

**The effect of nutrient rich water on the biological control of water hyacinth**

**by**

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**Submitted in partial fulfillment of the requirements of the degree**

**M.Inst. Agrar (Sustainable Insect Management)**

**in the Faculty of Natural & Agricultural Sciences.**

**University of Pretoria**

**Pretoria**

**June 2002**

"I declare that the dissertation, which I hereby submit for the degree M. Inst. Agrar.  
(Sustainable Insect Management) at the University of Pretoria, is my own work and has  
not previously been submitted by me for a degree at another university."



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## Introduction

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Poir.) B. & P., is native to South America, probably as an ornamental plant in the early 1900s (Hill & Clifton 1999). It has established throughout the country and is now considered to be the most serious weed in South Africa (Hill & Clifton, 1999). In 1973, biological control was initiated with the importation and the release of the first natural enemy of water hyacinth, the Australian weevil *Neochlamisus bebbianae* (Coleoptera: Curculionidae), attacking water hyacinth (Hill & Clifton 1999). This programme was terminated in 1977 but resumed in 1981 (Hill & Clifton 1999). Since then a further five natural enemy species have been released and are being evaluated.

## The effect of nutrient rich water on the biological control of water hyacinth.

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### Abstract

The effect of nutrient rich water on the biological control of water hyacinth was investigated at a field site and under laboratory conditions. Water hyacinth was sampled at three sites at Hammarsdale Dam (Kwa-Zulu Natal Province, South Africa) over an eighteen month period. Several plant growth and insect population parameters were measured. Water samples were analyzed for nitrate, nitrite (N) and phosphorus (P) concentrations. Daughter plant production and plant weight varied significantly at the sites. These differences in plant growth parameters correlated with both N and P concentrations in the water and biological control agent activity on the plants. A laboratory study in which three of the biological control agent species were individually exposed to plants growing in three different nutrient concentrations showed that although there was a significant difference between the insect treatments and the controls there was no significant difference between the different nutrient concentrations. This was ascribed to the nutrient concentrations being too high in the laboratory study. The impact of eutrophication on biological control of water hyacinth warrants further investigation.

### Introduction

Water hyacinth, *Eichhornia crassipes*, (Mart.) Solms-Laubach (Pontederiaceae), was introduced to South Africa, probably as an ornamental plant in the early 1900s (Cilliers, 1991). Since then it has established throughout the country and is now considered to be the most important aquatic weed in South Africa (Hill & Cilliers, 1999). In 1973 a biological control programme was initiated with the importation and the release of the first natural enemy, the weevil *Neochetina eichhornia* Warner (Coleoptera: Curculionidae) attacking water hyacinth (Cilliers, 1991, Hill & Cilliers 1999). This programme was terminated in 1977 but restarted in 1985 (Cilliers, 1991). Since then a further five natural enemy species have been released and have become established

in South Africa (Table 1). Biological control is regarded as the best long term, cost-effective and sustainable control option for water hyacinth in South Africa (Hill & Cilliers, 1999).

Despite considerable resources allocated to the water hyacinth biological control programme in South Africa, the results have been variable. Success has been achieved in some areas e.g. New Years Dam near Alicedale, Eastern Cape, where there was an 80% reduction in the population of water hyacinth some four years after the introduction of *Neochetina eichhorniae* and *N. bruchi* (Hill & Cilliers, 1999). However in other areas in the Western Cape Province, that are prone to seasonal flooding, mechanical removal of the weed and eutrophication has hampered the biological control of the weed (Hill & Cilliers, 1999). This contrasts the situation elsewhere in the world where effective biological control has been achieved, relatively quickly (less than 5 years) using only the two weevil species (e.g. Lake Victoria (Ochiel *et al.*, 2001) and Papua New Guinea (Julien & Orapa, 1999).

According to Hill & Olckers (2001) this variation in effectiveness of biological control of water hyacinth could be ascribed to:

- i) Trying to control the weed in high elevation areas characterized by cold winters in which the biological control agents are limited to a six month growth period (most of the examples of successful biological control comes from tropical areas). Currently there is research focussed on the thermal tolerance of the biological control agents released in South Africa with a view to import more cold adapted species (J.Coetzee, University of Witwatersrand, Pers.com.).
- ii) The interference from herbicide control programs. Ueckerman & Hill (2000) showed that some of the herbicides used to control water hyacinth in South Africa are toxic to the insects released as biological control agents. In addition, large-scale herbicidal application causes water hyacinth mats to collapse, decimating agent population.
- iii) The fact that many of the aquatic ecosystems in South Africa are enriched with nitrates and phosphorus that enhance water hyacinth growth.

Nutrient enriched water results in the stimulation of an array of symptomatic changes among which are the increase in the growth of algae and macrophytes and the deterioration of water quality (Bartsch, 1972). The nutrient enrichment of surface water is recognized as a global problem, most often associated with highly populated and developed areas with agricultural practices like fertilization, cattle dung and other nutrient rich practices that enrich the soil and



water and urban runoff. All of these add high levels of nutrients to water resources (United States Environmental Protection Agency (EPA) 1999, European Environmental Agency (EEA), 1998). The impacts of aquatic eutrophication on the environment in South Africa, include the increase in salination of surface and groundwater, changes in the ecological habitat, the lowering of aquatic bio-diversity and a general reduction in water quality (Palmer & O'Keeffe, 1990; O'Keeffe *et al.*, 1990; Walmsley, 2000).

In South Africa the potential problems of eutrophication have been under investigation since the Water Research Commission (WRC) initiated the National Institute for Water Research in 1972 (Walmsley & Butty, 1980). Phosphorus (P) and nitrogen (N) (present in water as ammonia, nitrates and nitrites) were identified as the key nutrients in the process of eutrophication. The amount of P present in the water was seen as the dominant factor influencing eutrophication in South Africa (Thornton & Walmsley, 1982). This led to a national standard for discharge of effluent of 1mg / l P effluent in sensitive catchments in 1985. This national standard for effluent discharge was expected to reduce general eutrophication problems in the catchments, but this does not seem to have occurred (Grobler & Silbauer, 1984; Department of Water Affairs and Forestry, 1999 in Walmsley 2000).

The aim of this study was to assess the impact of water quality on the biological control of water hyacinth under field conditions. However, as it is difficult to control all the possible variables that could influence field studies, we also investigated the impact of eutrophication on the biological control of water hyacinth under laboratory conditions.

## Methods

### Field study

#### Site description

The effect of eutrophication on the growth of water hyacinth plants and the effect of the natural enemies at different levels of eutrophication were studied at Hammarsdale Dam (Figure 1.). This dam is situated in a eutrophic catchment of the Sterkspruit River in Kwa-Zulu Natal (KZN) (29°48'18"S 30°39'50"E) (Pearce, 1987). The site is situated about halfway between Durban and Pietermaritzburg in a sub-tropical climate. Temperature and rainfall were measured on a daily basis at the wastewater treatment plant and this information is presented.

Inflow from the wastewater treatment plant on the edge of Hammarsdale Dam that includes the effluent from textile industries and the poultry farms in the area account for up to 90 % of the inflow into the dam in summer and up to 99% in winter (Wastewater treatment plant manager, Pers com., 2000.). These inflows are recognized sources of eutrophication of water bodies all over the world (Walmsley, 2000).

Water hyacinth has covered Hammarsdale Dam since the early 1980s. Natural enemies of water hyacinth were first introduced in 1989, when the two weevil species, *Neochetina eichhorniae* and *N. bruchi* as well as the pathogen *Cercospora piaropi* were introduced. In 1996 the mirid *Eccritotarsus catarinensis* was released. The moth *Niphograpta albiguttalis* and the mite *Orthogalumna terebrantis* were introduced in 1999.

Three sites were chosen on the dam, at the dam wall (site 1), close to the wastewater inlet into the dam (site 2) and upstream of the dam (site 3) (Figure 1). At each of these sites a sample of ten plants were removed, destructively sampled and several plant and biological control agent parameters were measured (Table 2). Samples were taken every six weeks between January 2000 and January 2001 and every four weeks between February 2001 and July 2001. In total there were 15 sampling occasions at Hammarsdale Dam. Water samples were taken at the dam wall (site 1) with every visit. The Department of Water Affairs and Forestry's Institute for Water Quality Studies (DWAF-IWQS) analyzed the samples. Weekly water samples were taken and analyzed by Umgeni Water at or close to the other two sites upstream in Sterkspruit (close to site 3) and at the inflow of the wastewater treatment release (close to site 2). These samples included a full range of nutrients present in the water and the quantities. The results of these samples were made available for the study.

A flood occurred in February 2000, that washed about 50% of the plants over the dam wall. This did not interfere with the sampling, as the plants on the 10-20 meter edge of the dam were undisturbed. Within two months after the flood, the water surface was covered again with water hyacinth plants. pH, N and P were measured for each visit at each site, to determine seasonal fluctuation. The means and standard error were calculated for each plant parameter and biological control enemy parameters (as mentioned in Table 2) for each site, for each sampling occasion.

In order to study the differences in plant growth parameters and insect damage between the three sites all the collected data for each site was grouped per site and a Canonical Variate Analysis



(CVA) was performed to determine the factor (s) in the data that were responsible for the difference. The CVA is also known as a linear discriminant analysis. Canonical Variate Analysis (CVA) is used when it is of more interest to show differences between groups of data rather than individual variables (in this case the grouped site data). The variability in a large number of variables is firstly reduced to a smaller set of variables that account for most of the variability. The new set of variables, called canonical variates, are linear combinations of the original measurements, and are thus given as vectors of loadings for the original measurements. With this approach a set of directions are obtained in such a way that the ratio of between group variability to within group variability in each direction is maximised. In this study the variables were the different plant and insect measurements.

