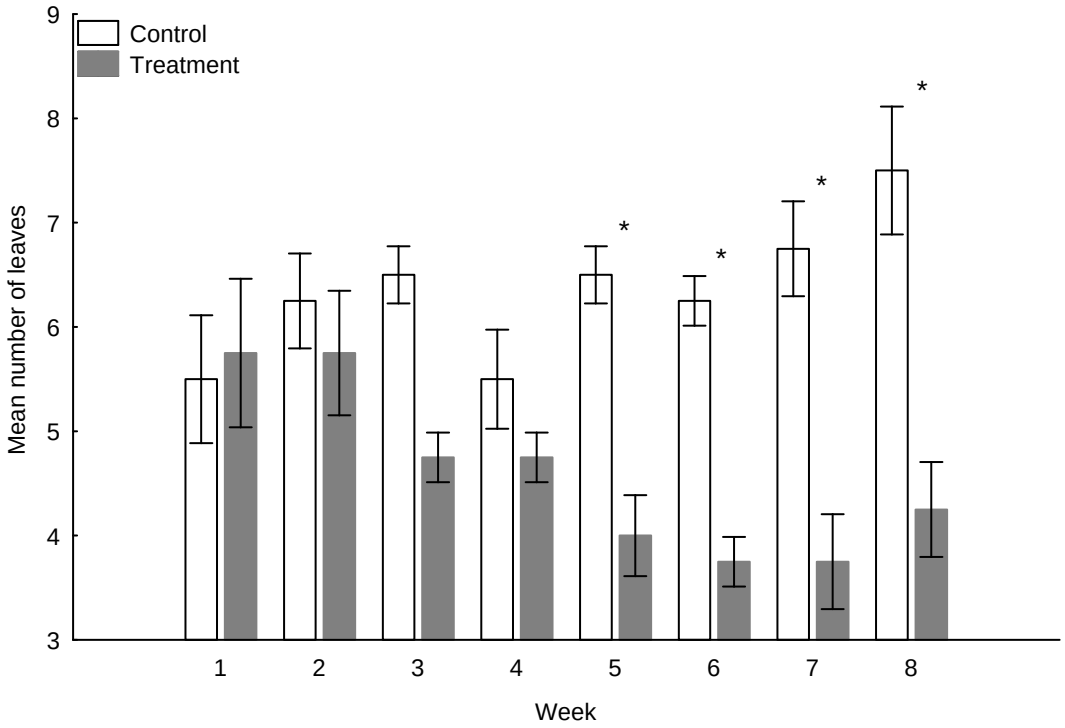


Figure 2.12: Number of leaves per water hyacinth plant (mean of three plants ±SE) in response to an eight-week exposure to low (Treatment) and moderate (Control) winter temperature regimes. Weeks with stars denote significant differences between the treatment and the control at $p < 0.05$ (ANOVA, Tukey HSD test).

2.4 Discussion

This study indicates that, as expected, temperature influences insects' performance on water hyacinth – defined in terms of feeding rates, mortality and reproduction – and suggests that their efficacy as biological control agents will be limited at low average temperatures. It also showed that water hyacinth growth rate and plant quality diminished with decreasing temperature, but was less affected in terms of plant mortality (which was not seen) and leaf recruitment, which stopped when the canopy temperature dropped below 10°C. The relative consequences at a population level were therefore less severe for the weed when compared to the insects because individuals appear not to be removed from the plant population during winter, indicating that water hyacinth has a greater tolerance of lower temperatures. This mismatch of tolerances increases the likelihood of a lack of synchrony between water hyacinth and its natural enemies in areas where temperature is low, as the weed can recover from a large number of overwintering individuals.

Goolsby et al. (2005) stress that biological control needs to make the transition from a purely empirical method to a predictive science. Predictions of agent efficacy prior to screening and release into a new country would validate the initial investment in that agent, and if predicted not to establish or be effective, could save agencies both time and money. For example, Bownes (2009) has shown that the water hyacinth biocontrol agent *Cornops aquaticum*, which has been in quarantine in South Africa since 1995,

will not feed at temperatures below 10°C, reducing its potential value as a control agent in high-lying areas and suggesting that no further development of this agent should take place. With increasing emphasis placed on integrated approaches to managing invasive plants, predicting an already established agent's efficacy with reference to its locality and seasonality will be valuable for timing other management interventions such as herbicide sprays.

The natural enemies on water hyacinth are assumed to be hampered by lower average temperatures, in terms of both their persistence and efficacy, especially in areas that are heavily frosted (Hill and Olckers, 2001). Taking the insects' thermal physiology into account, summer conditions throughout the country can be held not to be limiting on development or reproduction. For this reason, it is important to determine how winter affects the insects directly and, possibly more importantly, how well they are able to recover from these adverse conditions relative to water hyacinth. Differing tolerances towards temperature extremes could lead to asynchrony of the plant and insect population growth rates emerging from winter. Low insect feeding rates or delayed population increase following winter will severely limit the level of control achieved, the effects of which could last well into the more favourable summer months. In order to test these assumptions, models describing water hyacinth, *Neochetina*, and *E. catarinensis* population growth emerging from adverse winter conditions and based on accumulating degree-days were constructed utilising the above reported experimentally derived thresholds.

2.4.1 Phenological Model and Evaluation of Synchrony

To produce an accurate phenological model, appropriate boundaries must be established which will act as starting points after which degree-days can be accumulated (Herms, 2004). These boundaries or biofix points are usually based simply on a calendar date (Fidanza et al., 1996; Satake et al., 2006) derived on past observation of some sort of biologically relevant event such as planting dates, first trap catch or first occurrence of a pest (Zalom et al., 1983). Establishing a biofix point in a relatively unknown 'natural' system is more difficult than within the more controlled agricultural systems and accordingly, must therefore be based on some physiological threshold that is limiting to the organism involved. In this way, biofix points will be variable from year to year in terms of calendar date, but will be tailored to the specific conditions prevalent in a particular system at the time, allowing for more accurate predictions.

Measurement of the effects of low temperature and frost demonstrated that water hyacinth growth, at least in terms of leaf production, is largely determined by water temperature. Extrapolation of the regression line in Figure 2.7 suggests that leaf production ceases at a water temperature of 6.9°C. However, this temperature threshold seems unrealistically low given that plants within the controlled environmental chamber, maintained at a mean water temperature of 9.5°C, stopped leaf production and

showed increased rates of leaf senescence (Figure 2.12). This resulted in a steady decrease in the number of leaves per plant during the eight-week period. At the Delta Park sites leaf production ceased at mean daily water temperatures of 7.5°C at the lower dam and 11.0°C at the upper dam. By week 18, however, new leaves were recorded at the upper dam at water temperatures around a mean of 10.7°C. Plant growth can therefore be assumed to cease at water temperatures between 9.5°C and 10.7°C, which is consistent with Gopal's prediction of a developmental zero for water hyacinth of 10°C (Gopal, 1987). This is further justified by the fact that plant growth did not cease at the campus pool with mean daily water temperatures never dropping below 11.5°C (Figure 2.6). Similarly, plants maintained at a water temperature of 14°C remained actively growing for the duration of the experiment. Therefore, plants at the Delta Park sites are assumed to remain dormant throughout winter until a biofix point or onset of more favourable conditions in the form of rising mean water temperature is reached.

Water and canopy temperature at the lower dam was monitored through spring and into summer, providing a basis against which plant and insect recovery after winter could be modelled (Figures 2.13 and 2.14). Biofix points for the start of growth were set as the final day of the first consecutive seven days with a mean temperature above the relevant developmental threshold occurring after the coldest week in winter. Daily leaf production rate was modelled using the regression line equation derived from Figure 2.7, from mean daily water temperatures above a threshold of 10.1°C.

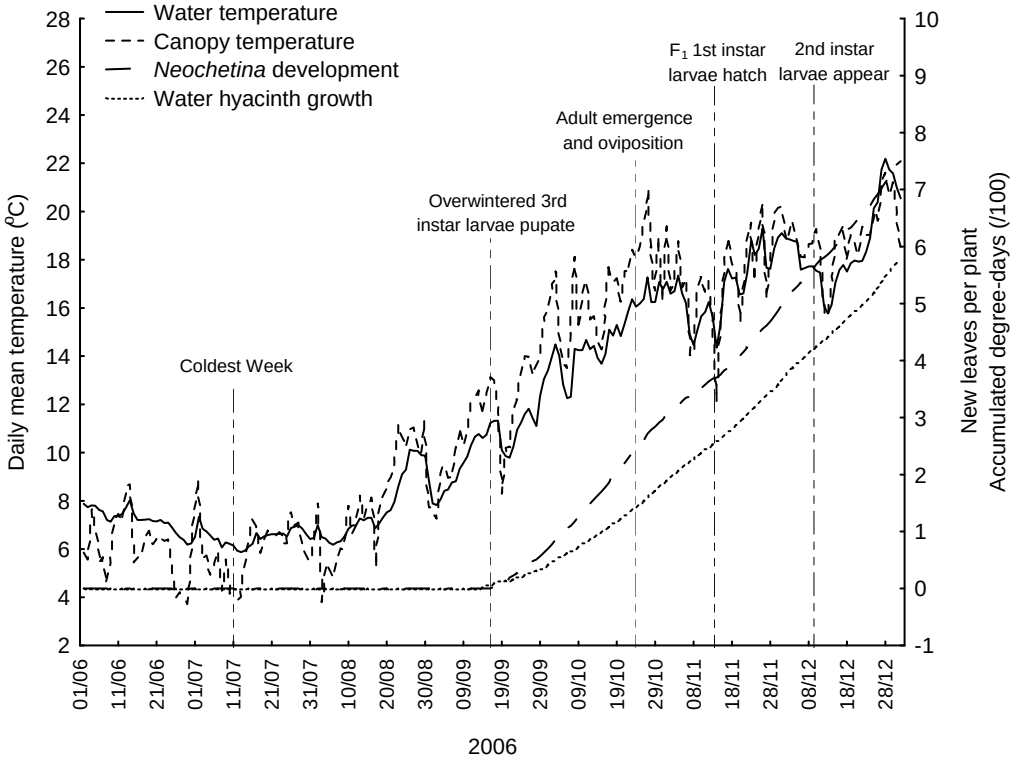


Figure 2.13: Predicted water hyacinth and *Neochetina* recovery following low temperatures during a highveld winter. Leaf production rate and *Neochetina* accumulation of degree-days during pupation are in response to water temperature. Subsequent *Neochetina* development is in response to plant canopy temperature.

From Figures 2.13 and 2.14, plant growth is predicted to commence on the 13th of September, producing new leaves at a rate that rises sharply in conjunction with rising water temperature to a rate of 0.56 new leaves per week by the end of December. Center (1980) found that leaf production rates in Florida summers could reach as high as 0.7 new leaves per week.

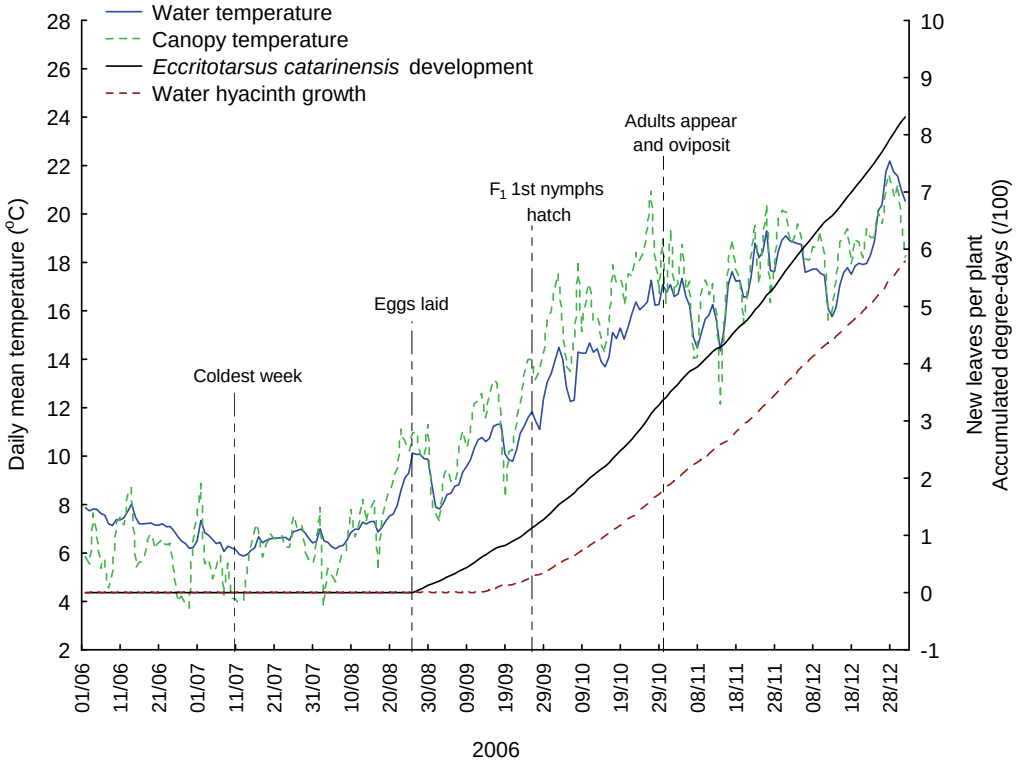


Figure 2.14: Predicted water hyacinth and *Eccritotarsus catarinensis* recovery following adverse conditions during a highveld winter. Leaf production rates are in response to water temperature. *Eccritotarsus catarinensis* accumulation of degree-days is in response to plant canopy temperature.

Water temperature can also be used to determine the biofix point for the onset of *N. bruchi* and *N. eichhorniae* recovery following winter. However, given the limited developmental dataset for both weevils, the fact that their immature stages are indistinguishable from one another, and the difficulty in estimating the relative proportion that each species contributes to a particular site's weevil population, it is necessary to consider both species collectively so as to maximise the applicability of the model.

Insects occupying the same or very similar ecological niches can be assumed to share a variety of similar adaptations to temperature (Ikemoto, 2003). Consequently, insect strains or closely related species are expected to have correspondingly similar developmental thresholds and durations of development. Ikemoto (2003) reports on a number of studies that found that K decreases with increasing *t* among related species mainly at order level but even at higher taxonomic categories. He shows that within a number of insect families and genera, at least mathematically, a common *t* and K does

in fact exist. He proposes that this threshold temperature and duration of development represent a physiological optimum that a group of closely related organisms possesses. *Neochetina bruchi* and *N. eichhorniae* are very closely related with similar physiology and behaviour, and occupy almost identical niches. Evidence of this can be seen from the rates of development of each life stage (Figure 2.1).

Combining the development times for both *Neochetina* species yielded a developmental threshold of 9.62°C with a thermal constant of 984.36°D which was consistent with estimates made for other tropical weevil species that live on aquatic plants. Mazzei et al. (1999) found that the milfoil weevil *Euhrychiopsis lecontei* required 309°D above a threshold temperature of 9.8°C to complete the development of its immature stages. Similarly, McConnachie (2004) found that the weevil *Stenopelmus rufinasus* released against *Azolla filoculoides* required 256°D above a threshold temperature of 9.2°C to complete its development. As all of the species were tropical in origin, developmental thresholds were expected to be roughly similar, but as *N. bruchi* and *N. eichhorniae* are larger in size than these species, the thermal constants are likely to be correspondingly larger (Chown and Nicolson, 2004).

It was evident from the evaluation of weevil performance that lower average temperatures promote high levels of mortality, up to 70% in as little as eight weeks. It must therefore be assumed that numbers of adult weevils able to survive through winter until conditions are more favourable will be very small and as such, their contribution to a recovering population through egg production will be negligible. Additionally, from the assessment of the effects of frost at the lower dam, plant damage as a result of heavy frosting is both severe and rapid. Of all observed plants, between 75 and 100% of the lamina area had browned and died in as little as three weeks. *Neochetina* eggs laid prior to these initial frost events, and normally found at the bases of laminas (DeLoach and Cordo, 1976), will almost certainly be killed, as well as the majority of both first and second instar larvae occupying the upper portions of petioles. Due to the progressive browning of petioles from lamina to crown observed during winter, only third instar larvae, normally occupying the crown (DeLoach and Cordo, 1976) are predicted to successfully overwinter in any meaningful numbers, and able to contribute to a post winter weevil population.

Setting a biofix point in the same way as for water hyacinth, overwintering third instar larvae are assumed to pupate after the coldest winter week, following the first consecutive seven days with a mean water temperature above the larval developmental threshold of 10.8°C. Water temperature rather than canopy temperature is used due to its close proximity to the plant crown, and thus the larvae. Therefore, larvae are estimated to pupate around the 16th of September at which point mean water temperature will be well above the pupal developmental threshold of 6.7°C (Figure 2.13). Note that before the weevils have accumulated 750°D of their required 984°D,

the water hyacinth plants will have added over seven new leaves. Degree-hours were calculated from hourly measures of water temperature for pupation and canopy temperatures were used for egg maturation and larval development, and these were averaged over 24 hours to determine the number of degree-days accumulating per day. The accumulation of degree-days utilising life history stage-specific developmental thresholds was then used to predict the rest of the weevils' post-winter phenology. Adult eclosion is predicted on the 24th of October, 1st instar larval hatch on the 13th of November and 2nd instar development 26 days later on the 9th of December. The first new generation of weevils will emerge almost three months after the plants have started to grow.

With daily mean canopy temperatures at the time of adult emergence ranging between 17°C and 21°C, adults are not limited by temperature, and are assumed to oviposit immediately. The *Neochetina* oviposition threshold was estimated from the environmental chamber experimentation to lie between 10.9°C and 14.1°C. As both weevil species are nocturnal and mean daily maximums in both the control and treatment chambers were similar, ranging between 17.7°C and 19.9°C, oviposition is most likely restricted by mean daily minimum temperatures. Furthermore, as eggs are primarily laid in the uppermost part of the petiole below the base of the lamina, the temperature to which weevils will be exposed to whilst laying can be assumed to be a combination of both canopy and air temperature. In this way, the threshold temperature for oviposition can be assumed to lie between 4.6°C and 10.0°C. However, it is unclear to what extent the weevils (or plants) are able to store thermal energy and thus the oviposition threshold may lie between 10.9°C and 14.1°C, determined from the daily mean canopy temperatures to which the weevils will primarily be exposed. This estimate is close to that suggested by DeLoach and Cordo (1976) of 10°C, and is further justification for not utilising the linear intercept method proposed by Cambell et al. (1974) when determining the thermal physiology of the weevils.

The model therefore predicts a lag period of roughly 42 days between the onset of water hyacinth growth and the time at which the plant will be subjected to adult weevil herbivory from overwintering larvae emerging as adults. Feeding damage at this stage will be restricted to the leaves with a consequently negligible impact because of limited weevil numbers and increasing leaf production rates. Realistically then, as the larval instars are the most damaging stage (DeLoach and Cordo, 1976), the weevils may only begin to significantly impact the weed population when the first instar F_1 generation hatches around 62 days later. Wilson, (2002) maintains that the late larval instars are the most damaging with 3rd instar larvae having the highest consumption rates. Accordingly, this lag period could extend into early January of the following year when 3rd instar larvae are predicted to appear.

As degree-days accumulate during weevil development at similar rates to leaf production, and the recovery of both weevil and plant populations commences at roughly the same time after winter, differential responses to temperature cannot be the sole factor promoting this lag period. Rather, it is the susceptibility of each *Neochetina* life stage to mortality brought on by low temperature and the consequences of frost for the plant that promotes this mismatch between plant and weevil phenology. Low temperature will hamper oviposition and cause overwintering mortality of all *Neochetina* life stages which will severely limit the number of individuals able to contribute to a post-winter population. Additionally, frost-induced leaf senescence will kill both eggs and early larval instars but does not lead to any significant plant mortality, leaving only a reduced 3rd instar population able to overwinter. Therefore, the need for an early season period for pupation, prolonged by slow development due to low temperature, allows the weed population to recover from winter largely unmolested by the weevils.

In addition to this lag period, the potential for population growth of *Neochetina* relative to water hyacinth must also be considered to explain the poor efficacy of the weevils in colder areas. The intrinsic rate of natural increase (r_m) has been identified as a key demographic parameter describing the population growth potential of an organism relative to particular environmental conditions (Pilkington and Hoddle, 2007). Roy et al. (2003) maintain that the outcome of predator-prey interactions will often depend on the relative r_m of the organisms involved. They showed that *Stethorus punctillum*, a coccinnellid predator of the mite *Tetranychus mcdanieli*, had a narrower range of favourable temperature for survival, reproduction and development relative to its prey. The authors conclude that because of this *S. punctillum* is unlikely to provide consistent control of the mite.

In contrast to the relatively slow population expansion of weevils (*K* selected), water hyacinth (*r* selected) has a far higher intrinsic rate of increase. K selected species are generally more suited to stable environments and, due to relatively slow reproductive rates and longer generation times, are far slower to recover from adverse conditions and are therefore unable to attain large population sizes in unfavourable and seasonal environments. Harris (1991) suggests that control is more likely to be achieved with biological control agents that attack their hosts early in a plant's lifecycle. Targeting water hyacinth early in its growth season, during post winter recovery, would have a far greater impact given the plant's slow growth rate. For this reason *r* selected natural enemies, better able to cope with more stochastic environments, should overcome this lag period, whereas K selected species require long periods of stability, which don't occur in water hyacinth systems which are either flooded out or deliberately cleared. Faster reproductive rates and shorter generation times facilitate rapid population increase, leading to more feeding damage more quickly. Center (1980) touched on this, observing that the moth *Niphograpta albiguttalis* (formerly *Sameodes*), which behaves

more like an *r* strategist, is responsible for a different type of age specific leaf mortality as compared to that initiated by *N. eichhorniae*.

Eccritotarsus catarinensis is an *r* strategist, having a short generation time (Coetzee, 2007a) and rapid reproductive rate as demonstrated in the laboratory evaluation (Figure 2.11). Accumulating degree-days for the mirid against the same temperature dataset used in Figure 2.13, it is evident that this different life history strategy may offer better control of water hyacinth at the lower Delta Park dam. A biofix point was chosen in the same way as for the plant and weevils although the canopy temperature profile and a developmental threshold of 10.3°C were used. As mean daily temperature within the water hyacinth canopy picked up far sooner than in the water temperature profile, this point occurred on the 26th of August, 19 days prior to the onset of plant growth which is dictated by cooler water temperatures (Figure 2.14). Mirid eggs laid at this time are predicted to hatch after a further 33 days and a full generation can be completed in as few as 66 days, around the 31st of October. Adult mirids are the most likely stage to successfully overwinter due to their high motility relative to the flightless nymphal instars. Consequently, nymphal mortality during winter is presumed to be high and their contribution to a recovering post winter population negligible.

Nevertheless, a successfully overwintering mirid population cannot be guaranteed. Although adult mortality was significantly lower in the warm control chamber relative to the cooler treatment chamber, high levels of mortality – up to 75% – were observed in both chambers by the end of the experiment. This suggests an adult life span of approximately seven weeks and is consistent with the estimate by Hill et al. (1999) of approximately 50 days, and suggests that a short adult life span, rather than temperature, was responsible for the observed mortality. Additionally, no reproduction was recorded at mean daily air temperatures of 10.3°C in the colder chamber for the duration of the seven-week period. Given that daily canopy temperature at the lower dam fluctuated around a mean of 8.4°C for 15 weeks prior to the biofix point, thus precluding any reproduction, and with no recruitment during winter, this short lifespan means that the mirid population will die out each winter. Indeed, repeated attempts to establish mirids and get them to survive through the winter at the lower dam site in Delta Park have failed, despite starting with viable populations on three consecutive summers (Coetzee, 2003).

In this way, these models can be seen as purely theoretical and will constitute a best case scenario for each organism emerging from adverse thermal conditions. A variety of factors that might shorten or prolong different stages of the models are omitted. Initial biofix points, as well as points of transition between growth rates or insect life stages, can only be estimated. For instance, following pupation it is assumed that emerging weevils are able to lay eggs immediately and thus don't require a maturation or mating period prior to oviposition. Increased survival of adult weevils and early larval instars

through winter could lead to larger population numbers and inclusion of more generations than predicted.

Up to now, both weevils have largely been considered together, but different proportions of each species at a site could influence the relative impact of this lag period on the plant. Although the difference was not significant, *N. eichhorniae* was able to lay more eggs in colder conditions relative to *N. bruchi* which may contribute to a larger, more damaging post-winter first instar population. This refutes DeLoach and Cordo's findings that *N. bruchi* laid more eggs at all their test temperatures relative to *N. eichhorniae* (DeLoach and Cordo, 1976). The environmental chamber evaluation of weevil performance also tentatively suggests that *N. bruchi* is better able to tolerate colder conditions in terms of adult survival and feeding. More feeding scars recorded in the warmer chamber could reflect the fact that mortality in the colder chamber was significantly higher translating into fewer feeding individuals. Differences in weekly accumulations of feeding scars only by week seven and eight provides further evidence of this. Feeding rates may be slower as a consequence of colder temperatures but the data suggest that this difference is not significant for either species, but rather temperature-dependent mortality is the most likely cause of insignificant levels of feeding and consequently diminished levels of control at colder sites.

Frost severity during winter is another compounding variable which will affect the realised impact of insect natural enemies on water hyacinth populations. Whilst water temperature dictates the rate of plant growth, frost damage is most often linked to plant and leaf mortality (Wilson, 2002). In this way, the severity of frosting has the potential to influence water hyacinth population density and thus any density-dependent plant processes as well. From the assessment of the effects of frost, one such example appears to be the production of ramets which continued throughout winter at all three sites.

Center and Spencer (1981) found that the vegetative regrowth of a water hyacinth population following defoliation by winter freezes in north-central Florida was characterised by three distinct phenological phases. The first was characterised by a reorganising of the distribution of biomass between the roots and the aerial parts. The second saw an increase in plant density in response to available interplant space and the third was characterised by an increase in plant size due to competition for light. Many plants respond morphologically to decreases in light, as a result of competition, in order to increase their potential to intercept light. These responses include elongation of stems and petioles, as well as increases in leaf area or resource portioning to leaves (Méthy et al., 1990), or the modification of photosynthetic rates (Méthy and Roy, 1993). In a study on the effects of light quality on the morphology and growth of water hyacinth, Méthy and Roy (1993) found that fewer ramets were produced under reduced levels of far-red radiation. In short, conditions simulating low light availability within a water hyacinth canopy do not stimulate the production of new ramets. Further evidence from Richards

(1982), showed that axillary buds of plants inside a water hyacinth mat rarely produced stolons whereas plants on the mat edge, characterised by shorter inflated petioles, produced numerous stolons. Thus, it can be assumed that increased light availability due to high levels of leaf senescence brought on by severe frosting (rather than low temperatures) stimulates the production of new ramets.

2.5 Conclusion

Although both water hyacinth and its natural enemies are negatively affected by lower average temperatures, the relative consequences for each at a population level are quite different. Similar thresholds of development mean that periods available for growth will be roughly the same for both the plant and insects in areas where winters are limiting. However, the reduced ability of control agents to overwinter successfully appears to the primary cause for limited control at colder sites. Reduced recruitment as well as high susceptibility to cold and frost induce mortality of all insect life history stages, which pushes their populations through a bottleneck each winter, or causes local extinctions. Surviving post-winter insect populations are therefore small, slow to recover in the case of the weevils, and consequently, the impact on recovering plants is negligible. Despite frost damage, plant populations lose few individuals during winter, while ramet production continues or even increases, setting up a new generation virtually free of weevils. Free from early season herbivory, water hyacinth populations are able to recover quickly and outpace the detrimental effects caused by insect feeding well into the new growth season. However, it should be noted that warm sites (such as Mkhadzi Spruit in the KNP) also suffer from poor levels of water hyacinth biocontrol.

It is therefore paramount to accurately predict the seasonality of both water hyacinth and its natural enemies to assure maximum success of any augmentative management interventions. Undoubtedly, this will be most important in regions where winters are limiting. In these areas, modelling will provide a means of predicting at least the first distinct, and most vital – according to Harris (1991) – post winter early season cohorts of both plant and insect populations. Subsequent cohorts or generations are likely to become less prominent and more difficult to predict as the year proceeds. Similarly, in regions with less severe winters and thus less distinct seasonality, these patterns will be less clear. However, combined with other findings of this project, herbicide interventions (whether lethal or sublethal) can now be applied with regard to the plants' and insects' worst and best interests, respectively, at heart.

CHAPTER THREE – PREDICTION OF WATER HYACINTH BIOLOGICAL CONTROL AGENT PHENOLOGY

3.1 Introduction

3.1.1 Degree-day Accumulation

Phenology can be described as the timing of events in an organism's life history. Degree-day values are used for modelling insect development rates because they can quantify phenological development (Snyder et al., 1999; Cesaraccio et al., 2001) and be used to make predictions about the timing of events. Degree-day calculations allow the duration of plant and insect development to be estimated by adding up the number of heat units that occur above the lower temperature threshold in a certain time period (Wagner et al., 1984; Pilkington and Hoddle, 2006). This physiological approach enables calculation of the theoretical duration of different life stages, the number of generations in a given time period (Pilkington and Hoddle, 2006), and the timing of phenological events (Fidanza et al., 1996). Due to its relative simplicity in terms of data input and formulation, this method is widely used, yielding approximately correct values which have shown good predictive capability in the field (Wagner et al. 1984; Skinner et al., 2006).

Daily degree-days are an estimate of the amount of heat that accumulates above a specific temperature threshold over a 24-hour period. One degree-day accumulates for every degree the mean daily temperature is above a given lower developmental threshold (Zalom et al., 1983; Herms, 2004). Summing these values in anticipation of a particular phenological event is known as 'degree-day accumulation'. Degree-day values are most often estimated from daily maximum and minimum temperatures (Snyder et al., 1999) as long-term weather data seldom includes hourly recordings (Purcell, 2003). For this reason, a variety of increasingly complex techniques have been developed to accurately approximate diurnal trends (Roltsh et al., 1999). In a review on the assumptions behind the degree-day approach, Higley et al. (1986) warned that by emphasizing calculation methods, other potentially more significant drawbacks could be overlooked.

Such drawbacks include the assumption that plant and insect development is always directly or linearly related to temperature (Herms, 2004). Higley et al. (1986) maintain that this relationship is not always linear. At a basic level, degree-day models use the assumption that the growth of an organism depends on the rate of various enzymatic reactions. However, the rates of such reactions can be influenced by the availability of water, nutrients and photosynthates for plants, and food and water for animals, and must be present in adequate amounts for optimal development which can then be driven by temperature. Similarly, the availability of enzymes, which are regulated hormonally, can also lead to reduced or unpredictable growth rates (Higley et al., 1986).

Higley et al. (1986) also cite a number of laboratory based factors that can promote error. The calculation of both the developmental minima and maxima for a given species are predominantly done in growth chambers at constant temperatures with little regard for other factors such as photoperiod, which may be critical under field conditions. Fornasari (1995) found that variable temperatures analogous to field conditions sped up the embryonic development of the beetle *Aphthona abdominalis* relative to constant temperatures. Variable temperatures will therefore lead to faster development provided that the high and low temperatures experienced fall within the thermal limits of the organism involved. Higley et al. (1986) maintain that fluctuating temperatures can change which enzymatic reactions are favoured and therefore affect enzyme availability. Thus, calculating degree-day values from constant temperature experimentation invariably introduces some inaccuracy. In addition, the developmental maxima are often not calculated leading to inflated degree-day accumulations, and with the minima, are normally calculated as single values but could vary between different life history stages. Despite this, single values or averages are most often used for thresholds to avoid undue complexity (Higley et al., 1986).

Another compounding variable is the assumption that poikilothermic organisms cannot regulate their body temperature. Among insects especially, many species use both behavioural (Herms, 2004) and physiological mechanisms for thermoregulation (Higley et al., 1986). One common behavioural mechanism is for an organism to seek a thermally favourable microhabitat. It is therefore important to consider whether or not the temperature data from which degree-days are accumulated accurately represent the actual temperatures the organism experiences. Indeed, McClay and Hughes (1995) warn that the use of standard meteorological data for this purpose is a drastic simplification. Furthermore, errors in the collection of temperature data, even if taken directly from a species' microhabitat, will also lead to inaccurate phenological predictions.

3.1.2 Monitoring Temperatures

Long-term climate data must be homogenous in order to draw accurate conclusions from any subsequent analyses. Aguilar et al. (2003) define homogenous climate data as that within which any variation is caused only by variations in climate. However, when dealing with real data sets, one must overcome a number of non-climatic factors that can make data unrepresentative of actual climatic variation. Changes in recording practices, data analysis techniques, and monitoring locations and environments; poorly calibrated instrumentation (Aguilar et al., 2003); missing data (Dunis and Karalis, 2003, Kotsiantis et al., 2006) and instrument exposure (Anderson and Baumgartner, 1998; Brunet et al., 2006), all affect the homogeneity of the data. Many studies have attempted to develop ways of identifying these non-climatic inhomogeneities, as well as methods to adjust the data in order to minimise the biases these variables cause. A variety of techniques has emerged regarding these adjustments but they differ widely in terms of method and complexity, necessitated by the specific needs of a particular study or

dataset (Aguilar et al., 2003). Nevertheless, data homogenising can be simplified into two main processes of replacing both missing data and erroneous values (Kotsiantis et al., 2006).

Complete or continuous temperature datasets are essential for developing accurate and applicable phenological models that are based on degree-days. As a result, a number of filling methods have been developed for reconstructing missing temperature data. These range from 'naïve' approaches, where missing values are replaced with the same day value as the previous year, through to complex algorithms (Dunis and Karalis, 2003) which are chosen at the user's discretion. A popular approach to filling missing temperature data is through the construction and subsequent comparison of the data to be homogenised with a reference time series predicted from historical data (Kotsiantis et al., 2006) or developed from a similar neighbouring weather station (Aguilar et al., 2003; Dunis and Karalis, 2003).

Using neighbouring or 'fallback' weather stations in order to replace missing data has become an established standard due to its relative simplicity (Dunis and Karalis, 2003). The fallback method is best suited to small gaps (up to 12 consecutive days) of data and involves establishing an average offset, between days from the data set to be homogenised and the neighbouring or fallback station. The mean difference between the datasets is calculated from 15 days prior to and after the gap, and the corresponding days from the fallback station. This average offset can then be applied to the fallback station data which falls within the missing data sequence and is inserted in the dataset being homogenised (i.e. missing day value=corresponding fallback station day value±average offset). The fallback method can also be used to fill gaps that are longer than 12 consecutive days. In these cases the average offset is calculated from 15 days prior to the gap, the corresponding missing days themselves and 15 days after the gap from the previous three years, preferably of the dataset being homogenised, although data from the fallback station can also be used (Dunis and Karalis, 2003). In a study to assess the accuracy of differing data-filling methodologies, Dunis and Karalis (2003) found the fallback method yielded the second most accurate imputation values, outperforming other more complex models. A PCA-based model provided far more accurate imputation values but requires that the data be correlated to at least four homogenised temperature datasets, which are not always available.

3.1.3 Aim

The aim of this study was to describe the seasonal population dynamics of the *Neochetina* weevils and *Eccritotarsus catarinensis* at a variety of water hyacinth field locations distributed over a wide range of climatic regions in South Africa. This links to the preceding chapter which determined lower temperature thresholds, below which water hyacinth and its biological control agents cannot develop. This section of the report concentrates on the biocontrol insect populations, and seeks to predict how many

generations per year they can produce at each field site. These predictions are then tested against real long-term data of insect numbers collected from the field. Degree-day models are employed to predict numbers of generations and are based on long-term (up to two years) temperature datasets which were recorded in such a way as to minimise some of the drawbacks associated with degree-day calculations.

Hourly temperature data was recorded in order to preserve diurnal temperature trends and three microhabitats were monitored at each site in an attempt to encompass temperatures directly experienced by the different life history stages of the insects. The homogenisation techniques used on the temperature data were also described and critically evaluated in terms of their biological relevance to the insects and their phenology. Subsequent predictions based on the phenological degree-day models were also evaluated against long-term, on-the-ground measurements at each of the field locations.

3.2 Materials and Methods

3.2.1 Study Sites

Study sites described in Chapter 1 were used for this study.

3.2.2 Temperature Data Collection

Temperature was recorded at three microsites within each field location, using Thermochron iButtons (DS1921G; Maxim Dallas Semiconductor Corporation) that have an operational temperature range of between -40°C and 85°C at a resolution of 1°C and a proven accuracy of ±1°C (Hubbart *et al.* 2005). Data were recorded every half hour and averaged hourly. Recordings of air temperature within the water hyacinth canopy, 11 cm above the water surface, and water temperature recordings 8 cm below the water surface were facilitated by a purpose built floating iButton housing. This device housed two ibuttons, one below the water surface in a watertight but conductive brass capsule, and the second in a vented capsule made from UV stable, engineering grade nylon with a low thermal conductivity. Water hyacinth plants were tethered to the floating device to ensure it remained within the plant canopy. Air temperature was recorded 1.2 m above the water surface immediately adjacent to the site, in such a way as to minimise the effect of radiative heating. Due to the relatively short memory capacity (up to 2048 data points), each iButton was replaced monthly in order to preserve the high resolution of data collection.

3.2.3 Homogenisation Techniques

Homogenisation techniques used on the temperature data were critically evaluated in terms of their biological relevance to the insects and their phenology. Subsequent predictions based on the phenological degree-day models were also evaluated against long-term, on-the-ground measurements at each of the field locations.

Hubbart *et al.* (2005) warn that Thermocron iButtons are prone to radiative heating errors if not shielded sufficiently. To homogenise the microsite data, a reference time series was constructed from data obtained from the closest available weather station. Microsite air temperature data was plotted in an attempt to visually identify possible discontinuities in the data as a result of radiative heating. The longest available sequence of microsite data, but not exceeding a year, that appeared to be free from radiative heating errors was then used to estimate an average offset. This offset was calculated as the mean difference between daily air temperature means calculated from microsite data and those calculated from weather station data. Directly subtracting this average offset temperature from the weather service data transformed that dataset into the desired reference time series. Mean monthly maximum temperatures for the microsite and the reference time series datasets were then compared and months where these maximums were significantly higher in the microsite data identified as containing radiative heating errors.

Data from these months were then corrected by averaging the affected hourly air temperature value with the corresponding hourly water temperature value for the hours from 09H00 to 16H00. Radiative heating errors within the canopy temperature profile were corrected in a similar way except only those months where the mean maximum petiole length was shorter than 15 cm were adjusted. Petioles longer than 15 cm were considered long enough to adequately 'shade' the canopy-monitoring iButton capsule from radiative heating. Using water temperature for correcting radiative heating errors provides a conservative estimate of 'real' temperatures and overcomes the difficulty in quantifying the relative extent of radiative heating between months and sites.

Periods of missing data were homogenised using a modified fallback approach. This consisted of using sequential series of reference time series to first fill missing data within the air temperature and then the canopy temperature profiles. As with the reference time series used to correct radiative heating errors, missing air temperature values were derived from values obtained from the closest available weather station. Due to the high proportion of missing data sequences longer than 12 consecutive days, average offsets were calculated from the longest period but not exceeding a year, of 'complete' data available and not from 15 days prior to and after the gap, as is done in the fallback method described earlier. The offset was calculated as the mean difference between daily air temperature means calculated from microsite data and those calculated from the corresponding period of weather station data. Weather station data only included a daily maximum and minimum temperature so daily means were calculated from these extremes. The average offset was then directly added to or subtracted from the necessary value obtained from the weather station data and inputted into the missing sequence of microsite air temperature data. To preserve a complete sequence of hourly temperature recordings for microsite data, this corrected daily mean value was inputted into each hour of the relevant day. Following the homogeneity

adjustments to the air temperature profile, an average offset between this dataset and the canopy temperature profile was calculated in the same way. Missing values from the canopy temperature dataset could then be filled using offset values derived from the air temperature profile and inputted in the same manner as before. This modified fallback approach was applied to all missing data sequences longer than 24 hours. Gaps shorter than this were filled using the linear trend between the observed values on either side of the gap.

As water reacts more slowly to changes in temperature than air, the variability within the water temperature dataset was expected to be far smaller than that in the air and canopy temperature datasets. Consequently, missing data sequences within the water temperature profile were filled using linear trends. Gaps were filled using the linear trend between the mean value obtained from five days before the gap and the opposite mean calculated from five days after the gap.

3.3 Results

Only two sites from the total of 14 had unbroken temperature records spanning the complete two-year sampling period. Both sites are in Johannesburg which illustrates the difficulty involved in remote coordination of field data collection across the country, and also the vagaries of man or nature, in causing the loss of temperature probes as a result of theft or floods. However, five other sites had only minor gaps in the data (Figure 3.1).

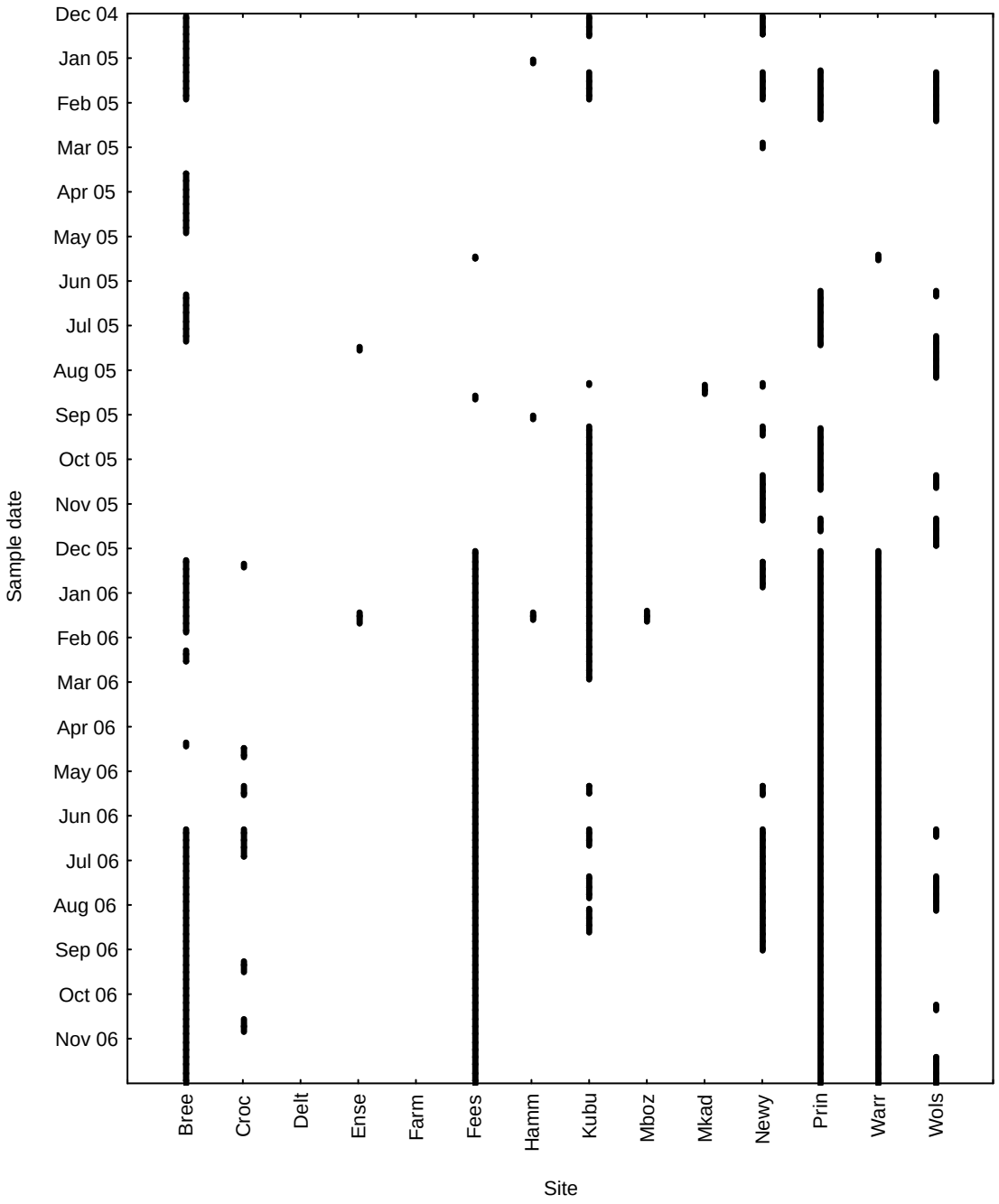


Figure 3.1: Frequency of missing temperature data, concurrently from three microsites (water, canopy and air temperature profiles) per site, during two years of monitoring at 14 water hyacinth-infested sites around South Africa. Black bar = data missing.

Homogenisation of the temperature data allowed accurate estimates of the number of insect generations at all sites (Figures 3.2-15). The error from the Princes Vlei data set, which had the most missing points, was less than half a generation per year for *Neochetina* (3.32 ±0.35 gens/yr; Table 3.1; Figure 3.13) and one generation per year for *E. catarinensis* (8.82 ±1.00 gens/yr; Table 3.2), Conversely, the best estimates were obtained for Delta Park (2.19 ±0.0 & 5.77 ±0.0 gens/yr, *Neochetina* and *Eccritotarsus* respectively) and Farm Dam (2.36 ±0.0 & 6.27 ±0.0 gens/yr *Neochetina* and *Eccritotarsus* respectively) with less than ±0.001 error (Tables 3.1 and 2). Across 14

sites, the average number of weevil generations was calculated to be 3.2 generations per year, with a minimum of 2.08 at Kubusi River (Figure 3.9) and a maximum of 5.03 at Mkadhzi Spruit (Figure 3.11). For the mirid these figures were 5.38 and 13.77 generations at the same respective sites.

Temperature profiles for the 14 field sites sampled showed a dramatic range of temperatures both within and between sites. High temperatures exceeded 38°C at both Mbozambo Swamp (Figure 3.10) and Mkadhzi Spruit (Figure 3.11), which is just above the LT_{50} for *E. catarinensis,* but is unlikely to be significant as the nymphs and adults are both motile and able to move to a more favourable microclimate on the plant. Both weevil species have an LT_{50} well above 38°C and are nocturnal and should not be adversely affected by these extremes. Conversely, low temperatures at many sites were physiologically significant for the biocontrol agents. Only Enseleni River (Figure 3.5) did not drop below the lower development threshold for the weevils or the mirid, while Mkadhzi Spruit (Figure 3.11) and Princess Vlei (Figure 3.13) were close to this limit, and the oviposition threshold of the weevils. Six sites (Crocodile River (Figure 3.3); Delta Park (Figure 3.4); Farm Dam (Figure 3.6); Feesgronde (Figure 3.7); Kubusi River (Figure 3.9); and Warrenton Weir (Figure 3.14)) all dropped below the CT_{min} of the weevils at some stage during the winter, and four of these (Delta park (Figure 3.4); Farm Dam (Figure 3.6); Feesgronde (Figure 3.7); and Kubusi River (Figure 3.9), fell below the CT_{min} of the mirid. At these extreme low temperatures the insects will go into torpor and are at risk of death through further temperature drops or incapacitation.

Canopy and air temperatures differed in the way they tracked each other at different sites reflecting the amount of plant material above the water to shade the temperature probe (c.f. Enseleni River (Figure 3.5) and Farm Dam (Figure 3.6)). Temperature profiles for each site also revealed events at each site, such as the mechanical clearing of plants at Feesgronde (Figure 3.7). Beetle developmental rates closely tracked temperature profiles for all sites, which is to be expected as they are calculated from heat accumulation. However, the occurrence of larval mines (petioles mined) closely tracked the °Day prediction for 11 of the 14 sites (Breede River (Figure 3.2); Delta Park (Figure 3.4); Farm Dam (Figure 3.6); Feesgronde (Figure 3.7); Kubusi River (Figure 3.9); Mbozambo Swamp (Figure 3.10); Mkadhzi Spruit (Figure 3.11), New Years Dam (Figure 3.12) Princess Vlei (Figure 3.13); Warrenton Weir (Figure 3.14) and Wolseley (Figure 3.15)), supporting the predictions of the °Day model, and indicating that counting larval mines is a good method for estimating weevil population size, especially when they occur at low numbers, as seen in all of these sites. However, larval numbers were consistently low by this measure, reaching a maximum of eight mines per 10 plants in two consecutive summers at Breede River (Figure 3.2), and declining to zero at most sites during spring or early summer, but generally averaging around three mines per 10 plants. This indicates that larval damage is minimal, translating into, at best, less

than one larva per plant, and at worst no larvae at all, allowing the plants to grow unscathed by the weevils.

Table 3.1: Mean error in yearly accumulation of degree-days, using the development threshold temperature of 9.62°C for *Neochetina* weevils, when calculated from incomplete sequences of canopy temperature data. Data gaps were filled with daily means fitted from nearby regional weather stations.

Site name	Number of gaps filled ($year^{-1}$)	Mean gap length (days / year ±SE)	Mean difference (°D / day)	Accumulated degree-days (°D / year ± error)	Number of generations ($year^{-1}$ ± error)
Breede River	3.5	57.62 ±26.63	0.15 ±3.69	2738.16 ±30.25	2.78 ±0.03
Crocodile River	3.5	12.58 ±2.92	1.44 ±4.15	3041.91 ±63.40	3.09 ±0.06
Delta Park	0	-	-	2152.12	2.19
Enseleni Nature Reserve	1.5	16.00	1.26 ±3.17	4669.56 ±30.24	4.74 ±0.03
Farm Dam	0	-	-	2324.47	2.36
Feesgronde	2	2.50	-1.32 ±3.50	3174.19 ±6.60	3.22 ±0.007
Hammarsdale Dam	2	4.5 ±0.29	1.19 ±2.92	2968.87 ±10.71	3.02 ±0.01
Kubusi River	4	38.47 ±19.94	1.42 ±3.38	2044.04 ±218.51	2.08 ±0.22
Mbozambo Swamp	0.5	4.00	0.44 ±3.53	4325.24 ±0.88	4.39 ± 9^{-4}
Mkadhzi Spruit	1	23.00	-0.21 ±3.45	4953.43 ±4.83	5.03 ±0.005
New Years Dam	4.5	39.75 ±18.67	-0.30 ±7.59	3109.08 ±53.66	3.16 ±0.05
Princess Vlei	4	61.75 ±24.61	-1.39 ±5.73	3264.07 ±343.33	3.32 ±0.35
Warrenton Weir	1	5.00	0.46 ±5.47	2985.35 ±2.30	3.03 ±0.002
Wolseley	4.5	16.38 ±5.34	-0.15 ±5.51	2548.36 ±11.06	2.59 ±0.01

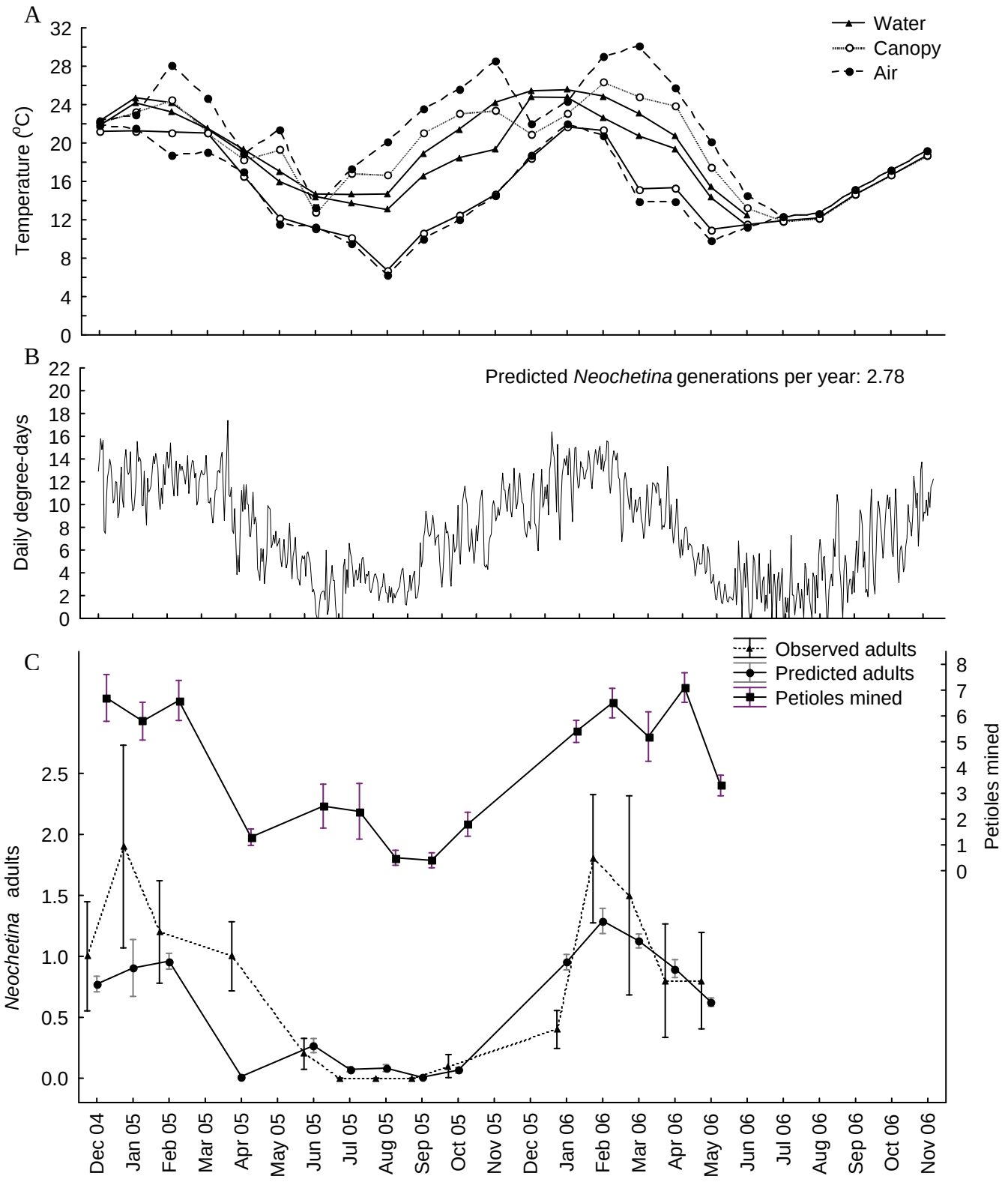


Figure 3.2: Breede River: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

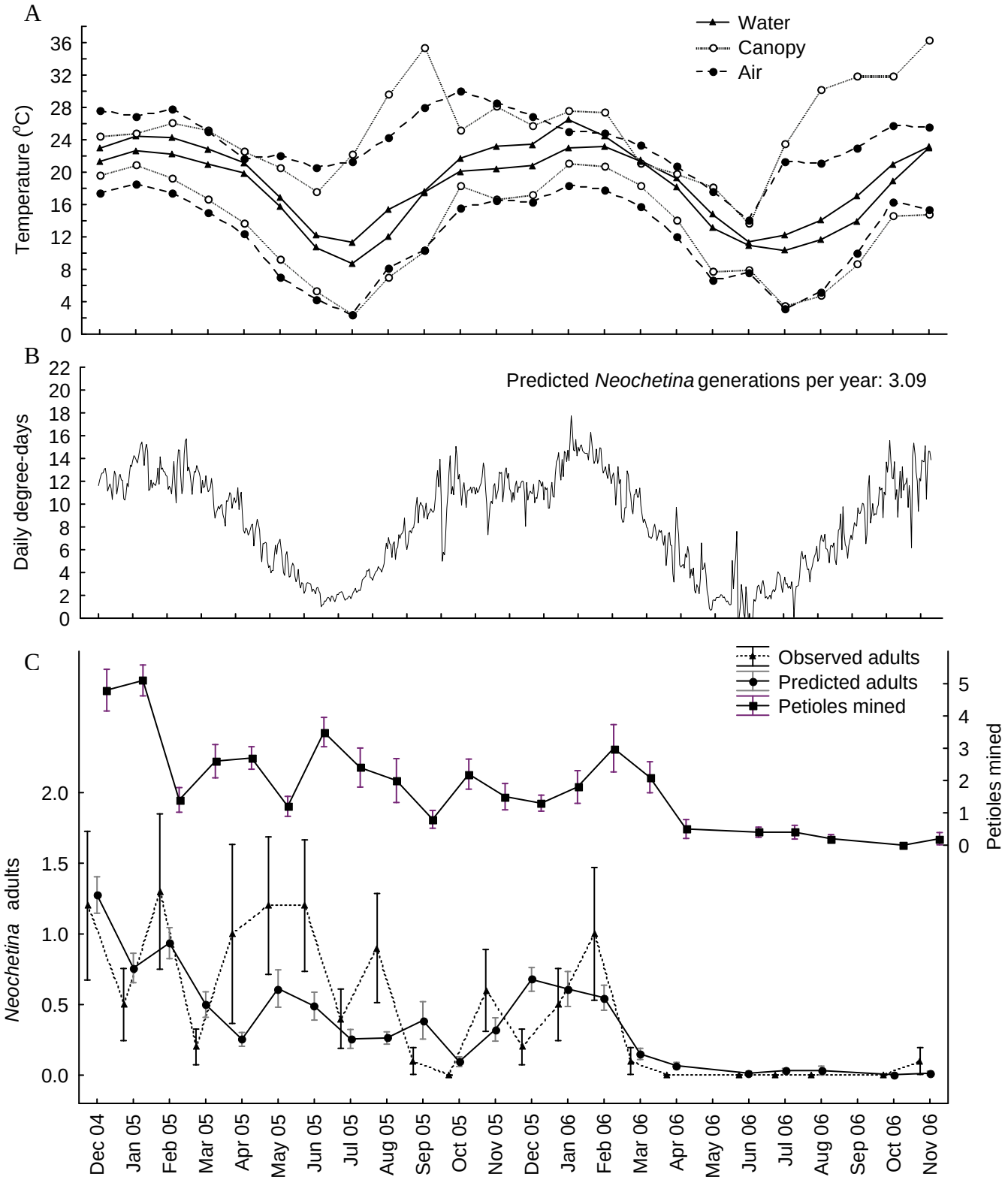


Figure 3.3: Crocodile River: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

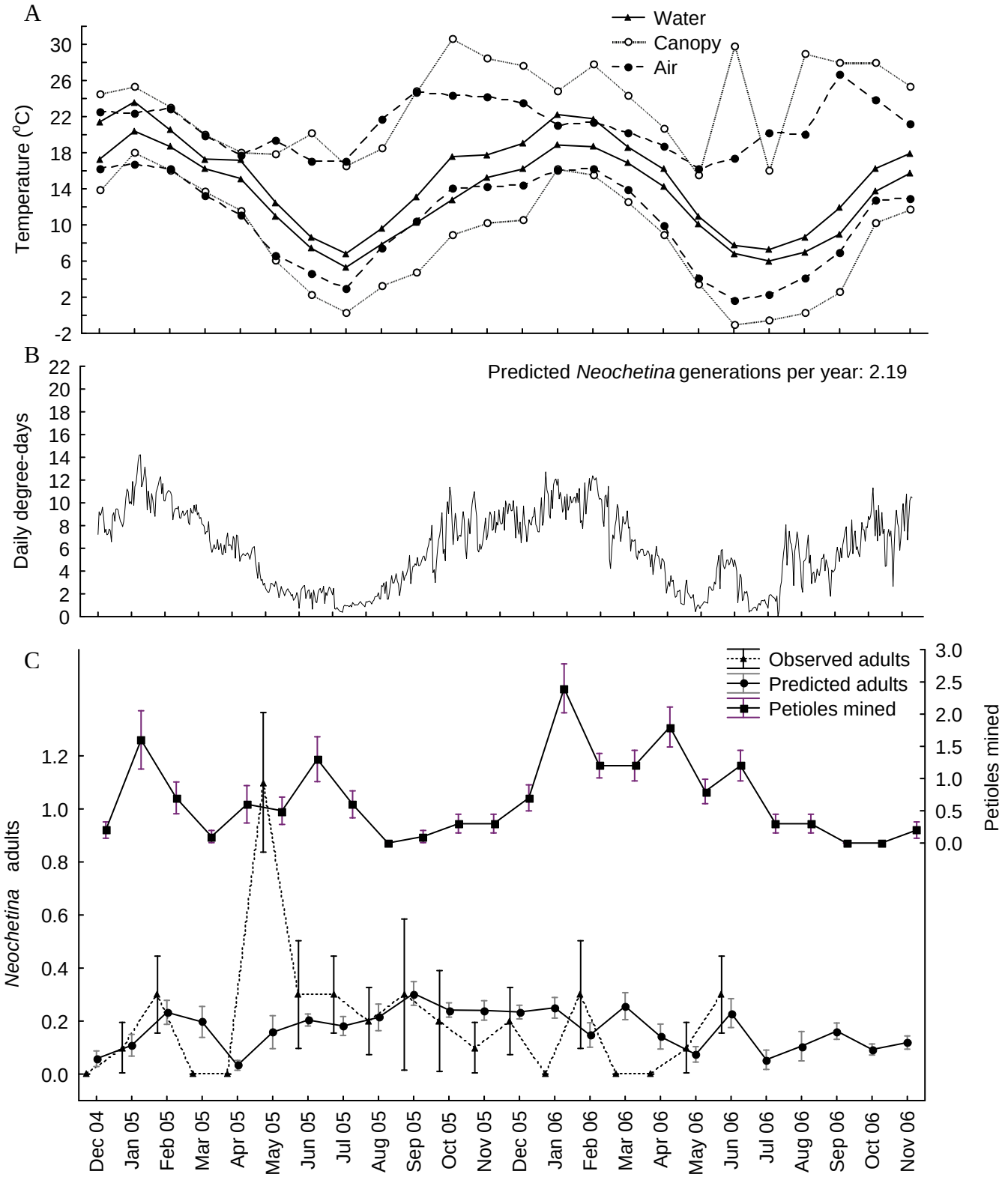


Figure 3.4: Delta Park: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-days (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

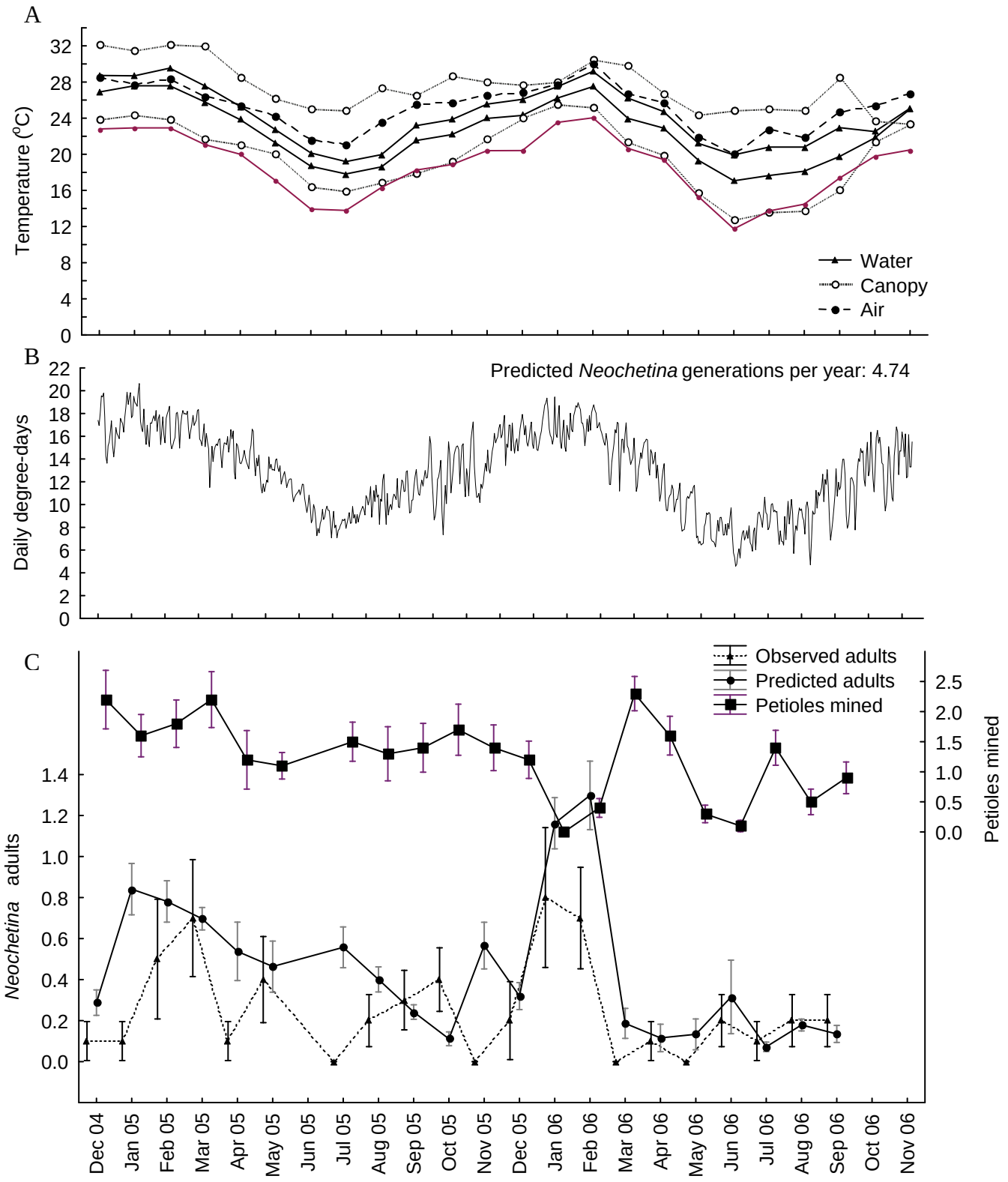


Figure 3.5: Enseleni Nature Reserve: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

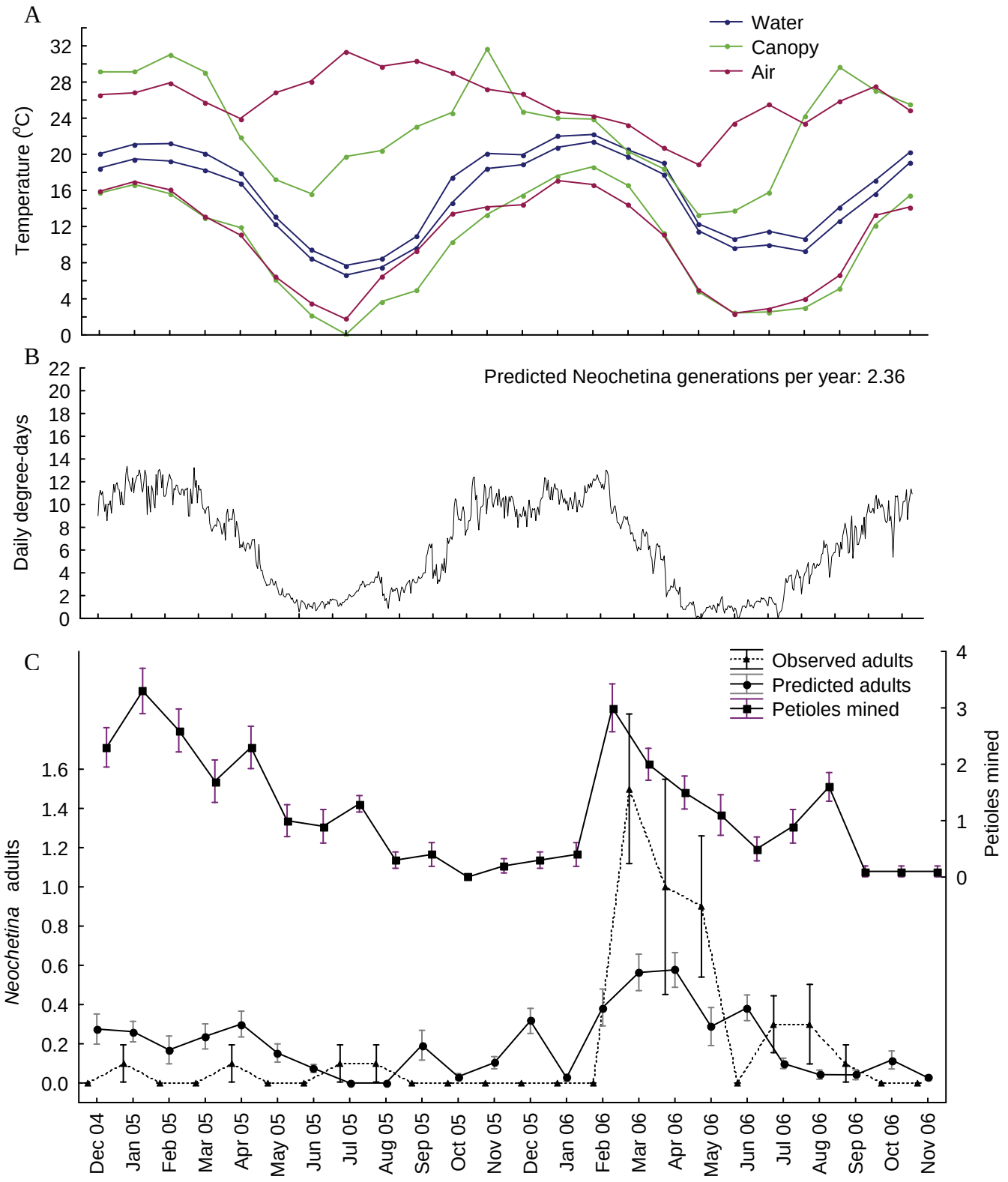


Figure 3.6: Farm Dam: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

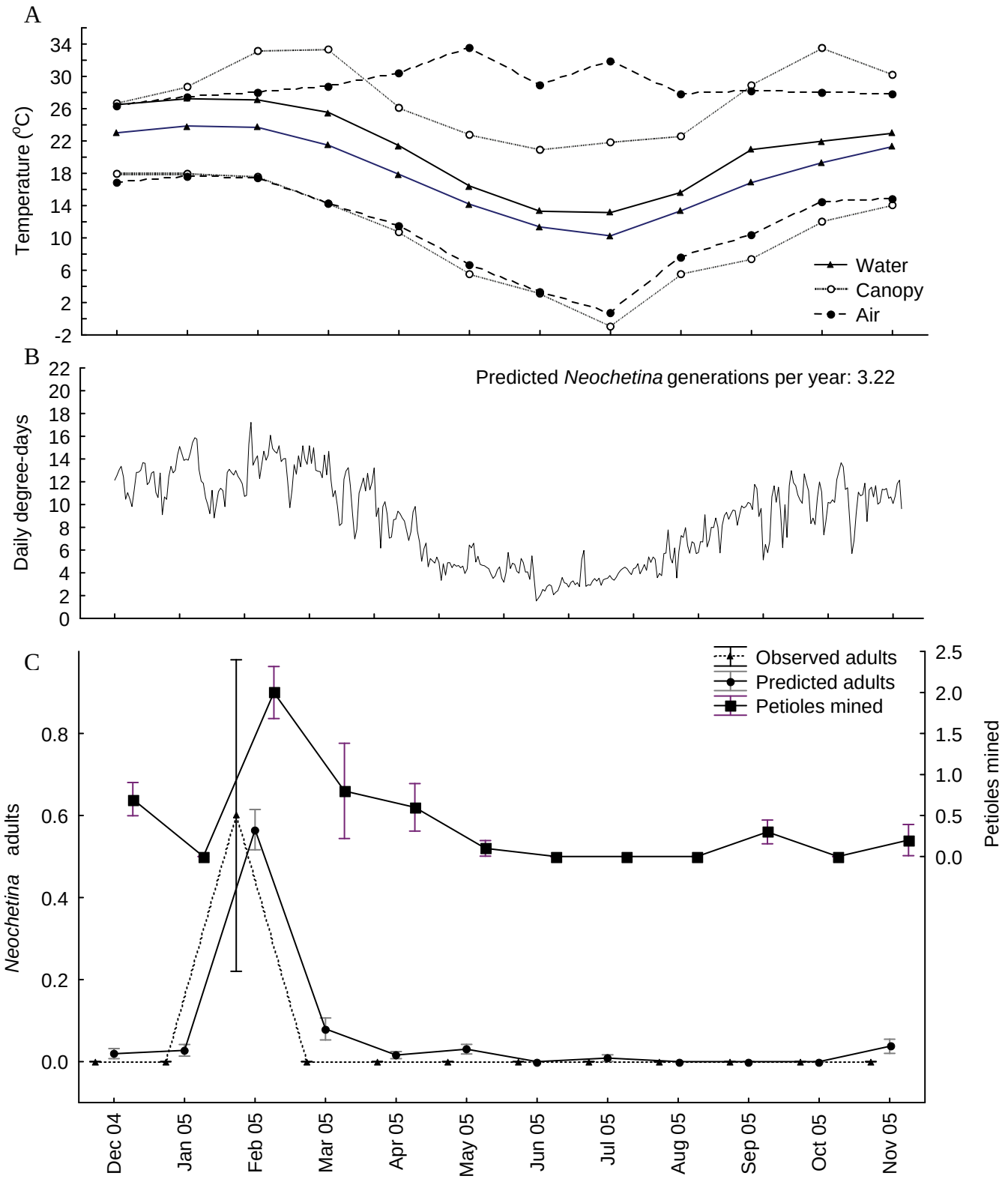


Figure 3.7: Feesgronde: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and(C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

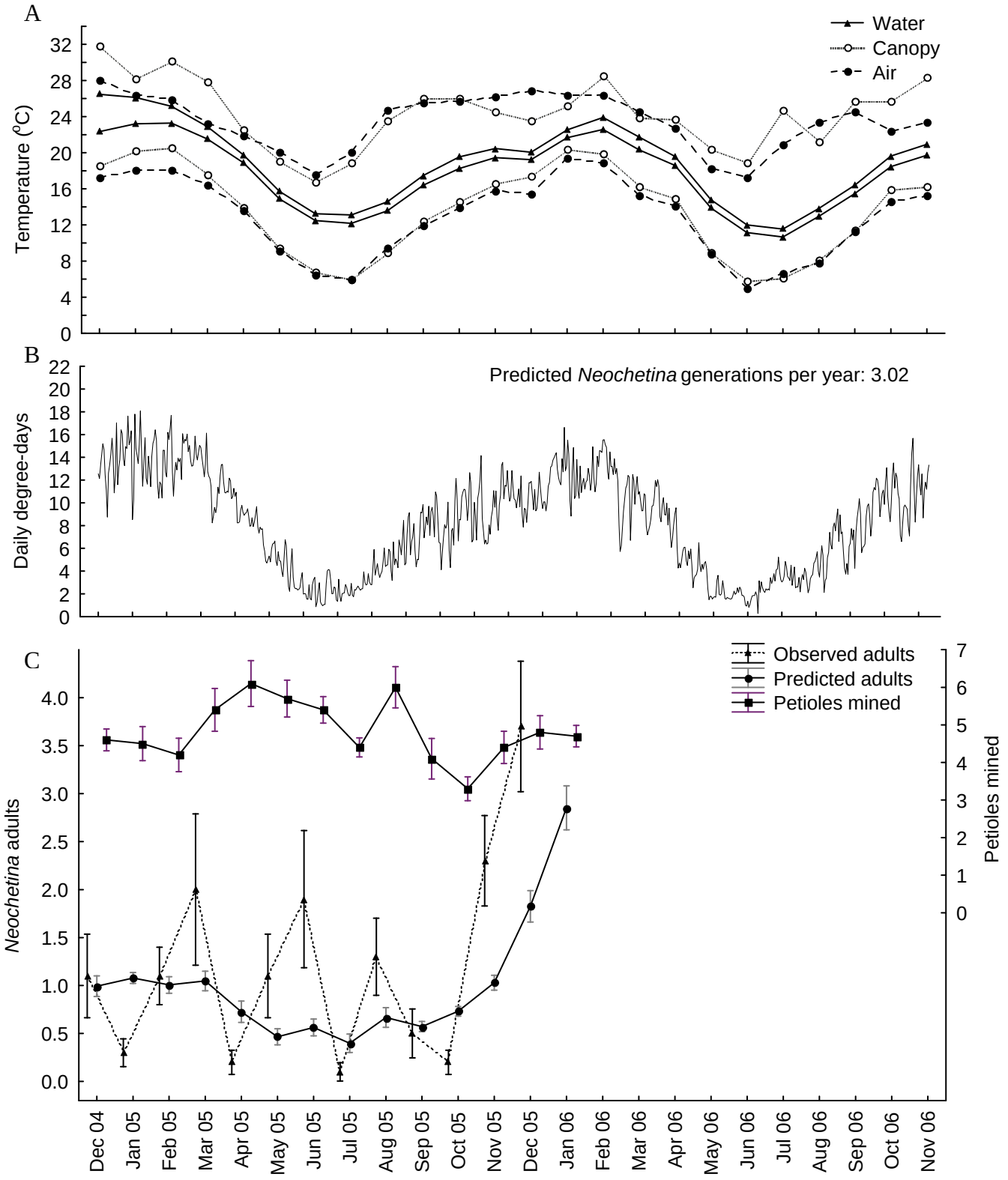


Figure 3.8: Hammarsdale Dam: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

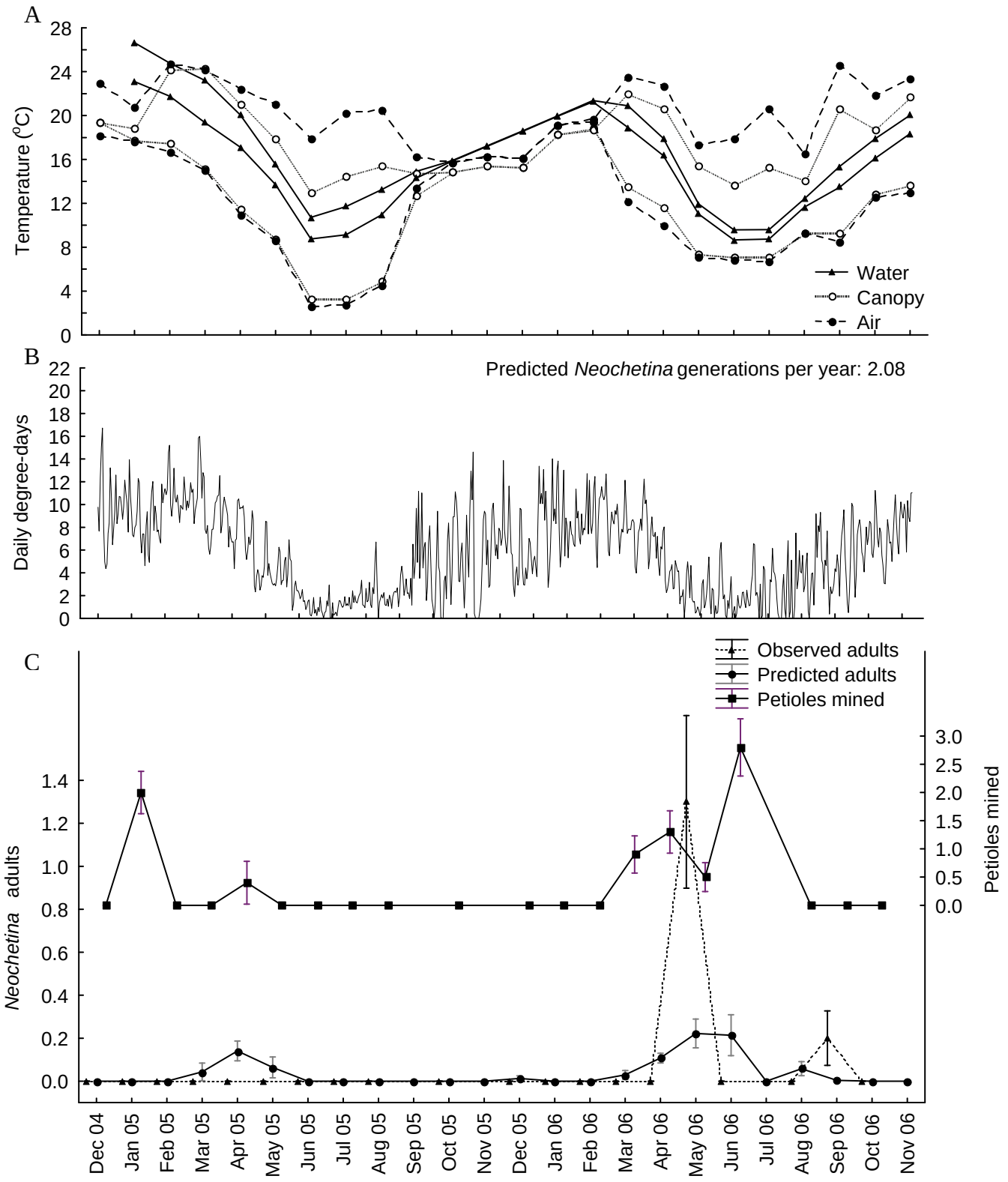


Figure 3.9: Kubusi River: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

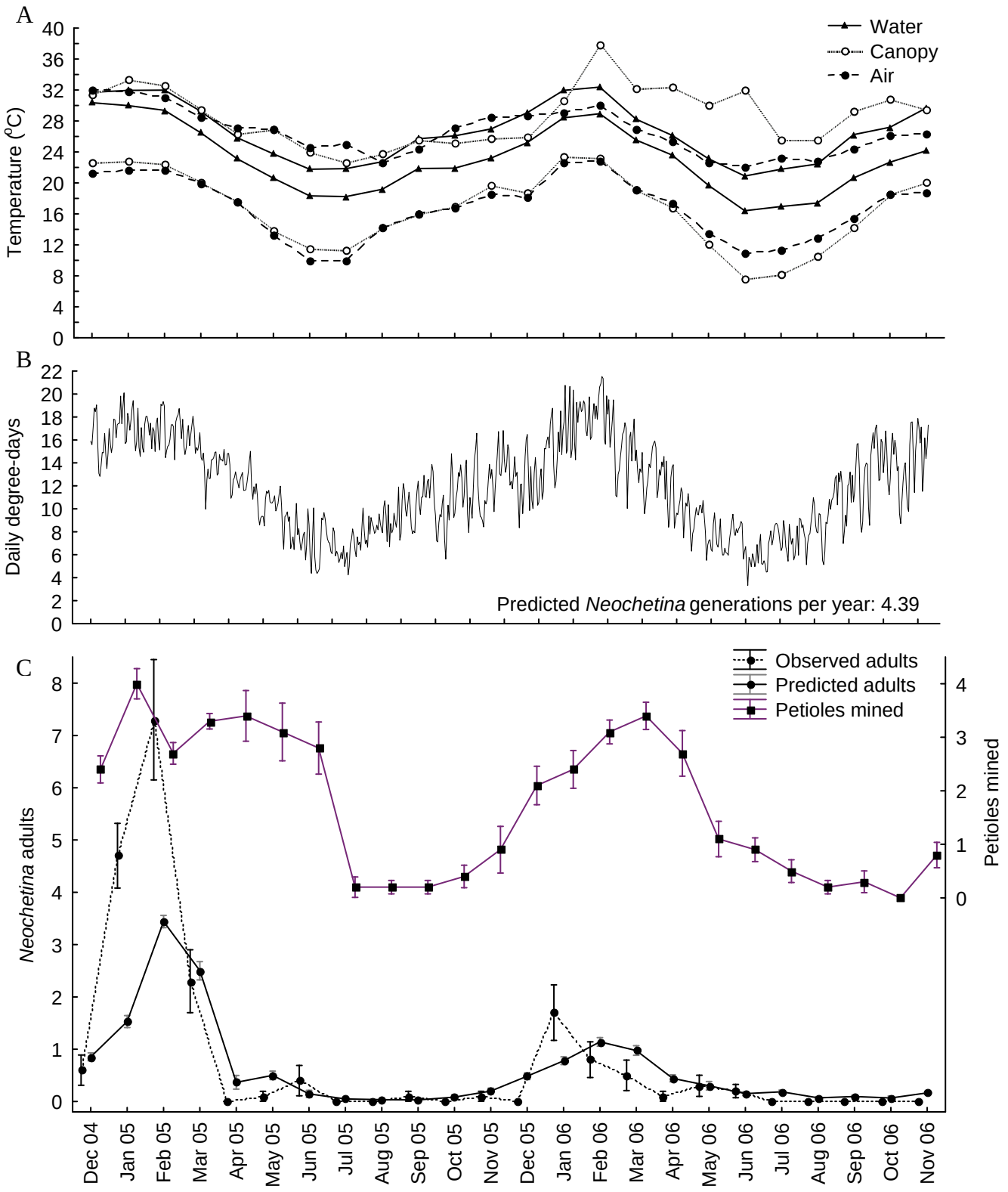


Figure 3.10: Mbozambo Swamp: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

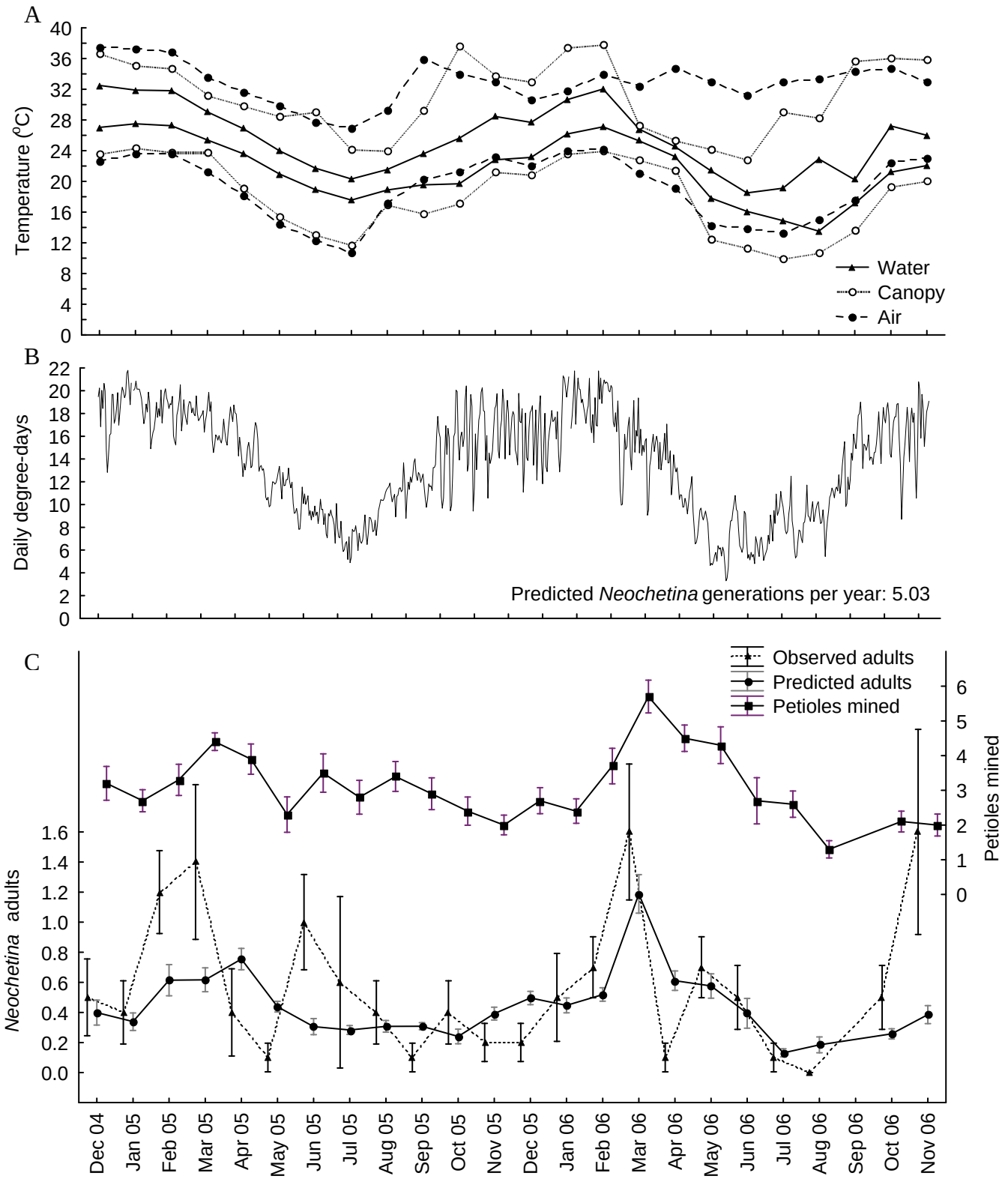


Figure 3.11: Mkadhzi Spruit: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

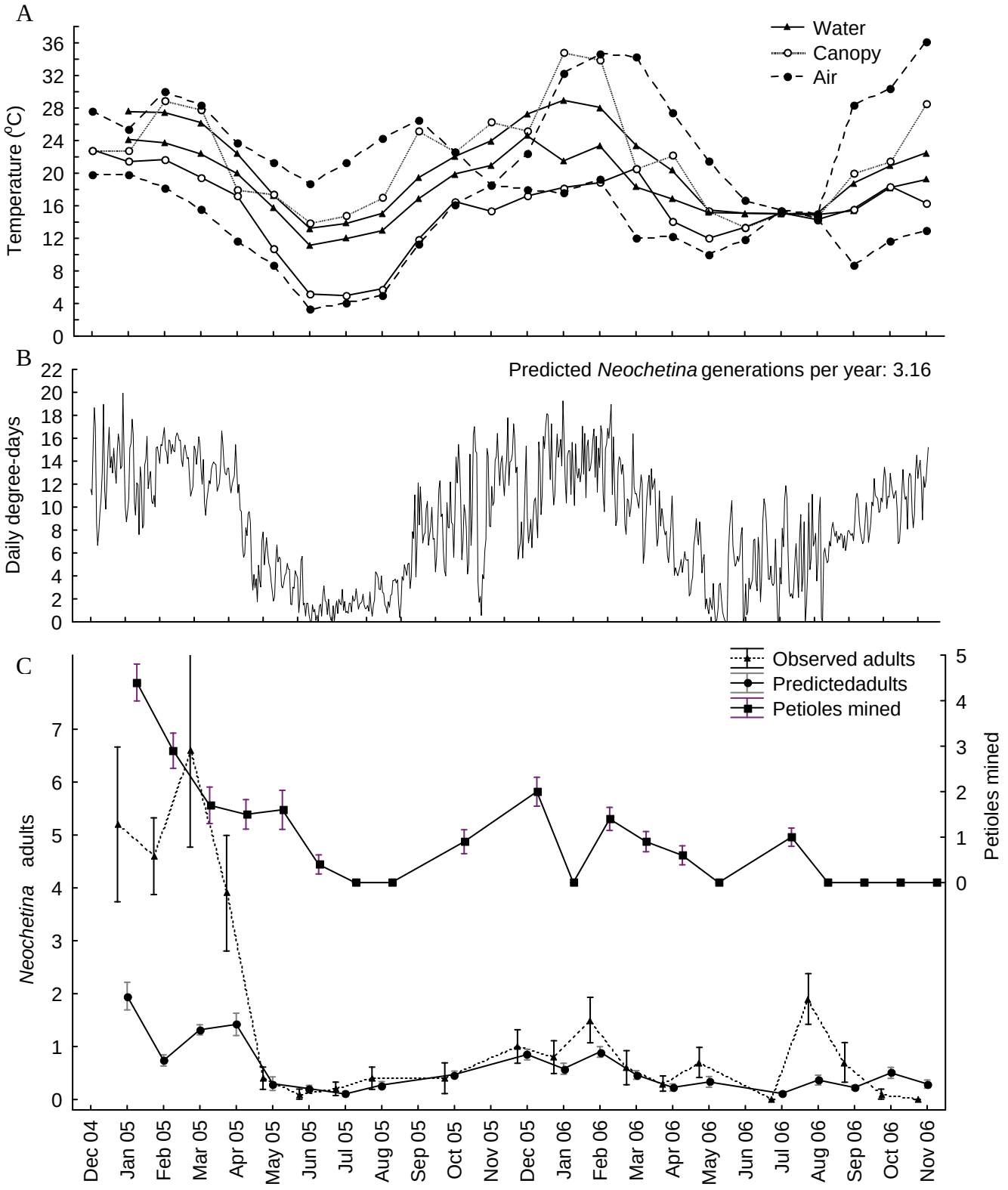


Figure 3.12: New Years Dam: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-days (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

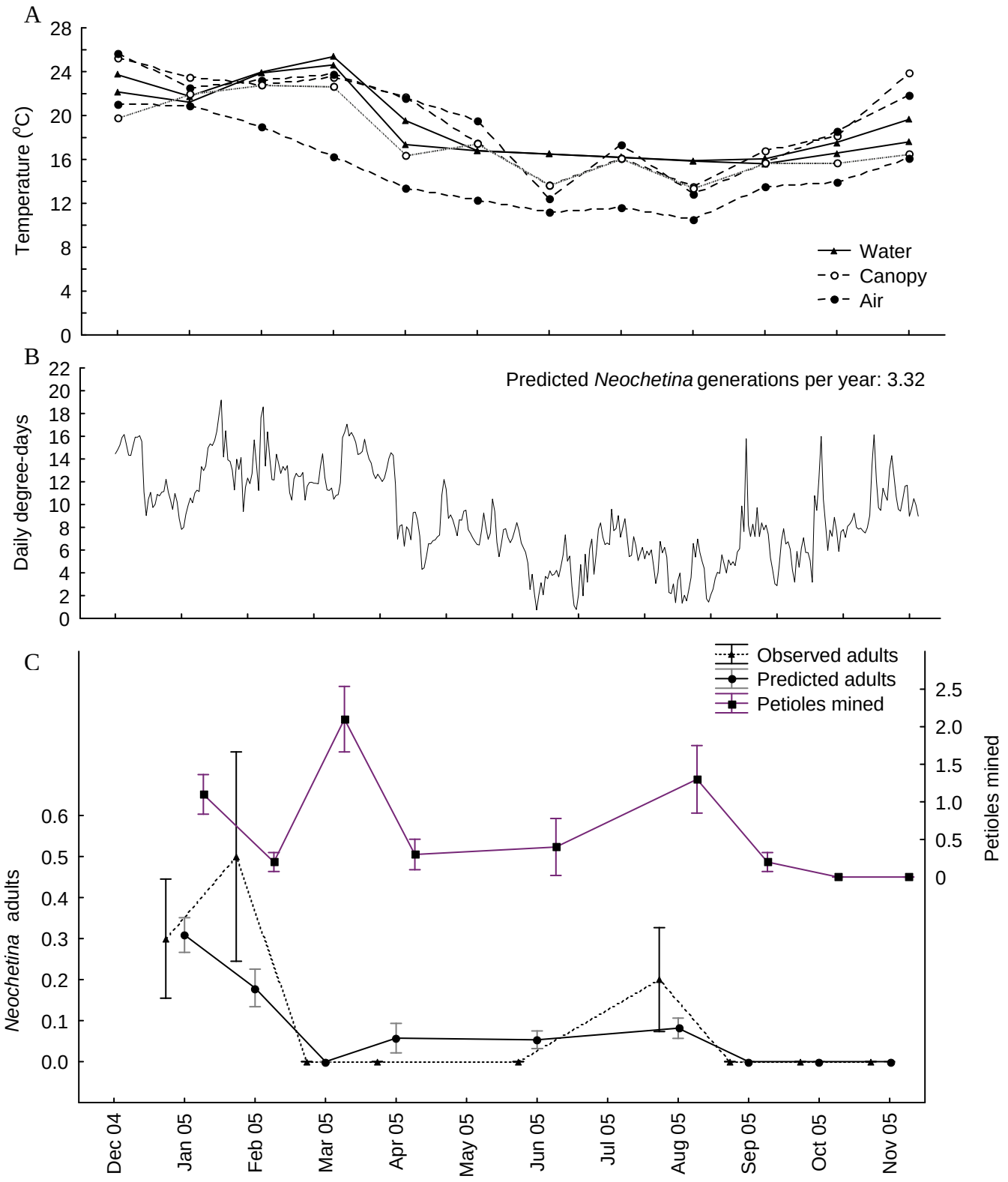


Figure 3.13: Princess Vlei: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and(C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

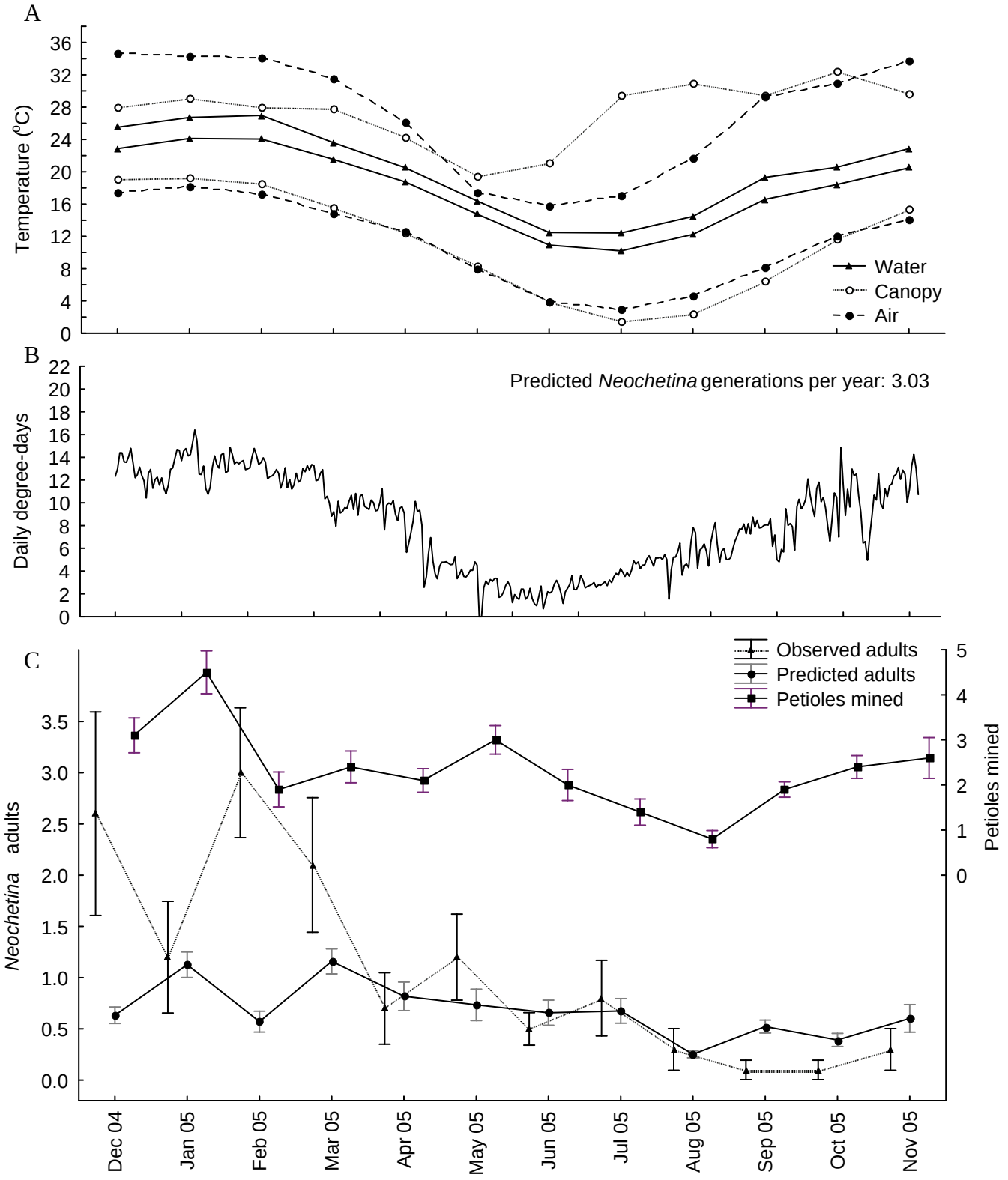


Figure 3.14: Warrenton Weir: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

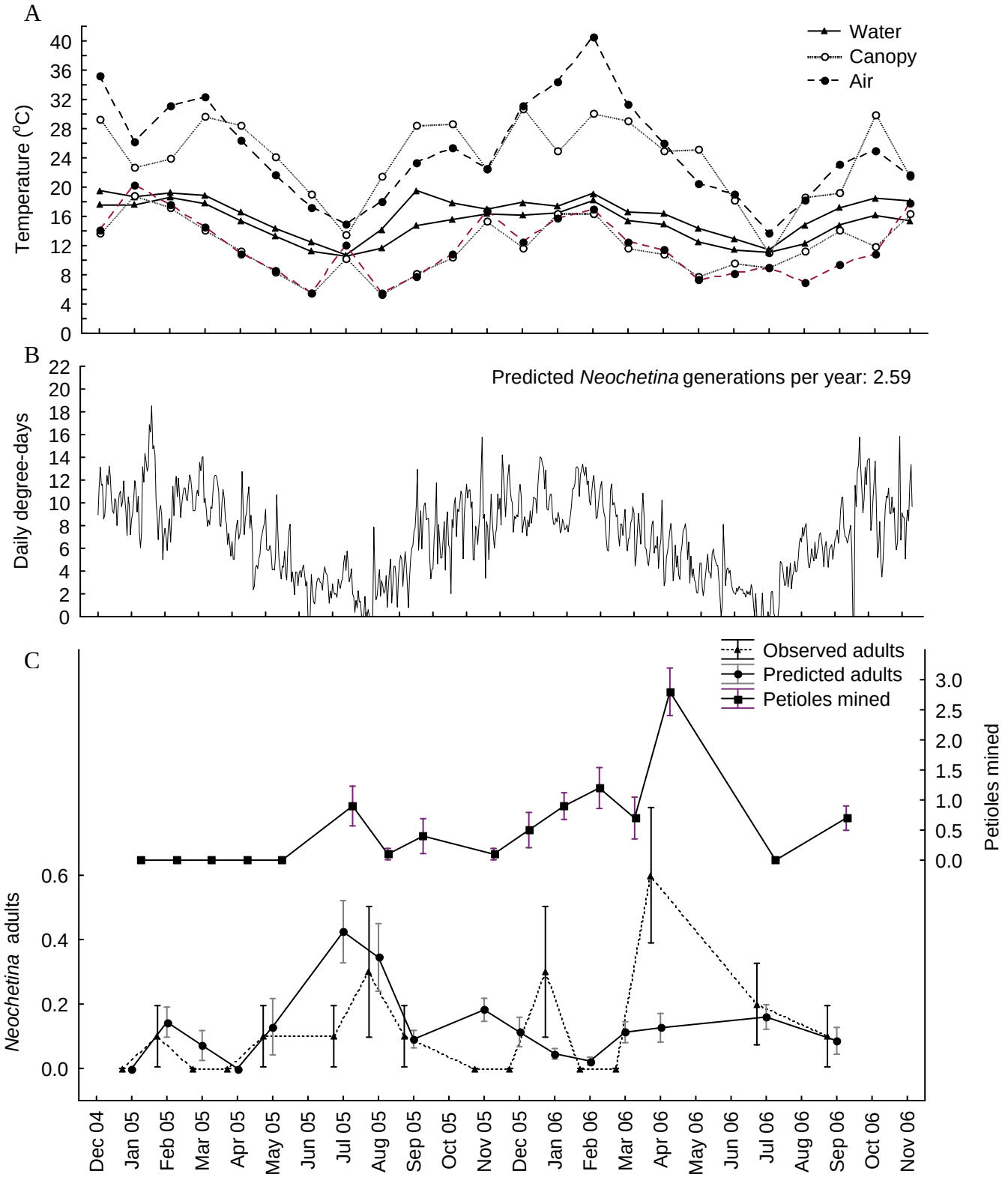


Figure 3.15: Wolseley: (A) Monthly mean daily maximum and minimum temperature for three microsites; (B) daily degree-day accumulation (t = 9.62°C) and subsequent number of generations (K = 984.36°D) derived from canopy temperature; and (C) *Neochetina* phenology from 2004 to 2006. Weevil phenology is based on counts of adults and larval mines, and predicted adult numbers are calculated from feeding scars (Mean from 10 plants ±SE).

Use of feeding scars to predict adult numbers worked well for a few sites, such as Feesgronde (Figure 3.7), Mbozambo Swamp (Figure 3.10) and New Years Dam (Figure 3.12), but most sites showed weak correspondence between the prediction derived from feeding scars and the actual numbers collected e.g. Hammarsdale Dam (Figure 3.8). Peaks in larval numbers (petioles mined) coincided with adult peaks for six sites (Crocodile River (Figure 3.3); Delta Park (Figure 3.4); Farm Dam (Figure 3.6); Kubusi River (Figure 3.9); New Years Dam (Figure 3.12) and Warrenton Weir (Figure 3.14)), and the larval peaks generally preceded the adult peaks, although low adult numbers again made this interpretation difficult and generally reflected no significant difference in numbers found between successive collection dates.

Table 3.2: Mean error in yearly accumulation of degree-days, using the development threshold temperature of 10.3°C for *Eccritotarsus catarinensis*, when calculated from incomplete sequences of canopy temperature data. Data gaps were filled with daily means fitted from nearby regional weather stations.

Site name	Number of gaps filled ($year^{-1}$)	Mean gap length (days / year ±SE)	Mean difference (°D / day)	Accumulated degree-days (°D / year ± error)	Number of generations ($year^{-1}$ ± error)
Breede River	3.5	57.62 ±26.63	0.18 ± 3.64	2506.74 ±36.30	7.33 ±0.11
Crocodile River	3.5	12.58 ±2.92	1.51 ± 4.03	2836.62 ±66.49	8.29 ±0.19
Delta Park	0	-	-	1972.38	5.77
Enseleni Nature Reserve	1.5	16.00	1.26 ± 3.17	4421.44 ±30.24	12.93 ±0.09
Farm Dam	0	-	-	2142.85	6.27
Feesgronde	2	2.50	-1.12 ± 3.54	2971.30 ±5.6	8.69 ±0.02
Hammarsdale Dam	2	4.5 ±0.29	1.26 ± 2.74	2753.31 ±11.34	8.05 ±0.03
Kubusi River	4	38.47 ±19.94	1.37 ± 3.32	1839.96 ±210.82	5.38 ±0.62
Mbozambo Swamp	0.5	4.00	0.50 ± 3.47	4085.42 ±1.00	11.95 ±0.003
Mkadhzi Spruit	1	23.00	-0.19 ± 3.43	4709.33 ±4.37	13.77 ±0.01
New Years Dam	4.5	39.75 ±18.67	-0.19 ± 7.32	2883.70 ±34.00	8.43 ±0.10
Princess Vlei	4	61.75 ±24.61	-1.39 ± 5.73	3015.97 ±343.33	8.82 ±1.00
Warrenton Weir	1	5.00	0.54 ± 5.36	2790.69 ±2.70	8.16 ±0.008
Wolseley	4.5	16.38 ±5.34	-0.13 ± 5.50	2336.04 ±9.58	6.83 ±0.03

3.4 Discussion

The temperature data presented above represents a very large data set from a very broad range of field sites. Loss of data points, creating gaps in the data series, has been shown not to be seriously detrimental to the utility of the data, although complete temperature records are obviously preferable to ones that have been repaired. Estimations of insect development based on complete and incomplete temperature data sets were shown to have a small error, resulting at most in a two in nine (mirid 8.82 ±1.00 gens/yr, Table 3.2), or 22% error in the estimation of generations per year. However, sites such as Farm Dam and Delta Park, which have no missing data and are consequently free of errors, correspond well with the estimates from less perfect sites. Nevertheless, the °Day estimates of generations appear to slightly, but consistently, overestimate the number of weevil generations produced per year when compared to actual numbers from the field (Table 3.3).

Table 3.3: Comparison of the predicted number of *Neochetina* weevil generations per year derived from °Day calculations, compared with an estimate of generations based on larval mines.

Site name	Number of generations predicted by °D model ($year^{-1}$± error)	Number of generations estimated from larval mines (petioles mined)
Breede River	2.78 ±0.03	2.5
Crocodile River	3.09 ±0.06	4
Delta Park	2.19	2
Enseleni River	4.74 ±0.03	3-4
Farm Dam	2.36	2-3
Feesgronde	3.22 ±0.007	-
Hammarsdale Dam	3.02 ±0.01	3
Kubusi River	2.08 ±0.22	2
Mbozambo Swamp	4.39 $\pm 9^{-4}$	-
Mkadhzi Spruit	5.03 ±0.005	3
New Years Dam	3.16 ±0.05	3
Princess Vlei	3.32 ±0.35	2
Warrenton Weir	3.03 ±0.002	3
Wolseley	2.59 ±0.01	2-3

Crocodile River is an exception, and in the second year appears to have had a collapse of the beetle population. The mechanical clearing of Feesgronde and herbicide treatment of Mbozambo Swamp had a similar effect, and no sensible estimate can be made from

the field data. Nevertheless, where estimates can be made they fit reasonably well with the theoretical value. Overestimation of the generation numbers could be due to lag periods in the beetles' lifecycles that have not been accounted for in the model. These include maturation feeding by females between emergence from the pupa and commencement of oviposition. A lower oviposition temperature threshold may also prevent egg-laying at cooler periods in the year. Oviposition thresholds are addressed in the next chapter of this report. Incorporation of more thresholds into the model may help to generate a biofix point at which the new generation can be considered to start, which will allow the daily °Day curves to be turned into monthly generation predictors.

The temperature profiles of the various sites reveal how varied the environment is in which these beetles persist and is testimony to their hardy nature, as they are able to survive and breed within a very broad temperature range. However, what is now required is similar data sets from Lake Victoria and other sites where the weevil has been successful in controlling the weed for comparison of extremes of temperature and the number of generations produced per year. Temperature data from beetle localities in South America would also indicate how the few (or maybe many) generations produced here in South Africa compare to those in its home range.

Feeding scars do not emerge as a good estimator of adult beetle numbers, which is not surprising as this is a cumulative measure that will persist or disappear at a rate dictated by the leaf turnover rate. Cold periods will accumulate scars simply because leaves are not being shed. A correction factor could possibly be introduced based on temperature and leaf turnover, but adult numbers remain so low that this would remain a largely academic exercise with a large error component.

Low numbers of adults and larvae are telling. Ajuonu et al. (2003) report up to 250 feeding scars per leaf on water hyacinth in Benin and six weevils per plant which was achieved only fleetingly at few of the South African field sites, which averaged below one weevil per plant for most of the year at most of the sites. These low numbers are all the more worrying when the lack of cumulative increase of adults or larvae is seen consistently at any of the sites. Some sites boom and bust in beetle numbers, which is odd given that *Neochetina* is assumed to be a K selected species, but none build to a clear population maximum during the course of the year. Wilson (2002) found that *Neochetina* larval numbers are density dependent, but suggested that cannibalism was not an important feature of *Neochetina* populations. Carnivory is known from many ostensibly herbivorous insects and is explained as a means of increasing nitrogen intake for insects living on a low nitrogen diet. Eating one's siblings might be a variation on this method for *Neochetina* larvae, seeking to boost their nitrogen intake, but given the low larval numbers it seems unlikely that they are short of space or food. Data from Atteridge (2009) suggests that cannibalism by *Neochetina* larvae is low or absent, but early instar larval mortality is high. Adults will lay up to 20 eggs per leaf in the

laboratory and high numbers have also been reported from the field, but petioles mined average about two per plant, rarely rising to five or six at some sites.

Predictions made by the degree-day model for *E. catarinensis* support the model generated by Coetzee et al. (2007) in that the number of generations completed at high-altitude cold sites (e.g. Delta Park and Farm Dam) is much lower than that predicted for warm subtropical sites (e.g. Mbozambo Swamp and Mkahdzi Spruit) because the inability to develop sufficiently rapidly during winter months appears to hinder overwintering of this insect. While this model does take into account the influence of microclimates, it does not explain why, for example, high numbers of mirids were present at the Kubusi site during midwinter, which is the site that has the greatest cold accumulation in this model. Behavioural responses by the mirids to cold temperatures are likely to play a role in their persistence through cold winters.

An important issue emerges from these data which illustrates the ephemeral nature of water hyacinth sites in South Africa. The majority of river sites experienced flooding at some time during the two year sample period. These floods often washed away the data-logger, knocking a hole in the data set for that site. More important, however, was the effect on the biocontrol agent populations swept away with the water hyacinth into the sea where they will certainly have perished. The weed mat returns rapidly from individual plants trapped in corners of the river or seeds in the substrate, at a rate that outstrips the agent growth rate (Chapter 1, this report). Water hyacinth is presumably supremely adapted to this lifestyle in its home range where perennial rivers flood massively every year, and this pioneer plant persists, in the presence of all of its natural enemies. Other enclosed sites, such as Hammarsdale Dam and Mbozambo Swamp, were sprayed with herbicide and the plants and insects were lost in a similar fashion to the flooding. High turnover-rate agents such as *E. catarinensis* released inundatively may be required in these situations.

These combined disturbance events should be picked out of these data sets to illustrate the transient nature of water hyacinth sites and may go some way to explaining why agent numbers never really build up to significant numbers. Nevertheless, at sites where they had both the time and the temperature, this has not happened.

CHAPTER FOUR – THE EFFECT OF TEMPERATURE ON THE REPRODUCTIVE STATUS OF THE WATER HYACINTH WEEVILS, *NEOCHETINA EICHHORNIAE* AND *N. BRUCHI*.

4.1 Introduction

4.1.1 Reproductive Status of Overwintering Females

The temperature and development model presented in the preceding section has shown that *Neochetina* species are able to produce between two and five generations per year at different sites around South Africa and that the weevils overwinter primarily as third instar larvae. The developmental model has revealed that, as a consequence of this, new generations of water hyacinth plants (as ramets produced over winter) will have up to three months of undisturbed growth before they are likely to be attacked by the new season's F_2 generation of beetle larvae. What has not been established is the effect of temperature on adult female beetles, which may overwinter and make an important contribution to the new season's F_1 generation.

The best measure of when the weevils start to reestablish their population in spring is when new individuals are introduced into the population at the onset of egg laying. Because beetle numbers are generally low in spring and the eggs are laid by inserting them into the leaf tissue, direct observation of this event in the field is virtually impossible. However, by assessing the state of the females' ovaries, the onset and cessation of oviposition can be recorded with some precision in the laboratory and compared with field observations of female reproductive status. Both species of *Neochetina* can undergo reversible flight muscle generation and degeneration – alternating with reproductive periods – whereby the ovarian follicles are reabsorbed when flight muscles are produced and vice versa (Buckingham and Passoa, 1985, Grodowitz et al., 1997). In many insect species ovarian development is regulated by temperature (Perez-Mendoza et al., 2004) and the condition of weevil ovaries has been found to vary with season (Grodowitz et al., 1997). Temperature has been found to influence flight muscle development, and in turn, the reproductive status in *Neochetina* weevils (Buckingham and Passoa, 1985); and so, by exposing weevils to various temperatures the temperature range at which there is a decline in their ability to produce eggs can be established.

4.1.2 Reproductive System Morphology

The water hyacinth weevils' reproductive system consists of two ovaries, each comprising of two ovarioles. The ovarioles themselves are separated into two parts: the germarium, where follicles are formed and the vitellarium, where the follicles develop and mature (Grodowitz et al., 1997). This layout reveals a progression of follicles in sequential states of maturity in functional, healthy ovaries, which allows assessment of the reproductive status and reproductive history of the individual being examined. The two ovarioles are connected via the lateral oviduct, which connect and lead into the

common oviduct, which unites both ovaries (Figure 4.1). The spermatheca, which stores sperm, joins to the common oviduct and each egg is fertilized as it passes through the common oviduct (Grodowitz et al., 1997).

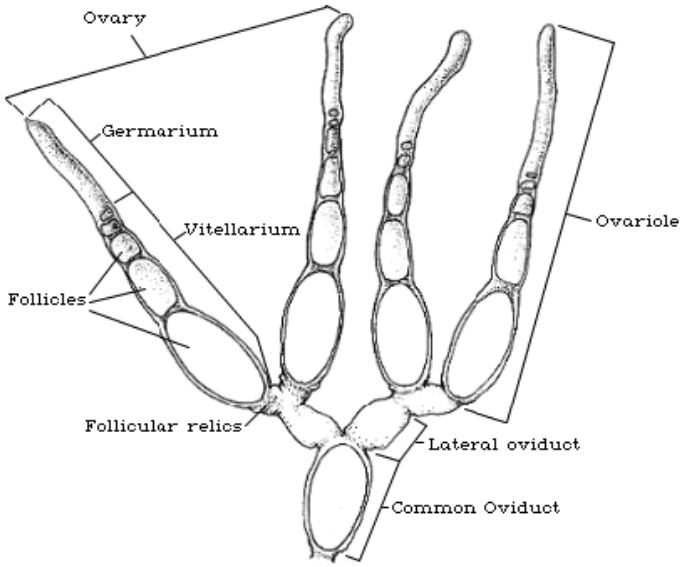


Figure 4.1. The reproductive system of *N. eichhorniae*. (From Grodowitz et al., 1997)

4.1.2.1 Follicular Relic Formation

The follicles (or eggs) are composed of a large central ovum surrounded by a layer of follicular epithelium. The entire set of developing follicles is housed within a thin ovariole sheath. Follicular relics form at the base of the ovarioles as a result of the follicle passing through the lateral oviduct, when the follicular epithelium surrounding the ova is sloughed off and accumulates in the ovariole base. As more ovulations occur, more follicular relics are formed. When follicles degenerate within the base of the ovarioles they also form follicular relics. Follicular degeneration is likely to occur when food sources fail to provide sufficient nutrition and/or when insects become too old to support further follicular development, or when oviposition stops due to low temperatures. There is no way to distinguish between follicular relics formed by ovulation or degeneration (Grodowitz et al., 1997), but they can be used to ascertain that the individual has reproduced.

When the ovaries contain swollen follicles they can be classified as fully functioning and/or capable of producing eggs (parous); if they have reduced or no follicles the individual is described as being non-functioning (nulliparous) (Figure 4.2). The presence of follicular relics in the same individual will then indicate that the weevil has reproduced before and that her ovaries have degenerated. If the follicular relics are absent, this reveals that she has not yet reproduced. Thus, the ovarian status of a weevil can be classified in one of four possible ways:

- Parous, no follicular relics – ovaries are fully functioning and eggs are present, but the weevil has never produced eggs.
- Parous, follicular relics present – fully functioning, eggs present and has reproduced.
- Nulliparous, no relics – ovaries not functional, no eggs present and weevil has not reproduced.
- Nulliparous, relics present – ovaries not functional, but has reproduced.

Figure 4.2: Photomicrograph contrasting (a) healthy (or parous) and (b) degenerative (or nulliparous) ovaries. Relics are also evident at the bases of each ovariole. (Bar=0.25 mm)

4.1.3 Aim

To determine the temperature range over which female water hyacinth weevils stop oviposition, resorb eggs and discontinue contributions to the biocontrol agent's population.

This temperature range will help to refine predictions of when the weevil population will start to reproduce at the start of spring and stop at the onset of winter. As shown above, water hyacinth continues to reproduce during winter, but if there is a preceding period in autumn during which the weevils have stopped laying eggs, this presents a window of time during which the weed can be treated with herbicide to stop asexual reproduction via ramets, with minimal impact on the biological control agents as few eggs or early instar larvae will be in the leaves.

4.2 Materials and Methods

The experiment was repeated three times in 2007, during May, July and September, allowing an autumn, midwinter and spring examination of the effect of temperature on ovariole development.

4.2.1 Experimental Setup

Adult *Neochetina* weevils were collected from water hyacinth grown in outdoor pools at either the University of the Witwatersrand, Johannesburg or PPRI Weeds Division, Pretoria and sorted according to sex. In May 2007, adult weevils were released onto whole water hyacinth plants housed in four 60 *l* plastic tubs filled with 40 *l* tap water, with standard nutrients added. Approximately 20 female and 10 male weevils were released into each tub which were then placed in constant temperature rooms maintained at five, 12, 15 and 20° C. Five weevils were removed from the initial population to act as a control or reference group. In the following three weeks, five female weevils were removed from each of the different temperatures each week and dissected to assess the reproductive state of their ovaries. Adequate numbers of weevils were not always found, resulting in variable sample sizes.

The second and third repeats of the experiment, during July and September respectively, used smaller, sealed containers, allowing for successful retrieval of equal numbers of weevils for each treatment. Adult weevils were placed into 1 *l* polythene containers. A sub-sample of the population at the beginning of the experiment was again collected to act as a control. In July, approximately 60 female weevils were randomly divided between each of the four temperatures, allowing for the removal of five weevils per temperature per week, for a period of three weeks. In September, the number of weevils placed at each temperature was increased to allow for the weekly removal of ten female weevils for an extended period of four weeks. Between five and ten males were added to each container for both the second and third experiments. Weevils were provided with fresh water hyacinth leaves daily. Each leaf was cut and kept turgid by wrapping the petiole in damp cotton wool and inserting it into a small, water-filled shell vial. For all three repeats, once female weevils were removed from their respective chamber they were stored in 70% alcohol until dissected.

In addition, weevils collected in the preceding year from 14 field sites around South Africa were also dissected, allowing for comparison of results obtained from the laboratory with actual field data. Samples from five sites were used, three of which were characterized as being ‘warm’ sites, in that they receive no frost throughout the year, and two of which were classified as ‘cold’ sites which regularly experience frost throughout winter. The warm sites were the Mkadhzi Spruit in the Kruger National Park, Enseleni River in northern KwaZulu-Natal and Mbozambo Swamp, near Stanger in KwaZulu-Natal. The cold sites, both situated in Gauteng, were Farm Dam and Delta

Park. *Neochetina eichhorniae* has established at all five sites, while *N. bruchi* has only established at Enseleni River and Farm Dam.

4.2.2 Dissection Technique

Female weevils were removed from alcohol and allowed to dry, until the species identity could be determined. A pin was inserted through the dorsal portion of the thorax, securely fixing the beetle in a water-filled wax dissection tray. Dissections were accomplished under water using 16X magnification. The elytra were removed using fine forceps, exposing the membranous wings, which were also removed. At this stage, the fat content of the weevil was assessed, to ensure that the ovaries had declined as a result of temperature, rather than food quality. Fat content was classified as either high or low; "high" individuals had enough fat bodies to obscure internal organs, in "low" fat individuals the organs were clearly visible. An incision was made along the median line of the abdominal tergites which were then slowly removed using fine forceps. Fat bodies were removed from the body cavity to reveal the ovaries which were then classified into one of the four categories above. The number of eggs present was counted in the third run of the experiment, to determine if this differed between ages or experimental temperature.

4.2.3 Analyses

Due to the categorical nature of the data (females were either capable of reproducing or not), contingency tables were constructed, comparing the count of reproducing females between weeks for each different temperature. A Chi-square test of association was run in conjunction with the contingency tables to reveal any significant difference between measures from different temperatures and/or any significant change in the proportion reproducing, taken over the weeks at each particular temperature. Statistica and SAS were used for all data analyses.

4.3 Results

Results from the three trails are presented as graphs showing the proportion of reproducing females for each different temperature over a series of weeks. They reveal a pattern of low to declining reproduction in autumn (May), which shows little response to increased temperature; low reproduction in winter (July), which responds to an experimental increase of temperature; and rising reproduction in spring (September), which was possible to reverse by exposure to low temperature.

The autumn sample showed 40% of beetles collected from the field to be reproductively active (Figure 4.3). This generally declined in all of the treatments, as all beetles stopped maturing eggs after three weeks at all temperatures except 15°C, where all individuals collected at weeks one and two had stopped producing eggs, but one of the two females collected at week three did have developing eggs in her ovaries. Beetles moved to 5°C stopped maturing eggs after one week, in contrast to the females placed at

20°C, where the proportion of reproducing females gradually declined until all females had stopped after three weeks.

The winter sample revealed that only 20% of female beetles collected from the field population had mature eggs at the beginning of the experiment (Figure 4.3). However, after being placed at 15°C and 20°C, this rose to 80% and 100% respectively. Beetles at placed at 12°C reached 60% ovariole development at two weeks, which then dropped to 0% at three weeks. At 5°C the proportion of females with mature eggs remained at around 20% for the duration of the experiment.

In spring all female beetles collected from the field possessed fully functioning ovaries with mature eggs (Figure 4.3.). Beetles exposed to 5°C retained a similarly high level of functioning ovaries for the duration of the experiment. Initially at 15°C and 20°C, the proportion of females with mature eggs declined to 30% and 50% respectively at week two. Thereafter, at week four, this increased to 70% and 80% respectively. At 12°C the proportion of females with mature eggs remained at 100% at week one and continued around that level for the following three weeks.

The combined results of all three experiments (Figure 4.4) indicates a general trend of increasing reproductive capabilities from 12°C upwards, with the greatest proportion of individual beetles producing eggs at 20°C. However, these values are not significantly different from one another given the large variation in the samples. Beetles exposed to 5°C displayed the greatest variation and did not fit the general trend when compared to the other temperatures. The elevated level of reproductive capability in this group can be explained by the extremely low temperature slowing the beetles' metabolism to the point at which it was suspended and the individual remained in the physiological state it was in at the start of the experiment.

The mean number of follicles produced by each female was not significantly different between different age categories of *Neochetina* weevils (Figure 4.5), although there was a general decrease as the weevils got progressively older. Weevils in the youngest age category show the greatest variation in the number of follicles produced. Large variations in the data influenced this outcome.

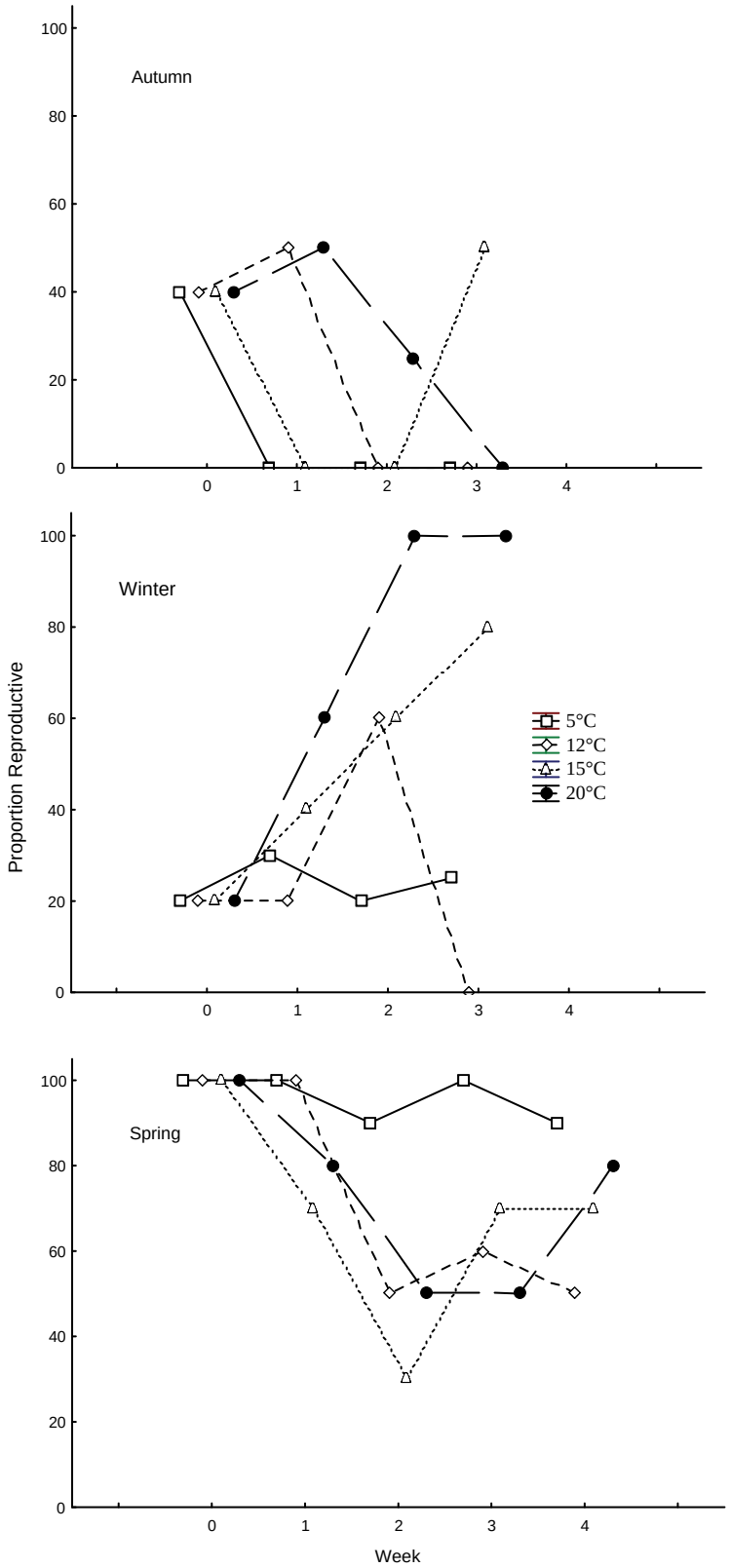


Figure 4.3: Proportion of *Neochetina* females reproducing after being collected from the field in different seasons then placed at the temperatures shown. Autumn = May 2007; Winter = July 2007; Spring = September 2007.

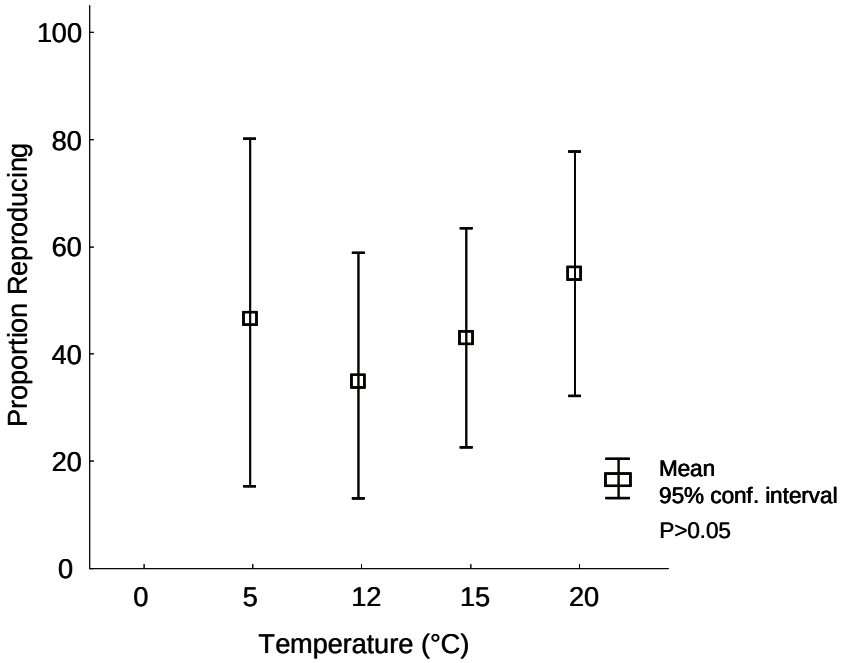


Figure 4.4: The proportion of field-collected *Neochetina* females with parous ovaries capable of producing eggs, after being maintained at a fixed temperature for three or four weeks.

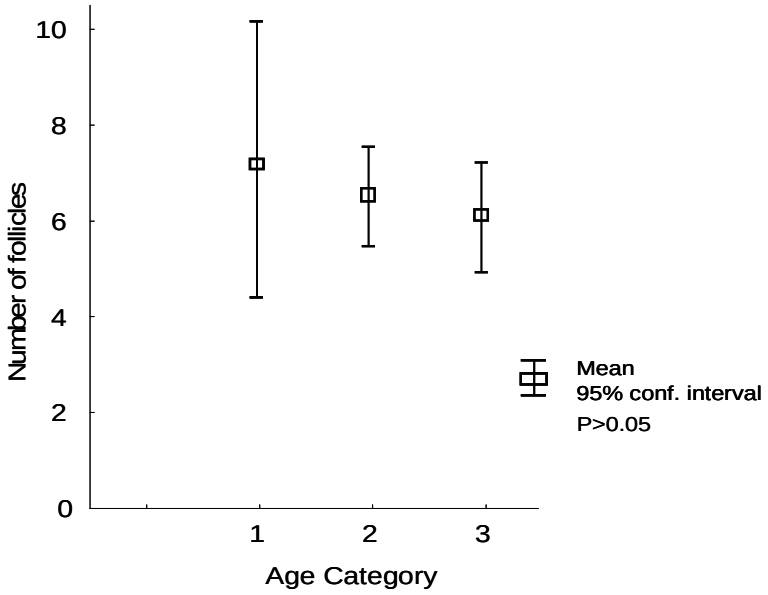


Figure 4.5: The average number of follicles produced by field collected *Neochetina* females in each age group after being maintained at a fixed temperatures of 5°C, 12°C, 15°C and 20°C for three or four weeks.

Neochetina weevils sampled from various field sites showed a large variation in their reproductive capabilities throughout the year (Figure 4.6). Notably during winter there is an almost complete dichotomy in the proportion of females who are reproductively

capable (none) at cold sites and those reproducing (most) at warm sites. In June, in the middle of winter, 100% of females were reproducing at the warm sites in comparison to none at the cold sites. By October females at both warm and cold sites were all reproducing.

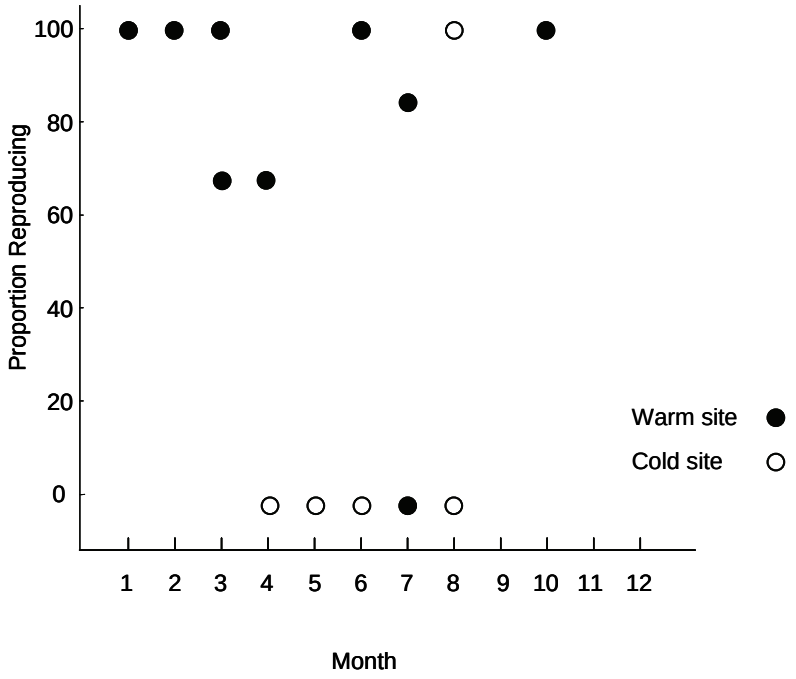


Figure 4.6: The proportion of *Neochetina* females with parous ovaries found at warm or cold water hyacinth field sites throughout the year. Two or more sites may occur in the same month.

4.4 Discussion

Measurements of temperature at water hyacinth field sites around South Africa have shown the thermal environment experienced by *Neochetina* weevils to be varied and often extreme (Chapters 1 and 2). The results of *Neochetina's* reproductive response to temperature presented here show that beetles enter winter in a reduced reproductive state, in advance of reproductive quiescence. This quiescence is not absolute, as some individuals collected from artificial ponds in Johannesburg and Pretoria were parous (Figure 4.3). However, beetles collected from the field in the same region were all nulliparous and had all entered what appears to be a complete reproductive diapause. In contrast, females from warmer lowveld sites were predominantly parous and still reproducing (Figure 4.6). The winter quiescence can be broken by moving beetles to higher temperatures, as shown by the winter trail where beetles quickly resumed follicle production over a period of three weeks (Figure 4.3), and beetles occurring at warm lowveld sites can continue to reproduce throughout the winter (Figure 4.6). By spring all the females are parous, but this state is still susceptible to a drop in temperature, which will decrease the numbers of reproductive individuals (Figure 4.3) and decrease the number of follicles (eggs) those females can produce. Because older beetles produce fewer follicles, female beetles which have overwintered as adults will have a lower

reproductive capability than newly-emerged females that overwintered as third instar larvae. *Neochetina* has been shown not to overwinter as pupae (Chapters 1 and 2).

The responses associated with seasonal changes in the environment are probably one of the best-studied aspects of insects' responses to varying conditions (Chown and Terblanche, 2007). Diapause and dormancy are two such reactions that have received much attention. Diapause is a hormonally mediated state of low metabolic activity, associated with reduced morphogenesis, increased resistance to environmental extremes, and altered or reduced activity. It is usually seen as a physiological state of developmental and reproductive suppression (Tauber et al., 1982) that occurs during a genetically determined stage of the life cycle and generally in response to token environmental cues that precede unfavourable conditions (Tauber and Tauber, 1981).

The fact that the females collected in autumn and placed at 20°C did not continue reproducing even though temperatures were favourable may be due to the fact that once diapause has begun, growth and development are suppressed even if favourable conditions of temperature, food and moisture persist (Tauber and Tauber, 1981). Diapause ends when the organism no longer responds to diapause-maintaining token stimuli. The time-period over which the weevils were exposed to the warm temperatures may not have been long enough for the *Neochetina* females to switch from diapause to reproduction. Otherwise, the weevils may need to experience a period of cold weather before they can revert to a non-diapause state. Females collected in winter and placed at 20°C responded immediately by resuming follicle production. The leaf miner, *Agromyza frontella*, was found to respond strongly to temperature, allowing it to switch from diapause to non-diapause mode late in the season – thereby possibly producing an additional late-season generation (Tauber et al., 1982). Some variation in the number of weevils producing eggs in these trials could be expected due to seasonal adaptations which vary between individuals within species (Tauber and Tauber, 1981). This may explain why the autumn 15°C population was found to be capable of producing follicles after three weeks of being subjected to temperatures that other *Neochetina* females did not find suitably high enough to warrant the reverse of diapause. However, the sample for the third week at 15°C consisted of only two females. It appears unlikely that a temperature of 15°C provides sufficient heat energy for almost any female weevil to produce and maintain eggs. Neither can they actively reabsorb eggs, and the females simply remain in their prior reproductive state.

The constant temperatures in a closed laboratory are not strictly equivalent to field conditions that the beetles will experience. Choice of microhabitat will affect weevil physiology and hence behaviour, which in turn will influence the ecology and distribution of the species (Chown and Nicolson, 2004). The significance of microhabitat selection lies in the temperature and moisture content of the environment, which is continuously modified by solar radiation and air movement and can be greatly

modified especially in amongst plants where leaves respire, provide shade and slow down air movement (Chown and Nicolson, 2004). Selection of sunlit patches will allow for regulation of body temperature (May, 1979), but laboratory populations do not have the opportunity to bask in direct sunlight. However, field-collected individuals largely mirrored results from laboratory held beetles (Figures 4.3 and 6). The decreasing proportion of females with healthy ovaries in autumn may be attributed to seasonal anticipation of declining environmental temperatures (Chown and Terblanche, 2007) or declining food quality (Grodowitz et al,. 1997). Microhabitats at cold sites will be restricted because frost removes water hyacinth leaves, adding to the difficulty of maintaining reproductive capacity. Weevils in the field will experience declining food quality as leaves are damaged and leaf turnover declines, while females in the laboratory trials were fed leaves from healthy growing plants, allowing us to conclude that the reproductive quiescence seen in the laboratory is driven by declining environmental temperatures.

4.5 Conclusion

The preparation of insects for winter can be considered a long-term programmed response to declining temperatures, prolonged periods of cold or, less commonly, to changing photoperiod or dietary cues (Chown and Nicolson, 2004), and although this programmed response does not necessarily lead to diapause, the two processes are often closely related. As the year progresses for *Neochetina* weevils, and temperature declines, the number of new individuals (in the form of eggs) being introduced into the weevil population falls. This population decline results in fewer weevils available to exert herbivory pressure on the water hyacinth population. Come summer, the population re-establishes itself from older females that have overwintered, or, much later, from third instar larvae that have completed development, pupated then emerged and become sexually mature (Chapter 2). By this stage the water hyacinth has asexually reproduced to a point beyond the control of the weevils alone (Chapters 2 and 5).

Spraying the water hyacinth with a sublethal/retardant dosage of herbicide, to effectively freeze the growth of the plants in spring, will allow the weevils to begin reproducing before the water hyacinth plants start to grow and increase the biomass of the mat. This study suggests that at a mean temperature of 12°C the average proportion of actively reproducing female weevils will be 40% or below. From this it is recommended that at cold sites, water hyacinth should be sprayed in autumn when the plants start to produce ramets (Chapter 2) and the average temperature drops to within a range of 12°C to 15°C for more than two weeks, when the weevils will begin to reabsorb follicles. However, spraying must take place before winter as water hyacinth will not take up sufficient herbicide if the plants are not metabolically active and the ambient temperature is below 19°C for three hours after spraying. However, the mean daily temperature must drop for sufficiently long to allow the weevils to respond and adjust their reproduction to the resulting lower temperatures, which will also allow time

for eggs already in the leaves to hatch and for larvae to migrate into the petioles. The response of insects to cold is complex and differs between individuals at different times of the year, as well as between populations in different years (Chown and Nicolson, 2004 and references therein). Therefore, the way in which a population responds to cold may differ from one winter to the next and thus, a successful spray regime should be altered yearly according to the temperatures experienced during that particular year.

While temperature plays a significant role in determining the reproductive capabilities of *Neochetina* weevils, it is unlikely to be the only limiting factor in determining the success of biological control of water hyacinth. This can be seen in the warm field sites and spraying regimes at these sites should largely be driven by plant growth, taking into account the effect that lower temperatures will have on the rate of follicle production and development of the weevil larvae.

4.6 Temperature – General Conclusions

The main points which can be drawn from these studies have already been presented in the summary at the beginning of this report. However, it is worth considering where these findings fit into the terms of reference of the larger project, which are: to determine the effect of climate on biological control; to determine the effect of nutrients on biological control; to integrate herbicides and biological control; and to develop an integrated management plan for water hyacinth.

To date, water hyacinth sites have been mapped around the country and 14 sites have been monitored over two years, measuring water hyacinth plants, insects, temperature and nutrients. This report is the first of its kind to show how water hyacinth and its biological control insects actually respond to temperature in the field. Speculation on the topic has underpinned previous conclusions about the shortcomings of water hyacinth control in South Africa (e.g. Hill and Olckers, 2001), and has been shown here to be largely correct. Now important details of the lifecycles of the organisms are known and will influence further management behaviour of the weed and further selection of biocontrol agents for its control. Significantly, the plant is shown to have the potential to grow throughout the year and most importantly to reproduce asexually during the winter. Conversely, the beetles are susceptible to low temperatures and will stop laying eggs between 12°C to15°C, but the larvae will continue to develop down to temperatures around 10°C. Consequently, the population largely overwinters as larvae, which establish the next generation in the new season as temperatures start to rise. This is biologically advantageous for the beetle which has numerous new hosts in spring, but disadvantageous for biological control because of asynchrony between the reproduction of the plant and the beetles. This asynchrony offers an opportunity for a tactical intervention with glyphosate herbicide in autumn, when plant reproduction can be halted without suppressing beetle numbers, and a second spray in spring to freeze any new plant growth, which will allow the new season's adults to produce an F_1

generation, which should suppress further plant growth as the weed recovers from the herbicide.

The conclusion therefore at this stage is optimistic, in that important progress has been made in working towards an integrated management plan for water hyacinth. What is disconcerting is the low beetle numbers – which remain low even at sites that produce more than two generations per season. Exponential growth of beetle numbers is not seen even at the most favourable sites, despite favourable temperatures and excess plant material. Cannibalism by early instar larvae is suggested to be unlikely to be responsible for low numbers (Wilson et al., 2001, Atteridge, 2009) and it is not clear how glyphosate herbicide sprays will alter this phenomenon. The effect of such sprays could be beneficial because glyphosate is known to enhance sugar production in other plants; or it could be detrimental in increasing cannibalism through reduction of the number of petioles available to the larvae. These topics are explored in Chapter 9 in this report.

CHAPTER FIVE – THE IMPACT OF NUTRIENTS ON THE BIOLOGICAL CONTROL OF WATER HYACINTH

5.1 Introduction

5.1.1 Nutrients in Water

Nutrient enrichment of surface water from anthropogenic (cultural) sources has long been recognized as a cause of eutrophication (Walmsley, 2000). “Eutrophication” is an ecological term used to describe the process by which a body of water becomes enriched with nutrients that promote plant growth. Nutrient enrichment is often found in highly populated and developed areas where water-borne sewage systems and agriculture contribute to elevated loads of nutrients, particularly nitrogen and phosphorus. The increase in nutrients causes water quality and user problems as it increases and promotes the development of both living and decaying biological material (Walmsley, 2000). Very few countries have escaped the problem of eutrophication and this includes South Africa, which has some of the most highly enriched surface waters in the world (Walmsley, 2000). South African water bodies are enriched with phosphorus in the form of orthophosphates, polyphosphates and organic phosphates. Nitrogen is also present in many forms such as ammonium, nitrates and nitrites. These forms of phosphorus and nitrogen are all as a result of agricultural run-off and input from waste-water treatment plants (Walmsley, 2000). Eutrophication causes a serious water quality problem in South Africa in terms of the increased occurrence of floating and rooted aquatic macrophytes and the first remedial step that has been taken is the promulgation of a 1 mgP/*l* standard to be implemented into water catchment areas (Grobler, 1984). This standard was introduced to reduce the quantity of orthophosphates available for plant growth so that waste water or effluent produced by, or resulting from, the use of water for industrial purposes cannot contain phosphates in a higher concentration than 1 mg/*l*. The achievement of the phosphorus standard is however very expensive and there is a wide non-compliance with the standard (Chutter, 1989). Water hyacinth growth is only limited when phosphorus levels drop below 0.1 mg/*l* (Haller and Sutton, 1973).

5.1.2 Water Hyacinth and Nutrients

The linkage between aquatic plant production, nutrients and human activity was first noted in the early part of this century (Walmsley, 2000). Water hyacinth growth can be directly correlated to water nutrient concentrations, particularly those of nitrogen and phosphorus (Heard and Winterton, 2000). Increasing concentrations of nitrogen and phosphorus result in increases in biomass accumulation, ramet production, shoot:root ratio and plant height (Reddy et al., 1989 and 1990).Wilson (2002), working from the literature, found that a nitrogen to phosphorus ratio of 7:1 was optimal for the growth of water hyacinth, but this has not yet been proven in the laboratory or field. Water hyacinth biomass has been shown to increase eightfold in nutrient-rich sites compared to sites that are nutrient-poor (Reddy et al., 1990). Gosset and Norris (1971) showed

that there is a positive correlation between the nitrogen and phosphorus content of water hyacinth tissues and the nitrogen and phosphorus content of the water bodies they were grown in.

Phosphorus is one of the major plant nutrients that can potentially affect growth and nutrient storage by water hyacinth. Reddy et al. (1990) showed that, with increasing phosphorus, all measures of water hyacinth growth increased. The rate of increase was not proportional to the increase in phosphorus, but phosphorus deficiency was found to be a limiting factor for growth and reproduction. Phosphorus concentration is thought to be the main factor underlying eutrophication in South Africa (Thornton and Walmsley, 1982) as well as the primary growth limiting nutrient of water hyacinth (Gosset and Norris, 1971). Reddy et al. (1990) showed the biomass yield of water hyacinth to be highest with an increase of phosphorus up to 1.06 mg/*l*. At low concentrations of 0.06 mg/*l* plant biomass decreased by 50% within the first week (Reddy et al., 1990). Haller and Sutton (1973) found that if the concentrations of phosphorus fell below 0.1 mg/*l* active growth of water hyacinth stops but concentrations above this allowed for growth as well as the uptake of nutrients in excess of the plants requirements. These values represent upper and lower limits within which the growth of water hyacinth can be predicted. Below 0.06 mg P/*l* the plants would be expected to die; between this value and 0.1 mg/*l* the plants will survive but not grow. From 0.1 mg/*l* to 1.06 mg P/*l* hyacinth will actively grow, but above 1.06 mg P/*l* no additional growth is expected for increasing levels of Phosphorus (Figure 5.1). The prevailing range of phosphorus levels in South Africa range from 0.001-2.00 mg P/*l* (Resource Quality Services-DWAF.)

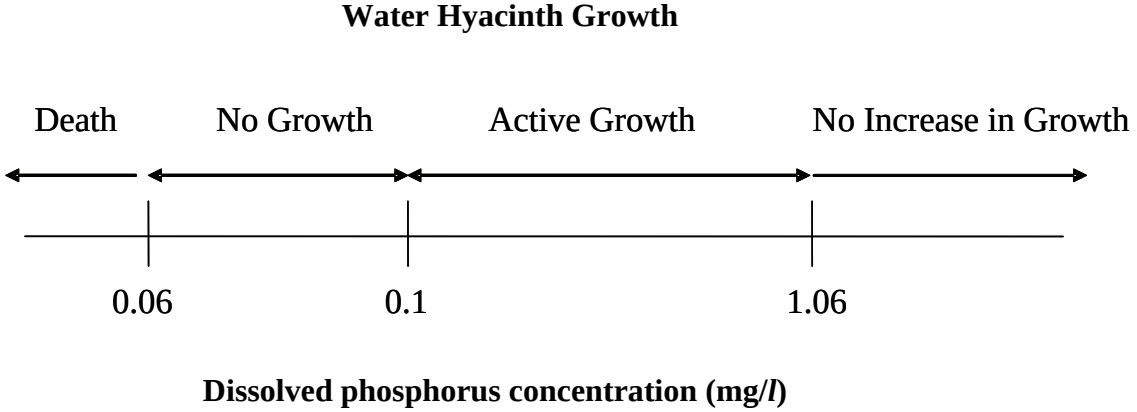


Figure 5.1: Phosphorus thresholds described in terms of their effect on water hyacinth growth.

Above 0.1 mg/*l* of phosphorus, nitrogen levels are not limiting (Haller and Sutton, 1973). Maximum growth is achieved at 21 mg N/*l* (Reddy et al., 1989). Chadwick and Obeid (1966) showed that an increase in nitrogen concentrations from 1 to 25 mg N/*l* increased the number of plants and total dry weight of the plants. Conversely, Reddy et al. (1989) showed that in nitrogen limited water, plant tissue decreased by 75% within

four weeks of growth. Prevailing levels of average total nitrogen in South African waters range from 0.01-2.1 mg N/*l* (Figure 5.2) (Resource Quality Services-DWAF).

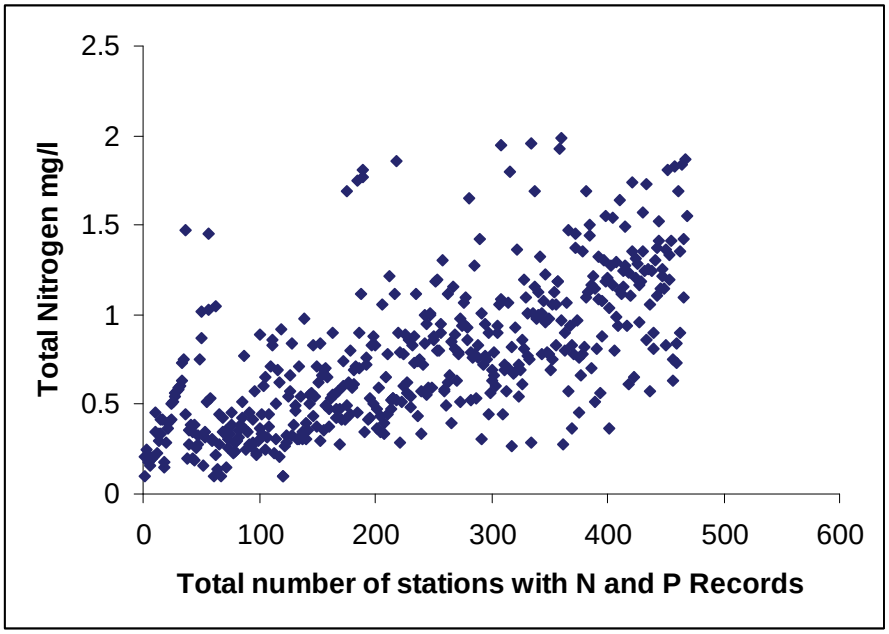


Figure 5.2: Plot of median values of total nitrogen concentration in South African waters at Institute for Water Quality Service (IWQS) recording stations. Field sites were chosen to be representative of this range of nitrogen.

5.1.3 Nutrients and Insects

Nitrogen has a central role in all metabolic processes as well as in cellular structure and genetic coding. Nitrogen is therefore the critical element in the growth of all organisms. Compared to plants, insects have vastly higher nitrogen requirements, in preference to carbohydrates (Mattson, 1980). Most insects have qualitatively similar nutritional requirements, most of which are met by their diet (Chapman, 1998, Mattson, 1980). Supplementary nitrogen promotes enhanced health, growth, reproduction and survival in many organisms (Mattson, 1980). Thus, the higher the concentration of nitrogen in plant tissue, the better the growth response of the insects should be (Mattson, 1980).

The concentration of nitrogen is known to vary widely in many species of plant (Mattson, 1980), and many occurrences of increase in insect pest populations when fertilizer has been added to crops have been recorded (Jones, 1976). Two cases are known in which increased nitrogen and phosphorus levels in the form of fertilizer were used to increase the population density of a biological control agent. The application of nitrogen to prickly pears resulted in increased damage by *Cactoblastis cactorum* (Heard and Winterton, 2000) and local augmentation of water nutrient concentrations facilitated establishment of the weevil *Cyrtobagos salviniae* released for control of *Salvinia*

molesta (Room and Thomas, 1985). In this case attack by the weevil increased the nitrogen concentration of the plant, leading to an escalation in plant damage due to increased reproduction as well as higher feeding rates by the weevil (Forno and Semple, 1987).

Herbivorous insect populations may increase in response to higher quality plants such as those that are found in eutrophic waters (Room, 1990). Water hyacinth, however, has rapid leaf production and hence the effect of some herbivores may be negated due to leaf turnover outstripping growth in agent numbers. Weevil effectiveness against water hyacinth varies, but it seems to be related to plant quality and the preference shown by the two weevil species for plants of different phenologies (Center and Wright, 1991). In a field study by Heard and Winterton (2000), *N. bruchi* was more sensitive to plant quality, resulting in adults feeding on plant tissue that was higher in nitrogen and leaves and plants that were younger in age. A higher number of *N. eichhorniae* adults were found on mature plants rather than developing parts of the plants or young plants (Heard and Winterton, 2000) It is notable that *N. bruchi* showed a higher feeding rate on lush plants growing in nutrient rich water (Center and Dray, 1992). Heard and Winterton (2000) showed that *N. bruchi* performed better at higher nutrient concentrations by inflicting more damage on these plants than on plants grown at lower concentrations. *Eccritiotarsus catarinensis* showed an increase in body size with increasing nutrient concentrations but high concentrations showed no other visible effect on the mirid. At medium levels of nitrogen and phosphorus mirid numbers were the greatest (Coetzee et al., 2007). Herbivory by *E. catarinensis* at higher nutrient concentrations did not have an effect on water hyacinth vigour, although there was a significant reduction in the production of ramets and chlorophyll content of leaves at lower nutrient levels (Coetzee et al., 2007).

5.1.4 Trophic Status of Southern African Waters and Site Selection

In Southern Africa, water pollution has increased due to population distribution, land-use, industrial activity and agricultural activity (Walmsley, 2000). In Lake Chivero, Zimbabwe – the capital's main water supply – water quality is optimal for the growth of aquatic macrophytes. These values averaged 13.5±2 mg/*l* of nitrogen and 2.6±0.6 mg/*l* of phosphorus, exceeding World Health Organisation limits (Nhapi and Tirivarombo, 2004).

Selection of sites for this project to monitor the effect of climate on water hyacinth has necessarily also included a range of nutrient states (Chapter 1). The selected sites encompass a range from oligotrophic to hypertrophic conditions as described by the South African water quality guidelines (Table 5.1). Walmsley (2000) used OECD (Organisation for Economic Co-operation and Development, 1982) criteria to classify the trophic status of water bodies in South Africa (Table 5.2). Thus the water hyacinth monitoring sites that were selected encompass nutrient conditions based on international

guidelines and South African water quality guidelines. These sites were monitored in terms of water quality on a monthly basis to measure seasonal fluctuations in nutrients with the intention of correlating plant and insect performance.

Table 5.1: Criteria used for assessing the trophic status of water bodies in South Africa (South African Water Quality Guidelines DWAF 1996).

Effects	**Average Inorganic Nitrogen Concentration mg/*l***	**Average Inorganic Phosphorus Concentration mg/*l***
Oligotrophic	**<0.5**	**<0.005**
Mesotrophic	**0.5-2.5**	**0.005-0.025**
Eutrophic	**2.5-10**	**0.025-0.25**
Hypertrophic	**>10**	**> 0.25**

Table 5.2: Criteria used for assessing the trophic status of water bodies (OECD 1982) based on phosphorus as the limiting nutrient.

Classification	**Average Total Phosphorus Concentration mg/*l***
Oligotrophic	**0.004-0.01**
Mesotrophic	**0.01-0.035**
Eutrophic	**0.035-0.1**
Hypertrophic	**>0.1**

According to the above criteria, South Africa has a range of water hyacinth-infested sites which range from oligotrophic to hypertrophic. Past studies on water hyacinth growth and nutrients show widely varying levels of nutrients used in experimental trials (Table 5.3). Most of these levels are applicable to the physiological requirements of the plant but not necessarily to South African water conditions.

Table 5.3: Concentrations of nitrogen and phosphorus characterized as “high” and “low” in published studies on water hyacinth growth.

Treatment	Nitrogen		Phosphorus	
	High mg/*l*	**Low mg/*l***	**High mg/*l***	**Low mg/*l***
Half saturation coefficient. N&P concentration at which growth is half maximum rate. (Wilson, 2002)	**0.5**		**0.08**	
Larval density trials. (Wilson et al., 2006)	**4**	**0.4**	**0.57**	**0.057**
Gradually increasing nutrient concentrations. (Xie et al., 2004)	**10**	**5**	**1**	**0.5**
Nitrogen supply rates. Effect on growth and nutrient storage.	**50.5**	**0.5**	**3**	**3**
Phosphorus supply rates. Effect on the growth and nutrient storage. (Reddy et al., 1989,1990)	**20**	**20**	**10.06**	**0.06**
Weevil herbivory. Effects of nutrients. (Heard and Winterton, 2000)	**1.6**	**0.4**	**1**	**0.025**
Mirid Herbivory. Effects of nutrients.(Coetzee et al., 2007)				

Despite the existence of 500 water quality monitoring sites around the country, serviced by Resource Quality Services (formerly Institute for Water Quality Studies), very little is known about the precise nutrient status of water hyacinth-infested waters in South Africa, the effect these nutrients have on the plants in the field, or their populations of biological control agents.

With a view to incorporating nutrient level recommendations in the integrated management plan for the control of water hyacinth the following key questions will be addressed to increase our understanding of the relationship between nutrient status, plant growth and insect population dynamics of water hyacinth-infested waters.

5.1.5 Research Aims

1. Characterize South African water hyacinth sites in terms of nitrogen (N) and phosphorus (P) levels at selected water bodies, and determine if a common ratio exists between these nutrient levels at each site.

2. Characterize the seasonal fluctuation in N and P in water hyacinth-infested waters.

3. Estimate nutrient levels at which biological control can be expected to be effective.

4. Make recommendations on where biological control should be supplemented with herbicidal intervention.

5.2 Materials and Methods

5.2.1 Nutrient Profile of South African Waters

Water quality data (N and P levels) for the last five years from all the monitoring sites in South Africa have been obtained from the Institute for Water Quality Services. These were used to calculate the average nutrient levels at these sites which were compared to growth of water hyacinth at the sites.

5.2.2 Seasonal Fluctuation in Nutrients

At each of the 14 water hyacinth monitoring sites, a water sample was collected at a depth of 15 cm below the water surface approximately every four weeks. This sample was then sent to the Resource Quality Services for analysis. Unfortunately the data set is incomplete for most sites.

Plant leaf tissue samples (leaf three) were also taken every four to six weeks, then analysed to determine total nitrogen (Kjeldahl digestion in Benton Jones, 2001), and phosphorus content (Ulrich et al., 1959 in Benton Jones, 2001). At each site plant and insect parameters were measured as described in Chapter 2.

5.3 Results

5.3.1 Characterization of Water Hyacinth Site N and P Levels

The sites for which data are available reveal that, from a sample of six to nine months per site, all the sites are hypertrophic for phosphorus in the water (Figure 5.3) and eutrophic for total nitrogen, except New Years Dam and Mkadhzi Spruit which are both mesotrophic (Figure 5.4).

Nitrogen:phosphorus ratios are above the 7:1 level, which is favourable for water hyacinth growth, at all sites except Mbozambo Swamp (Figure 5.5). This is a consequence of the site having such extreme levels of phosphorus (Figure 5.4) that its ratio lessens the extraordinarily high levels of nitrogen. Needless to say, this site must be suitable for water hyacinth growth, which is confirmed by the size of the plants measured at that site (Figure 5.6).

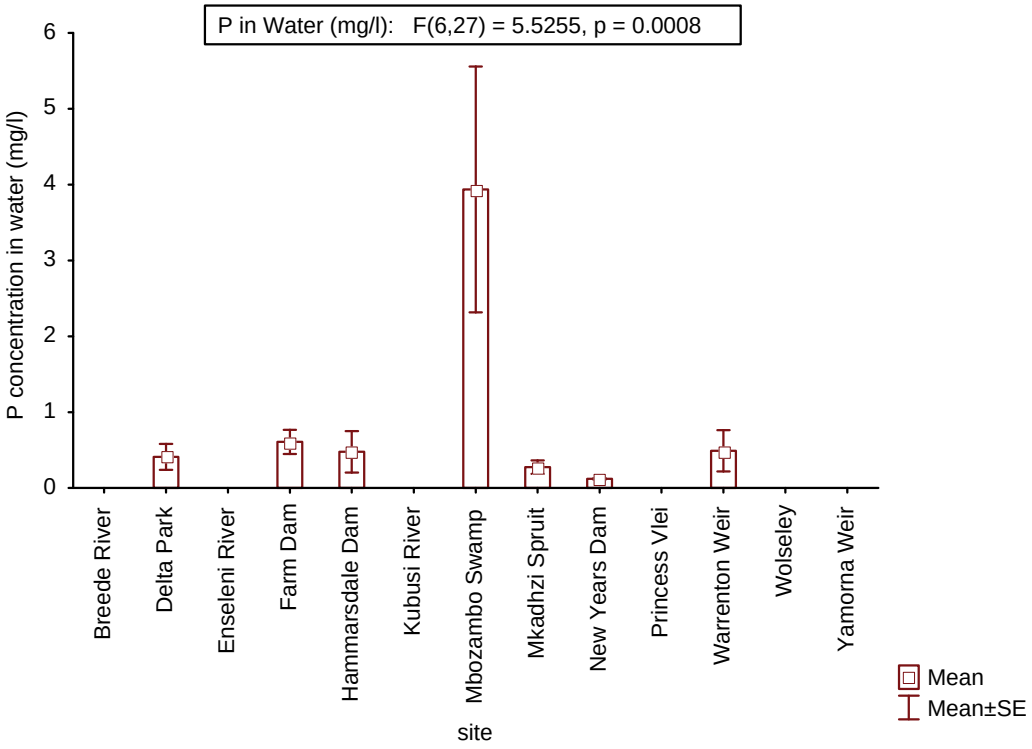


Figure 5.3: Phosphorus concentration in water at different field sites. (n = 6-9 monthly samples per site).

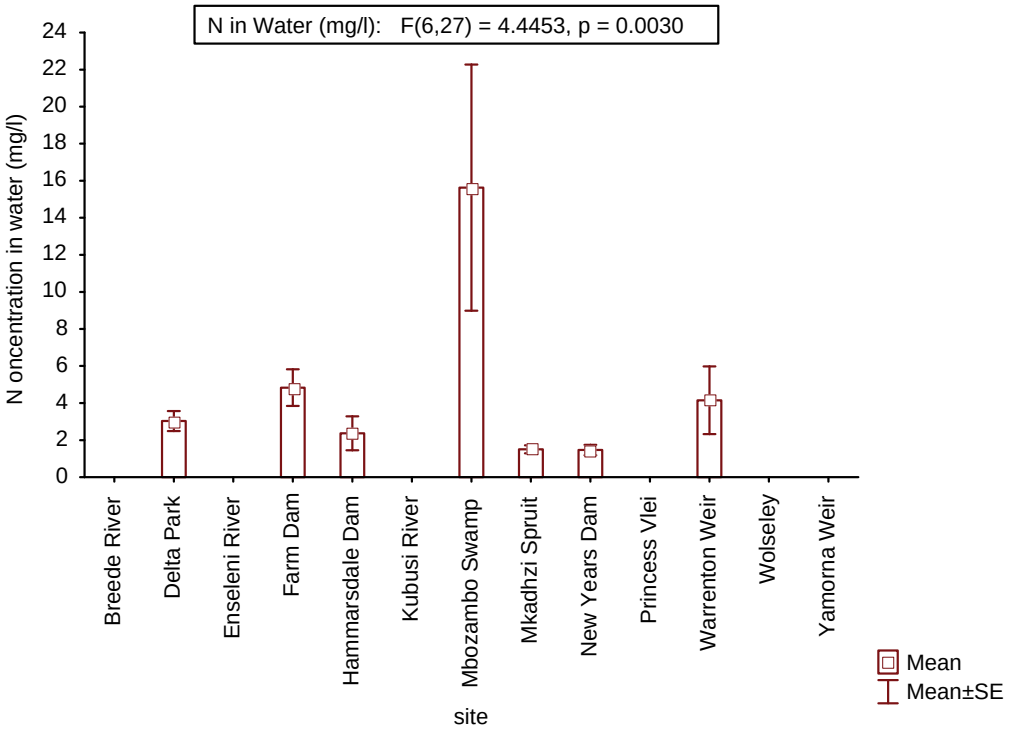


Figure 5.4: Nitrogen concentration in water at different field sites. (n = 6-9 monthly samples per site).

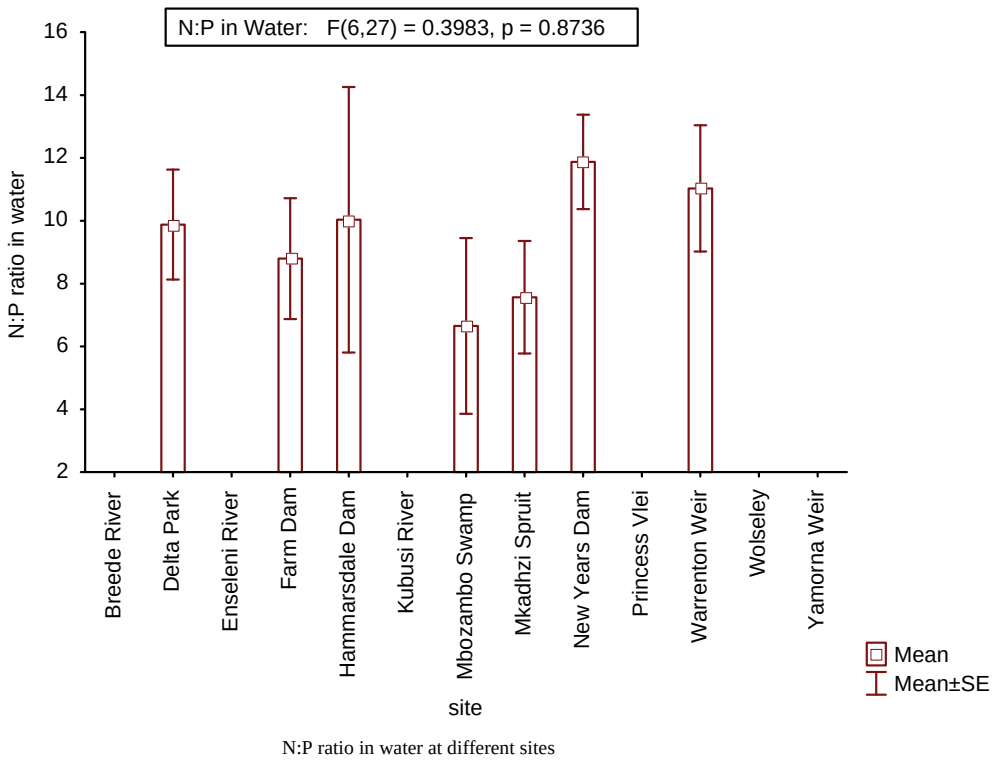


Figure 5.5: Water nitrogen:phosphorus ratios at different field sites. (n = 6-9 monthly samples per site).

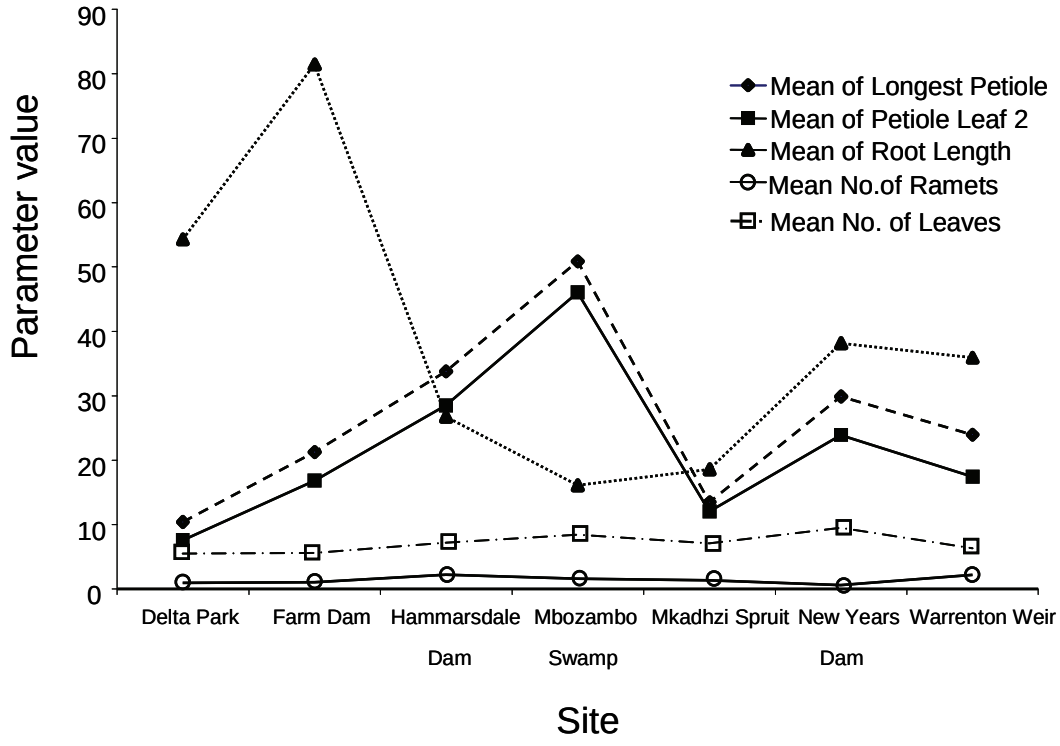


Figure 5.6: Water hyacinth plant measures at different field sites. Means of 20-25 monthly samples. Error bars are omitted and site points are joined for clarity.

Despite large differences in levels of water phosphorus between sites, the concentration is fairly constant in the plants across all sites (Figure 5.7), suggesting that the plants accumulate phosphorus up to a certain limit, beyond which they are incapable of storing more. This is supported by the N:P ratio, which fluctuates from month to month in the water but remains fairly constant in the plant, at about 1 (Figure 5.8).

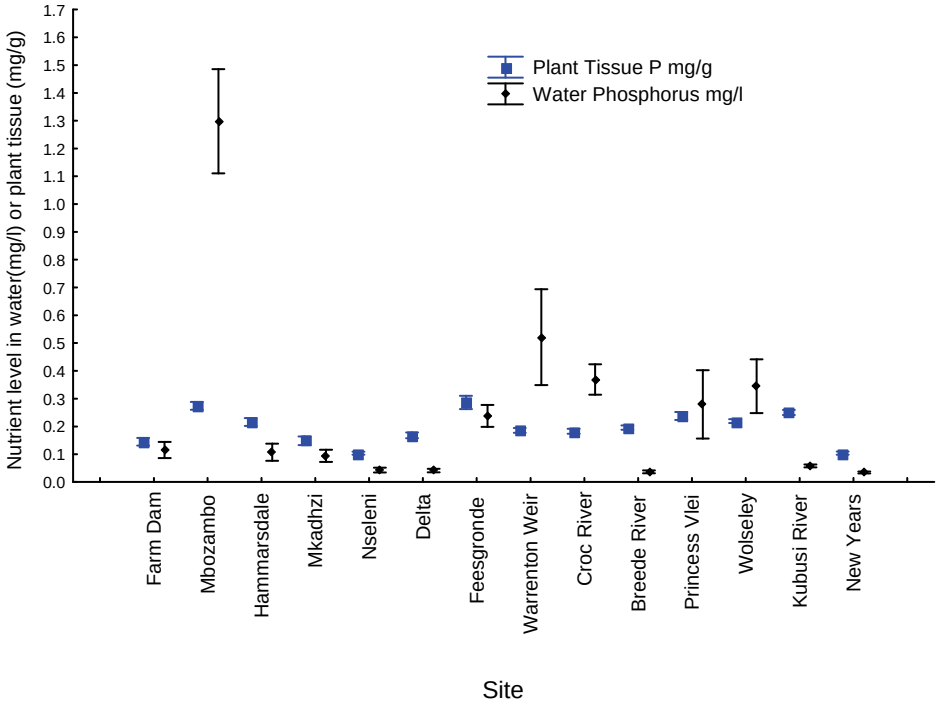


Figure 5.7: The relative (mean) amounts of phosphorus in the water and in water hyacinth tissue at 14 different field sites. Means are from monthly samples taken from between six and nine months depending on the site.