A two-dimensional representation of the mean scores (Figure2) of the sites of the first two canonical variates gave a clear picture of the contrasts or groupings between the three sites at Hammarisdale Dam. The scores found for each of the canonical variates are then correlated with the original variates to find those that are the most important in discriminating between the sites.

When the CVA was done and the variables causing the difference between the sites determined, it was deemed necessary to study the individual variables separately.

A one-way analysis of variance (ANOVA) was used to determine differences between the different plant and insect parameters at the different sites. The plant and biological control agent data (individual parameters) were analyzed using the program GenStat (2000). The data were normal with homogeneous treatment variances, except for the insect counts. Logarithms of the insect counts were used to stabilize the site variances. Site means were separated using Fishers' protected t-test least significant difference (LSD) at the 5 % level of significance, if the F-probability from the ANOVA was significant at 5% (Snedecor & Cochran, 1980).

### Laboratory study

In order to investigate the impact of the varying N and P concentrations in the water on the biological control of water hyacinth, a laboratory experiment was performed. This experiment was conducted in a glasshouse with an average photo-period of 13 hours. Mean minimum temperature was 18°C, with a lowest measurement of 10°C. Mean maximum temperature was 30°C, with a 37°C maximum temperature measured. Five free-floating healthy water hyacinth

plants were placed in each of 12 tubs, measuring 730mm x 520mm x 620 mm in size and were sleeved covered with gauze. Each cage was filled with 30 litres of tap water.

The water was fertilized with: Sasol® (N:P:K) 1:0:1 (with 360 grams of active ingredients per kg) and Kynoch® Superphosphate (10.5 grams of active ingredients per kg) were mixed to obtain a (N:P:K) 1:1:1 ratio, then added to the water at three different concentrations. In the low nutrient treatment 10 g, in the medium treatment 50 g and in the high treatment 100 g of fertilizer mixture were added to the water in each cage. These values were selected after planning and deliberation with specialists in the field of water quality. The idea was that these values would represent eutrophic conditions in the field, similar to what was found at Hammarsdale Dam. Since the water that was used was low in iron, one gram of iron chelate was added to each treatment and control, 12 cages in total. (Refer to Table 5 for the different concentrations used.)

There were three different insect treatments, *Neochetina eichhorniae*, *N. bruchi*, *Eccritotarsus catarinensis* and one control group, without insects and each treatment was replicated three times. For the purposes of this experiment, we considered the insect species separately and we did not investigate combined effects. The insects were stocked at a rate of five adult weevils and twenty mirids per plant. After seven days the water in each cage was cleaned and the tubs were drained, refilled and re-fertilized. Water samples were taken at the beginning of the experiment and weekly intervals prior to the cages being drained. The water was analyzed by DWAF-IWQS.

Prior to the introduction of the insects, the following plant growth parameters were measured: the wet weight, maximum petiole length, root length, leaf 2 petiole length and leaf 2 area. The same measurements were taken on the completion of the experiment after four weeks. The insect feeding damage was also recorded at the same time. This experiment was replicated on three occasions.

## Results

### Field study (Hammarsdale Dam)

A biological control programme was initiated at the dam in 1989. It was thought to be an ideal site for biological control because of the favourable climate and the absence of herbicidal and mechanical control. The natural enemies, *Neochetina eichhorniae*, *N. bruchi* and the mirid *Eccritotarsus catarinensis*, have become well established at the dam. The impact of the natural enemies is visible on every plant, but has not been quantified before this study.



The area has an annual rainfall of over 800 mm per year. The mean minimum temperature for the year 2000 was 13°C, with the lowest recorded temperature of 6°C in May 2000. The mean maximum temperature for the same year was 30°C, with the highest recorded temperature of 36°C in December 2000.

After the eighteen month study period, the plant and natural enemy data for the different sites, were grouped per site. A Canonical Variate Analysis (CVA) determined the variables that were responsible for the difference between the sites. When the data was grouped together the sites were found to be different at the 95% confidence level (Figure 2). The variables that were responsible for the difference between the sites were identified as N ( $r = -0.77$ ) and pH ( $r = -0.66$ ) for CV1 and P ( $r = +0.80$ ) and to a lesser extent daughter plants ( $r = 0.52$ ) with CV2, as all these correlate strongly with the scores (Figure 2). The values between brackets indicate how strong the correlation is, while the + or - indicate the linear direction of the correlation. A plot of these two canonical variates (= variable) mean scores shows the grouping of the sites.

The level of N at Hammarsdale Dam was different at the three sites, throughout the study period (Figure 4 and Table 3a). The lowest average (eighteen month period) was 0.418 mg/l at site 1 and the highest average was 1.561 mg / l at site 2. At site 3 the average was 0.968 mg / l. According to the South African Water Quality Guidelines (SAWQG) (DWA, 1996) site 1 would be categorized as oligotrophic, defined as a low nutrient level present with no water quality problems (Walmsley, 2000). While site 2 and 3 would be categorized as mesotrophic that is defined as intermediate levels of nutrients present, with emerging signs of water quality problems (Walmsley, 2000). The definition of Walmsley (2000) suggests no or little water quality problems, yet there is an abundance of water hyacinth. This disparity is due to the ability of the plant to utilize nutrients effectively and filtering the N from the water (Musil, 1977; Reddy *et al.* 1989). The high average N at site 2 is ascribed to the effluent being pumped into the dam from the waste water treatment plant close to where the sampling was done. The N level at site 3 is ascribed to water flowing into the dam, from the town of Mpumalanga's waste water treatment plant and commercial agriculture, 5- 10 km upstream (Grobler & Silbauer, 1984).

The measured levels of P are also highest at site 2 (average of 0.731mg / l) (Table 3a; Figure 5) due to treated effluent being pumped in to the dam, close to site 2. According to SAWQG (DWA, 1996) site 1 is eutrophic, defined as a site with high levels of nutrients present in the

water and an increased frequency of water quality problems. Sites 2 and 3 are considered to be hypertrophic and are defined as water with excessive levels of nutrients where plant production is governed by physical factors and water quality problems are almost continuous (Walmsley, 2000).

The N:P ratio is important for the growth of water hyacinth, the phosphorus uptake is in direct proportion to N availability (Reddy *et.al.* 1990). The N:P ratio at the three sites were 1:0.412 for site 1; 1:0.468 for site 2 and 1:0.214 for site 3. The levels of N and P are more than enough to satisfy the minimum requirements for the growth of water hyacinth as evidenced by the luxuriant growth of all the plants at all 3 sites (Figures 6, 7, 8, 9 and 10).

The three sites differed significantly in terms of mean plant wet weight (figure 6) while they show differences in regard to the mean area of leaf 2 (figure 9) and daughter plant production (figure 10), indicating best plant growth at site 2. This shows a good correlation with the N and P levels that also differed significantly between the sites with site 2 having significantly higher levels of both nutrients (Figures 3 to 12).

While the plants were heaviest at site 2, the insect activity was significantly lower at this site. There were more *Neochetina eichhorniae* present than *N. bruchi* at all three sites and more weevils were recorded at site 3 than at site 1 and 2 (Table 3b and Figure 11).

With the onset of spring (September / October) and an increase in temperature, it is expected that water hyacinth plants will grow throughout summer, until the onset of autumn (June) and then halt growth through winter (Julien *et al*, 2001). This corresponds with what was found at Hammarsdale Dam. The wet weight (Figure 6), maximum petiole length (Figure 7), area of leaf 2 (Figure 9) are highest in late summer and lowest in late winter /early spring. The mirid population follows the same trend, showing that the mirid population probably corresponds to the change in season and are linked to temperature. The weevil population, measured by the number of feeding scars present on leaf 2 has a peak in late summer, which also indicate the importance of temperature on insect population. The number of feeding scars is higher throughout the year at site 3 (Figure 11), indicating the presence of a more stable insect population.

Mean number of daughter plants (Figure 10) produced per plant is at its highest in late winter (August) and at its lowest in summer (January). At site 3, in summer (Figure 10) when the insect



populations (Figure 11) are highest, there were no daughter plants present, even with the nutrient rich waters, while there were daughter plants at the other sites. The larvae of the weevils burrow in to the crown of the plant and prevent daughter plant development (Pers.com. T. Center) explaining the lack of daughter plants when weevil population are high. Root length (Figure 8) showed a maximum peak in winter and a minimum peak in late summer, this corresponds with the N and P availability in the water (Figure 4 and 5).

### Laboratory study

Water hyacinth growth responds positively up to the level of 5.5mg / l N and 13.8 mg / l P (Reddy *et.al.* 1989 and Reddy *et. al* 1990). Higher than these levels the plants does not show significant increase in biomass production (Reddy *et.al.* 1989 and Reddy *et. al* 1990). Throughout the trial the concentration of nutrients being used exceeded these levels (Table 5) and the plant growth at the different nutrient concentrations showed no significant difference in growth (Tables 4a and 4b). The only variable that showed any difference was the production of daughter plants, where the low nutrient level showed the highest production of daughter plants and the high concentration showed the lowest. Reddy *et.al.* (1989) and Reddy *et.al* (1990) found that very high levels of N and P can have a detrimental effect on the growth of the plants and the production of daughter plants.

When compared to the control there is a clear indication that the presence of the insects (weevils and mirids) had a definite impact on all the plant growth parameters measured (Table 4b). The two weevil species, when compared to the control, showed significant impacts on the maximum petiole length, root length, leaf two petiole length, the number of leaves produced, and the area of leaf 2. This is consistent with what other research has shown (e.g. Center 1987, Center & Spencer 1981, Center & Van 1989 and Center *et. al.* 1999a). The impacts of the adult mirids, when compared to the control, were significant on plant growth (root development, number of leaves and the area of leaf two), over a one month period of study. Indicating the ability of the mirid to stress the water hyacinth plant.