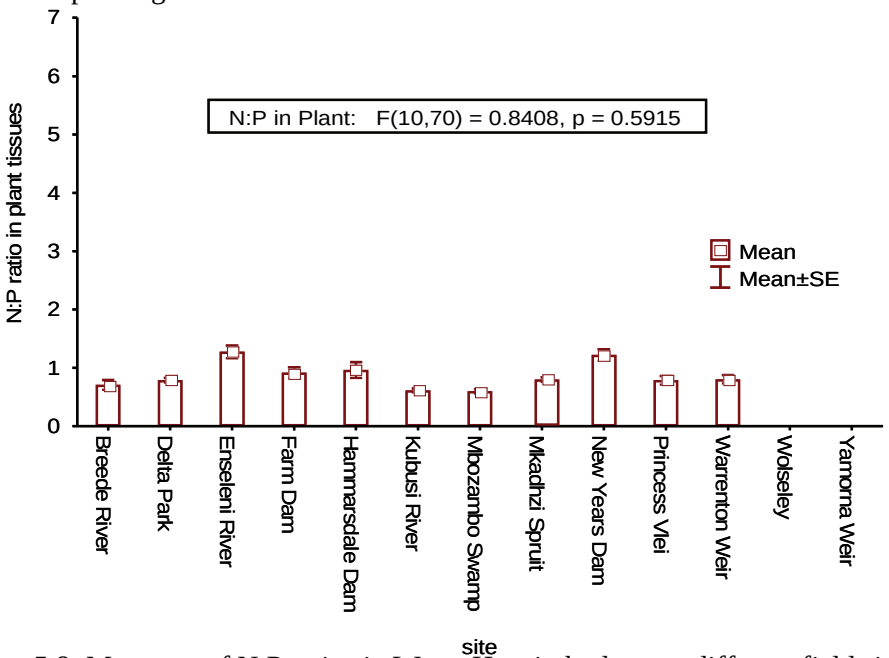


Figure 5.8: Measures of N:P ratios in Water Hyacinth plants at different field sites.

Monthly measures of plant biomass from 11 of the field sites for which at least a year's data had been taken (in most cases two years'), showed how different the sites were in terms of biomass (Figures 5.9a-5.9k). However, some patterns do emerge which are common to all sites. Firstly, virtually all sites reveal a decline in biomass over the approximately two-year sampling period, implying that herbivory pressure from biocontrol agents is capable of reducing the weed population.

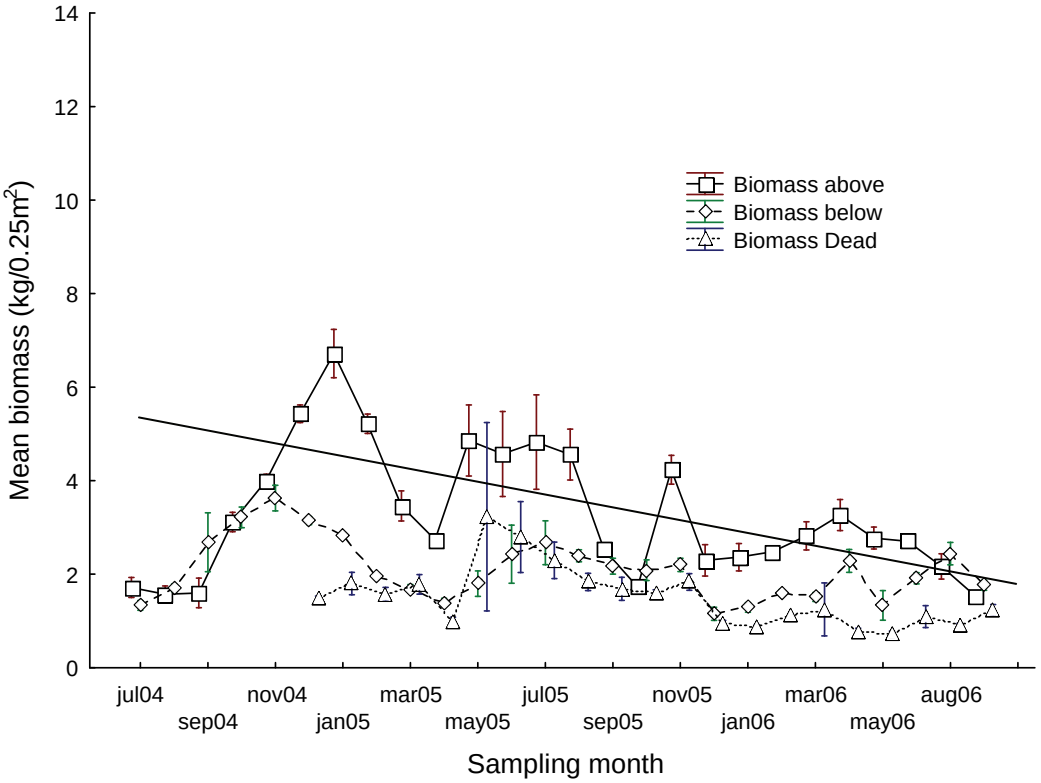


Figure 5.9a: Monthly measures of biomass of water hyacinth plants at Crocodile River. Linear regression of abovewater biomass.

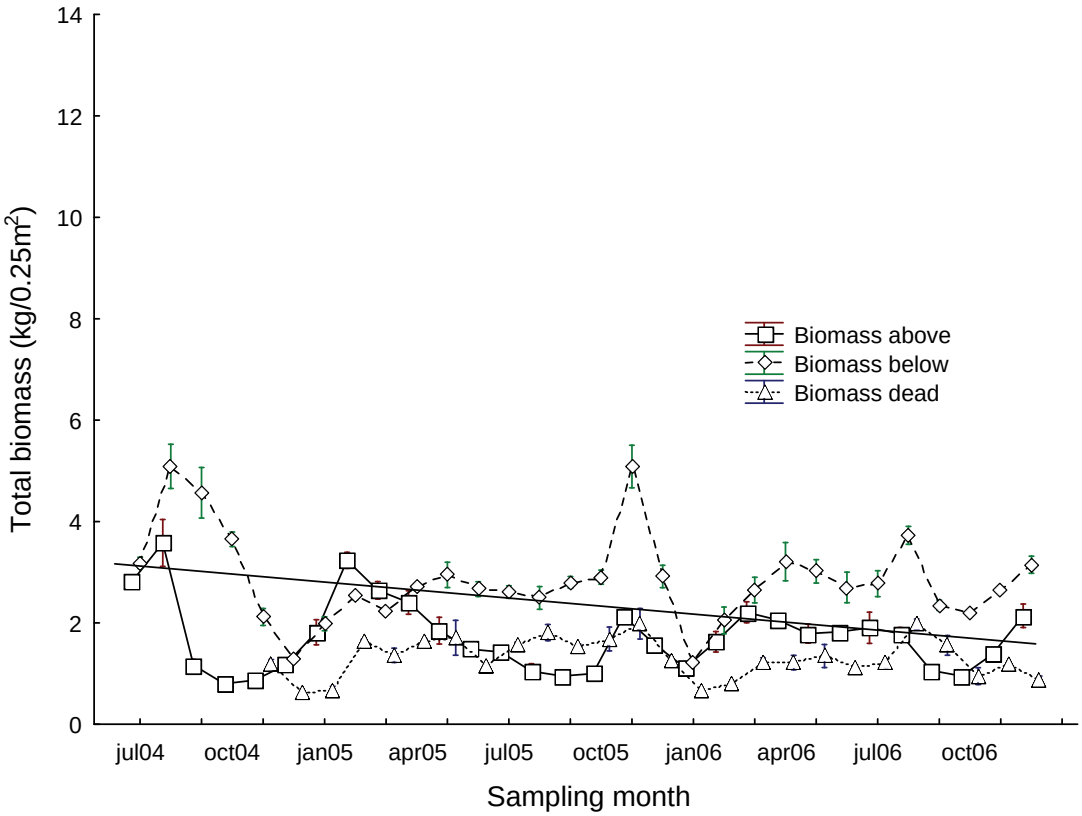


Figure 5.9b: Monthly measures of biomass of water hyacinth plants at Delta Park. Linear regression of abovewater biomass.

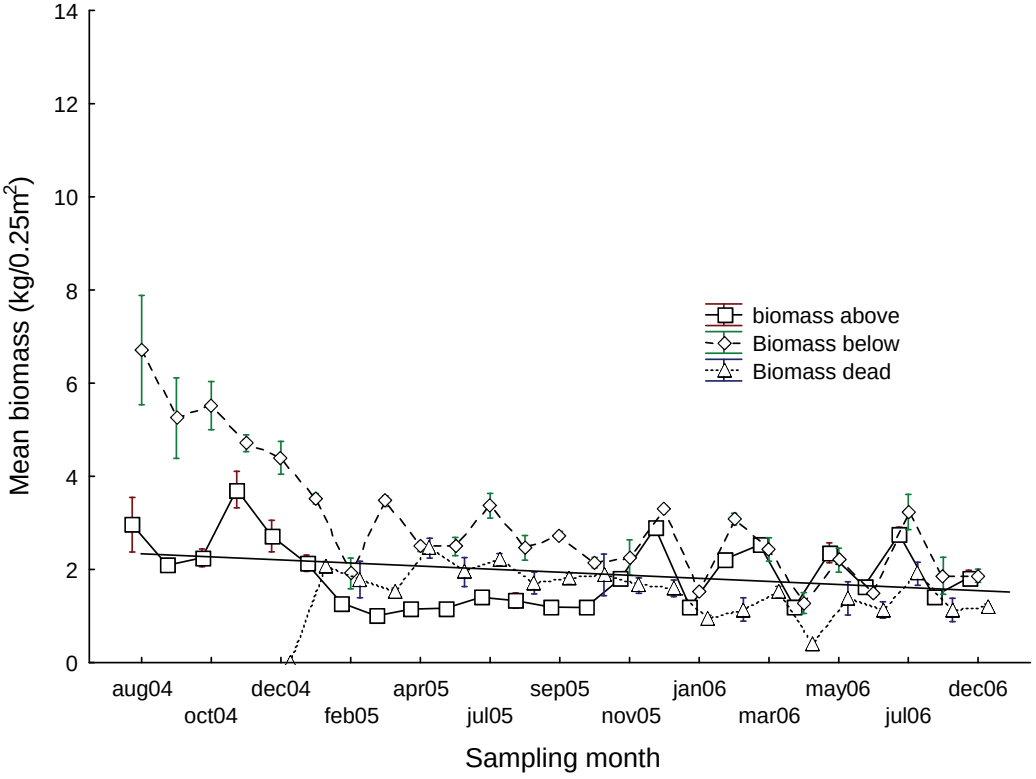


Figure 5.9c. Monthly measures of biomass of water hyacinth plants at Enseleni River. Linear regression of abovewater biomass.

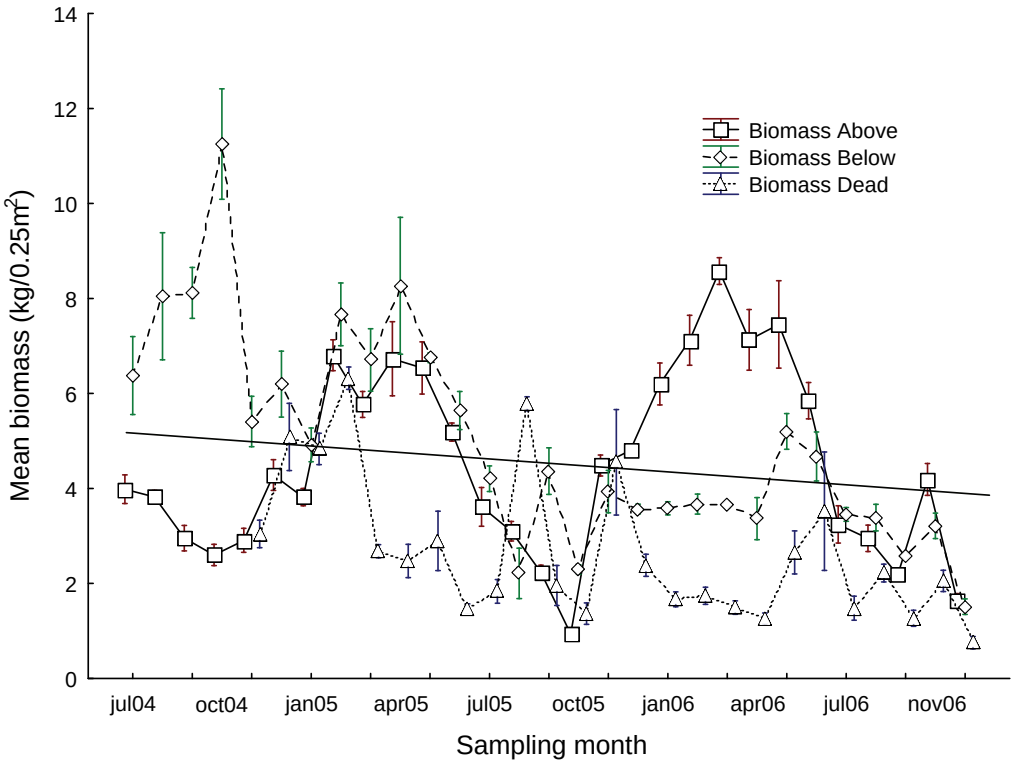


Figure 5.9d: Monthly measures of biomass of water hyacinth plants at Farm Dam. Linear regression of abovewater biomass.

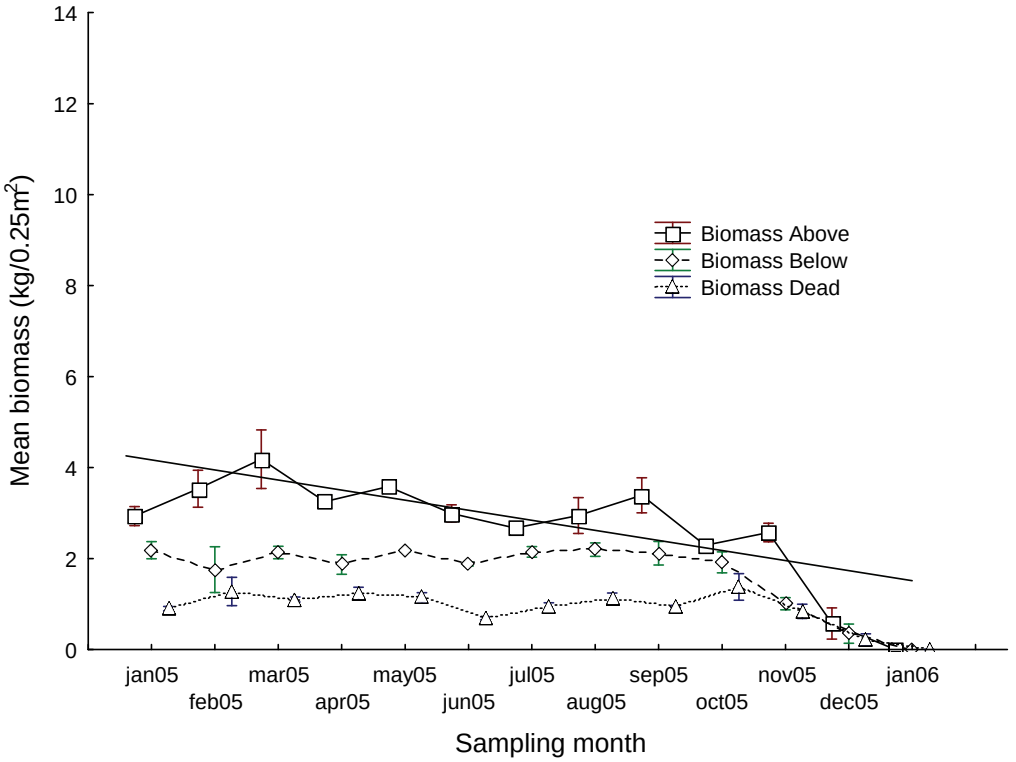


Figure 5.9e: Monthly measures of biomass of water hyacinth plants at Hammarsdale Dam. Linear regression of abovewater biomass.

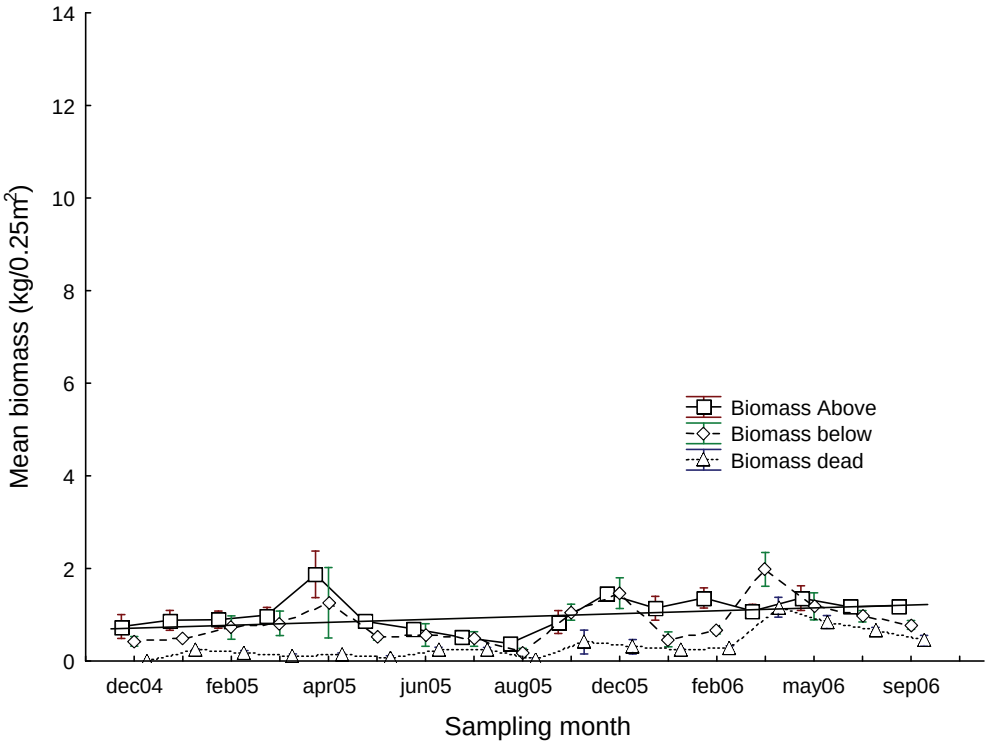


Figure 5.9f: Monthly measures of biomass of water hyacinth plants at Kubusi River. Linear regression of abovewater biomass.

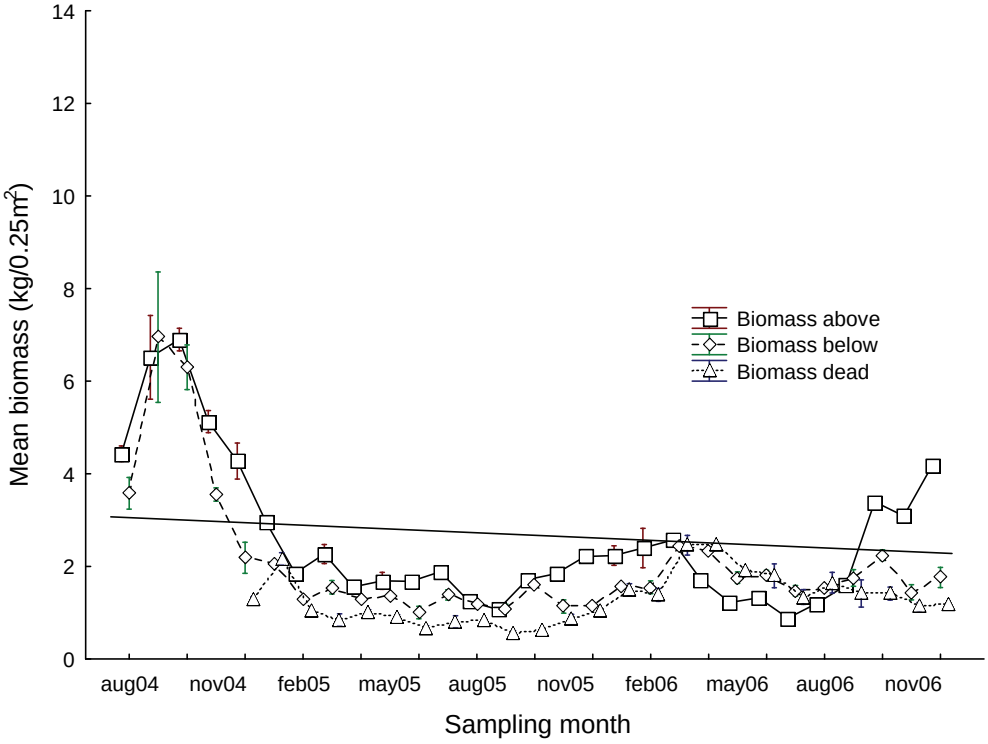


Figure 5.9g: Monthly measures of biomass of water hyacinth plants at Mbozambo Swamp. Linear regression of abovewater biomass.

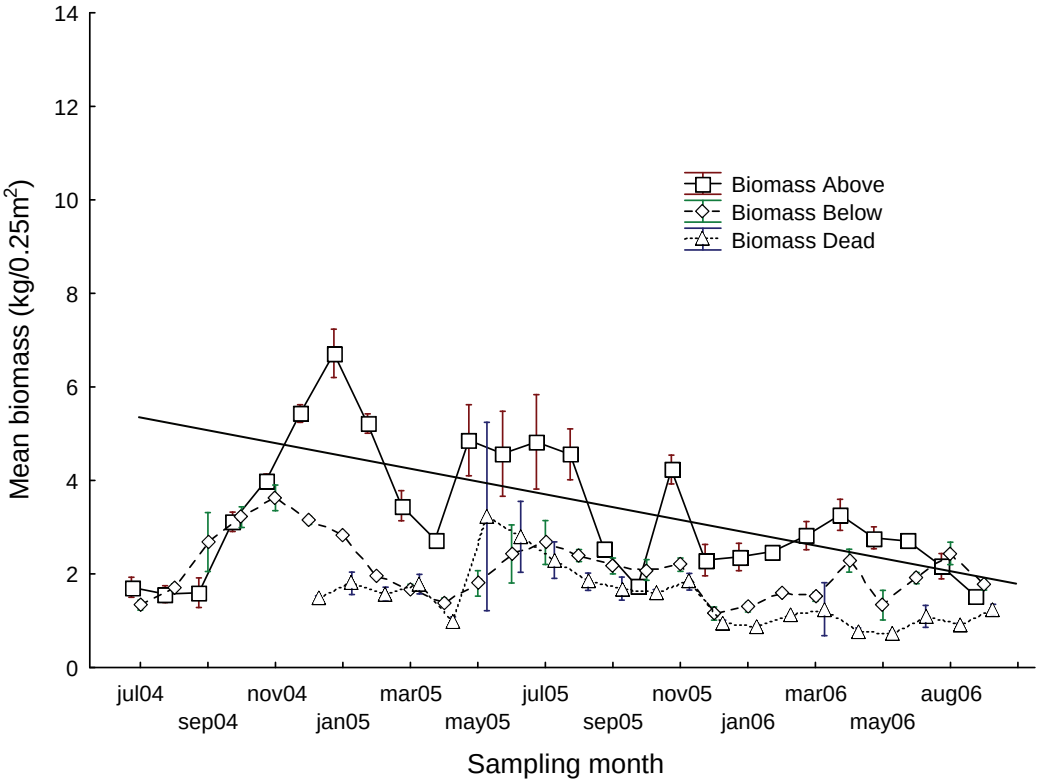


Figure 5.9h: Monthly measures of biomass of water hyacinth plants at Mkadhzi Spruit. Linear regression of above water biomass

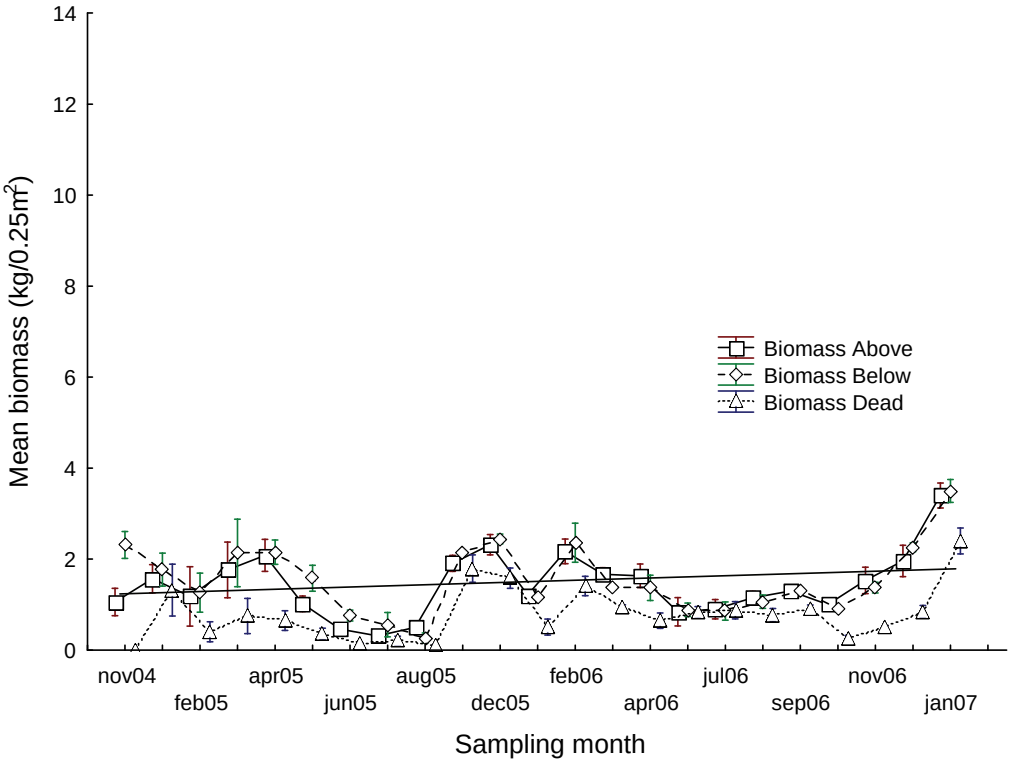


Figure 5.9i: Monthly measures of biomass of water hyacinth plants at New Years Dam. Linear regression of above water biomass.

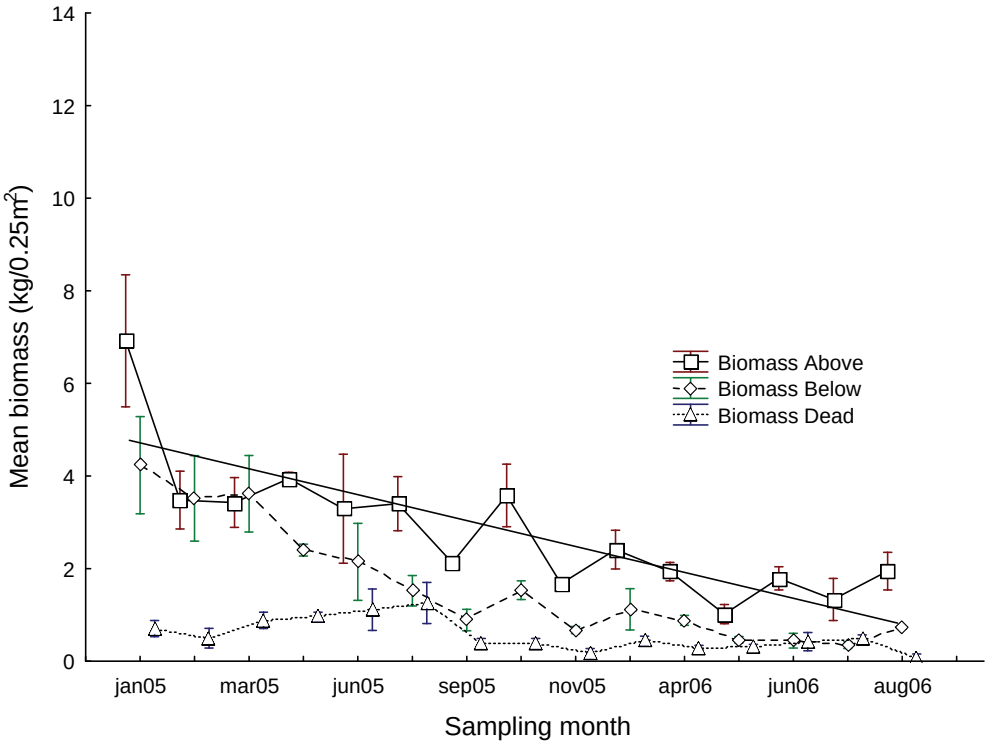


Figure 5.9j: Monthly measures of biomass of water hyacinth plants at Princess Vlei. Linear regression of abovewater biomass.

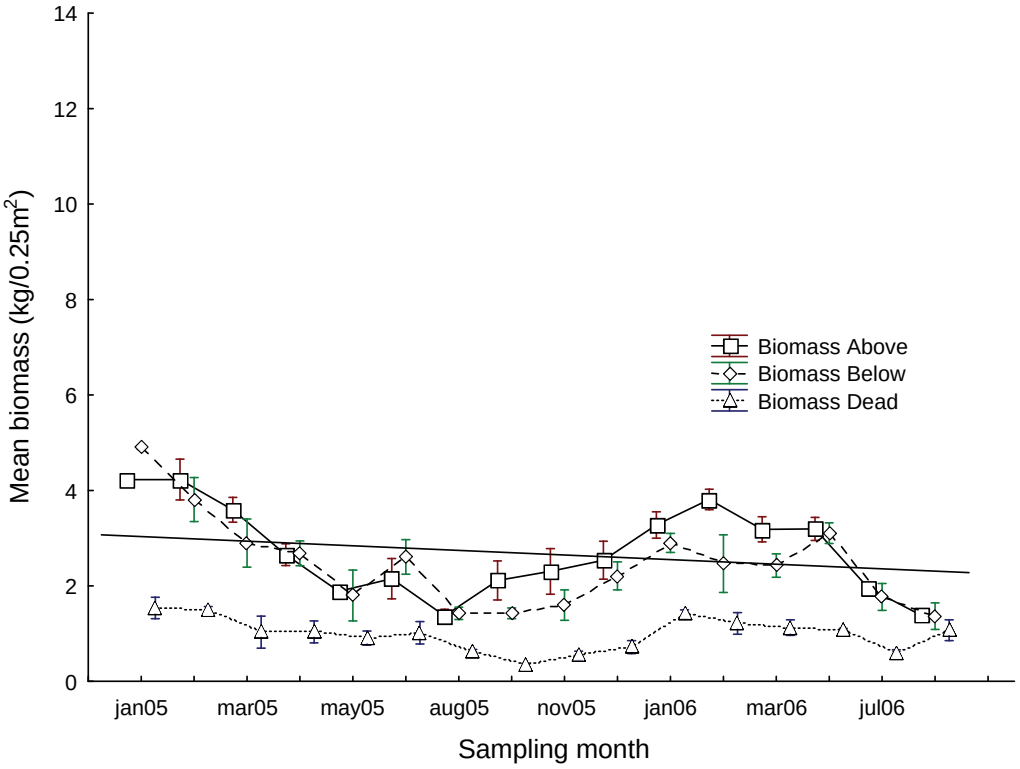


Figure 5.9k: Monthly measures of biomass of water hyacinth plants at Wolseley. Linear regression of abovewater biomass.

Combining nutrient data from all the field sites showed that water hyacinth responds to increased water nutrients by producing longer petioles (Figure 5.10) and shorter roots (Figure 5.11).

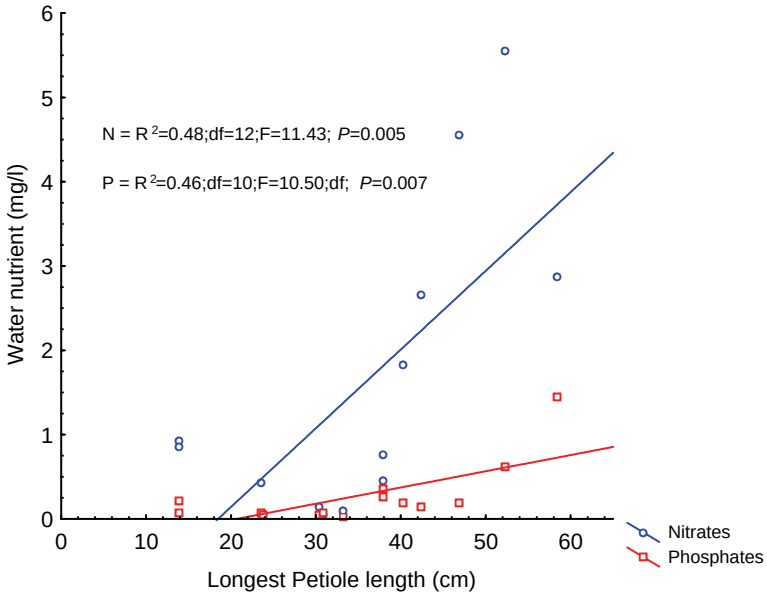


Figure 5.10: The effect of water nutrients on water hyacinth petiole growth. Data taken from field sites shown above.

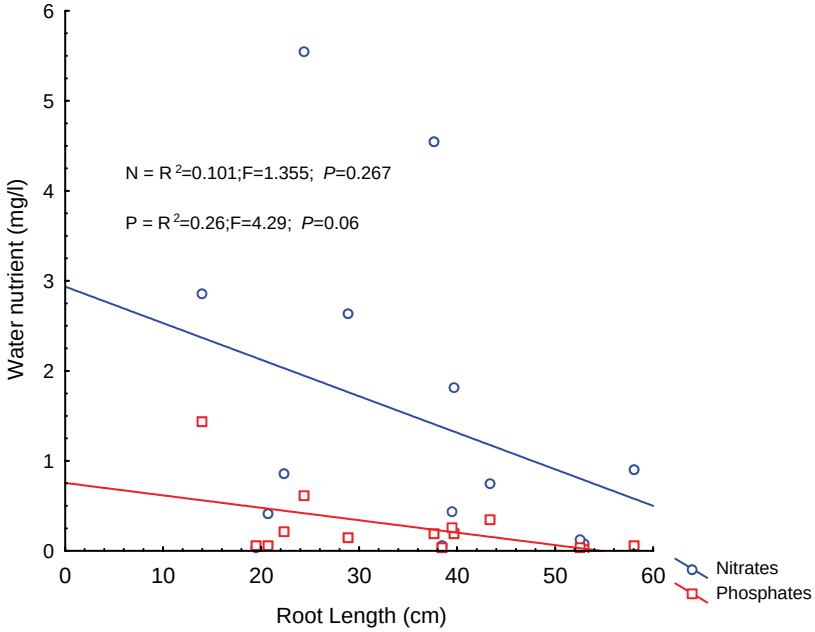


Figure 5.11: The effect of water nutrients on water hyacinth root growth. Data taken from field sites shown above.

5.4 Discussion

The selected field sites encompass a range of nutrient regimes across South Africa, however, with regard to phosphorus levels all sites can be classified as hypertrophic. New Years Dam and Mkadhzi Spruit are the only two sites that can be considered as mesotrophic for nitrogen, while all the others sites are eutrophic. New Years Dam (Figure 5.9i) is under biological control which may be due to very low phosphorus levels (Figure 5.3) which dropped below the 0.1 mg/*l* threshold for water hyacinth growth on two occasions, when active growth of water hyacinth is expected to cease (Haller and Sutton, 1973). All other monitoring sites remain above this threshold, indicating that phosphorus is not a limiting nutrient for water hyacinth in South African waters. Hypertrophic sites such as Mbozambo Swamp (Figures 5.2 and 5.9g) show large changes in levels of water nutrients which may be related to rainfall or effluent inflows from a nearby paper mill; nevertheless, the level of nitrogen and phosphorus in the plants is fairly constant across all sites.

Despite the eutrophic nature of all of the field sites, the general trend shows either stability or a slight reduction in water hyacinth biomass (Figure 5.9). This cannot be directly attributed to biocontrol as there are no sites without agents which would act as an experimental control, and with which comparisons could be made. Nevertheless, seven of 11 sites showed a negative trend in water hyacinth biomass over a two-year period, and each of the three other sites each has a very low average water hyacinth biomass and suggests a stable rather than increasing biomass trend. Wilson et al. (2001) suggest that biomass is the best population measure of water hyacinth occurrence. Biomass measures fluctuate widely at each site and between sites, largely because plant density can be affected by wind and water flow, which may pack or loosen the hyacinth mat overnight. The number of leaves and ramets per plant is fairly consistent across sites, and within sites, as the plant appears to accumulate biomass by lengthening the petioles (Figure 5.6) rather than growing more densely.

Given the eutrophic nature of the field sites and South African fresh water in general (Walmsley, 2000), the apparent reduction in water hyacinth biomass is encouraging. However, Coetzee et al. (2007b; 2009) have shown that the water hyacinth mirid *Eccritotarsus catarinensis* is unable to curtail the growth of the weed under high-nutrient conditions. Bownes (2009) has reached a similar conclusion for the water hyacinth grasshopper *Cornops aquaticum*: despite its voracious feeding behaviour in the laboratory, it might not be able to satisfactorily suppress the weed's growth at densities of the insect expected in the field. New Years Dam is the only low-nutrient site of the selection with average water nitrogen levels at about 2 mg/*l* and phosphorus levels of 0.1 mg/*l*. This is the only site which is considered to be under good biological control, and this is attributed to its oligotrophic status, but given eutrophication trends in this country and worldwide (Harding, 2008), biocontrol agents of aquatic weeds will face an increasingly difficult task. Lake Victoria is also considered to be a biocontrol success

story; and it also has extremely low levels of nitrogen and phosphorus, estimated to be 0.640 mg/*l* and 0.074 mg/*l* respectively (COWI 2000). However, these conditions are unlikely to persist at either New Years Dam or Lake Victoria. Lake Victoria, for instance, has shown an increase in chlorophyll-a from 70 ug/*l* in near-shore areas of the lake to 170 ug/*l* over the last 40 years, resulting in a twofold reduction in the secchi transparency of the lake (Talling and Talling, 1965; Talling, 1966; Rutagemwa et al., 2006).

Even if the downward trend seen in water hyacinth biomass at the majority of field sites can be regarded as evidence of biocontrol, the slope of each reduction is generally low, indicating a slow decline of the weed at these sites. Given the instability of the sites, brought about by weather – cold winters and severe flooding – or inappropriate management interventions, which may severely reduce biocontrol agent populations, a management tool is required to assist the biocontrol agents in rapidly reducing hyacinth populations when required, but without stimulating see-saw swings of both the weed and agents. Herbicides have a potential role as a hyacinth management tool (Ueckermann and Hill, 2001), but it remains to be seen if they can be utilised alongside biocontrol agents, to maintain water hyacinth at levels which will be acceptable to water users and managers.

CHAPTER SIX – BIOLOGICAL CONTROL OR HERBICIDES FOR WATER HYACINTH CONTROL? OR BOTH?

6.1 General Introduction

Water hyacinth has earned its reputation as one of the world's most damaging aquatic weeds in terms of its invasive potential and impact on aquatic ecosystems (Cock et al., 2000), where it threatens the socio-economic development of Africa (Chikwenhere and Phiri, 1999). The development of biological control has been motivated by a need to reduce the abundance and distribution of this invasive alien where chemical and mechanical controls alone were not cost effective (Harris, 1993). Effective and sustained use of biocontrol as a tool to manage water hyacinth infestations across the world includes examples from Africa, South Africa, Australia, USA, Sudan, India and Papua New Guinea (Julien et al., 1999).

6.1.1 Biological Control of Water Hyacinth on Lake Victoria (East Africa): A Biocontrol Success Story

Lake Victoria, bordering Kenya (6%), Tanzania (51%) and Uganda (43%) is the second largest freshwater lake in the world, with a surface area of 68,800 km^2 and a catchment of 258,700 km^2. This enormous lake is, however, shallow, with a mean depth of 40 m, which makes it sensitive to nutrient loading (Hecky et al., 1994). As the economic backbone for the riparian states, the lake provides food, water, transport, recreation and tourism. By 1990, water hyacinth covered an estimated 15,000 ha along Lake Victoria's shoreline of Kenya, Tanzania and Uganda (Cilliers et al., 2003). Various control measures such as mechanical clearing and use of herbicides such as glyphosate and 2,4-D, although practiced, were not feasible in the long run due to high costs. The herbicides posed long term effects such as fish kills resulting from deoxygenation; and more importantly, the lake is a source of potable water to the shore-line communities and the use of herbicides compromised the quality of available water (Mallya, 1999).

Efforts to combat water hyacinth infestations culminated in the importation of *Neochetina bruchi* Hustache (Coleoptera, Curculionidae) and *N. eichhorniae* Warner into Tanzania in 1995. By 1998, the extent of weed infestation on the Ugandan shoreline had been reduced by an estimated 80% (Cock et al., 2000) and by 1999, the infestations had been reduced by 80% on the Kenyan shoreline, with adult weevil numbers varying from zero to 32 per plant. By 2001, the hyacinth infestations on the lake had been reduced to about 2000 ha (Cilliers et al., 2003). This massive decline coincided with an increased weevil population and the El Nino rains that lashed the riparian countries in late 1997 (Cilliers et al., 2003). The severe weather caused the lake level to rise 1.8 m, creating high winds and wave action that supported the breakdown of plants already weakened by biocontrol agents. The unusual weather – including reduced sunshine – rather than the weevils has been credited for the demise of the weed by some commentators (Williams et al., 2005). However, this view has been strongly

refuted by an international group of biocontrol adherents (Wilson et al., 2006). Nevertheless, Lake Victoria, where data exists, is known to be low in dissolved nutrients, specifically phosphorus at < 0.1 mg/*l* which limits the growth of weeds (Gikuma-Njuru and Hecky, 2004). This would be exaggerated by the dramatic water level rise and consequent further dilution of dissolved solutes. The favourable annual mean tropical temperature of 26°C may also have contributed to the success of biocontrol through accelerated weevil development.

6.1.2 Biological Control of Water Hyacinth in Benin (West Africa): Another Biocontrol Success

Water hyacinth was first observed in Benin in 1977 (Van Thielen et al., 1994) and by the late 1980s had gained the status of a major aquatic weed, affecting socio-economic development of rural populations. The use of herbicides remains constrained because the rivers support huge populations of rural communities that depend on the water source for domestic use, including drinking and other activities like fishing, transport and agriculture (Ajuonu, personal communication). Biological control culminated with the release of the two weevils, *Neochetina bruchi* and *N. eichhorniae,* a pyralid moth *Niphograpta albiguttalis* Warren (Lepidoptera, Pyralidae) and a mirid, *Eccritotarsus catarinensis* Carvalho (Hemiptera, Miridae) between 1991 and 1999. The mirid and the moth have failed to establish, apparently displaced by a local generalist species. The weevil release effort was fairly modest: 6500 adults and 17000 immatures of *N. eichhorniae* and around 2000 adults and 5500 immatures of *N. bruchi,* both of which have established (Van Thielen et al., 1994; Neuenschwander et al., 1996). Water hyacinth cover was reduced from 100% to about 5% at Tevedji, Lihu and Kafedji on the Oueme River within eight years, while in just five years the same level of control was achieved on Lake Azili, where the weevils had dispersed on their own from the nearest release site 15 km away (Cilliers et al., 2003). The two weevils have established at all release sites, with *N. eichhorniae* being the dominant species in terms of numbers (Cilliers et al., 2003).

The success of biological control has resulted in an increased income of US$ 30.5 million per year, which translates to a benefit to cost ratio of 124:1 (De Groot et al., 2003). The effectiveness of biocontrol in Benin has been attributed to weevil numbers continuing to build up, possibly due to favourable environmental conditions such as annual temperature ranging from 24°C to 31°C, low salinity, water flow and wind and wave action which break up mats of the weed (Ajuonu et al., 2003). The nutrient status of the water bodies is not thoroughly researched and is therefore not available.

6.1.3 Biological Control of Water Hyacinth in Nigeria and Elsewhere

The rapid spread of water hyacinth in Nigeria was attributed to the interconnection of water bodies, receiving an annual influx from the Niger River (Cilliers et al., 2003). Information pertaining to biocontrol agent release and establishment from this region is sparse, presumably because no internal bodies have taken it upon themselves to address biocontrol as a potential solution to the problem. At the onset, water hyacinth control efforts involved physical removal of the weed, but the results were unsatisfactory and expensive (Farri and Boroffice,1999). Biological control was effected by the release of *Neochetina* weevils between 1994 and 1995. It is reported that the water hyacinth infestation was visibly reduced by 2001 (Cilliers et al., 2003), possibly assisted by annual temperatures ranging from 23° C to 32°C.

Successful biocontrol initiatives have also been reported from Niger, Ghana, on Lake Kariba and Shire River in Malawi, and Côte d'Ivoire (Cilliers et al., 2003). In the absence of hard data, is reasonable to assume that the water nutrient load in these developing countries will be lower than that of South Africa, where the agro-industry and heavy industries contribute substantially to eutrophication of water bodies (Walmsley 2000).

In conclusion, the success of biological control in Africa is associated with favourable temperatures ranging from 23°C to 32°C that maintain high rates of development of biocontrol agents allowing them to reach damaging population numbers (up to 32 per plant) (Cilliers et al., 2003). The oligotrophic or mesotrophic status of the water bodies with phosphorus levels below 0.1 mg/*l* limits the growth of the weed as a result of which the biocontrol agents are able to overcome it. However, in South Africa, biocontrol has not enjoyed the same success rate because of low, unfavourable temperatures and eutrophic waterways (Hill and Olckers, 2001; Hill and Cilliers, 1999).

6.1.4 Biological Control of Water Hyacinth in South Africa

Biological control has been effective at New Years Dam in the Eastern Cape Province, and at Hammarsdale Dam in KwaZulu-Natal Province (Hill, 2003). Control is usually attributed to favourable temperatures and low nutrients at these sites. New Years Dam is temperate with an annual mean temperature of 20°C (Chapter 2) and is oligotrophic with phosphorus levels below 0.1 mg/*l* (Chapter 3) as a result of which the agent population overcomes the weed. Conversely, at Hammarsdale Dam, although the annual mean temperature of 18°C is fairly low, the site is not cold enough for winter frost and is eutrophic with phosphorus level above 0.1 mg/*l*. Biocontrol appeared to be suppressing the weed infestations, but the site was cleared with herbicide in February 2006 so this hypothesis could not be tested. However, in the majority of the country, where temperatures range from -1°C to 35°C biocontrol remains hampered by the inability of the biocontrol agents to establish or flourish at infestation sites with cold winters and frost (Chapter 2; Hill, 2003).

6.1.5 Integrated Control of Water Hyacinth on the Enseleni Water System, South Africa

Water hyacinth was first recorded on the Enseleni/Mposa Rivers and Lake Nsezi (northern KwaZulu-Natal) in 1982, covering approximately 1.5 million square meters of water (Ashton, 1982). Glyphosate at recommended doses was sprayed on an ad hoc basis between 1983 and 1995, with limited success (Jones and Cilliers, 1999)
An "Integrated Water Hyacinth Control Program" was initiated in 1995, using a holistic approach incorporating the existing chemical and biological control options. The river system was divided into eight management units which were sprayed, whilst selected water hyacinth "islands" within these units were not sprayed to support biocontrol agent populations. Floating cable booms were strung across the river, to prevent re-infestation by wind-driven plants moving up and downstream, and to allow monitoring of the plant populations (Jones and Cilliers, 1999). The integrated approach, using a recommended dose of glyphosate, was instrumental in clearing the weed from 22 km of the river and the cleared units now only require occasional and carefully managed follow-up herbicidal sprays two or three times a year to control any re-growth (Jones and Cilliers, 1999). The favourable mean annual temperature of 22°C and the mesotrophic status of the water body have also contributed to its success.

The success of biological control in Africa generally coincides with favourable climatic conditions and oligotrophic waterways. However, in South Africa, which as an emerging economy is faced with challenging socio-economic and environmental conditions, biological control is a particularly cost-effective, efficient and sustainable management tool, provided it is effectively integrated into existing control options, as demonstrated by the Enseleni system. However, this success at a low-altitude, warm, well-managed site is tempered by high-altitude boom and bust populations of the weed, which are managed by extermination sprays costing over R10m/annum (Rael Hughes, Working for Water, personal communication). The challenge lies in taking successful elements of the Enseleni method and coupling them with reduced dosages of glyphosate to give the biocontrol agents an edge over the weed.

Glyphosate used at recommended doses has garnered bad press over its effects on aquatic wildlife (Relyea, 2005a,b,c; Relyea et al., 2005). Notwithstanding these environmental concerns, which are worthy of further investigation, Ueckermann and Hill (2001) have concluded that glyphosate is non-toxic to the water hyacinth weevils and the mirids. If the weed growth can be constrained by low, non-lethal doses of glyphosate, an environmentally friendlier and low-management control method is envisaged whereby the insect populations survive herbicidal control sprays and persist in holding the weed back to acceptable levels (Jadhav et al., 2007).

This is the ongoing goal of this research and this report advocates a technique that uses a sublethal or retardant dose of glyphosate which constrains the weed without

undermining the biocontrol agent population. This technique will add another tool to the biological control armoury used against water hyacinth.

6.1.6 Key Question

The objective of this part of the study was to determine the effect of a sublethal or retardant dose of glyphosate on water hyacinth and its biological control agents, *Neochetina eichhorniae and N. bruchi*.

This chapter is broken down into several sections, representing a logical progression of questions and answers, which arise from the use of a low dose of herbicide on water hyacinth and the consequences of this for the plant and its biological control agents.

The sections are organised as follows:

6.2 Laboratory Trials
- 6.2.1 What constitutes an effective low dose of herbicide?
- 6.2.2 What influence do nutrients have over the effect of this herbicide dose?
- 6.2.3 What are the ecotoxological effects of this herbicide dose?
- 6.2.4 What is the effect of a low dose of herbicide on the nutrient status of the weed?

6.3 Field Trials
- 6.3.1 When should a low dose of herbicide be applied in the field?
- 6.3.2 How effective is a low dose of herbicide in the field?

6.2 Laboratory Trials

6.2.1 What constitutes an effective low dose of herbicide?

6.2.1.1 Introduction

This research has been published as a paper (Jadhav et al., 2008). The core findings are presented here, with the methods and conclusions.

The objective of this study was to identify a retardant dose of glyphosate which will not kill the water hyacinth mat but will retard the vegetative growth, in terms of ramet (daughter plant) and leaf production, so that the agent population can persist, offering some degree of permanent control.

6.2.1.2 Materials and Methods

In each of the experiments described below, four medium-sized water hyacinth plants, two of which were tagged with plastic labels on leaf one (i.e. the innermost, youngest leaf), were placed in circular 50 *l* (52 cm diameter) plastic tubs, containing 42 *l* of water, outdoors at the University of the Witwatersrand, Johannesburg, South Africa. The plants were medium to tall phenotypes and formed 100% cover of the water surface in the tubs. The nutrient levels of the water in the tubs were adjusted to 1.5 mg N/*l* (as ammonium nitrate) and 0.22 mg P/*l* (as potassium dihydrogen orthophosphate). These

levels approximated those typically found under local conditions during country-wide surveys of water quality performed by the South African Institute for Water Quality Service. The herbicide treatment consisted of applications of a broad spectrum, glyphosate-based herbicide, Roundup (active ingredient, 360 g/*l* glyphosate, containing 480 g isopropylamine salt of glyphosate/*l*) with the surfactant polyethoxylated tallowamine, supplied by Monsanto Pty. Ltd., South Africa, which was sprayed on the hyacinth plants in the prescribed dosages. A buffer (2% ammonium sulphate) was added to the spray solution to maintain pH at between 5 and 5.5. A battery operated (12 V) pressurized spray rig (Multispray, South Africa) was calibrated to spray 150 *l*/ha using Tee Jet TP (TP11020) nozzles (Tee Jet Technologies, USA). The recommended lethal dose for Roundup on water hyacinth is 3%.

Twenty-one tubs were set up, as described above, and divided into seven groups of three. At the outset, glyphosate was applied at concentrations of 0.1%, 0.3%, 0.5%, 0.8%, 1% and 1.5% with active ingredient values (g m^2) of 0.01, 0.04, 0.07, 0.11, 0.14 and 0.21, respectively. Three tubs were used as controls and were not sprayed with glyphosate. Over a period of eight weeks, weekly measurements were made on the tagged water hyacinth plants to record: total number of leaves; position of leaf one; total number of ramets; second leaf petiole length; and longest petiole length. Endpoint analysis, using One-Way Analysis of Variance and student's t-test (STATISTICA, version 6, StatSoft, Southern Africa, 2001) was carried out on each of the plant parameters measured at the end of the experiment and the results were considered significant at the 0.05 probability level.

6.2.1.2.1 Effect of Glyphosate on *N. eichhorniae* and *N. bruchi*

Trials were carried out during spring of 2005. Twenty four tubs were set up as described above, 12 for *N. eichhorniae* and 12 for *N. bruchi*. Each set was sub-divided into four groups of three. Three of the groups were treated with herbicide (0.8%, 1.5% and 2%, details above) while one group served as a control which was not treated with herbicide but did contain insects. Four pairs of adult weevils were released onto the plants in each tub, giving an initial weevil density of two weevils per plant. Each tub was then enclosed in a net canopy (mesh size: 0.8-0.5 mm) to confine the weevils, which were then allowed to acclimatize for one week after which glyphosate was sprayed on the plants (day zero). Two water hyacinth rosettes were randomly chosen in each of the tubs and tagged, so that fortnightly measurement of the feeding intensity on the second-youngest leaf could be measured by counting the number of feeding scars. The lamina area of the second-youngest leaf was measured by scanning and digitizing an outline of the leaf drawn on paper in order to determine the leaf area, which was then used to calculate the number of feeding scars per square centimetre. The proportion of petioles mined and the number of adult weevils (both *N. eichhorniae* and *N. bruchi*) and larvae found were counted by dissecting the tagged plants within each tub at the end of the experiment. The crown of the plant was not cut open to count or recover any late-instar

larvae. The experiment ran for eight weeks.

Student's t-tests, using STATISTICA program, version 6 (StatSoft, Southern Africa), was carried out on each of the parameters measured. Means obtained for insect parameters were considered significant at the 0.05 probability level. Analysis of Covariance (ANCOVA) was used to test the effect of a covariate (number of leaves) on the number of feeding scars. A contingency table analysis (StatSoft, Southern Africa) was used to compare the proportions of petioles mined between the treated and unsprayed (control) plants.

6.2.1.3 Results

A mean (±SE) of 1.5 (±0.80) ramets per plant was produced on the plants sprayed with 0.8% concentration of herbicide. This was significantly lower ($t10 = 2.19$; P = 0.05) than the mean number of ramets produced by the unsprayed, control plants (Figure 6.1). Ramet production in the plants sprayed with 1% and 1.5% herbicide declined as the mother plant lost condition but the mean number of ramets produced by plants sprayed with 0.5% and 0.3% concentrations of herbicide were not significantly different from the unsprayed, control plants ($F2,15 = 1.02$; $p = 0.38$). The total number of leaves produced by plants sprayed with 0.8% concentration of herbicide was significantly lower ($t10 = 8.62$; $p << 0.0001$) compared to the unsprayed, control plants (Figure 6.2). Although they produced fewer than the control, plants sprayed with 0.5% concentration of herbicide produced significantly more ($F2,15 = 9.51$; $p = 0.002$) leaves than those treated with 0.8% and 1% concentrations. Plants sprayed with 1.5% concentrations lost all their leaves and died. After adding one leaf, the plants sprayed with the 0.8% concentration did not produce any more new leaves and maintained their leaf numbers throughout the sampling period, as shown by the position of the tagged leaf (Figure 6.3), which remained at leaf two position. Plants sprayed with a 0.5% concentration continued to add leaves during the study, while those sprayed with the 1% and 1.5% concentrations initially added a leaf, in some cases two, and then lost leaves due to mortality.

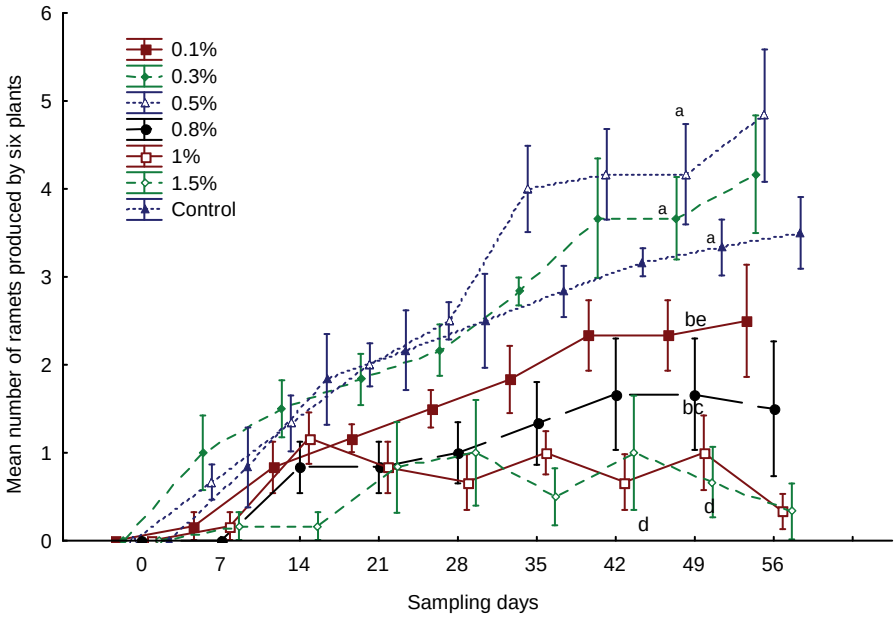


Figure 6.1: Mean number of ramets produced by water hyacinth plants sprayed with different doses of glyphosate herbicide at 140 *l*/ha. Error bars = standard error of the mean, n=6. Different letters indicate significant differences at P< 0.05.

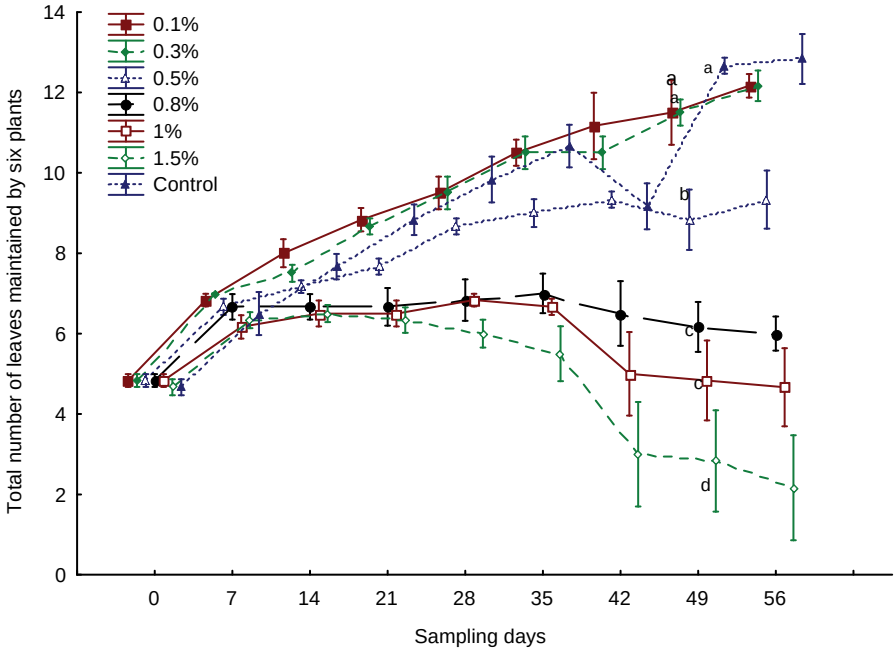


Figure 6.2: Total number of leaves produced by water hyacinth plants sprayed with different doses of glyphosate herbicide at 140 *l*/ha. Error bars = standard error of the mean, n= 6. Different letters indicate significant differences at P< 0.05.

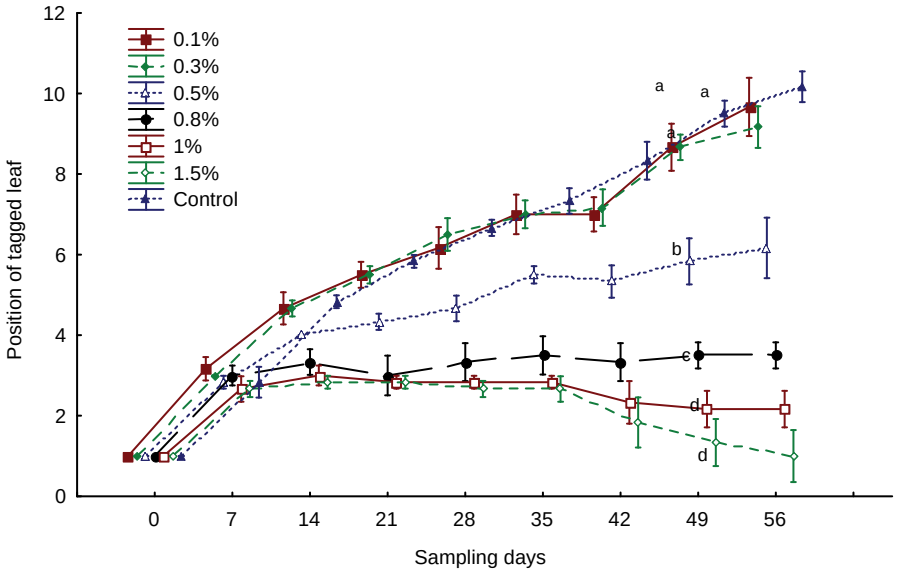


Figure 6.3: Leaf turnover in water hyacinth plants sprayed with different doses of glyphosate herbicide at 140 *l*/ha. Error bars = standard error of the mean, n=6. Different letters indicate significant differences at P< 0.05.

6.2.1.3.1 Effect of a Retardant Dose of Glyphosate on *N. eichhorniae* and *N. bruchi*

Glyphosate applied at doses of 1.5% and 2% killed the weed and resulted in the demise of the weevils as their host plant disappeared. The water hyacinth plants treated with a 0.8% concentration of glyphosate were still alive at the end of the eight weeks of the experiment and only these (treated) plants along with the control (untreated) plants were considered for further analysis. There were no significant differences, at day 60, between the mean numbers of adults found on the treated and control plants (Figure 6.4) (for *N. eichhorniae*, $t10 = 2.076$, $p = 0.06$, and for *N. bruchi*, $t10 = 2.07$; $p = 0.065$).

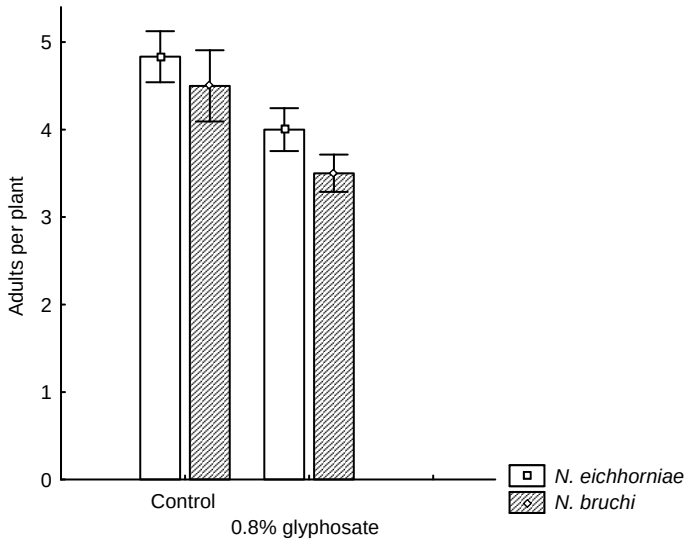


Figure 6.4: Mean (±SE) number of *Neochetina eichhorniae* (unshaded bars) and *Neochetina bruchi* (diagonally-hatched bars) adults harvested from water hyacinth plants 60 days after treatment.

The total number of feeding scars per square centimetre was significantly higher on the treated plants than the control plants at day 60 (Figures 6.5A and B) (for *N. eichhorniae*, $t10 = 5.83$; $p = 0.0001$; and for *N. bruchi*, $t10 = 3.59$; $p = 0.004$) but not on the earlier sample days. ANCOVA results showed that the number of leaves was not a significant covariate ($F1,8 = 0.037$, $p = 0.85$) and hence the increase in number of feeding scars was the effect of the treatment alone.

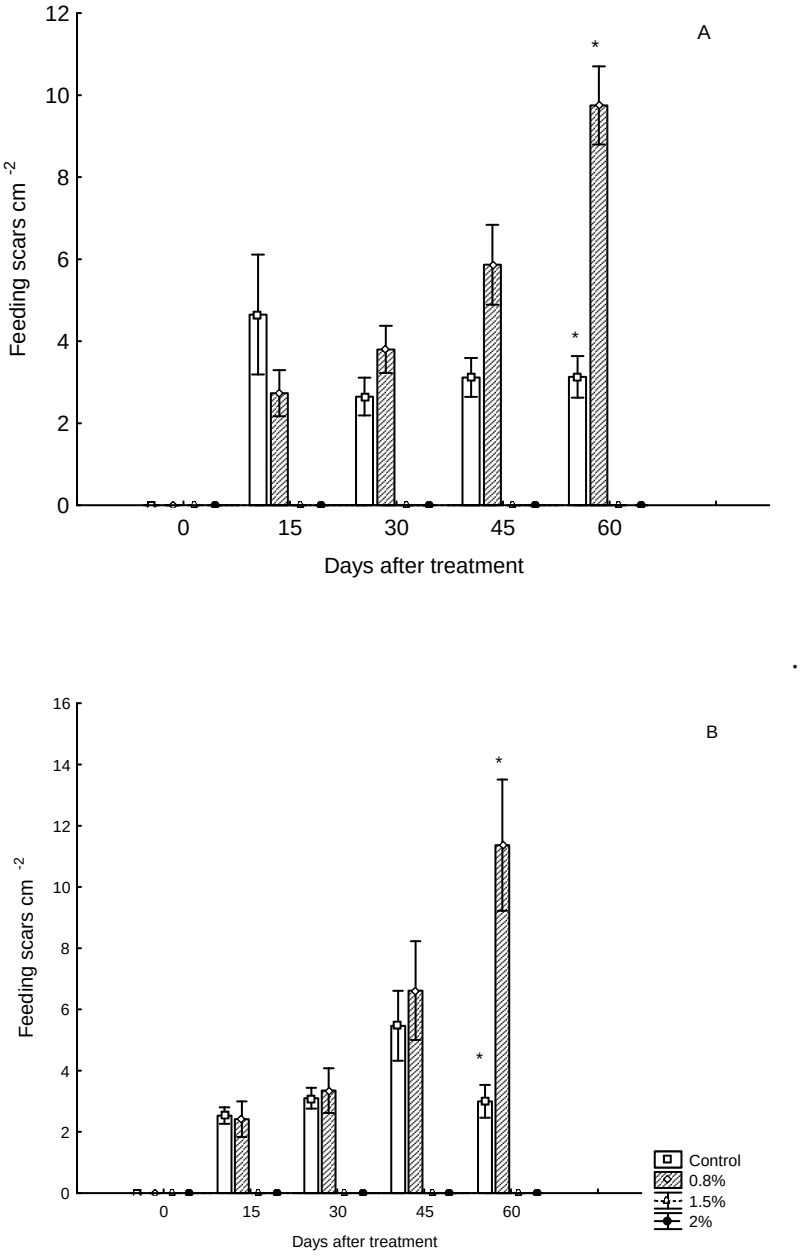


Figure 6.5: Mean (± SE) number of feeding scars cm^{-2} on water hyacinth plants harbouring *N. eichhorniae* (A) and *N. bruchi* (B). Hatched bars = plants sprayed with glyphosate; unshaded bars = control plants. * = significant difference at $p < 0.05$ for comparisons of pairs on each sample date.

The proportion of petioles mined by weevil larvae was significantly greater on the treated plants than on the control plants for both *N. eichhorniae* ($v2 = 4.51$, $p = 0.03$) and *N. bruchi* ($v2 = 6.02$, $p = 0.01$) (Table 6.1). There were no significant differences between the mean numbers of first- and second-instar larvae obtained from the treated plants and control plants at the end of the experiment for both *N. eichhorniae* (1st instars: $t10 = 1.53$, $p = 0.15$; 2nd instars: $t10 = 0.62$, $p = 0.54$) and *N. bruchi* (1st instars: $t10 = 0.0$, $p = 1.00$; 2nd instars: $t10 = 1.58$, $p = 0.144$) (Figures 6.6A and B). No third-instar larvae were found on treated plants, possibly due to movement of the instars into the crown to avoid competition as food resources became depleted (Center, 1987).

Table 6.1. The percentage of petioles with mining damage caused by *Neochetina spp.* larvae on treated and untreated (=control) *Eichhornia crassipes* plants.

	N. eichhorniae	*N. bruchi*
Treated	71.6	75
Untreated	45	41.6

6.2.1.4 Discussion

Haag (1986a) and Ueckermann and Hill (2001) demonstrated that the glyphosate-based formulation, Roundup, was neither lethal nor a feeding retardant for either *N. eichhorniae* or *N. bruchi* but, to the best of our knowledge, this study is the first to identify a retardant dose of a glyphosate-based herbicide on water hyacinth and to test this particular dosage on the biocontrol agents, *N. eichhorniae* and *N. bruchi*. Several other studies have demonstrated cases where herbicides have not affected insect herbivores used as biological control agents of different terrestrial invasive plant species (Rees and Fay, 1989; Lym and Carlson, 1990; Lindgren et al., 1998, 1999; Nelson and Lym, 2003).

Increased feeding levels by the *Neochetina spp*. in this study could be due to glyphosate-induced inhibition of the synthesis of phenylalanine-derived phenols and secondary metabolites that are feeding deterrents in many plants (Ainsworth, 2003). It is also possible that a very low dose of glyphosate increases the sugar content in the sprayed plants, thereby making the plants more palatable. Wright and Bourne (1990) showed that 2,4-D amine improved water hyacinth plant quality by decreasing leaf and petiole hardness, thereby improving plant quality for larval stages of the moth, *Niphograpta albiguttalis* Warren and the two *Neochetina* species.

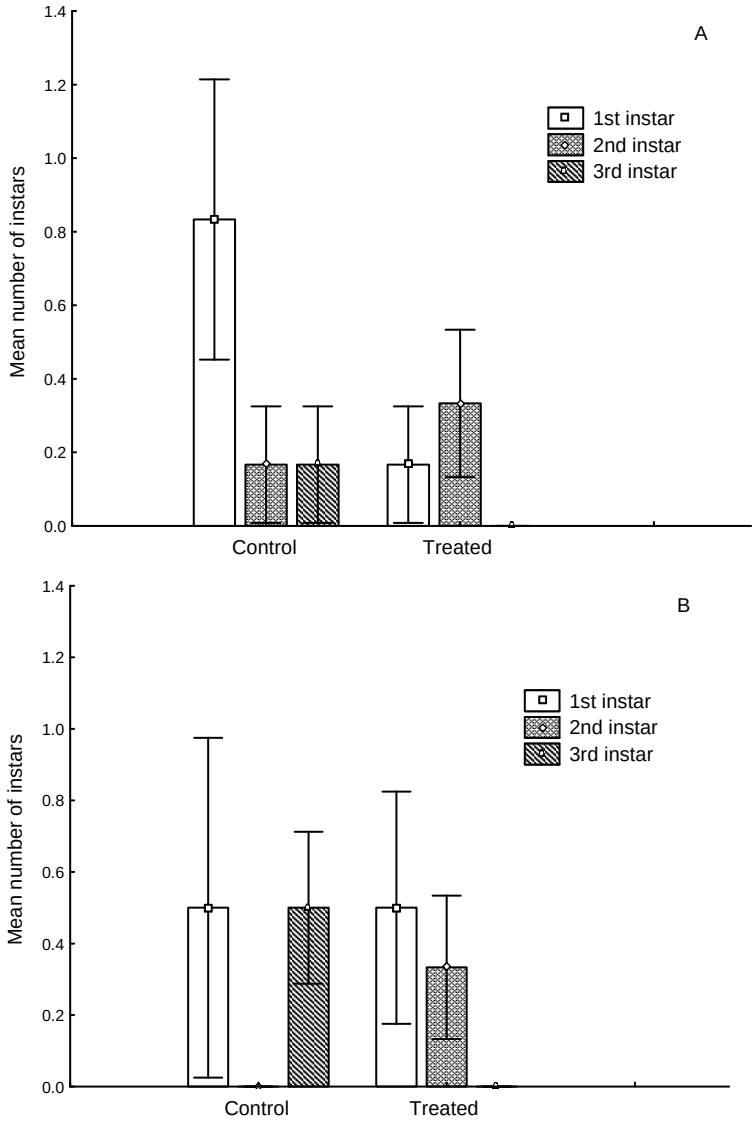


Figure 6.6: Mean (± SE) numbers of first- (unshaded bars), second- (diagonally hatched bars) and third-instar (stippled bars) larvae of *N. eichhorniae* (A) and *N. bruchi* (B) on treated and control water hyacinth plants.

The reproductive capacity of the weevils was not compromised by the herbicide and newly-hatched larvae were able to establish in the petioles, as evidenced by the mined petioles and the early-instar larval counts. The palatability and suitability of the sprayed plants allowed the weevils to persist in our trial, despite fewer oviposition sites being available to them due to reduced numbers of leaves. Wilson et al. (2006) indicated that early larval stages of the *N. eichhorniae* experienced density-dependent mortality when larval densities were high and there was disruption of leaf dynamics. We predict that when populations of *Neochetina spp.* reach high levels, severe damage to the plants by larval feeding will lead to competition among the crowded larvae which in turn may cause a decline in adult weevil numbers, but not a total loss of the weevil populations, as would result from a lethal spray.

South African ecosystems have been characterized by unstable 'boom-and-bust' water hyacinth populations which preclude the build up of damaging numbers of biocontrol agents (Hill and Olckers, 2001). Integration of a retardant dose of glyphosate with biological control offers a potential tool with which to manage water hyacinth. The low dose does not kill the water hyacinth mat and should preserve the habitat for immature and immobile stages of the weevils, allowing the adult populations to persist at damaging levels. The herbicide-induced curb on vegetative growth of the water hyacinth plants will result in disproportionate levels of damage by the weevils and further suppression of the weed through continued herbivory. Jones and Cilliers (1999) have successfully implemented an integrated management system for water hyacinth using a full (3%), lethal dose of glyphosate by sub-dividing the infestation into smaller areas and maintaining herbicide-free insect refuges. This, however, requires a level of management not currently available for many water hyacinth-infested African waterways, and requires a high dose of glyphosate, which is under scrutiny for its detrimental effects on other aquatic organisms (Relyea et al., 2005).

If the full suite of water hyacinth biological control agents available in South Africa can be shown to be compatible with low doses of glyphosate over extended periods, this procedure offers potential for integrated control of the weed. One of the key aspects of successful implementation of an integrated management approach is the optimal timing of the herbicide application (Cullen, 1996). The ongoing goal of our work will be to determine the best seasonal spray regime to manage water hyacinth infestations and to determine whether sublethal doses of herbicide can be used on a practical scale in the field.

6.2.2 What Effect do Nutrients have on the Efficacy of a Low Herbicide Dose in Controlling Water Hyacinth?

6.2.2.1 Introduction

The aim of any weed management programme is to maintain the target weed community at an acceptable level. To this effect, the development of biological control and its agents has been motivated by a need to reduce the abundance and distribution of an invasive alien where chemical and mechanical controls alone were not cost effective (Harris, 1993). Nevertheless, under certain circumstances such as elevated nutrients and low temperatures, biocontrol alone may be insufficient to effect control. The use of sublethal dosages of herbicide in conjunction with biocontrol agents has been shown to constrain growth and reproduction of the weed (Section 6.2.1). However, this synergy of biotic and abiotic pressure on the weed may be influenced by different water nutrient levels in which the water hyacinth is growing. The work that follows in this section addresses that question, by showing that the low herbicide dose is effective across a range of nutrient conditions.

6.2.2.1.1 Eutrophication

Nutrient enrichment of surface water from anthropogenic (cultural) sources has long been recognized as a cause of eutrophication (Walmsley, 2000). “Eutrophication” is an ecological term used to describe the process by which a body of water becomes enriched with nutrients that promote plant growth. Nutrient enrichment is often found in highly populated and developed areas where water-borne sewage systems and agriculture contribute to elevated loads of nutrients, particularly nitrogen and phosphorus. The increase in nutrients causes water quality and user problems as it increases and promotes the development of both living and decaying biological material (Walmsley, 2000). Very few countries have escaped the problem of eutrophication and this includes South Africa, which has some of the most highly enriched surface waters in the world (Walmsley, 2000). South African water bodies are enriched with phosphorus in the form of orthophosphates, polyphosphates and organic phosphates. Nitrogen is also present in many forms such as ammonium, nitrates and nitrites. These forms of phosphorus and nitrogen originate from agricultural run-off and input from waste-water treatment plants (Walmsley, 2000).

Eutrophication causes a serious water quality problem in South Africa in terms of the increased occurrence of floating and rooted aquatic macrophytes and has an important role in biological control. The biological control agents can reach very high numbers under these conditions, but the rate at which the plants grow due to increased nutrients in the water is greater than the rate at which the agents reduce the growth of the plant (Heard and Winterton, 2000; Gutiérrez et al., 2001; Center et al., 2002). Typical examples include Hammersdale Dam, KwaZulu-Natal, which occurs next to a water treatment facility, and Mbozambo Swamp, KwaZulu-Natal, located at the exit point of an effluent pipeline. These water bodies have severe water hyacinth infestations in spite of the large numbers of biocontrol agents, such as *N. eichhorniae* weevils and *E. catarinensis* mirids (Hill and Cilliers, 1999).

Nitrogen and phosphorus levels are correlated with increasing water hyacinth growth (Heard and Winterton, 2000). Increasing levels of nitrogen and phosphorus result in an increase in the growth activity of the plant, leading to an increase in its biomass (Reddy et al., 1989 and 1990). Water hyacinth plants grow actively at phosphorus levels between 0.1 mg/*l* and 1.06 mg/*l*, with no additional growth at levels above 1.06 mg P/*l*, and at these levels nitrogen is not limiting (Reddy et al., 1990). Maximum water hyacinth growth occurs at 25 mg N/*l* (Reddy et al., 1989).

In South Africa phosphorus levels range from 0.001 mg/*l* to 2.5 mg/*l* and nitrogen levels range from 0.01 mg/*l* to 7 mg/*l* and in some cases exceed this (Figure 6.7). Therefore, the effects of nitrogen and phosphorus are of particular interest in the control of water hyacinth. Evidence has been presented that suggests that herbicides can change the quality of the weed as a food source for herbivores (Wright and Bourne, 1990),

although whether this is a result of alterations in the nutrient content of the plant material is not clear. Therefore, it is possible that the available nutrients in the water may result in different levels of control if a sub lethal dose of herbicide is applied to the plants.

In some cases, herbicides have been used at sites where biological control agents are already established, mainly because the managers of those sites felt that the biological control was taking too long, or could not identify the control effect of the agents (Center et al., 1999). Herbicidal control is costly, especially considering that the programmes must continue indefinitely (Wright and Bourne, 1990). The hazardous effects of herbicides on the environment may be brought about by complex, indirect pathways, which are not always properly understood, and may only be discovered some time into the future (Wright and Bourne, 1990; Wang et al., 1994; Relyea et al., 2005). It would therefore be optimal to reduce the input of herbicides into the environment as much as possible. Biological control is much less costly, but not always suitable. Therefore, integrating the two types of control in order to obtain a more effective and more cost-effective means of control has been considered (Haag, 1986a; Wright and Bourne, 1990; Center et al., 1999; Hill and Cilliers, 1999).

6.2.2.1.2 Integrated Control

To date, herbicide application used in conjunction with biological control has not been very effective against water hyacinth (Center et al., 1999; Haag, 1986a,b). The herbicides can kill severely damage the plant (Wright and Bourne, 1990), but dosages recommended by the herbicide manufacturers are often higher than necessary to ensure that all of the plants are killed (Haag, 1986). Death of the plants can occur 5-10 days after the chemicals have been applied (Haag, 1986). Some herbicides affect water hyacinth biological control agents, causing mortality, and reducing feeding and fecundity (Ueckermann and Hill, 2001). For example, the herbicide Midstream® at the recommended dosage causes total mortality of water hyacinth mirids (*E. caterinensis*) after only 48 hours, and causes a significantly higher percentage of mortality in the weevils (*N. bruchi* and *N. eichhorniae*) after 120 hours compared to many other herbicides (Ueckermann and Hill, 2001). On the other hand, Roundup® causes significantly lower mortality of the mirid, even when applied at well above the recommended dosage, and almost no mortality of the weevils (Ueckermann and Hill, 2001).

Killing, and therefore removing, large mats of water hyacinth also removes the habitat and food source of the biological control agents (Haag, 1986b), whereas healthy plants that missed herbicide application, or seeds that germinated due to an increase in light, can quickly grow to re-infest the empty water created by the loss of the water hyacinth mat (Center et al., 1999). Biological control agents take much longer to recover than the plants and any biological control achieved at the site is lost (Haag, 1986b; Hill and

Cilliers, 1999). To overcome this, the creation of refuges has been suggested, where only a certain percentage of the water hyacinth plants should be sprayed at a particular site, leaving a portion of the mat undamaged, to which the biological control agents can move in order to avoid the herbicide and the sinking mat (Haag et al., 1988; Center et al., 1999; Hill and Cilliers, 1999). This method has been very successfully employed on the Enseleni River to control water hyacinth, but requires active monitoring of the weed, and has failed at most other sites around the country (Jones and Cilliers, 1999).

The refuge strategy in integrated control relies heavily upon implementers who spray the herbicide, and their judgement of which section of the plant population to leave unsprayed. The size and shape of the refuge relative to the size and shape of the infestation is also important, and this is not always easy to determine (Haag et al., 1988). It has been shown that the adult *N. eichhorniae* weevils will tend to move away from herbicide sprayed plants to unsprayed plants (Haag, 1986b; Ueckermann and Hill, 2001). But many of the water hyacinth biological control agents have stages in their lifecycle that are slow moving or sessile (Center et al., 2002). Often one of the stages preceding the adult stage is most damaging to the plant (Center et al., 2002). The larvae of water hyacinth weevils are embedded in the tissue of the plant, and are unable to move easily to a neighbouring plant; and the pupae are enclosed in a cocoon that is attached to a root below the water surface (Center et al., 2002). So if the plants in which these stages of the weevils occur sink, these immatures will sink with them (Center et al., 2002; Haag, 1986b). Therefore, since the agent's immature stages cannot move across to unsprayed plants, the biological control agents will still suffer severe losses to their numbers if herbicides at the recommended dosages are applied, even if refuges are provided (Haag, 1986b). The large amount of plant matter that sinks also decreases the oxygen levels of the water, which results in poor water quality.

A possible way to integrate biological control and herbicidal control is to apply sublethal dosages of certain herbicides, which are compatible with biological control agents (Ueckermann and Hill, 2001; Wright and Bourne, 1990). Ideally, these would stop plant growth and prevent problems resulting from sinking plant material. The quantity of herbicide and the frequency of application would also be reduced, making this form of control more cost-effective. However the nutrient levels of the water may play a role in this type of control.

6.2.2.1.3 Key Question

The objective of this study was to determine the effects that nutrients have on the growth of water hyacinth treated with a sublethal dosage of a glyphosate herbicide, and what the effects of these treatments are on the biological control agent, *N. eichhorniae*.

6.2.2.2 Methods

Experiments were carried out during the summer of 2005, outdoors at the University of the Witwatersrand. Water hyacinth plants (four per tub), were placed into circular 50 L

plastic tubs containing 42 L of water and a mix of nutrients. Three nutrient levels were tested: low, 0.5 mg N/*l* and 0.08 mg P/*l*; medium, 1.5 mg N/*l* and 0.22 mg P/*l*; and high, 3 mg N/*l* and 0.43 mg P/*l*. These levels were chosen using data from the country-wide water quality analyses performed by the South African Institute for Water Quality Service (IWQS) which included levels from 14 selected water hyacinth monitoring sites country wide (Figure 6.7) (Chapter 1). The ratio of nitrogen to phosphorus was approximately 7:1 in each experimental nutrient level, which has been suggested to yield optimum growth of water hyacinth plants (Wilson, 2002), and mimics conditions found at the field sites (Figure 6.7).

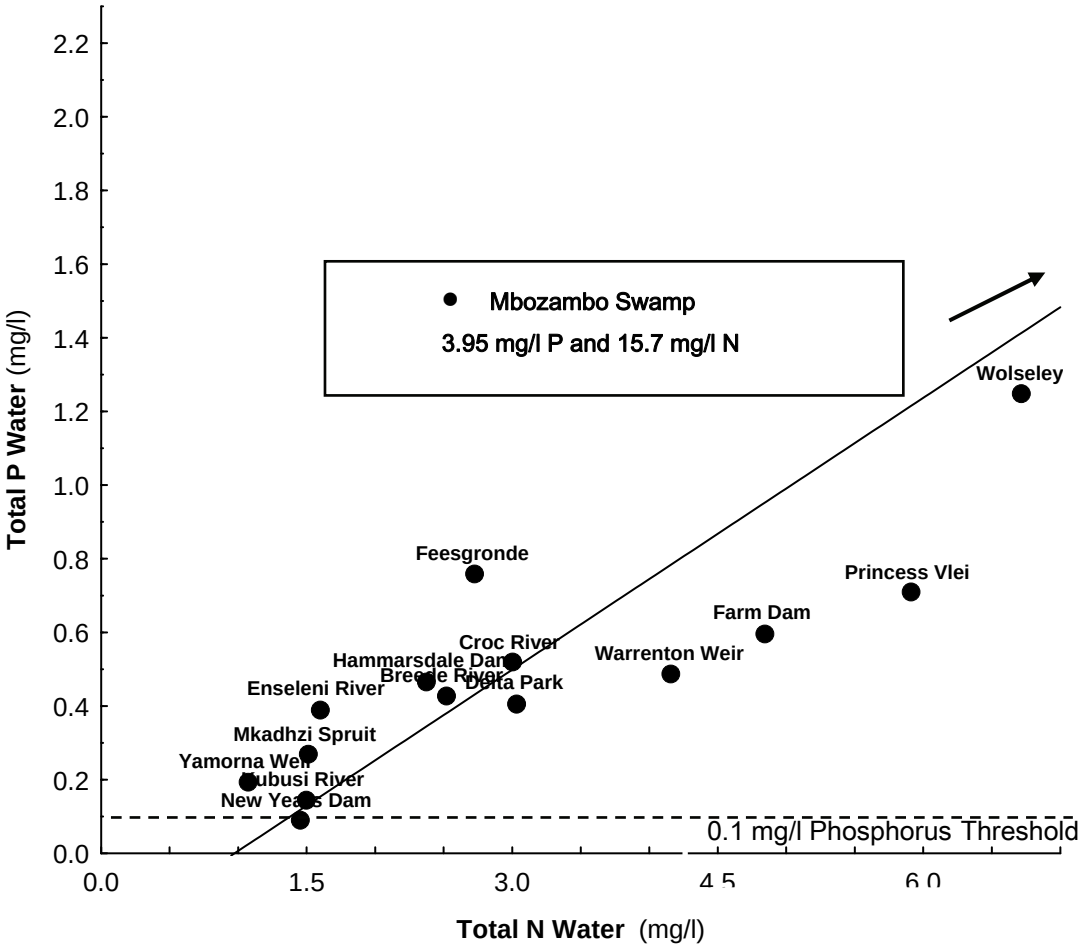


Figure 6.7: Mean values of total nitrogen and total phosphorus recorded over approximately 12 months from the water of 15 water hyacinth-infested sites in South Africa. (R^2= 0.9314; p< 0.0034). Nitrogen:phosphorus = 7: 1.45

Five experimental tubs and five control tubs were set up for each nutrient level; 30 tubs were used in total. Water and nutrients were replaced weekly (Center et al., 1982; Coetzee et al., 2007b). The plastic tubs were enclosed in a net canopy to ensure that the weevils remained on the plants. Two pairs of *N. eichhorniae* were released onto each plant in all 30 tubs, giving an initial weevil density of four weevils/plant and 16

weevils/tub. These infestation rates match the observed rates in the field in South Africa (Chapter 3).

Roundup (active ingredient isopropylamine salt (360 g/*l*) with surfactant polyethoxylated tallowamine (POAE)) was supplied and sprayed by Monsanto one week after the weevils had been released, at 0.8% with a spray volume of 155 *l*/ha. A buffer (2% ammonium sulphate) was added to the spray solution to maintain the pH between 5 and 5.5. Spraying of the plants was carried out by Anton Swanepoel from Monsanto.

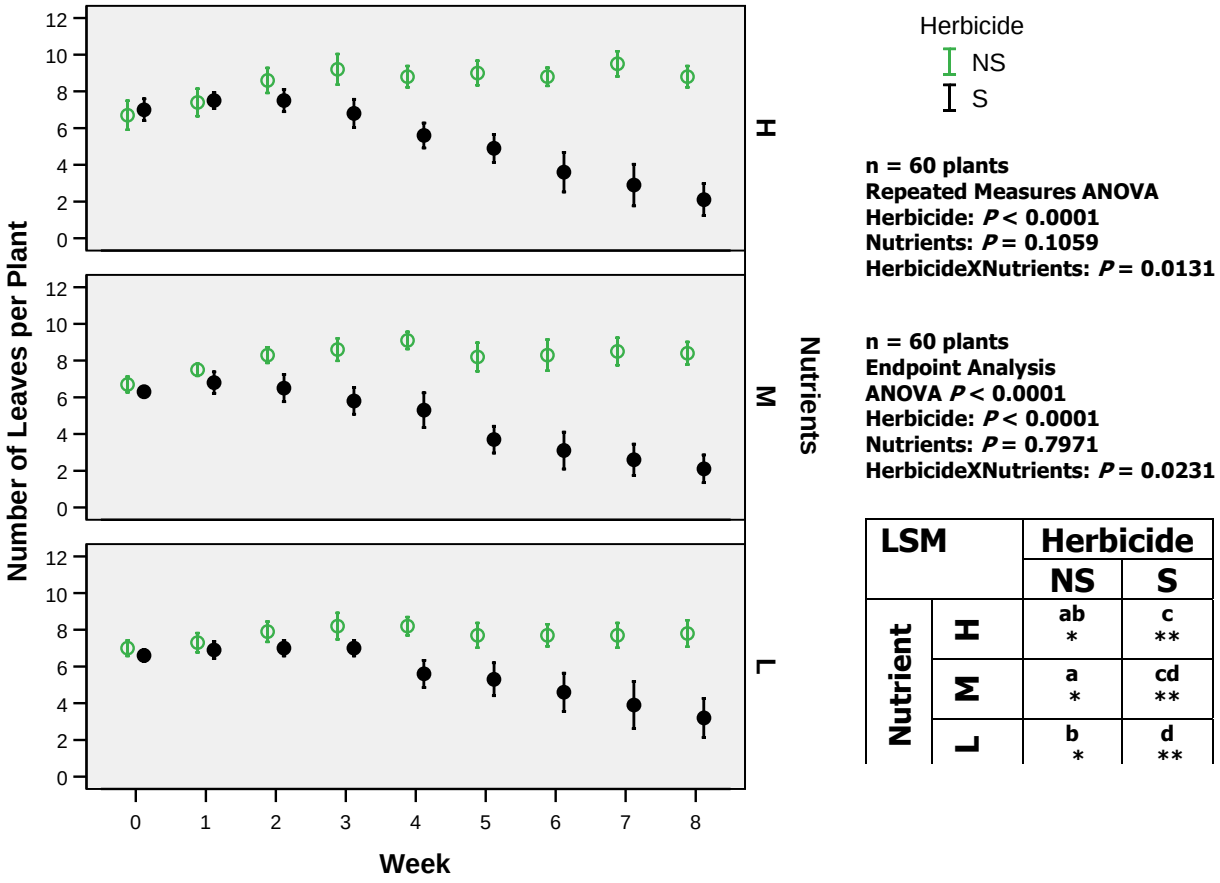
Two water hyacinth rosettes were randomly chosen from each of the tubs and tagged, so that weekly measurements could be made on these plants. The number of leaves, number of ramets and the number of inflorescences were counted on each tagged rosette. The length of the longest petiole, the length of the petiole of the second youngest leaf, and the length of the longest strand of root, was measured on each of the tagged plants. The lamina area of the second-youngest leaf was measured, in order to determine the number of feeding scars per cm^2. The experiment ran for eight weeks. Measurements were compared between treatments by using repeated measures Analysis of Variance. Endpoint analysis, via Analysis of Variance (SAS: version 8), was also carried out on each of the parameters using the data obtained at the last measurement occasion. Plots of the data were produced using SPSS (version 14.0). All analyses using repeated measures Analysis of Variance were carried out using the SAS procedure, Proc Mixed. Differences between treatments were calculated using Least Squares means.

The feeding intensity was measured weekly, while the number of live adult weevils and the presence or absence of larvae was determined for each tub by dissecting all plants within a tub at the end of the experiment. These counts were grouped into a contingency table and were compared between treatments by using a Chi-square test. All the plants from each tub were weighed to get the total wet biomass of plant material for each tub. These measurements were compared between treatments by using Analysis of Variance. These analyses allowed for comparison of plant performance at different nutrient levels under herbicide treatments, and examination of the effects of available nutrients.

6.2.2.3 Results

6.2.2.3.1 Plant Parameters

Most of the plant parameters measured indicated that there were significant differences in growth between plants sprayed with a sublethal dose and unsprayed plants. The herbicide treatment significantly suppressed the number of leaves on the plants (Figure 6.8), and the biomass of the plant material (Figure 6.9), and this was shown across three different nutrient levels. Plots of the mean number of leaves over time (Figure 6.8) for the different nutrient treatments show that the addition and loss of leaves is similar for the sprayed plants. The leaf-number plots for the three nutrient levels (Figure 6.8) are also similar within the unsprayed plant treatment, but there are noticeable differences in that the high nutrient level maintains the highest mean number of leaves over time compared to the medium and low nutrient levels, giving a significant difference between the high and low nutrient treatments. The slopes of the mean number of leaves per plant over time for the unsprayed plants are relatively level, indicating that there was an equilibrium between the addition and loss of leaves for the unsprayed plants; for the sprayed plants, the slopes were clearly negative, indicating a net loss of leaves during the course of the experiment (Figure 6.8). The differences between nutrient treatments were more marked in the unsprayed plants than in the sprayed plants (Figure 6.8). The significant difference between the low and medium nutrient treatments of the sprayed plants may be due to the slightly greater number of leaves on plants under the low nutrient treatment seen in week eight. Endpoint analysis gave similar results to the repeated measures analysis (Figure 6.8), but revealed no significant difference between the low and medium nutrients treatments of the sprayed plants. Resprouting from the crown of sprayed plants was observed towards the end of the experiment, but because these leaves did not fully unfurl this growth was not recorded.

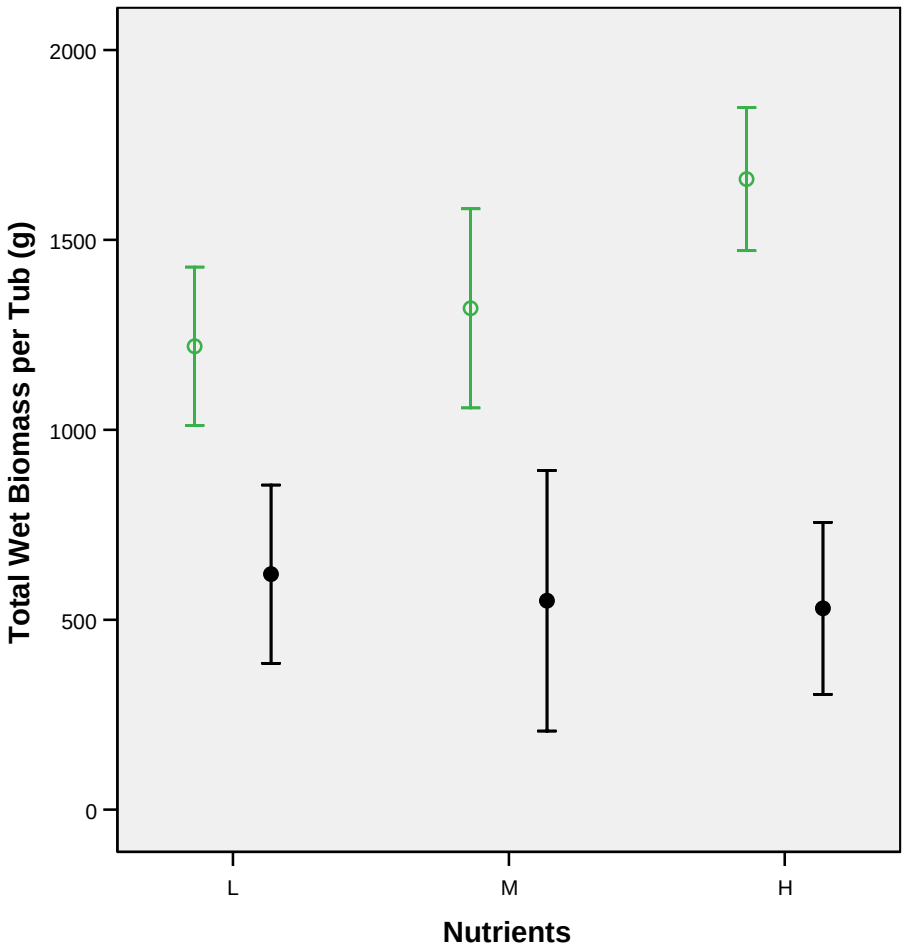


LSM		Herbicide	
		NS	S
Nutrient	H	ab *	c **
	M	a *	cd **
	L	b *	d **

Least Squares Means (LSM) comparison table: Treatments that have the same letter are not significantly different at $p = 0.05$ for the repeated measures Analysis of Variance. Treatments that have the same number of *'s are not significantly different at $p = 0.05$ for the endpoint Analysis of Variance.

Figure 6.8: Mean number of leaves maintained on water hyacinth plants over time, either sprayed (S) or not sprayed (NS) with 0.8% glyphosate herbicide, grown at three nutrient levels: high (H), medium (M) and low (L). Bars = standard error.

Similarly, the mean final wet biomass of the three nutrient levels of the sprayed plants are very similar ($p > 0.05$), but for the unsprayed plants, those under the high nutrient level treatment had significantly greater final wet biomass than those under medium or low nutrient level treatments (Figure 6.9). Within all three nutrient treatments, the sprayed plants had significantly lower wet biomass than the unsprayed plants, and in each case the mean biomass of the sprayed plants was approximately three times less than the mean biomass of the unsprayed plants (Figure 6.9).

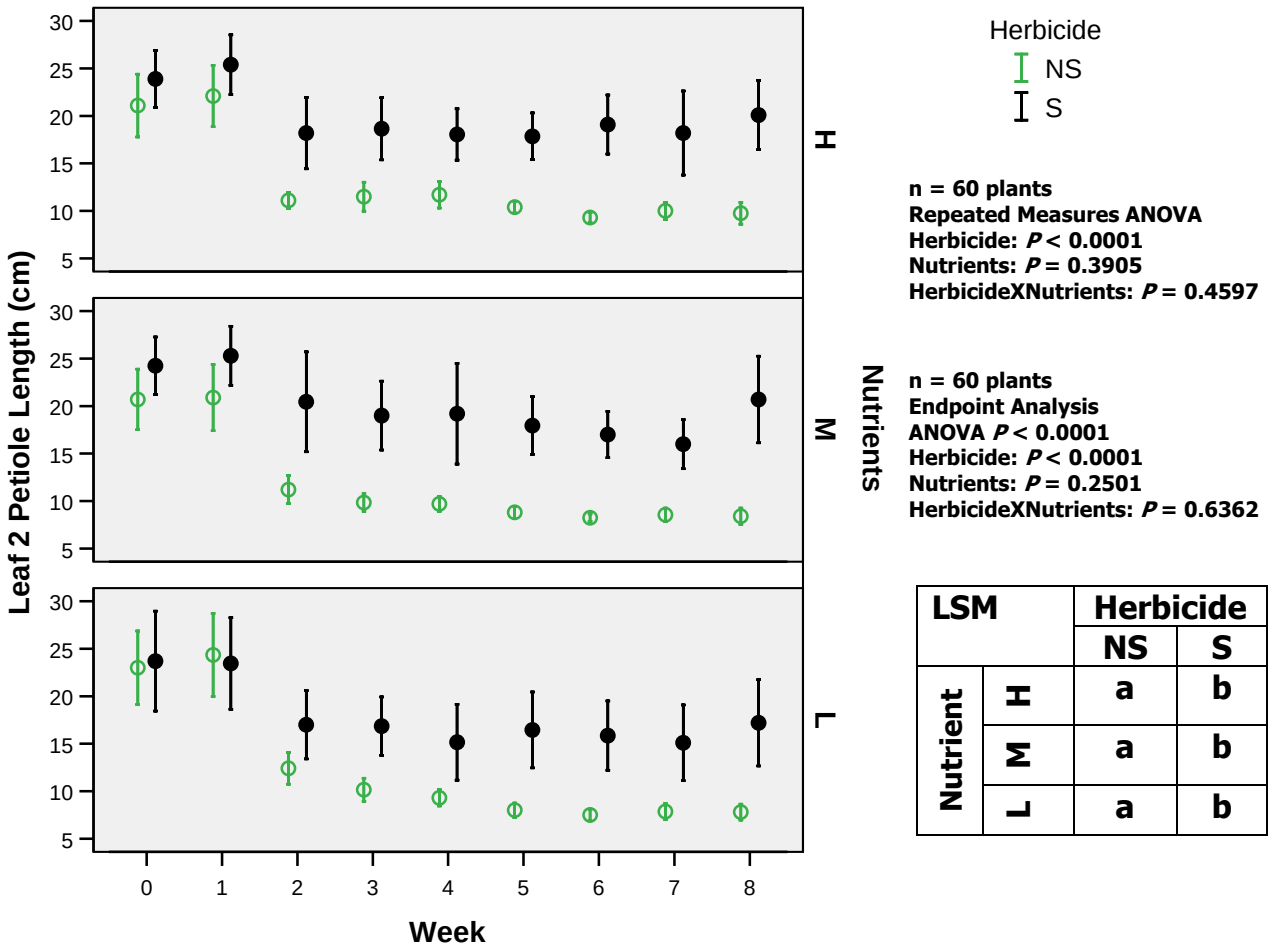


LSM		Herbicide	
		NS	S
Nutrient	H	a	c
	M	b	c
	L	b	c

Least Squares Means (LSM) comparison table: Treatments that have the same letter are not significantly different at P = 0.05 for the two-way Analysis of Variance.

Figure 6.9: Mean wet biomass of water hyacinth plants, either sprayed (S) or not sprayed (NS) with 0.8% glyphosate herbicide, after growing for eight weeks at three nutrient levels: high (H), medium (M) and low (L). Bars = standard error.

Both the repeated measures Analysis of Variance and the endpoint Analysis of Variance showed that the petiole lengths of the second-youngest leaves (Figure 6.10) and the numbers of ramets (Figure 6.11) were significantly different between the herbicide treatments, but not between the nutrient treatments, indicating that the herbicide treatment halted vegetative and reproductive growth of the water hyacinth equally, regardless of the nutrient status of the water. The sprayed plants had significantly higher mean petiole lengths for the second-youngest leaf compared to the unsprayed plants (Figure 6.10), because the unsprayed plants produced a new leaf at leaf position two each week, whereas the sprayed plants generally retained the same leaf at position two for the course of the experiment. The statistical evidence indicates that growth of the petiole of the second-youngest leaf depends only on whether or not the plants were sprayed, and not on the nutrient level.

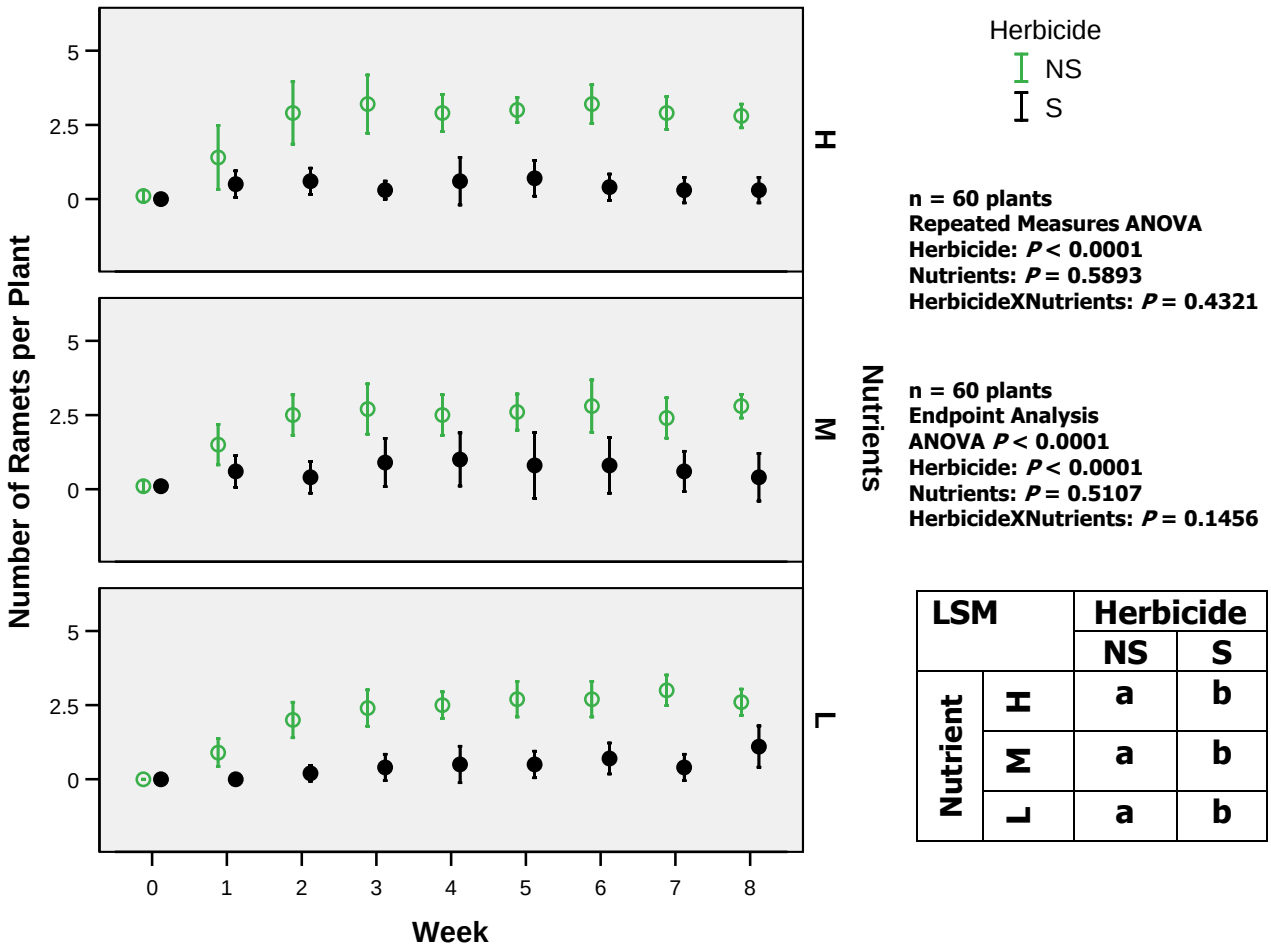


LSM		Herbicide	
		NS	S
Nutrient	H	a	b
	M	a	b
	L	a	b

Least Squares Means (LSM) comparison table: Treatments that have the same letter are not significantly different at P = 0.05 for repeated measures Analysis of Variance or for the endpoint Analysis of Variance.

Figure 6.10: Mean petiole length of the second-youngest leaf of water hyacinth plants either sprayed (S) or not sprayed (NS) with 0.8% glyphosate herbicide, and grown at three nutrient levels: high (H), medium (M) and low (L). Bars = standard error.

The mean number of ramets produced by the unsprayed plants was significantly higher than the sprayed plants (Figure 6.11). Sprayed plants produced less than one new ramet per plant throughout the experiment, compared to three produced by the unsprayed plants, and ramet production was also unaffected by the nutrient levels (Figure 6.11).



LSM		Herbicide	
		NS	S
Nutrient	H	a	b
	M	a	b
	L	a	b

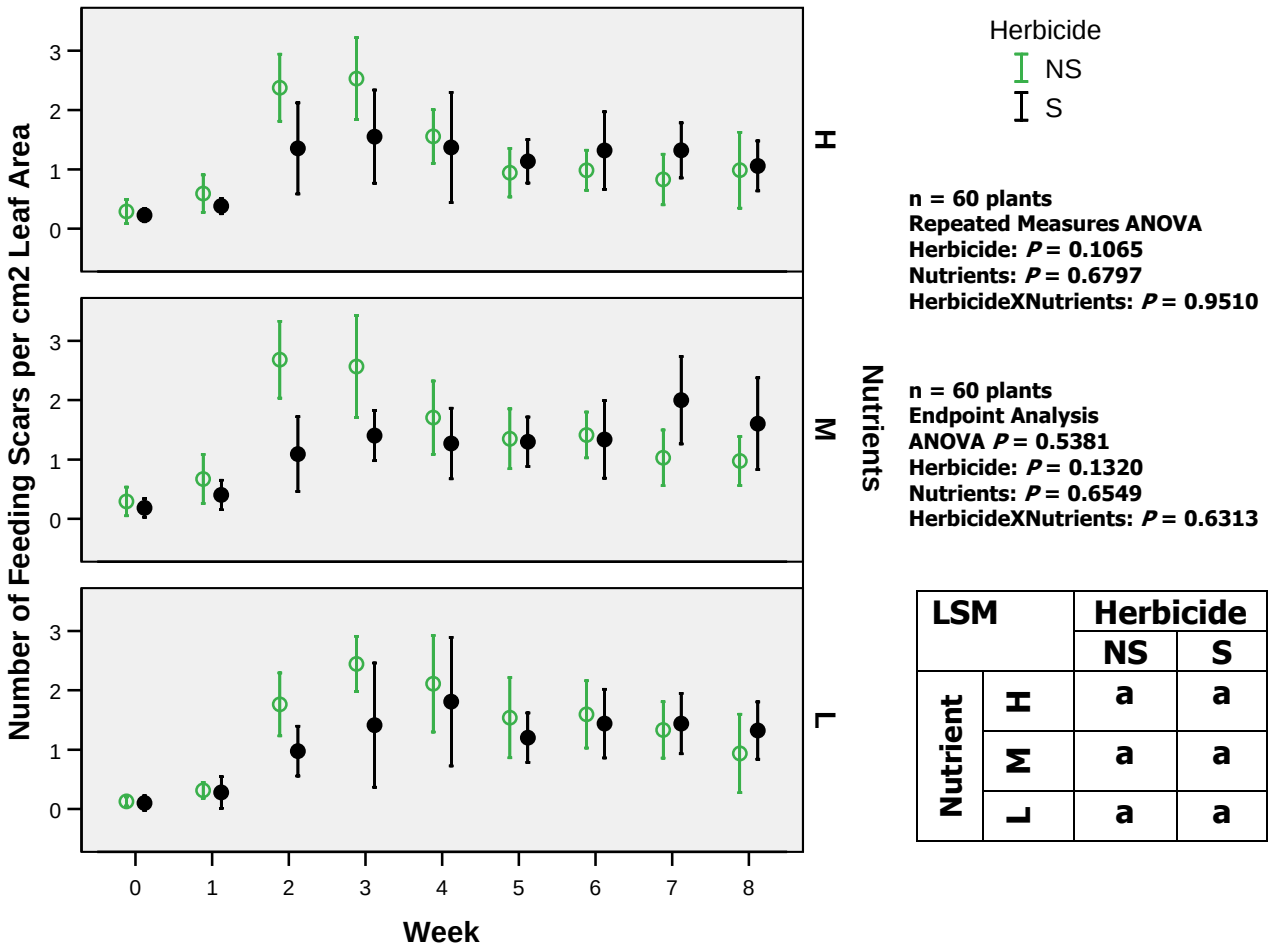
Least Squares Means (LSM) comparison table: Treatments that have the same letter are not significantly different at P = 0.05 for repeated measures Analysis of Variance or for the endpoint Analysis of Variance.

Figure 6.11: Mean number of ramets per water hyacinth plant either sprayed (S) or not sprayed (NS) with 0.8% glyphosate herbicide, and grown at three nutrient levels: high (H), medium (M) and low (L). Bars = standard error.

6.2.2.3.2 Insect Parameters

The insect parameters did not indicate any significant differences between herbicide treatments, or between the three nutrient levels.

There was no significant difference between the mean numbers of feeding scars per cm^2 leaf area in any of the treatments (Figure 6.12), which suggests that the feeding intensities did not differ between the sprayed and unsprayed plants, or between any of the nutrient levels. Plots of the data indicate that the feeding intensity reached a peak and then levelled off, but the peaks of the unsprayed plants were higher for all three nutrient levels (Figure 6.12).



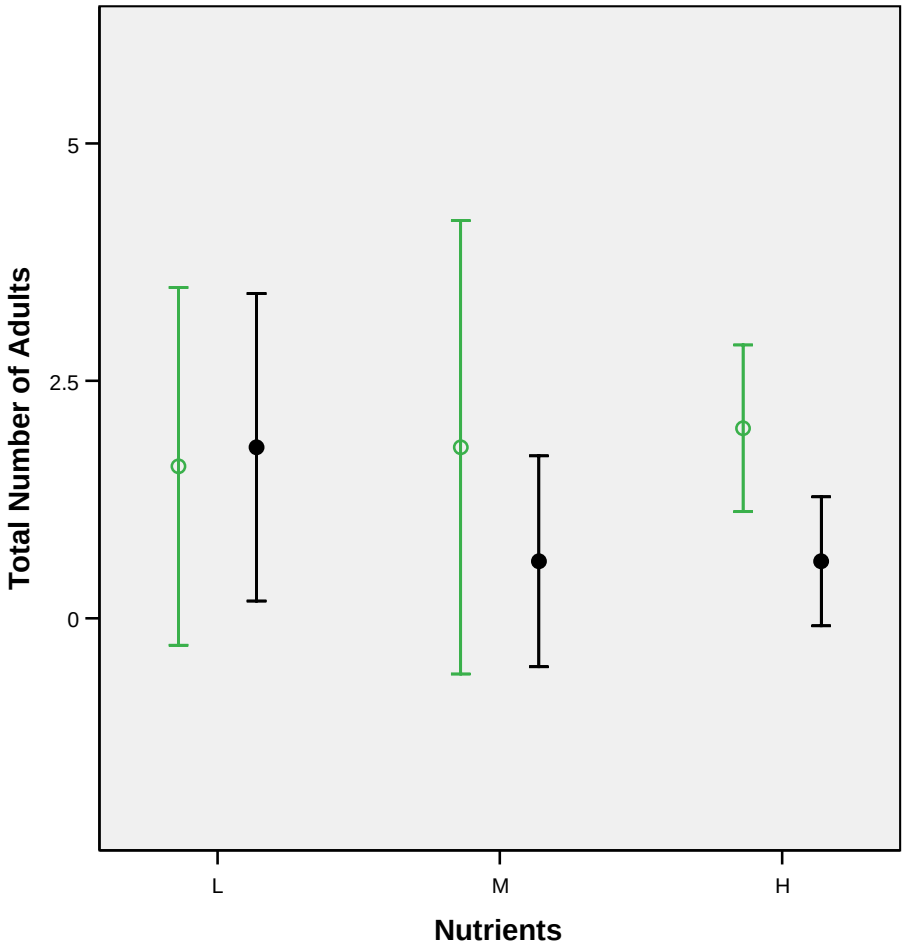
LSM		Herbicide	
		NS	S
Nutrient	H	a	a
	M	a	a
	L	a	a

Least squares means comparison table LSM: Treatments that have the same letter are not significantly different at P = 0.05 for repeated measures Analysis of Variance or for the endpoint Analysis of Variance.

Figure 6.12: Mean number of feeding scars per water hyacinth plant either sprayed (S) or not sprayed (NS) with 0.8% glyphosate herbicide, and grown at three nutrient levels: high (H), medium (M) and low (L). Bars = standard error.

The number of adult weevils that were found at the end of the experiment was not significantly different for any of the treatments, indicating that the total numbers were not affected by whether the plant was sprayed or not, or under which nutrient regime the plant grew (Figure 6.13). If the nutrient effect is discounted, the unsprayed plants had significantly more adults than the sprayed plants (one-sided Binomial test, $p = 0.0442$), but if biomass is taken into account by calculating the number of adults per gram of plant material, no significant difference is obtained (ANOVA, $p = 0.3614$), and in fact, the mean number of adults per gram of plant material is higher for the sprayed plants (0.0018291 adults/g) than for the unsprayed plants (0.0012417 adults/g). Plots of the data indicate the mean number of adults found on high-nutrient, unsprayed plants was relatively higher than the mean number of adults found on the high-nutrient, sprayed plants, compared to the medium and low nutrient treatments (Figure 6.13), but the small

number of adults found overall (only 42 out of the initial 480 adults) must be taken into consideration. The presence or absence of larval mines was noted; mines were found in all plants. Therefore it would appear that life cycle of the insect was not affected by either herbicide application or the nutrient level.



LSM		Herbicide	
		NS	S
Nutrient	H	a	a
	M	a	a
	L	a	a

Least squares means comparison table LSM: Treatments that have the same letter are not significantly different at P = 0.05 for the Chi-square tests.

Figure 6.13: Total number of adults per water hyacinth plant either sprayed (S) or not sprayed (NS) with 0.8% glyphosate herbicide, and grown at three nutrient levels: high (H), medium (M) and low (L). Bars = standard error.

6.2.2.3.3 Summary of Results

The sublethal dose (0.8%) of the herbicide Roundup retarded the growth of leaves on water hyacinth plants grown at different nutrient levels (Figure 6.10). The sublethal dose also halted the production of daughter plants at all three nutrient levels (Figure 6.11). The nutrient treatment was not significant for either of these plant parameters; herbicidal treatment determined leaf turnover and ramet production.

The feeding intensity and adult weevil numbers was not affected either by the sublethal concentration of the herbicide, or by the nutrient regime. This suggests that the weevils

will survive on water hyacinth plants that have been sprayed with a 0.8% dose of glyphosate herbicide.

6.2.2.4 Discussion

The extent and rate of spread of water hyacinth infestations in South Africa justify its herbicidal control, which is most often chosen because of the immediate impact it can have on weed populations. It is usually regarded as the primary control option because water managers need a quick solution to a pressing problem. However, planning and implementation are complicated by the effects of floods, drought and pollution, requiring a pro-active allocation of resources and continuity of control activities, a strategy that costs millions of Rands. Any improvement in the efficacy and/or reduction in spray frequency and dosage rates (by using retardant doses) will be important and will provide a more cost-effective control strategy.

Excess use of herbicides may have complex hazardous effects which act via indirect pathways, and may still not be properly understood, despite years of use and research (Wright and Bourne, 1990; Wang et al., 1994). This is exemplified by the work of Relyea et al., (2005), who are uncovering toxic effects of glyphosate on North American amphibian tadpoles. In South Africa, where most – if not all – water bodies are used for potable water, it is advisable to reduce the input of herbicides into the environment as much as possible. Ecotoxological effects of a low dose of glyphosate are covered in Chapter 6, Section 2.3 of this report.

Commercial formulations of glyphosate, such as Mamba, Round Up and Round Ultra are readily available. Mamba (Dow Agro Chemicals) is the preferred herbicide for hyacinth control because it is the cheapest of all the herbicides registered for use against water hyacinth in South Africa (Kathleen Saunders, Implementation Officer, Working for Water**,** personal communication). However, Mamba requires the use of additives like “Mist Control”, which may have a detrimental effect on the biocontrol agents, and the death of biocontrol agents after spraying has been anecdotally noted (Kathleen Saunders, Implementation Officer, Working for Water, personal communication). Although, the Monsanto glyphosate formulation uses a surfactant, our study indicates that it is non-toxic to the weevil *Neochetina eichhorniae* over an eight-week period. However, these findings will have to be extended to longer periods of observation in the field and are discussed in Chapter 6, Section 3.2 of this report. The results presented here indicate that a sublethal dose of 0.8% Roundup could be integrated into the existing biological control methods for water hyacinth in South Africa. The integrated approach is already regarded as cost-effective in terms of per-hectare weed infestation cleared, where the monetary investment has been calculated to be R 277/ ha, in contrast to solely herbicidal control where the cost would be R 1481/ ha because of expenditure on herbicides and follow-up regimes involved (Van Wyk and Van Wilgen, 2002).

Most importantly, given the eutrophic nature of South African fresh waters (which are ideal for water hyacinth growth, being generally above the 0.1 mg/*l* phosphorus lower threshold, with corresponding nitrogen levels in a 7:1 ratio), a sublethal dose of 0.8% Roundup will retard the plant's growth over a wide range of water nutrients. Our findings indicate that nutrient levels do not override the effects of the herbicide in the short- to medium-term. This type of integrated approach may thus contribute to control of unrestricted water hyacinth growth at eutrophic sites, by maintaining the biocontrol agents in the system, on retarded plants. Ultimately, nutrient pollution will have to be addressed in South African fresh water systems, which are ripe for invasion by alien weeds that can take advantage of this nutrient-rich habitat.

6.2.3 What Are The Ecotoxicological Effects of a Sublethal Dose of Glyphosate Herbicide?

6.2.3.1 Introduction

The conclusion drawn from Section 6.2.1 was that a sublethal dose of glyphosate has the potential to be used in the field to control populations of water hyacinth. However, glyphosate has attracted a great deal of negative attention recently because of the harmful effects it can have on larval amphibians (Relyea, 2005a,b,c). Therefore, consideration was given to the effects that glyphosate, at a lethal or sublethal dose, might have on other non-target organisms when used on South African water bodies.

The following experiments are from a manuscript being prepared for publication. The introduction and discussion have been abbreviated to avoid repetition of other sections of this report.

The objective was to modify the methods of Relyea (2005a) using an indigenous African frog species to determine what effect different glyphosate dosages have on *Xenopus laevis* tadpole survival and growth. *X. laevis*, the Platanna or African clawed toad, is permanently aquatic in all its life stages, with a widespread distribution through South Africa, and is therefore a good representative organism for testing the toxicity of water pollutants (Haywood et al., 2004), including herbicides.

Jadhav et al. (2008) have identified a sublethal dose of glyphosate which retards the growth of water hyacinth by inhibiting the production of daughter plants without affecting the survival and reproductive capacity of the biocontrol agents. This offers a sustainable management tool if biocontrol can be integrated effectively with a non-lethal or retardant dose of glyphosate. However, the glyphosate-based herbicide Roundup® has garnered bad press for its non-target toxic effects on various species of amphibians (Relyea, 2005a,b,c; Relyea et al., 2005).

Roundup® is a commercial formulation of glyphosate which is combined with a surfactant, polyethoxylated tallowamine (POEA) to facilitate active penetration of the

herbicide into the leaf cuticles. The half-lives of glyphosate and POEA are 7 to 70 days and 21 to 28 days respectively (Giesy et al., 2000 and Relyea, 2005 c). The maximum concentration of glyphosate expected in the environment is 3.7 mg active ingredient (a.i)/*l* (Giesy et al., 2000) and in natural habitats, Roundup has been detected at concentrations of 0.1 to 2.3 mg a.i/*l* (Relyea, 2005 c). Respective LC_{50} values of 1 to 10 mg and 0.1 to 1 mg a.i/*l* means that Roundup at these concentrations is moderately to highly toxic (Giesy et al., 2000). The impact of Roundup is likely to be a direct toxicity, possibly by damaging the respiratory surfaces of the tadpoles (Edington et al., 2004).

In view of the ecological pressure on freshwater ecosystems, and in particular the global decline of many amphibian species (Houlahan et al., 2000), the impact of glyphosate is of concern to environmentalists and water managers. Therefore, the aim of this experiment was to test the ecotoxic effects of a retardant dose of glyphosate-based formulation Roundup®, on *Xenopus laevis*. It is an ideal test organism because of its fecundity and its ability to obtain embryos throughout the year. Moreover, it occurs throughout South Africa (Passmore and Carruthers, 1995; Channing, 2001) and is entirely aquatic in its lifecycle.

6.2.3.2 Materials and Methods

6.2.3.2.1 Animal Care

Housing and husbandry were under the supervision of the Central Animal Services (CAS) of The University of the Witwatersrand and care and treatment of the animals were in accordance with the guidelines of University of the Witwatersrand Animal Ethics Screening Committee (clearance number: 2006/98/2A). Ten *X. laevis* adults (five males and five females) were held in 10 L polythene water tanks with screen lids, and fed with chicken liver and fish food.

Xenopus laevis mating was induced by injecting a single priming dose of 300 i.u. of Folligon into the dorsal lymph sac of five adult, female frogs in order to induce ovulation. Two days later, a second dose of 750 i.u. human chorionic gonadotropin was injected in order to induce egg laying. Five males were injected with 200 i.u. human chorionic gonadotropin on the day of the females' second dose, for gonadotropin stimulation. Males and females were then paired up and placed in 10 *l* polythene breeding tanks filled with dechlorinated water. Tanks were fitted with wire grating held approximately 30 mm off the bottom of the tank to allow fertilised eggs to drop through. Amplexus, egg laying and fertilization occurred within 24 hours in a darkened room. After amplexus, the frogs were removed from the breeding tanks and the eggs were allowed to hatch. Tadpoles hatched from a single clutch were used for the experiment. All tadpoles used were at Gosner-stage 25 in their development (Gosner, 1960).

6.2.3.2.2 Experimental Design and Procedure

Trials were conducted outdoors at the University of the Witwatersrand, Johannesburg, South Africa. A total of 18 tubs (50 *l* plastic, with a diameter of 52 cm containing 42 *l* of water) were set up, nine with five medium-sized water hyacinth plants in each (+WH), and nine without plants (–WH). Two herbicidal treatments: 0.8% (0.00288 mg a.i/*l*) and 3% (0.0108 mg a.i/*l*) with three replicates each were applied to tubs with and without plants. Three tubs with plants and three without were not sprayed with glyphosate and served as control treatments. Fifteen tadpoles were released into each tub at week 0, where they fed on suspended algal blooms inoculated into the tubs from pond water.

The glyphosate-based herbicide, Roundup® (active ingredient, 360 g/*l* glyphosate (acid equivalent a.e)/*l*, containing 480 g isopropylamine salt of glyphosate/*l*) with the surfactant polyethoxylated tallowamine (POAE), supplied by Monsanto Pty. Ltd. South Africa, was sprayed at either 0.8% or 3% (week 0), using a knapsack sprayer (Multispray, South Africa) calibrated at 140 L/ha, using Tee Jet nozzles (8003E) (Tee Jet Technologies, USA).

The experiment ran for a total of three weeks. Five tadpoles were collected from each tub per week and fixed with 4% alcohol. The body lengths of all tadpoles were measured using a clear ruler.

Two-way ANOVA (Statistica, v6) was used to test the effect of water hyacinth and its different herbicidal treatments on tadpole survival and development in terms of body length within treatments. A student t-test (Statistica, v6) was used to test the effect of herbicide concentrations between treatments. Results were considered significant at the 0.05 probability level.

6.2.3.3 Results

After one week, the number of live tadpoles collected and their mean body lengths in the +WH treatments (+WH control, +WH 0.8% and +WH 3%) were lower compared to -WH treatments (–WH control, –WH 0.8% and –WH 3%), and after two weeks tadpole mortality in all +WH treatments was 100% (Figure 6.14).

6.2.3.3.1 Mortality

After one week, no mortality was observed in any –WH treatments (–WH control, –WH 0.8% and –WH 3%). However, at week two, fewer live tadpoles remained in the –WH 3% treatment (2.66±1.45 SE), but not significantly less than the –WH control (5±0.0 SE) or –WH 0.8% (5±0.0 SE) treatments ($F_{(2,6)} = 2.57$, $p = 0.15$). There were no significant differences between the mean numbers of live tadpoles collected from the –WH control and –WH 0.8% treatments.

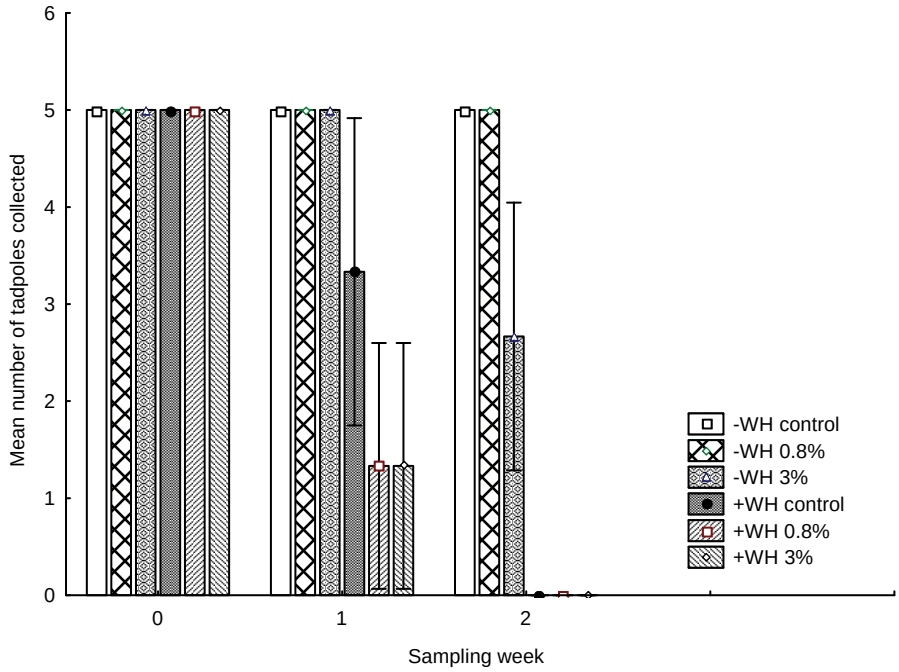


Figure 6.14: The effect of glyphosate herbicide and water hyacinth on survival of *Xenopus laevis* tadpoles in tubs, treated with no (control), 0.8% and 3% glyphosate herbicide. +WH: water hyacinth plants present. -WH: water hyacinth plants absent. Mean (± SE)

At week one, the mean numbers of live tadpoles collected from +WH 0.8% (1.33±1.33 SE), +WH 3% (1.33±1.33 SE) and +WH control (3.3±1.6 SE) were not significantly different ($F_{(2,6)} = 0.63$, $p = 0.56$), despite lower numbers of tadpoles being collected from the +WH0.8% and +WH3% treatments (Figure 6.14). No tadpoles in the +WH treatments survived to week two (Figure 6.14), either with (+WH 0.8% or +WH 3%) or without (+WH control) herbicide applications.

6.2.3.3.2 Body Lengths

After one week, there were no significant differences between the mean body lengths of the tadpoles collected from the –WH control (1.69 cm±0.08 SE), –WH 0.8% (1.65 cm±0.06 SE) and –WH 3% (1.54 cm±0.09 SE) ($F_{(2,42)} = 0.98$, $p = 0.38$) (Figure 6.15). At week two, the mean body length of the –WH 3% treatment was significantly less (1.62 cm±0.42 SE) than that of the –WH control (3.54 cm±0.13 SE), or the –WH 0.8% (3.77 cm ±0.09 SE) treatments ($F_{(2,42)} = 20.22$, $p = 0.000$). However, there were no significant differences between the mean body lengths of the –WH control and –WH 0.8% treatments (t_{28}= -1.35, p= 0.18) (Figure 6.15).

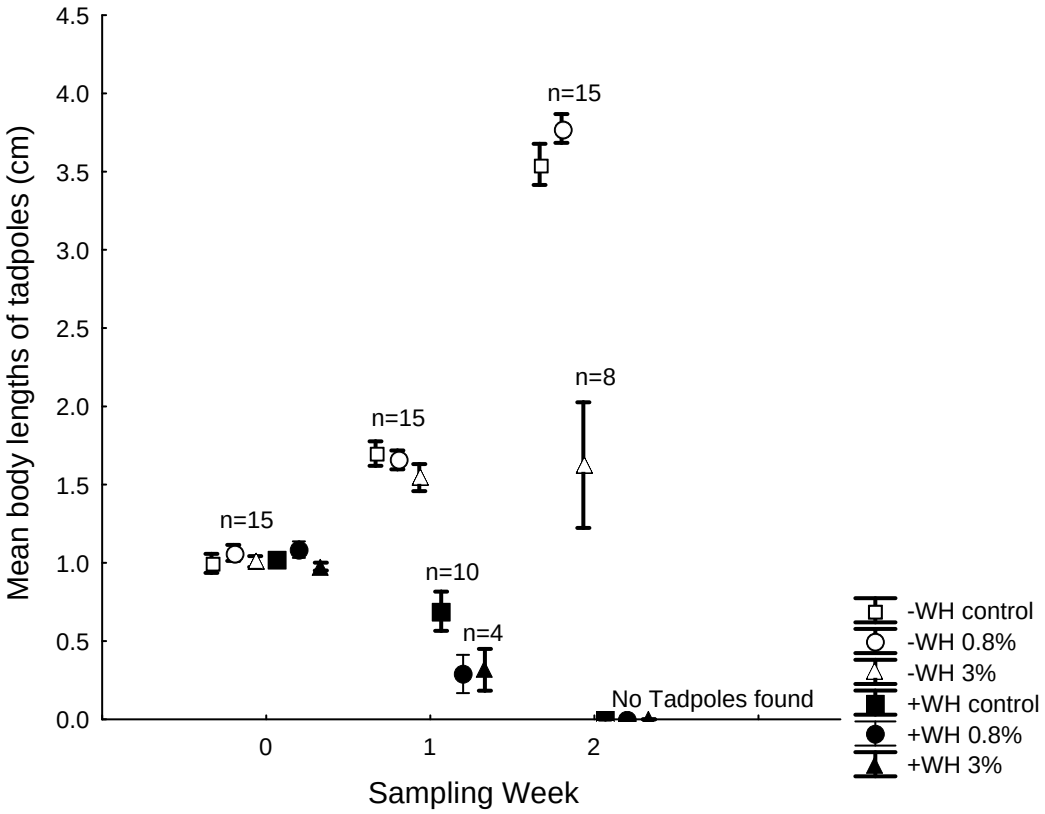


Figure 6.15: The effect of glyphosate herbicide and water hyacinth on the body length of *Xenopus laevis* tadpoles in tubs, treated with no (control), 0.8% and 3% glyphosate herbicide. +WH: water hyacinth plants present = closed symbols; –WH: water hyacinth plants absent = open symbols. Mean (± SE)

After one week all of the +WH treatments showed a much shorter mean body length, which was associated with increased mortality in these treatments. The +WH 0.8% (0.29 cm±0.12 SE) and +WH 3% (0.31 cm±0.14 SE) tadpoles were significantly shorter than the +WH control (0.69 cm±0.13 SE), ($F_{(2,42)} = 2.80$, $p = 0.07$) (Figure 6.15), which was in turn significantly shorter than all –WH treatments. All remaining tadpoles in the +WH treatments had died by week two so no body length measurements could be made.

The presence of water hyacinth cover (+WH) in the experimental tubs had a clear and significant effect on the survival and the body lengths of the tadpoles as delineated by Two-way ANOVA analysis (Body lengths: F= 386.70, df=1, P< 0.0001; tadpole survival: F=54.49, df=1, P< 0.0001).

6.2.3.4 Discussion

The most notable result from this experiment is that, alone or with an application of glyphosate herbicide, water hyacinth is potentially lethal to aquatic amphibians. All *X. laevis* tadpoles died in the treatments containing water hyacinth regardless of whether they were sprayed with herbicide or not. No significant mortality was seen in the herbicide treatments without water hyacinth. *Xenopus laevis* tadpoles feed by filtering

suspended particles out of the water column, while holding themselves in midwater. The water hyacinth had a detrimental effect on the tadpoles, presumably by shading out sunlight which stimulates the growth of the phytoplankton on which the tadpoles feed. Some mortality was seen in the –WH 3% treatment which, given the effect of water hyacinth on plankton, could have been caused by glyphosate reducing the phytoplankton population. Hildebrand et al., (1980) found that Roundup® treatments at concentrations up to 220 kg/ha did not significantly affect the survival of *Daphnia magna* or its food base of diatoms under laboratory conditions. Simenstad et al., (1996) found no significant differences between benthic communities of algae and invertebrates on untreated mudflats and mudflats treated with the glyphosate formulation Rodeo® (which is reported to have no surfactant) and X-77 Spreader®. Nevertheless, water hyacinth has been shown here to present a threat to aquatic wildlife beyond that which the herbicide alone presents. The detrimental effect of alien weeds on insect fauna is well-documented for terrestrial weeds (Kinvig and Samways, 2000; Samways and Moore, 1991), and water hyacinth has been shown to suppress aquatic invertebrate fauna in South Africa (Midgley et al., 2006; Jones, 2009). However, this is the first time that the weed has been shown in laboratory trials to be potentially more lethal than its herbicidal control.

Water hyacinth can act through other indirect routes by lowering the water temperature, bicarbonate alkalinity and dissolved oxygen content and increasing the pH, free carbon dioxide content, biological oxygen demand (BOD) and nutrient levels (Gopal, 1987; van Wyk and van Wilgen, 2002). Edington et al. (2004) have shown that the toxicity of Vision herbicide (glyphosate-based) to *X. laevis, R. clamitans, R. pipiens* and *B. americanus* is enhanced by an increase in pH. In addition, glyphosate photo-degrades (Tu et al., 2001) and disappears from water within three days of treatment when exposed to sunlight (Wang et al., 1994). However, the shading produced by the water hyacinth canopy could have interfered with this photo-degradation, leading to a prolonged residence in the treated water.

An application of the label-recommended dose (3%) and a retardant dose (0.8%) of glyphosate based formulation Roundup® did not kill *X. laevis* tadpoles, and apparently did not interfere with the development of the tadpoles as measured by body length. Wojtaszek et al. (2004) concluded that Vision, (glyphosate isopropyl amine salt, 356 g/*l* plus MON 0818 surfactant blend 15% by weight; Monsanto, Canada) applied to mesocosms at an "expected environmental concentration" of 4.61 mg/*l* did not cause any significant mortality to larval leopard frogs, *Rana pipiens* or green frogs, *R. clamitans*. However other studies have shown that lower concentrations of Roundup reduced *R. pipiens* tadpole survival by 94%, tree frog, *Hyla versicolor*, survival by 73%, and American toad, *Bufo americanus*, tadpole survival by 97% (Relyea, 2005 b – Roundup® at a concentration of 3.8 mg of a.i./*l*). These contradictory results could be

due to the details of each experimental setup, including the precise way in which the herbicide was applied, which formulation was used, and what other ions and molecules were present in the water body. Various glyphosate formulations have differential toxicity towards amphibian species. For example Touchdown 480 and Roundup Biactive at label doses did not cause amphibian mortality (Howe et al., 2004). But Howe et al. (2004) concluded that Roundup Original (MON 78078) was toxic to *R. pipiens*, *R. sylvatica* and *B. americanus*, and LC_{50} values at 24hrs were 11.9, 18.1 mg and 13.5 mg/*l* respectively. Conversely, Relyea (2005c) determined that the 16-day LC_{50} values for Roundup® "Weed and Grass Killer" for six species of North American amphibian larvae (*R. sylvatica, R. pipiens, R. clamitans, R. catesbeiana, B. americanus* and *H. versicolor*) ranged from 0.5 to 2.5 mg a.i/*l*. Perkins et al. (2000) noted that a concentration of 12.4 mg/*l* a.i. of Roundup caused 50% mortality of *X. laevis* at 96 hours.

The surfactant POEA has been implicated as the main toxic ingredient in these formulations (Mann and Bidwell, 1999; Giesy et al., 2000; Perkins et al., 2000; Lajmanovich et al., 2003, Howe et al., 2004), and may account for much of the variation in published results. However, two important points are worth making. Firstly, the concentration of active ingredient of a retardant dose is 0.00288 mg/*l*, much less than that of any of the preceding studies. Secondly, the concentration of the surfactant, POEA, is also correspondingly lower as much less of the product is used. Reduction in the percentage of POEA surfactant has been shown to reduce the toxicity of glyphosate formulation (Wan et al., 1989).

Giesy et al. (2000) concluded that, provided that factors such as application rate, depth of water, vegetation density, and overall rehabilitation goals are considered, aquatic habitat restoration using Roundup® will not lead to unreasonable adverse effects to the environment and amphibians. Integrated control methods, such as a sublethal herbicide dose in combination with biocontrol agents, will reduce the concentration of the herbicide and POEA (to 60% less than the label-recommended dosage) and its associated effects on non-target organisms including other waterside vegetation. Bearing in mind that water hyacinth alone may be more deadly than any control measures, control by any means may be better than no action.

6.2.4 What is the Effect of a Low dose of Herbicide on the Nutrient Status of the Weed?

The preceding sections have shown sublethal doses of glyphosate herbicide to be effective in the laboratory at suppressing the growth and reproduction of water hyacinth, while being less detrimental to aquatic fauna than water hyacinth itself is. The effect of the herbicide on populations of *Neochetina* weevils resident on the plants appears to be neutral or even positive in some cases (Sections 6.2.1 and 6.2.2). Because glyphosate interferes with amino acid synthesis, low doses could possibly increase the availability of nitrogen to weevils feeding on treated plants, which in turn should promote the growth of immature stages. This section, which is being prepared as a paper, reports on experiments to measure the levels and availability of nitrogen in water hyacinth plants in the field, which have been treated with a sublethal dose of glyphosate.

6.2.4.1 Introduction

Plants vary in their response to damage, whether it is physical, such as herbivory or mowing; or chemical, such as herbicides. Stress can have indirect effects on insect herbivores by altering the nutrition and defence metabolism of the host plant (De Bruyn et al., 2002; Huberty and Denno, 2004; Nykanen and Koricheva, 2004). Nutrients are among the most important resources that influence plant community biomass, which is usually controlled by nitrogen and phosphorus (Morris, 1991). Low levels of leaf nitrogen are associated with decreased feeding preference by insect herbivores (Moran and Hamilton, 1980). Wilson et al. (2006) have shown that water hyacinth plant quality affects *N. eichhorniae* pupal size. However, improved plant quality does not necessarily result in increased plant damage by herbivores: if the quantity of nutrients obtained from petiole material, for example, is proportional to larval size and petioles are low in nutritive quality, more leaves and more time are required to reach pupation under low nutrient conditions (Moran and Hamilton, 1980).

Integrated control methods have been developed for several terrestrial weed systems. Application of glyphosate Roundup® at 2% (Lindgren et al., 1999) during late bloom stage, did not affect survival or establishment of *Galerucella calmariensis* L. (Coleoptera, Chrysomelidae), a biocontrol agent of purple loosestrife. Autumn application of 2,4-D and Picloram did not interfere with the survival capacity and the overwintering fitness of the leafy spurge biocontrol agent, *Aphthona* sp. (Coleoptera, Chrysomelidae), and this integrated strategy was considered to be economical when compared to biocontrol alone (Lym 1998; Lym et al., 1996; Lym and Nelson 2002; Nelson and Lym, 2003). Similarly, Wilson et al. (2004) concluded that integrated control of diffuse knapweed with low rates of Picloram and Clopyarid combined with *Sphenoptera jugoslavica* (Coleoptera: Buprestidae) was more efficient than control with *S. jugoslavica* alone. Integration of herbicides with biocontrol has been successful against leafy spurge where 2.2 kg ae/ha (ae: acid equivalent) of 2, 4-D and Picloram did not hinder the establishment of the larvae of leafy spurge hawk moth, *Hyles euphorbiae*

(Rees and Fay, 1989), and long-term establishment of *Spurgia esulae* Gagne (Diptera, Cecidonyiidae) larval populations was not affected by applications of 2,4-D, Imazethapyr and Picloram plus 2,4-D at 1.1 kg, 0.28 kg and 0.28+1.1 kg respectively (Lym and Carlson, 1990). Low doses of Picloram did not limit the establishment of *Cyphocleonus achates*, a biocontrol agent of spotted knapweed (Jacobs et al., 2000). Application of 2,4-D and clopyralid during autumn proved to be compatible with *Cyphocleonus achates* (Coleoptera: Curculionidae) and *Agapeta zoegana* (Lepidoptera: Tortricidae) for the control of spotted knapweed, whereas spring applications interfered with the successful establishment of the biocontrol agents (Story and Stougaard, 2006). The biocontrol agents *Urophora affinis* Frauenfeld and *U. quadrifaciata* (Meigen) (Diptera: Tephritidae) have been successfully integrated with 2,4-D and Picloram for spotted knapweed control.

It is therefore evident that "the desirability of integrating biological control agents with other techniques has been frequently and convincingly explained by several authors" (Ainsworth, 2003), and for several orders of insects on a variety of plants. A sublethal dose of glyphosate has repeatedly been shown to be compatible with both the weevil and mirid water hyacinth biocontrol agents, in that it suppresses growth of the weed without harming the biocontrol agent populations (Jadhav et al., 2008). In some cases it even seemed to boost agent populations.

An additional benefit of integrating herbicidal and chemical control could be a paradoxical improvement in plant quality from the insects' point of view, stimulated by a low dose of herbicide. Wright and Bourne (1990) showed that 2,4-D amine improved water hyacinth plant quality by decreasing leaf and petiole hardness, thereby improving plant quality for larval stages of the moth, *Niphograpta albiguttalis* and the two *Neochetina* species. Glyphosate acts by inhibiting the enzyme 5-enoylpyruvylshikimate-3-phosphate synthase, thereby blocking the shikimic acid pathway and disrupting the synthesis of aromatic amino acids. Protein synthesis is therefore affected, and the synthesis of phenylalanine-derived phenols. These include secondary metabolites which can act as feeding deterrents or digestion inhibitors (Westwood and Biesboer, 1986). Therefore glyphosate has the potential to change nitrogen metabolism in the plant, which could be beneficial to growing larvae and adult females maturing eggs. Reduction in phenols could make the plants more palatable to biocontrol agents and other herbivores.

Water hyacinth grows as a whorl, from the centre outwards, allowing leaf age to be determined by its position. *Neochetina* weevils preferentially use leaf two and leaf three for feeding and oviposition. Hatching larvae then work their way down the leaf petiole to the plant crown where the third instar larvae are found. In this way leaf and beetle phenology are linked and could be affected by differential nitrogen distribution in the plant.

6.2.4.2 Methods

Field trials were run to discover which of the above-water parts of the water hyacinth plant should be sampled, and when, to best understand the effect of glyphosate on nitrogen and carbon levels in the plant. These data were then used to examine the effect of glyphosate on the carbon:nitrogen ratio of the plant material with regard to its nutritional value to the weevils.

6.2.4.2.1 Field Setup

Trials were carried out at Delta Park and Farm Dam, Johannesburg in spring, November 2008. At each site a strip of water hyacinth plants, approximately 12x3 metres, was restrained between two cables fitted with buoys. Cables perpendicular to these divided the strip into two equal plots of 6x3 m, each comprising a treatment plot and a control plot. Both plots were each further subdivided into three blocks 2x3 m, forming three replicates per plot.

The treatment plots at each site were sprayed with 0.8% Roundup (glyphosate; active ingredient: 360 g/*l*, Monsanto Pty. Ltd. South Africa) at a spray volume of 140 *l*/ha, giving an active ingredient of 0.07 g/m^2 (Jadhav et al., 2008), while the control plot was untreated. A 12V battery-operated boom sprayer (Multispray, Midrand, South Africa) with three spray tips (Tee Jet even flat: TP65015E) was used to spray the weed from a motor boat moving at 4 km/h.

Two weeks after spraying, four plants were collected from the sprayed plots and from the control plots. These plants were broken up into leaf one, leaf two, leaf three and the crown. These were oven-dried at 60ºC for 18 hours. Each plant part sample was replicated three times and each replicate weighed at least two grams. Samples from both sites were sent to BemLab, Stellenbosch for nitrogen and carbon analysis. Four weeks after spraying, another batch of samples prepared in the same way as the above were sent to BemLab for analysis.

6.2.4.2.2 Statistical Analysis

Data were analyzed by two-way Analysis of Variance (ANOVA). If significant differences were found, Tukey's HSD multiple comparisons test was performed to determine where the differences lay. To compare different values between sites or phenostages, a student's t-test was used.

6.2.4.3 Results

Within sites, minor differences were found in the nitrogen content of different water hyacinth leaves on a plant, and the carbon values followed a similar pattern. However, plant crowns contained significantly less nitrogen and carbon than leaves, and substantial differences in both elements were found between plants from different sites.

Spraying with a sublethal dose of herbicide reduced the nitrogen content of plants in all cases.

The small plants at Delta Park showed no significant difference in nitrogen content between leaves of the same plant (Control leaves, $F_{(2,6)} = 1.067$; $p = 0.4011$; Treatment leaves, $F_{(2,6)} = 0.127$; $p = 0.8830$), but had significantly lower levels of nitrogen in the crown and in plants that had been sprayed with a sublethal dose of glyphosate sampled at week two (Control *vs.* Treatment leaves, $F_{(2,6)} = 32.400$; $p = 0.00471$), but crowns did not differ in their nitrogen content (Control *vs.* Treatment crowns, $F_{(1,4)} = 0.0670$; $p = 0.8085$) (Figure 6.16A). These patterns were maintained in plants from the same site sampled at week four (Control leaves, $F_{(2,6)} = 3.898$; $p = 0.0822$; Treatment leaves, $F_{(2,6)} = 3.946$; $p = 0.0806$; Control *vs.* Treatment leaves, $F_{(2,6)} = 24.075$; $p = 0.0080$), except that the nitrogen levels in the treated crowns became significantly lower than those in the control crowns (Control *vs.* Treatment crowns, $F_{(1,4)} = 9.9214$; $p = 0.0345$) (Figure 6.16B). However, carbon values in the plants were less affected by the herbicide (Control *vs.* Treatment leaves, week 2, $F_{(2,6)} = 0.7280$; $p = 0.4416$, week 4, $F_{(2,6)} = 4.2956$; $p = 0.1069$) and significant differences were only noted between sprayed and unsprayed crowns at week two where the treated plants had a higher carbon content than the control (Control *vs.* Treatment crowns, week 2, $F_{(1,4)} = 21.794$; $p = 0.0095$; week 4, $F_{(1,4)} = 0.5009$; $p = 0.5182$) (Figure 6.17).

Larger plants from Farm Dam had overall higher levels of nitrogen than those at Delta Park but followed similar patterns in that the nitrogen content of consecutive leaves did not differ (Control leaves, $F_{(2,6)} = 0.0196$; $p = 0.9806$; Treatment leaves, $F_{(2,6)} = 1.5481$; $p = 0.2870$), but was significantly greater than that of the crowns, which had significantly higher nitrogen levels in the treatment at week two (Control *vs.* Treatment crowns, $F_{(1,4)} = 50.9253$; $p = 0.0020$) (Figure 6.18A). However, at week four, nitrogen values generally dropped, but remained higher in the untreated plants' leaves (Control *vs.* Treatment leaves, $F_{(2,6)} = 15.6796$; $p = 0.0166$) , except for the crown values, which equalised (Control *vs.* Treatment crowns, $F_{(1,4)} = 1.1250$; $p = 0.3486$) (Figure 6.18B). Carbon content was similar to the Delta Park plants and changed little between treatments (Control *vs.* Treatment leaves, week 2, $F_{(2,6)} = 1.78801$; $p = 0.2521$, crowns $F_{(1,4)} = 3.2786$; $p = 0.1444$). However, it did significantly increase in the leaves, though not the crowns, of untreated plants on week four(Control vs. Treatment leaves week 4, $F_{(2,6)} = 10.9155$; $p = 0.0298$, crowns $F_{(1,4)} = 61.2896$; $p = 0.0014$) (Figure 6.19).

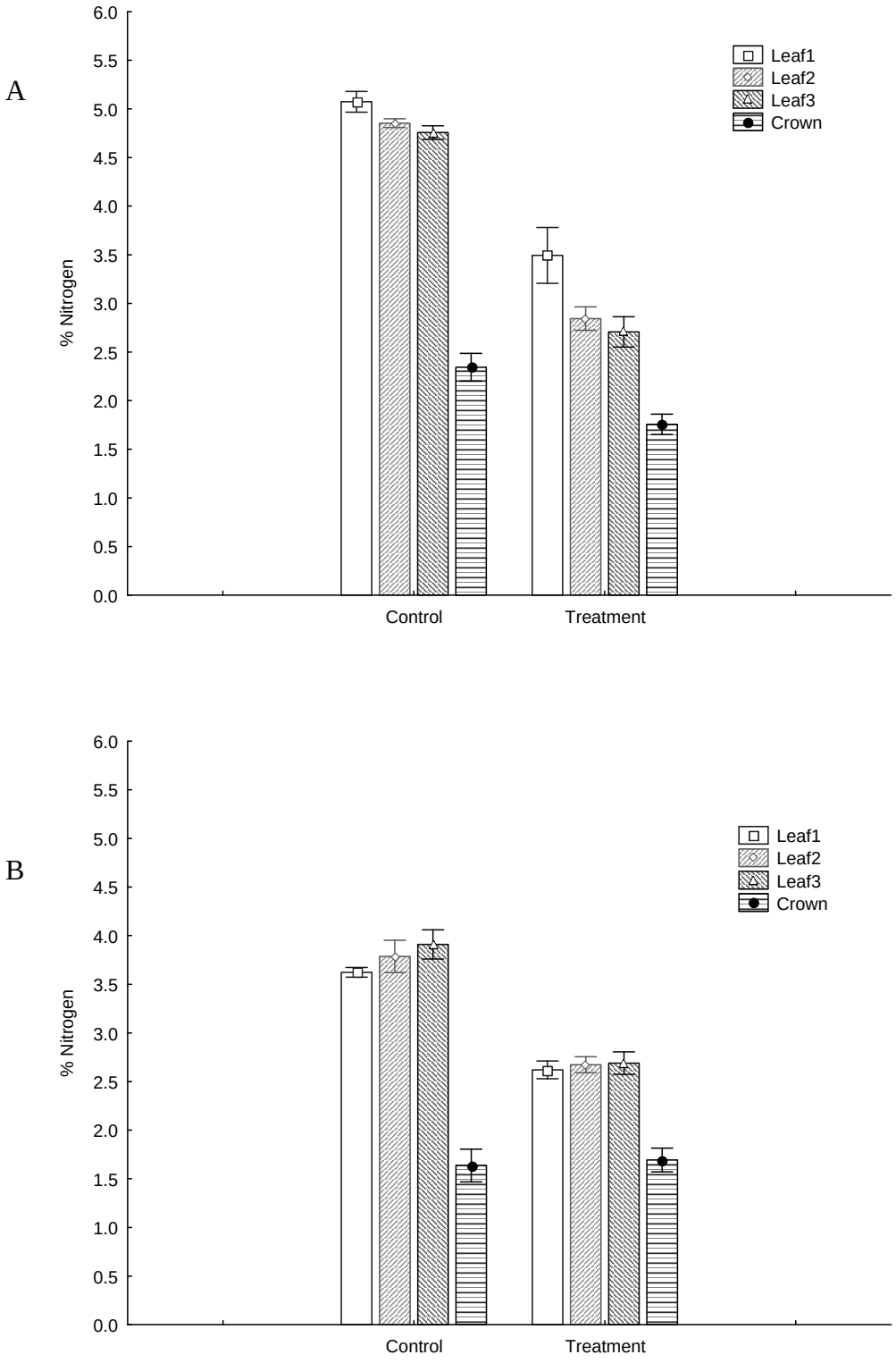


Figure 6.16: Nitrogen content of different parts of water hyacinth plants from Delta Park, sampled two (A) and four (B) weeks after spraying with Roundup; glyphosate 360 at 0.8%, 140 *l*/ha.

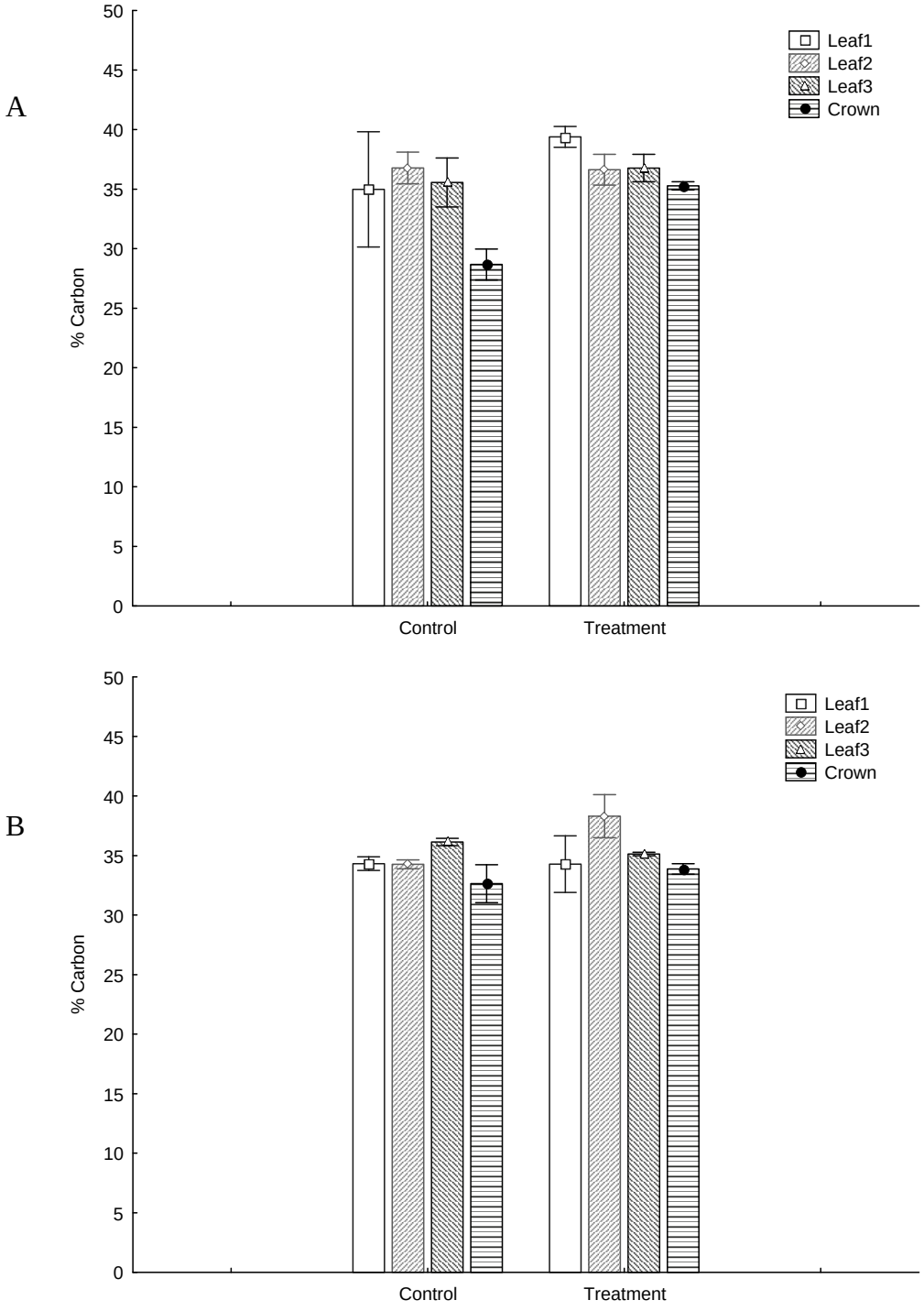


Figure 6.17: Carbon content of different parts of water hyacinth plants from Delta Park, sampled two (A) and four (B) weeks after spraying with Roundup; glyphosate 360 at 0.8%, 140 *l*/ha.

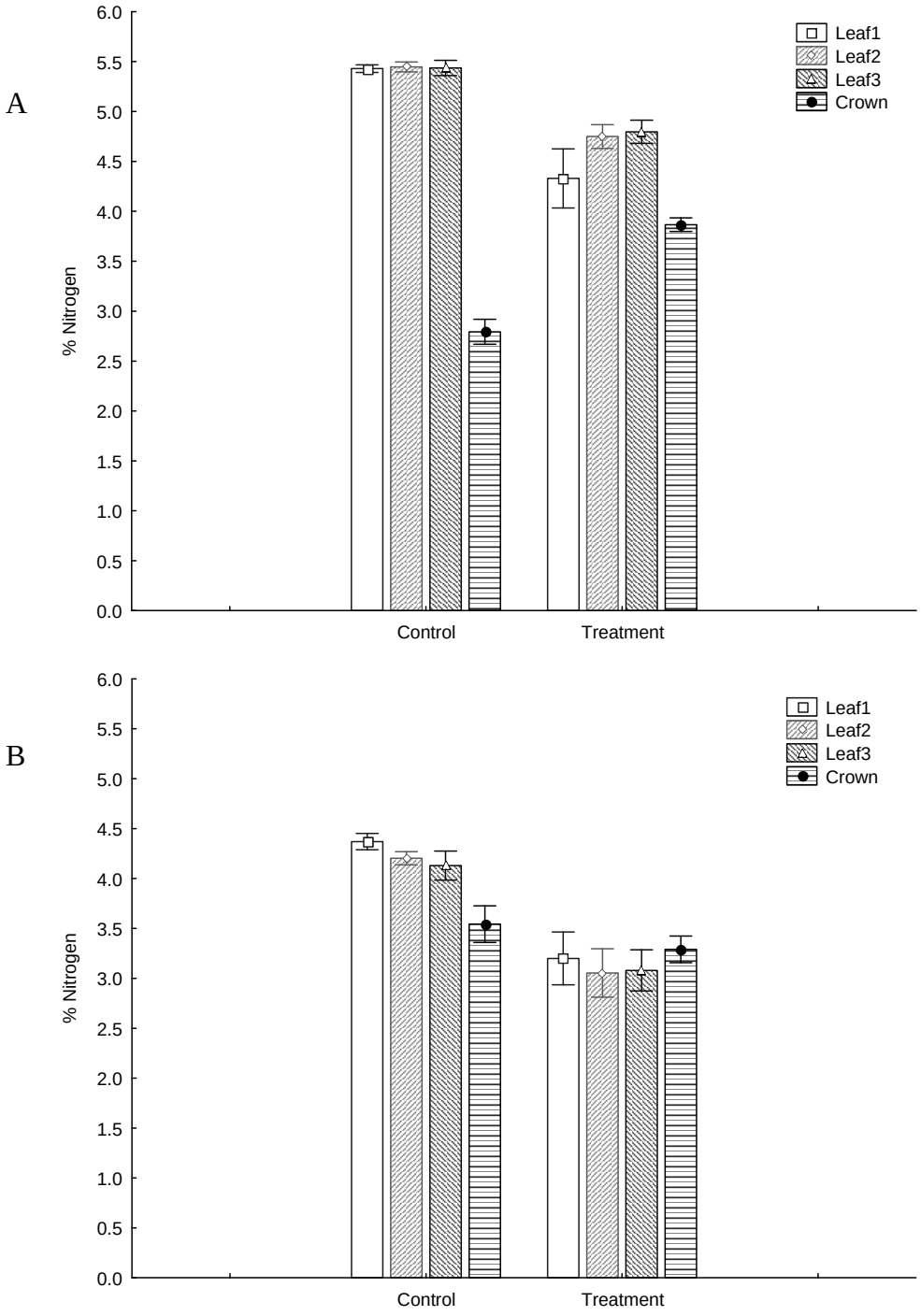


Figure 6.18: Nitrogen content of different parts of water hyacinth plants from Farm Dam, sampled two (A) and four (B) weeks after spraying with Roundup; glyphosate 360 at 0.8%, 140 *l*/ha.

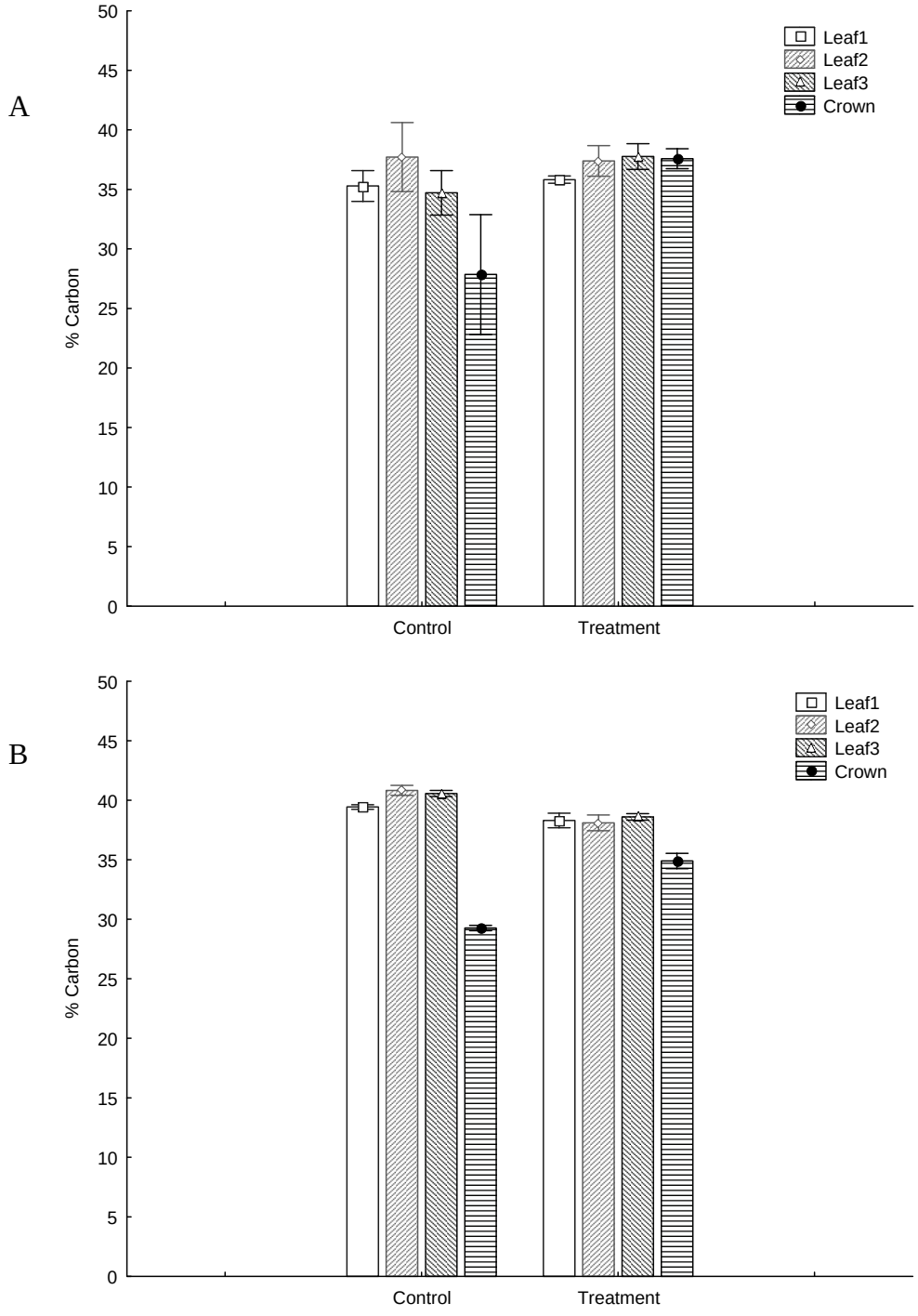


Figure 6.19: Carbon content of different parts of water hyacinth plants from Farm Dam, sampled two (A) and four (B) weeks after spraying with Roundup; glyphosate 360 at 0.8%, 140 *l*/ha.

Plant data from Delta Park were pooled to compare carbon, nitrogen and phosphorus content of the plants, which showed that treatment with a sublethal dose of glyphosate significantly reduced the nitrogen content of both crowns and leaves of water hyacinth and also the phosphorus content of the leaves, without altering the carbon content (Figure 6.20). Consequently, the glyphosate treatment significantly increases the carbon:nitrogen ratio of treated plants (Figure 6.21).

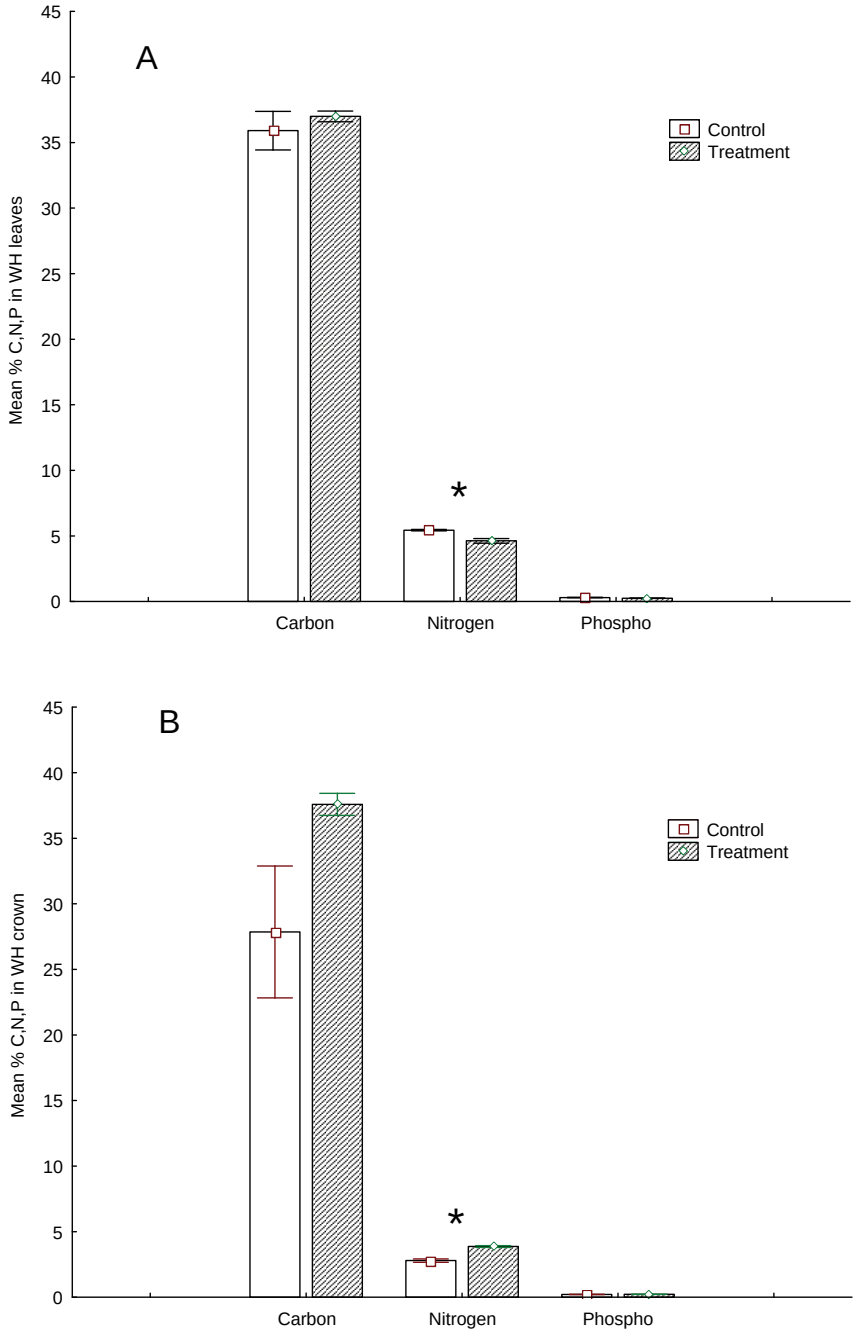


Figure 6.20: Nitrogen, carbon and phosphorus content of water hyacinth plants from Farm Dam, leaves (A) and crowns (B) two weeks after spraying with Roundup; glyphosate 360 at 0.8%, 140 *l* /ha. * pairs of means are significantly different at P<0.05.