This trial will be redone with water quality levels that are indicative of field conditions in South African rivers and dams.



## Discussion

The growth of water hyacinth is correlated to the availability of nutrients in the water. The more nutrients, especially N and P, available the more luxuriant the growth of the plant (Reddy *et.al.* 1989; Reddy *et. al.* 1990 and Gopal, 1987). Under ideal situations the time to double the biomass of the water hyacinth plant is 6 – 14 days and as soon as the available water surface is covered, the plants start to compete for available resources, like sunlight and they become taller (Penfound & Earl, 1948).

These sites were statistically different according to the Canonical Variate Analysis and this was ascribed to N, pH and P. The general trend for Hammarsdale Dam was that the site with the highest nutrient concentration (site 2) had the heaviest plants that grows the best. While the sites with the recorded highest insect activity (site 3) had the smallest plants. However it is difficult to separate nutrient effects from the insect effects. The field study was done with no control., complicating matters even more. However, the laboratory study showed that even when there are very high levels of nutrients in the water, the insects are able to suppress plant growth. It is however difficult to predict what the plants at Hammarsdale Dam might look like in the absence of the bio-control agents.

The biological control programme at Hammarsdale Dam has not yielded good results. The three natural enemies (*Neochetina eichhornia*, *N. bruchi* and *Eccritotarsus catarinensis*) that are well established and abundant during 10 months of the year, did not reduce the plant population below 100% cover of the dam. Situated in the sub-tropics, with relatively warm winters the expectation was that after 5 years there would be a reduction in the weed population (Julien, 2001).

We assumed that Hammarsdale Dam was a highly eutrophic site and yet the results of the water chemistry analysis showed that the levels of N were not excessive, while the levels of P were very high. Water chemistry analysis need to be interpreted with care as they offer only one sample in every period (month), while water quality changes on an hourly basis. Water resource managers would be better served to use biotic indicators for the water quality (e.g. the growth of aquatic macrophytes or benthic invertebrate diversity and abundance). These indicators would have to be correlated before they could be implemented.

At site 1 the plants were more prone to be flushed out of the dam at the slightest increase of water level and at site 2 there was a steady flow of effluent water being pumped into the dam, moving

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Mipidae	<i>Eurichorda eichhorniae</i> Curvalles	Water hyacinth sap-sucking bug	1996
Acantho- Colombidae	<i>Orthogalium laticornis</i>	Water hyacinth root	1996
Psyllids	<i>Cercospora perniciosa</i> & <i>C. malinaria</i>	Cercospora	1996

Table 1. The different parameters of the water hyacinth plant and the biological control insects collected at Hammarsdale Dam per individual plant, per sample.

Plant parameters	Biological control insect parameters
Wet weight	Number <i>Neochetina eichhorniae</i> and <i>N. bruchi</i> present, male and female
Maximum petiole length	Presence of the <i>Neochetina eichhorniae</i> and <i>N. bruchi</i> larvae
Leaf length	Number of weevil feeding scars on leaf 3
Leaf 2 petiole length	Number of <i>E. crassipes</i> third instar on leaf 3
Area of leaf 2	Number of <i>E. crassipes</i> nymphs
Number of daughter plants	Number of <i>E. crassipes</i> adult
Number of petioles above water surface	Number of <i>Orthogalium</i> on leaf 4
Number of petioles below water surface	The estimated percentage of the root damage by the mite



**Table 1:** Natural enemy species introduced on water hyacinth, into South Africa.

Family	Species	Common name	Year of introduction
Curculionidae	<i>Neochetina eichhorniae</i> Warner	Water hyacinth weevil	1974
Curculionidae	<i>Neochetina bruchi</i> Hustache	Chevroned hyacinth weevil	1990
Pyralidae	<i>Niphograpta albiguttalis</i> Warren	Water hyacinth moth	1990
Miridae	<i>Eccritotarsus catarinensis</i> Carvalho	Water hyacinth sap-sucking bug	1996
Acarina: Galumnidae	<i>Orthogalumna terebrantis</i> Wallwork	Water hyacinth mite	1989
Pathogen	<i>Cercospora piaropi</i> & <i>C. rodmanii</i> Tharp	Cercospora	1986

**Table 2:** The different parameters of the water hyacinth plant and the biological control insects collected at Hammarsdale Dam per individual plant, per sample.

Plant parameters	Biological control insect parameters
Wet weight	Number <i>Neochetina eichhorniae</i> and <i>N. bruchi</i> present, male and female
Maximum petiole length	Presence of the <i>Neochetina eichhornia</i> and <i>N. bruchi</i> larvae
Root length	Number of weevil feeding scars on leaf 2
Leaf 2 petiole length	Number of <i>E.catarinensis</i> frass marks on leaf 3
Area of leaf 2	Number of <i>E.catarinensis</i> nymphs
Number of daughter plants	Number of <i>E.catarinensis</i> adult
Number of petioles above water surface	Number of <i>O.terebrantis</i> on leaf 4
Number of petioles below water surface	The estimated percentage of the leaf damaged by the mite

**Table 3a** : One-way analysis of variance (ANOVA) on the plant parameters and pH, N and P for the three sites at Hammarsdale Dam.

Site	Mean wet weight per plant (kg)	Mean maximum petiole length per plant (mm)	Mean root length per plant (mm)	Shoot: Root ratio per plant	Petiole leaf 2 per plant (mm)	Leaf 2 area per plant	Daughter plants per plant	pH	N mg/l	P mg/l
1	0.716 b	573.3 a	322.7 b	1: 1.901a	402.5a	125.6 a	2.487 a	7.123 c	0.418 c	0.399 b
2	0.835 a	589.4 a	324.4 b	1: 1.958a	418.1a	129.6 a	2.633 a	7.579 b	1.561 a	0.731 a
3	0.571 c	577.1 a	344.4 a	1: 1.868a	396.3a	97.2 b	0.933 b	7.638 a	0.968 b	0.207 c
SEM *	0.025	9.09	7.12	0.06	13.35	3.44	0.140	0.025	0.054	0.021
F Probability	p< 0.001	p= 0.423	p= 0.057	p= 0.558	p= 0.493	p< 0.001	p< 0.001	p< 0.001	p< 0.001	p< 0.001
LSD (5%) +	0.069	-	-	-	-	3.44	0.390	0.069	0.151	0.060

\*SEM is the standard error of the mean

+ Means were separated using Fishers protected t-test, least significant difference at the 5% level.

Means per column with different letters were significantly different at the 5% level as indicated.

Means per column with different letters were significantly different at the 5% level as indicated.

**Table 3b:** One-way analysis of variance (ANOVA) on the insect parameters for the three sites at Hammarsdale Dam.

Site	Mean number of insects per plant				Number of weevil feeding scars	<i>Eccritotarsus catarinensis</i> frass marks on leaf 3	<i>E. catarinensis</i> nymphs on plant	<i>E. catarinensis</i> adults on plant
	<i>Neochetina eichhorniae</i> Male	<i>N.eichhorniae</i> Female	<i>N. bruchi</i> Male	<i>N. bruchi</i> Female				
1	0.271 b	0.245 b	0.044 c	0.061 b	3.405 b	2.753 b	0.824 b	0.684a
2	0.165 c	0.149 c	0.091 b	0.095 b	2.595 c	2.490 c	0.793 b	0.506 b
3	0.454 a	0.394 a	0.212 a	0.199 a	4.424 a	3.312 a	1.088 a	0.763 a
SEM *	0.064	0.035	0.023	0.024	0.096	0.129	0.094	0.076
F Probability	p< 0.001	p< 0.001	p< 0.001	p< 0.001	p< 0.001	p< 0.001	p= 0.052	p= 0.048
LSD (5%) +	0.101	0.096	0.064	0.065	0.267	0.361	-	0.209

\*SEM is the standard error of the mean

+means were separated using Fishers protected t-test, least significant difference at the 5% level.

Means per column with different letters were significantly different at the 5% level as indicated.



**Table 4a:** One-way analysis of variance (ANOVA) on the different applications of the laboratory study when measuring plant parameters

Concentration	Mean wet weight per plant (kg)	Mean maximum petiole length per plant (mm)	Mean root length per plant (mm)	Petiole leaf 2 per plant (mm)	Number of leaves per plant	Leaf 2 area per plant	Daughter plants per plant	Log of feeding damage #
Low	0.128 a	423 a	471 a	355 a	36.17 a	129.3 a	5.58 a	5.69 a (296)
Medium	0.108 a	417 a	414 a	363 a	35.58 a	107.9 a	3.92 ab	5.89 a (361)
High	0.119 a	433 a	437 a	378 a	36.58 a	114.0 a	3.25 b	5.90 a (365)
SEM *	0.903	22	18.7	24.7	0.802	8.55	0.625	0.183
F Probability	p=0.314	p=0.879	p=0.120	p=0.794	p=0.680	p=0.211	P=0.041	p=0.674
LSD + (5%)	-	-	-	-	-	-	1.833	-
CV%	26.4%	18%	14.7 %	23.4 %	7.7%	25.3 %	51 %	9.4 %
Grand mean	118.3	424	441	365	36.11	117.1	4.25	5.83

# Means were used. Actual values in ( ). Ln Damage:  $y = \ln x$  and transferred value  $x = e^y$

\* SEM is the standard error of the mean

+ means were separated using Fishers protected t-test, least significant difference at the 5% level.

Means per column with different letters were significantly different at the 5% level as indicated.