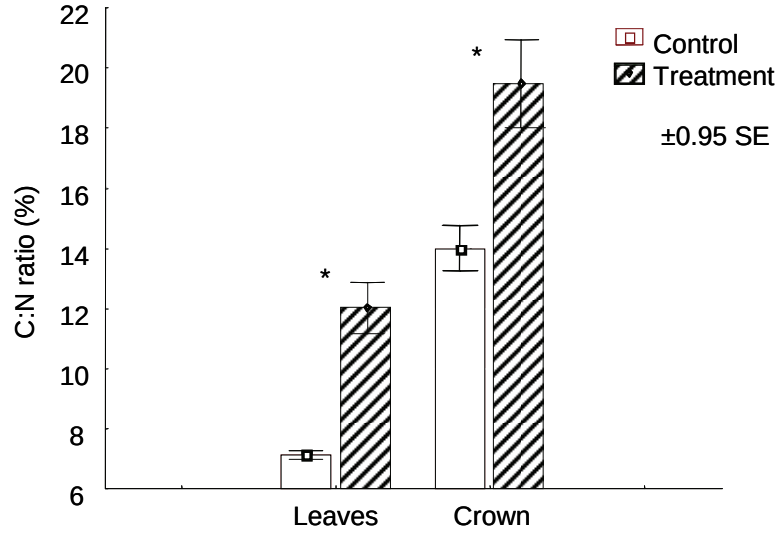


Figure 6.21: Carbon:Nitrogen ratios of water hyacinth plants from Farm Dam, two weeks after spraying with Roundup; glyphosate 360 at 0.8%, 140 *l* /ha. * pairs of means are significantly different at P<0.05.

6.2.4.4 Discussion

Contrary to expectations, treatment with glyphosate reduced the levels of nitrogen found in water hyacinth. Because the carbon content of the plants is unaffected by glyphosate, the overall result is a higher carbon:nitrogen ratio which presumably makes it more difficult for the weevils to access the nitrogen (Elser et al., 2000). However, results from the effect of glyphosate on weevil populations has been equivocal (Sections 6.2.1 and 6.2.2, this report), not demonstrating any obvious beneficial or detrimental effects. Therefore this clear result with nitrogen levels suggests that the larval weevils might not be limited by nitrogen, or that free nitrogen might be unaffected even though the overall levels drop, although this seems unlikely. It is also possible that plant defences such as leaf hardness or phenol production, which were not measured in this study, might be suppressed in some way which counters the drop in nitrogen recorded in treated plants. Anecdotal evidence from bird feeding at Farm Dam showed treated plants were preferentially grazed by water birds.

Nitrogen or phosphorus deficiency generally results in the accumulation of carbohydrates in the leaves. This rerouting of primary metabolism and resources increases the transportation of sugars to the roots, resulting in a higher allocation of carbon to the roots, which in turn increases the root:shoot (R:S) biomass ratio (Hermans et al., 2006). This could be advantageous to late-stage larvae, which are generally found in the crowns of the plant, where they accumulate fat in preparation for pupation. Increased levels of carbon in the plant crown, seen at both sample dates at Farm Dam

and the first at Delta Park, might go some way to explain apparently beneficial effects for the weevils seen on some of the treatment dates.

Plants primarily store nitrogen as free amino acids (Schneider et al., 1996) and specific proteins (Wetzel et al., 1989). Phosphorus is found in water hyacinth and other perennials that overwinter using vegetative, non-reproductive biomass, stored as organic molecules (esters, lipids and nucleic acids) (Schachtman et al., 1998) in vacuoles. Seasonal allocation patterns of both critical nutrients and carbohydrate reserves follow clear patterns relative to the growth phase of aquatic plants and allocation patterns vary seasonally both for the entire plant, and for individual organs throughout the year (Madsen, 1991). Carbohydrate reserves are important to the overwintering and competitive ability of many macrophytes: a typical pattern for carbohydrate storage indicates high levels in spring before intensive plant growth, followed by a consumptive period through which reserves are depleted through the early and mid growing season. This could be responsible for the decline in nitrogen levels seen at Farm Dam between the two sampling dates. Plants at Delta Park were very small and may not have started their spring burst of growth.

After the active growth has ceased, the plant allocates fixed carbon to storage for overwintering (Best and Visser, 1987). Similarly, production of propagules follows a seasonal cycle. In general, propagule development follows the period of active biomass accumulation, coinciding with seed and flower formation. Through either environmental or physiological cues, the plant diverts energy and fixed carbon from biomass production to flowers, seeds and the formation of asexual propagules. No major changes were seen in carbon allocation in these trails which could be explained by the plants not flowering and having done the majority of their asexual reproduction by ramet production in winter. In many species, the propagule is the only remaining part of the plant during winter. This does apply to water hyacinth, where daughter plants remain attached to the mother plant through winter and into spring, but the fate of the mother plant has not been followed to determine if it dies in summer. Anecdotal observations of nodal scars on the roots suggest that this is not the case. Although the proportion of resources distributed to sexual reproduction of water hyacinth is probably minimal, plants continue to produce daughter plants throughout winter (Chapter 2), showing considerable investment of resources to asexual reproduction.

The unfavourable carbon:nitrogen ratios caused by sublethal doses of glyphosate are clear. However, the seasonality of this effect remains unexplored, as does the potentially differential seasonal effect on the water hyacinth weevils as their phenology changes through the growing season, developing from early instar larvae feeding in the leaves to late instar larvae feeding in the plant crowns. Nevertheless, it is predicted that a spring/summer sublethal spray of glyphosate which reduces leaf nitrogen, while not killing these insects, is more likely to be detrimental to the development of the

population, which will be feeding in the leaves and petioles of the plants, than a late season autumn spray, which will generally increase nitrogen levels in the plant crowns and may even increase sugar content, favouring late instar larvae as they prepare to overwinter in the plants.

6.3 Field Trials

6.3.1 When Should a Low Dose of Herbicide be Applied in the Field?

The following experiments are presented in the form of a manuscript which is being prepared for publication. To avoid repetition within this report some sections of the introduction and discussion have been abbreviated.

The objective was to field-test laboratory-developed methods which have already been published (Jadhav et al., 2008), to test their efficacy on wild populations of water hyacinth. Field application will require that herbicide intervention should take place at a particular point in the growth cycle of the weed. This paper sets out to determine at what stage of the plant and insect growth cycle herbicide should be applied, and then to test what effect this would have on a population of water hyacinth grown outdoors.

6.3.1.1 Introduction

Jadhav et al. (2008) have shown that a concentration of 0.8% glyphosate suppresses the growth of water hyacinth without causing direct detrimental effects to water hyacinth biocontrol agents. These results demonstrate that application of retardant doses of herbicide might be used for integrated control of *E. crassipes*. However, one of the key aspects of the successful implementation of an integrated management approach is the optimal timing of the herbicide application, which requires information on the growth patterns of the weed and its biocontrol agents (Cullen, 1996).

Consideration of the effects of temperature on both the plants and the biocontrol agents is crucial for predicting senescence or growth patterns associated with the plant and insect phenology, which will enable efficacious timing of herbicide sprays. The aims of this investigation were:

(1) to study seasonal plant and insect phenology at two weed-infested sites with differing climatic conditions, in order to determine at what time of year herbicide should be applied;

(2) to test the seasonal effect of the retardant dose of glyphosate on water hyacinth and its biocontrol agents, *N. eichhorniae and N. bruchi*, under semi-field conditions.

6.3.1.2 Materials and Methods

6.3.1.2.1 Seasonal Plant and Insect Phenology at Two Climatically Different Sites

Water hyacinth sites were identified using Principal Component Analysis (PCA) of climate data from Schulze et al., (1997), using daily maximum and minimum temperatures, average number of days with frost, and altitude (Chapter 1).

Consequently, two sites, one a high-altitude interior site with limiting cold winters (Farm Dam) and the other a warmer, lower coastal site (Mbozambo Swamp), were monitored for a period of 24 months. Plant biomass was taken from wet weight measures of above- and below-water live plant material in three 0.5x0.5 m quadrats, removed from the water hyacinth mat at each site every month. In addition, 10 plants per site were randomly selected and plant growth parameters such as the number of ramets produced were recorded, after which the plants were destructively sampled to record insect parameters such as feeding scars on the second youngest leaf, and number of petioles mined. These measures were used to infer adult and larval (respectively) weevil populations at each site.

At each site temperature dataloggers (Thermochron iButtons) were placed at three microsites; in the water, within the water hyacinth canopy and at 1.5 m above ground (King et al., 2005; WRC Deliverable 3). Temperatures were recorded at 30-minute intervals. Daily maximum and minimum temperatures were averaged and plotted to discern any seasonal trends.

6.3.1.2.2 Seasonal Effect of the Retardant Dose of Glyphosate on Water Hyacinth and its Biocontrol Agents, *Neochetina spp*.

Trials were carried out during autumn (April) and spring (September-October) of 2007. The methods described apply to both trials.

Two experimental pools (3 m diameter), each with a full mat of water hyacinth, were maintained outdoors at the Weeds Division, Plant Protection Research Institute, Pretoria, South Africa. A plastic rope divided each pool into two equal sections. One section was sprayed with a concentration of 0.8% glyphosate (active ingredient= 0.11 g/m²). The other half was covered during spraying and formed the control. These pools were maintained as part of a mass rearing programme at the Plant Protection Research Institute and thus contained a healthy population of adult weevils and various developmental stages of the weevils, *Neochetina eichhorniae* and *N. bruchi*. Six medium-sized water hyacinth plants (for the autumn trial) and five plants (for the spring trial) per pool per section were randomly selected and tagged. These plants were then measured weekly during the sampling period (four weeks for the autumn trial and three weeks for the spring trial).

A broad spectrum, glyphosate-based herbicide, Roundup® (active ingredient isopropylamine salt (360 g/*l*) with the surfactant polyethoxylated tallowamine (POAE), supplied by Monsanto Pty Ltd, South Africa, was sprayed at the abovementioned concentration using a knapsack sprayer (Multispray, South Africa) calibrated at 140 *l*/ha, using Tee Jet nozzles (8003E) (Tee Jet Technologies, USA).

The following parameters were recorded on the tagged plants: total number of ramets and leaves, number of adult weevils, instars, feeding scars, and petioles mined. Wet weights were also recorded at the start and the end of the experiment.
Endpoint analysis to compare mean values of the above parameters (at day 21 for spring trial and at day 28 for autumn trial), using student's t-test (Statistica, version 6) was carried out on each of the parameters measured and the results were considered significant at the 0.05 probability level.

6.3.1.3 Results

6.3.1.3.1 Seasonal Plant and Insect Phenology at Two Climatically Different Sites

Low winter water temperatures (9.1 ±0.13°C) and frequent frosting at the high-altitude interior field site correlated with reduced plant biomass each winter, while conversely, ramet production increased during winter as temperature declined (Figure 6.22A). Similar patterns were observed at the warm coastal site, where water temperatures remained sufficiently warm in winter (19.5 ±0.13°C) to allow active plant growth throughout the year (Figure 6..22B).

At both field sites a distinct lack of synchrony was observed between the onset of water hyacinth growth and increasing levels of *Neochetina* activity following winter, leading into spring. A lag period of approximately one month was evident between the start of living plant biomass accumulation and the onset of *Neochetina* herbivory during post-winter recovery (Figures 6.23A and 6.23B).

6.3.1.3.2 Seasonal Effect of a Retardant Dose of Glyphosate on Water Hyacinth and its Biocontrol Agents, *N. eichhorniae* and *N. bruchi*

The mean number of ramets produced by plants sprayed with 0.8% glyphosate during the autumn and spring spray regimes was significantly lower (autumn: t_{22}= 2.916; p=0.008; spring: t_{18}= 6.772941; p=0.000002) than those produced by the unsprayed control plants (Figures 6.24A and 6.24B respectively). In addition, the mean number of leaves produced by the sprayed plants in autumn and spring was significantly lower (autumn: t_{22}=2.22; p= 0.03; spring: t_{18}=3.28; p=0.004) than those of the control plants (Figures 6.25A and 6.25B).

There were no significant differences between the mean numbers of weevil larvae found on the sprayed plants compared to the unsprayed plants at the end of the autumn or spring season (autumn: t_{22}= 0.3978, p= 0.69; spring: t_{18}= -1.29, p= 0.21) (Figures 6.26A and 6.26B) and the number of petioles mined was not significantly different between the treatment and control plants during autumn (t_{22}= -0.395, p= 0.69). However, the mean number of petioles mined during spring was significantly higher in the sprayed plants than in the unsprayed plants (t_{18}= -3.65, p= 0.001), (Figures 6.27A and 6.27B).

Although low numbers of adult *Neochetina* weevils were found on the sprayed plants compared to the unsprayed plants, the numbers were not significantly different between treatments for either the autumn or the spring trials (autumn: t_{22}= 0.61, p= 0.54; spring: t_{18}= 0.42, p= 0.67) (Figures 6.28A and 6.28B).

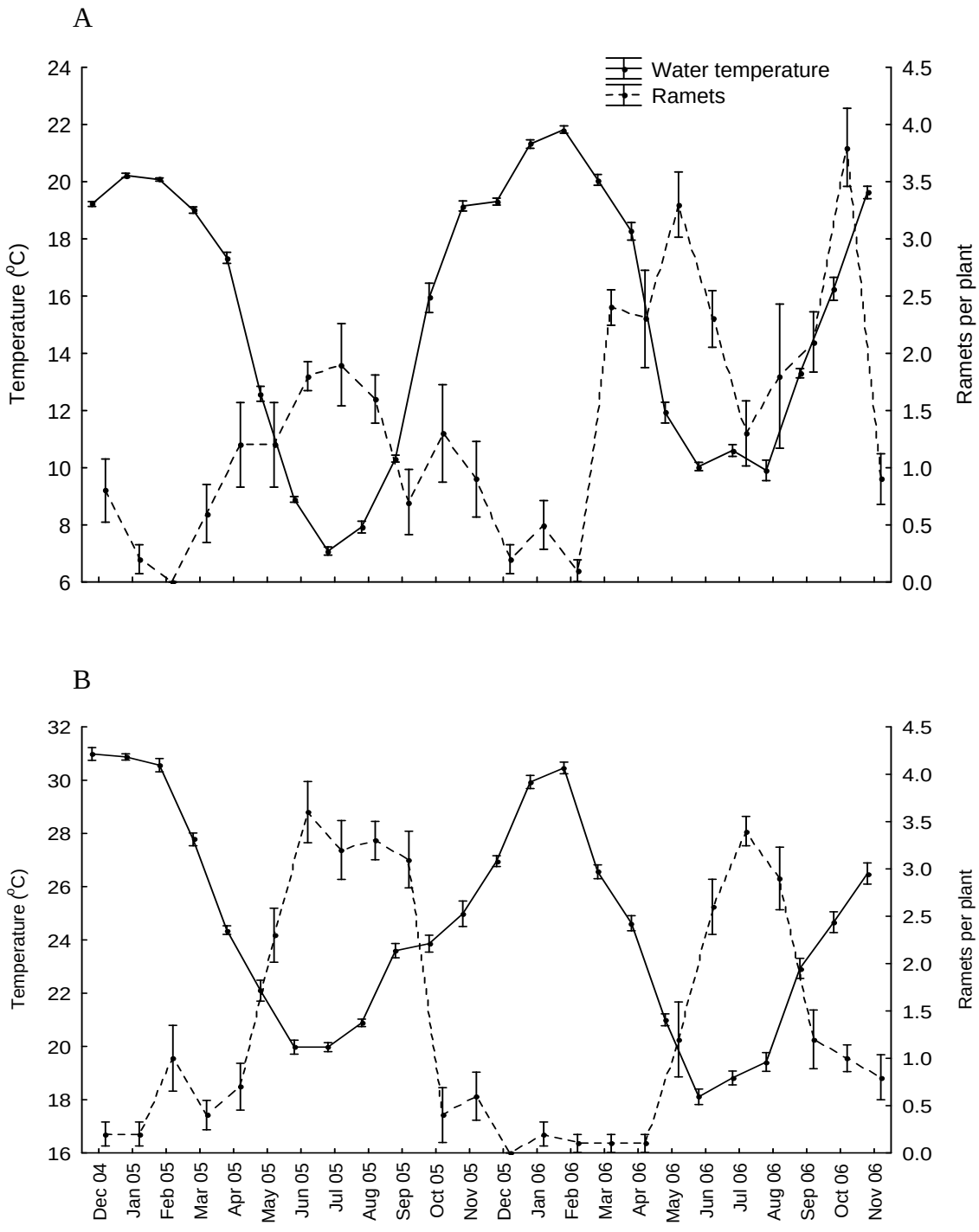


Figure 6.22: Seasonal fluctuation in water temperature (monthly mean ±SE) and water hyacinth ramet production (Mean of 10 plants ±SE) over 24 months, at a cold, high-altitude inland site (A – Farm Dam), and a warmer, low-altitude coastal site (B – Mbozambo Swamp).

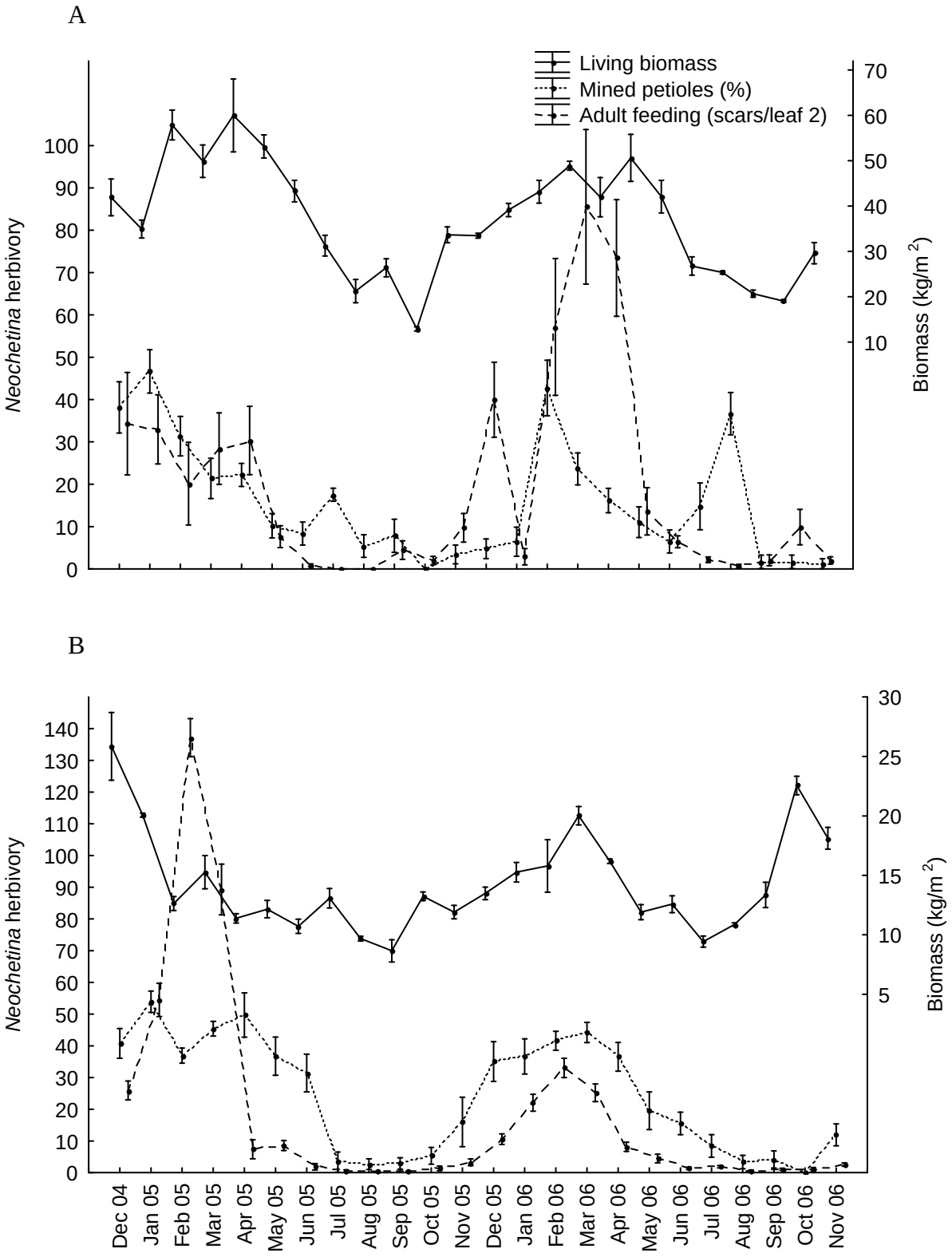


Figure 6.23: Seasonal fluctuation in adult and larval herbivory by the water hyacinth weevils *Neochetina spp.,* (mean of 10 plants ±SE) relative to fluctuations in living water hyacinth biomass (Mean of three quadrats ±SE) over 24 months, at a cold, high-altitude inland site (A – Farm Dam), and a warmer, low-altitude coastal site (B – Mbozambo Swamp). (Adult feeding scars for B have been fitted by multiplying mean values by 0.1 to fit on to the left Y axis, with petioles mined).

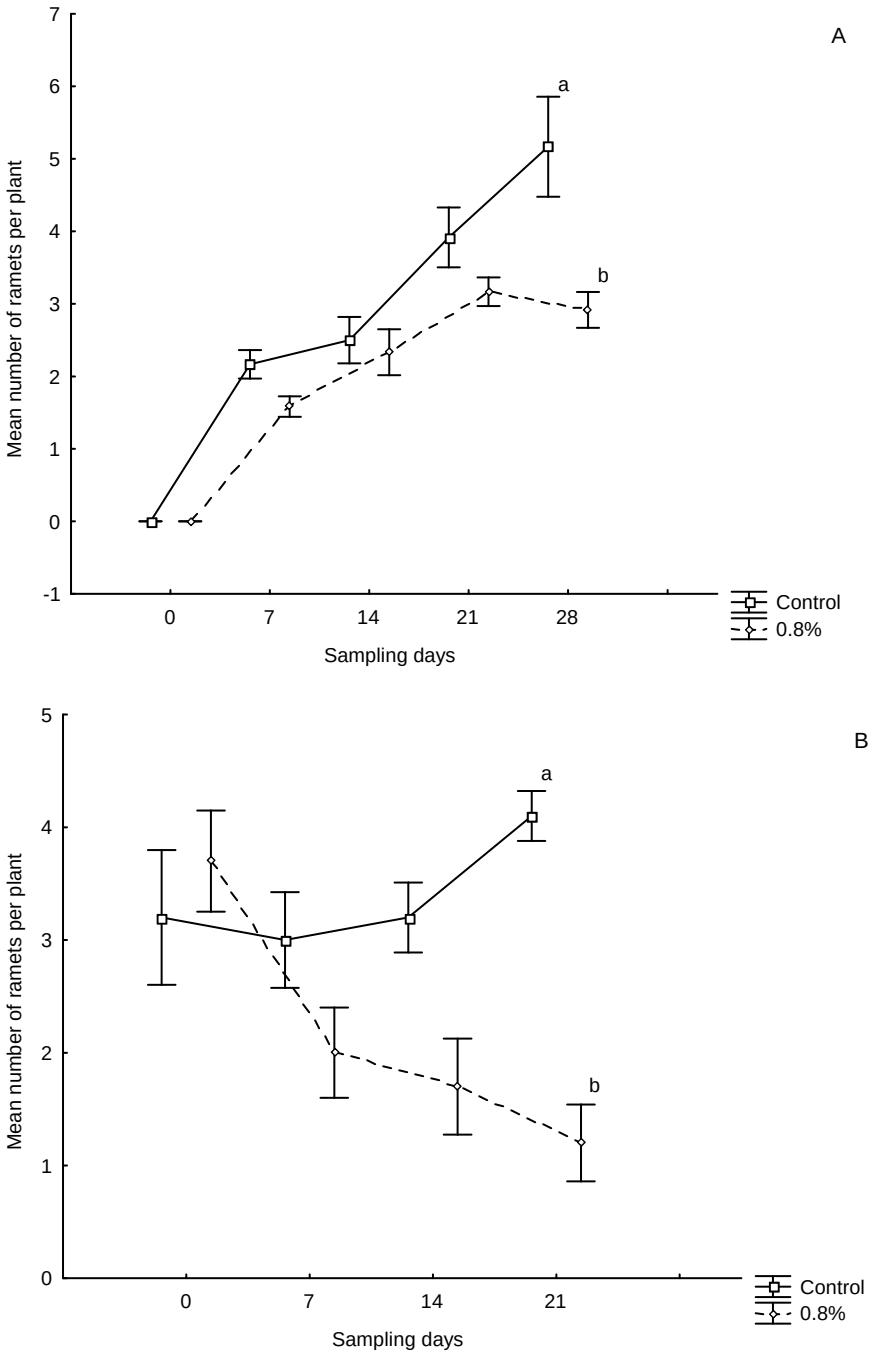


Figure 6.24: The effect of 0.8% glyphosate herbicide sprayed at 140 *l*/ha on the mean number of ramets produced by water hyacinth plants carrying *Neochetina spp*. weevils, during (A) autumn and (B) spring. Error bars = standard error of the mean. End point means followed by different letters indicate significant differences at $p < 0.05$.

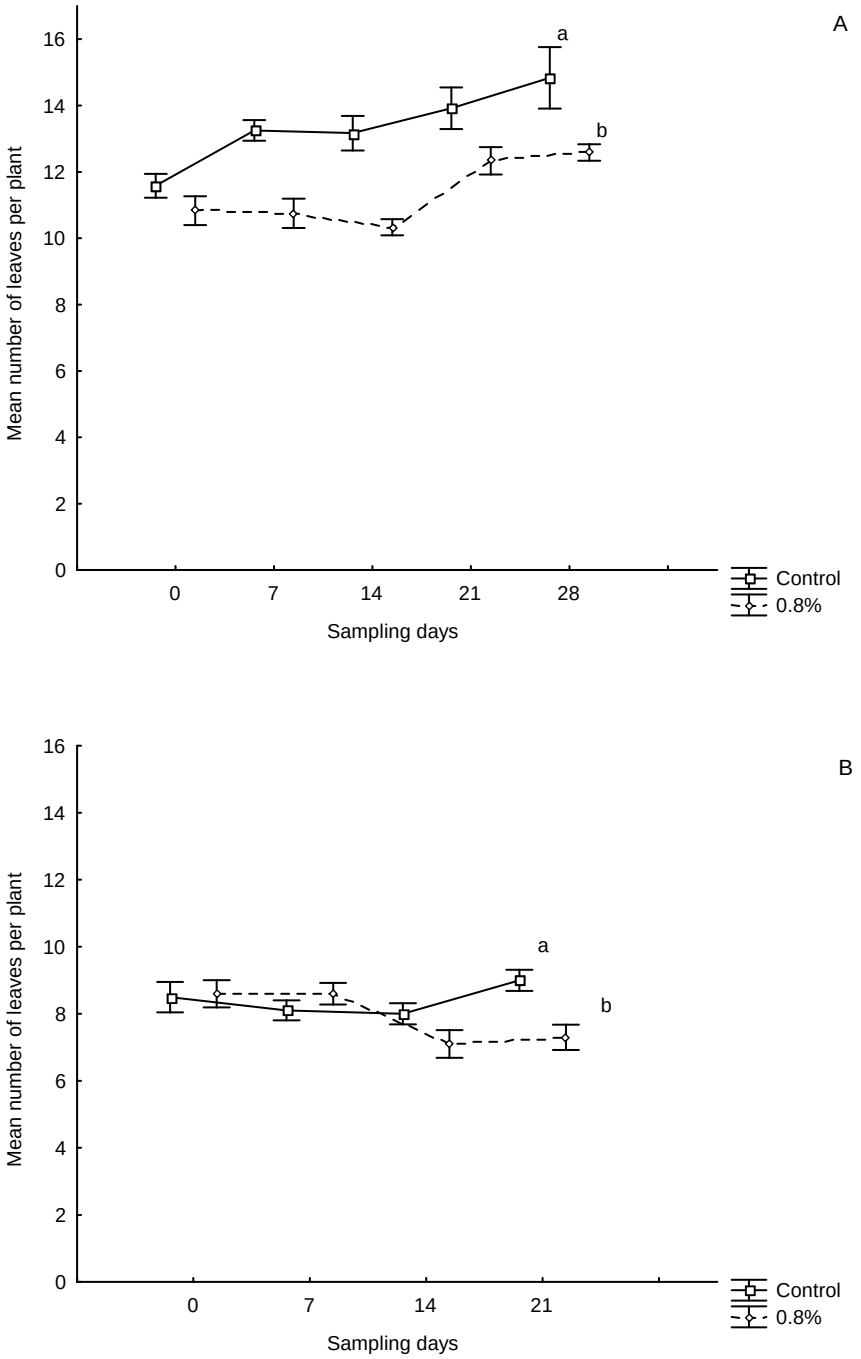


Figure 6.25: The effect of 0.8% glyphosate herbicide sprayed at 140 *l*/ha on the mean number of leaves produced by water hyacinth plants carrying *Neochetina spp*. weevils, during (A) autumn and (B) spring. Error bars = standard error of the mean. End point means followed by different letters indicate significant differences at $p < 0.05$.

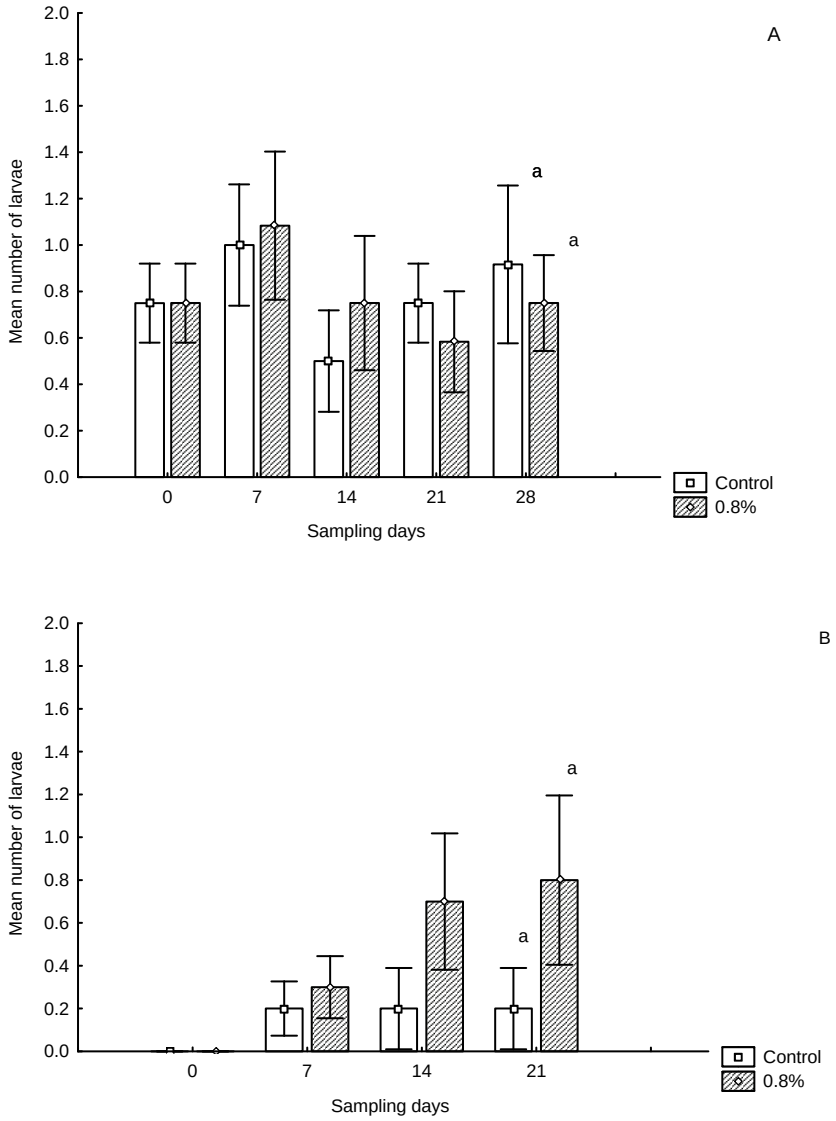


Figure 6.26: The effect of 0.8% glyphosate herbicide sprayed at 140 *l*/ha on the mean number of *Neochetina spp.* larvae on water hyacinth plants, during (A) autumn and (B) spring. Error bars = standard error of the mean. End point means followed by different letters indicate significant differences at $p < 0.05$.

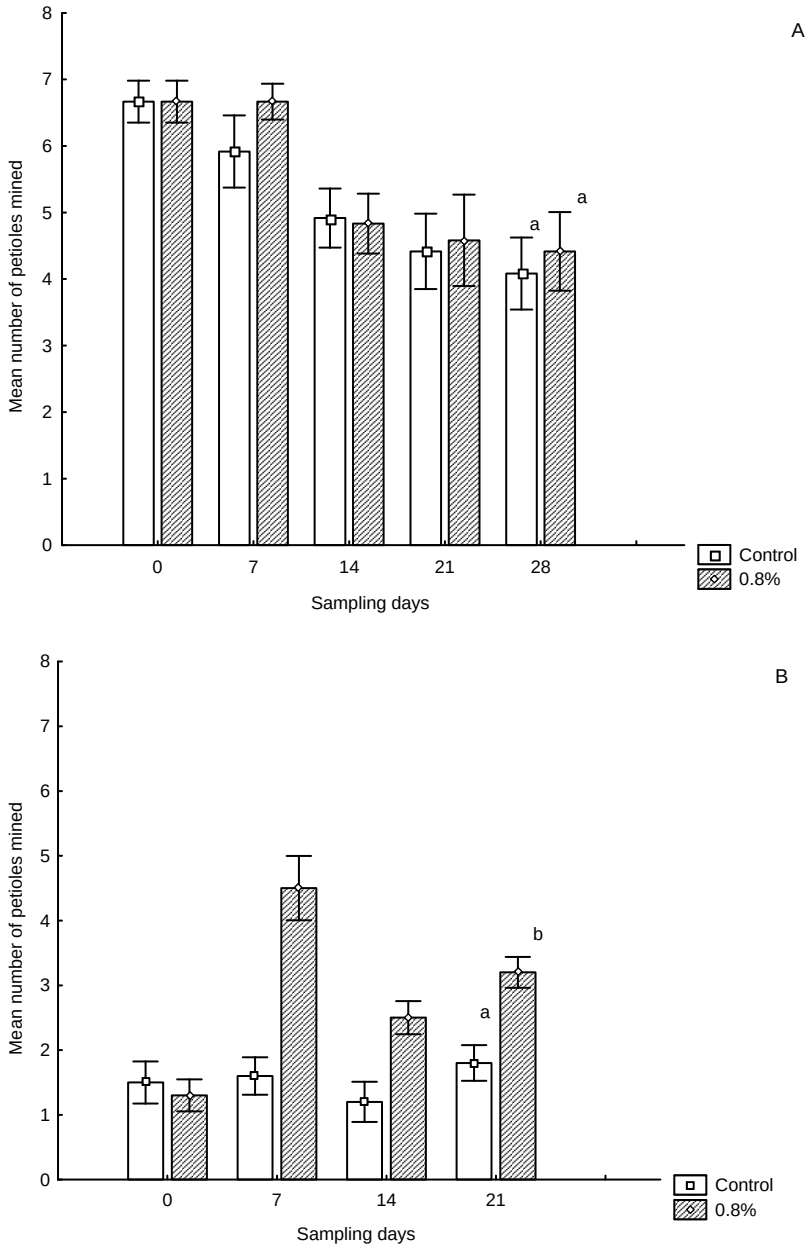


Figure 6.27: The effect of 0.8% glyphosate herbicide sprayed at 140 *l*/ha on the mean number of petioles mined on water hyacinth plants carrying *Neochetina spp*. weevils, during (A) autumn and (B) spring. Error bars = standard error of the mean. End point means followed by different letters indicate significant differences at $p < 0.05$.

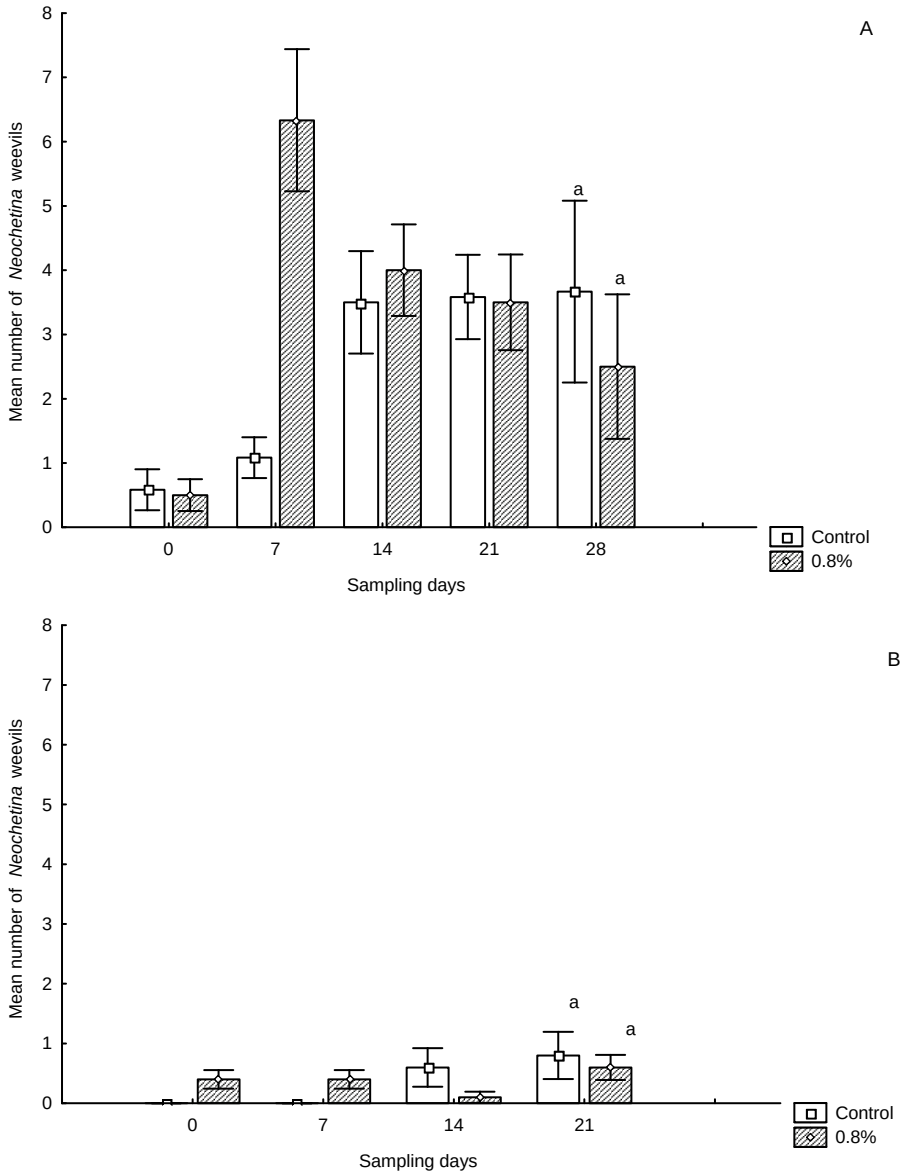


Figure 6.28: The effect of 0.8% glyphosate herbicide sprayed at 140 *l*/ha on the mean number of *Neochetina spp*. adults on water hyacinth plants, during (A) autumn and (B) spring. Error bars = standard error of the mean. End point means followed by different letters indicate significant differences at $p < 0.05$.

6.3.1.4 Discussion

Water hyacinth plants at two widely differing field sites, measured over two years, were seen to reproduce asexually by production of ramets each winter, and to accumulate new living biomass during the summer months. On the other hand, weevil numbers fluctuated more extensively and tended to lag behind summer increases in plant biomass, and showed no response to the winter production of ramets at either site. Successful weed control requires an understanding of the target weed's ecology (Cruttwell McFadyen, 1998) and especially differing natural enemy and target weed ecologies, which, driven by a variety of ecoclimatic factors, can lead to seasonal asynchrony. This in turn may allow a pest to escape early season regulation so that it

reaches damaging levels before control agents can accumulate sufficient numbers to inflict any meaningful harm on the population (Muller et al., 1990).

Our results show strong seasonal trends in the phenology of the weed at two climatically differing sites, and asynchronous growth patterns between the weed and its natural enemies, *Neochetina spp*. At both sites, ramet production increased during winter, even though temperatures were declining. This reproduction is thought to be triggered by increased light penetration through the plant canopy which stimulates the plant crown to produce ramets (Méthy and Roy, 1993). At the colder interior site, following the initial drop in temperature that coincided with the onset of ramet production, water temperature remained well above the lower growth threshold of about 10°C for water hyacinth (Gopal, 1987; Chapter 2 this report). Because the plants actively grow during this period from February into April (autumn) by adding ramets even though they are actually losing total biomass, this phase could serve as a window of opportunity for a late season herbicide intervention which requires active plant growth for herbicide uptake, while beetle numbers are low and more likely to be in the petioles and crowns than the leaves.

Spring is the other point in the plant/insect growth cycle that could be used to tactically suppress plant growth by herbicide application, allowing the insect population to build up to damaging levels before the newly produced ramets break free from the mother plants and contribute to the increasing biomass of the water hyacinth mat. At both the colder, high-altitude and the warmer, low-altitude site, a lag period was apparent between the start of living plant biomass accumulation and the onset of *Neochetina* herbivory following winter, leading into spring. Free from early season herbivory and aided by ramet production through winter, the water hyacinth populations were able to recover quickly from winter and outpace the effects of insect feeding well into the growth season. Prioritising herbicide intervention during this period would delay early season plant growth and allow it to overlap with higher levels of herbivory caused by increased numbers of weevils later into the growing season.

Seasonal application of herbicide in outdoor pools resulted in significant suppression of vegetative growth of leaves and ramets of water hyacinth, without negatively influencing the numbers of either adult or larval *Neochetina spp*., or their feeding activities. These results largely mirror those of Jadhav et al., (2008) who showed in tub trials that low doses of glyphosate can retard the growth of water hyacinth. Combined with the largely neutral effect of the herbicide on *Neochetina* larvae and adults, the method offers the opportunity to integrate herbicidal and biological control where the weed growth can be suppressed without removing the biological control agents from the infestation.

Other plant/insect systems have shown tolerance to the application of low (sublethal) doses of herbicide to the plants, without affecting establishment or survival of associated insect herbivores (Lindgren et al., 1999; Lym and Carlson, 1990; McCaffrey and Callihan, 1988; Nelson and Lym, 2003; Rees and Fay, 1989; Story and Stougaard, 2006). Specifically, glyphosate has been successfully used in conjunction with the insect biocontrol agent *Galerucella calmariensis* (Coleoptera, Chrysomelidae), against the terrestrial weed, purple loosestrife (Lym 1998; Lym et al., 1996). Our results show no detrimental effects on *Neochetina* species living on water hyacinth in an aquatic system, and may even be beneficial to the insects by altering the plant chemistry as a result of mobilizing nutrients such as nitrogen, or reducing defence chemicals such as phenols. Because glyphosate (at high dosages) has been shown to cause mortality of tadpoles in aquatic "microcosms" (Relyea, 2005a,b,c), reducing the dose from 3% to 0.8% in a sublethal spray would be expected to diminish the toxic effects on aquatic fauna (Chapter 4 this report). Ideally, the combined effect of herbicide and biocontrol agent should be greater than that of the biocontrol agent alone; this remains to be tested.

The application of herbicide, once in autumn and again in spring, could adjust the asynchrony between the growth pattern of the weed and herbivory by its biocontrol agents, by retarding ramet growth in autumn and reducing biomass accumulation in spring. Since fewer ramets and leaves are produced as a result of the herbicidal spray with a retardant dose (Jadhav et al., 2008), the biocontrol agents should be better equipped to outpace the weed's growth. However, the weevils have presumably adapted their growth strategy to the plant phenology over millennia, so that it remains to be seen if seasonal slowing of the plant growth is detrimental to the weevil populations. Nevertheless, reducing the herbicide spray dosage will decrease the non-target effects of the herbicide caused by overspraying and spray drift, and lessen any ecotoxicological effects of glyphosate on the local aquatic fauna.

6.3.2 How Effective is a Low Dose of Herbicide In the Field?

Following the findings of Section 6.3.1, that seasonal application applications of the herbicide could possibly be used against water hyacinth, and that a low dose of herbicide had no effect on tadpole development (Section 6.2.3) it was decided to test the method on a larger scale in the field. Field trials were conducted at two sites, on three different "seasonal" occasions to test the proposal that water hyacinth populations in the field could be controlled by seasonally timed applications of a sublethal dose of glyphosate. These trials are the first field tests of the sublethal herbicide method.

6.3.2.1 Introduction

Center et al. (1999) and Haag (1986a,b) have shown that herbicides can be integrated with biocontrol agents if an island of water hyacinth mat is left unsprayed to facilitate the migration of the adult *Neochetina* weevils from the herbicide-sprayed plants to unsprayed plants. In contrast, the sublethal dose method recommends broadcast

spraying of all plants which are then retarded in both growth and reproduction, while the biocontrol agents remain on the plants to further suppress population growth.

Jadhav et al. (2008) have shown the feasibility of using a sublethal dose of glyphosate to retard growth of water hyacinth plants in the laboratory, while retaining populations of adults and larval *Neochetina spp*. in the treated plants. The preceding section has shown that this method also works under semi-field conditions at different seasons of the year. It remains to test this method on a larger scale in the field, in natural water bodies subject to the normal fluctuations of temperature, nutrients and other factors such as water level. The following experiments tested the effect of a sublethal dose of glyphosate, applied from a boat, on two small mats of water hyacinth at Delta Park and Farm Dam, Johannesburg.

6.3.2.2 Methods

6.3.2.2.1 Field Setup

Trials were carried out at Delta Park and Farm Dam Johannesburg, where populations of water hyacinth with *Neochetina spp*. weevils have persisted for more than five years. At each site a strip of water hyacinth plants, approximately 12x3 meters, was restrained between two cables fitted with buoys. Similar cables were inserted as perpendicular separators to divide the strip into two equal plots of 6x3 m, comprising a treatment plot and a control plot. Both plots were each further subdivided into three blocks 2x3 m, forming three replicates per plot.

The treatment plot was sprayed with 0.8% Roundup (glyphosate; active ingredient: 360 g /*l*, Monsanto Pty. Ltd. South Africa) at a spray volume of 140 *l*/ha (Jadhav et al., 2008), while the control plot was untreated. A 12V battery-operated boom sprayer (Multispray, Midrand, South Africa) with three spray tips (Tee Jet even flat: TP65015E) was used to spray the weed from a motor boat moving at 4 km/h.

Data was collected weekly from the second week after spraying, for eight weeks. Base measures were taken immediately before herbicide application. The same experimental design and protocol was used at both sites. Spraying was done in autumn, spring and summer and the same plants were treated as the control and treatment on each occasion to simulate a managed water body being repeatedly sprayed.

6.3.2.2.2 Plant Measurements

Nine water hyacinth plants were randomly selected from each plot, from which the longest petiole length, leaf two petiole length, maximum root length, number of ramets, number of flowers, and number of leaves were recorded.

A 50x50 cm quadrat was randomly thrown into each of the three blocks in both treated and untreated plots. All the plants in each quadrat were counted, dismembered and

weighed to give the biomass of living material above water, living material below water and dead material.

6.3.2.2.3 Insect Measurements

Destructive sampling was conducted on three plants randomly selected from each of the three blocks, yielding nine plants from each treatment plot. Adult feeding scars were counted on the second-youngest leaf. Plants were pulled apart to search for adult weevils and larvae and the petioles were slit open along their length to look for larvae and record the number of mined petioles.

6.3.2.3 Results

A selection of the above measurements is presented here, and is adequate to illustrate the overall trends seen in the experimental plants in response to a sublethal dose of herbicide. In general, the retardant effect of spraying 0.8% glyphosate at 140 *l*/ha, which was seen in laboratory trials, was also repeated in the field trials over three seasons at two field sites. Some differences were noted in the effect on ramet production and occasionally the effect of the herbicide was not significant.

The autumn spray trial generally retarded plant growth at both sites, without having a detrimental effect on the weevil population. A sublethal dose of glyphosate slowed down the growth of water hyacinth plants at both sites by reducing the plant density (Figure 6.29) and above-water biomass (Figure 6.30. Ramet production was significantly reduced only at Farm Dam (Figure 6.31). This may be because the crowns of the smaller plants at Delta Park are more exposed to sunlight, which is assumed to stimulate ramet production, than those at Farm Dam, which has a population of bigger, taller plants at a lower initial density. Adult weevil feeding scars varied at each sampling date and were extremely low at Farm Dam (Figure 6.32). No adults were found at Farm Dam and only low numbers were recorded at Delta Park (Figure 6.33).

In spring the plants at Delta Park were very numerous but small compared to those at Farm Dam, which is reflected in their density and biomass (Figures 6.34 and 35). The retardant spray, therefore, did not reduce the number of plants – which were probably ramets which had overwintered – but did significantly check the growth of sprayed plants. Addition of ramets was generally reduced at both sites, but not significantly so (Figure 6.36). Weevil feeding also differed dramatically between sites but was not generally adversely affected (or stimulated) by the herbicide treatment except on the last sampling date at Farm Dam, where feeding damage was seen to suddenly increase (Figure 6.37). Almost the converse was seen in the number of larvae collected at both sites, where Delta Park showed significantly high numbers of larvae on small plants while Farm Dam yielded half as many larvae on its larger plants, with significantly more being found on the untreated plants (Figure 6.38). No adult weevils were collected at either site.

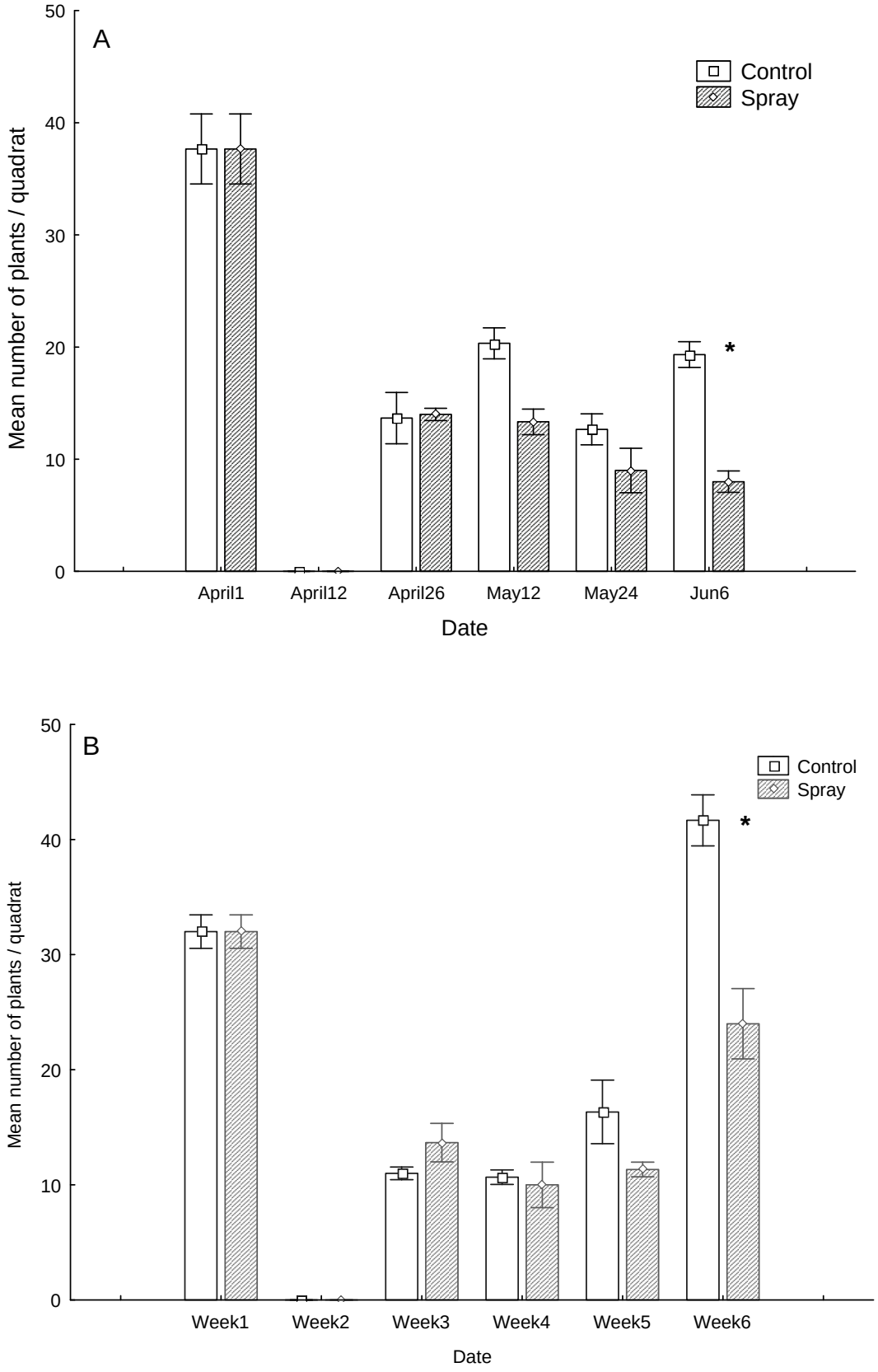


Figure 6.29: The effect on plant density of spraying water hyacinth plants in the field with a sublethal glyphosate dose (0.8% at 140 *l*/ha), in autumn 2008. A: Delta Park; B: Farm Dam. * end point means are significantly different at $p < 0.05$. Bars = S.E.

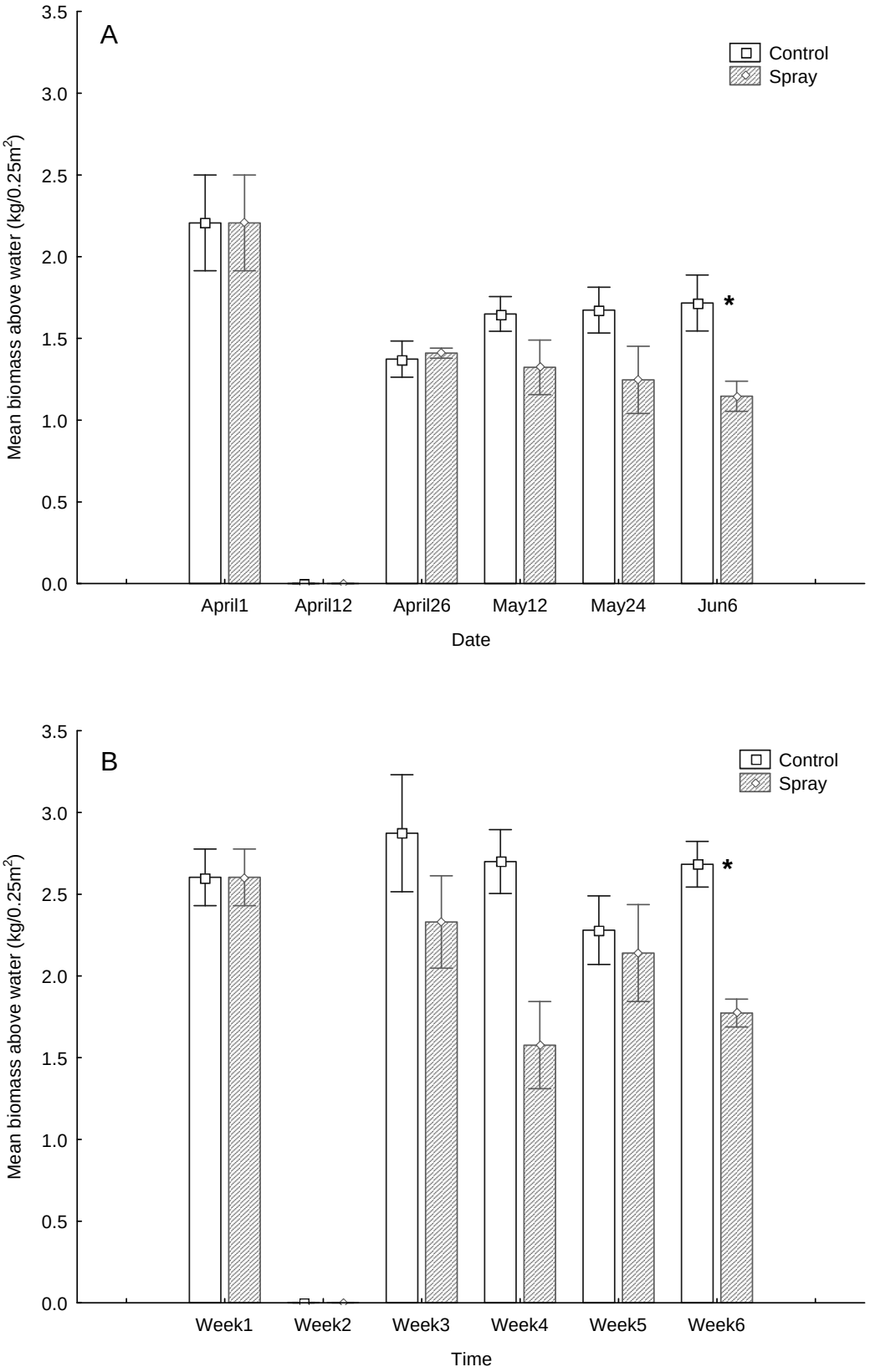


Figure 6.30 The effect on plant biomass above water of spraying water hyacinth plants in the field with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in autumn 2008. A: Delta Park; B: Farm Dam.* end point means are significantly different at $p < 0.05$. Bars = S.E.

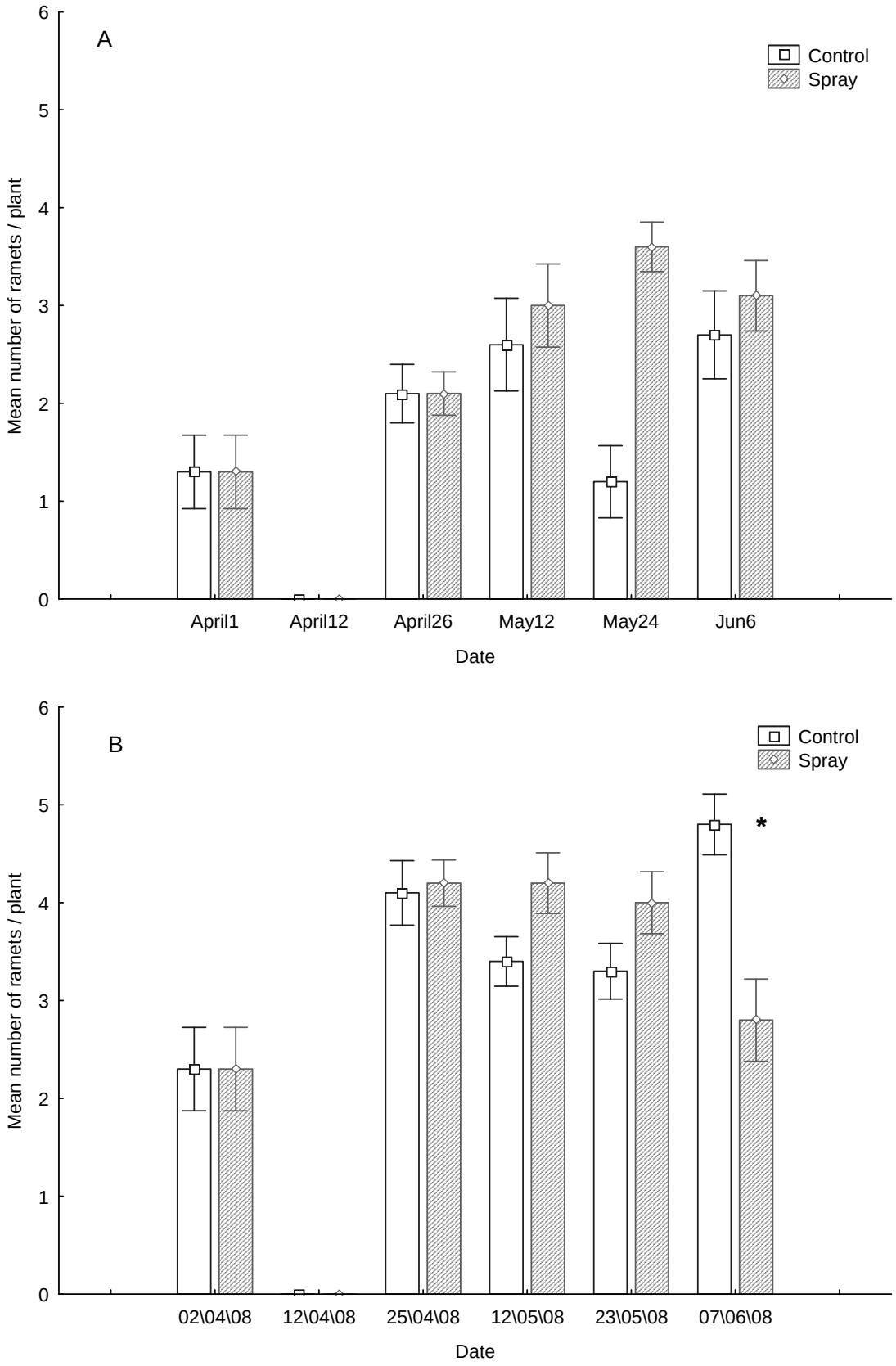


Figure 6.31: The effect on ramet production of spraying water hyacinth plants in the field with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in autumn 2008. A: Delta Park; B: Farm Dam. * end point means are significantly different at $p < 0.05$. Bars = S.E.

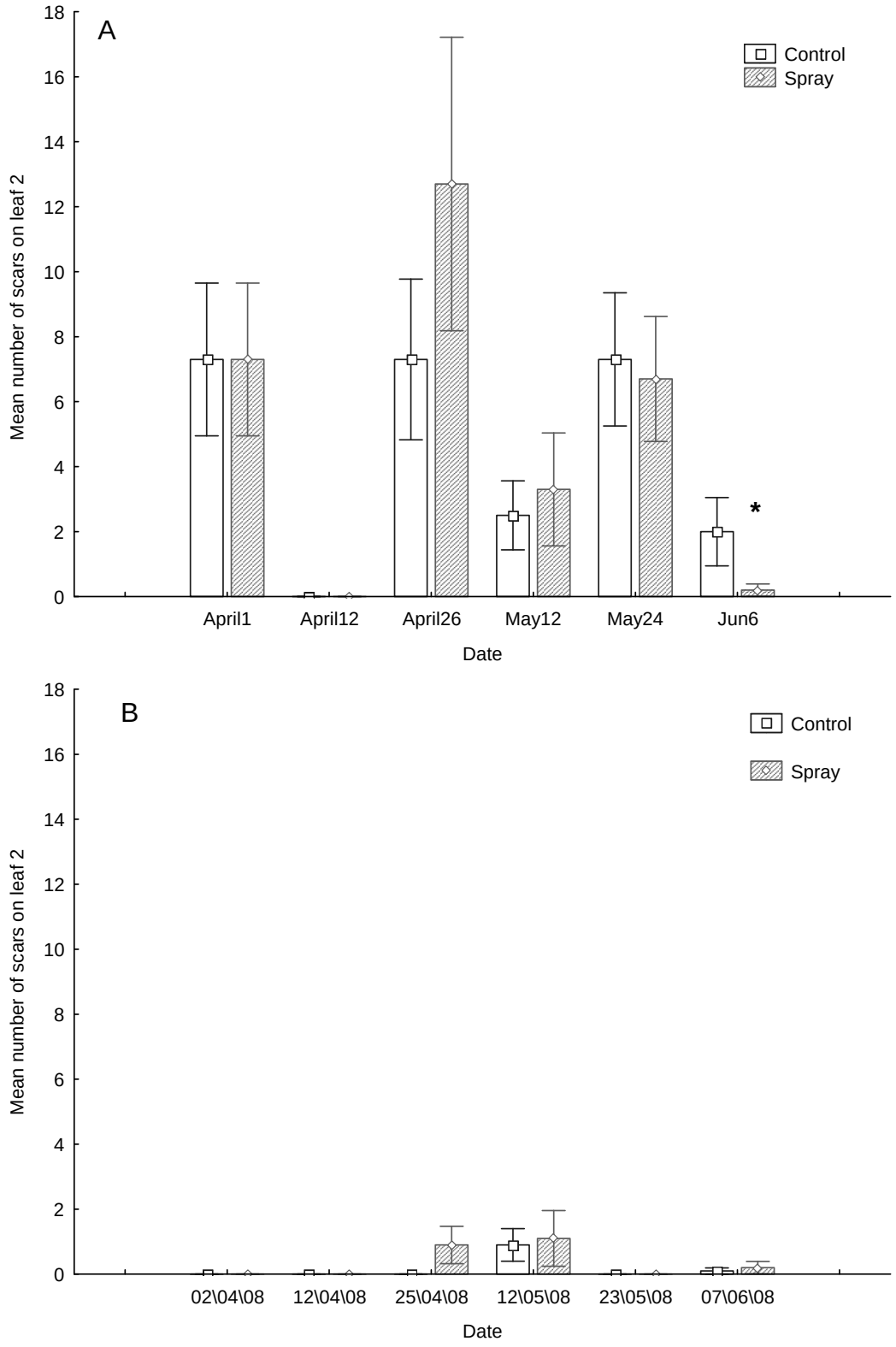


Figure 6.32: The effect on adult weevil feeding scars of spraying water hyacinth plants in the field with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in autumn 2008. A: Delta Park; B: Farm Dam. * end point means are significantly different at $p < 0.05$. Bars = S.E.

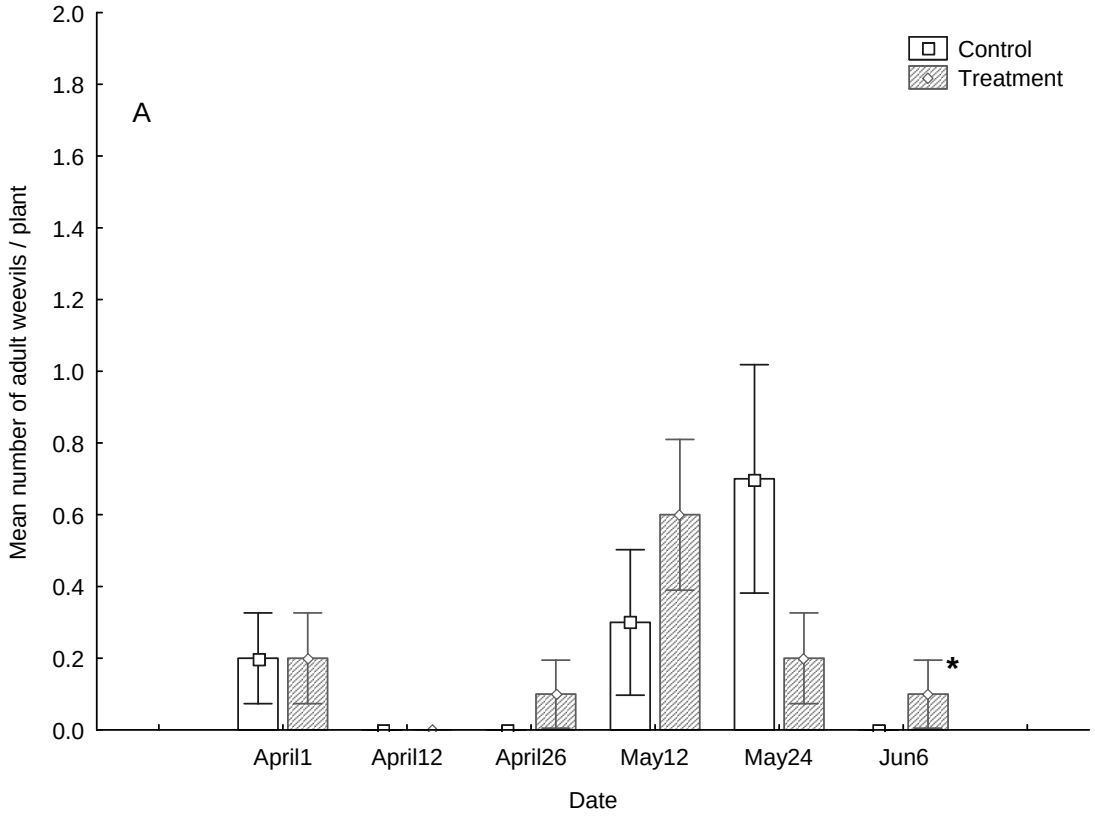


Figure 6.33: The effect on adult weevil numbers of spraying water hyacinth plants in the field with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in autumn 2008. A: Delta Park. * end point means are significantly different at $p < 0.05$. Bars = S.E.

Summer data were collected only at Delta Park because heavy rains broke the dam wall at Farm Dam, sweeping the water hyacinth out of the site. Again, the sublethal herbicide dose did not reduce the number of plants but did reduce their overall size (Figure 6.39). Ramet numbers were generally reduced by the herbicide treatment (Figure 6.40A), but adult weevil feeding was also reduced, which may be due to leaf growth being inhibited by the treatment (Figure 6.40B). Surprisingly, both adult and larval weevil numbers were significantly reduced by the herbicide treatment (Figure 6.41). This may be due to the very poor quality of the plants, which were small, and stunted by repeated spraying.

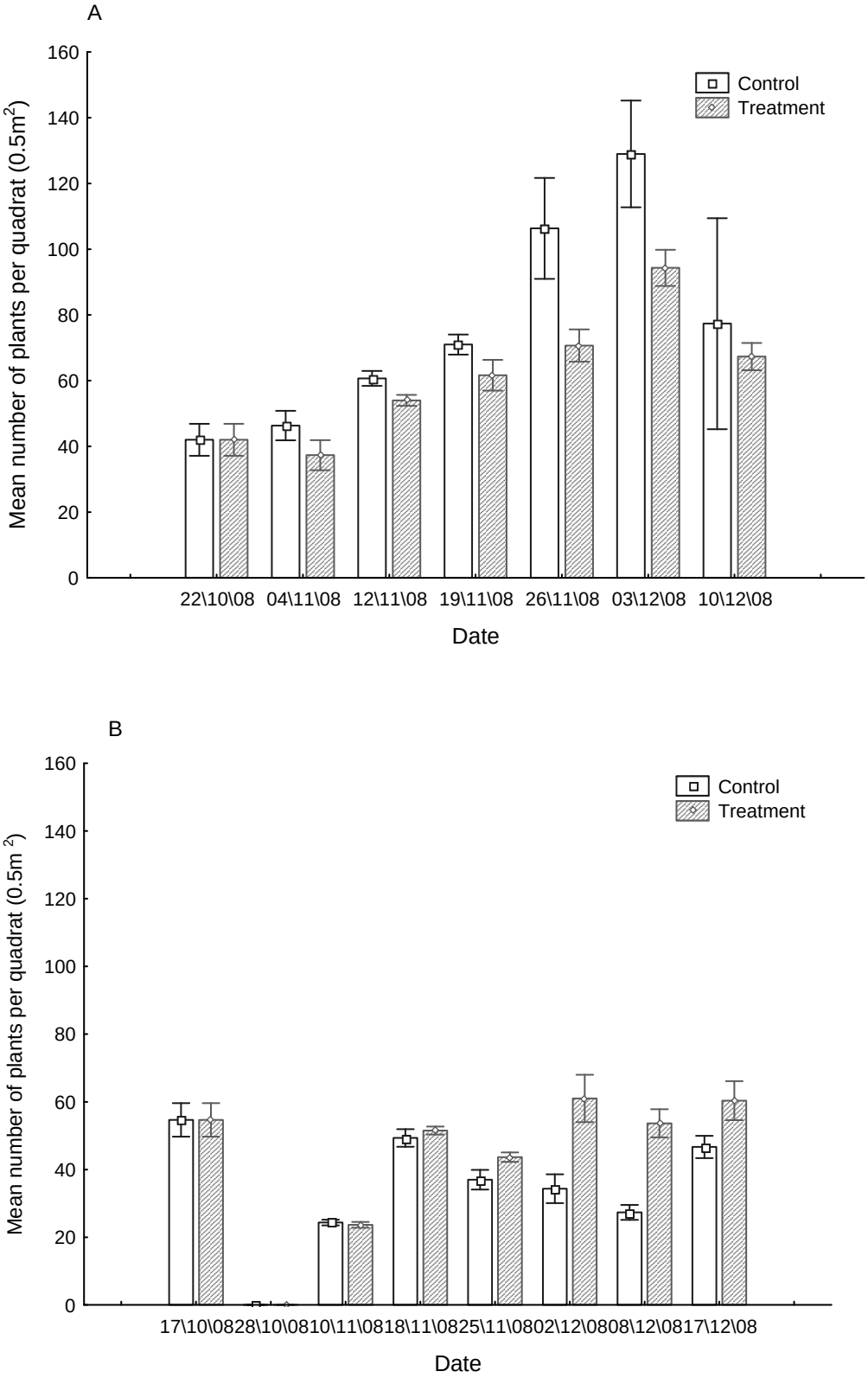


Figure 6.34: The effect on plant density of spraying water hyacinth plants in the field with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in spring 2008. A: Delta Park; B: Farm Dam. No sample taken 28/10. * end point means are significantly different at $p < 0.05$. Bars = S.E.

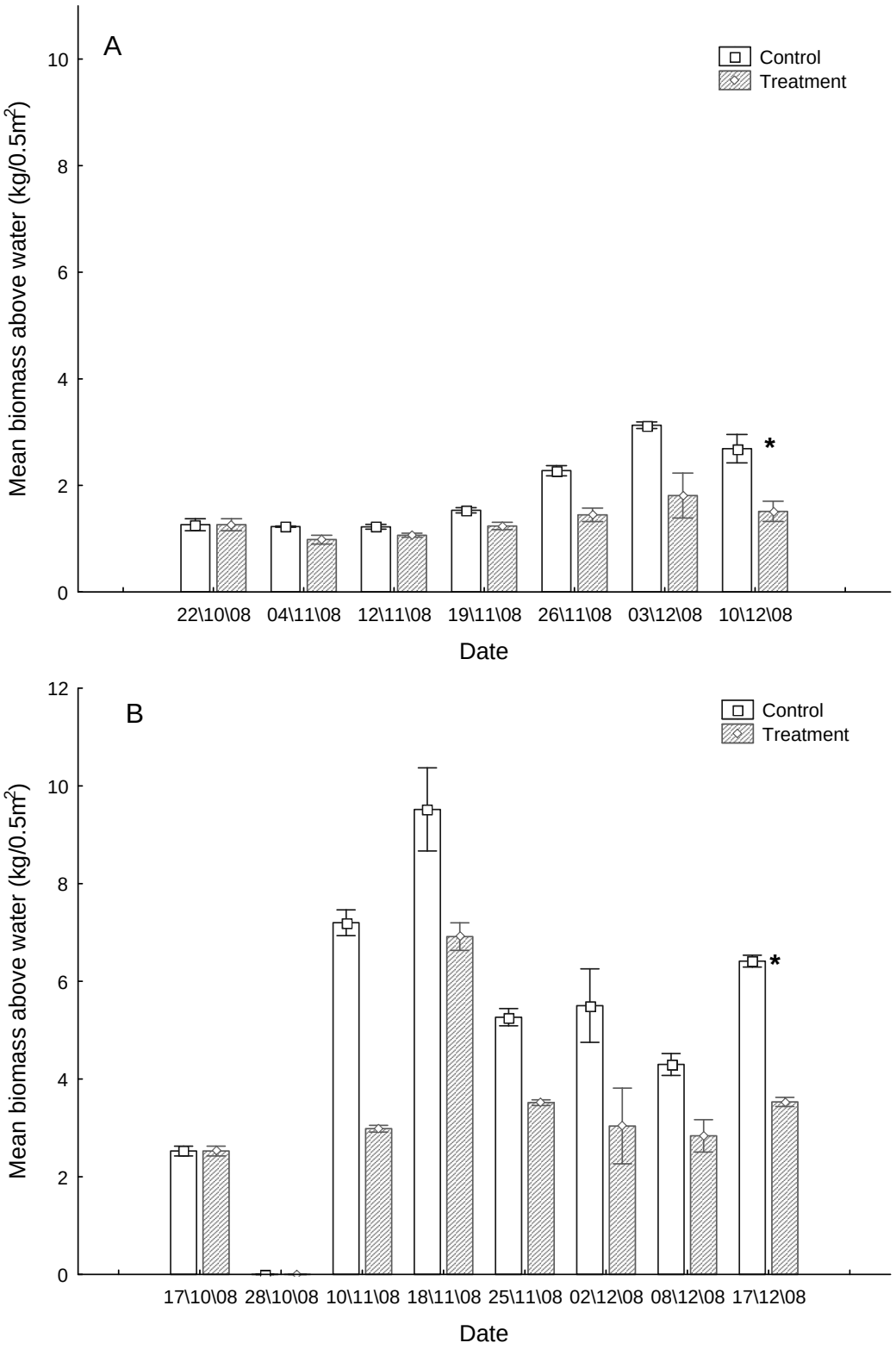


Figure 6.35: The effect on plant biomass of spraying water hyacinth plants in the field with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in spring 2008. A: Delta Park; B: Farm Dam. No sample taken 28/10.* end point means are significantly different at $p < 0.05$. Bars = S.E.

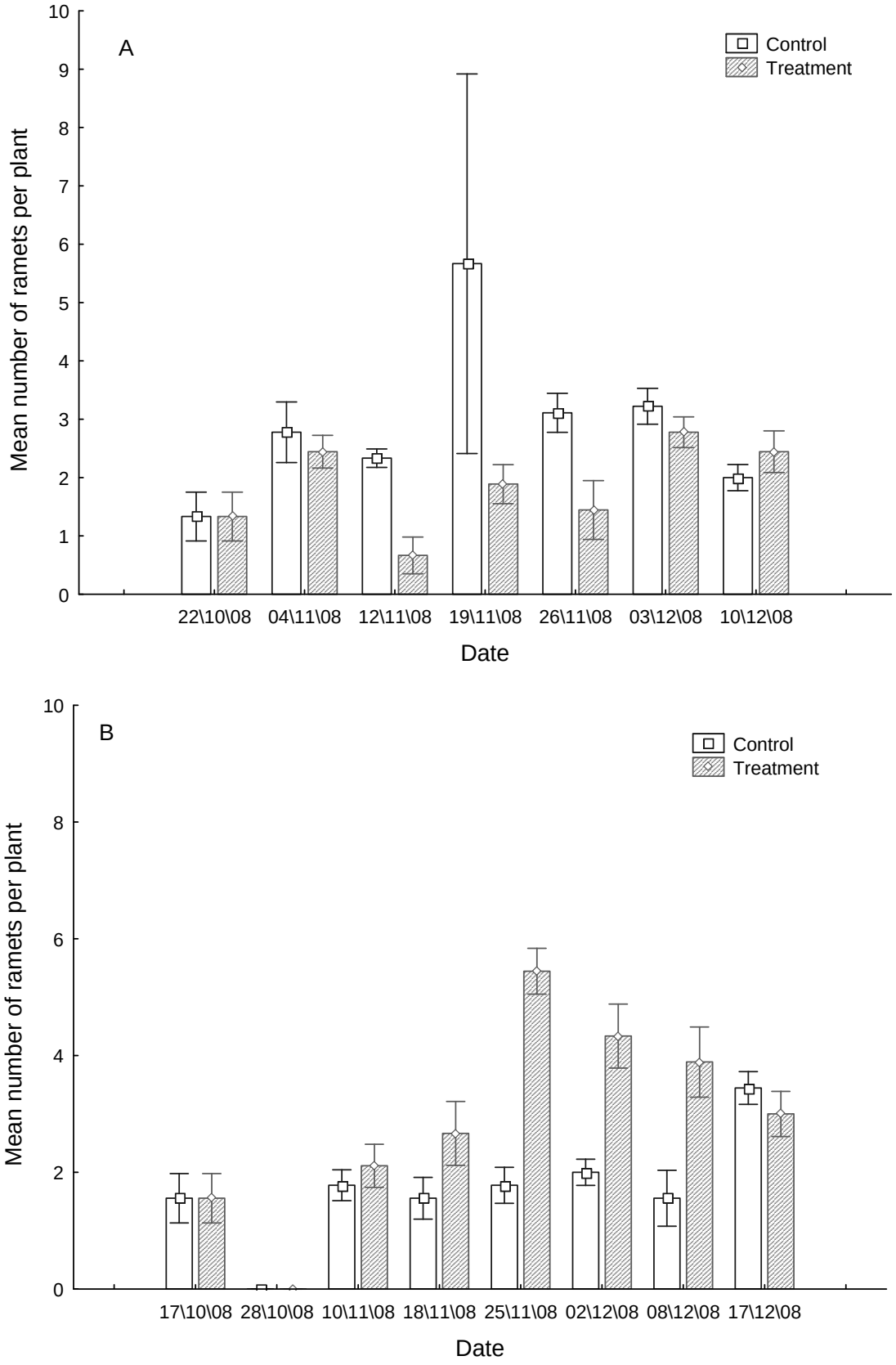


Figure 6.36: The effect on ramet production of spraying water hyacinth plants in the field with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in spring 2008. A: Delta Park; B: Farm Dam. No sample taken 28/10.* end point means are significantly different at $p < 0.05$. Bars = S.E.

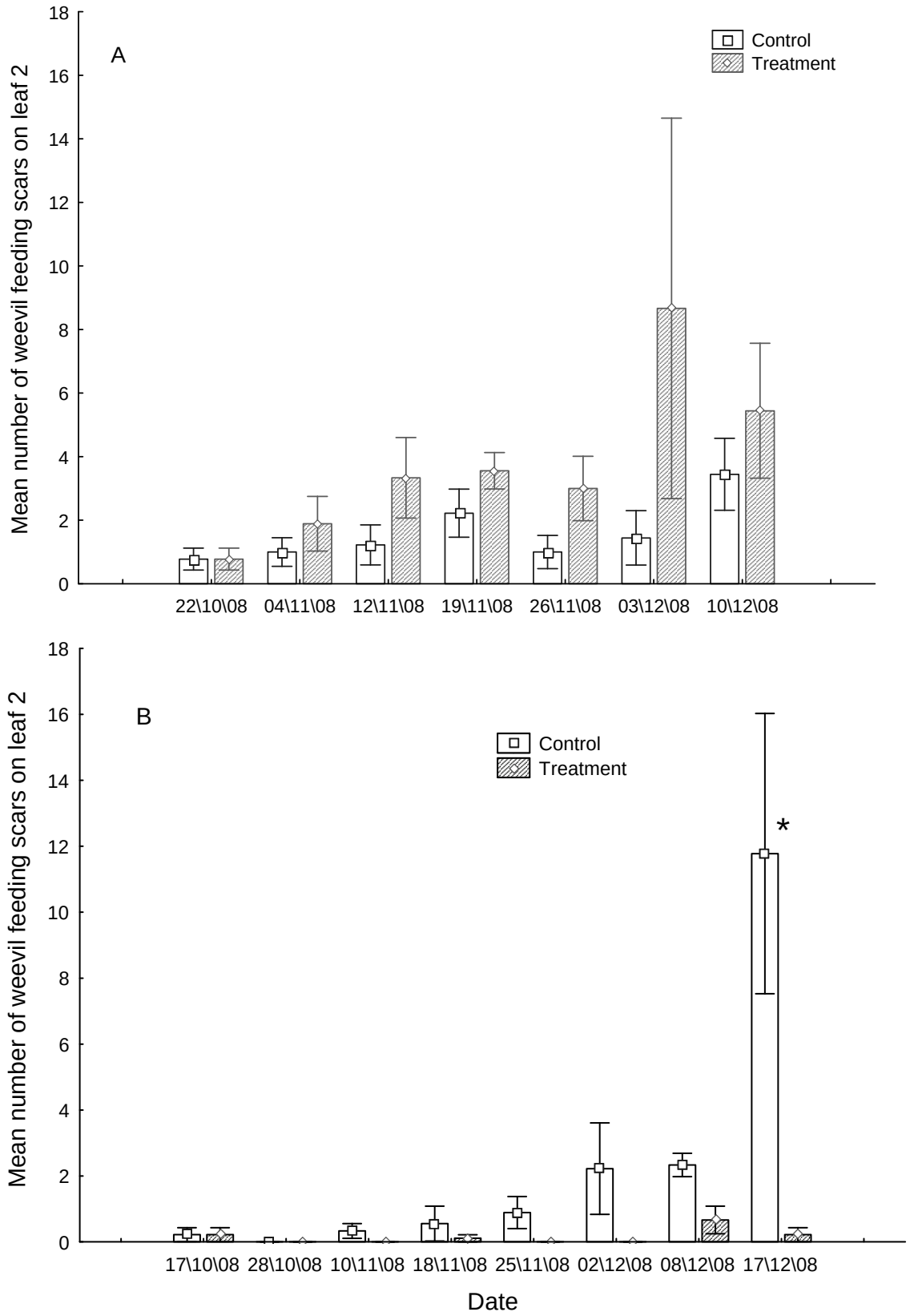


Figure 6.37: The effect on adult weevil feeding of spraying water hyacinth plants in the field with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in spring 2008. A: Delta Park; B: Farm Dam. * end point means are significantly different at $p < 0.05$. Bars = S.E.

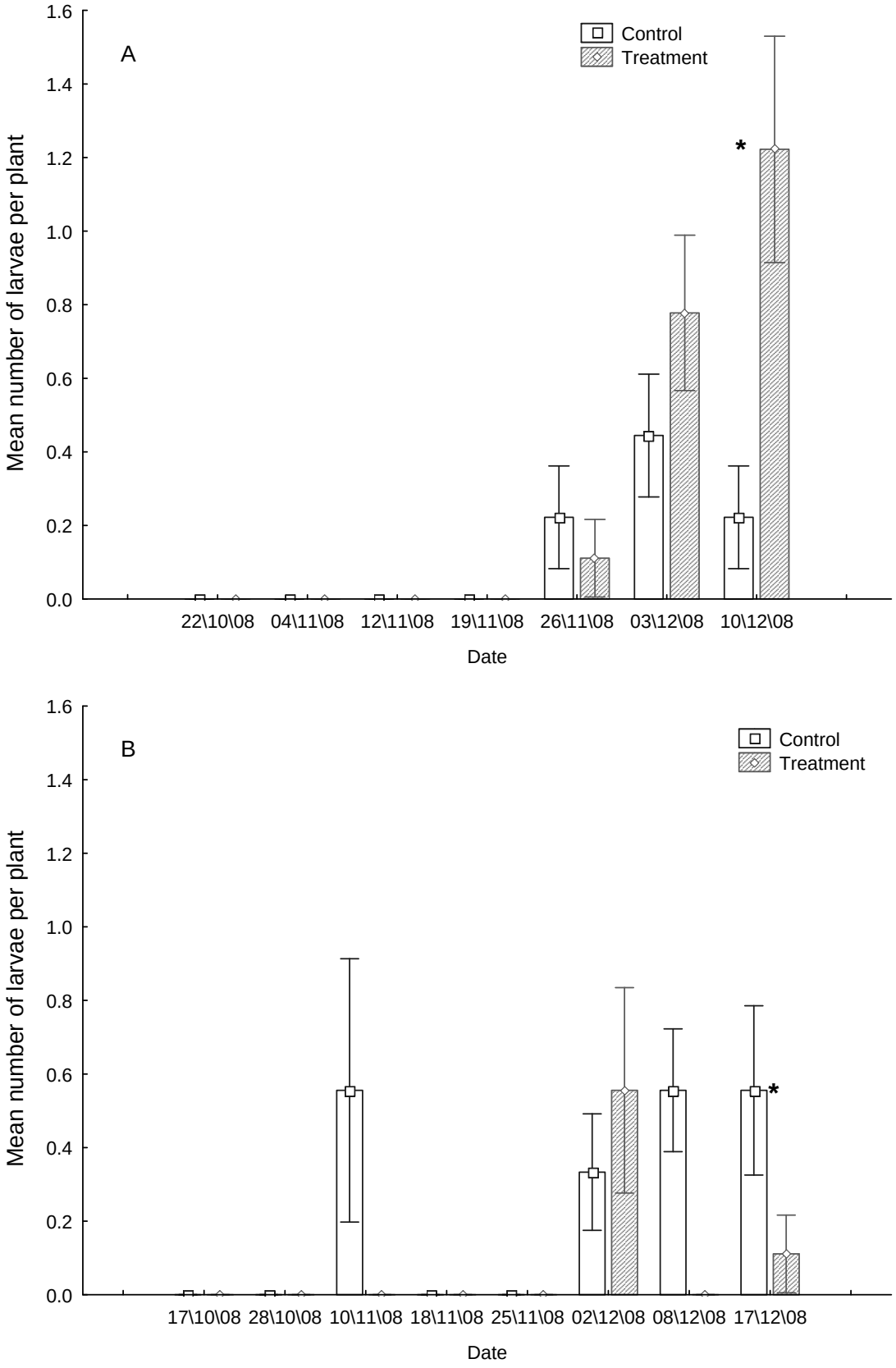


Figure 6.38: The effect on weevil larvae of spraying water hyacinth plants in the field with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in spring 2008. A: Delta Park; B: Farm Dam. * end point means are significantly different at $p < 0.05$. Bars = S.E.

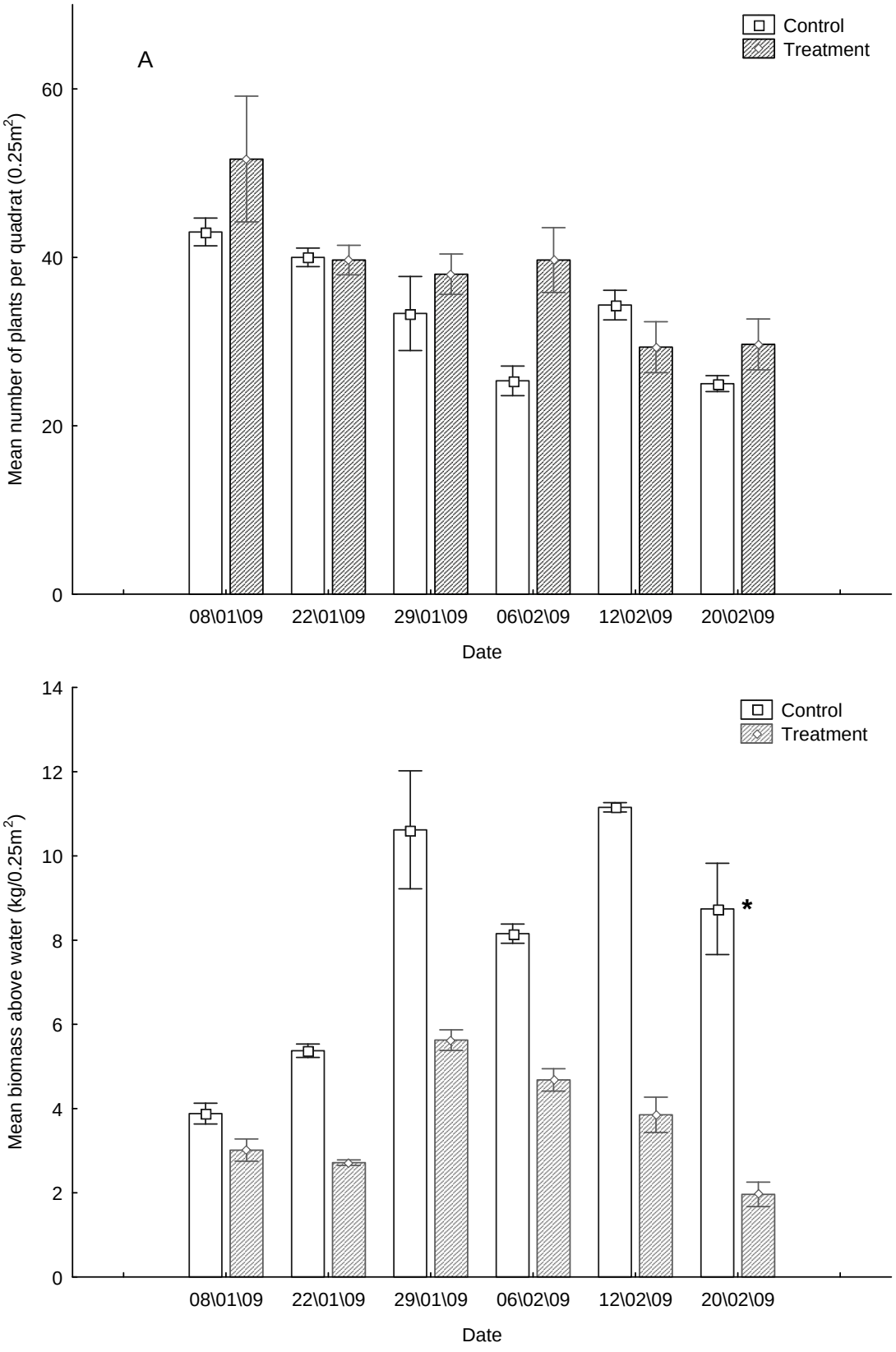


Figure 6.39: The effect on water hyacinth of spraying plants with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in the field at Delta Park in summer 2009. A: plant density; B: plant biomass. * end point means are significantly different at $p < 0.05$. Bars = S.E.

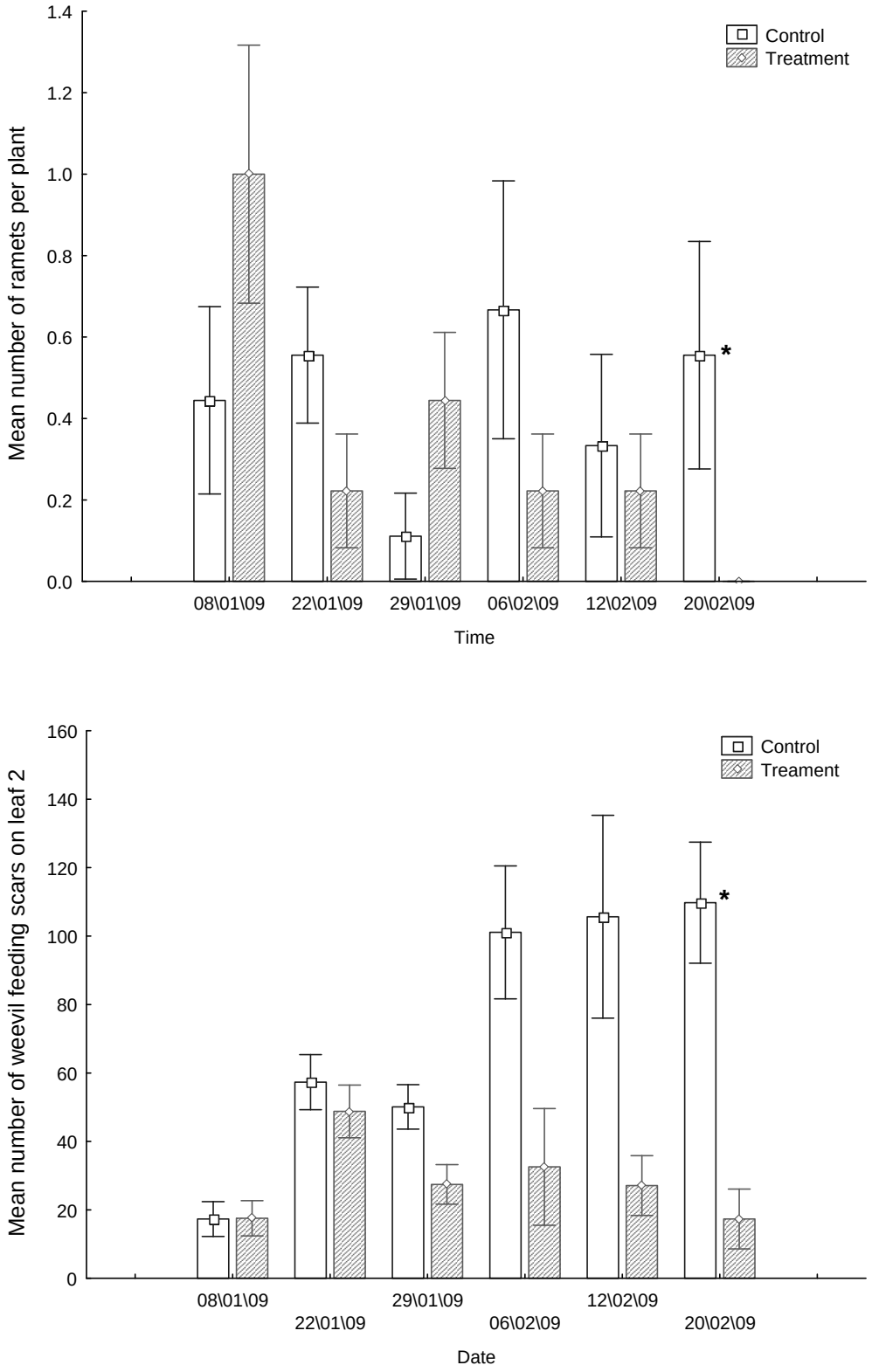


Figure 6.40: The effect on water hyacinth of spraying plants with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in the field at Delta Park in summer 2009. A: number of ramets produced; B: weevil feeding damage. * end point means are significantly different at $p < 0.05$. Bars = S.E.

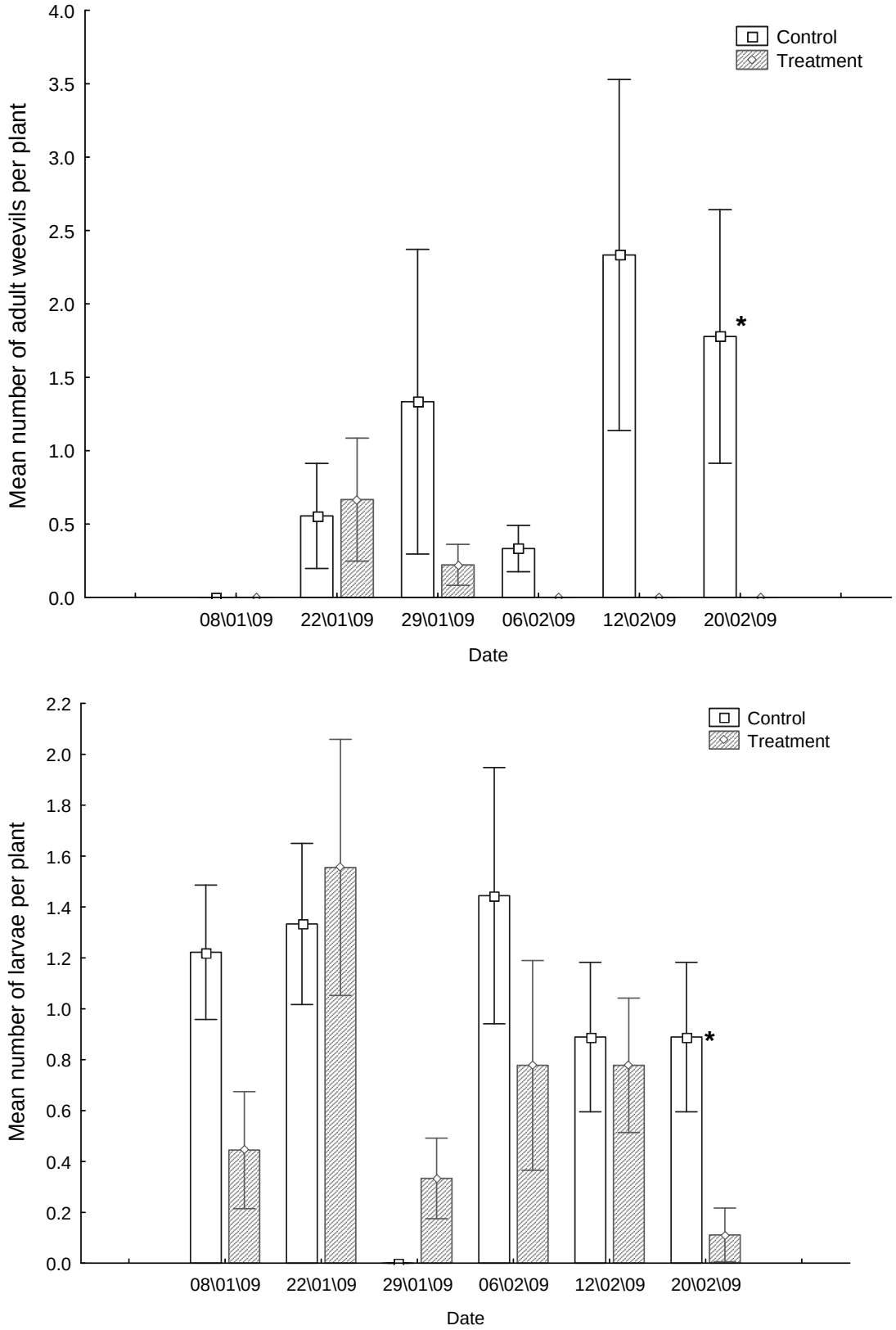


Figure 6.41: The effect on water hyacinth weevils of spraying plants with a sublethal glyphosate dose (0.8% at 140 *l*/ha) in the field at Delta Park in summer 2009. A: number of adult weevils; B: number of larval weevils. * end point means are significantly different at $p < 0.05$. Bars = S.E.

6.3.2.4 Discussion

Seasonal application of herbicide at two field sites over three seasons, although not as clear-cut as laboratory data, showed that a sublethal dose of glyphosate herbicide integrated with *Neochetina* weevils as biological control agents could be used to control water hyacinth populations. These results are gratifying as they largely mirror those of Jadhav et al. (2008) and Chapter 4 of this report. However, it is notable that the effects are more variable and the trends in plant growth are not as clear as those seen in the laboratory trials.

An autumn spray of water hyacinth populations was recommended because water hyacinth in South Africa has been shown to reproduce by ramet production in winter (Chapter 1). Even though vegetative growth was restricted at both sites, reducing plant numbers and biomass, ramet production was only significantly retarded at Farm Dam. Insect numbers were low at both sites but not significantly reduced by the herbicide treatment. However, neither were they particularly stimulated by the treatment, which had been suggested by the laboratory trials (Chapter 4) and results from other integrated weed programmes (Story and Stougaard, 2006).

Applying a low dose of herbicide in spring was recommended because plants typically start to increase their biomass during this period. These field trials showed no significant effect of the spray on plant density or ramet growth, but did reduce the accumulation of plant biomass at both sites as intended. The effect on weevil populations was less clear cut as numbers of adults and larvae were generally low. However, it again appears that the herbicide had no detrimental effect on the biocontrol agents. Large fluctuations at both sites are probably due to the vagaries of collection at low population densities. This was compounded by the sprayed plants varying greatly in size as the experiment progressed, caused by differential dosages applied to the treatment plot which were delivered from a hand-held spray-rig in a moving boat. This was set-up was not suitable to deliver a precise spray volume over a larger field site.

The summer spray treatment at Delta Park clearly held the small plants under control in terms of restricting vegetative growth. Many plants in the treatment plot were extremely small, stunted by the combination of insect attack and herbicide, and ramet production was slowed to a stop. These small plants were not particularly suitable for the biological control agents as the adult weevils were absent from the samples. However, adult feeding scars and larval numbers are comparable with those of the control on a plant weight basis and indicate that the weevils were still able to survive on these plants.

6.4 General Conclusion

The feasibility of integrating a low dose of herbicide with biological control has now been proven in small-scale field trials. This outcome was expected, given the extensive laboratory testing of the method and the careful determination of the herbicide dosage required to freeze plant growth (Jadhav et al., 2008). The principal advantage of this technique will be its application on large-scale, permanent hyacinth infestations where the success of biological control has been hampered by ad hoc application of lethal herbicide doses, which remove virtually all of the plants and the complete population of biological control agents (Cilliers, 1991; Hill and Cilliers, 1999; Hill and Olckers, 2001). Low doses of glyphosate herbicide are now known to be largely benign in their effect on *Neochetina spp*. and other aquatic fauna (Chapter 4, this report), and also on the water hyacinth mirid, *Eccritotarsus catarinensis* (unreported data), which will allow the combined use of insects and herbicides to smooth out the boom and bust populations of water hyacinth which have appeared in recent years. However, two aspects of this technique remain to be resolved before it can be recommended for use on a large scale. The first issue revolves around tailoring a retardant dose to the size (biomass) of the plants being sprayed; and the second obvious caveat requires that water managers should go to extensive lengths to garner resources such as herbicides, helicopters and labour teams, with the express purpose of carefully meting out the herbicide so as not to kill their target weed – this is improbable.

Water hyacinth can vary in size from mature but tiny 5 cm tall bulbous plants, to giants over one metre tall, weighing ten times more than their minuscule counterparts. This presents a problem for water managers who will have to weigh and measure plants to calculate spray volumes to deliver the required amount of active ingredient per gram of plant tissue to freeze the plant growth. This calculation will have to take into account plant surface area exposed to the spray, which changes as the infestation varies from a two-dimensional mat of bulbous plants to a three-dimensional canopy of tall weeds. This underlines why the manufacturer's recommended lethal dose is 3% at 140 *l*/ha, which is three times the 1% actually required to kill the average water hyacinth plant, and explains why plant and insect measures varied more widely in these field trials than they did in the equivalent laboratory experiments. Because the retardant effect varies with the dose of herbicide received by the plant, the technique will be complicated as described above by differing field conditions. However, this apparent problem could be turned into a benefit if we consider more broadly the circumstances under which herbicide is applied to water hyacinth mats in South Africa.

Asking a water manager to devote resources to spray water hyacinth with the express intention of not killing the plant has always been a concern underlying the sublethal dose concept. Clear rivers and dams are the most obvious measure of a manager's efficiency. However, integrated control requires a residual population of water hyacinth to sustain its biological control agents. Spraying a sublethal does of glyphosate onto the

weed will allow this, but does not necessarily have to be done by careful calculation of dosage rates, or by holding back on clearing a booming population of the weed. Instead, strip-spraying the water hyacinth mat with a 3% dose, confined to the centre of the mat, will inevitably generate spray drift onto the adjacent water hyacinth without endangering fringing non-target vegetation alongside the water body. The spray drift will deliver a sublethal dose of herbicide to adjacent water hyacinth which will decline with distance from the central strip spray. We know from Jadhav et al. (2008) that a sublethal dose of glyphosate down to 0.5%, sprayed at 140 *l*/ha onto medium-sized plants, will stop the addition of new leaves over eight weeks. We also know from Chapter 4 that nutrient levels will not affect this outcome. Therefore, spraying with a lethal dose of glyphosate, while deliberately leaving the plants at the perimeter of the infestation to the retardant effects of a sublethal herbicide dose, or weevils migrating off the lethally sprayed plants (Ueckermann and Hill 2001), will allow clearance of any large infestation or water hyacinth outbreak, but leave biocontrol agents in place, where they can suppress growth of the herbicide-weakened plants and contain a resurgence of the weed after spraying.

Tu et al. (2001) recommend that only one third to one half of any water body be treated with glyphosate as an aquatic herbicide at any one time, to prevent fish kills caused by dissolved oxygen depletion. Applying the same restriction will also allow biocontrol agents to survive and continue their work in suppressing water hyacinth. All that is required is a change in the mindset to accept a fringing water hyacinth population of 10-20% surface cover, as a “naturalised” part of the South African aquatic landscape.

CHAPTER 7 – MULTISPECTRAL SATELLITE REMOTE SENSING OF WATER HYACINTH ON SMALL WATER BODIES

7.1 Introduction

Invasions by non-indigenous species result in damage to ecosystem function, diversity and economic value (Hulme, 2003). Although progress has been made in the control of invasive species, inadequate post-control evaluation is done, which limits further management of problem weeds. Identifying, mapping and monitoring of invasive species are key in their management (Hulme, 2003).

Mapping of invasive species can be problematic as no common spatial mapping unit exists. Since invasions are dynamic in nature, the spatial extent as well as the spatial and temporal resolution of the surveys is important. Remote sensing has often been used to map net primary production on a broad scale (Greegor, 1986, Field et al., 1995, Defries and Townshend, 1999); however recent advances in the field of remote sensing have resulted in higher spatial and spectral resolution imagery becoming available for non-military use, and the ability to distinguish between certain plant species is now possible (Nagendra and Gadgil, 1999; Turner et al., 2003). Although mapping invasive species at coarse scales does have advantages, because the extent of occurrence (distribution) can be measured, fine-scale mapping is necessary to determine the area of occupancy. In the case of water hyacinth, the extent of occurrence is the whole of South Africa and the area of occupancy is restricted to water bodies, making the mapping of the weed relatively easy since the distribution is somewhat limited.

With regard to remotely sensed imagery, the size of the smallest unit on the image is known as the grain; and the extent refers to the overall size of the study area (Wiens, 1989). These spatial concepts are important when planning a mapping project because the correct spatial scale relative to the species of interest must be used (Hulme, 2003). Water hyacinth infestations can range from 0 % to 100 % cover of a water body. The size of the water body and the resolution of the image will be factors limiting the mapping of water hyacinth infestations. The grain of a satellite image therefore needs at least to be smaller than the size of the water body. The sizes of the smaller water hyacinth-infested water bodies sampled monthly in this project are under 160 ha; therefore images with resolutions ranging from 10 m to 30 m should be adequate to map water hyacinth on these water bodies. In this study, the use of satellite remote sensing at resolutions of 10 m to 30 m is proposed as a tool to monitor water hyacinth populations in South Africa at small extents (under 160 ha), in terms of the size and health of infestations.

Resolution of images is usually defined as high, medium and low; however these definitions are relative to the extent of the study area. In this study, resolutions under

10 m will be referred to as high, resolutions between 10 m and 30 m as medium, and resolutions greater than 30 m (30 m to 1 km) as low.

7.1.1 Water Hyacinth

Integrated management planning (IMP) is a possible solution to water hyacinth infestations and involves integrating the different types of control mechanisms that are least harmful to the environment. Control techniques include biocontrol, mechanical removal and chemical control using herbicides (van Wyk and van Wilgen, 2002). The current focus of integrated control of water hyacinth in South Africa is a combination of biocontrol and herbicide control (Ueckermann and Hill, 2001) with chemical mowing, whereby retardant doses of herbicide are used to slow the vegetative growth of plants, is proposed (Jadhav et al., 2008). Chemical mowing of water hyacinth has been seen to be ineffective when no biocontrol agents are present on the plants, and must therefore be used in conjunction with biocontrol, when it has a possible synergistic and additive effect on the biocontrol agents (Center et al., 1982).

7.1.2 Remote Sensing of Water Hyacinth

The process of remote sensing involves obtaining information about an object without being in physical contact with it (Lillesand et al., 2004). The sun emits radiation, which is reflected back into the atmosphere when this radiation 'bounces' off a surface. All objects with a temperature above 0K reflect electromagnetic (EM) energy which includes visible light as well as infrared energy.

Unique reflectance characteristics of water and vegetation are important for remote sensing of water hyacinth using satellite images. Leaf properties influence reflectance characteristics of plants and include leaf pigmentation, leaf thickness and the amount of water that is contained in the leaf (Woldai, 2004). More chlorophyll contained in a leaf causes higher absorption of red light while more water in the leaf causes higher reflection of Near Infrared (NIR) light (Lillesand et al., 2004). This last property should make it easy to identify the spectral image of aquatic plants, which have a higher NIR reflectance than terrestrial plants (Everitt et al., 1999).

Everitt et al. (1999) found that water hyacinth has a relatively high reflectance of NIR and can be differentiated from other aquatic plants such as the largely submerged aquatic weed hydrilla (*Hydrilla verticillata*). If the plant is dead or has dried out, there is little to no photosynthesis so reflectance of red light is higher. Dead or dried plants will also have a lower reflectance in the NIR. It is therefore possible to determine the health of plants based on their water content (NIR reflectance) and amount of chlorophyll (red light reflectance) using the Normalized Difference Vegetation Index (NDVI = NIR – Red / NIR + Red) (Woldai, 2004). Water hyacinth can be distinguished from water, which has a high absorption of NIR (Woldai, 2004), making it possible to delineate water bodies according to this spectral characteristic (Lillesand et al., 2004). The NDVI obtained from low resolution imagery has been shown to correlate with dry biomass of

grasses in the Sahel; however, due to the relatively coarse spatial and temporal resolutions of the satellite imagery used (80 m to 1100 m spatial resolution), flowering and budding of vegetation is often not discernible (Roller and Colwell, 1986). Venugopal (1998) used NDVI to quantify the change in health of water hyacinth mats over time; however this research was not ground-truthed.

7.1.3 Case Studies

Remote sensing of water hyacinth has been successful in many regions of the world, including Lake Victoria (Albright et al., 2004), Lake Kariba (Pettersson and Namakando, 2002), India (Verma et al., 2003; Chopra et al., 2001) and North America (Everitt et al., 1999, Nelson et al., 2006). However, the spatial scale of these investigations has always been large, ranging from 43.86 ha (Verma et al., 2003; Chopra et al., 2001) to 68 000 km^2 (Albright et al., 2004). Often only one image per site per year was obtained (Albright et al., 2004; Everitt et al., 1999), and only occasionally were seasonal images obtained (Verma et al., 2003), depending on the objective of the study. The aims of these studies were either to map the distribution of water hyacinth, to monitor change in the water hyacinth populations, or to monitor the change in the extent of the water hyacinth.

The study of Lake Victoria included the Kagera River Basin, with the overall extent of the water body being 68 000 km^2 (Albright et al., 2004). Since the objective of the study was to determine the abundance and distribution of water hyacinth in Lake Victoria and the Kagera River Basin, and not the change in the water hyacinth populations, repeat images were not necessary. The resolution of the satellite images used in the study ranged from 4 m to 100 m resolution (Table 7.1). Albright et al. (2004) were successful in determining the area of the lake covered by water hyacinth. However, due to the majority of the images having a low resolution (between 25 m and 100 m), and only a few images having a high resolution (4 m) (Table 7.1), the larger mats of water hyacinth that could be detected at the lower resolutions were included in the survey, and the number of smaller mats identified was minimal. Thus there may have been an under-estimation of the area covered by water hyacinth (Albright et al., 2004).

Table 7.1: Satellite image sources and resolutions used to monitor water hyacinth on Lake Victoria and the Kagera River Basin (After Albright et al., 2004).

Satellite image	Resolution (m)
Radarsat ScanSar Wide B	100
JERS	100
Radarsat ScanSar Narrow B	50
Landsat 5 TM	30
Landsat 7 ETM+	30
Radarsat Standard Beam 1	25
Ikonos	4

Verma et al. (2003) studied six water hyacinth-infested water bodies in Bangalore City, India. Indian Remote Sensing Satellite (IRS) LISS-II and -III images (36 m and 23 m resolution respectively) were used to determine percentage cover of water hyacinth and detect change in the cover of water hyacinth over time (1988-2001) using annual and seasonal images. Sizes of the water bodies ranged from 43.9 ha to 135.7 ha with the area covered by water hyacinth ranging from 0 ha to 21 ha. Verma et al. (2003) achieved 87.7% accuracy in correctly classifying water hyacinth and were able to monitor change in the size of water hyacinth mats.

Satellite remote sensing has seen many improvements in the past 20 years including an increase in resolution and improved methods for characterizing types of land cover (vegetation); and remote sensing has subsequently become useful for both static and dynamic monitoring of land cover types (Defries and Townshend, 1999). Remote sensing provides an avenue through which to study landscape ecology which has its base in understanding spatial patterns of features in the landscape. Temporal changes in landscapes can also be studied, and from this the biotic and abiotic processes occurring at the landscape level can be derived (Turner et al., 2001). Remote sensing is a technique that is used to monitor or understand landscapes; therefore it is only a means to an end. The information obtained from images must then be interpreted and related to the ecology or biology of the study subject.

When an image is taken by a satellite, atmospheric and topographic distortions of the images occur. Curvature of the earth, and variations in the altitude and velocity of the satellite result in geometric distortions. These errors are corrected in order to make the image usable and comparable to maps. Georeferencing is performed in order to place the satellite image in the correct position on the earth's surface and orthorectification is done to add elevation to the images, as images are taken in two dimensions and geometric distortion results (all points are in their true relative horizontal position; however, their terrain placement is not true) (Lillesand et al., 2004). Atmospheric conditions also add error to an image because particles in the atmosphere reflect and refract light, which is corrected for with a series of algorithms. A further adjustment of

the image is needed because, as well as containing atmospheric and topographic errors, an image is not recorded as reflectance values, but rather as digital numbers. A process called radiometric normalization is used to convert the digital numbers into reflectance values (Lillesand and Kiefer, 1994).

7.1.4 Remote Sensing for This Project

Two years of monthly sampling of water hyacinth growth at 15 sites around South Africa have required many hours of field work, driving (± 100 000 kms) and associated activities getting to and from field sites. The data gathered reveal important insights into how water hyacinth grows under different temperature and nutrient regimes and provides a valuable foundation on which this project's management plan is built. However, management interventions of infested sites will inevitably require monitoring of sites, which could be done by remote sensing if the technique allows. If low- to medium-resolution satellite images can be used to track the change in area of water hyacinth infestations at different sites, then this will provide management information for intervention if required. Remote sensing of water hyacinth will in any case prove to be a valuable tool to any water manager wishing to track the growth of water hyacinth. If plant health is also remotely measurable as suggested, then this will add to the information that can be gathered in this way.

It is envisaged that a herbicide spray will be applied to intractable sites just before the plants start to reproduce asexually in winter. Different local growing conditions, which have been categorized in an expert system model (Wilson et al., 2006; Chapter 8, this report) will affect the timing of this event at different sites which are under a variety of nutrient and climatic regimes. If remote sensing of sites is feasible, and also reveals the state of the weed in terms of its growth and reproduction, then this method could be used as a management monitoring tool, to be incorporated in to the integrated management plan for control of water hyacinth.

7.1.5 Aims

This study seeks to determine whether satellite remote sensing, using existing imagery at medium resolutions (10 m-30 m resolution), can be used to monitor the extent of water hyacinth populations on a small scale in South Africa. To address this aim the following questions will be asked:

1. How accurately can the area of water hyacinth infestations be measured at small extents and at medium resolutions (10 m-30 m)?

   1a. What errors occur in measuring the area of water hyacinth at decreasing resolutions?

   1b. How do shape and size of the water body and the patches of water hyacinth affect the classification accuracy of water hyacinth at medium resolutions?

2. Can satellite remote sensing be used as part of a tool for water hyacinth management?

   2a. How do the images relate to in situ measurements of water hyacinth growth (plant and insect parameters, biomass, temperature and nutrients)?

   2b. What is the feasibility of using satellite remote sensing as a monitoring tool for water hyacinth in terms of time and cost?

7.2 Materials and Methods

7.2.1 Field Sites

Water hyacinth was monitored on a monthly basis at 15 sites around South Africa (Figure 7.1). Sites ranged in size from 0.3 ha to 154 ha and were situated on either rivers or dams. Each month abiotic and biotic parameters were measured in the field. Water and plant tissue nutrient levels, as well as water, air and water hyacinth canopy temperatures were measured (Chapter 1).

7.2.2 Satellite Images

Existing Landsat 5 TM (Thematic Mapper) and SPOT IV satellite images are freely available for research purposes from the Satellite Applications Centre (SAC) of the CSIR in South Africa (www.csir.co.za). Therefore all existing Landsat 5 TM and SPOT IV images for 2005 were requested for the 15 water hyacinth monitoring sites in South Africa in order to compare information about the water hyacinth measured from the images to the water hyacinth data collected in the field each month. Although field sampling continued during 2006, new satellite images were not ordered due to the possible time constraints of receiving and processing the images.

In total, 46 satellite images were obtained for the study, although four of the SPOT IV 20 m resolution images did not cover any of the 15 monthly monitoring sites (Table 7.2). A panchromatic merge had been applied to the nine SPOT IV 10 m resolution images. This is a type of Intensity-Hue-Saturation transformation where the intensity data are used from the 10 m resolution panchromatic band, and the hue and saturation data are taken from the 20 m resolution multispectral bands, resulting in a 10 m resolution multispectral image (Lillesand and Kiefer, 1994).

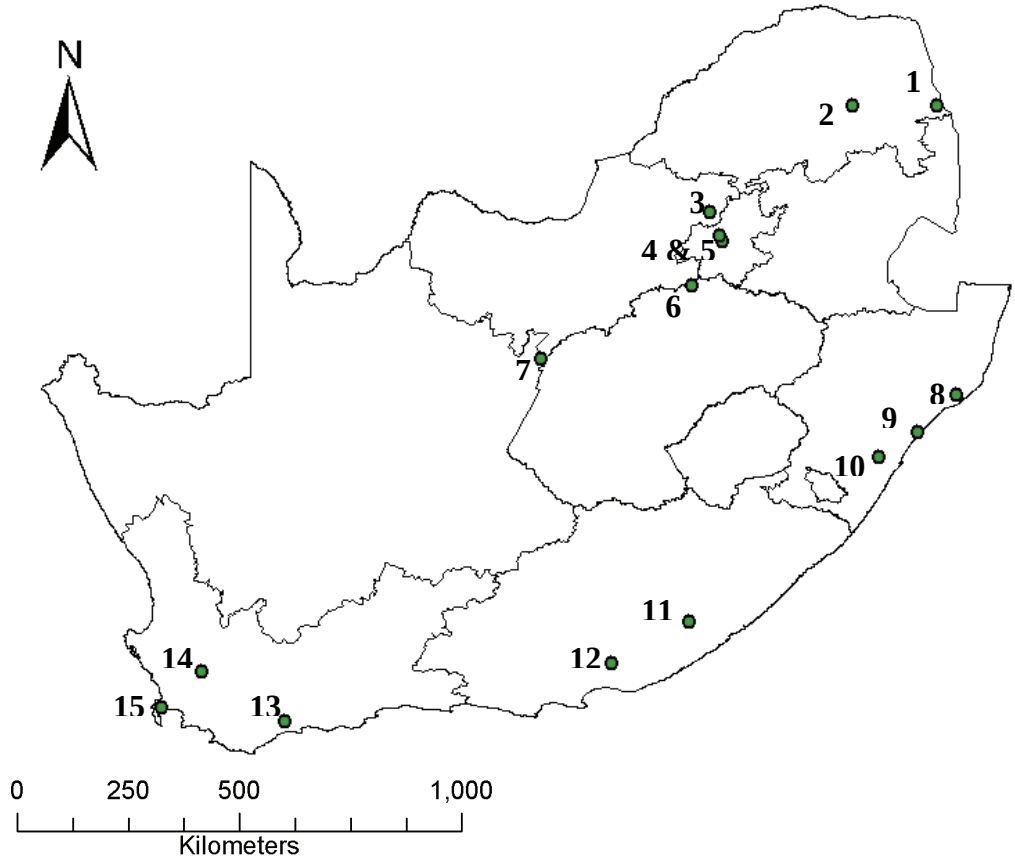


Figure 7.1: Fifteen water hyacinth sites monitored monthly in South Africa (1: Mkadhzi Spruit, 2: Yamorna Weir, 3: Crocodile River, 4 & 5: Delta Park and Farm Dam, 6: Feesgronde, 7: Warrenton Weir, 8: Enseleni River, 9: Mbozambo Swamp, 10: Hammarsdale Dam, 11: Kubusi River, 12: New Years Dam, 13: Breede River, 14: Wolseley, 15: Princess Vlei)

Certain practical problems were experienced during the image acquisition and pre-processing stages of this project. The Landsat 5 TM satellite was launched in 1982 and subsequently the sensor has degraded resulting in banding on the images (http://edc.usgs.gov). Inaccuracies in the orbital model parameters of the satellite cause difficulties when performing atmospheric correction; thus the Landsat 5 TM images were not atmospherically corrected. The 19 SPOT IV 20 m resolution images could not be pre-processed as they were at processing level 2A, which means that no orbital information about the satellite is included in the image and atmospheric corrections cannot be performed. SPOT IV images in level 2A are geometrically corrected to the UTM WGS84 projection (www.spot.com). South Africa covers three UTM zones – 34S, 35S and 36S; however, all the SPOT IV 20 m resolution images obtained for this project were projected in UTM zone 0, which does not exist. Although this problem was brought to the attention of the Satellite Applications Centre, it was not solved, which resulted in the images not being used. SPOT IV images should be requested in level 1A, which allows for atmospheric corrections to be made.

Table 7.2: Satellite images of 15 water hyacinth monitoring sites in South Africa (Landsat 5 TM images are 30 m resolution, SPOT IV images are 20 m resolution; * indicates SPOT IV images at 10 m resolution)

Site Name	Image type	Date
Breede River	Landsat 5 TM	12/04/2006
	SPOT IV	08/09/2005
Crocodile River	Landsat 5 TM	25/07/2005
	Landsat 5 TM	18/07/2005
	SPOT IV	17/12/2005
Delta Park	SPOT IV *	10/12/2003
Enseleni River	Landsat 5 TM	15/09/2005
Farm Dam	SPOT IV *	10/12/2003
Feesgronde	Landsat 5 TM	09/07/2005
	Landsat 5 TM	25/07/2005
	Landsat 5 TM	18/07/2005
	Landsat 5 TM	16/06/2005
	SPOT IV	23/05/2005
	SPOT IV	19/08/2005
	SPOT IV *	16/05/2003
Hammarsdale Dam	Landsat 5 TM	01/05/2005
Kubusi River	Landsat 5 TM	18/07/2005
	Landsat 5 TM	18/07/2005
	Landsat 5 TM	08/05/2005
	SPOT IV	26/08/2005
	SPOT IV	03/06/2005
	SPOT IV	03/06/2005
	SPOT IV	16/09/2005
Mbozambo Swamp	Landsat 5 TM	18/06/2005
	SPOT IV *	21/09/2005
Mkadhzi Spruit	Landsat 5 TM	20/07/2005
New Years Dam	Landsat 5 TM	18/07/2005
	SPOT IV	13/12/2005
	SPOT IV *	19/11/2003
Princess Vlei	Landsat 5 TM	15/03/2005
	SPOT IV	13/09/2005
	SPOT IV	12/07/2005
	SPOT IV *	11/05/2005
Warrenton Weir	Landsat 5 TM	16/07/2005
Wolseley	Landsat 5 TM	15/03/2005
	SPOT IV	07/02/2005
	SPOT IV	16/09/2005
	SPOT IV *	07/02/2005
Yamorna Weir	Landsat 5 TM	13/09/2005
	SPOT IV *	18/08/2003

Although the Landsat 5 TM images were not atmospherically corrected, they can still be used to calculate relative area of water hyacinth, and they can also be used to determine characteristics which make field sites classifiable. However, information on images taken on different dates cannot be compared as the atmospheric conditions may affect the results. Of the nine SPOT IV 10 m resolution images that were pre-processed, water hyacinth was only visible at six sites.

7.2.3 Identifying the Site on the Satellite Image

Often no clear water is visible at the edge of a site; therefore the boundaries of each of the 15 sites needed to be determined. This was done using the high resolution images (3-10 m resolution) from Google Earth™ (http://earth.google.com) to determine fixed landmarks; and expert help from members of the water hyacinth sampling team who were familiar with the sites and surrounding vegetation. The sites used for this study were actual field monitoring sites, so the percentage cover obtained from the monthly monitoring could be compared to the percent cover measured from the images. Often the monitoring sites were in tributaries along a river because they are more accessible than the main river; however, these tributaries are usually quite small and cannot be seen on the image as riparian trees cover the waterway. This was the case at Feesgronde (Figure 7.2), but since the image was from 2003 and information extracted from the image could not be compared to the monthly monitoring data, water hyacinth in the main river channel was measured.

Figure 7.2: SPOT IV satellite image (10 m resolution) of Feesgronde (RGB = 4, 3, 2) (site = 47.7 ha, 1: 130). The monthly water hyacinth monitoring site is circled in white; the site used to determine characteristics of water hyacinth from the image is circled in black.

7.2.4 Assessing the Accuracy of Classifying Water Hyacinth

In order to determine the accuracy with which water hyacinth can be classified at 10 m, 20 m and 30 m resolutions, the area of water hyacinth was measured at four sites at each resolution (Figure 7.3). Although water hyacinth was present at six of the monthly monitoring sites in the SPOT IV 10 m resolution satellite images, it was only possible to classify water hyacinth at four sites as the other sites (Farm Dam and Delta Park) were too small for the boundaries to be clearly defined.

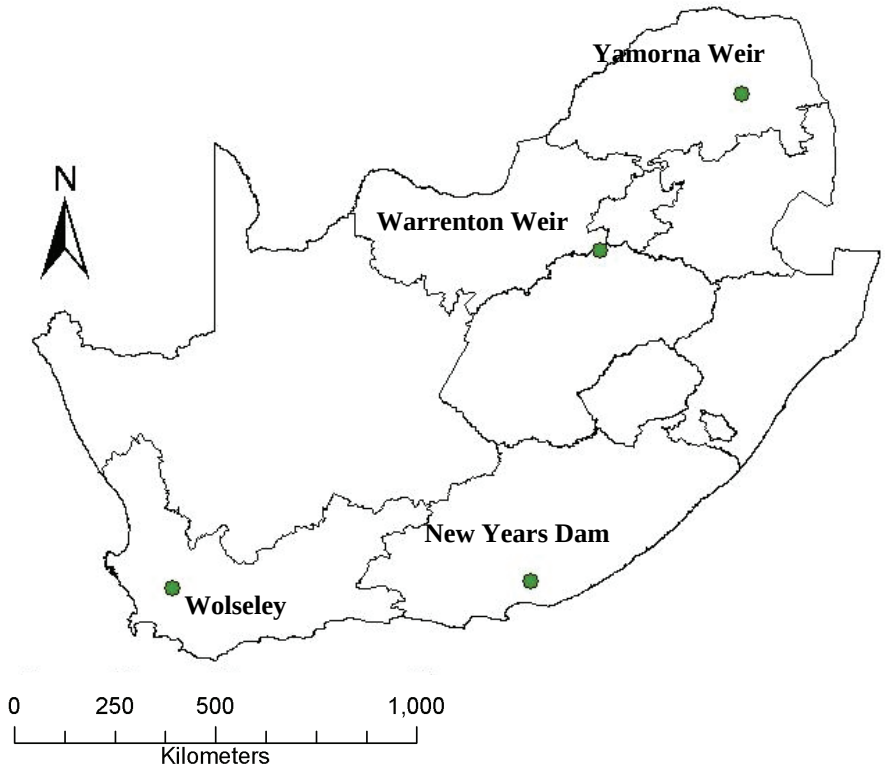


Figure 7.3: Four water hyacinth monitoring sites in South Africa, monitored monthly on the ground, and used to classify satellite images from remote sensing.

7.2.4.1 Aggregation

In order to compare the area of water hyacinth measured at different resolutions the 10 m resolution SPOT IV images were aggregated to produce 20 m and 30 m images for comparison. The function of aggregation is to increase the pixel size of an image. This is an acceptable procedure to decrease resolution as the rules used in the aggregation mimic the way an image is taken by a satellite. Therefore, the 20 m and 30 m resolution aggregated images are comparable to SPOT IV 20 m resolution and Landsat 5 TM 30 m resolution images respectively. The programme ArcMap™ 9.1 was used. First the images were split into their component layers (each multi-spectral band on the satellite image is a separate layer) and the following command was used in the Raster Calculator function of Spatial Analyst on each layer:

Aggregate[image_layer1,2,MEAN,TRUNCATE,DATA]

To aggregate the image from 10 m to 20 m resolution, the cell factor used is 2, which translates into four pixels being grouped. The mean of these four pixels was taken to be the value of the new larger pixel. If the cell factor (2 for aggregating from 10 m to 30 m resolution, 3 for aggregating from 10 m to 30 m resolution) does not divide evenly into the number of columns and rows of the image, there will be a remainder of pixels. The truncate option was chosen, thereby removing the remaining pixels from the image.

Finally, a pixel with a NoData value was ignored. This translates loosely into the majority rule for calculating the new value of the larger pixel (ArcMap™ 9.1). The component layers were then restacked using ERDAS Imagine V8.7® (ERDAS, 2005).

7.2.4.2 Classifying Water Hyacinth

Image interpretation requires that patterns in the landscape, which are seen on the image, be identified and recognized. The patterns are formed by the spectral signature (reflectance of wavelengths) of the pixels and the position, size and shape of clustered pixels (pixels with similar spectral signatures) in the image (Molenaar, 1996). In order to measure the area of water hyacinth in the images, the water hyacinth must be identified, or classified, according to its spectral signature and geometry (position, size and shape).

Image enhancement tools were used to view the patches of water hyacinth; these included changing the brightness and contrast of the image using the Contrast function in Raster tools in ERDAS Imagine V8.7®, as well as applying a 5 x 5 edge enhancement (Convolution filtering in Raster tools in ERDAS Imagine V8.7®). An edge enhancement is a spatial filtering technique that enhances the contrast in the image in order to emphasize either linear features or edges (Lillesand and Kiefer, 1994). This technique enhanced the patches of water hyacinth in the image, and a 5 x 5 edge enhancement was found to be the most practical; however, 3 x 3 or 7 x 7 edge enhancements can also be used. A 7 x 7 enhancement will exaggerate the edges more than a 3 x 3 enhancement. Since image enhancement techniques are subjective, the correct level to use depends on the image and the person processing the image.

Two types of classification processes can be used: supervised or unsupervised. A supervised classification is used when knowledge about the area to be classified is already known and is used to aid in the classification (Molenaar, 1996). Three steps make up a supervised classification process: training stage, classification stage and output stage. In the training stage the analyst identifies clusters of typical land-cover types, or of the land cover of interest, and creates a numerical signature for these areas based on their spectral attributes. The classification stage is automated and each pixel in the image is then assigned to the different land-cover types identified by the analyst. The resulting output can then be used as a GIS (geographical information systems) input and can be analyzed (Lillesand and Kiefer, 1994). An Iterative Self-Organizing Data Analysis (ISODATA) is an unsupervised classification and requires no knowledge of the area; therefore, there is no training stage. Instead, algorithms are used that aggregate the pixels according to the natural clusters in the image, based on their spatial and spectral attributes. The analyst can decide on the number of classes used (Lillesand and Kiefer, 1994).

Since the sites had been physically sampled, they had effectively been ground-truthed; and expert knowledge about the areas was available from the members of the monitoring team; therefore supervised classification could be used. Monotypic areas of water hyacinth were selected, using the AOI (Area of Interest) polygon tool, then used as the training sample. These water hyacinth signatures were then saved. It was found that if only the signature of water hyacinth was used in the classification, then other features in the landscape were classified as water hyacinth. Therefore, signatures of other features in the image, such as water, grass and trees, were extracted to distinguish water hyacinth from these features. The classification was restricted to the actual field sites, and not applied to the entire image, by creating a polygon around the site using the AOI tool in ERDAS Imagine V8.7®, which was used to perform the classifications.

An unsupervised classification using the ISODATA algorithm was also performed on the images, to compare the results to the supervised classification. The supervised classification was found to be superior in identifying water hyacinth, as the unsupervised classification was unable to distinguish the water hyacinth from other vegetation.

Since the programme identifies where spectral signatures occur in the image, a decision rule is required to tell the programme how to find the areas with the same signatures. The space decision rule feature (non-parametric) was used in the supervised classification. This classification then identifies all areas of water hyacinth using the chosen water hyacinth signatures. This procedure was repeated for each image, obtaining the signature of water hyacinth each time at the different resolutions.

7.2.4.3 Patterns in the Landscape

The accuracy with which water hyacinth can be classified is a scale-related issue. As the resolution of the image decreases, the boundaries of land-cover types blur and affect classification accuracy (Turner et al., 2001). The size and shape of the patches of water hyacinth at four sites were determined using Fragstats (McGarigal et al., 2002). The area covered by water hyacinth, the shape index, perimeter:area (PARA), patch density and landscape shape index were calculated. The shape index is a measurement of shape complexity: a value of one indicates the simplest, most compact shape, and the index increases to infinity with an increase in shape complexity. Perimeter:area is the measurement of perimeter relative to area. This value increases with an increase in shape complexity. Patch density is the density of the patches of water hyacinth in the landscape, and landscape shape index (LSI) is a measurement of the overall shape of the water hyacinth infestation at the site, with the same scale as shape index. LSI is also inversely proportional to the aggregation index; therefore, as shape complexity decreases, so the aggregation or amount of clumping in the image will increase. The above parameters were chosen to determine classification accuracy because they relate

to the boundaries of the water hyacinth patches (shape index and PARA) as well as the arrangement of the water hyacinth in the landscape (patch density and LSI).

a. b.

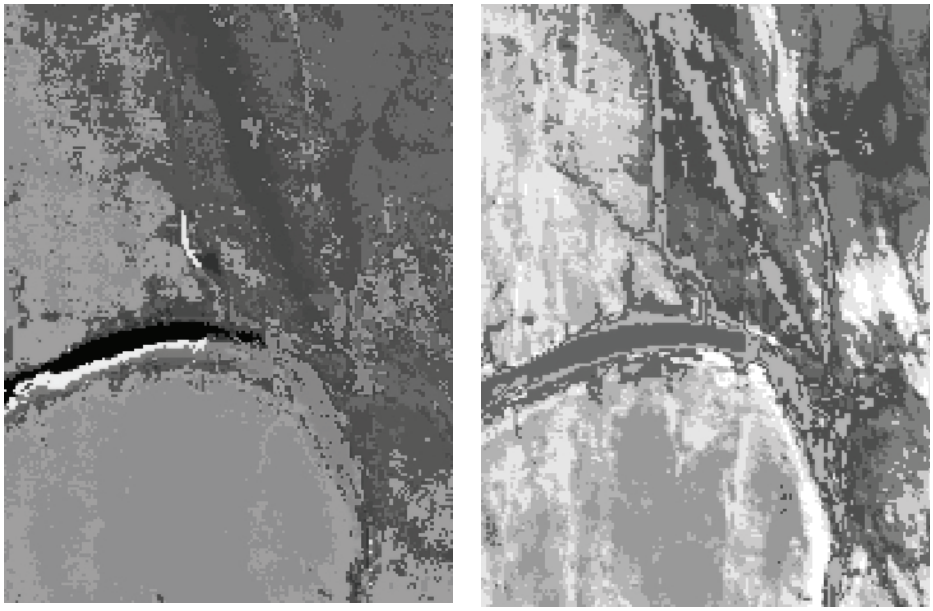


Figure 7.4: Landsat 5 TM image (30 m resolution) of Mkadhzi Spruit: a. Supervised classification (white is water hyacinth, black is water); b. Unsupervised classification (dark grey is water, pale grey is vegetation, including water hyacinth and other riparian vegetation) (site = 9.42 ha, 1:313).

Although water hyacinth was present at each of the 15 field monitoring sites, spatial properties of the site might have made it impractical to classify water hyacinth. To determine which attributes of the water body affect classification accuracy of water hyacinth, measurements of size and shape (area (ha), perimeter:area, width, length and length/width) of the 15 sampling sites (for example, Figure 7.5) were determined from the satellite images (Landsat 5 TM and SPOT IV 10 m and 20 m resolution images, Table 7.2) using the measuring tool in ERDAS Imagine V8.7®. Perimeter:area was calculated using the following formula:

$$\text{PARA} = \text{perimeter (of site)} / \text{area (of site)}$$

Percentage cover of water hyacinth was included in the analysis but was obtained from the monthly field data collected at each site. In addition, the surrounding land cover was visually assessed from the satellite images.

7.2.5 Data Analysis

All statistical analyses used Statistica V6 (StatSoft Inc. 2001). Scatter plots were drawn to determine how the relationship of the area of water hyacinth changed when measured at different resolutions at each site. To compare the slope of the line at each site an Analysis of Covariance (ANCOVA) should be used; however, one of the assumptions of an ANCOVA is that the correlations of the homogeneity of the regression coefficients are the same (http://carbon.cudenver.edu/). The slopes for three sites were positive, but the slope at the fourth site was negative, thereby violating an assumption of the ANCOVA. Therefore, no formal statistics were used to compare the slopes of the regressions. To compare the difference in area measured between resolutions, the difference in areas between 10 m and 20 m, 10 m and 30 m, and 20 m and 30 m resolutions was calculated and compared using a non-parametric sign test.

To determine if the spatial configuration of water hyacinth mats affected the classification accuracy and therefore the accuracy of the measurement of area of the water hyacinth at different resolutions, non-metric multi-dimensional scaling (NMDS) was performed using Primer V5 (Clarke and Gorley, 2001). This process clusters the SPOT IV images (images of Wolseley, Yamorna Weir, Feesgronde and New Years Dam) at different resolutions (10 m, 20 m and 30 m resolution) using the Euclidean distance according to the landscape patterns of water hyacinth (shape, PARA, patch density and LSI). An NMDS was used because there is no restriction on the relationship between the variables to be linear or even multivariate normal (Statistica 6; StatSoft Inc. 2001). PARA, shape index, patch density and LSI were used to determine the clusters.

An NMDS was also used to cluster all 15 monthly water hyacinth monitoring sites (by the size of the site, the perimeter:area of the site, the width of the site, the length/width ratio and the percentage cover of water hyacinth) into those at which water hyacinth could be classified and those at which water hyacinth could not be classified. A square root transformation was applied to the data due to the large differences between the units of the variables. The surrounding land cover at a site was assessed if the site did not cluster according to whether or not water hyacinth could be classified at the site.

In order to determine the accuracy of the remotely sensed measurements of area covered, the remotely sensed percentage cover of water hyacinth was compared to the visual estimates taken from the monthly field sampling. Monthly field sampling only commenced in January 2005; therefore, remotely sensed estimates from the 2003 images could not be compared to the field estimates of cover. The percentage cover of water hyacinth at a site can change rapidly, either decreasing (due to flooding) or increasing (fast regeneration rate when colonizing a new area (Everitt et al., 1999)). The visual estimates of percentage cover and the remotely sensed measurements of percentage cover of water hyacinth were compared at six sites (Wolseley, Mkadhzi Spruit, Enseleni River, Mbozambo Swamp, New Years Dam and Warrenton Weir). The

sites were chosen for comparison if the date on which the image was taken was within 15 days of the date on which the site was sampled. Since area of water hyacinth could be measured on un-pre-processed images, Mkadhzi Spruit, Enseleni River, Mbozambo Swamp, New Years Dam and Warrenton Weir were used in the analysis. The remotely sensed and visual estimates of percentage cover of water hyacinth were compared using a paired sample t-test as the data were normally distributed. A linear regression, of the visual or remotely sensed percentage cover estimates against the size of the water body, was used to determine if the size of the water body affected the estimate of percentage cover, either visual or remotely sensed.

7.2.5.1 Health Status of Plants

To determine the health of the plants the Normalized Difference Vegetation Index (NDVI) was used. This is calculated as follows:

$$NDVI = \frac{\text{Near-infrared} - \text{Red}}{\text{Near-infrared} + \text{Red}}$$

where Near Infrared (NIR) is the measure of the amount of water in the plant (the higher the water content, the higher the reflection of NIR), and Red is the measure of the amount of chlorophyll contained in the leaf tissue (the more chlorophyll contained in the leaf, the lower the reflection of red light). NDVI was calculated using the Model Maker in ERDAS Imagine V8.7®. The values for NDVI range from '-1' to '1', with '1' being a healthy, unstressed plant, and '-1' being a stressed, possibly dead plant (Lillesand et al., 2004). NDVI can be used to classify land-cover types (Soriano and Paruelo, 1992); however, due to the small extent of the sampling sites, the water hyacinth could not be distinguished according to the health of the water hyacinth plants compared to the health of surrounding vegetation. Waterways can be detected on the images as the vegetation growing in and around them is healthier; however, the riparian and aquatic vegetation could not be distinguished from one another based on their health (Figure 7.4). Therefore only NDVI values for known water hyacinth mats were used in the analysis.

a. b.

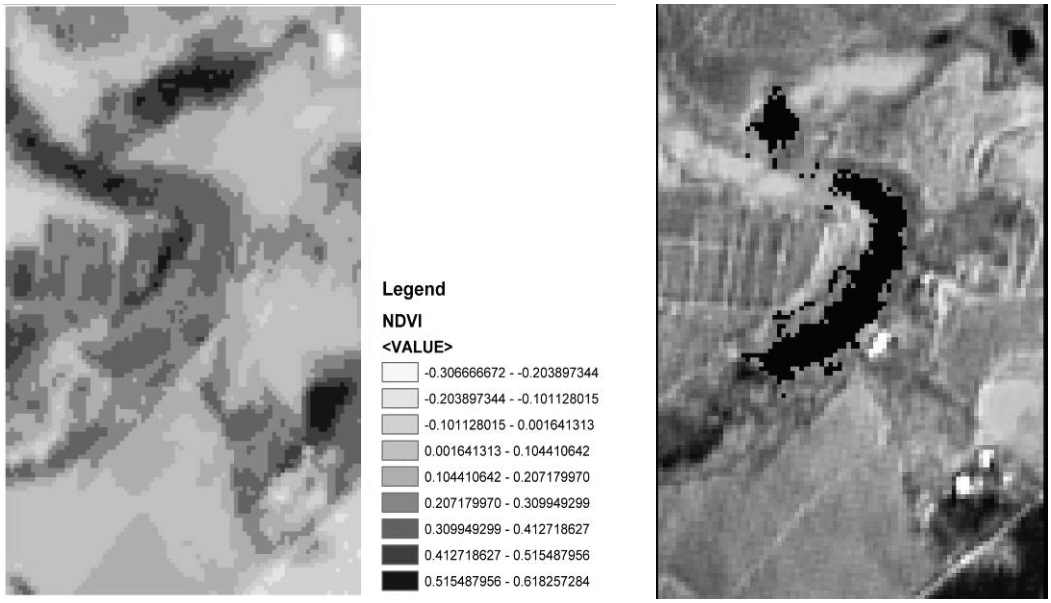


Figure 7.5: Comparison of vegetation classification using a. the Normalized Difference Vegetation Index (NDVI) and b. supervised classification (RGB = 4, 3, 2, black areas are water hyacinth) at Yamorna Weir (site = 10 ha, 1: 61).

7.2.5.2 Ground Truthing NDVI Data Analysis

The NDVI of water hyacinth from four satellite images (three sites: Warrenton Weir, Mkadhzi Spruit and Wolseley (SPOT IV 10 m resolution image and SPOT IV 20 m resolution image – 16/09/2005, Table 7.2)) was compared to the corresponding number of petioles mined per plant; the number of weevil feeding scars per unit leaf area; the biomass of the plants (kg/m^2); mean minimum canopy temperature; mean maximum canopy temperature; and water nutrients (NO_3, NO_2, PO_4 and NH_4) measured in the field, to establish the relationships between NDVI and the plant and insect parameters, water nutrients and water hyacinth canopy temperatures. The relationships were established using a correlation matrix because the variables have different units and cannot be directly compared. The correlation matrix contains the Pearson R coefficient, which consists of values that range between '-1' and '1'. Zero indicates there is no correlation, '-1' indicates a strong negative correlation (as one value increases the other value decreases) and '1' indicates a strong positive correlation (as one value increases so does the other) (StatSoft Inc., 2001).

Sites were divided into those that experience frost, and those that do not. Water hyacinth biomass values were log transformed in order to normalize the data. The change in biomass was analyzed using a one-way Analysis of Variance (ANOVA) to

determine the monthly growth pattern for 2005. A Tukey HSD post-hoc test was performed to detect where the differences in biomass lay between months.

7.2.5.3 Feasibility

Existing SPOT IV and Landsat 5 TM images were chosen for this study because they were freely available for research purposes from the Satellite Applications Centre (SAC) of the CSIR in South Africa. Although existing satellite images were useful to determine whether satellite remote sensing of water hyacinth in South Africa is possible, future monitoring of water hyacinth will depend on contemporary images. While medium-resolution imagery is adequate to monitor water hyacinth, it may not be the most cost-effective. The price/pixel of eight different satellite images (Quickbird, IKONOS, SPOT 5 (2.5 m, 5 m, 10 m and 20 m resolutions), Formosat-2, SPOT 1, 2 and IV (existing images), SPOT IV (10 m and 20 m resolution), Landsat 5 TM and Landsat 7 ETM+) was calculated. The price of the Formosat-2 images was obtained from www.spotimage.fr, IKONOS prices were obtained from www.landinfo.com, and all other image prices were obtained from SAC (www.csir.co.za). Prices quoted in foreign currency were converted to Rands based on the exchange rate on 17 October 2006 (www.sabcnews.com). Acquisition time and pre-processing costs of the satellite images were taken into account. The costs of a field trip to the Kruger National Park (Yamorna Weir and Mkadhzi Spruit), to KwaZulu-Natal (Mbozambo Swamp, Hammarsdale Dam and Enseleni River) and to the sampling sites in Gauteng (Delta Park and Farm Dam) are given for comparison to the cost of satellite images.

7.2.5.4 Cost:Benefit Analysis

A linear regression of spatial resolution against the price/pixel of eight different satellite images was done to determine the relationship between the two variables.

7.3 Results

7.3.1 Assessing the Accuracy of Water Hyacinth Classification

The area of the water hyacinth infestation, calculated for Wolseley, Yamorna Weir, Feesgronde and New Years Dam from SPOT IV satellite images, increased with a decrease in resolution at three of the sites and decreased with a decrease in resolution at one of the sites (Figure 7.6).

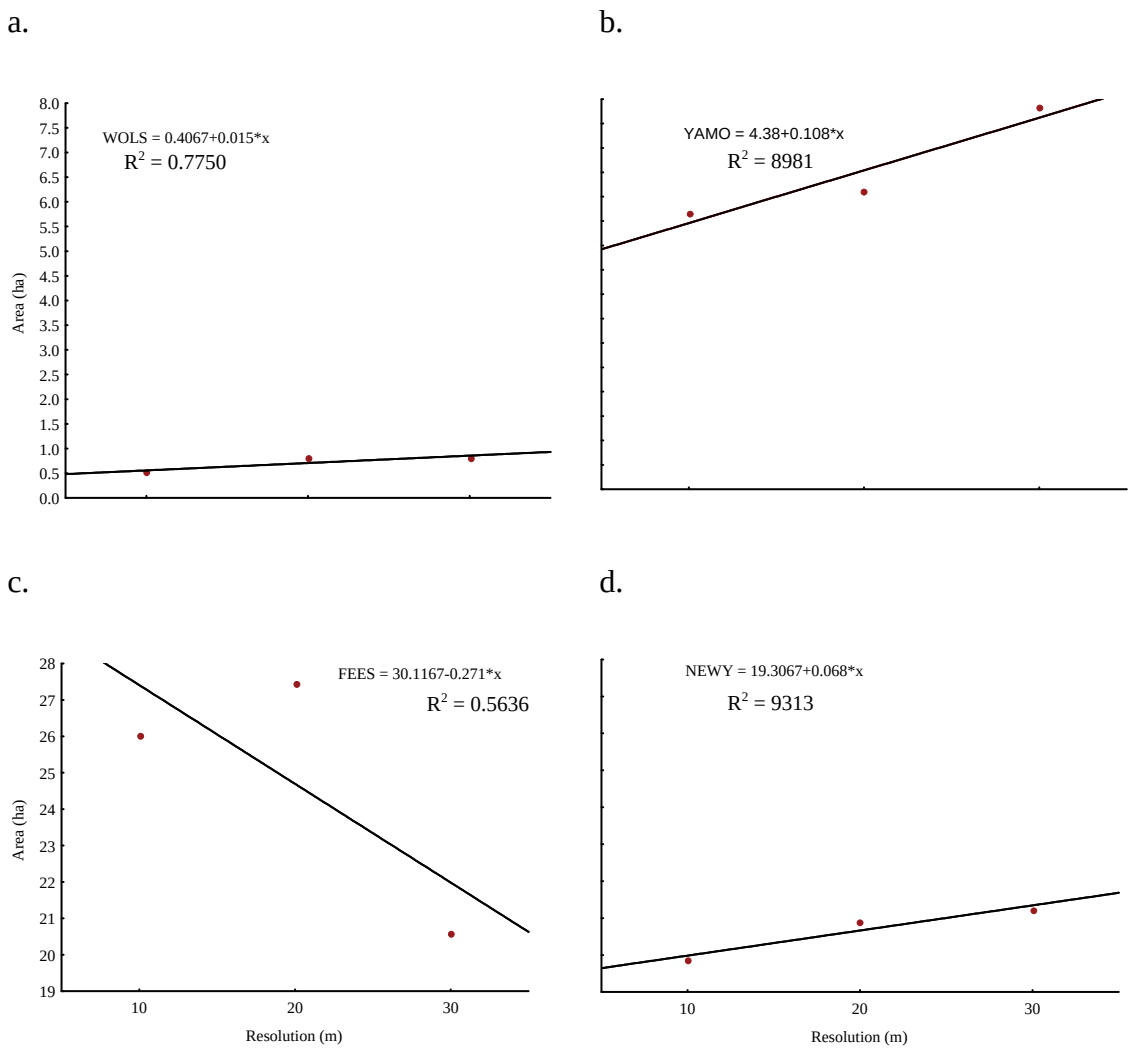


Figure 7.6: The area of water hyacinth measured at decreasing resolution from 10 m to 30 m resolution at four sites on SPOT IV satellite images (a. Wolseley (site size = 0.7 ha, n = 3), b. Yamorna Weir (site size = 10 ha, n = 3), c. Feesgronde (site size = 48 ha, n = 3) and d. New Years Dam (site size = 131 ha, n = 3)).

The mean difference in area of water hyacinth measured between 10 m and 20 m resolution is smaller, and has a smaller variance, than the difference in area measured between 10 m and 30 m, and 20 m and 30 m resolutions (Figure 7.7). The sign test indicated that mean differences in area calculated from different resolution images were not significantly different from one another at $p < 0.05$ (Sign test: ANOVA Chi Square $_{(4, 2)} = 3.5$; p = 0.17).

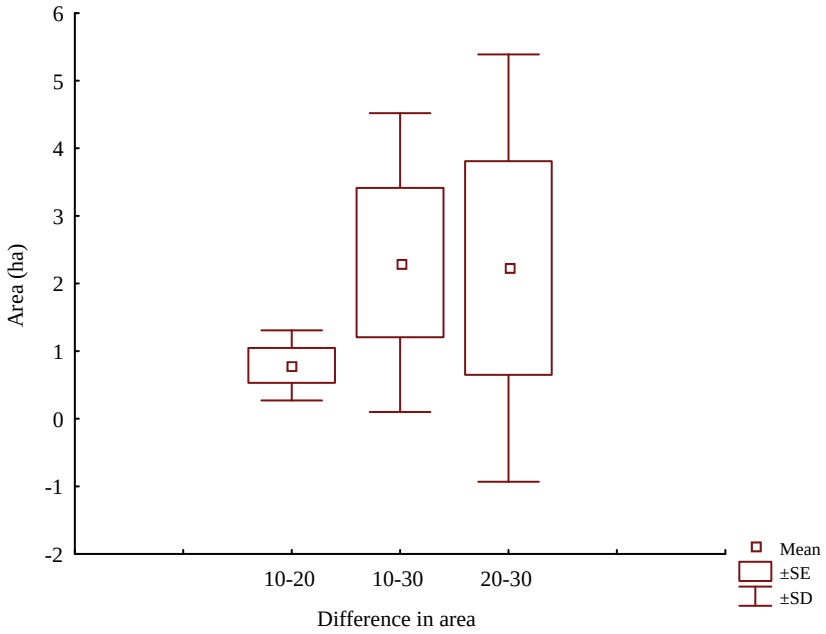


Figure 7.7: The mean difference in area of water hyacinth measured at four sites (Wolseley, Yamorna Weir, Feesgronde and New Years Dam) on SPOT IV images between 10 m, 20 m and 30 m resolutions (n = 12) (10 m-20 m, 10 m-30 m and 20 m-30 m)

The four sites at which the area of water hyacinth was measured at decreasing resolutions (SPOT IV satellite images (Figure 7.3)) cluster according to patterns of the water hyacinth mats in the landscape, rather than site locality. The exceptions are WOLS_20 and WOLS_30, which form their own cluster (Figure 7.8). The stress level for this analysis is 0.01, indicating that the sites cluster well into a 2-dimensional arrangement based on the variables used.

Size, shape and the percentage cover of water hyacinth do not affect whether or not water hyacinth can be classified at the 15 monthly monitoring sites; the sites at which water hyacinth can and cannot be classified do not cluster according to these variables (Figure 7.9).

A low stress value for this NMDS (0.05) indicates the sites cluster well into a 2-dimensional arrangement based on the variables used. Farm (no), Delt (no) and Wols (yes) do form a cluster, and are all small sites. Mkad (yes) and Bree (no) also form a cluster; however, none of the other sites form clusters (Figure 7.9).

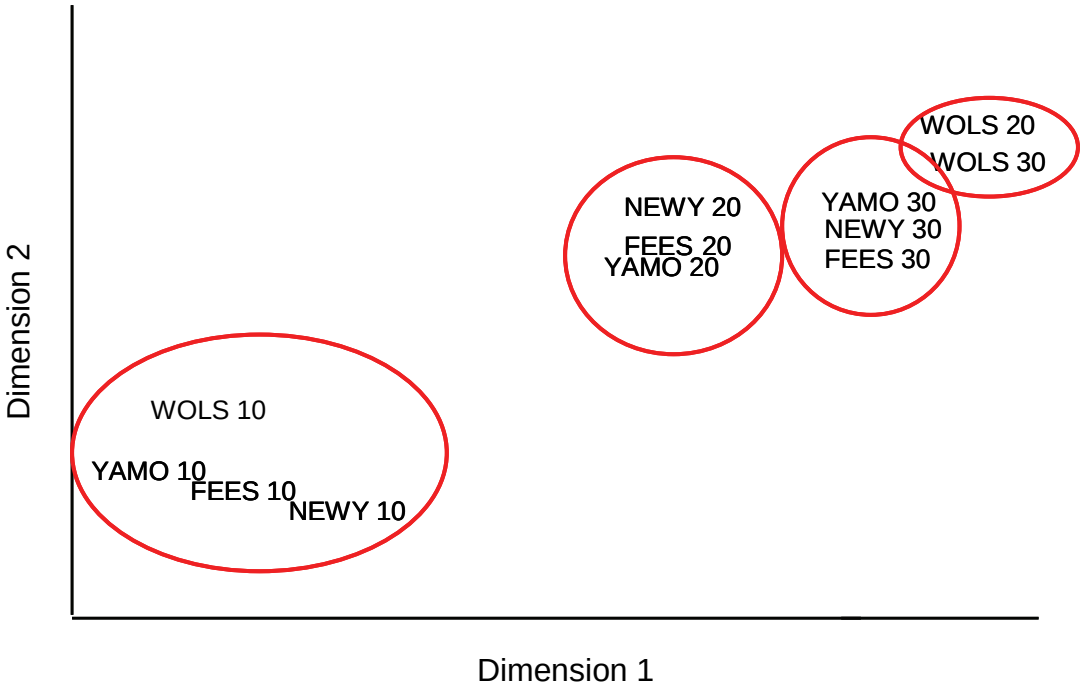


Figure 7.8: Non-metric multi-dimensional scaling (NMDS) clustering of four sites (Wolseley = WOLS, Yamorna Weir = YAMO, Feesgronde = FEES and New Years Dam = NEWY) at different resolutions (10 m, 20 m and 30 m resolutions) according to patterns of water hyacinth in the landscape (shape, perimeter:area, patch density and landscape shape index).

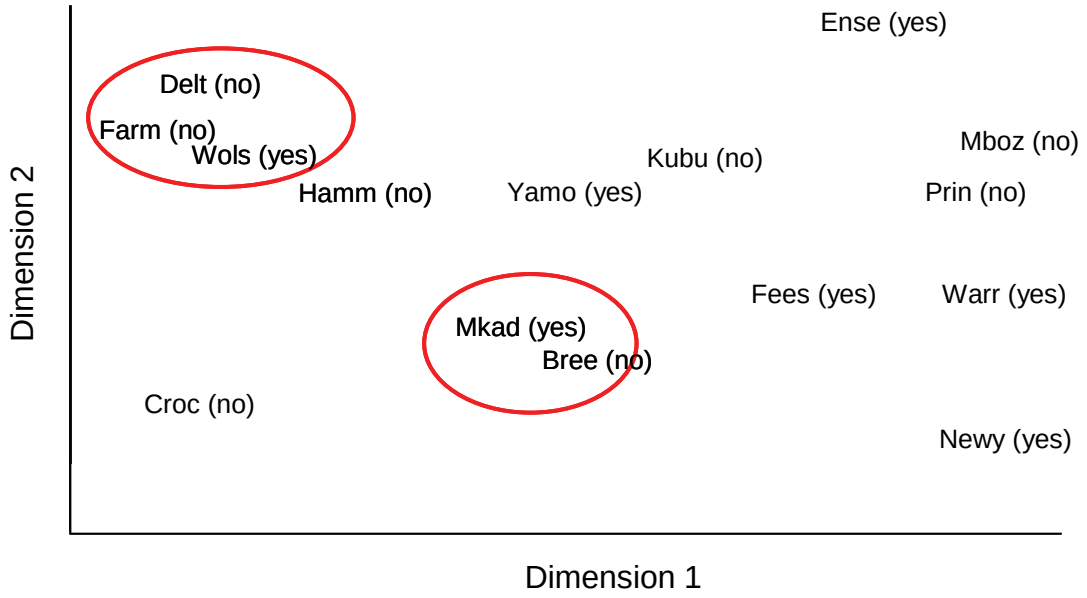


Figure 7.9: Non-metric multi-dimensional scaling (NMDS) clustering of 15 water bodies where water hyacinth is monitored monthly, according to their shape and size (perimeter:area, width, length/width and percentage cover of water hyacinth). Yes or no indicates whether the water hyacinth could be detected in the image or not.

Although the 15 monthly water hyacinth monitoring sites do not cluster well according to whether or not water hyacinth can be classified at the site (Figure 7.9), attributes of the site such as surrounding land cover can be assessed visually from the satellite

images and may explain why water hyacinth can be classified at some sites and not at others. For instance, Farm Dam (Figure 7.10g) and Delta Park (Figure 7.10f) have a high percentage cover of water hyacinth, but are very small (0.3 ha and 0.65 ha respectively). Wolseley (Figure 7.10a) and Delta Park are comparable sizes (0.68 ha and 0.65 ha respectively), but water hyacinth could not be classified at Delta Park because the boundary of the site could not be distinguished. The site at Wolseley is more visible because of the surrounding land cover (Figures 7.10a and 7.10f respectively). Yamorna Weir, New Years Dam (Figures 7.10b and 7.10h respectively) and Warrenton Weir (Figure 7.11g) all have extensive water hyacinth infestations, and the water hyacinth at New Years Dam contrasts to the surrounding land cover (water hyacinth in bright green on the dam).

The other sites are all long, thin river sites (Figure 7.11). However, Mkadhzi Spruit (Figure 7.11c), which is only 84 m wide, has an extensive water hyacinth infestation, while the other sites do not, and despite being the second-narrowest site, was the only one at which water hyacinth could be classified. The Crocodile River site (Figure 7.11e) is just over one pixel wide, and water hyacinth could not be identified or classified at this site. The sites at Enseleni River (Figure 7.11f), Mbozambo Swamp (Figure 7.10d) and Princess Vlei (Figure 7.10c) are large (3.77 ha, 7.8 ha and 26.6 ha respectively), but have low levels of water hyacinth.

Visual estimates of percentage cover of water hyacinth and remotely sensed percentage cover of water hyacinth do not differ significantly (T- test: $T_{(6, 5)} = 1.2056$; $p = 0.28$) at the 5% significance level. At small sites (< 20 ha) for all levels of cover, and at large sites (> 120 ha) for low levels of cover (> 10 %), the visual estimate of percent cover is greater than the remotely sensed cover (Figure 7.12). At large sites (> 120 ha) with moderate cover (approximately 30 %), the remotely sensed calculation was greater than the visual estimate of cover (Figure 7.12).

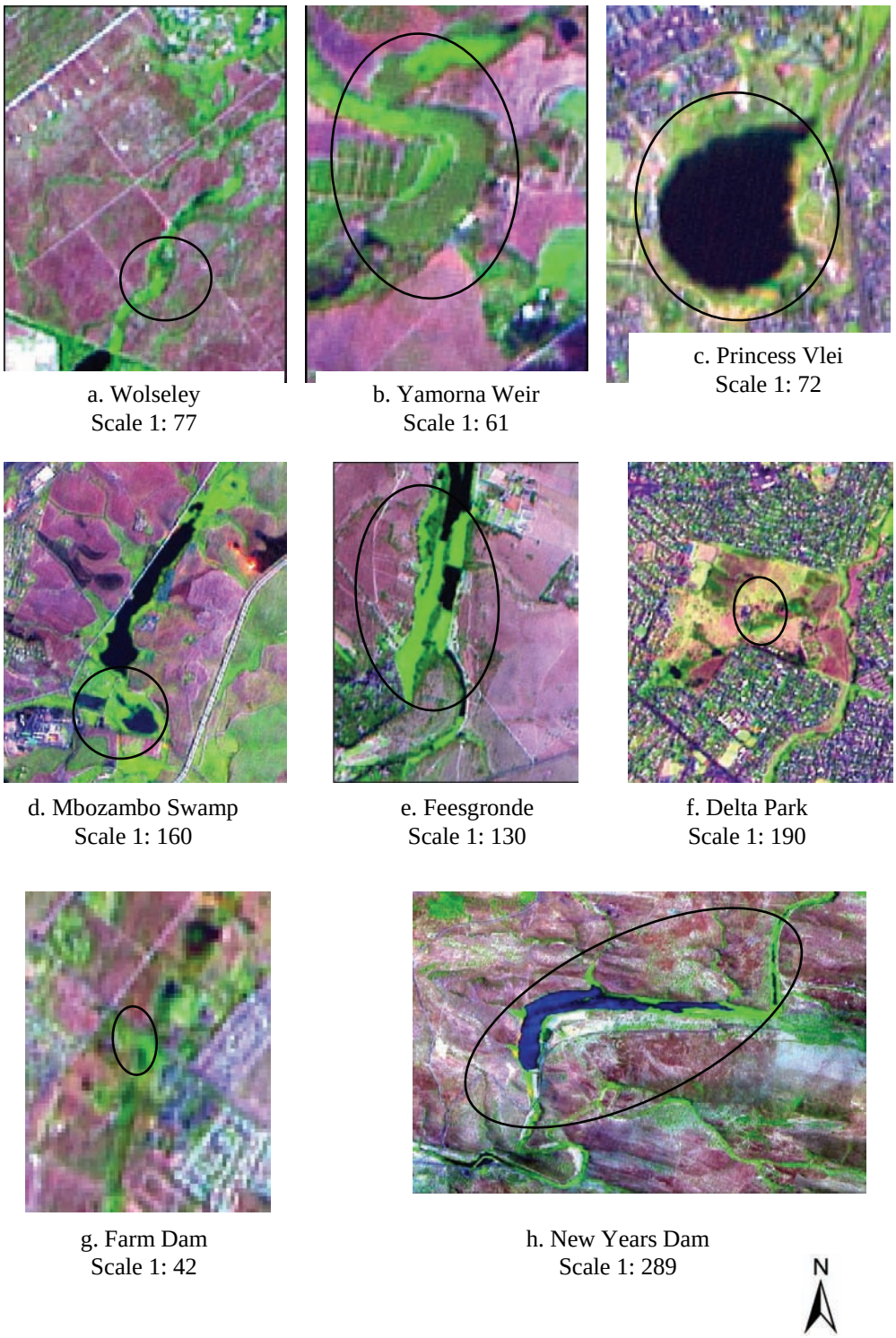


Figure 7.10: SPOT IV (10 m resolution) satellite images of eight water hyacinth monitoring sites in South Africa. The sampling sites are circled in black (Band combination: R, G, B = 4, 3, 2).

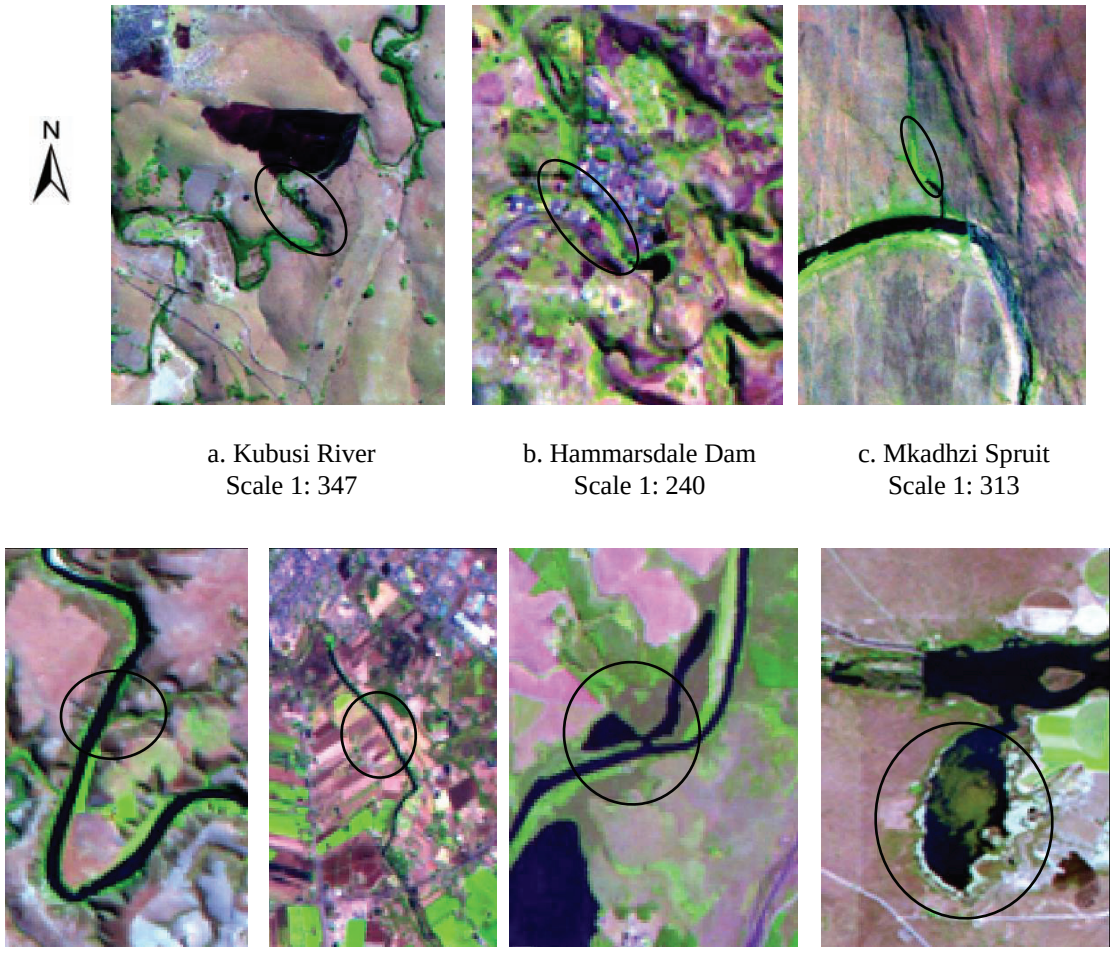


Figure 7.11: Landsat 5 TM (30 m resolution) satellite images of seven water hyacinth monitoring sites in South Africa. Sampling sites are circled in black (Band combination: R, G, B = 5, 4, 3)

7.3.2 Potential of Satellite Imagery for Monitoring Water Hyacinth

The health of water hyacinth plants measured on satellite images using the Normalized Difference Vegetation Index (NDVI) shows a positive correlation with minimum water hyacinth canopy temperature (Table 7.3). A negative correlation was found between NDVI and weevil feeding scar density, and NDVI and ammonium. Therefore, these variables could be used to indicate the health of the water hyacinth plants (Table 7.3).

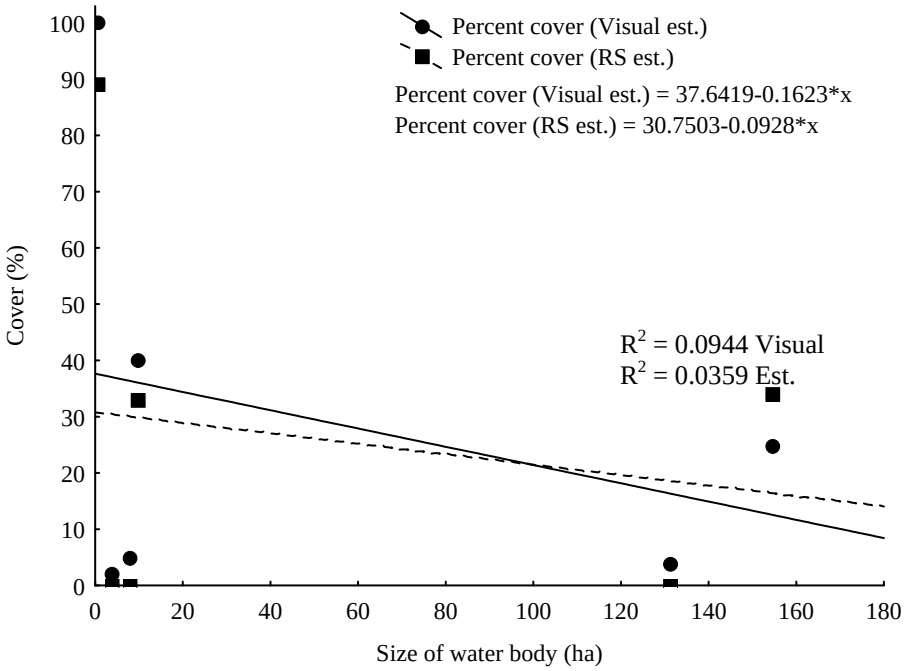


Figure 7.12: Comparison of percentage cover of water hyacinth obtained from remotely sensed images, with percentage cover of water hyacinth obtained from visual estimates of the size of six water bodies (n = 12).