**Table 4b:** One-way analysis of variance (ANOVA) on the insects parameters of the laboratory study

Natural enemy	Mean wet weight per plant (kg)	Mean maximum petiole length per plant (mm)	Mean root length per plant (mm)	Length, petiole leaf 2 per plant (mm)	Mean number of leaves per plant	Leaf 2 area per plant	Number of daughter plants per plant	Log of damage per plant #
<i>Neochetina eichhorniae</i>	111.6 a	366 b	414 b	302 b	33.33 b	90.8 c	3.78 a	5.33 b (296)
<i>N. bruchi</i>	120.9 a	377 b	460 ab	297 b	35.11 b	92.5 c	3.56 a	5.15 b (172)
<i>Eccritotarsus catarinensis</i>	99.8 a	461 a	401 b	419 a	35.78 b	124.6 b	3.89 a	7.00 a (1097)
Control	140.8 a	494 a	487 a	443 a	40.22 a	160.4 a	5.78 a	No damage
SEM	10.42	25.4	21.6	28.5	0.926	9.87	0.722	0.183
F Probability	P=0.066	p=0.003	p=0.034	p=0.001	p<0.001	p<0.001	p=0.139	p<0.001
LSD (5%)	-	74.5	-	83.5	2.715	28.96	-	0.547
CV%	26.4%	18 %	14.7 %	23.4%	7.7 %	25.3 %	51 %	9.4 %
Grand mean	118.3	424	441	365	36.11	117.1	4.25	5.83

# Means were used. Actual values in ( ). Ln Damage:  $y = \ln x$  and transferred value  $x = e^y$

\*SEM is the standard error of the mean

+ Means were separated using Fishers protected t-test, least significant difference at the 5% level

Means per column with different letters were significantly different at the 5% level as indicated

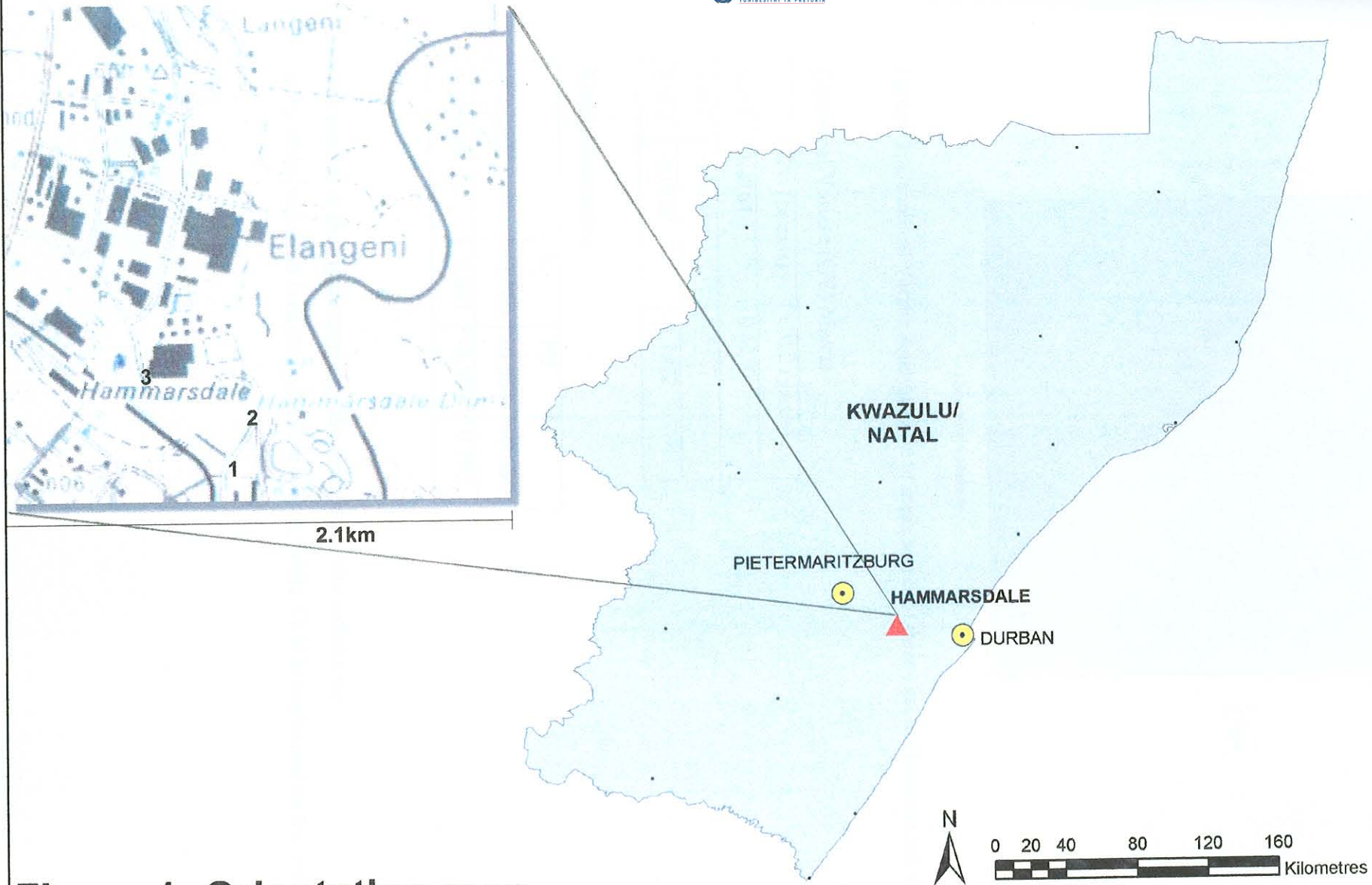
**Table 5: The different concentrations used in the laboratory study and the mean values for the different nutrients throughout the study period.**

Concentration	N (mg/l)	Classification according to Water Quality Guidelines (DWAf, 1996)	P (mg/l)	Classification according to Water Quality Guidelines (DWAf, 1996)
Low	9,59	Eutrophic	20,29	Eutrophic
Medium	51,9	Hypertrophic	132	Hypertrophic
High	101,4	Hypertrophic	241,8	Hypertrophic

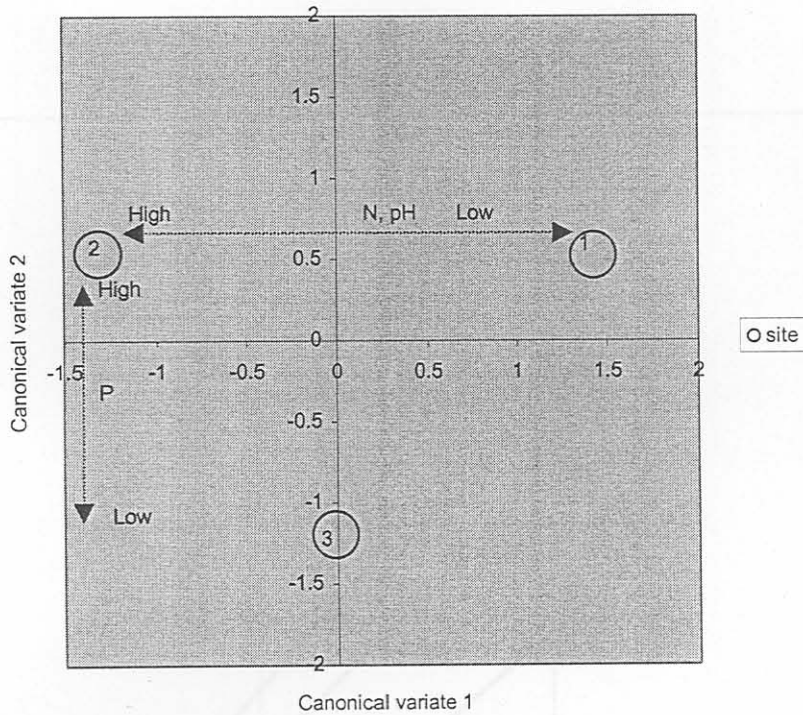


**Figure 1: Orientation map**





**Figure 1: Orientation map**



**Figure 2:** Canonical Variate Analysis, plot of mean scores of three sites at Hammarsdale Dam

#### Canonical Variate (CV) Means

	CV 1(x axis)	CV 2 (y axis)
Site 1	1.4264	0.5250
Site 2	-1.3141	0.5387
Site 3	-0.0122	-1.1972

#### Correlation matrix

	N	PH	P
U scores	-0.769	-0.664	-0.535
U scores	0.061	-0.509	0.795

The arrows in the figure represent the direction of the correlation.

67% of the variation is explained on the x-axis, while 33 % is explained on the y-axis.