Table 7.3: Correlations of biotic and abiotic variables related to the health of water hyacinth plants measured on satellite images using the Normalized Difference Vegetation Index (NDVI) (numbers are Pearson's R, significant relationships are indicated in **bold**) (n = 4).

Biotic and abiotic variables	**Average NDVI**
% petioles mined	0.1148
Biomass	0.2650
Scars/area	**-0.8670**
NH_4	**-0.8858**
NO_2 & NO_3	0.0646
PO_4	0.5779
Maximum canopy temperature	0.2308
Minimum canopy temperature	**0.8754**

The mean biomass of water hyacinth (kg/m^2) decreased in winter months at sites that experience frost (Figure 7.13), with significant differences between monthly biomass measures (ANOVA: $F_{(11,492)}$=3.0367; p= 0.0061). Mean biomass starts decreasing in May and only starts increasing again in October. A Tukey HSD post-hoc test showed significant differences in biomass between September, January, February, April and May.

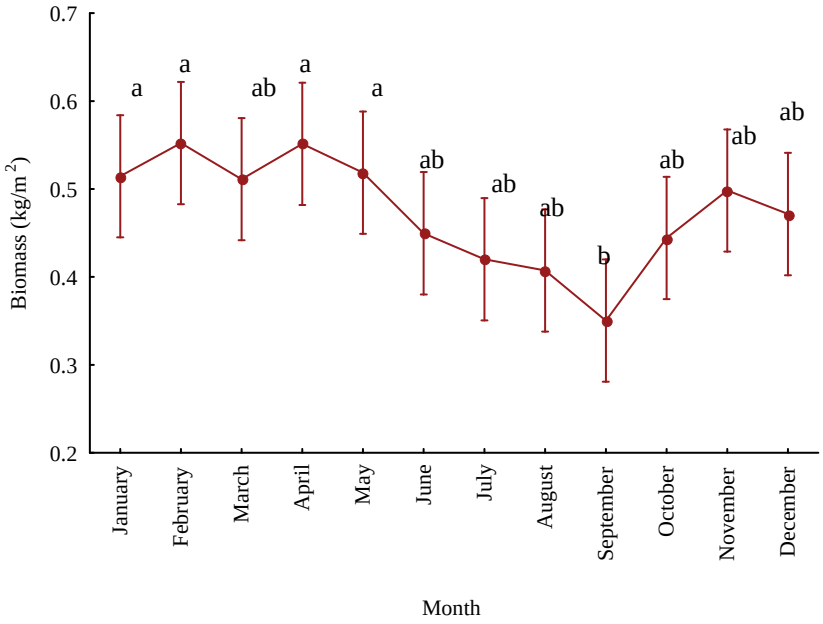


Figure 7.13: Mean monthly biomass measurements from 2005 for sites that experience frost in winter. Error bars denote 95% confidence interval. Means followed by the same letter are not significantly different (P>0.05).

At sites which are not frosted, the mean biomass of water hyacinth plants is also significantly different between months (ANOVA: $F_{(11,492)}$=8.959; p<0.0000) (Figure 7.14). Mean biomass decreases in May and starts increasing again in July, with a slight decrease again in October and November. The January biomass of water hyacinth is significantly greater than that in the months May through to December, and the biomass in February and April is significantly greater that of May, June, October and November. The biomass in March is significantly greater than the biomass in May, June and November.

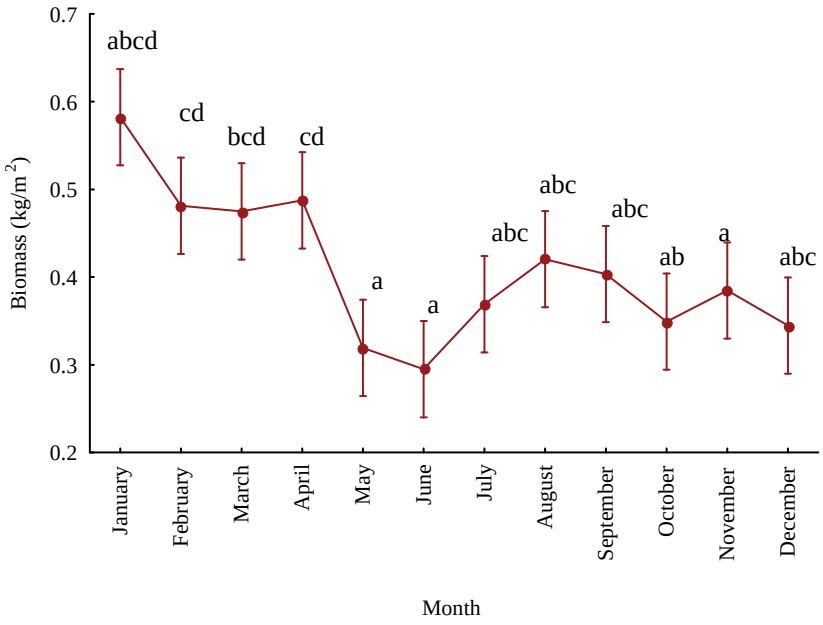


Figure 7.14: Mean monthly biomass for 2005 for sites that do not experience frost in winter. Error bars denote 95% confidence interval. Means followed by the same letter are not significantly different (P>0.05)

7.3.3 Feasibility

IKONOS imagery offers the lowest price/pixel of the high resolution images. The cost of a 10 m resolution SPOT IV pixel is less than the cost of a 20 m resolution SPOT IV pixel (Figure7.13). Although an IKONOS image is cheaper per pixel than a SPOT IV image, more IKONOS pixels are needed to cover the same extent (3 IKONOS pixels:1 SPOT IV 10 m resolution pixel). SPOT IV images also cover a larger area (60 km^2) than IKONOS (10 km^2), and are also cheaper per image (Table 7.4). Existing SPOT 1, 2 and IV (20 m resolution), and existing and current Landsat 5 TM and Landsat 7 ETM+ images are free (Table 7.4). In addition to the cost of the images, a standard charge of R1 500 is added to each image for pre-processing by the Satellite Applications Centre. Current SPOT IV (20 m resolution) satellite images (Table 7.4) work out to be cheaper than the cost of the monthly water hyacinth monitoring field trips that require a day travelling to and from the site (Table 7.5).

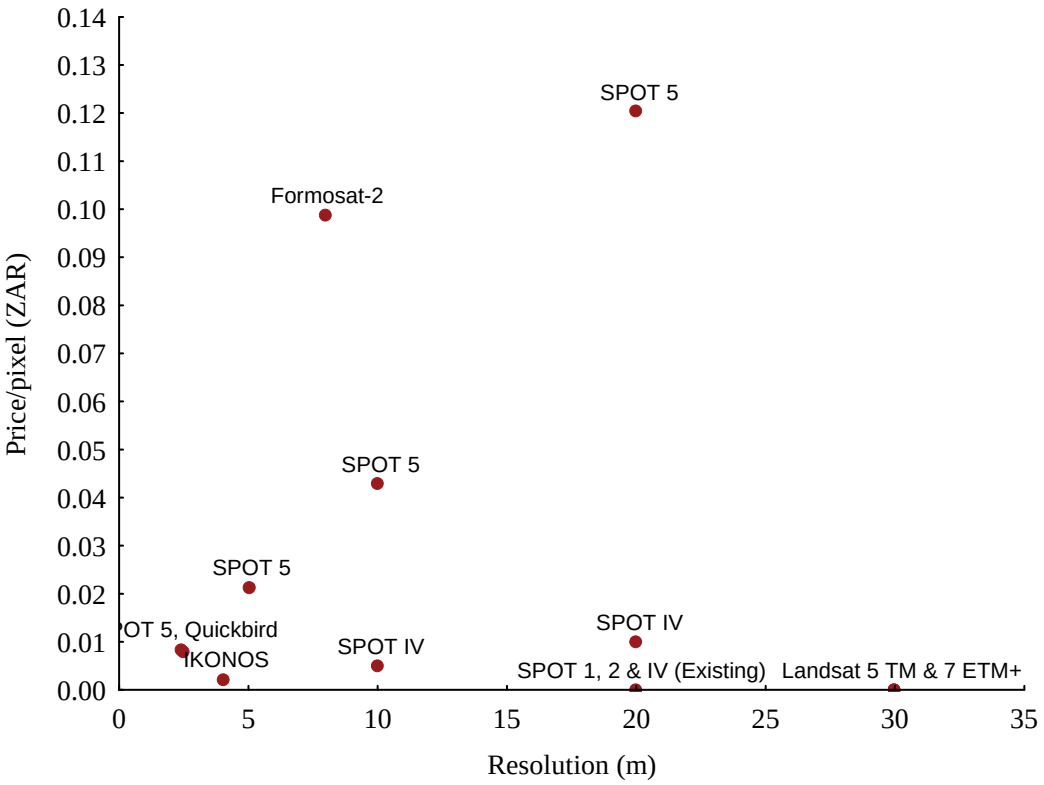


Figure 7.15: The price/pixel with increasing spatial resolution for eight different satellite images (n = 14).

Once an image is received (approximately five days after the image has been taken) the time taken to work with each image and extract the water hyacinth information is approximately one and a half days. The time taken for a field trip to KwaZulu-Natal is three days, a Kruger National Park field trip takes 1.5 days and a Gauteng field trip takes about one day. Therefore the total time taken to sample seven sites (Table 7.5) is six days and the total time taken to monitor the same seven sites remotely is 10.5 days. Although sampling in the field takes fewer days, the travel that it involves is inconvenient, and extra time (that is not included in the six days) is needed, both before and after the field trips, to pack and organize. If this time taken to organize the trips is taken into account, the total time taken to sample seven sites is nine days (Table 7.5).

Table 7.4: Spatial extent and price per image of seven satellite image types.

Satellite image source	Price (ZAR)	Spatial extent (km^2)
Landsat 5 TM (30 m resolution)	0	180
Landsat 7 ETM+ (30 m resolution)	0	180
SPOT 1, 2 and IV (Existing images)	0	60
SPOT IV (20 m resolution)	1 500	60
SPOT IV (10 m resolution)	3 000	60
IKONOS (4 m resolution)	13 610.7	10
SPOT 5 (20 m resolution)	18 046.77	60
SPOT 5 (10 m resolution)	25 645.41	60
Quickbird (2.4 m resolution)	37 021.104	25
Formosat-2 (7.5 m resolution)	37 043.37	24
SPOT 5 (5 m resolution)	51 290.82	60
SPOT 5 (2.5 m resolution)	76 936.23	60

Table 7.5: The approximate cost of three monthly field trips including travel expenses, labour on trip, labour to process plant nutrient samples and cost to process water nutrient samples

Field Trip	Sites sampled	Time spent travelling	Approximate cost/trip
KwaZulu-Natal	Hammarsdale Dam Mbozambo Swamp Enseleni River	3 days	R 6 500.00
Kruger National Park	Yamorna Weir Mkadhzi Spruit	1.5 days	R 4 800.00
Gauteng	Farm Dam Delta Park	1.5 hours	R 1 000.00

7.4 Discussion

Medium resolution satellite remote sensing of water hyacinth over small extents is possible, but is dependent on the resolution of the images available, as well as the properties of the site (size, shape and surroundings). The health of the water hyacinth plants measured on the image can be related to measurements of water hyacinth parameters taken in the field. Monitoring water hyacinth via satellite images biannually is more cost effective than field sampling.

7.4.1 Assessing Accuracy of Water Hyacinth Classification

Although the sample size is small (four sites), the area of water hyacinth measured at three sites (Wolseley, Yamorna Weir and New Years Dam) is seen to increase as resolution decreases (Figure 7.6a, Figure 7.6b and Figure7.5d). This makes intuitive sense because as the grain increases, detail in the image is lost and the image becomes more generalized (Figure 7.15).

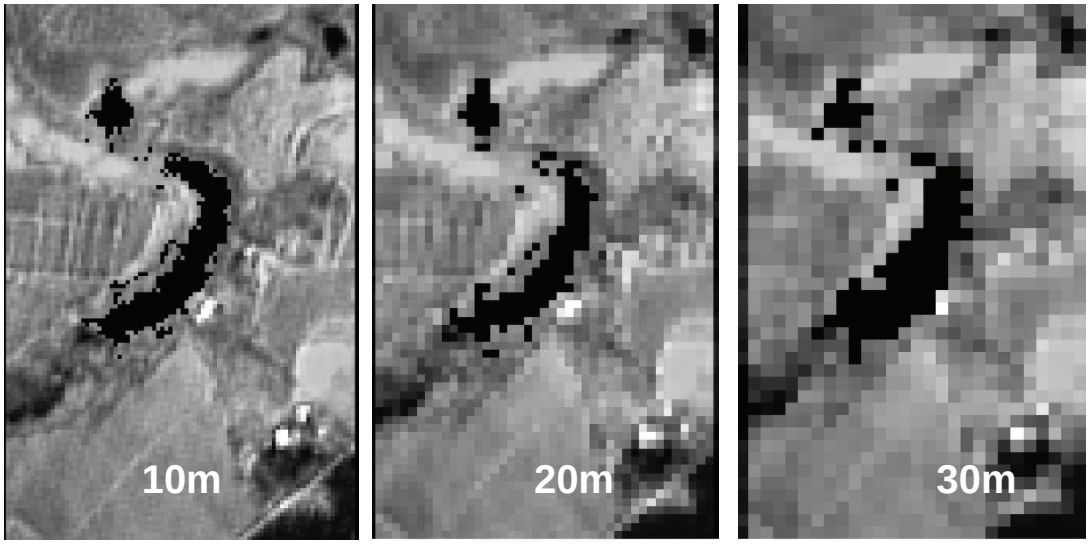


Figure 7.16: SPOT IV satellite images of Yamorna Weir at 10 m, 20 m and 30 m resolution. The black areas are classified water hyacinth. (RGB = 4, 3, 2) (site = 10 ha, 1: 61)

The exception is Feesgronde (Figure 7.6c) where the area of water hyacinth measured increases from 10 m to 20 m resolutions, but then shows a decrease at 30 m resolution. The decrease in area measured is also a result of the resolution decreasing. The contradictory response of the area of water hyacinth measured relative to changes in resolution can be explained in the following way: if a landscape has two cover types, a and b (water and water hyacinth), then the proportion of a and b in the landscape is *p* and 1 – *p* respectively (Turner et al., 2001). When the resolution of the image is decreased (aggregated), if the new cell contains cover types a and b in equal proportions (50% each) then the 50% rule will be used and the value of the new aggregated cell will be assigned randomly to either cover type; otherwise, if one cover type is in the majority then that cover type will become the cover type of the new cell such that the new proportion of a in the landscape is:

$$p= p^4 + 3(p^3 (1-p)) + 3(p^2 (1-p) + p(1-p)^3)$$

The rule described above is known as the averaging rule, and is generally taken to be accurate. Since nine pixels are aggregated to one pixel when decreasing the resolution of an image from 10 m to 30 m, the 50% rule will not be used as there is an odd number of pixels in the aggregation (3 x 3), resulting in only the majority rule being used with the new proportion of a in the landscape being:

$$p = p^4 + 4p^3(1-p) + 6p^2(1-p)^2$$

Ideally, if both land cover types occur in similar proportions (as is the case at Feesgronde (Figure 7.10e)), then cover type a and b should be assigned randomly to

both land cover types using the averaging rule when aggregating from 10 m to 20 m resolution. However, when aggregating from 10 m to 30 m, the land cover type that is only slightly more dominant will become the new dominant land cover in the larger pixel as a result of using the majority rule (Turner et al., 2001). Due to these errors made when decreasing the resolution, it is possible for the area of water hyacinth measured to either increase or decrease at lower resolutions. It must be stressed that these errors do not only occur when aggregating an image, but also occur when an image is taken by the satellite at lower resolutions. As a result, higher resolution images will be more accurate than lower resolution images.

The difference in the area of water hyacinth measured at decreasing resolutions is greater between 10 m and 30 m resolutions and between 20 m and 30 m resolution than between 10 m and 20 m resolutions, although this result is not significant (Figure 7.7). The mean and standard deviation of the difference between area measured at 10 m and 20 m resolution is the smallest of the three (Figure 7.7). A larger error lies in the difference in area measured between 20 m and 30 m resolutions resulting in area measurements at 10 m and 20 m resolution being more accurate. The exception is at Wolseley where the area measured at 20 m and 30 m resolution was similar (Figure 7.6a). Wolseley is the smallest site and the rule used in the aggregation process explains how the water hyacinth, the dominant land cover type at Wolseley, will become increasingly dominant as resolution increases. Therefore, for monitoring smaller sites ($\leq$ 10 ha, the size of sites smaller than Yamorna Weir at which 20 m resolution becomes more accurate than 30 m resolution for measuring area and thus an improvement in classification accuracy), the images used must have a resolution of 10 m or better. Although the area of water hyacinth measured at 10 m resolution is seen to be the most accurate for all sites, this is a relative scale and accuracy of measuring the area covered by water hyacinth will increase with an increase in resolution. Since the least error occurs in area measured between 10 m and 20 m resolution, 20 m resolution is also adequate to determine change in the area covered by water hyacinth.

A major source of error in classification accuracy occurs at the boundaries of land cover types, and is usually due to mixed pixels which cover more than one vegetation type (Foody, 2002). The errors in measuring the area covered by water hyacinth at decreasing resolutions is affected by shape index, PARA, number of patches and landscape shape index (LSI) (Figure 7.8). With a decrease in resolution, shape becomes simpler, and PARA and number of patches decrease, as does LSI. The aggregation therefore increases as resolution decreases (Figure 7.15). Wolseley sites WOLS_20 and WOLS_30 do not cluster with the other sites. This could be because of the sites' small size, and the fact that the areas measured at 20 m and 30 m resolutions were both greater than the size of the site (Figure 7.6a).

The size and shape of the sites did not influence whether or not the water hyacinth infestation could be detected and therefore classified according to the NMDS (Figure 7.12). However, to be directly comparable, the sites should all be at the same resolution, because water hyacinth on river sites at 30 m resolution could have been detected/classified if the image were at 10 m resolution. Although percentage cover of water hyacinth at a site (obtained from the monthly monitoring data) was not a defining variable in the NMDS, when assessing site properties visually, the level of water hyacinth infestation did appear to be more important than the size and shape of the site (Figures 7.10, 7.11). All sites where the water hyacinth covered a large area were classifiable (Wolseley, Yamorna Weir, Feesgronde and New Years Dam (Figures 7.10a, b, e and h respectively), Mkadhzi Spruit and Warrenton Weir (Figures 7.11c and g respectively). Mkadhzi Spruit (Figure 7.11c) is narrower than Kubusi River, Hammarsdale Dam and Breede River (Figures 7.11a, b and d respectively); however, because it has a large area covered by water hyacinth, it was possible to classify the water hyacinth. The size and shape of the site is important; for example, the site on Crocodile River (Figure 7.11e) is just over one pixel wide at 30 m resolution (43 m), and riparian vegetation will most probably obscure our view of the water hyacinth in the image. Three pixels is likely to be the minimum width of a site for water hyacinth to be successfully classified at the site; however, this does depend on the occurrence and abundance of riparian vegetation.

Maheu-Giroux and de Blois (2005) had difficulty classifying populations of *Phragmites australis* distributed in linear wetland corridors, where the weed was growing less vigorously than other populations of *P. australis*. Although the sites at Delta Park and Wolseley are similar in size (0.65 ha and 0.68 ha respectively), and both have a substantial water hyacinth infestation, the inability to classify water hyacinth at Delta Park might be caused by the water hyacinth growing less vigorously than at Wolseley, as determined by NDVI values (0.16 and 0.54 respectively). The NDVI values coincided with on-site measurements of plants at Delta Park, which are usually short and small with inflated petioles, while at Wolseley the plants are medium to tall with attenuated petioles. On average, the weed's biomass is also slightly higher at Wolseley than at Delta Park. Contrast between the vegetation type to be classified and the surrounding vegetation will also determine the ability to classify the weed (Maheu-Giroux and de Blois 2005). The water hyacinth at Farm Dam does not contrast strongly with the surrounding vegetation, which limited the ability to define the site on the image and thereby classify the water hyacinth (Figure 7.10g).

The percentage cover of water hyacinth estimated visually on the ground and the percentage cover measured on the satellite images did not differ significantly (P=0.92); however, on small water bodies for all levels of cover, and on large water bodies with low levels of water hyacinth cover, the remotely sensed cover is greater than the visual estimate of cover. The mat of water hyacinth at Warrenton Weir (Figure 7.11g) is in the

centre of an open water body and has a lower perimeter:area ratio than occurs at the smaller sites, while the lower percentage coverage results in fewer mixed pixels around the edge of the mat. Therefore, more of the water hyacinth is classified correctly, leading to a greater remotely sensed estimate cover than the on-ground assessment. Small sites with low percentage cover and high perimeter:area ratios will have more mixed pixels around the edge of the mat resulting in the remotely sensed cover being smaller than the visual estimate. Therefore, at small sites (< 20 ha) and at low percentage cover (< 10% at all sites), the visual estimate will be more accurate than the remotely sensed cover.

Visual estimates of water hyacinth cover over large extents are generally less accurate than remote sensing estimates. The area covered by water hyacinth on Lake Victoria was over-estimated by up to 70 000 ha in visual estimates, and this was at a low resolution of 30 m-100 m, which would only detect larger mats of water hyacinth, thereby already under-estimating the area covered (Albright et al. 2004). Visual estimates of area covered by water hyacinth are subjective and the people estimating cover are often sensitive to the fact that water hyacinth negatively affects their livelihood; and therefore the consequences of its presence are large. In such circumstances, visual estimates of area covered by water hyacinth are more likely to be over-estimates, and remotely sensed measurements will be more objective and therefore inherently more accurate.

7.4.2 Potential for Monitoring Water Hyacinth with Satellite Imagery

Although knowing the area covered by water hyacinth can useful for initiating a management intervention, it is more useful if the information derived from the images can also be used to establish the health of the plants and the abiotic variables affecting the plants. NDVI (measurement of plant stress) is positively correlated to the mean minimum canopy temperature (R = 0.89, Table 7.3) which means that sites with higher minimum temperatures have generally healthier plants (as minimum temperature increases, NDVI increases). NDVI is also negatively correlated to the number of weevil scars per unit leaf area and ammonium concentrations in the water. The Normalized Difference Vegetation Index has been used by Soriano and Paruelo (1992) to determine biozones, which are vegetational or environmental units defined by their functional characteristics. Soriano and Paruelo (1992) found that the NDVI of terrestrial plants in a region in Patagonia increased going into summer and decreased going into winter. Accordingly, NDVI increases with an increase in the mean minimum canopy temperature (Table 7.3) (as would occur in spring).

Feeding scar density correlating with NDVI may not necessarily indicate that the health of the plants is adversely affected. Feeding damage reduces chlorophyll in the leaves which will cause a decrease in the NDVI, but may not necessarily mean the overall vigour of the plant is being negatively affected (Ripley et al., 2008) Although mining of

petioles by weevil larvae damages the plants, this variable is not strongly correlated to NDVI (R = 0.11) (Table 7.3). This could be because there is no change in the amount of water and chlorophyll in the plant tissues, which would mean that the NDVI did not change either.

The effect of frost on water hyacinth leaves will be particularly apparent in NDVI values as the leaves dry out and turn brown. Being able to predict the canopy temperature at a site is useful in terms of the management model because temperature is a factor used to determine the correct management strategy of either biocontrol, chemical control, or both.

Since changes in the biomass of water hyacinth occur in winter and summer, monitoring of water hyacinth infestations should be done twice a year, in autumn and in spring, to monitor the growth of the plants when the populations are increasing in number due to asexual reproduction (autumn), and when they are increasing in biomass (spring). Although water hyacinth populations can double in biomass every six to 18 days (Everitt et al., 1999), this usually only occurs on open water bodies during the initial colonizing phase, and it is not feasible to monitor a water body on this time scale. Sites that experience frost could be monitored in May and September (Figure 7.13), and sites that do not experience frost could be monitored in May and July or August (Figure 7.14).

7.4.3 Feasibility

For satellite remote sensing to be a feasible method for water hyacinth monitoring, it must be cost-effective in terms of both time and money. Existing and current Landsat 5 TM and Landsat 7 ETM+ images are free for research purposes (Figure 7.15, Table 7.4) and therefore obviously cost-effective. However, they will be constrained when monitoring relatively small water bodies because the low (30 m) resolution induces greater errors in estimating coverage. Although IKONOS imagery is the cheapest per pixel of the high-resolution imagery, and is cheaper per pixel than SPOT IV imagery (Figure 7.15), current SPOT IV 20 m resolution imagery is more cost-effective for an entire image (R1 500) (Table 7.4) and is cheaper than field trips that require a day or more of travelling (Table 7.5). For sites smaller than 1 ha (such as Wolseley, Delta Park and Farm Dam, Figures 7.10a, f and g respectively), IKONOS 4 m resolution imagery could be used. IKONOS imagery is only feasible if the sites are remote and inaccessible and the cost and time taken to monitor the sites is greater than the cost of the image (R13 610.70) (Table 7.5).

Feasibility will depend on what an image will be used for. If the current extent of a water hyacinth infestation needs to be determined then existing Landsat 5 TM, Landsat 7 ETM+ and SPOT 1, 2 and IV imagery is adequate and comes at no cost if used for research purposes. Existing imagery includes all images taken before the current date,

while new images will need to be commissioned for specific appropriate dates in the future. SPOT IV images would be most appropriate for water bodies greater than approximately 5 ha and IKONOS images can be used for smaller water bodies. However, these images may be more costly than visiting the site. To precisely establish the change in the extent of an infestation at high temporal and spatial resolution will require regular high-resolution images, for which IKONOS imagery will be useful. Other high-resolution imagery, such as SPOT 5 and Quickbird (2.5 m resolution), is available; these are relatively cheap per pixel (Figure7.14). However, the cost of an entire image is high (R 76 936.23 and R 37 043.37 respectively (Table 7.4)).

The cumulative time taken to process an image may be greater than the number of days taken to physically sample a site. Conversely, for management, water hyacinth populations probably do not need to be monitored more than biannually. Presently each site will occur independently on a separate image. However, if remote sensing is used as a national monitoring tool then the price of monitoring per site will decrease because more than one site may occur on an image, since the spatial extent of some images is large (Landsat 5 TM and Landsat 7 ETM+ = 180 km^2, SPOT IV = 60 km^2 (Table 7.4)). IKONOS imagery has been used in conjunction with Landsat to map non-native invasive species on freshwater systems (Turner et al., 2003) and is recommended here to be used in conjunction with SPOT IV imagery if necessary.

7.4.4 Advantages of Monitoring Invasive Species Using Remote Sensing

An additional advantage of using satellite remote sensing to monitor a weed such as water hyacinth is that the past dynamics of water hyacinth at a site can be measured. For instance, at New Years Dam, where biocontrol has been successful, existing SPOT IV and Landsat 5 TM satellite images from 1982 and 1986 are available from the Satellite Applications Centre. Long-term data sets from satellite images may help track change in weed populations in the face of global climate change.

Invasive plants will be favoured by several aspects of global climate change (Dukes and Moony, 1999). Water hyacinth is limited in its distribution by low temperature (Owens and Madsen, 1995), but since global minimum temperatures are increasing at twice the rate of global maximum temperatures (Alward et al., 1999), minimum temperature may cease to be a limiting factor. Global climate change scenarios have also predicted an increase in the metabolic rate of plants with an increase in atmospheric CO_2, which may increase uptake rates of herbicides (Edis et al., 1996), or conversely plants might develop thicker cuticles and/or larger canopies which will decrease herbicide efficacy (Coakley et al., 1999).

Databases with information about the distribution and biology of invasive species are a step in effective management of non-indigenous invasive species (Hulme, 2003). Ricciardi et al. (2000) advocate the construction of a Global Information System for

invasive species to facilitate the flow of information, especially in such a dynamic field as invasion biology. Remote sensing of water hyacinth, therefore, would become more feasible if it was done in addition to the mapping of other invasive species. What is more, NDVI could be used to monitor the effects of alien invasive plants on national net primary production.

7.4.5 Hyperspectral Remote Sensing of Water Hyacinth

An alternative to the multispectral RS used in this study is hyperspectral RS, which has a spectral resolution of approximately 10nm compared to the 100nm resolution used in multispectral RS (Lillesand et al., 2004). Hyperspectral devices record reflectance spectra between 400nm and 2500nm, and the instruments can either be hand-held, or fitted onto aircraft or satellites (Goetz et al., 1985). The spectral reflectance of objects such as vegetation or minerals can then be entered into a spectral reflectance library for future reference and identification (Goetz et al., 1985). The uses of hyperspectral RS range from detecting and distinguishing between types of minerals and different plant species, conducting species-specific land cover classification (Turner et al., 2003), and detecting plant stress and the relevant stress factors such as insect damage (Goetz et al., 1985). Mapping and monitoring of invasive plants using hyperspectral imagery is becoming a widespread practice (Glenn et al., 2005, Lawrence et al., 2006, Miao et al., 2006), and insect damage to crops can be quantified using spectral vegetation indices (SVI's) (Mirik et al., 2006).

Canopy and leaf structure have been found to be more useful than plant water content in discriminating between species (Schmidt and Skidmore, 2003). Leaf pigments such as chlorophyll, carotenoids and anthocyanins are positioned in the leaf such that light absorption in particular wavebands can be easily assessed using spectral reflectance, and physiological properties of the plant such as nutrient status can then be determined (Gamon and Surfus, 1999). The Normalized Difference Vegetation Index (NDVI) is an SVI calculated from multispectral imagery; however, this index is not sensitive enough to differentiate vegetation species (Figure 7.5). NDVI is calculated using the red and NIR bands; however, the red band (600nm to 700nm), which is related to chlorophyll, is too broad to pick up differences between species. Indices calculated from two narrow bands in the red part of the electromagnetic spectrum are more sensitive to plant pigments than those calculated using the entire red and NIR bands, and thus allow for the discrimination of species (Dobrowski et al., 2005). Two bands (690nm and 740nm) characterize the emission spectra of chlorophyll, and the ratio between the two bands is inversely related to the chlorophyll content of the plant, thus making species discernible (Dobrowski et al., 2005).

Thenkabail et al. (2004) compared the usefulness of hyperspectral and multispectral sensors for modelling biomass and classifying forest land use and land change (LULC) classes. Models using data from a hyperspectral sensor (Hyperion, 30 m resolution)

explained 36-83 % more variation in the data than the models created using the data obtained from the multispectral sensor (IKONOS, 4 m resolution); and the accuracy in classifying LULC classes was 45 -52 % greater using the hyperspectral images, even though the Hyperion sensor records information at a lower spatial resolution (Thenkabail et al., 2004).

The superior ability of hyperspectral sensors to discriminate and detect physiological change in species (Dobrowski et al., 2005), should warrant their use for monitoring water hyacinth to detect the change in extent and physiological status of the plants. Higher spatial and spectral resolution sensors will allow determination of the nature of change in infestations' extent. The use of hyperspectral RS will allow for the detection of where in the mat plants are growing more vigorously, where insect damage is the greatest, and whether or not the shape of the mat affects these trends. Jakubauskas et al. (2002) used an Analytical Spectral Devices (ASD) spectroradiometer to take spectral measurements of water hyacinth, recording 512 discrete spectral bands ranging from 330nm to 1055nm. They were then able to determine the relationship between percentage cover, biomass and spectral reflectance of the weed. Wilson et al. (2001) argued that percentage cover and biomass of water hyacinth are the two most important variables to measure in a monitoring programme because the growth of water hyacinth populations can be modelled using these variables. If a water body has phosphorus levels at concentrations less than the 0.1 mg/*l* threshold proposed by Haller and Sutton (1973), then biocontrol is a viable option as the water hyacinth will no longer be actively growing. Phosphorus levels below the 0.1 mg/*l* threshold limit the growth and reproduction of the plants (Reddy et al., 1990).

Hyperspectral sensors on satellites are not as numerous as multispectral sensors; however, there is still a range of spatial resolutions available, ranging from 3.5 m resolution (HyMap) to 30 m resolution (Hyperion) (edc.usgs.gov). Although the 30 m resolution Hyperion sensor (hyperspectral) is superior to 4 m resolution IKONOS sensors (multispectral) (Thenkabail et al., 2004), the higher spatial resolution sensor is recommended for future monitoring of water hyacinth as increased spatial and spectral resolution will be advantageous in detecting population change.

In terms of cost, low-resolution (30 m) hyperspectral imagery is more expensive than multispectral imagery because 30 m resolution multispectral imagery is free (Table 7.6). Hyperion images are cheaper than IKONOS multispectral images and, even though they have a lower spatial resolution, the data obtained from Hyperion images are superior to those obtained from IKONOS images (Thenkabail et al., 2004). AVIRIS imagery is the most expensive type of hyperspectral imagery, and is mostly used for military applications (Lillesand et al., 2004). HyMap imagery, which is recommended for future use in monitoring the nature of change in water hyacinth populations, is relatively

expensive (Table 7.6); however, the high spatial and spectral resolution it provides is likely to be superior.

Table 7.6: Comparison between the cost and spatial extent of three hyperspectral satellite image types.

Satellite image source (resolution)	Satellite image type	Number of bands	Spatial extent (km2)	Price (ZAR)
Hyperion (30 m)*	Hyperspectral	±210	18	9 450
AVIRIS (20 m)*	Hyperspectral	±210	10	226 800
HyMap (3.5 m)*	Hyperspectral	±210	7	37 800

* edc.usgs.gov

7.5 Conclusion

Monitoring of water hyacinth using existing satellite imagery is possible at small extents and at medium resolutions. The spatial properties of the site affect the ability to identify and classify water hyacinth, so grain and extent need to be considered when planning a remote sensing project to ensure the grain is not larger than the object of interest, since classification accuracy increases with an increase in resolution.

Satellite remote sensing at medium resolutions is only possible if details about the site are known. Latitude and longitude points of the exact boundary of the site to be monitored are essential to restrict analysis to the particular water body. Information about surrounding land cover (urban or rural), riparian vegetation and other aquatic plants occurring on the same water body is necessary and could affect the accuracy of classification of water hyacinth. Due to the increased occurrence of mixed pixels at small sites, which affects classification accuracy, it is recommended that the smallest site to be monitored accurately at medium resolutions be 5 ha.

To determine the extent of a current water hyacinth infestation, existing SPOT IV 10 m resolution imagery is recommended; however, if the change in the extent of cover is needed, then new SPOT IV images should be commissioned and a pan-merge applied to them to view the images as 10 m multispectral images. For large water bodies (< 50 ha), SPOT IV 10 m resolution imagery is adequate to assess the nature of change in the water hyacinth mat; however, for smaller water bodies, IKONOS imagery is recommended. The temporal scale of the images should be objective-specific; however, since changes in biomass occur in spring and autumn, biannual monitoring is adequate to detect change in extent of the mat.

IKONOS or SPOT IV imagery is recommended for future studies. Real-time field sampling should take place on the same date as the image is taken to accurately ground-

truth the data obtained from the image. Hyperspectral remote sensing is recommended because the effects of nutrients, temperature and insect damage on the plants can be determined using a hand-held hyperspectral device, and the results can then be applied to hyperspectral satellite images. Spectral signatures of species found in the image can be compared to those in a spectral reference library and the species can be identified with little to no prior knowledge area under study.

CHAPTER EIGHT – AN ADAPTIVE MANAGEMENT MODEL FOR BIOLOGICAL CONTROL OF WATER HYACINTH

8.1 Introduction

There are several population models used to describe water hyacinth growth and its control (Akbay et al., 1991; Guitiérrez et al., 2001; Lorber et al., 1984; Mitsch, 1976; Musil and Breen, 1985; Polprasert and Khatiwada, 1998; Wilson, 2002; Wilson et al., 2005). However, these often require extensive parameterisation and lack flexibility. In complex situations, decisions have to be made quickly and management tools often have to be adaptive. They have to incorporate new data as they become available, and this information is often observational. Therefore, there is an increasing shift towards using both known relationships and prior knowledge to form predictions, and for these predictions to be updated as comparisons with real systems are made (Wilhere, 2002).

Here, the classical biological control of water hyacinth using *Neochetina spp*. is addressed to answer three questions about water hyacinth dynamics at a given site:

- What will the water hyacinth population look like if no control measures are used?
- What type of control will classical biological control using *Neochetina spp*. give?
- Where would integrated control be recommended?

The basic relationships between abiotic conditions, water hyacinth growth, and water hyacinth control by *Neochetina spp*. are developed based on a review of the literature, some basic testable hypotheses, and data collected as part of this project. These relationships are then used to develop qualitative predictions for how water hyacinth will grow and how well classical biological control under different scenarios will work.

It is assumed here that classical biological control is an environmentally benign, cost-effective, and long-lasting method of weed control. If it promises to provide some control, then biological control should be used and integrated with other control measures. It is taken for granted that water hyacinth is not a problem if it covers <5% (or occasionally up to 10%) of the water surface of a specified bay or area. While particular areas of large lakes should not be considered in isolation from the rest of the system (Wilson et al., 2007), the centre of the lake, where there is significant wave action, may never be covered in plants. Consequently, the percentage coverage should refer to a bay or discrete area as opposed to the whole lake. For example, water hyacinth covered at most about 0.3% of the total surface area of Lake Victoria. However, certain bays were completely covered and this prevented boat travel and hampered shipping.

Based on these premises, five management scenarios are considered:

- Water hyacinth cannot persist.
- Water hyacinth will persist, but only at low levels, so no control will be required.
- Water hyacinth will be a problem and biological control using weevils will be ineffective. Plants should be controlled using herbicides, mechanical methods, or other biological control agents.
- Water hyacinth will be a problem and biological control using weevils will significantly reduce plant populations but not to a satisfactory level. Therefore, integrated pest management will be required. Herbicides or mechanical methods should occasionally be applied, but in a way that is complementary to *Neochetina spp*. as this will save money and resources.
- Water hyacinth will be a problem, but will be reduced to a low, stable level by classical biological control (<10% coverage on a small water-body or in a part or bay of a large lake).

8.2 Methods

After deciding on a suitable currency by which to measure plant populations, the abiotic factors most likely to influence water hyacinth growth and control were identified, and the basic general relationships between these were then proposed. Predictions were based on these relationships and then mapped onto appropriate management options. The predictions were also compared with observations collated from sites in South Africa and around the world.

8.2.1 Measures for Describing Water Hyacinth Populations

Percentage cover of a water body was used in preference to individual plant density or biomass density. To describe water hyacinth populations according to individual plant density can be highly misleading, because a single plant in its life-time can vary in weight by over two orders of magnitude (<10 g to >1 kg and back to <10 g). Biomass density is arguably the most relevant variable. The majority of problems caused by water hyacinth is due to the sheer weight of biomass, and biomass density has been shown to respond in a predictable manner to abiotic conditions and biological control insects (Wilson et al., 2005; Wilson et al., 2006). However, biomass is harder to define and measure (Is the material alive or dead? Must the large plants in the middle of the mat be measured, or the smaller ones at the edge?). By far the easiest variable to estimate is percentage cover. Percentage cover is also required if density estimates of biomass, number of individuals, and number of weevils are to be converted into a total population size for a site, and if reductions in light penetration into a water-body, and the concurrent reduction in water quality, are to be understood. Biomass should be considered if the effect of water hyacinth populations on the nutrient dynamics; sedimentation rates; the threat posed to river transport; or the possible damage caused by plants during flooding (e.g. to bridges) is of interest. However, to retain simplicity and ease of use, populations are described here in terms of percentage cover (with the

explicit assumption that this relates to the problems caused, with percent coverage in some instances acting as a proxy for biomass).

Two measures are used to describe the water hyacinth population at a site: maximum percentage of the water surface covered, and how variable the water surface coverage is. Each measure is split into categories, with the choice of categories reflecting in part the natural variation in water hyacinth populations, but primarily scenarios that require different management considerations.

Maximum Density (4 categories):

- **0%**: the site is unsuitable for water hyacinth to grow;
- **1–5%:** sites typified by a narrow strip of plants fringing the bank, or in sheltered bays;
- **~20%:** plants grow out several metres from the bank, or completely cover small sheltered areas, but either a channel in the river is always clear, or the centre of the lake remains clear;
- **100%:** the whole water body is covered in water hyacinth mats and associated vegetation.

Variability (3 categories): this is defined as the absolute difference between the maximum and minimum percentage coverage of a water-body by water hyacinth over a 2–3 year period.

- **0–5%:** the coverage is always in the same category of maximum density;
- **5–20%:** there are noticeable differences in the coverage, perhaps seasonally;
- **20–100%**, water hyacinth can occasionally cover most of a water-body, but is also occasionally brought to a low percentage coverage (e.g. by a flood).

The maximum density of water hyacinth at a site limits the possible options for how variable the population is. For sites unsuitable for plants, variability is undefined.

8.2.2 Abiotic Factors Affecting Water Hyacinth Growth

Water hyacinth growth and control is assumed to be driven by temperature, frost, nutrients, the flow or current, and the depth of the water body (Wilson et al., 2005; Wilson et al., 2006). Under certain conditions other factors determine water hyacinth population dynamics – e.g. pH and salinity – but these are generally atypical situations, or are readily identifiable (e.g. an estuarine lagoon).

Not considered directly are less frequent floods (less than one every three years), mechanical removal, and drought. The management of water hyacinth after an event of this kind, where almost the whole plant population is removed but at a very irregular interval, is discussed separately. This situation may be similar to a newly-invaded water body and both the speed of reinvasion and re-establishment of control agents must be considered.

The effects of abiotic conditions on water hyacinth growth are categorised. The aim of this categorisation is that within a defined set of conditions, all known water hyacinth populations can be accurately described by the same maximum density and variability.

Formally, each **factor** that is known to affect water hyacinth growth is defined as an abiotic feature (some biotic factors are considered). **Categories** describe the general conditions at a site that affect the management of water hyacinth. Each **category** can be distinguished based on **levels**. The **levels** should be easily measurable conditions. Therefore, at a given site the nutrients (**factor**) may be good (**category**) for water hyacinth growth, which is concluded from knowing that the nitrogen concentration is around 3–8 $mg.L^{-1}$ and phosphorus is 0.5–2 $mg.L^{-1}$ (high **level**s).

8.2.2.1 Air Temperature

Categories

i) Always good, never extreme
ii) Good, seasonally low, but minimum temperature rarely cold
iii) Seasonal, 2–8 days of cold temperatures
iv) Short winter, 2–4 months of cold temperatures
v) Long winter, >4 months of cold temperatures

Levels

a) Extremely high — >35°C
b) Good — 20–35°C
c) Low — 8–20°C
d) Cold (no plant growth) — <8°C minimum daily temperature

Reason for Inclusion

Water hyacinth growth increases with temperature (Imaoka and Teranishi, 1988; Knipling et al., 1970; Kumar et al., 1985). Consequently, the speed with which plants regrow after chemical or mechanical control is affected by temperature. All the biological control agents are expected to be affected by temperature.

Suggested Levels

The general proposed relationship between temperature and water hyacinth growth is shown below (Wilson et al., 2005) (Figure 8.1).

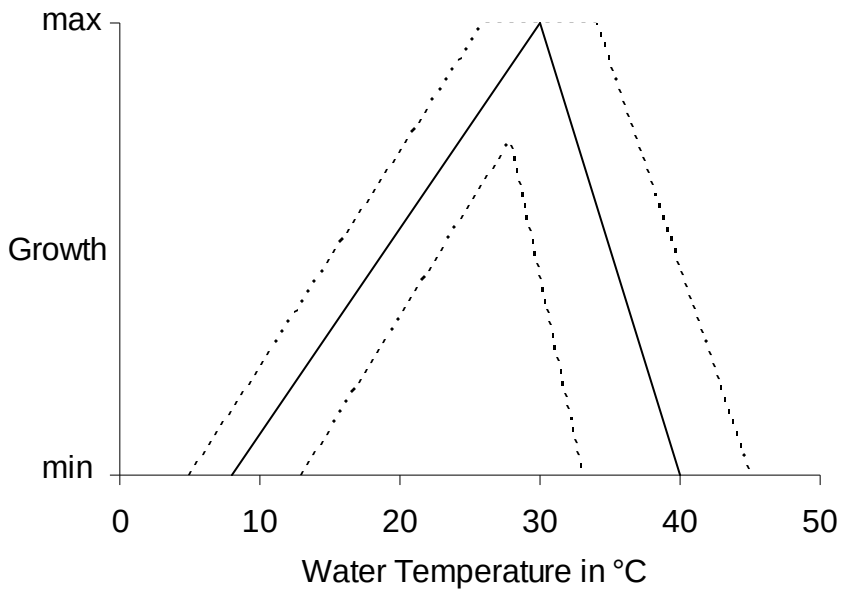


Figure 8.1: The proposed relationship between temperature and water hyacinth growth. The solid line indicates the best estimate; the dotted lines indicate the upper and lower bounds on this estimate.

Other Issues

The water temperature is not the same as the air temperature (Chapter 2). Water bodies act as a buffer.

There is no observed interaction between temperature and nutrients on water hyacinth growth; the effects are simply additive (Sastroutomo et al., 1978; Wilson, 2002).

8.2.2.2 Frost Damage

Categories

i) None: no frost damage to leaves
ii) Minor damage
iii) Most leaves are heavily damaged, but some areas (at least 5% of leaves) are not or only slightly affected
iv) All leaves heavily damaged

Levels

a) No damage — leaves stay almost completely green
b) Minor damage — leaves brown and curl, but still some green (at least 20% of the leaf or petiole is still green)
c) Heavy damage — leaves completely brown (<10% green) and die-back

Reason for Inclusion

Frost causes damage to water hyacinth leaves and is the major cause of plant mortality in temperate regions (Bock, 1966; Ueki, 1978). Frost can also affect the biological control agents either directly (by killing them) or indirectly (by removing their

habitat/food source). The effectiveness of foliar herbicides also depends on leaves being present.

Suggested Levels

The number of frost days (measured at a meteorological station) will not necessarily relate to the amount of damage to the plant. Damage depends on local microclimatic conditions; e.g. how sheltered the water body is; whether the site is windy or not; how much shade there is. Therefore, a visual inspection of the plants may provide a better indication of the effect of frost. If a small area of plants remains undamaged, this can act as a refuge for biological control agents. It may also act as a source for plant regrowth. This is therefore included in the levels.

Other Issues

Clearly, the number and severity of frosts will change from year to year. However, the success of biological control agents may depend on the frosts from several years. If one year is unusually mild, it may be important to remember the severity of the frost the previous year when assessing the population dynamics of the biological control agents.

Plants can survive as rhizomes for short sharp frosts (e.g. 24 hours at -16°C); but sustained periods of cold prove fatal (e.g. three weeks of near-freezing temperatures) (Owens and Madsen, 1995). Seeds may survive frosts that kill off all the plants (Ueki, 1978).

8.2.2.3 Water Nutrients

Categories

i) Good (both nitrogen and phosphorus always high)
ii) Flushes (low or medium, but high nutrient flushes during the growing season)
iii) Medium (always at least medium nitrogen and medium phosphorus)
iv) Low (always low nitrogen or low phosphorus)

Levels

a) Low — <0.1 $mg.l^{-1}$ N; <0.02 $mg.l^{-1}$ P
b) Medium — 0.1–1 $mg.l^{-1}$ N; 0.02–0.2 $mg.l^{-1}$ P
c) High — >1 $mg.l^{-1}$ N; >0.2 $mg.l^{-1}$ P

Reason for Inclusion

Water hyacinth growth increases with nutrient concentration (Wilson, 2002). This means that plants regrow faster once the main growing season starts; and faster after chemical or mechanical control. Nutrients also affect the rate of growth of biological control agents and, arguably, the level of control they provide (Heard and Winterton, 2000).

Suggested Levels

Based on the levels of nutrients in plant tissue, and published work on plant growth (Reddy et al., 1989, 1990), it is assumed that nitrogen is limiting if its concentration is less than seven times that of phosphorus. The general relationship between nitrogen and water hyacinth growth is shown below, (Figure 8.2).

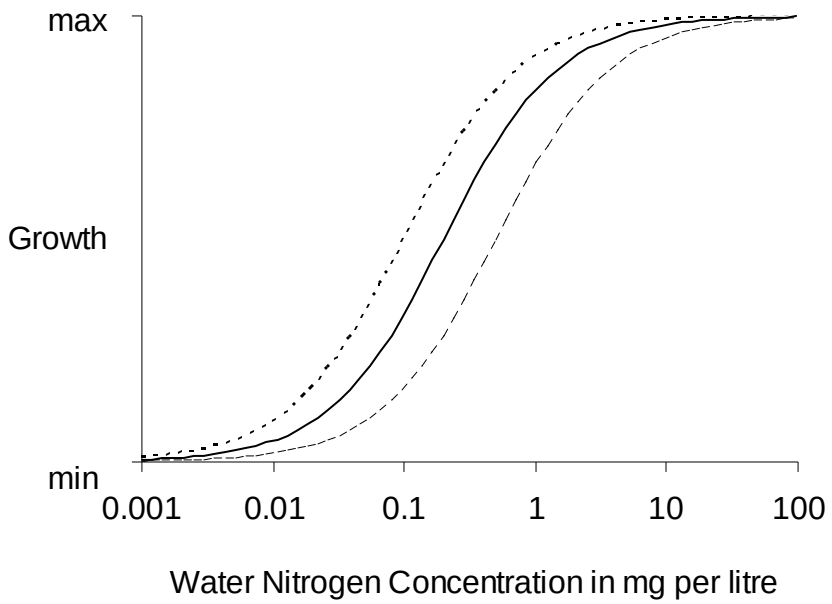


Figure 8.2: The proposed relationship between nitrogen concentration and water hyacinth growth. The solid line indicates the best estimate; the dotted lines indicate the upper and lower bounds on this estimate.

The levels for the categories reflect this graph and field levels. If the nitrogen concentration is less than 0.1 mg/*l*, then plant growth is less than 50% of the maximum; if nitrogen is greater than 1 mg/*l*, then plant growth is at least 75%. A similar relationship is proposed for phosphorus.

Nutrient uptake is a dynamic process. The size of plants will reflect the nutrient conditions for about the last month (the oldest leaves will have reached maturity under the nutrient conditions of several weeks earlier). Water hyacinth plants can also store more nutrients than are required for immediate growth. Therefore, it is important to know if nutrient levels occasionally become high, as plants will be expected to respond to these surges in nutrients.

Other Issues

The relationship between growth and nutrients is calculated as the total nitrogen concentration of the water. There may be differences between the uptake of different forms of nitrogen (ammonia, nitrates, nitrites, et cetera (Ueki, 1978)); however, the general relationship should hold. Other nutrients can limit growth (see Newman and Haller, 1988). However, nitrogen and phosphorus are expected to be the most likely limiting resources.

Water flow may be expected to increase the mixing of water, and so water immediately around the plants' roots will not become depleted of nutrients. Decomposing plant material will also release nutrients that can be reused by the weed. These effects are not considered here.

The levels here are slightly different from the South African Water Quality Guide-Line Levels:

Oligotrophic <0.5 mg.l^{-1} N; 0.005 mg.l^{-1} P
Mesotrophic 0.5–2.5 mg.l^{-1} N; 0.025 mg.l^{-1} P
Eutrophic 2.5–10 mg.l^{-1} N; >0.25 mg.l^{-1} P

These levels are for average summer inorganic nitrogen concentration and average summer inorganic phosphorus concentration.

8.2.2.4 Flow and Swell

Categories

i) Small or sheltered site; no or small current.
ii) If clear of plants, there would be some current or wave action.
iii) If clear of plants, water would flow quickly or there is regular strong wave action.

Levels

a) No or small current <x m.s^{-1}; no wave action. x still to be determined.
b) Some current x–x m.s^{-1}. A basic swimmer could easily swim against the current.
c) Rapid current >, fast flowing; if large the water body would be unsafe to cross.
d) Some wave action; waves often noticeable and can have chop, but no break.
e) Potentially strong wave action; if very windy waves occasionally break; wind fetch of at least 2 km.

Reason for Inclusion

If plants are likely to be moved around, this will influence how easily a mat could develop. Two factors that can move plants around have been combined — current (e.g. a slow-flowing swamp versus a strong river); and wind/wave action (e.g. a small protected dam versus a large lake suitable for sailing). Plants can be controlled by strong river currents and the rise and fall of the water level during flooding (Olivares and Colonnello, 2000). If there is a current, water hyacinth mats often break up after insect or herbicide damage. Plants can be swept downstream or buffeted about. Most control measures reduce plant buoyancy.

Suggested Levels

If a water body is still, mats can grow out from the sides over the water-body. This is only halted by a physical barrier, or if control acts to reduce buoyancy. The levels are chosen so that they can be readily understood and estimated.

8.2.2.5 Other Factors

8.2.2.5.1 Water Depth

Categories

i) Marshy site; >50% plants are rooted in mud or decomposing plant material.
ii) Shallow site, or there are only seasonally some marshy areas.
iii) All areas at least moderately deep; <5% of area is shallow or marshy.
iv) Deep with steep banks.

Levels

a) Marshy; 0–30 cm water and soft sediment (likely to be turbid).
b) Shallow; 0–30 cm water and hard sediment (water probably clear).
c) Moderately deep; 30–100 cm water.
d) Deep; >1 m water.

Reason for Inclusion

If the water is deep, then plants can sink to the bottom after damage.

Other Issues

The effect of water flow is linked to the effect of swell and the size of the water body. In marshy conditions, after herbicidal control, dead water hyacinth plants may remain and provide nutrients for regrowth. If the weed's roots are trapped in silt then this may affect the ability of weevil biological control agents to pupate.

8.2.2.5.2 Presence of Other Species

Categories

i) Mono-culture.
ii) Other floating aquatic weeds present.
iii) Other floating aquatic species present, but no other weeds.
iv) Water hyacinth mat covered with reeds and other emergent aquatic plants.

Levels

a) Other species rare.
b) Other species equally dominant.
c) Other species dominant.

Deep; >1 m water

Reason for Inclusion

The presence of other vegetation may mean certain control measures are unsuitable.

Suggested Levels

Other Issues

In some instances, water hyacinth may be replaced by native vegetation. This native vegetation may naturally have grown in that habitat and so this would be a restoration of the natural flora. Open water, without floating macrophytes, is the natural state of most medium to large water bodies, so these circumstances would not normally pertain here. In some instances, however, water hyacinth may have acted as a transformer. By trapping sediment and preventing free-flow, an infestation of water hyacinth can convert a shallow water body to a marsh (Sastroutomo et al., 1978).

Water hyacinth is one of the most competitive aquatic plants known and it often suppresses other aquatic weeds, such as water lettuce and salvinia (Kariba weed) (Bond and Roberts, 1978; Buckingham, 1994). If water hyacinth is brought down to an acceptable level, then, in the absence of suitable control measures, other weeds can take over.

8.2.2.6 Other Factors Not Considered

Reason for Non-inclusion

These factors are believed to only affect water hyacinth when their levels are very extreme, or only in particular areas.

pH

Water hyacinth grows in water at a variety of pHs.

CO_2 Concentration

With climate change, this may affect plant growth.

Light Levels

Water hyacinth obviously requires light for growth.

Humidity

Low humidity has been seen to cause significant damage to plants. However, a suspicion is that pot experiments can create problems with humidity due to the clothes-line effect. A plant growing in a bucket can hang over the edge and so the surface area over which transpiration occurs is much higher than if the plants were level with the top of the bucket (Allen et al., 1997).

Salinity

Salinity is a major constraint on water hyacinth growth in coastal regions. Salinities of <0.1% (w/v) have no effect on water hyacinth growth (Kola, 1988), but above 0.3% all plants die (Haller et al., 1974). A field survey in the Orinoco found water hyacinth surviving at salinities of 0.13% and 0.19%, but not at 0.34% (Olivares and Colonnello, 2000). However, it is clear that plants can survive short periods in salty conditions. Often, high densities of the weed are observed out at sea or in coastal lagoons, when they have been swept downstream.

Upstream Effects

Plants are swept downstream, continuously adding to a population that might be under management. Division of infestations into physical management units, using either

floating booms or existing structures such as weirs and bridges, provides information on the origin of plants – either local or immigrant – and allows different management techniques, such as spraying, integrated control and pure biocontrol, to be practised at different points along a particular catchment.

8.2.3 General Rules for Water Hyacinth Populations with No Control Measures

The maximum plant density without any control is relatively simple (Figure 8.3). Plant density is expected to increase with increasing temperature, decreasing frost, increasing nutrients, and decreasing water flow rates. These general trends are consistent, but how important a factor is will depend on its interactions with the other factors. If conditions are eutrophic, a strong water flow tends not to reduce water hyacinth densities as much as it would in oligotropic conditions, when it is much less likely for plants to be able to completely cover and block a water-body (thereby reducing the flow, allowing a large mat to develop).

The variability in water hyacinth populations is much less straight forward. Given that variability (as defined here) requires a consideration of maximum density, the two (density and variability) are linked. Variability does, however, provide important management implications. A population that usually covers about 5-10% of the water surface, but occasionally reaches 100% coverage, should be managed in a manner differently from a population that, without control measures, permanently covers a water-way (a stable 95-100% coverage).

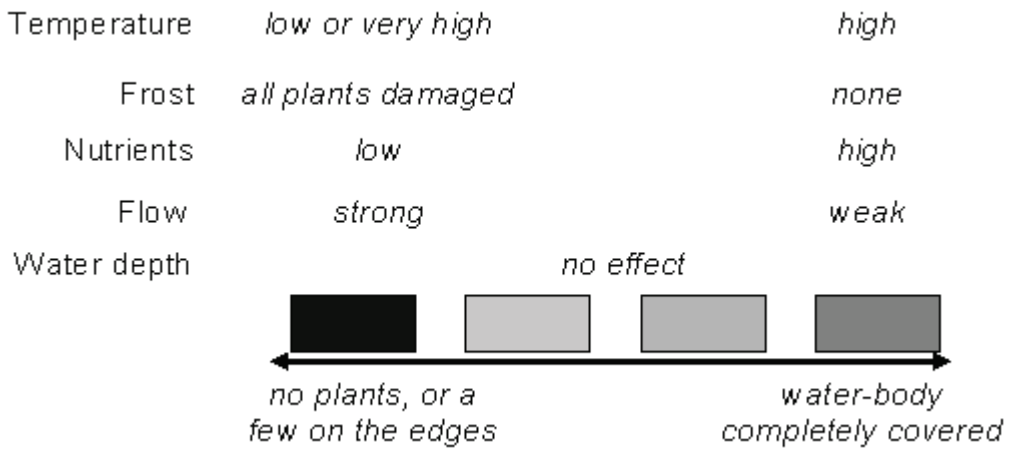


Figure 8.3: Effects of abiotic conditions on water hyacinth densities in the absence of control measures. Levels for abiotic conditions have values specified in the preceding text.

8.2.4 General Rules for Water Hyacinth Populations with Weevil Biological Control Agents Present

The maximum plant density with weevil biological control agents is a little less simple than the preceding example, as it will depend not only on how the weevils and the plants respond to the abiotic conditions, but also on how abiotic factors affect the plant-insect interaction. The conditions at which maximum weevil density, weevil survival,

and weevil development rate are all higher, are also those conditions that favour water hyacinth growth (Figure 8.4).

If conditions are fairly stable, the effect of abiotic conditions on the weevils will relate directly to the level of control. Control will be slower if nutrients are low, but at the same time plants will do less well, so that even in oligotrophic conditions, given time, the weevils will be expected to reduce plants to a low density.

Seasonally low temperatures have indirect and direct effects on the level of control observed. Water hyacinth has a lower temperature developmental threshold than the weevils (Chapter 2). Moreover, damage by weevils is primarily caused by late third instar larvae, so there is a developmental delay between conditions becoming suitable for the insects and the establishment of a high herbivore pressure. These factors mean that water hyacinth can temporarily escape from herbivore pressure early in the season. This reprieve can be augmented by eutrophic conditions as, although the developmental delay in the weevils will be shorter, the plants will be growing more quickly. In a more oligotrophic environment, the weevils have a little more time to respond.

A related problem is that of frost. While frost damages leaves, much of the root-stock of plants can remain unaffected. However, it has been shown that population regulation in *Neochetina eichhorniae* can occur through young larval competition for space in leaves (Wilson et al., 2006). If there are few leaves, this competition is more intense, and fewer large, damaging larvae survive. Therefore frost, by increasing larval mortality, reduces weevil populations and provides more herbivore-free time for plant populations to regrow after winter.

Control is also affected by the flow and depth of water. In regions where water flow is good, relatively small amounts of weevil damage can reduce the buoyancy of plants, and normal flows can sweep plants downstream. In slow-flowing areas, higher levels of damage are required before plants are swept away or sink. Similarly, if the area is marshy, plants tend not to be removed after damage and instead they rot, forming a putrid mat which provides a site for water hyacinth seeds to germinate. Weevil pupation success may also be adversely affected by high turbidities, or marshy substrates. Therefore, although the general trends for weevil populations are largely similar to those outlined in Figure 8.3, the relationship between, for example, temperature and weevil population growth is not the same as between temperature and plant growth. Therefore, the trends illustrated in Figure 8.4 do not necessarily translate directly into an observed level of control.

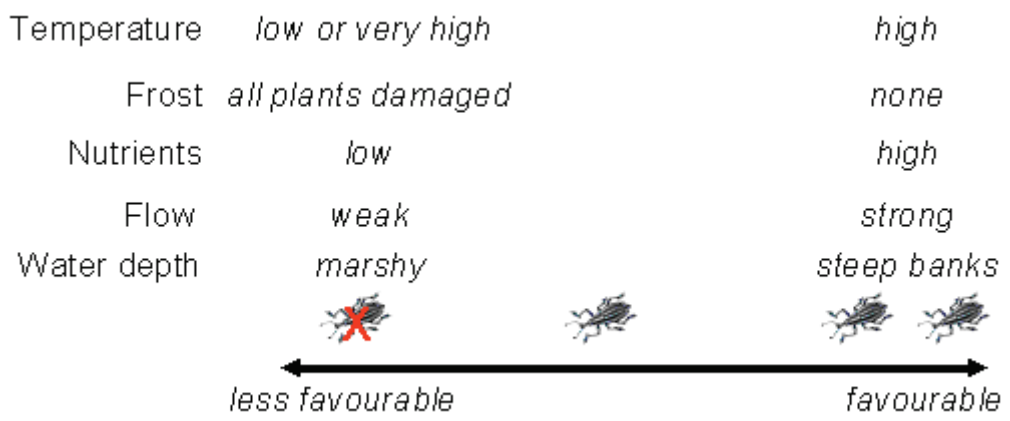


Figure 8.4: The effect of abiotic conditions on water hyacinth weevils. These general trends were developed from a review of the biology of *Neochetina eichhorniae* and *N. bruchi* (Wilson, 2002).

8.2.5 Comparison of Predictions with Observations

Sites in South Africa were identified for monitoring (Chapter 1), and were then categorised based on measurements of temperature, nutrients, and plant and insect populations. A prediction for the model was obtained from this information, and was qualitatively compared with the physical observations of the plant population at each site. Water hyacinth sites were also identified from around the world, and these data were used to verify the model.

8.3 Results

8.3.1 Management Recommendations

Based on the expected plant populations it was possible to evaluate where each strategy was most appropriate (Figure 8.5).

Where Figure 8.5 indicates biocontrol only, the expectation is that the weevil (or other biocontrol agents) will eventually provide an adequate and permanent reduction in the plant population to only a few fringing plants covering at most 5% of the water surface. However, an integrated management plan may be required before this stage is reached.

8.3.2 Comparison of Predictions with Observations

The expected water hyacinth coverage, given the simple rules outlined above, is shown in Table 8.1. Observed and predicted densities show some differences, but it must be noted that many sites may be experiencing transient dynamics or have been disrupted by non-integrated control, and so the level observed does not reflect the potential for dynamics over the longer term.

The information for the world sites is shown in Table 8.2.

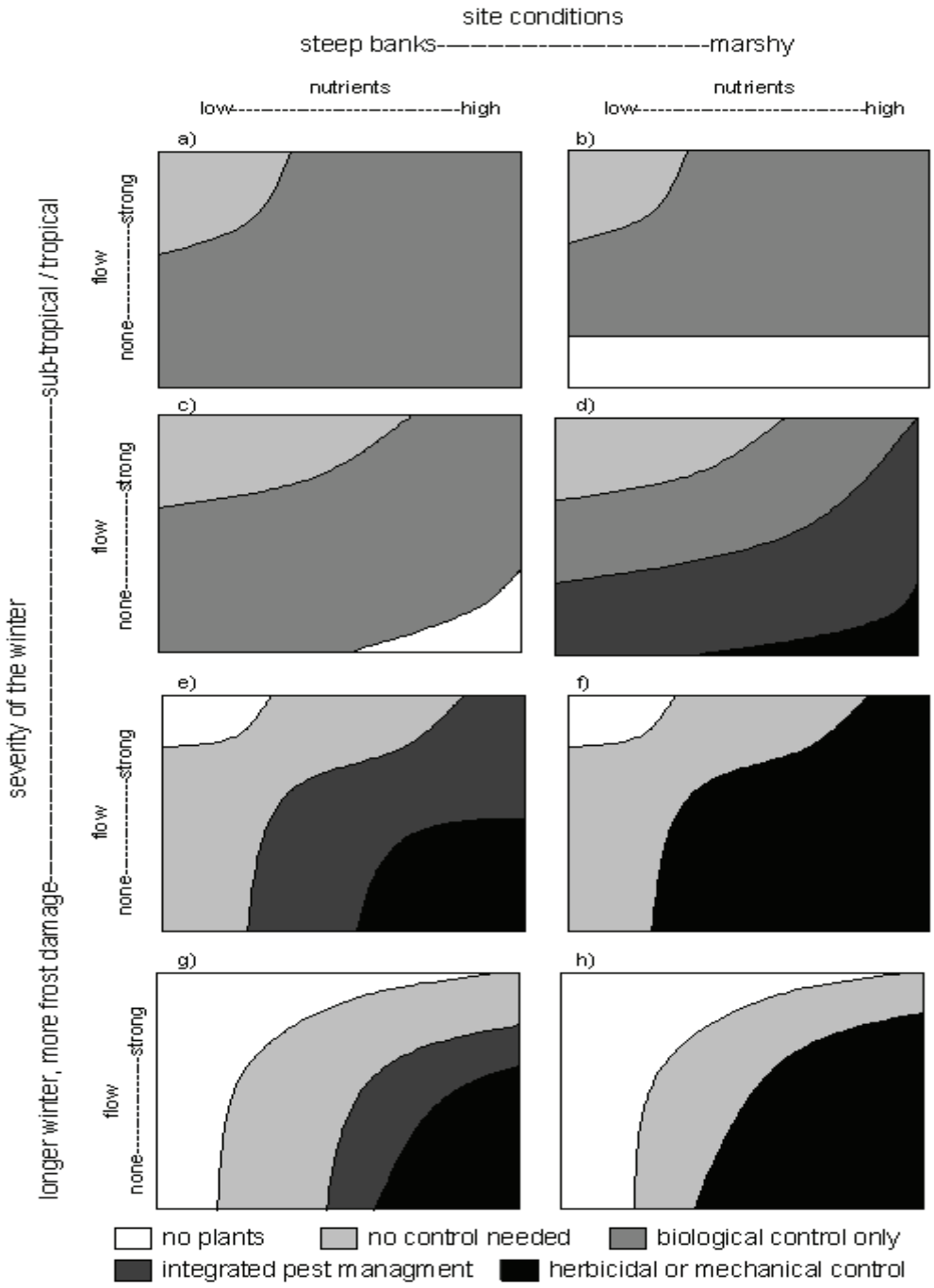


Figure 8.5: Broad management recommendations under different abiotic conditions.
The conditions in the panels are:

a) Temperature always good or very good; banks deep or with some shallows or some marsh.

b) Temperature always good or very good; marshy.

c) Seasonal or short winter; no or minor frost damage; deep or some shallows.

d) Seasonal or short winter; no or minor frost damage; some or all marshy.

e) Seasonal or short winter; major or severe frost damage; deep or some shallows.

f) Seasonal or short winter; major or severe frost damage; some or all marshy.

g) Short or long winter; minor or most frost damage; deep or some shallows.

h) Short or long winter; minor or most frost damage; some or all marshy.

Table 8.1: Observations and predictions for water hyacinth population at sites in South Africa. Weevils are present at all the sites. The categorisation of abiotic conditions is based on data collected at the sites from this project. *ipm* = Integrated Pest Management; *bc* = biological control; *none* = no control is required,; *herb* = the weevils do not provide substantial control, and other control methods should be used without consideration for the control agents.

1) Breede River, Malgas;
2) Crocodile River, Brits;
3) Delta Park, Randburg;
4) Enseleni River, Richards Bay;
5) Farm Dam, North Riding;
6) Feesgronde, Parys;
7) Hammarsdale Dam, Pietermaritzburg;
8) Kubusi River, Stutterheim;
9) Mbozambo Swamp, Stanger;
10) Mkadhzi Spruit, Kruger National Park;
11) New Years Dam, Alicedale;
12) Princess Vlei, Muizenberg;
13) Warrenton Weir, Warrenton;
14) Wolseley, Kluitjies Kraal;
15) Yamorna Weir, Tzaneen.

site	temp	frost	nutrient	flow	water depth	observation	prediction with no weevils	prediction with weevils	action
1	seasonal / short winter	minor	flushes / high	weak	moderately deep	tall & healthy	90–100%	0–40%	ipm
2	good / seasonal	minor	high	none / weak	deep	tall & healthy	95–100%	5%	bc
3	short winter	all	flushes / medium	none	shallow / marshy	small & old	5–20%	no effect	none
4	always good	none	high / medium	some waves	deep	tall/med & old	95–100%	5%	bc
5	short winter	all	high	none / weak	moderately deep	med & old/hlthy	80–100%	no effect	herb
6	short winter	most	high	weak	moderately deep	small & healthy	80–100%	5–100%	ipm
7	good / rarely cold	minor	high	none	deep	tall & damaged	95–100%	5%	bc
8	short winter	most	flushes / medium	some	shallow	med & hlthy/dmgd	10–20%	5–20%	none
9	always good	none	high	none	deep	tall & healthy	95–100%	5%	bc
10	always good	none	medium	some	deep	sml/med & hlthy/dmgd	95–100%	5%	bc
11	short winter	most	medium	none / weak	deep	med & old/dmgd	10–20%	5–20%	none
12	seasonal / short winter	minor	high	none	marshy	vtall/tall & hlthy	80–100%	20–100%	herb
13	short winter	all	high	weak	deep	tall/med & old	15–20%	no effect	none
14	short winter	all	high	none	moderately deep	tall/med & old	80–100%	no effect	herb
15	always good	none	high	none	moderately deep	med & healthy	95–100%	5%	bc

8.4 Discussion

Three broad management recommendations are made here, which are: biological control on its own; integrated weed management; and primarily herbicidal control.

In tropical situations where there the temperature rarely drops below 20°C, *Neochetina spp*. have controlled many water hyacinth infestations (Figure 8.5 panels a–d; Table 8.2). There are a few situations where plant densities reach over 20% – e.g. marshy areas, or where plants are rooted in substrate – but many tropical lakes and rivers have achieved excellent control using the weevils (Julien and Orapa, 1999). But, if plant and insect populations are disturbed by weather or human interference, the level of control is often poor. This is best exemplified by reports of efforts to tidy up areas by removing all the unhealthy-looking plants (i.e. those damaged by weevils), which releases the weed from herbivore pressure. The long-term strategy in tropical and sub-tropical climate regions should be to rely solely on biological control and no other interference. However, the release effort of biocontrol agents should be as intense as resources allow, aiming to place as many agents as possible onto water hyacinth infestations as quickly as possible, instead of relying on a slow, natural build-up of agents by reproduction from a small nucleus of founder individuals. The latter technique takes time and is therefore susceptible to unforeseen or unusual circumstances; for example, flooding or site contamination from pollutants such as sewage, minerals or herbicides. During the establishment period when the weevil population is still too low to show marked effects, healthy water hyacinth plants should be sprayed, either with sublethal doses of herbicide or leaving small areas of plants for weevil population to build up. Efforts to eradicate the weed will result in a resurgence of the problem several years later, and a small permanent water hyacinth population has to be accepted as part of the management plan.

In more temperate areas, the strategy is different, requiring herbicide intervention in addition to biocontrol in marshy, eutrophic water bodies, if most or a few leaves are damaged by winter frost. If there is a severe winter and most of the leaves are damaged, then the weevils are predicted to have little effect on the plants. However, in some of these scenarios water hyacinth does not reach high densities, and clearly no or little additional control should be required because the current biological control options are sufficient. Agents for cooler climates are being sought, but until such an agent has been shown to be suitable, supplemental herbicidal or mechanical control should be seasonally applied at a time when it will have least impact on weevil populations (late autumn and spring) if water hyacinth is problematic.

In sub-tropical regions with steep banks, or in more marshy tropical regions, the weevils will generally provide some control, but usually not enough. Integrated control should be implemented, i.e. chemical and mechanical methods that reduce the population of water hyacinth but do not completely disrupt weevil populations.

Table 8.2: Parameterisation using sites from around the world. Data are from literature sources and personal communication.

Country / Lake	Location		Size (ha)	Notes	Ref
Lake Victoria	Winam Gulf			reductions from 18,000 ha to <2000 ha	(Albright et al., 2004)
	Murchison Bay, Uganda			from 2000 ha to <100 ha	(Albright et al., 2004)
Argentina	Dique Los Sauces (15 km from La Rioja)	29°20'S 67°0'W	120	hot and dry temp -5–45°C, ecologically isolated. Cover 25–75%. After 4 years with *N. bruchi* stable at 4–8%	(Deloach & Cordo, 1983)
Australia	Coraki			*N. eichhorniae* present since 1983 and *N. bruchi* since 1990 data from 1991-1997 coverage 80–100%	(Wright & Stegeman, 2002)
	Emigrant Creek			30–100%	(Wright & Stegeman, 2002)
	Junction Hill			50–100%	(Wright & Stegeman, 2002)
	Kempsey			55–100%	(Wright & Stegeman, 2002)
	Mackay			5–90%	(Wright & Stegeman, 2002)
	Maitland			35–100%	(Wright & Stegeman, 2002)
	Mutdapilly			40–90%	(Wright & Stegeman, 2002)
	Nambour			5–35%	(Wright & Stegeman, 2002)
	Shiralee			55–100%	(Wright & Stegeman, 2002)
	Gleeson Weir, Townsville				(Wright & Stegeman, 2002)
Benin	Banikora				
	Lihu	07°09'N 02°25'E		reduction from 100% to somewhere around 5%	(Ajuonu et al., 2003)
	Tévédjii	07°05' N 02°24' E		reduction from 100% to 0-50%. Also sites at Godolo, Akpome, Gouri, Sekanmey, Togbodan, Lake Azili, Savalou, Kafedji, Kpokissa, Azowlissa	(Ajuonu et al., 2003)
Egypt	Jebel Aulia Dam			"drastic reductions"; need to find reference	(Bashir & Bennett, 1984)
India	Hebbal, Bangalore		20	5–100% coverage (lack of rains), with *N. eichhorniae* suppressed to 5%	(Jayanth, 1988)
	Hebbal, Bangalore		50–60	Used remote sensing. Weevils present, no mention made of other control efforts. 0–40% coverage, measured December and March 1993 & 1998	(Verma, Singh & Ganesh Raj, 2003)
	DB Sandra		34–42	Used remote sensing. Weevils present, no mention made of other control efforts 0–5% coverage, measured March and December 1993 & 1998	(Verma et al., 2003)
	Yelahanka		71–108	0–30% coverage	(Verma et al., 2003)
	Jakkur		40–68	10–20% coverage	(Verma et al., 2003)
	Rachenahalli		24–50	0–55% coverage	(Verma et al., 2003)
	Nagavara		30.7–41.5	0–5% coverage	(Verma et al., 2003)
Kenya	Lake Naivasha		10,000–16,000	from 1988 ~5% by 1998 100% (replaced *Salvinia*) eutrophic. 2001 no *Neochetina*	(Adams et al., 2002)
Mozambique	Cabora Bassa			were the weevils introduced?	(Bond & Roberts, 1978)
Malawi	Lake Kariba			Info needed	
Mexico	Batamote Dike, Sinaloa		134	Before weevils (Oct 94), coverage 100%; after weevils (Mar 98), 2%. Weevils released Jan 95–Aug 96	(Aguilar et al., 2003)

Country / Lake	Location	Size (ha)	Notes	Ref
	Arroyo Prieto Dike, Sinaloa	42	before 100% after 1%	(Aguilar et al., 2003)
	Hilda Dike	12	before 100% after 1%	(Aguilar et al., 2003)
	Mariquita Dike	492	before 80% after 20%	(Aguilar et al., 2003)
	Andrew Weiss Diversion	53	before 100% after 0%	(Aguilar et al., 2003)
	Adolfo López M. Dam	6393	before 30% after 15%	(Aguilar et al., 2003)
	Sanalona Dam	2443	before 20% after 5%	(Aguilar et al., 2003)
Papua New Guinea	Kuriva, region 7, site 18		1994–98 biomass density at Kurivera and Tomanto decreased by ~66% some *N. bruchi* mostly *N. eichhorniae*; plants 0.88–1.8%N, 0.3–0.5%P; water temperature 26–37°C	(Julien & Orapa, 1999)
	Gerehu, region 4, site 6		more Ne; 27–31°C; plants 1.2–1.7%N; 0.4–0.7%P	(Julien & Orapa, 1999)
	Tomano, region 7, site 36		more Ne; 25–30°C; plants 1.2–2.8%N; 0.3–0.6%P	(Julien & Orapa, 1999)
	Waiga, region 4, site 37		more Nb; 29–32°C; plants 1.7–2.5%N; 0.4–0.7%P	(Julien & Orapa, 1999)
Rwanda	Lake Mihindi		between 200 and 600 ha before weevils released, situation now?	(Albright et al., 2004)
Sri Lanka			plenty of info on *Salvinia*, not much on water hyacinth	(Room & Fernando, 1992)
Uganda	Lake Kyoga		lake nearly free of weed	(Julien et al., 1999)
U.S.A	California, Sacremento Delta		generally bad levels of control? (JRUW to talk to Californians)	(Spencer & Ksander, 2004)
	Florida		see some of the Center papers	(Center et al., 1999)
	Florids, Miner's Lodge Pond		Nitrogen 2.1ppm and phosphorus 0.8ppm (equivalent to mg per l?) decline from 95% to <5%but some minor frost damage on plants	(Haag & Center, 1988)
	Louisiana		general state-wide decline in plant cover after introduction of the weevils, some indication of seasonal trends	(Cofrancesco et al., 1984; Goyer & Stark, 1984)
	Texas		some good (have weather data)	(Grodowitz et al., 1991)
	Texas, Lake Conroe		caused by fungi?	(Martyn, 1985)
Zimbabwe	Manyame River		weed cover reduced by 55%	(Chickwenhere, 1999)
	Lake Chivero		weed cover reduced by 85%? Still plenty of plants? Nutrient data from Rommens.	(Chickwenhere, 1999; Marshall, 1991; Rommens et al., 2003)

Importantly, until biocontrol is widely established, water managers often need to do something. If control strategies have not been integrated or there have been extreme ecological events, then even though control agents may have been long established and small populations have been maintained, the biological control agents may still need several years of minimal disruption to be effective. Therefore, comparisons between observed levels of control and predicted levels need to take these factors into account. Figure 8.6 illustrates how unstable South African water hyacinth sites are, which will limit the effectiveness of *Neochetina spp*. weevils by resetting their populations to low starting sizes almost annually.

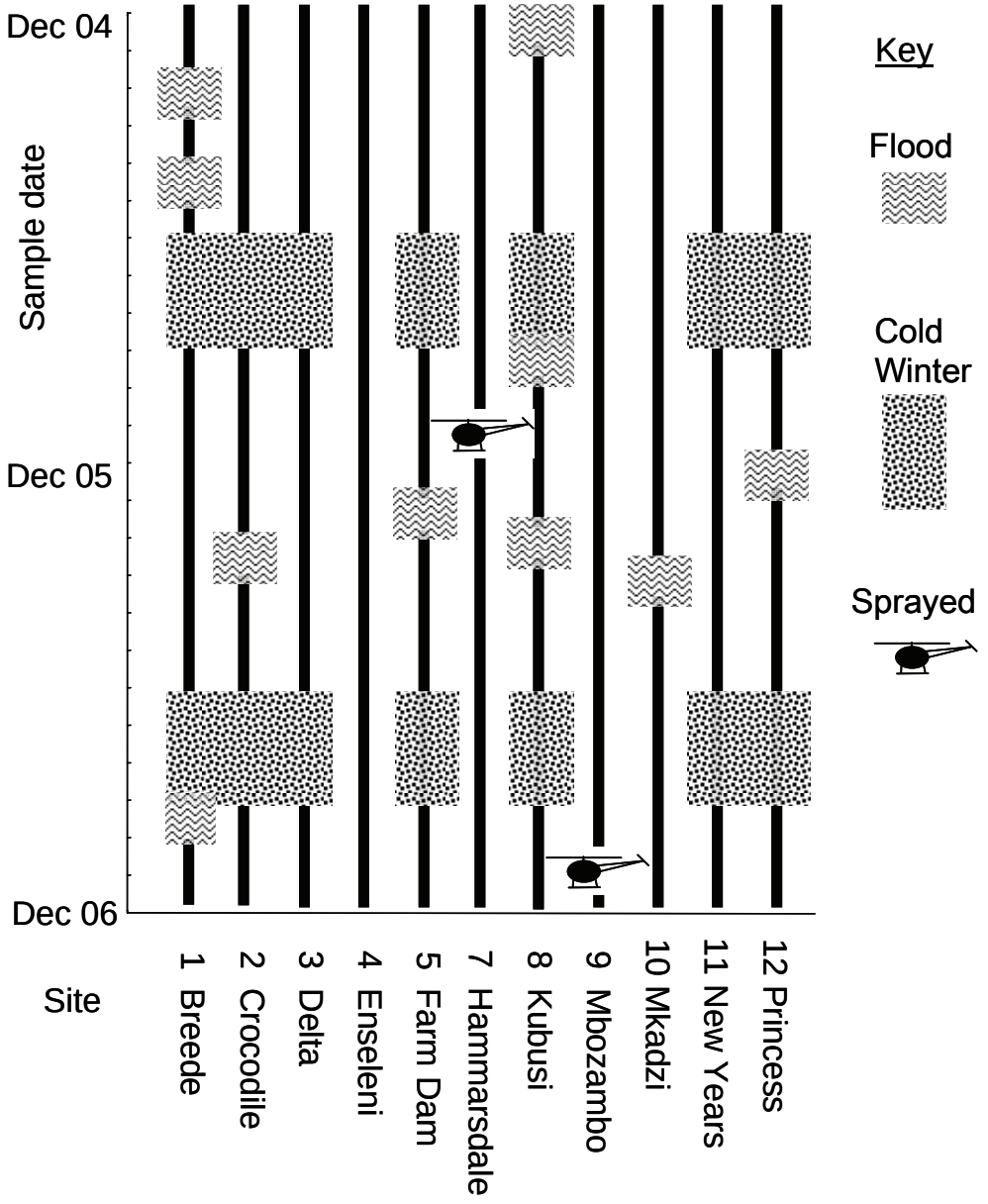


Figure 8.6: The disturbance history of 11 water hyacinth sites sampled over a two-year period in South Africa. Four other sites were not included as they had not been continuously sampled for the whole period.

The instability of South African water hyacinth infestations could well be an important factor contributing to poor biological control in many areas. Julien et al. (1999) recommend three to five years of uninterrupted biological control of water hyacinth for satisfactory control to take place. This long lead time is often unacceptable to many people affected by the weed, who expect rapid, conspicuous results, and have an unrealistic expectation of extermination, rather than acceptance of controlled water hyacinth populations as an eventually naturalised part of the South African landscape. Therefore, the first step in any control scenario should be to decide on what level of water hyacinth site coverage will be acceptable to the local water users (5-20% is a reasonable range), followed by an explanation of what control options are available, with the long-term objective of maintaining a healthy population of biocontrol insects which require the weed to live on.

The next step is aggressive release efforts to boost biological control agents to saturated population levels, rather than waiting for a natural build-up from a tiny starting number. Recent surveys by the Rhodes University water hyacinth team have revealed woefully few water hyacinth sites with a full suite of the six biological control agents available in South Africa (Figure 8.7), and an average of only 2.7 species of biocontrol agents present across the country. Many sites have no agents present, despite more than 30 years of water hyacinth biological control in South Africa. This fact suggests that the agents have been exterminated by site instability, or that the agents were never put there in the first place. Integrated water hyacinth management will require that agents are actively pushed onto all sites as the first line of attack in any control plan.

One factor not considered here is how connected a site may be to other waterways. The movement of plants around a catchment can easily cloud impressions of the size of the problem. When examined in terms of local areas, the success of *Neochetina spp*. in controlling water hyacinth on Lake Victoria appeared equivocal. Plant populations appeared to show dramatic resurgences in some regions, and poor control in others, while control occurred too fast for the weevils to possibly have been responsible in others. However, if the whole lake was considered, a clear consistent reduction in water hyacinth populations was seen, similar to the rate of control seen in other similar infestations. It was simply that water currents and prevailing winds moved huge mats of water hyacinth around the lake, making regional assessments of the problem very misleading (Wilson et al., 2006). Obviously, any water catchment should be assessed as a whole, and the recommendations here must be adjusted for how likely plants are to arrive from upstream. Jones and Cilliers (1999) provide an example of how a river and lake can be divided into management units to address this problem.