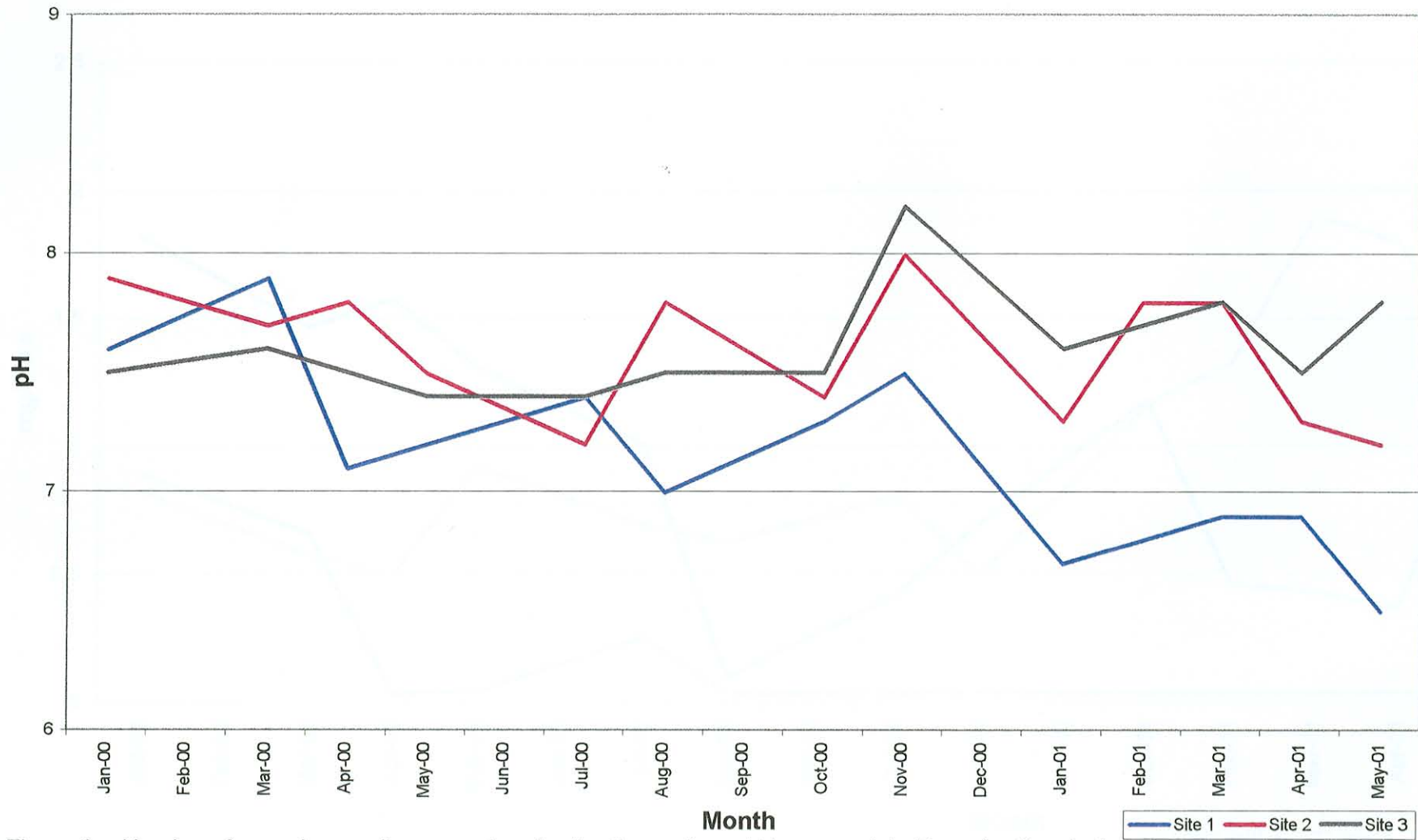


Figure 3: pH values for each sampling occasion, for the three sites at Hammarsdale Dam, for the study period



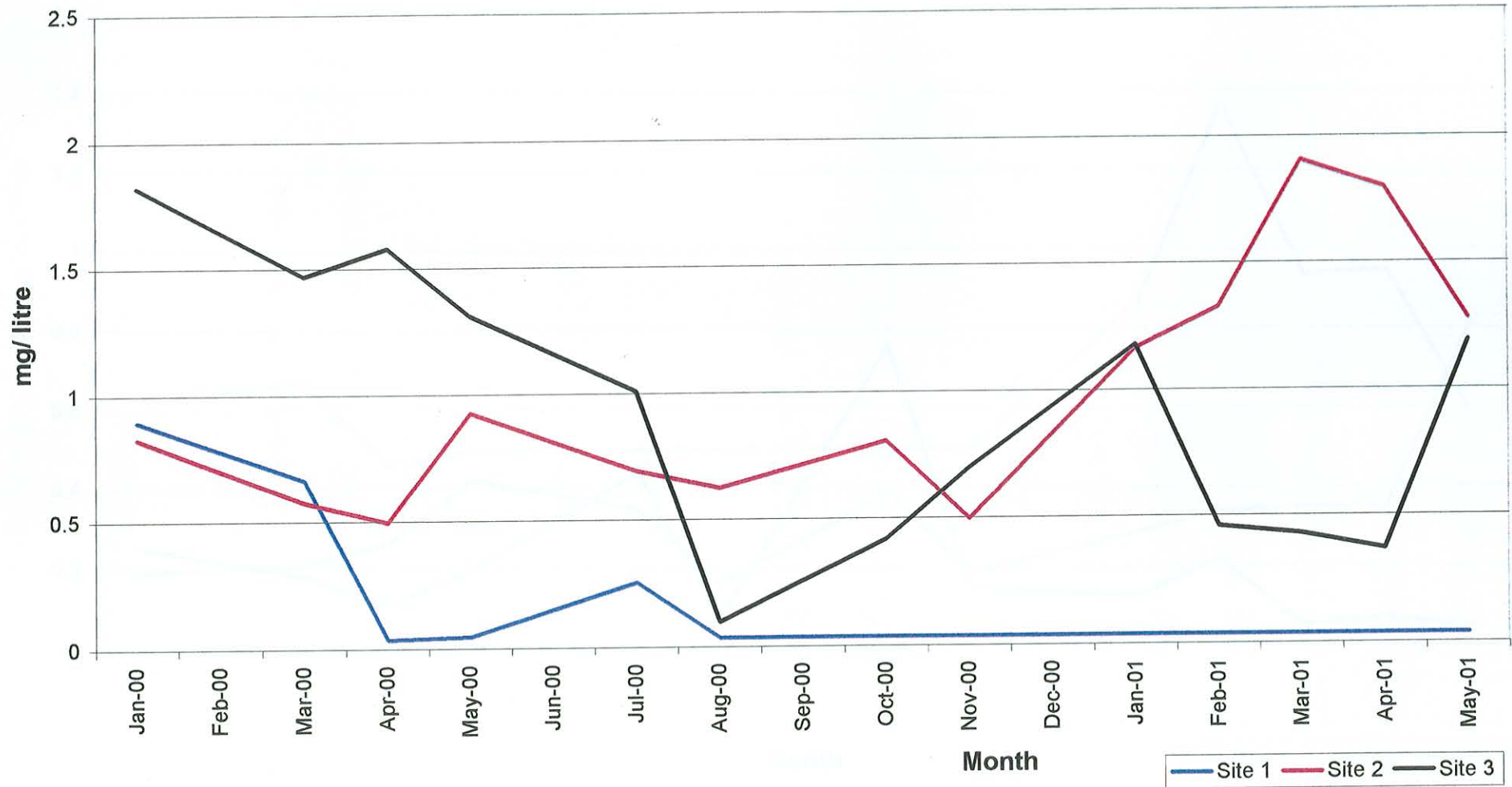


Figure 4: Total N, for each sampling occasion, for three sites at Hammarsdale Dam, for the study period

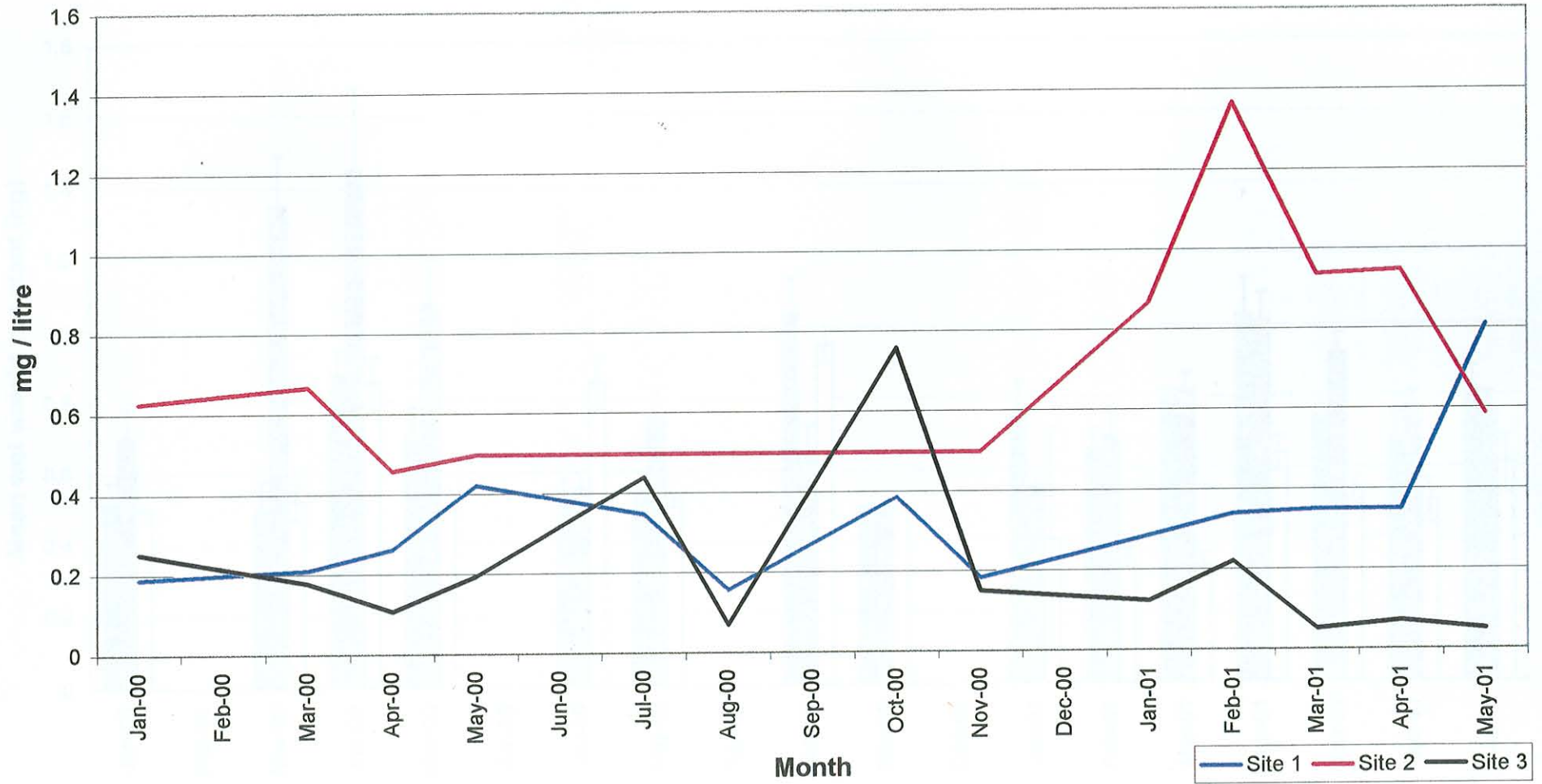


Figure 5: Total P, for each sampling occasion, for the three sites at Hammarsdale Dam, for the study period

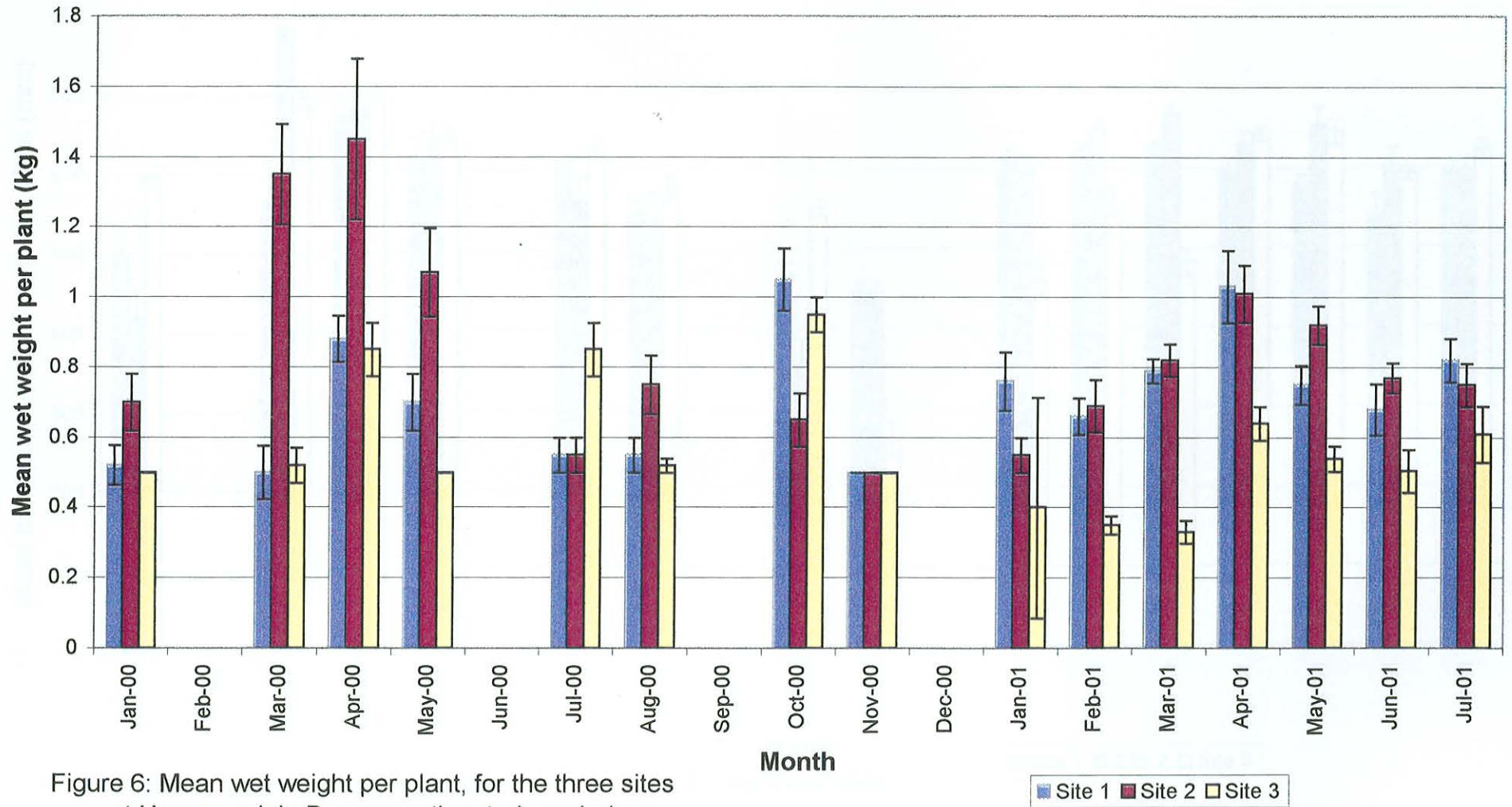


Figure 6: Mean wet weight per plant, for the three sites at Hammarsdale Dam, over the study period



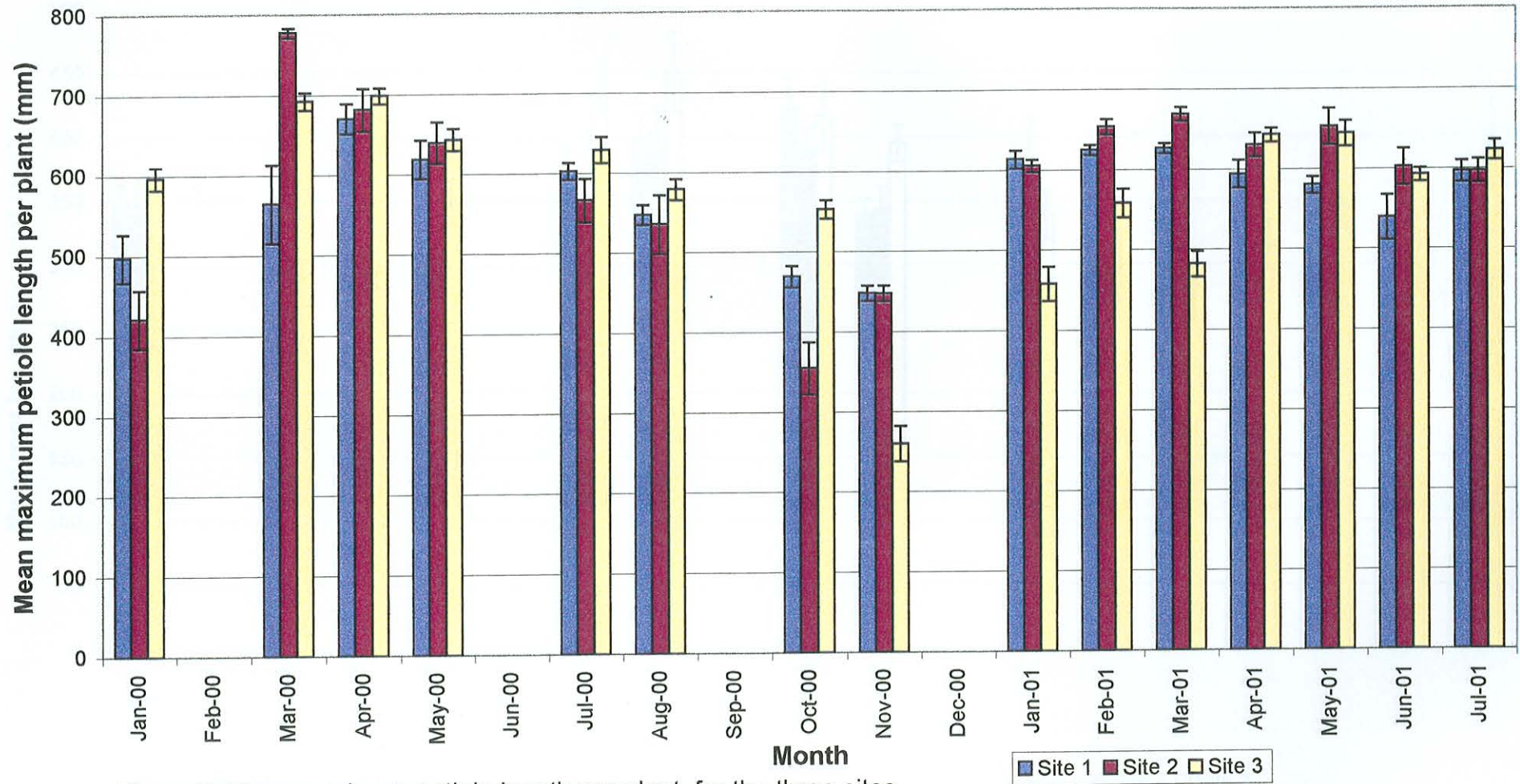


Figure 7: Mean maximum petiole length per plant, for the three sites at Hammarsdale Dam, for the study period