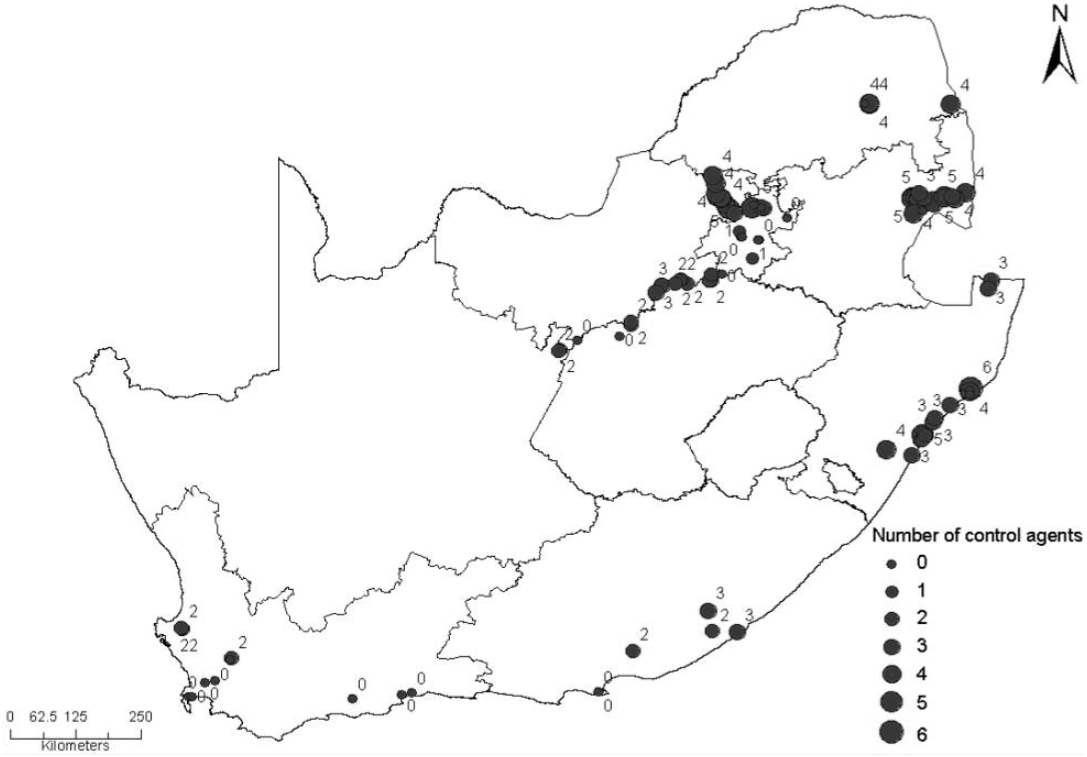


Figure 8.7: Numbers of species of water hyacinth biocontrol agents present on water hyacinth infestation site around South Africa, surveyed in May 2009 by Rhodes University. (Julie Coetzee unpublished data.)

Finally, water hyacinth management should not exist in isolation from general water management. A water hyacinth infestation may be indicative of poor water quality and sedimentation, and if water hyacinth is controlled it may be replaced by another invasive aquatic weed (e.g. *Salvinia* or *Pistia*) or an algal bloom. However, in many instances, floating aquatic invasive species are not replaced by anything and did not replace anything (at least in the middle of a lake). These species, when moved outside their native range, occupy a vacant ecological niche (open water) that they may not have occupied in their native range. While successful classical biological control does not provide proof of the natural enemy release hypothesis (Keane and Crawley, 2002), the success of invasive aquatic plants in covering water bodies and their subsequent control by herbivory is strongly suggestive of herbivore pressure preventing any plant species from completely occupying the ecological niche that is open fresh water. Therefore, unless a manager wishes to change the ecosystem, all invasive floating aquatic weeds should be controlled.

This is an adaptive management tool. If a site does not fit into the scheme proposed here, then the scheme is wrong. However, where information on a site is poor, the confidence in the predictions may be poor; other sites may simply be hard to predict. This conceptual model is limited structurally by what were perceived to be the most important abiotic factors. Which factors should be considered is still a matter of debate, but where

observations do not match predictions, insights may be gained into important factors which were not considered but are important in determining water hyacinth dynamics. The data gathered during the course of this project will generate more insights when they have been subject to more in-depth analysis, which is ongoing. The scenarios presented here can then be updated and criticised as more sites come under proper management and control.

8.5 Conclusions

Given time, water hyacinth can be controlled by water hyacinth weevils in many tropical situations. However, in sub-tropical and temperate regions (e.g. much of South Africa) there are many conditions under which water hyacinth still prospers (most notably, high nutrient conditions), and the weevils can provide little control. In these areas, current biological control should be part of a long-term integrated control strategy using herbicides and more aggressive establishment of agents, or it should be discounted. The approach taken here sets goals for new biological control agents, and a frame-work within which they can be assessed. It also formalises current thinking into hypotheses that should be tested.

CHAPTER NINE – CAPACITY BUILDING

9.1 Student Researchers

Nine students from Wits University have been directly involved in research projects on biological and integrated control of water hyacinth, for the WRC Project K5/1487 (Table 9.1). Two of these students, Ryan Brudvig (Working for Water) and Antony King (Plant Protection Research Institute, Weeds Division), are now employed on biological control of alien weeds. This is a direct result of the experience and exposure they gained on the WRC Project K5/1487, both working in the field and presenting their findings at scientific meetings.

Table 9.1: Name, sex, race, and academic registration, source of bursary funds, and current status of students who are, or were, involved with WRC Project K5/1487.

Name	**Race Sex**	**Academic status on project**	**Source of funds**	**Current Status**
Ryan Brudvig	W M	MSc registration	WRC	Employed, WfW – Weed Biocontrol
Jolene Fisher	W F	BSc Hons Graduate	NRF	Student, Wits – Resource Conservation
Ashwini Jadhav	I F	PhD registration	WRC	Student, Wits – this project
Naweji Katembo	B M	MSc registration	WRC	Student, Wits – this project
Bronwen Keiller	W F	BSc Hons Graduate	Working for Water	Student, Wits – Resource Conservation
Anthony King	W M	MSc registration	WRC	Employed, PPRI – Weed Control
Alecia Kirton	W F	BSc Hons Graduate	Working for Water	Employed, CSIR – Statiscian
Noxolo Mtembu	B F	BSc Hons Graduate	Working for Water	Employed – Environmental Management
Rory Atteridge	W M	BSc Hons	WRC	Student, Wits – this project

9.2 Laboratory Assistance

The project, because of its size and duration, helped pull funds from other sources, such as the Working for Water Capacity Building fund, which helped to pay for an undergraduate "lab. assistant" who worked about five hours per week, entering data into the project data base and assisting on field trips (Table 9.2). This experience of real research inspired the majority of these students to remain in the biological sciences field, and two of them, Ayanda Nongogo and Karen Tunley, have continued to work on alien

organisms. Both Jolene Fisher and Noxolo Mtembu got a taste of research as the undergraduate assistant and then joined the lab as Honours students in the following year. Both have remained in the environmental and biological fields; Jo as a student who has expanded her Hons project into an MSc., and Noxolo, as an environmental officer for Basil Read, Civil Engineering company, tasked, among other things, with controlling alien invasive weeds alongside highway construction sites.

Table 9.2: Academic or employment fate of Wits undergraduate students who were appointed as technical assistants to help with the WRC Project, K5/1487, doing field work and data capture. Working for Water capacity building money paid for the appointments up to 2007, when the WRC project budget took over.

Technical Assistants	**Student**	**Race Sex**	**Degree completed /registration**	**Current status**
Technical Assistant 1st Appointment	Dire Mamogale	B M	MBA Completed 2007	Employed by Deloitte and Touche
Technical Assistant 2005 2nd Appointment	Jolene Fisher	W F	Wits BSc Hons Completed 2006	MSc project on Remote Sensing
Technical Assistant 3rd Appointment	Ayanda Nongogo	B F	Wits BSc Hons Completed 2006	Appointed by PPRI Weeds division
Technical Assistant 2006 4th Appointment	Karen Tunley	W F	Wits BSc. Hons UCT Completed 2007	Alien invasive marine organisms
Technical Assistant 2006 5th Appointment	Noxolo Mthembu	B F	Wits BSc Hons Completed 2007	Env. Officer Basil Read
Technical Assistant 2006 6th Appointment	Chileshe Mphele	B M	Wits BSc. 2007. MBBCh reg., 2008	Wits Medical Student
Technical Assistant 2007 7th Appointment	Rosemary Edeling	W F	Wits BSc Completed 2008	Employed
Technical Assistant 2008 8th Appointment	Welma	B F	Wits BSc 2009	Degree in abeyance; due to recommence BSc chemistry 2010.
Technical Assistant 2008 8th Appointment	Solomon Newete	B M	Asmara BSc; Wits MSc 2009	Wits MSc 2009, about to submit.

9.3 Academic Progress

Table 9.3 summarises the degrees either already conferred or being pursued by students directly associated with this project. One white female student graduated with First Class Honours in 2005, and one with First Class Honours in 2006. Two female students – one black and one white – and one black male graduated with Honours in 2007. All conducted their Honours research projects as part of WRC Project K5/1487. One black male has now registered for an MSc as part of the project. A further female PhD and three male MSc students are associated with the project; two of whom are black.

Table 9.3: The academic progress of students who have been part of the research team for the WRC Project K5/1487.

Degree	**Status***	**Black**		**White**		**Total**	
		men	women	men	women	C	P
PhD	C	0	0	0	0	0	
	P	0	1	0	0		1
MSC	C	0	0	0	0	0	
	P	1	0	2	0		3
Hons	C	1	1	0	3	5	
	P	0	0	1	0		1
Totals	C	**1**	**1**	**0**	**3**	**5**	
10	P	**1**	**1**	**3**	**0**		**5**

*Status: C = degree completed; P= degree in progress. NB: one black male has progressed from BSc Hons to MSc with the project and therefore appears twice.

9.4 Other Graduations Associated with the Project

PhD

2009. Ms Angela Bownes. Title: Evaluation of a plant-herbivore system in determining potential efficacy of a candidate biological control agent, *Cornops aquaticum* for water hyacinth, *Eichhornia crassipes*. Rhodes University, Co-supervised with Prof M. Hill, Rhodes University.

MSc

2009. Mr Ajuonu Obinna. Title: A study on the interaction between two weevils *Neochetina eichhorniae* and *N. bruchi*, and the mirid *Eccritotarsus catarinensis,* as biological control agents of water hyacinth *Eichhornia crassipes*. Co-supervised with Prof M. Hill, Rhodes University.

CHAPTER TEN – COMMUNICATION

10.1 Introduction

The progress and results of the project to date have been widely publicized, to the local weed biocontrol community in particular, but also to ecological researchers in the Kruger National Park and in industry publications. Some of the scientific findings have been published in international journals and also presented as talks and posters at international and local conferences and workshops.

Two scientific papers, two articles and 28 conference presentations can be directly attributed to this project; their details along with some other associated papers are listed below.

10.2 Publications

10.2.1 ISI Rated Scientific Journals

Jadhav, A., Hill, M. and Byrne, M., 2008. Identification of a retardant dose of glyphosate with potential for integrated control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach. *Biological Control* 47:154–158

10.2.2 Peer-reviewed Conference Proceedings or Other Journals

Jadhav, A., King, A., Brudvig, R., Hill, M., and Byrne, M., 2007. Integrated weed control using a retardant dose of glyphosate: a new management tool for water hyacinth? *Outlooks on Pest Management* 18: 213–216.

Coetzee, J.A., Hill, M.P. and Byrne, M.J., 2008. Ten years after release of the water hyacinth mirid, *Eccritotarsus catarinensis* in South Africa: What have we learnt? XII International Symposium on Biological Control of Weeds, 22-27 April, 2007. Montpellier, France.

10.2.3 Popular Articles/ Industry Publications

Knoll, C., 2008. Integrated Management of Water Hyacinth. *Environmental Management* 3: 28-29.

Lorentz, K.B.M. and Byrne, M.J., 2006. Working Towards a Site-Specific IPM Strategy for Control of Water Hyacinth in South Africa. *Biocontrol News and Information* 27(3), 47N–62N pestscience.com

10.3 Conferences and Workshops

EMAPI 10: 10th International Conference, Ecology and Management of Alien Plant Invasions. 23-27 August, 2009. Stellenbosch, South Africa.

Byrne, M.J., Coetzee, J.A., Hill. M.P., King, A., and Brudvig, R. Management of Water Hyacinth in South Africa. Options for Biocontrol and IPM.

XXIII International Congress of Entomology. 6-12 July, 2008. Durban, South Africa.

Jadhav, A., Hill, M., Katembo, N. and Byrne, M., 2008. Effect of a retardant dose of glyphosate on biocontrol agents of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach. Oral.

Lorentz, M., Hill, M., Byrne, M. and Ripley, B., 2008. The effects of biological-control-agent herbivory on water hyacinth plants: Resource and nutrient allocation; herbivore-induced plant defenses; and photosynthetic mechanisms and outputs. Poster.

Lukac, D., Byrne, M. and Hill, M. 2008. Impact of herbivory by the mite, *Orthogalumna terebrantis* (Acarina: Galumnidae), on water hyacinth (*Eichhornia crassipes*) growth parameters and leaf chlorophyll content. Poster.

XII International Symposium on Biological Control of Weeds, 22-27 April, 2007. Montpellier, France.

Coetzee, J.A., Hill, M.P. and Byrne, M.J**.,** 2007. Ten years after release of the water hyacinth mirid, *Eccritotarsus catarinensis*, in South Africa: What have we learnt? Oral.

King, A.M., Hill, M.P. and Byrne., M.J., 2007. Microclimate effects on biological control: Water hyacinth in South Africa. Oral.

Jadhav, A.M., Kirton, A., Hill, M.P. and Byrne, M.J., 2007. Integrated weed control using a retardant dose of glyphosate: a new management tool for water hyacinth. Poster.

Fisher, J.T., Erasmus, B.F.N. and Byrne, M.J., 2007. Multispectral satellite remote sensing of water hyacinth at small extents – a monitoring tool? Poster.

36th Annual Workshop on Biological and Integrated Control of Weeds, 6th to 9th May 2008. Goudini Spa.

King, A. The effects of microclimate on the biocontrol of water hyacinth.

Brudvig, R. The effects of water nutrients on the biocontrol of water hyacinth.

Jadhav, A. Ecotoxicity to tadpoles, of sublethal doses of glyphosate used for integrated control of water hyacinth.

Byrne, M. Integrated control of water hyacinth: Summary of findings to date.

35th Annual Weeds Workshop, May 2007. KwaZulu-Natal.
Katembo, N. The impact of a sublethal dose of herbicide on the mirid (*Eccritotarsus caterinensis*), a biological control agent of water hyacinth.

Mtembu, N. The effect of different spray volumes of glyphosate herbicide on three phenostages of water hyacinth.

Keiller, B. The effect of temperature on the reproductive status of the weevils *Neochetina eichorniae* and *N. bruchi*.

Byrne, M. An Overview of Progress with the Water Research Commission Water Hyacinth project

34th Annual Weeds Workshop, May 2006. Klein Kariba.
Brudvig, R. Can nutrients limit the biological control of water hyacinth?

King, A. How do highveld winters hamper the biological control of water hyacinth?

Jadhav, A. Integrating a retardant dose of glyphosate with biocontrol of water hyacinth.

Fisher, J. Remote sensing of water hyacinth – an IMP tool.

Byrne, M. Aquatic weed control in the face of increasing water pollution.

The 15th Congress of the Entomological Society of Southern Africa, July 2005. Grahamstown.
Byrne, M.J., Hill, M.P., and Robertson, M.P., 2005. Integrated management plan for control of water hyacinth. Oral.

33rd Annual Weeds Workshop, July, 2005. Grahamstown.
King, A. Temperature and IPM of Water Hyacinth.

Jadhav, A. Herbicides and IPM of Water Hyacinth.

Kirton, A. Herbicides and IPM of Water Hyacinth.

Brudvig, R. Nutrients and IPM of Water Hyacinth.

32nd Annual Weeds Workshop, May, 2004. Golden Gate National Park.
Kirton, A. Integrated control of water hyacinth (*Eichhornia crassipes*) incorporating biological control and herbicidal control.

Byrne, M. An integrated weed management programme for water hyacinth: the plan.

Kruger National Park Project Feedback Workshops in 2004 and 2005
Two papers covering the Mkadhzi Spruit aspects of this work were presented to the Kruger National Park project feedback workshops in 2004 and 2005.

10.4 Planning and Training Workshops

Water Hyacinth Workshop, June 2004. Enseleni River.
A workshop was held at Enseleni River in June 2004 to plan the immediate details of the project and to train the project team in field methods. Mr Roy Jones of Ezemvelo Wildlife along with Prof. Martin Hill and Mr Hardi Oberholzer of PPRI demonstrated techniques for measuring and controlling water hyacinth.

Water Hyacinth Workshop, January 2007. Vaal River.
Ten participants, all student researchers or supervisors, reviewed the progress of the project to date, and got valuable advice from consultant Dr Jim Findlay, who later trained Ms Jadhav in the finer points of herbicide application. Key experiments were planned for 2007-08.

10.5 Webpage

The page was created in 2006 to advertise the objectives and progress of the project. It garnered several hundred visitors in 2006 and was updated in 2007. However, since then it has languished in a backwater of the Wits computer servers, where, unbeknownst to visitors from within the University system, it was actually hidden from the general public. This problem has been fixed but the site is badly in need of an update.

The experience of erecting the site was valuable, but brought home the value of having a dedicated “webmaster” of some description, who can look after the technical needs of such a site and also ensure that the content is updated regularly. Some degree of nervousness was felt about putting original, unpublished research results on the site,

which will always be a consideration for future efforts of this kind. As a means of promoting the final report, the website could be valuable and is at this stage being retained.

10.6 Conclusion

The practice of spreading the word as the project progressed has served two important purposes. Firstly as part of the capacity building process, in which students have been encouraged to present their work to the scientific community in a formal scientific manner. Then, secondly to keep the biocontrol community, especially in South Africa, abreast of developments in trying to integrate herbicides and biocontrol, as they took place. The result of this has been a general acceptance of the methods proposed in this report, as the benign effects of glyphosate used in integrated water hyacinth management became apparent, from both this work and application by extension officers in the field. A chicken-or-egg first? situation has arisen in which it is difficult to ascribe the acceptance and implementation of the integrated control methods as one of the successes of this project; or was the project riding on the back of a new awareness amongst WfW implementation officers that herbicides could be integrated with biocontrol? Whatever conclusion is reached, the energetic and positive presence of Roy Jones, who along with Carina Cillers and Martin Hill who have strongly promoted, and proven integrated management on the Enseleni River, showed that integrated management can work on a large water system. Nevertheless, our findings have been well publicized and further scientific publications are envisioned from the data gathered during the course of this project.

REFERENCES

Abjar, Z.E. and Bashir, M.O. (1984) Biology and life-tables of *Neochetina bruchi* Hustache (Coleoptera, Curculionidae) introduced to the White Nile, Sudan for the biological control of water hyacinth. *Zeitschrift fur Angewandte Entomologie* **97:** 282–286.

Adams, C. S., Boar, R. R., Hubble, D. S., Gikungu, M, M.,Harper M.., Hickley. P. and Tarras-Wahlberg N. (2002): The dynamics and ecology of exotic tropical species in floating plant mats: Lake Naivasha, Kenya. In: Hydrobiologia 488 (1/3), November 2002, National Institute of Informatics. Kluwer, Academic Publishers.

Addo-Bediako, A., Chown, S.L. and Gaston, K.J. (2000). Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society London* **267:** 739-745.

Addo-Bediako, A., Chown, S.L. and Gaston, K.J. (2002). Metabolic cold adaptation in insects: a large-scale perspective. *Functional Ecology* **16:** 332-338.

Aguilar, E., Auer, I., Brunet, M., Peterson T.C. and Wieringa, J. (2003). Guidelines on climate metadata and homogenization. WCDMP-No. 53, WMO-TD No. 1186. World Meteorological Organization: Geneva.

Ainsworth, N. (2003). Integration of herbicides with arthropod biocontrol agents for weed control. *Biocontrol, Science and Technology* **13:** 547–570.

Ajuonu, O., Schade, V., Veltman, B., Sedjro, K. and Neuenschwander, P. (2003) Impact of the weevils *Neochetina eichhorniae* and *N. bruchi* (Coleoptera: Curculionidae) on water hyacinth, *Eichhornia crassipes* (Pontederiaceae) in Benin, West Africa. *African Entomology* **11:** 153–162.

Akbay, K.S., Howell, F.G. and Wooten, J.W. (1991) A computer simulation model of water hyacinth and weevil interactions. *Journal of Aquatic Plant Management*, **29:** 15-20

Albright, T.P., Moorhouse, T.G. and McNabb, T. (2004) The Rise and Fall of Water Hyacinth in Lake Victoria and the Kagera River Basin, 1989-2001. *Journal of Aquatic Plant Management* **42**: 73-84

Allen, L.H., Sinclair, T.R. and Bennett, J.M. (1997) Evapotranspiration of vegetation of Florida: perpetuated misconceptions versus mechanistic processes. *Proceedings of the Soil and Crop Science Society of Florida* **56:** 1–10.

Alward, R.D., Detling, J.K. and Milchunas, D.G. (1999) Grassland vegetation changes and nocturnal global warming. *Science* **238**: 229-231

Anderson, S.P. and Baumgartner, M.F. (1998) Radiative heating errors in naturally ventilated air temperature measurements made from buoys. *Journal of Atmospheric and Oceanic Technology* **15:** 157-173.

Ashton, P.J. (1982) Report to Empangeni Municipality- A visit to the Enseleni River and Lake Nsezi to the water hyacinth problem. *Council for Scientific and Industrial Research National Council for Water Research.*

Ashton, P.J., Scott, W.E., Steyn, D.J. and Wells, R.J. (1979) The chemical control programme against the water hyacinth *Eichhornia crassipes* (Mart) Solms on Hartbeespoort Dam: historical and practical aspects. *South African Journal of Science* **75:** 303–306.

Atteridge, R. (2009) Sibling Cannibalism: An Evolved Advantage? Unpublished Honours dissertation. University of the Witwatersrand, Johannesburg, South Africa.

Bashir, M.O. & Bennett, F.D. (1984) Biological control of water hyacinth on the White Nile, Sudan. pp. 491–496 in Delfosse, E.S. (Ed.) Proceedings of the VI International Symposium on Biological Control of Weeds, 1986, Vancouver, Canada, Agriculture Canada.

Benton Jones, J. (2001) Laboratory guide for conducting soil tests and plant analysis. CRC Press, New York, pp 191-227.

Berry, D.I. and Kent, E.C. (2005) The effect of instrument exposure on marine air temperatures: an assessment using VOSCLIM data. *International Journal of Climatology*. **25:** 1007-1022.

Best, E.P.H. and Visser, H.W.C. (1987) Seasonal growth of the submerged macrophyte *Ceratophyllum demersum* L. in the mesotrophic Lake Vechten in relation to insolation, temperature and reserve carbohydrates. *Hydrobiologia* **148:** 231-243

Block, W., Wharton, D.A. and Sinclair, B.J. (1998) Cold tolerance of a New Zealand alpine cockroach, *Celatoblatta quinquemaculata* (Dictyoptera, Blattidae). *Physiological Entomology* **23:** 1-6.

Bock, J.H. (1966) An Ecological study of *Eichhornia crassipes* with special emphasis on its reproductive biology. Unpublished PhD Thesis. University of California, Berkeley, USA.

Bond, W.J. and Roberts, M.G. (1978) The colonisation of Cabora Bassa, Moçambique, a new man-made lake, by floating aquatic macrophytes. *Hydrobiologica,* **60**(3): 243- 59.

Bownes, A. (2009) Evaluation of a plant-herbivore system in determining potential efficacy of a candidate biological control agent, *Cornops aquaticum*, for water hyacinth, *Eichhornia crassipes*. Unpublished PhD Thesis. Rhodes University, Grahamstown, South Africa.

Brudvig, R., Byrne, M., Hill, M. and Robertson, M. (2005) Literature review and research plan for management of water hyacinth under different nutrient regimes, WRC Project K5/1487 deliverable 4. Water Research Commission, Pretoria, South Africa, 23pp.

Brunet, M., Saladié, O., Jones, P., Sigró, J. Aguilar, E., Moberg, A., Lister, D., Walther, A., Lopez, D. and Almarza, C. (2006) The development of a new dataset of Spanish daily adjusted temperature series (SDATS) (1850-2003). *International Journal of Climatology.*

Buckingham. G.R. (1994) Biological control of aquatic weeds. In: D. Rosen, F.D. Bennett and J.L. Capinera (eds) *Pest Management in the subtropics: biological control a Florida perspective*, (Intercept, Andover, U.K. pp. 413-81.

Buckingham, G. and Passoa, S. (1985) Flight muscle and egg development in water hyacinth weevils. In: Delfosse, E.S. (ed.) *Proceedings of the VI International Symposium on Biological Control of Weeds*, Agriculture Canada, Vancouver, Canada. pp. 497-510.

Byrne, M.J., Currin, S. and Hill, M.P. (2002) The influence of climate on the establishment and success of the biological control agent *Gratiana spadicea*, released on *Solanum sisymbriifolium* in South Africa. *Biological Control*, **24:** 128-134.

Campbell, A., Frazer, B.D., Gilbert, N., Gutierrez, A.P. and Mackauer, M. (1974) Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology* **11:** 431-438.

Center, T.D. (1980). Biological control and its effect on production and survival of water hyacinth leaves. Proceedings of the V International Symposium on the Biological Control of Weeds, Brisbane, Australia. pp 393-410.

Center, T.D. (1987) Do water hyacinth leaf age and ontogeny affect intra-plant dispersion of *Neochetina eichhorniae* (Coleoptera: Curculionidae) eggs and larvae? *Environmental Entomology* **16**(3): 699-707.

Center, T.D. and Dray, F.A. (1992) Associations between water hyacinth weevils (*N. Eichhorniae & N. bruchi*) and phenological stages of water hyacinth in Southern Florida. *Florida Entomologist* **75:**196-211.

Center, T.D., Dray, F.A., Jubinsky, G.P. and Grodowitz, M.J. (1999) Biological control of water hyacinth under conditions of maintenance control: can herbicides and insects be integrated? *Environmental Management* **23:** 241-256.

Center, T.D., Hill, M.P., Cordo, H. and Julien, M.H. (2002) Water hyacinth In: van Driesche, R., Lyon, S., Blossey, B., Hoddle, M. and Reardon, R. (Technical Coordinators), *Biological control of invasive plants in the Eastern United States.* USDA Forest Service Publication, Chapter 4.

Center, T.D. and Spencer, N.R. (1981) The phenology and growth of water hyacinth (*Eichhornia crassipes* (Mart.) solms) in a eutrophic north-central Florida lake. *Aquatic Botany* **10:** 1-32.

Center, T.D., Steward, K.K. and Bruner, M.C. (1982) Control of water hyacinth (*Eichhornia crassipes*) with *Neochetina eichhorniae* (Coleoptera: Curculionidae) and a growth retardant. *Weed Science* **30:** 453-457

Center, T.D. and Wright, A.D. (1991) Age and phytochemical composition of water hyacinth leaves determine their acceptability to *N. eichhorniae*. *Environmental Entomology* **20:** 323-334.

Cesaraccio, C., Spano, D., Duce, P. and Snyder, R.L. (2001) An improved model for determining degree-day values from daily temperature data. *International Journal of Biometeorology*. **45:**161–169.

Chadwick, M.J. and Obeid, H. (1966). Some observations on the ecology of water hyacinth. Annual report of the Hydrobiological Research Unit. University of Khorton. **8:**23-28.

Channing, A. (2001) *Amphibians of Central and Southern Africa*. Protea Bookhouse, Pretoria.

Chapman, R.F. (1998). *Nutrition* (Chapter 4): *In The Insects – Structure and Function* 4th ed. Cambridge University Press, Cambridge. pp69-89.

Cherrill, A.J. and Begon, M. (1989) Timing of life cycles in a seasonal environment: the temperature-dependence of embryogenesis and diapause in a grasshopper (*Chorthippus brunneus* Thunberg). *Oecologica* **78:** 237-241.

Chikwenhere, G.P and Phiri, G. (1999) History of water hyacinth and its control efforts on Lake Chivero in Zimbabwe. In: Hill, M.P., Julien, M.H and Center, T.D. (eds) Proceedings of the first IOBC global working group meeting for the biological and integrated control of water hyacinth. 16-19 November, 1999, Harare, Zimbabwe.

Chikwenhere, G.P. (2000) Biological control of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach: an evaluation of factors affecting population growth of *Neochetina bruchi* Hustache (Coleoptera: Curculionidae). Department of Ecology, Copenhagen: The Royal Veterinary and Agricultural University.

Chopra, R., Verma, V.K. and Sharma, P.K. (2001) Mapping, monitoring and conservation of Harike wetland ecosystem, Punjab, India, through remote sensing. *International Journal of Remote Sensing* **22**(1): 89-98

Chong, J.H., Oetting, R.D. and Van Iersel, M.W. (2003) Temperature effects of the development, survival, and reproduction of the madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), on Chrysanthemum. *Annals of the Entomological Society of America* **96**(4): 539-543.

Chown, S.L., Addo-Bediako, A. and Gaston, K.J. (2002) Physiological variation in insects: large-scale patterns and their implications. Comparative Biochemistry and Physiology, Part B, *Biochemistry and Molecular Biology*. **131**(4): 587-602.

Chown, S.L. and Nicolson, S.W. (2004) *Insect physiological ecology: Mechanisms and Patterns.* Oxford University Press. Oxford.

Chown, S.L. and Terblanche, J.S. (2007) Physiological diversity in insects: ecological and evolutionary contexts. *Advances in Insect Physiology* **33:** 50-152.

Chutter, F.M. (1989). Evaluation of the Impact of the 1 mg/*l* Phosphate standard on the Water Quality and Trophic State of Hartbeespoort Dam. WRC Report No. 181/1/89.

Chytry, M., Maskell, L.C., Pino, J., Pysek, P., Vilà, M., Font, X. and Smart, S.M. (2008) Habitat invasions by alien plants: a quantitative comparison among Mediterranean, subcontinental and oceanic regions of Europe. *Journal of Applied Ecology* **45:** 448–458

Cilliers, C.J. (1991). Biological control of water hyacinth, *Eichhornia crassipes* in South Africa. *Agriculture, Ecosystems and the Environment* 37, 207-217.

Cilliers, C.J. (1991) Biological Control of water hyacinth in South Africa. *Agricultural Ecosystems and Environment*. Special issue: Biological control of weeds in South Africa. 37. Elsevier. pp1-3.

Cilliers, C.J., Hill, M.P., Ogwang, J.A. and Ajuonu, O. (2003). Aquatic weeds in Africa and their control. In: Neuenschwander P, Borgemeister C and Langewald J (eds) *Biological Control in IPM Systems in Africa*, pp. 161–178. CAB International, Wallingford (GB).

Clarke, A. (1996) The influence of climate change on the distribution and evolution of organisms In: Johnston, I.A. and Bennett, A.F. (eds) *Animals and Temperature: Phenotypic and Evolutionary Adaptation*. Cambridge University Press, Cambridge.

Clarke, K.R. and Gorley, R.N. (2001) PRIMER v5: User Manual/Tutorial. PRIMER-E, Plymouth UK, 95pp.

Coakley, S.M., Scherm, H. and Chakraborty, S. (1999) Climate change and plant disease management. *Annual Review of Phytopathology* **37:** 399-426.

Cock, M., Day, R., Herren, H., Hill, M.P., Julien, M.H., Neuenschwander, P. and Ogwang, J. (2000). Harvesters get that sinking feeling. *Biocontrol News and Information*, **21:**1-8.

Coetzee, J.A. (2003) Suitability of *Eccritotarsus catarinensis* as a biological control agent of water hyacinth. Unpublished PhD thesis. University of the Witwatersrand, Johannesburg, South Africa.

Coetzee, J.A., Byrne, M.J. and Hill, M.P. (2007a) Predicting the distribution of *Eccritotarsus catarinensis* , a natural enemy released on water hyacinth in South Africa. *Entomologia Experimentalis et Applicata* **125:** 237–247.

Coetzee, J.A., Byrne, M.J. and Hill, M.P. (2007b) Impact of nutrients and herbivory by *Eccritotarsus catarinensis* on the biological control of water hyacinth, *Eichhornia crassipes*. *Aquatic Botany* **86:** 179–186.

Coetzee, J.A., Byrne, M.J. and. Hill, M.P. (2009) Should the mirid *Eccritotarsus catarinensis*, (Heteroptera : Miridae) be considered for release against water hyacinth in the United States of America? *Biocontrol Science and Technology* **19:** 103-111.

Cofrancesco, A.F., Stewart, R.M. & Sanders, D.R. (1984). The impact of *Neochetina eichhorniae (Coleoptera: Curculionidae)* on Waterhyacinth in Louisiana. In *Proceedings of the VI International Symposium on Biological Control of Weeds* (ed E.S. Delfosse), pp. 525-35. Agriculture Canada, Vancouver, Canada.

Cordo, H.A. and DeLoach, C.J. (1976) Biology of the water hyacinth mite *Orthogalumna terebrantis* in Argentina. *Weed Science* **24:** 245-249.

COWI (2000) Integrated Water Quality/Limnology Study for Lake Victoria, Final Report Part II: Technical Report. LVEMP, Dar es Salaam (unpublished).

Cruttwell McFadyen, R.E. (1998) Biological control of weeds. *Annual Review of Entomology* **43:** 369-393.

Cullen, J.M. (1996) Integrated control and management. In: Moran, V.C., Hoffmann, J.H. (eds.), *Proceedings of the 1X International Symposium on Biological Control of Weeds.* Stellenbosch, South Africa, pp. 483–486.

Danneberger, T.K. and Vargas, J.M. Jr. (1984) Annual bluegrass seedhead emergence as predicted by Degree-day accumulation. *Agronomy Journal* **76:** 756-758.

Davies, P.M., Brenes, M. and Allee, L.L. (1996) Temperature dependent models to predict regional differences in corn rootworm (Coleoptera: Chrysomelidae) phenology. *Environmental Entomology* **25**(4): 767-775.

De Bruyn, L., Scheirs, J. and Verhagen, R. (2002) Nutrient stress, host plant quality and herbivore performance of a leaf mining fly on grass, *Oecologia* **130:** 594–599

DeGroote, H., Ajuonu, O., Attignon, S., Djessou, R. and Neuenschwander, P. (2003) Economic impact of biological control of water hyacinth in southern Benin. *Ecological Economics* **45:** 105–117.

Defries, R.S. and Townshend, J.R.G. (1999) Global land cover characterization from satellite data: from research to operational implementation? *Global Ecology and Biogeography*. **8**: 367-379

DeLoach, C.J. and Cordo, H.A. (1976) Life cycle and biology of *Neochetina Bruchii*, a weevil attacking water hyacinth in Argentina, with notes on *N. eichhorniae*. *Annals of the Entomological Society of America*. **69** (4): 643-652.

DeLoach, C.J. and Cordo, H.A. (1978) Life history and ecology of the moth *Sameodes albiguttalis*, a candidate for biological control of water hyacinth. *Environmental Entomology*. **7**(2): 309-321.

Deloach, C.J. & Cordo, H.A. (1983) Control of water hyacinth by *Neochetina bruchi* (Coleoptera: Curculionidae: Bagoini) in Argentina. *Environmental Entomology* 12, 19–23.

Dobrowski, S.Z., Pushnik, J.C., Zarco-Tejada, P.J. and Ustin, S.L. (2005) Simple reflectance indices track heat and water stress-induced changes in steady-state chlorophyll fluorescence at the canopy scale. *Remote Sensing of the Environment* **97**: 403-414

Dukes, J.S. and Mooney, H.A. (1999) Does global change increase the success of biological invaders? *Trends in Evolution and Ecology*. **14**(4): 135-139

Dunis, C.L. and Karalis, V. (2003) Weather derivatives pricing and filling analysis for missing temperature data. Liverpool Business School & CIBEF.

Edington, A.N., Sheridan, P.M., Stephenson, G.R., Thompson, D.G. and Boermans, H.J. (2004). Comparitive effects of pH and Vision herbicide on two life stages of four anuran amphibian species. *Environmental Toxicology and Chemistry* **23:** 815-822.

Edis, D., Hull, M.R., Cobb, A.H. and Sanders-Mills, G.E. (1996) A study of herbicide action and resistance at elevated levels of carbon dioxide. *Journal of Applied Biology* **45**: 205-210.

Edwards, D., and Musil, C.J. (1975) *Eichhornia crassipes* in South Africa – A general review. *Journal of the Limnological Society of Southern Africa* **7**(1): 23-27.

Ehrlich, P.R., Breedlove, D.E., Brussard, P.F. and Sharp, M.A. (1972) Weather and the regulation of subalpine populations. *Ecology* **53:** 243-247.

Elser, J. J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A., Interlandi, S., Kilham, S.S., Mc-Cauley, E., Schulz, K.L., Siemann, E.H. and Sterner, R.W. (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature* **408:**578–580.

ERDAS (2005): *ERDAS Software version 8.7 of ERDAS IMAGINE tour guide, field guide and VirtualGIS.* Atlanta, G.A

Everitt, J.H., Yang, C., Escobar, D.E., Webster, C.F., Lonard, R.I. and Davis, M.R. (1999) Using Remote Sensing and Spatial Information Technologies to Detect and Map Two Aquatic Macrophytes. *Journal of Aquatic Plant Management* **37**: 71-80

Farri, T.A. and Boroffice, R.A. (1999) An overview on the status and control of water hyacinth in Nigeria. In: Hill, M.P., Julien, M.H and Center, T.D. (eds) *Proceedings of the first IOBC global working group meeting for the biological and integrated control of water hyacinth.* 16-19 November, 1999, Harare, Zimbabwe.

Field, C.B., Randerson, J.T. and Malmstrom, C.M. (1995) Global net primary production: Combining ecology and remote sensing. *Remote Sensing of the Environment*. **51**: 74-88

Fidanza, M.A., Dernoeden, P.H. and Zhang, M. (1996) Degree-days for predicting smooth crabgrass emergence in cool-season Turfgrasses. *Crop Science* **36:** 990-996.

Fornasari, L. (1995) Temperature effects on the embryonic development of *Aphthona abdominalis* (Coleoptera: Chrysomelidae), a natural enemy of *Euphorbia esula* (Euphorbiales: Euphorbiaceae). *Environmental Entomology* **24**(3): 720-723.

Forno, I.W. and Semple, J.L. (1987). Response of *Salvinia molesta* to insect damage: changes in nitrogen, phosphorus and potassium content. *Oecologia* **73:**71-74

Foody, G.M. (2002) Status of land cover classification accuracy assessment. *Remote Sensing of the Environment* **80**: 185-201

Gamon, J.A. and Surfus, J.S. (1999) Assessing leaf pigment content and activity with a reflectometer. *New Phytologist* **143**(1): 105-117

Gasvoda, D.S., Tinus, R.W. and Burr, K.E. (2003) Monitor tree seedling temperature inexpensively with the thermochron iButton data logger. *Tree Planters Notes*. **50**(1): 14-17.

Geden, C.J. (1997) Development models for the filth fly parasitoids *Spalangia gemina, S. cameroni*, and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) under constant and variable temperatures. *Biological Control* **9:** 185-192.

Giesy, J.P., Dobson, S. and Solomon, K.R. (2000) Ecotoxicological risk assessment for Roundup herbicide. *Review of Contamination and Toxicology* **167:** 35-120.

Gikuma-Njuru, P. and Hecky, R.E. (2004) Nutrient concentrations in Nyanza Gulf, Kenya: light limits algal demand and abundance. *Hydrobiologia* **534**: 131-140

Glenn, N.F., Mundt, J.T., Weber, K.T., Prather, T.S., Lass, L.W and Pettingill, J. (2005) Hyperspectral data processing for repeat detection of small infestations of leafy spurge. *Remote Sensing of the Environment* **95**: 399-412

Goetz, A.F.H., Vane, G., Solomon, J.E. and Rock, B.N. (1985) Imaging spectrometry for earth remote sensing. *Science* **228**(4704): 1147-1153

Goolsby, J.A., DeBarro, P.J., Kirk, A.A., Sutherst, R.W., Canas, L., Ciomperlik, M.A., Ellsworth, P.C., Gould, J.R., Hartley, D.M., Hoelmer, K.A., Naranjo, S.E., Rose, M., Roltsch, W.J., Ruiz, R.A., Pickett, C.H., and Vacek, D.C. (2005) Post-release evaluation of biological control of *Bemisia tabaci* biotype 'B' in the USA and the development of predictive tools to guide introductions for other countries. *Biological Control* **32:** 70-77.

Gopal, B. (1987) *Aquatic plant studies 1: Water Hyacinth.* Elsevier, Amsterdam.

Gordan, D.R. (1998) Effects of invasive, non-indigenous plant species on ecosystem processes: lessons from Florida. *Applied Ecology* **8:** 975-989.

Gosner, K. L. (1960) A simplified table for staging anuran embryos and larvae with notes on their identification. *Herpetologica* **16:** 183–90.

Gosset, D.R. and Norris, W.E. (1971). Relationship between nutrient availability and the content of nitrogen and phosphorus in the tissue of the aquatic macrophyte, *Eichhornia crassipes. Hydrobiologia*. **38:**15-28

Goyer, R.A. and Stark, J.D. (1984) The impact of *Neochetina eichhorniae* on water hyacinth in Southern Louisiana. *Journal of Aquatic Plant Management*, **22:** 57-61.

Greegor, D.H. Jr. (1986) Ecology from space. *Bioscience* **36**(7): 429-432

Grodowitz, M.J., Center, T.D. and Freedman, J.E. (1997) A Physiological Age-Grading System for *Neochetina eichhorniae* (Warner) (Coleoptera: Curculionidae), a biological control agent of Water Hyacinth, *Eichhornia crassipes. Biological Control* **9:**89-105.

Grodowitz, M.J., Stewart, R.M. and Cofrancesco, A.F. (1991) Population dynamics of water hyacinth and the biological control agent *Neochetina eichhorniae* (Coleoptera: Curculionidae) at a Southeast Texas location. *Environmental Entomology* **20**(2): 652-60.

Grobler, D.C. (1984). Impact of eutrophication control measures on the trophic status of South African Impoundments. WRC Report No. 130/1/84.

Gutiérrez, E.L., Ruiz, E.F., Uribe, E.G. and Martinez, J.M. (2001) Biomass and productivity of water hyacinth and their application in control programs. In: Julien, M.H., Hill, M.P., Center, T.D., and Jianqing, D. (eds.) *Biological and integrated control of water hyacinth*, Eichhornia crassipes. Proceedings of the 2nd Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth. 9-12 October, 2000. Beijing, China. ACIAR Proceedings **102:** 109-119.

Harley, K.L.S. (1990) The role of biological control in the management of water hyacinth, *Eichhornia crassipes*. *Biocontrol News and Information* 11 (**1**): 11-22

Haag, K.H. (1986a) Effective control of water hyacinth using *Neochetina* and limited herbicide application. *Journal of Aquatic Plant Management* **24**: 70-75

Haag, K.H. (1986b) Effects of herbicidal application on mortality and dispersive behaviour of the water hyacinth weevils, *Neochetina eichhorniae* and *Neochetina bruchi* (Coleoptera: Curculionidae). *Environmental Entomology* **15**:1193-1198

Haag, K. H., Glenn, M. S. and Jordan, J. C., (1988) Selective patterns of herbicide application for improved biological control of water hyacinth. *Journal of Aquatic Plant Management* **26:** 17-19.

Haag, K.H. and Center, T.D. (1988) Successful biocontrol of water hyacinth: a documented example. *Aquatics* **10**(3): 19-22.

Haller, W.T. and Sutton, D.L. (1973) Effect of pH and high phosphorus concentration on the growth of water hyacinth. *Hyacinth Control Journal* **11**: 59-61

Haller, W.T., Sutton, D.L. and Barlow, W.C. (1974) Effects of salinity on growth of several aquatic macrophytes. *Ecology* **55:** 891-94.

Harding, W.R. (2008) The determination of annual phosphorus loading limits for South African dams. WRC report No. 1687/1/08. Water Research Commission, Pretoria, South Africa.

Harley, K.L.S., Kassulke, R.C., Sands, D.P.A. and Day, M.D. (1990) Biological control of water lettuce by *Neohydronomus affinis*. *Entomophaga* **35:** 363-374.

Harris, P. (1991) Classical biocontrol of weeds: Its definition, selection of effective agents, and administrative-political problems. *Canadian Entomologist* **123:** 827-849.

Harris, P. (1993) Effects, constraints and the future of weed biocontrol. *Agriculture, Ecosystem and Environment* **46:** 289-303.

Haywood, LK., Alexander, G.J., Byrne, M.J. & Cukrowska, E. (2004). *Xenopus laevis* embryos and tadpoles as models for testing for pollution by zinc, copper, lead and cadmium. *African Zoology*. **39**. 163-174.

Heard, T.A. and Winterton, S.L. (2000) Interactions between nutrient status and weevil herbivory in the biological control of water hyacinth. *Journal of Applied Ecology* **37:** 117-27.

Hecky, R.E., Bugenyi, F.W.B., Ochumba, P., Talling, J.F., Mugidde, R., Gophen, M. and Kaufman, L. (1994) Deoxygenation of the deep water of Lake Victoria, East Africa. *Limnology and Oceanography* **39**: 1476–1481.

Henderson, L. (1998) Southern African Plant Invaders Atlas (SAPIA). *Applied Plant Sciences*. **12:** 31-32.

Henderson, L. (2001) *Alien Weeds and Invasive Plants. A Complete Guide to Declared Weeds and Invaders in South Africa*. Plant Protection Institute Handbook no. 12. ARC-PPRI, Pretoria.

Hermans, C., Hammond, J.P., White, P.J. and Verbruggen, N. (2006) How do plants respond to nutrient shortage by biomass allocation? *Trends in Plant Science* **11**(12): 610-617

Herms, D.A. (2004). Using degree-days and phenology to predict pest activity. In: Krischik, V., Davidson, J. (eds.) Integrated pest management of Midwest landscapes. *Minnesota Agricultural Experiment Station Publication* SB-07645. pp. 49-59.

Higley, L.G., Pedigo, L.P. and Ostlie, K.R. (1986) DEGDAY: A program for calculating degree-days, and assumptions behind the degree-day approach. *Environmental Entomology* **15**(5): 999-1005.

Hildebrand, L. D., Sullivan, D.S. and Sullivan, T.P. (1980) Effects of Roundup herbicide on populations of *Daphnia magna* in a forest pond. *Bulletin of Environmental Contamination and Toxicology* **25:**353-357.

Hill, M.P. (2003) The impact and control of alien aquatic vegetation in South African aquatic ecosystems. *African Journal of Aquatic Science* **28:** 19–24.

Hill, M.P. and Cilliers, C.J. (1999) A review of the arthropod natural enemies, and the factors that influence their efficacy, in the biological control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae), in South Africa. In: Olckers, T. and Hill, M.P. (eds.) Biological Control of Weeds in South Africa (1990-1998). *African entomology Memoir* **1:**103-112.

Hill, M.P., Cilliers, C.J. and Neser, S. (1999) Life history and laboratory host range of *Eccritotarsus catarinensis* (Carvalho) (Heteroptera: Miridae), a new natural enemy released on water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laub.) (Pontederiaceae) in South Africa. *Biological Control* 14: 127–133.

Hill, M. P. and Coetzee, J. A. (2008) Integrated control of water hyacinth in Africa. *Bulletin OEPP/EPPO* **38:** 452–457.

Hill, M.P. and Olckers, T. (2001) Biological control initiatives against water hyacinth in South Africa: constraining factors, success and new courses of action In: Julien, M., Hill, M.P., Center,T. and Ding, J. (eds.) *Proceedings of the Meeting of the Global Working Group for the Biological Control and Integrated Control of Water Hyacinth*. 9-12 October, 2000. Beijing, China. Australian Centre for International Agricultural Research, Canberra, Australia. Proceedings 102, pp. 33-38.

Hoagland, D.R. and Arnon, D.I. (1950). The water culture method of growing plants without soil. *California Agriculture Experiment Station Circular* 347.

Hoelmer, K.A. and Kirk, A.A. (2005) Selecting arthropod biological control agents against arthropod pests: Can the science be improved to decrease the risk of releasing ineffective agents? *Biological Control* **34:** 255-264.

Houlahan, J.E, Findlay, C.S, Schmidt, B.R, Meyer, A.H. and Kuzmin, S.L. (2000) Quantitative evidence for global amphibian declines. *Nature* **404:** 752-755.

Howe, C.M., Berrill, M., Pauli, B.D., Helbring, C.C., Werry, K. and Veldhoen, N. (2004) Toxicity of glyphosate- cased pesticides to four North American frog species. *Environmental Toxicology and Chemistry* **23**: 1928-1938.

Hubbart, J., Link, T., Campbell, C. and Cobos, D. (2005) Evaluation of a low-cost temperature measurement system for environmental applications. *Hydrological Processes*. **19:** 1517–1523.

Huberty, A.F. and Denno, R.F. (2004) Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology* **85:** 1383–1398

Hulme, P.E. (2003) Biological invasions: winning the science battles but losing the conservation war? *Oryx*. **37**(2): 178-193

Ikemoto, T. and Takai, K. (2000) A new linearized formula for the law of total effective temperature and the evaluation of line-fitting methods with both variables subject to error. *Environmental Entomology* **29**(4): 671-682.

Ikemoto, T. (2003) Possible existence of a common temperature and a common duration of development among members of a taxonomic group of arthropods that underwent speciational adaptation to temperature. *Applied Entomology and Zoology* **31**(4): 487-492.

Imaoka, T. and Teranishi, S. (1988) Rates of nutrient uptake and growth of the water hyacinth (*Eichhornia crassipes* (Mart.) Solms). *Water Research* **22**(8): 943-51.

Inouye, D.W. (2000) The ecological and evolutionary significance of frost in the context of climate change. *Ecology Letters* **3:** 457-463.

Jacobs, J.S., Shelley, R.L. and Story, J.M. (2000) Use of Picloram to enhance establishment of *Cyphocleonus achates* (Coleoptera: Curculionidae). *Environmental Entomology* **29:** 349-354.

Jadhav, A., King, A., Brudvig, R., Hill, M., and Byrne, M. (2007). Integrated weed control using a retardant dose of glyphosate: a new management tool for water hyacinth? *Outlooks on Pest Management*.

Jadhav, A., Hill, M. and Byrne, M. (2008) Identification of a retardant dose of glyphosate with potential for integrated control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach. *Biological Control* **47:**154–158.

Jakubauskas, Peterson, D., Campbell, S.W., Campbell, S.D., Penny, D. and deNoyelles, F. (2002) Remote sensing of invasive aquatic plant obstruction in navigable waterways. *Proceedings of the 2002 ASPRS-ACSM Annual Conference and FIG XXII Congress*. 22-26 April, 2002. Washington, D.C., USA.

James, F.C. and McCulloch, C.E. (1990) Multivariate analysis in ecology and systematics: panacea or pandora's box? *Annual Review of Ecology and Systematics* **21:** 129-166.

Jayanth, K.P. (1988) Successful biological control of water hyacinth (*Eichhornia crassipes*) by *Neochetina eichhorniae* (Coleoptera: Curculionidae) in Bangalore, India. *Tropical Pest Management* **34**(3): 263-66.

Jones, F.G.W. (1976) Pests, resistance and fertilizers. In: *Fertilizer use and plant health. Proceedings of the 12th colloquium of the International Potash Institute,* Izmir/Turkey, 1976. International Potash Institute (Worblaufen-Bern). Switzerland. pp233-258.

Jones, R. and Cilliers, C.J. (1999) Integrated control of water hyacinth on the Enseleni / Mposa Rivers and Lake Nsezi in KwaZulu-Natal, South Africa. In: Hill, M.P., Julien, M.H and Center, T.D. (eds.) *Proceedings of the first IOBC global working group meeting for the biological and integrated control of water hyacinth*. 16-19 November, 1999. Harare, Zimbabwe. pp. 160-167.

Jones, R.W. (2001) Integrated control of water hyacinth on the Enseleni/Mposa Rivers and Lake Nsezi, KwaZulu-Natal, South Africa. In: Julian, M.H., Hill, M.P., Center, T.D., and Ding Jianqing (eds.) Biological and integrated control of water hyacinth, *Eichhornia crassipes*. . *ACIAR Proceedings* **102:**123-129.

Jones, R.W.J. (2009) The impact on biodiversity, and integrated control, of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) on the Lake Nsezi/Enseleni River System. Unpublished MSc dissertation. Rhodes University, Grahamstown, South Africa.

Julien, M.H. (2001) Biological control of water hyacinth with arthropods: A review to 2000. In: Julian, M.H., Hill, M.P., Center, T.D., and Ding Jianqing (eds.) Biological and integrated control of water hyacinth, *Eichhornia crassipes*. *ACIAR Proceedings* **102**: 8-20.

Julien, M.H. and Griffiths, M.W. (1998) *Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds* 4th edn. CABI Publishing, CAB International, Wallingford (GB).

Julien, M.H., Griffiths, M.W. and Wright, A.D. (1999) Biological control of water hyacinth. The weevils *Neochetina bruchi* and *N. eichhorniae:* biologies, host ranges, and rearing, releasing and monitoring techniques for biological control of *Eichhornia crassipes*. Australian Centre for International Agricultural Research (ACIAR), Canberra.

Julien, M.H., Harley, K.L.S., Wright, A.D., Cilliers, C.J., Hill, M.P., Center, T.D., Cordo, H.A. and Coffrancesco, A.F. (1996) International co-operation and linkages in the management of water hyacinth with emphasis on biological control. *Proceedings of the IX International Symposium on Biological Control of Weeds*, pp. 273-282.

Julien, M.H. and Orapa, W. (1999) Successful biological control of water hyacinth (*Eichhornia crassipes*) in Papua New Guinea by the weevils *Neochetina bruchi* and *Neochetina eichhorniae* (Coleoptera: Curculionidae). In: Spencer, N.R. (ed.) *Proceedings of the X International Symposium on Biological Control of Weeds.* Montana State University, Bozeman, Montana, U.S.A. (p 1027.

Keane, R.M. and Crawley, M.J. (2002) Exotic Plant Invasions and the Enemy Release Hypothesis. *Trends in Ecology & Evolution* **17**(4): 164-70.

Kinvig, R. G. and Samways, M. J. (2000) Conserving dragonflies (Odonata) along streams running through commercial forestry. *Odonatologica* **29:** 195 –208.

Klok, C.J. and Chown, S.L. (2005) Inertia in physiological traits: *Embryonopsis halticella* caterpillars (Yponomeutidae) across the Antarctic Polar Frontal Zone. *Journal of Insect Physiology*. **51:** 87-97.

Knipling, E.B., West, S.H. and Haller, W.T. (1970) Growth characteristics, yield potential and nutritive content of water hyacinth. *Soil and Crop Science Society of Florida* **30:** 51-63.

Kola, K. (1988). Aspects of the ecology of water hyacinth *Eichhornia crassipes* (Martins) Solms. in the Lagos Lagoon System. In: Oke, O.L., Imevbore, A.M.A. and Farri, T.A. (eds.) *International Workshop on Water Hyacinth – Menace and Resource.* Nigerian Federal Ministry of Science and Technology, Lagos, Nigeria. pp. 80-84.

Kotsiantis, S., Kostoulas, A., Lykoudis, S., Argiriou, A., and Menagias, K. (2006) Filling missing temperature values in weather data banks. 2nd IEE International Conference on Intelligent Environments. 5-6 July, 2006. Athens, Greece. **1:** 327-334.

Kumar, L., Singh, S.P. and Pahuja, S.S. (1985) Studies on vegetative reproduction rate of water hyacinth and water chestnut. *Indian Journal of Agricultural Research* **19**(1): 54-58.

Lajmanovich, R.C., Sandoval, M.T. and Peltzer, P.M. (2003) Induction of mortality and malformation in Scinax nasicus tadpoles exposed to glyphosate formulations. *Bulletin of Environmental Contamination and Toxicology* **70:** 612-618.

Lawrence, R.L., Wood, S.D. and Sheley, R.L. (2006) Mapping invasive plants using hyperspectral imagery and Breiman Cutler classifications (RandomForest). *Remote Sensing of Environment* **100**: 356-362

Lillesand, T.M. and Kiefer, R.W. (1994) *Remote sensing and image interpretation (3rd Edition)*. John Wiley & Sons, Inc. New York

Lillesand, T.M., Kiefer, R.W. and Chipman, J.W. (2004) *Remote Sensing and Image Interpretation*, 5th Edition. John Wiley & Sons, Inc. New York

Lindgren, C.J., Gabor, T.S. and Murkin, H.R. 1998. Impact of Triclopyr Amine on *Galerucella calamariensis* L. (Coleoptera: Chrysomelidae) and a step toward Integrated Management of Purple Loosestrife, *Lythrum salicaria*. *Biological Control* 12: 14-19.

Lindgren, C.J., Gabor, T.S. and Murkin, H.R. (1999) Compatibility of glyphosate with *Galerucella calamariensis*; a biological control agent for purple loosestrife (*Lythrum salicaria*). *Journal of Aquatic Plant Management* **37:** 44-48.

Liu, S.S., Zhang, G.M. and Zhu, J. (1995) Influences of temperature variations on rate of development in insects: Analysis of case studies from entomological literature. *Annals of the Entomological Society of America* **88**(2): 107-119.

Lombardero, M.J., Ayres, M.P., Ayres, B.D. and Reeve, J.D. (2000) Cold tolerance of four species of bark beetle (Coleoptera: Scolytidae) in North America. *Environmental Entomology* **29**(3): 421-432.

Lorber, M.N., Mishoe, J.W. and Reddy, P.R. (1984) Modelling and analysis of water hyacinth biomass. *Ecological Modelling* **24:** 61-77.

Lym**,** R. G. (1998) The biology and integrated management of leafy spurge (*Euphorbia esula*) on North Dakota rangeland. *Weed Technology* **12**:367–373.

Lym, R.G. and Carlson, R.B. (1990) Effect of herbicide treatment on leafy spurge gall midge population. *Weed Technology* **8:** 285-288.

Lym, R. G., R. B. Carlson, K. M. Christianson, D. A. Mundal, and C. G. Messersmith. (1996). Integration of herbicides with *Aphthona* spp. flea beetles for leafy spurge control. In Pouteau, K. (ed.). *Proceedings of the Leafy Spurge Symposium.* Brandon, Manitoba, Canada.

Madsen, J. D. (1991) Resource allocation at the individual plant level. *Aquatic Botany*. 41: 67-86.

Maheu-Giroux, M. and de Blois, S. (2005) Mapping the invasive species *Phragmites australis* in linear wetland corridors. *Aquatic Botany* 83(**4**): 310-320

Malik, A. (2007) Environmental challenge *vis à vis* opportunity: The case of water hyacinth. *Environmental International* **33**: 122-138

Mallya, G.A. (1999) Water hyacinth in Tanzania. In: Hill, M.P., Julien, M.H. and Center, T.D. (eds.). *Proceedings of the first IOBC global working group meeting for the biological and integrated control of water hyacinth*. Harare, Zimbabwe. (eds Hill MP, Julien MH & Center TD) 16-19 November 1998, Zimbabwe, Plant Protection Research Institute, Pretoria, South Africa.

Manly, B.F.J. (1994) Principal components analysis In: *Multivariate statistical methods: a primer*. Chapman & Hall, London. pp 76-92.

Mann, R.M. and Bidwell, J.R. (1999) The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs. *Archives of Environmental Contamination and Toxicology* **36:** 193-199.

Marshall, B.E. (1991). Floating water-weeds in Zimbabwe, with special reference to the problems of water hyacinth in Lake Chivero. In: Greathead, A. and De Groot, P.J. (eds.) *Control of Africa's Floating Water Weeds* CAB International, Zimbabwe. pp. 23-29.

Martyn, R.D. (1985) Water hyacinth decline in Texas caused by *Cercospora piaropi*. *Journal of Aquatic Plant Management*, **23:** 29-32.

Mattson, W.J. (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* **11:**119-161.

May, M. L. (1979) Insect thermoregulation. *Annual Review of Entomology*. **24**: 313–349.

Mazzei, K.C., Newman, R.M., Loos, A. and Ragsdale, D.W. (1999) Developmental rates of the native milfoil weevil, *Euhrychiopsis lecontei*, and damage to Eurasian watermilfoil at constant temperatures. *Biological Control* **16:** 139-143.

McAvoy, T.J. and Kok, L.T. (1999) Effects of temperature on eggs, fecundity, and adult longevity of *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a biological control agent of purple loosestrife. *Biological control* **15:** 162-167.

McCaffrey, J.P. and Callihan, R.H. (1988) Compatibility of picloram and 2,4-D with *Urophora affinis* and *U. quadrifasciata* for spotted knapweed control. *Environmental Entomology* **17:** 785-788.

McClay, A.S. (1996) Biological control in a cold climate: Temperature responses and climatic adaptation of weed biocontrol agents. *Proceedings of the IX International Symposium on Biological Control of Weeds* pp. 377-383. Moran VC, Hoffmann JH, eds.. *9th International Symposium for the Biological Control of Weeds.* Stellenbosch: University Press, Cape Town. South Africa. 563 pp.

McClay, A.S. and Hughes, R.B. (1995) Effects of temperature on developmental rate, distribution, and establishment of *Calophasia lunula* (Lepidoptera: Noctuidae), a biocontrol agent for toadflax (*Linaria spp.*). *Biological Control* **5:** 368-377.

McConnachie, A.J. (2004) Post release evaluation of *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) – a natural enemy released against the red water fern, *Azolla filiculoides* Lamarck (Pteridophyta: Azollaceae) in South Africa. Unpublished PhD thesis, University of the Witwatersrand, Johannesburg.

McEvoy, P.B. and Coombs, E.M. (2000) Why things bite back: Unintended consequences biological weed control. In: Follett, P.A., and Duan, J.J. (eds.) *Non-Target Effects of Biological Control*, Kluwer Academic. pp. 167-194.

McGarigal, K., Cushman, S. A., Neel, M. C. and Ene, E. (2002) FRAGSTATS: Spatial Pattern Analysis Program for Categorical Maps. Computer software program produced by the authors at the University of Massachusetts, Amherst. Available at the following web site: www.umass.edu/landeco/research/fragstats/fragstats.html

Méthy, M., Alpert, P. and Roy, J. (1990) Effects of light quality and quantity on growth of the clonal plant *Eichhornia crassipes*. *Oecologia* **84:** 265-271.

Méthy, M. and Roy, J. (1993) Morhogenic changes induced by low red:far-red ratio and their growth consequences in water hyacinth (*Eichhornia crassipes*). *Journal of Experimental Botany* **44**(265): 1275-1280.

Miao, X., Gong, P., Swope, S., Pu, R., Curruthers, R., Anderson, G.L., Heaton, J.S. and Tracey, C.R. (2006) Estimation of yellow starthistle abundance through CASI-2 hyperspectral imagery using linear spectral mixture models. *Remote Sensing of Environment* **101**: 329-341

Midgley, J.M., Hill, M.P. and Villet, M.H. (2006) The effect of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae), on benthic biodiversity in two impoundments on the New Year's River, South Africa. *African Journal of Aquatic Science* **31**(1): 25–30.

Miller, J.C. (1992) Temperature-dependent development of the convergent lady beetle (Coleoptera: Coccinellidae) *Environmental Entomology*. **21**(1): 197-201.

Mirik, M., Michels, G.J. Jr., Kassymzhanova-Mirik, S., Elliott, N.C., Catana, V., Jones, D.B. and Bowling, R. (2006) Using digital image analysis and spectral reflectance data to quantify damage by greenbug (Hemiptera: Aphididae) in winter wheat. *Computers and Electronics in Agriculture* **51**: 86-98

Mitchell, J.D., Hewitt, P.H. and De K.Van Der Linde, T.C. (1993) Critical thermal limits and temperature tolerance in the harvester termite *Hodotermes mossambicus* (Hagen). *Journal of Insect Physiology* **39**(6): 523-528.

Mitsch, W.J. (1976) Ecosystem modelling of water hyacinth management in Lake Alice, Florida. *Ecological Modelling* **2:** 69-89.

Molenaar, M. (1996) Digital image interpretation In: Buiten, H.J. and Clevers, J.G.P.W. (eds.) *Land observation by remote sensing – Theory and applications.* Gordon and Breach Science Publishers, Singapore

Moran, H.A. and Hamilton, D.W. (1980) Low nutritive quality as a defense against herbivores. *Journal of Theoretical Biology* **86:** 247-254

Morris, J.T. (1991) Effects of nitrogen loading on wetland ecosystems with particular reference to atmospheric depositions. *Annual Review of Ecology and Systematics* **22:** 257-279

Muller, H., Nuessly, G.S. and Goeden, R.D. (1990). Natural enemies and host-plant asynchrony contributing to the failure of the introduced moth, *Coleophora parthenica* Meyrick (Lepidoptera: Coleophoridae), to control Russian thistle. *Agriculture, Ecosystems and Environment* **32:** 143-154.

Musil, C.F. and Breen, C.M. (1985) The development from kinetic coefficients of a predictive model for the growth of *Eichhornia crassipes* in the field. I. Generating kinetic coefficients for the model in greenhouse culture. *Bothalia*, **15**(3 & 4): 689-703.

Nagendra, H. and Gadgil, M. (1999) Satellite imagery as a tool for monitoring species diversity: An assessment. *The Journal of Applied Ecology*. **36**(3): 388-397

Nelson, S.A.C., Cheruvelil, K.S. and Soranno, P.A. (2006) Satellite remote sensing of freshwater macrophytes and the influence of water clarity. *Aquatic Botany* (In press).

Nelson, J.A. and Lym, R.G. (2003) Interactive effects of *Aphthona nigriscutis* and picloram plus 2,4-D in leafy spurge. *Weed Science* **51:** 118-124.

Neuenschwander, P., Ajuonu, O. and Schade, V. (1996) Biological control of water hyacinth in Benin West Africa. In: Charudattan R., Labrada R., Center T. D. and Kelly-Begazo C. (eds.) *Strategies for Water hyacinth Control. Report of a Panel of Experts Meeting*, 11-14 September, 1995. Fort Lauderdale, Florida USA. FAO Rome, 1996. pp.15-25.

Newman, S. and Haller, W.T. (1988) Mineral deficiency symptoms of water hyacinth. *Journal of Aquatic Plant Management* **26:** 55-58.

Nhapi, I. and Tirivarombo, S. (2004). Sewage discharges and nutrient levels in Marimba River, Zimbabwe. *Water S.A.* **30**(1):107-113.

Nykanen, H. and Koricheva, J. (2004) Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. *Oikos* **104:** 247–268
Obeid, M. and Taggel, M. (1976) Factors affecting dormancy and germination of seeds of *Eichhornia crassipes* (Mart.) Solms, from the Nile. *Weed Resource* **16**: 71- 80

Olivares, E. and Colonnello, G. (2000) Salinity gradient in the Mánamo River, a dammed distributary of the Orinoco Delta, and its influence on the presence of *Eichhornia crassipes* and *Paspalum repens*. *Interciencia* **25**(5): 242-48.

Owens, C.S. and Madsen, J.D. (1995) Low temperature limits of water hyacinth. *Journal of Aquatic Plant Management* **33:** 63-68.

Passmore, N.I. and Carruthers, V.C. (1995) *South African Frogs: A Complete Guide*. Southern Book Publishers, Witwatersrand University Press, Johannesburg.

Penfound, W.T. and Earle, T.T. (1948) Biology of the water hyacinth. *Ecological Monographs* **18**(4): 447-472.

Perez-Mendoza, J., Throne, J.E. and Baker, J.E. (2004) Ovarian physiology and age-grading in the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) *Journal of Stored Products Research* **40:** 179–196

Perkins, P.J, Boermans, H.J. and Stephenson, G.R. (2000) Toxicity of glyphosate and triclopyr using the frog embryo teratogenesis assay- *Xenopus*. *Environmental Toxicology Chemistry* **19:** 940-945.

Pettersson, U.T. and Namakando, C. (2002) Water hyacinth monitoring in Lake Kariba using satellite images – A study of the eastern basin. Zambezi River Authority.

Pilkington, L.J. and Hoddle, M.S. (2006) Use of life table statistics and degree-day values to predict the invasion success of *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae), an egg parasitiod of *Homalodisca coagulate* (Hymenoptera: Cicadellidae), in California. *Biological Control* **37:** 276-283.

Pilkington, L.J. and Hoddle, M.S. (2007) Use of life tables to quantify reproductive and developmental biology of *Gonatocerus triguttatus* (Hymenoptera: Mymaridae), an egg parasitoid of *Homalodisca vitripennis* (Hemiptera: Cicadellidae). *Biological Control* **42:** 1-8.

Polprasert, C. and Khatiwada, N.R. (1998) An integrated kinetic model for water hyacinth ponds used for wastewater treatment. *Water Research* **32**(1): 179-85.

Purcell, L.C. (2003) Comparison of thermal units derived from daily and hourly temperatures. *Crop Science* **43:** 1874-1879.

Reddy, K.R., Agami, M. and Tucker, J.C. (1989) Influence of nitrogen supply rates on growth and nutrient storage by Water Hyacinth (*Eichhornia crassipes*) plants. *Aquatic Botany* **36**: 33-43.

Reddy, K.R., Agami, M. and Tucker, J.C. (1990) Influence of phosphorus supply on growth and nutrient storage by water hyacinth. *Aquatic Botany* **37:** 355-65.
Rees, N.E. and Fay, P.K. (1989) Survival of leafy spurge hawk moths when larvae are exposed to 2,4-D or picloram. *Weed Technology* **3**: 429–431

Relyea, R. A. (2005 a) The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications* **15**(2): 618-627.

Relyea, R.A. (2005 b) The lethal impact of Roundup on aquatic and terrestrial amphibians. *Ecological Applications* **15**(4): 1118-1124.

Relyea, R.A. (2005 c) The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. *Environmental Contamination and Toxicology* **48**: 351-357.

Relyea, R.A., Schoeppner, N.M. and Hoverman, J.T. (2005) Pesticides and amphibians: the importance of community context, *Ecological Applications* **15:** 1125-1134.

Ricciardi, A., Steiner, W.W.M., Mack, R.N. and Simberloff, D. (2000) Toward a global information system for invasive species. *BioScience* **50**(3): 239-244

Richards, J.H. (1982) Developmental potential of axillary buds of water hyacinth, *Eichhornia crassipes* solms. (Pontederiaceae). *American Journal of Botany* **69**(4): 615-622.

Ripley, B.S., De Wet, L. and Hill, M.P. (2008) Herbivory-induced reduction in photosynthetic productivity of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae), is not directly related to reduction in photosynthetic leaf area. *African Entomology* **16**(1): 140–142

Robertson, M., Hill, M. and Byrne, M. (2004) Production of a GIS map of water hyacinth sites in South Africa. WRC Project K5/1487. Integrated Management Plan for control of water hyacinth, Deliverable 1

Roller, N.E.G. and Colwell, J.E. (1986) Coarse-resolution satellite data for ecological surveys. *BioScience*. **36**(7): 468-475

Roltsch, W.J., Zalom, F.G., Strawn, A.J., Strand, J.F. and Pitcairn, M.J. (1999) Evaluation of several degree-day estimation methods in California. *International Journal of Biometeorology* **42**: 169-176.

Rommens, W., Maes, J., Dekeza, N., Inghelbrecht, P., Nhiwatiwa, T., Holsters, E., Ollevier, F., Marshall, B. and Brendonck, L. (2003) The impact of water hyacinth (*Eichhornia crassipes*) in a eutrophic subtropical impoundment (Lake Chivero, Zimbabwe). I. Water quality. *Archiv fur Hydrobiologie*, **158**(3): 373-88.

Room, P.M. (1990) Ecology of a simple plant-herbivore system: biological control of *Salvinia*. *Trends in Ecology and Evolution* **5:** 74-79.

Room, P.M. and Fernando, I.V.S. (1992) Weed invasions countered by biological control *Salvinia molesta* and *Eichhornia crassipes* in Sri Lanka. *Aquatic Botany* **42:** 99-107.

Room, P.M. and Thomas, P.A. (1985) Nitrogen and the establishment of a beetle for biological control of the floating weed *Salvinia* in Papua New Guinea. *Journal of Applied Ecology* **22:**139-156.

Roy, M., Brodeur, J. and Cloutier, C. (2003) Effect of temperature on intrinsic rates of natural increase(r_m) of a coccinellid and its spider mite prey. *BioControl* **48:** 57-72.

Roy, M., Brodeur, J. and Cloutier, C. (2005) Seasonal activity of the spider mite predators *Stethorus punctillum* (Coleoptera: Coccinellidae) and *Neoseiulus fallacies* (Acarina: Phytoseiidae) in raspberry, two predators of *Tetranychus mcdanieli* (Acarina: Tetranychidae). *Biological Control* **34**(1): 47-57.

Rutagemwa, D. K., Myanza, O. I. and Mwanuzi, F. (2006) Water Quality Synthesis Report – Lake Monitoring. Lake Victoria Environmental Project, Dar es Salaam (unpublished).

Samways, M.J. and Moore, S.D. (1991). Influence of exotic conifer patches on grasshopper (Orthoptera) assemblages in a grassland matrix at a recreational resort, Natal, South Africa. *Biological Conservation* **57**: 117–137.

Sastroutomo, S.S., Ikusima, I. and Numata, M. (1978) Ecological studies of water hyacinth (*Eichhornia crassipes* Mart. Solms) with special emphasis on their growth. *Japanese Journal of Ecology* **28:** 191-97.

Satake, A., Ohgushi, T., Urano, S. and Uchimura, K. (2006) Modelling population dynamics of a tea pest with temperature-dependent development: predicting emergence timing and potential damage. *Ecological Research* **21**(1): 107-116.

Schachtman, D., Reid, R. and Ayling, S.M. (1998) Phosphorus uptake by plants: from soil to cell. *Plant Physiology* **116:** 447-453

Schmidt, K.S. and Skidmore, A.K. (2003) Spectral discrimination of vegetation types in a coastal wetland. *Remote Sensing of Environment* **85**: 92-108

Schneider, S., Ge□ler, A., Weber, P., von Sengsbusch, D., Habemann, U. and Renenburg, H. (1996). Soluble N compounds in trees exposed to high loads of N: a comparison of spruce (*Piscea abies*) and beech (*Fagus sylvatica*) grown under field conditions. *New Phytology* **134**: 103-114

Schulze, R.E., Maharaj, M., Lynch, S.D., Howe, B.J. and Melvil-Thomson, B. (1997) *South African Atlas of Agrohydrology and Climatology*. Water Research Commission, Pretoria.

Shih, C.I.T., Chen, H.Y., Wang, C.J. and Chang, H.Y. (1994) Life history, feeding and oviposition preferences of water hyacinth weevils, *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae). *Chinese Journal of Entomology* **14:** 207–216. (in Chinese).

Simenstad, C.A., Cordell, J.R., Tear, C., Tear, L., Weitkamp, L.A., Paveglio, F.L., Kilbride, K.M., Fresh, K.L. and Grue, C.E. (1996) Use of Rodeo and X-77 spreader to control smooth cordgrass (*Spartina alterniflora*) in a southwestern Washington estuary: 2. Effects on benthic microflora and invertebrates. *Environmental Toxicology and Chemistry* **15**(6):969-978.

Sinclair, B.J., Vernon, P., Klok, C.J. and Chown, S.L. (2003) Insects at low temperatures: an ecological perspective. *Trends in Ecology and Evolution* **18**(5): 257-262.

Skinner, L.C., Ragsdale, D.W., Hansen, R.W., Chandler, M.A. and Spoden, G. (2006) Phenology of first and peak emergence of *Aphthona lacertosa* and *A. nigriscutis*: Two flea beetles introduced for biological control of leafy spurge, *Euphorbia esula* L. *Biological Control* **37**(3): 382-291.

Skirvin, D.J. and Fenlon, J.S. (2003) The effect of temperature on the functional response of *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Experimental and Applied Acarology* **31:** 37-49.

Snyder, R.L., Spano, D., Cesaraccio, C. and Duce, P. (1999) Determining degree-day thresholds from field observations. *International Journal of Biometeorology* **42:** 177-182.

South African Water Quality Guidelines DWAF (1996). Volume 7: Aquatic ecosystems, 1st Edition.

Soriano, A. and Paruelo, J.M. (1992) Biozones: Vegetation units defined by functional characters identifiable with the aid of satellite sensor images. *Global Ecology and Biogeography Letters* **2**(3): 82-89.

Sosa, A.J., Remse Lenicov, A., Marinani, R. and Cordo, H.A. (2004) Redescription of *Megamellus scutellaris* Berg (Hemiptera: Delphacidae), a candidate for biological of water hyacinth. *Annals of the Entomological Society of America* **97**: 271-275

Sosa, A.J., Cordo, H.A. and Sacco, J. (2007) Preliminary evaluation of *Megamelus scutellarsis* Berg (Hemiptera:Delphacidae), a candidate for biological control of water hyacinth. *Biological Control* **42:** 129–138

Spencer, D.E. and Ksander, G.G. (2004) Do tissue carbon and nitrogen limit population growth of weevils introduced to control water hyacinth at a site in the Sacramento-San Joaquin Delta, California? *Journal of Aquatic Plant Management*, **42:** 45-48.

Stark, J.D. and Goyer, R.A. (1983) Life cycle and behaviour of *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) in Louisiana: A biological control agent of water hyacinth. *Environmental Entomology* **12**(1): 147-150.

StatSoft, Inc. (2001). STATISTICA (data analysis software system), version 6. www.statsoft.com.

Stedinger, J.R. and Shoemaker, C.A. (1985) A stochastic model of insect phenology for a population with spatially variable developmental rates. *Biometrics* **41:** 691-701.

Stewart, C.A., Emberson, R.M. and Syrett, P. (1996) Temperature effects on the alligator weed flea-beetle, *Agasicles hygrophila* (Coleoptera: Chrysomelidae): implications for biological control in New Zealand. *Proceedings of the IX International Symposium on Biological Control of Weeds*, pp. 393-398.

Story, J.M. and Stougaard, R.N. (2006) Compatibility of two herbicides with *Cyphocleonus achates* and *Agapeta zoegana*, two root insects introduced for biological control of spotted knapweed. *Environmental Entomology* **35**: 373-378.

Sutherst, R.W. and Maywald, G.F. (1985) A computerised system for matching climates in ecology. *Agriculture, Ecosystems, and Environment* **13:** 281-299.

Talling, J.F. (1966). The annual cycles of stratification and phytoplankton growth in Lake Victoria (East Africa). *Int. Revue ges. Hydro biol*. **51**: 545-621.

Talling, J.F. and Talling, I.B. (1965). The chemical composition of African Lake Waters.. *Int. Revue ges. Hydro biol*. **50**: 1-32.

Tauber, C.A. and Tauber, M.J. (1981) Insect seasonal cycles: genetics and evolution. *Annual Review of Ecological Systematics* **12:** 281-308.

Tauber, M.J., Tauber, C.A., Nechols, J.R. and Helgesen, R.G. (1982) A New Role for Temperature in Insect Dormancy: Cold Maintains Diapause in Temperate Zone Diptera. *Science* **218**: 690-691.

The Mathworks, Inc. 2000. *Matlab version 6.* The Mathworks Inc., Natick.

Thenkabail, P.S., Enclona, E.A., Ashton, M.S., Legg, C. and De Dieu, M.J. (2004) Hyperion, IKONOS, ALI, and ETM+ sensors in the study of African rainforests. *Remote Sensing of Environment* **90**: 23-43

Thomas, M.B. and Willis, A.J. (1998) Biocontrol – Risky but necessary? *Trends in Ecology and Evolution* **13**(8): 325-328.

Thornton, J.A. and Walmsley, R.D. (1982) Applicability of phosphorus budget models to South African man-made lakes. *Hydrobiologia* **89:**237-245.

Tu, M., Hurd, C. and Randall, J.M. (2001) Weed control methods handbook: tools and techniques for use in natural areas. Wildland Invasive Species Program, *The Nature Conservancy* 7E.1-7E.10.

Turner, M.G., Gardner, R.H. and O'Neill, R.V. (2001) *Landscape ecology in theory and practice – pattern and process.* Springer-Verlag, New York, Inc.

Turner, W., Spector, S., Gardiner, N., Fladeland, M., Sterling, E. and Steininger, M. (2003) Remote Sensing for biodiversity science and conservation. *TRENDS in Ecology and Evolution* **18:** 306- 314

Ueckermann, C. and Hill, M.P. (2001) Impact of herbicides used in water hyacinth control on natural enemies released against the weed for biological control. WRC report No. 915/1/01. Water Research Commission, Pretoria, South Africa.

Ueki, K. (1978) Habitat and nutrition of water hyacinth. *Japan Agricultural Research Quarterly,* **12**(3): 121-27.

Ugerer, M.J., Ayres, M.P. and Lombardero, M.J. (1999) Climate and the northern distribution limits of *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae). *Journal of Biogeography* **26:** 1133-1145.

Van der Heide, T., Roijackers, R.M., Peeters, E.T. and Van Nes, E. (2006) Experiments with duckweed-moth systems suggest that global warming may reduce rather than promote herbivory. *Freshwater Biology* **51**: 110-116.

Van Thielen, R., Ajuonu, O., Schade, V., Neuenschwander, P., Adite, A. and Lomer, C.J. (1994) Importation, release and establishment of *Neochetina spp*. for the biological control of water hyacinth in Benin, West Africa. *Entomophaga* **39:** 179-188.

Van Wyk, E. and van Wilgen, B.W. (2002) The cost of water hyacinth control in South Africa: A case study of three options. *African Journal of Aquatic Science* **27**: 141-149

Venugopal, G. (1998) Monitoring the effects of biological control of water hyacinth using remotely sensed data: A case study of Bangalore, India. *Singapore Journal of Tropical Geography* **19**(1): 92-105

Verma, R., Singh, S.P. and Ganesh Raj, K. (2003) An assessment of changes in water hyacinth coverage of water bodies in northern part of Bangalore city using temporal remote sensing data. *Current Science* **84**(6): 795-804.

Wagner, T.L., Wu, H.I., Sharpe, P.J.H., Schoolfield, R.M. and Coulson, R.N. (1984) Modeling insect development rates: A literature review and application of a biophysical model. *Annals of the Entomological Society of America* **77**(2): 208-217.

Walmsley, R.D. (2000) Perspectives on eutrophication of surface waters: Policy/research needs in South Africa. WRC report No. KV129/00. Water Research Commission, Pretoria, South Africa.

Wan, M.T, Watts, R.G, and Moul, D.J. (1989) Effects of different dilution water types on the acute toxicity to juvenile Pacific salmonids and rainbow trout of glyphosate and its formulated products. *Bulletin of Environmental Contamination and Toxicology* **43:** 378-385.

Wang, Y., Jaw, C. and Chen, Y. (1994) Accumulation of 2,4-D and glyphosate in fish and water hyacinth. *Water, Air and Soil Pollution* **74:** 397-403.

Westwood, A.H. and Biesboer, D.D. (1986) The influence of glyphosate on endogenous levels of free and conjugated IAA and phenolic compounds in leafy spurge, *Euphorbia esula* L. *American Journal of Botany* **73:** 725

Wetzel, S., Demmers, C. and Greenwood, J.S. (1989) Seasonally fluctuating bark proteins are a potential form of nitrogen storage in three temperate hardwoods. *Planta* **178:** 275-281.

Wiens, J.A. (1989) Spatial scaling in ecology. *Functional Ecology* **3**:385-397

Wilhere, G.F. (2002) Adaptive management in habitat conservation plans. *Conservation Biology*, **16**(1): 20-29.

Williams, A.E., Duthie, H.C. and Hecky, R.E. (2005) Water hyacinth in Lake Victoria: Why did it vanish so quickly and will it return? *Aquatic Botany* **81:** 300-314

Wilson, J.R. (2002) Modelling the dynamics and control of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach. Unpublished PhD thesis. Imperial College London, Silwood Park.

Wilson, J.R., Rees, M., Holst, N., Thomas, M.B. and Hill, G. (2001) Water hyacinth population dynamics. In: Julien, M.H., Hill, M.P., Center, T.D. and Jianqing, D. (eds.) Biological and Integrated Control of Water Hyacinth, *Eichhornia crassipes*. *ACIAR Proceedings* **102**: 96-104

Wilson, J.R., Holst, N. and Rees, M. (2005) Determinants and patterns of population growth in water hyacinth. *Aquatic Botany* **81**(1): 51-67.

Wilson, J.R.U., Ajuonu, O., Center, T.D., Hill, M.P., Julien, M.H., Katagria, F.F., Neuenschwander, P., Njoka, S.W., Ogwang, J., Reeder, R.H. and Van, T.K. (2007) The decline of water hyacinth on Lake Victoria was due to biological control by *Neochetina spp*. *Aquatic Botany*. **87**. 90-93.

Wilson, J.R.U., Rees, M. and Ajuonu, O. (2006) Population regulation of a classical biological control agent in its introduced range: Larval density dependence in *Neochetina eichhorniae*, a biological control agent of water hyacinth *Eichhornia crassipes*. *Bulletin of Entomological Research* **96:** 145–52.

Wojtaszek, B.F., Staznik, B., Chartrand, D.T., Stephenson, G.R. and Thompson, D.G. (2004) Effects of Vision herbicide on mortality, avoidance response, and growth of amphibian larvae in two forest wetlands. *Environmental Contamination and Toxicology* **23**: 832-842.

Woldai, T. (2004) Electromagnetic Energy and Remote Sensing In: Kerle, N., Janssen, L.L.F. and Huurnemon, G.C. (eds.) *Principles of Remote Sensing – An introductory textbook, 3rd Edition*. The International Institute for Geo-Information Science and Earth Observation (ITC).

Wright, A.D. and Bourne, A.S. (1990) Effect of 2, 4-D on the quality of water hyacinth as food for insects. *Plant Protection Quarterly* **5**: 139-141.

Wright, A.D. and Stegeman, A. (2002). Personal Communication. In: *CSIRO Entomology*, Brisbane.

Xie, Y., Wen, M., Yu, D. (2004) Growth and resource allocation of water hyacinth as affected by gradually increasing nutrient concentrations. *Aquatic Botany* **79:** 257-266.

Zalom, F.G., Goodell, P.B., Wilson, L.T., Barnett, W.W. and Bentley, W.J. (1983) Degree-days: The calculation and use of heat units in pest management. Division of Agriculture and Natural Resources. University of California. Leaflet 21373.

Zilahi-Balogh, G.M.G., Salom, S.M. and Kok, L.T. (2003). Temperature-dependent development of the specialist predator *Laricobius nigrinus* (Coleoptera: Derodontidae). *Environmental Entomology* **32**(6): 1322-1328.

Zimmermann, H.G. and Neser, S. (1999) Trends and prospects for biological control of weeds in South Africa. *African Entomology Memoir* **1**: 165-173.

Webpages

http://www.csir.co.za/plsql/ptl0002/PTL0002_PGE100_LOOSE_CONTENT?LOOSE_PAGE_NO=7141747 (Accessed on 20 April 2006)

http://www.spot.com/html/SICORP/ (Accessed on 20 April 2006)

http://earth.google.com/

www.yorku.ca/eye/spectra.htm (Accessed on 25 February 2006)

http://www.spotimage.fr/html/_167_.php (Accessed on 20 April 2006)

http://edc.usgs.gov/products/satellite/tm.html#description (Accessed on 15 October 2006)

www.sabcnews.com/ (Accessed on 17 October 2006)

http://carbon.cudenver.edu/~lsherry/rem/ancova.html (Accessed on 23 October 2006)

http://www.landinfo.com/satprices.html (Accessed on 15 October 2006)

http://edc.usgs.gov/products/satellite/eol.html.prices