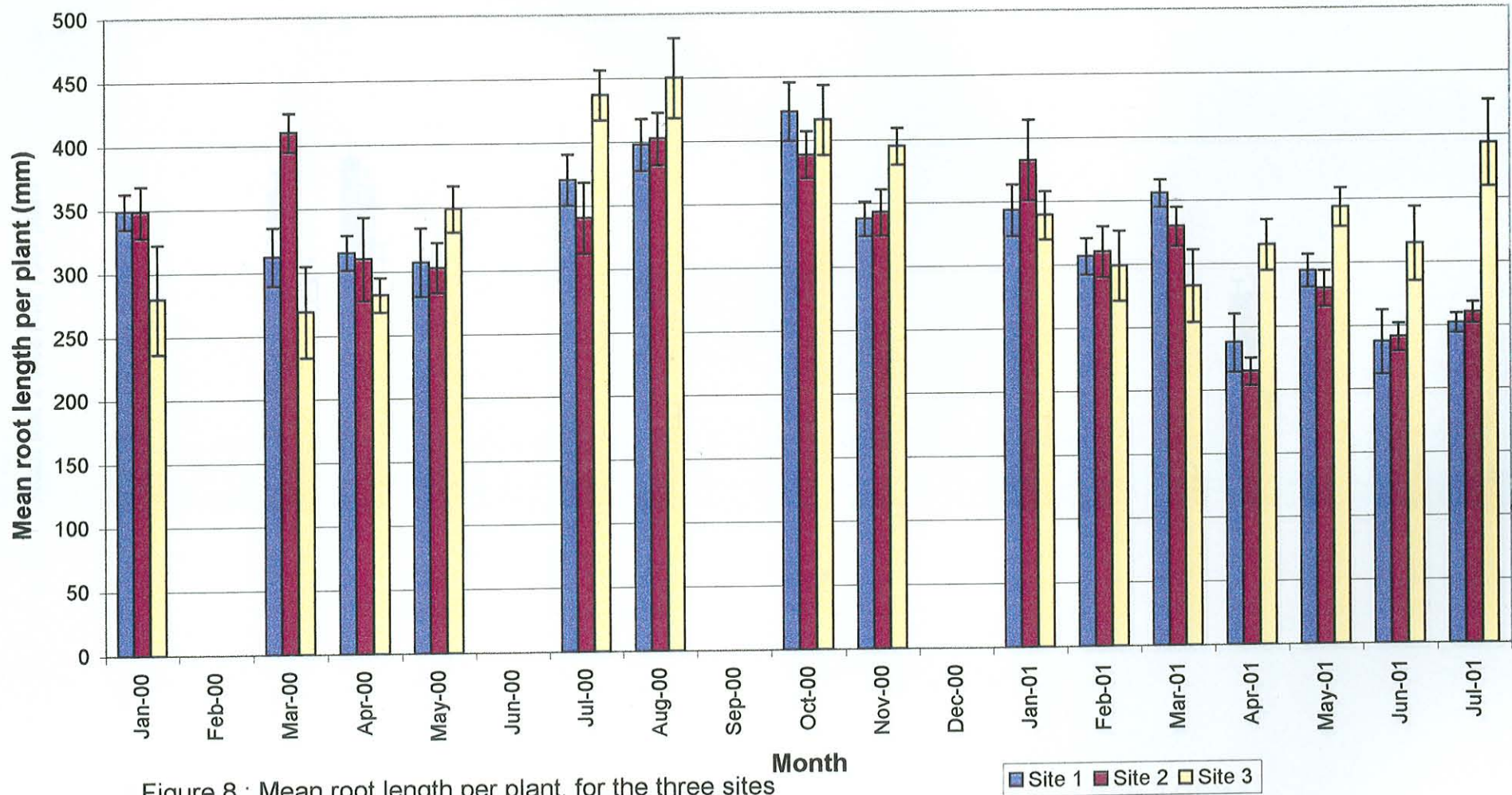


Figure 8 : Mean root length per plant, for the three sites at Hammarsdale Dam, for the study period



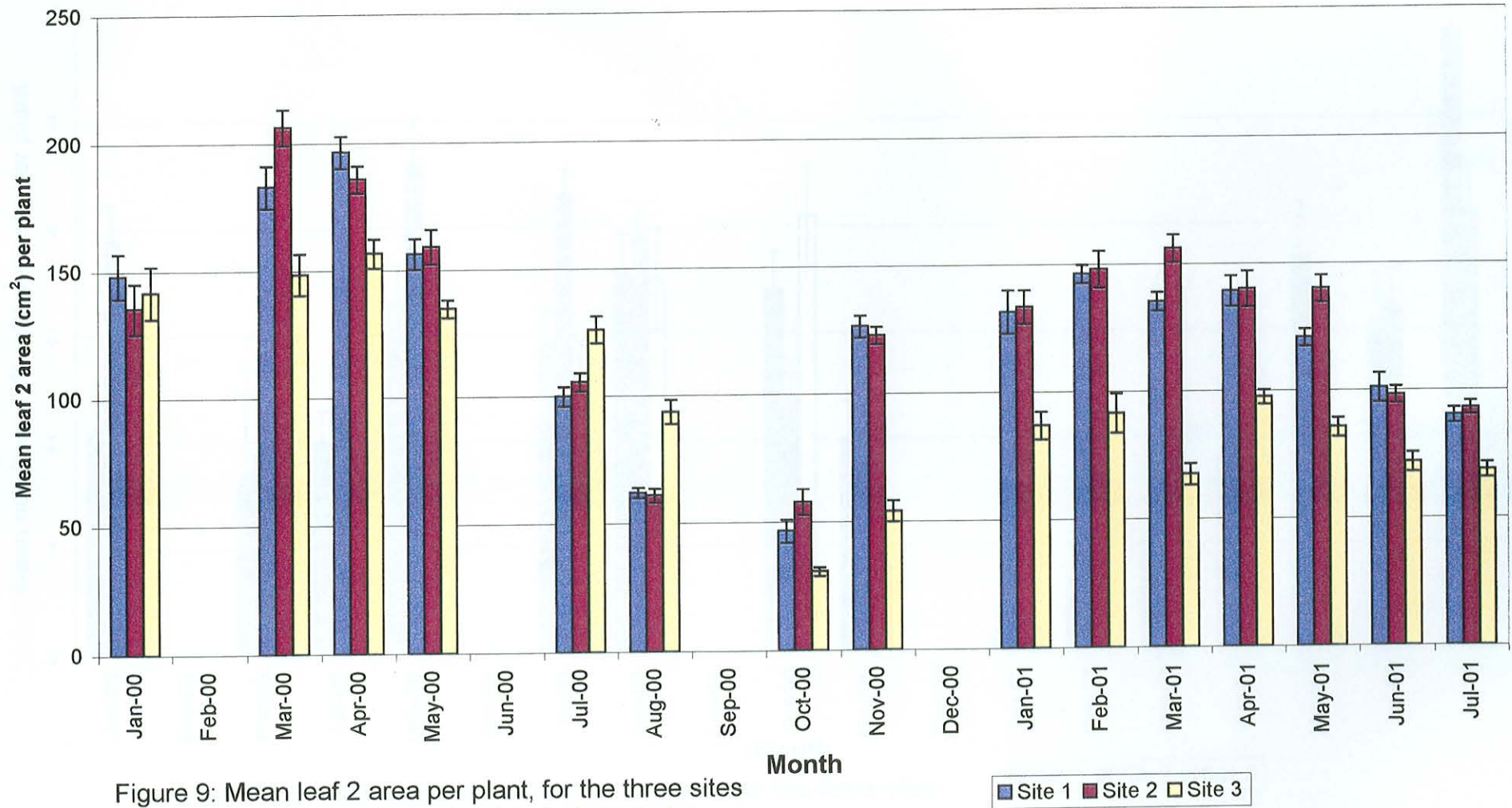


Figure 9: Mean leaf 2 area per plant, for the three sites at Hammarsdale Dam, for the study period



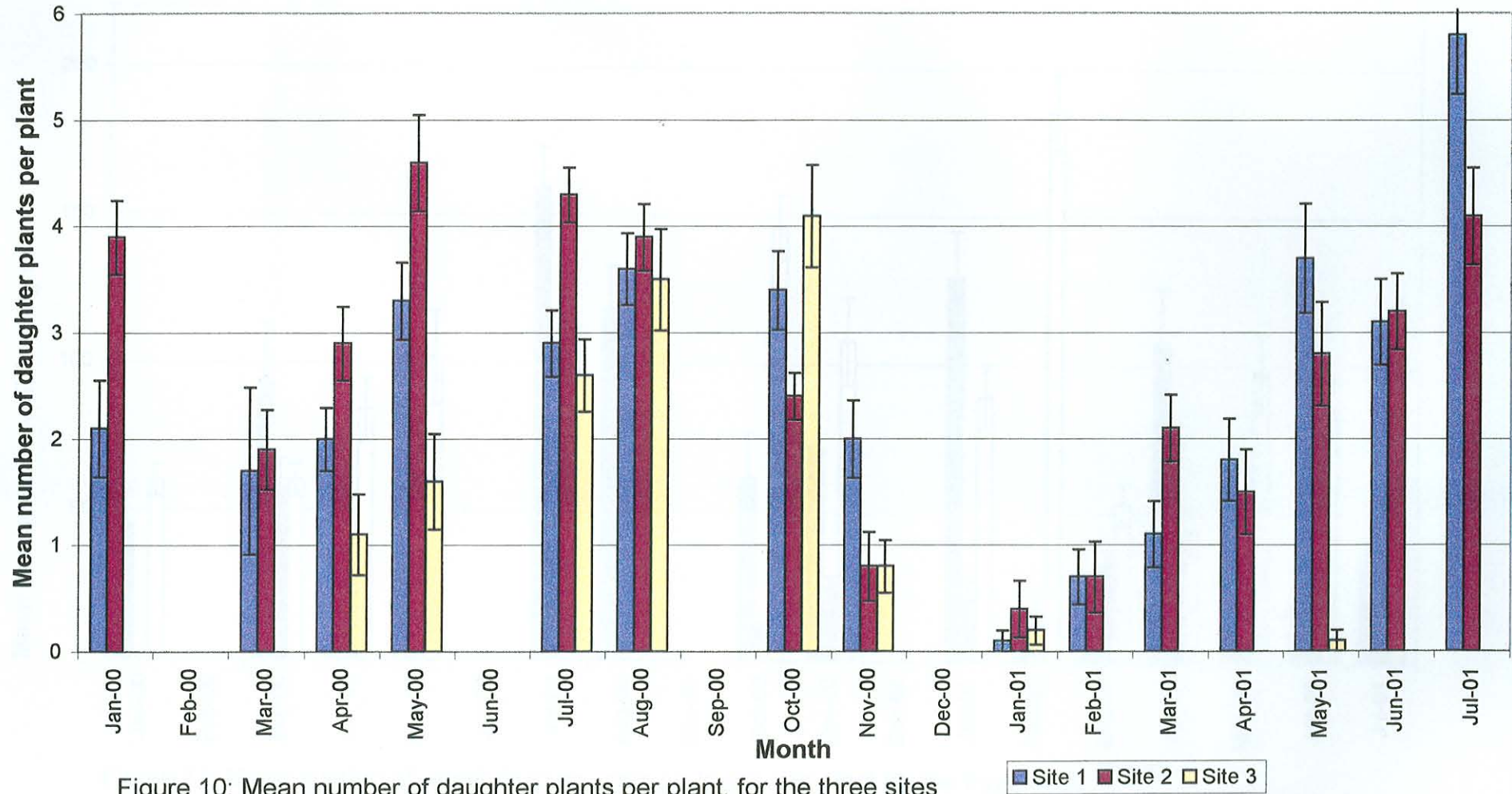


Figure 10: Mean number of daughter plants per plant, for the three sites at Hammarsdale Dam, for the study period

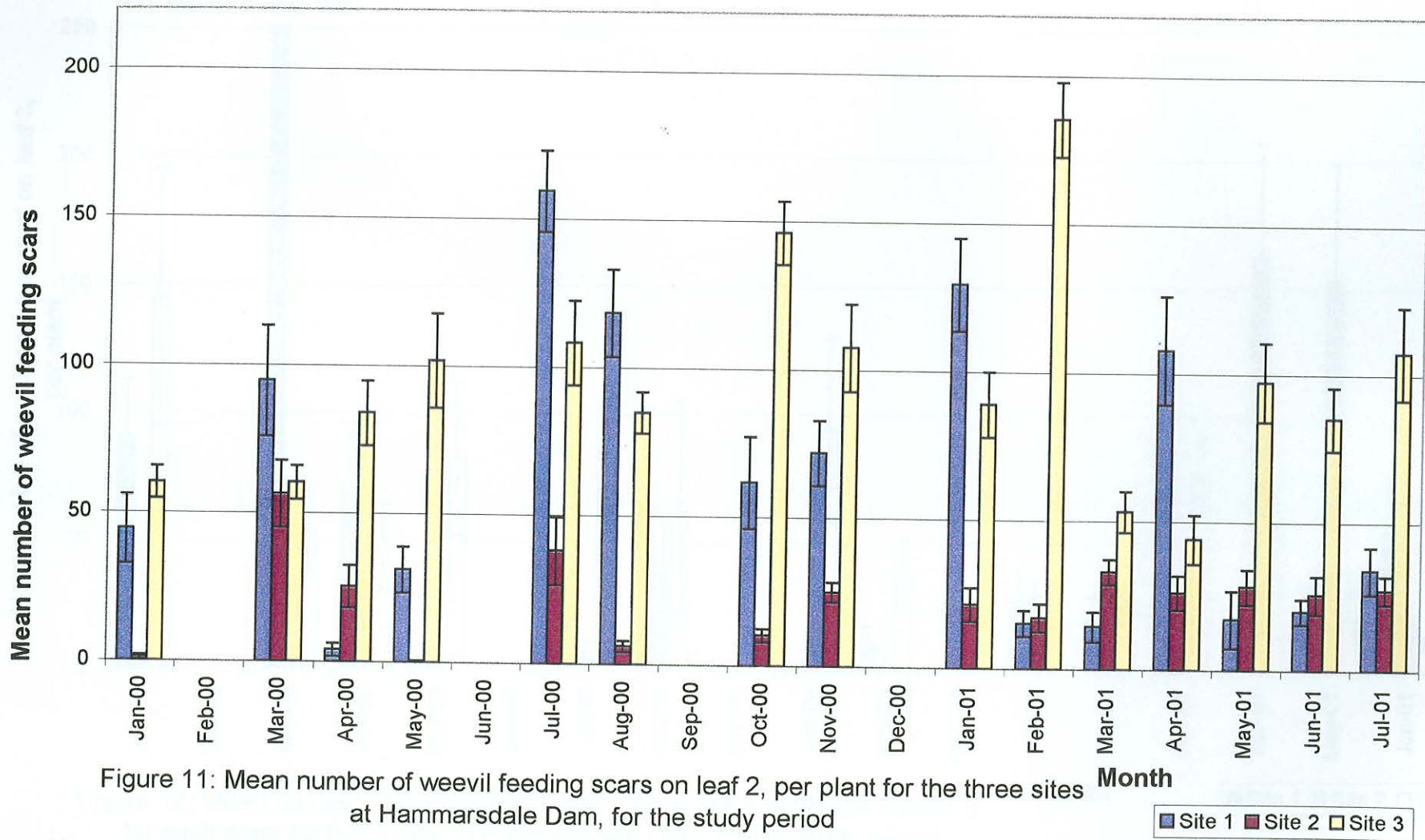


Figure 11: Mean number of weevil feeding scars on leaf 2, per plant for the three sites at Hammarsdale Dam, for the study period

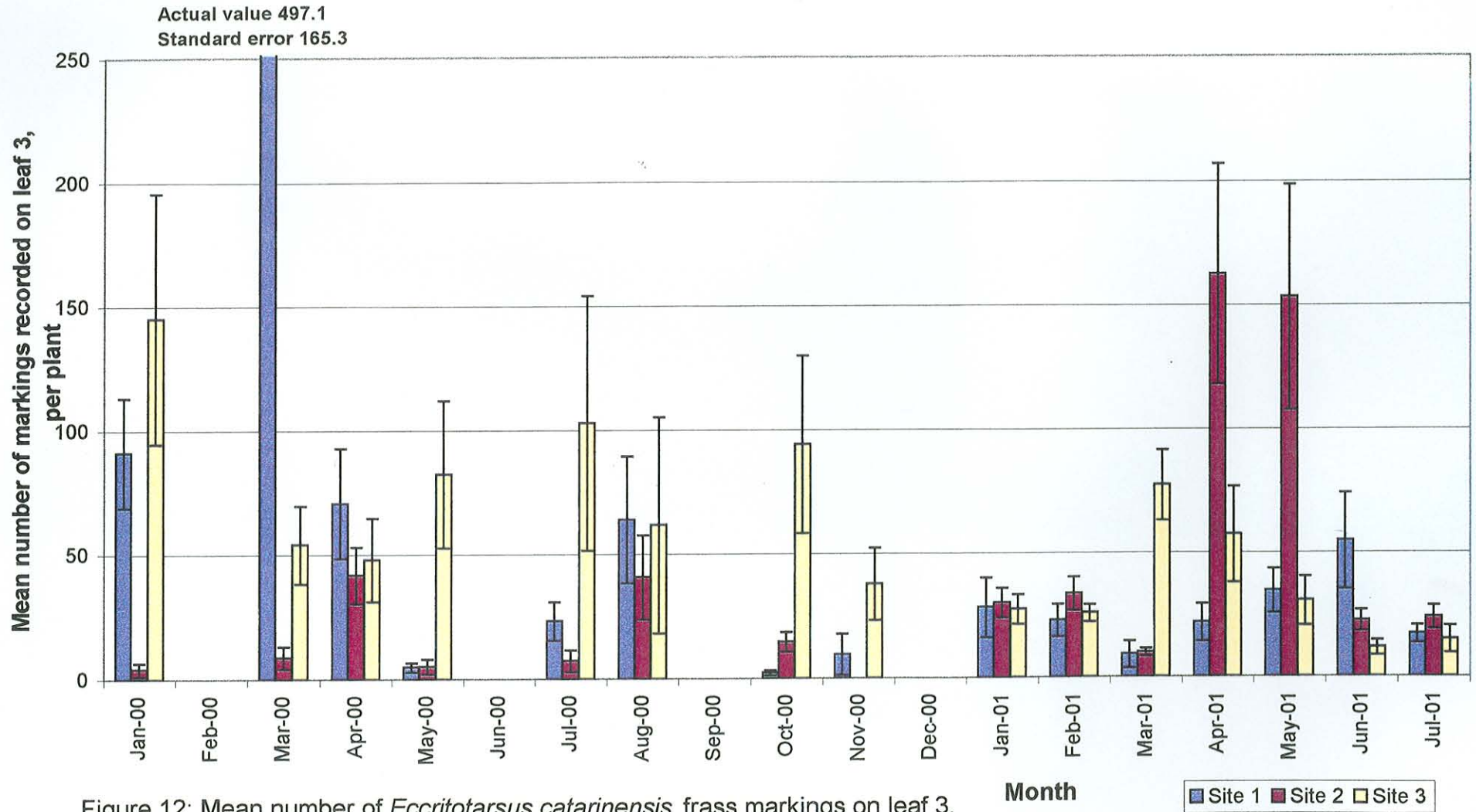


Figure 12: Mean number of *Eccritotarsus catarinensis* frass markings on leaf 3, for each plant for the 3 sites at Hammarsdale Dam, for the study period